Progress Report to CDPR:

Determination of Efficacy of Chlorophacinone Treated Artichoke Bracts, Zinc Phosphide Treated Artichoke Bracts, and Rozol® Pellets for Controlling California Voles in Artichokes.

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Abstract: California meadow voles (*Microtus californicus*) are the primary vertebrate pest in artichoke fields around Castroville, CA. For many years, chlorophacinone treated artichoke bracts, chlorophacinone grain pellets (Rozol®), and zinc phosphide treated bracts have been the primary rodenticides used for vole control in artichokes. However, there has been much ambiguity about the current efficacy of these baits given known resistance to chlorophacinone in some voles in the local population, and an apparent lack of bait acceptance of zinc phosphide treated bracts by many voles in the population. Therefore, we initiated a study in winter 2011 to assess the efficacy of these baits to provide quantifiable data on this issue. We found that chlorophacinone treated bracts provided the greatest efficacy of the tested rodenticides. Rozol was intermediate in efficacy, with zinc phosphide bracts least effective. Collectively, these results indicate that chlorophacinone treated bracts can still be an effective tool for helping to control vole populations in artichokes, while use of zinc phosphide treated bracts does not appear to be effective. Research into additional rodenticides is suggested to find other alternatives to chlorophacinone, as relying solely on this pesticide will likely lead to further resistance issues in the future.

INTRODUCTION

Nearly one hundred percent of all artichokes grown commercially in the United States are grown in California. While artichokes are a small industry compared to other crops (e.g., broccoli, grapes, lettuce), they add over $50 million to the economy of the state. Approximately 75% of the state's total acreage of artichokes lies within Monterey County. Nowhere else in the world is there such a concentrated area of production, consistently yielding nearly 4 million cartons of artichokes annually.

California meadow voles (*Microtus californicus*) are the primary vertebrate pest in artichoke fields around Castroville, CA. For years, the main control method for voles was chlorophacinone treated artichoke bracts. However, in 2001, artichoke growers began to notice an uncharacteristic increase in vole populations (Salmon and Lawrence 2005). In response to this, a research project was conducted to develop baiting strategies for voles in artichokes with the hope that improved baiting strategies would address the increased populations. This study

indicated that the Castroville vole population had become resistant to chlorophacinone (Salmon and Lawrence 2005).

Faced with vole populations that were resistant to chlorophacinone, the artichoke growers, USDA, and researchers with the support of the Vertebrate Pest Control Research Advisory Committee (VPCRAC) worked to develop and register zinc phosphide for use on artichoke bracts. However, this baiting strategy has not proven to be as efficacious as was anticipated. This is of particular concern as growers are faced with the possibility of losing the chlorophacinone treated bracts due to resistance, while zinc phosphide treated bracts do not appear to be accepted at a level high enough to effectively control voles in a field setting. Without effective control options, growers will continue to suffer increasing losses, extensively damaging the artichoke industry in California. Therefore, we established a study to test the efficacy of the three rodenticides currently available for controlling voles in artichokes: 1) chlorophacinone (active ingredient [AI] = 0.01%) treated artichoke bracts, 2) Rozol® pellets (AI = 0.005% chlorophacinone), and 3) zinc phosphide treated artichoke bracts (AI = 0.54%). This study will provide insight into the current efficacy of these baits and will provide data needed to guide future baiting strategies for artichoke growers.

STUDY AREA AND METHODS

Site location

This investigation was conducted in the Castroville area of Monterey County, CA. This location is the heart of artichoke production in CA, as well as for the U.S. Efficacy trials were conducted from mid-January through mid March, 2011.

Indexing vole activity

To determine the efficacy of the selected rodenticides at controlling vole populations, we first had to develop a method for monitoring changes in population size and activity. For this, we used non-toxic wax blocks to monitor chewing activity, as such chewing indices were successfully correlated to minimum estimates of population size for voles ($r = 0.90$, $p = 0.03$), deer mice (*Peromyscus* spp.; $r = 0.86$, $p = 0.06$), and house mice (*Mus musculus*; $r = 0.95$, $p = 0.02$; R. Baldwin, unpublished data). This method has proven effective for monitoring vole populations in other studies as well (e.g., Whisson et al. 2005, Engeman and Whisson 2006). The indexing protocol entails placing wax blocks in a grid pattern; for our study, we followed a 6 x 6 grid pattern with blocks placed at 5-m intervals underneath artichoke plants. The blocks were collected 48 hours after initial placement and were weighed to determine mass removed by rodents. These 48-hour indexing plots will be referred to as full day plots hereafter.

Although voles, deer mice, and house mice often occurred in the same areas, we were still able to relate the potential reduction in chewing after treatment to a reduction in vole population size given the strong correlation of population size for all three rodent species to the amount removed from wax blocks. Nonetheless, we also wanted to use a method that would essentially eliminate deer and house mice from our index. Voles are active throughout the day, while deer and house mice are only active at night. This was verified through 7 days of daytime and nighttime trapping, as we never captured either mouse species during the daytime ($n = 29$ and 10 for

nighttime trapping of deer mice and house mice, respectively), while voles were frequently captured during both daytime (n = 16) and nighttime (n = 18). Therefore, to provide an additional method to assess efficacy for only vole populations, we placed blocks out at approximately 08:00 and removed them at 17:00 for weighing; this procedure was conducted for two days (hereafter referred to as daytime plots).

Determining efficacy of rodenticides

We established one replicate of each treatment (chlorophacinone treated artichoke bract, zinc phosphide treated artichoke bract, and Rozol pellets) plus a control in each of 5 fields (i.e., n = 5 for each treatment type). Following the indexing protocol, wax blocks were placed at 5-m intervals underneath artichoke plants following a 6 x 6 grid pattern to formulate a sampling grid. Two sampling grids were placed per treatment type per block. These grids were operated for 2 consecutive days pre-treatment, with one of these sampling grids operated for the full 48-hour period (full day plots), while the other grid was operated for the daytime period for both days (daytime plots). Indexing activities associated with these grids concluded 1–2 days prior to bait application. The chlorophacinone bract, Rozol, and control plots were resurveyed for 2 consecutive days 7 (post-treatment session 1) and 14 (post-treatment session 2) days after treatment, while the zinc phosphide bract plots were surveyed 3 (post-treatment session 1) and 10 (post-treatment session 2) days post-treatment. The zinc phosphide plots were surveyed at shorter intervals given the fast acting nature of this acute toxicant.

Efficacy (percent reduction in amount of block chewed) was determined through the following equations:

$$\text{Equation 1: } \frac{(\bar{x}\text{ mass of whole block} - \bar{x}\text{ mass of chewed blocks})}{\bar{x}\text{ mass of whole block}} \times 100 = \%\text{ Chewed}$$

$$\text{Equation 2: } (1 - [\%\text{ treatment block chewed} / \%\text{ control block chewed}]) \times 100 = \%\text{ Efficacy}$$

Before trials were initiated, 20 non-chewed blocks were weighed to determine mean ($\bar{x}$) mass of non-chewed (whole) blocks.

Natural changes in population size and rodent activity can occur irrespective of the application of a rodenticide, thereby potentially biasing results from baiting trials. To account for this possibility, we tested for differences in wax block consumption for control plots across pre-treatment and post-treatment sessions 1 and 2 using paired t-tests. If a significant difference was observed, we applied a correction factor to the baiting trials conducted within that same field. The correction factor was calculated as follows:

$$\text{Equation 3: } \frac{\text{Grams of block removed pre-treatment (treated plot)}}{\text{Grams of block removed pre-treatment (control plot)}} = \frac{\text{Expected grams of block removed if no treatment applied to treated plot}}{\text{Grams of block removed post-treatment (control plot)}}$$

Equation 4: $$\frac{\text{Grams of block removed post-treatment (treated plot)}}{\text{Expected grams of block removed if no treatment to treated plot}} \times 100 = \text{Adjusted \% remaining}$$

Equation 5: 100 – Adjusted % remaining = % Adjusted control

This correction factor was modified from equations provided by O'Connell and Clark (1992) to allow for the use of wax blocks to monitor populations rather than animal counts. If no significant differences were noted between pre-treatment and post-treatment sessions for control plots, no corrections were made.

Fields were assessed separately to determine performance across replicates, and they were combined to develop mean and variance estimates for each treatment type. Finally, the efficacy of each treatment type was compared using a Kruskal-Wallis test to determine potential differences (Zar 1999).

RESULTS

For full day control plots, we observed significant differences ($p < 0.05$) for 2 of 5 fields (Sella and Gianini; Fig. 1a) with greater chewing activity occurring after pre-treatment indexing trials were conducted. For daytime control plots, we observed greater chewing activity during post-treatment indexing trials for 2 of 5 fields (Sella 2 and Gianini), less chewing activity for 1 field (Mulligan 3), and no difference for 2 fields (Molera 4 and Sella; Fig. 1b). Results from treatment applications for fields that exhibited significant differences for control plots in chewing activity between pre- and post-treatment sessions were all adjusted appropriately to account for the potential impact of natural population fluctuations on associated control estimates.

For the full day plots, the chlorophacinone treated bracts scored higher than all other treatment types for each field and each post-treatment sampling session, except for Sella, where it was the lowest scoring for the first post-treatment session (Fig. 2a) and was outscored by Rozol during the second post-treatment session (Fig. 2b). Zinc phosphide treated bracts exhibited the lowest efficacy for all fields and post-treatment sessions except for Sella and Gianini fields during the first post-treatment session (Fig. 2a). Collectively, chlorophacinone treated bracts and Rozol exhibited greater efficacy than zinc phosphide during both post-treatment sampling periods (Fig. 3a), although this value was only significant for the second session (first session: $H_2 = 2.0$, $p = 0.368$; second session: $H_2 = 6.5$, $p = 0.039$). Additionally, the chlorophacinone bract and Rozol plots tended to exhibit greater efficacy 14-days post-treatment (Fig. 3a), whereas efficacy for the zinc phosphide plots was greatest 3-days post-treatment (Fig. 3a). It should be noted that all chlorophacinone bract plots exhibited good to great control (> 70% efficacy) based on the second sampling session ($\bar{x} = 85.5\%$, SE = 4.8), while 3 of 5 Rozol plots exhibited similar control levels ($\bar{x} = 70.5\%$, SE = 7.9); only 1 zinc phosphide plot achieved the desired level of control ($\bar{x} = 30.9\%$, SE = 17.3).

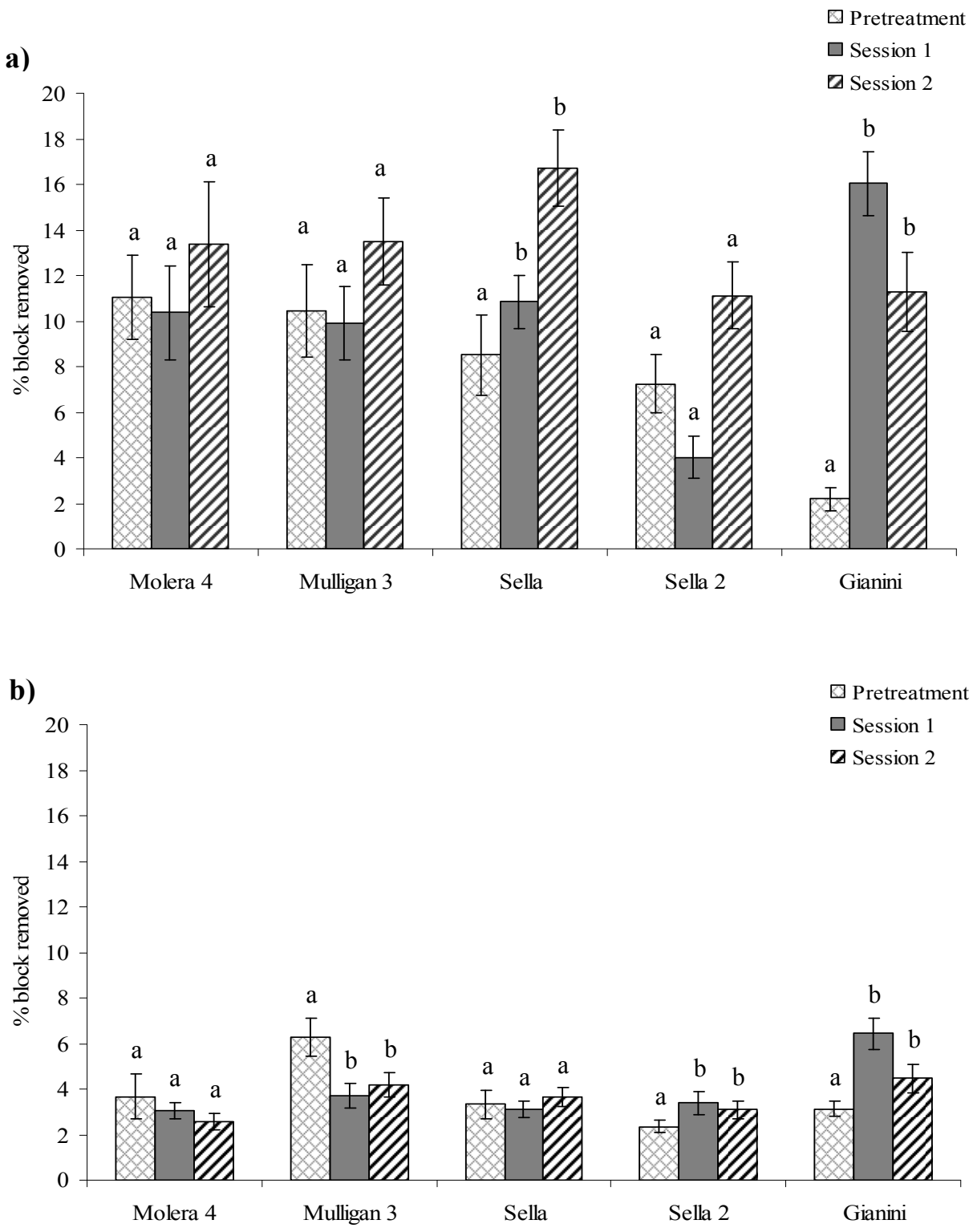

Figure 1. Graphs showing mean and standard error values for the percent of block removed from control blocks during pre-treatment and post-treatment sessions 1 and 2 during a) full day, and b) daytime indexing for 5 artichoke fields in Monterey County, CA. Significant differences between pre-and post-treatment index values are represented by different letters.

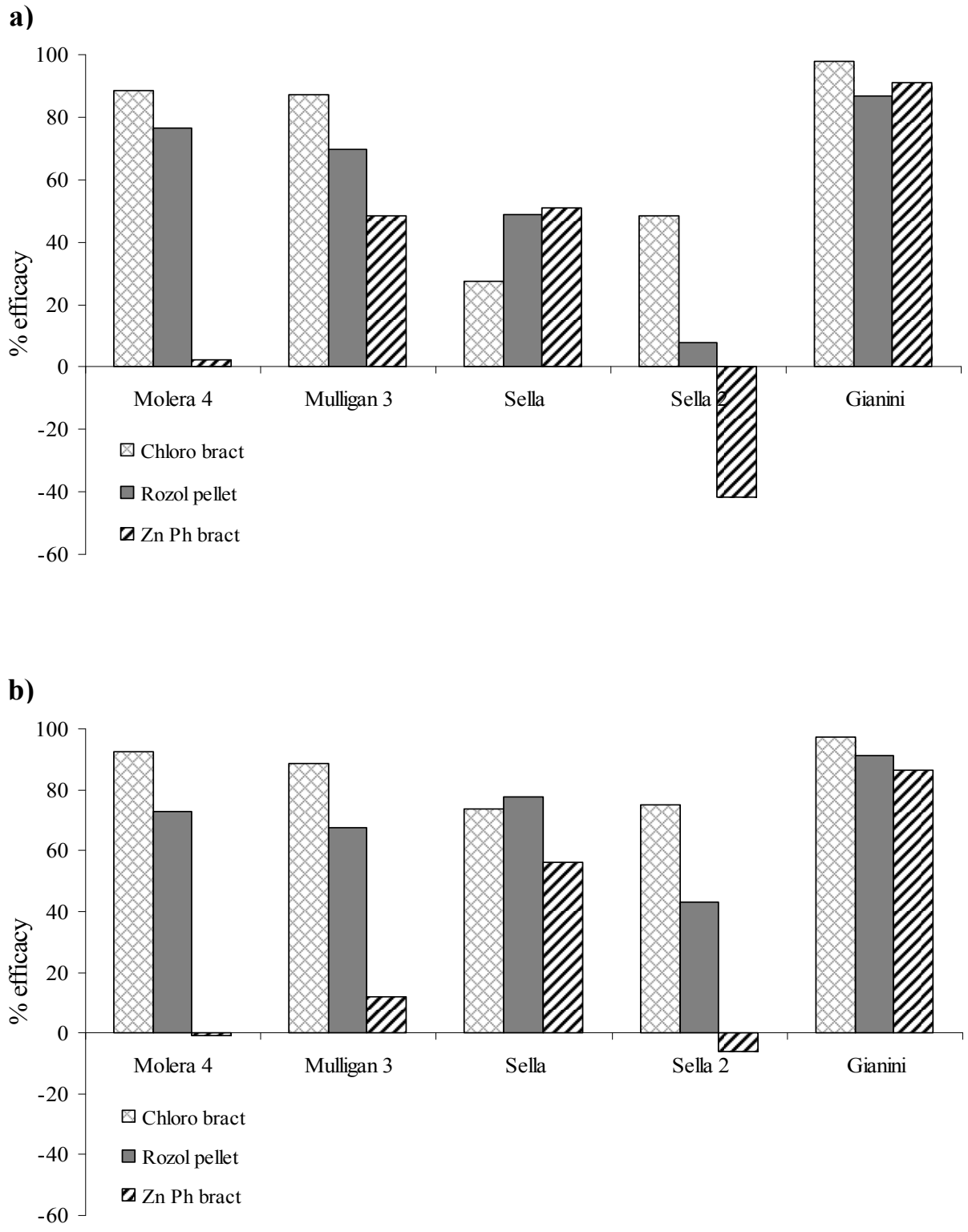

Figure 2. Percent efficacy for 3 rodenticides for controlling voles in 5 artichoke fields in Monterey County as determined by full day indexing during: a) post-treatment session 1, and b) post-treatment session 2.

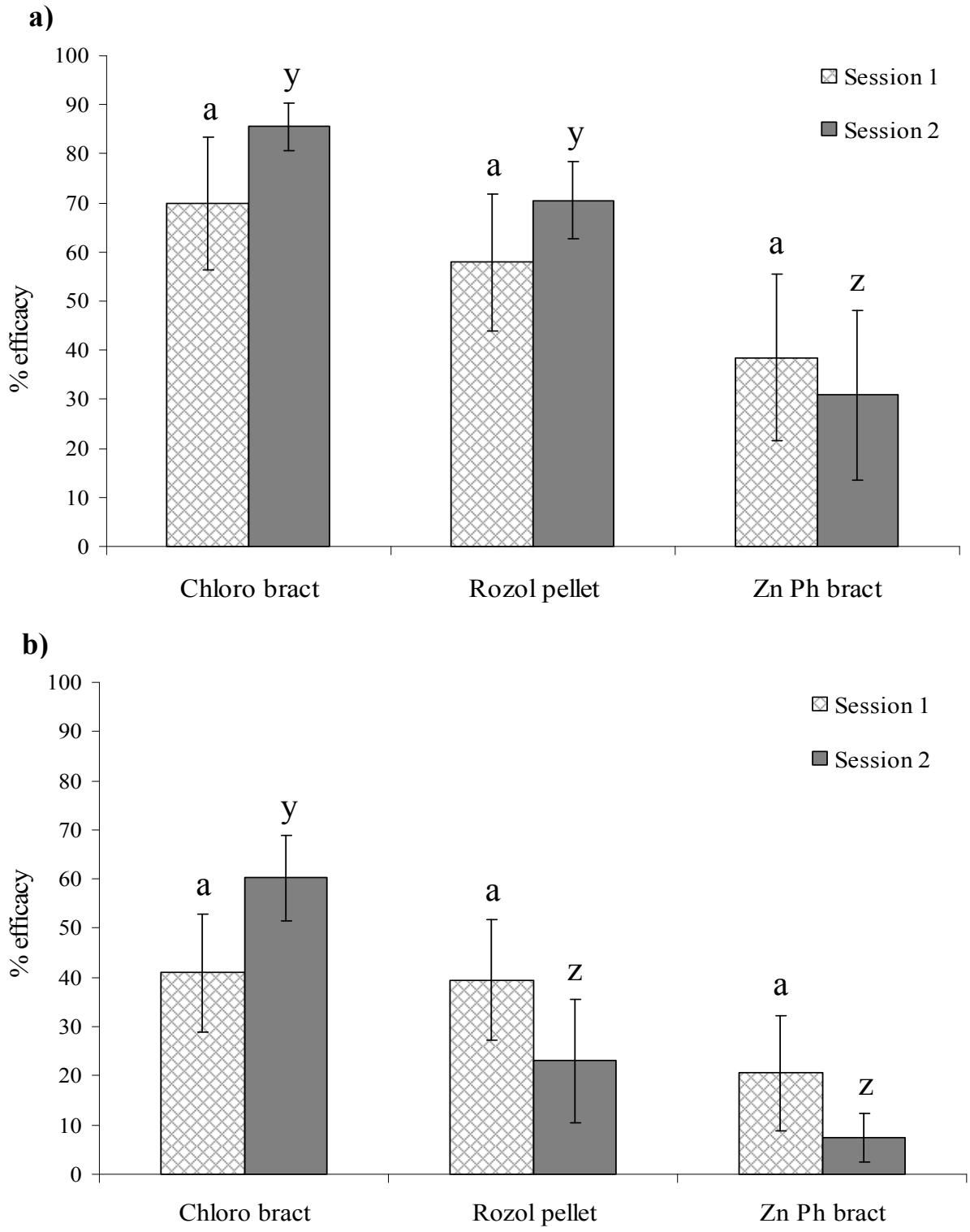

Figure 3. Mean efficacy and standard errors of 3 rodenticides applied to control voles across 5 artichoke fields in Monterey County. Efficacy was determined through: a) full day indexing, and b) daytime indexing. Significant differences among rodenticides for post-treatment sessions 1 and 2, respectively, are represented by different letters.

For daytime plots, the chlorophacinone treated bracts again scored higher than all other rodenticides during the second sampling session (Fig. 4b), although these results were a little more variable for the first post-treatment period (Fig. 4a). The zinc phosphide plots exhibited the lowest scores for all fields and sampling sessions except for the first post-treatment session for Sella 2 (Fig. 4a) and both post-treatment sessions for Gianini (Fig. 4). As with the full day plots, the chlorophacinone treated bracts were the rodenticide that yielded the greatest control for the second post-treatment session ($H_2 = 7.7$, $p = 0.021$; Fig. 3b); there was no significant difference in efficacy among rodenticides for the first post-treatment session ($H_2 = 2.2$, $p = 0.337$; Fig. 3b). Efficacy was again substantially higher for chlorophacinone bracts and lower for zinc phosphide bracts during the second post-treatment indexing sessions (Fig. 3b). However, in contrast to the data observed at the full day plots, Rozol efficacy was lower during the second post-treatment session (Fig. 3b). For daytime plots, overall efficacy (chlorophacinone bracts: $\bar{x} = 60.2\%$, SE = 8.7; Rozol: $\bar{x} = 23.1\%$, SE = 12.6; zinc phosphide bracts: $\bar{x} = 7.3\%$, SE = 5.0) after 10–14 days was substantially lower than levels reported for the full day plots (chlorophacinone bracts: $\bar{x} = 85.5\%$, SE = 4.8; Rozol: $\bar{x} = 70.5\%$, SE = 7.9; zinc phosphide bracts: $\bar{x} = 30.9\%$, SE = 17.3).

DISCUSSION

Our findings clearly indicate that chlorophacinone treated artichoke bracts are the most efficacious rodenticide that we tested, as they were the highest scoring rodenticide for both indexing methods (i.e., full day and daytime) and sampling sessions. The chlorophacinone treated bracts have long been the control method of choice for vole control in artichokes in Monterey County. However, past investigations and grower comments indicated that the chlorophacinone bracts were not as effective as they previously had been (Salmon and Lawrence 2005). Subsequent studies indicated that some voles had developed a resistance to chlorophacinone given its long-standing use as the sole rodenticide registered for such application in artichokes (Salmon and Lawrence 2005). This led to the development of a zinc phosphide alternative to control resistant voles.

Initial trials of zinc phosphide treated bracts indicated good success (90–99% control; Salmon and Lawrence 2005). However, subsequent applications by growers were considered ineffective (Pers. Comm., D. Huss, Ocean Mist Farms). Our findings back this assertion. Reasons for the current low efficacy of the zinc phosphide bract are unclear. It has been well documented that many rodents have an aversion to zinc phosphide given its strong garlic-like odor and taste (Marsh 1987). This can often lead to bait acceptance issues, particularly if the rodents have consumed a sub-lethal dose of the bait. This will cause the rodent to suffer deleterious effects from the toxin but will not be enough to kill them. These sickness-like symptoms are then often associated with consumption of this pungent food source and typically results in future avoidance of zinc phosphide treated bracts.

We may have observed evidence of the consumption of sub-lethal doses of zinc phosphide in our study, as less chewing typically occurred 3-days after bait application as compared to 10-days post-treatment. This may indicate that some voles consumed a sub-lethal dose of the bait, became sick and relatively immobile for several days, then resumed normal activities (i.e.,

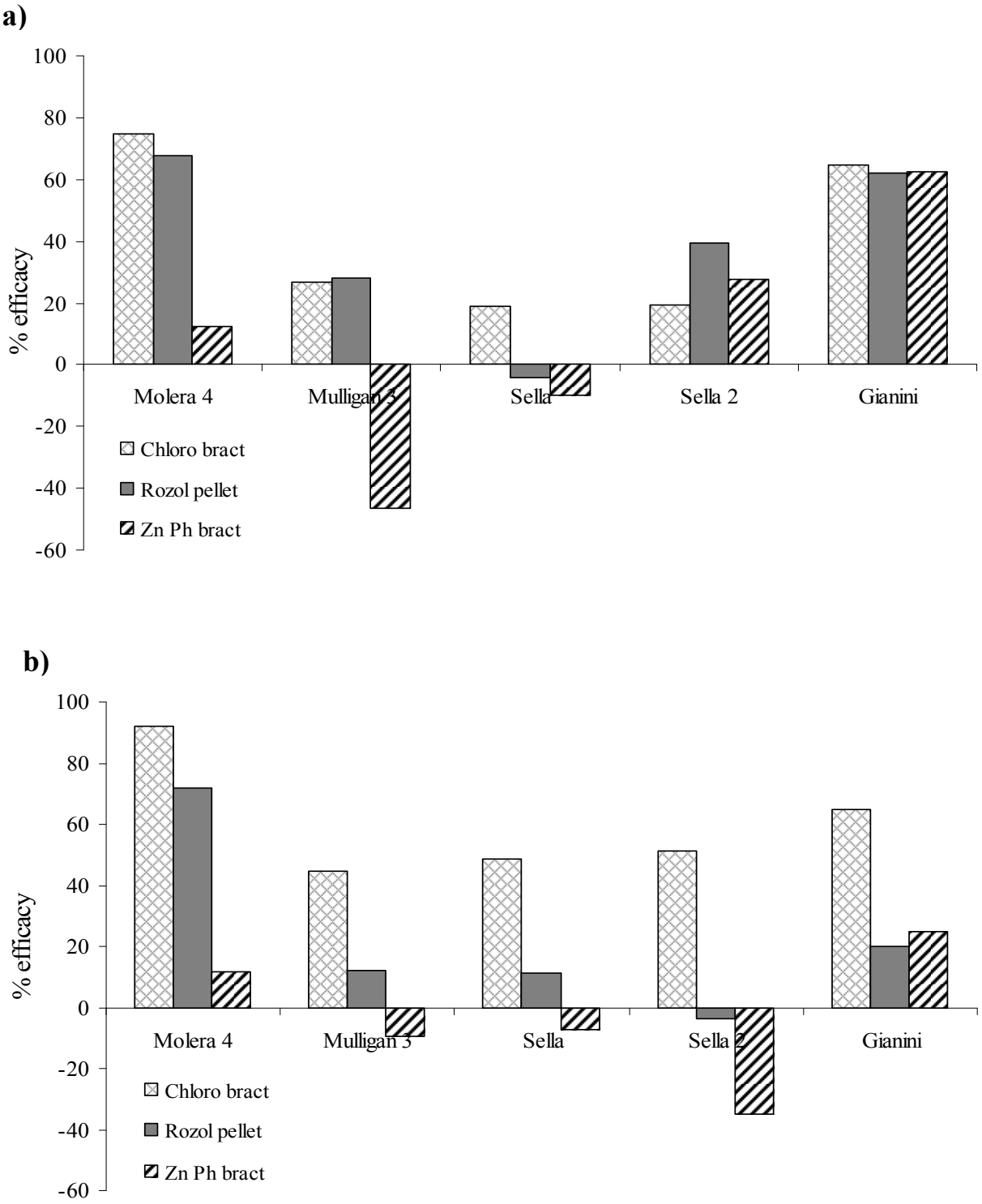

Figure 4. Percent efficacy for 3 rodenticides for controlling voles in 5 artichoke fields in Monterey County as determined by daytime indexing during: a) post-treatment session 1, and b) post-treatment session 2.

increased chewing in monitoring blocks). Because these baits have now been used for several years, similar reactions over time could have led to an increasingly large proportion of the vole population that is now bait shy, thereby substantially lowering the efficacy of this rodenticide. This is purely hypothetical, but does seem to be a plausible explanation for the reduction in efficacy observed since the bait was first developed.

The purpose behind developing the zinc phosphide treated bract was primarily to offset the loss in efficacy associated with the chlorophacinone bract due to the development of resistance in local vole populations. However, based on our findings with the full day indexing methodology, we observed relatively high efficacy with the chlorophacinone bract, whereas we observed substantially lower efficacy values from our daytime sampling. Reasons for this are unclear. One likely explanation for at least some of the variation observed between the two sampling time-periods is directly related to sampling protocol. With the daytime index, the blocks were not available for chewing for as lengthy of a period of time (i.e., total of 18 hours vs. 26 hours for the daytime and full day plots, respectively). Therefore, there will be less overall chewing on monitoring blocks located in the daytime plots. Further, through the placement and removal of blocks, there will always be some incidental loss in mass from handling the blocks, weather factors, etc. This incidental loss will have a larger effect on efficacy estimates if the total amount removed by rodents is smaller (i.e., a larger proportion of the amount removed from incidental loss will result in a greater level of unaccounted variability in estimates), which was certainly the case in our investigation. As such, we are currently working to provide a method to account for this variability. Once completed, this will hopefully clear up some of the observed differences between the two sampling protocols.

Another possible explanation could be directly related to the chewing activity of mice (both deer and house mice). As mentioned earlier, mice were responsible for some chewing on the monitoring blocks for the full day plots, but not for the daytime plots. If the voles were somewhat resistant to chlorophacinone, but the mice were not, then much of the reduction in chewing could be due to a large removal of mice, and a small to moderate population reduction of voles. There is evidence to support this supposition, as control levels were substantially lower for the daytime plots. However, mice have been present in the fields with voles since the onset of chlorophacinone bait applications, so it seems plausible that they could have developed a similar resistance, although no one has assessed this possibility. Further, even if mice are more susceptible to the chlorophacinone, it does not explain why control of voles was so high in the Molera 4 field (% control = 92%) which is believed to be one of the sites with the greatest number of resistant voles. This does not appear to be a spurious result, as efficacy from the Rozol treatments were also high (% control = 72%), indicating that chlorophacinone resistance did not appear to be an issue at that time.

A number of factors could lead to the increased efficacy observed at the Molera site including weather and alternative food/cover sources. Weather does not appear to be an overriding factor, as both the Molera 4 and Mulligan 3 sites were baited at the same times with differing results for the daytime plots. However, alternative food/cover sources could play a part in the observed differences. The Molera 4 site was sprayed with an herbicide 7–10 days before the baiting trial commenced. This resulted in the die-off of yellow oxalis (*Oxalis pes-caprae*) which is a preferred food and cover source for voles (Marsh et al. 1985). It is possible that this removal

either increased the desirability of voles to consume the chlorophacinone baits, or increased their susceptibility to them. We plan to explore this possibility further in the coming fall and early winter, 2011.

CONCLUSIONS

At least in the short-term, the use of chlorophacinone treated artichoke bracts appears to be the best available rodenticide for vole control in artichokes. The use of Rozol pellets appears to be a somewhat less effective but viable alternative when bracts are not available for use. However, resistance is already present in the vole population in the Castroville area of Monterey County. A new alternative is needed to help offset this resistance. Unfortunately, at least in the manner that they are currently formulated, zinc phosphide treated artichoke bracts do not appear to be the answer. It is imperative, however, that a new material be found to use in concert with chlorophacinone to control these vole populations. As history has shown us repeatedly, relying on a single toxicant usually results in the development of resistant pest populations (Jutsum et al. 1998). Current research is underway to find a new, viable option for field use in artichokes. Even if a suitable alternative is found, we strongly recommend that chlorophacinone be kept as an option for vole control so that the use of these toxicants can be cycled back and forth to reduce the possibility of resistance (Roush 1989). Otherwise, resistance is likely to again develop with any new rodenticide in the future.

Acknowledgments

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