PROJECT REPORT

Project Title: An Investigation of the Effects of Vitamin K-Rich Plant Food on the Efficacy of Diphacinone on Voles

Research Agency: National Wildlife Research Center

Principal Investigator: Gary Witmer

Budget: **$**22,623.00

Background:

Introduction

There are numerous species of microtines (Subfamily *Microtinae*) throughout the northern hemisphere and, particularly at high population densities, several are known to be 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). Specifically, Californian (*M. californicus*) and montane (*M. montanus*) voles cause significant agricultural damage in California (Clark 1994). California is the top agricultural producing state, generating nearly $39 billion worth of produce annually (Shwiff et al. 2009). Meanwhile, damage to crops from rodents (e.g., including voles) and birds consistently results in annual losses of jobs (2,106-6,317) and revenue ($168-504 million). 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'Brein 1994). Additionally, most species of voles exhibit strong population cycles whereby they reach extremely high densities (>1,000/ac) every 3–5 years (Ford and Pitelka 1984, Hornfeldt et al. 1986, Krebs 1996, Stenseth 1999, Ylonen et al. 2003). Severe damage to agriculture and forestry resources commonly occurs during the peak of the cycle (Witmer and VerCauteren 2001, Witmer et al. 2007). Therefore, many growers relied on anticoagulant rodenticide baits to reduce the populations of voles, and subsequently reduce the damage to crops (O'Brien 1994). Recently however, observations have shown a reduction in the efficacy of those rodenticides (Salmon and Lawrence 2006a), thereby resulting in high levels of damage.

One hypothesis for the reduced efficacy of anticoagulant rodenticides is that voles are counteracting the toxicant by inadvertently ingesting elevated levels of the antidote for anticoagulants (i.e., vitamin K) along with their daily diet. Vitamin K, a compound found in many green leafy plants, is ingested by most herbivores through their daily diet of plants (Robbins 1993). Vitamin K is then physiologically processed for the production of blood clotting proteins, among other uses. For this reason, vitamin K is the antidote for poisoning from an anticoagulant compound (e.g., Machintosh et al. 1988, Tasheva 1995).

There are several chemical formulations of vitamin K, which all are required in metabolic activity in the liver where blood-clotting proteins are synthesized (Robbins 1993; Tasheva 1995). Vitamin K1 is phytomenadione and it is produced by green plants. Vitamin K2 compounds (1-13, depending on the number of isoprenoid residues) are menaquinones and they are produced by bacteria. Vitamin K3 is menadione; this form is produced commercially and is added to animal feeds. The vitamin K requirements of vertebrates are met by dietary intake and microbial synthesis in the digestive system, but also by "recycling" of vitamin K in the liver (Hadler and Buckle 1992; Robbins 1993). Vertebrates require a constant supply of vitamin K. However, there is a limit to the amount to vitamin K an animal can utilize, and the excess will be excreted (Harrington *et al.* 2007).

There have been conflicting findings regarding if diets enhanced with vitamin K-rich plants can reduce the efficacy of anticoagulant rodenticides for rodents (e.g., Chaudhary and Tripathi 2004, Witmer and Burke 2009). However in these studies, the variations in the toxicants (i.e., active ingredients) and the species of rodents tested may explain the inconsistent findings. Witmer and Burke (2009) found that treating Norway rats (*Rattus norvegicus*), black rats (*R. rattus*), and house mice (*Mus musculus*) fed green plants with high levels of vitamin K; (i.e., collard greens [0.62 mg vitamin K/100 g food] and Brussels Sprouts [0.19 mg/100 g]) did not seem to reduce the efficacy of brodifacoum or diphacinone anticoagulant rodenticides. Before that study, Chaudhary and Tripathi (2004) conducted a study of Indian gerbils (*Tatera indica*) and found a high dose of vitamin K (i.e., 2 mg/kg body weight for 15 days) indeed reversed the effects of an anticoagulant rodenticide (i.e., difethialone), while a smaller dose (i.e., 1 mg/kg) body weight did not. Several researchers have noted that some feeds for commercial livestock are supplemented with vitamin K (Partridge 1980b; MacNicholl and Gill 1993). They suggested that the availability of that vitamin K enriched food source may lessen the effectiveness of anticoagulant rodenticides.

Because of the observed reductions in efficacy of anticoagulants in California, other research has been conducted to identify the problem (e.g., Salmon and Lawrence 2006a). Those researchers identified a region of California where *M. californicus* showed evidence of genetic-resistance to chlorophacinone. However, Salmon and Lawrence (2006a) reported that the findings were unique to the area (i.e., 3 artichoke fields in Castroville county), and we surmised that the resistant voles were not likely exposed to high levels of vitamin K (artichoke = 0.01 mg/100 g; calculated from USDA website mentioned below). Therefore, other regions of California that grow crops, particularly crops that are rich with vitamin K, remained "in question" regarding the reduction in efficacy of anticoagulant rodenticides.

The United States Department of Agriculture (USDA) provides a listing of the vitamin K content for a large number of foods on the following website: (www.nal.usda.gov/fnic/foodcomp/Data/SR21/nutrlist/sr21w430.pdf). Vitamin K-rich plants are readily available to voles in California and, in many cases, may be the crop the growers are trying to protect from vole damage by using the anticoagulant rodenticides. Voles have been reported to cause significant amounts of damage to many types in agricultural fields (Clark 1984; Hines 1993, 1997; Hygnstrom et al. 1996, 2000; Hines and Hygnstrom 2000). Some examples of vitamin K-rich plants that are cultivated in California and consumed by voles are collards (*Brassica Oleracae*), spinach (*Spinacea*

oleracea), and sugar beets (*Beta vulgaris*; Clark 1994). Additionally, some non-cultivated plants (e.g., the common dandelion [*Taraxacum officinale*]) also contain high amounts of vitamin K and have been introduced throughout the world (Gleason 1952), including California. To our knowledge no studies have examined if the efficacy of anticoagulant rodenticides for voles can be reduced by vitamin K-rich diets.

We examined if a diet rich in vitamin K could reduce the efficacy of two commonly-used first-generation anticoagulant rodenticides, (i.e., 0.005% chlorophacinone [Rozol® pellets, Liphatec, Inc., Milwaukee, WI]; and 0.005% diphacinone [Ramik® brown nuggets, Haaco, Inc., Randolph, WI]) for *M. montanus*, in controlled laboratory trials. The Environmental Protection Agency (EPA) standard for desired efficacy of rodenticides in a 2-choice laboratory trial is 90% mortality (Schneider 1982). We considered the EPA standard as our guideline for acceptable efficacy in our trials. To make the study realistic to California, we used vitamin K-rich plants that were commonly cultivated in California as the maintenance food and alternative 2-choice diet. We predicted that both anticoagulant rodenticides would have lower than acceptable efficacy when fed to voles that were maintained on diets rich with vitamin K.

Objectives:

1. Determine if vitamin K-rich plant foods can reduce the efficacy of a major vole control tools (diphacinone and chlorophacinone rodenticides) in some situations.

2. If reduced efficacy does occur, determine actions to remedy or prevent this situation from occurring.

Summary:

The following is an abstract from the final report:

Voles (*Microtus* spp.) can cause significant losses to vegetable production in California. Growers rely on anticoagulant rodenticide baits to reduce populations and damage. In recent years, the efficacy of chlorophacinone and diphacinone rodenticides has been noticeably decreasing. Several reasons may account for this arising situation, including: genetic resistance, or the possibility that many of the green leafy vegetables that are consumed by voles contain high levels of vitamin K (i.e., the antidote to anticoagulants) and counteract the effects of the rodenticides. We tested the latter hypothesis using 3 groups of voles fed either: 1) chlorophacinone bait, 2) diphacinone bait, or 3) control (rodent chow) in a laboratory trial. All voles were fed plant diets with high vitamin K content for 30 days, along with the rodenticide bait for 10 day, and monitored for mortality. Despite the high vitamin K diet, we found the chlorophacinone bait remained highly efficacious (100% mortality), however the diphacinone bait showed low efficacy (60% mortality). In a separate, no-choice, trial (during which voles were not fed vitamin K-rich food, but were offered the same rodenticides) we found that neither bait had acceptable efficacy (compared to the EPA standard of 90%), although sample sizes were small. We surmised that a diet rich with vitamin K did not negate the effects of the

chlorophacinone rodenticide for voles, and we recommend the continued use of chlorophacinone baits in California to reduce damage by voles. We suspect that the diphacinone bait could have shown some reduced efficacy from the vitamin K-rich diet. We found evidence to suggest that diphacinone baits are marginally effective for vole control, although the results warrant more investigation.

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