

# Oriental disruption of codling moth, *Cydia pomonella* (L.) (Lep., Tortricidae), by concentrated formulations of microencapsulated pheromone in flight tunnel assays

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Ms. received: April 13, 2005; accepted: August 17, 2005

**Abstract:** The effects of two formulations of microencapsulated pheromone (CheckMate CM-F), containing 14.3% (*E,E*)-8,10-dodecadien-1-ol (codlemone), on the behaviour of the male codling moth, *Cydia pomonella* (L.), were evaluated in a flight tunnel after several periods of formulation ageing. The two treatments of CheckMate CM-F evaluated consisted of the label-recommended field rate of 50 g active ingredient (a.i.)/ha diluted in: (1) a standard 1000 l of water (low concentration 0.05 g/l), and (2) a low volume of 100 l of water (high concentration 0.5 g/l). The low-concentration treatment was formulated by diluting 0.071 ml of CheckMate CM-F in 500 ml of water and the high-concentration treatment contained 0.71 ml of CheckMate CM-F in 500 ml of water. Wax-paper strips (2.1 × 20 cm) were treated at 0.06 ml of solution/cm<sup>2</sup>. The mean (±SE) number of CheckMate CM-F microcapsules adhering to treated wax-paper strips in the high-concentration treatment (398 ± 38) was sevenfold greater than that (57 ± 5) counted on wax-paper strips treated with the low concentration. Both low- and high-concentration treatments prevented anemotactic orientation of male codling to an adjacent 0.1 mg codlemone lure for up to 24 h after application. These moths flew out of the release cages, but exhibited erratic and short flights not restricted to any plume and ending at the tunnel walls or the floor. This occurred with approximately 60 and 400 microcapsules per wax-paper strip in the low- and high-concentration treatments, releasing codlemone at approximately 0.15 and 1.5 µg/h respectively. After 2 days of ageing, the low-concentration treatment no longer interfered with the ability of males to find the codlemone lure. However, the number of males contacting the lure was significantly reduced for up to 6 days with the high-concentration treatment relative to the control and low-concentration treatments. The high-concentration treatment no longer impeded normal orientational flight after 2–6 days of ageing; but, it diverted males from the codlemone lure by causing them to land on the adjacent treated wax-paper strips. This occurred at a release rate of approximately 0.7 µg codlemone/h from approximately 400 microcapsules per wax-paper strip distributed as clumps of approximately 30 microcapsules per 14 mm<sup>2</sup>. We suggest that an initial but short-lived disruption mechanism like camouflage is followed by a longer period of false-plume following to clumps of microcapsules. The low-volume, concentrated application method for disseminating pheromone microcapsules warrants further investigation for moth codling, as well as other pests because this approach may improve the efficacy without the need for increasing the field application rate.

**Key words:** camouflage, CheckMate CM-F, false-plume following, mating disruption, pheromone microcapsules, sprayable pheromone, tree fruit

## 1 Introduction

The codling moth, *Cydia pomonella* (L.), is a major worldwide pest of apples, pears and walnuts (BARNES, 1991), and is widely resistant to broad-spectrum organophosphorous insecticides (RIEDL et al., 1985; WELTER et al., 1991; CROFT and RIEDL, 1992; VARELA et al., 1993; KNIGHT et al., 1994; CHAPMAN and BARRETT, 1997; REUVENY and COHEN, 2004). Pheromone-based mating disruption has become an important component of pest management programmes targeting codling moth. Pheromones supplement, and in some

cases replace, broad-spectrum insecticides (CALKINS, 1998; GUT and BRUNNER, 1998; THOMSON et al., 1998). Mating disruption is achieved by broadcasting synthetic pheromones into the crop atmosphere to disrupt mate finding (CARDÉ and MINKS, 1995). Several pheromone-delivery formulations and release devices have been developed for mating disruption of codling moth. These include Isomate polyethylene-tube dispensers (THOMSON et al., 2001), puffers and micro-sprayers (SHOREY and GERBER, 1996; ISAACS et al., 1999), hollow fibres (MOFFITT and WESTIGARD, 1984; KNIGHT,

2003) and microcapsules (MOFFITT, 1978; KNIGHT, 2000).

Microencapsulated pheromone formulations offer several advantages over other currently marketed, pheromone dispensers. Chiefly, they can be applied with conventional spray equipment. This reduces labour cost associated with manual application of reservoir-type devices and increases flexibility to regulate application rate and frequency (HALL and MARRS, 1989; GUT et al., 2004). In addition, these formulations are compatible, and can be concurrently applied with miticides and fertilizers, which require frequent sprayings in certain tree-fruit growing regions (EPSTEIN et al., 2003). Finally, microencapsulated pheromone formulations offer the possibility of economical multi-species disruption in tree-fruit systems harbouring a complex of multiple pests (GUT et al., 2004).

Recent studies have shown that sprayable, microencapsulated formulations of pheromone successfully disrupt some moth species including the Oriental fruit moth, *Grapholita molesta* (Busck) (TRIMBLE et al., 2004), the *Sparganothis* fruitworm, *Sparganothis sulfureana* (Clemens) (POLAVARAPU et al., 2001), and the grape berry moth, *Endopiza viteana* (Clemens) (TRIMBLE et al., 2003). However, results with codling moth control using microencapsulated pheromones have been variable (KNIGHT and LARSEN, 2001; EPSTEIN et al., 2003; KNIGHT, 2005). The main drawbacks are the relatively short lifespan of microencapsulated formulations in the field and the ineffectiveness of disrupting high population densities of codling moth (CAMPION, 1976; KNIGHT and LARSEN, 2001, 2004; EPSTEIN et al., 2003). This has been attributed, in part, to the instability of the conjugated diene (*E,E*)-8,10-dodecadien-1-ol codling moth pheromone (MILLAR, 1995). Attempts to improve the efficacy of microencapsulated pheromones against codling moth have included the addition of: UV screens and antioxidants to improve stability (ENG et al., 2003), stickers to improve rainfastness (KNIGHT et al., 2004), and the modification of spray application methods (KNIGHT and LARSEN, 2001; HULL et al., 2004; LIGHT et al., 2004). Most recently, an 'ultra low volume' application of microencapsulated pheromone (CheckMate CM-F; Suterra LLC, Bend, OR, USA) that is approximately 10 times more concentrated than the standard air-blast application method was shown to significantly improve mating disruption of codling moth using microencapsulated pheromone (KNIGHT and LARSEN, 2004; KNIGHT, 2005).

In the current laboratory flight-tunnel study, we attempted to uncover some of the possible mechanisms that might mediate disruption of codling moth exposed to CheckMate CM-F (Suterra LLC) microencapsulated pheromone. The specific objectives were: (1) to determine how an experimental, highly concentrated formulation of CheckMate CM-F affects the behaviour of codling moth males compared with the standard formulation that is 10 times less concentrated, (2) determine the duration of activity of both high- and low-concentration formulations of CheckMate CM-F on the behaviour of male codling moth, and (3) quantify the number of microcapsules applied and

their associated release rate for the two concentrations of CheckMate CM-F evaluated.

## 2 Materials and Methods

### 2.1 Insects

Codling moth males were selected from 1- and 5-year-old laboratory colonies originally collected as pupae from untreated apple orchards in Michigan and North Carolina, USA respectively. Moths from North Carolina were supplied by Benzoin Research (Carlisle, PA, USA). Moths were reared at 24°C and 60% relative humidity (RH) on pinto bean-based diet (SHOREY and HALE, 1965) under a 16 : 8 h [light : dark (L : D)] photoperiod. Pupae were sorted by sex and adults emerged in 1-l plastic cages containing 5% sucrose in plastic cups with cotton dental wicks protruding from their lids.

### 2.2 Flight tunnel

Behavioural assays were conducted in a Plexiglas sustained-flight tunnel (STELINSKI et al., 2004a). The flight tunnel measured 1.3 × 0.8 m in cross section, and 2.4 m in length. It was housed in a temperature-controlled room maintained at 21–23°C and 50–70% RH. Light at 30–40 lux inside the tunnel was generated by two fluorescent bulbs (95 W; Philips model F96T12; Philips, Bothel, WA, USA) mounted 22 cm above the top of the tunnel. A variable speed, blower-type fan (Dayton 5C090C, Northbrook, IL, USA) pushed air through the tunnel at 0.3 m/s. The pheromone plume emerging from the tunnel was expelled from the building, via a downwind fan, through a roof-mounted stack. The upwind fan pushed air first through both a Vari-flow II filter for fine, particulate matter and a Vari-Pure high capacity, activated carbon filter; both filters were obtained from Airguard (Airguard, Louisville, KY, USA). Finally, air was pushed through a 1.3 × 0.8 × 0.2 m hardwood frame attached tightly to the upwind end of the tunnel and enclosed with cloth dampening screens (20 mesh/cm) stretched tightly across each opening. The downwind end of the tunnel was enclosed with wire-mesh screen.

### 2.3 Treatments evaluated (CheckMate CM-F microencapsulated pheromone)

We evaluated two concentrations of a microencapsulated, sprayable formulation of pheromone (CheckMate CM-F) manufactured by Suterra LLC. This formulation contains 14.3% (*E,E*)-8,10-dodecadien-1-ol by volume. The treatments consisted of the label-recommended field rate of 50 g active ingredient (a.i.)/ha diluted in: (1) a standard 1000 l of water (low concentration 0.05 g/l) and (2) a low volume of 100 l of water (high concentration 0.5 g/l). The low-concentration treatment was formulated by diluting 0.071 ml CheckMate CM-F in 500 ml of water and the high-concentration treatment was a solution of 0.71 ml CheckMate CM-F in 500 ml of water. Solutions were formulated in 700 ml chemically resistant, high-volume spray bottles (Consolidated Plastics Company, Inc., Hadson, OH, USA). These mixtures were prepared 30 min prior to application. Spray bottles were vigorously shaken for 5 min immediately prior to application of sprayable pheromone. The pheromone spray was applied to wax-paper strips (Meijer, Inc, Grand Rapids, MI, USA), 2.1 cm wide and 20.0 cm long, hung from a horizontally positioned 25.4 × 25.4 cm piece of wire-mesh grid, the openings of which were a uniform 1.3 cm<sup>2</sup>. Twelve

wax-paper strips were attached in three rows spaced 8.0 cm apart. The three rows contained four strips each, spaced 4.0 cm apart. The strips were attached to the frame with a clear cellophane tape.

Pheromone was applied in three spritzes per row, for a total of nine spritzes per frame. Spritzes expelled 2.7 ml of CheckMate CM-F solution on average. The treated wax-paper strips received approximately 0.06 ml of solution/cm<sup>2</sup>. Behavioural assays with pheromone-treated, wax-paper strips took place after several intervals of ageing to determine longevity of activity. Wax-paper strips were aged in a fume hood at 22°C for 1 h and the intervals shown in fig. 1 before the flight-tunnel bioassays.

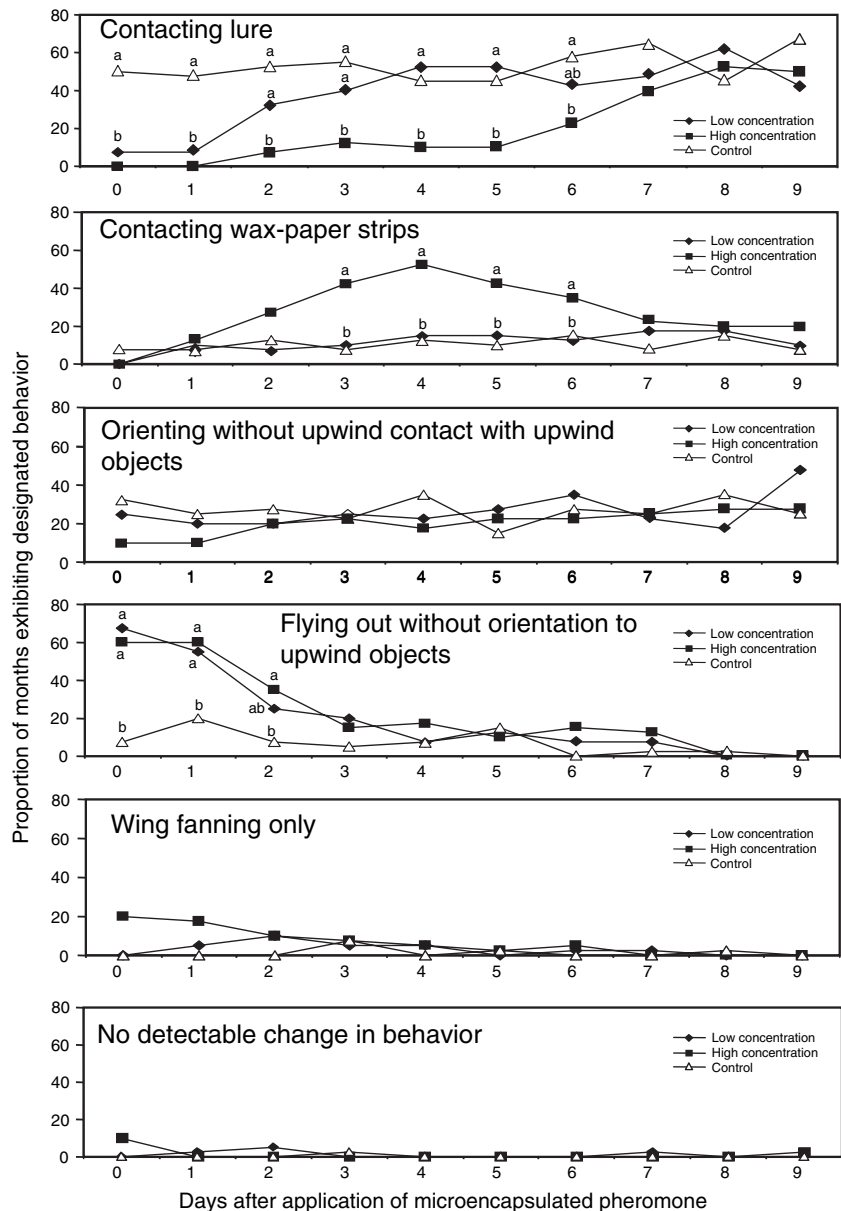
**2.4 Flight-tunnel assay procedures**

A rubber septum lure containing 0.1 mg of (*E,E*)-8,10-dodecadien-1-ol (>98% isomeric and chemical purity; Suterra LLC) was positioned centrally in the flight tunnel, 63.6 cm from either side wall and 25.4 cm from the floor. Immediately adjacent to each side of the lure, we hung a set of wax-paper strips sprayed with: (1) water only (control) or with the (2) low- or (3) high-concentration treatments of

CheckMate CM-F. The treated wax-paper strips were hung from horizontally mounted, wire-mesh frames so that the centre of each wax-paper strip was 25.4 cm from the tunnel floor. They were hung so that the interior rows of paper strips were 9 cm from either side of the rubber septum lure.

Male codling moths, 2–3 days old, were collected 0.5 h prior to the end of a 16-h photophase and placed into cylindrical (8 cm long × 8 cm diameter) release cages made of window screening. Each cage, containing two moths, was placed into the flight tunnel for 0.5 h of acclimation prior to assays. At the upwind end of the tunnel, pheromone lures were placed 1 cm above a 7.5 × 12.5 cm yellow card (American Scholar, Bay Shore, NY, USA) attached to a horizontally clamped 9-cm glass rod attached to a steel ring-stand. Wire-mesh release cages holding the codling moth males were placed at the down-wind end of the tunnel at a height matching that of the pheromone lure. The treatments were evaluated after the ageing intervals given above. Moths were allowed 3 min to respond to the inserted pheromone lure. The behaviours recorded were: wing-fanning only; non-anemotactic flight from the release cage; upwind anemotactic flight without touching the lure or wax-paper strips; upwind anemotactic flight followed by landing on the wax-paper

**Fig. 1.** Behaviour of male codling moth in response to lures containing 0.1 mg of codlemone placed 9 cm cross-wind from wax-paper strips treated with low or high concentrations of CheckMate CM-F microencapsulated pheromone. Flight-tunnel assays were conducted at 10 intervals after application of CheckMate CM-F and 40 moths were tested per treatment at each interval. The control treatment consisted of water without CheckMate CM-F. On day 0, treated wax-paper strips were aged for 1 h. Means within a panel and for a particular ageing interval followed by the same letter are not significantly different ( $\alpha < 0.05$ )



strips; upwind anemotactic flight followed by landing on the release platform and touching the lure. In addition, the number of individuals exhibiting no detectable behavioural change were recorded. Twenty replicates of two moths were assayed per treatment at each ageing interval.

To avoid bias caused by possible slight variations between days, all treatment combinations aged for the same duration were assayed on the same day. Treatment order was randomized daily to equalize any effect of time after the onset of scotophase. Release cages, ring-stands, and glass rods were thoroughly washed with acetone after daily use. The interior of the wind tunnel was also briefly scrubbed with a cloth wetted with acetone and immediately rinsed with water so as not to damage the plexiglas. The exhaust fan also ran for at least 2 h after assays were completed.

### 2.5 Response of male codling moth to treated-wax paper strips in the absence of a lure

An additional experiment was performed to determine whether male codling moths respond to treated wax-paper strips in the absence of a pheromone lure. Wax paper strips were treated with: (1) water only (control) or with the (2) low- or (3) high-concentration treatments of CheckMate CM-F as described above and aged for 4 days. This period of ageing was chosen because previous tests suggested that the highest level of codling moth attraction to treated wax-paper strips took place after 4 days (fig. 1). A single metal grid containing wax-paper strips was placed centrally at the upwind end of the flight tunnel as described above. Treatments applied to wax-paper were inserted at random. Wire-mesh release cages containing moths were inserted at the downwind end at a height matching the mid-point of vertically hanging wax-paper strips. Codling moths were assayed identically as described above except that five moths were released from cages concurrently. Twelve replicates were completed for each treatment.

### 2.6 Quantification of microcapsule number applied per treatment

Wax-paper strips were treated with the low and high concentrations of CheckMate CM-F as described above. The number of microcapsules adhering to wax-paper strips after each treatment was quantified using a protocol slightly modified from that described by WALDSTEIN and GUT (2003). After 3 h of post-application drying, wax-paper strips were dipped for 20 s in a 0.2% solution of rose bengal (certified dye content 93%; Aldrich, Inc., Milwaukee, WI, USA). Three hours later, these strips were dipped for 20 s in a 0.2% solution of brilliant cresyl blue (certified dye content 66%, Aldrich, Inc.). As observed by WALDSTEIN and GUT (2003), the combination of rose bengal and brilliant cresyl blue was absorbed into the microcapsule wall rendering them clearly visible by light microscopy.

Microcapsules were counted within 48 h of the final dye treatment under a dissecting microscope with 50× magnification and a 14 mm<sup>2</sup> field of view. Twenty wax-paper strips were examined per treatment. The entire surface area of both sides of each treated wax-paper strip was examined.

Four additional wax-paper strips were sprayed with either the low- or high-concentration treatment as described above and subsequently examined to determine the mean number of microcapsules per 14 mm<sup>2</sup> of microscope field. Each side of each wax-paper strip was partitioned into 30 fields of view, each 14 mm<sup>2</sup>. The number of microcapsules within each of 60 fields of view per wax-paper strip was counted. The CheckMate CM-F formulation diluted in water beaded upon the

surface of wax-paper strips after spray application. Consequently, microcapsule distribution was clumped. For each wax-paper strip, we quantified both the density of microscopic fields containing microcapsules as well as the density of microcapsules per positive field.

### 2.7 Quantification of pheromone release rate from microcapsules

Release rate of pheromone from CheckMate CM-F microcapsules was measured gravimetrically. Five blank Whatman no. 1 (VWR International, West Chester, PA, USA) filter discs (1.5 cm diameter) were placed within a Petri dish (9 cm internal diameter) and dried for 1 h at 100°C. CheckMate CM-F (3 ml) was thoroughly mixed in 20 ml of deionized water. Diluted CheckMate CM-F (200 µl) was applied to four replicates of oven-dried filter discs. The same amount of deionized water (blank control) was applied to a fifth disc. The discs were subsequently dried at approximately 22°C for 4 h (no measurable loss of codlemone occurred during this time as measured by gas chromatography). The discs were weighed using a Mettler M3 (Mettler-Toledo, Inc., Columbus, OH, USA, ±0.001 mg) balance to determine the amount of codlemone in the samples at the start of the test. All samples were aged in an environmental chamber (Baxter Ultratech WJ 301G; Ultratech, San Jose, CA, USA) set at 22°C and with a linear airflow rate of 0.40–0.52 m/s. Actual temperature within the chamber fluctuated between 22 and 24°C. Weight loss of material on filter discs was measured 1, 2, 3, 6, 7, 8, 9 and 10 days after application.

### 2.8 Statistical analysis

The number of moths contacting lures, wax-paper strips, or flying out of release cages without orienting (per batch of 40; fig. 1) at each ageing interval was transformed ( $\log[x + 1]$ ) and submitted to analysis of variance (ANOVA) followed by Tukey's test (SAS Institute, 2000) for separation of mean values. In the experiments without lures, the number of males contacting wax-paper strips, orienting without contact, flying out of release cages without orienting, wing fanning, or exhibiting no detectable behavioural change was transformed as above and also analysed by ANOVA followed by Tukey's mean separation. The number of microcapsules per wax-paper strip for each treatment was analysed by *t*-test. In all cases, the significance level was set at  $\alpha < 0.05$ .

## 3 Results

### 3.1 Effect of CheckMate CM-F on behaviour of male codling moth

The response of the male codling moths to lures in the control treatment was substantial at each ageing interval; 48–65% of male codling moths contacted lures and 23–35% of the remaining males oriented to lures without contact in the control treatment (fig. 1). From 1 h to 1 day of ageing, both CheckMate CM-F treatments suppressed male codling moth sexual responses (fig. 1); significantly fewer males contacted lures and significantly more moths flew out of release cages without orienting to pheromone plumes compared with the control treatment. The proportion of males contacting lures adjacent to wax-paper strips with the low-concentration treatment was not

significantly different from the control treatment by 2 days of ageing (fig. 1). However, the proportion of males contacting lures in the high-concentration treatment was significantly reduced compared with the control and low-concentration treatments for up to 6 days (fig. 1). The proportion of males contacting wax-paper strips treated with the high concentration of CheckMate CM-F was significantly higher at 3 to 6 days of ageing, compared with the low-concentration and control treatments at those ageing intervals (fig. 1). By 7 days, there were no significant differences in moth behaviour among the three treatments (fig. 1).

**3.2 Response of male codling moth to wax-paper strips in the absence of a lure**

Significantly more codling moth males contacted and oriented without source contact to wax-paper strips treated with the high-concentration treatment of CheckMate CM-F compared with the low-concentration or control treatments (fig. 2). No males oriented in response to these latter two treatments. Significantly fewer males exhibited no behavioural change in response to the high-concentration treatment compared with the low-concentration treatment or the control (fig. 2).

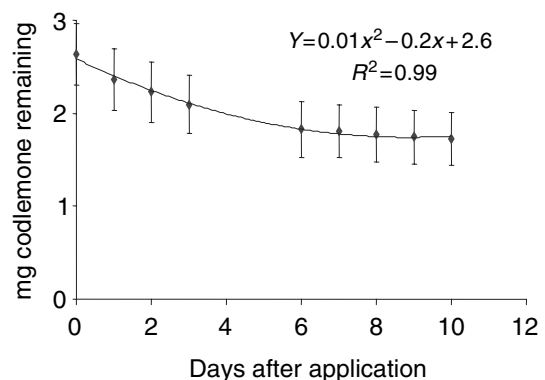
**3.3 Number of microcapsules per treatment**

The mean ( $\pm$ SE) number of CheckMate CM-F microcapsules adhering to treated wax-paper strips in the high-concentration treatment ( $398 \pm 38$ ) was sevenfold ( $P < 0.0001$ ) greater than that ( $57 \pm 5$ ) counted on wax-paper strips treated with the low-concentration treatment. The mean number of microcapsules per  $14 \text{ mm}^2$  in the high-concentration treatment ( $0.01 \pm 0.003$ ) was significantly ( $P < 0.0001$ ) greater than that ( $0.001 \pm 0.003$ ) per  $14 \text{ mm}^2$  in the low-concentration treatment. In the high-concentration treatment, approximately 22% of the

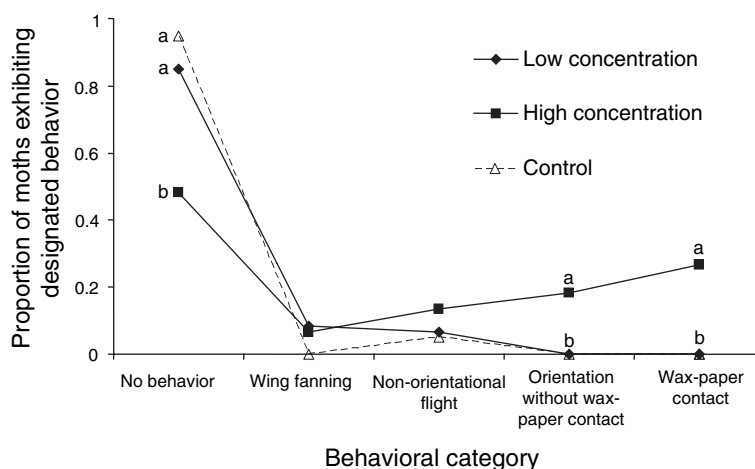
fields of view per wax-paper strip examined contained clumps of approximately 30 microcapsules. In the low-concentration treatment, approximately 48% of the fields of view examined per wax-paper strip contained one to two microcapsules. This distribution was highly clumped and regular. When considering only microcapsule-positive fields, the mean ( $\pm$ SE) number of CheckMate CM-F microcapsules per  $14 \text{ mm}^2$  in the high-concentration treatment ( $32.0 \pm 4.7$ ) was significantly ( $P < 0.0001$ ) greater than that ( $1.9 \pm 0.3$ ) per  $14 \text{ mm}^2$  in the low-concentration treatment.

**3.4 Gravimetrically measured release profile of Checkmate CM-F microcapsules**

The release profile of codlemone from CheckMate CM-F microcapsules fit a polynomial decay curve (fig. 3). Pheromone was released most rapidly during day 1 at approximately 0.3 mg/h. Release rate did not change much during days 2 to 6 (0.13–0.14 mg/h), after which release became undetectable by weighing. A substantial amount of pheromone remained in the capsules after 10 days when the release rate was minimal.



**Fig. 3.** Release profile of CheckMate CM-F microcapsules fitted to a polynomial decay curve



**Fig. 2.** Behaviour of male codling moth in response to wax-paper strips treated with low- (0.05 g/l) and high-concentration (0.5 g/l) treatments of Checkmate CM-F applied at 0.06 ml of solution per  $\text{cm}^2$ . The control treatment consisted of water without CheckMate CM-F and 60 moths were tested per treatment. Mean values for a particular treatment followed by the same letter are not significantly different ( $\alpha < 0.05$ )

## 4 Discussion

One to 24 h after application, both the low- and high-concentration treatments of CheckMate CM-F impeded normal orientation of male codling moths to the codlemone lure deployed in a flight tunnel. The majority of these moths became activated and flew out of the release cages, but exhibited erratic and short flights not restricted to any plume and ending at the tunnel walls or floor. This suggests that the amount of codlemone released from microcapsules up to 24 h after application was sufficient to, e.g. (1) camouflage the codlemone plume emanating from the lure, (2) desensitize the moths' olfactory receptors, or (3) both mechanisms may have been operative. Flights to lures were significantly reduced when microcapsules were releasing codlemone at approximately 1.5 and 0.15  $\mu\text{g}/\text{h}$  in the high- and low-concentration treatments, respectively. Thus a mechanism of false-plume following is not supported. After 2 days of ageing and when the release rate stabilized at approximately 0.7 and 0.07  $\mu\text{g}/\text{h}$  in the high- and low-concentration treatments, respectively, the low-concentration treatment no longer precluded lure-finding. However, the high-concentration treatment continued to prevent the majority of males from contacting the lure; it reduced lure contact for up to 6 days. But, the mechanism by which lure contact was prevented differed from that observed at 1–24 h after application of CheckMate CM-F. The high-concentration treatment no longer impeded normal orientational flight; but rather, diverted males from the codlemone lure by causing them to land on the treated wax-paper strips. This occurred at an approximate release rate of 0.7  $\mu\text{g}$  codlemone/h from approximately 400 microcapsules per wax-paper strip distributed in clumps of approximately 30 microcapsules per 14 mm<sup>2</sup> on average.

KNIGHT and LARSEN (2004) recently showed that a highly concentrated, 'low volume' application of CheckMate CM-F (approximately 50 g a.i./ha applied in 49 l of water) disrupted orientation of male codling moth to virgin-female baited traps while a standard air-blast application of the same amount of active ingredient per hectare applied in 1000 l of water did not. Furthermore, these authors found that more microcapsules were deposited per leaf with the low-volume method than the air-blast method. Moreover, artificial leaves containing 80–160 CheckMate CM-F microcapsules attracted male codling moth to sticky traps for up to 5 weeks. The authors suggested that the improved efficacy of the low-volume, concentrated-spray application method may have been due to an increased 'importance of false-trial following as a mechanism for disruption' given that attractive point sources were likely created by high densities of microcapsules on leaves (KNIGHT and LARSEN, 2004). Our flight tunnel data support this hypothesis; wax-paper strips with approximately 400 adhering microcapsules diverted male codling moths from an adjacent, highly attractive codlemone lure. Furthermore, wax-paper strips treated in this manner attracted male codling moth in the flight tunnel in the absence of a pheromone lure. The 'long-term' effectiveness of the

high-concentration treatment in preventing males from reaching the highly attractive codlemone lure in the flight tunnel was most parsimoniously explained by competitive attraction between clumps of microcapsules and the lure. In our experimental context, we define competitive attraction as decreased frequency of male moths contacting a highly attractive pheromone lure because they are diverted from orienting to this source because of attraction to other nearby plumes emanating from aggregations of pheromone microcapsules.

In our flight-tunnel experiments, CheckMate CM-F microencapsulated pheromone disrupted codling moth by more than one mechanism. From 1–24 h after application, and at a release rate of approximately 1.5  $\mu\text{g}$  codlemone/h, approximately 400 microcapsules/wax-paper strip prevented male codling moths from anemotactically orienting along the plume emanating from the codlemone lure. SANDERS (1997) and DOANE (1999) have suggested that sprayable, microencapsulated formulations achieve disruption in the field by creating a 'cloud or fog' of pheromone that camouflages authentic female plumes. Our data suggest that such a mechanism is most likely to occur soon after microcapsules are applied and when they are initially releasing pheromone at a high rate. However, as the release rate of codlemone stabilized at approximately 0.7 and 0.07  $\mu\text{g}$  of codlemone/h, both the high and low concentrations of CheckMate CM-F no longer prevented males from orienting to the lure in the flight tunnel. This suggests that the short residual disruption activity of microencapsulated formulations targeting codling moth applied at standard dilutions (approximately 20–50 g a.i./ha in 1000 l water) (CAMPION, 1976; KNIGHT and LARSEN, 2001, 2004; EPSTEIN et al., 2003) may result from insufficient airborne concentrations of pheromone to camouflage female-produced plumes. However, results in the field may differ from what was observed in the current flight-tunnel investigation given that feral moths are likely to be in direct contact with microencapsulated pheromone for much longer intervals.

The longer-lasting mechanism that disrupted male codling moth in the flight tunnel was competitive attraction between CheckMate CM-F (approximately 400 microcapsules/wax-paper strip) and the codlemone lure. False-plume following has been recently underscored as an important mechanism mediating disruption of tortricid moths using polyethylene, reservoir-type dispensers (STELINSKI et al., 2004a,b; 2005) and may be a primary mechanism in cases where uniform and highly concentrated coverage of pheromone is difficult to achieve or uneconomical. In addition, codling moth males attracted to clumps of microcapsules may receive sufficient pheromone exposure to induce other disruptive mechanisms such as desensitization. Although levels of disruption can be improved for some species by increasing the amount of active ingredient applied using microencapsulated formulations (POLAVARAPU et al., 2001; TRIMBLE et al., 2003), this approach increases cost. Our results support the idea that more research is warranted towards

development of application methods that concentrate pheromone microcapsules into discrete point sources attractive to males for prolonged periods.

### Acknowledgements

We thank Krista Buehrer and Elizabeth Steere for diligent maintenance of insect colonies and for help with counting microcapsules and conducting flight-tunnel assays. We acknowledge Kevin Vogel for help in developing the flight-tunnel assay procedure and Jesse Stark for performing the microcapsule pheromone release-rate test.

### References

- BARNES, M. M., 1991: Codling moth occurrence, host race formation, and damage. In: *Tortricid Pests: Their Biology, Natural Enemies, and Control*. Ed. by VAN DER GEEST, L. P. S.; EVENHUIS, H. H. Amsterdam: Elsevier, 313–328.
- CALKINS, C. O., 1998: Review of the codling moth area-wide suppression program in the western United States. *J. Agric. Entomol.* **15**, 327–333.
- CAMPION, D. G., 1976: Sex pheromones for the control of lepidopteran pests using microencapsulated and dispenser techniques. *Pestic. Sci.* **7**, 636.
- CARDÉ, R. T.; MINKS, A. K., 1995: Control of moth pests by mating disruption: successes and constraints. *Ann. Rev. Entomol.* **40**, 559–585.
- CHAPMAN, K. L.; BARRETT, B. A., 1997: Susceptibility of male codling moth (Lepidoptera: Tortricidae) to azinphosmethyl in Missouri. *J. Agric. Entomol.* **14**, 441–447.
- CROFT, B. A.; RIEDL, H. W., 1992: Chemical control and resistance to insecticides of the codling moth. In: *Tortricid Pests: Their Biology, Natural Enemies, and Control*. Ed. by VAN DER GEEST, L. P. S.; EVENHUIS, H. H. Amsterdam: Elsevier, 313–328.
- DOANE, C. C., 1999: Controlled-release devices for pheromones. In: *Controlled-release Delivery Systems for Pesticides*. Ed. by SCHER, H. B. New York: Marcel Dekker, Inc, 295–317.
- ENG, J. A.; HOLMES, E.; LARSEN, T.; STADLMANN, S.; KETNER, K., 2003: Effects of sunlight on encapsulated sprayable codling moth pheromone. In: *Proceedings of the 77th Annual Western Orchard Pest and Disease Management Conference*, Portland, OR, 15–17 January. Pullma, WA: Washington State University, 15–17.
- EPSTEIN, D.; GUT, L.; MCGHEE, P.; HAAS, M., 2003: Evaluation of new mating disruption formulations. In: *Proceedings of the 77th Annual Western Orchard Pest and Disease Management Conference*, Portland, OR, 15–17 January. Pullma, WA: Washington State University, 1–6.
- GUT, L. J.; BRUNNER, J. F., 1998: Pheromone-based management of codling moth (Lepidoptera: Tortricidae) in Washington apple orchards. *J. Agric. Entomol.* **15**, 387–405.
- GUT, L. J.; STELINSKI, L. L.; THOMPSON, D. R.; MILLER, J. R., 2004: In: *Behavior Modifying Chemicals: Prospects and Constraints in IPM. Integrated Pest Management: Potential, Constraints, and Challenges*. Ed. by KOUL, O.; DHALIWAL, G. S.; CUPERUS, G. Wallingford, UK: CABI Press, 73–121.
- HALL, D. R.; MARRS, G. J., 1989: Microcapsules. In: *Insect Pheromones in Plant Protection*. Ed. by JUTSUM, A. R.; GORDON, R. F. S. New York: Wiley, 199–248.
- HULL, L. A.; MYERS, C.; KRAWCZYK, G., 2004: Low rate-frequent applications of sprayable pheromones for oriental fruit moth and codling moth mating disruption in Pennsylvania. In: *Proceedings of the 78th Annual Western Orchard Pest and Disease Management Conference*, Portland, OR, 14–16 January. Pullma, WA: Washington State University, 5.
- ISAACS, R.; ULCZYNSKI, M.; WRIGHT, B.; GUT, L. J.; MILLER, J. R., 1999: Performance of the microsprayer, with application for pheromone-mediated control of insect pests. *J. Econ. Entomol.* **92**, 1157–1164.
- KNIGHT, A. L., 2000: Applying sex pheromones - from puffers to air-blast sprayers. *Proc. Wash. Hortic. Assoc.* **96**, 161–163.
- KNIGHT, A. L., 2003: Testing an attracticide hollow fibre formulation for control of codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae). *J. Entomol. Soc. Br. Columbia* **100**, 71–78.
- KNIGHT, A. L., 2005: Developing ultra low volume microencapsulated sprayables for codling moth. In: *Proceedings of the 79th Annual Western Orchard Pest and Disease Management Conference*, Portland, OR, 5–7 January. Pullma, WA: Washington State University, 31.
- KNIGHT, A. L.; LARSEN, T., 2001: The influence of spray application method on the efficacy of a microencapsulated sprayable codling moth pheromone. In: *Proceedings of the 75th Annual Western Orchard Pest and Disease Management Conference*, Portland, OR, 10–12 January. Pullman, WA: Washington State University, 139–140.
- KNIGHT, A. L.; LARSEN, T. E., 2004: Improved deposition and performance of a microencapsulated sex pheromone formulation for codling moth (Lepidoptera: Tortricidae). *J. Entomol. Soc. Br. Columbia* **101**, 109–116.
- KNIGHT, A. L.; BRUNNER, J. F.; ALSTON, D., 1994: Survey of azinphosmethyl resistant codling moth (Lepidoptera: Tortricidae). *J. Econ. Entomol.* **93**, 955–962.
- KNIGHT, A. L.; LARSEN, T. E.; KETNER, K. C., 2004: Rainfastness of a microencapsulated sex pheromone formulation for codling moth (Lepidoptera: Tortricidae). *J. Econ. Entomol.* **97**, 1987–1992.
- LIGHT, D.; REYNOLDS, K.; FRITTS, R.; LINGREN, B., 2004: Advances in use and augmentation of lower rates of sprayable pheromone for mating disruption of codling moths in walnuts. In: *Proceedings of the 78th Annual Western Orchard Pest and Disease Management Conference*, Portland, OR, 14–16 January. Pullma, WA: Washington State University, 4.
- MILLAR, J. G., 1995: Degradation and stabilization of (*E,E*)-8,10-dodecadien-1-ol, the major component of the sex pheromone of the codling moth (Lepidoptera: Tortricidae). *J. Econ. Entomol.* **88**, 1425–1432.
- MOFFITT, H. R., 1978: Control of the codling moth through mating disruption with the sex pheromone. *Mitt. Biol. Bundesanst. Land. Forstwirtschaft. Berlin Dahlem* **180**, 42.
- MOFFITT, H. R.; WESTIGARD, P. H., 1984: Suppression of the codling moth (Lepidoptera: Tortricidae) population on pears in southern Oregon through mating disruption with sex pheromone. *J. Econ. Entomol.* **77**, 1513–1519.
- POLAVARAPU, S.; LONERGAN, G.; PENG, H.; NEILSEN, K., 2001: Potential for mating disruption of *Sparganothis sulfureana* (Lepidoptera: Tortricidae) in cranberries. *J. Econ. Entomol.* **94**, 658–665.
- REUVENY, H.; COHEN, E., 2004: Resistance of the codling moth *Cydia pomonella* (L.) (Lep., Tortricidae) to pesticides in Israel. *J. Appl. Entomol.* **128**, 645–651.
- RIEDL, H.; SEAMAN, A.; HERNIE, F., 1985: Monitoring susceptibility to azinphosmethyl in field populations of the codling moth (Lepidoptera: Tortricidae) with pheromone traps. *J. Econ. Entomol.* **78**, 692–699.
- SANDERS, C. J., 1997: Mechanisms of mating disruption in moths. In: *Insect Pheromone Research New Directions*.



- Ed. by CARDÉ, R. T.; MINKS, A. K. New York: Chapman and Hall, 333–346.
- SAS Institute, 2000: SAS/STAT User's Guide, version 6, 4th edn, Vol. 1. Cary, NC: SAS Institute.
- SHOREY, H. H.; GERBER, R. G., 1996: Use of puffers for disruption of sex pheromone communication of codling moths (Lepidoptera: Tortricidae) in walnut orchards. *Environ. Entomol.* **25**, 1398–1400.
- SHOREY, H. H.; HALE, R. L., 1965: Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. *J. Econ. Entomol.* **58**, 522–524.
- STELINSKI, L. L.; VOGEL, K. J.; GUT, L. J.; MILLER, J. R., 2004a: Behaviors of naïve and pheromone pre-exposed leafroller moths in plumes of high-dose pheromone dispensers in a sustained-flight wind tunnel: implications for pheromone-based mating disruption of these species. *J. Insect Behav.* **17**, 533–553.
- STELINSKI, L. L.; GUT, L. J.; PIERZCHALA, A. V.; MILLER, J. R., 2004b: Field observations quantifying attraction of four tortricid moth species to high-dosage, polyethylene-tube pheromone dispensers in untreated and pheromone-treated orchards. *Entomol. Exp. Appl.* **113**, 187–196.
- STELINSKI, L. L.; GUT, L. J.; EPSTEIN, D.; MILLER, J. R., 2005: Attraction of four tortricid moth species to high dosage pheromone rope dispensers: observations implicating false plume following as an important factor in mating disruption. *IOBC/WPRS Bull.* (in press).
- THOMSON, D. R.; GUT, L. J.; JENKINS, J. W., 1998: Pheromones for insect control. In: *Methods in Biotechnology, Vol 5: Biopesticides: Use and Delivery*. Ed. by HALL, F. R.; MENN, J. J. Totowa, NJ: Humana Press, 385–412.
- THOMSON, D. R.; BRUNNER, J. F.; GUT, L. J.; JUDD, G. J. R.; KNIGHT, A. L., 2001: Ten years implementing codling moth mating disruption in orchards of Washington and British Columbia: starting right and managing for success. *IOBC/WPRS Bull.* **24**, 23–30.
- TRIMBLE, R. M.; VICKERS, P. M.; NIELSON, K. E.; BARINSHTEYN, G., 2003: Sprayable pheromone for controlling the North American grape berry moth by mating disruption. *Agric. For. Entomol.* **5**, 263–268.
- TRIMBLE, R. M.; PREE, D. J.; BARSZCZ, E. S.; CARTER, N. J., 2004: Comparison of a sprayable pheromone formulation and two hand-applied pheromone dispensers for use in the integrated control of Oriental fruit moth (Lepidoptera: Tortricidae). *J. Econ. Entomol.* **97**, 482–489.
- VARELA, L. G.; WELTER, S. C.; JONES, V. P.; BRUNNER, J. F.; RIEDL, H., 1993: Monitoring and characterization of insecticide resistance in codling moth (Lepidoptera: Tortricidae) in four western states. *J. Econ. Entomol.* **86**, 1–10.
- WALDSTEIN, D. E.; GUT, L. J., 2003: Comparison of microcapsule density with various apple tissues and formulations of oriental fruit moth (Lepidoptera: Tortricidae) sprayable pheromone. *J. Econ. Entomol.* **96**, 58–63.
- WELTER, S. C.; VARELA, L.; FREEMAN, R., 1991: Codling moth resistance to azinphosmethyl in California. *Resist. Pest Manag. Newslett.* **3**, 12.

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