Evaluation of Alternative Pheromone Dispensing Technologies

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Field Trials in Orchards with Scentry Fibers.

Pheromone mating disruption has proven highly effective for selective management of codling moth in the smaller canopies of pears and apples. However, larger canopies, e.g. mature walnut trees have proven difficult logistically using hand-applied dispensers, including the Shin-Etsu ropes or Suttera membranes. One alternative device for pheromone mating disruption for larger canopies includes mechanical aerosol emitters, which have proven successful in pear orchards in Lake County, CA. However, issues of potential mechanical failure combined with the limited number of dispensers per acre have made adoption slow to date in walnuts and some localities of pears.

Application of hollow fibers (e.g. NoMate CM fibers) using modified ground or aerial application equipment may provide a suitable alternative to hand-applied dispensers. Applications by air using fixed wing or helicopters also have the added advantage of reaching the upper portions of the tall canopies for more complete diffusion of the pheromone throughout the canopy. One disadvantage of this application technique might be photodegration of the pheromone from sunlight within fibers that fail to fall into the shaded portions of the tree canopy. One unique feature of this approach is the use of small fibers (tubules) that are mixed with an adhesive material within a hopper before direct application to the crop. The fibers are pushed by a turning screw onto a rapidly spinning cone outside of the hopper. The fibers are then flung at random from the applicator; eventually falling and attaching to the crop foliage. The pheromone diffuses from the open ends of the fiber and is emitted over time into the orchard. The application requires specialized equipment and has the additional challenges associated with application of very sticky fibers to the canopy.

Given that this was the first year of testing with the product, several areas were identified that needed to be resolved:

a) Do applications of the Scentry fibers effectively suppress codling moth traps as proxies of female codling moths in orchards?
b) Does trap suppression correlate with additional damage suppression if the NoMate fibers are added to the existing conventional insecticide programs. This combination approach mitigated the risk of high damage levels within the first year of trials.

We had anticipated applications to both walnut and pear orchards in 2004. Given the fact that the fibers need to be applied with specialized apparatus attached to helicopters or fixed wing, we were only able to secure one applicator willing and able to apply the material. The distance between the home base for our applicator in Richvale, CA and the nearest pears precluded a trial in pears in 2004. Hopefully, the successes observed in 2004 will increase our opportunities for applications in pears. One development in progress in WA is the design of applicators for ground applications of the fibers.

Materials and Methods

Field Trials in Walnut Orchards with Scentry Fibers. We evaluated the NoMate CM Fiber (Scentry Biologicals, Inc., Billings, Montana) in three walnut orchards in the Sacramento Valley. The trial was initially designed to demonstrate the impact of a Scentry fiber application on codling moth trap catch. Scentry treated plots were set up in conventional treated grower blocks. The entire block received the same grower treatment, with a codling moth pheromone supplement on acreage within the block. Thus, the only treatment difference was the pheromone supplement. Given that many walnut orchards often experience 1-2% damage by codling moth, the benefit of adding the pheromone fibers would be expressed as the difference between the 2 treatments.

Experimental plots were set up in three walnut orchards characterized as follows: 1) Glenn orchard was approximately 80 acres of Chandler variety, in a 40’ X 20’ offset planting, young trees approximately 15 – 20 feet tall, open canopy. Grower applications for insect control were Lorsban at 4 pts per acre on July 1st 2) Chico orchard was a 40 acre block of Vina variety, 35-40 ft mature trees with full canopy. Insecticide applications were Lorsban on May 13, Intrepid on June 10 and August 1, Perm-up on August 28. 3) Corning orchard was a 141 acre block of Tehema variety, 24’ X 48’ offset planting, 25-35 ft, mature trees with full canopy. Applications for insect control were Lorsban on July 13 and August 4, and Perm-up on August 27.

Aerial applications of Scentry No-mate fibers were made on April 16 -19 with second applications made on June 25, 2004. Fifteen-acre Scentry treated plots (Glenn and Chico) received two treatments at 10 gm ai / acre, while the Corning site received 20 gm ai / acre on 9.5 acres for first application and 10 gm ai / acre fro the 2nd application on 19 acres (which included the original plot plus contiguous acreage). NoMate Fibers were mixed with an adhesive material, Bio-Tac, as a sticker/carrier and applied at a rate of 100 gm fibers / 1 qt Bio-Tac / acre. The sticker is available in different viscosities; one site (Glenn) used a 100:300 weight blend on both application dates, but application proved difficult as foaming in the sticker hampered extrusion from the application pod. Substituting 25 weight Bio-Tac in a ratio of 7 parts (100:300 weight) to 1 part (25 weight) significantly improved the extrusion. Application required the use of a specialized “pod” provided by Scentry. Two “pods” mounted on a frame (developed by Craig Compton, AVAG, Inc., Richvale, CA) were carried by helicopter for application.

Two study sites (Chico and Corning) had additional untreated controls that received no codling moth control. The success of the pheromone treatment to shut down a standard CM trap was monitored by three traps placed in each Scentry and grower standard plot. The trap sample was increased to six for each treatment in the Corning site when the plot size was increased for the second application. Traps (Pherocon ® Delta VI, Trece, Inc., Adair, OK) were baited with Codling Moth 1X Biolure (Suterra, Inc., Bend, OR), monitored weekly and lures changed at 6-week intervals. Codling moth damage was assessed pre-harvest by ground inspection of 1000 nuts per treatment plot, and at harvest by sampling 1000 nuts following shake. Collected nuts were cracked out and damage was recorded.

Results

Field Trials in Walnut Orchards with Scentry Fibers. The Scentry applications appeared to effectively shut down standard pheromone baited traps in all sites (Figure 1, 2). A single codling moth was trapped in the Scentry treated plot in the Corning orchard prior to the second application in June. Codling moth flights were sufficiently reflected in traps placed in the grower standard plots (Figure 1). Trap catches indicate orchards varied in population pressure with the Glenn site appearing to have the greatest pressure and the Chico site the least (Figure 2).

Trap suppression may not correlate with damage suppression. A pre-harvest canopy sample was conducted in two orchards by inspecting fruit that could be reached from the ground (Figure 3). In both the Corning and Glenn sites, damage in Scentry treated plots was less than the grower standards with the greatest difference in the Glenn orchard (0.3% Scentry, 2.6% grower). (Canopy height prevented pre-harvest assessment of the Chico site.) Codling moth damage observed in canopy samples does not necessarily correspond to harvest damage for several reasons: worms sometimes complete development by feeding only on the husk and nuts damaged from early flights can be blown out during the harvest process and not included in a final sample.

Codling moth control in all sites was good based on the harvest sample crack-out with a maximum damage of 3.6% observed in the Chico grower standard (Figure 4). Overall, average damage in the Scentry plots (1.7%) was less than that observed in the grower standard samples (2.2%) though rank of treatment in individual sites varied. We observed 0.7% nut damage in the Scentry plot compared to 0.4% in the grower plot at the Glenn orchard. In all other sites, damage in the added Scentry treated plots was equal to (Corning single application area) or less than the grower standard. Damage observed in the untreated controls was less that the grower standards in both sites and may reflect the impact of localized population pressure (or lack) and the much smaller size and limited sampling area of these controls.

Controlled Application Field Exposure and Aging Studies

Our ability to evaluate new pheromone dispensing devices/formulations is largely restricted to field evaluations with trap and damage suppression being the primary criteria for evaluation. Obviously, damage suppression is the key element for determining commercial success, but these types of trials are difficult to replicate, vary by location and year, and lengthy. Approaches that rely on extraction of residual pheromone over time provide another measure, but the extraction process may yield different results depending on the product. Thus, inter-product comparisons become difficult. More direct measures of pheromone emissions rates using direct measurements of pheromone concentrations in an air stream are perhaps the most direct and accurate, but are technically difficult and expensive.

An alternative is the use of a device called an electroantennogram which records the electrical signals from a living antenna in response to different chemicals, e.g. pheromones or plant volatiles. The relative response of the antenna to different odor streams provides an indirect measure of the activity of the compound in the air stream. While this technique does not provide a quantitative estimate of pheromone concentration, it does provide a quick and relatively easy means to compare dispensers under different conditions, over time to evaluate the longevity of a dispenser, or to contrast dispenser types under a fixed set of conditions. As such, new products or formulations can be compared within a few months for improvement in product quality or as compared to industry standards.

In 2004, we are evaluating the ability of codling moth to respond to aged residues and dispensers of 3M and Suterra sprayable CM formulations, Scentry fibers, and Hercon flakes. Discs treated with dilute solutions of the sprayable formulations (3M and Suterra) or individual dispensing units of Scentry or Hercon products were aged in direct sun or complete shade to provide samples to test the stability of the products over time. An electroantennogram (EAG) (Syntech, Netherlands) measured codling moth antennal response to the pheromone-treated disc samples. The EAG "spike" that is recorded is the sum of the neural response of all receptors in the sample antenna firing after exposure to the compound. Thus, the amplitude of the spike is an indication of the relative number of receptors detecting the pheromone and is an indirect measure of the concentration. Antennal activity is one measure of pheromone response, but these data should be corroborated with direct assays for residues, behavioral data, and ultimately damage suppression. However, if the discs are not emitting pheromone, then there is little opportunity for program success.

Controlled Field Aging. Aging chambers were constructed of PVC pipe with a wire mesh bottom and a cover which protected pheromone samples from rain and wind. The chambers permitted two different light treatments. The light exposed chambers had a clear plastic film cover that permitted direct solar radiation. Dark chambers each had two nested aluminum covers, each with two side vents oriented on different axis to prevent direct exposure to light and to minimize heat buildup within the chamber. A 2-channel HOBO H8 (Onset Computer Co. Pocasset, MA USA) data recorder was placed inside one light and one dark chamber to record hourly temperature data both inside and outside the chambers. These indicated no differential heating between chamber types that might impact rates of pheromone degradation.

Filter paper discs pinned to a balsa wood rack were treated with pheromone (detailed below). Four discs from each treatment (light and dark) and for each compound were collected on the day of setup and weekly thereafter. Collected discs were placed individually into sample tubes and frozen until evaluations could be conducted. Samples were set up for the following products: MEC-CM (3M, St. Paul, MN), Checkmate CM-F (Suterra LLC, Bend, OR), Hercon DISRUPT Micro-Flake® CM (Hercon Environmental, Emigsville, PA), and Scentry CM NoMate Fibers (Scentry Biologicals, Inc., Billings, MT). Sprayable formulations were diluted such that a 50 microliter aliquot deposited 72 micrograms AI pheromone per 2.3 cm diameter Whatman # 3 filter paper disc (Whatman® CAT No. 1003323). Hercon flakes were attached to the filter paper, one flake per disc, using the sticker Gelva® Multipolymer Emulsion 2333 (Surface Specialties, Inc. Smyrna, GA). Scentry fibers were attached, one fiber per disc, using the sticker Bio-Tac. Initial application of the Scentry fibers failed when the Bio-Tac was absorbed by the filter paper within a week. The Scentry trial was restarted using one inch diameter plastic discs cut from a vinyl sheet protector (product #61013, C-Line Products, Inc., Mt. Prospect, IL). The disc samples were subject to the conditions detailed above and collected weekly for ten weeks. The trial was conducted in a pear orchard near Marysville, CA.

EAG analysis. A single antenna is removed from a male codling moth, the distal end is cut, and each end is embedded in electrode gel on the contact points of the antenna holder. EAG settings are set as follows: gain = 100, sample and reference times = 0.3 seconds, pause = 10 seconds, TC = 2. Green leaf alcohol (product # 101, Bedoukian, CT) dissolved in paraffin oil (5% AI /10 microliter solution) is placed on a polypropylene cap and set in the reference chamber of the EAG. When a stable signal from the antennae is displayed (2-10 minutes), sampling is commenced. Pheromone samples were run at two minute intervals to allow the antennae to recover between exposures. For each product, a test run consisted of exposing one antenna to one disc sample from each time period of the treatment being evaluated. The range of ages examined for each product or exposure was determined by preliminary testing. If residues of a particular age failed to elicit a response from antennae, older residues were not examined. An untreated disc (filter paper or plastic) served as a control for all trials. Discs were presented in a random order for each antenna. To date, we have evaluated the light exposed discs of all four products, and the dark exposed discs of the Hercon and Scentry products (Suterra and 3M dark exposure data not yet completed).

Field Application Exposure and Aging Studies

Sprayable pheromone leaf application and residue evaluation. Solutions of 3M and Suterra products were applied to pear leaves using a Crown Spra-Tool®. For each product, two shoots on each of eight trees were flagged for treatment. One shoot of each set had south side sun exposure and the other was within the canopy for shaded exposure. Each product was diluted to the same rate as the controlled residue study described above so that degradation curves for the leaf samples could be directly compared. The spray concentration of each product was 1.4 gm ai / liter, and sprays were applied to drip. The initial sample (wk 0) was taken after residues dried and then weekly for 6 weeks. Leaf samples were taken by removing one leaf from all eight shoots for each exposure and product. Leaves were individually bagged and frozen for later analysis. These EAG evaluations are yet to be completed.

Field application of Hercon Flakes – distribution and retention. An aerial application of the Hercon Flakes was made in a young block of Bartlett pears after harvest. Application was postponed until post harvest.   Application was made at a rate of 4.4 lb (formulated) / acre onto two rows. Four passes were flown at a rate of 1.1 lb / acre each pass. Percent interception of flakes by the tree canopy was estimated by comparing the number of flakes deposited on 3.89 square foot poster board samples placed under the canopy to samples placed in canopy gaps within the rows and between the treatment rows. A total of 18 poster boards were distributed across the three placements. Retention of flakes through time was estimated by locating and flagging leaves with a flake, and monitoring these leaves weekly for 10 weeks.

Field application of Scentry CM NoMate Fibers – distribution and retention. Inspection of a walnut orchard was made following aerial application of the Scentry CM NoMate Fibers. Few fibers could be located, thus, formal counts for distribution and retention were not conducted.

Results

Controlled Application Field Exposure and Aging Studies.

The results of the EAG analysis need to be interpreted in a relative context. The response of an antenna to the aged fibers was always compared against the activity of a known standard, the plant alcohol. The initial reading at week zero provides the baseline for comparing all other dispensers. In addition, plastic discs without any fiber served as a second type of control. As the ratio of the source/reference changes (S/R), the relative amount of pheromone to which the antenna is being exposed also is presumably changing.

For both light exposed and shaded Scentry fibers, the highest levels of activity were observed after no aging (week 0) (Fig 5 and 6). The largest drop occurred after one week presumably due to pheromone on the outside of the fibers during preparation, but the emission rates remained consistent for the next 6 weeks. A decline was noted after week 6 in the light exposed fibers, whereas emissions were detected for the entire 9 weeks of the trial.

A similar pattern was observed for the Scentry fibers kept in the dark with the highest emission rate at week 0. No strong decline in emission rates were observed from week 4 to 10 with significant emissions noted at week 10 compared to the untreated controls. In general, a stronger signal was detected from fibers late in the study that were kept shaded compared to the light exposed fibers. Given the overall similarity in patterns for both types of dispensers, it appears that the fiber is able to provide sufficient protection from UV degradation by sunlight.

The results for emissions from the Hercon microflakes are shown in Figures 7 and 8. The microflakes that were shaded showed a slow, but steady decline in emission rates from week 0 to week 8. In contrast, the light exposed flakes declined more rapidly and reached a similar release rate to the untreated controls by week 2. As such, fibers in full sunlight do not appear to provide complete protection from photodegradation.

For the microencapsulated formulations, the results are similar to the past in that microcapsules exposed to full sunlight are generally not as well protected from degradation by sunlight (Figures 9 and 10). For the Suterra formulation, emissions as measured by antennal activity had virtually disappeared by week 1, whereas the 3M formulation indicated more significant activity at week 1, but this also disappeared by week 2. While the shaded capsules have yet to be evaluated, historically, capsules kept in the shade lasted between 4-6 weeks depending on other conditions. The field aging directly on leaves also need to be evaluated this winter.

Field application of Hercon Flakes – distribution and retention.

Distribution of flakes from the poster board spray cards indicated that approximately two-thirds of the Micro-Flakes were intercepted by the pear canopy. Cards placed adjacent the trunks of six trees intercepted an average 1.8 flakes (sd=0.98) while those with the rows and between rows captured an average of 6.0 (sd=3.52) and 5.3 (sd=1.63), respectively (Figure 11).

Flakes were difficult to locate on leaves following application. Sixty three flakes on leaves were flagged following the aerial application and then monitored for 10 weeks. An unknown number of flakes appeared to have inadequate sticker coating. We estimated that 15-20 % of the flakes we initially found were knocked off while flagging and thus were not included in the retention study. Either the sticker or pheromone from the flake appeared to have a localized impact on leaf tissue as necrosis was observed at and immediately surrounding the contact point. Retention following application was 84% after 15 days and was 70% after 49 days (Figure 12). There appeared to be a sharper decline in retention by day 56 with a drop to about 50%. We terminated the study at day 70 when orchard maintenance and pruning removed part of our sample.

Discussion

Overall, trap suppression using the Scentry fibers was excellent with complete trap shutdown in 2 orchards and a single moth caught in the third. In contrast, the grower trap counts averaged from 7.3 to 121.7, which indicate significant flights in 2 of the 3 orchards. Comparisons of codling moth damage at pre-harvest timing suggested a stronger suppressive effect, but this difference either was lost or became obscured at harvest. The damage levels between the grower standard and the plots treated with a combination of the grower standard practices and the pheromone application were not significantly different. Because of the unreplicated nature of the Corning plot, the increased suppression in the plot using 20 gm ai of codling moth pheromone per acre compared to 10gm ai per acre cannot be attributed to the treatment.

From a speed of application perspective, the ability to treat orchards from the air has a distinct time advantage. However, our ability to utilize this approach will depend on applicator acceptance of the apparatus and the difficulties associated with the Bio-Tac sticker.

The EAG data suggests at least 10 weeks of effective and relatively consistent emissions for both the sunlight exposed and non-exposed Scentry fibers. As such, two applications would be expected to have at least 140 days of effective emission. This notion was supported by the strong trap suppression all season. While the suppression of damage was promising at the pre-harvest canopy counts, the pattern was not as strong at harvest. Damage suppression trials in 2005 will need to focus on increased replication across orchards with codling moth pressure.

Less stability was observed with the Hercon microflake for the units exposed to full sunlight. However, greater longevity was observed for the shaded microflakes with 4-6 weeks of emissions noted. Similarly, a steady decline in retention of the flakes from their position over time was noted, such that efforts to increase adherence of the microflake should increase the performance or efficiency of the program. No significant improvement in the stability of codlemone in microencapsulated formulations was observed with detectable emissions lasting less than 2 weeks from both formulations under full sunlight conditions and for less than 1 week with the Suterra formulation. However, positive results for trap and moderate suppression of codling moth in walnut orchards suggest significant release in the more heavily shaded walnut canopies.

Our ability to suppress traps and presumably long-range orientation of codling moth to females appears to be fairly easy compared to suppression of other possible mechanisms of orientation. Current speculation is that other mechanisms (visual cues, larval aggregation behavior, larval pheromones) are bringing male and females together under high pressure situations. Understanding how these individuals are successfully circumventing efforts to disrupt their mating remains one of the key challenges currently facing more effective pest suppression.

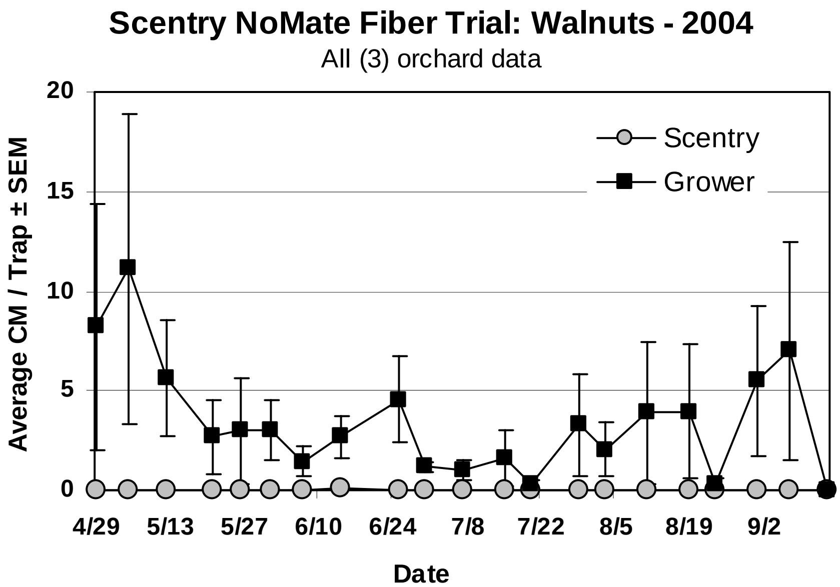

Figure 1. Average codling moth trap catch in Scentry NoMate Fiber treated vs grower standard plots from three walnut orchards, Sacramento Valley, CA.

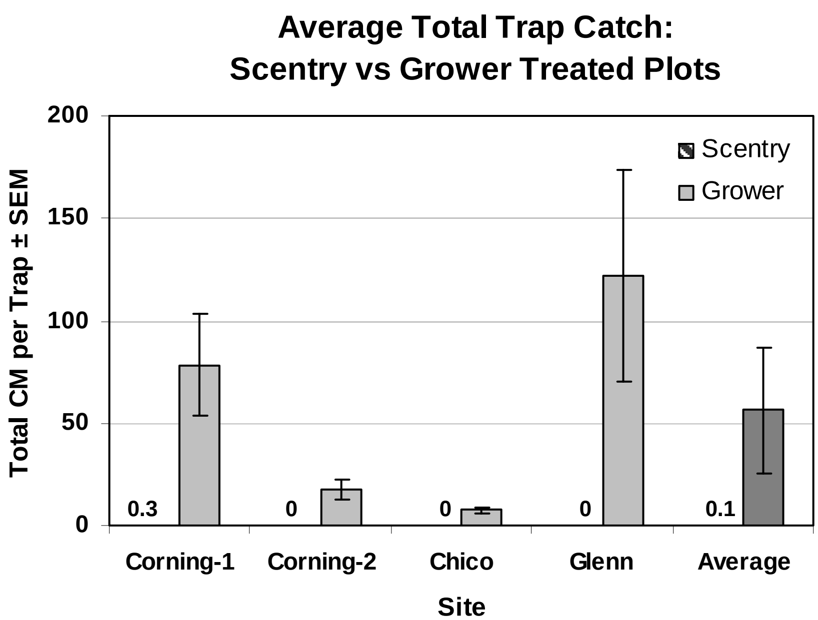

Figure 2. Average season total trap catch for standard codling moth pheromone traps in Scentry treated and grower standard plots. The Corning-2 site reflects data accumulation beginning late June in an expanded treatment area adjacent Corning-1.

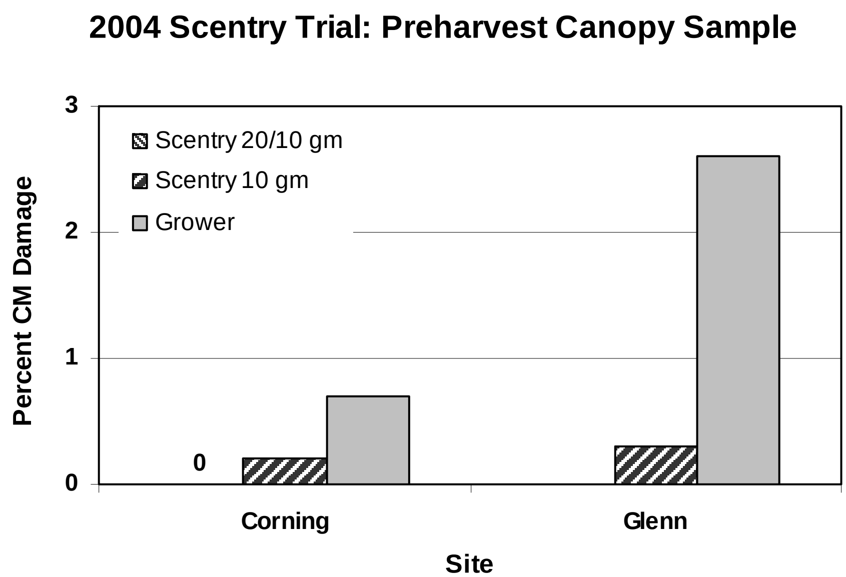

Figure 3. Pre-harvest canopy sample damage in two Scentry treated orchard sites.

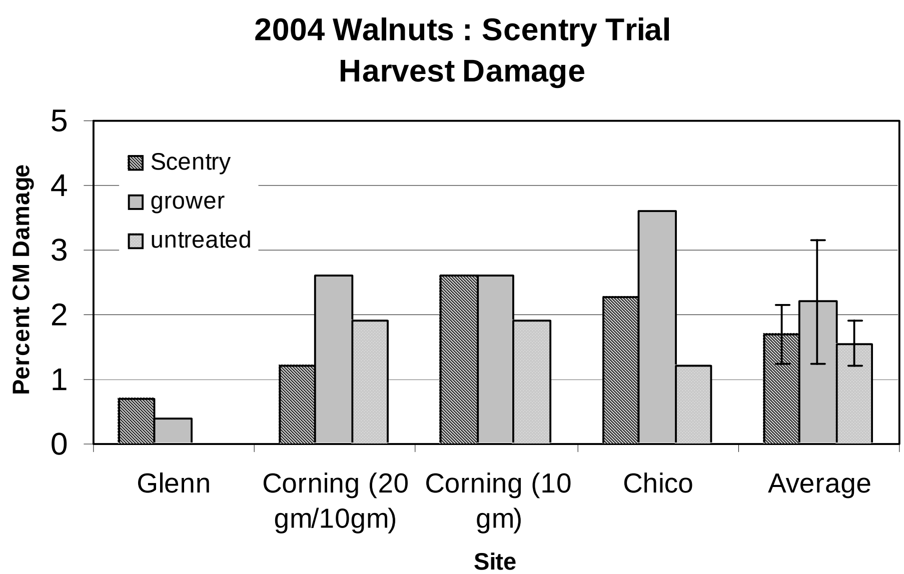

Figure 4. Walnut damage at harvest in Scentry treated, grower standard, and untreated plots. Data indicate in-shell damage by codling moth.

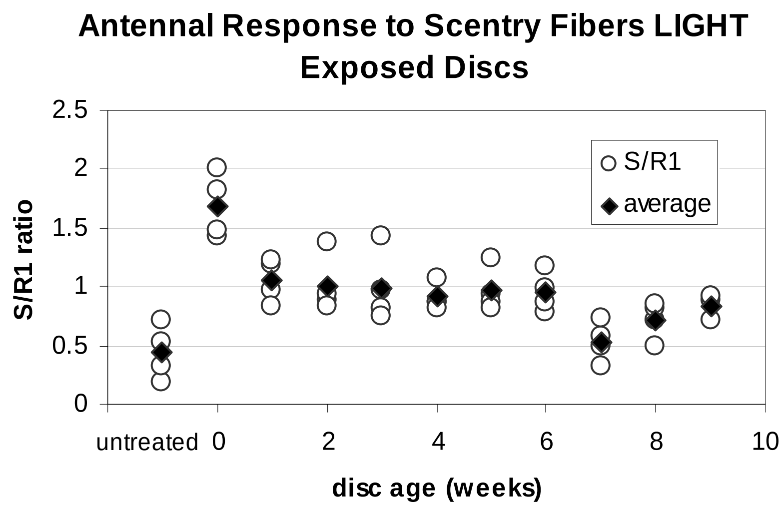

Figure 5. Relative antennal activity over time of codling moth antenna exposed to an air stream passing over sunlight-exposed discs with a Scentry fiber attached. The untreated disc serves as a negative control, whereas week 0 sets the baseline for highest potential emission rates.

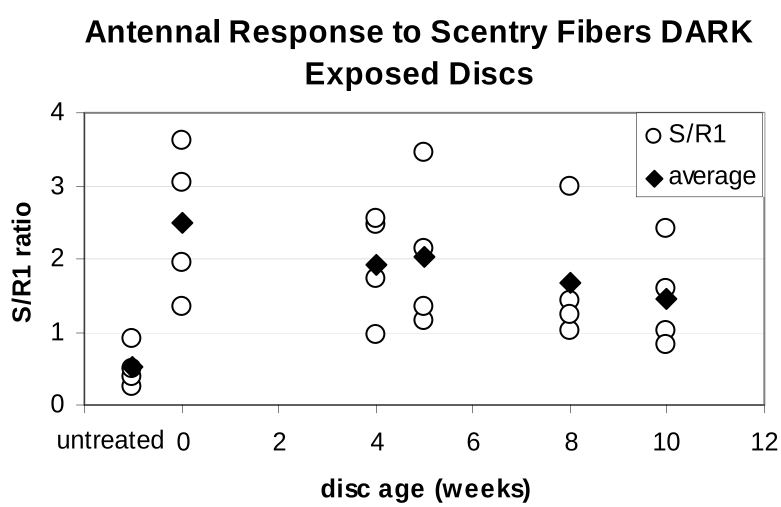

Figure 6. Relative antennal activity over time of codling moth antenna exposed to an air stream passing over shaded discs with a codlemone-filled Scentry fiber attached. The untreated disc serves as a negative control, whereas week 0 sets the baseline for highest potential emission rates.

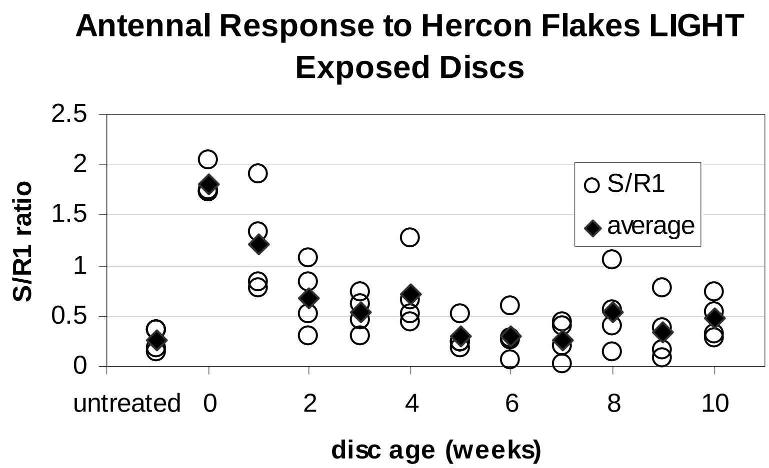

Figure 7. Relative antennal activity over time of codling moth antenna exposed to an air stream passing over sunlight-exposed discs with a codlemone-impregnated Hercon microflake attached. The untreated disc serves as a negative control, whereas week 0 sets the baseline for highest potential emission rates.

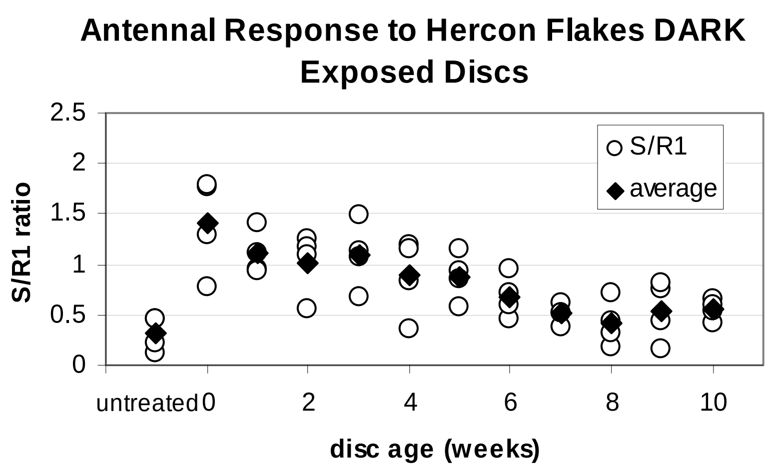

Figure 8. Relative antennal activity over time of codling moth antenna exposed to an air stream passing over shaded discs with a codlemone impregnated Hercon microflake attached. The untreated disc serves as a negative control, whereas week 0 sets the baseline for highest potential emission rates.

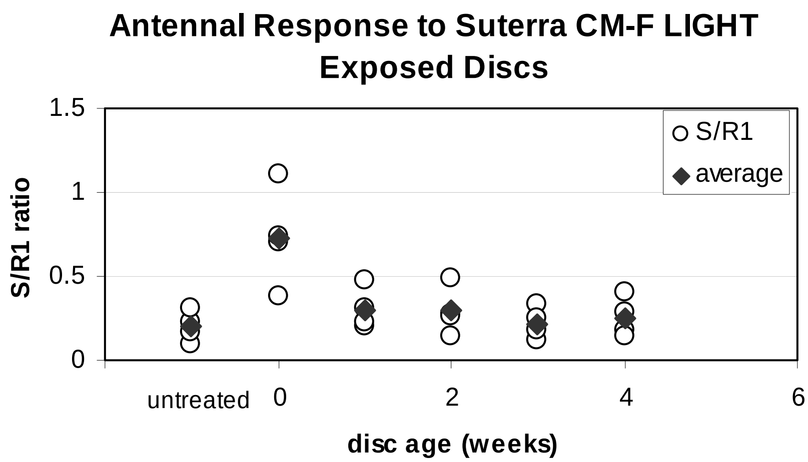

Figure 9. Relative antennal activity over time of codling moth antenna exposed to an air stream passing over sunlight exposed discs treated with Suterra microencapsulated codlemone. The untreated disc serves as a negative control, whereas week 0 sets the baseline for highest potential emission rates.

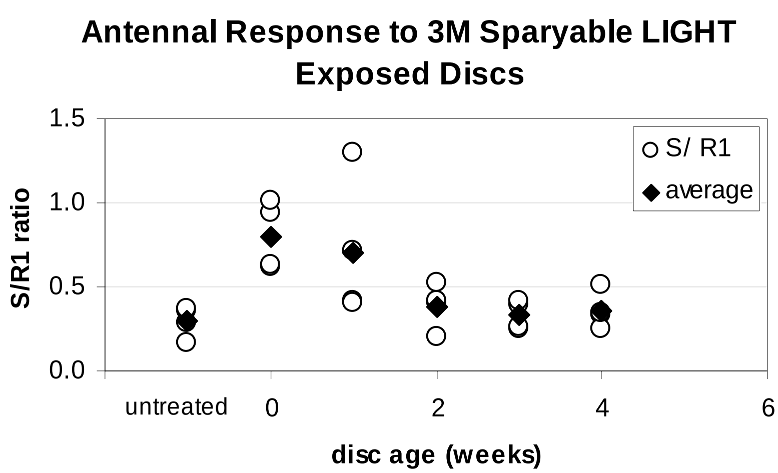

Figure 10. Relative antennal activity over time of codling moth antenna exposed to an air stream passing over sunlight exposed discs treated with 3M microencapsulated codlemone. The untreated disc serves as a negative control, whereas week 0 sets the baseline for highest potential emission rates.

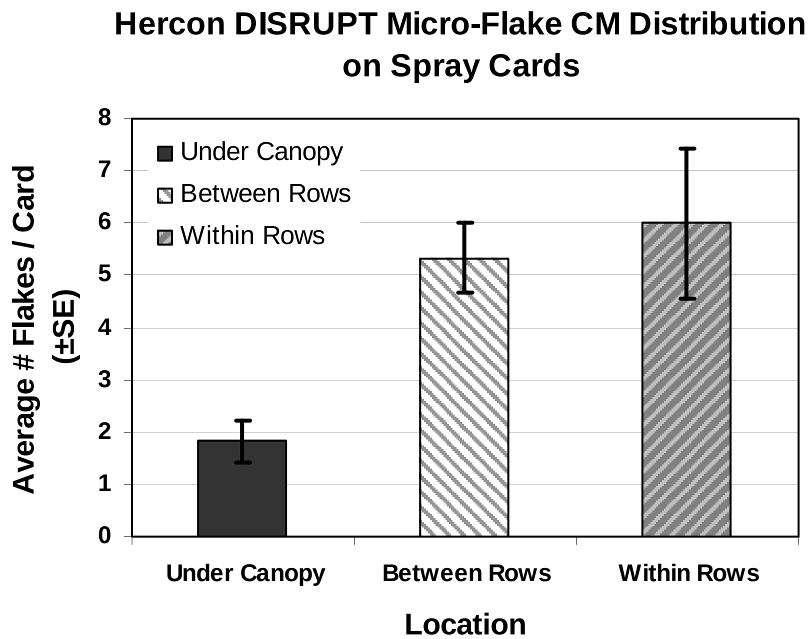

Figure 11. The effect of canopy on the interception of the Hercon flake with pear orchards in Marysville.

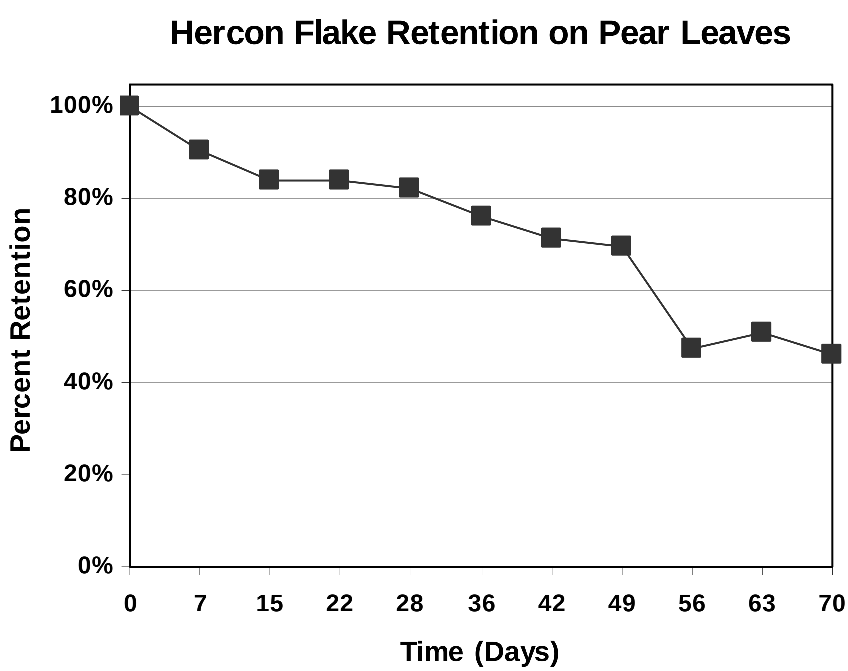

Figure 12. Retention rates of aerially applied Hercon flakes to a pear orchard in Marysville, 2004.