I. WHY PUFFERS WORK: DETERMINING THE EFFECTS OF RESIDUAL RELEASES ON CONTROL OF CODLING MOTH

II. OPTIMIZING "MESO-PHEROMONE" EMITTERS FOR CODLING MOTH MANAGEMENT IN WALNUTS

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ABSTRACT

Our results suggest that the level of pheromone applied per "puff" by the aerosol "puffer" dispenser can be reduced dramatically without any detectable change in the size or shape of the pheromone plume. Reductions by 50% in the active ingredient per puff did not result in any increased trap capture of sterile codling moths downwind of the puffer unit. A substantial, but smaller and narrower, plume was observed when rates fell to 10% of the standard active ingredient (ai) per puff. No clear plume was detectable at 1% of the standard ai per puff. Hence, there appears to be the potential for significant cost savings if lower amounts of pheromone can be used per puff, which leads to lower amounts of pheromone per acre. Within an unreplicated trial, no clear plume was observed when hand-applied dispensers were clustered together into a single location to produce an emission rate comparable to puffer emitting at 50% of the standard emission rate.

Meso emitters provided significant suppression of codling moth traps and damage in all trials, but when pressures from codling moth were high, the ring dispenser applied at rates comparable to 50% of the standard program failed to provide similar levels of control. If codling moth pressures are high, then the number of ring dispensers would need to be increased to higher levels in the orchard. Excellent trap suppression was observed using the Suterra membrane dispensers at 20 per acre, which is roughly comparable to the traditional amounts of pheromone per acre as the standard hand-applied programs.

INTRODUCTION

Pheromone mating disruption has been shown to provide excellent control of low-moderate populations of codling moths using passive hand-applied pheromone dispensers or aerosol based "puffers". While the performance is no longer questioned, issues of overall program costs and labor requirements remain a source of concern. In the past 3 years, our efforts have shifted to overall cost reductions (materials or labor) for both types of delivery devices. Based on earlier research, new hand-applied dispensers were developed which rely on a reduced number of dispensers per acre, and are referred to generically as "meso-dispensers". Two commercial products have been developed; the Suterra meso membrane dispenser and the Pacific Biocontrol "Ring" dispenser. The primary advantage for these products has been the ease of application and reduced labor costs because only 20-40 units are placed per acre.

As outlined below, research on puffers has focused on optimizing the product with potential changes in the timing of release or in the levels of active pheromone required per "puff". The hope for this area of research will be reductions in overall program costs if the levels of pheromone required per acre can be reduced. Therefore, studies to understand how changes in the levels of pheromone per puff influenced plume size and efficacy were the primary targets for 2010.

Puffer Trials: Optimizing Aerosol Based Pheromone Systems

High dose aerosol pheromone emitters, “puffers”, have been used successfully for a number of years for control of codling moth in pears, and are emerging as a leading mating disruption strategy in walnuts. While their success has been documented in large acreages, the exact mechanism by which they operate has not been adequately studied. Understanding how this type of dispenser works may provide opportunities for improving program performance as well as decreasing overall costs. In 2009, a series of studies were initiated which attempted to define the effective area of the pheromone plume from a puffer; understand its impact on female mate location; and enhance our understanding of the effects of direct release of pheromone from the unit as well as secondary release from foliage.

In 2010 we continued on the same line of research by conducting laboratory and field studies related to the results from the previous year. Most of the activity in 2010 focused in continuing the wind tunnel studies of orientation of codling moth males towards pheromone-exposed foliage, and the field comparison of the effect of pheromone release rate by puffers on the plume shape and behavior. Additionally, we ran an assay to test for influence of pheromone puffers on within orchard movement of males, and a preliminary comparison of an aerosol device and a passive one with equivalent pheromone emission rate. In essence, we are attempting to address a fundamental issue that surprisingly remains unresolved for mating disruption as follows: “Does the intermittent delivery of the pheromone as an aerosol change the fundamental properties of the pheromone plume compared to a constant low level diffusion from the hand applied dispensers?” Data from both walnuts and pears are presented for the puffer plume studies since the larger combined datasets from similar types in both crops provides a more robust set of conclusions.

OBJECTIVES

1. To study the attraction of male codling moth males toward pheromone-exposed leaves in the wind tunnel.
2. To compare the shape of the plume of puffers releasing the standard pheromone dose, and others releasing lower doses;
3. To initiate studies comparing the plumes of puffers and that of a passive device with equivalent release rates.
4. To determine the effects of puffers on the within orchard movement of codling moth males.

EXPERIMENT 1. Upwind attraction of codling moth males to pear and walnut leaves from puffer-treated orchards.

Leaves exposed to pheromone in mating disrupted orchards have been reported to adsorb sex pheromones and elicit antennal responses in conspecific males (Karg et al., 1994; Suckling et al., 1996), but no influence on male behavior in wind tunnel has been previously shown. Furthermore, it has been reported that mating disruption may be influenced by the abundance of foliage on the crop (Suckling et al., 1996). What is not clear is how the secondary release of pheromone from the foliage might influence the overall performance of the program and more specifically, is this an important part of how puffers work. The theory is that the foliage may in fact be acting as a passive reservoir for the pheromone such that the number of point sources in a puffer exposed orchard might be dramatically underestimated, given that each leaf might be an effective point source. The first step was to determine if the pheromone was being release from exposed foliage could elicit a behavioral response in male codling moths. A second goal was to determine if foliage re-emissions can be used as a proxy for determining the size and strength of a pheromone plume. In our report in 2009 we showed partial results for attraction to pear leaves and very preliminary ones in attraction to walnut leaves. We herein present the more definitive results that we have obtained.

Material and methods

Pear foliage

In early-September 2009 a puffer loaded with a standard codling moth pheromone canister (Puffer® CM-O, Suterra LLC, Bend, OR, USA) was deployed (12-h mode; 5 pm to 5 am) in a Bartlett pear orchard near Freeport, CA (38º 25’ 59” N, 121º 31’ 24” W). No other pheromone or pest management techniques were in use in this orchard. Three weeks later, foliar samples were taken at increasing distances downwind from the puffer (<1, 7, 35, 55, 75, 100, 115, and 140 m). An extra sampling point in the same orchard at 300 m perpendicular to the main wind direction from the puffer was added as a blank (negative control). Samples consisted of pear cuttings bearing 6 or 7 green leaves were collected around 2.5 m above ground. These samples were taken into the laboratory as soon as possible, where they were stored in ziplock bags in the freezer (-20 ºC) until their use. Additional replicates were used in 2010 from the same sampling period.

Pear cuttings were assayed in a wind tunnel for codling moth male attraction. The cuttings were removed from the freezer, allowed to reach room temperature (ca. 15 min) and then attached to a stainless steel pole, at ca. 25 cm above the floor of the wind tunnel. Batches of 14 to 20 codling moth males (replicate) were flown to each cutting. Males were flown individually, and they were allowed to respond for up to 3 min. However, if they started an oriented flight, they were allowed to finish their response, and then replaced with a fresh male.

Male behaviors were recorded as follows: taking-off (TO), the male left the releasing platform and started flying; oriented (OR), the male finds the plume and starts the oriented flight; F1, oriented flight up to one half of the wind tunnel (around 1 m); F2, oriented flight up to 4/5th of the wind tunnel length (around 1.7 m); close-in (CI), the male approaches the cutting and zigzags closely (a few centimeters away); contact (CN), the male touches the cutting; and walking and wing-fanning (WW), the male lands on the cutting and starts walking and wing-fanning excited. Five replicates per leaf cutting distance (accounting for between 94 and 100 individuals) were conducted.

Proportions of males responding to leaves at the different distances were analyzed by fitting a GLM to each behavior in the sequence. A step-wise model simplification was performed until reaching the minimal significant model. The groups with the smallest difference on effect size were aggregated, and loss of explanatory power was checked. A logistic link function and a quasibinomial error distribution were used. Data analysis was performed with the open-source R statistical data analysis environment (R Development Core Team, 2009).

Walnut foliage

Similarly to the above described for the pear foliage assay, in early-June 2010, a puffer loaded with a standard codling moth pheromone canister (Puffer® CM-O, Suterra LLC, Bend, OR, USA) was deployed (12-h mode; 5 pm to 5 am) in a walnut orchard near Linden, CA (37º 59’ 59” N, 121º 07’ 52” W). No additional pheromone techniques for pest management were in use in the orchard. Approximately three weeks later foliar samples were taken at increasing distances downwind from the puffer (2, 7, 25, 45, 100, 145, 190 and 290 m). An extra sampling point in the same orchard at 600 m perpendicular to the main wind direction from the puffer was added as a blank (negative control). Samples consisted of the three top leaflets of green leaves, collected around 4 m above ground (low canopy). These samples were taken to the laboratory as soon as possible, where they were stored in ziplock bags in the freezer (-20 ºC) until their use.
Samples were assayed in wind tunnel for codling moth male attraction, following the same procedure described for the pear twigs. Four replicates per distance (accounting for between 78 and 80 individuals) were conducted. Data analysis was analogous to the analyses of the data obtained with pear cuttings.

Results

Pear foliage

The responses of codling moth to a pheromone odor are a series of sequential steps that the male must undertake to successfully find a female. The question raised by this experiment was “Will codling moth successfully orient to a leaf exposed to a pheromone plume in the same way that the male would orient to a female emitting her pheromone?”

Using a leaf exposed to the pheromone plume as a lure, a gradient of responses was seen with fewer males finishing the complete sequence in response to foliage collected at greater distances from the puffer. The codling moth males responded by flying upwind to cuttings of all the sampling distances to some extent (Figure 1A). Upwind orientation up to at least 50% of the wind tunnel length was observed in response to foliage collected up to 115 meters away from the puffer (Figure 1B). Thus, these indirect effects from the pheromone plume can cause male orientation to foliage collected at lengths in excess of a football field. The most complete responses were recorded to cuttings from the closest points (<1 and 7 m), achieving a 12.5 and 11.22 % contact rate, respectively. Fewer complete flights occurred with cuttings from 55, 100 and 140 m downwind (Figure 1D). Responses to the control cuttings tended to be the lowest of all sampling points for all the behavioral steps with the exemption of taking-off. Similarly, insects flown to negative controls never approached them closely, whereas some did to samples from most of the other positions. Male responses showed an important degree of variability. However, a tendency to decreasing response levels with increasing distances can be seen.

In many cases males showed problems to orient upwind. They often changed directions while orienting, behavior that is typical of orientation towards suboptimal pheromone lures (e.g. Willis and Baker, 1988). Nonetheless, our data shows that the amount of pheromone released from pear cuttings exposed to a puffer at several distances is enough to elicit a behavioral response in codling moth males. The exact effects of this finding under field conditions, where the amount of leaves is extremely large, are difficult to predict and could include phenomena of both false-trail-following and camouflage.

Walnut foliage

Data obtained with walnut samples widely agreed to that of pear cuttings. Codling moth males also oriented to a greater extent to walnut leaflets in the pheromone plume at any distance than to control leaflets (Figure 2A-D). However, contacts were only reached with samples from the closest of the distances (2 m). Close-in approaches were observed to sampling distances ranging from 2 to 145 m, whereas they were not for the distances of 190 and 290, and the control leaflets. Responses to the control leaflets were the lower of all sampling points for all the behavioral steps recorded. Male responses again showed a high degree of variability, and as had been seen for the pear cuttings, in many cases males showed problems to orient upwind.

EXPERIMENT 2. Effect of rate of pheromone emission by puffers on their area of influence, and initial comparison of puffers and passive devices.

The pheromone load of the commercial aerosol canisters was originally set empirically. It was selected to match the release amount of the most widely applied mating disruption device for codling moth, which is the hand-applied polyethylene rope of Shin-Etsu Inc. at a rate of 400 ac^{-1}. Taking into account that puffers are deployed at a rate of 1 per acre, and that their area of influence widely exceeds one acre, the system may be redundant in the amount of pheromone used. We tried to determine the impact in the area of influence of the puffer (from the point of view of trap suppression) of reducing pheromone load. Initially this experiment was carried out with wild populations of codling moth. However, meaningful results require high and homogeneous population densities that rarely occur in orchards. For that reason we also incorporated releases of sterile moths later in the season.

Materials and methods

Fields

Rate emission assays were conducted in 2010 in three different orchards, namely Big Valley, Scotts Valley, and Podesta. Big Valley was an abandoned pear orchard close to Finley, CA (38º 59’ 53” N, 122º 52’ 18” W) with a surface of approximately 9.5 ac. Scotts Valley was a commercial pear orchard close to Lakeport, CA (39º 04’ 27” N, 122º 56’ 45” W). It was ca. 27 ac in size, and no pheromone based pest control techniques were carried out. Finally, Podesta was a commercial walnut orchard close to

Linden, CA (37º 59' 49" N, 121º 07' 47" W) with a surface of approximately 80 ac, and no pheromone-based control techniques were used by the grower.

Traps

We used orange delta traps (LPD, Suterra LLC) baited with pheromone lures (CM 1X, 1 mg, Suterra LLC) in our field assays. Rather uniform grids of traps were set across the whole surface of the three orchards. A total of 30 traps at Big Valley, 100 at Scotts Valley, and 158 at Podesta were deployed. In the pear orchards traps were hung between 2 and 2.5 m above ground, whereas in the walnut orchard, they were at a height around 4.5 m (low canopy).

Traps were checked twice per week all through the growing season. Their positions were geo-referenced, and interpolation surfaces of captures were created, using the open-source R statistical data analysis environment (R Development Core Team, 2009).

Wind conditions

Predominant wind direction during puffer operational time (5 pm to 5 am) was determined in the three orchards. In Podesta a wind station provided with data logger was installed, and wind direction and wind speed measurements were recorded at 15 min intervals through the whole season. In Scotts Valley, hourly wind direction and speed were available from a weather station kept by the owner in this orchard. Finally, in the case of Big Valley, data from a nearby weather station (ca. 3 km east) had to be used. Wind data for Scotts Valley and Big Valley were obtained online from Western Weather Group (www.westernwx.com/lakeco/).

Puffers and canisters

The puffer cabinets used in the assay were the standard commercial units (Suterra LLC). Volume of aerosol released per puff (40 µl) and puff frequency (1 every 15 minute) were kept constant for all puffers used in the assay. The different rates of pheromone application were achieved by varying the pheromone load of the aerosol canisters. The commercial canisters for codling moth contain 69.33 g of active ingredient. Suterra LLC produced canisters loaded with the full load of active ingredient (a.i) (100 %), as well as cans with increasing dilute formulations of only 50, 10 and 1 % of the full a.i. These amounts correspond to releases of 7.22, 3.61, 0.72, and 0.07 mg per puff.

Due to the large size of the puffer plumes, the capacity to combine different puffers in the same orchard without interaction is very limited. For this reason puffer rates used through the assay rotated through time to accommodate a limited amount of space. Puffers were placed close to the upwind end of the different orchards to allow the study of their plume for a long distance. When puffers were rotated, the new one was deployed 3 to 4 days after the withdrawal of the previous one to avoid the influence of residual effects across treatments. In the pear orchards, puffers were hung at approx. 3-3.5 m above ground, whereas in the walnut orchard, they were at ca. 6 m.

Sterile insect releases

As an effort to increase and homogenize insect density in the orchards, four sterile insect releases were performed. Sterile codling moths were purchased from the Okanagan-Kootenay Sterile Insect Facility (Osoyoos, BC, Canada). Insects were packed in Petri dishes containing ca. 800 individuals each (50:50 sex ratio) inside cold storage and received the day after leaving the facility. Upon arrival they were taken to the fields for their release. Releases were carried out on August 12th, 19th, and 27th, and September 3rd.

A release point was set 7 to 10 m downwind from each trap. The release points consisted of paper bags (cut at a height of 3 inches) attached to the trunk of trees by two staples. Petri dishes (still cold) were opened and insects were poured into the bags.

Sterile insects had been reared on artificial diet containing a colorant that turns the hemolymph pinkish. This allowed for discrimination between wild and sterile individuals during trap readings.

Results

Wind direction

Wind conditions are summarized in Figure 3. In Scotts Valley wind blowing from the west was clearly predominant. At Podesta most of the wind was from Southwest and West-Southwest components. Finally, at Big Valley predominant wind direction was less clear, but winds coming both from South and West were most common.

Wild insects

Despite our efforts to find orchards with high codling moth densities, only Big Valley produced enough wild males to obtain meaningful results. At Scotts Valley, codling moth density was very low across the whole orchard, with weekly captures averaging 0.39 ± 0.04 males/trap from May 1st to August 6th. Lastly, in Podesta density was higher than in Scotts Valley, but it was mainly due to high densities at the border of the orchard, especially along the western edge. From May 9th to May 25th (before any puffer was placed in the orchard) weekly captures in Podesta averaged 4.00 ± 0.66 and 1.20 ± 0.09 males/trap for outer and inner traps, respectively. Hence, wild insect data from Podesta could be used, but interpreted with extreme care.

Effect of Pheromone Concentration per puff

A summary of all pheromone plume trials is shown in Table 1. The density of insects at Big Valley was very high. From May 1st to May 11th, the orchard was pheromone-free and weekly captures averaged 36.56 ± 3.39 males/trap. Male density was higher at the northeastern corner (downwind edge), but captures were really high all over the field (Figure 4). All four pheromone rates were tested in random order in this orchard: 1%, from July 26th to August 3rd; 10%, from June 7th to June 14th; 50%, from July 13th to 20th; and 100%, between June 28th and July 6th. Results are shown in Figure 5. The 1% rate had little to no-impact on the captures. All traps around the puffer were able to attract males and there was not a clear plume. Some of the traps at the east limit of the orchard did not catch during this trial, but this is most likely due to the distribution of the individuals in the field, or the influence of the neighboring orchard, than to any effect of the puffer.

For the 10% rate a plume can already be seen from the puffer position towards the east. However this plume is much narrower than those for the 50 and 100% rates. For the two higher rates, captures were strongly suppressed in a large portion of the orchards, to the east and north, following the directions of the predominant winds (Figure 3).

In Podesta, from July 8th to August 7th, three puffers at rates 10, 50 and 100 % were placed next to the upwind edge of the orchard. Results obtained are similar to those from Big Valley, but as mentioned above we need to be cautious when interpreting them (Figure 6). The three rates within a single orchard appeared to affect trap performance with the edges appearing to overlap. Plumes are apparent for all of them, and captures upwind from the puffers (more west) are much higher than those downwind. Nonetheless, we have to keep in mind the important border effect found at the beginning of the season. Trap suppression downwind from the 100% rate puffer was total up to the end of the orchard, whereas in the cases of 50 and 10% some captures occurred around 200 m downwind from the puffer. Furthermore, traps near the 50% canister puffer unit did not capture any males, whereas some captures occurred around the 10% puffer (Figure 6).

Despite the large size of the Podesta orchard (80 ac), interactions among puffer plumes seem to have occurred (Figure 6). This supports the large area of effect for puffers, even with pheromone loads lower than the standard commercial units. This also highlights that ideally, only one puffer should be in the orchard at a time to do this kind of assay. However, time and orchard availability, and labor needs represent large limitations for such an approach.

Sterile insects

The use of sterile insects helped to achieve clearer and more reliable results. In general, most of the insect releases were successful. Insects were healthy, and they were recaptured in high numbers at Podesta and Big Valley. However, for unknown reasons the releases performed at Scotts Valley did not work despite using the same methodology as for the other two sites. Only in the release on August 27th was the recapture rate slightly satisfactory, but due to the failure of the other dates no comparisons are possible. In that release the puffer (100 % rate) was close to the western end of the orchard and trap captures were nothing in a wide and long area towards the east and the southeast, with captures increasing towards the northeast.

At Big Valley three of the releases were successful. In the fourth release, few insects were recovered. This orchard was abandoned, and there was no irrigation through the entire summer. By the date of the last release the trees did not have much foliage left. This probably greatly affected the performance of the sterile insects. Each of the other three releases was performed with a single puffer in the field in the same position as previously shown for the wild insects. The pheromone rates used were 1, 10, and 50%.

Results for the sterile insect releases at Big Valley are shown in Figure 7. With the 1% rate in the field, total suppression was recorded in a few positions towards the northeast of the puffer. A plume can be estimated, but captures still occurred in most of the orchard. For the 10% rate, the effects were stronger, and trap suppression happened in most of the area towards the northeast. On the other hand no influence took place towards the south. Lastly, the 50% rate puffer totally suppressed captures in most of the eastern part of the orchard, and a gradient of effect is very apparent towards both the north and the south directions (Figure 7).

All four sterile insect releases at Podesta were successful. In the first release, three puffers were placed in the orchard loaded each with one of 10, 50, and 100% rate canisters. A clear plume was observed for all three rates. However, trap suppression was more intense in the plumes of 50 and 100%, as the size of their isolines for 0.5 captures/day show (Figure 8). Despite Podesta being ca. 80 acre; the area of effect of the puffers was such that the plumes touched each other. Hence, we decided to reduce the number of puffer positions to two for subsequent releases.

For the second release, one 50 and one 100 % rate puffers were used. Again both puffers showed a clear plume of great dimensions (Figure 9). However, some differences can be seen. On the one hand, captures were not recorded at all inside the 100 % plume, and the center of the plume was “clean” until the downwind limit of the orchard. Alternatively, some captures were recorded inside the 50 % plume, “breaking” the continuity of the “clean” area.

Similar to the second release, for the third release, a 10 % rate puffer was compared to a 100 % rate one. Once more, both puffers showed a clear plume (Figure 10). Also similar to the difference between 50 and 100 %, the plume of the 10% puffer was narrower, and shorter than that of the 100% puffer. In the case of the 10% canister plume, the end of the plume was indeed very clear after ca. 200 m.

Active Aerosol Emitters versus Passive Hand-applied Devices at Equivalent Release Rates

Finally, in the last release of sterile insects at Podesta we made the first comparison between puffers and a passive pheromone emission device. The plume from a puffer emitting 50% of the normal full rate was compared to the plume from 10 units of Isomate®-CM Ring (Pacific Biocontrol Corp., Vancouver, WA, USA) tied together as a single point emitter. The 10 rings have a combined daily emission comparable to one half of a standard puffer (the 50% rate in our study). The differences in the results obtained by the two different types of device were surprising (Figure 11). The 50% rate puffer strongly depressed captures downwind, and trap suppression was 100% within much of the plume. On the contrary, the 10 rings emitting a comparable level of pheromone per day also reduced captures downwind, but this effect was much weaker and more localized to a small patch near the rings.

As only one replicate of this comparison was made, we must be very cautious interpreting the results. Nonetheless, if these results are repeated in the future, then they would strongly suggest that delivering the pheromone as an aerosol or by diffusion deeply influences the behavior of the pheromone in the environment and the way that it interacts with the insects.

EXPERIMENT 3. Influence of a puffer on the within orchard movement of codling moth males.

The effects of high amounts of sex pheromone (from puffers or others) on codling moth movement are not very well known. We thought that moths may orient to the pheromone for a portion of the plume, with some moths showing arrested movement as the source is approached. This type of “super-female” effect has been described in movement from untreated to pheromone-permeated orchards (Witzgall et al. 1996; 1999). To track adult movement in the orchard we adapted an immunomarking technique developed by Jones and co-workers (2006). This technique basically consists of spraying a limited area of the experimental field with egg protein (or other alternative proteins), and testing captured insects for presence of egg protein by indirect ELISA. Moths that score positive for protein marking are then known to have either originated from or at least visited the treated area of the orchards.

PROCEDURES

Pasteurized and homogenized egg whites (Michael Foods Inc., Minnetonka, MN, USA) were mixed with water to form a slurry with 10% egg content. The slurry was then sprayed into the canopy ensuring an appropriate coverage in a volume of ca. 250 gal/ac. Egg sprays were done early in the morning, when wind is weak to avoid drift as much as possible.

The study was conducted in an unfarmed pear orchard of approximately 17 ac near Freeport, CA (38º 25’ 59” N, 121º 31’ 24” W). A total of 45 pheromone-baited delta traps (LPD, Suterra LLC) were distributed through the orchard following a fairly regular pattern. They were hung at 2 to 2.5 m above ground.

Two egg protein sprays were conducted on June 15th and August 11th, 2010, respectively. The surface sprayed was approx. 0.5 ac (2.9 % of the total area) in the center of the orchard. Following each application, trap liners of all traps were replaced weekly for 5 weeks. Liners were removed, individually isolated with plastic wrap, and brought to the laboratory for processing.

For the first collection date, no puffer or other mating disruption devices were used in the field, and data represent codling moth male movement under natural conditions. At the second week, a puffer loaded with Puffer® CM-O (Suterra LLC, Bend, OR) was placed approximately 4.5 m above ground at the southwest corner of the orchard (upwind end). During the second spray, an extra lure of pear ester and sex pheromone (Pherocon® CM-DA Combo™, Trace Inc., Adair, OK) was added to the 20 traps in an effort to increase captures in the puffer plume. Wind direction and speed were recorded every 15 min from May 8th to September 29th by a wind station.

Once in the laboratory, liners were removed one-by-one from the freezer, and then gradually unwrapped as insects were removed from the glue and transferred into 1.5 ml vials (USA Scientific Inc., Orlando, FL, USA) with the help of toothpicks. Special effort was made to try to remove the entire insect from the glue, and never touch more than one individual with the same toothpick. Once in the vials, they were rinsed for 3 minutes in 1 ml of TBS (Tris-Buffered Sline, ph 8.0, Sigma-Aldrich, St. Louis, MO, USA), containing 0.3 g/l sodium (tetra) ethylenediamine tetra acetate (EDTA, Sigma-

Aldrich). After the rinse, moths were removed and discarded; and the vials were frozen until the ELISA analyses.

Indirect ELISA analyses were conducted by following slightly modified procedures from Jones et al. (2006). In each ELISA plate, six wells were occupied by negative controls consisting of rinses of laboratory reared codling moth males that had not been exposed to egg. After the ELISA analyses, the optical density (OD) between 450 and 490 nm of the wells in the plate was read and recorded using an Emax Endpoint Microplate Reader (Molecular Devices Inc., Sunnyvale, CA, USA).

Positive determination was made following Sivakoff et al. (2010). This methodology uses the values of OD for negative controls and their standard deviation to define an OD-threshold. Samples with OD larger than the threshold are considered positive, otherwise they are negative. The methodology proposed by Sivakoff et al. (2010) largely emphasizes the reduction of false positives, and it is very conservative in the sense that accepting as positive an actual negative is much more difficult than the other way around.

RESULTS

The predominant wind in the orchard was from the southwest. Nonetheless winds also blew often from south and west (Figure 2). A total of 4,889 and 867 males were captured after the first and second sprays, respectively. Out of these, 3,272 and 838 have been respectively analyzed. The number of captures scored as positives was 88 (2.7 %) and 9 (1.1 %) for the first and the second sprays, respectively.

In the absence of the puffer we captured a high number of individuals all over the orchard (Figure 12-A), and most of the marked insects were captured inside or close by the treated area. Some positives were found far from the sprayed patch, but they were scarce (Figure 12-B). This suggests that in natural conditions males tend to be sedentary with a small proportion of them making longer flights.

Unfortunately, in the replicate with the puffer emitting in the field the addition of the combo lures did not help to increase our captures. Hence, in most of the center of the orchard we failed at capturing insects (Figure 13-C). This issue prevented us from obtaining meaningful results in movement of males exposed to puffers. Other approaches are needed to capture males in numbers high enough to conduct the protein marking tests under mating disruption, such us the use of light traps.

Overview

During these two years, the different assays conducted have helped to improve the knowledge about aerosol puffers and their use in mating disruption. It is clear that foliage plays an important role in the performance of the technique. We have shown that the amount of pheromone re-released by pear and walnut foliage is enough to elicit behavioral responses in codling moth males. Our wind tunnel data showed that upwind attraction to foliage is greater on leaves exposed to pheromone than in control leaves. Nonetheless, the real impact of this secondary release on the behavior of the males is difficult to predict, and will need further research using different approaches.

Our data also suggests that there is room for economic optimization of the aerosol puffers. Little difference was observed between standard puffers, and puffers loaded with a 50% rate of pheromone. However, more replicates need to be conducted, and assays including damage on fruit at harvest will be needed to avoid the exposure of growers to unwarranted risk. Nonetheless, our data strongly supports that reductions in the pheromone content of the canisters are possible.

The comparison between passive and aerosol devices suggested deep differences in the behavior of the pheromone under field conditions depending on the way that it is dispensed. We are aware that our data on this topic very limited, but it is nonetheless an interesting field that will require further studies. Furthermore, this differences in the behavior of the pheromone may explain why puffers have such a large area of effect, whereas in passive devices the number of release points is perhaps more critical.

Finally, our movement study was only a partial success. We succeeded at showing male movement pattern under natural conditions. On the other hand, we failed at capturing enough insects with a puffer in the field and this prevented us from finding a movement pattern.

MESO EMITTERS: SUTERRA MEMBRANE EMITTERS AND PACIFIC BIOCONTROL RING

The development of meso emitters has been on-going for 4-5 years with the culmination of a new registration for the Pacific Biocontrol and with federal registration of the Suterra meso completed with California's registration pending. The primary advantage of the meso emitters will be their ease of application given that only 20-40 dispensers per acre are used. The two products are emitting at higher rates of pheromone per dispenser such that the Suterra Meso emitter at 20 units per acre and the Pacific Biocontrol Ring at 40 units per acre are roughly equivalent to traditional hand-applied dispensers at 160-200 ties per acre. The speed of application of 20-40 units is improved dramatically in pear orchards and becomes a more reasonable alternative for walnut growers.

In essence, the question being addressed with these studies is "As we move to larger plots with higher codling moth pressures, will we continue to see comparable performance between the meso and standard pheromone treatments". One fact to note though is that the ring treatments made at 20 units per acre have only 50% of the pheromone per acre compared to the standard pheromone programs. Thus, both the number of point sources and the amount of pheromone were reduced in the Walnut Grove and Upper Lake orchards.

OBJECTIVES

1. Evaluation of the meso-style emitters in field plots for suppression of codling moth damage.

PROCEDURES

Large plot field trials of "meso-emitters" for codling moth control. In 2010 we continued our efforts to test the reliability of the reduced point source pheromone applications for codling moth management with additional replication across years, sites, and varied codling moth pressures. Products used were the Suterra Meso (Suterra LLC, Bend, OR 97702) and the Isomate Ring (Pacific Biocontrol Corporation, Vancouver, WA).

Traditional mating disruption programs for codling moth have assumed that higher numbers of dispensers are needed for a successful program. The meso-emitter trials challenge this idea by reducing the number of pheromone release points to rates as low as 18 to 20 units per acre. The rate of pheromone emission per dispenser is increased to offset the fewer units per acre. The dispenser products are 1) a large membrane dispenser referred to as the Suterra Meso in this report utilizes the membrane the technology of their CM-XL1000 dispenser, and 2) the Isomate Ring consisting of uncut five-unit lengths of the Isomate-C TT dispensers that form a single large circular unit.

Efficacy trials for the meso-emitters were evaluated in replicated plots of three Bartlett pear orchards located in the Sacramento River delta region and Lake County (Table 2). The Suterra Meso was used in one site (Lakeport) and the Isomate Ring was used in two sites (Upper Lake and Walnut grove). Isomate®-C TT was applied at a rate of 200 dispensers per acre as a standard pheromone comparison at all three sites. Both Lake County sites were unmanaged in 2010, and thus provided untreated controls. The Walnut Grove orchard was the only pear site to receive supplemental insecticide treatments (Table 3). Insecticide treatments were applied across all pheromone treatments at the discretion of the grower and the only contrast was between the ring and standard pheromone application as the entire site was under mating disruption. All pheromone applications were completed March 25 in Walnut Grove and April 8-16, 2010 in Lake County.

The Suterra meso was deployed at 18 units per acre and the Isomate Ring at 20 units per acre. Deployment of the Isomate ring product at the rate of 20 per acre is equivalent to a 100 Isomate-C TT per acre application which is half the full standard rate. Dispensers were distributed in a uniform grid pattern through treatment plots of five to twenty-three acres. All emitters were placed in the upper third of the tree canopy in pears. Codling moth flight activity was monitored with large plastic delta traps (Suterra) for five acre treatment and control plots or up to 12 traps for larger 20 acre plots. Traps were baited with 1X Biolures (Suterra) or Pherocon® CM-DA COMBO™ lures (Trécé, Inc., Adair, OK 74330). Traps baited with 1x lures were hung low, and with combo lures high in the canopy. Traps were read weekly and lures changed on the recommended schedule. Damage assessments were made at harvest time or prior to pick.

RESULTS AND DISCUSSION

Efficacy trials using modified dispensers for codling moth control.

Pears: codling moth flight curves.

Similar to our results in the past, the meso treatments are somewhat less effective in suppressing traps as the standard pheromone programs (Figure 13). Moth flights were substantial in all orchards with the grower standards averaging between 200 and 300 moths for the season in the grower standards in Lakeport and Upper Lake, and greater than 700 moths in the Walnut Grove grower standard. As such, the pressures were higher than normally would be challenged with a pheromone program, especially as a stand-alone program. Using the 1X lures, the meso programs suppressed codling moth counts compared to the grower standard in the Lakeport and Upper Lake orchards by 90 and 95%, respectively. The codling moth counts in traps baited with the 1X lures in the standard pheromone programs were suppressed by 98 and 100%.

The seasonal flight curves for the Suterra Meso treatments in Lakeport are compared to the flights in the standard pheromone and untreated control. The flights were highly suppressed during entire season (Figures 14 and 15) for the 1X baited traps. In contrast, strong flights were observed in all plots using the combination lures.

In contrast, the ring dispensers in the Walnut Grove site failed to suppress the moths effectively during the growing season with consistent catches during the growing season (Figure 16). However, we also noted the standard pheromone program also had sporadic captures during the growing season with repeated catches in July. The results from the combination lures are shown in Figure 17. The overall counts were very high with peak flights up to almost 100 moths per trap per week in the combination lure baited traps. Given that the rings at 20 units per acre only emit 50% of the standard pheromone program and the high pressure levels, it is not all that surprising to see more limited trap suppression.

For the Upper Lake site, the rings looked better overall with minor catches periodically during the growing season (Figures 18 and 19) in the 1X lures and some strong flights in the combination lures. While the combination lures had a peak in the grower standard of 35 moths per trap per week, the overall counts were not as high as expected for an untreated orchard. However, one concern was that the lack of any trap capture in the 1X lures in the standard CTT treated plots did not predict the damage observed at harvest (see Figure 20).

***Pears: fruit damage in efficacy trials due to codling moth*.**

The effects on codling moth damage are seen in Figure 20 for the Upper Lake and Walnut Grove sites. No results are shown for the Lakeport site because the number of fruit per acre was too low to achieve meaningful results. The site has been under low input management for a while and the lack of inputs such as water severely limited the yield.

The results from Upper Lake showed very high damage levels in the untreated control at ca. 22% compared to the Isomate ring at 12% and the standard pheromone program at ca. 7.5% damage. Thus both pheromone treatments appear to provide suppression of the moth, but neither program gave commercially acceptable levels of control as a stand-alone program. Again, it is no surprise that pheromone mating disruption alone would not provide sufficient control given the pressure in this orchard. Given the pressure, the higher number of rings at 40 per acre would be a more likely choice for this level of pressure, because it result in the same amount of pheromone per acre as the standard pheromone program.

A similar pattern was observed for the Walnut Grove site where the lower number of rings per acre and the lower amount of pheromone per acre failed to provide suppression comparable to the standard pheromone program. Given that the combination lures showed a seasonal total of >600 moths in both treatments, we would not have expected these programs to provide commercially acceptable control as stand-alone programs. What was surprising was this orchard also received 4 insecticide applications over the entire orchard during the growing season and even in combination with the standard pheromone program, codling moth damage remained high.

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Table 1. Pheromone Plume Studies – Summary.

Site	Crop	Dates	No Puffer[$]	Puffer Release Rates 1 %	10 %	50 %	100 %	Rings[§]	Codling Moth Wild	Sterile
Big Valley	pear	May 1 to 11	X						X	
		May 13 to 24				X[#]			X	
		June 7 to 23			X				X	
		June 28 to July 6					X		X	
		July 13 to 20				X			X	
		July 26 to Aug. 5		X					X	
		Aug. 13 to 17		X					X	X
		Aug. 20 to 24			X				X	X
		Aug. 27 to Sep. 1				X			X	X
		Sep. 3 to 8		X					X	X
		Sep. 8 to 15					X		X	X[+]
Scotts Valley	pear	May 1 to Aug. 3	X						X	
		Aug. 13 to 17			X				X	X
		Aug. 20 to 24				X			X	X
		Aug. 27 to Sep. 1					X		X	X
		Sep. 3 to 8			X				X	X
		Sep. 8 to 15			X				X	
Podesta	walnut	May 14 to June 1	X						X	
		June 4 to 25		X	X	X	X		X	
		July 2 to Aug. 7			X	X	X		X	
		Aug. 12 to 16			X	X	X		X	X
		Aug. 19 to 23				X	X		X	X
		Aug. 27 to 31			X		X		X	X
		Sep. 3 to 10				X		X	X	X
		Sep. 10 to 18				X		X	X	

[$] No puffer in the field.
[§] Ten Isomate Rings were tied together as a single point lure.
[#] The puffer was placed in an unsuitable position.
[+] No specific sterile insect release was performed, but a few remained from the previous one.

Table 2. Summary of 2010 codling moth pheromone field trials.

	Crop	Site	Treatment Plots (number of acres) Meso	Ring	Pheromone Std	Conventional Grower Std
Efficacy Trials	Pears	Walnut Grove		1 (23)	1(5)	na
		Lakeport	1 (8.4)		1 (2.8)	1 (Untreated Control) (5.6)
		Upper Lake		1 (5.1)	1 (4.4)	1 (Untreated Control) (4.2)
	Total number of plots = **8 (58.5 ac)**		**1 (8.4)**	**2 (28.1)**	**3 (12.2)**	**2 (9.8)**

- Number of acres in parentheses for each plot.

Table 3. Pear site efficacy trial locations and grower applied insecticide treatments.

Site	Grower Standard Treatments
Walnut Grove	Altacor 4.5 oz/ac (5/12/10) Altacor 4.5 oz/ac (6/3/10) Delegate 5 oz/ac +2 qt oil ((7/2/10) Delegate 6 oz/ac + 1 qt oil (pre-harvest)
Upper Lake	No sprays / Unmanaged site
Lakeport	No sprays / Unmanaged site

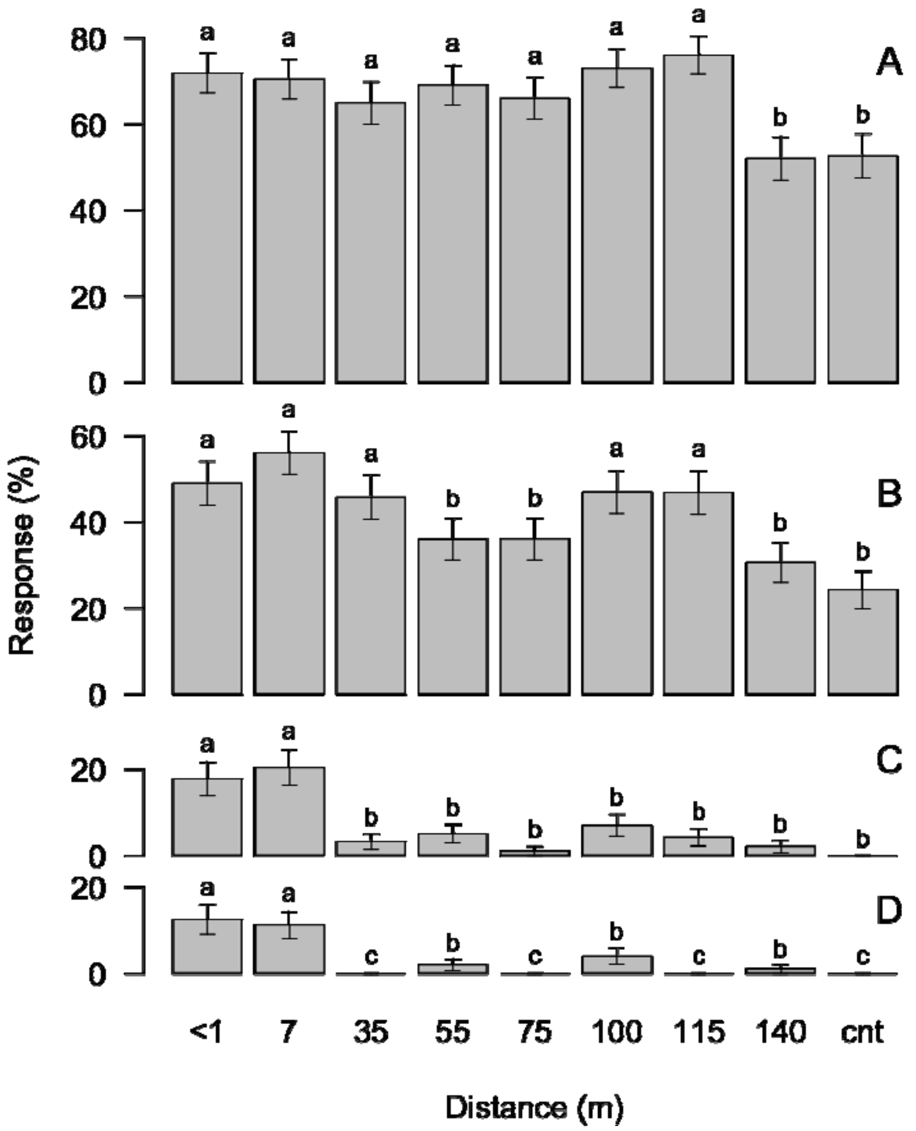

Figure 1. Average behavioral responses (± SE) of males of codling moth to pear cuttings, collected at different distances from a puffer. Distance groups with different letters within behaviors differed significantly. Responses shown are *A*, beginning of oriented flight; *B*, oriented (sustained) flight up to more than one half of the wind tunnel; *C*, close-in approach to the cutting; and *D*, contact with the cutting. N=5; 94 to 100 males.

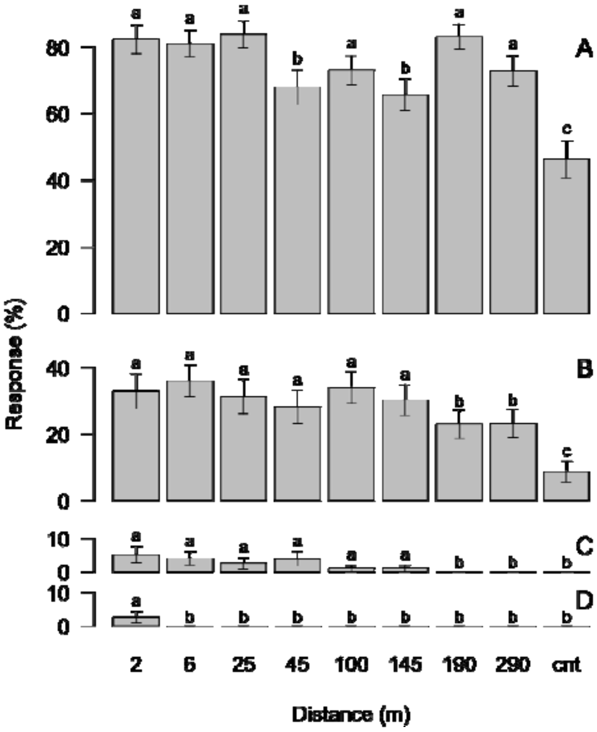

Figure 2. Average behavioral responses (± SE) of males of codling moth to walnut cuttings, collected at different distances from a puffer. Distance groups with different letters within behaviors differed significantly. Responses shown are *A*, beginning of oriented flight; *B*, oriented (sustained) flight up to more than one half of the wind tunnel; *C*, close-in approach to the leaflets; and *D*, contact with the leaflets. N=4; 78 to 80 males.

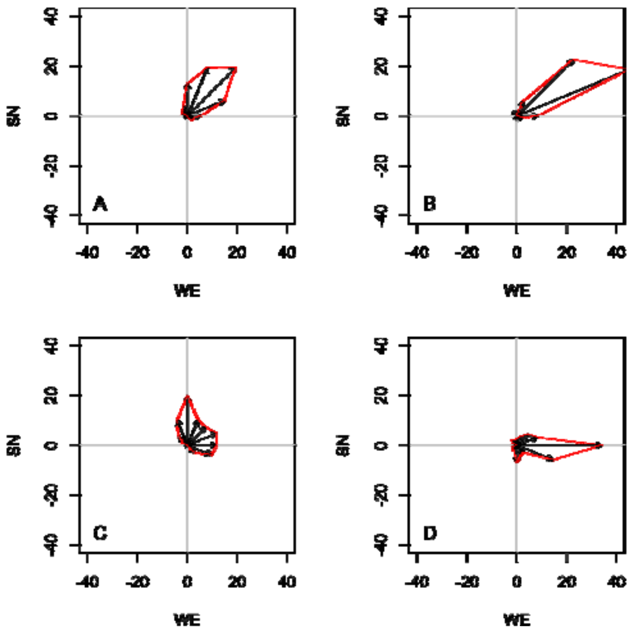

Figure 3. Wind direction and intensity at the four experimental fields in 2010: A, Freeport; B, Podesta; C, Big Valley; and D, Scotts Valley. Arrow size is proportional to the sum of speeds recorded for a given direction. Hence it depends on both, number of readings and speed of the wind.

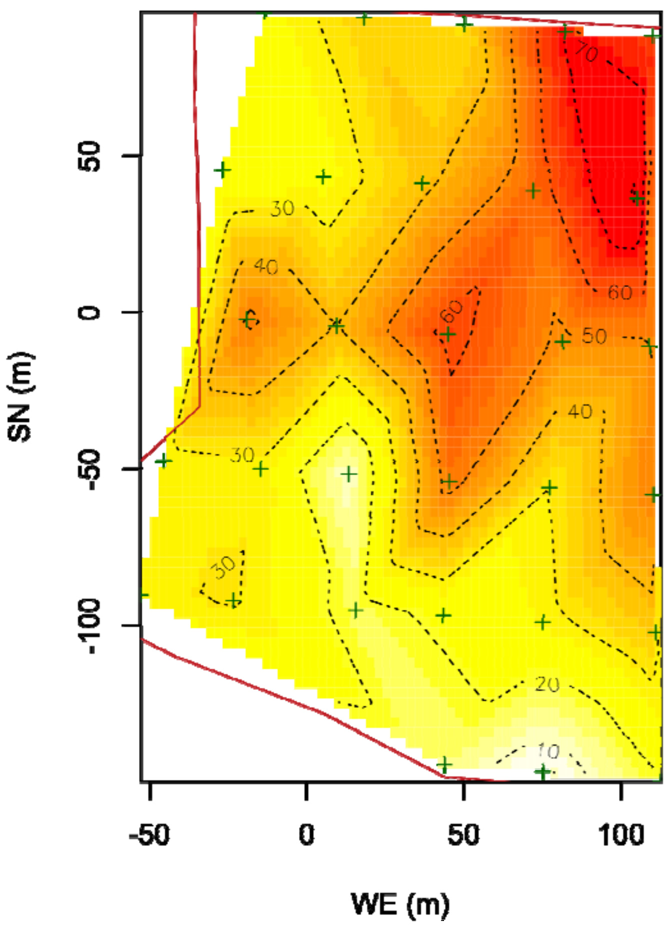

Figure 4. Weekly captures of codling moth males by pheromone-baited traps in the pear orchard, Big Valley. Between May 1st and 11th, in absence of puffer. Result of interpolating data from 30 traps. Crosses signal actual trap positions.

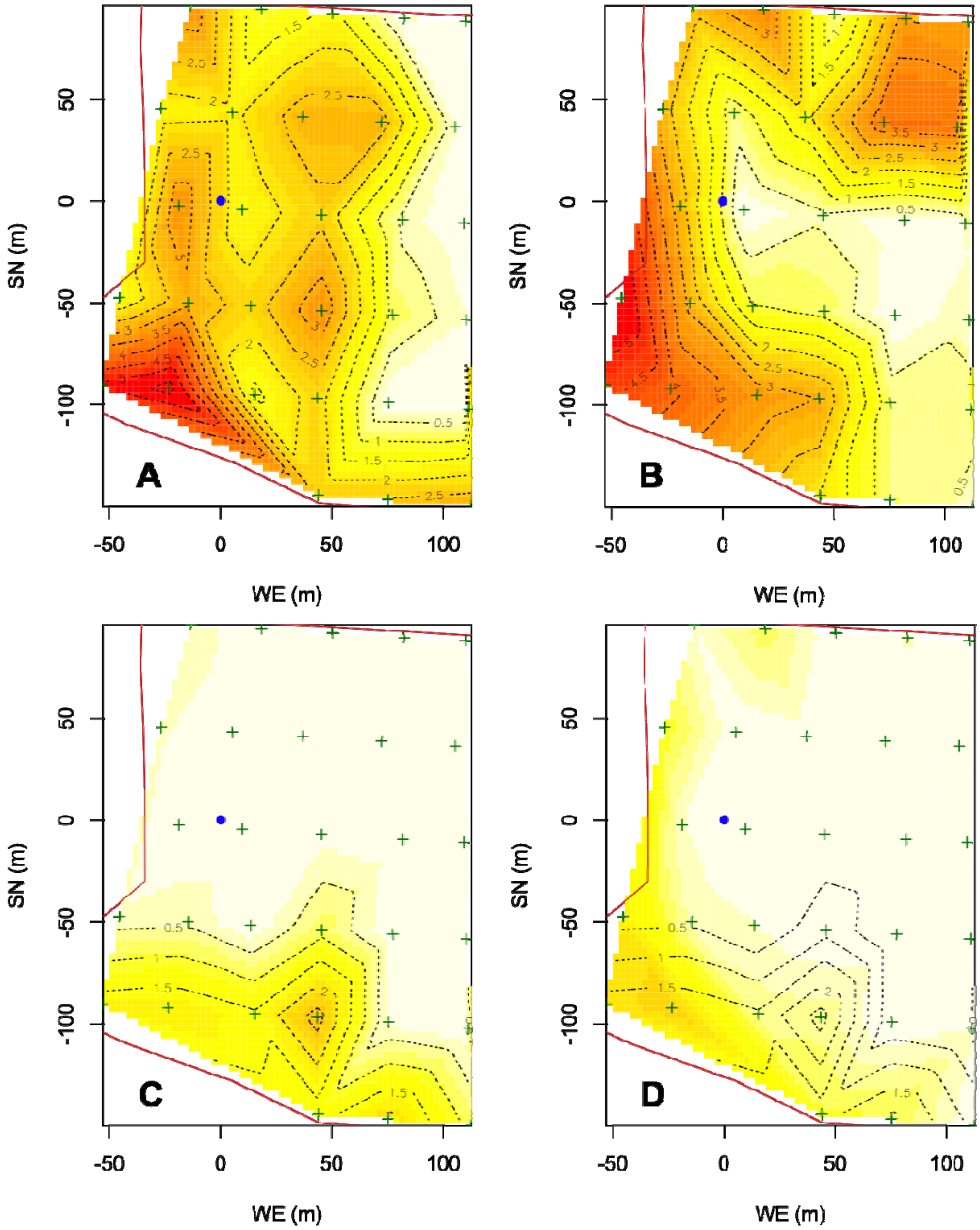

Figure 5. Daily captures of wild codling moth males by pheromone-baited traps in the pear orchard, Big Valley under the effect of puffers with different release rates: A, 1%; B, 10%; C, 50%; and D, 100%. Different periods between June 7th and August 3rd, 2010. Result of interpolating data from 30 traps. Crosses signal actual trap positions, and the dot at (0, 0) the position of the puffer.

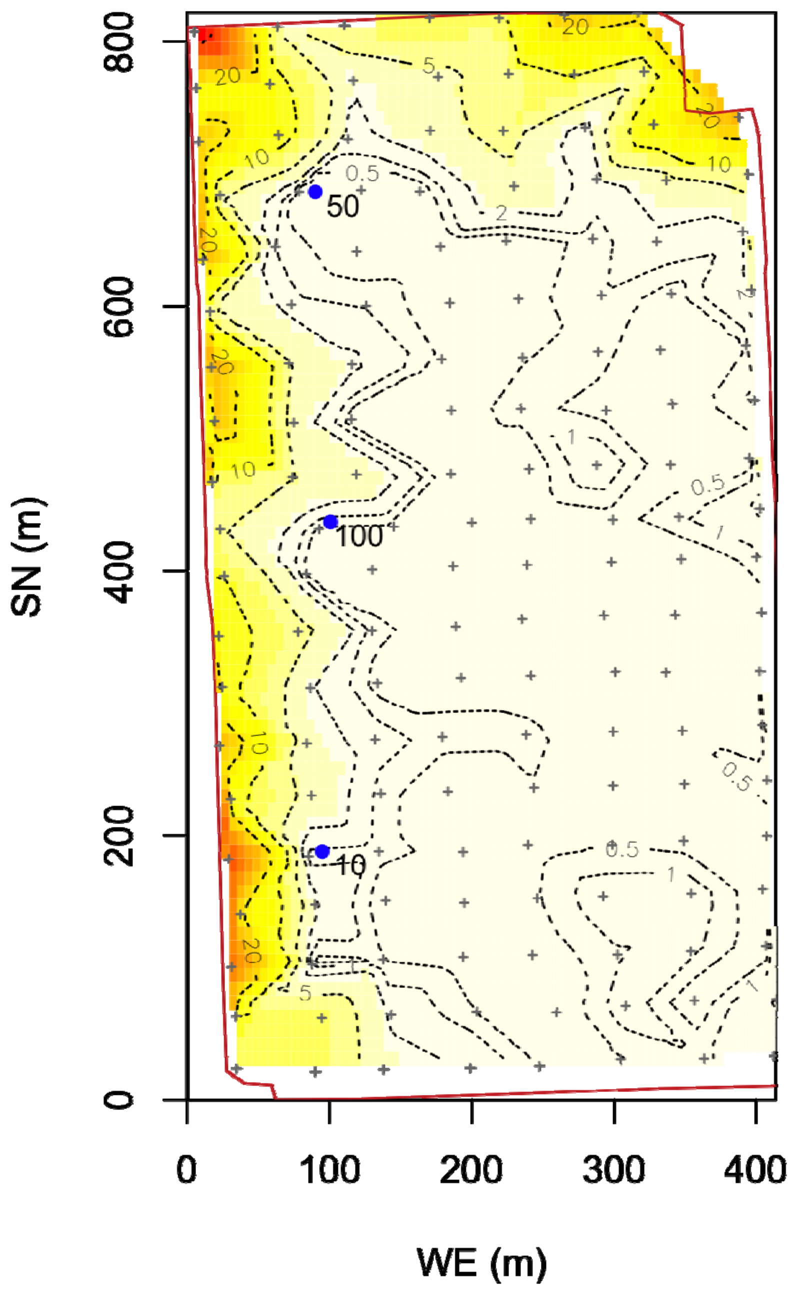

Figure 6. Daily captures, from July 8th to August 7th 2010, of wild codling moth males by pheromone-baited traps in the walnut orchard, Podesta. Three puffers of different release rates were in the field: 50, 100 and 10 %, from North to South, respectively. Image obtained by interpolation of 158 data points. Crosses signal actual trap positions, and dots signal the position of the puffers.

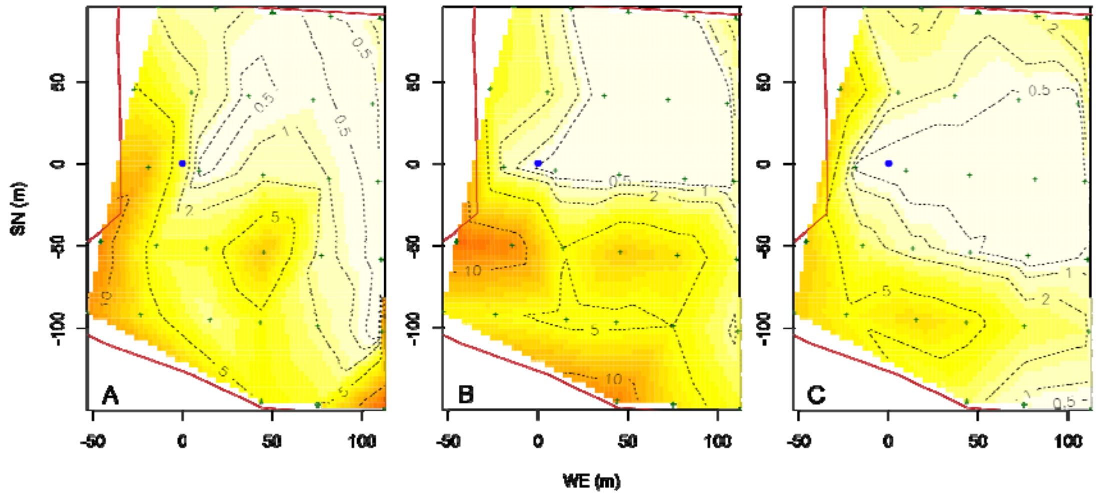

Figure 7. Daily captures of sterile codling moth males by pheromone-baited traps in the pear orchard, Big Valley under the effect of puffers with different release rates: A, 1%; B, 10%; and C, 50%. Different periods from August 12th to August 31st, 2010. Images resulted from interpolating 30 data points each. Crosses signal actual trap positions, and the dot at (0, 0) signals the puffer position.

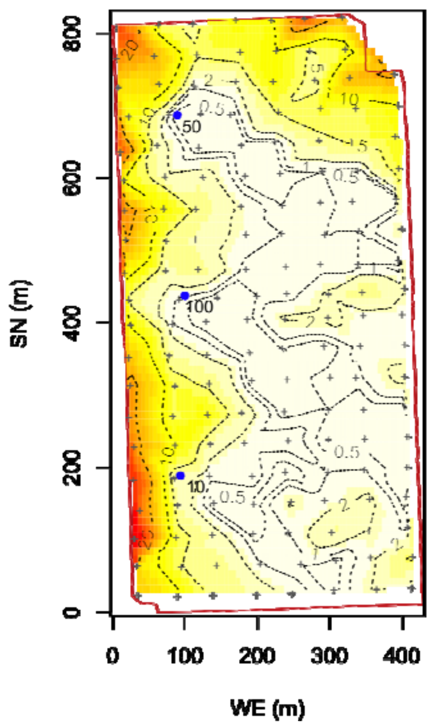

Figure 8. Daily captures, from August 12th to August 16th 2010, of sterile codling moth males by pheromone-baited traps in the walnut orchard, Podesta. Three puffers of different release rates were in the field: 50, 100 and 10 %, from North to South. Image obtained by interpolation of 158 data points.

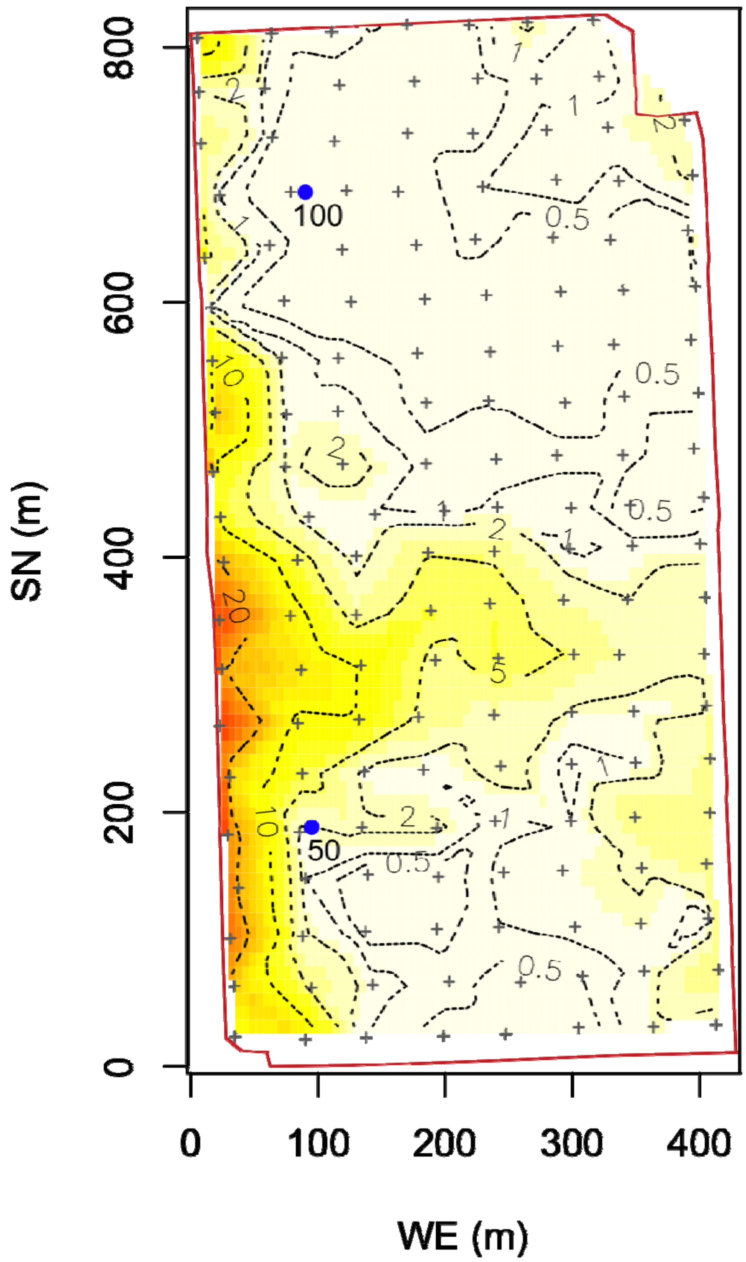

Figure 9. Daily captures, from August 19th to August 23rd 2010, of sterile codling moth males by pheromone-baited traps in the walnut orchard, Podesta. Two puffers of different release rates were in the field: 100 and 50 %, from North to South. Image obtained by interpolation of 158 data points. Crosses signal actual trap positions, and dots signal the position of the puffers.

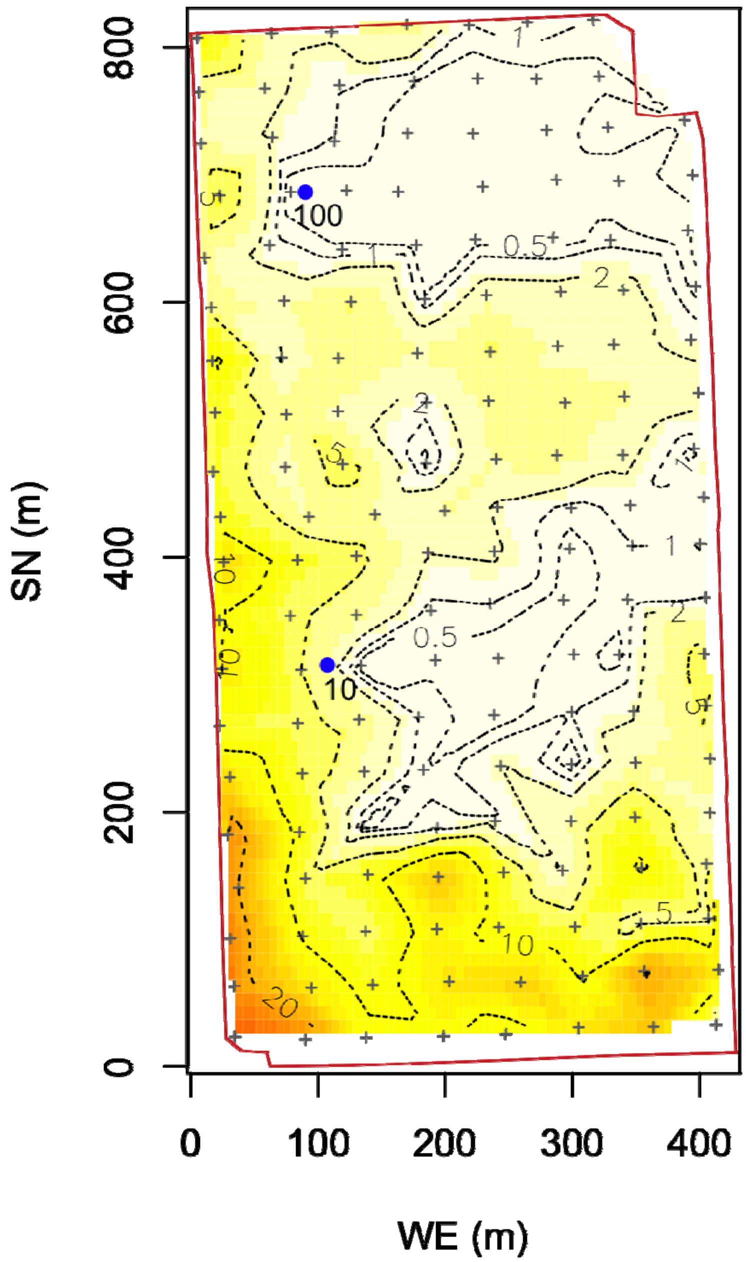

Figure 10. Daily captures, from August 27th to August 31st 2010, of sterile codling moth males by pheromone-baited traps in the walnut orchard, Podesta. Two puffers of different release rates were in the field: 100 and 10 %, from North to South. Image obtained by interpolation of 158 data points. Crosses signal actual trap positions, and dots signal the position of the puffers.

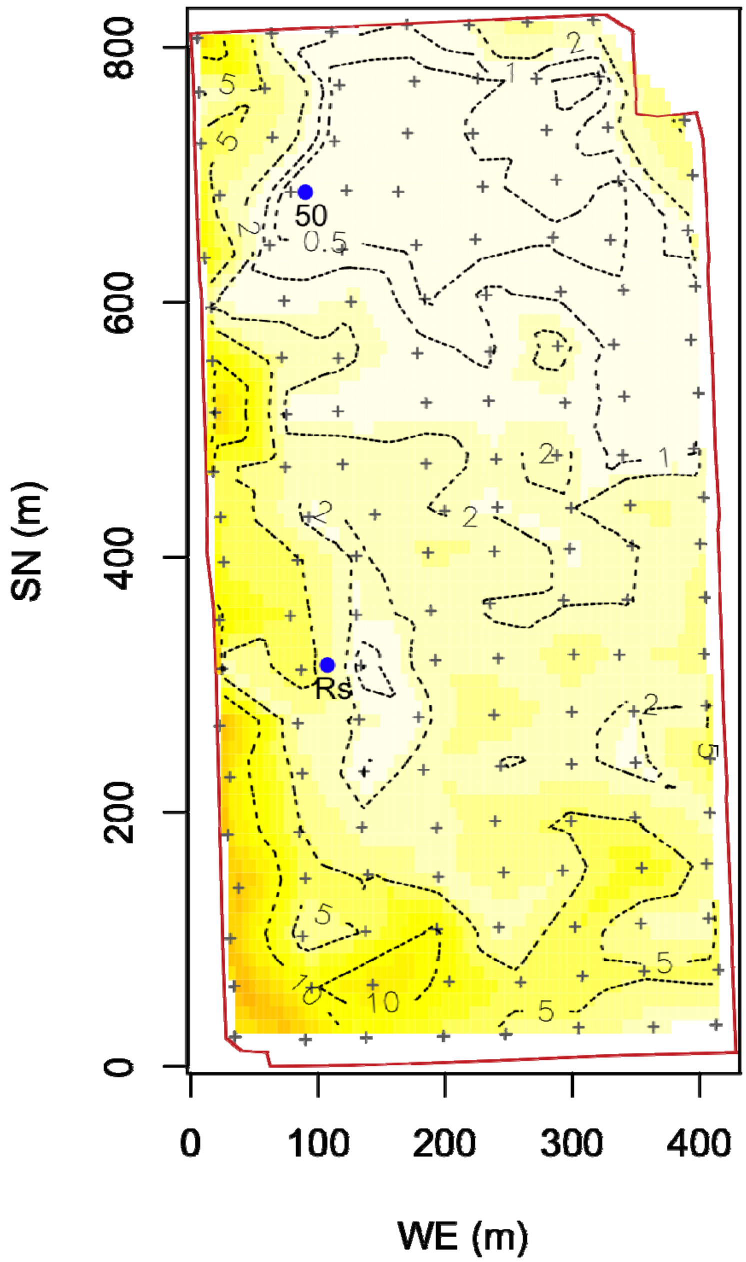

Figure 11. Daily captures, from September 3rd to September 10th 2010, of sterile codling moth males by pheromone-baited traps in the walnut orchard, Podesta. One puffer at 50% rate (North) was compared to an equivalent-rate passive emitter (10 Isomate®-CM Rings, Rs, South). Image obtained by interpolation of 158 data points. Crosses signal actual trap positions, and dots signal the position of the dispensers.

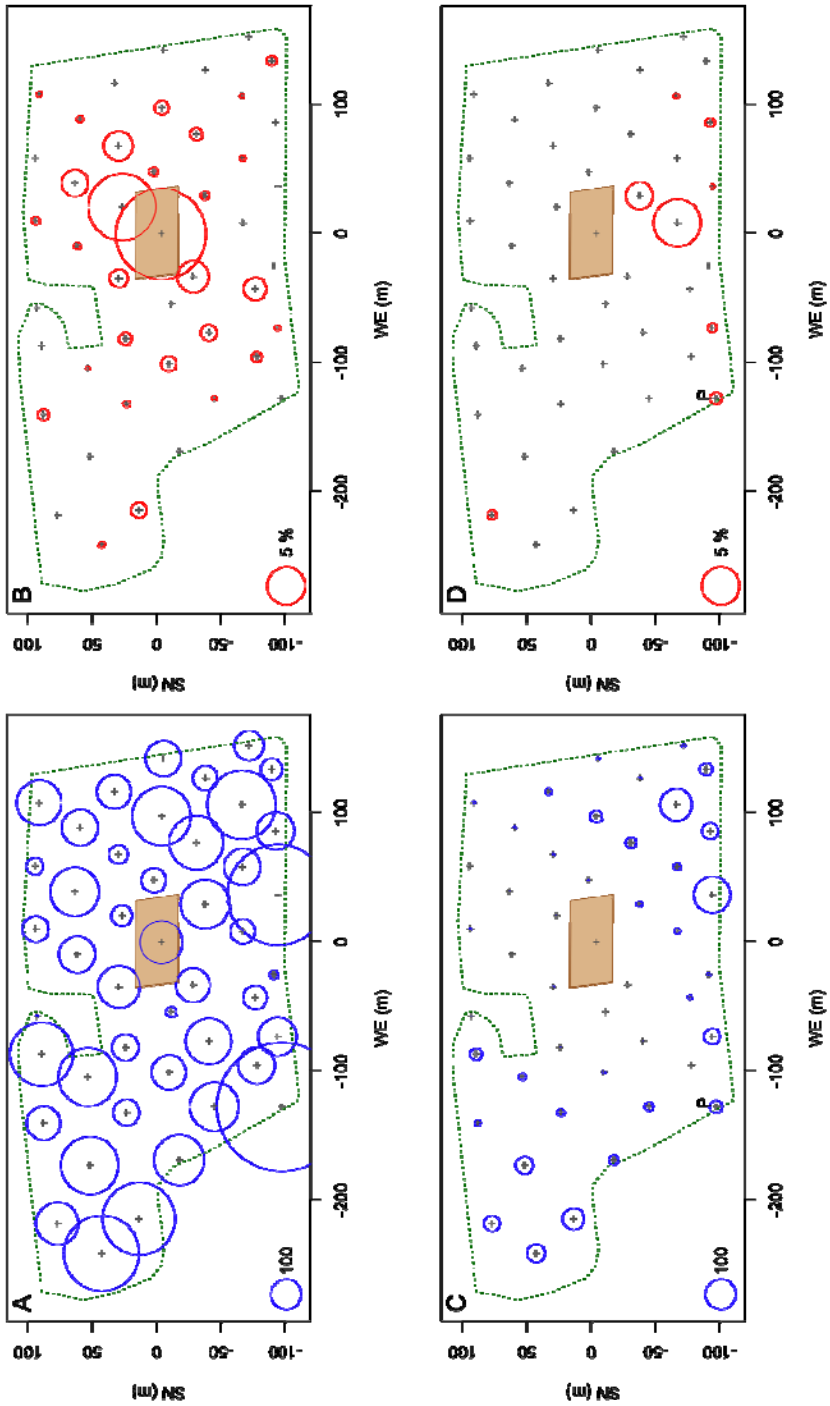

Figure 12. Experimental design and results from the movement assay at Freeport. A and B show data with no puffer: A, total number of insects captured; and B, percentage of insects scoring positively for egg protein. C and D show data with puffer (P): C, total number of insects captured; and D, percentage of insects scoring positively for egg protein. The brown area is the sprayed area. The grey crosses correspond to trap positions, and circles are proportional in size to number of insects (A-C), or percentage of positives (B-D).

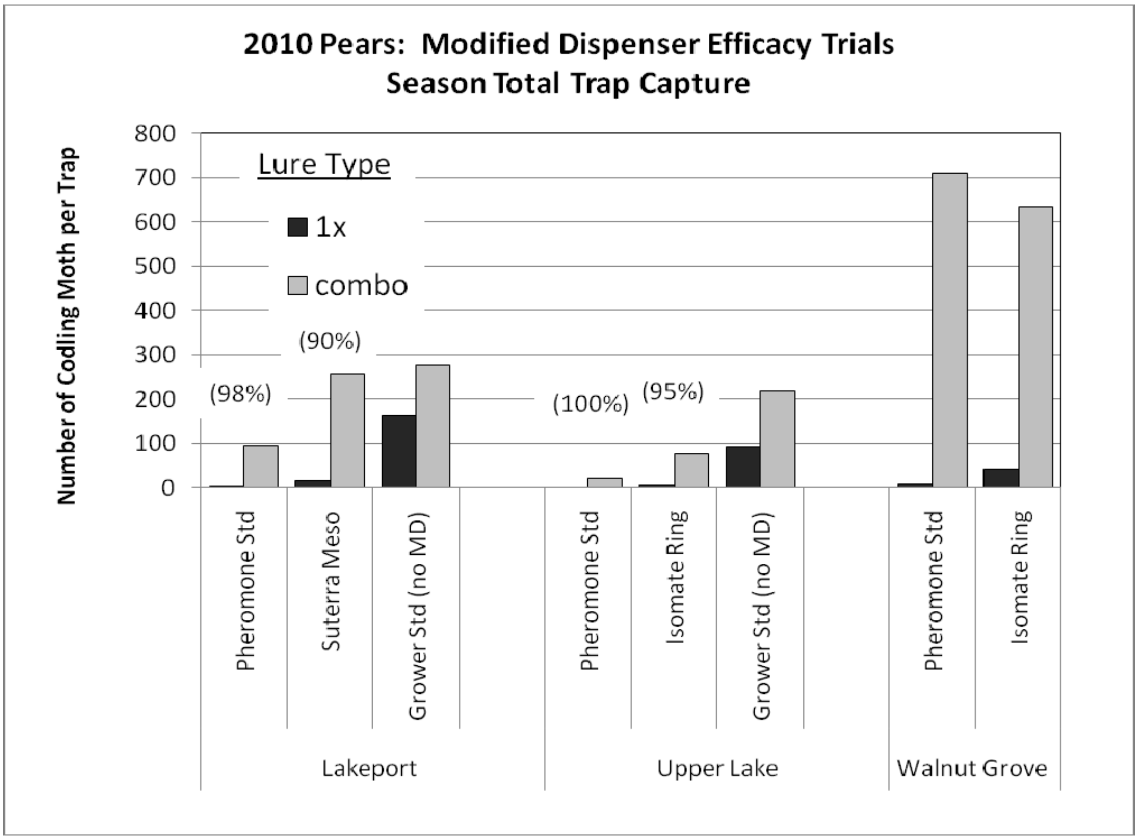

Fig. 13. Season total codling moth capture in 1x- and combo/DA-baited traps. Pear orchard plots treated with the Suterra Meso or Isomate Ring dispensers were compared with a standard pheromone application (Isomate-C TT) and untreated controls (Lakeport and Upper Lake sites only). Shutdown of 1x-baited traps in pheromone treatments relative to the grower standard (no pheromone) is indicated within the parentheses above each category when available.

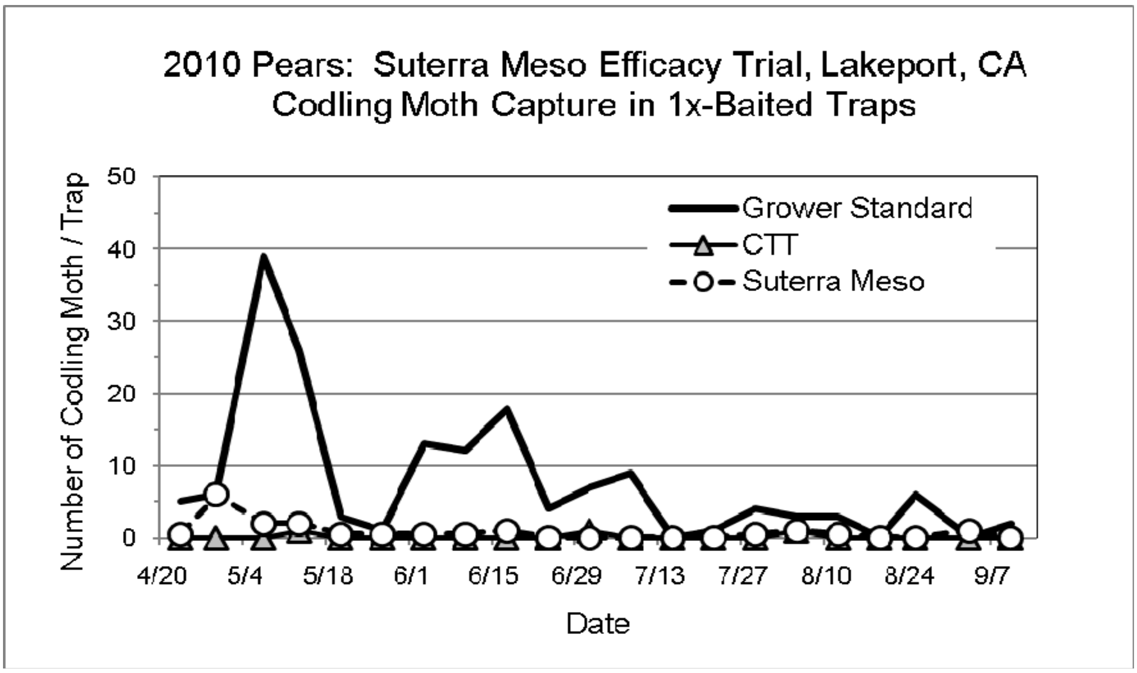

Fig.14. Seasonal flight patterns indicated by 1x-baited traps for codling moth in a Suterra Meso efficacy trial plot located in Lakeport, CA.

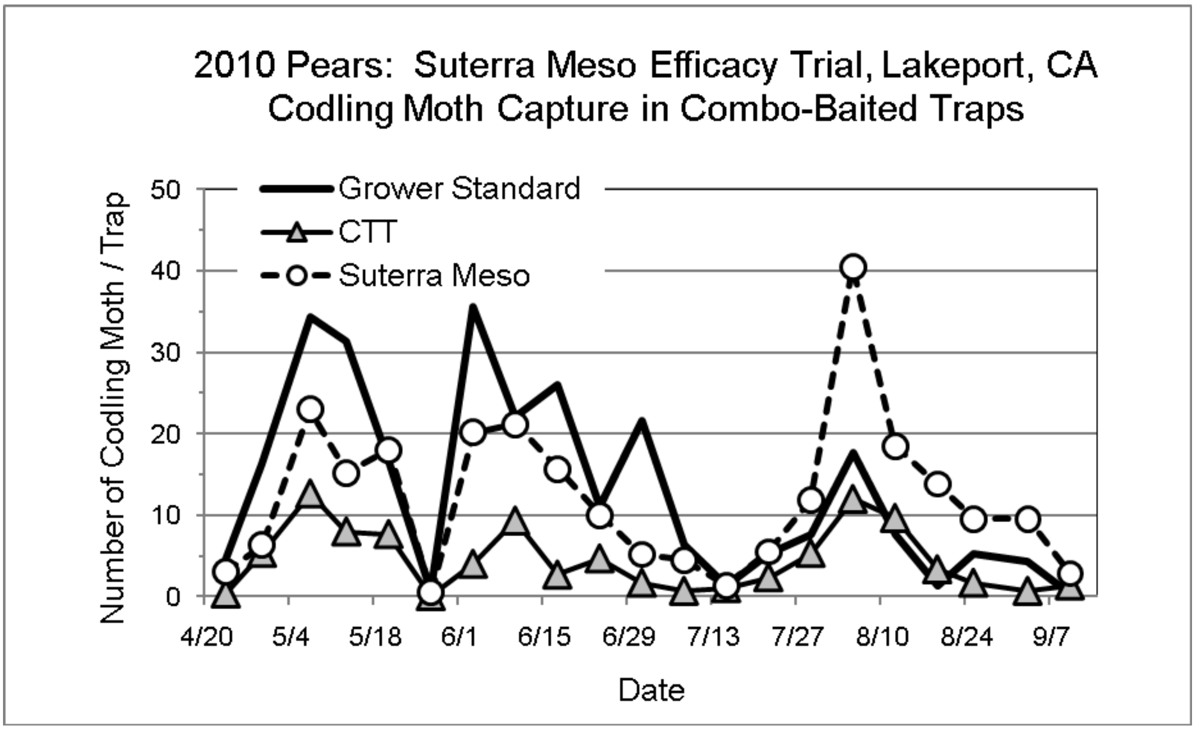

Fig.15. Seasonal flight patterns indicated by combo/DA-baited traps for codling moth moth in a Suterra Meso efficacy trial plot located in Lakeport, CA.

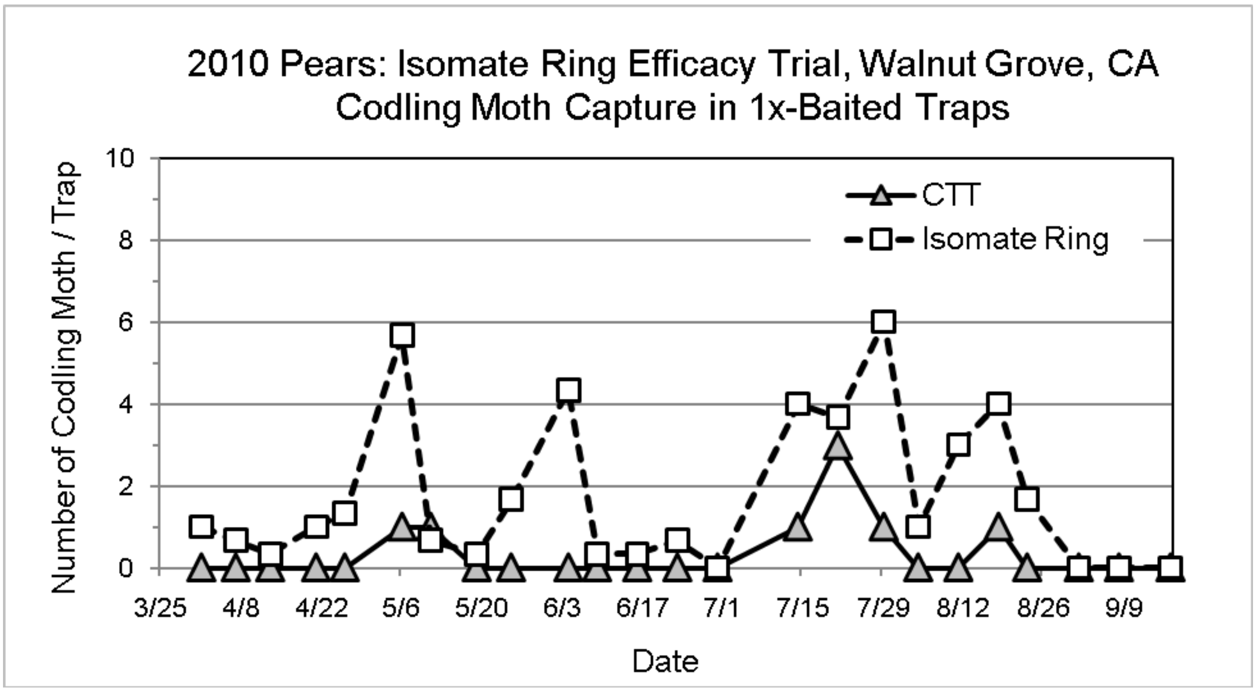

Fig. 16. Seasonal flight patterns indicated by 1x-baited traps for codling moth in an Isomate Ring efficacy trial plot located in Walnut Grove, CA.

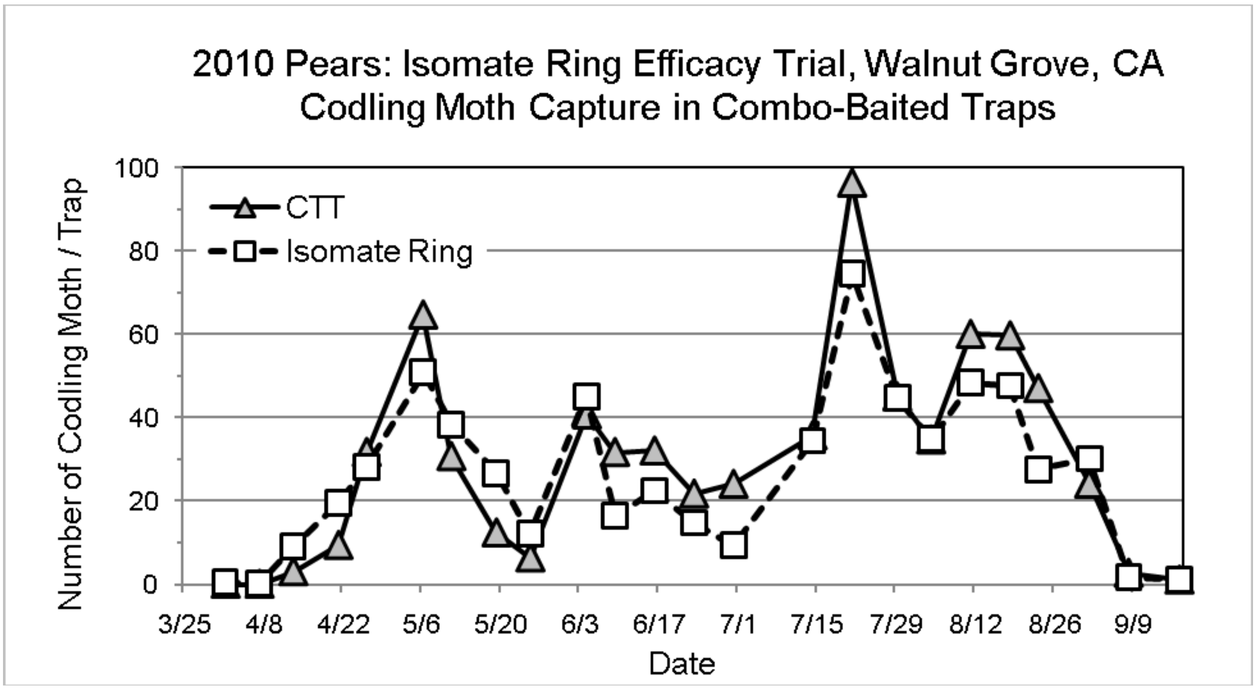

Fig. 17. Seasonal flight patterns indicated by combo/DA-baited traps for codling moth in an Isomate Ring efficacy trial plot located in Walnut Grove, CA.

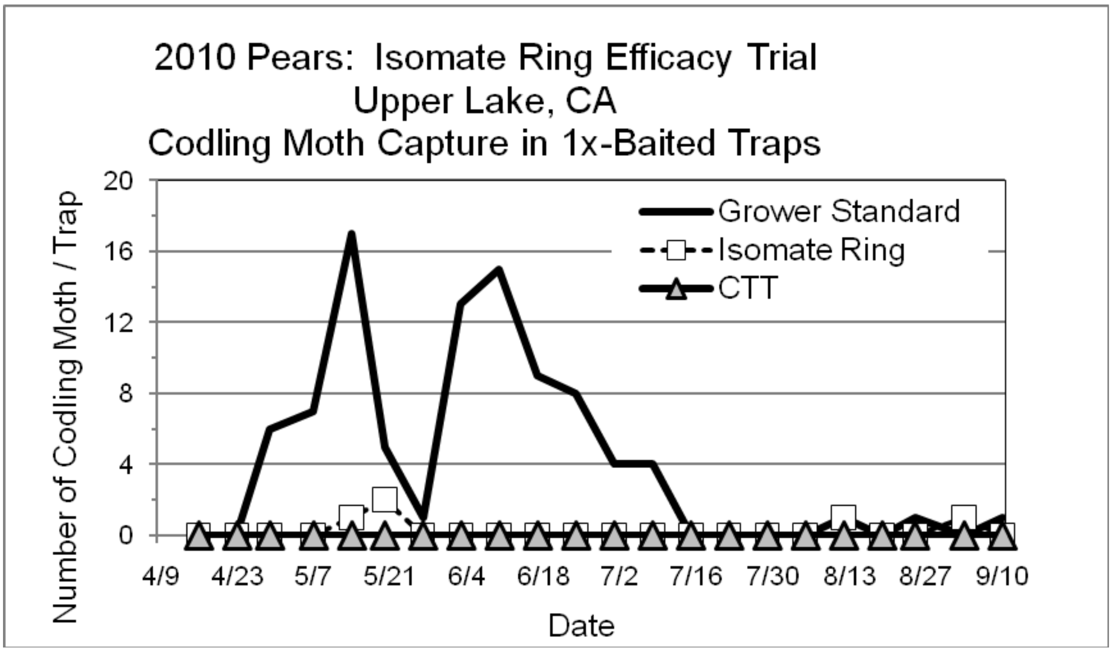

Fig.18. Seasonal flight patterns indicated by 1x-baited traps for codling moth in an Isomate Ring efficacy trial plot located in Upper Lake, CA.

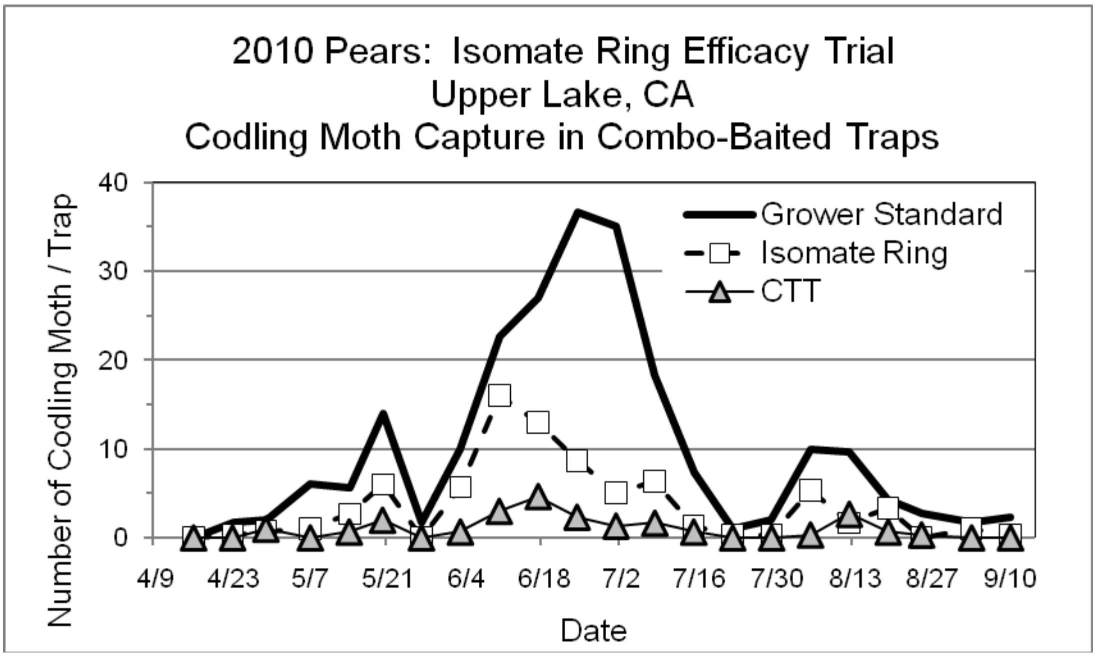

Fig.19. Seasonal flight patterns indicated by combo/DA-baited traps for codling moth in an Isomate Ring efficacy trial plot located in Upper Lake, CA.

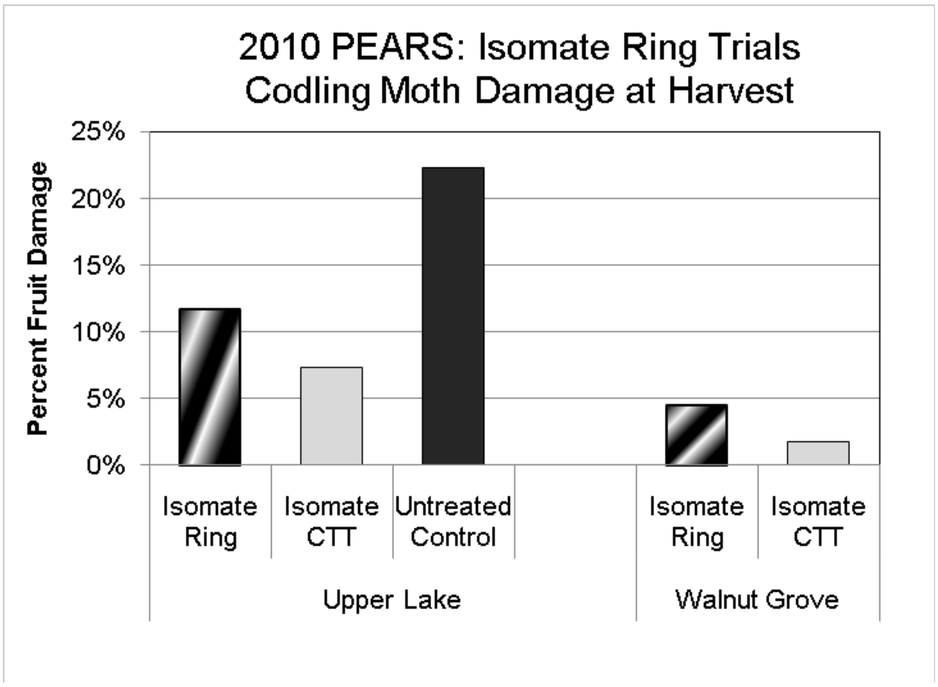

Fig. 20. Estimated codling moth damage at harvest in Isomate Ring efficacy trial plots. A harvest sample was not conducted in the Lakeport plot because of the limited fruit set in this unmanaged site.