Are Cholecalciferol plus Anticoagulant Rodenticides a Viable Option for Field Rodents?

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ABSTRACT: Burrowing rodents, such as pocket gophers (Geomyidae) and voles (*Microtus* spp.), often cause extensive damage in agricultural, urban/residential, and natural resource areas. Effective management of burrowing rodents typically follows an integrated pest management (IPM) approach that involves a number of tools including rodenticide baiting. However, some of the more commonly used rodenticides have limitations including the development of resistance (e.g., first-generation anticoagulants and strychnine), secondary-toxicity concerns (e.g., anticoagulants), and limited availability (e.g., strychnine). Initial research with combination cholecalciferol plus anticoagulant rodenticides has indicated potential promise at overcoming some of these limitations. As such, we tested the efficacy of several different cholecalciferol plus anticoagulant combinations to determine if they were efficacious in managing Botta's pocket gophers and California voles in both cage and field trials. Two-choice cage trials for California voles indicated that both pelletized (0.03% cholecalciferol plus 0.005% diphacinone, efficacy $\bar{x}$ = 80%) and bract baits (0.012% cholecalciferol plus 0.002% diphacinone, efficacy $\bar{x}$ = 70%) containing cholecalciferol plus diphacinone (C+D) were efficacious. Further field testing indicated that C+D-coated bract baits (0.014% cholecalciferol plus 0.003% diphacinone) were highly efficacious for vole control (efficacy $\bar{x}$ = 85%), while pelletized baits were less promising (efficacy $\bar{x}$ = 60%). Cage trials indicated that both C+D (0.03% cholecalciferol plus 0.005% diphacinone, efficacy $\bar{x}$ = 80%) and two concentrations of cholecalciferol plus brodifacoum (C+B1 = 0.015% cholecalciferol plus 0.0025% brodifacoum, efficacy $\bar{x}$ = 100%; C+B2 = 0.03% cholecalciferol plus 0.0025% brodifacoum, efficacy $\bar{x}$ = 100%) pelleted baits showed promise as pocket gopher rodenticides. Further field testing of C+D and C+B2 resulted in efficacy significantly >70% (efficacy $\bar{x}$ = 83% and 75%, respectively), although strychnine (0.5%) applications were the most efficacious (efficacy $\bar{x}$ = 100%). Collectively, these results suggest that cholecalciferol plus anticoagulant rodenticides are effective options for managing burrowing rodent populations; they deserve further consideration for registration against these potentially damaging species.

KEY WORDS: brodifacoum, cholecalciferol, diphacinone, integrated pest management, *Microtus* spp., pocket gopher, rodenticide, strychnine, *Thomomys* spp., vole

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INTRODUCTION

Field rodents such as pocket gophers (Geomyidae) and voles (*Microtus* spp.) cause extensive damage in a variety of agricultural and natural resource environments including, but not limited to, loss of crop production (e.g., Gebhardt et al. 2011, Baldwin et al. 2014), food safety concerns (Meerburg and Kijlstra 2007, Kilonzo et al. 2013), disease transmittance (see Williams and Barker 2001 for examples), and damage to irrigation and water storage infrastructure (Ordeñana et al. 2012, Baldwin et al. 2014). The use of an integrated pest management (IPM) approach is preferred to mitigate these damage situations (Engeman and Witmer 2000, Baldwin et al. 2014). IPM strategies for field rodents often include a combination of management tools including habitat modification, cultural practices, exclusion, trapping, burrow fumigation, and rodenticides.

Of these tools, rodenticides are often one of the preferred tools as they are usually the quickest and easiest method for population reduction, and they are generally very effective (Engeman and Witmer 2000, Baldwin et al. 2014). However, current rodenticides do have some limitations. For example, rodents can develop a resistance to some rodenticides [e.g., California voles (*Microtus californicus*) and Chlorophacinone – Salmon and Lawrence 2006, Horak et al. 2015; pocket gophers (*Thomomys* spp.) and strychnine – Lee et al. 1990, Marsh 1992], thereby rendering them ineffective.

Secondly, some rodenticides pose secondary toxicity risks. Second-generation anticoagulants (e.g., brodifacoum, bromadiolone, difethialone, and difenacoum) are widely known to pose the greatest risk, given their high potency and long half-lives in various tissues within the target species (Eason et al. 2010). First-generation anticoagulants and strychnine also pose some risk, although these risks are generally considered much less either due to lower toxicity and shorter half-lives (Crowell et al. 2013) or due to target species [e.g., strychnine is only used in burrow systems of pocket gophers, which spend the vast majority of their lives below ground (Gettinger 1984), thereby limiting their availability to predators/scavengers].

Furthermore, not all rodenticides are readily available when needed. Strychnine baits, for example, are available in limited supplies given minimal amounts of strychnine currently imported into the U.S. (Baldwin et al. 2016b). Without a sufficient supply, it does not matter how effective a rodenticide is; if it is not available, it is not a practical management tool. Because of these shortcomings, there is a definite opportunity for an alternative field-use rodenticide to mitigate some of these concerns.

One possible alternative is a combination of cholecalciferol plus an anticoagulant. With this combination rodenticide, the anticoagulant generally acts as the synergist, increasing the potency of cholecalciferol (Eason and Ogilvie 2009). This allows a lower concentration of cholecalciferol in the combination bait than when used by itself, which is beneficial, given that rodents often show avoidance of cholecalciferol at higher concentrations (Pospischil and Schnorbach 1994). It also lowers potential costs, which is important, given that cholecalciferol is more expensive than most other rodenticides (Eason and Ogilvie 2009).

Initial research indicated that a combination of cholecalciferol plus coumatetralyl was effective against anticoagulant-resistant Norway rats (*Rattus norvegicus*) and house mice (*Mus musculus*). This combination was initially pursued in New Zealand as a potential toxicant for possums (*Trichosurus vulpecula*) and rats (*Rattus* spp.) and proved to be as efficacious as brodifacoum, which is generally considered to be the most efficacious anticoagulant rodenticide (Eason and Ogilvie 2009). However, the pursuit of this registration was eventually dropped in favor of a combination of cholecalciferol plus diphacinone (C+D; active ingredient concentrations vary depending on the species), given lower secondary toxicity risk associated with diphacinone when compared to coumatetralyl while maintaining high efficacy (C. Eason, pers. comm.). The positive performance of C+D was important, as diphacinone is registered for use in the U.S. while coumatetralyl is not.

Although C+D may have great utility for many field rodents, it might not be as effective against pocket gophers, as pocket gophers do not always accept grain or pelletized baits at a high rate, given that their normal diet consists of roots and green vegetation; this can reduce general efficacy of pocket gopher baits (Marsh 1992). Higher concentration baits may prove more effective, given the need to consume less bait to obtain a lethal dose. As previously mentioned, brodifacoum is considered the most efficacious anticoagulant rodenticide (Eason and Ogilvie 2009). Combining brodifacoum with cholecalciferol (C+B) might yield superior efficacy when compared to C+D and is worth exploring. Therefore, we set up a series of studies to look at the efficacy and potential utility of using a combination of cholecalciferol plus anticoagulant rodenticides for California vole and Botta's pocket gopher (*Thomomys bottae*) management. A brief summary of our findings are highlighted below. See Baldwin et al. (2016a, 2016b), Witmer et al. (2013), and Witmer and Baldwin (2014) for a complete review of these projects.

VOLE LAB TRIALS

Voles can cause extreme damage in artichokes (Clark 1984, Salmon and Lawrence 2006). Historically, chlorophacinone-treated bracts have been the preferred tool for managing voles in this crop, but voles have begun to develop a resistance to chlorophacinone in some fields (Salmon and Lawrence 2006, Horak et al. 2015). A combination C+D bait, either on bracts or on pellets, may prove to be a more effective option. To test this, we live-trapped voles in artichoke fields in Monterey County, CA, during April 2012 and transported them to the National Wildlife Research Center in Fort Collins, CO, to conduct cage trials. We initially conducted no-choice trials using both bract and pellet baits. For bract baits, we tested three different dilutions of a 7.8% cholecalciferol and 1.3% diphacinone solution: 30:1, 50:1, and 60:1. Bracts were coated in the dilution and fed to voles *ad libitum.* Pellet baits (0.03% cholecalciferol plus 0.005% diphacinone) were also offered to voles *ad libitum*. Efficacy was 100% for all trials except for the highest concentration of C+D on the bract baits, which was 80%.

Given the positive results from the no-choice trials, we proceeded to two-choice trials where voles were provided both the combination bait and a standard maintenance diet. For two-choice trials, we tested the lowest concentration C+D bract bait (60:1 dilution), the C+D pellets, as well as a 0.075% cholecalciferol pellet bait for comparative purposes. Although the cholecalciferol bait was completely ineffective, both the pellet and bract C+D baits proved efficacious (80% and 70% mortality, respectively). Mean time-to-death was 6.1 and 6.5 days for the bract and pellet baits, respectively, which is shorter than what is typically observed for anticoagulant-only baits (e.g., Witmer et al. 2013). Based on our positive results from lab trials, we decided field trials were warranted. Greater detail on this study can be found in Witmer et al. (2013).

VOLE FIELD TRIALS

We established three 0.025-ha enclosures in artichoke fields in Monterey County, CA, to house voles for field tests. Each enclosure was randomly assigned to one of the following: bract application (0.014% cholecalciferol plus 0.003% diphacinone), pellet application (0.03% cholecalciferol plus 0.005% diphacinone), or control. For these trials, voles were captured by hand (see Baldwin et al. 2015 for description of technique) and radiocollared to monitor survival. Voles were then released into one of the three enclosures and were given at least 1-2 days to acclimate to the enclosure before commencement of rodenticide application.

For application, five coated bracts or 4-6 grams of pellets were placed at the base of every other artichoke plant within their respective treatment enclosures. Voles were then monitored for survival for up to 15 days post-treatment. These trials were repeated three times between November 2013 and January 2014 to determine mean efficacy of each treatment type. Treated bract baits proved more efficacious than the pelletized bait (efficacy $\bar{x}$ = 85% and 60%, respectively), with a mean time-to-death that was also somewhat shorter ($\bar{x}$ = 6.9 and 8.8 days, respectively), although not significantly so. C+D bract baits appear to be an efficacious option for vole control in artichoke fields; pelletized baits may hold less promise. For greater detail on this study, please see Baldwin et al. (2016a).

POCKET GOPHER LAB TRIALS

Strychnine has generally been the most effective rodenticide for pocket gopher management (Marsh 1992), but strychnine bait supply is dwindling in the U.S. A new alternative may soon be needed to replace strychnine if supplies do not increase, and even if supplies do increase, the availability of an effective alternative rodenticide

would be of great use to land managers and pest control professionals to help mitigate potential strychnine resistance (Lee et al. 1990, Marsh 1992). As such, we established a cage trial to test four different cholecalciferol plus anticoagulant baits (C+D = 0.03% cholecalciferol plus 0.005% diphacinone, C+B1 = 0.015% cholecalciferol plus 0.0025% brodifacoum, C+B2 = 0.03% cholecalciferol plus 0.0025% brodifacoum, C+B3 = 0.015% cholecalciferol plus 0.00125% brodifacoum) to determine the potential utility of these products as a pocket gopher rodenticide.

To supply pocket gophers for the study, we initially live-trapped and transported wild pocket gophers from two different locations (San Diego and Sonoma Counties) in CA and transported them to the National Wildlife Research Center in Fort Collins, CO, during February through March 2014. After an acclimation period of at least two weeks, two-choice trials commenced with combination baits and standard maintenance diets provided to the pocket gophers *ad libitum*. The C+B1, C+B2, and C+D baits were all considered highly efficacious ($\bar{x}$ efficacy = 100%, 100%, and 80%, respectively), while the C+D3 product was less effective ($\bar{x}$ efficacy = 60%). Greater detail on this study can be found in Witmer and Baldwin (2014).

POCKET GOPHER FIELD TRIALS

Given the effectiveness of C+B1, C+B2, and C+D baits in cage trials, we decided to set up a field study (with concentrations same as in cage trials) to determine how effective they would be in a more realistic setting. We compared these baits to 0.5% strychnine bait (Avalon Gopher Grain Bait, RCO International, Inc., Harrisburg, OR) to serve as a comparison. For this investigation, we established three study sites in vineyards in San Joaquin County, CA during summer 2015. Each study site was divided into four treatment blocks and a control block that were 1 ha in size, with a 0.4-ha treatment plot located in the center. Nine 9.1×9.1-m monitoring plots were established in a 3×3 grid structure within each treatment plot. We used the open-hole method to monitor pocket gopher activity (Engeman et al. 1993, 1999). This approach involved opening a hole into the pocket gopher burrow system and then checking to see if the hole was plugged 48 hours later. Two holes were opened per monitoring plot. If any of the holes were plugged, the plot was considered occupied; if unplugged, the plot was considered unoccupied. This allowed us to compare occupancy before and after treatment to assess efficacy for each rodenticide.

For bait application, we used the funnel-and-spoon method that involved poking a hole into a pocket gopher tunnel system. We then inserted a funnel into the opening and poured the appropriate amount of bait into the opening (C+D, C+B1, and C+B2 = 10-11 g; strychnine = 5 g). The hole was then sealed with a wad of toilet paper and covered with loose soil. Tunnels were treated 1-3 times depending on the estimated size of the burrow system. Baits were applied in all active burrow systems within the 0.4 ha-treatment plot and extended 9.1 m beyond the treatment plot on all sides to help limit reinvasion before efficacy could be assessed. Applications occurred twice, separated by approximately three weeks. Two treatment periods are often needed for pocket gophers to account for their variable mounding, as 20-30% of pocket gophers are often missed following the initial treatment period given a lack of fresh mounds associated with those individuals (Richens 1965). Rodenticides were considered efficacious if mean efficacy values were significantly >70% (Schneider 1982).

All rodenticides yielded mean efficacy values >70% after two treatments, although C+B1 was not significantly >70% given substantial variability in efficacy across treatment plots (C+D: efficacy $\bar{x}$ = 83%, SD = 7; C+B1: efficacy $\bar{x}$ = 85%, SD = 17; C+B2: efficacy $\bar{x}$ = 75%, SD = 0; strychnine: efficacy $\bar{x}$ = 100%, SD = 0). The strychnine product was most efficacious, indicating that it is still an effective option when available. Although C+D and C+B2 were effective options, C+D may be the more practical combination, given the use of a first-generation anticoagulant as the synergist, as opposed to brodifacoum, which is a second-generation anticoagulant. Additional details on this study can be found in Baldwin et al. (2016b).

DISCUSSION

Combination cholecalciferol plus anticoagulant rodenticides seem to hold real promise for vole and pocket gopher management, and perhaps for other field rodents as well. They have proven highly efficacious and apparently work well against anticoagulant and strychnine resistant rodents (Pospischil and Schnorbach 1994, Witmer and Baldwin 2014); they generally exhibit shorter times-to-death than reported for anticoagulants (Eason and Ogilvie 2009, Witmer and Baldwin 2014), which should reduce the chance of secondary toxicity; and they typically utilize lower concentrations of active ingredients, which further reduces the chance of secondary toxicity, increases palatability, and reduces cost (Pospischil and Schnorbach 1994, Eason and Ogilvie 2009). Given these positive attributes, we feel that consideration should be given to registration of these combination rodenticides for use against field rodents.

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