Pheromone traps for monitoring *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) in the presence of mating disruption

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Abstract

High-dose pheromone lures have proved useful for monitoring some lepidopteran pests in the presence of mating disruption, but not others. We performed experiments in commercial and pilot scale facilities to examine the effect of pheromone dose on detection of Indianmeal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae), in the presence of mating disruption. When *P. interpunctella* males were released into 1000 m3 rooms containing traps baited with 0, 1, or 10 mg (Z,E)-9,12-tetradecadienyl acetate (Z9,E12-14:Ac), traps containing 10 mg captured more than those baited with 1 mg in both the presence and absence of mating disruption. Traps baited with 1 mg captured fewer males in the presence of mating disruption than in its absence, but the opposite was observed with traps baited with 10 mg. When males released into 73 m3 rooms were exposed sequentially to blank traps, traps baited with unmated females, and traps baited with 0.1 mg and then 1.0 mg Z9, E12-14:Ac in the presence or absence of mating disruption, 92% of trapped males were captured in female-baited traps in the absence of mating disruption, whereas in the presence of mating disruption 72% of males captured were caught in synthetic pheromone traps. These data suggest that pheromone lures can be used for monitoring *P. interpunctella* in the presence of mating disruption. Implications of these data for mass trapping are also discussed.

Keywords: *Plodia interpunctella*, Mating disruption, Monitoring, Pheromone lures, Mass trapping

1. Introduction

Plodia interpunctella (Hübner) shares (Z,E)-9,12-tetradecadienyl acetate (Z9,E12-14:Ac), as the primary component in its sex pheromone (El-Sayed, 2009) with several other stored product pests of the subfamily Phycitinae. This compound has been used successfully by itself in integrated management of these species, both for monitoring (Nansen and Phillips, 2004; Nansen et al., 2006; Witzgall et al., 2010) and for mating disruption (Ryne et al., 2006, 2007; Sieminska et al., 2009).

Reduced utility of pheromone traps for monitoring can be a barrier to adoption for mating disruption. The degree to which mating disruption prevents use of pheromone traps for monitoring depends on neurobehavioral characteristics of the response of the target species to its sex pheromone. Pheromone lures can be used to monitor the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), in the presence of mating disruption, albeit only with higher loads of pheromone in the lure and then at reduced efficiency compared to optimized lures in a non-mating disruption situation (Charmillot, 1990). In contrast, males of the oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) are more repelled by higher concentrations of sex pheromone (Roelofs et al., 1973; Cardé and Minks, 1995). Higher pheromone concentrations on lures do not increase the number of males captured, either in the presence of mating disruption or in its absence (Kovanci et al., 2005). Here we report experiments examining the effect of pheromone (Z9, E12-14:Ac) dosage on lures on the number of *P. interpunctella* captured in either the absence or the presence of mating disruption with Z9, E12-14:Ac.

2. Materials and methods

2.1. Insects, lures, and pheromone dispensers

Insects used for these tests came from a laboratory strain of *P. interpunctella* founded with larvae collected at a dried-fruit packer in fall 2007. The laboratory culture was maintained on a wheat brain diet (Tebbets et al., 1978) in walk-in environmental chambers maintained at 26°C, a photophase of 16:8 L:D, and 60% r.h. Unmated males and females were obtained by isolating adults within 30 minutes of emergence. Dissection of many moths demonstrated that females thus obtained were consistently unmated. When unmated females were used as lures, three recently emerged females were sealed into plastic mesh bags. When adults that eclosed on multiple days were used in an experiment, they were held at 10°C until used since previous research showed no loss of fertility at this temperature for two weeks (Johnson et al., 1997). Synthetic pheromone lures containing 0, 0.1, 1 or 10 mg pheromone were prepared by applying 300 µl hexane with the appropriate concentration of Z9,Z12-14:Ac (P6050-93, Bedoukian, Danbury, CT USA) into vacuum-extracted 11 mm gray rubber septa (West Pharmaceuticals, Lionville, PA USA). CheckMate SPM dispensers (Suterra LLC, Bend, OR USA) were used to examine the effect of permeation with Z9, Z12-14:Ac on the number of males captured in traps.

2.2. Experiments

The first experiment was conducted in paired 1000 m3 rooms (floor 12 × 12 m; ceiling 7 m). They belonged to a dried fruit processer and were constructed as fumigation chambers; they are also used for product storage. Each had a small window over the roll-up door, allowing weak daylight and a natural photoperiod. They were not heated or cooled. Temperature loggers (Hobo U10, Onset Instruments, Pocasset, MA USA) placed in the rooms over the test period indicated a minimum 27°C and maximum 36°C temperature. One of the two chambers was treated with 16 CheckMate SPM dispensers, placed at ceiling level at equal intervals on the perimeter of the room. After the pheromone dispensers had been in place for a day, twelve delta traps (LPD, Suterra, Bend, OR USA) were placed in each of the two rooms. Three treatments were assigned randomly to trap positions in the two rooms: no bait, or bait consisting of gray rubber septa loaded with 1 or 10 mg Z9, E12-14:Ac. In each room, two to four jars containing a total of 160 unmated males, aged 0-2 d post eclosion, were placed on the floor near the center, opened, and then the entrance to the room were quickly closed. Three d later, the traps were removed and captured adults were counted.

A second experiment was performed using identical 73 m3 structures (Open bay security offices, Mini Mobile Inc., Phoenix, AZ USA). The interior floor area was 2.4 x 12.2 m, and the ceiling was 2.4 m high. Four wing traps (Suterra LLC, Bend, OR USA) were suspended 1.5 m from the floor, midway between the two long walls, 1.5 m from the two short walls, and 3 m apart. Each long wall had three closed windows covered with blinds, allowing diffuse natural light. When mating disruption was used, two CheckMate SPM dispensers were placed 15 cm from the ceiling on opposite long walls, 3 m from the short wall.

This second experiment consisted of a series of trials examining the number of males captured following the release of forty newly eclosed unmated males from a jar on the floor in the center of the room. Two preliminary trials examined the number of males captured over six nights when both rooms contained traps baited with 1 mg septa, and neither room was treated with mating disruption. In these trials, all males quickly exited the jars; but subsequently we found it necessary to place the release jar on a cafeteria tray covered with mineral oil to avoid ant predation.

Three subsequent trials represented three replications of the second experiment. One of rooms was treated with CheckMate SPM dispensers as previously described, and these were left in place in the same room for the 28 d required for the three trails. In each trial new wing traps were put in the room prior to releasing the males in the late afternoon. Males were released once at the beginning of a series of lure changes, starting with empty traps (no lure). After two nights, plastic mesh bags containing three virgin females were placed in each trap. After an additional two nights the unmated females were replaced with 0.1 mg septa, and after two more nights these were replaced with 1.0 mg septa. After two more nights the traps and septa were removed, and the room was left empty 2 d prior to the beginning of the next trial. Males in traps were counted each morning, and liners were changed when unmated females were removed and replaced with 0.1 mg septa.

The rooms were not heated or cooled, and the temperature inside fluctuated with outdoor temperature and solar radiation. The minimum and maximum temperatures (°C) for trials 1-5 were, respectively: 18, 47; 10, 39, 6, 37, 4, 28; and 4, 28.

2.4. Data analysis

The first experiment was analyzed using a two-way ANOVA, with males per trap (transformed as squareroot[x + 1]) as the dependent variable and mating disruption treatment and septa dose as categorical independent variables. A Tukey multiple range test was used for comparisons. The second experiment was analyzed using a cumulative logit model (Agresti, 2007), with the sequential pheromone treatments (blank, unmated females, 0.1 mg, and 1 mg) as ordinal responses and the mating disruption treatment (presence, absence) as a categorical predictor. This model tests the null hypothesis that there is no difference in ordinal location between the two categories examined. This type of analysis was more appropriate than ANOVA for this experiment because the sequential application of pheromone lure strengths created autocorrelation between these treatments and therefore made ANOVA inappropriate. Student's t-test was also used to compare, between mating disruption treatments, the total number of males captured in traps and the oil trays used to counter ants. For comparison with other studies, percent trap suppression for traps baited with unmated females was calculated as:

% Suppression = {(Countuntreated – Countmating disruption)/Countuntreated} × 100.

Untransformed means and standard errors are used in the figures.

3. Results

In first experiment, a total of 85% of released males were captured in the 1000 m3 room treated with mating disruption dispensers, compared to 71% in the identical untreated room. ANOVA revealed significant differences in males per trap due to pheromone dose on the lure ($F_{2,18} = 38.5$, $P < 0.001$), but not due to mating disruption treatment ($F_{1,18} = 0.03$, $P = 0.86$) or the interaction of lure dose×mating disruption treatment ($F_{2,18} = 2.2$, $P = 0.14$). The number of males captured using blank traps was significantly lower than in traps baited with 1 mg septa ($P < 0.05$) (Fig. 1), and 10 mg-baited traps captured much higher numbers than 1 mg septa-baited traps ($P < 0.001$) (Fig. 1). Fewer males were captured in traps baited with 1 mg septa in the room treated with mating disruption treatment compared to the untreated comparison whereas the converse was true for the traps baited with 10 mg septa (Fig. 1). However, this implied interaction was not significant ($P < 0.05$).

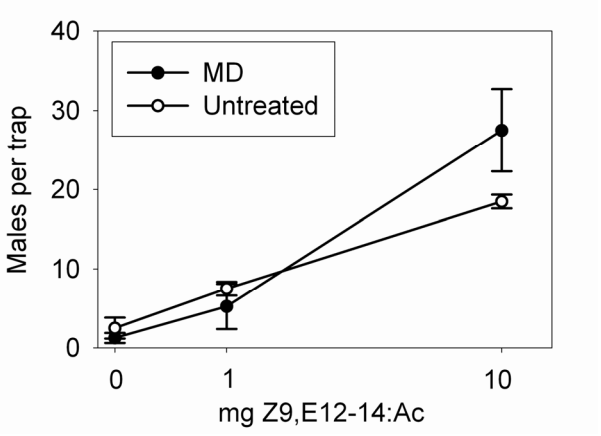

Figure 1 *Plodia interpunctella* males per trap (mean and SE) in empty traps, or traps baited with gray rubber septa loaded with 0, 1 or 10 mg Z9,E12-14:Ac, with or without mating disruption (MD). Traps were exposed simultaneously to 160 males released into each of two 1000 m3 rooms.

For the second experiment, in two preliminary trials examining trap capture with 1 mg septa after 40 males were released into 73 m3 rooms without mating disruption, 38 males were captured each time from the room which was subsequently treated with mating disruption, and 34 and 35 were recaptured

from the other room. These observations suggest that the number of males captured was similar between the two rooms in the absence of mating disruption, and that any possible difference did not result in fewer males captured in the room that subsequently received mating disruption treatment. After applying mating disruption treatment of one of the rooms, fewer males were captured overall in traps the treated room (27 ± 1.7 v. 33 ± 5.4) (mean ± SE) and conversely more were captured in the oil pans placed on the floor to protect the release jar (6 ± 1.7 v. 3 ± 1.9). These differences were not statistically significant ($t4 = 4$; $P > 0.05$). Males captured in traps were significantly more likely to be captured in the blank trap or the trap baited with unmated females in the untreated room compared to the room treated with mating disruption (Wald $\chi 2 = 25.5$, $df = 1$; $P < 0.001$) (Fig. 2).

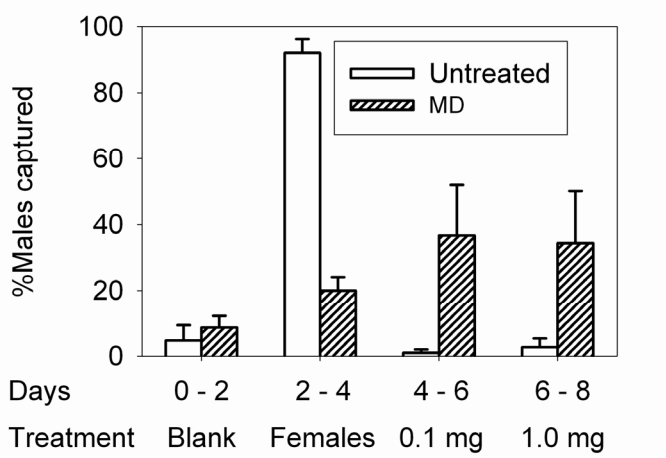

Figure 2 Percent (mean and SE) of *Plodia interpunctella* males captured by various lures in 73 m3 an untreated room, or one treated with mating disruption (MD). Males were exposed successively, over 2 day intervals, to wing traps baited with empty gray rubber septa (blank), unmated *P. interpunctella* females, or gray rubber septa loaded with 0.1 or 1.0 mg Z9,E12-14:Ac.

3. Discussion

These data demonstrate that pheromone permeation resulting in substantial suppression of males captured in traps baited with unmated females, *P. interpunctella* males can be nonetheless be captured in traps baited with septa containing Z9,Z12-14:Ac over a wide range of concentrations. In this study, traps with septa containing as little as 0.1 mg captured males not captured by traps containing unmated females, and trap capture increased as septa loads were increased to 10 mg. Many commercial lures contain 1 mg of Z9,Z12-14:Ac (Nansen et al., 2006). Among lepidopteran orchard pests managed with mating disruption, pheromone lures with loads higher than those usually used for monitoring are useful in the presence of mating disruption against *C. pomonella* but not G. molesta (Roelofs et al., 1973; Charmillot, 1990; Kovanci et al., 2005). These data indicate that *P. interpunctella* is more like *C. pomonella* than *G. molesta* in this regard, and even suggest that standard monitoring lures could provide useful information in structures treated with mating disruption.

The data in this study could alternatively be viewed as suggesting that the mating disruption product used was ineffective. Three observations suggest that this is not the case. First, mating disruption is generally density-dependent; i.e., it is usually more effective at lower population abundance (Cardé and Minks, 1995), and often lower amounts can be used with lower population abundance. We purposely used relatively high population densities to ensure that we could distinguish among treatments. Second, the reduction by mating disruption of the number of males captured in traps baited with unmated females seen in this study, 82%, is similar to the range of reduction in mating seen in a previous study of mating disruption targeted against *P. interpunctella* (Ryne et al., 2001). In that study, which found a range of 80-95% mating suppression over 24 h, no significant differences among treatments were observed when Z9,Z12-14:Ac, alone or with three other components, was applied in small arenas at rates of 0.005, 0.045, or 0.2 mg/d/100 m3 to population densities of 1 or 3 pairs/m3. These densities bracket the density

of males in the 73 m3 rooms in the current study. Subsequent studies in industrial settings found evidence of long-term population reduction of P. interpunctella (Ryne et al., 2007), as well as *Cadra cautella* (Walker) and *Ephestia kuehniella* (Zeller) (both Lepidoptera: Pyralidae) (Ryne et al., 2006; Sieminska et al., 2009), when dispensers emitting 2-3 mg/d were used for every 100 m3 of treated space. Third, while CheckMate SPM dispensers are not currently registered for use in California, data supporting their efficacy has been obtained in other jurisdictions (C.C.R., unpublished data).

In other species of Lepidoptera, an increase in response of males to sex pheromone has been observed for the first several days after eclosion (McNeil, 1991). It is therefore possible that, in the second experiment, age as well as behavioral response to different doses contributed to the observation that traps with baited with synthetic pheromone lures captured males not captured when the traps were baited with virgin females. In the first experiment, however, younger males (0-2 days post eclosion) were exposed to lures loaded with 1 mg of Z9,Z12-14:Ac (the upper dose in the second experiment) and were captured in similar numbers in the presence or in the absence of the mating disruption treatment. Thus the two experiments offer reinforcing evidence that a mating disruption treatment sufficient to substantially suppress male capture in traps baited with virgin females was much less effective at suppressing male capture with lures loaded with 1 mg of Z9, Z12-14:Ac.

This and previous studies provide seemingly contradictory data concerning the effect of concentration of Z9, Z12-14:Ac on attraction of *P. interpunctella* males. On the one hand, septa with pheromone loads of 0.01 to 2 mg all captured males as effectively as calling females when known numbers of unmated males were released in a building with a grid of traps (Nansen et al., 2006). On the other hand, when a 1.46 m3 wind tunnel was used to examine responses to gel drops containing 2.4 or 4.8 mg Z9, Z12-14:Ac, unmated males examined for 15 minutes were significantly less likely to fly upwind, land on a container platform, or make contact with the gel drop with 4.8 compared to 2.4 mg (Nansen and Phillips, 2004). In the current study, using known concentrations of males in a large structure over three nights, we found more males captured in sticky traps baited with septa containing 10 mg Z9,Z12-14:Ac compared to 1 mg. This apparent discrepancy may be due to differences in time and scale between short term wind tunnel assays and assays on a larger scale and over longer time in industrial- or pilot-scale structures. It may also be that *P. interpunctella* males approach concentrated pheromone sources closely enough to be captured on sticky traps, but not closely enough to make contact with lure-and-kill formulations.

A recent simulation study (Byers, 2007) and reviews (El-Sayed et al., 2006, 2009) suggest that, for attractive pheromone formulations, mass trapping or lure-and-kill formulations should reduce populations more efficiently than mating disruption. Particularly for mass trapping, it is useful to maximize the effective attraction radius (EAR) of lures (Byers et al., 1989, El-Sayed et al., 2006). Some previous work has examined lure load for P. interpunctella in the context of optimizing the ability to locate sources of infestation using a trap grid (e.g., Nansen et al., 2006), for which a smaller EAR might be beneficial. However, other studies indicate reduction of population of pyralid stored product moths over time using commercial lures loaded with ≤2 mg Z9,E12-14:Ac with sticky traps (Pierce, 1994) or funnel traps (Trematerra and Gentile, 2010).

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References

Agresti, A., 2007. An Introduction to Categorical Data Analysis. Wiley & Sons, New York.

Byers, J.A., Anderbrandt, O., Löfqvist, J., 1989. Effect attraction radius: a method for comparing species attractants and determining densities of flying insects. Journal of Chemical Ecology 15, 749-765.

Byers, J.A., 2007. Simulation of mating disruption and mass trapping with competitive attraction and camouflage. Environmental Entomology 36, 1328-1338.

Cardé, R.T., Minks, A.K., 1995. Control of moth pests by mating disruption: successes and constraints. Annual Review of Entomology 40, 559-585.

Charmillot, P.J., 1990. Mating disruption technique to control codling moth in Western Switzerland. In: Ridgway, R.L., Silverstein, R.M., Inscoe, M.N. (Eds), Behaviour-modifying Chemicals for Pest Management: Applications of Pheromones and Other Attractants. Marcel Decker, New York, USA. pp. 165-182.

El-Sayed, A.M., 2009. The Pherobase: Database of Insect Pheromones and Semiochemicals. http://www.pherobase.com

El-Sayed, A.M., Suckling, D.M., Wearing, C.H., Byers, J.A., 2006. Potential of mass trapping for long-term pest management and eradication of invasive species. Journal of Economic Entomology 99, 1550-1564.

El-Sayed, A.M., Suckling, D.M., Byers, J.A., Jang, E.B., Wearing, C.H., 2009. Potential of lure and kill in long-term pest management and eradication of invasive species. Journal of Economic Entomology 102, 815-835.

Johnson, J.A., Valero, K.A., Hannel, M.M., 1997. Effect of low temperature storage on survival and reproduction of Indianmeal moth (Lepidoptera: Pyralidae). Crop Protection 16, 519-523.

Kovanci, O.B., Schal, C., Walgenbach, J.F. & Kennedy, G.G., 2005. Comparison of mating disruption with pesticides for management of Oriental fruit moth (Lepidoptera: Tortricidae) in North Carolina apple orchards. Journal of Economic Entomology 98, 1248-1258.

McNeil, J.N., 1991. Behavioral ecology of pheromone-mediated communication in moths and its importance in the use of pheromone traps. Annual Review of Entomology 36, 407-430.

Nansen, C., Phillips, T.W., 2004. Attractancy and toxicity of an attracticide for Indianmeal moth, Plodia interpunctella (Lepidoptera: Pyralidae). Journal of Economic Entomology 97, 703-710.

Nansen, C., Phillips, T.W. Morton, P.K., Bonjour, E.L., 2006. Spatial analysis of pheromone-baited trap captures from controlled releases of male Indianmeal moths. Environmental Entomology 35, 516-523.

Pierce, L.H., 1994. Using pheromones for location and suppression of phyticid moths and cigarette beetles in Hawaii--a five-year study. In: Highley, E., Wright, E.J., Banks, H.J., Champ, B.R., (Eds), Stored Product Protection, Proceedings of the sixth International Working Conference on Stored-Product Protection, 17-23 April, 1994, Canabera, Australia. CAB International, Oxford, UK, pp. 439-443.

Roelofs, W.L., Cardé, R.T., Tette, J.P., 1973. Oriental fruit moth attractant synergists. Environmental Entomology 2, 252-254.

Ryne, C., Svensson, G.P., Löfstedt, C., 2001. Mating disruption of *Plodia interpunctella* in small-scale plots: effects of pheromone blend, emission rates, and population density. Journal of Chemical Ecology 27, 2109-2124.

Ryne, C., Ekeberg, M., Jonzén, N., Oehlschlager, C., Löfstedt, C., Anderbrant, O., 2006. Reduction in an almond moth *Ephestia cautella* (Lepidoptera: Pyralidae) population by means of mating disruption. Pest Management Science 62, 912-918.

Ryne, C., Svensson, G.P., Anderbrant, O., Löfstedt, C., 2007. Evaluation of long-term mating disruption of Ephestia kuehniella and *Plodia interpunctella* (Lepidoptera: Pyralidae) in indoor storage facilities by pheromone traps and monitoring of relative aerial concentrations of pheromone. Journal of Economic Entomology 100, 1017-1025.

Sieminska, E., Ryne, C., Löfstedt, C., Anderbrant, O., 2009. Long-term pheromone-mediated mating disruption of the Mediterranean flour moth, Ephestia kuehniella, in a flourmill. Entomologia Experimentalis et Applicata 131, 294-299.

Tebbets, J.S., Curtis, C.E., Fries, R.D., 1978. Mortality of immature stages of the navel orangeworm stored at 3.5C. Journal of Economic Entomology 71, 875-876.

Trematerra, P., Gentile, P., 2010. Five years of mass trapping of *Ephestia kuehniella* Zeller: a component of IPM in a flour mill. Journal of Applied Entomology, 134, 149-156.

Witzgall, P., Kirsch, P., Cork, A., 2010. Sex pheromones and their impact on pest management. Journal of Chemical Ecology 36, 80-100.