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ORIGINAL PAPER

Cholecalciferol plus diphacinone baits for vole control: a novel approach to a historic problem

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Abstract Combination baits containing cholecalciferol plus an anticoagulant are effective against commensal rodents resistant to anticoagulants, and they likely pose less risk than anticoagulant-only rodenticides due to lower concentrations of active ingredients and shorter time to death. However, these combination baits have not been tested for agricultural rodent pests. Therefore, we established a study to test the efficacy of cholecalciferol plus diphacinone artichoke bract and pellet baits to determine their ability to manage California voles *Microtus californicus* in artichokes, where resistance to anticoagulants is known to occur. Field tests using radiocollared voles indicated that bract baits were highly efficacious (85 %), although pellet baits were less effective (60 %). Low observed efficacy of pellet baits may have resulted from poor weather following application during the second sampling period; further testing may yield more positive results. We observed a bimodal distribution in timing of death, with one group of voles dying between 4.3 and 5.8 days post-consumption; the other group died between 9.0 and 14.5 days post-consumption. Deaths in the first group were attributed to cholecalciferol, while deaths in the second group were likely due to chronic anticoagulant exposure. Almost double the proportion of voles that died from bract consumption did so during the early period when compared to their pellet plot counterparts. This suggests that voles were consuming greater quantities of bract baits over a shorter period of time when compared to the pellet bait. Collectively, these findings indicate that baiting with cholecalciferol plus diphacinone coated bracts is an effective method for controlling vole populations in artichokes.

Keywords Artichoke California vole Cholecalciferol Diphacinone *Microtus californicus* Resistance

Key message

- Cholecalciferol plus diphacinone could be an effective alternative to chlorophacinone for managing California vole populations but has not been field tested
- Our findings indicate that cholecalciferol plus diphacinone bract baits were very effective at reducing vole populations in artichoke fields; pellet baits were less effective
- Time to death is quicker with cholecalciferol plus anticoagulant baits than with anticoagulants alone, thereby reducing secondary poisoning hazards
- This combination bait shows promise for use in field applications.

Introduction

Rodenticides are frequently used to manage damaging rodent populations in agriculture around the world. Chronic-exposure anticoagulants (e.g., chlorophacinone and diphacinone) and the acute toxicant, zinc phosphide, are currently the only rodenticides used for aboveground application in agricultural

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fields in the U.S. Anticoagulants generally are considered the safest rodenticides to use given the availability of an antidote (Vitamin K) combined with their multiple-feed requirement. However, rodents can develop a resistance to anticoagulants (e.g., Myllymäki 1995; Salmon and Lawrence 2006), and anticoagulants can pose some risk to non-target scavengers and predators, although this risk is primarily attributed to second-generation anticoagulants (Stone et al. 2003; Gabriel et al. 2012; Tosh et al. 2012). Zinc phosphide poses very little risk to non-target scavengers and predators (Eason et al. 2010) but does have a high risk of toxicity to non-target species that might consume the bait directly (Marsh 1987). Additionally, zinc phosphide often suffers from poor bait acceptance and bait shyness (Marsh 1987). An alternative toxicant that minimizes the negative attributes of these current field-use rodenticides could be a real benefit to agricultural producers world-wide.

One potential alternative that shows initial promise is cholecalciferol plus anticoagulant baits. In the early 1990s, a combination rodenticide containing cholecalciferol plus coumatetralyl (C+C) proved effective at controlling anticoagulant-resistant rats and mice (Pospischil and Schnorbach 1994). Death typically occurred around 5 days. The primary toxic effects of this combination rodenticide resulted from hypercalcemia (i.e., cholecalciferol poisoning) with the anticoagulant acting as a synergist. More recently, this combination has been tested in New Zealand and may be registered for use there in the future (Eason and Ogilvie 2009). Results from studies in New Zealand have indicated that C+C is similar in efficacy to brodifacoum but is less persistent in the environment (Eason and Ogilvie 2009). In fact, C+C baits can often kill after a single feeding, which is not typically accomplished with either active ingredient alone (Pospischil and Schnorbach 1994). However, coumatetralyl is not widely used in the U.S. and is more persistent than diphacinone (Crowell et al. 2013). Therefore, a combination of cholecalciferol plus diphacinone (C+D) would be more practical for use in the U.S. Initial laboratory study of the efficacy of C+D baits has shown promise for Norway rats *Rattus norvegicus* (efficacy = 100 %; C. Eason, unpublished data) and California voles *Microtus californicus* (efficacy = 70–100 %; Witmer et al. 2014) suggesting potential use for these species.

In addition to high efficacy, cholecalciferol plus anticoagulant baits have additional positive attributes including lower concentrations of active ingredients when compared to baits containing just one of the active ingredients. These lower concentrations of active ingredients reduce the risk of secondary toxicity to non-target predatory and scavenging species (Eason and Ogilvie 2009). High levels of cholecalciferol can also lead to bait shyness (Pospischil and Schnorbach 1994), so reducing concentrations of cholecalciferol should increase the palatability of these baits. Additionally, cholecalciferol is very expensive, so a reduction in cholecalciferol usage should result in cheaper products (Eason and Ogilvie 2009). Clearly, cholecalciferol plus anticoagulant combination baits have many positive attributes for use in field applications. Further testing of C+D is warranted to determine the utility of this rodenticide combination in the field.

Rodenticide baiting is often included in Integrated Pest Management (IPM) programs given their overall effectiveness, relatively low application cost, and quick knockdown times (Engeman and Witmer 2000; Baldwin et al. 2014b). For some species, few effective alternatives have historically been available for managing population outbreaks. For example, with California voles, trapping and burrow fumigation are not practical over large areas given the large size of many vole populations. Repellents are generally considered ineffective, and in some crops, habitat modification is of limited use as the crop is the habitat (e.g., alfalfa *Medicago sativa* fields; Baldwin 2011). These limitations are particularly relevant in globe artichokes *Cynara cardunculus* var. *scolymus*, where damage from voles can be extreme (Clark 1984; Salmon and Lawrence 2006). In the U.S., >99 % of artichoke production occurs in California, with the bulk of this production occurring in the Castroville area of Monterey County. Historically, vole control in artichokes has relied on 0.01 % chlorophacinone-treated artichoke bracts, and to a lesser extent, 0.005 % chlorophacinone pellets (Salmon and Lawrence 2006; Baldwin and Stetson 2011). This approach was highly successful for many years, but eventually the local vole population began to develop a resistance to chlorophacinone (Salmon and Lawrence 2006). The development of an alternative toxicant is needed to rotate with chlorophacinone to prevent further resistance to this anticoagulant while still providing effective control of voles in artichoke fields. Cholecalciferol plus diphacinone baits could be a good fit as a rotational rodenticide given the known efficacy of cholecalciferol plus anticoagulant rodenticides against anticoagulant-resistant rodents (Pospischil and Schnorbach 1994). Therefore, we devised a study to test the efficacy of C+D bract and pellet baits to determine if they are effective at managing vole populations in artichokes. If field testing of C+D baits is successful, these baits could provide a more effective and potentially less-hazardous alternative to current field-use rodenticides. At a minimum, they would provide a good rotational option with chlorophacinone baits currently used in artichoke fields.

Materials and methods

Study area

All field activities occurred at a single field owned by Sea Mist Farms. The study site was located approximately

3.2 km southeast of the town of Castroville in Monterey County, California. The field was devoid of voles prior to the initiation of this study. Rows of artichoke plants were located in the middle of berms with broad ditches in between rows. *Oxalis* spp. were found throughout the berms but were not present in the ditches due to herbicide applications.

Enclosures

We initially attempted to test C+D baits in a non-enclosure field setting. However, vole activity was too low to determine if baits were efficacious (only 1 remote-triggered camera out of 50 indicated vole presence; RA Baldwin, unpublished data). Vole abundance can be quite cyclical (Pugh et al. 2003); given low vole abundance at the time of our study, we constructed enclosures to house individuals in an area devoid of voles. Using an unpopulated field allowed us to control the number of voles in an enclosure (stocking rate equivalent to 160–400 ha^{-1}), thereby allowing for a sufficient number of voles to determine effect size, while eliminating the potential for unrealistic densities in defined areas (other studies have shown densities of 600 to >10,000 ha^{-1}; Batzli 1968; Heske 1987; Pugh et al. 2003; Whisson et al. 2005). That being said, voles were housed in enclosures for <3 weeks with abundant food and shelter available, so density likely would not have impacted results appreciably. To determine efficacy, we radiocollared voles, as radiocollared individuals allow for a more direct measure of survival than when relying on indices or estimates of population size (e.g., Sorensen and Powell 1998). Although efficacy trials were conducted in enclosed pens, the enclosures were located in production artichoke fields with growing conditions identical to those available to vole populations in the study area. As such, we considered our approach representative of a standard field study, but with the advantage of greater sensitivity of response rate of study animals to the rodenticides due to known fate of collared individuals, while requiring substantially smaller study areas, which was important given area restrictions (<4.05 ha) for testing novel pesticides in the U.S.

For vole enclosures, we dug trenches that were 46 cm in depth and 20 cm wide using shovels. The trenches were dug in a square pattern and were 16 m in length on all sides (enclosure size = 0.025 ha). Once dug, we placed 91 cm wide sections of 0.6 cm galvanized hardware cloth into the trenches. The bottom 15 cm of the hardware cloth was bent toward the enclosure area at a 90 angle. This bend was designed to keep voles from digging down and around the fencing structure. The trench was then filled in with loose soil, and wooden stakes were driven into the ground at approximately 3 m intervals and attached to the fencing to provide stability and structure to the fence. This left approximately 30 cm of fencing above ground. All artichoke plants that touched or were hanging over the fencing were trimmed to reduce the potential of voles escaping the enclosure. Several voles escaped from the enclosures after the initial release, presumably by climbing out of the structure. We noticed a vole using the corners to assist with climbing, so we added individual sections of overhanging mesh in the corners to prevent this in the future. We also bent the top 5 cm of the hardware cloth toward the enclosure area at a 90 angle to help prevent future escapes. A total of three enclosures were constructed, with baits and control designations randomly assigned to each enclosure. We maintained the same treatment strategy (i.e., bract bait, pellet bait, or control) for each enclosure throughout the study to eliminate the possibility of cross-contamination from a previous treatment.

Capture and collaring

For collaring activities, we utilized a hand-capture method where vole burrow openings were identified, and voles were dug out and captured by hand. This approach allowed for a greater number of captures in areas where vole population size was low (R. Baldwin, unpublished data). It also sped up the capture process thereby limiting the number of days that voles had to be held captive before initiating the study. All voles were captured in identically managed artichoke fields within 2 km of the field trial area so food and shelter resources were similar between capture and release locations. After capture, we weighed and identified gender of voles. We sedated voles with an isoflurane nose cone following procedures outlined by Parker et al. (2008). The nose cone was administered for ~10 to 15 s, depending on the response of the animal. Once the vole was unresponsive, we used a cable tie to fix the transmitter (PIP3 Ag376, mass = 1.4 g; Biotrack Ltd., Wareham, UK) to the vole. If the vole became too active to complete the process, we administered the nose cone for an additional 5–10 s. Once the transmitter was attached, we placed the vole into a holding container for 15–30 min to make sure it resumed normal health and movement. Voles were then randomly assigned to the various treatment plots, although we did attempt to maintain roughly equal sex-ratios for each plot. We tested for differences in the proportion of male and female radiocollared voles using a binomial exact test (Zar 1999) to help characterize our sampled vole population.

Radiotracking

Once voles were released, we initially commenced tracking on a daily basis; locations were obtained during the morning. If a vole was found outside of the enclosure, we

recaptured the individual and placed it back into the enclosure. We never had an individual vole escape more than once. During the course of the first sampling period, we observed a large number of censored individuals (mostly from escaped individuals and predated/scavenged voles) potentially due in part to the voles being released into a new environment. We felt that we would minimize these losses by checking twice daily. Therefore, for the second and third sampling periods, we checked locations both during the morning and afternoon.

When tracking, we identified exact locations and looked for above-ground movement when present. Exact locations were marked with wire flags, so that we knew when voles changed locations between sampling periods. Because of the small size of the voles, we could not add a mortality switch to the radiotransmitters. Therefore, we relied on this movement to assess time to death. If a vole did not move for several days, we attempted to dig up the carcass. If the vole was alive, we resumed normal tracking procedures. If it was dead, we estimated time to death by using the median date between the last known date the vole was alive and the recovery date. Sometimes, voles were found dead on the surface of the ground. When this occurred, we also used the median date between the last known date it was alive and the recovery date. However, if the last known date that a vole was alive was $\leq$3 days post-treatment, we used day 3 as the minimum potential time to death given that we did not observe any earlier dates nor did Witmer et al. (2014) from lab trials. This truncation minimized the chance of an overly low bias on time to death estimates.

Because there was a level of uncertainty for the time to death for each vole, we incorporated this uncertainty in our variance estimates of the mean. For this, we estimated the SE for each time to death observation by dividing the known interval between when the vole was last observed moving and when it was recovered (i.e., confidence interval) by the critical t value for $\alpha = 0.001$. We used this α given our certainty of this timeframe. We then calculated the mean for the combined SE's for each individual time to death estimate for use in bootstrapping models. This SE estimate is essentially a nested measure of variance within the overall variance of the mean value of time to death. We then combined the mean time to death value, the SE for this mean value, and the mean SE for each individual time to death value into a bootstrap equation to calculate an overall SE for the mean (Efron and Tibshirani 1993). Lastly, we utilized a randomization test (bootstrapping; Efron and Tibshirani 1993) to determine if the time to death differed between the two treatment types. We ran 1000 bootstrap iterations of the mean difference in time to death between the treatment types and determined the proportion of values in the resultant ranked frequency distribution below 0. We multiplied this value by two to represent a two-tailed test. This proportion indicated the probability of a difference in the time to death between the two bait types.

Bait application

The C+D pellets and oil concentrate for bract baits were provided by Connovation, Ltd. (Manukau, New Zealand). The pellets were extruded products and contained 0.03 % cholecalciferol and 0.005 % diphacinone. The concentrate contained 7.8 % cholecalciferol and 1.5 % diphacinone. The concentrate was diluted with a 50/1 solution of mineral oil to reach an approximate concentration of 0.156 % cholecalciferol and 0.03 % diphacinone; previous lab research indicated that this concentration was very effective against voles (Witmer et al. 2014). Through lab testing, we determined that the oil mixture accounted for 9.06 % (SE = 0.32) of the coated-bract weight (Baldwin et al. 2014a). Therefore, once the oil mixture was added to the bracts, the estimated concentration of cholecalciferol and diphacinone was approximately 0.014 and 0.003 %, respectively. To coat the bracts, the 50/1 solution was added to bracts and mixed in an industrial cement mixer (see Salmon and Lawrence 2005 for further description).

For application in the bract plot, we placed five bracts at the base of every other artichoke plant, while for the pellet plot, we placed 4–6 g of pellets at the base of every other plant. No pellets or bracts were added to the control plot. Bait application occurred 1–2 days after the last voles were released into their respective enclosures. Tracking during the first and third sampling periods was halted 15 days post-treatment. Tracking during the second sampling period was truncated 14 days post-treatment due to time constraints. Any voles alive at that the end of each sampling period were recaptured and euthanized. We combined all non-censored individuals for each respective treatment to determine efficacy. Efficacy was determined by dividing the number of voles that died by the number of uncensored voles in each treatment plot. We used Fisher's exact test (Zar 1999) to determine if gender of the vole influenced efficacy. All field activities occurred during November 2013 through January 2014. All animal care and use procedures were approved by the National Wildlife Research Center's Institute of Animal Care and Use Committee (Study Protocol QA-2087).

Results

We radiocollared 58 voles during this project. We did not observe a difference in the proportion of males ($n = 33$) and females ($n = 25$) in this population (exact binomial test $p = 0.358$). Of these voles, a large number ($n = 23$) were censored due to escape events, predation/scavenging,

inclement weather, and malfunctioning collars. Of these censored individuals, all but 5 went missing within 2 days post-application indicating that although we did observe losses due to searching behaviors in their new environment, voles acclimated to their new environment quickly. Of the remaining 5 censored individuals, 3 (2 in bract plot and 1 in pellet plot) signals went missing on day 10 post-treatment during the first baiting session when we were only checking for locations once a day in the morning. These three individuals had stopped moving 1–3 days prior to signal loss. This substantial number of signal losses occurring in a single day at a time when we would expect mortality from the rodenticide application, combined with the fact that each of these voles had not moved for 1–3 days prior, suggests that these voles may have been scavenged after death. If these censored individuals did in fact die from rodenticide exposure, then our reported efficacy may in fact be lower than what actually occurred. The remaining two voles (one in control plot and one in bract plot) that were censored escaped on day 11 post-treatment after an irrigation pipe had been mistakenly placed by field workers over the vole enclosures. This likely allowed voles to escape from the enclosure along the pipe.

Of the remaining voles, 13, 15, and 7 were located in the bract, pellet, and control plots, respectively. The numbers in each treatment plot varied across the three sampling periods depending on the number of voles that we were able to capture for each period and the number of voles that were not censored due to reasons described previously (Table 1). We attempted to place approximately equal numbers of voles into both the bract and pellet plots. We did not place any voles in the control plot during the first sampling period due to low numbers captured. Nonetheless, we observed no mortality from any voles located in the control plot during the other two sampling periods (Table 1). Therefore, we are confident that the results from the treatment plots are representative of the efficacy of the two bait types.

Of the two treatment plots, the bract bait was by far the most effective, with a mean observed efficacy of 85 %. This is well above the 70 % threshold required by U.S. EPA to consider the rodenticide effective. Efficacy for the pelletized bait was below this 70 % threshold (60 %), primarily due to low observed efficacy during the second sampling period (Table 1) when weather was cold and rainy approximately 24 h after bait application. Efficacy of both bait types was not impacted by gender of the collared voles (bract bait: Fisher's exact $p = 0.487$; pellet bait: Fisher's exact $p = 0.580$).

Overall, mean time to death was slightly quicker with the bract bait ($x = 6.9$ days, SE = 2.4) than with the pellets ($x = 8.8$ days, SE = 2.8) although this difference was not significant ($p = 0.318$). However, there was a noticeable bimodal distribution in time to death for voles that consumed lethal doses of bait. One group died relatively quickly after bait application ($x = 4.9$ days, SE = 0.9, range 4.3–5.8, $n = 11$, i.e., death attributed to the acute toxicant cholecalciferol). The other group required a longer period of time to succumb to the rodenticide ($x = 11.6$ days, SE = 1.8, range 9.0–14.5, $n = 8$, i.e., death attributed to chronic exposure to diphacinone). The difference in mean time to death between these two periods was significant ($p < 0.001$). Almost double the proportion of voles that died from bract consumption did so during the early period (8 out of 11) when compared to their pellet plot counterparts (3 out of 8). The observed difference was not significant (Fisher's exact $p = 0.181$), although small samples sizes limited the power of this test. Regardless, most voles that consumed the bract baits died in the early period, indicating that cholecalciferol was the primary killing agent, with sufficient consumption perhaps occurring after a single feeding.

Discussion

The use of rodenticide baits is often the preferred method for managing damaging vole populations in agricultural fields (Baldwin et al. 2014a, b). The C+D bract bait we tested was very effective at managing voles in artichoke fields and corroborates a previous lab study that also

Table 1 The number of censored individuals, the number of mortalities versus the number of radiocollared voles per plot (Mortality/total), and the percent efficacy for control, bract, and pellet plots across three trial periods (Trial no.) when testing cholecalciferol plus diphacinone baits for California vole control in artichoke fields in Monterey County, California

Trial no.	Control		Bract		Pellet	
	Censored	Mortality/total	Censored	Mortality/total	Censored	Mortality/total
1			7	2/3	4	4/5
2	2	0/5	0	6/7	3	1/4
3	2	0/2	3	3/3	2	4/6
% efficacy		0		85		60

indicated high efficacy for this bait (Witmer et al. 2014). Our C+D formulation contained substantially lower concentrations of cholecalciferol typically used in rodenticide baits (this study: 0.014 %; typical products: 0.075 %), while also reducing the level of anticoagulant (this study: 0.003 %; typical products: 0.005–0.01 %). Past research conducted by Pospischil and Schnorbach (1994) suggested that efficacy was more dependent on sufficient levels of anticoagulant rather than cholecalciferol. As such, further reduction of diphacinone levels may not be possible, although this merits further exploration. Regardless, the lower level of anticoagulant used in the C+D baits when compared to the chlorophacinone bract baits should reduce potential impacts to non-target species. The shorter time to death observed with the C+D bract baits limits the amount of anticoagulant that can be consumed by the rodent, further limiting potential secondary hazards. Furthermore, the risk of secondary toxicity is generally considered fairly minimal with first-generation anticoagulants (Silberhorn et al. 2006; McMillin et al. 2008; Lima and Salmon 2010), so little negative impact to predators or scavengers is expected from the combination bait if applied appropriately.

Our results closely mirrored those obtained in other studies when comparing 0.01 % chlorophacinone bract baits ($x = 88$ %, Salmon and Gibson 2003; $x = 86$ %, Baldwin and Stetson 2011) and 0.005 % chlorophacinone pellet baits ($x = 71$ %, Baldwin and Stetson 2011), indicating that both C+D and chlorophacinone baits can be effective at managing vole populations in artichokes. However, voles have started to develop resistance to chlorophacinone in the study population (Salmon and Lawrence 2006). As such, efficacy of chlorophacinone baits is expected to diminish over time unless an alternative active ingredient is rotationally applied to counteract this resistance. The C+D bract bait we tested would provide an effective alternative to rotate into IPM programs to counter this resistance pattern in the local population.

We did not observe any impact of gender on efficacy, which is counter to what was reported by Witmer et al. (2014) in an initial lab study of these combo baits. The difference observed in the lab study was likely driven by small samples sizes (Witmer et al. 2014). The C+D bract bait appears to be equally effective for both males and females, which is essential for effective management of rodent species.

Although the C+D bract baits proved very effective against voles, the pellet baits were less effective. Historically, pellet baits have been less effective than bract baits for vole control in artichokes (Marsh et al. 1984; Baldwin and Stetson 2011), likely due to the familiarity of the vole population to the local food source. However, in a lab investigation comparing the C+D pellet and bract baits, both proved to be highly effective (bracts: efficacy = 70–100 %, pellets: efficacy = 80–100 %; Witmer et al. 2014). We feel that the observed lower efficacy of the field trial may have been driven in part by inclement weather after bait application during the second sampling period, as substantial rainfall, wind, and low temperatures likely reduced vole activity and diminished the palatability of the pelleted bait following application. Previous positive lab trial results (efficacy = 80–100 %; Witmer et al. 2014), combined with the favorable results from the other two applications in more favorable weather conditions (x efficacy = 73 %), suggest that further tests of these pellets may be warranted.

However, even if the efficacy of pellet baits can be increased, they may not ever be as efficacious as the bract baits. For example, applications of the bract baits occurred at the same time as the pellet baits, yet bract applications were highly successful during the second sampling period ($x = 86$ %, Table 1). Additionally, mortality appeared to be impacted primarily by cholecalciferol given the short time to death for the majority of the voles in the bract plots. Cholecalciferol plus an anticoagulant has the ability to kill after a single feeding if sufficient quantities are consumed; this was not the case when each active ingredient is consumed separately (Pospischil and Schnorbach 1994). Given the longer times to death observed for the pellet baits, it appears that voles are often not consuming enough of the bait to kill after a single feeding, perhaps due to reduced palatability of the pellets. This longer time to death has several negative ramifications including greater potential plant damage caused by voles before death and a potentially elevated risk of secondary hazards due to greater consumption of bait over time. Further investigation into the mean residual levels of cholecalciferol and diphacinone in poisoned voles from both baiting strategies, as well as the cause of slower time to death for pellet baits, could provide insight into whether or not pellets pose a greater secondary toxicity risk than bract baits, while also potentially yielding a pelletized bait that is sufficiently effective to control voles in artichokes. Regardless of the outcome of such an investigation, baiting with C+D-coated bracts appears to be an effective method for controlling vole populations in artichokes. Registration of this product could be pursued to add an additional tool to current IPM programs for managing voles. This addition would likely reduce the impact of chlorophacinone resistance in the local vole population, dramatically increasing the sustainability of vole management in this important crop.

Author contribution

RAB and GWW designed this study. RAB, RM, and GWW were involved in data collection. RAB was responsible for

data analysis and writing. All authors reviewed the manuscript.

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