

# **FURTHER INVESTIGATIONS ON PUFFER PERFORMANCE IN CODLING MOTH MATING DISRUPTION: IMPLICATIONS OF PHEROMONE LOAD AND AEROSOL FORM, AND UPWIND MOVEMENT OF MALES**

Stephen Welter, Daniel Casado and Frances Cave

## **INTRODUCTION**

High dose aerosol emitters of pheromone, “puffers”, have been used successfully for over 15 years for control of codling moth in pears, and more recently have emerged as the leading mating disruption strategy in walnuts. While their efficacy is well documented, the mechanisms by which they operate are still not properly understood. Understanding how this type of dispenser works may provide opportunities for improving program performance as well as decreasing overall costs. During 2009 and 2010, a series of studies were conducted which attempted to define the area of influence of the pheromone plume from a puffer; understand its impact on mate location; and enhance our understanding of the effects of secondary pheromone release from foliage. Furthermore, in 2010 we conducted preliminary studies that aimed to determine how the pheromone content (ai) in the puffers affects the area of influence of the puffers; evaluate within-orchard movement of codling moth with and without the presence of a puffer; and compare the plumes from aerosol devices and passive dispensers with similar pheromone emission rates.

In 2011 we have focused our efforts on the development of an extensive comparison of plumes generated by puffers loaded with different concentrations of codling moth sex pheromone, and a high emitting passive device; and evaluation of upwind attraction of codling moth males to puffers under field conditions. Data from both walnuts and pears are presented collectively since the larger combined datasets allow for more meaningful inferences.

## **OBJECTIVES**

1. To compare the shape and efficacy of the pheromone plume from puffers releasing the standard pheromone dose, reduced pheromone doses, and from a passive device.
2. To determine the effect of puffers on the within-orchard movement of codling moth males.

## **EXPERIMENT 1. Pheromone Plumes from Single Point Pheromone Emitters: Comparison of Puffers with Different Rates and Passive Emitters.**

The pheromone rate of the commercial aerosol cans for codling moth mating disruption was originally selected empirically. It was chosen to roughly match the release amount of the more widely applied mating disruption hand-applied polyethylene ropes of Shin-Etsu Inc. at a rate of  $400 \text{ ac}^{-1}$ . Given that puffers are

deployed at rates ranging from 0.5 to 1 ac<sup>-1</sup>, and that we know from previous experiences that their area of influence widely exceeds one acre, it seems that current application practices may be redundant as to the amount of pheromone used. In 2010, we ran trials to estimate the effect of a reduction of pheromone content (ai) on the area of influence of the puffers (understood as trap suppression). However, the following difficulties were encountered in that season: 1) initial trials were carried out with wild populations, which turned out to be too low or excessively patchy to render meaningful results; 2) sterile insect releases (SIR) were made later in the season, but their timing was not fully appropriate; and 3) the areas of influence were larger than expected (even with lower pheromone rates) and plume overlap occurred. Nonetheless, we obtained promising results that permitted us to continue these investigations. In 2011, we addressed these issues with the following experimental modifications: SIRs were used for all trials rather than relying on unpredictable wild populations; we improved the plot design to avoid overlapped pheromone plumes; and we included the appropriate negative controls.

In 2010, one SIR was dedicated to observe the plume generated from a group of passive dispensers placed at a single point. Based on a replicate, the observed plume was quite different from those created by puffers. For this reason we decided to include a passive-emitter treatment in our experimental design for 2011. If the plume from an aerosol emitter appeared to be dramatically different than from a device emitting the same level of pheromone over longer periods passively, then it would infer that the puffers might be working with a different mechanism than the passive devices.

## ***Materials and methods***

### *Fields*

Plume comparison trials were conducted in 2011 in 4 different orchards; Big Valley, Burger Line, Dondero, and Podesta. Big Valley was an abandoned pear orchard close to Finley, CA (38° 59' 53" N, 122° 52' 18" W) with a surface of approximately 9.5 ac. Its shape was pseudo-rectangular, with the SW corner being "horned"; distance was approx. 250 m S-N, and between 140 and 200 m W-E. Burger Line was a t pear orchard north of Lakeport, CA (39° 04' 53" N, 122° 56' 20" W) that had experienced significant codling moth flights in the past. It was an almost perfect square with sides of ca. 210 m (12 ac in size). Dondero and Podesta were both commercial walnut orchards. Dondero was south of Linden, CA (37° 57' 57" N, 121° 03' 35" W) and had a surface almost 140 ac, 370 m W-E and over 1,400 m S-N. Podesta was also near Linden (37° 59' 49" N, 121° 07' 47" W) and was smaller than Dondero in size but very similar in width, approximately 80 ac in a rectangular shape, 370 m W-E and 820 m S-N.

### *Traps*

We used orange delta traps (LPD, Suterra LLC, Bend OR) baited with pheromone lures (CM 1X Biolure, 1 mg, Suterra LLC) in all the field assays. In the pear orchards, traps were hung between 2 and 2.5 m above ground,

whereas in the walnuts they were at a height around 4.5 m (low-canopy in Podesta, mid- to high-canopy in Dondero).

#### *Wind conditions*

Hourly data on wind (direction and speed) and temperature during the puffer operational time (5 pm to 5 am) were obtained online from the Western Weather Group ([www.westernwx.com/lakeco/](http://www.westernwx.com/lakeco/), and <http://www.westernwx.com/lwwwc/>). For Big Valley, data from a wind station ca. 2 km to the south (38° 58' 47" N, 122° 51' 58" W) were used. In the case of Burger Line the station was ca. 1.2 km to the southwest (39° 04' 28" N, 122° 56' 58" W). Finally, for both Podesta and Dondero data from a weather station to the north of Linden (38° 02' 34" N, 121° 07' 21" W) were used.

The overall wind patterns for the 3 stations are shown in Figure 1. Average wind directions for the different replicates were computed as follows: first the records with average temperature under 15 °C (the lower flight threshold for codling moth males) were eliminated; then each record was represented by a vector of modulus equal to wind speed and angle the wind direction; vectors were decomposed into their south-north ( $V_n$ ) and west-east ( $V_e$ ) components; these components were averaged independently and the average wind vector was rebuilt from them.

The estimated average wind directions (referenced to the north) varied between 227 and 273°, 251 and 292°, and 279 and 305° for Big Valley, Burger Line, and the Linden area, respectively. This means that at Big Valley the wind usually blew from the SW-W arch, at Burger Line in the WSW-WNW one, and between the WNW and NW at the orchards around Linden.

#### *Puffers and pheromone cans*

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 minutes, from 5 pm to 5 am) were kept constant for all the puffers used in the assay. The different rates of pheromone application were due to differences in the pheromone content of the aerosol cans. The commercial cans for codling moth contain 69.33 g of active ingredient. Suterra LLC supplied cans loaded with the full load of active ingredient (a.i) (100 %), as well as cans with diluted formulations at 50, 25, and 10 % of the full a.i. These amounts correspond to releases of 306, 153, 76.5 and 30.6 mg/day and unit, respectively.

#### *Passive emitters*

A passive emitter was made using the Isomate®-CM Ring (Pacific Biocontrol Corp., Vancouver, WA). These emitters are normally uniformly distributed at a rate of 20 to 40 ac<sup>-1</sup>, but as our goal was to contrast the area of influence created from a single point release of active (puffers) and passive (rings) emitters, we hung a group of rings from a single hanger (Figure 2).

On the basis of data provided by Pacific Biocontrol, we decided to make 40-ring groups. We estimated emission from 40 rings to be ca. 320 mg/day. This amount is very similar to the emissions from a full rate (100%) puffer. A clear difference exists between the two dispenser types; rings are emitting 24 hours of the day, whereas the puffer emits only 12 hours. Hence during the 12-hour period of a puffer's activity, a 100% unit would emit roughly twice as much pheromone as the rings, and thus a fairer comparison might be between the rings and the 50%-rate puffer.

Two different groups of rings were used to conduct the experiments. Each group was aged under field conditions for 7 to 10 days before using it in the experiments. The rings were weighed twice to estimate actual pheromone emission; once after the aging period, and again after the ring-trials were over (ca. 1 month later). Average weight loss was 442 and 515.6 mg/day for the 2 groups, respectively. Assuming that weight loss is basically due to pheromone emission, and that all the components in the rings were emitted at the same rate, those weight losses translate into 243.3 and 283.8 mg of codlemone/day, respectively. While less than expected, these rates were still between the 50 and 100%-rate puffer emissions for the full day or the 25 and 50%-rate puffer emissions, if only a 12 hour-emission period is considered.

### *Sterile insects*

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 they left the Canadian facility. Upon arrival they were taken to the fields for their release.

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

### *Experimental design and procedures*

Grids of traps were set up at the different sites following an approximately uniform distribution. In Big Valley the irregular corner at the southwest was left out, and a grid of 56 traps at distances of approx. 25 m covered the remaining pseudo-rectangular plot of 8.6 ac. In Burger Line, a 64-trap grid was deployed throughout the entire orchard, with a distance of ca. 25 m between traps. In the case of both walnut orchards, the grid was formed by 72 traps at a distance between 40 and 45 m. In Dondero the size of the grid was 330 x 370 m, and in Podesta it was 360 x 370 m (Figure 3).

In order to calculate trap suppression it is necessary to designate control traps that lie out of the area of pheromone influence. For the walnut orchards, space was not a limiting factor and 3 extra (control) traps were situated between 100 and 120 m to the north of the border of the grid. In Big Valley 3 extra (control) traps were also placed. In this case they were at the southwest corner that had

been left out of the grid. Finally in the case of Burger Line no extra space was available and the west-most row of traps was used as control (Figure 3).

Due to the large size of puffer plumes, the capacity to combine different puffers in the same plot (or orchard) without interaction is very limited. For this reason the different puffer rates were rotated through time to accommodate them into a limited amount of space. Puffers were placed close (45 m in walnuts, and 25-30 m in pears) to the upwind border of the different orchards in the west-east direction, and approximately centered in the south-north one (Figure 3). This decision was based on our previous knowledge of the wind directions to allow for a good accommodation of the plumes in the orchards. In the pear orchards, puffers were hung at approx. 3-3.5 m above ground (top canopy), whereas in the walnut orchards they were at ca. 6 m (mid- to high-canopy). The groups of rings were placed at same spots, when it was their turn in the rotation.

The rotation of treatments (puffer rates, rings and control) through the season was assigned randomly, and the operational sequence was as follows: a) in day 0 the first treatment was deployed, and allowed to “stabilize” for 9 days; b) on day 9 traps were zeroed and a SIR was performed; c) we recaptured insects for 5 days, and on day 14 the traps were read and the next treatment was deployed starting a new sequence. A total of 6 SIRs were performed at each site (one per treatment). The dates of the releases were as follows: a) June 16 and 30, July 14 and 28, and August 11 and 25 in the pear orchards; and b) June 9 and 23, July 8 and 21, and August 4 and 18 in the walnut orchards. To conduct the SIRs, a dish of moths (see above) was released 8-12 m downwind from each trap. Moths were poured while still cold into paper bags (#6, Duro Bag Mfg. Co., Florence, KY) that had been stapled to the trunk of trees at ca. 1.5 m above ground. Insects were seen to both climb up the trunks and fly out of the bags within a few minutes.

### *Data analyses*

Trap positions were geo-referenced and interpolation surfaces of sterile males captured per day were performed using geostatistical models for each of the site-treatment combinations. For normality purposes the data were log-transformed,  $\ln(x+1)$ , prior to the analyses. Once the surfaces were obtained there were transformed from captures/day into percent of trap suppression for easier comparison among treatments and concordance with regular mating disruption data. To perform the transformation the following formula was used:

$$TS_i = \frac{C_c - C_i}{C_c} \times 100$$

Where  $TS_i$  was percentage of trap suppression at the position  $i$ ;  $C_c$  was the average number of captures in the control traps; and  $C_i$  was the number of captures predicted at the position  $i$ .

The same models used to construct the interpolation surfaces were further used to generate a series of 1,000 conditional simulations for each site-treatment combination. These simulations allowed estimating the average and 95%

confidence intervals for the area size with trap suppression over 75 and 90% (arbitrary, but illustrating thresholds). The R statistical data analysis environment (R Development Core Team, 2009) was used to conduct all the data analyses.

## ***Results and discussion***

### *Sterile insect recapture*

The use of sterile insects helps to achieve clearer and more reliable results in plume imaging. In general, all the SIRs carried out in 2011 were successful. Insects were healthy, and recapture rates were acceptable. . The total number of sterile males recovered at the different treatment-site combinations, and the percentage of recapture are shown in Table 1. As expected there was variability among both sites and dates. Overall percentages of recapture ranged from 2.1 to 29.1 %. As a general trend, more insects were recaptured in the control than in the other treatments, probably due to the action of the pheromone. The exception was the control at Burger Line, where the recapture was surprisingly low. Comparing the different sites, the recapture rate was generally higher in Dondero, whereas in the rest of orchards it was rather similar.

Despite the variation, recapture was enough to render meaningful results in all trials. Even at the lower recapture rates, the average was over 8 males/trap in a 5-day period. This amount is high enough, given that it is an orchard-wide mean that does not account for the effect of the pheromone, which suppressed captures in large areas.

### *Control treatments*

Control (no pheromone) replicates are very important not only to validate the results from the other treatments, but also because they allow us to identify patterns in insect distribution and recapture across the different orchards. Space is highly heterogeneous and under field conditions there are multiple variables that cannot be controlled, but can influence the outcome of the trials, resulting in patchiness or distribution trends even in the absence of pheromone. These trends must be identified and taken into account when interpreting the results.

The interpolation surfaces of sterile males recaptured per day at the different control treatments are shown in Figure 4. These results illustrate very clearly how male recapture was never uniform across the orchard, despite the initial homogeneous distribution of the males. In both walnut orchards there was a clear west to east gradient of recaptures, with more males being recaptured towards the west, which was the up-wind border (Figure 4E). In the case of Big Valley the pattern was similar, but in this case it was less clear. A possible reason is the border versus size ratio, which was much higher in the case of this orchard. Finally, in the case of Burger Line, recaptures at the northeast quadrant of the orchard were lower than in the rest. The connection between wind direction and the pattern of recapture is not clear in this case, and other

factors may have been involved. Nonetheless this pattern with lower recapture at the northeast quadrant in Burger Line was present in all the treatments independently of the treatment and wind patterns (see below).

It is not clear how these observations on recapture patterns can be included in modeling the plumes of the rest of treatments, but it is necessary to keep them in mind when interpreting the results. Neglecting this kind of pattern may result in an overestimation of the size of the area of influence of the puffers, or other data misinterpretations.

#### *Effect of Pheromone Rate*

Clear plumes (meaning depressions in male recaptures downwind from the puffer) were recorded for the 4 pheromone-rates (10 through 100%) tested. As expected some variation occurred among orchards and dates, but in all cases the plumes were large and clear. However we did not observe a clear dose-response relationship; on the contrary plumes look very similar regardless the rate used.

At Big Valley (Figure 5) all the treatments with puffers showed a clear plume towards the east or northeast in concordance with the wind direction. Opposite, in the control, trap suppression was at 0% almost in the entire field. In all the puffer treatments the plume reached the downwind end of the orchard with a width of 50 m or more. In this orchard the narrowest plume was achieved with the 50% dose, but this was probably due to differences among SIRs.

As we saw earlier, for Burger Line a clear pattern of recapture was observed in the control. Figure 6 shows how high values of suppression (i.e. low recapture) were found at the northeast quadrant in all the situations. Furthermore, Figure 6 also suggests that the lower level of recapture in that area of the orchard cannot be related to the action of the pheromone because the wind direction follows a different direction. Hence we should be aware that in the simulations for this orchard, plume size would be overestimated. Northeast quadrant apart, plumes are still clear for all 4 puffer-rates. In this case the area of influence seems larger in the case of the 100%, while the rest of rates showed a lesser impact in captures. Nonetheless for all the rates, the area of influence was over 1 acre, which is the area that currently one puffer is supposed to cover for in pears.

In Dondero (Figure 7) the recorded plumes were very similar for the 10, 25 and 100% treatments. In all these cases the areas of influence clearly exceeded the 2 acres, and they reached the downwind end of the orchard. However the effect was less intense after roughly 200 m. For the 50% puffer, trap suppression was abnormally large and roughly 10 acres showed suppression over 90%. Probably other factors besides the puffer contributed to this strong suppression. As the models used for the simulations are based on the original data, predictions for plume size in this last case may be overestimated.

Finally, in Podesta (Figure 8) the pattern was similar to Dondero, however in this case the accordance between wind direction and plume shape was poorer.

In the case of the 100% puffer the area of influence seemed extremely large, like-wise in the 50% at Dondero (Figure 7D). These 2 replicates were run in different dates, and factors involved in their similar results must be other than weather conditions or insect quality. In general, the plumes recorded at Podesta were messier than those from Dondero, but a clear effect of all the rates was still present. The less clear plumes may be related to the fact that Podesta is a closed canopy orchard, whereas Dondero is still open. Closed canopies impact wind permeability and disrupt wind direction, weakening the relationship between wind direction and plume shape. The results from the simulations summarize all the above-mentioned in a simpler way. When a threshold of 75% trap suppression was considered, the area estimated was above 1 acre for all the puffer rates in both pear orchards (Figure 9A,B). In the case of Burger Line, the control also had over 1 acre with suppression above 75%. This was due to the effect in the northeast quadrant that we pointed out earlier. Nonetheless the areas estimated for the rest of treatments were much larger (Figure 9B). In the walnut orchards, predictions differed considerably. In the one hand, in Dondero (Figure 9C) all puffer treatments were largely different from the control. The areas predicted for 10, 25 and 100% were above 3 acres, and for the 50% the area was extremely large. Nonetheless as commented earlier we should be cautious in the 50% case, as it is most likely an overestimation. In contrast, at Podesta the prediction for the control treatment was very high. This is related to the gradient observed in captures in the control (Figure 4D). Due to this high prediction in the control, only the 10 and 100% treatments were clearly different from the former (Figure 9D). Despite this, the shape of a plume was also clear with the 25 and 50% puffers (Figure 8C,D), unlike in the control (Figure 8A). While the reason is not clear at this point, the effects of the pheromone plume in walnuts does appear to be greater than puffers releasing similar amounts of pheromone in pears. This may limit our ability to pool the two datasets when developing programmatic deployment decisions.

When the more stringent threshold of 90% trap suppression is used, the areas of impact become smaller, but the difference between the puffers and the control becomes more distinct for all puffer treatments except in one site. Only at Podesta, the area for the 50% rate is not strongly distinguished from the control (Figure 10).

#### *Active (aerosol) emitters versus passive devices at similar release rates*

Despite the 40 rings releasing as much pheromone as a 50% puffer (see above), the impact on trap captures was different than with the puffers in 2 cases. In Dondero (Figure 7F) no plume was recorded for the passive treatment, although some suppression occurred in the neighborhood of the pheromone source. Similarly, a small plume was recorded at Big Valley (Figure 5F), where trap suppression (up to 75%) was detected downwind from the source point, but faded away after ca. 70 m. In Burger, it is hard to distinguish between the effect of the rings, and the lower recapture reiteratively detected at the northeast quadrant. Especially unfortunate was the fact that wind direction was towards the east or east-southeast in all the replicates but the one with the rings. In the latter wind blew more towards the east-northeast or northeast, making it impossible to discriminate the ring plume (if any) from the typical

depression in recapture at the northeast quadrant of this orchard (Figure 6). The strongest plume from the rings was recorded at Podesta. In this last orchard the plume reached the downwind edge, and was similar in size to the 50% puffer (Figure 8F).

### *Remarks from Experiment 1*

Despite the variability inherent to field trials, our results show that all the pheromone rates in puffer assays (10, 25, 50 and 100%) generate large plumes, and suppress downwind captures in large areas in both pear and walnut orchards. Surprisingly we did not find any clear dose-response relationship, and plume behavior was similar regardless of the pheromone rate. Our results strongly suggest that a large reduction in the pheromone content of puffer cans for codling moth mating disruption is possible. Nonetheless, these trials were performed with single point sources, and efficacy trials, with an adequate distribution of puffers are required before applying pheromone reductions at the commercial level.

Our data also suggest that pheromone releases from puffers and passive devices behave differently. When we used passive devices placed at a single point to release amounts of pheromone similar to those of puffers, the plumes resulting were in general weaker, and some times totally absent. The differences may be due to several factors: a) pheromone bursts versus a continuous leak of pheromone, b) the propellants used in the cans, c) physical properties of the aerosol formulations, or d) a better match between insect behavior window and the period of pheromone release. Further studies are needed in this topic.

We have started to use conditional simulation to assess size of the area of influence of puffers. We are still working in the technique, which we think has great potential, but further improvements have to be made. Here we report our simulations taking into account the entire size of our plots, but pheromone is indeed moving basically in the wind direction. The area that is away from the main pheromone “channel” is a source of noise, which makes estimations look too similar to the control treatments in some cases, despite the interpolation showing very different patterns. We are still working in this approach and we hope to have great improvements soon.

### **EXPERIMENT 2. Influence of a puffer in 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 well known. We hypothesized that male moths may be attracted to pheromone puffers from medium or long distances. This type of “super-female” effect has been described in movement from untreated to pheromone-treated orchards (e.g. Witzgall et al. 1996). Similarly McGee and Gut (2011) suggested that codling moth sterile moths were dragged to puffer plots from downwind adjacent plots. In 2010 we tried to track adult movement in the orchard using an immunomarking technique (Jones et al. 2006). Briefly, the technique consisted of marking existing feral codling moth with egg proteins,

capturing them and using ELISA procedures to detect the protein on them. Despite locating the trial in a heavily infested orchard, the number of marked insects captured was too low to draw meaningful results, and made us question the adequacy of the technique for our purposes. For this reason we tried a different approach this year using sterile insects.

## ***Material and methods***

### *Field*

The study was conducted in an unfarmed pear orchard of approximately 17 ac near Freeport, CA (38° 25' 59" N, 121° 31' 24" W). In this orchard dominant wind directions are from the southwest and the west-southwest.

### *Experimental Design*

Six orange delta traps (LPD, Suterra LLC) baited with standard pheromone lures (CM 1X, 1 mg, Suterra LLC) were placed at the southwest corner of the orchard (upwind corner). Traps were hung between 2 and 2.5 m above ground, and spaced at ca. 30 m from each other. Just downwind from the inner most trap a "puffer-position" was defined, and 150 m further downwind a release-position was designated (Figure 11).

Six SIRs were conducted during the season (June 17, July 1, 15 and 29, and August 12 and 26). In each of the releases approximately 10,000 codling moth males were released at the release position, and 7 days later recaptures at the upwind traps were recorded. Three of the releases were conducted as control and the other 3 as puffer treatment. In the puffer treatment replicates, a single puffer loaded with Puffer® CM-O (Suterra LLC) was placed at the puffer-position, approximately 4.5 m above ground (top-canopy). In the control replicates the puffer-position was left empty. Before each release the orchard had been under the appropriate regime (puffer or control) for 7 days.

### *Data analyses*

The number of sterile males per trap and day were compared between the control and the puffer treatment using a mixed effects model after log-transformation,  $\ln(x+1)$ . Treatment (puffer/no puffer) was a fixed effect, and date and trap were considered as random effects.

## ***Results and discussion***

Sterile codling moth males were successfully recaptured at the upwind corner of the orchard. A total of 131, 47 and 26 males were recaptured in each of the 3 control releases, respectively; and 179, 11, and 50 in the 3 releases with the puffer. The average number of males per day and trap (and standard error) was 2.14 (1.00) in the replicates with puffer, and 1.68 (0.32) in the control replicates.

No significant differences were found between puffer and control (L-ratio = 0.481,  $df = 1$ ,  $p = 0.488$ ).

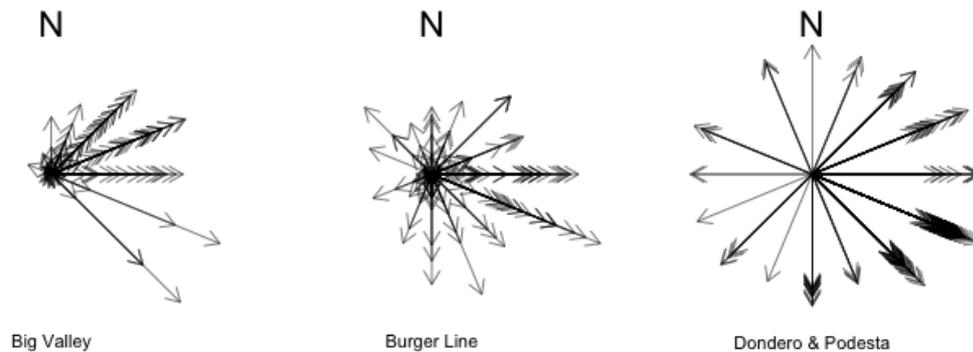
These results suggest that codling moth males move upwind, but the presence of a single puffer (high-concentration pheromone source) does not affect their upwind movement, at least for medium distance movements (150 m in this case). The results obtained in this trial are also in agreement with observations from the first experiment, where we observed a tendency to have higher counts of moths in the upwind edges of our control treatments. These data did not support differential upwind movement as a strong driver of the patterns of trap suppression observed in the pheromone plume studies. With only 3 replicates of each treatment, additional trials are warranted. What is important to note though is that by defining the question as “Is codling moth more likely to move upwind toward a puffer source than moths in an untreated orchard” may generate a different answer than if the question is “Does codling moth upwind towards a puffer source”. Inclusion of a control treatment allows us to separate out the 2 questions such that our data would support the upwind movement, but not the differential movement towards a puffer source.

## References

- Jones, V. P., J. R. Hagler, J. F. Brunner, C. C. Baker, and T. D. Wilburn. 2006. An inexpensive immunomarking technique for studying movement patterns of naturally occurring insect populations. *Environmental Entomology* 35: 827-836.
- McGhee, P., and L. Gut. 2011. Movement of male codling moths in puffer treated apple orchards, pp. 56, 2011 Western Orchard Pest and Disease Management Conference, Portland, Oregon.
- Witzgall, P., A. C. Bäckman, M. Svensson, M. Bengtsson, C. R. Unelius, J. Vrkoc, A. Kirsch, C. Ioriatti, and J. Löfqvist. 1996. Potential of a blend of E8,E10-12OH and E8,E10-12Ac for mating disruption of codling moth, *Cydia pomonella* L. (Lep., Tortricidae). *Journal of Applied Entomology* 120: 611-614.

**Table 1. Total numbers (and % of number released) of sterile codling moth males recaptured at the different trials during 2011.**

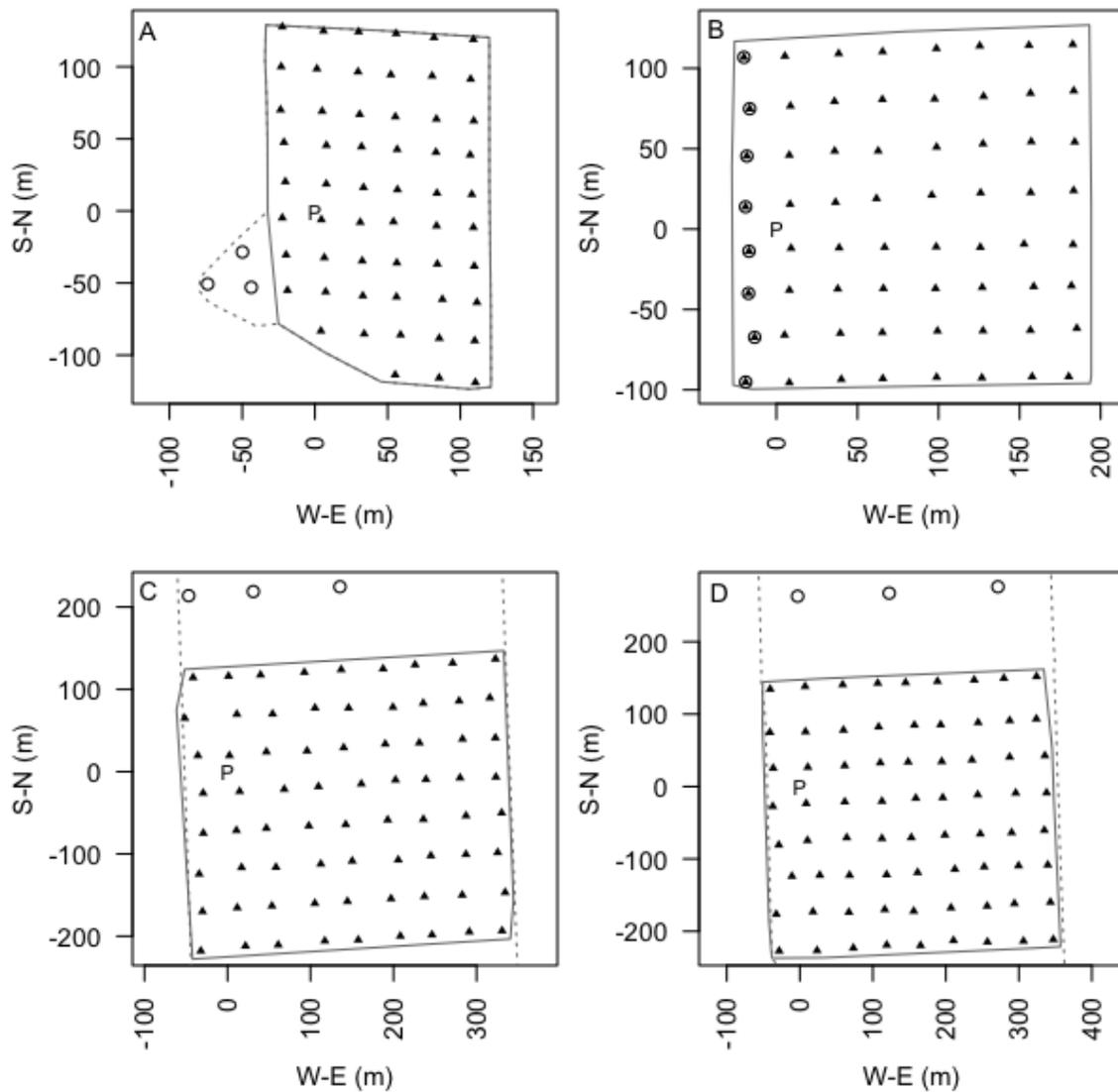
| Treatment | Site              |                    |                |                |
|-----------|-------------------|--------------------|----------------|----------------|
|           | <i>Big Valley</i> | <i>Burger Line</i> | <i>Dondero</i> | <i>Podesta</i> |
| Control   | 1,634 (6.9)       | 565 (2.2)          | 8,375 (29.1)   | 2,474 (8.6)    |
| 10 %      | 928 (3.9)         | 1,135 (4.4)        | 1044 (3.6)     | 1,772 (6.2)    |
| 25 %      | 585 (2.5)         | 2,280 (8.9)        | 4,002 (13.9)   | 596 (2.1)      |
| 50 %      | 785 (3.3)         | 693 (2.7)          | 885 (3.1)      | 2,155 (7.5)    |
| 100 %     | 709 (3.0)         | 1,375 (5.4)        | 3,721 (12.9)   | 591 (2.1)      |
| 40 Rings  | 2,925 (12.4)      | 725 (2.8)          | 4,513 (15.7)   | 875 (3.0)      |
| Average   | 1,261 (5.3)       | 1,129 (4.4)        | 3,757 (13.1)   | 1,411 (4.9)    |



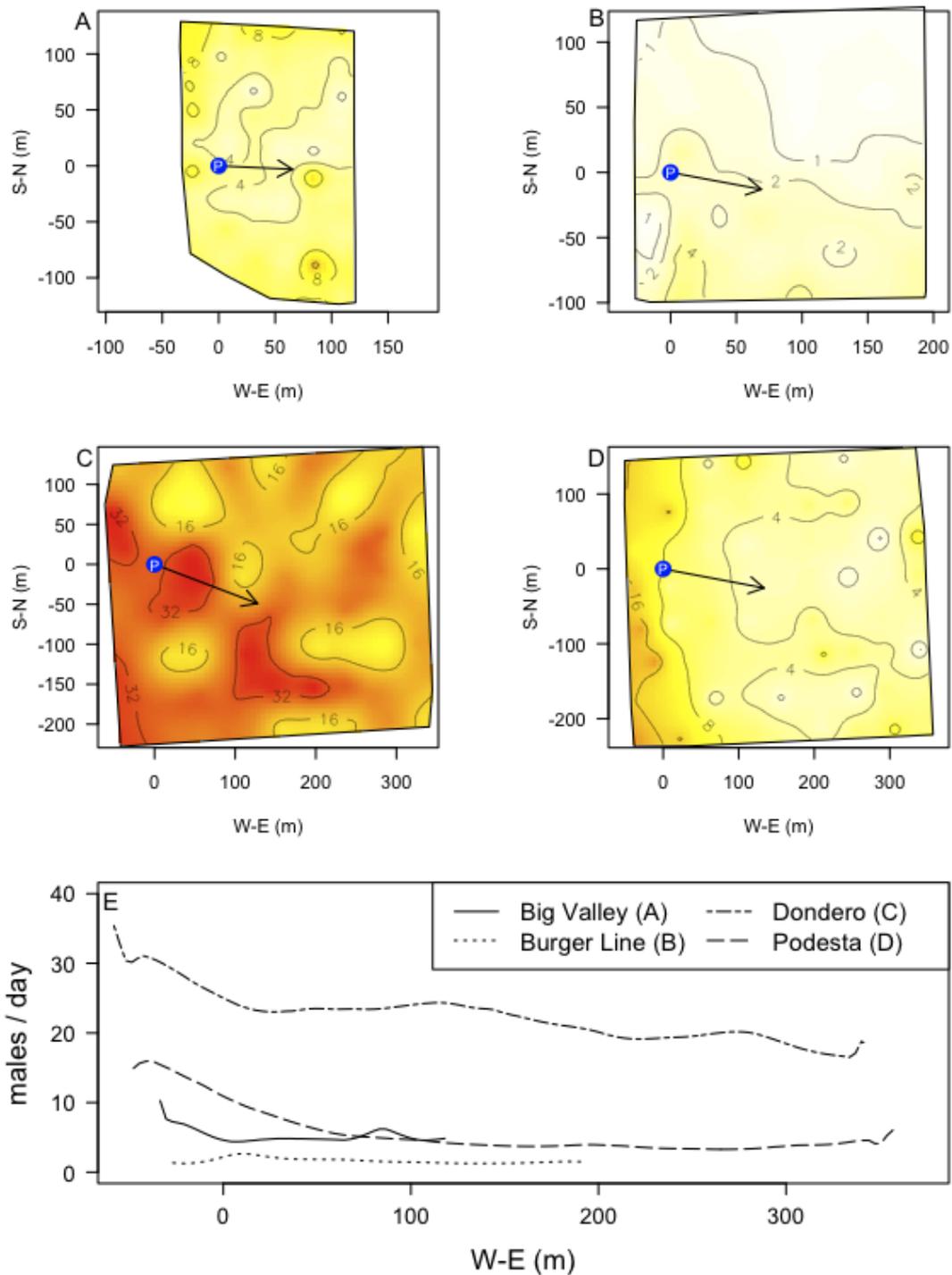
**Figure 1. Wind direction and intensity patterns at the different sites in 2011.** Arrow lengths are proportional to wind speed (within each wind rose). Number of arrowheads proportional to hourly counts in each direction.



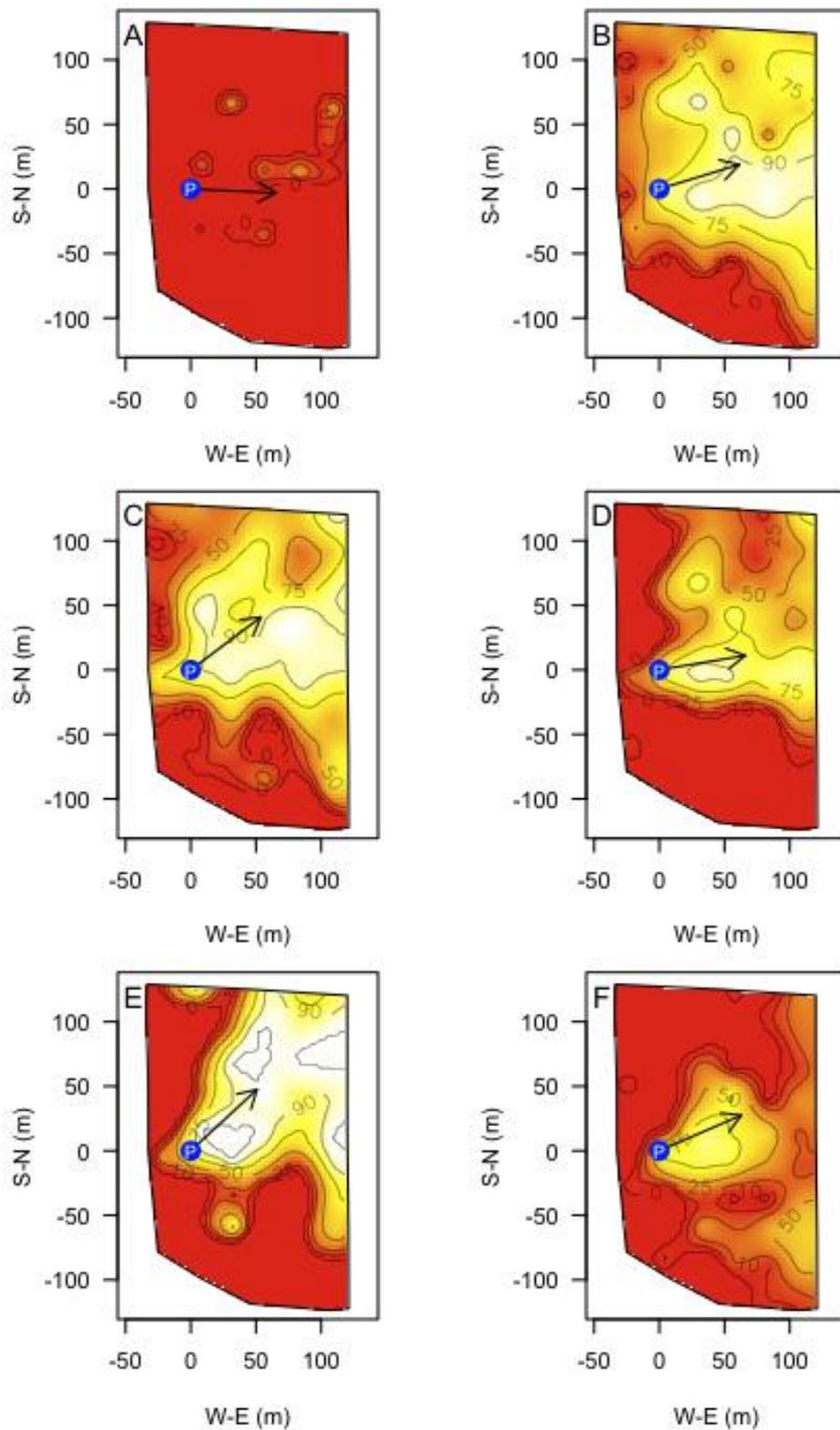
**Figure 2. Picture of 40 Isomate®-CM Rings as a single emission point.** The 40 rings were hung on a puffer hook, and prevented from falling by a double tied rope.



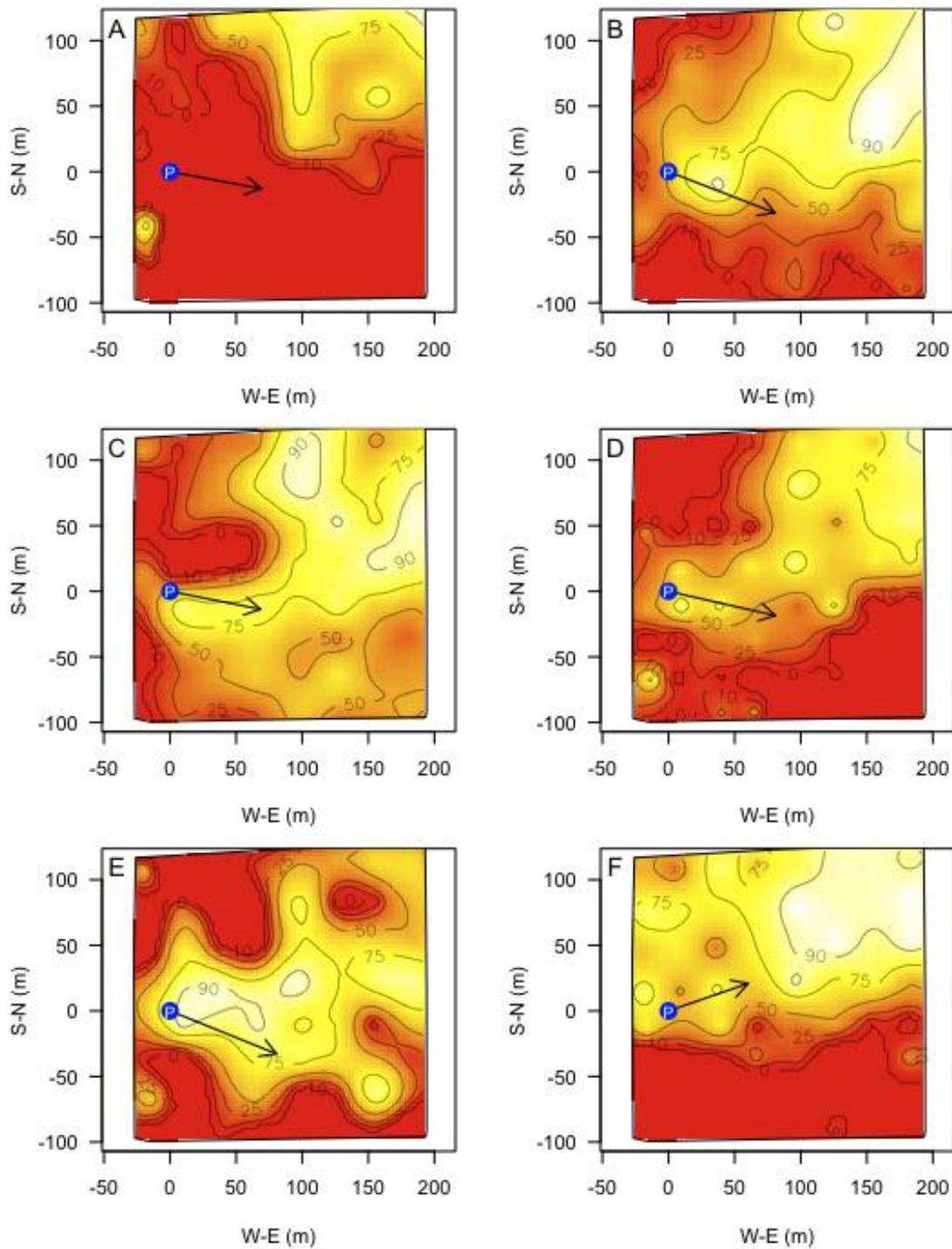
**Figure 3. Experimental design and trap displays in the 4 sites for the pheromone-rate, and passive *versus* active comparisons (Experiment 1).** Dotted lines represent orchard edges, and solid lines experimental plot limits. Triangles stand for within plot traps, and circles for control traps (for trap suppression calculations). P is the position assigned to the puffer/rings. A, Big Valley; B, Burger Line; C, Dondero; and D, Podesta.



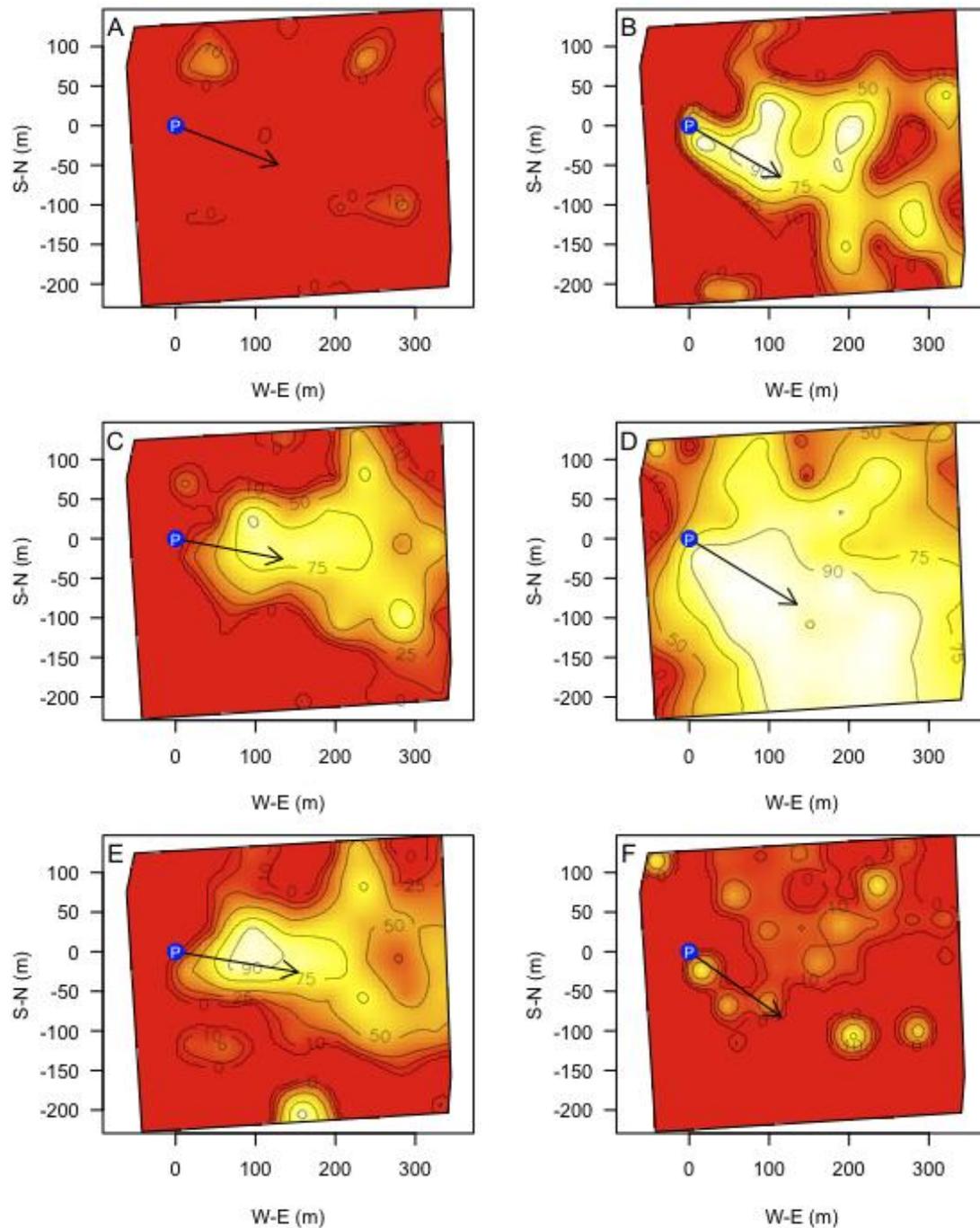
**Figure 4. Spatial predictions (A-D) of daily capture of sterile codling moth males in the 4 control (no pheromone) replicates by 1x pheromone-baited traps, and average captures in the west-east direction (E). Obtained by geostatistical interpolation. P signals the position for puffers (empty in these instances). The arrows show estimated average wind direction. A, Big Valley; B, Burger Line; C, Dondero; and D, Podesta.**



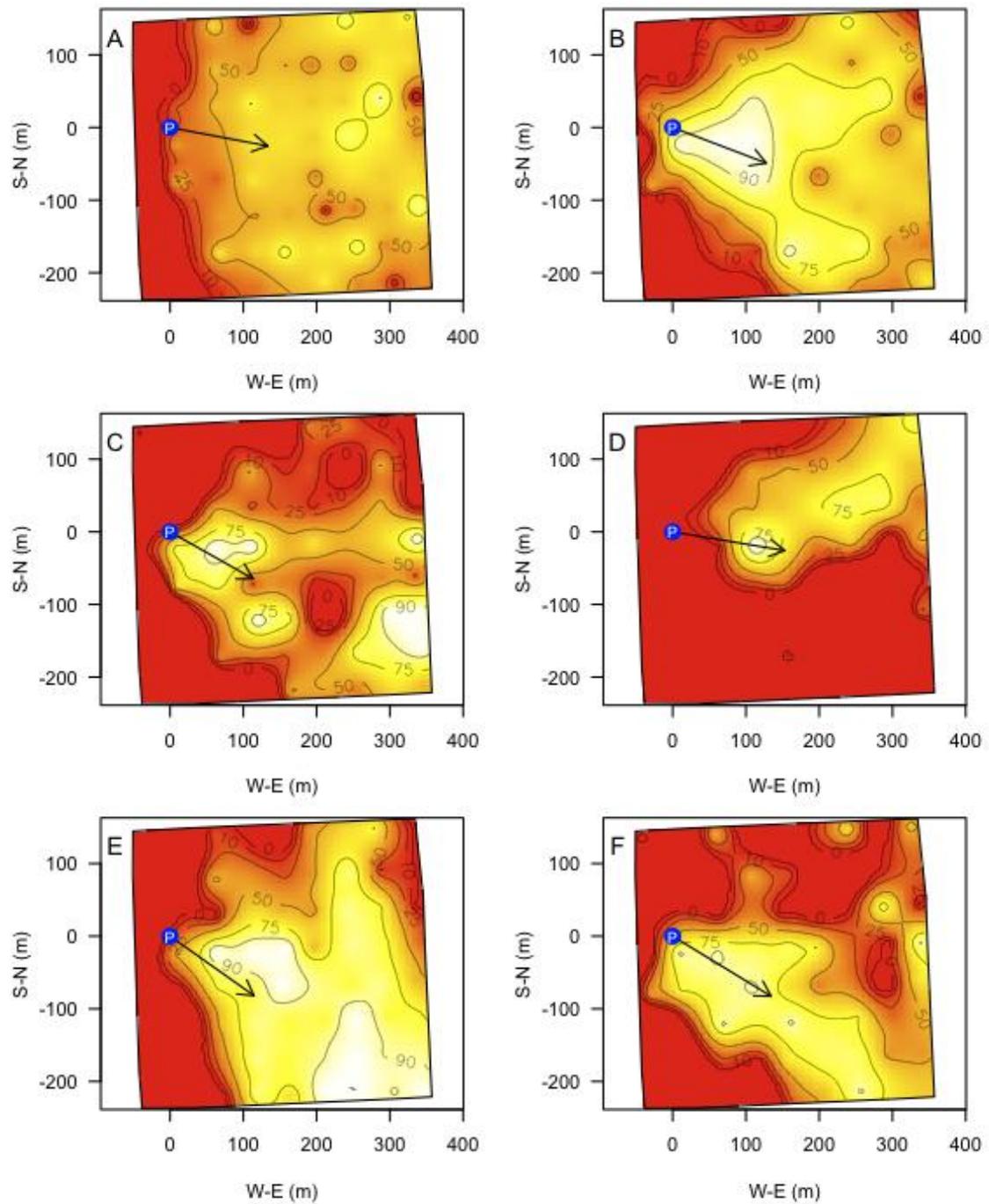
**Figure 5. Trap suppression (%) patterns at the trials in Big Valley (pear).** Obtained by geostatistical interpolation of 1x pheromone-baited traps. P signals the position of the puffer/rings. The arrows show estimated average wind direction. A, control; B, 10%-rate puffer; C, 25%-rate puffer; D, 50%-rate puffer; E, 100%-rate puffer; and F, 40 Isomate®-CM rings.



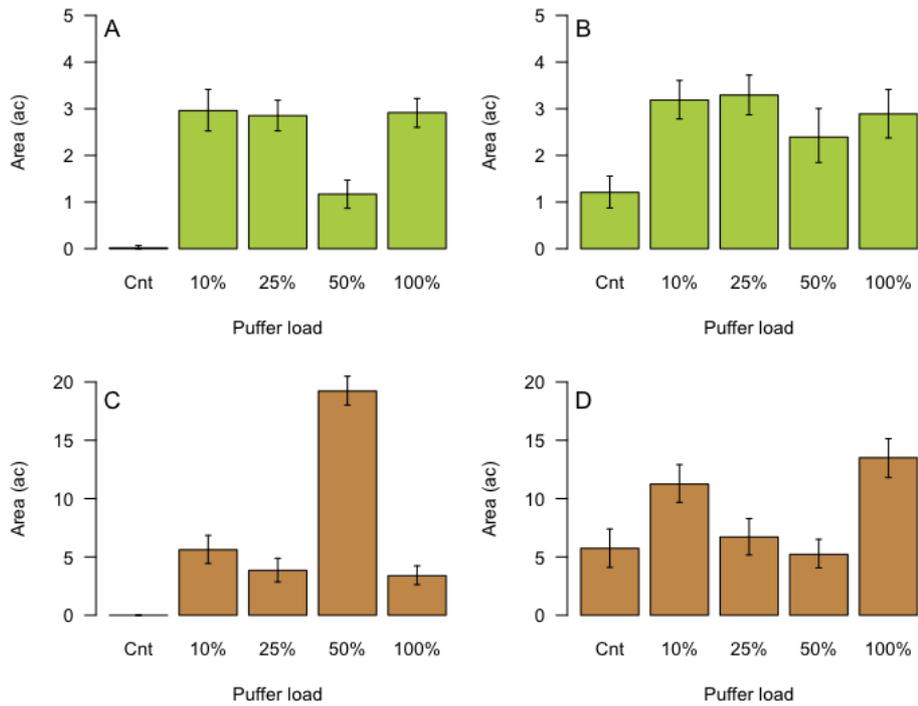
**Figure 6. Trap suppression (%) patterns at the trials in Burger Line (pear).** Obtained by geostatistical interpolation of 1x pheromone-baited traps. P signals the position of the puffer/rings. The arrows show estimated average wind direction. A, control; B, 10%-rate puffer; C, 25%-rate puffer; D, 50%-rate puffer; E, 100%-rate puffer; and F, 40 Isomate®-CM rings.



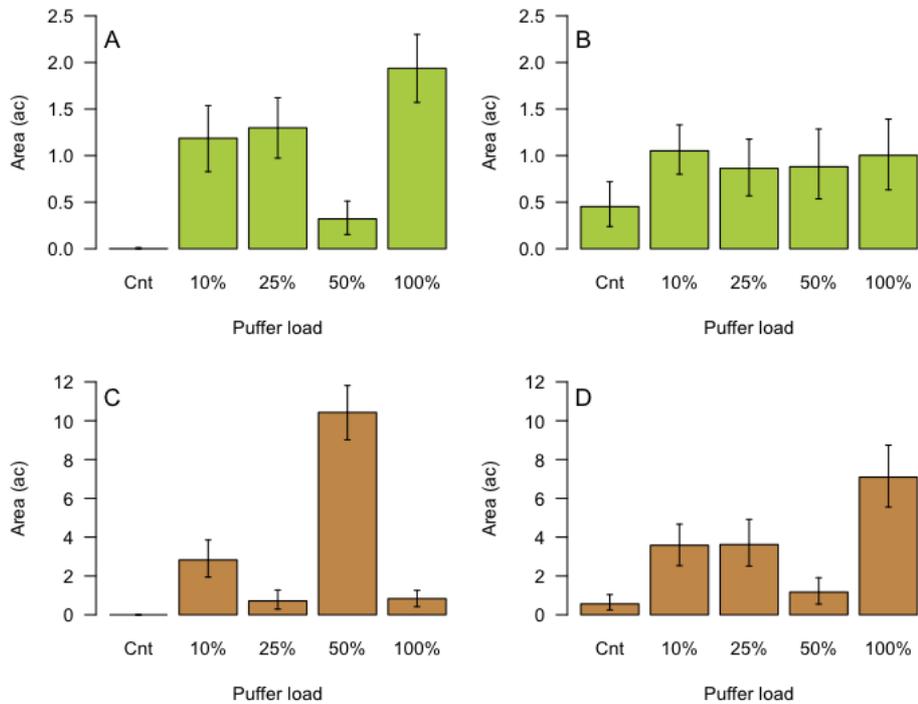
**Figure 7. Trap suppression (%) patterns at the trials in Dondero (walnut).** Obtained by geostatistical interpolation of 1x pheromone-baited traps. P signals the position of the puffer/rings. The arrows show estimated average wind direction. A, control; B, 10%-rate puffer; C, 25%-rate puffer; D, 50%-rate puffer; E, 100%-rate puffer; and F; 40 Isomate®-CM rings.



**Figure 8. Trap suppression (%) patterns at the trials in Podesta (walnut).** Obtained by geostatistical interpolation of 1x pheromone-baited traps. P signals the position of the puffer/rings. The arrows show estimated average wind direction. A, control; B, 10%-rate puffer; C, 25%-rate puffer; D, 50%-rate puffer; E, 100%-rate puffer; and F; 40 Isomate®-CM rings.



**Figure 9. Average area (and 95% confidence intervals) with trap suppression over 75% estimated by conditional simulation for the different sites and pheromone rates. A, Big Valley (8.6 ac); B, Burger Line (11.9 ac); C, Dondero (34 ac); and D, Podesta (37.5 ac).**



**Figure 10. Average area (and 95% confidence intervals) with trap suppression over 90% estimated by conditional simulation for the different sites and pheromone rates. A, Big Valley (8.6 ac); B, Burger Line (11.9 ac); C, Dondero (34 ac); and D, Podesta (37.5 ac).**



**Figure 11. Experimental design used for the movement assay at Freeport CA.** Blue dashed line denotes the orchard limits; blue circle shows the release-point; red circle shows the puffer-position; and orange triangles show the 1x pheromone-baited trap positions.