

MATING DISRUPTION OF *Cadra cautella* IN THE PRESENCE OF SYNTHETIC SEX PHEROMONE (Z, E)-9, 12-TETRADECADIENYL ACETATE (ZETA)

Sammani AMP, Dissanayaka DMSK, Wijyaratne LKW*, Egodawatta WCP

Department of Plant Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka,
Puliyankulama, Anuradhapura, Sri Lanka

ABSTRACT

The almond moth *Cadra cautella* (Walk.) (Lepidoptera: Pyralidae) is difficult to control using insecticides. Female moth releases a sex pheromone identified as (Z, E)-9, 12-tetradecadienyl acetate (ZETA). Information on population management of *C. cautella* using pheromone is limited. Objectives of this study were to determine the effect of pheromone dose, population size and air flow on mating status of *C. cautella*. The unmated male and female adults were introduced into a cubicle, allowed mating, recaptured, and the females dissected to determine the presence of spermatophores indicative of mating. The moths exposed to pheromone doses had lower mating than untreated control. Mating disruption (MD) was higher at ZETA 4.5 mg/45 mL than other doses. For each population size tested, moths exposed to synthetic pheromone had lower mating than their respective control. Insect population sizes 10 and 20 had higher MD with the natural air flow than 30 moths/3.375 m³. In contrast, under forced air flow, MD was similar across all the population sizes tested. This study reveals that MD using ZETA is possible for controlling *C. cautella*. Future research should address its usage under warehouse conditions having different complexities.

Keywords: Air movement, *Cadra cautella*, Mating disruption, Pheromone dose, Population size

INTRODUCTION

Loss of durable food commodities including cereals, pulses and nuts continues when the yield is stored following harvest to cater the consumer demand at different spatial and temporal profiles. Numerous quantitative and qualitative losses caused by living organisms including insects in raw and processed commodities during different post-harvest operations impose a great challenge for food security (Hill, 1990; Hagstrum and Subramanyam, 2006). Stored-product insects cause 10%-50% direct and indirect losses of grains and grain-based products throughout the world (Wijyaratne *et al.*, 2018). In Sri Lanka, 80% of grain loss during storage is caused by insects (Wijyaratne *et al.*, 2009). The Almond

moth *Cadra cautella* (Lepidoptera: Pyralidae) is a major pest of stored products throughout the warmer parts of the world and in heated stores in temperate regions. The larvae feed on grains, flour, nuts, dried fruits, seeds and other food products causing economic loss (Hill, 1990).

The current control methods for stored-product pests include the use of contact insecticide Pyrethroids (Ghimire *et al.*, 2016) and fumigation with phosphine (Ridley *et al.*, 2011) or sulfuryl fluoride (Fields, 2012). However, resistance development by certain stored-product insect species (Arthur *et al.*, 1988; Opit *et al.*, 2012), adverse effects on non-target organisms and environment (Fields, 1992; Arthur, 1996; Phillips and

*Corresponding author: wollylk@yahoo.com

Throne, 2010; Wijayaratne *et al.*, 2018) impose limitations for the continuous use of synthetic chemical insecticides. Alternatives include use of high (Brijwani *et al.*, 2012) or low temperature (Eliopoulos *et al.*, 2010), hermetic storage (Sanon *et al.*, 2011; Hasaranga *et al.*, 2018; Wijayaratne *et al.*, 2019), modified atmosphere control (Wijayaratne *et al.*, 2009), botanicals (Dissanayaka *et al.*, 2018b), use of safer insecticides alone or in combination with other management practices (Wijayaratne and Fields, 2010; Wijayaratne *et al.*, 2012a,b; Wijayaratne and Rajapakse, 2018; Wijayaratne *et al.*, 2018). Besides, biorational pest management is emphasized in stored product protection (Phillips and Throne, 2010) as alternative to synthetic chemicals.

Pheromones have been identified from over 35 species of stored-product insects. Pheromones, low-molecular-weight volatile organic compounds released by insects, are used in intraspecific communication. These compounds can be used as attractants in traps for detecting and monitoring pests in storage facilities (Phillips, 1997). There is a renewed interest on the use of pheromones in stored-product protection (Burks and Kuenen, 2012; Trematerra, 2012; Campos and Phillips, 2014; Dissanayaka *et al.*, 2018a). The female *C. cautella* adult moth releases a sex pheromone complex including three compounds (Z, E)-9, 12-tetradecadienyl acetate (ZETA), (Z)-9-tetradecadien-1-yl acetate (ZTA) and (Z, E)-9, 12-tetradecadien-1-ol (ZETOH) to attract the males for mating (Kuwahara *et al.*, 1971; Kuwahara and Casida, 1973; Brady, 1973) and the ratio in the natural pheromone blend is 10:5:1 respectively (Read and Beevor, 1976). ZETA attracts the male insects. The ZTA acts as a synergist for ZETA. ZETOH is an inhibitor of the attraction (Read and Haines, 1976). Response by the male moth is directly proportional to the pheromone releasing rate; high releasing rates simply catch more insects than low rates (Vick *et al.*, 1981). However, the upwind flight of male moths towards ZETA can be disrupted by the availability of high doses of synthetic pheromone. This

process is termed mating disruption (MD) (Ryne *et al.*, 2006).

The MD has been successfully used for population management in certain field insects such as the Oriental fruit moth *Grapholita molesta* (Carde and Minks, 1995) and the pink bollworm *Pectinophora gossypiella* (Staten *et al.*, 1987). Certain factors affecting the success of MD have been identified: pheromone formula (Evenden *et al.*, 1999), the number and type of dispensers (Sauer and Karg, 1998; Thorpe *et al.*, 1999) and population dynamics of the target species (Carde and Minks 1995). However, MD of many stored-product moths and the effect of factors on its success remain undiscovered. Therefore, the objectives of current study were to determine the effect of ZETA dose, population size and the air movement on the mating status in *C. cautella*.

MATERIALS AND METHODS

Rearing of Almond moth

C. cautella adults were captured from a rice milling center at Puliyankulama, Anuradhapura, Sri Lanka and reared in the laboratory nearly one year were used in the experiments. The moth adults were introduced to 250 g of rice flour in plastic bottles (1 L), covered with a thin cloth material and maintained inside the incubator (FH-1200 LED T8, HiPoint Laboratory, Taiwan) at $33\pm 1^\circ\text{C}$ and $60\pm 5\%$ relative humidity). At the pupal stage, the flour medium was sieved through 850 μm and 2 mm mesh (ASTM E11, W.S. Tyler Industrial Group, Mentor, U.S.A.). The male and female pupae were separated using a microscope (Zhu *et al.*, 1999) (Optika, Triace, Italy), and each pupa was introduced into a separate plastic vial (3.6 cm diameter and 6.2 cm height) having 5 g rice flour using an artist's brush (No. 3). The vial having the pupa was covered with a thin piece of cloth and maintained inside the incubator at $33\pm 1^\circ\text{C}$ temperature and $60\pm 5\%$ relative humidity until the adults emerged. The adult moths aged 2-4 days were used in the experiments.

Wind tunnel construction

Four cubicles (each 1.5 m×1.5 m× 1.5 m) with a metal frame were constructed on a cement floor. Each cubicle was housed in a separate room. Two opposite sides, top and bottom of the cubicle were covered using transparent polythene (25 mm thickness). The remaining two opposite sides were covered using an insect proof net which facilitates air circulation. A zip entry was arranged to permit the introduction of moths into the wind tunnel and recapture them following the exposure period. A domestic exhaust fan (12.0 m³/min air delivery, 1,200 rpm speed) (XFN-E002, Orel Corporation Pvt Ltd, PR China) was connected to one side having the insect-proof net, and close to the window of the room for easy direction of air out. This was to facilitate testing the impact of forced air flow condition on the mating status and removal of contaminated air inside the wind tunnel. The wind speed was measured by using Professional Standard Environmental meter (13/464/0, S. Brannan and Sons, Cumbria, UK).

Preparation of pheromone doses

Commercially-available pheromone ZETA was used in the study. The pheromone was kept inside the refrigerator (4°C) until used. Four solutions of ZETA (10%) were prepared using the stock solution of the commercial pheromone (Insects Ltd. Inc., Westfield, USA) diluted in hexane solution (100%). Accordingly, 10% pheromone dose (4.5 mg of pheromone) was prepared mixing 4.5 mL of pheromone solution in 45 mL of hexane solution. Subsequently, the 10% pheromone doses having 1 mg, 0.1 mg and 0.05 mg were prepared by diluting 1 mL, 0.1 mL and 0.05 mL pheromone in 10 mL, 1 mL and 0.5 mL of hexane, respectively. These four pheromone doses were added by micropipette (Labnet International, Inc, Poland) into separate glass Petri dishes and covered by individually the net material.

Experimental design

Experiments were conducted as Completely Randomized Design (CRD). The effects of pheromone dose, insect population size and the status of air movement on MD were tested. Each treatment had four replicates. All tests were conducted simultaneously under the same ambient environmental conditions (33±1°C and 60±5% relative humidity).

Experiment 1: Effect of pheromone dose on mating status

The glass Petri dish having a particular pheromone dose (0.05, 0.1, 1 or 4.5 mg) was placed inside a monitoring trap (Storgard kit insect monitoring system, Trece, Inc., Adair, OK, U.S.A.) and hung to be at the middle of wind tunnel (75 cm distance from top or bottom) 24 hours before introducing the adult moths. Hobo data loggers (Onset Computer Corporation, MA, U.S.A.) were placed both inside and outside of the wind tunnel to record the temperature and humidity of the environment. Three population sizes (10, 20 or 30) of *C. cautella* adults having equal number of males and females were introduced separately into the wind tunnel. The moths were maintained for 24 hours inside the wind tunnel following introduction (Ryne *et al.* 2001) and subsequently collected into a conical flask (250 mL) using an aspirator, frozen (-10°C for 2 hours) inside a freezer and the female moths dissected under a microscope (Optika, Triace, Italy) to determine the presence/absence of spermatophores to ascertain the mating status (Mafra-Neto and Baker, 1996). As the spermatophores are maintained large by freezing up to 2 hrs (Ryne *et al.*, 2001) and thus to avoid spermatophores deteriorated with the lapse of time following mating (Drummond, 1984), the females were dissected within 2 hrs following their removal from the wind tunnel. The mating status of moths was determined by counting the mated females. Mating status for a particular pheromone dose was conducted under natural air flow and forced air flow conditions. The effective pheromone dose ob-

tained in this experiment 1 with natural/forced air flow was subsequently used in the experiment 2 to find out the effective population size under that particular air flow status. Control experiments were conducted by using hexane solution (with the natural or forced air flow, as required).

Experiment 2: Effect of population size on mating status

Petri dish having the effective pheromone dose (found in experiment 1) was placed in the middle of the wind tunnel at approximately 75 cm distance from top or bottom of the wind tunnel by using a monitoring trap 24 hours before the introduction of moths, as practiced in the experiment 1. Temperature and humidity inside and outside the wind tunnel were measured using Hobo data loggers (Onset Computer Corporation, MA, U.S.A). The male and female adults of *C. cautella* (aged 2-4 days), emerged individually in separate vials were introduced into the wind tunnel at three different population sizes (Table 1), maintained for 24 hours (Ryne *et al.*, 2001), and captured from the walls of wind tunnel into a conical flask (250 mL) using an aspirator. Similar to the previous practice in experiment 1, the insects were frozen (at -10°C) for 2 hours and the females dissected under a microscope (Optika, Triace, Italy) to determine the presence or absence of spermatophores. Each experiment with a particular insect population size was conducted either with the natural or forced air flow. The control experiments were conducted using hexane alone (with the natural or forced air flow, as required).

Table 1: Different population sizes of *Cadra cautella* used in the experiment.

Instance	Males	Females	Population size
1	5	5	10
2	10	10	20
3	15	15	30

Statistical analysis

The percentages of mated females were transformed using the square root of the arcsine value to accommodate the unequal variances associated with the percentage data (Ryne *et al.*, 2001; Svensson *et al.*, 2002; Burks and Kuenen, 2012; Trematerra *et al.*, 2013; Toews *et al.*, 2010; Dissanayaka *et al.*, 2018a; Wijayarathne *et al.*, 2019). These transformed data were analyzed using ANOVA procedures of Statistical Analysis system (SAS) (SAS Institute, 2002-2008). Mean separation of pheromone doses, population sizes and natural/forced air flow was done by Tukey's test. The significance was tested at $P=0.05$.

RESULTS AND DISCUSSION

Environmental conditions and moth identification

The average temperature and relative humidity inside and outside of the wind tunnels were $30\pm 1^{\circ}\text{C}$, $63.5\pm 1\%$ and $31\pm 1^{\circ}\text{C}$, $64\pm 2\%$ respectively. The average wind speed inside the wind tunnel under forced air condition was 1.35 m/s. Under natural air flow, the average wind speed 0.2 m/s. Male pupa has two nodes close to the genital scar on the ventral side of the 8th body segment (Zhu *et al.*, 1999). The mated females were identified by the spermatophores in white color and located as clusters in the bursa copulatrix (Ryne *et al.*, 2001).

Effect of pheromone dose and air flow on mating status

Overall, there was a significant effect of pheromone dose ($F=55.53$; $df=3, 27$; $P<0.0001$) and air flow ($F=7.05$; $df=1, 27$; $P=0.0131$) on mating status of *C. cautella*. Thus, in general, the percentage mated females was lower when the pheromone (ZETA) was present than when it was not present (in the hexane control). Under natural air flow, the percentage mated females at 4.5 mg of ZETA (30%) was lower than other pheromone doses (Figure 1). This decreased mated status at 4.5 mg of ZETA means that the highest MD occurred at that dose. However, the mated female percentages 65%, 60% and 50% obtained at 0.05,

0.1 and 1 mg of ZETA, respectively did not differ from each other. A similar pattern of mating status was observed when forced air flow was maintained as well. The mating status (22.5%) at 4.5 mg was lower than those at other three ZETA doses; 60%, 55% and 45% with 0.05, 0.1 and 1 mg, respectively. Furthermore, the three mating percentages at 0.05, 0.1 and 1 mg of ZETA respectively did not differ from each other (Figure 2). Accordingly, the highest MD was observed in 4.5 mg

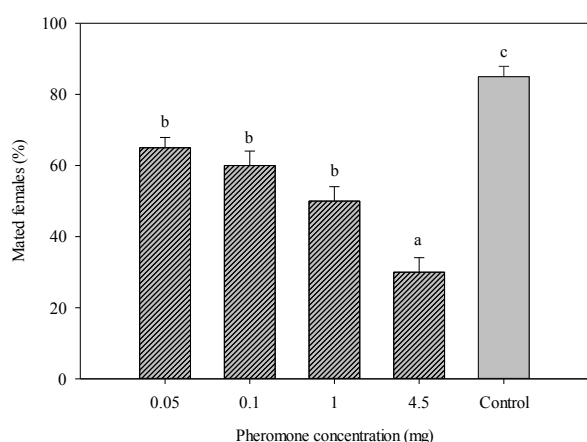


Figure 1: Percentage of mated females (mean±SE) at different pheromone concentrations (natural air flow condition). Means followed by the same letter are not significantly different at $P=0.05$ according to Tukey's test following ANOVA.

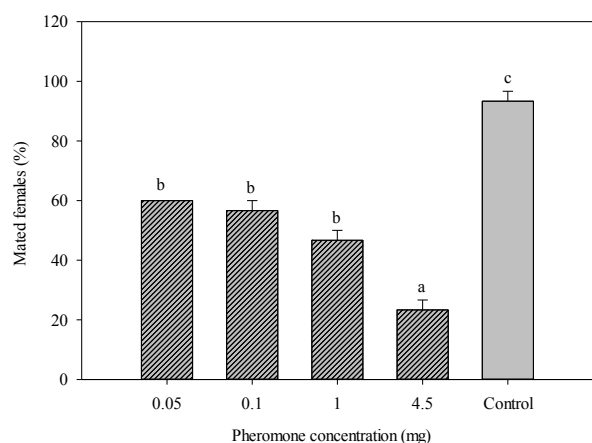


Figure 2: Percentage of mated females (mean±SE) at different pheromone concentrations (forced air flow condition). Means followed by the same letter are not significantly different at $P=0.05$ according to Tukey's test following ANOVA.

of ZETA. The increased MD was under forced air flow condition may possibly be due to the increased dispersal of pheromone by the air flow.

The mating status is determined by the presence of spermatophores in bursa copulatrix of the female moth (Ryne *et al.*, 2001). Similarly in the current study, spermatophores of mated females were clearly observed. The mating of *Plodia interpunctella* can be suppressed by 93% by using synthetic pheromones (Ryne *et al.*, 2001). In the current study, the highest MD was at 4.5 mg of ZETA.

Effect of insect population size on mating status

In all the population sizes tested, mating percentages of moths exposed to ZETA were significantly lower than their respective controls both under natural and forced air flow conditions. (Figure 3 and 4). Furthermore, there was a significant effect of insect population size on mating status ($F=50.86$; $df=5, 41$; $P<0.0001$).

Under natural air flow, the percentage of mated females at population sizes 10 (5 males+5

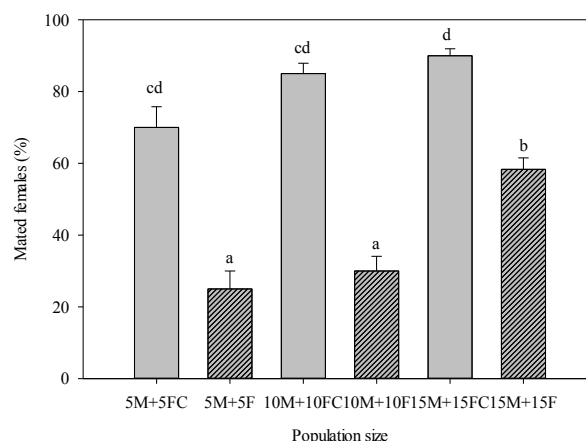


Figure 3: Percentage of mated females (mean±SE) at different insect population sizes (natural air flow condition). Different male (M) and female (F) populations sizes followed by C indicates the mating percentages obtained in the respective control. Means followed by the same letter are not significantly different at $P=0.05$ according to Tukey's test following ANOVA.

females) and 20 (10 males+10 females) was lower than 30 (15 males+15 females) (Figure 3). Thus, the MD was higher in low population sizes (25% of mated females for 10 population size and 30% of mated females for 20 population size). Under forced-air flow, there was no significant difference in the percentage of mated females among the insect population sizes 10 (20% of mated females), 20 (22.5% of mated females) and 30 (48.33% of mated females) (Figure 4). No differences in the mating status among population sizes under forced air flow condition in contrast to the alteration of mating status under natural air flow indicates that the air movement would have helped the dispersion of the pheromone throughout the space.

According to the current study, low population sizes *C. cautella* are better controlled by ZETA under natural air flow whereas all population sizes of *C. cautella* are equally controlled by ZETA under forced-air flow condition. This agrees with the previous studies on controlling of mating in low populations of *C. cautella* (Sower and Whitmer, 1977). Furthermore, another previous study indicates that

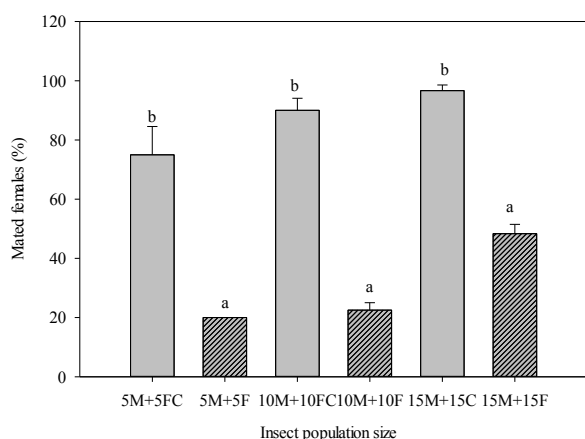


Figure 4: Percentage of mated females (mean±SE) at different insect population sizes (forced air flow condition). Different male (M) and female (F) populations sizes followed by C indicates the mating percentages obtained in the respective control. Means followed by the same letter are not significantly different at P=0.05 according to Tukey's test following ANOVA.

the mating of *C. cautella* is controlled 100% in the low population densities tested (Mafra-Neto and Baker, 1996). In contrast, in the current research, the maximum mating percentage (approximately 58%) of *C. cautella* moths was observed at high population size under natural air flow condition.

In general, the combination of high doses of ZETA and low population sizes of *C. cautella* ensure effective suppression of mating. This condition can be achieved when the insect population of *C. cautella* has been already controlled by using the conventional controlling methods (Hodges *et al.*, 1984). During this experiment, the moths were aggregated in dark areas in the wind tunnel. Aggregation to specific areas increases the chance of individual moths to be mated (Ryne *et al.*, 2001). The wind tunnel used in our experiment was simply a cubicle without modifications. However, the complexity of the wind tunnel increases the aggregation spots which may facilitate reduced mate finding (increase MD) (Ryne *et al.*, 2001). The MD is mediated through different mechanisms: local adaptation of antennal receptors, habituation on a central level, false trail following, or camouflage of the female's pheromone plume (Carde and Minks, 1995). The MD in some other Pyralidae moths such as *Plodia interpunctella* has already been demonstrated (Burks and Kuenen, 2012). However, as *Plodia interpunctella* undergoes larval diapause (Wijayarathne and Fields, 2012), the effect of MD might be altered. This is worthy of investigation.

The current findings open up new avenues of further research on MD. Testing MD under high populations sizes that represent in actual warehouse conditions, exploration of the exact mechanism underlying MD, further testing with ZETA at different doses to maximize MD; influence of different sex pheromone components and their ratios, botanicals and other insecticides on MD in *C. cautella* and other Pyralidae moths would be useful inves-

tigations to expand the practical use of MD technology in the population monitoring and subsequent pest management programs.

CONCLUSION

Mating disruption of *C. cautella* increases at high doses (4.5 mg) of ZETA, low population sizes (5-10 moths in 3.375 m³) and forced air flow condition. Therefore, while being an effective monitoring tool, ZETA can effectively be used in the management strategies of *C. cautella* populations. Further exploration on different components of ZETA and incorporation them into existing pest management strategies for *C. cautella* would strengthen integrated pest management practices for this species.

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