

**“Chem herding”: Adapting existing semiochemical delivery technologies to
improve the precision of weed biocontrol agents**

Report – October 25, 2012

Summary

This project was proposed to show the potential for using commercial semiochemical formulation technology to actively manipulate populations of the northern Tamarisk beetle *Diorhabda carinulata* Desbrochers (Dc hereafter). The project goals required the synthesis of a known pheromone of this species and commercial formulation of this and of a known host plant attractant into field ready formulations. Specific objectives required measurement of laboratory and field emission rates of these compounds and proof of concept trials at a field location.

The project has been complicated by two fundamental issues. The first is the difficulty in obtaining adequate quantities of pheromone and the second is the request for payment to prepare the formulations by the commercial manufacturers. It was anticipated that the synthesis of the pheromone would not be limiting and that the target companies would partner in the formulation. Neither of these was correct and together these require funds beyond the scope of the initial award.

To date, we have quantified the release rate from commercially-available rubber septa treated with three doses of (2E,4Z)-2,4-heptadien-1-ol. Release rates from these septa were quantified on multiple dates after exposure in incubators maintained at 25°C. Release rates were also collected from rubber septa impregnated with a single dose of pheromone or with a single dose of the known host plant compound (Z)-3-hexenal across three temperatures (20°C, 25°C and 30°C). These data were all collected under laboratory conditions. We have also tested beetle displacement using pheromone and host plant impregnated rubber septa in the field in a *Tamarix* infestation near Lovell, WY. The site had a low population of Dc and was suitable for testing limited quantities of pheromone or host plant attractant lures.

The first key finding was that the release rate of pheromone from commercial septa is quite rapid across treatment dose and across three temperatures. The release rate of host plant attractant is also quite rapid across the three temperatures. Impregnated septa are not an adequate media for deployment of these compounds. The longevity of the host plant treated septa was greater than that for pheromone treated septa, which was surprising. This pheromone is an atypically volatile compound.

The other key finding is that these short-lived pheromone lures effectively attract beetles to host trees when they are newly-deployed, but are no longer effective after a week in the field. There was no evidence that the host plant compound with greater longevity attracted the beetles to *Tamarix* trees, and deploying both pheromone and host plant treated septa was no better than pheromone alone. The goal of this project was to attract adult beetles to trees using semiochemicals, not to capture the insects in traps baited with semiochemical lures. This is an important distinction, because establishing the

beetles in the trees is very different from the single attract-and-capture events typically used to optimize blends and formulations.

Thus both the negative and positive results from these experiments support that further research is merited, but additional funds will be required. These additional funds will be used to facilitate synthesis of larger amounts of pheromone and to pay for the costs of preparing formulations, in addition to basic support for the field and laboratory research.

If supported, amounts of pheromone adequate for preparing two types of commercially available products will be produced. These will be tested to determine their feasibility to increase and maintain local abundance of Dc. The products targeted are Scentry® impregnated hollow microfibers and the ISCA® technologies wax based formula SPLAT™. Both of these products were originally developed to deliver pheromones for mating disruption. These products will be tested because of their potential of maintaining a constant release rate over long periods of time and ease of application. This proposal is focused on the long term relocation of beetles. This is different from previously conducted research that has focused on trapping experiments to demonstrate the attractiveness of compounds to Dc.

SPLAT™ is a mixture of water, wax and vegetable oils and contains common preservatives and emulsifiers. SPLAT™ is most commonly applied aerially but can also be applied by hand dispenser or by use of a local-scale mechanical spray implement. All ingredients in SPLAT™ are listed by the Environmental Protection Agency as safe and non-toxic. After SPLAT™ is applied, it dries and clings to the trees and begins to release its pheromone. Once it comes in contact with the soil, the oils and wax are quickly degraded. SPLAT™ has been used in both agricultural and forest settings.

The Scentry® microfibers allow for season long mating disruption (45-70 days). They do not photodegrade and are not negatively impacted during rainfall events. They are applied mechanically and results in increased point source dispersion compared to other controlled release products used in crop and orchard settings.

Aggregation Pheromone

Two male-produced aggregation pheromones have been identified for Dc: (2E,4Z)-2,4-heptadienal and (2E,4Z)-2,4-heptadien-1-ol, both produced antennal responses in both males and females. These two pheromones are very different from other known chrysomelid pheromones because they are small, simple molecules. These compounds can also be found in the foliage of *Tamarix* and are also released when the beetles feed on the foliage. The small amount of these compounds released by the females is believed to originate in foliage and is not produced by the females. The males control the emission of the two compounds, suggesting biosynthesis of these pheromones in body tissue rather than acquisition from the foliage.

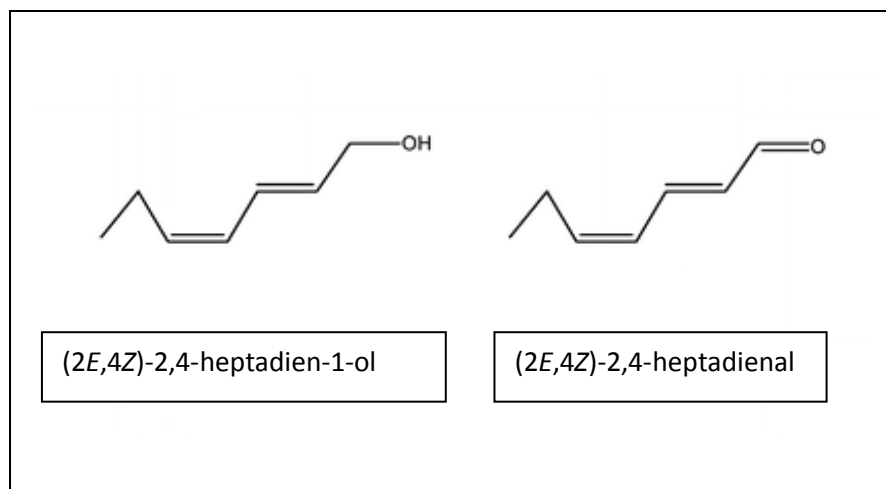


Figure 1. Dc Pheromones

(2E,4Z)-2,4-heptadien-1-ol is much more attractive than (2E,4Z)-2,4-heptadienal and as attractive as a 1:1 blend of the two pheromones. (2E,4Z)-2,4-heptadien-1-ol attracted roughly equal numbers of males and females, which suggests this is an aggregation pheromone and not a sex pheromone. Thus, we used only the alcohol form of the pheromone in our research.

Initial Project Objectives

- 1) Determine release rates of (2E,4Z)-2,4-heptadien-1-ol from rubber septa (10 mm Precision Seal® rubber septa , Sigma-Aldrich Chemical Co. Milwaukee, Wisconsin)
- 2) Determine release rates of plant volatile (Z)-3-hexenal from rubber septa
- 3) Show proof of concept in field trials with *Tamarix* stands that have Dc present.

Results from these project objectives will be the basis for a larger scale trial using the commercially available formulations when there are greater amounts of pheromone available

Methods

Laboratory trials to quantify release rates:

Milligram scale amounts of the pheromone (2E,4Z)-2,4-heptadien-1-ol were synthesized according to the published method but with minor modifications to improve efficiency. (2E,4Z)-2,4-heptadien-1-ol has been shown to be one component of the Dc pheromone and captures equivalent numbers of insects when compared to a paired pheromone combination in field trials using baited sticky traps.

Rubber septa, which are composed of standard rubber, accelerator, oxidizer, hard resin acid, anti-ager, lime carbonate and dye, were impregnated with (2E,4Z)-2,4-heptadien-1-ol by adding a dichloromethane solution (125µl) to the septa. Three solutions were prepared, containing either 250µg,

500µg or 1000µg of (2E,4Z)-2,4-heptadien-1-ol per 125µl aliquot. After evaporation of the dichloromethane, wire paper clips were inserted into the rubber septa to individually suspend the septa in an incubator to ensure that there was no contact between them. The septa were in three separate incubators at 25°C, each incubator receiving one of the three different concentrations. Each incubator was vented three times a day to prevent accumulation of volatiles in the incubator. Volatiles were collected at three, six, nine, fourteen, twenty one and twenty eight days to determine the release rate of the pheromone in the rubber septa. Collections were made in glass volatile collection chambers (inner diameter 35mm, length 625mm) over a two hour period. Volatile collection traps containing 30mg of Super-Q (Alltech Associates, Inc.) adsorbent were fixed in place at the apical opening of the glass chamber. Purified air (humidified) was delivered at a rate of 100 ml/min. Collected volatiles were eluted from the traps into vials using methylene chloride (200µl) and the samples were spiked with 10µL of a .73ng/µL solution of nonene in hexane as an internal standard. Volatiles were analyzed using a GC (Agilent 6890 instrument; Agilent Technologies, Santa Clara, California) coupled to a mass selective detector (MSD, Agilent 5973 instrument). Quantification of compounds was made relative to the internal standard.

An experiment to test the effects of temperature on release rates from the septa was conducted. This experiment also included the key component in the host plant attractive blend, (Z)-3-hexenal, which is commercially available at high purity. A formulation of this host plant compound was also tested using these rubber septa. Nine septa were impregnated with 4 mg of (Z)-3-hexenal per 125µL of dichloromethane and nine septa were impregnated with 5 mg of (2E,4Z)-2, 4-heptadien-1-ol per 125µL of dichloromethane. These lures were then split among incubators maintained at either 20°C, 25°C or 30°C. The lures were held in incubators vented three times a day over a fifteen day period. Volatiles were collected from the lures at four day intervals using the methods described above.

Field trials to determine the effect on Dc

Field experiments were carried out between July and September 2012 in *Tamarix* stands along the Big Horn River southwest of Lovell, WY, USA. The field site (44° 46'30", 108° 11'8") received beetles from Fukang, China in 2001 and currently has a marginal population of Dc.

The first experiments measured the effect of the compounds on Dc distribution and were conducted from July 2nd through August 6th, 2012. Treatments consisted of rubber septa treated with 5 mg of (2E,4Z)-2,4-heptadien-1-ol, with 4 mg of (Z)-3-hexenal or a solvent treated control. A random design was used for each of four experimental treatment dates. On each experimental date a discrete area received four treatments via rubber septa prepared as described above. Four trees were baited with 5 mg of pheromone; four trees were baited with 5 mg of pheromone plus 4 mg of the plant compound (as two separate lures) and four trees were baited with only the plant compound. Two trees were baited with solvent treated lures as controls. All lures were isolated in transportation and carefully suspended to prevent cross contamination of the septa. Overall, there were 8 replicates of each treatment and 4 replicates of the untreated control.

The septa were placed in *Tamarix* trees that were at least five meters apart. Due to natural variation, it was not possible to fully control for similar sizes, foliage density and age of trees. These parameters were recorded to correct for tree to tree bias. Temperature and humidity dataloggers were also deployed. Beetle counts were taken using sweep nets before the lures were placed, one week after the lures had been deployed, and one week after the lures had been removed from the tree. Trees were sampled in the morning (9:30am-12:00 noon) before peak flight hours (mid-to-late afternoon) to maximize the number of beetles on vegetation. Sweep samples were taken using a canvas sweep net with an opening diameter of 30.5cm and a depth of 61cm. One sweep consisted of a 1.25m upward arc. At the end of each arc, a quick and fluid twist of the net was used to prevent the escape of captured Dc. Each tree was swept three times. After the three sweeps the captured Dc adults and larva were counted. All individuals were placed back on the tree to minimize disturbance.

After one week in the field, the first set of lures were removed, isolated and returned to the laboratory. Volatiles were then collected from these field-exposed lures to determine the release rates of the different compounds. The septa that had been deployed in the field for a week were then stored at -30°C until they were used in the field again at a later date. When the first set of lures was removed, a second set of fresh lures were deployed. After a week of field exposure, these were also returned to the lab for volatile collection. The first sets of lures (already subjected to a week of field exposure) were returned to the field. After another week of field exposure, the lures were collected for laboratory determination of release rates. These were then replaced by the second set of lures that had experienced one week of field conditions. After a week these lures were removed for volatile collection in the laboratory. Therefore, there were also 8 replicates of each treatment and 4 replicates of the untreated control for the week old lures.

The following data was collected weekly starting July 2 and ending August 6

- Baited trees: number of larvae and adults of Dc
- Percent herbivore damage (Dc and other possible herbivore damage)
- Number of potential predators
- Plant volume
- Plant morphology and condition: green foliage, senescing, and dead
- Reproductive status: flowers (y/n), seeds (y/n)
- Temperature
- Relative humidity
- Latitudinal and longitudinal coordinates of each tree

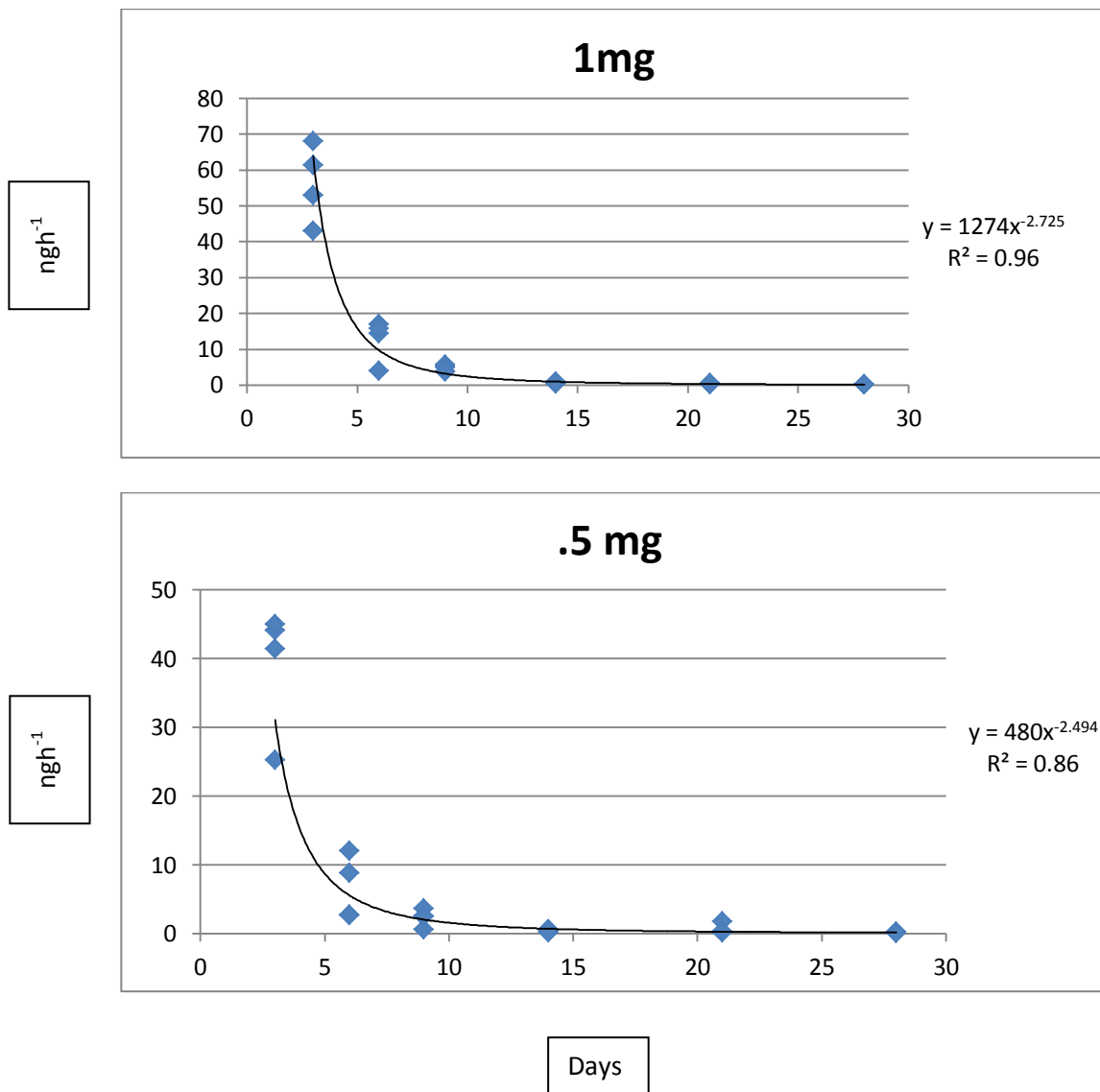
Other field data was collected on August 6, 2012 and again on September 14, 2012. A full census was taken to determine the numbers of Dc across the entire *Tamarix* population. This was conducted to assess if the lures impacted the final distribution of the Dc population. The stand was sampled once before the beetles entered reproductive diapause (August 6th) and once after the beetles had entered reproductive diapause (September 14th) but when they were still active on the foliage. Every tree was swept using the methods described above to determine the density of Dc on the tree and the

corresponding coordinates were noted. Contour maps were generated to evaluate the distribution of the Dc adults relative to the treated trees.

Results:

Laboratory trials to quantify release rates

The first experiment determined the release rate of commercial rubber septa with different concentrations of the pheromone. The pheromone was released at an exponential rate of decay (Figure 1). Rubber septa, as a cheap and readily available dispenser, do not appear to be a viable option for a slow release formulation of the pheromone because most of the compound evaporates from the septa in the first few days.



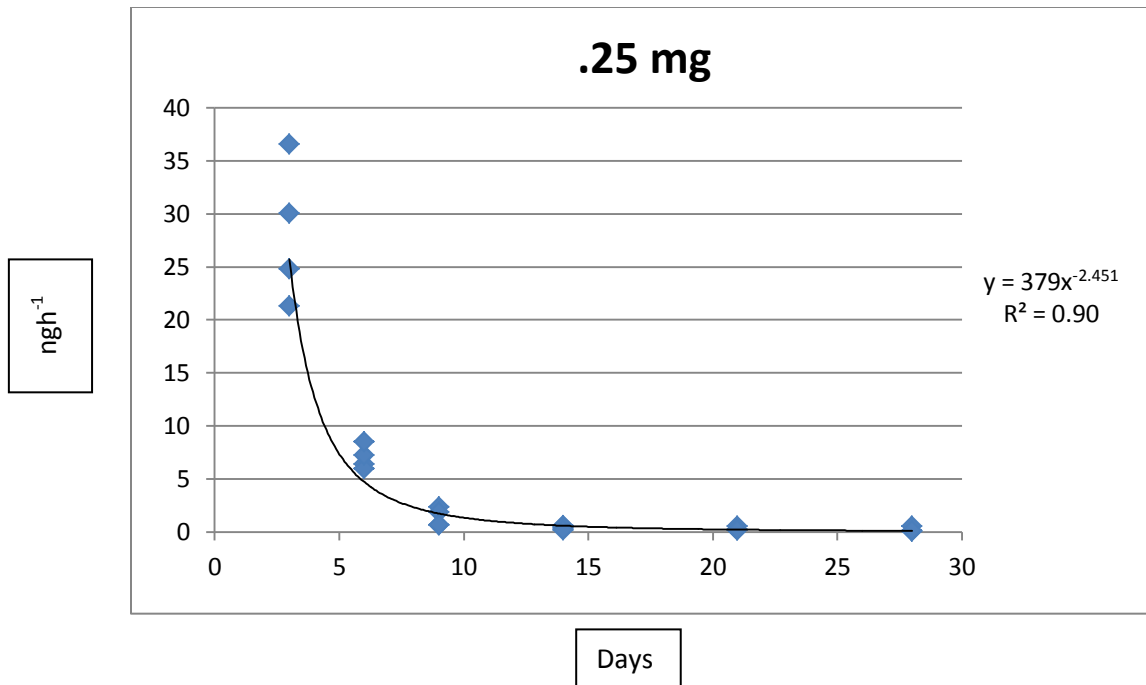


Figure 2. The release of (2E,4Z)-2,4-heptadien-1-ol from impregnated rubber septa at 0.25, 0.5, and 1 mg in the laboratory.

Table 1. Mean release rates with standard deviation

<u>Means for 1 mg</u>						
Days:	3	6	9	14	21	28
Mean:	56.31± 10.78	12.77±5.94	4.53±0.9	0.70±0.23	0.29± 0.10	0.18±0.03
<u>Means for .5mg</u>						
Days:	3	6	9	14	21	28
Mean:	38.93± 9.24	6.55±4.66	2.31±1.25	0.43±0.22	0.58±0.76	0.17±0.08
<u>Means for 0.25mg</u>						
Days:	3	6	9	14	21	28
Mean:	28.16±6.66	7.00±4.66	1.38±.87	0.41±0.20	0.27±0.18	0.24±0.24

Laboratory Trials to Determine Temperature Effects

The second laboratory experiment evaluated the effect of temperature on the release rates of the rubber septa impregnated with the pheromone and the key plant volatile. Rubber septa were impregnated with 5 mg of pheromone and 4 mg of the plant volatile. The septa were incubated in separate incubators at 20, 25, and 30°C. The pheromone concentrations measured once again showed an exponential rate of decay in release rates at all temperatures while the plant compound release rate was more linear. Mean release rates of (2*E*,4*Z*)-2,4-heptadien-1-ol for day four at 25°C could not be determined because of saturation of the GC-MS sensor by the unexpectedly large amount of compound collected. It was expected that release rates from the rubber septa would be higher with increasing temperature. However, from the trials conducted, it appears that the pattern of release rates from septa are similar over the three temperatures tested (Fig 3, 4), only the (*Z*)-3-hexenal impregnated septa incubated at 20°C appears to deviate from this pattern.

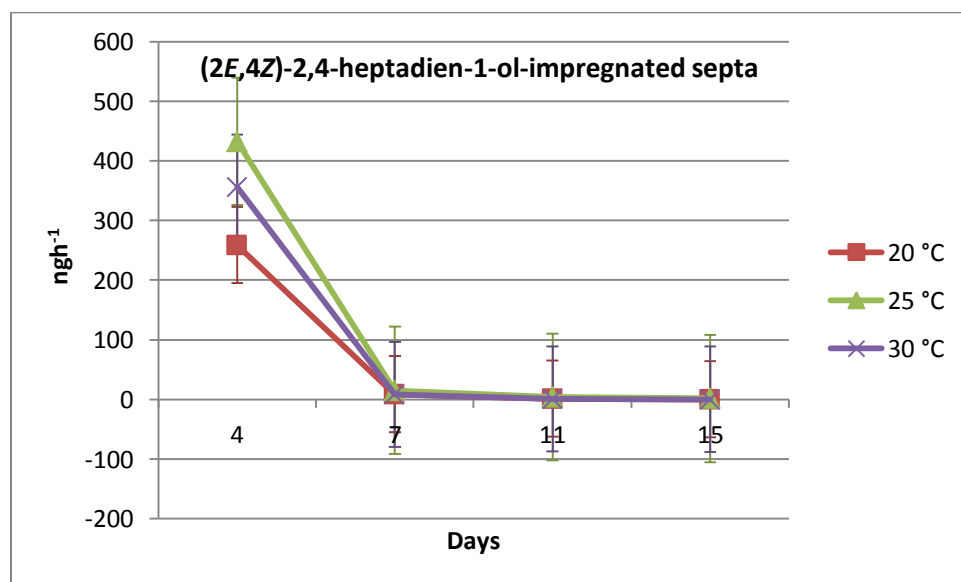


Figure 3. Mean release rate of (2*E*,4*Z*)-2,4-heptadien-1-ol at 20°C, 25°C, and 30°C

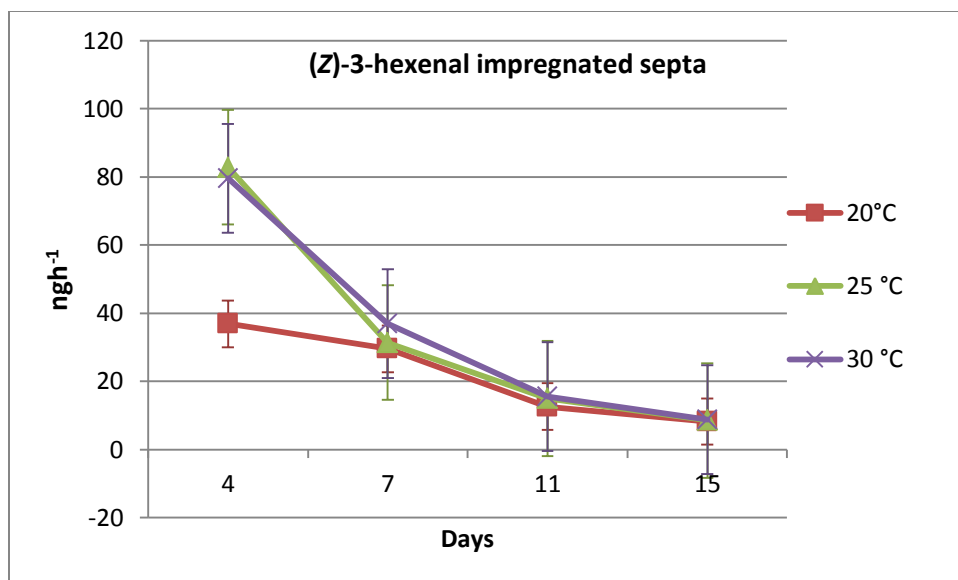


Figure 4. Mean release rate of (Z)-3-hexenal at 20°C, 25°C, and 30°C

Table 2. Mean release rate for (2E,4Z)-2,4-heptadien-1-ol and (Z)-3-hexenal

Mean (2E,4Z)-2,4-heptadien-1-ol						
days	20 °C	SD	25 °C	SD	30 °C	SD
4	258.89	±60.74	-----	-----	355.64	±158.20
7	8.74	±3.03	15.22	±3.88	8.28	±7.12
11	1.03	±0.14	3.89	±1.09	0.69	±2.89
15	0.29	±0.09	1.47	±0.72	0.26	±1.14
Mean (Z)-3-hexenal						
days	20 °C	SD	25 °C	SD	30 °C	SD
4	36.86	±1.41	82.85	±3.35	79.56	±8.82
7	29.54	±2.47	31.35	±0.89	36.94	±4.20
11	12.59	±1.65	14.93	±0.43	15.47	±1.24
15	8.19	±1.39	8.48	±0.54	8.83	±1.05

Field Trials

After only one week in the field, the lures released the pheromone at a rate of $1.5 \text{ ngh}^{-1} (\pm 0.47)$. According to the literature, a single beetle releases approximately 5 ngh^{-1} . Thus, after one week in the field, each lure was releasing pheromone at a rate approximating one fifth of a beetle. The lures released the plant compound at $7.6 \text{ ngh}^{-1} (\pm 1.69)$. After the second week in the field, the pheromone lures did not release a detectable amount of pheromone, but released the plant compound at $3.8 \text{ ngh}^{-1} (\pm 1.00)$.

For each initial deployment date, all baited trees were sampled using a sweep net at three times, once at the time of placement to establish a baseline, once after the lures had been in the field for a week and finally another week after the lures had been removed to determine the long term relocation potential of the lures for Dc.

The area selected for the first deployment (Fig 7) did not have any Dc before lure placement. After a week with fresh lures, 12 beetles were swept using the method described above. 10 beetles were on the trees baited with pheromone only, while 2 were found on trees with the pheromone and plant compound. No Dc were found on the control trees or on those baited with the plant compound only. The week after the lures were removed, no Dc were found. This indicates that the lures were attracting adults from the surrounding area and concentrating these on specific trees (Fig 4). However, after the lures were removed, the adults dispersed.

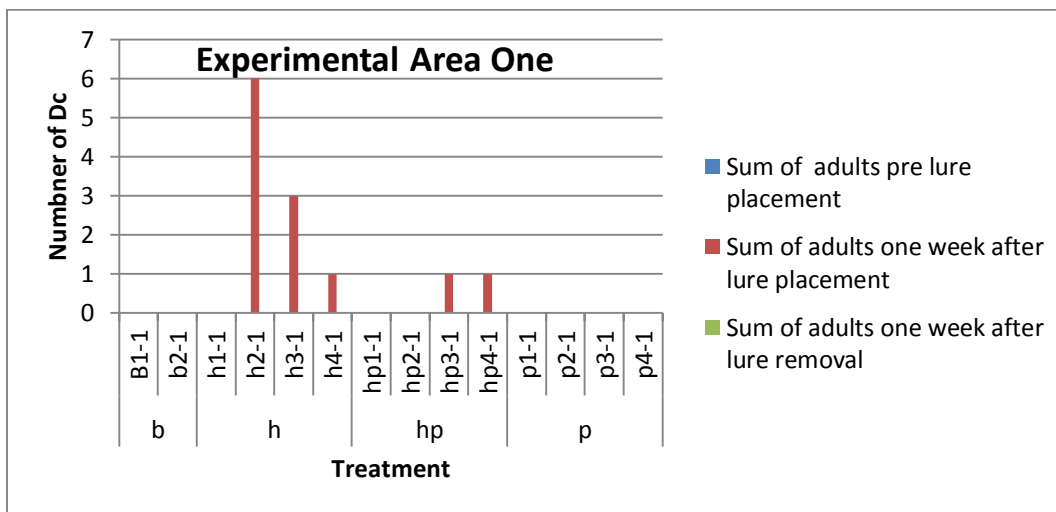


Figure 5. Number of beetles captured per baited tree before lures were placed, one week after lures were placed and one week after lures were removed for experimental area one. B = control tree, h = pheromone baited tree, hp = pheromone and plant volatile baited tree, p = plant volatile baited tree.

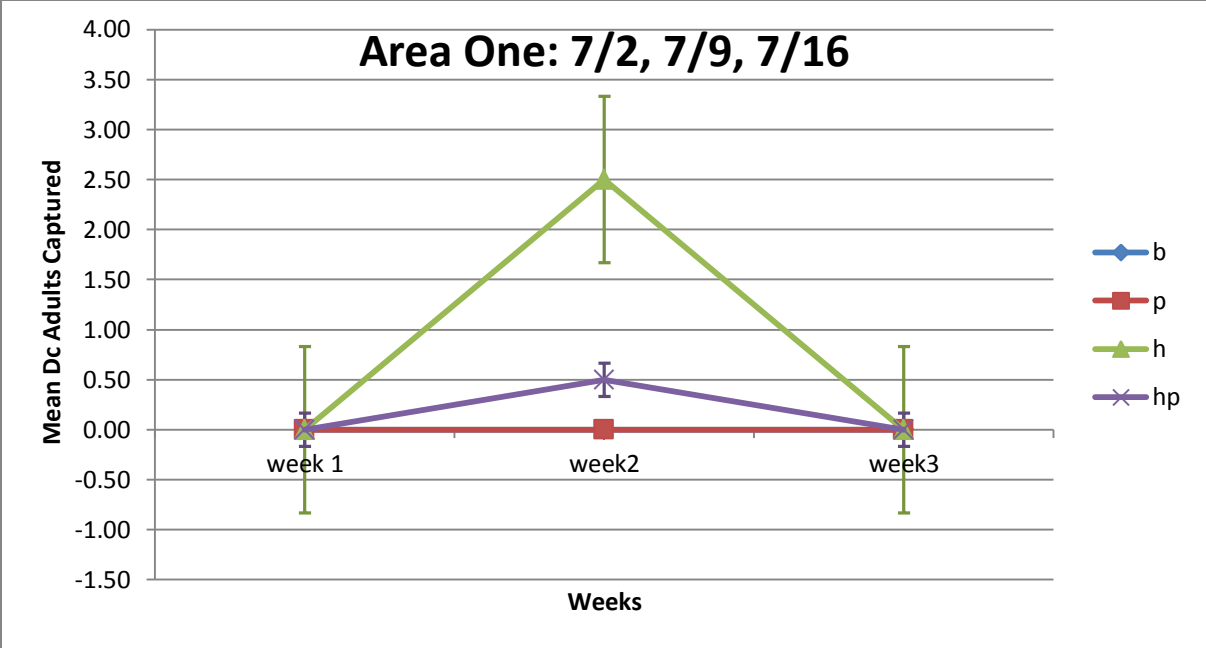


Figure 6. Mean number of adult Dc captured per week. b = control tree, h = pheromone baited tree, hp = pheromone and plant volatile baited tree, p = plant volatile baited tree.

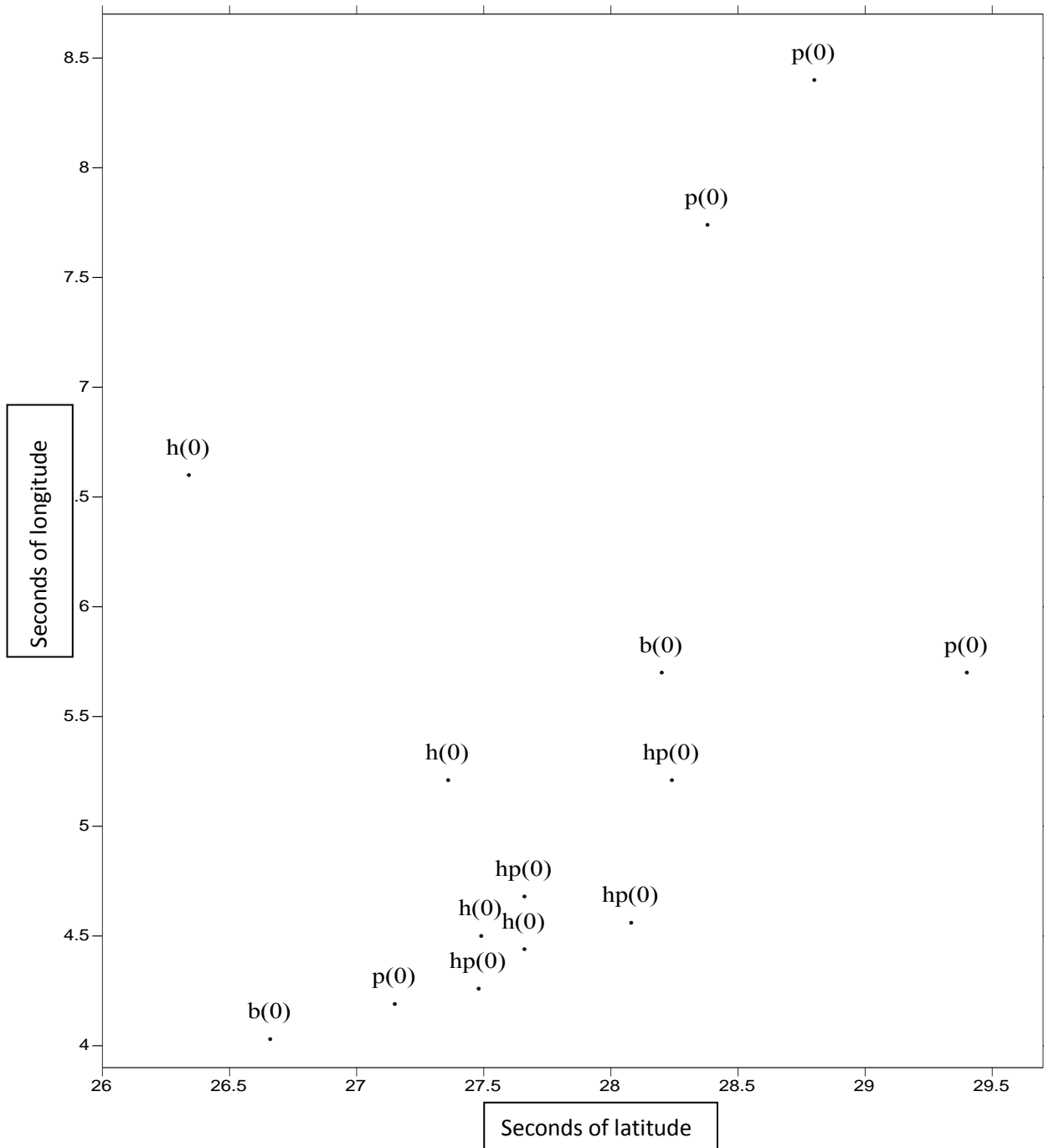


Figure 7. A posting map of experimental area one Dc counts before lure placement.

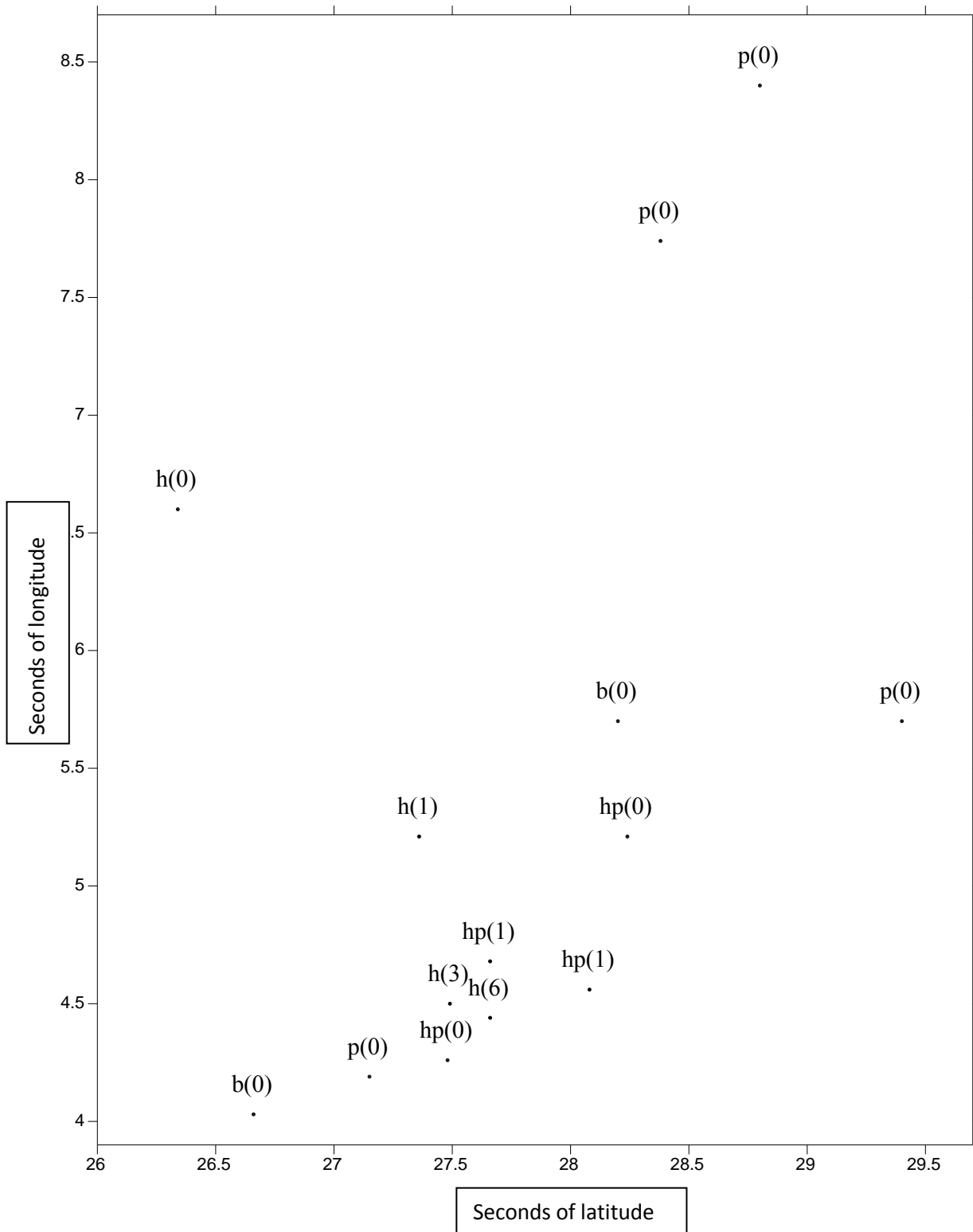


Figure 8. A posting map of experimental area one Dc counts immediately after lure removal.

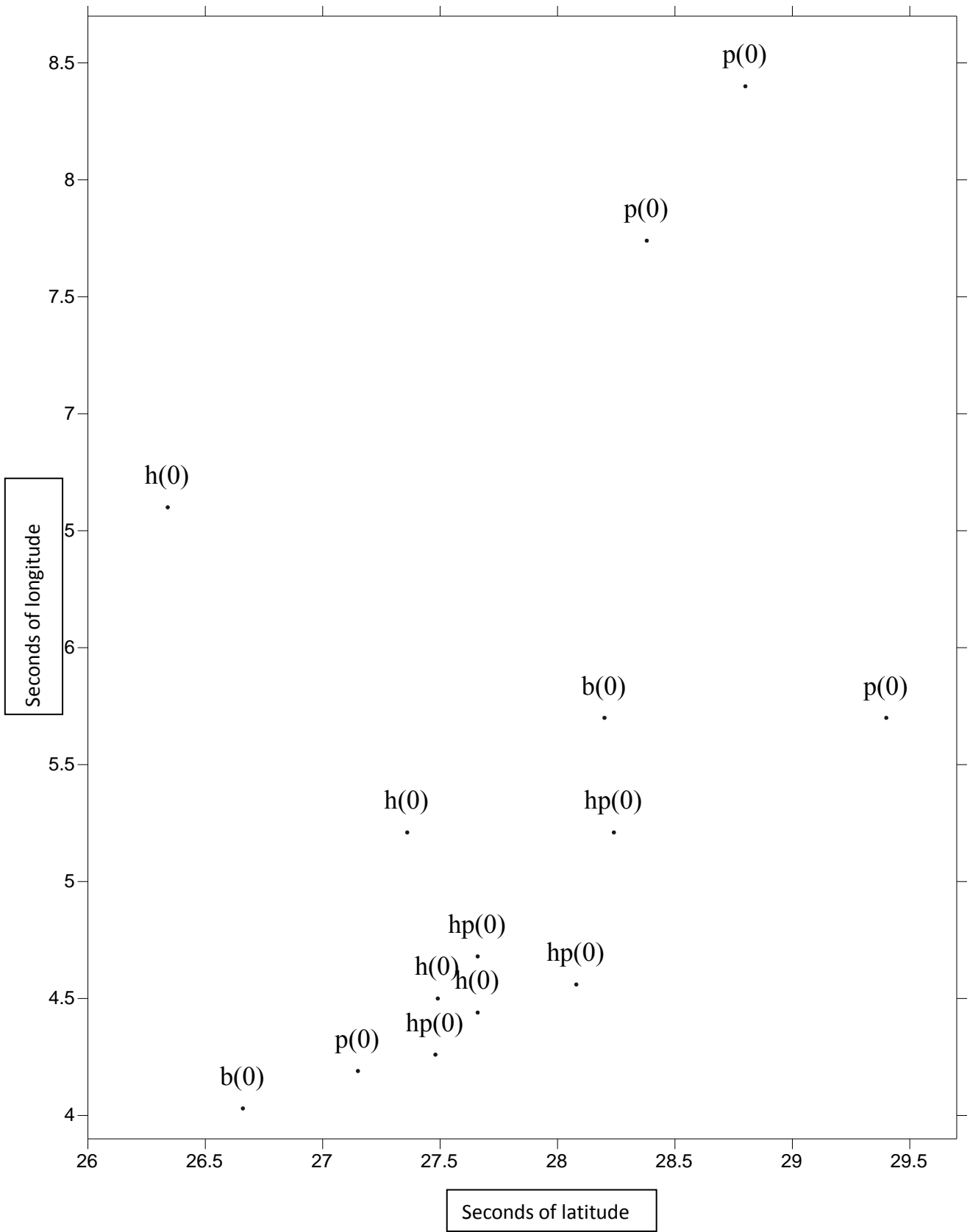


Figure 9. A posting map of experimental area one Dc counts the week after lure removal.

The area selected for experiment two (Fig 10) had an adult Dc on a tree that subsequently received a pheromone lure. After a week of exposure to fresh lures, seven beetles were collected. Four were found on pheromone baited trees while three were found on pheromone and plant baited trees. The control and plant baited trees had no beetles. The week after the lures were removed, 2 adults were found. One was on a pheromone treated tree, and one on a control. Experimental area two also shows proof of concept with the relocation of adults to trees that previously had no adults present (Fig 11).

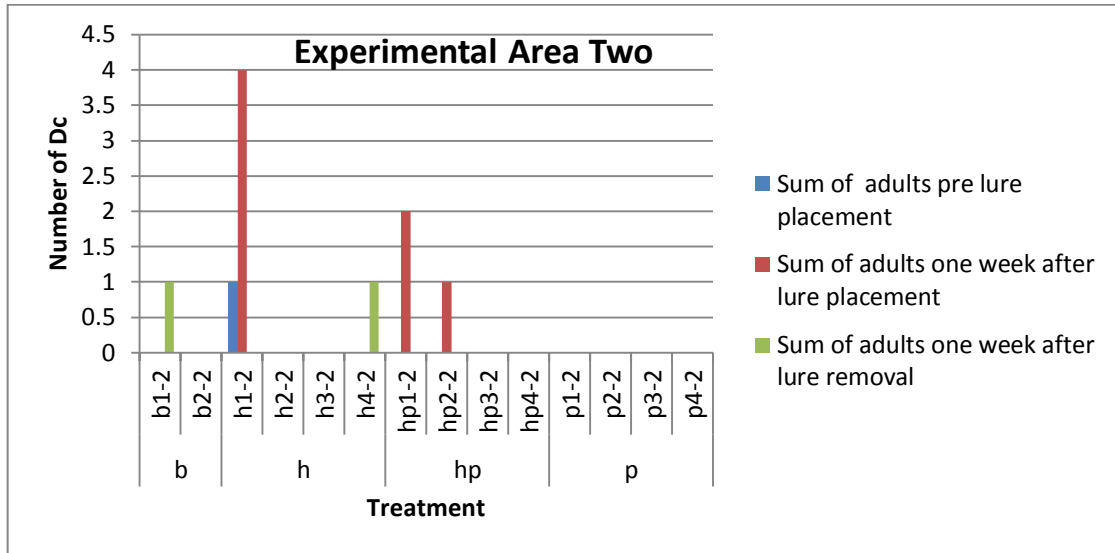


Figure 10. Number of beetles captured per baited tree before lures were placed, one week after lures were placed and one week after lures were removed for experimental area two. B = control tree, h = pheromone baited tree, hp = pheromone and plant volatile baited tree, p = plant volatile baited tree.

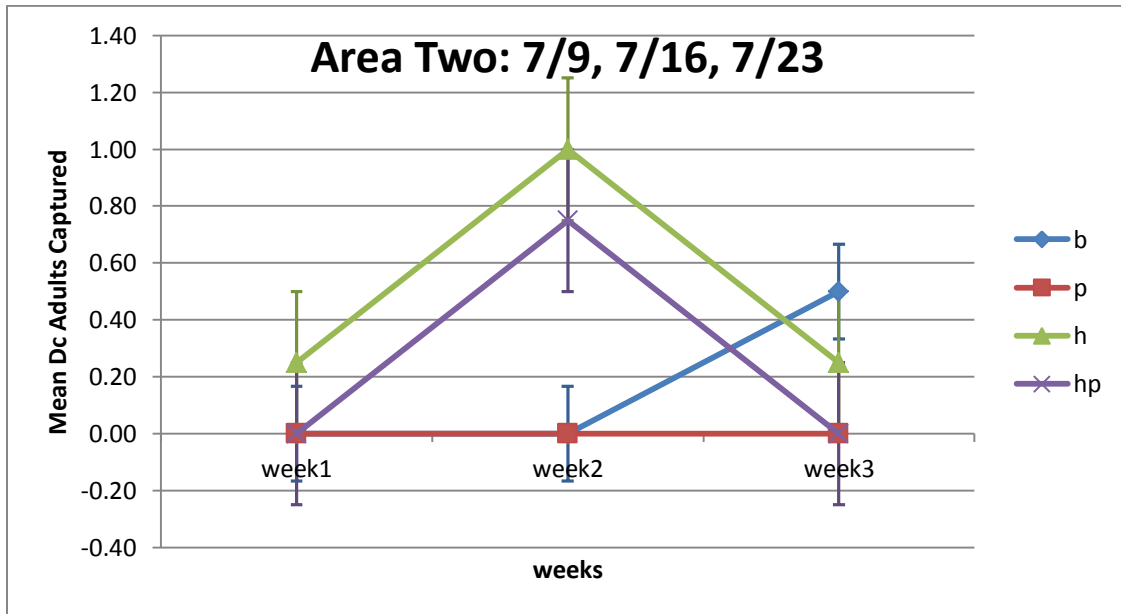


Figure 11. Mean number of adult Dc captured per week. b = control tree, h = pheromone baited tree, hp = pheromone and plant volatile baited tree, p = plant volatile baited tree.

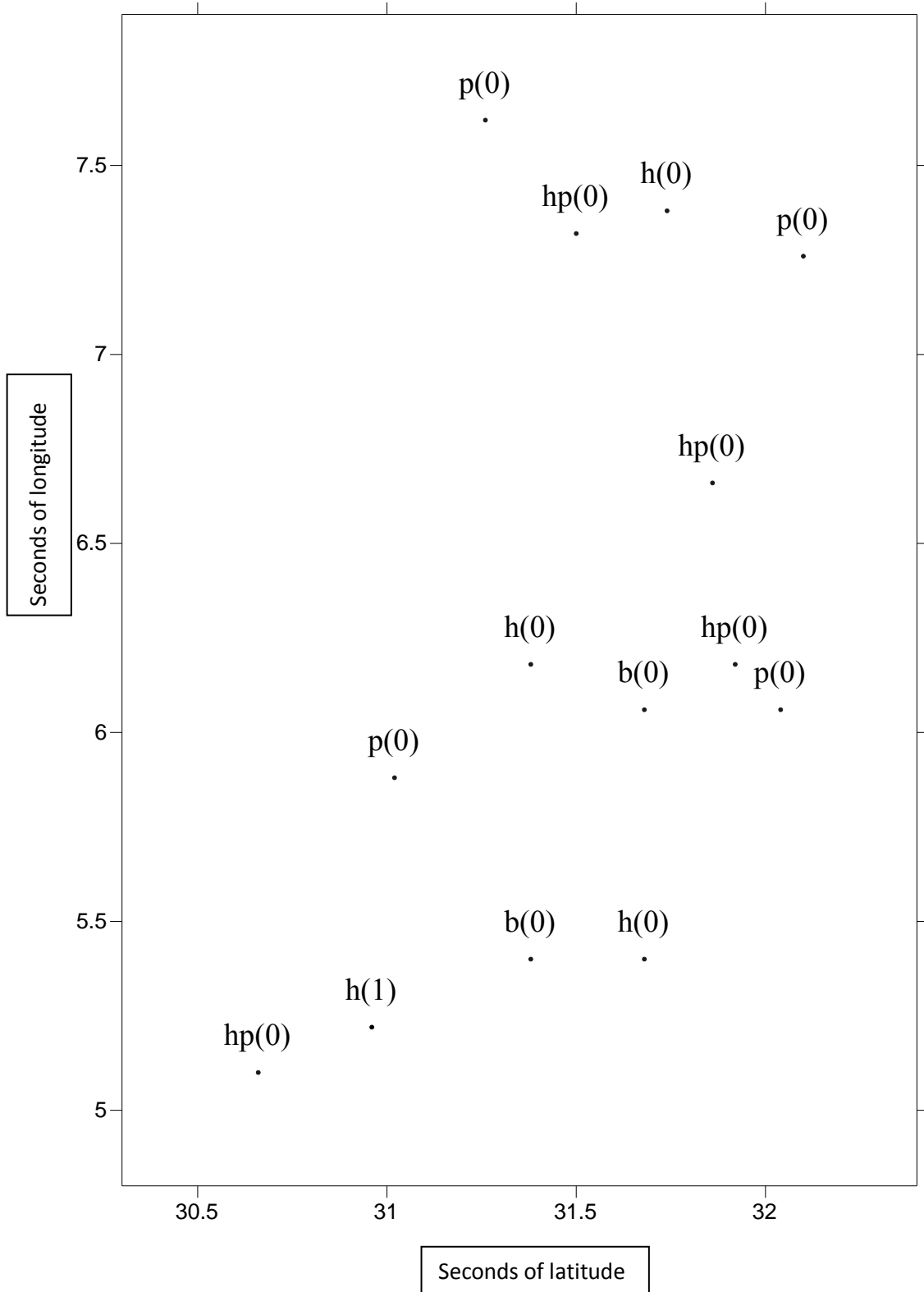


Figure 12. A posting map of experimental area two Dc counts before lure placement.

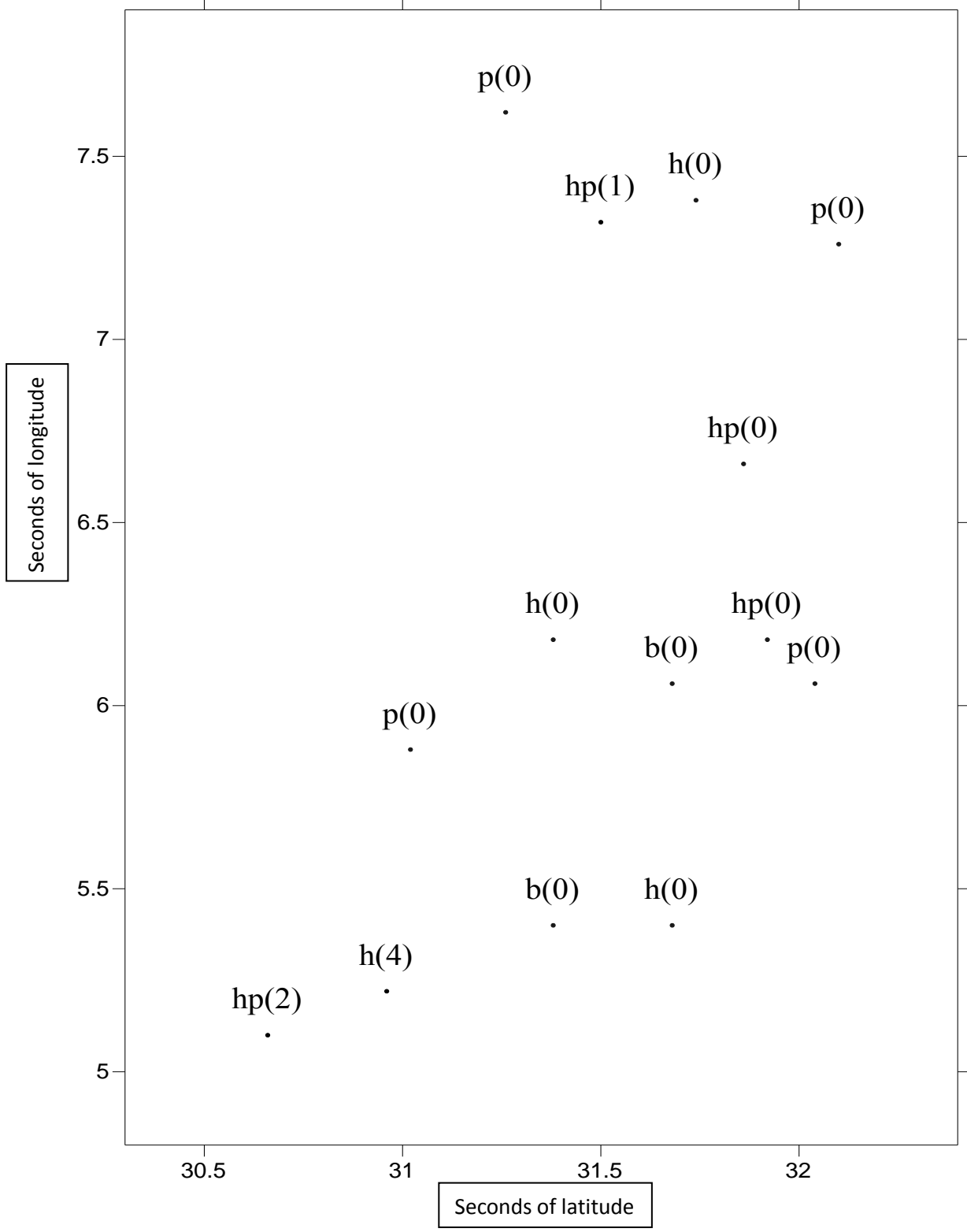


Figure 13. A posting map of experimental area two Dc counts immediately after lure removal.

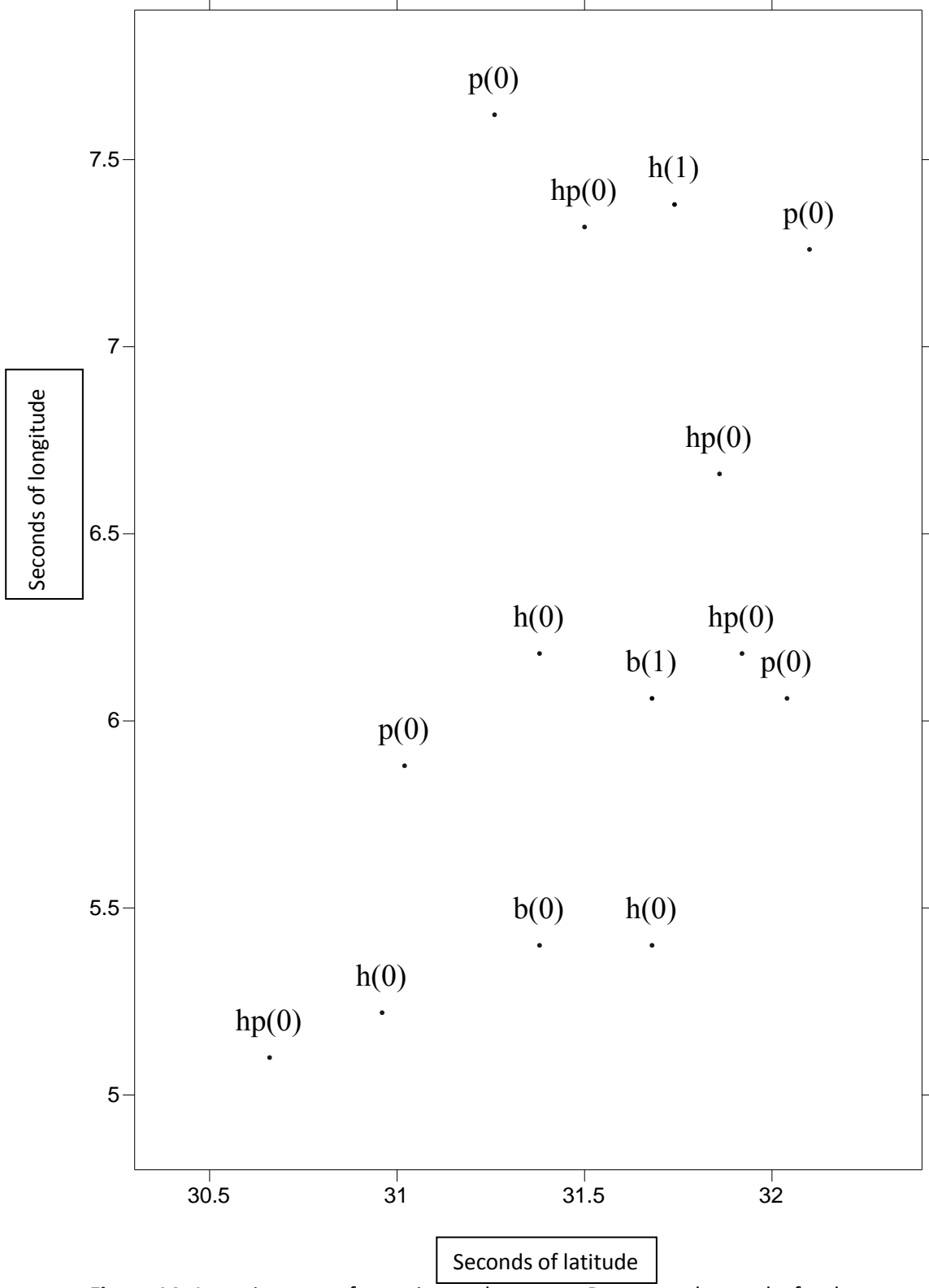


Figure 14. A posting map of experimental area two Dc counts the week after lure removal.

In the area selected for experiment three (Fig 15), there were 14 adult beetles found at the time of lure placement. 10 were found on trees that subsequently received both pheromone lures and plant lures (six on one tree and four on another tree) and four were found on a single plant compound baited trees. After the lures previously exposed in the field (for one week) were deployed for a week, 12 adults were found. One adult was found on a control tree, two were found on pheromone baited trees, and 9 were found on pheromone and plant baited trees. One week after these lures were removed, the area yielded no adults during the sweeping. Area three was the only experimental area that had a comparable number of adults before and immediately after baiting with previously-exposed lures. There are several factors that could account for the lack response to the lure by Dc. The lures used were already exposed in the field for one week, so the release rate of the pheromone was very low. Also, since there was a higher density of adults before the lures were deployed, it is likely that any slight potential effect of the lure was masked by many beetles releasing pheromone. It appears that the numerical response to the lure is very time dependent in the beetle life cycle. In areas one and two, fresh lures were deployed during a time of relatively low adult density and the lure increased the density of beetles on pheromone baited trees. In experimental area three (Fig 16) and four (Fig 21), lures that were already a week old were deployed at a time of higher adult density and there was no evidence of any pheromone effect.

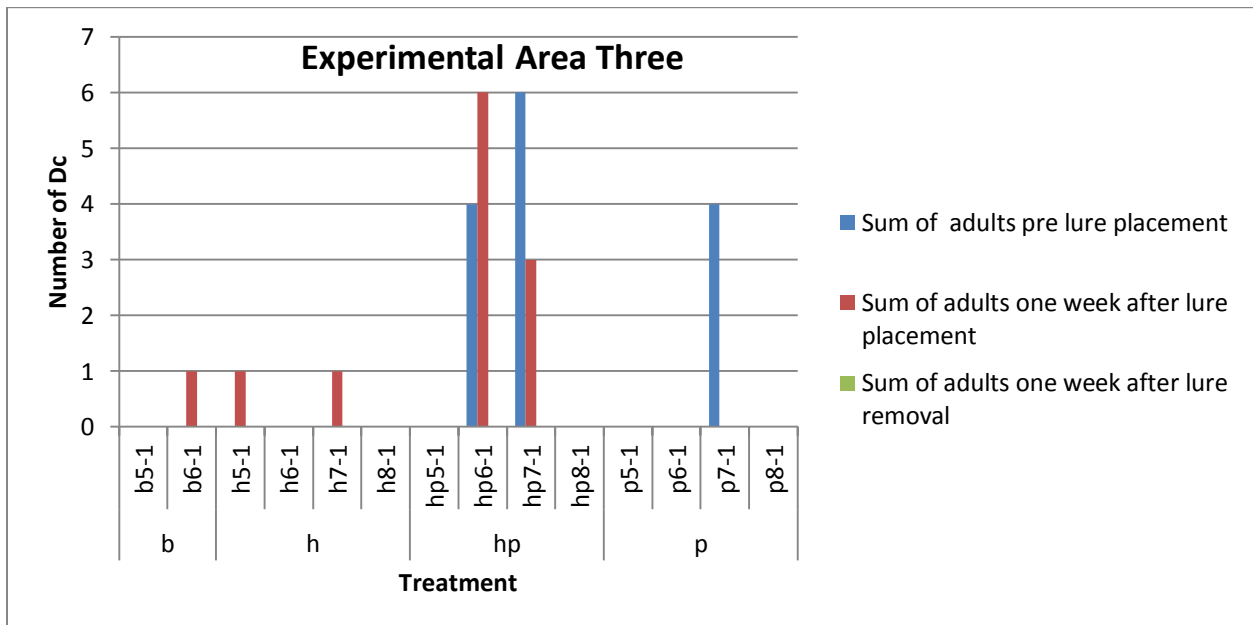


Figure 15. Number of beetles captured per baited tree before lures were placed, one week after lures were placed and one week after lures were removed for experimental area three. b = control tree, h = pheromone baited tree, hp = pheromone and plant volatile baited tree, p = plant volatile baited tree.

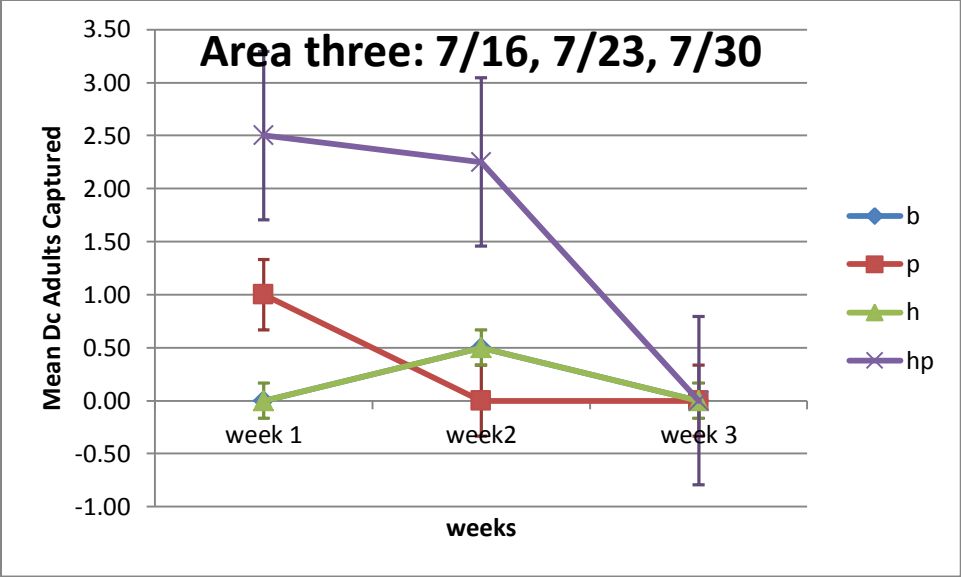


Figure 16. Mean number of adult Dc captured per week. b = control tree, h= pheromone baited tree, hp= pheromone and plant volatile baited tree, p= plant volatile baited tree.

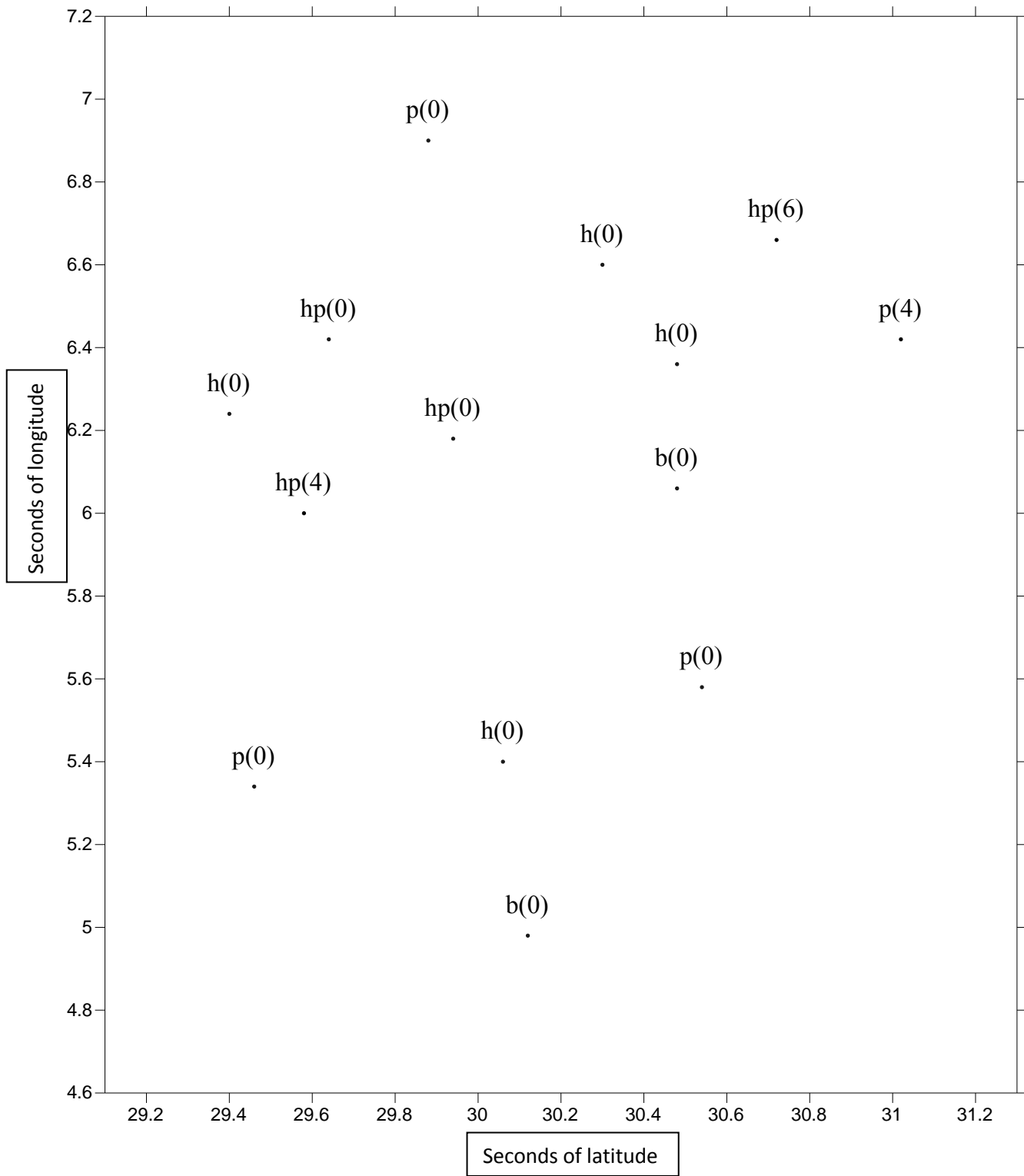


Figure 17. A posting map of experimental area three Dc counts before lure placement

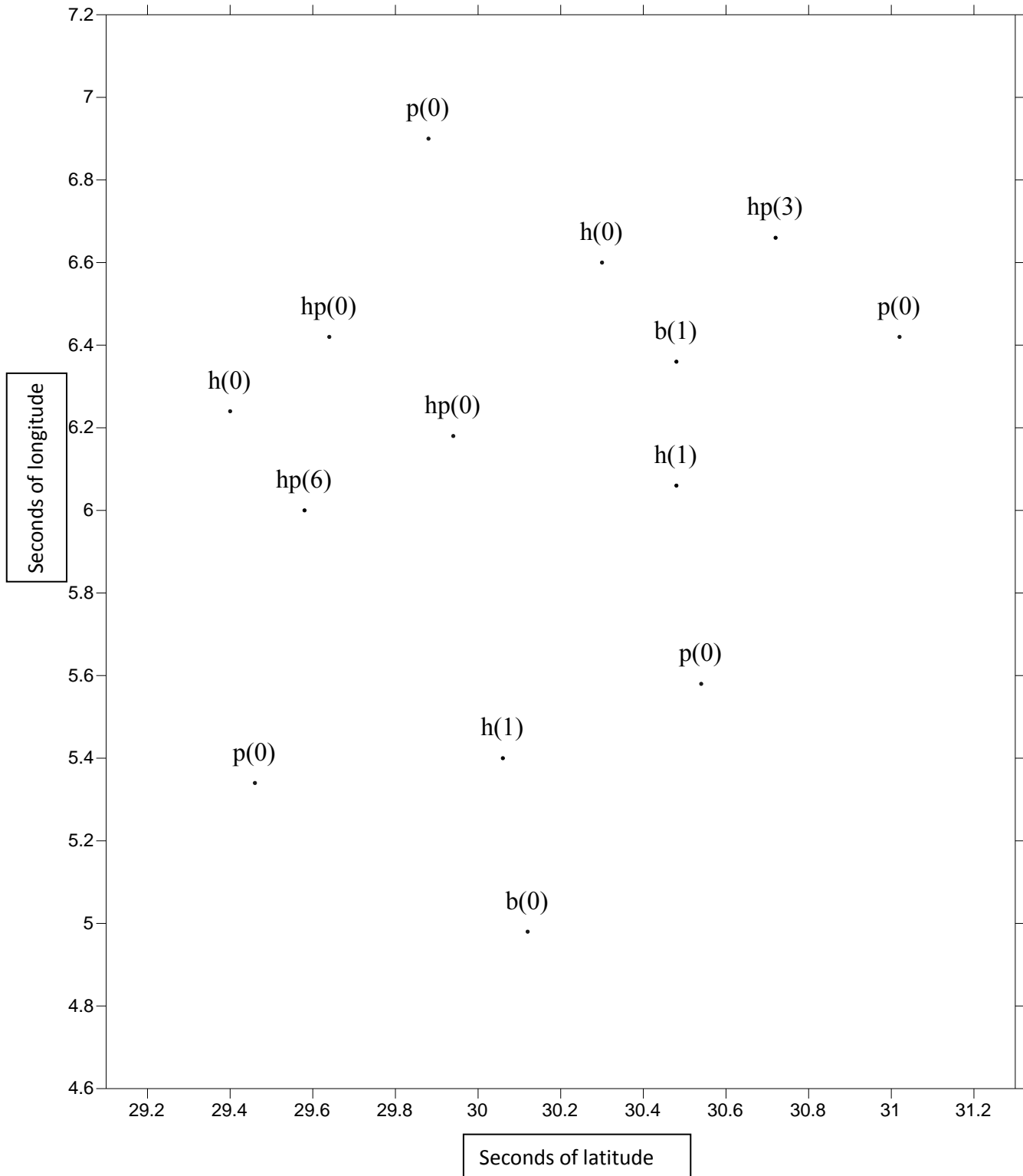


Figure 18. A posting map of experimental area three Dc counts immediately after lure removal.

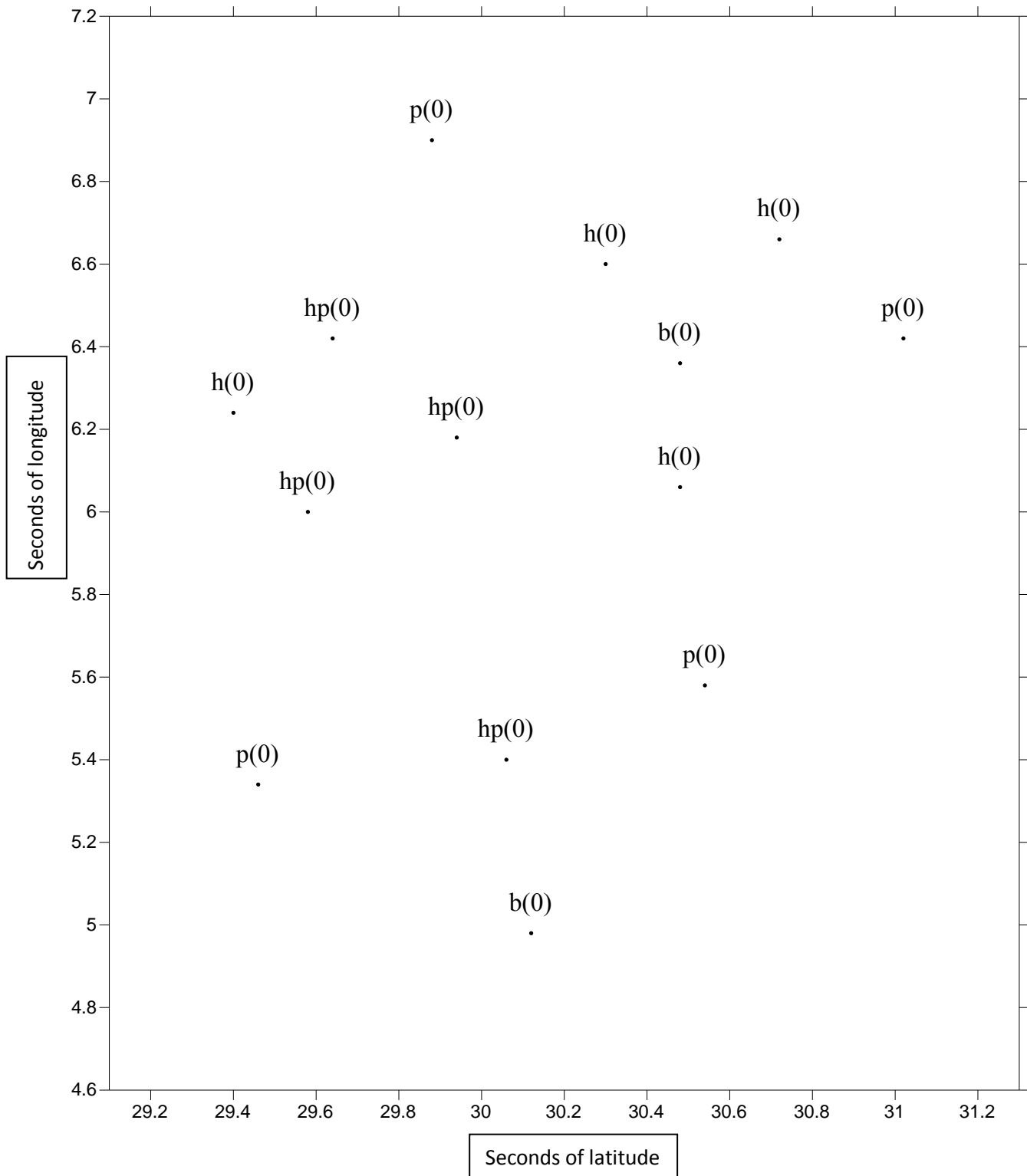


Figure 19. A posting map of experimental area three Dc counts the week after lure removal.

No adults were captured before lure placement in the area selected for experiment four (Fig 20). After one week of deployment of the previously exposed lures, 2 adults were found on pheromone baited trees and one on a pheromone and plant compound baited tree. A week after the lures were removed, four adults were found. Three were found on pheromone baited trees, one was found on a plant baited tree. Although it appears that there might be slight evidence of an aggregation effect from the semiochemical lures in area four, it is not clear. The pheromone release rate of older adults is not known, so there is no way to ascertain if the lure was delivering a signal greater than the background (Fig 21).

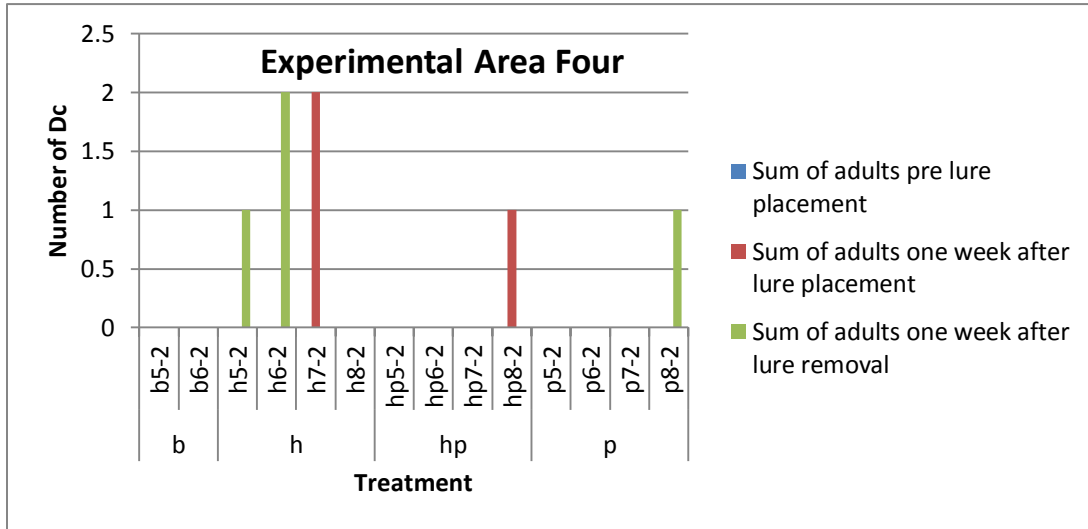


Figure 20. Number of beetles captured per baited tree before lures were placed, one week after lures were placed and one week after lures were removed for experimental area four. b = control tree, h = pheromone baited tree, hp = pheromone and plant volatile baited tree, p = plant volatile baited tree.

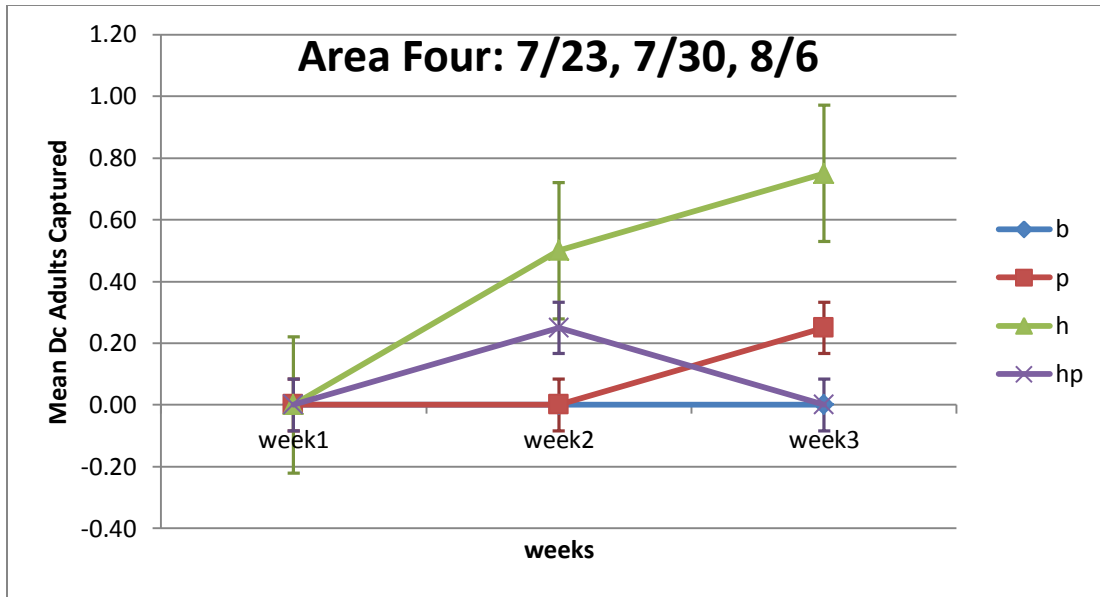


Figure 21. Mean number of adult Dc captured per week. b = control tree, h = pheromone baited tree, hp = pheromone and plant volatile baited tree, p = plant volatile baited tree.

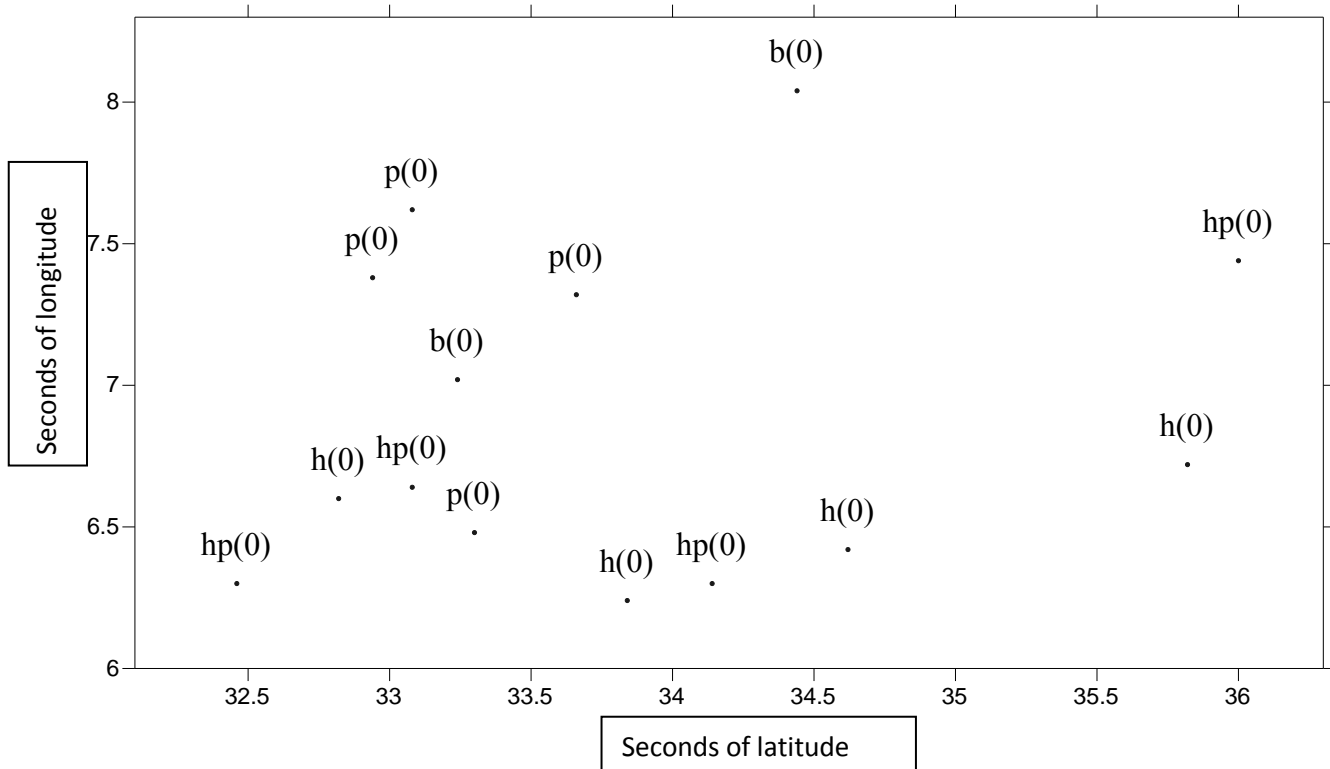


Figure 22. A posting map of experimental area four Dc counts before lure placement.

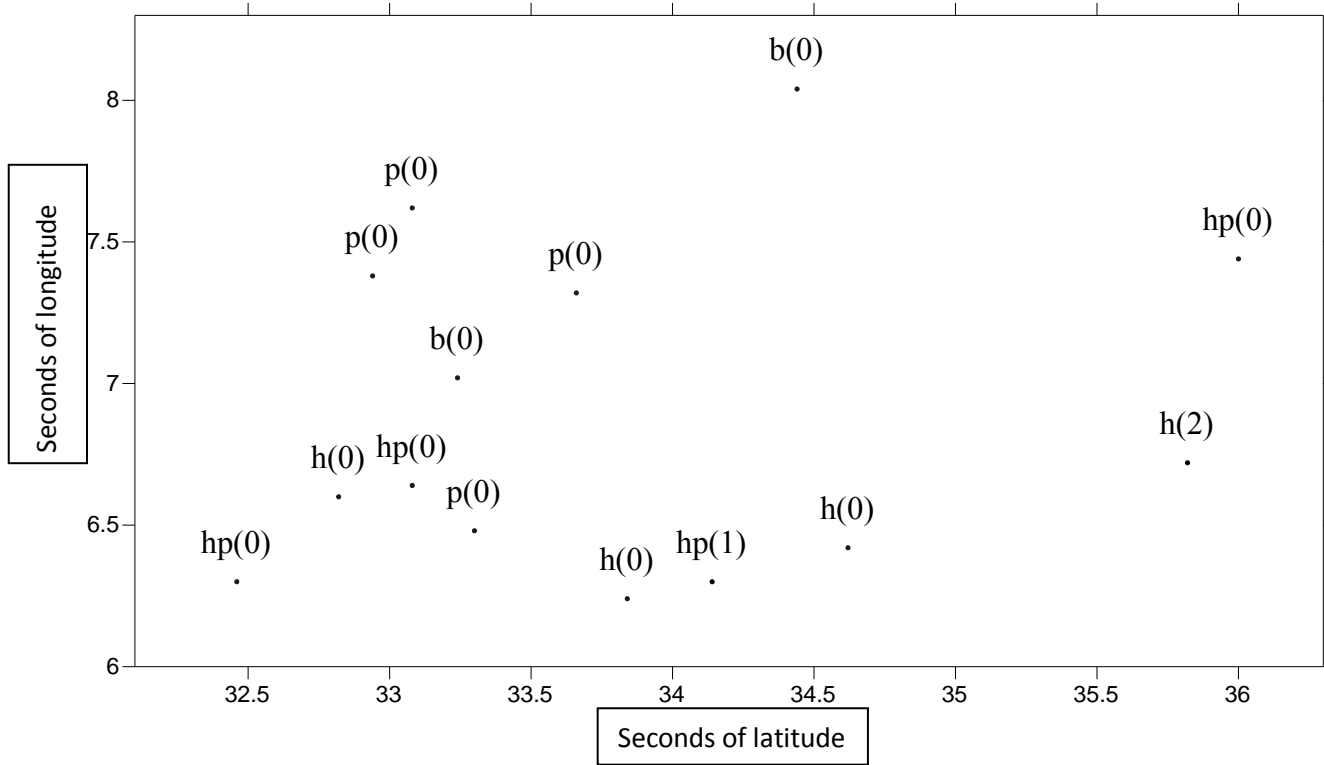


Figure 23. A posting map of experimental area four Dc counts immediately after lure removal.

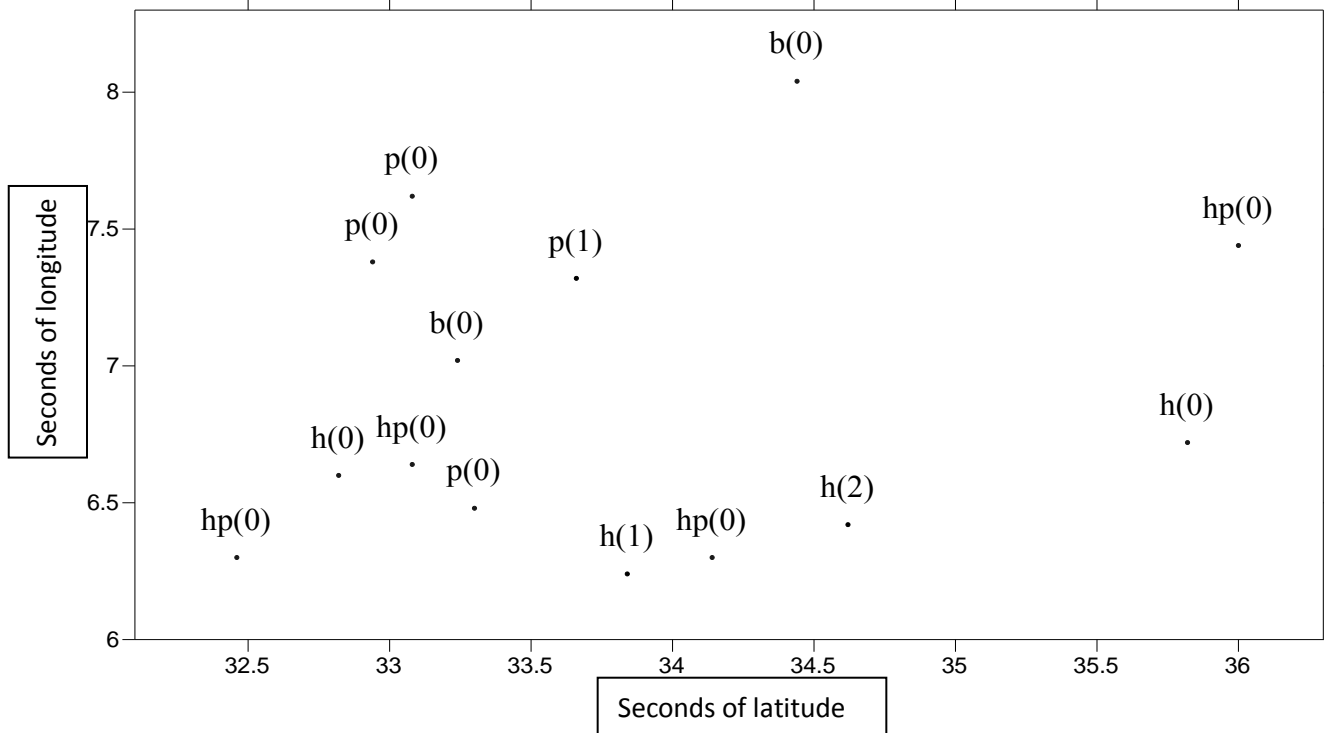


Figure 24. A posting map of experimental area four Dc counts the week after lure removal.

When the numbers of Dc captured from each area are considered over time (Fig 25), data from areas one and two show an initial proof of concept with an increase in Dc capture following a week of fresh lures placed on the 2nd and 9th of July. In contrast, areas three and four received lures that had already been exposed in the field and do not show the same trend, probably due to a low release rate of the attractant compounds. The decrease in capture that is seen on the 23rd and 30th of July is likely due to beetles moving among unmonitored trees.

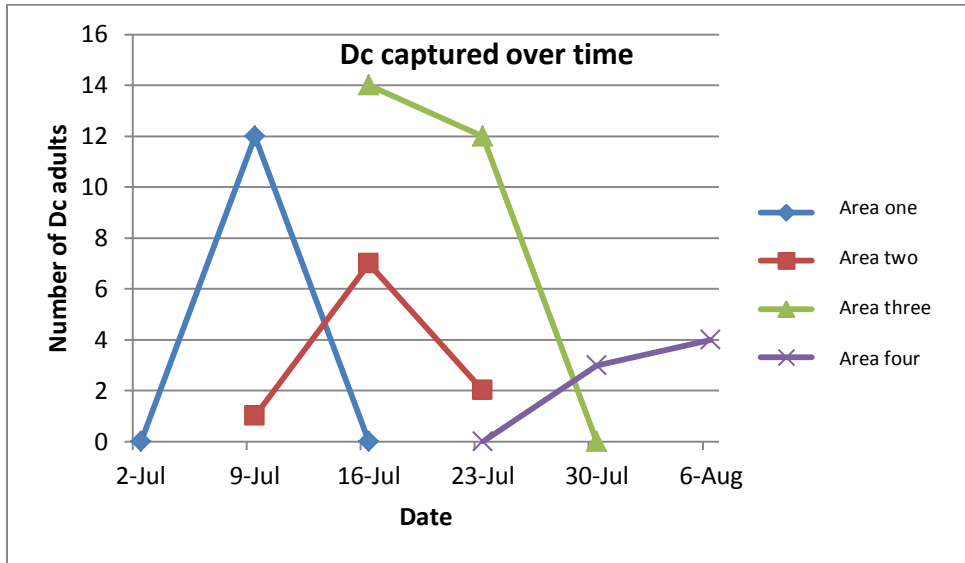


Figure 25. Number of Dc adults captured over the course of the study.

A complete census of all *Tamarix* at the field site on August 6th shows that the density of Dc adults varies and adults tend to be highly aggregated (Fig 26). Another census was taken on September 14th, but very few adults were found. These were typically in aggregations, as well.

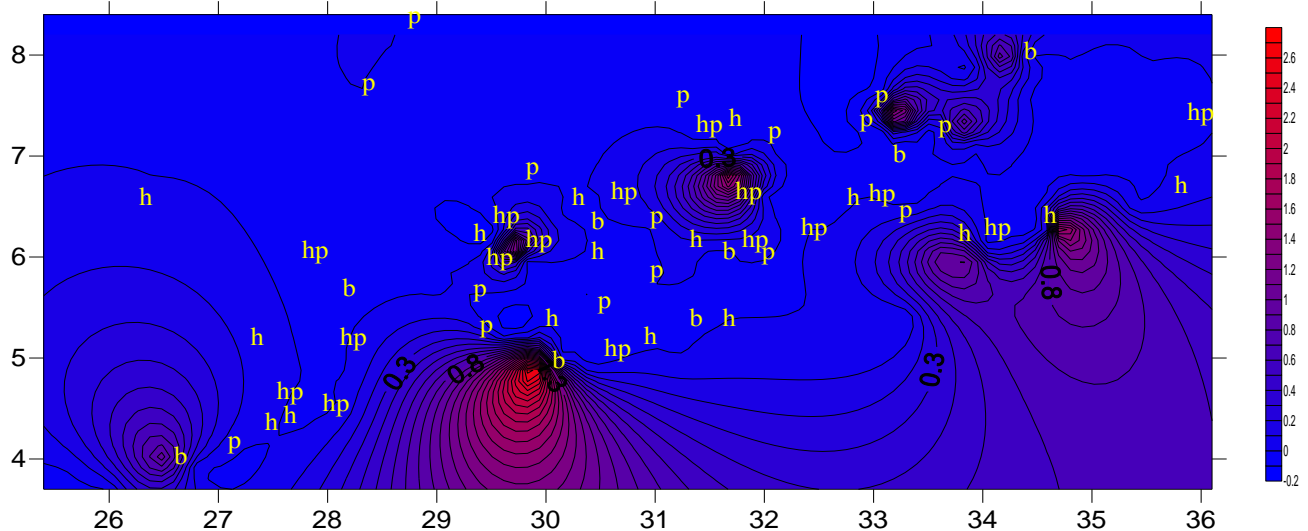


Figure 26. Contour maps of distribution of Dc adults on August 6, 2012: p = plant baited tree, hp = pheromone plant baited tree, h = pheromone baited tree, b = control tree.

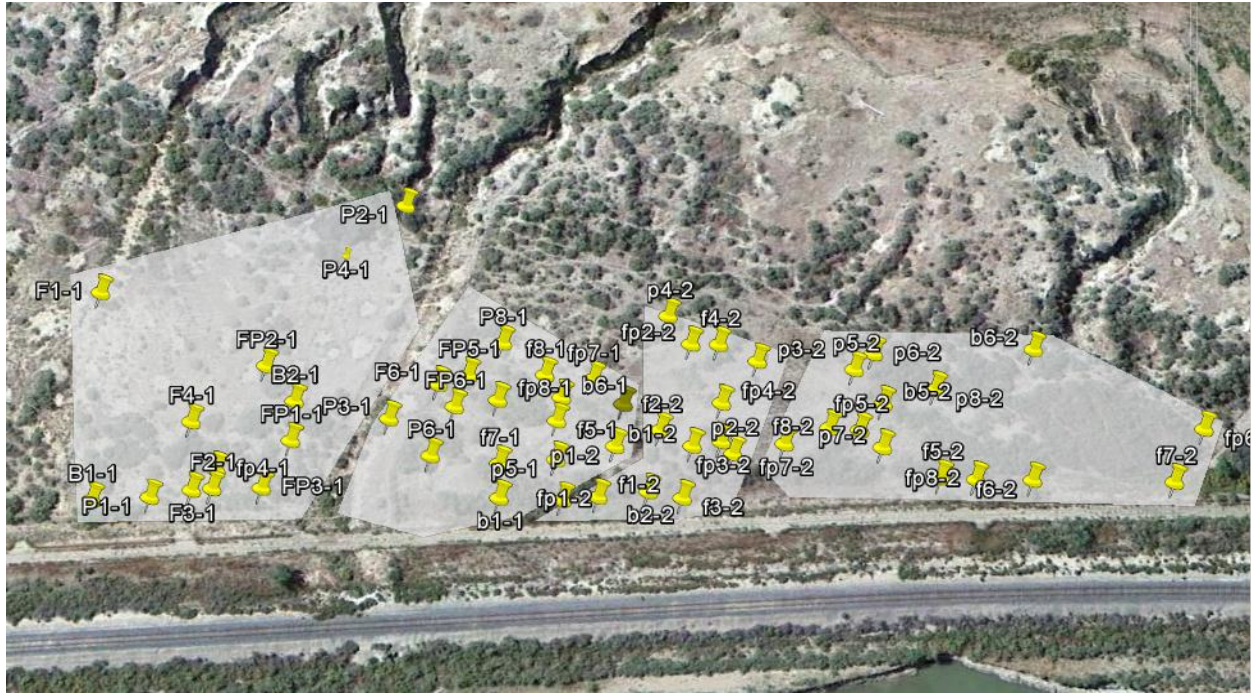


Figure 27. Image of field site with shaded blocks and pinned treatment trees

Discussion.

Analysis of the release rates of (2*E*, 4*Z*)-2,4-hetadien-1-ol from rubber septa demonstrate that rubber septa are not a viable option to increase local aggregations of *Dc*. This is because lure longevity in the field is limited to a few days. After one week in the field, the septa released (2*E*, 4*Z*)-2,4-hetadien-1-ol at an average of 1.5 ng/hour. This is much too low to facilitate aggregation of *Dc*; a single male beetle is reported to be able to release about five times the amount of (2*E*, 4*Z*)-2,4-hetadien-1-ol. The placement of the septa is very time sensitive since they have a very limited window of effectiveness. If the septa were placed while the beetle pupae were in the soil, it is possible that the emergence of the beetles could be asynchronous with the lure activity. It is also possible that many of the beetles that were attracted to the baited trees dispersed to other trees once the release rate from the septa dropped below a certain threshold. Sampling weekly may therefore greatly underestimate the potential of these lures to attract beetles. Greater lure longevity via more widely used controlled release media must be explored to minimize loss of efficacy due to attraction being too short lived.

It was expected that release rates from the rubber septa would be greater with increasing temperature. However, from the trials conducted, it appears that the release rates from the septa, after an initial large burst, are similar over the three temperatures tested.

From the field trials we can see that trees that received fresh lures were able to facilitate aggregation of the beetles to baited trees. Fresh lures were placed in area one on the second of July, so these lures were able to attract newly emerging adults as peak emergence might be expected to occur near the 13th of July. Area two received fresh lures on the ninth of July and these also attracted adults. By the time lures were placed in areas three and four, field emergence had peaked. When lures already exposed to field conditions for a week were placed within a higher density of adults, the aggregation of beetles to baited trees was not readily evident. Since the lures were releasing attractants at such a low rate and peak emergence had already occurred, the effect of the lures would be minimized.

In previously reported studies, a synergistic effect was observed between the pheromone and the plant volatile. The plant volatile by itself was also highly attractive to Dc. Neither were observed during this study. Throughout the field trials, an increase in Dc adults did not occur on trees baited with the host plant compound. In area one and two, trees that received only pheromone attracted more beetles than trees that were baited with both the pheromone and the host plant compound, which is also contrary to previous reports. This could be due to several factors. In previous studies, a blend of plant volatiles was used, although this was found not be more attractive than the single component we used. This blend of volatiles was also deployed in *Tamarix* stands that had seen major defoliation. The current study used a single plant volatile and was conducted in a *Tamarix* stand that had minimal defoliation and a low beetle population.

Future work

The utilization of controlled release formulations of pheromones to control and manipulate weed biological control agents has great potential. However, it does not appear that rubber septa, an inexpensive and commonly used media for pheromone dispersal, are a viable option. Therefore two types of commercially available products will be tested to determine their feasibility in being used for long term increase in local abundance of Dc: Scentry® sprayable impregnated hollow microfibers and ISCA® technologies wax based formula SPLAT™. This project will continue the current effort being conducted to develop a scientific basis for semiochemical manipulation of biological control agents. The following represent studies that will be conducted next summer:

- Develop pheromone impregnated media (SPLAT™ and Scentry® microfibers) to be used in manipulating populations of Dc and determine the release rate of the pheromone from these media
- Develop similar host plant volatile impregnated media to be used in conjunction with pheromone impregnated media and determine the release rate of the plant volatile from this media
- Determine if pheromone and plant volatile impregnated media can be used to facilitate long term intentional aggregation of Dc.

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