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Field and Laboratory Responses of Male Codling Moth (Lepidoptera: Tortricidae) to a Pheromone-Based Attract-and-Kill Strategy

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Environ. Entomol. 31(2): 189–197 (2002)

ABSTRACT A case study of a pheromone-based attract-and-kill management strategy for codling moth, *Cydia pomonella* (L.), was conducted to examine key insect behavioral factors mitigating the possible effectiveness of this strategy. Last Call CM is a newly registered attracticide product that combines the primary component of codling moth sex pheromone with the insecticide permethrin. Studies of competition between pheromone point sources within caged trees showed individual attracticide droplets were significantly more attractive to male moths than calling females. In commercial orchard blocks, marked male moths were recaptured after visiting attracticide droplets applied at rates of 50, 100, and 200 droplets/ha, although no marked moths were recaptured in plots with 500 droplets/ha. This experiment also revealed no significant differences among 0, 50, 100, and 200 droplets/ha in suppressing total catch in female-baited traps, nor were total numbers of females attracting at least one male reduced significantly. In plots with 500 droplets/ha applied, male moth catch was suppressed significantly compared with catches in untreated control plots, and the number of females attracting at least one male was reduced significantly as well. Experiments investigating sublethal physiological effects of attracticide exposure upon mating competency of male codling moths demonstrated male leg autotomy at 1, 24, 48, and 72 h after exposure. Male codling moth at 1, 24, 48, and 72 h after exposure placed near calling virgin females exhibited significant behavioral differences from sham-treated males in courtship and mating. These results clarify some of the possible mechanisms, and strengths and weaknesses of this attract-and-kill management strategy for codling moth.

KEY WORDS *Cydia pomonella,* attracticide, behavior, pheromone, orchard

INSECT SEX PHEROMONES are selective and valuable pest management tools in techniques ranging from monitoring of populations to disruption of the pheromone-mediated mating sequence. Recently, an attract-and-kill system combining the sex pheromones of insects with the insecticide permethrin has been developed. This technology has shown efficacy in the control of a number of important lepidopteran pests including pink bollworm, *Pectinophora gossypiella* (Saunders) (Hofer and Angst 1995), light brown apple moth, *Epiphyas postvittana* (Walker) (Suckling and Brockerhoff 1999), and codling moth, *Cydia pomonella* (L.) (Charmillot et al. 1996, Charmillot and Hofer 1997). However, like many studies of efficacy, the aforementioned investigations focused primarily on end-results of the application, such as trap catch or crop damage. Often, inferences regarding the mating, oviposition, and other behaviors of the target insects are made based on these data. Nevertheless, the long-term efficacy of any behavior-based management strategy is dependent on a stable and, to some extent, predictable suite of responses on the part of the target insect. Therefore, in any behavior-modifying pest management strategy, the behaviors in question should be studied with an eye toward long-term goals under varying ecological situations.

Broad-spectrum neuroactive insecticides are reliable pest management tools, with modes of action that are well documented through nearly half a century of extensive use. However, if we want to combine some of these same insecticides with an additional component that is attractive to the target insect, a host of other factors are brought into play. These factors may be crucial in determining the efficacy of the attracticide, and will be different for each attracticide/insect combination. For example, in a study of Mediterranean fruit fly food bait sprays by Prokopy et al. (1992), the authors found that the physiological and experiential status of the flies were important in mediating effectiveness of sprays. In other cases, sublethal insecticidal effects may play a large role. Although there is no denying the visual appeal of high LD50s to the

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pest manager, they are not necessarily a prerequisite for efficacy in the case of an attracticide. Irreversible sublethal effects, such as an inability of the insect to perceive and/or respond to potential mates, food sources, or other stimuli, will effectively remove the affected individual from the population with the same net result as a kill.

Sublethal effects may be subtle, but may often be critical components in the success of a behavior-modifying pest management strategy. Here, we attempt to analyze these behaviors in a case study of one attract-and-kill strategy, Last Call CM. Last Call CM has been developed by Novartis (Basel, Switzerland) and is newly registered for use in the control of codling moth in South Africa, Europe, and recently the United States.

Codling moth is a key pest of pome fruits in many areas of the world, including the large contiguous apple-growing regions of western North America, which include portions of British Columbia in Canada, and Washington, and Oregon in the United States. Both the United States and Canada are in the process of establishing more stringent limitations on the use of organophosphate insecticides (Calkins 1998), the primary tools for codling moth management over the past 35–40 yr (Croft and Riedl 1991). These limitations will necessitate a shift to alternate technologies, such as more selective insecticides, pheromone-based mating disruption (Charmillot 1990, Pfeiffer et al. 1993, Judd et al. 1997), and/or other pheromone-based methods, such as attract-and-kill. The combination of pheromones with insecticide has been proposed by a number of authors for a variety of pests (Butler and Las 1983, Bariola and Lingren 1984) in an attempt to achieve enhanced levels of control over pheromone release alone. These reports have been complemented by studies investigating the effects of insecticide poisoning on pheromone-mediated behavior of the pink bollworm (Floyd and Crowder 1981, Haynes and Baker 1985, Haynes et al. 1986, Miller et al. 1990), the oriental fruit moth, *Grapholita molesta* (Busck) (Linn and Roelofs 1984), and the cabbage looper, *Trichoplusia ni* (Hubner) (Rider and Berger 1985). However, the mode of action of attract-and-kill strategies has not been critically evaluated until quite recently (Brockerhoff and Suckling 1999) in the development of an attracticide for *E. postvittana.* Although Last Call CM is currently registered for use in U.S. orchards, no North American research data have been published on this new technology.

The primary purpose of this article is to serve as a case study in the examination of a novel pheromone-based pest management strategy by focusing upon the behavioral effects of the attract-and-kill strategy on the target insect—in this case, the male codling moth. In contrast with traditional broadcast insecticides—which kill a fixed proportion of the population regardless of density—attract-and-kill methods may be highly inversely density dependent, due to competition with natural attractant point sources, and this hypothesis is examined. In addition, sublethal effects are also examined within the framework of the male codling moth sequence of mating behaviors—from mate location through courtship, and finally copulation—under influence of Last Call CM.

Materials and Methods

Insects. All moths used in the experiments described were laboratory reared on a sawdust-based artificial diet (Brinton et al. 1969). Pupae were removed from the diet, sexed, and transferred individually to 29-ml plastic Solo cups (Rap-id paper, Kelowna, BC). Males and females were held in different rooms under identical conditions of 25°C, 65% RH, and a photoperiod of 16:8 (L:D) h fluorescent lighting. For laboratory experiments, insects were held at a photoperiod of 8:16 (L:D) h reversed scotophase regime. Pupae were checked daily for eclosion, and moths were provided with distilled water ad libitum from cotton dental wicks. All insects used in experiments were chilled for 10 min at 0°C to facilitate handling. In experiments involving mass releases of moths into orchards, adults were reared at the Okanagan-Kootenay Sterile Insect Release (SIR) production facility in Osoyoos, BC, where they were sterilized in a cobalt-60 irradiator at 35 krad. Chilled moths used in these experiments were dispensed from all-terrain vehicles that ejected the moths directly onto the ground in orchards.

Last Call CM. All of the Last Call CM used in experiments was manufactured in Basel, Switzerland, by Novartis AG, and was dispensed by hand using the pump-type applicator supplied. All droplets were weighed to 50.0 mg and all formulations were prepared in a fume hood before use in the field. Droplets were double-sealed and placed in a −20°C freezer until 24 h before use to minimize release of pheromone and photodegradation before experimental trials. Unless otherwise noted, droplets were not used more than once.

Field Cage Experiments. To assess the effectiveness of Last Call CM in attracting CM males while in the presence of calling virgin females, four pear trees were each caged individually within 3.60 by 3.60 by 3.60-m cages. Tests were conducted from 5 to 14 June 1998. Cages were placed in a pear orchard at the Agriculture and Agri-Food Canada Research Center at Summerland, BC. Trees were separated by at least 50 m, and SIR field releases and monitoring traps in the orchard were discontinued for the duration of the experiment, to reduce the chances of additional pheromone sources being present adjacent to caged trees. This orchard had been reported free of wild codling moth infestations for the three seasons before the experiment. Treatments were randomly assigned and rerandomized over time, so that each treatment occupied each plot for one time period. Thirty 24-h-old fertile CM males were placed in each cage 24 h before deployment of pheromone sources to allow them to acclimate to the field and distribute throughout the tree canopy. Males were released from 29-ml plastic cups suspended in the tree canopy, and all males released were marked with Day-Glo UV fluorescent powder (Switzer, Cleveland, OH) to ensure that males released for each replicate were readily identifiable. Last Call CM droplets were placed on the bottom of inverted 29-ml plastic cups. Virgin 48-h-old codling moth females were placed individually in fiberglass mesh bags (7 by 4 cm). Both droplets and females were placed in wing traps (Phero Tech, Delta, BC). Cups with Last Call CM were placed on the trap bottom, and females in mesh bags were suspended from the center of the trap top using copper wire, so that Last Call CM droplets and females were both approximately in the center of the trap interior. Trap bottoms were coated with Stickem Special (Phero Tech) to capture attracted male moths. Wing traps containing pheromone sources were placed 1.5–1.7 m above the ground, with all trap openings oriented north–south, and trap locations marked and maintained between replicates. All wing traps were baited at the study site in early morning to allow female codling moth maximum acclimation time before the onset of scotophase. Females were placed a minimum of 30 cm from any other wing trap.

There were four treatments in this experiment: a single 50-mg Last Call CM droplet (designated control) in a trap, one droplet in trap + two female codling moth (each in one trap), one droplet in trap + four female codling moth (each in one trap), and one droplet in trap + eight female codling moth (each in one trap). Each replicate consisted of a single 24-h exposure of males to the given treatment. All sticky trap bottoms were collected and captured males were counted at the end of the 24-h exposure period.

Small Plot Field Experiments. *Total Males Caught/Females Mated.* All experiments were conducted in the southern Okanagan Valley, in commercial orchards in Summerland, BC, between 27 July and 5 September 1998. The manufacturer of Last Call CM suggests densities of 1,800–3,000 droplets/ha, depending on pest pressure. The low rates (50–500 droplets/ha) used in this experiment were chosen to reveal mechanisms of Last Call CM at sub-optimal dose levels. All plots were within conventional plantings of mature apple trees, 3.0 ± 0.5 m in height for droplet densities of 0, 50, 100, and 200 droplets/ha. A second experiment was conducted comparing a control (0 droplets) with a 500-droplets/ha treatment later in the season. Treatments were randomly assigned to plots and rerandomized over time. Each of the four experimental plots used were located in different orchards, and each plot was 0.1 ha in size, and located at least 10 m from the edge of the orchard.

Virgin nonirradiated 48-h-old female moths were held in wing traps, at a density of 16 cages/plot. Numbers and placement of females was held constant throughout the experiment. Each replicate was run over two consecutive nights, with females replaced following each replicate. Male insects captured in the female-baited traps were counted at the end of each experiment. Sterile male insects were released into plots at known densities ranging between 1,500 and 2,000 males/ha by all-terrain vehicles 1 d before the initiation of the experiment to allow males to acclimate to ambient orchard conditions.

Marked Males. To determine whether males were contacting attracticide droplets before capture in female-baited traps, a system to reliably mark males responding to droplets in the field was developed. This method was a modification of that developed by Haynes and Baker (1988). Each marking station consisted of a single 50-mg droplet placed in the center of a plastic 29-ml cup lid measuring 6.5 cm in diameter. UV fluorescent powder was applied around the periphery of each droplet. This powder is extremely fine and adheres to moth scales, eyes, and tarsi very readily, so only 0.5 mg of UV powder was required at each station. Moths captured on the bottoms of female-baited wing traps were examined under UV light to determine whether they were marked. Fluorescent powder colors at each station were rotated to denote the day of each trial. Wing trap locations were held constant in each plot and all traps placed at 2.0 m above the ground in the tree canopy. Last Call CM stations were also placed at 2.0 m above ground, and affixed to tree branches using pushpins. Droplets and stations were discarded and replaced between replicates. Sticky-trap bottoms from female-baited traps were used to assess Last Call CM with the following three parameters: (1) the numbers of males caught in each plot; (2) the numbers of females that attracted at least one male in each plot, i.e., the number of females becoming 'mated;' and (3) the numbers of UV-marked males captured in each plot, i.e., those that had visited droplets before capture in female-baited traps.

Sublethal Dose Experiments. *Dosing Methods.* In all cases, 24-h-old males were chilled briefly at 0°C before handling. Chilled males were handled using forceps to grasp the forewings of moths. For attracticide-exposed moths, chilled moths were exposed to a single 50-mg droplet by moving the immobilized insect toward the droplet until contact was made with the tip of one tarsus. Moths were then returned to 29-ml cups until bioassay. Males designated as "sham" in each case were chilled, handled with forceps, and then returned to cups until bioassay.

Mortality, Autotomy, and Mating Behavior Assessments. Mortality was assessed at intervals of 1, 24, 48, and 72 h after exposure to Last Call CM. In addition, males were surveyed daily for leg autotomy to determine whether the insecticide effects included physical disabling of the insects. To determine effects of exposure on mating competency, males were held in isolation from females at identical temperature and light conditions until bioassay, and attracticide-exposed males and sham males in each group had mating behaviors observed and catalogued. These experiments were conducted in 10 cm^3 plexiglas cages. One wall of each cage was constructed of fiberglass mesh to ventilate the cage. One wall of each cage was inserted into grooves, enabling the insertion of moths by sliding this wall of the cage upward. In each case, 48-h-old female CM were inserted into the cage within 15 min to 1 h of the onset of scotophase and observed until the initiation of calling behavior. After the onset of calling a single male was introduced into the chamber by carefully placing the open 29-ml cup containing the male into the cage. This facilitated introduction of the male with minimal disturbance to both the calling female and the male. Timing of the encounter was initiated after insertion of the male into the cage. Male response was observed for 5 min, or until the pair copulated. Among copulating pairs, the length of time spent in copula was also recorded. Males that did not copulate successfully had responses characterized and placed in one of the following five categories: (1) no response, (2) wing fanning and antennal elevation but no movement, (3) nonoriented search behavior, (4) lock-on to female/following female, and (5) coordinated copulation attempts.

Statistical Analyses. Field cage data were expressed as proportions of total responders in each replicate, and were arcsine-square root transformed to satisfy conditions of normality and homoscedasticity (Zar 1996). Analysis of variance (ANOVA) (ANOVA, α = 0.05) was performed on transformed data, followed by Tukey-Kramer honestly significant difference (HSD) test (α = 0.05) (SAS Institute 1995). Data collected in the field experiment were expressed as proportions of total moths caught/total released in each plot, and were arcsine-square root transformed to satisfy conditions of normality and homoscedasticity (Zar 1996). Data were analyzed to compare numbers of males captured in each of the five treatments. ANOVA was performed on transformed data, followed by Tukey-Kramer HSD test (α = 0.05) (SAS Institute 1995). Behavioral data collected in the sublethal dose experiment were analyzed using a heterogeneity chi-square analysis to test whether the variables of treatment with Last Call CM and age were independent of behavioral response (Zar 1996).

Results and Discussion

Field Cage Experiments. Under the attracticide droplet to calling virgin female ratios tested, Last Call CM, baited traps consistently captured the majority of responders, with the total numbers of responders ranging from 26 to 30. However, when eight females were in the cage, a significant proportion of the moths were attracted to the females, i.e., away from the droplet-baited traps (Fig. 1).

The principal factor favoring the attractiveness of attracticide-baited traps may be the release of pheromone from the droplets even while females are not yet calling. High concentrations of male moths were observed at entrances of these traps before the onset of the female calling period. Although all traps/trees could not be observed concurrently, the majority of males caught in Last Call CM-baited traps observed were captured during this period. The larger response window of the male moths, extending beyond the calling period of female codling moth, may have resulted in the vast majority of responders in each treatment being captured in the attracticide-baited traps. This overlap of the male response period with female pheromone production may be an adaptation to the

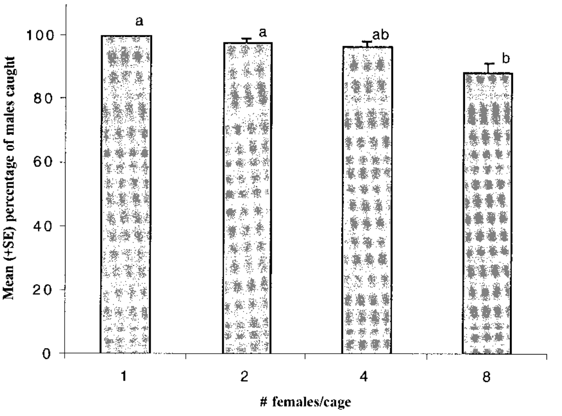

Fig. 1. Mean percentage (±SE) of codling moth males captured in field cages by Last Call CM-baited traps with 0, 2, 4, and 8 females present in each field cage. Bars with the same letter superscript are not significantly different, Tukey-Kramer HSD test, α = 0.05. n = 4.

polygamous trait of male codling moths, which are able to produce up to five spermatophores during their adult life (Howell et al. 1978, Howell 1988). This leads to scramble competition among males, defined by early search for and swift location of mates (Anderson and Iwasa 1996). Under such conditions, the earliest responding males would have a competitive advantage in securing a mate. Males may advance their diel period of response in the presence of pheromone sources, and Last Call CM may serve to activate male response behaviors before the onset of female calling. This advancement in diel rhythm has been documented in pink bollworm males released into cotton fields containing high-dose mating disruption point sources (Cardé et al. 1998). Similarly, Last Call CM may be facilitating phenotypic plasticity of male codling moth behavior by eliciting a generally unexpressed portion of the male behavioral reaction pattern (Stearns 1989).

Another explanation for the low, but pervasive, captures of males by females in each treatment may stem from the behavior of male moths in response to intense scramble competition. Although there have been no studies documenting the mate-seeking behavior of male codling moths under such conditions, males unsuccessful early in the mating period may have relocated to a different area of the tree canopy, increasing the likelihood of encountering a female-produced pheromone plume.

In evaluating these data, it is important to note that responding males were trapped within wing traps and not allowed to contact pheromone sources freely. As such, the insecticidal feature of Last Call CM was not evaluated, and these results represent only an investigation of the attractiveness of the compound placed in wing traps in competition with natural pheromone sources.

Small Plot Field Experiments. *Total Males Caught.* Although the numbers of males caught tended to decrease with increasing droplet densities, there were no

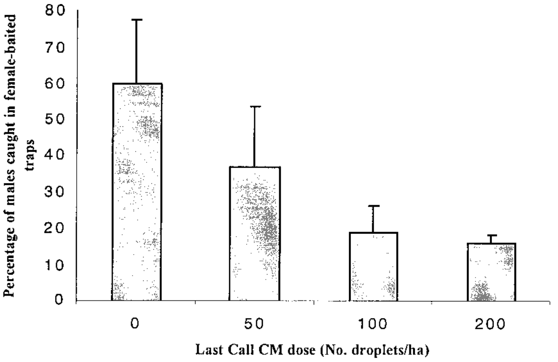

Fig. 2. Mean percentage (±SE) of total codling moth males captured in virgin codling moth female-baited traps in plots treated with 0, 50, 100, and 200 droplets of Last Call CM/hectare. Numbers of males captured are expressed as a percentage of total sterile males released before the experiment. ANOVA, $P > 0.05$, $n = 4$.

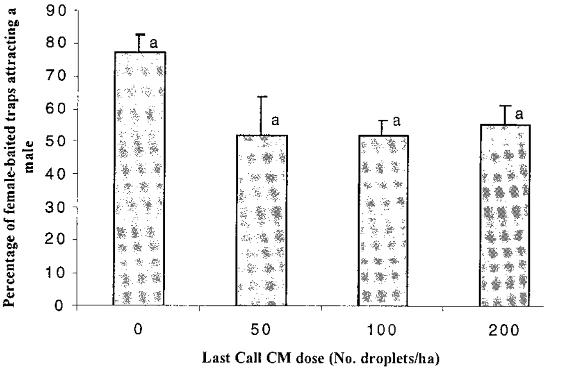

Fig. 4. Mean percentage (±SE) of total codling moth virgin females in traps capturing at least one male in trap in plots treated with 0, 50, 100, and 200 droplets of Last Call CM/hectare. ANOVA, $P > 0.05$, $n = 4$.

significant differences in the total numbers of males caught in the 0, 50, 100, and 200-droplet treatments. The low number of replicates and subsequent low power of this test should be noted ($\beta = 0.63$) (Fig. 2). However, in the second experiment, which consisted of a 500-droplets/ha treatment and a 0 droplet control, there was a significant reduction in catch (Fig. 3). This second experiment was conducted later in the season than the 50, 100, and 200-droplet treatments (which were applied concurrently), and night temperatures were cooler, possibly resulting in lower percentages of released moths flying to females. Even so, the dramatic reduction in catch in the 500-droplet treatment suggests that the number of point sources may be key in the ability of Last Call CM to reduce numbers of males attracted to calling females. The mode of action may be a function of the ratio of attracticide droplets to calling females. If this is the case, a ratio favoring the former results in a higher frequency of males contacting the toxic pheromone source, eventually resulting in their death or paralysis. For example, at the highest droplet densities tested, ratios of 50 droplets:16 females would be observed in each plot—representing ≈3:1 ratio favoring the attracticide.

Total Numbers of Females 'Mated.' At densities of 0, 50, 100, and 200-droplets/ha, no significant difference was observed between treatments ($\beta = 0.61$) (Fig. 4). However, at the 500-droplet/ha dose compared with a control later in the season there were significant reductions in the number of females mating as measured by the capture of at least one male in the trap bottom (Fig. 5). A similar study conducted by Suckling and Brockerhoff (1999) with light brown apple moth reported a 75% reduction in visits to female-baited traps with 450 droplets/ha. The results presented here are key from a management standpoint, because each female needs to attract only one male,

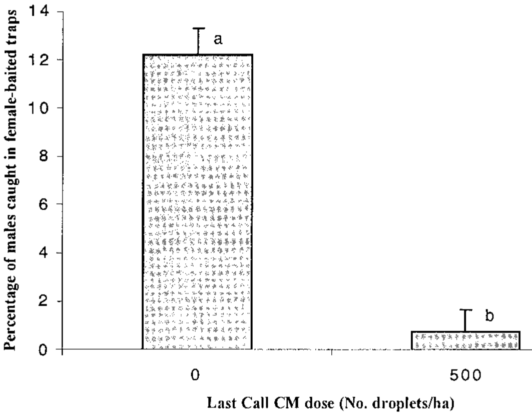

Fig. 3. Mean percentage (±SE) of total codling moth males captured in virgin female-baited traps in plots treated with 0 and 500 droplets of Last Call CM/hectare. Numbers of males captured are expressed as a percentage of total sterile males released before the experiment. Bars with the same letter superscript are not significantly different, Tukey-Kramer HSD test, $\alpha = 0.05$, $n = 4$.

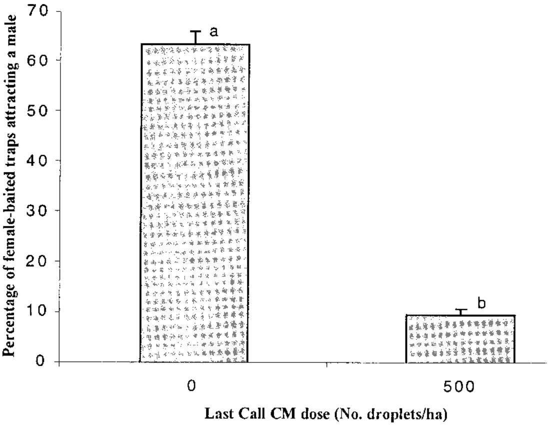

Fig. 5. Mean percentage (±SE) of total codling moth virgin females in traps capturing at least one male in trap in plots treated with 0 and 500 droplets of Last Call CM/hectare. Bars with the same letter superscript are not significantly different, Tukey-Kramer HSD test, $\alpha = 0.05$. $n = 4$.

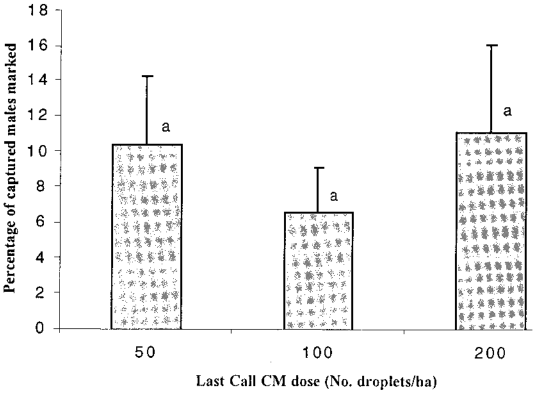

Fig. 6. Mean percentage (±SE) of total codling moth males that were marked with Day-Glo UV-fluorescent powder and recaptured in female-baited traps in plots treated with 50, 100, and 200 droplets of Last Call CM/hectare. ANOVA, $P > 0.05$, $n = 4$.

and one mated female in an orchard block can do significant localized damage.

In interpreting these results, it is important to note that calling females were held in wing traps. This may have affected results in a number of ways. The design of wing traps, as outlined for an earlier experiment, may present physical limitations to males attempting to enter the trap and respond to females within. Plume structure is an important variable in the orientation of lepidopteran males to calling females (Baker and Haynes 1989, Willis et al. 1994). Here, the distribution of the female-produced plume was limited—and perhaps distorted—by the trap opening, a phenomenon documented in another tortricid species, the *E. postvittana* (Foster et al. 1991). In contrast, attracticide droplets placed on top of inverted plastic cups were exposed on all sides, and freely contacted by male moths. Realizing that the experimental methods used (due to the necessity of marking and capturing males) bias results somewhat in favor of Last Call CM, these results should not be regarded as evidence of efficacy of this compound in a commercial orchard setting—this was not the purpose of our investigation. Additional experiments at droplet densities approaching the recommended range, with experiments using tethered females to assess mating success, are required to ascertain whether a shutdown in mating is achievable in an orchard using Last Call CM. In addition, the short duration of the experiment (2 d) prevents the drawing of any conclusions regarding season-long control. This experiment elucidates the mechanism behind this attract-and-kill product on a night-by-night, individual insect basis. Although inferences can be made based on this snapshot, additional work for longer periods should be conducted.

Numbers of Marked Males Captured. In the field experiment, marked male moths were captured at droplet densities of 50, 100, and 200 droplets/ha (Fig. 6). In the second experiment, at 500 droplets/ha, no marked males were captured in the female-baited traps ($n = 48$).

Although the marked males captured at the lower droplet densities represented a relatively small percentage of the total catch, this demonstrates that some males responding to Last Call CM were able to detect and respond to calling females after exposure to the toxic compound. It is impossible to state with certainty whether all of these marked males did indeed contact the droplets, however, wind-tunnel bioassay results suggest that at least one brief contact was made (C.H.K., unpublished data). The catch of marked males by virgin female-baited traps may represent only a fraction of the responders to the attracticide droplets. Studies on pink bollworm have shown that male moths exposed to sublethal doses of pyrethroids have been able to contact a pheromone source after exposure (Haynes et al. 1986, Moore 1988). However, absence of marked males in the 500-droplet/ha treatment is further evidence of the density-dependent nature of the mode of action of the attract-and-kill strategy. These data suggest that males will contact Last Call CM droplets repeatedly, and a ratio which favors an encounter with a droplet over an encounter with a calling female will result in a greater killing efficiency of the compound on an area-wide level. Similar research using pink bollworm attracticide formulations revealed that male visitation to individual attracticide point sources increased with the densities deployed (Miller et al. 1990).

Sublethal Effects. In examining any attract-and-kill strategy that seeks to reduce the incidence of mating among target insects, it is important to stress that mate location often does not conclude the premating sequence of behaviors (Hutt and White 1977; Castrovillo and Cardé 1980). In the case of codling moth, males must be able to visually orient to, and court prospective mates before female acceptance, mating and spermatophore transfer occur. The importance of this suite of highly stereotyped and specific courtship behaviors make them vulnerable to sublethal effects of a toxicant, where even minor deviations from "normal" codling moth behavior may result in the inability of the affected individual to obtain a mate. Sublethal effects upon these individuals may act as a prophylactic treatment if these effects reduce the incidence of successful mating in affected individuals.

Mortality rates among treated males increased over time throughout the observation period, and were greatest after 72 h. No mortality was observed in the sham-treated groups. It should be noted here that males were not allowed to contact the material freely, as the principal objective of this experiment was to expose the male moths as briefly as possible to observe sublethal effects, and not to quantify the killing efficiency of the compound.

Autotomy of thoracic legs was noted in all observational categories among toxin-exposed insects, and the percentage of males exhibiting leg autotomy remained constant after 24 h following exposure. There was no autotomy noted in the control group of moths (Fig. 7). Other researchers have proposed that this self-amputation of limbs is an adaptive trait to avoid uptake of toxins (Moore et al. 1989). In our discussion

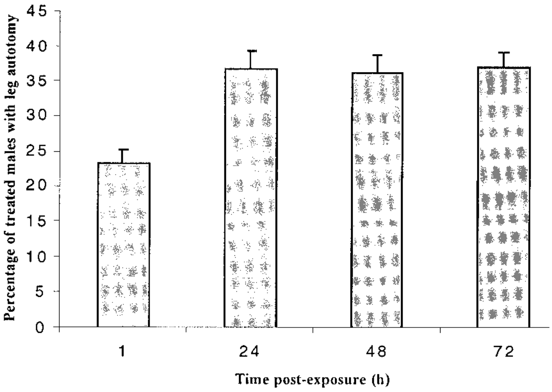

Fig. 7. Cumulative percent autotomy of codling moth males in Last Call CM-treated groups at 1, 24, 48, and 72 h after treatment. $n = 30$.

of attract-and-kill efficacy, this phenomenon may translate into a lower level of mating success for affected males.

There was no effect of age upon the exhibition of various mating behaviors, in either the sham or Last Call CM-treated insects, although highly significant differences between sham and treated insects were noted in each of the behavioral categories (Table 1). In total, only two successful copulations were observed among treated males, compared with 98 in the sham treatment (Table 1). One of the copulations in the former group lasted only 18 min in duration, well below both the 36-min mean reported for sham-treated mating males in this experiment ($n = 98$), and a 37-min mean reported by other researchers investigating codling moth mating behavior in the laboratory (Howell et al. 1978). Thus, it is unlikely that a full spermatophore was transferred in this case. These data present strong evidence of the behavioral effects of sublethal exposure to Last Call CM in interfering with the mating sequence. Commonly observed specific symptoms included an inability of autotomized male moths to climb vertically while following the female, inability to sustain orientation to females, and 'misdirected' copulation attempts, where the abdomen of males immediately adjacent to calling females was directed in a direction opposite to the female. These symptoms of generally uncoordinated behavior are in agreement with published accounts of the general effects of pyrethroids (Coats 1982) and with more specific accounts of behavior of male moths exposed to pyrethroids followed by exposure to sex pheromone (Floyd and Crowder 1981, Linn and Roelofs 1984, Haynes and Baker 1985, Haynes et al. 1986). The ability of males to compete with conspecifics is a key factor mitigating reproductive success, and male moths exhibiting uncoordinated behaviors may have difficulty not only in closing the distance between themselves and the female, but also in influencing the female's choice of a courting male—a principal function of courtship (Alexander et al. 1997, McNeil 1992). This is of particular importance in the case of autotomized males who may have difficulty climbing and pursuing a female with less than the full complement of legs. Male moths in each of the treated groups did appear to perceive the female-produced pheromone (i.e., exhibited some behavioral response), suggesting that the sensory system of males was still functioning at some level. However, there were males in each Last Call CM treatment that demonstrated no response when confronted with a calling female (Table 1). Similar sublethal experiments using permethrin were conducted using pink bollworm males (Haynes and Baker 1985), who postulated that permethrin may be acting at both the peripheral nervous system level, by blocking the receptor sites due to continuous firing; and at the central nervous system, by changing qualitatively or quantitatively the signal from the sensory system. The former may result in a total lack of response on the part of males, while the latter may cause males to experience difficulties completing various phases of the pheromone-mediated sequence of courtship behaviors. The behaviors documented in sublethally exposed moths may be due to the combination of permethrin, codlemone, and the viscous carrier; there is no way to separate these effects from one another with the protocol used in this experiment.

Table 1. Influence of sublethal exposure to Last Call CM on subsequent behavioral responses of male codling moth to calling female moths

Time after treatment, h	Treatment	Degree of response elicited in response to calling female CM[a]					
		No responses	Wing fan	Non-oriented search	Coord. pursuit	Copulation attempts	% mating success
1	Sham***	0	0	0	3	27	90
	Last Call CM	16	4	6	3	1	0
24	Sham***	0	0	0	2	28	90
	Last Call CM	15	10	4	0	1	0
48	Sham***	0	0	0	3	27	90
	Last Call CM	15	6	2	5	2	3.3
72	Sham***	0	0	0	1	29	93.3
	Last Call CM	16	6	6	0	2	3.3

Comparisons made between treatments for individual time categories; and between time categories within each treatment (sham versus control; chi-square test for heterogeneity; *** $P < 0.001$. Data presented as numbers of responders in each behavioral category. The sample size at each time/treatment level combination is 30 males.

[a] Behavioral categories are mutually exclusive, each male placed in only one category.

A unique aspect of Last Call CM is its tendency (due to its viscous nature) to adhere to the tarsi of male moths in small amounts. This feature is ostensibly to enhance the killing efficiency of the contact insecticide. However, it would be useful to document the effects of continuous exposure of the males to the pheromone component of the attracticide, to determine whether the small amount of pheromone present on the tarsi would be sufficient to alter subsequent pheromone-mediated behaviors in any way. This exposure may have played a role in the results reported here.

From the results reported above, it is clear that sublethal exposure to Last Call CM does exert significant debilitating effects upon the mating behavior of male CM. These results may appear incongruous with the results presented above, where marked (i.e., Last Call CM-exposed) males were recaptured in virgin female-baited traps, suggesting that these males were able to locate and fly to calling females. This observation may be due to one of two factors: (1) "false marking"—the males did not actually contact the droplet—contacting only the dust on the periphery of the droplet; (2) males did not receive a sufficient dose to impair sensory and motor function used in maintaining anemotactic flight immediately after exposure. The former explanation appears unlikely, in light of the results of our wind tunnel study testing the reliability of the marking system. Anemotactic flight immediately after contact with Last Call CM droplets was documented in the wind tunnel, and may have occurred in the field as well, explaining the catches of marked males in female-baited traps. Further evidence of contact with attracticide droplets are several observed instances of the classical symptoms of pyrethroid poisoning, namely hyperexcitation, convulsions, and trembling of appendages (Coats 1982) among living, marked males stuck on trap bottoms on the morning after the field experiment. Even in the absence of a trap (i.e., free access to calling female), these males may have been unable to complete the mating sequence, as the results presented here suggest.

The preceding experiments were designed to elucidate some of the mechanisms at work in the Last Call CM management strategy by focusing upon behaviors of individual male moths, both in the laboratory and in the field. This emphasis on insect behavior will help expand our understanding of the effects that pest management strategies have upon target organisms. For example, our results suggest that sublethal exposure to attracticide droplets is sufficient to remove males from the viable mating population; a factor that may have been overlooked by studies focusing solely upon trap catch or mortality as predictors of field efficacy. Other researchers have noted that the addition of pyrethroids to attracticide formulations for pink bollworm appear to have sublethal modification of mating behaviors as their primary mode of action, rather than direct lethal effects (Haynes et al. 1986).

As with any new technology, attract-and-kill strategies in general are in need of further development and refinement. We have shown the importance of including high-resolution studies of the behavior of target insects in studies of new attract-and-kill strategies. The theory behind the attract-and-kill technique represents a fusion of 'old' and 'new' technologies, and it is this type of integration that may prove to be a necessity in coping with the pest management challenges of the future.

Acknowledgments

We thank the orchardists for use of their properties in field research. Thanks to M. L. Evenden for advice on moth-marking methods, and to M.G.T. Gardiner for technical advice and support. We thank the Okanagan-Kootenay Sterile Insect Release Program for coordinating insect releases, especially R. Gauthier and A. McCluskey. Thanks to integrated pest management Technologies for supplying Last Call CM batches for these experiments. This research was supported by the Science Council of British Columbia, the British Columbia Fruit Growers' Association, NSERC and Simon Fraser University.

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Received for publication 27 December 2000; accepted 19 September 2001.