

Behavior of Codling Moth (Lepidoptera: Tortricidae) Neonate Larvae on Surfaces Treated With Microencapsulated Pear Ester

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ABSTRACT Codling moth, *Cydia pomonella* (L.), larvae cause severe internal feeding damage to apples, pears, and walnuts worldwide. Research has demonstrated that codling moth neonate first instar larvae are attracted to a pear-derived kairomone, ethyl (2*E*,4*Z*)-2,4-decadienoate, the pear ester (PE). Reported here are the behavioral activities of neonate codling moth larvae to microencapsulated pear ester (MEC-PE) applied in aqueous solutions to both filter paper and apple leaf surfaces that were evaluated over a period of up to 20 d of aging. In dual-choice tests the MEC-PE treatment elicited attraction to and longer time spent on treated zones of filter papers relative to water-treated control zones for up to 14 d of aging. A higher concentration of MEC-PE caused no preferential response to the treated zone for the first 5 d of aging followed by significant responses through day 20 of aging, suggesting sensory adaptation as an initial concentration factor. Estimated emission levels of PE from treated filter papers were experimentally calculated for the observed behavioral thresholds evident over the aging period. When applied to apple leaves, MEC-PE changed neonate walking behavior by eliciting more frequent and longer time periods of arrestment and affected their ability to find the leaf base and stem or petiole. Effects of MEC-PE on extended walking time and arrestment by codling moth larvae would increase temporal and spatial exposure of neonates while on leaves; thereby potentially disrupting fruit or nut finding and enhancing mortality by increasing the exposure to insecticides, predation, and abiotic factors.

KEY WORDS larvae, arrestment, kairomone, pear ester, microencapsulated

Lepidopterous larvae that internally feed on their host plants pose a serious challenge to pest management because of the short period of their vulnerability to insecticidal control; the time between hatching on plant surfaces and penetration of their target fruits, vegetables, or nuts. Their ability to quickly find a host shortens their exposure to mortality factors of desiccation, predation, and the toxicity of applied insecticides. Neonate first instar larvae are aided in locating their hosts through detection of and orientation to host-plant kairomones (Jones and Coaker 1978, Hanson 1983). Identifying larval attractants for the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) has been a key research focus for over 70 yr (Wingo and Brown 1942). Codling moth larvae cause severe internal feeding damage to apples, *Malus domestica* Borkhausen; pears, *Pyrus communis* L.; and walnuts, *Juglans regia* L., worldwide as well as the introduction of molds and spoilage micro-organisms.

Codling moth females lay single eggs aggregated near fruits or nuts (Putman 1962, Geier 1963, Wearing et al. 1973, Jackson 1979, Thiery et al. 1995, Blomefield et al. 1997). In unmanaged apple orchards, Jackson

(1979) found that 92% of codling moth eggs were oviposited on foliage and 91% are laid within 20 cm of fruit. Within a 2 h period of hatching, neonate codling moth were typically able to crawl these distances, find, and penetrate a host fruit (Geier 1963, Jackson and Harwood 1980, Jackson 1982). In laboratory bioassays neonate codling moth were attracted to apple odors (Sutherland 1972, Landolt et al. 1998) particularly the predominant apple volatile, (*E,E*)- α -farnesene (Sutherland and Hutchins 1972, 1973; Sutherland et al. 1974; Suski and Sokolowski 1985; Bradley and Suckling 1995; Knight and Light 2001). In these prior in vitro experiments the extracts or compounds were applied as a droplet to a substrate or impregnated into a slower-release material (e.g., rubber septa). Thus, in these experimental designs kairomones were emitted from discrete point-sources that then evoked orientation and taxis responses from neonate larvae. Conversely, application of these kairomones, apple extract and (*E,E*)- α -farnesene, in a more diffuse or broadcast manner to larger areas of a substrate has demonstrated arrestment of neonate codling moth larvae and increases in the time taken by larvae to locate an apple fruit (Hughes et al. 2003). These effects led Hughes et al. (2003) to suggest that with broad substrate appli-

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cations of host kairomones “it may be possible to disrupt the host location behavior” of neonate larvae.

Autoxidative instability of (*E,E*)- α -farnesene (Cavill and Coggiola 1971) has hindered its development as a larval and adult attractant or disruptant for control; but, likewise has fostered the identification of other potential codling moth kairomones (Landolt et al. 1999). For example, a kairomone derived from pears that displays better stability and potency was identified as the pear ester (PE), ethyl (2*E*,4*Z*)-2,4-decadienoate (Light et al. 2001). Similar to (*E,E*)- α -farnesene, PE attracts both female and male adults (Light et al. 2001, Light and Knight 2005) and neonate codling moth larvae (Knight and Light 2001), while being specific to adult codling moth and congeneric *Cydia* species (Knight and Light 2004a, Schmidt et al. 2007). PE also stimulates oviposition (Knight and Light 2004b). Knight and Light (2001) demonstrated in laboratory bioassays that neonate larvae responded to point sources of PE by both increased speed of walking upwind and chemotaxis in still air.

To test its potential for larval control tactics in the field, PE was recently formulated in controlled-release microcapsules (MEC-PE) with emission rates at pg/h level that follow first-order power decay dynamics for up to 3 wk (Light and Beck 2010). Preliminary laboratory studies have demonstrated that dilute doses of MEC-PE will preferentially attract neonate codling moth larvae (Vitagliano et al. 2007, Light and Beck 2010). Moreover, kairomones have recently been tested as spray additives with insecticides. Ballard et al. (2000) demonstrated in field trials that (*E,E*)- α -farnesene tank-mixed as a spray additive with *C. pomonella* granulovirus significantly reduced deep-feeding damage of apples by codling moth. Similarly, MEC-PE has recently been tank-mixed with virus and various synthetic insecticides and found to improve their efficacy against codling moth infestation (Pasqualini et al. 2005a, Light 2007, Arthurs et al. 2007, Schmidt et al. 2008, Light and Knight 2011). Additionally, these field spray studies have investigated the application of dilute MEC-PE with no insecticide and demonstrated direct disruptive effects with decreases in fruit injury and increases in larval mortality in apple and pear orchards (Pasqualini et al. 2005a, Arthurs et al. 2007, Schmidt et al. 2008). No significant effects were noted in walnut orchard studies (Light 2007, Light and Knight 2011). However, to properly use such kairomones as spray adjuvants and to minimize the cost to the growers, the underlying mode of action, sensitivity, and specific influences on larval behavior should be resolved for this controlled release formulation and its broadcast-spray application.

The present laboratory study investigated the mode of action of MEC-PE through observation of orientation and taxis responses of neonate codling moth larvae to MEC-PE broadcast applied at simulated field rates to both filter paper and apple leaf substrates that were evaluated over periods of substrate residual-aging of up to 20 d. Dual-choice bioassays were conducted in open petri dish arenas to assess preferential responses of neonate larvae to zones of filter papers

treated with either water controls or MEC-PE. A prior, similar dual-choice MEC-PE study (Light and Beck 2010) was preliminary and limited to simply four aging-intervals and a single experimental dose rate, while this expanded study exposed differential behavioral effects and derived response thresholds by comparing two applied dose rates and their residual activity over 14 aging-intervals spanning up to 20 d of substrate aging. No-choice bioassays using whole, treated apple leaves compared neonate orientation and taxis behavior upon control water-treated leaves versus leaves treated with MEC-PE.

Materials and Methods

Microencapsulated Pear Ester. MEC-PE was produced by Trécé, Inc. (Adair, OK) using purified ($\approx 98\%$) ethyl (2*E*,4*Z*)-2,4-decadienoate. Previously used field application rate was equivalent to 1.5 g PE AI/ha, using a dose of 30 ml MEC-PE formulation tank-mixed in usually 9.4 hl water or a vol:vol dilution of the formulation of $\approx 1/32,000$ (Light and Knight 2011). MEC-PE formulation was removed from cold storage, warmed to room temperature and then thoroughly shaken before dilution mixing. Treatment rates used in the larval bioassays were the dilute field application rate of 1/32,000 and a more concentrated 1/1,000 mixture of MEC-PE with water.

Insects. Codling moth eggs laid upon wax-paper and diet were supplied weekly from an established colony at USDA-ARS San Joaquin Valley Agricultural Center, Parlier, CA. Additional eggs were laid by females reared in the laboratory on diet. Waxed-paper sheets with eggs were cut into squares (2×2 cm), placed in glass petri dishes with moist filter paper, sealed with parafilm against desiccation, and then refrigerated (13°C) until use. Single dishes with mature eggs were removed from refrigerator and warmed to room temperature (28°C), upon which eggs would hatch. Unfed, neonate first-instar larvae were used in bioassay experiments immediately upon hatching or within 1 h.

Host-Plant Leaf Surface Areas. Apple (Golden Delicious variety), pear (Bartlett variety), and walnut (Hartley variety) had leaf samples picked from five trees at mid-season from orchards in Winters, CA. Average surface areas were determined to be 29.0 ± 1.9 cm² (mean \pm SEM, range 12.9–43.2 cm², $n = 25$) for apple leaves, 16.1 ± 0.7 cm² (range, 9.7–23.9 cm², $n = 38$) for pear leaves, and 321.9 ± 26.5 cm² (range, 194.9–471.0 cm², $n = 10$) for walnut compound leaves. Larval bioassays were conducted on treated filter papers of pear leaf size and intact apple leaves. The average volume of water to moisten 16 cm² areas of filter paper (Whatman No. 1) was determined to be ≈ 200 μl , while ≥ 0.5 ml was required to wet the area of either the top or bottom of an average apple leaf.

Dual-Choice Tests, Filter Paper Arenas with Treated Versus Untreated Zones. Solutions of dilute and concentrated (1/32,000 and 1/1,000 dilutions, respectively) of MEC-PE formulation were mixed in volumes of 10 ml distilled water to which was added 12 μl of yellow food coloring (FD&C Yellow 5; Mc-

Cormick Inc., Hunt Valley, MD). A control solution of water and yellow dye was also mixed. Filter papers (9 cm, No. 2370-0900; Ahlstrom Inc., Mt. Holly Springs, PA) were first entirely wetted with distilled water and allowed to dry. Aliquots of 200 μl of the two test and control solutions were pipetted, as 20 droplets of 10 μl each, onto $\approx 60\%$ of the area of one hemisphere-side of the filter papers ($n = 4$), creating a half circular treatment-zone of 3.2 cm radius and approximate area of 16 cm^2 . Treatment zones were a faint yellow color and upon drying their edges were outlined with a faint No. 3 graphite pencil line. The opposite side of the filter paper was considered a blank zone and remained untreated. Estimated amount of encapsulated PE loaded upon a treatment zone was ≈ 320 ng for the dilute treatment and 10 μg for the concentrated treatment, representing ≈ 20 and 625 ng/cm^2 on treated filter paper, respectively. Treated filter papers set flat in the open lid of petri dishes (10 cm, No. 351029, Becton, Dickinson & Co., Franklin Lakes, NJ) were placed in a fume hood for drying (30°C) and aging. Because neonates are photopositive (Sutherland 1972), all bioassays were conducted in a warm (25–28°C, 60–70% RH) interior room without windows and solely illuminated by a single 60 W frosted-white light bulb (powered at 80 VDC rectified, ≈ 58 lux) hung directly over the bioassay table. For testing, single filter paper treatments in open dish lids were placed upon a rotatable stand (rotated manually $\approx 90^\circ$ every 20 s) positioned 1 m below the light source. Single newly hatched neonate larvae were placed via a fine tip sable-hair brush (No. 000; Princeton Art & Brush Co., Princeton, NJ) at the center point of the filter paper, the interface of the dual zones. Active walking neonates were each observed for a 300 s period while video recorded by a camera mounted ≈ 12 cm above arena (Video Flex 7600, Ken-A-Vision Mfg. Co., Inc., Kansas City, MO) followed by storage and analysis on a PC computer (Applied Vision software, ver. 2.1.0d, Ken-A-Vision). Number of crossing entries and exits of the zones was observed and time spent walking within a zone determined. Larvae that climbed upon the petri dish ($\approx 10\%$) were repositioned to center point of the filter paper, while inactive nonwalking larvae ($\approx 2\%$) were discarded. Bioassays were conducted on treated filter papers dried for 4 h, and then after progressive aging from 1 to 20 d in a fume hood, with 4 to 16 replicate runs per aging day for each MEC-PE treatment (dilute rate $n = 139$, concentrated rate $n = 161$) and water controls ($n = 66$). Shapiro-Wilk Normality test was used to determine that data sets were normally distributed. One- and two-way analysis of variance (ANOVA) were used to compare treatment effects in all studies (version 4.0, SigmaStat, 2008). Where necessary, the proportional data were arcsine transformed before analysis. Significant *F*-ratio means were further separated with the Tukey test for multiple comparisons, $P < 0.05$.

Estimation of PE Release Rates From MEC-PE Dose Applications. Collection of headspace emissions and their quantification were reported in Light and Beck (2010) and have been further analyzed here to

interpolate and extrapolate daily release rates (pg/h) of PE from the concentrated and dilute dose rates of MEC-PE applied to filter paper substrates. Briefly, filter papers (24 cm^2 , apple leaf size) placed in containment jars were wetted with 200 μl doses of MEC-PE at aqueous dilution rates of 1/100, 1/1,000, and 1/3,200 ($n = 3$) then opened jars were place in a vented oven (32°C) and allowed to evaporatively age over a 14 d period. Jars were removed and temporarily capped while solid-phase microextraction (SPME) headspace collections (60 min) were taken at 4 h after first drying and then 24, 48, 72, 96, 168, and 336 h later. SPME collected volatiles were analyzed by standard GC-MS procedures (Light and Beck 2010). Emission curves were generated, plotted, and used to interpolate and extrapolate the dynamics of PE emission over time.

No-Choice Tests, MEC-PE Treated Apple Leaves. Apple branches (Fuji variety) were cut from trees in Davis, CA, and their cut ends immediately immersed in a water bucket and transported to the laboratory. A solution of dilute MEC-PE formulation (1:32,000 dilution) was mixed in 10 ml distilled water that had 15 μl of red food coloring added (FD&C Red 5). A control solution of water and red dye was also mixed. Leaves with their petiole or stem intact were cut, placed in individual glass petri dishes, and then wetted to run-off on both the top and bottom leaf surfaces with either the water control or the dilute MEC-PE solution. Treatments were applied by pipetting 0.6 ml of a test solution, as a series of 20 μl droplets evenly distributed upon each side of the leaves ($n = 8$). Treated leaves were placed in a fume hood to dry followed by aging for an 8 d period over which the leaves were repeatedly used in daily bioassay runs. For testing, individual leaves were placed in deep petri dishes (9 cm) at $\approx 45^\circ$ angle with their top surface upward and petiole end upright. Single newly hatched larvae were placed via a fine tip sable-hair brush on the top leaf blade surface ≈ 1 cm from the leaf tip, and observed for a 10 min period. Movements, attained locations, and time sequence of their walking paths were observed and video recorded for analysis as previously described. On both artificial filter paper and natural apple leaf substrates neonate larvae were observed to stop or walk for periods of time in relatively straight tracks and various degrees of turning. Walking neonates made turns that were gradual to abrupt, defined as ranging in track angle from a simple directional change of $\geq 45^\circ$ in 10 s period to more complex closed loops of $\geq 360^\circ$. Stopping and restarting walking by the neonates ranged from short pauses to extended stopped or arrestment periods. Walking activities recorded were the occurrences and time duration of (1) relatively straight or nonturning forward tracks, (2) turnings, (3) loopings, and (4) stopping. Location of neonates on leaves were noted as they changed or progressed and defined as: on the top, bottom, or edge surface; progressing two-thirds up the leaf; reaching the leaf base; walking onto the petiole; and reaching the petiole's terminal cut-end. In total, 31 replicate runs were conducted for both control and MEC-PE

treated leaves. For the particular days of aging of treated leaves the number of replicate runs were as follows: d-0 ($n = 3$), d-1 aging ($n = 8$), d-4 aging ($n = 5$), d-5 aging ($n = 4$), d-6 aging ($n = 6$), and d-7 aging ($n = 5$). By day 7 of aging the cut leaves were obviously desiccating and in senescence, thus testing was terminated. The same room conditions and data analysis were used as described previously.

Results

Dual-Choice Tests, Filter Paper Arenas With Treated Versus Untreated Zones. Controls. For each of the 14 aging intervals tested (4 h to 20 d) there were no significant differences ($P > 0.05$) observed between the time larvae spent on either the water treated zone or untreated zone of the control filter papers. For the 300 s test period the average was (mean \pm SEM) 147.2 ± 3.2 s (range: 139.1 ± 5.9 – 157.0 ± 6.0 s) on the water treated zone; nearly equal to the unbiased (50/50) expectation of 150 s duration on each side. This essentially unbiased response activity of codling moth neonates to the control water treatment continued throughout 20 d of filter paper aging ($F = 0.455$; $df = 12, 64$; $P = 0.93$) (Fig. 1A). Number of entries (and exits) by neonates into the treated zone did not differ ($F = 0.882$; $df = 2, 390$; $P = 0.45$) between the control treatment (2.27 ± 0.14) and either the dilute or concentrated MEC-PE treatments (2.54 ± 0.09 and 2.49 ± 0.14 , respectively).

Dilute Rate MEC-PE. Time larvae spent on MEC-PE treated zone of the filter paper versus the untreated zone was on average 196.5 ± 4.5 s (range: 175.6 ± 10.4 – 215.6 ± 5.0 s) for the first 14 d of filter paper treatment aging and remained fairly consistent without significant change ($F = 0.711$; $df = 10, 91$; $P = 0.712$) over that period (Fig. 1B). For each day of testing over the initial 14 d aging period, the accumulated time spent by larvae on zones of filter paper treated with MEC-PE was significantly greater than for the control filter papers with zones treated with water (Table 1). However, the time neonate larvae spent in the dilute rate MEC-PE treated zone decreased to an average of 169.1 ± 7.1 s during the period from 16 to 20 d of filter paper aging (Fig. 1B), not significantly different from the time spent by neonates on the water control treatment ($P > 0.40$, Table 1), but significantly different from the pooled response levels during the first 14 d of filter paper aging of the MEC-PE treatment ($t = 3.335$; $df = 130$; $P = 0.001$).

Concentrated Rate MEC-PE. Accumulated time spent by neonates in the MEC-PE treated zone for the concentrated rate (Fig. 1C) was not significantly different from that in the water control treated zone for the first 5 d of filter paper aging (Table 1). On the sixth day of aging the time spent by neonate larvae in the concentrated rate MEC-PE treated zone was significantly ($P > 0.001$) greater than on the water treated zone of control filter papers. These significant temporal increases continued to be observed for the remainder of the test duration from day 6 to day 20 of filter paper aging (Table 1). In addition, the pooled

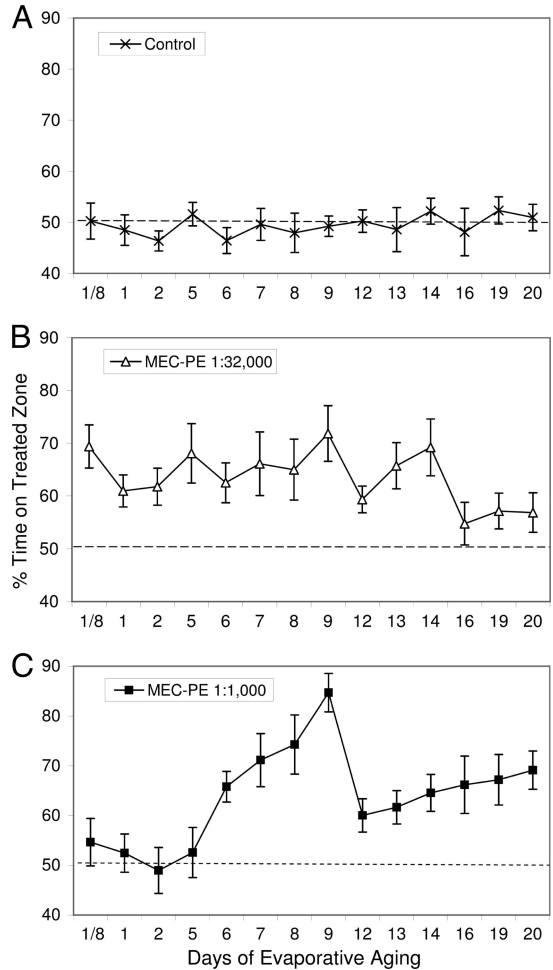


Fig. 1. Percentage time spent by neonate codling moth larvae upon zones of filter papers treated with (A) water control, (B) MEC-PE at a dilute rate (1/32,000 dilution), and (C) MEC-PE at a concentrated rate (1/1,000 dilution) ($N = 4$ – 16 per test date for each treatment).

response levels over the period of day 6 to day 20 were significantly greater than during the period of day 0 through day 5 ($t = 5.116$; $df = 159$; $P < 0.001$) for the MEC-PE treatment at the concentrated rate. Moreover, the accumulated retention times were significantly greater for the MEC-PE treatment at the dilute rate over the concentrated rate for the initial day of testing and for day 2 and 5 of filter paper aging, while the opposite trend was observed for day 9 and 20 of aging (Table 1).

Estimated Release Rates of PE From MEC-PE Dose Applications. The emission rate of PE from filter papers treated with the concentrated dose (1/1,000 dilution) of MEC-PE were found to be best described by a power curve equation ($y = 471.43x^{-0.7697}$) that was used to interpolate an estimated daily emission curve (Fig. 2A). Interpolation of the change in PE emission rate during the period that appears critical to the response behavior of neonate larvae (Fig. 1C) re-

Table 1. Statistical analyses of accumulated time responses of codling moth neonate larvae to separate treatment tests of water controls and MEC-PE at dilute and concentrated rates applied to filter papers then aged and tested over a 20 d period ($N = 4-16$)

Days of aging	Mean differences, ANOVA	Pairwise statistical comparisons ^a		
		Dilute rate MEC-PE vs control	Concentrated rate MEC-PE vs control	Dilute rate vs concentrated rate MEC-PE
4 h	$F = 7.57; df = 2, 21; P = 0.003$	$P = 0.004$	NS ($P = 0.66$)	$P = 0.026$
Day 1	$F = 4.00; df = 2, 21; P = 0.034$	$P = 0.035$	NS ($P = 0.83$)	NS ($P = 0.11$)
Day 2	$F = 4.37; df = 2, 21; P = 0.026$	$P = 0.024$	NS ($P = 0.81$)	$P = 0.037$
Day 5	$F = 3.97; df = 2, 27; P = 0.031$	$P = 0.049$	NS ($P = 0.93$)	$P = 0.045$
Day 6	$F = 9.98; df = 2, 26; P < 0.001$	$P = 0.011$	$P < 0.001$	NS ($P = 0.69$)
Day 7	$F = 6.06; df = 2, 27; P = 0.007$	$P = 0.037$	$P = 0.006$	NS ($P = 0.46$)
Day 8	$F = 8.22; df = 2, 23; P = 0.002$	$P = 0.041$	$P = 0.002$	NS ($P = 0.34$)
Day 9	$F = 21.72; df = 2, 29; P < 0.001$	$P = 0.001$	$P < 0.001$	$P = 0.048$
Day 12	$F = 4.21; df = 2, 32; P = 0.024$	$P = 0.048$	$P = 0.027$	NS ($P = 0.98$)
Day 13	$F = 4.32; df = 2, 35; P = 0.021$	$P = 0.025$	$P = 0.049$	NS ($P = 0.73$)
Day 14	$F = 8.08; df = 2, 29; P = 0.002$	$P = 0.002$	$P = 0.020$	NS ($P = 0.65$)
Day 16	$F = 4.09; df = 2, 21; P = 0.026$	NS ($P = 0.70$)	$P = 0.024$	NS ($P = 0.14$)
Day 19	$F = 4.80; df = 2, 40; P = 0.014$	NS ($P = 0.44$)	$P = 0.011$	NS ($P = 0.15$)
Day 20	$F = 6.26; df = 2, 35; P = 0.005$	NS ($P = 0.44$)	$P = 0.004$	$P = 0.043$

^a Pairwise statistical comparisons were by the Tukey test, $P < 0.05$.

solved an estimated emission rate of PE was 136 pg/h on day 5 of aging, dropping to 118 pg/h on day 6 (inset, Fig. 2A). However, the emission rate estimates were based on SPME volatile collections that allow for relative quantifications based on calibration curves and not actual emission quantities from such filter paper substrates (Romeo 2009). Similar data analysis of reported emission dynamics for MEC-PE dilutions at ratios of 1/100, 1/1,000, and 1/3,200 (Light and Beck

2010) were used to generate an extrapolative PE emission for the dilute rate (1/32,000 dilution) deriving similarly a power decay curve dynamic ($y = 75.89x^{-0.1806}$) (Fig. 2B). Using this equation the extrapolated PE emissions suggests that at day 14 the rate would be ≈ 47 pg PE/h and then would drop slightly to 46 pg PE/h at day 16 of aging (inset, Fig. 2B), the date when behavioral responses dropped to a level not significant from controls (Fig. 1B; Table 1). However, these are calculated estimates in rate of emission, and verification of these extrapolated limits would need to be performed on an instrument with sufficiently lower detection limits.

No-Choice Tests, Water Treated, or MEC-PE Treated Apple Leaves. Neonate larvae released on apple leaves walked most often in an upward direction (Fig. 3). As observed on the water treated control leaves, once neonates encountered a minor leaf vein or rib they would follow it by walking, either immediately beside or directly upon it, to the central-longitudinal main vein of the leaf, then following it upward toward the base of the leaf and the petiole or stem (Fig. 2A-D). Between apple leaves treated with the water and leaves treated with MEC-PE no significant differences were found in observed occurrence and numbers of straight tracks, turns, loops, crossings of the central main leaf vein, or stops by walking codling moth neonates (Fig. 2; Table 2). Accumulated time spent in each of these particular index behaviors was relatively consistent over the 7 d of aging and testing with no significant differences in the time values between each day of aging or testing for both the water control treatment (two-way ANOVA; $F = 1.73; df = 5, 144; P = 0.13$) and the MEC-PE treatment ($F = 1.12; df = 5, 144; P = 0.35$). Because no significant differences were determined for the factor of days of leaf aging, the accumulative time data were pooled over the dates for each observed behavior for analysis of the averaged treatment effects of the MEC-PE versus water controls (Table 2). Significant increases were found for MEC-PE treated leaves over water control treated

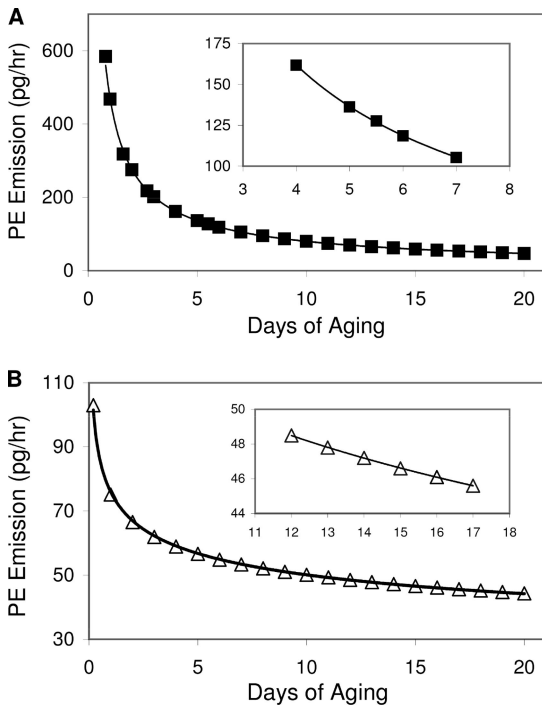


Fig. 2. Interpolated and extrapolated PE emission rates over days of filter paper substrate aging (32°C vented oven) for MEC-PE applied at (A) a concentrated rate (1/1,000 dilution) and (B) a dilute rate (1/32,000 dilution). Derived from data reported in Light and Beck (2010).

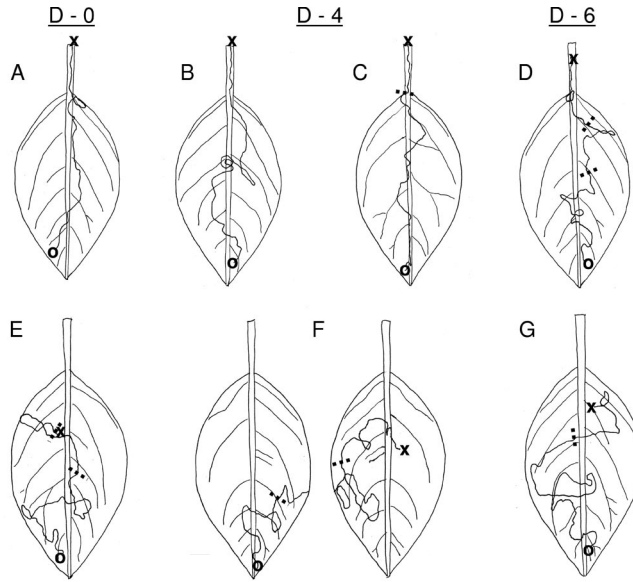


Fig. 3. Examples of walking tracks of neonate codling moth larvae on apple leaves treated over entire surface with water-control (A–D) or aqueous dilute MEC-PE (1/32,000 dilution) (E–G), with testing on the treatment day (d–0) and after 4 or 6 d of aging. Location of release of larvae on leaf top depicted by (O), stopping points by a dotted line (---), and the test termination point (X) after 600 s, except runs in which the end of the petiole was reached after 325 s (A), 445 s (B), and 575 s (C). These walking tracks were upon the top surface of leaves except for (F) that preceded from top to bottom surface.

leaves in the accumulated time larvae spent: (1) stopping ($P < 0.01$; increase of 1.9 times), (2) progressing two-thirds the distance up the leaf ($P < 0.001$; increase of 1.7 times), and (3) progressing up to the leaf base ($P < 0.05$; increase of 1.3 times). Additionally, significantly higher proportions of tested larvae walking on the water treated control leaves versus on the MEC-PE treated leaves were able to progress up the leaf and reach the leaf base, walking onto the petiole, and reaching the cut end of the petiole (Fig. 2; Table 2).

Discussion

Ambulatory responses of lepidopteran larvae to point source emissions of kairomones and aggregating pheromones are well established. Point sources of these semiochemicals elicit directed responses of attraction, both chemotaxis and anemotaxis, as well as orthokinesis (undirected stimulation of rate of locomotion); as demonstrated for codling moth neonate larvae responding to (*E,E*)- α -farnesene (Sutherland

Table 2. Occurrence and duration (means \pm SEM) of walking behaviors of neonate codling moth larvae released for a 600 s period on apple leaves (Fuji variety) wetted on both top and bottom blade surfaces with either control-water treatment or MEC-PE treatment at the field application rate ($N = 31$)

Observed behavior	Control-water treated		MEC-PE treated	
	Accumulated time, s ^a	No. occurrences	Accumulated time, s	No. occurrences
Straight tracks	243.7 \pm 14.6	14.6 \pm 0.7	211.4 \pm 12.3	16.4 \pm 0.9
Turns and loops	156.7 \pm 13.6	13.1 \pm 0.7	198.9 \pm 16.0	14.8 \pm 0.9
Stops	90.3 \pm 14.5b	0.97 \pm 0.13	174.8 \pm 24.5b	1.7 \pm 0.19
Central vein crossings	N/A	2.5 \pm 0.9	N/A	2.2 \pm 0.6
	Accumulated time, s ^a	Proportion of individuals ^b	Accumulated time, s	Proportion of individuals
On leaf top	363.8 \pm 32.5	1.00	365.2 \pm 32.5	1.00
On leaf bottom	170.6 \pm 33.7	0.53	218.9 \pm 30.9	0.70
Progress two-thirds up leaf	244.9 \pm 23.5a	1.00	405.6 \pm 36.3a	0.83
To leaf base	447.9 \pm 27.8c	0.80e	569.3 \pm 24.4c	0.10e
On petiole	124.0 \pm 15.4	0.70d	0	0d
To end petiole	568.0 \pm 27.9	0.47e	0	0e

^a Row values for accumulated time followed by the same letter are by Student *t*-test analysis significantly different at: a, $P < 0.001$; b, $P < 0.01$; c, $P < 0.05$.

^b Row values for proportion of individuals followed by the same letter are by χ^2 analysis significantly different at: d, $P < 0.01$; e, $P < 0.05$.

1972; Sutherland and Hutchins 1972, 1973; Sutherland et al. 1974; Bradley and Suckling 1995; Landolt et al. 1998; Knight and Light 2001) and PE (Knight and Light 2001); and for pupation site-seeking, fifth-instar codling moth larvae by the aggregation pheromone of cocoon-spinning conspecific larvae (Jumean et al. 2008).

However, elicited behaviors are fundamentally different when codling moth neonate larvae are placed upon areas of broadcast application of kairomones rather than specific point source emissions, as first shown by Hughes et al. (2003) and elaborated upon here. Hughes et al. (2003) reported that an apple extract, and (*E,E*)- α -farnesene to a lesser degree, caused the arrestment of neonate larvae and their retention in the treated zone, though the duration of activity of such applied droplets was ephemeral because of evaporation. To demonstrate field practicality of kairomone use, we have used a microencapsulation formulation for the prolonged and controlled release (Light and Beck 2010) of PE from discrete zones of treated filter papers and from broadcast applications to leaves that simulate full-coverage spraying. Both types of MEC-PE application were found to similarly elicit arrestment from neonate codling moth larvae. Whereas the work of Hughes et al. (2003) applied 10 μ g neat doses resulting in neonates spending a majority time (59%) within the treated zone, our investigation with MEC-PE provided smaller amounts of encapsulated PE, estimated at \approx 317 ng per treated zone, and evoked similarly a dominant percentage of time the larvae spent within the PE treated zone ($65.5\% \pm 4.5\%$ SEM). More importantly, this preference response to PE treated zones persisted through 14 d of substrate aging. Thus, application of the MEC-PE at field rate provoked larval arrestment responses over a 2 wk residue period of laboratory aging which supports the potential use of MEC-PE as a spray adjuvant with insecticides, which often have similar residual activity periods of weeks in the field.

A key factor of the current study is the 20 d aging and subsequent behavioral testing of the treated filter papers. This prolonged period of aging allowed PE emission dynamics to progress (Light and Beck 2010) and thereby resolve different larval behaviors elicited by the two loading rates of MEC-PE. The dilute rate (field equivalent) of MEC-PE caused larvae to preferentially spend greater time in the treated zone relative to controls for the first 14 d of aging. For the concentrated rate (32 times greater) of MEC-PE, preferential responses from neonate larvae were not observed for the first 5 d; however, this reversed by day 6 and their preference for the treated zone was maintained through day 20 of aging. This nonexpression of larval responsiveness initially for 5 d is suggestive of a suppressive effect, perhaps olfactory sensory adaptation of larval chemoreceptors that might lead to CNS habituation of responsiveness with prolonged exposure to a high or over-dosing emission rate of PE from the concentration of microcapsules.

Light and Beck (2010) have characterized PE emission of the MEC formulation at the concentrations

used in this study. The dual choice bioassays using the concentrated dose (1/1,000 dilution) of MEC-PE show that the period between day 5 and day 6 appears critical to the response behavior of neonate larvae, changing from nonpreference to a significant preference in attraction (Fig. 1C). Interpolative analysis of the power decay curve emissions of PE from filter papers treated with the concentrated rate of MEC-PE suggest that the emission rate of PE on day 5 of aging was 136 pg/h and dropped to 118 pg/h on day 6 (inset, Fig. 2A). Thereby, the implied upper limit threshold for larval responsiveness to MEC-PE emissions is suggested to be approximately <136 pg PE/h, while higher emission rates of PE could possibly have caused sensory adaptation and thus a nonresponse. Moreover, behavior of neonate larvae to the dilute rate treatment of MEC-PE (1/32,000 dilution) was observed to change from a significant attraction preference to MEC-PE treated zones on day 14 to a nonpreference response between treated and control zones by day 16 of testing (Fig. 1B). Analysis here generated an extrapolative PE emission for the dilute rate would drop to 46 pg PE/h at day 16 of aging (inset, Fig. 2B). Thereby, the estimated lower limit threshold for observed larval responsiveness to MEC-PE emissions, from a surface the size of an apple leaf, is ca. ≥ 46 pg PE/h. Consequently, behavioral responses of codling moth neonates to PE may require a subtle and fairly narrow emission range, such as an activity range of between ≥ 46 and ≤ 136 pg/h PE emission. This subtle emission range would incite and maintain attraction and arrestment behaviors by codling moth neonate larvae, while not provoking chemoreceptive sensory adaptation and habituation by continual exposure to higher over-loading stimulus rates.

Similarly, on the natural surface of apple leaves broadcast full-coverage applications of dilute MEC-PE caused neonate codling moth larvae to increase the incidence and time of arrestment over that of control leaves, while occurrence and time spent in other observed walking behaviors on leaves were not affected (Fig. 3; Table 2). Furthermore, codling moth larvae took a significantly longer time to walk toward the petiole on MEC-PE treated leaves than controls. Codling moth females lay single eggs, primarily on foliage (92%) and within 20 cm of fruit or nuts (91%) (Geier 1963, Jackson 1979, Thiery et al. 1995, Blomefield et al. 1997). Upon eclosion, the innate behavior of neonate codling moth larvae is to walk immediately up the leaf, onto the petiole and branch, and within a 2 h period find and penetrate a host fruit or nut (Geier 1963, Jackson and Harwood 1980, Jackson 1982). Here we observed with broadcast application of MEC-PE fewer (eight times less) test larvae could find their way up to the leaf base, while none reached the petiole (Fig. 3E-G; Table 2). In contrast, 70% of the larvae on control leaves reached the petiole and 47% reached the goal of the end of the cut petiole within the 10 min testing period (Fig. 3A-D; Table 2). Thus, the presence of MEC-PE on leaves elicits arrestment and prolonged retention of codling moth larvae and appears to disrupt or counteract their innate behavior of exiting

from leaves. These arrestment, retention, and disruptive effects evoked by MEC-PE on neonate larvae could be the basis of the decreased fruit injury and increased larval mortality reported for dilute spray applications of MEC-PE alone in apple and pear orchard studies (Pasqualini et al. 2005a, Arthurs et al. 2007, Schmidt et al. 2008).

Kairomones not only affect larval behavior but also influence oviposition by female codling moth and the placement of eggs in relation to host fruit location. Hughes et al. (2003) reported that point sources of apple extract caused eggs to be laid competitively away from a natural apple host. Similarly, Pasqualini et al. (2005b) demonstrated that applications of PE in a gel formulation caused females to lay eggs on leaves further from fruit than with control treatments in apple and pear orchards.

Because the time interval from neonate hatching to their boring within a fruit or nut usually spans only 2 h (Geier 1963, Jackson and Harwood 1980, Jackson 1982), this short period of neonate walking or host seeking is the critical target window of pest vulnerability and a challenge of insecticidal controls. MEC-PE elicits behavior that may retard or disrupt taxis by neonate larvae. In addition, foliar applications of MEC-PE may cause female codling moth to oviposit on leaves at distances further away and disassociated from fruit location, thus increasing the distance larvae must traverse to find host fruit or nuts (Pasqualini et al. 2005b). In concert, these effects evoked by MEC-PE application could disrupt host fruit or nut finding by neonate codling moth larvae, thereby increasing the temporal and spatial exposure of larvae to various potential mortality factors, including biotic factors of predation and abiotic factors of desiccation and contact/adsorption/ingestion of applied insecticides (Pasqualini et al. 2005a, Arthurs et al. 2007, Light 2007, Schmidt et al. 2008, Light and Knight 2011).

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