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An efficacy test of cholecalciferol plus diphacinone rodenticide baits for California voles (*Microtus californicus* Peale) to replace ineffective chlorophacinone baits

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California voles cause damage to pastures and rangelands, orchards and nurseries, and a wide variety of field crops, including artichokes. The anticoagulant rodenticides (chlorophacinone and diphacinone) typically used for voles are becoming less effective in controlling their populations. Consequently, there is a need to identify new rodenticides that will have a high efficacy on California voles so that agricultural production losses to rodents can be substantially reduced. We tested a new formulation containing two active ingredients (cholecalciferol and diphacinone) as a control method for California voles. Both a pelleted bait and an oil-coated artichoke bract bait were very palatable and efficacious against wild-caught, captive California voles. Efficacy levels of 70%–80% were achieved in the two-choice feeding trials. Additionally, the days-to-death (5–6 days) were less than the time-to-death with anticoagulant only baits. We recommend that a field efficacy study be conducted with cholecalciferol plus diphacinone bait formulations to determine their field performance in the reduction of agricultural damage by California voles.

Keywords: California vole; cholecalciferol; diphacinone; *Microtus californicus*; rodenticide; wildlife damage

1. Introduction

There are numerous species of microtines (Subfamily Microtinae) throughout the northern hemisphere and, at high population densities, several become serious pests (Nowak 1991). In North America, many of the pest species belong to the genus *Microtus*, commonly called voles or meadow mice (Clark 1984; Edge et al. 1995). The biology, ecology, management, and distribution of voles, along with the types of damage caused, have been summarized by O'Brien (1994) and Pugh et al. (2003). Two species (*M. californicus* and *M. montanus*) cause significant agricultural damage in California (Clark 1994). Voles cause damage to pastures and rangelands, orchards and nurseries, and a wide variety of field crops including alfalfa, grains, clover, potatoes, sugar beets, artichokes, carrots, Brussels sprouts, cauliflower, and tomatoes (Clark 1994; O'Brien 1994). Additionally, most species of voles exhibit strong population cycles, whereby they reach very high densities (>2471/ha) every 3–5 years. Severe damage to agricultural and forestry resources occurs at these peak densities (Witmer & VerCauteren 2001; Witmer et al. 2007; Witmer & Proulx 2010).

First-generation anticoagulants (chlorophacinone and diphacinone) and acute (zinc phosphide) rodenticides are currently used in California to control vole populations, primarily by placing bait in runways near burrow openings, by spot-baiting or broadcasting bait over the infested area. In California artichoke fields, the rodenticides are often applied through an oil-based coating on artichoke bracts given the availability of culled artichoke bracts

combined with the voles' strong preference for these bracts (Marsh et al. 1985). It appears, however, that the efficacy of the first generation anticoagulants for vole control has declined in recent years (Salmon & Lawrence 2006). It is possible that voles in California's intensive vegetable production areas have developed a genetic or physiological resistance to some anticoagulants (Horak K, 2012, personal communication). It is also possible that the high vitamin K content of green vegetables may reduce the effectiveness of anticoagulant rodenticides because vitamin K is the antidote to anticoagulant poisoning (Witmer et al. 2013). Witmer et al. (2013), however, found that voles from the Fort Collins area fed a diet high in vitamin K-rich plants along with the anticoagulant rodenticides and did not reduce the efficacy of chlorophacinone baits, but may have reduced the efficacy of diphacinone baits. And finally, it could be that there is a palatability or formulation issue with the currently used commercial baits. Consequently, there is a need to identify new rodenticide formulations that will have a high efficacy on California voles so that agricultural production losses to rodents can be substantially reduced.

Researchers in New Zealand are investigating a combination of existing rodenticide, one with two active ingredients (cholecalciferol and coumatetralyl) has had promising results against rats and mice (Eason et al. 2010). Interestingly, they were able to obtain high efficacy with lower concentrations of the active ingredients than the concentrations used when either active ingredient alone is used. Hence, there may be some synergistic effect

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with the anticoagulants causing hemorrhaging and the cholecalciferol causing hypercalcemia. This is noteworthy because if lower concentrations can be used to effectively control rodent populations, there could be a lower risk to non-target animals and less environmental contamination. Additionally, lower concentrations of active ingredients may increase the palatability of a bait formulation. Currently, there are no rodenticide products registered for use in the United States that combine two active ingredients.

The objective of this study was to determine the efficacy of a cholecalciferol plus diphacinone rodenticide bait on California voles in both no-choice and two-choice trials, using wild-caught voles in a controlled setting. We selected these two active ingredients because each is registered as a single active ingredient rodenticide in the United States (US). Hence, the US Environmental Protection Agency (which regulates pesticide use in the US) is familiar with these compounds and has extensive data sets on each of them. We tested a pelleted bait and an oil bait (for artichoke bract dipping). We expected that the test baits would exhibit a high ($\geq 80\%$ mortality) efficacy when presented to wild-caught California voles.

2. Materials and methods

2.1. Vole trapping and maintenance

Voies used in this study were California voles (*Microtus californicus*) live-trapped in Monterey County, CA, and transported to the USDA National Wildlife Research Center (NWRC), Fort Collins, CO. Animals were maintained and used in compliance with the requirements of the United States' Animal Welfare Act. Voies were kept in individual numbered shoebox cages in an animal room at NWRC. They were fed a maintenance diet of rodent chow pellets (Formulab 5008, PMI Nutrition International, Inc., Brentwood, TN) and apple slices, and received water *ad libitum*. They were provided with bedding and a den tube. There was a two-week quarantine and acclimation period before the trials were started. When females had litters, the young were euthanized.

2.2. Treatment materials and procedures

There were seven trials conducted, each involving a different treatment (Table 1). Voies were randomly assigned

to the treatment and control groups. The C+D pelleted bait used in this study was provided by Connovation Ltd. (Manukau, New Zealand). These pellets contained 0.03% cholecalciferol and 0.005% diphacinone (henceforth, "C+D pellets"). Connovation Ltd. also provided 150 ml of the C+D oil concentrate which contained 11.68 g cholecalciferol and 1.95 g diphacinone. We used consumer-grade canola oil from a grocery store for our dilutions of the concentrate (henceforth, "C+D-coated bracts"). In the bract trials, we dipped four fresh artichoke bracts in the oil and placed them in each vole cage. The voies were fed untreated bracts the day before the trial began. The untreated bracts were readily fed upon by the voies which had been captured in artichoke fields in California. Hence, the voies had previous exposure to artichoke plants as a food source. However, before arrival, the voies had not been previously exposed to the maintenance diet or the C+D pellets. The cholecalciferol pellets used in this study contained 0.075% cholecalciferol. Additionally, a control group of voies was maintained on the maintenance diet so that their mortality levels could be compared with those of the treatment groups.

The weight, sex, cage number, and treatment of each vole were recorded before the initiation of a trial. On day 1 of the trial, all food was removed from the cages and was replaced with a weighed amount of the bait alone (no-choice) or a weighed amount of the bait plus rodent chow (two-choice) as per the treatments described in Table 1. They continued to receive water *ad libitum* throughout all trials. Foods were replenished as needed, so that it was always available to the treatment voies during the next 10 days (i.e., throughout the rodenticide exposure period). In the two-choice trials, this also included replenishment of the maintenance diet. Uneaten pelleted foods in the cages were gathered at the end of the 10-day exposure period and weighed. This allowed us to determine the total amount of pelleted rodenticide bait consumed during the trial. However, we did not determine the daily consumption rates. Additionally, we did not account for any drying of the baits due to room conditions over the exposure period. On day 11, remaining voies were put into clean, individual cages with the maintenance diet for another 10 days of observation (post-exposure period). We did not estimate the weight of bracts

Table 1. Numbers and gender of voies, rodenticide types, trial type, and exposure periods for the seven rodenticide treatments used in this study.

Treatment number	Number of voies (females/males)	Trial type	Rodenticide type	Parts canola oil: parts oil concentrate	Exposure period (days)	Post-exposure period (days)
1	10 (5/5)	No-choice	C+D pellet	N/A	10	10
2	10 (5/5)	Two-choice	C+D pellet	N/A	10	10
3	5 (3/2)	No-choice	C+D oil-coated bract	30:1	10	10
4	5 (3/2)	No-choice	C+D oil-coated bract	50:1	10	10
5	5 (3/2)	No-choice	C+D oil-coated bract	60:1	10	10
6	10 (6/4)	Two-choice	C+D oil-coated bract	60:1	10	10
7	5 (4/1)	Two-choice	Cholecalciferol pellet	N/A	10	10

Note: C+D = cholecalciferol plus diphacinone; N/A = not applicable.

eaten as they tended to dry out quickly and we wanted to minimize disturbance of the voles during the trial.

Voies were examined twice daily, but not handled, and their condition and any mortalities were recorded. Dead voies were placed in individual, labeled zip-lock bags and refrigerated for later necropsy. When necropsied, they were examined for signs of anticoagulant poisoning as described by Stone et al. 1999. Carcasses were later incinerated. All surviving voies at the end of the study were euthanized and incinerated.

2.3. Statistical analyses

The percent mortality of each treatment group was compared to the mortality of the control group using Fisher's exact test. Days-to-death between groups and pelleted bait consumption between groups was compared using a *T*-test. We considered a $P \leq 0.05$ to represent a significant difference.

3. Results and discussion

3.1. Bait acceptance

The C+D pellets seemed to be very palatable as there was no significant difference ($P = 0.555$) between their consumption in the no-choice trial (ave. of 8.1 g per vole over the 10-day exposure period; Table 2) and the consumption of the pellets in the two-choice trial (ave. of 7.1 g per vole over the 10-day exposure period; Table 2). The consumption of the cholecalciferol pelleted bait was low (ave. of 2.4 g per vole over the 10-day exposure period) and was significantly lower ($P = 0.014$) than the consumption of the C+D pelleted bait (ave. of 7.1 g per vole over the 10-day exposure period) in our two-choice trial (Table 2). This may have been a palatability issue with the cholecalciferol bait from the commercial formulation or perhaps because the higher concentration of the active ingredient resulted in lower bait acceptance which has been demonstrated in the past (Marsh et al. 1985). As mentioned in Section 2, we were not able to accurately quantify the amount of the coated bracts that the voies consumed. However, all bracts had been fed on; the voies seem to particularly prefer the "fleshy" part of the bract near its base. Typically, the "fleshy" part of the bract comprises only about 18%–20% of the bract's entire weight.

3.2. Efficacy and mortality

We first tried C+D pellets in a no-choice trial to see if this would be an effective rodenticide with voies. The efficacy was 100% so we proceeded with a two-choice trial (Table 2). In this case, the C+D pellets were still highly efficacious with 80% mortality (Table 2). In neither of these trials nor any of the subsequent trials did any control animals die so the mortality level for that group was always 0%. The average days-to-death were nearly the same for the voies in these two trials (6.0 versus 6.5; Table 2). Generally, rodents exposed to an anticoagulant rodenticide do not start dying until day 7 or so, and most do not die before 10–12 days have passed since first exposure (Timm 1994). We found this to also be true for voies in an earlier trial using either a chlorophacinone or diphacinone bait, whereby the average days-to-death was 9.4 (Witmer et al. 2013). This was a significantly longer period of time ($F = 3.58$, $P = 0.048$) than what we observed with the C+B bracts and the C+D pellets in the study being reported here. Upon necropsy, a number of voies had white nodules on organs which we suspected might be calcium deposits because over intoxication of cholecalciferol (vitamin D) causes hypercalcemia (Eason et al. 2000). We speculated that their death might primarily have been due to the acute cholecalciferol toxicant that was dispatching the voies sooner than expected for anticoagulants. Hence, we put a group of voies on a two-choice trial with a pelleted cholecalciferol bait which contained 60% more cholecalciferol than our C+D pelleted bait. All five voies survived the trial (Table 2).

Our first three C+D oil-coated artichoke bract trials were designed to determine a minimum dilution of the C+D oil concentrate to use that would achieve a high level of efficacy. Using less active ingredients, while achieving high efficacy, also reduces the cost of rodenticide production and purchasing by users in many cases. We expected high efficacy with the 30:1 dilution of the oil concentrate and that did occur (80%). The follow-up dilutions of 50:1 and 60:1 were also highly efficacious (100% in both trials; Table 2). It was then important to test whether or not the 60:1 dilution would still be effective when presented with an alternative food source (i.e., the maintenance diet). In that two-choice trial, the efficacy was 70% which was somewhat lower than anticipated

Table 2. Mortalities, days-to-death, and average rodenticide bait consumption over a 10-day exposure period by California voies by rodenticide bait type (pellet versus oil-coated).

Treatment	Number of dead voies	Ave. days-to-death (S.D.)	Pelleted bait consumption (g) (S.D.)
C+D pellets (no-choice)	10 of 10	6.00 (1.89)	8.13 (4.35)
C+D pellets (two-choice)	8 of 10	6.50 (2.00)	7.07 (3.56)
30:1 C+D oil treated artichoke bracts (no-choice)	4 of 5	4.50 (0.58)	N/A
50:1 C+D oil treated artichoke bracts (no-choice)	5 of 5	5.80 (0.84)	N/A
60:1 C+D oil treated artichoke bracts (no-choice)	5 of 5	5.40 (1.14)	N/A
60:1 C+D oil treated artichoke bracts (two-choice)	7 of 10	6.14 (2.73)	N/A
Cholecalciferol pellets (two-choice)	0 of 5	N/A	2.44 (0.81)

Note: S.D. = Standard deviation; N/A = Not applicable.

(Table 2). Although we did not conduct a two-choice trial with the 50:1 dilution, we recommend that the 50:1 dilution of the oil concentrate be used if a field trial is conducted to help assure adequate efficacy.

All trials of the C+D pellets and C+D oil-coated artichoke bracts resulted in significantly higher (all values of $P \leq 0.024$) mortality than in the control group. The average days-to-death of the C+D pelleted bait (6.3 days) versus the C+D oil dipped bracts (5.5 days) was not significantly different ($P = 0.215$; Table 2). Additionally, the days-to-death was not significantly different ($T = 0.51$, $P = 0.6215$) between females and males.

We found that the mortality level in males (100%) was significantly greater ($P = 0.0298$) than for females (55%). While this result may be from the small sample sizes in the study, it needs to be investigated further because it is important that a rodenticide be highly effective for the female component of the pest population to reduce future reproductive potential. Fisher (2005) noted in a review of house mouse (*Mus musculus*) susceptibility to anticoagulant rodenticides that female mice seem to be less susceptible than male mice.

Both tested two-active ingredient formulations have potential for control of anticoagulant resistant voles in artichoke fields in California. Some of the advantages of two active ingredient rodenticide are increased efficacy and reduced concentrations of active ingredients over those currently being used in single active ingredient rodenticides. It has also been suggested that the acute toxicant, because of its more rapid “knock down” time, might result in sickened animals retreating to burrows or other refugia before the anticoagulant takes effect and causes their death. Morgan et al. (2013) noted that dosing brush-tail possums (*Trichosurus vulpecula*) with cholecalciferol combined with aspirin (an anticoagulant) resulted in more rapid mortality than dosing with aspirin alone. In the case of burrowing rodents such as voles, this could potentially reduce the risk of predators and scavengers having access to poisoned carcasses.

We recommend that a field efficacy study be conducted with these two-active ingredient formulations to confirm their value to the reduction of agricultural damage by California voles.

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