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# An efficacy test of a cholecalciferol plus diphacinone rodenticide bait for California voles resistant to chlorophacinone baits

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We tested a new rodenticide containing two active ingredients (cholecalciferol and diphacinone) as a control method for California voles which cause substantial damage to commercial artichoke production in California. The voles in the area have apparently become resistant to the first generation anticoagulant chlorophacinone. Both a pelleted bait and an oil-dipped artichoke bract bait were very palatable and efficacious in controlled wild-caught, captive California voles. We recommend that a field efficacy study be conducted with these rodenticide baits to confirm their value to the reduction of agricultural damage by California voles.

## 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 Pugh et al. (2003) and O'Brien (1994). There are two species of voles (*M. californicus, M. montanus*) that 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 (>1,000/ac) every 3-5 years. Severe damage to agricultural and forestry resources occurs at these peak densities (Witmer and Proulx 2010, Witmer and VerCauteren 2001, Witmer et al. 2007).

Anticoagulant (chlorophacinone and diphacinone) and acute rodenticides (zinc phosphide) are used to control vole populations, primarily by placing bait in runways near burrow openings, or by spot-baiting or broadcasting over the infested area. In California artichoke fields, the rodenticides are often applied through an oil-based coating on artichoke bracts. It appears, however, that the efficacy of anticoagulants for vole control has dropped off in recent years (Salmon and Lawrence 2006). It is possible that voles in California's intensive vegetable production areas have developed a genetic or physiological resistance to some anticoagulants (Katherine Horak, NWRC, personnel 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 (2010), however, found that voles from the Fort Collins area fed a diet high in vitamin K-rich plants along with the anticoagulant rodenticides did not reduce the efficacy of chlorophacinone baits, but may have reduced the efficacy of diphacinone baits. 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.

Researchers in New Zealand are investigating a new "combination" rodenticide, one having 2 active ingredients (cholecalciferol and coumatetralyl) and have found promising results with rats and mice (Eason et al. 2010). Interestingly, they are able to obtain high efficacy with lower concentrations of the active ingredients than the concentrations used when either active ingredient are used as the only rodenticide in a commercial bait. Hence, there may be some synergistic effect. This is noteworthy because if lower concentrations can be used to effectively control rodent populations, there could be a lower risk of harm to non-target animals.

The objective of this study was to determine the efficacy of a cholecalciferol plus diphacinone rodenticide baits on California voles in both no-choice and 2-choice trials, using wild-caught voles in a controlled setting. We tested a pelleted bait and an oil bait (for artichoke bract dipping). We hypothesized that the test baits would exhibit a high ($\geq$ 90% mortality) efficacy when presented to wild-caught California voles.

## Methods

Voles used in this study were California voles (*Microtis californicus*) live-trapped in Monterey County, California and transported to the USDA National Wildlife Research Center (NWRC), Fort Collins, Colorado. Voles were kept in individual numbered shoebox cages an animal room at NWRC. They were fed a maintenance diet of rodent chow pellets and apple slices, and received water *ad libitum.* They were provided with bedding and a den tube. There was a two week quarantine period before the trials were started. There were six trials conducted, each involving a different treatment:

1. A no-choice cholecalciferol plus diphacinone pelleted bait trial. This was to determine the efficacy of this new bait at concentrations of 0.03% cholecalciferol and 0.005% diphacinone pelleted bait (henceforth, "C+D pellets").
2. A two-choice cholecalciferol plus diphacinone pelleted bait trial. This was to determine the palatability of the new rodenticide bait given that an alternative food was available (i.e., the maintenance diet).
3. A no-choice cholecalciferol plus diphacinone oil concentrate diluted 30:1 with canola oil which was used to dip artichoke bracts in before presentation to the voles (henceforth, "C+D dipped bracts"). This was to determine the appropriate dilution of the concentrate needed for high efficacy.
4. A no-choice cholecalciferol plus diphacinone oil concentrate diluted 50:1 with canola oil before dipping the bracts.
5. A no-choice cholecalciferol plus diphacinone oil concentrate diluted 60:1 with canola oil before dipping the bracts.
6. A two-choice cholecalciferol plus diphacinone oil concentrate diluted 60:1 with canola oil which was used to dip artichoke bracts in before presentation to the voles. This was to determine the palatability of the new rodenticide-dipped bracts given that an alternative food was available (i.e., the maintenance diet).
7. A two-choice cholecalciferol pelleted bait trial. This bait contained no diphacinone and was conducted to see if the relatively rapid time-to-death of voles on the C+D pelleted bait was from the effects of the cholecalciferol alone.

The C+D pelleted bait used in this trial was provided by Duncan MacMorran and Dr. Charlie Eason. Dr. Eason is affiliated with Lincoln University, Lincoln, New Zealand and with Connovation Ltd., Manukau, New Zealand; Duncan MacMorran is affiliated with Connovation, Ltd. They also provided 150 g of an C+D oil concentrate. The concentrate contains 11.68 g (7.8%) of cholecalciferol and 1.95 g (1.3%) of diphacinone. He also provided a table showing the percentages of the two active ingredients when the concentrate is diluted from 10:1 to as much as 60:1 with a vegetable oil. We used consumer-grade canola oil from a grocery store for our dilutions. In the bract trials, we dipped 4 fresh artichoke bracts in the oil and placed them in each vole cage. The voles were fed untreated bracts the day before the trial began. The bracts were readily fed upon by the voles which had been captured in artichoke fields in California.

Additionally, a control group of voles was maintained on the maintenance diet so that mortality levels could be compared with those of the treatment groups. Voles were randomly assigned to treatment groups. Ten voles of mixed sexes were used in each of the first two trials (C+D pelleted bait trials), and in the sixth trial (dipped bracts, two-choice trial). For trials 3-5 (no-choice dipped bracts to determine the appropriate dilution of the C+D oil concentrate), we used 5 voles in each. Likewise, with trial 7 (cholecalciferol pelleted bait) we used 5 voles.

The weight, sex, cage number, and treatment of each vole was recorded before the initiation of a trial. Rodents were fed the maintenance diet before the start of the trial. On Day 1 of the trial, all food was removed from the cages and was be 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 above. They continued to receive water *ad libitum* throughout all trials. Foods were replenished as needed, so that it was always available to the treatment voles during the next 10 days (i.e., throughout the rodenticide exposure period). 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. On day 11, remaining voles 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 eaten and they tended to dry out quickly.

Voles were examined twice daily and their condition and any mortalities were recorded. Dead voles 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 voles at the end of the study were euthanized and incinerated.

Voles were randomly assigned to the treatment and control groups. 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.

## Results and Discussion

<u>Pellet Trials.</u> We first tried C+D pellets in a no-choice trial to see if this would be an effective rodenticide with voles that were somehow resistant to the customary anticoagulant rodenticides being used in artichoke fields in California. The efficacy was 100% so we proceeded with a two-choice trial (Fig. 1). In this case, the C+D pellets were still highly efficacious with 80% mortality (Fig. 1). 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 8.2 g per vole; Table 2) and the consumption of the pellets in the two-choice trial (ave. of 7.1 g per vole; Table 3). The average days-to-death were nearly the same for the voles in these 2 trials (6.0 versus 6.5; Table 1). Generally, rodents exposed to an anticoagulant rodenticide don't start dying until day 7 or so and most don't die before 10-12 days have passed since first exposure. Upon necropsy, a number of voles had white nodules on organs which we suspected might be calcium deposits because over intoxication of cholecalciferol (vitamin D) causes hypercalcemia. We speculated that their death might primarily have been due to the acute cholecalciferol toxicant that was dispatching the voles so soon. Hence, we put a group of voles on a two-choice trial with a pelleted cholecalciferol (0.075% which is the standard concentration of commercial cholecalciferol baits). All 5 voles survived the trial (Table 4). The consumption of the cholecalciferol pelleted bait was low (ave. of 2.4 g per vole; Table 4) and was significantly lower (P = 0.014) than the consumption of the C+D pelleted bait (ave. of 7.1 g per vole) in our two-choice trial (Table 3). This may have been a palatability issue with the cholecalciferol bait from

the commercial formulation or perhaps because of the higher concentration of the active ingredient (0.075% versus 0.03% cholecalciferol in the C+D pelleted bait).

Oil dipped artichoke bract trials. Our first 3 C+D oil `dipped artichoke bract trials were to determine an appropriate dilution of the C+D oil concentrate to use that would achieve a high level of efficacy. This was important because it is prudent to minimize the amount of toxicants placed in the environment. 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 occurred (80%). The follow-up dilutions of 50:1 and 60:1 were also highly efficacious (100% in both trials; Fig. 1). 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 trial, the efficacy was 70% which was somewhat lower than anticipated (Fig. 1). Hence, if a field trial is conducted, we recommend that the 50:1 dilution of the oil concentrate be used.

All trials of the C+D pellets and C+D oil dipped artichoke bracts resulted in significantly higher (all values of $P \leq 0.024$) mortality than in the control group. (Note: no control animals died during the course of the study so the mortality for that group was 0%). 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 1). Hence, both rodenticide formulations seem to have potential for control of anticoagulant resistant voles in artichoke fields in California. We recommend that a field efficacy be conducted with these rodenticide baits to confirm their value to the reduction of agricultural damage by California voles.

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**Literature Cited**

Clark, J. 1984. Vole control in field crops. Proc. Vertebr. Pest Conf. 11:5-6.

Clark, J. 1994. Vertebrate pest control handbook. 4th Ed. California Department of Food and Agriculture, Sacramento. 803 pp.

Eason, C., R. Henderson, S. Hix, D. MacMorran, A. Miller, E. Murphy, J. Ross, and S. Ogilvie. 2010. Alternatives to brodifacoum and 1080 for possum and rodent control—how and why? New Zealand Journal of Zoology 37:175-183.

Edge, W., J. Wolff, and R. Carey. 1995. Density-dependent responses of gray-tailed voles to mowing. J. Wildl. Manage. 59:245-251.

Nowak, R. 1991. Walker's mammals of the world. 5th Ed. Vol. II. Johns Hopkins Press, Baltimore. 1,629 pp.

O'Brien, J. 1994. Voles. Pages B-177 - B-182 *In:* S. Hygnstrom, R. Timm, and G. Larsen, eds. Prevention and control of wildlife damage. Cooperative Extension Division, University of Nebraska, Lincoln.

Pugh, S., S. Johnson, and R. Tamarin. 2003. Voles. Pages 349-370 *In:* G. Feldhamer, B. Thompson, and J. Chapman, eds. Wild mammals of North America. The Johns Hopkins University Press, Baltimore.

Salmon, T., and S. Lawerence. 2006. Anticoagulant resistance in meadow voles. Proc. of the Vertebr. Pest Conf. 22:156-160.

Stone, W., J. Okoniewski, and J. Stedelin. 1999. Poisoning of wildlife with anticoagulant rodenticides in New York. J. Wildl. Diseases 35:187-193.

Witmer, G. 2010. The effects of vitamin K-rich plant foods on the efficacy of two anticoagulant rodenticides for voles (*Microtus montanus*). Final Report, QA-1629. USDA National Wildlife Research Center, Fort Collins, CO. 11 pp.

Witmer, G., and G. Proulx. 2010. Rodent outbreaks in North America. Pp. 253-267 *In*: G. Singleton, S. Belmain, P. Brown, and B. Hardy, eds. Rodent outbreaks: ecology and impacts. Los Banos, Philippines: International Rice Research Institute.

Witmer, G., and K. VerCauteren. 2001. Understanding vole problems in direct seeding–strategies for management. Pages 104-110 *In:* R. Veseth, ed. Proc. of the Northwest Direct Seed Cropping Systems Conference. Northwest Direct Seed Conference, Pasco WA.

Witmer, G., R. Sayler, D. Huggins, and J. Capelli. 2007. Ecology and management of rodents in no-till agriculture in Washington, USA. Integrative Zoology 2:154-164.

Figure 1. Percent efficacy of various cholecalciferol plus diphacinone (C+D) rodenticide baits using wild-caught California voles. The first two bars represent pelleted baits, whereas the last four bars represent dipped artichoke bracts. Details on the baits are presented in the text.

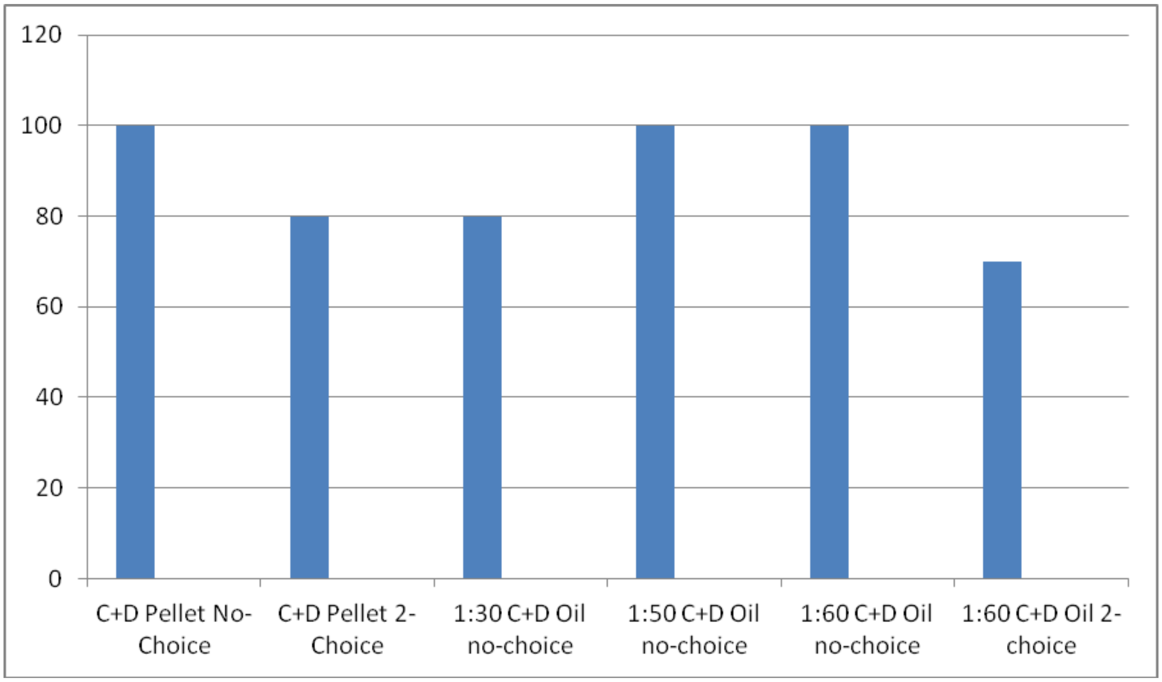

Table 1. Days-to-death of California voles by rodenticide bait type (pellet versus oil).

| Treatment | Number of Dead Voles | Ave. Days to Death | S.D. Days to Death |
|---|---|---|---|
| C+D Pellets (no-choice) | 10 of 10 | 6.00 | 1.89 |
| C+D Pellets (two-choice) | 8 of 10 | 6.50 | 2.00 |
| 30:1 C+D Oil Treated Artichoke Bracts (no-choice) | 4 of 5 | 4.50 | 0.58 |
| 50:1 C+D Oil Treated Artichoke Bracts (no-choice) | 5 of 5 | 5.80 | 0.84 |
| 60:1 C+D Oil Treated Artichoke Bracts (no-choice) | 5 of 5 | 5.40 | 1.14 |
| 60:1 C+D Oil Treated Artichoke Bracts (two-choice) | 7 of 10 | 6.14 | 2.73 |

Table 2. Food consumption and fate of California voles in the C+D pellet no-choice trial.

| Animal ID | Weight of Food IN (g) | Date IN | Weight of Food OUT (g) | Date OUT | Amount Consumed (g) | Fate of Animal | Date of Fate | Number of Days Exposed |
|---|---|---|---|---|---|---|---|---|
| 03M | 40.09 | 5/16 | 20.54 | 5/25 | 19.55 | died | 5/26 | 10 |
| 05F | 39.99 | 5/16 | 33.94 | 5/21 | 6.05 | died | 5/21 | 6 |
| 08M | 39.99 | 5/16 | 34.70 | 5/21 | 5.29 | died | 5/21 | 6 |
| 13M | 39.99 | 5/16 | 33.80 | 5/19 | 6.19 | died | 5/19 | 4 |
| 15F | 40.10 | 5/16 | 32.42 | 5/21 | 7.68 | died | 5/21 | 6 |
| 24M | 40.03 | 5/16 | 30.67 | 5/21 | 9.36 | died | 5/21 | 6 |
| 27M | 39.95 | 5/16 | 30.95 | 5/21 | 9.00 | died | 5/21 | 6 |
| 30F | 40.02 | 5/16 | 31.97 | 5/21 | 8.05 | died | 5/20 | 5 |
| 34F | 39.96 | 5/16 | 35.97 | 5/21 | 3.99 | died | 5/20 | 5 |
| 39F | 39.97 | 5/16 | 33.83 | 5/21 | 6.14 | died | 5/20 | 5 |
| | | | | Ave | 8.13 | | Ave | 5.90 |
| | | | | SD | 4.35 | | SD | 1.60 |

Table 3. Food consumption and fate of California voles in the C+D pellet two-choice trial.

| Animal ID | Weight of C+D IN (g) | Date C+D IN | Weight of C+D OUT (g) | Date C+D OUT | Amount C+D bait Consumed (g) | Weight of Rodent Chow IN (g) | Date Rodent Chow IN | Weight of Rodent Chow OUT (g) | Date Rodent Chow OUT | Amount Rodent Chow Consumed (g) | Fate of Animal | Date of Fate | Number of Days Exposed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 04F | 40.05 | 5/23 | 29.63 | 6/1 | 10.42 | 40.32 | 5/23 | 36.94 | 6/1 | 3.38 | died | 6/4 | 10 |
| 07F | 40.10 | 5/23 | 33.12 | 6/1 | 6.98 | 40.28 | 5/23 | 40.28 | 6/1 | 0.00 | alive | N/A | 10 |
| 09F | 39.94 | 5/23 | 37.28 | 6/1 | 2.66 | 40.12; 40 | 5/23; 5/30 | 59.21 | 6/1 | 20.91 | alive | N/A | 10 |
| 10M | 40.11; 40.00 | 5/23; 5/27 | 66.43 | 5/30 | 13.68 | 40.41 | 5/23 | 39.05 | 5/30 | 1.36 | died | 5/30 | 8 |
| 11M | 39.98 | 5/23 | 37.83 | 5/29 | 2.15 | 39.05 | 5/23 | 39.13 | 5/29 | -0.08 | died | 5/26 | 4 |
| 16M | 39.94 | 5/23 | 34.63 | 5/29 | 5.31 | 39.86 | 5/23 | 36.26 | 5/29 | 3.60 | died | 5/28 | 6 |
| 28F | 39.98 | 5/23 | 34.24 | 5/29 | 5.74 | 40.22 | 5/23 | 36.81 | 5/29 | 3.41 | died | 5/26 | 4 |
| 37M | 40.15; 40.00 | 5/23; 5/27 | 71.34 | 5/30 | 8.81 | 40.29 | 5/23 | 37.03 | 5/30 | 3.26 | died | 5/29 | 7 |
| 43F | 39.97 | 5/23 | 34.36 | 5/29 | 5.61 | 40.04 | 5/23 | 36.03 | 5/29 | 4.01 | died | 5/28 | 6 |
| 48M | 40.02 | 5/23 | 30.67 | 5/29 | 9.35 | 39.58 | 5/23 | 36.23 | 5/29 | 3.35 | died | 5/29 | 7 |
| | | | | Ave | 7.07 | | | | Ave | 4.32 | | Ave | 7.20 |
| | | | | SD | 3.56 | | | | SD | 6.02 | | SD | 2.30 |

Table 4. Food consumption and fate of California voles in the cholecalciferol pellet two-choice trial.

| Animal ID | Weight of Food IN (g) | Date IN | Weight of Food OUT (g) | Date OUT | Amount Consumed (g) | Fate of Animal | Date of Fate | Number of Days Exposed |
|---|---|---|---|---|---|---|---|---|
| 18F | 40.03 | 9/4 | 36.28 | 9/14 | 3.75 | Alive | N/A | 7 |
| 52F | 40.06 | 9/4 | 38.10 | 9/14 | 1.96 | Alive | N/A | 7 |
| 54F | 39.97 | 9/4 | 37.26 | 9/14 | 2.71 | Alive | N/A | 7 |
| 53M | 39.99 | 9/4 | 38.20 | 9/14 | 1.79 | Alive | N/A | 7 |
| 46F | 40.05 | 9/4 | 38.06 | 9/14 | 1.99 | Alive | N/A | 7 |
| | | | | Ave. | 2.44 | | | |
| | | | | S.D. | 0.81 | | | |