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Published Version Information

Citation: Campos, M., & Phillips, T. W. (2013). Laboratory evaluation of attract-and-kill formulations against the Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). Journal of Stored Products Research, 52, 12-20.

Digital Object Identifier (DOI): doi:10.1016/j.jspr.2012.09.005

Publisher's Link:
http://www.sciencedirect.com/science/article/pii/S0022474X1200077X

Journal of Stored Products Research

LABORATORY EVALUATION OF ATTRACT-AND-KILL FORMULATIONS AGAINST THE INDIANMEAL MOTH, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)

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Abstract

Pheromone-based population suppression methods for stored-product insects can reduce or eliminate application of chemical insecticides near finished food products. The responses of adult male Indianmeal moth males (IMM), *Plodia interpunctella* (Hübner), to the attract-and-kill formulations of a gel, a flat wax panel, and a plastic cylinder device, mixed or sprayed with the pyrethroid insecticides permethrin, cyfluthrin, or organically compliant natural pyrethrin, combined with the synthetic female sex pheromone *(Z,E)* -9,12 tetradecadienyl acetate, were evaluated in a laboratory wind tunnel. The wax panel and cylinder, which utilized controlled-release pheromone lures, were more attractive to IMM males over the course of an eight-week aging period than was the gel, which had the pheromone incorporated into the gel matrix. The contact time for responding males was longer on the wax panel and plastic cylinder than on the gel formulation. The percentage of mortality of males was higher with wax panels formulated with cyfluthrin at 6.0% AI, permethrin at 6.0% AI and the cylinder formulated with cyfluthrin at 2.0% AI, compared to the gel over the eight-week study. These same formulations had the greatest impact on egg-laying by females paired with treated males and on the percent of eggs that hatched. Of all the attract-and-kill formulations tested, the most promising for field applications to suppress IMM pest populations was the wax panel containing 6.0% AI of either cyfluthrin or permethrin.

Key words: Wind tunnel, attracticide, pheromone, stored-products, residual insecticides

1. Introduction

Semiochemically-based pest management techniques such as mating disruption, mass trapping and attract-and-kill have been developed as alternatives to traditional insecticides applications to control important pest Lepidoptera. Many of these techniques use synthetic sex pheromones to attract males in close contact with killing agent (attract-and-kill), but not necessary in close contact with the pheromone source (mass-trapping, El-Sayed et al., 2006), or otherwise interrupt male mating behavior so that females go unmated and the population declines. However, the most common use of synthetic pheromones for stored product moths is for monitoring populations, and this has become part of the integrated pest management programs for these pests (Burkholder & Ma, 1985; Vick et al., 1981; 1986; Arthur et al., 1991; Phillips et al., 2000, Phillips and Throne, 2010). The predominate female pheromone of *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae) is *(Z,E)* -9,12 tetradecadienyl acetate, is commonly referred to as ZETA (Brady et al., 1971; Kuwahara et al., 1971; Kuwahara & Casida, 1973; Sower et al., 1974; Soderstrom et al., 1980; Teal et al., 1995; Zhu et al., 1999).

The attract-and-kill, or "attracticide", method of pest control incorporates an attractant of a target insect species with an insecticide in order to kill large numbers of responding insects and ultimately reduce a pest population (Lanier, 1990). The LastCall® gel (IPM Tech, Inc., Portland, OR), which is a combination of a synthetic sex pheromone with the synthetic pyrethroid permethrin in a gel matrix, was formulated to control Oriental fruit moth, *Grapholita molesta* (Evenden & McLaughlin, 2004, 2005; Evenden et al., 2005), Codling moth, *Cydia pomonella* (L.) (Krupke et al., 2002; Evenden & McLaughlin, 2005), and it showed promise for the Indianmeal moth, *Plodia interpunctella* (Nansen & Phillips, 2004).

The Indianmeal moth, *Plodia interpunctella* (Hübner), is one of the most serious stored-product insect pests of value-added food products worldwide, and there is substantial interest in developing safe and effective alternatives to traditional residual and fumigant chemical control of this pest (Arthur & Phillips, 2003; Phillips, 2006). Efficacy of the attract-and-kill method, in which reproduction is impacted after large numbers of males are killed following contact with point-sources that have pheromone combined with an effective contact insecticide, was demonstrated for *P. interpunctella* by Nansen & Phillips (2004). However, that study examined only one attract-and-kill gel-based formulation, and it did not assess the activity of the tested gel formulation over time. A study with aged gel formulations would have helped predict the time period over which it would remain active in practical pest control applications.

The broad objective of our work has been to develop an effective attract-and-kill technology for *P.* interpunctella. We initially evaluated a variety of contact insecticides against male *P. interpunctella* for residual activity, and determined that natural pyrethrum and synthetic pyrethroids had very good activity in simple surface-contact bioassays (Campos and Phillips 2010). In the current study described below we evaluated the efficacy of three types of attract-and-kill devices, each with previously determined active insecticide formulations, in a wind tunnel activity against *P. interpunctella* males. The efficacy of these attract-and-kill formulations was based on male contact with a treated substrate following upwind flight to a pheromone lure, male mortality, and reproductive fitness of surviving males when paired with females. We evaluated the residual activity of these attract-and-kill formulations at five different times over a period of eight weeks.

2. Materials And Methods

2.1. Insects.

P. interpunctella male and female adults from the laboratory culture at Oklahoma State University were reared on a diet containing corn meal, chick starter, egg crumbles and glycerol (4:2:2:1) in 425-ml glass jars (Alltrista, Muncie, IN) placed in a growth chamber at 28 °C, 60-70 % r.h., and L16:D8 photoperiod. Corrugated cardboard rolls (1×5 cm) were placed into the culture jars for the last-stage wandering larvae to crawl into and pupate. The pupae were removed from the cardboard rolls, separated by sex and placed individually into 1.0-dram vials with ventilated plastic caps (Fisher Scientific, Pittsburg, PA, USA) and returned to the growth chamber until they emerged as adults. For these experiments, 1-2- day-old virgin adults were used and each adult was used only once.

2.2. Wind tunnel

The wind tunnel used consisted of a wood frame (W = 91 cm, H = 91 cm, L = 182 cm) with side walls and roof made of rigid Plexiglass®. The floor of the tunnel was an aluminum sheet and the two ends of the tunnel were covered by conventional window fly screening to prevent escape of moths. The down-wind end of the tunnel had a plenum that reduced the 91- × 91-cm square-opening to a circular-opening (38-cm i.d.) with an exhaust fan driven by an electric motor equipped with a rheostat to adjust exhaust wind speed. Air was exhausted from the tunnel via a pipe (38-cm i.d.) directly out of the room and to the exterior of the building so that contaminated air could not re-enter the tunnel. Room air was drawn into the tunnel at the upwind end by the suction of the exhaust fan and passed through an activated charcoal-impregnated filter to provide relatively clean air to the tunnel for flight assays. Wind speed in the tunnel was measured with

112 smoke tests using titanium tetrachloride (J.T.Baker, Phillipsburg, NJ, USA) and was set at 60 cm
113 per second for all tests, which was observed to give relatively even laminar flow through the
114 central core of the tunnel from upwind to downwind end.
115 Insects and test materials were prepared in a separate room, and only brought into the wind
116 tunnel room when a specific test was to be conducted to minimize contamination of room air
117 between assays. Controlled conditions maintained in the wind tunnel room were 26-28 °C, 50-
118 60% r.h., and lights provided by four fluorescent tubes, 60 W each, suspended over the tunnel
119 roof.
120
121 *2.3. Formulations tested and experimental procedures*
122 Three sets of experiments, each one with a different type of attract-and-kill formulation, were
123 conducted. The first tested was LastCall® gel (IPM Tech, Inc., Portland, OR, USA) with the
124 following formulations that each contained the synthetic female pheromone *Z,E* -9, 12-
125 tetradecadienyl acetate at 0.16% by weight; permethrin 6.0% active ingredient (AI), pyrethrin
126 6.0% AI, and gel with no insecticide but with the synthetic female pheromone only as an
127 attractant to serve as a non-insecticide "blank" control. These formulations were tested as
128 droplet sizes of 50- or 100-mg applied to the surface of a glass microscope slide (7.6 × 2.5 cm,
129 Sargent-Welch, USA) and held in place at the upwind end of the tunnel with a small binder clip
130 (ACCO, USA) suspended from a laboratory stand.
131 The second attract-and-kill formulation was a wax panel (20 × 13 cm; Suterra, Bend, OR,
132 USA) that contained the AI cyfluthrin at 0.01, 0.1, 1.0 and 6.0%, or permethrin at 6.0% AI and
133 deployed with a controlled release pheromone lure containing the synthetic female pheromone
134 (Biolure® by Suterra, Bend, OR, USA) placed in the center of the wax panel; and a control wax

panel, with no insecticide, deployed with the pheromone lure "blank". The material structure of the wax panel was a paper fiberboard panel that was coated with a mixture of paraffin and oil that contained the insecticide. The Biolure® pheromone release device was a sealed, thin foil pouch for which the bottom and most of the top surface were impermeable film that contained a reservoir of liquid pheromone, and the pheromone was evaporated through a semi-permeable membrane that controlled the release rate by its size and structure.

Finally, the third device was a plastic mesh cylinder (7 mm mesh; 35-cm height ×10-cm i.d.; Uniek Co., USA). Insecticides were sprayed onto the cylinders until run-off with an artist's air brush (Paasche, USA), and were either permethrin (FMC Co., Philadelphia, PA, USA), cyfluthrin (Bayer, Kansas City, MO, USA) or organically-compliant pyrethrin without the synergist PBO (Pyperonyl Butoxide; McLaughlin Gormley King Co., Minneapolis, MN, USA), each at 2.0% AI in the final mix and deployed with a Suterra Biolure® in the middle of the cylinder. A cylinder without insecticide, but with a pheromone lure was used as a control "blank". Attract-and-kill devices were suspended on a laboratory stand at the mid-point of the upwind end of the wind tunnel.

Two-day old virgin adult male *P. interpunctella* were released from a cage held on a laboratory stand at the middle of the downwind end of the tunnel. Five adult males were released inidividually in the wind tunnel and bioassayed against each replicate of each device type. Each male moth was given a maximum of 5 min to take flight and respond upwind to the device and contact it. Moths that did not touch the device in 5 min were considered as "no response" and scored 0 for analysis of the males that landed on the device only; those males that contacted the device were scored as responders.

157 The percentage of moths in a test group contacting each device, and time each male was in
158 contact with a device, were recorded. Once a male finished contact and flew away from the
159 device it was captured and placed into a 425-ml glass jar with a virgin female moth and 15 g of
160 cracked wheat kernels as a substrate for egg laying. Every male-female pair was kept for 24 h in
161 a growth chamber at 28 °C, 60-70 % r.h., and L16:D8 photoperiod.
162 Male mortality was recorded after 24 h. Eggs laid in the wheat were carefully separated from
163 the wheat using a U.S. no. 14 sieve (Seedburo Equipment Company, USA), counted and placed
164 on double-sided tape on a 9-cm-diameter black filter paper (Ahlstrom, Mt Holly Springs, PA,
165 USA) in a 9-cm-diameter Plastic Petri dishes (Fisher Scientific, Canada). The eggs were placed
166 into a growth chamber at 28 °C, 60-70 % r.h., and L16:D8 photoperiod for 5 days, after which
167 the the number hatched eggs was recorded.
168
169 *2.4. Statistical Analysis*
170 Data for each of the three attract-and-kill formulations were analyzed as three separate
171 experiments within a time period, and comparisons were made for each specific formulation
172 (e.g., applied insecticide concentration of a particular device type) across time periods. Each
173 device formulation type was treated with different concentrations of insecticides and four
174 replicates of each device type-insecticide concentration were established. A total of 20 males
175 were tested within four blocks of each device type. Each adult male in a group of five was
176 released individually and used only once.
177 The attract-and-kill formulations were tested in the wind tunnel at 0, 4, 6, and 8 weeks after
178 being established, and they were held and aged in a room separate from the wind tunnel between

testing times. The experimental design used for each attract-and-kill formulation was a randomized complete block design with four replicates. Each replicate was treated as a block.

The observations assessed were the percentage of released males that landed on and made contact with the device, the time in seconds each adult male was in contact with a given device (contact time), the percentage of male mortality of those that made contact, the number of eggs laid per female, and the percentage of these eggs that hatched per female. Proportions (percentages) were transformed by the arcsine-square root function prior to analysis. Data were analyzed with the procedure PROC MIXED in SAS/STAT 9 for Windows (SAS Institute, 2005), and the repeated measures option assuming an autoregressive covariance structure was used. Every attract-and-kill device type was analyzed separately. Every treatment was compared across the test period times (0, 2, 4, 6, or 8 weeks) and treatment differences were compared within each time period. Treatments compared across and within each time period were analyzed with pair wise t-tests and comparisons were protected by examining the SLICE OPTION within the Least Square Means statement at $\alpha = 0.05$ level.

3. Results

3.1. Device contact

Fig. 1 shows the mean percentage of *P. interpunctella* adult males that contacted the attract-and-kill devices within five minutes in the wind tunnel. Statistical analyses of the LastCall® gel formulations (Fig. 1a) across the entire eight-week experiment did not show significant differences for contact behavior among the two insecticide active ingredients and blank gels ($F_{2, 567} = 1.82$; $P = 0.1633$), or the amount tested, 50 mg or 100 mg each, for Permethrin and Pyrethrin in the gels ($F_{1, 567} = 0.04$; $P = 0.8492$). Also, significant differences were not found on the interactions of insecticide treatment and weeks ($F_{8, 567} = 1.69$; $P = 0.0970$), interactions of amounts of the two insecticide gels and weeks ($F_{4, 567} = 0.58$; $P = 0.6781$), and interactions among insecticide active ingredient, amount of the gel used and weeks of aging of the gel formulations ($F_{8, 567} = 1.24$; $P = 0.2750$). However, there was a significant interaction effect of active ingredient tested and the two amounts of gel for each AI ($F_{2, 567} = 4.73$; $P = 0.0092$).

Landing and contact responses of moths varied significantly in some cases when compared across gel types within a given bioassay week, and also across weeks within a particular gel type. At week 0, the highest percentage of landing by male moths was 55% for the Blank 100 mg and Permethrin 50 mg, and the lowest was on Permethrin 100 mg at 20%. At week 2, 4, and 6, there was no significant difference in percent contact among treatments. By week 8 responses to the gels were very low, but with some difference among treatments (Fig. 1a). When comparing across bioassay times the maximum landing by males on the gel formulations was observed at week 2, when responses ranged from 70 to 85%.

Analysis of the percentage of males landing on the wax panel devices (Fig. 1b) showed no statistical difference among treatments for the whole experiment ($F_{5, 567} = 1.01$; $P = 0.4117$), nor for treatments within weeks ($F_{20, 567} = 1.30$; $P = 0.1733$). The comparison of the moths that contacted the panels across the eight-week period shows that at week 0, less than 25% of moths landed on the device. This response increased to 90 to 100% from week 2 to the end of the experiment. At week 0, the formulations Blank and Cyfluthrin at 0.01 and 0.1% elicited 25% or less of landing, which were statistically different from the Cyfluthrin 1.0 and 6.0%, and Permethrin 6.0%, at 5% landing for each (Fig. 1b). However, Cyfluthrin 0.1% was statistically similar to Cyfluthrin 1.0%. At week 2, all treatments elicited 90 to 100% landing by males and it was similar up to the end of the experiment at week 8.

The percentage of moths landing on the plastic cylinder (Fig. 1c) was observed to be significantly different among treatments overall ($F_{3, 377} = 3.74$; $P = 0.0113$) and treatments within weeks ($F_{12, 377} = 2.18$; $P = 0.0121$). At week 0, Blank and Cyfluthrin 2.0% showed attractiveness of 50 and 60% respectively, significantly greater than the other treatments. At week 2, Cyfluthrin and Pyrethrin 2.0% elicited 85 and 80% landing, respectively, and were statistically similar. Pyrethrin 2.0% did not differ from Blank (65% landing). However, these treatments differed from Permethrin 2.0%, which showed the lowest landing rate of 45% in week 2. From week 4 to the end of the experiment at week 8, all treatments elicited 100% landing by tested males onto the plastic cylinder devices. These plastic cylinder devices used the same commercial pheromone lures as the wax panel formulations, and similar patterns of response were observed during other weeks for the two devices. At week 0, there was low response and from week 4 to the end of the experiment there was 100% landing of all 20 males (5 males in four replicates) for all wax panel and cylinder devices.

239 *3.2. Contact time*

240 The contact time, which was the time in seconds that adult males were in contact with
241 devices tested, is shown in Fig. 2. The gel-like formulations (Fig. 2a) all had relatively short
242 contact times and did not show significant differences among amounts of gel (i.e., 50 mg vs. 100
243 mg; $F_{1, 570} = 0.19$; $P = 0.6594$), in the interaction of gel amount and week of the bioassay ($F_{4, 570}$
244 $= 0.96$; $P = 0.4300$), or in the interaction among treatment AI, amount of gel and week ($F_{8, 570} =$
245 1.66; $P = 0.1059$) for the whole experiment. The AI treatments were significantly different ($F_{2,}$
246 $_{570} = 3.69$; $P = 0.0255$), AI treatments within weeks among gel types ($F_{8, 570} = 2.20$; $P = 0.0259$)
247 and amount of gel within weeks ($F_{2, 570} = 5.67$; $P = 0.0036$) for moth contact time over the whole
248 experiment.

249 All gel treatments, when analyzed across the eight-week period, showed the highest contact
250 time at week 2, and they were significantly different from the rest of the weeks. At week 0 the
251 Blank (100 mg) and Permethrin (50 mg) gel formulations were statistically similar and showed
252 the highest contact time (0.8 and 0.75 seconds, respectively), but they differed statistically from
253 Pyrethrin 50 mg and Permethrin 100 mg, which had the lowest contact times. All these
254 treatments were statistically similar to the rest of the treatments. At week 2 Permethrin 50 mg
255 showed the longest contact time, with a mean of 1.5 seconds, and it was significantly different
256 from the formulations with Pyrethrin 50 and 100 mg. At week 4, all treatments were statistically
257 similar, and at weeks 6 and 8 the contact times were very brief and differences were slight
258 among gel types, though statistically significant.

259 Analysis of male contact time on the wax panel formulations (Fig. 2b) revealed that there
260 were significant differences among AI treatments overall ($F_{5, 570} = 2.23$=; $P = 0.0498$) and among
261 treatments within weeks ($F_{20,570} = 3.44$; $P < 0.0001$). At week 0, when pheromone lures were

fresh from their storage packages and residual insecticides were recently applied, all wax panel treatments had very short contact times when compared to the rest of the weeks, which were statistically similar to each other. At week 2, the panels with Cyfluthrin at 6% had the longest mean contact time of 13.8 seconds, while Permethrin at 6.0% had the shortest time of 7.1 seconds. Conversely, at week 4 Permethrin at 6.0% had the longest contact time at 12.4 seconds, while Cyfluthrin at 0.01% had the lowest contact time at 5.6 seconds. At week 6, Cyfluthrin 0.01%, Cyfluthrin 0.1% and Permethrin 6.0% did not differ statistically, but they were significantly different from Cyfluthrin 1.0% and 6.0%. Cyfluthrin 6.0% and Permethrin 6.0% did not differ statistically from the rest of the treatments at week 6. Contact times on wax panels at week 8 were statistically similar to those observed at week 6.

Analysis of contact times for the plastic cylindrical (Fig. 2c) device formulations showed a significant difference among AI treatments overall ($F_{3, 380} = 8.58$; $P < 0.0001$) and AI treatments within weeks ($F_{12, 380} = 1.82$; $P = 0.0436$). As with wax panels, contact times on plastic cylinders were short at time 0 and then were longer in most cases from bioassay time 2 weeks through 8 weeks, with the longest mean contact time observed for males on cylinders with Cyflthrin at 2.0% AI.

3.3. Male mortality after contact

Fig. 4 shows the percentage mortality of adult male *P. interpunctella* 24-h after contacting the attract-and-kill devices. For the gel formulations (Fig. 3a) there were no significant difference among amounts of gel ($F_{1, 567} = 0.60$; $P = 0.4380$), interaction of gel amounts within week ($F_{4, 567} = 0.14$; $P = 0.9664$), interaction of AI treatment by gel amount ($F_{2, 567} = 1.96$; $P = 0.1422$), or AI treatment by gel amount by week ($F_{8, 567} = 1.62$; $P = 0.1152$). However, there were significant differences among treatments ($F_{2, 567} = 35.86$; $P < 0.0001$) and treatments within

285 weeks ($F_{8, 567} = 6.99$; $P < 0.0001$). Regardless of overall differences, the highest mortality was
286 observed only in week 2 with gel containing 6.0% Permethrin, at 70%, and in subsequent
287 bioassay times the male mortality levels were relatively low, ranging from 0% to 40%.
288 The analysis of wax panel formulations (Fig. 4b) revealed significant differences among AI
289 treatments ($F_{5, 567} = 196.37$; $P < 0.0001$) and for the interaction of AI treatments by weeks ($F_{20,}$
290 $_{567} = 12.11$; $P < 0.0001$). At week 0, all AI treatments were statistically similar with very low
291 mortality. However, from week 2 to the end of the experiment at week 8 the wax panel
292 formulations based on Cyfluthrin and Permethrin both at 6.0% AI, which were statistically
293 similar, killed over 85% of the adult males, followed by Cyfluthrin 1.0%, which differed
294 statistically from the rest of the treatments, which had only 0% to 10% mortality. The attract-
295 and-kill formulations based on the plastic cylinder showed a significant difference in male
296 mortality among AI treatments ($F_{3, 380} = 78.15$; $P < 0.0001$), but the interaction of AI treatments
297 by weeks was not significantly different ($F_{12, 380} = 1.38$; $P = 0.1732$).
298 The cylinder device sprayed with Cyfluthrin 2.0% elicited significantly higher levels of
299 mortality compared to the other treatments, and it killed 75% or more of the adult males during
300 the whole experiment, except for week 0 in which it killed 50% on average (Fig. 3c).
301
302 *3.4. Egg-laying*
303 Fig. 4 shows the mean egg-laying per female *P. interpunctella* that were paired for 24-h with
304 males that had contacted attract-and-kill devices in wind tunnel bioassays. The statistical
305 analysis for the gel formulation (Fig. 4a) showed that there was no significant difference among
306 AI treatments ($F_{2, 570} = 2.75$; $P = 0.6877$), amount of gel ($F_{1, 570} = 0.20$; $P = 0.6558$), interaction
307 of AI treatment by amount of gel ($F_{2, 570} = 0.008$; $P = 0.9247$), interaction of AI treatment by

week ($F_{8, 570} = 0.70$; $P = 0.6877$), interaction of amount of gel by week ($F_{4, 570} = 0.41$; $P = 0.7981$) and interaction of AI treatment by amount of gel by week ($F_{8, 570} = 0.28$; $P = 0.9721$). Treatment differences were found only for week 2, in which the formulations with Pyrethrin 100 mg and Permethrin 100 mg showed the lowest averages of egg laying and were significantly different from the Blank formulations (50 and 100 mg). However, all the remaining formulations did not differ from each other and the numbers of eggs laid by females paired to males that had contacted gels were relatively high.

In the case of the wax panel (Fig. 4b), there was a significant difference among AI treatments ($F_{5, 570} = 35.85$; $P < 0.0001$) and with the interaction of AI treatments by weeks ($F_{20, 570} = 3.28$; $P < 0.0001$). At week 0, there was no significant difference among treatments. From week 2 to week 8 the wax panel formulations with Cyfluthrin 1.0% and 6.0%, and Permethrin 6.0% were statistically similar and elicited low egg laying averages compared to Blank and the formulations with low percentage of Cyfluthrin (0.01 and 0.1% AI), which averaged over 35 eggs laid per female.

Analysis of females paired with males that had been bioassayed against the cylinder devices (Fig. 4c) showed a significant difference in egg laying among the AI treatments ($F_{3, 380} = 28.98$; $P < 0.0001$), but there was no significant interaction effect of the AI treatments by weeks ($F_{12, 380} = 0.87$; $P = 0.5746$). In the whole experiment, the Blank treatment showed the highest egg laying and was significantly different from the rest of the treatments, except at week 8, in which it was similar to Permethrin 2.0%. The Cyfluthrin 2.0% generally had the most suppressive effect on number of eggs laid per female.

3.5. Egg hatching.

331 Analysis of responses to the gel formulations revealed a significant difference among AI
332 treatments ($F_{2, 569} = 10.21$; $P < 0.0001$) in the percentage of eggs that hatched from those laid by
333 females paired with males from bioassays (Fig. 5a). However, there were no significant
334 differences among gel amount ($F_{1, 569} = 0.01$; $P = 0.9492$), interaction of AI treatment by gel
335 amount ($F_{2, 569} = 1.79$; $P = 0.1679$), interaction of AI treatment by week ($F_{8, 569} = 1.82$; $P =$
336 0.0706), interaction of gel amount by week ($F_{4, 569} = 0.40$; $P = 0.8098$) and interaction of AI
337 treatment by gel amount by week ($F_{8, 569} = 0.38$; $P = 0.9299$). There were no significant
338 differences among AI treatments at weeks 0 and 8. In the other weeks there were statistically
339 significant reductions in egg hatching in clutches from insecticide-treated gels, but these were
340 not substantial.
341 The experiment with the wax panel (Fig. 5b) showed significant differences in egg hatch
342 among AI treatments ($F_{5, 570} = 45.57$; $P < 0.0001$) and in the interaction of AI treatments by week
343 ($F_{20, 570} = 4.05$; $P < 0.0001$). Permethrin 6.0% and Cyfluthrin 1.0% and 6.0% were the
344 treatments with lower percentage of hatched eggs in most of the dates and these three were
345 statistically similar at the eight-week period. In general, high concentrations of Cyfluthrin and
346 Permethrin on wax panels were associated with lower percent of egg hatching compared to the
347 Blank and low percent AI of Cyfluthrin.
348 The cylinder formulation analysis (Fig. 5c) showed experiment-wide significant differences
349 in egg hatching among AI treatments ($F_{3, 377} = 37.38$; $P < 0.0001$). However, there was no
350 significant interaction of AI treatments by weeks ($F_{12, 377} = 1.12$; $P = 0.3419$). The percentage of
351 hatching of the insecticide treatments was significantly lower than Blank in weeks 4, 6, and 8.
352 Hatch rates were the lowest resulting from AI treatments of Cyfluthrin 2.0% in week 2 and 4,

being 8.4% and 8.0 %, respectively, and hatching ranged from 16.9% to 67.7% in other AI treatments.

4. **Discussion**

The experiments reported here will help determine the optimal device design, pheromone release technology and insecticide formulation to pursue further for development of an attract-and-kill technology to control pest populations of *P. interpunctella* in commercial settings, some of which were tested in subsequent research (Campos 2008). The eight-week time period studied here was employed to examine a realistic time period in which a pest control company might apply a typical treatment to a facility, such as frequency of insecticide sprays or aerosols "fogging" applications (e.g., Arthur and Phillips, 2003), for Indianmeal moth control. Since the ultimate goal of the attract-and-kill strategy is to kill enough males in a population to cause a negative impact on reproduction, these experiments provided an estimate of reproductive impact by killing or otherwise incapacitating male moths so that mating and reproduction with females could be reduced. The reproductive fitness of individual males that had contacted an attract-and-kill device was manifested by how many eggs were laid and 1st instar larvae (percentage of eggs that hatched) produced when they were paired with a virgin female immediately after treatment.

It is important to note that the percentage of males landing on and maintaining contact with any of the three devices was consistently low at time 0, but then improved in subsequent weeks as the formulations aged. This delayed activity was probably due to the pheromone dispensing system being newly exposed to air at time 0. The commercial pheromone lures (Biolure® lures) were opened from sealed storage packages and the LastCall® gel was applied from tubes just before conducting the time-0 assays. We submit that there was a relatively high release of synthetic pheromone at time 0 compared to later times such that orientation to the point source and sustained contact by responding males was inhibited or otherwise less than optimal. Although percent contact and contact time were low at time 0, we noted that most males

379 approached the attract-and-kill device in a zig-zag flight pattern; they landed within a few
380 centimeters from the pheromone source and walked around with abdomens curved ready to mate,
381 which would be adequate to be captured in sticky traps for which the slow-release lures are
382 intended for use. Our results suggest that such lures and gels should be aged between 0 and 2
383 weeks for best use in attract-and-kill applications agains Indianmela moth. Work with
384 pheromone lures of other species has shown that initial low or high release of pheromone can
385 cause a lack of complete response or inactivation instead of full attractive response and sustained
386 contact with the source (Baker and Roelofs, 1981; Baker *et al*., 1981; Kuenen and Baker, 1982;
387 Hussain *et al.,* 1994, personal observations).
388 The wax panel and cylinder devices were clearly superior to the gel formulation for
389 achieving desired moth responses. Gel formulations elicited very low contact response (20-55%)
390 at time 0, peak responses at the 2-week bioassay (70-85%), and then had a sharp decline in
391 activity from week 4 to week 8. Thus, the gel formulation could not sustain activity for
392 substantial male-killing through the eight-week study, which was not addressed in the
393 experiments by Nansen and Phillips (2004), and our data suggest this gel would probably be
394 ineffective in a practical application for 8 weeks. Alternatively, the Biolure® pheromone lures
395 used with the wax panel and cylinder devices had characteristic low activity for contact only at
396 time 0, but showed increased and sustained activity for male response from week 2 onward, with
397 essentially 100% male contact and contact times of several seconds. Contact time with the
398 devices was similarly much higher for the wax panels and cylinders that were baited with
399 Biolure®, compared to the gel formulation, and this was maintained from week 2 until the 8-
400 week end of the study.

401 Higher and sustained mortality levels for certain formulations of wax panels and plastic
402 cylinders compared to the gel can be attributed to the more effective pheromone lure system, but
403 also probably to the overall larger surface area of the device itself, compared to the small amount
404 of material presented by the gel formulations. High contact times were recorded for moths
405 responding to wax panels and cylinders, and it was observed that during these times the male
406 moths would move around over the surface of the device, which probably contributed to better
407 contact with insecticide and the ultimate toxicity. Higher male mortality levels, specifically on
408 the wax panels with 6.0% Cyfluthrin and 6.0% Permethrin, and on the plastic cylinder with 2.0%
409 Cyfluthrin, corresponded to high male mortality, subsequent low levels of egg laying and
410 ultimately low hatch rates of those eggs. These results suggest that the wax panel formulation
411 would be effective for Indianmeal moth suppression in practical applications. The results
412 indicate that higher concentrations, greater than 1.0%, of the synthetic pyrethroids Cyfluthrin and
413 Permethrin, result in the most effective attract-and-kill devices when the wax panel and plastic
414 cylinder were used.
415 Organically-compliant natural Pyrethrin at 2.0% was not effective enough on the plastic
416 cylinder at any bioassay time during the eight-week period to pursue further applied research.
417 Permethrin at 2.0% on the cylinder was also not effective compared to 2.0% Cyfluthrin, and this
418 may have been due to physical or chemical interaction with the substrate that resulted in lowered
419 activity compared to that of the same compound on another substrate (Campos and Phillips,
420 2010). Future research will need to involve studies with formulations of high concentration
421 Cyfluthrin or Permethrin on wax panels, or Cyfluthrin on plastic cylinders with *P. interpunctella*
422 populations in experimental or commercial food establishments.

Pheromone-based pest management technologies are gaining popularity with stored-product systems because of their relative safety for food, workers and the environment, and the reduction or elimination of synthetic insecticides from these systems (Phillips and Throne, 2010). Mating disruption of stored-product moth species using the same synthetic pheromone used in this current work has been well studied (e.g., Ryne et al., 2007) and the method was registered for pest control by the lead regulatory agency of the USA (EPA, 2006). The attract-and-kill method studied here, like similar systems studied in other agricultural settings (e.g., the fruit pest systems of Evenden et al., 2005, and Krupke et al. 2002), uses sysnthetic sex pheromone at release levels similar to those used in moth monitoring traps, and deploys very small amounts of synthetic insecticide precisely placed point sources that can be retrieed at the end for the control program, thus leaving no or very little residue at the site. The most active insecticide treatments determined in the current work, the pyrethroids Cyfluthrin and Permethrin, are already widely registered for use in stored-product and food environments (Arthur and Phillips, 2003), thus we project that attract-and-kill systems for storage moths like those studied here might readily receive regulatory approval if developed into commercial pest control products.

Aknowledgements

The authors thank Dr. Mark Payton for advice on statistical analyses and also Drs. Jack Dillwith, Brad Kard and Mark Payton for comments on earlier drafts of this manuscript. Technical assistance and logistical support was provided by Edmond Bonjour and his staff for maintenance of insect colonies. We appreciate the generous supplying of test materials from IPM Technologies of Portland, Orgeon, USA and also from Suterra in Bend, Oregon, USA. Financial support was provided by grants from the USDA Risk Avoidance and Mitigation Program

(Project No. S05035), the Oklahoma Agricultural Experiment Station, and the Kansas Agricultural Experiment Station.

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Figure Legends

Fig. 1. Mean percentage (%) of *P. interpunctella* adult males (±SE) that landed onto three attract-and-kill formulations [a) Gel, b) Wax panel and c) Plastic cylinder] in a wind tunnel during an eight-week aging period. Means across weeks per each formulation followed by the same letter (Upper case) and means for all formulations at given week followed by the same letter (Lower case) are not significantly different at $P < 0.05$. Analysis was conducted separately for each attract-and-kill formulation. Py = Pyrethrin, Pe = Permethrin, Cy = Cyfluthrin, Blank = Pheromone only. All treatments for week 2-8 of wax panel formulation were A,a.

Fig. 2. Mean time in seconds (±SE) that *P. interpunctella* adult males were in contact with the attract-and-kill formulations [a) Gel, b) Wax panel and c) Plastic cylinder] in a wind tunnel during an eight-week aging period. Means across weeks per each formulation followed by the same letter (Upper case) and means for all formulations at given week followed by the same letter (Lower case) are not significantly different at $P < 0.05$. Analysis was conducted separately for each attract-and-kill formulation. Py = Pyrethrin, Pe = Permethrin, Cy = Cyfluthrin, Blank = Pheromone only.

Fig. 3. Mean percent (%) mortality of *P. interpunctella* adult males (±SE) for each attract-and-kill formulation [a) Gel, b) Wax panel and c) Plastic cylinder] in a wind tunnel during an eight-week aging period. Means across weeks per each formulation followed by the same letter (Upper case) and means for all formulations at given week followed by the same letter (Lower case) are not significantly different at $P < 0.05$. Analysis was conducted separately for each attract-and-kill formulation. Py = Pyrethrin, Pe = Permethrin, Cy = Cyfluthrin, Blank = Pheromone only.

565

566 Fig. 4. Mean number (±SE) of laid eggs per *P. interpunctella* female for each attract-and-kill
567 formulation [a) Gel, b) Wax panel and c) Plastic cylinder] in a wind tunnel during an eight-week
568 aging period. Means across weeks per each formulation followed by the same letter (Upper case)
569 and means for all formulations at given week followed by the same letter (Lower case) are not
570 significantly different at $P < 0.05$. Analysis was conducted separately for each attract-and-kill
571 formulation. Py = Pyrethrin, Pe = Permethrin, Cy = Cyfluthrin, Blank = Pheromone only.

572

573 Fig. 5. Mean percent (%) of hatched egg (±SE) of *P. interpunctella* for each attract-and-kill
574 formulation [a) Gel, b) Wax panel and c) Plastic cylinder] in a wind tunnel during an eight-week
575 aging period. Means across weeks per each formulation followed by the same letter (Upper case)
576 and means for all formulations at given week followed by the same letter (Lower case) are not
577 significantly different at $P < 0.05$. Analysis was conducted separately for each attract-and-kill
578 formulation. Py = Pyrethrin, Pe = Permethrin, Cy = Cyfluthrin, Blank = Pheromone only.

579

580 Supp. Fig. 1. Attract and kill formulations tested under in a wind tunnel: a) Gel with pheromone
581 and insecticide on a microscope slide (left) with moth responding upwind from right; b) Wax
582 panel impregnated with insecticide (note pheromone lure in center hole of panel); c) Plastic
583 cylinder coated with insecticide. Male moth in each photo is about 6.0 mm long.

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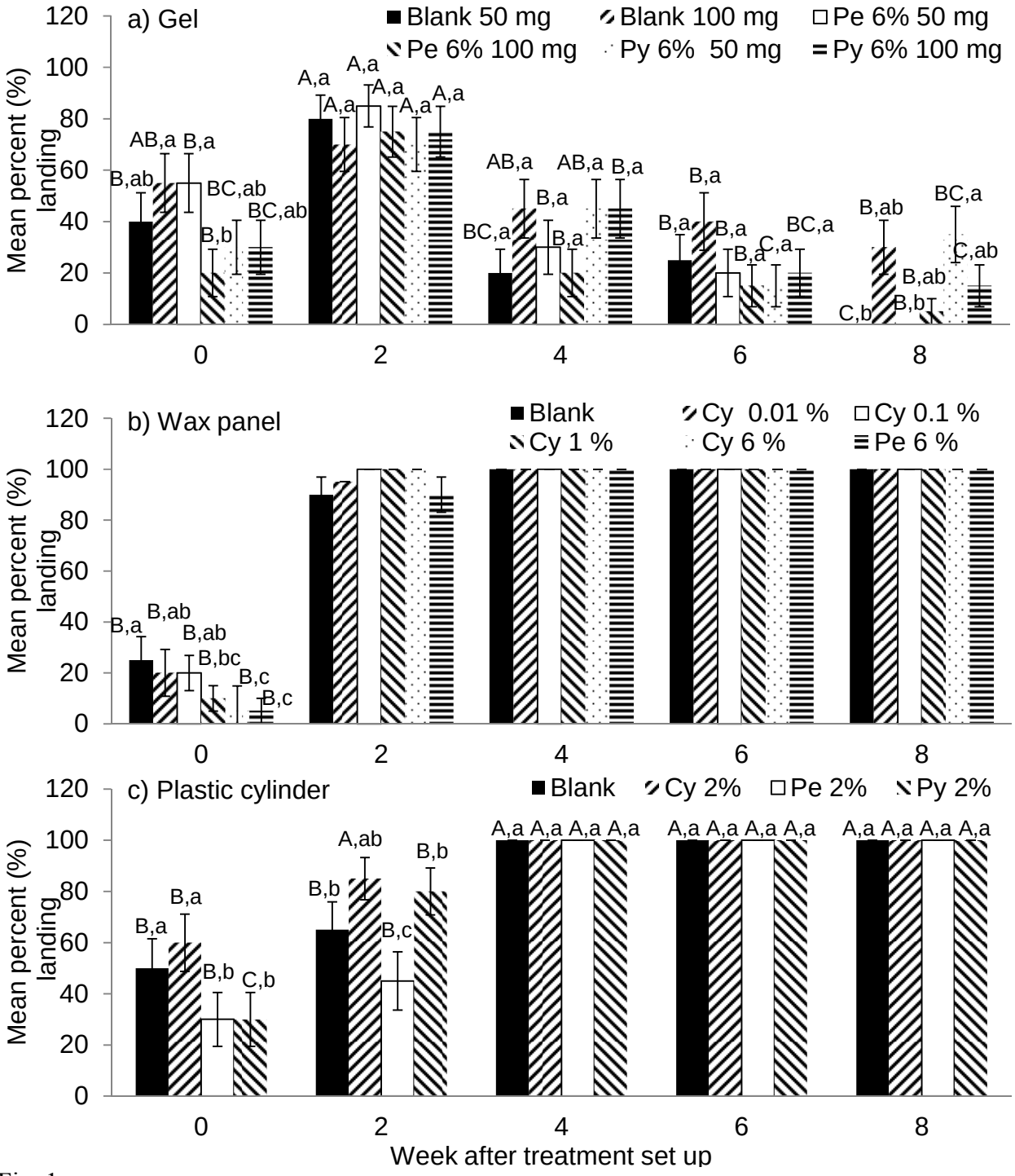
a) Gel
Blank 50 mg
Blank 100 mg
Pe 6% 50 mg
Pe 6% 100 mg
Py 6% 50 mg
Py 6% 100 mg
Mean percent (%) landing
b) Wax panel
Blank
Cy 0.01 %
Cy 0.1 %
Cy 1 %
Cy 6 %
Pe 6 %
c) Plastic cylinder
Blank
Cy 2%
Pe 2%
Py 2%
Week after treatment set up

Fig. 1.

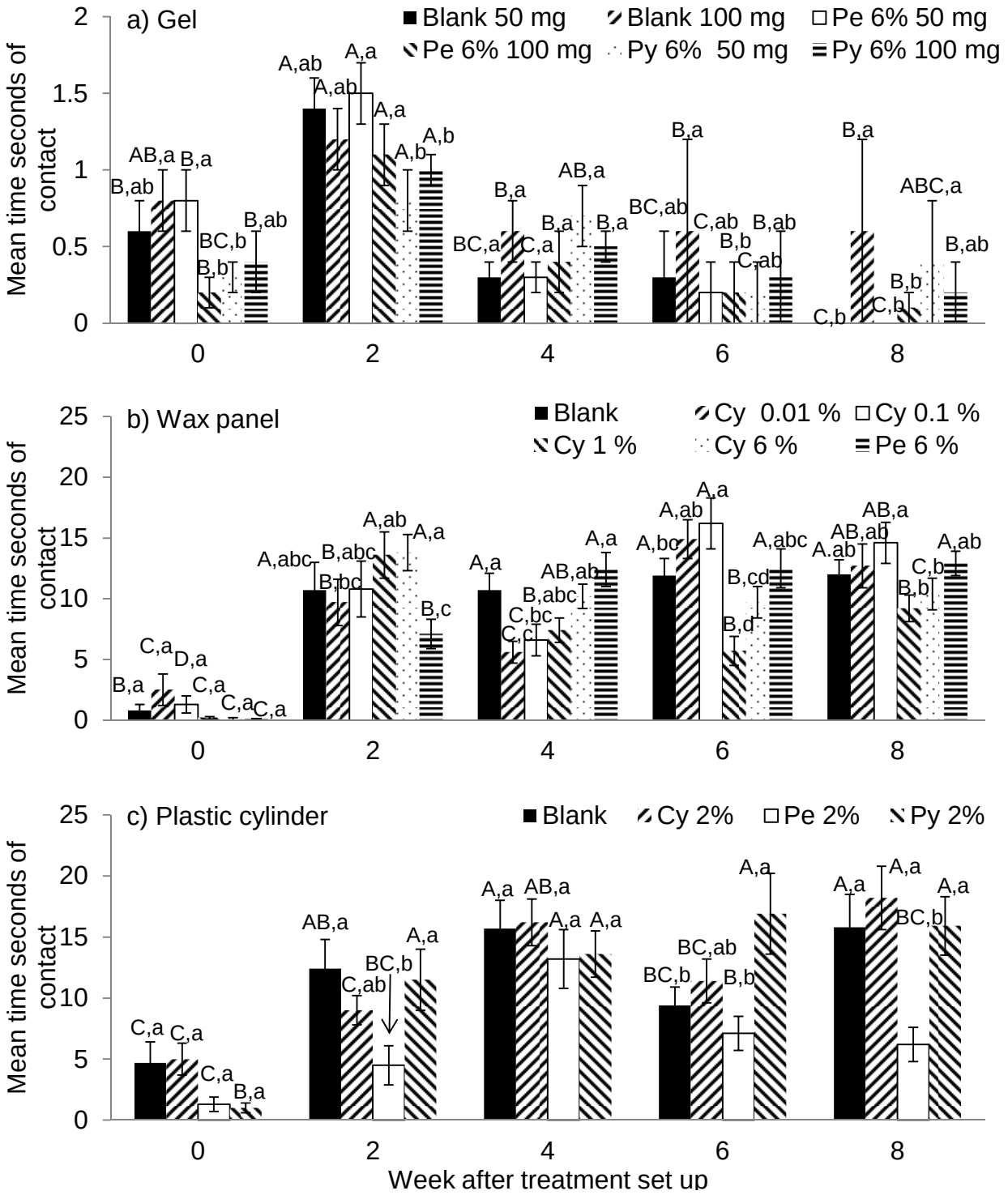
a) Gel
Blank 50 mg
Blank 100 mg
Pe 6% 50 mg
Pe 6% 100 mg
Py 6% 50 mg
Py 6% 100 mg
Mean time seconds of contact
b) Wax panel
Blank
Cy 0.01 %
Cy 0.1 %
Cy 1 %
Cy 6 %
Pe 6 %
Mean time seconds of contact
c) Plastic cylinder
Blank
Cy 2%
Pe 2%
Py 2%
Mean time seconds of contact
Week after treatment set up

Fig. 2.

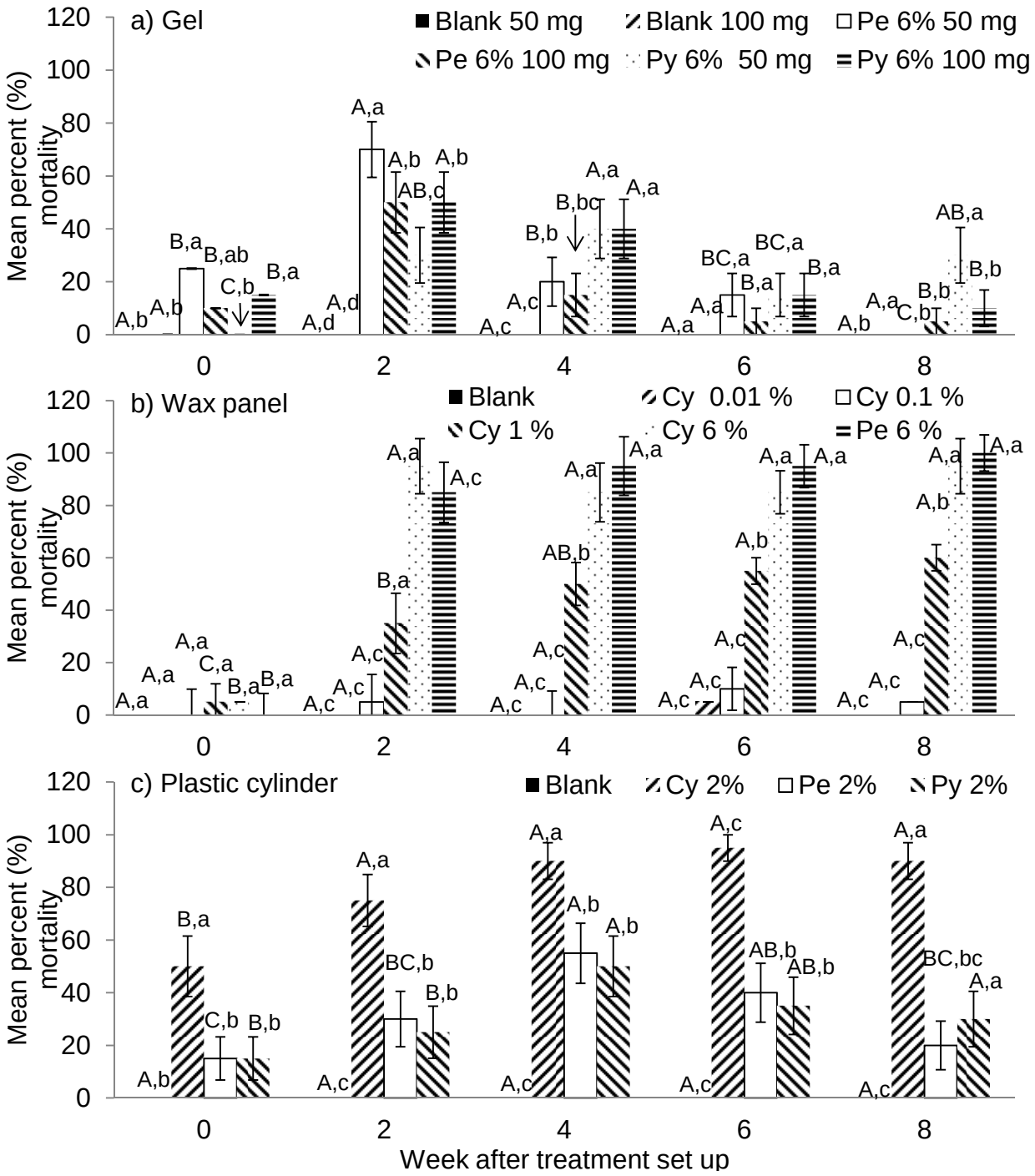
a) Gel
Blank 50 mg
Blank 100 mg
Pe 6% 50 mg
Pe 6% 100 mg
Py 6% 50 mg
Py 6% 100 mg
Mean percent (%) mortality
b) Wax panel
Blank
Cy 0.01 %
Cy 0.1 %
Cy 1 %
Cy 6 %
Pe 6 %
c) Plastic cylinder
Blank
Cy 2%
Pe 2%
Py 2%
Week after treatment set up

Fig. 3.

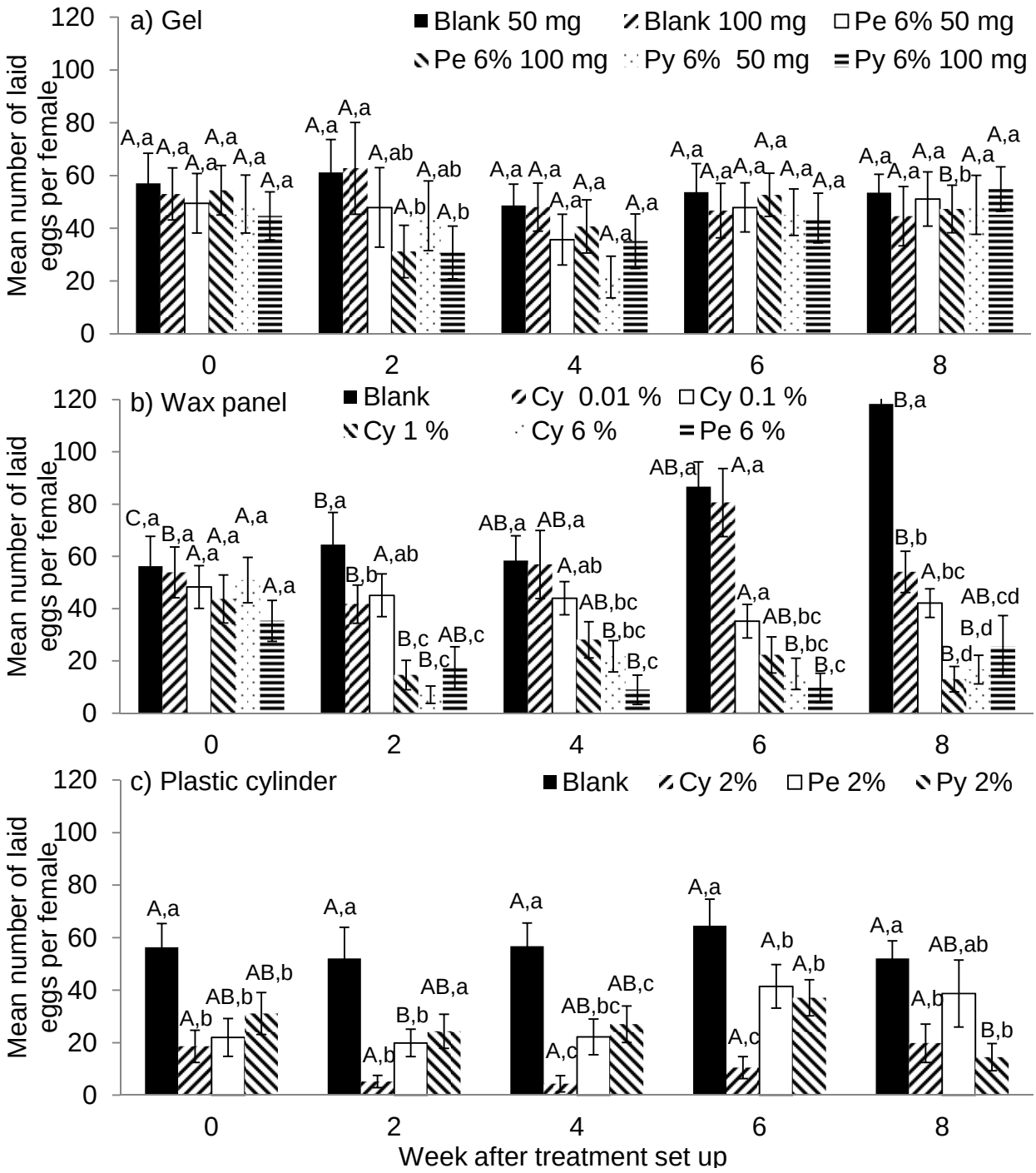
a) Gel
Blank 50 mg
Blank 100 mg
Pe 6% 50 mg
Pe 6% 100 mg
Py 6% 50 mg
Py 6% 100 mg
Mean number of laid eggs per female
b) Wax panel
Blank
Cy 0.01 %
Cy 0.1 %
Cy 1 %
Cy 6 %
Pe 6 %
c) Plastic cylinder
Blank
Cy 2%
Pe 2%
Py 2%
Week after treatment set up

Fig. 4.

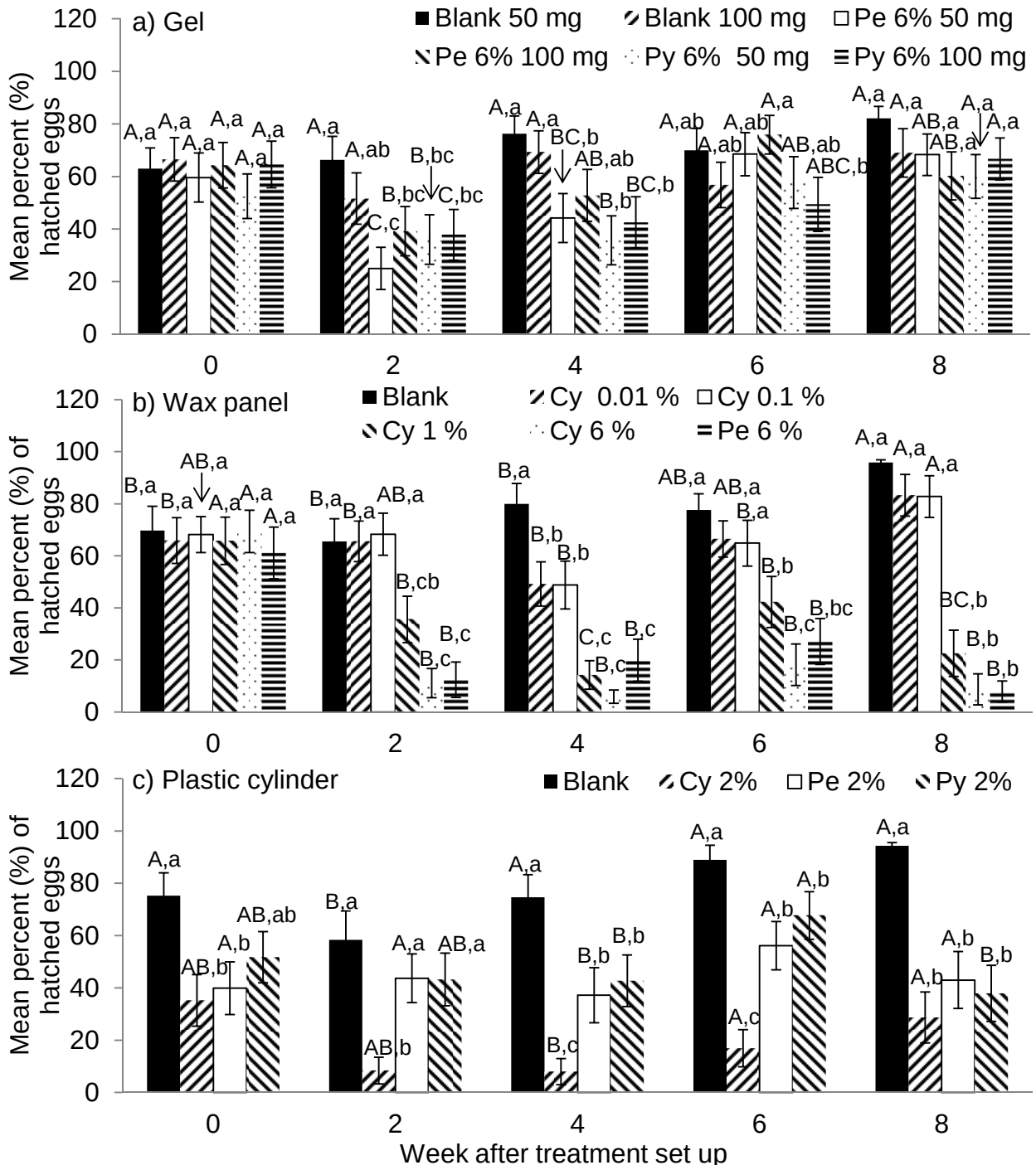
a) Gel
Blank 50 mg
Blank 100 mg
Pe 6% 50 mg
Pe 6% 100 mg
Py 6% 50 mg
Py 6% 100 mg
Mean percent (%) hatched eggs
b) Wax panel
Blank
Cy 0.01 %
Cy 0.1 %
Cy 1 %
Cy 6 %
Pe 6 %
Mean percent (%) of hatched eggs
c) Plastic cylinder
Blank
Cy 2%
Pe 2%
Py 2%
Mean percent (%) of hatched eggs
Week after treatment set up

Fig. 5.