

An Abstract of the Thesis of

Guiming Wang for the degree of Doctor of Philosophy in Wildlife Science

presented on January 14, 2000. Title: Improving Ecological Risk Assessment of

Pesticides for Nontarget Terrestrial Vertebrates.

Abstract approved:

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The Quotient Method (QM) is used by the United States Environmental Protection Agency (USEPA) in ecological risk assessments of pesticides for nontarget organisms. The QM requires several assumptions regarding exposure and hazards of pesticides to wildlife; several of these assumptions have not been tested. During 1997-99, I conducted three experiments using the gray-tailed vole (*Microtus canicaudus*) as a model species to test three assumptions of the QM. The experiments were conducted in 24 0.2-ha fenced vole-proof enclosures. In Experiment 1, I tracked voles using radio-telemetry and found that animals did not move from contaminated to uncontaminated habitat to avoid exposure to a pesticide, thus supporting one assumption of the QM. In Experiment 2, I studied demographic responses of gray-tailed voles and northern bobwhite quail (*Colinus virginianus*) to liquid and granular formulations of diazinon. The results of Experiment 2 indicated that quail were more susceptible to granular diazinon than

to liquid diazinon because of direct consumption of diazinon granules. Neither formulation of diazinon at 0.55 or 1.55 kg AI/ha adversely affected vole demography. In Experiment 3, I used sprinklers to simulate a 0.25-cm rainfall to test the assumption that the expected environmental concentration (EEC) of a pesticide is estimated immediately after application, and that rainfall does not modify the risk of pesticides to animals. The 0.25-cm rainfall may have reduced the risk of voles to Guthion® 2S either by improving the dry season habitat or by washing more pesticide residues down to the soil and reducing exposure of the animals. This experiment did not support the assumption of the QM that weather would not affect the EEC of pesticides. Last, I used a Ricker model incorporating demographic stochasticity to simulate the 1998 and 1999 vole populations and a single pesticide application at different population sizes. The simulations demonstrate that demographic stochasticity could cause uncertainties in predictions of significant effects of pesticide on voles, especially for small populations. These simulations suggest that ecological risk assessments of pesticides to nontarget wildlife should consider demographic characteristics of wildlife species to minimize the uncertainty of predictions.

**Improving Ecological Risk Assessment of Pesticides for Nontarget Terrestrial  
Vertebrates**

by

**Guiming Wang**

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## Contribution of Authors

This thesis is prepared in manuscript format with each chapter except for the first and last, prepared for submission to an international peer-review journal. Dr. W. Daniel Edge and Dr. Jerry O. Wolff were involved in the research design of each experiment and in writing of each manuscript, and helped in the field data collection during the study.

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# Improving Ecological Risk Assessment of Pesticides for Nontarget Terrestrial Vertebrates

## Chapter 1

### Introduction

Ecological risk of pesticides to nontarget organisms has drawn the attention of conservation biologists for over three decades (Carson, 1962; Hoffman et al., 1990; Kendall and Akerman, 1992; Pimentel et al., 1992). Since the 1940's, pesticides have been used to control agricultural pests and enhance agricultural productivity worldwide. However, pesticides also cause environmental problems, including adverse effects on wildlife. Direct toxic effects (e.g., physiological impairment, sublethal and lethal effects) and indirect adverse effects (e.g., declines in food availability and habitat deterioration) of pesticides on wildlife have been demonstrated repeatedly (Ratcliffe, 1967; Grue et al., 1983; Potts, 1986; Walker et al., 1996). Pesticides may affect organisms at multiple levels, including molecular, physiological, individual, population, and community levels (Newman, 1996).

### **Toxic Effects of Organophosphorus Pesticides on Wildlife**

#### *Physiological Effects of Organophosphorus Pesticides*

Organophosphorus pesticides (OPs) inhibit activation of acetylcholinesterase (AChE). AChE is an enzyme that breaks down acetylcholine,

which functions as a chemical messenger at nerve synapses. When acetylcholine accumulates at a nerve synapse, it causes overstimulation of the receptor and continues to engender a signal after this nervous stimulation should have stopped. As a consequence, the synapse is blocked and no additional signals can be relayed (Walker et al., 1996). Therefore, OPs disrupt central and peripheral nervous system functions of target pests and birds and mammals. This nervous system disruption in turn results in the failure of physiological functions and causes behavioral abnormalities. Respiratory paralysis caused by the depression of AChE is the immediate cause of death in birds (Murphy, 1975). Meyers and Wolff (1994) found that brain AChE activity of gray-tailed voles (*Microtus canicaudus*) was reduced by 40-50% for animals that died during laboratory tests. Generally, the physiological effects of pesticides are the underlying mechanisms for the toxic effects observed at other levels (e.g., behavioral and population levels).

### *Behavioral Responses of Wildlife to Organophosphorus Pesticides*

Behaviors of an organism represent the integrated result of multiple biochemical and physiological processes. Numerous studies have examined the behavioral effects of OPs (Peakall, 1985). Behavioral responses have been observed in birds when AChE activity is depressed to <50% of normal (Grue et al., 1983; Grue and Mineau, 1991). Starlings (*Sturnus vulgaris*) exposed to chlorfenvinphos (2-chloro-1-(2,4-dichlorophenyl) ethenyl diethyl phosphate) spent less time standing on one leg (Hart, 1993), singing and flying (Grue and Shipley,

1981), spent more time perching (Grue and Shipley, 1981), and reduced their ability to defend territories and care for offspring (Hart, 1993). House sparrows (*Passer domesticus*) exposed to chlorfenvinphos dropped 30% more seed than unexposed sparrows (Fryday et al., 1994). Common shrews (*Sorex araneus*) exposed to sublethal doses of dimethoate (*O,O*-dimethyl *S*-[2-(methylamino)-2-oxoethyl] phosphorodithioate) reduced their young rearing, exploring, sniffing, and locomotor activities (Dell'Omo et al., 1997). Exposure to dimethoate also depressed the locomotor activity, young rearing, grooming, and sniffing of wood mice (*Apodemus sylvaticus*) (Dell'Omo and Shore, 1996a, 1996b). In summary, behavioral responses may be a good indicator or measurement of the exposure and physiological impairment of birds and mammals to OPs.

#### *Demographic Responses of Wildlife to the Exposure of Organophosphorus Pesticides*

Physiological impairments and behavioral abnormalities caused by OPs can result in death and declines in reproduction of wildlife (Grue et al, 1983; Smith, 1993). Brewer et al. (1996) reported that exposure to terbufos (*S*-[[[1,1-dimethylethyl)thio]methyl] *O,O*-diethyl phosphorodithioate), an OP, at 21 mg/kg of body weight, led to 44% mortality in wild northern bobwhite quail (*Colinus virginianus*). Buerger et al. (1991) found that survival rate of sublethal-dosed northern bobwhites decreased compared to wild controls and caged controls, suggesting that behavioral changes made the birds more susceptible to predation. Application of Guthion® 2S (*O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-

yl)methyl] phosphorodithioate) at 1.55-4.67 kg/ha (2-6 times the recommended rates) decreased male and female survival rates of gray-tailed voles for 2-6 weeks, but significant effects on reproductive performance and juvenile recruitment were not detected (Edge et al., 1996). Schauber et al. (1997) found that azinphos-methyl applied at 3.61 kg/ha caused severe effects on population size and growth, and recruitment of voles, but no detectable effects on reproductive activity of females.

Individual vital parameters (e.g., survival rate, mortality and reproductive activity) of wildlife may not be comprehensive enough to reflect the effects of pesticides on wildlife. An increase in mortality in a population can be compensated by an increase in reproduction or immigration (Sibly, 1996). Each vital rate also has different sensitivity to changes in population size and growth rate of a structured population (Caswell, 1989, 1996). However, declines in population size and population growth rate provide unambiguous evidence for the adverse effects of a chemical. Therefore, demographic parameters of a population remain important for assessing ecological effects of pesticides and the mechanisms for population dynamics under contamination. The relationship between toxic effects on vital rates of individuals and subsequent adverse effects on a population is a crucial question in ecotoxicology.

### *Risk Assessment of Pesticides and the Quotient Method*

The U.S. Environmental Protection Agency (EPA) attempts to minimize the effects of pesticides on nontarget species through implementation of the Federal

Insecticide, Fungicide and Rodenticide Act of 1988 (FIFRA), which sets regulations on the acceptance and use of chemical pesticides. Even under these regulations, some chemicals are used that result in detrimental effects on nontarget birds and mammals (Kendall and Ackerman, 1992). One criterion originally required by FIFRA was to field test certain pesticides prior to registration. However, in 1992 the regulations were revised and field tests are no longer required (Norton et al., 1992; USEPA, 1992). However, without field tests, greater uncertainty exists regarding the potential impact of pesticides on nontarget species (Rattner and Fairbrother, 1991; Kendall and Lacher, 1994; Tiebout and Brugger, 1995). This is especially true in that exposure of animals to pesticides in the field depends on a suite of extrinsic and intrinsic factors that cannot be estimated in the laboratory.

The revision of EPA's registration requirements also raises a question from the view point of scientific methodology or the scientific basis of current ecological risk assessment. With respect to the comprehensiveness and integration of methodology, field or microcosm studies can integrate data from different levels (e.g., physiological, individual, population, and community levels). In contrast, laboratory data usually have a single attribute (Nabholz et al., 1997). In addition, extrapolation of ecological conclusions or patterns from one spatial or biological scale to another scale is still a challenge faced by both ecotoxicologists and ecologists (Levin, 1992). Experimental mesocosm or field studies could provide

valuable information for ecological risk assessment of pesticides that cannot be obtained in laboratory studies.

Currently, the EPA relies extensively on the use of the Quotient Method (QM) for estimating risk to wildlife. The QM is a simple formula that estimates risk based on the estimated environmental concentration (EEC) divided by the LC<sub>50</sub> or LD<sub>50</sub> (median-lethal concentration or dose of chemicals to the nontarget species). The QM consists of four parts (Urban and Cook, 1986; Fite, 1994): the nontarget species assumed to be at risk; the hazard to that species (or usually a surrogate), estimated by the LC<sub>50</sub> or LD<sub>50</sub>; the expected concentration of the pesticide in the nontarget individual's diet, estimated from the chemical's application rate; and the risk factor, estimated by the formula:  $\text{risk} = \text{EEC}/\text{LC}_{50}$ . Current policy lists a risk factor of  $<0.2$  as acceptable.

Tiebout and Brugger (1995) outline 12 assumptions of the QM with an emphasis on avian species, all of which leave considerable uncertainty with respect to its effectiveness. These 12 assumptions are: (1) nontarget species remain in the spray zone; (2) toxicity is equivalent for nontarget and reference species; (3) pesticide toxicity is equivalent in laboratory and field; (4) surface residues on food are the only exposure vector; (5) all ingested food items are contaminated with residues; (6) only direct toxicity has an impact; (7) food intake is based on dry weight measure; (8) food intake of wild and caged animals is equivalent; (9) EECs are measured immediately after pesticide application; (10) maximum residues remain on food items; (11) animals are exposed to only a single pesticide

application; and (12) risk is based only on a lethal endpoint. These assumptions cause problems when the data collected in the laboratory are extrapolated into the field. Other sublethal endpoints (e.g., behavioral abnormalities), demographic responses other than mortality of populations, and the interaction between chemical and the environment are ignored in current assessment methods.

Since 1992, the small mammal research group at Oregon State University has conducted a series of field and laboratory experiments to test the validity of the QM for accurately predicting exposure and effects of pesticides to quail and small mammals. Previous research has shown that toxicity varies considerably among nontarget taxa (assumption 2; Meyers and Wolff, 1994); exposure is different in the field than in the laboratory (assumption 3; Edge et al., 1996; Schaubert et al., 1997); food is not the only avenue of exposure (assumption 4; Schaubert, 1994; Schaubert et al., 1997); all ingested items are not contaminated equally (assumption 5; Meyer and J. Wolff, 1994); exposure has alternative routes besides food (assumption 6; Schaubert et al., 1997); residue degradation is rapid and affects exposure differently through time (assumption 9; Bennett et al., 1994; Schaubert et al., 1995); residue distribution is heterogeneous (assumption 10; Bennett et al., 1994; Schaubert et al., 1995); several applications have a greater effect than a single applications (assumption 11; Peterson, 1996); and endpoints other than death, such as activity and reproduction, are affected by pesticide exposure (assumption 12; Edge et al., 1996; Peterson, 1996; Schaubert et al., 1997).

Some important assumptions of the QM regarding avoidance behavior, different application formulation (granular or flowable), and extrinsic environmental factors (e.g., weather) have not been tested. Dissimilar life styles of voles and birds may affect exposure differently in these two groups of vertebrates with regard to application formulation. Birds may eat the granules of pesticides as a source of natural grit (Best and Gionfriddo, 1994; Best et al., 1996), and direct consumption of pesticide granules has caused bird die-offs (Balcom et al., 1984). However, herbivorous voles may not consume granules directly. These differences in life style can modify the exposure of birds and voles to granular and flowable pesticides. That is, herbivorous voles might be exposed to flowable chemicals more than to granular chemicals, while birds might have higher exposure to granular chemicals.

In the QM, the expected exposure concentration (EEC) is estimated on the basis of the application rate of a pesticide. However, physical environmental factors (terrain, vegetation type and structure, and weather) may affect the distribution of a pesticide at the microhabitat scale of an animal, and subsequently affect the exposure. Schauber et al. (1997) found that when an application was followed by a light rainfall, vole population size decreased by 50%, and suggested that rainfall might have washed the pesticide down to ground level, increasing the exposure of the voles above a period of no precipitation. A cause and effect relationship, however, has not been demonstrated.

Tiebout and Brugger (1995) point out that one unanswered question regarding response of terrestrial vertebrates to chemical exposure is whether or not animals will detect and move out of a contaminated area if an adjacent uncontaminated area is available. Movements of animals within or among habitats, however, are a function of the season and various aspects of the social system of a species. For instance, during nonbreeding seasons, many bird species often move freely over wide areas. During the breeding season, however, they are confined to well-defined territories where they nest and rear young. Large mammals are more mobile than are small ones, and therefore small mammals, those often inhabiting agricultural areas, may be more susceptible to chemical contaminants than larger mammals. Also, during the breeding season, female small mammals are typically confined to relatively small home ranges where they defend territories, occupy burrow systems, and defend their young and nest sites (Osfield, 1985; Wolff, 1993a). Males are often more mobile, but still may remain in well-defined home ranges and may be limited in their movements by a social fence of territorial adults (Hestbeck, 1982; Wolff, 1993b, 1994). Therefore, due to the spacing of individuals and well-defined social structure of small mammals, individuals that occupy habitats exposed to a chemical contaminant may not be able to move to avoid a contaminated habitat. Whether mammals will move or not is unknown.

## Objectives

The objectives of my research are to conduct three independent, but related studies on the ecotoxicology of birds and mammals that address several major concerns listed by Tiebout and Brugger (1995). I concentrate my research efforts on three basic questions.

1. *Given an alternative, will animals move away from a spray zone into an unsprayed area long enough to reduce exposure and risk?* H<sub>1</sub>: I hypothesize that gray-tailed voles will not move from established home ranges to avoid contaminated vegetation. H<sub>2</sub>: my alternative hypothesis regarding this question is that voles will move out of the sprayed zone when pesticide concentration is sufficient to decrease survival and reproductive success of voles. Under this situation, the risk to stay in the sprayed zone is higher than the cost of emigration.
2. *Will birds and mammals respond differently to equivalent concentrations of a pesticide applied in granular and flowable formulations?* H: I hypothesize that a granular application will have a greater negative impact on birds and less of an impact on mammals than will a flowable application.
3. *What are the effects of environmental variables such as rainfall on exposure of mammals to a pesticide?* H: I hypothesize that rainfall shortly after application will cause a greater negative impact on voles than would dry conditions.

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Wolff JO (1994) More on juvenile dispersal in mammals. *Oikos* 71: 349-351

## Chapter 2

Gray-tailed Voles Do not Move to Avoid Exposure to the Insecticide  
Guthion® 2S

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Gray-tailed Voles Do not Move to Avoid Exposure to the Insecticide  
Guthion® 2S

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## Abstract

We used gray-tailed voles, *Microtus canicaudus*, as an experimental model species to test an assumption of the Quotient Method that wildlife do not move out of a contaminated area to avoid exposure to potentially harmful agricultural chemicals. In May 1997, we placed voles into 12, 0.2-ha enclosures planted with a mixture of pasture grasses. In late July, we applied 1.5 kg/ha of the insecticide Guthion<sup>®</sup> 2S (azinphos-methyl) in three treatments; full spray (all of the habitat sprayed with Guthion<sup>®</sup> 2S), half-spray (one-half of the habitat sprayed with Guthion<sup>®</sup> 2S and one half with water), and a control (all habitat sprayed with water). Five replicates were used for the half-spray and control, and two replicates for the full-spray. We radio-tracked 44 females and three males before and after the spray treatment. None of the 47 animals moved out of their established home ranges after treatment and no animals moved from the contaminated to uncontaminated areas. Additionally, no biologically meaningful differences occurred in home range size, mean maximum distance moved, or average distance between two successive radio locations. Reproducing adult voles were relatively sedentary and did not leave their established home ranges in response to insecticide exposure. These results suggest that small mammals are not likely to reduce exposure by moving from the contaminated area, which supports the assumption of the Quotient Method that exposure to small mammals is a function of the spray application. However, behavioral responses such as contamination avoidance may be specific to the chemical, species and habitat.

Key Words: *Azinphos-methyl*, *Microtus canicaudus*, *Movements*, *Quotient Method*, *Radio Telemetry*

## **Introduction**

The adverse effects of pesticides on wildlife have been a major concern of conservation biologists for three decades [1-4]. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the U.S. Environmental Protection Agency (U.S. EPA) conducts ecological risk assessment of pesticides to minimize the effects of these contaminants on nontarget species. The current method extensively used by the U.S. EPA is the Quotient Method (QM) [5]. The QM uses the ratio of the expected environmental concentration (EEC)/ median-lethal dose or concentration of chemicals to the nontarget species ( $LD_{50}$  or  $LC_{50}$ ) of chemicals to assess the risk of pesticides to nontarget wildlife [6]. In the Quotient Method, the  $LD_{50}$  or  $LC_{50}$ , which usually is estimated on the basis of a dose-acute response curve of a surrogate animal in a laboratory, measures the hazard of a chemical to wildlife species; the chemical's EEC is used to indicate the exposure of the species to the contaminant. The EEC presumably is a direct function of application rate, and is estimated by a nomogram derived from a database of residues measured on crops [6]. A low QM ratio ( $< 0.2$ ) is considered comparatively low risk and acceptable for pesticide registration [7]. The quotient is relatively simple to calculate; however its use implies numerous underlying assumptions and

uncertainties related to intrinsic and extrinsic factors affecting variation in exposure of animals to pesticides, which may undermine the effectiveness of the QM [8,9].

An important component of the QM is the assumption that the dietary intake is a direct function of the application rate. The QM also assumes that wildlife will not move away from the contaminated zone to avoid exposure [8]. However, this assumption has not been tested. Several factors can affect whether or not an individual (or species) will move to avoid exposure. Many species have the ability to detect chemical contamination in their habitats or home ranges [10,11]. However, whether detection is sufficient to cause dispersal or avoidance is unknown. Also, various aspects of the social system can affect movements. Aggression by territorial small mammals may form a social fence, preventing animals from immigrating, and thus confining animals to their own home ranges [12-14]. Also, reproducing females are confined to relatively small home ranges where they defend territories, occupy burrow systems, and defend their young and nests [15-18]. Therefore, moving out of well-established home ranges may not be a viable option for many species of small mammals, at least during the breeding season. Whether small mammals, which are common inhabitants of agricultural areas, are able to assess the costs and benefits associated with exposure to chemical contaminants and respond accordingly is unknown.

Since 1991, our research group has been conducting ecological risk assessments of the effects of organophosphorus (OP) insecticides on small mammals [e.g., 9,10,19]. We primarily have used the gray-tailed vole, *Microtus*

*canicaudus*, as our experimental model species. Gray-tailed voles are a common small mammal species of grasslands and agricultural areas in the Willamette Valley of western Oregon. Breeding occurs from March through November, adult female gray-tailed voles are territorial, adult males have large home ranges that overlap those of several females, and dispersal occurs primarily among young males [20]. Furthermore, the genus *Microtus* has a worldwide distribution and our results may be applicable to species in different geographical areas. For our test chemical we use Guthion<sup>®</sup> 2S (azinphos-methyl; *O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-(4*H*)-yl)methyl] phosphorodithioate). The LC<sub>50</sub> is 297 ppm and the LD<sub>50</sub> is 48 mg/kg for gray-tailed voles [10]. This compound has been identified by the Office of Pesticide Programs as causing avian die-offs in the field that were not predicted by their QM risk assessment [19,21]. Guthion<sup>®</sup> 2S has a significant short-term depression on the survival and population size of gray-tailed voles at application rates of 1.5 and 2.25 kg/ha (2 and 3 times label rate; [9,19,22]).

Our objective was to determine whether or not gray-tailed voles will move away from an area contaminated with Guthion<sup>®</sup> 2S at the maximum allowable label rate into an adjacent unsprayed area long enough to reduce exposure and risk. We hypothesized that gray-tailed voles would not move away from established home ranges to avoid chemical contamination.

## **Materials and Methods**

### *Study Site and Enclosures*

The research site is located at the Hyslop Agronomy Farm of Oregon State University, approximately 10 km north of Corvallis, Oregon (123°12'W, 44°38'N). The site has a well-drained, silty-clay, loam soil with level topography and an elevation of about 70 m. Average annual precipitation was about 108 cm. During this experiment, the study site did not receive any rain. Twenty-four 0.2-ha enclosures have been constructed at the research site. Each enclosure is 45 x 45 m and is constructed of galvanized sheet metal approximately 90 cm high above ground and buried 90 cm deep to prevent escape or entry by burrowing animals. Each enclosure is planted with a mixture of pasture grasses and is similar to the natural habitat of gray-tailed voles. We used 12 of the 24 enclosures in this study. The 12 enclosures were randomly chosen. A 1-m wide strip along the inside of the fence within each enclosure is kept bare to minimize the use by the voles. Eighty-one, large-size Sherman live traps were placed in each enclosure in a 9 x 9 array with 5-m trap spacing.

### *Establishment of Experimental Populations and Chemical Application*

In mid-May, six adult male and six adult female wild-caught gray-tailed voles were introduced into each of the 12 enclosures to start the experiment. Voles were trapped to determine their home range locations and to fit with radiocollars

before the radio tracking. The 12 enclosures were assigned randomly to three treatments; full spray (all of the habitat was sprayed with Guthion<sup>®</sup> 2S), half-spray (one-half of the habitat was sprayed with Guthion<sup>®</sup> 2S and the other half with water), and a control (all habitat sprayed with an equal volume of water). Five replicates were used for the control and half-spray and two replicates for the full spray. The unbalanced design was due to limitations in availability of the chemical and number of enclosures. We were unable to obtain sufficient quantities of Guthion<sup>®</sup> 2S within the time frame of our experiment. The recommended application rate for Guthion<sup>®</sup> 2S is 0.77 kg active ingredient (AI)/ha on grass-like crops; we used the maximum allowable application rate for any purpose (1.5 kg AI/ha). This application rate had negative demographic consequences in our previous studies [19,22]. On July 24, Guthion<sup>®</sup> 2S was applied by a licensed applicator using a small tractor and trailer tank with a 7.6-m spray boom. The speed of the tractor and the pressure of the trailer tank were calibrated before the spray to deliver the desired amount of liquid mixture or water within the enclosures (114 l/ha).

### *Radio Telemetry*

We radiocollared (SM1 transmitters, AVM Instrument Company, Livermore, CA, USA) 45 female and three male voles to monitor movements before and after spray application. We collared four adult females weighing

40-45 g in each enclosure; except for two adult males and two adult females in one half-sprayed enclosure, and three adult females and one adult male in another half-sprayed enclosure. One radiocollared female in a half-sprayed enclosure died before we applied the chemicals, leaving a total of 44 females and three males tracked during the experiment. The radiocollared voles in the half-sprayed enclosures had their entire home range located within the half-sprayed area. All radiocollared animals in each enclosure were chosen so that they had nonoverlapping home ranges. Transmitters weighed about 2 g (5% of body weight) and were attached around the neck of the voles with a plastic collar. The collared animals were released at the same trap station where they were caught and given 1 to 2 days to adapt to the radiocollar before the tracking began. No trapping was conducted during the radio-tracking period to avoid trapping interference with the radio tracking.

Radio-collared voles were tracked on foot [23] to within 5 m of their location using an AVM radio receiver and hand-held three-element Yagi antenna. We located each animal twice a day starting about 0500 and 1930h. Radio tracking was conducted for two periods, 4 ½ consecutive days just before and 4 ½ consecutive days immediately after the chemical application. Final radio fixes were estimated to the nearest 1 m on a grid map referenced by trap locations.

We compared geometric centers of the home ranges of individual animals between the two tracking periods (before and after the chemical spraying) to test for home range shifts. Home range size was estimated with the Minimum Convex

Polygon Method [24]. Average distance moved between successive locations (ADMABL) was calculated as the mean of all distances between two successive locations for each animal during each tracking period (i.e., before and after the chemical application) to measure movements. The mean maximum distance moved (MMDM) was calculated as the average of the maximum straight line distance between all radio locations during a radio-tracking period [25]. MMDM was used as a relative index of activity. The mean of each parameter for the radiocollared females in each enclosure was used in statistical analyses.

### *Statistical Analysis*

All data were analyzed using the Statistical Analysis System [26]. Repeated measures ANOVA was used to test for differences in home range size, MMDM, and ADMABL between the two tracking periods (before and after), among treatments (control, half-spray, and full-spray), and for a time by treatment interaction. When the interaction of time by treatment was detected, we calculated the difference of the parameter between the two time periods and used a one-way ANOVA to detect the differences among the treatments. Fisher's LSD was used to make multiple comparisons of the means among the treatments when a detectable difference was found in the one-way ANOVA. We had five replicates for the control and for the half-spray and two replicates for the full-spray. To enhance the power of statistical analyses, we set  $\alpha = 0.1$  to detect biologically meaningful

results [27]. In ANOVAs, we used type III sum of squares as is appropriate for an unbalanced design without empty cells [28].

## Results

### *Home Range Location and Shift*

All 44 radiocollared female and three male voles in the control, half-sprayed, and full-sprayed enclosures remained in their originally established home ranges during both tracking periods. After the spray, geometric centers of individual home ranges were located approximately in the same place (within 1 m) as before the spray. No home range shifts were found for any of the 47 radiocollared animals, including three adult males in two half-sprayed enclosures. In half-spray enclosures, the collared voles had an adjacent uncontaminated area available, but no vole moved its home range from the contaminated to the uncontaminated area. This result supports our hypothesis that gray-tailed voles would not move away from established home ranges to avoid chemical contamination.

### *Home Range Size*

The mean home range sizes of adult female gray-tailed voles in the control, half-sprayed, and full-sprayed enclosures (Figure 2.1) did not differ by time (period) ( $F_{1,9} = 0.80, P = 0.395$ ), treatment ( $F_{2,9} = 0.04, P = 0.960$ ), nor was a time

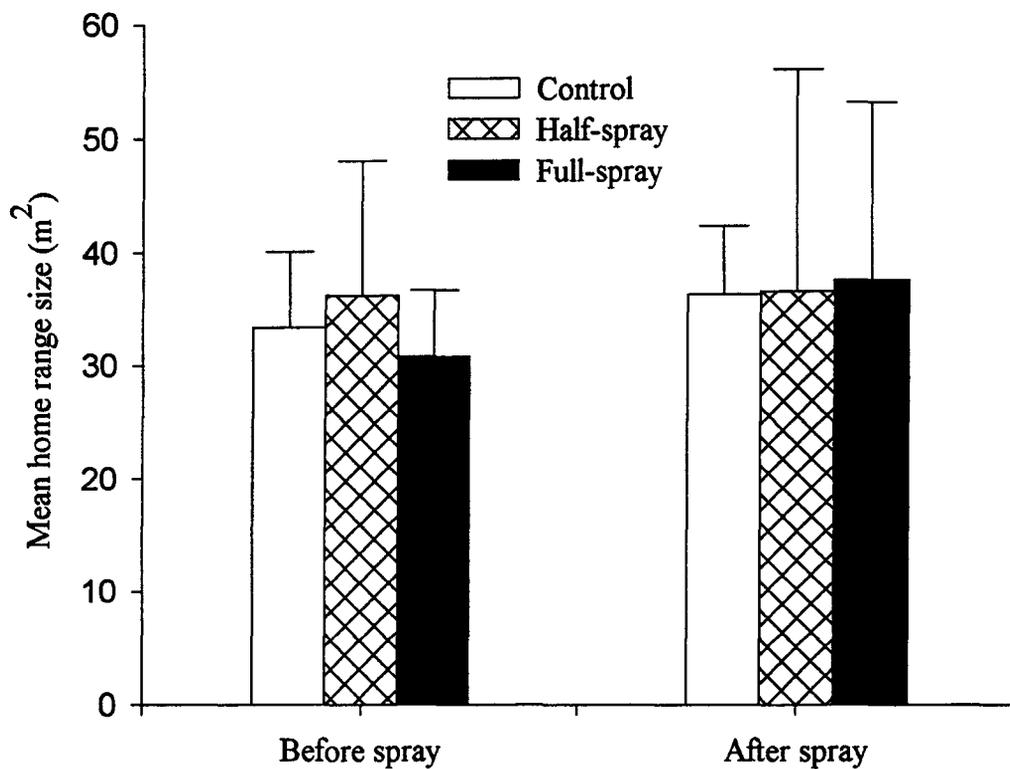


Figure 2.1. Mean home range size of adult female gray-tailed voles before and after exposure to Guthion<sup>®</sup> 2S in three spray treatments. Vertical lines are one standard deviation.

by treatment interaction ( $F_{2,9} = 0.21, P = 0.818$ ) detected. Therefore, the half-spray and full-spray Guthion<sup>®</sup> 2S treatments did not affect the mean home range sizes of female gray-tailed voles over time.

### *Movements*

The MMDM of the adult females in the control, half-spray, and full-spray enclosures did not differ by treatment ( $F_{2,9} = 0.05, P = 0.945$ ) or time ( $F_{1,9} = 0.66, P = 0.437$ ), but we did detect a time by treatment interaction ( $F_{2,9} = 3.43, P = 0.078$ ). A difference in the contrasts among the treatments was found between the full-spray and half-spray, and between the control and half-spray using Fisher's LSD. Thus, although no main effects of treatment or time on MMDM were detected, the MMDM in the half-spray enclosures tended to decrease after the spray while the MMDM in the control and full-spray enclosures tended to increase (Figure 2.2). MMDM in the control enclosures increased from 10.6 ( $\pm 0.94$  SD) m to 11.3 ( $\pm 0.91$  SD) m while MMDM in the half-spray enclosures decreased from 11.1 ( $\pm 1.96$  SD) m to 10.3 ( $\pm 1.86$  SD) m. Also, MMDM increased in the full-spray enclosures ( $10.4 \pm 0.20$  SD m to  $11.3 \pm 1.10$  SD m) over the same time period, similar to the control.

The ADMBL of adult females for the control, half-spray, and full-spray enclosures (Figure 2.3) did not differ by treatment ( $F_{2,9} = 0.09, P = 0.916$ ) or time ( $F_{1,9} = 0.65, P = 0.442$ ), nor did we detect a time by treatment interaction

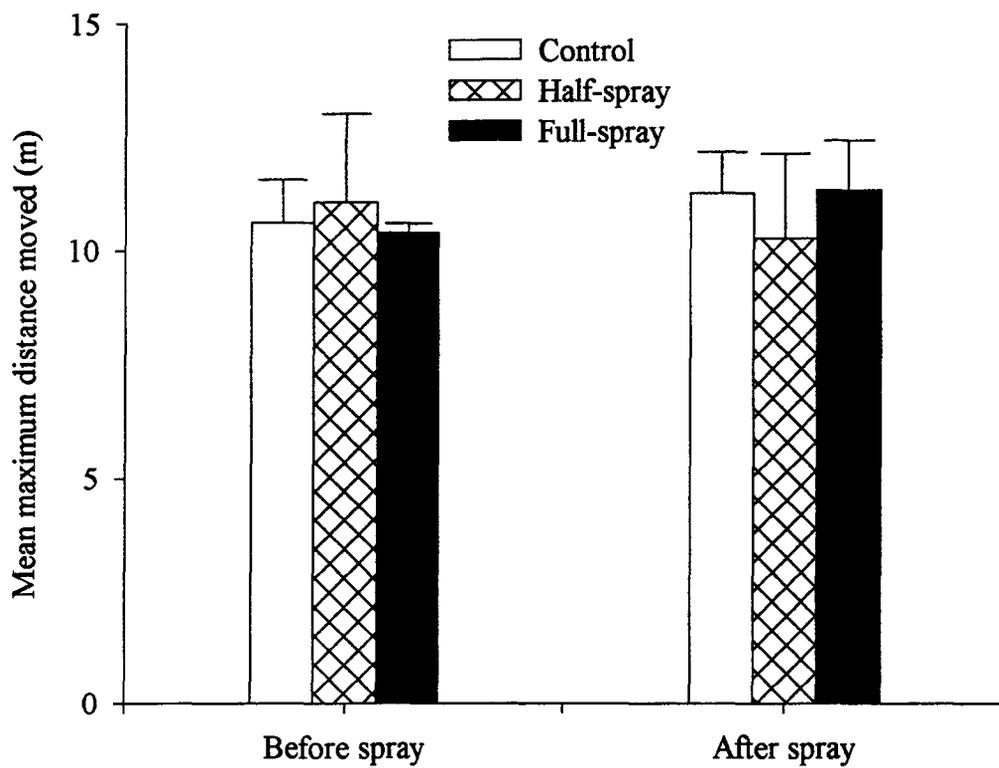


Figure 2.2. Mean maximum distance moved of adult female gray-tailed voles before and after exposure to three Guthion<sup>®</sup> 2S spray treatments. Vertical lines are one standard deviation.

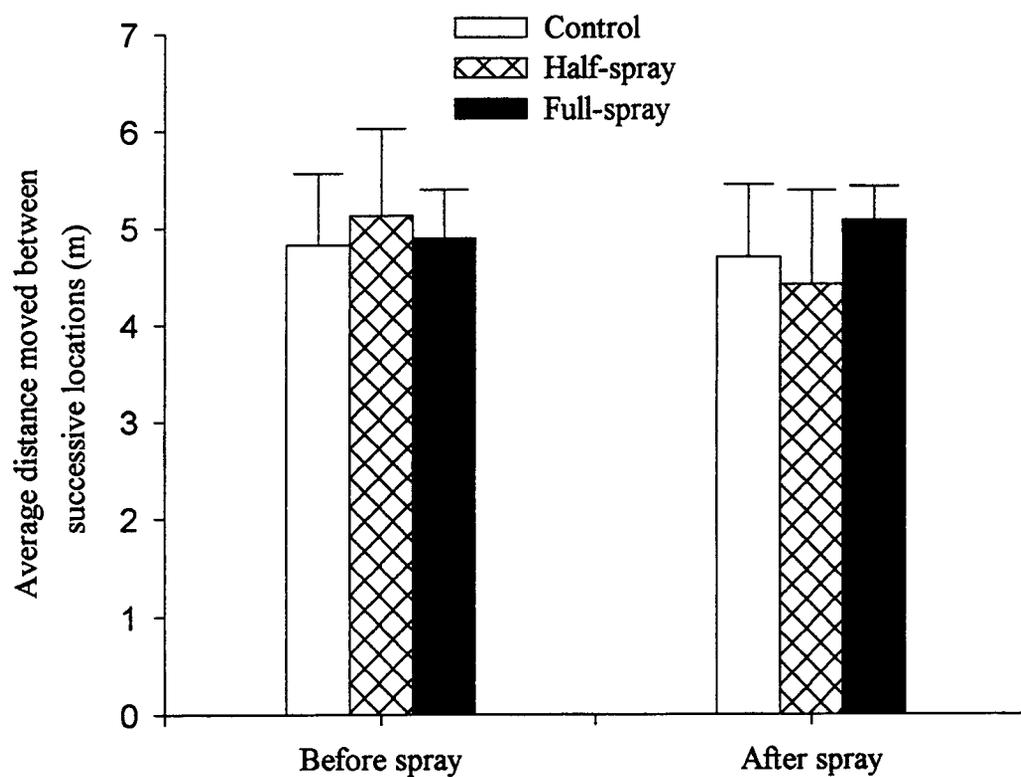


Figure 2.3. Average distance moved between successive radio locations of adult female gray-tailed voles before and after exposure to Guthion<sup>®</sup> 2S spray treatments. Vertical lines are one standard deviation.

( $F_{2,9} = 0.95$ ,  $P = 0.429$ ). Thus, average movements of adult females were not different among the treatments over the time.

## Discussion

Our results support the hypothesis that given access to uncontaminated habitat, gray-tailed voles would not move away from contaminated habitat to avoid chemical exposure. Voles did not shift the geometric center of their home ranges or alter their daily movements in response to chemical exposure. The only difference in movements we detected was a time by treatment interaction in MMDM, in which voles in the half-spray treatment decreased movement distances after spray by 0.8 m while controls increased by 0.7 m. This < 1-m difference before and after the spray in each treatment group does not appear to be biologically meaningful. Also, during this same time period, voles in the full-spray enclosures increased their MMDM by 0.9 m, similar to that in the control. Thus, the spray itself did not seem to affect vole movements.

The voles' failure to move away from the contaminated areas may be a function of their social system. Gray-tailed voles, like other *Microtus* species, are relatively sedentary as adults [16,17,29]. Females occupy individual territories that are exclusive to other unrelated females, where they have extensive burrow systems and underground nests [20]. During the breeding season, March-November, females are pregnant and/or nursing young almost continuously [30]. Of the 44 radiocollared adult females, 22 were lactating and 13 were pregnant when radio

transmitters were attached. Moving out of the established home ranges would mean losing their young, nest sites, and breeding space. Therefore, female voles would incur a high reproductive cost in abandoning their current residence. Also, because the uncontaminated areas were occupied by territorial female voles, emigration would have been deterred by aggression of resident females attempting to prevent immigration of dispersing voles [12,13,16,18]. We radio-tracked only three male voles, but they also did not change home range locations and may have been deterred from immigrating to areas inhabited by other adult males. Thus, the dependency on extensive burrow systems, territoriality, and the restrictions of reproducing and raising young make it difficult for voles to abandon their residence, even when exposed to contaminants.

The application rate used in this experiment, 1.5 kg AI/ha, was two times the normal application rate of 0.77 kg/ha for grassland/alfalfa habitat and represented the maximum allowable rate for any use [31]. Previous experiments in our enclosures detected decreased survival and population size at this same application rate [19,22]. Peterson [22] detected nearly a 40% decrease in population size and growth rates of gray-tailed voles exposed to 1.55 kg/ha in the enclosures planted with alfalfa. In these previous studies, the spray tank contents were sampled before the spraying and were resampled after the spraying. The analysis results of the tank content samples conformed to their nominal application rates (1.55kg/ha). The quotient of Guthion<sup>®</sup> 2S at the rate of 1.55 kg AI/ha for *M. canicaudus* in grass habitats is about 0.39, predicting a risk that may be mitigated

by restricted uses [5]. A quotient of 0.39 predicts a mortality rate of 29% based on the probit analysis and dose-response curve of gray-tailed voles to Guthion<sup>®</sup> 2S [10]. The actual exposures to Guthion<sup>®</sup> 2S in this study may have been significantly different than those of the previous studies and our proposed rate. However, none of these previous studies documented significant changes in MMDM associated with exposure to Guthion<sup>®</sup> 2S. The half-life of Guthion<sup>®</sup> 2S is <5 days [32] so we did not expect any long-term effects on the movement of the gray-tailed voles. However, we radiotracked voles within 8 hours of spraying and observed no significant change in movements, or avoidance of contaminated areas. Voles may be able to reduce exposure by increasing use of underground burrow systems. But, this behavior should have been expressed in differences in the measured parameters between controls and full spray treatment. Meyers and Wolff [10] reported that gray-tailed voles can detect and avoid eating Guthion<sup>®</sup> 2S-contaminated foods below the LC<sub>50</sub> level. Consequently, voles may have been able to avoid the worst effects of chemical contamination by selective foraging within their home ranges.

The gray-tailed voles did not move out of the Guthion<sup>®</sup> 2S-contaminated grassland habitat to avoid exposure in this study, nor did they alter daily movement measured by the ADMBL to respond to the application of Guthion<sup>®</sup>. However, numerous studies have examined the behavioral effects of OPs on mammals and birds [e.g., 33-35]. Behavioral responses have been observed when acetylcholinesterase (AChE) activity is depressed to <50% of normal in birds

[21,36,37] and in mammals (e.g., gray-tailed voles, [10]). Wood mice, *Apodemus sylvaticus*, injected intraperitoneally with dimethoate, another AChE inhibitor, significantly decreased locomotor activity in field and laboratory experiments [38,39]. Sublethal effects, e.g., behavioral abnormalities of agricultural chemicals on terrestrial vertebrates are important factors that need to be considered in the ecological risk assessment [8]. For gray-tailed voles, we tested only one of 12 factors listed by Tiebout and Brugger [8] that could affect the assumptions of the QM. Further field studies on behavioral, physiological, and demographic responses to chemical contaminants are needed to assess the validity of the assumptions and uncertainties associated with the QM and for making ecological risk assessments of agrichemicals on nontarget wildlife.

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## Chapter 3

### Bird and Mammal Response to Granular and Flowable Insecticide Applications

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**Bird and Mammal Response to Granular and Flowable Insecticide Applications**

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## Abstract

We used gray-tailed voles (*Microtus canicaudus*) and northern bobwhite quail (*Colinus virginianus*) as experimental model species to field test whether small mammals and birds respond differently to equivalent concentrations of a pesticide applied in granular and flowable formulations. In mid-May 1998, we placed voles into 15, 0.2-ha enclosures planted with a mixture of pasture grasses. In mid-July, we placed quail into the same enclosures with the voles. In late July, we applied the organophosphorus insecticide diazinon in five treatments; a control (all habitats sprayed with water), liquid formulation of diazinon at 0.55 kg/ha, liquid formulation of diazinon at 1.11 kg/ha, broadcast of granular diazinon at 1.11 kg/ha, and broadcast of granular diazinon at 2.22 kg/ha. The diazinon treatment in liquid and granular formulations did not depress population size or growth rate, or survival rate of voles. We found a significant difference in the survival rate of the quail between the controls and treatments; granular diazinon caused a measurable decline of quail survival, while the liquid application at an equivalent rate did not significantly affect quail survival. Our results suggest that ground-feeding birds are more susceptible to granular insecticides than flowable applications, but voles were not susceptible to either formulation at the rate we used.

Key Words: Bobwhite quail, demographic response, granular application, gray-tailed vole, liquid application, organophosphorus insecticide, quotient method.

## Introduction

The Quotient Method (QM) (Urban and Cook 1986) has been extensively used for ecological risk assessment of pesticides to wildlife by the United States Environment Protection Agency (USEPA) under the Federal Insecticide, Fungicide, and Rodenticide Act. In 1992, the regulations were revised, eliminating field tests for pesticide registration (Norton *et al.* 1992; USEPA 1992a). However, without field tests, uncertainty exists regarding the potential impact of pesticides on nontarget species (Tiebout and Brugger 1995). The lack of field tests causes concern among environmental biologists and ecologists (Nabholz *et al.* 1997).

The QM uses the ratio of the expected exposure concentration (EEC) of chemicals divided by the median-lethal dose or concentration of chemicals to the nontarget species ( $LD_{50}$  or  $LC_{50}$ ) to assess the risk of pesticides to wildlife (Urban and Cook 1986). In the QM, the  $LD_{50}$  or  $LC_{50}$ , which usually is estimated on the basis of a dose-acute response curve of a surrogate animal in a laboratory, measures the hazard of a chemical to wildlife species. The chemical's EEC then is used to measure the exposure of the species to the contaminant. The EEC is assumed to be a direct function of application rate, and is estimated by a nomogram derived from a database of residues measured on crops (Hoerger and Kenaga 1972). For granular pesticides, the granule number/ft<sup>2</sup> after applications is estimated and the risk index is expressed as the percent  $LD_{50}$  per square foot (USEPA 1992b). A low QM ratio ( $< 0.2$ ) is considered comparatively low risk and acceptable for pesticide registration (National Research Council 1983). Quotients between 0.2 and 0.5

indicate risk that may be mitigated by restricted use. A quotient  $>0.5$  is interpreted to indicate a high level of risk. The quotient is relatively simple to calculate; however, the quotient is affected by differences in intrinsic toxicity as well as in exposure estimations (Tiebout and Brugger 1995). Often, toxicity thresholds are extrapolated incorrectly to untested species.

Pesticide formulations and animal foraging behaviors are two factors that may affect the potential dose of pesticides to wildlife species. Different formulations of pesticides may have different primary exposure routes to wildlife species with different foraging behaviors. The QM does not incorporate formulations (*e.g.*, liquid and granular) of pesticides and foraging behaviors of wildlife into the assessment. Avian species use sand-size rocks as grit, and several avian species directly consume pesticide granules (Stafford and Best 1997). Direct consumption of pesticide granules may be one of the primary exposure routes of birds to granular pesticides and put birds at great risk (Hill and Camardess 1984). However, herbivorous species such as voles (*Microtus* spp.) mainly eat green plants during the growing season (Batzli 1985). Consumption of contaminated plants may increase the exposure of herbivorous species to liquid-formulation pesticides. These differences in foraging patterns may modify the exposure of granivorous birds and herbivorous mammals to granular and liquid organophosphorus (OP) insecticides. Whether or not populations of birds or herbivorous small mammals differentially respond to granular and liquid OP insecticides is unclear.

In this study, we used gray-tailed voles (*Microtus canicaudus*) and northern bobwhite quail (*Colinus virginianus*) as experimental species to investigate the effects of different insecticide formulations on the demography of small mammals and birds. Microtine species are distributed worldwide, and many are common herbivorous species in agricultural crop fields. Northern bobwhite quail (here after bobwhite) are ground foragers; seeds comprise 70% of summer diets and 90% of winter diets (Rosene 1969). The objective of our study was to test the hypotheses that the granular OP insecticides have greater negative effects on bobwhites than liquid pesticides, while the liquid OP insecticides would have greater negative effects on gray-tailed vole populations than granular OP insecticides.

## **Materials and Methods**

### *Study Site and Enclosures*

The research site is located at the Hyslop Agronomy Farm of Oregon State University, approximately 10 km north of Corvallis, Oregon (123°12'W, 44°38'N). Twenty-four 0.2-ha enclosures have been constructed at the research site. Each enclosure is 45 x 45 m and is constructed of galvanized sheet metal approximately 90 cm above ground and buried 90 cm deep to prevent escape or entry by burrowing animals. Each enclosure was planted with a mixture of pasture grasses, composed of fawn tall fescue (*Festuca arundinacea*), Linn perennial ryegrass (*Lolium perenne*), perennial tetraploid ryegrass (*Lolium perenne*), annual ryegrass

(*Lolium multiflorum*), and Potomac orchardgrass (*Dactylis glomerata*), which is similar to the natural habitat of gray-tailed voles. The coverage of grasses in all enclosures was 95-100%. A 1-m strip along the inside of each fence was kept bare by mowing to minimize small mammal activity near the fence. Average annual precipitation was 108 cm. The research site did not receive rain after 21 July during this study. Eighty-one, large-size Sherman live traps were placed in each enclosure in a 9 x 9 array with 5-m trap spacing. We randomly chose 15 enclosures for this study.

#### *Study Species and Test Chemical*

Since 1992, our research group has conducted ecological risk assessments of the effects of organophosphorus (OP) insecticides on small mammals, primarily gray-tailed voles (*e.g.*, Meyers and Wolff 1994; Edge *et al.* 1996; Schauber *et al.* 1997). We used gray-tailed voles and bobwhite as our model species in this study. We used diazinon (*O, O*-diethyl *O*-[6-methyl-2-(1-methylethy)-4-pyrimidinyl] phosphorothioate) as our test chemical. Diazinon is used extensively in both liquid and granular formulations to control nematodes and soil insects in croplands, golf courses, and grasslands. The maximum allowable application rate in grasslands is 1.11 kg AI/ha. This compound has caused avian die-offs in the field (Hill and Camardess 1984).

*Establishment of Experimental Populations and Chemical Application*

In mid-May 1998, 10 adult male and 10 adult female wild-caught gray-tailed voles were introduced into each of the 15 enclosures to start the experiment. Six healthy adult quail of similar body weight, purchased from a local distributor, were released into each of the 15 enclosures five days prior to the chemical application, allowing the quail to adjust to the enclosures. Quail were censused by resighting them one day before the chemical application. All quail were alive and active in each enclosure. The 15 enclosures were assigned randomly to five treatments: control (sprayed with an equal volume of water); 1-X liquid diazinon treatment (diazinon in liquid formulation at the recommended application rate for grasslands [1.11 kg AI/ha]); 2-X liquid diazinon treatment (diazinon in liquid formulation at two times the recommended application rate [2.22 kg AI/ha]); 1-X granular treatment (granular diazinon at the recommended application rate for grasslands [1.11 kg AI/ha]); and 2-X granular treatment (granular diazinon at two times the recommended application rate for grasslands [2.22 kg AI/h]). Three replicate enclosures were used for the control and each treatment group. We predicted population-level effects of liquid diazinon on voles would be greater than that of granular diazinon, while granular diazinon would have greater negative impacts on bobwhite survival than liquid diazinon. On 21 July 1998, liquid diazinon was applied in the early morning (about 0600 h) by a licensed applicator using a small tractor and trailer tank with a 7.6-m wide spray boom. The boom was set at a height of 60 cm, approximately 20 cm above the top of the vegetation. The

speed of the tractor and the pressure of the trailer tank were calibrated before the spray to deliver the desired amount of liquid mixture or water within the enclosures. Water was sprayed first in the controls, and then the diazinon-water mixture was sprayed in the treatment grids to avoid diazinon contamination in the control grids. Windless weather in early morning prevented chemical drift to the control enclosures. At the same time, granular diazinon was applied with a hand broadcast spreader. We adjusted our walking speed and cranking rate to apply the desired amount of diazinon granules for each application rate by practice trials before the application. We walked parallel to the fence, and adjusted the intervals between passes so as to broadcast the granules evenly over the whole grid area without overlapping applications. Spray tank contents were sampled before spraying the first enclosure and resampled after spraying the last of the three grids for each application rate. The actual mean sample concentration for planned 1-X liquid treatment was 0.55 kg AI/ha. The actual mean sample concentration for planned 2-X liquid treatment was 1.55 kg AI/ha. We reported actual application rate in our analyses in this paper (*i.e.*, 0.5-X liquid treatment and 1-X liquid treatment).

### *Trapping Procedures for Gray-tailed Voles*

Voles were trapped in the enclosures for 4 consecutive days (trap period) at 2-week intervals from mid-May through mid-September 1998 using mark-recapture procedures (Edge *et al.* 1996). Traps were baited with oats and sunflower

seeds, set just before sunset and checked once a day at sunrise. All captured animals were ear-tagged for identification, and data on body mass, age, sex, reproductive condition, and trap location were recorded for each capture. Voles with holes or rips in their right ears were assumed to have lost an ear-tag and were retagged with a new tag. Previous tag numbers were identified by similarities in sex, body mass and trap location between previous captures and the newly tagged animal. Females were considered in reproductive condition if they were lactating (larger nipples and white mammary tissue surrounding the nipples) or pregnant (obviously swollen abdomen) or had widely open pubic symphysis. Field personnel were trained for a 2-week period in accordance to an approved quality control plan. For the purpose of our analysis, period 1 began on 19 May 1999, and the study ended at period 9 (10 September 1998).

### *Census of Bobwhite Quail*

Quail were counted by resighting two times each day, early morning and late afternoon. The counting started one day before the pesticide application and lasted until 14 days after the application. The presence or absence of quail was recorded for each survey.

### *Population Parameters and Statistical Analyses*

*Gray-tailed voles*--we used capture-recapture methodology to estimate survival rates and population sizes of gray-tailed voles (Rexstad and Burnham 1992). As an index of activity, we calculated the mean maximum distance moved

(MMDM) as the average of the maximum straight line distance between trap locations for all captures within a trap period (Wilson and Anderson 1985). Sex-specific survival rates were estimated using derivations of Cormack-Jolly-Seber's method (Cormack 1964; Jolly 1965; Seber 1965) with programs RELEASE (Burnham *et al.* 1987) and SURGE (Pradel and Lebreton 1991). We measured recruitment by the number of newly tagged voles captured in an enclosure per adult female captured in the same enclosure 4 weeks (two trap periods) earlier. The time lag allowed recruits to reach trappable size. We used multivariate repeated measures ANOVA to test for differences in population size, population growth rate, juvenile recruitment, proportion of adult females in reproductive condition, and MMDM among treatments over time, and for detecting time by treatment interactions. In this study, we applied the contaminant part way through the study, so the treatment-time interaction was the primary effect of interest (Paine and Paine 1996). We used a whole-plot, split-plot ANOVA design to incorporate population size as a covariate in our analysis of MMDM. Natural variation in population demographic variables in our previous experiments has always been high. To enhance the power of statistical analyses, we set  $\alpha = 0.1$  to detect biologically meaningful results (Schauber and Edge 1999).

*Bobwhite quail*--we used data on the presence and absence of the quail to estimate the survival rate after treatment with derivations of Cormack-Jolly-Seber's method (Cormack 1964; Jolly 1965; Seber 1965). Programs RELEASE (Burnham *et al.* 1987) and SURGE (Pradel and Lebreton 1991) were used to determine the

best survival model for quail and test for differences in the survival rate of birds among treatments. We used one-way ANOVA to detect difference in the number of dead and missing quail among controls and treatments. When a difference was found in the one-way ANOVA, Least Significant Difference (LSD) was used to detect differences in the number of dead and missing quail among treatment groups.

## **Results**

### *Vole Population Size*

We captured 2,128 voles 7,560 times between May and September 1998. Populations grew from the initial 20 animals at the beginning of our experiment to a mean maximum of 139 animals in August (trap period 7) and declined slightly in September (Fig. 3.1). Population sizes differed by time ( $F_{8,13} = 71.836$ ,  $P = 0.002$ ), but no time by treatment interaction was detected ( $F_{32,13} = 1.073$ ,  $P = 0.468$ ). Population size declines were not detected after the spray of diazinon (Fig. 3.1).

### *Vole Population Growth Rate*

Population growth rates of gray-tailed voles fluctuated throughout the study (Fig. 3.2). Population growth rates differed statistically over time ( $F_{7,4} = 43.673$ ,

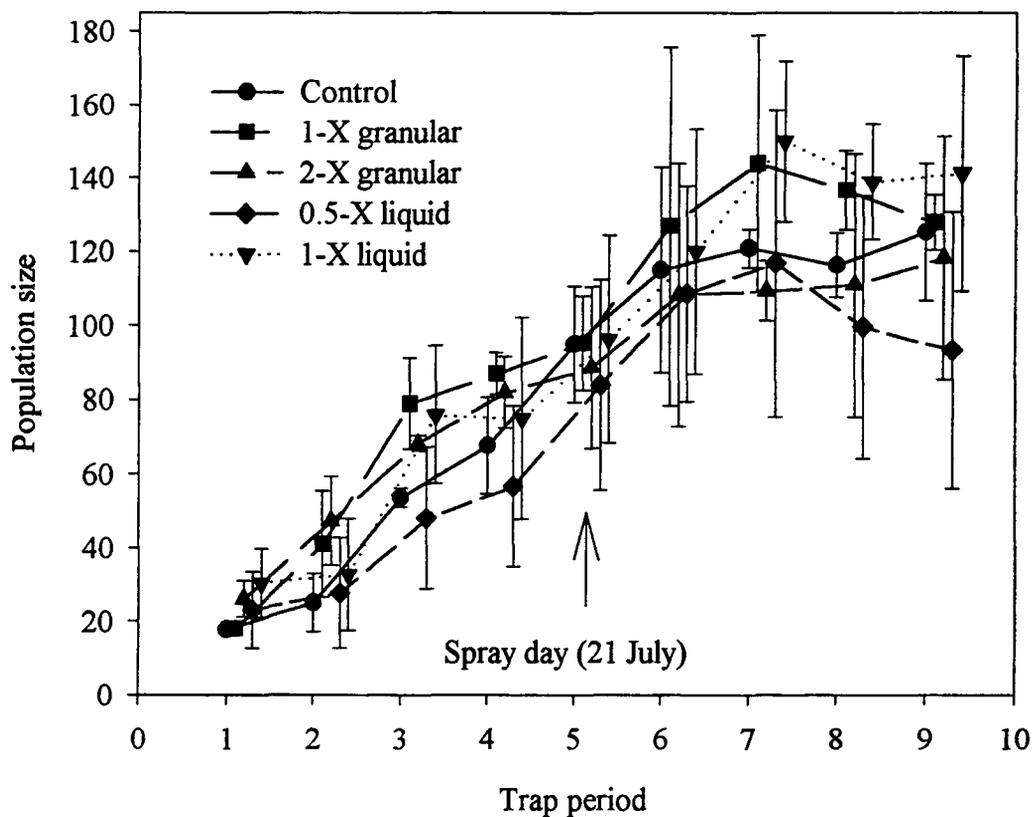


Figure 3.1. Mean population size of gray-tailed voles in control, 1-X granular, 2-X granular, 0.5-X liquid and 1-X liquid enclosures at Hyslop Agronomy Farm, Benton County, Oregon, May-September 1998. Vertical lines are one standard deviation.

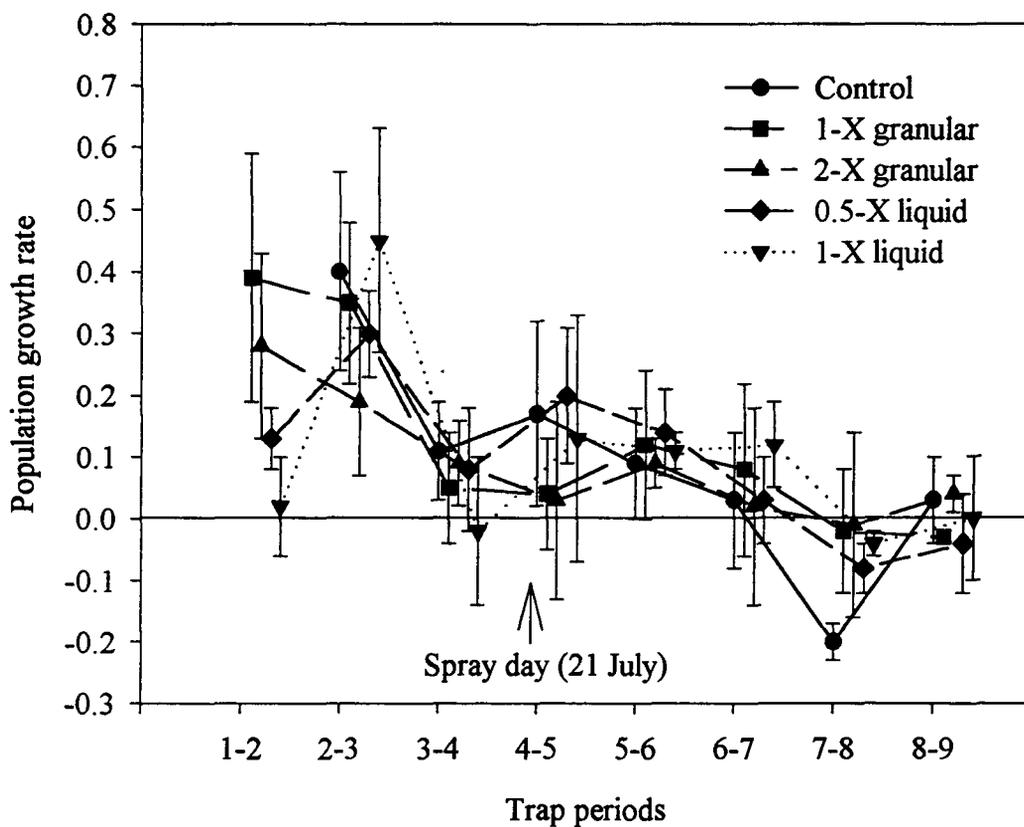


Figure 3.2. Mean population growth rate per week of gray-tailed voles in control, 1-X granular, 2-X granular, 0.5-X liquid and 1-X liquid enclosures at Hyslop Agronomy Farm, Benton County, Oregon, May-September 1998. Vertical lines are one standard deviation.

$P = 0.001$ ), however, we did not detect a time by treatment interaction ( $F_{28,16} = 1.351$ ,  $P = 0.268$ ). The spraying of diazinon did not negatively impact the growth rate of gray-tailed vole populations.

### *Vole Reproductive Activity and Recruitment*

Mean proportion of adult female voles in reproductive condition ranged from 0.32 to 1.00 and gradually declined after trap week three in both controls and treatments (Fig. 3.3). The proportion of adult female voles in reproductive condition differed over time ( $F_{8,3} = 43.673$ ,  $P = 0.003$ ), and we detected a time by treatment interaction ( $F_{32,13} = 1.351$ ,  $P = 0.056$ ). However, the contrasts of the proportion of adult female voles in reproductive condition between each two successive trapping weeks following the spray were not significantly different in one-way ANOVAs ( $F_{4,10} = 0.65$ ,  $P = 0.639$ ;  $F_{4,10} = 0.35$ ,  $P = 0.835$ ;  $F_{4,10} = 0.22$ ,  $P = 0.922$ ;  $F_{4,10} = 1.02$ ,  $P = 0.443$ ).

The numbers of juvenile recruits/adult female generally were greatest early in the summer (trap period 3, June) and declined toward September (Fig. 3.4). We detected a difference in juvenile recruitment over time ( $F_{6,5} = 59.4367$ ,  $P = 0.0002$ ), but not a time by treatment interaction ( $F_{24,19} = 0.7660$ ,  $P = 0.7338$ ). Seasonal variation was the cause for the statistical difference in the recruitment and proportion of adult female voles in reproductive condition over time. The difference was not related to the chemical application because no treatment-time

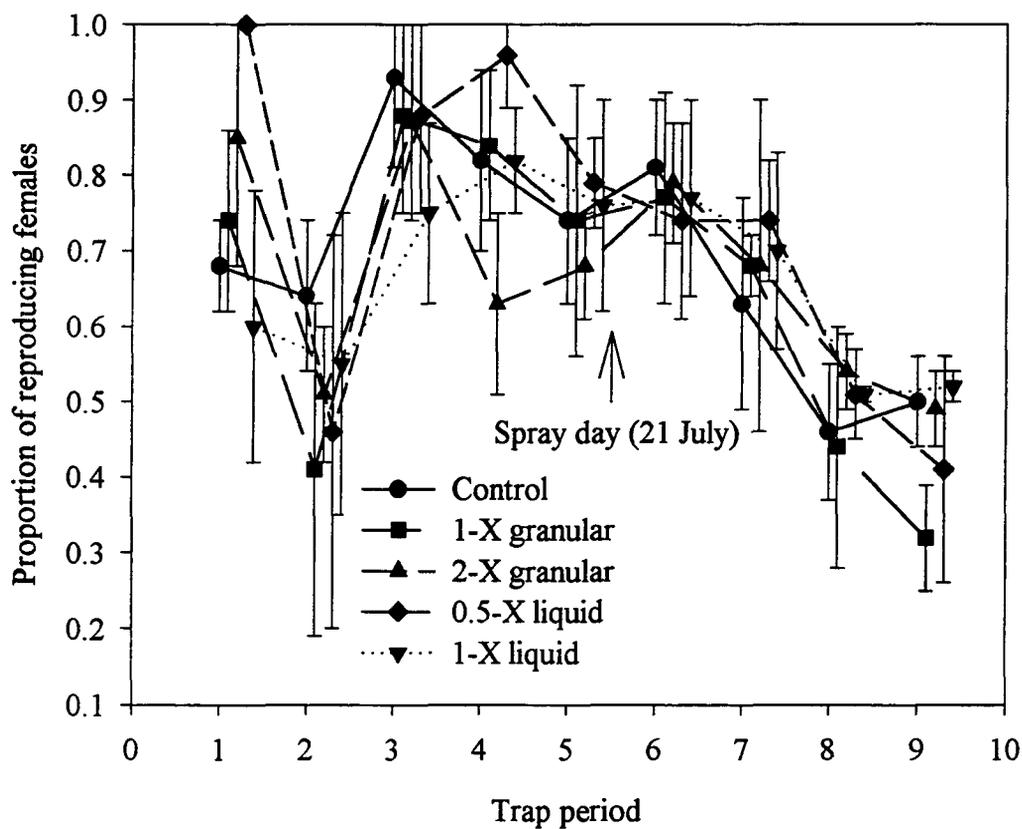


Figure 3.3. Mean proportion of reproducing female gray-tailed voles in control, 1-X granular, 2-X granular, 0.5-X liquid and 1-X liquid enclosures at Hyslop Agronomy Farm, Benton County, Oregon, May-September 1998. Vertical lines are one standard deviation.

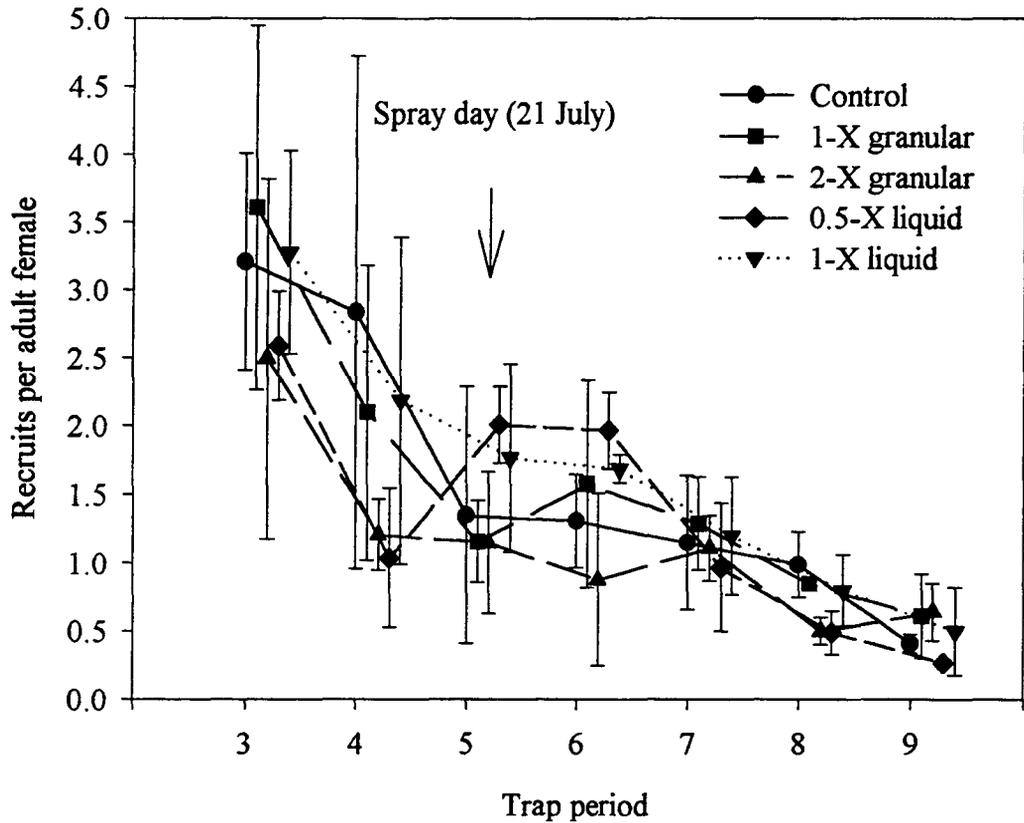


Figure 3.4. Mean number of recruits per adult female gray-tailed voles in control, 1-X granular, 2-X granular, 0.5-X liquid and 1-X liquid enclosures at Hyslop Agronomy Farm, Benton County, Oregon, May-September 1998. Vertical lines are one standard deviation.

interaction was detected. Therefore, the chemical treatment did not negatively affect reproductive activity or recruitment.

#### *Mean Maximum Distance Moved*

MMDM of gray-tailed voles differed by time ( $F_{23, 8} = 23.447$ ,  $P = 0.012$ ). We did not detect a time by treatment interaction ( $F_{1, 32} = 12.659$ ,  $P = 0.355$ ). Population size was not a significant covariate for treatment in the whole-plot ANOVA ( $F_{1, 9} = 1.97$ ,  $P = 0.194$ ) and in the split-plot ANOVA ( $F_{1, 79} = 0.407$ ,  $P = 0.532$ ). Thus, chemical treatment did not exert negative effects on MMDM over time.

#### *Vole Survival Rate*

In preliminary analyses, the survival probabilities of vole populations in each replicate of control and treatment groups could be modeled with the same best model, and survival estimates after replicates were combined were consistent with our preliminary findings. Sixteen models incorporating treatment and time factors were compared to test treatment effects of diazinon. The best models were those of time-constant survival rates or time-dependent survival without treatment factors (*i.e.*, the survival rates were different over time but the same among treatments). Our preferred models suggest that the application of diazinon did not cause any difference in vole survival rates between the control and treatment.

### *Quail Survival Rate*

None of the quail released in control and treatment enclosures died before the spray. We recovered one, two, and three carcasses of quail in the three 2-X granular diazinon treatment enclosures, respectively, during the census of the quail 10 hours after the spray. Diazinon granules were found in the crops of these dead quail. We also observed abnormal behavior (*e.g.*, lethargy, wing drop, ataxia, and hyporeactivity) typical of intoxicated quail in the granular-treatment enclosures. We found two intoxicated quail hiding in grass cover and recovered their carcasses there later. However, all other carcasses were recovered in the 1-m bare ground strip along the enclosure fences. One dead quail each was recovered in one control and one 0.5-X liquid diazinon treatment enclosure after the spray. These two quail were killed by either a Sherman trap or the tractor used for chemical application. We did not observe any mortality caused by nonchemical factors in the granular diazinon treatment enclosures. The quail in the control and liquid treatment enclosures did not display any abnormal behaviors during this study. Data on the presence and absence of quail were pooled by treatment group to estimate survival. The best model indicated a difference in quail survival rate between the granular enclosures and nongranular enclosures after the spray (Fig. 3.5). Quail survival rates in the granular enclosures were significantly lower than that of quail in the nongranular enclosures after the spray. No difference in quail survival rate was found between the control and liquid diazinon treatment enclosures, nor between the two liquid-application treatments. We found a significant difference in the

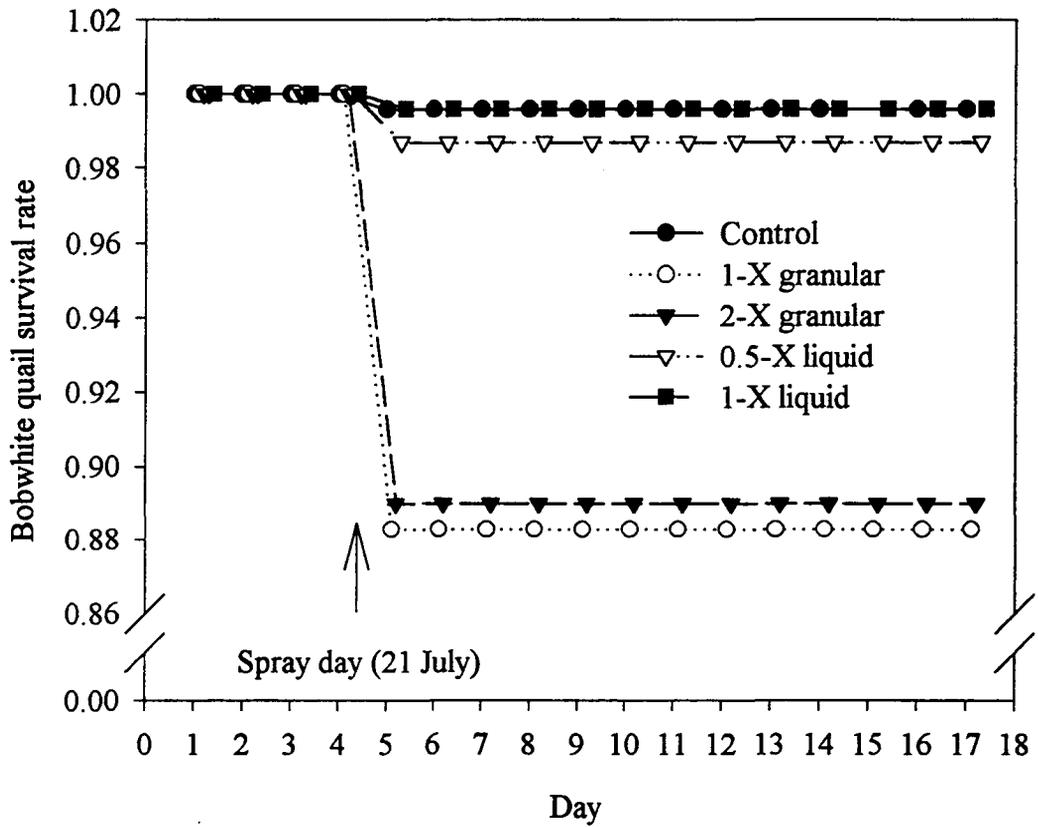


Figure 3.5. Daily survival rate of bobwhite quail in control, 1-X granular, 2-X granular, 0.5-X liquid and 1-X liquid enclosures at Hyslop Agronomy Farm, Benton County, Oregon, July 1998.

number of dead and missing quail between control and treatments in one-way ANOVA ( $F_{4, 14} = 8.65$ ,  $P = 0.0028$ ). The number of dead and missing quail in 1-X and 2-X granular treatment enclosures was significantly greater than in control enclosures and 0.5-X and 1-X liquid treatment enclosures ( $P < 0.1$ ). However, we did not detect differences in the number of dead and missing quail between 1-X and 2-X granular treatments, or between 0.5-X and 1-X liquid treatments, or among control and liquid treatments ( $P > 0.1$ ) (Fig. 3.6).

## Discussion

Our results support our hypothesis concerning pesticide formulation and quail, but did not support our hypothesis concerning voles. A granular application of diazinon had a greater negative impact on quail than did a flowable application. A granular application of diazinon at 1-X and 2-X label rate for grasslands caused a detectable decline in bobwhite survival. The intoxicated quail displayed abnormal behaviors in the granular-treatment enclosures. Diazinon, an OP insecticide, inhibits cholinesterase. Cholinesterase inhibition can cause excessive stimulation of central and peripheral nerve systems of wildlife, and result in lethal (death) or sublethal (abnormal behavior, reproductive impairment) effects on exposed wildlife (Grue *et al.* 1997). Most recovered quail carcasses in the granular treatment enclosures were found in 1-m bare ground strip along the enclosure fences. Hawkes *et al.* (1996) reported that bobwhite receiving a lethal dose of the acetylcholinesterase inhibitor, aldicarb, were limited in their cover-seeking

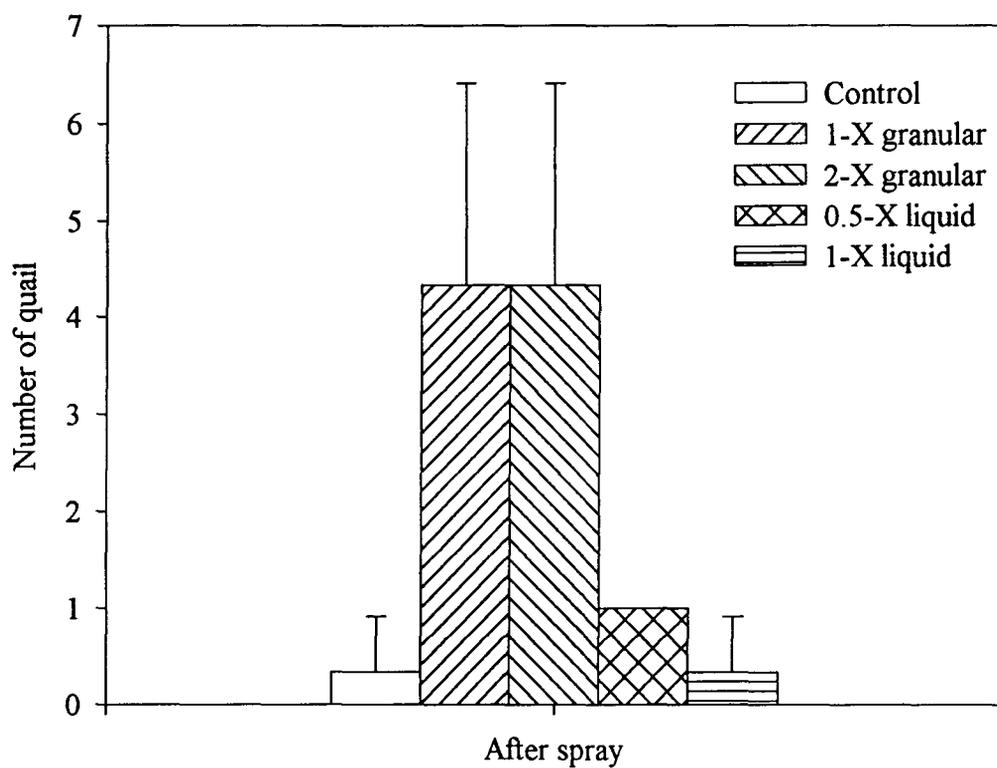


Figure 3.6. Mean number of dead and missing quail after diazinon application in control, 1-X granular, 2-X granular, 0.5-X liquid and 1-X liquid enclosures at Hyslop Agronomy Farm, Benton County, Oregon, July-August 1998. Vertical lines are one standard deviation.

behavior. The difference in the survival rate of the quail between granular and flowable diazinon applications suggests that direct ingestion of diazinon granules is the main exposure route causing the mortality of quail. Ground-feeding birds were reported to pick up pesticide granules as seeds or grit for helping food digestion by facilitating grinding of the food in the gizzard (Best and Gionfriddo 1994). Quail in the liquid treatment may be exposed to the diazinon through inhalation or dermal absorption (Tank *et al.* 1993). However, we saw no indication of this in our study. The survival rate of quail in the liquid treatments was not affected by a diazinon application of 1.55 kg AI/ha, the rate at which the granular application of diazinon suppressed bobwhite survival. Thus, ground-feeding birds are more susceptible to granular OP insecticides than to flowable insecticides.

Neither flowable nor granular applications of diazinon at either rate had measurable impacts on vole demography. Voles are herbivores (Batzli 1985), and consumption of contaminated plants is one of the main exposure routes for these rodents. We did not quantify diazinon residues on the grasses following the application in this study. However, previous studies in the same enclosures demonstrated that vegetation structure affects the residue distribution among vegetation strata. Tall plants tend to intercept more pesticide residue (Bennett *et al.* 1994; Schauber *et al.* 1995). Grasses in the enclosures were approximately 40 cm tall in this study, and the average coverage of grasses was >95%. Therefore, much of the residue likely accumulated in the upper strata of grasses and did not reach ground level. Accumulation of pesticides on the tall grass may reduce the potential

exposure of the voles to pesticides. Wang *et al.* (1999) found that an application of Guthion<sup>®</sup> 2S at the rate of 1.55 kg/ha caused fewer effects on gray-tailed vole demography in grasslands than previous experiments with the same application rate in alfalfa (Edge *et al.* 1996; Schauber *et al.* 1997). We hypothesized the difference in the vole responses between Schauber *et al.*'s (1997) and Edge *et al.*'s (1996) or Wang *et al.*'s (1999) was a function of vegetation structure between these two habitat types. Diazinon rapidly degrades in the field; the half life is less than 14 days under the field conditions. We trapped voles for four trap periods after the application, allowing newborn voles to reach catchable weight. However, we did not find any measurable differences in the juvenile recruitment between liquid treatments and controls following the application. Therefore, the liquid application of diazinon at the rates of 0.55 and 1.11 kg/ha did not affect vole demography in the grass habitats.

Our results demonstrate that ground-foraging seed-eating birds are more susceptible to granular insecticides than to flowable insecticides, while our results did not support our prediction that flowable diazinon would have greater adverse effects on voles. Frank *et al.* (1991) reported die-offs of Canada geese at a golf course that applied liquid diazinon. Geese were vulnerable to liquid diazinon because grazed on grasses. Because grass was not a significant component of quail diets (Rosene 1969), liquid diazinon did not negatively affect quail survival in this study. Differences in foraging behavior among avian species may result in differences in the potential hazard of pesticides.

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## Chapter 4

## Rainfall and Guthion® 2S Interactions Affect Gray-tailed Vole Demography

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Rainfall and Guthion® 2S Interactions Affect Gray-tailed Vole Demography

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## Abstract

The Quotient Method (QM), a pesticide risk assessment model used by the U.S. Environmental Protection Agency (USEPA), assumes that the expected exposure concentration of a contaminant is a function of application rate immediately after pesticide application. The QM does not take into account weather conditions (*e.g.*, rainfall) at the time of spray. We used gray-tailed voles (*Microtus canicaudus*) as an experimental model species to field test this assumption of the QM by simulating a 0.25-cm rainfall. In June 1999, we placed voles into 16, 0.2-ha enclosures planted with a mixture of pasture grasses. In early August, we applied 2.44 kg/ha of the insecticide Guthion<sup>®</sup> 2S (azinphos-methyl) in four treatments; a dry control, wet control (“rain”), dry treatment (sprayed with Guthion<sup>®</sup> 2S, no “rain”), and wet treatment (sprayed with Guthion<sup>®</sup> 2S and “rain” within 24 hours). We used four replicate populations for each treatment. Survival rates of male voles in dry treatment enclosures declined throughout the rest of study following pesticide application, while male survival rates displayed short-term increases in other treatments. Rainfall improved male survival and may have mitigated the adverse effects of Guthion<sup>®</sup> 2S. We also detected significant time by treatment interactions on population size and population growth rates of voles. Our results indicate that the Guthion<sup>®</sup> 2S treatment depressed population size and growth rate in the dry treatment. However, rainfall may have reduced the risk of Guthion<sup>®</sup> 2S to voles. The interaction between rainfall and Guthion<sup>®</sup> 2S application resulted in a deviation from the predicted risk by the QM.

Key words: Ecological risk assessment, gray-tailed voles, Guthion® 2S, quotient method, weather conditions

## Introduction

Ecological risk of agrichemicals to wildlife is a function of hazard ( $LC_{50}$  or  $LD_{50}$ ) and expected exposure concentration (EEC) of potential contaminant in the Quotient Method (QM) (Urban and Cook 1986). EEC is estimated as a direct function of application rate of agrichemicals on the basis of a nomogram derived from a database of residues measured on crops (Hoerger and Kenaga 1972). EEC measures the exposure of wildlife to agrichemicals in the QM. However, the EEC of the QM does not incorporate factors extrinsic to wildlife populations, which may affect the exposure of wildlife to agrichemicals (Tiebout and Brugger 1995).

Weather conditions are an important factor determining the fate and distribution of agrichemical residues in the environment. The fate and distribution, in turn, could influence the risk of chemicals to wildlife.

Rainfall following applications of agrichemicals may affect the distribution of contaminants in the environment. The top layers of plants or crops intercept a majority of chemical residues (Bennett *et al.* 1994; Schauber *et al.* 1995). A light rainfall shortly after a pesticide application could redistribute chemical residues by washing some of the contaminant from the crop canopy down to lower vegetation layers or ground level. The redistribution of chemicals caused by a light rainfall could cause the risk to wildlife to deviate from the risk predicted by the QM based

on application rates and the EEC. Schaubert *et al.* (1997) found population declines of up to 50% in gray-tailed voles (*Microtus canicaudus*) when an application of Guthion® 2S was followed by light rainfall, and hypothesized that rain might have washed the chemical residues down to ground level, increasing the exposure of voles. This hypothesis reveals an uncertainty of the QM assumption that EECs represent residue concentrations immediately after pesticide application (Tiebout and Brugger 1995). Nevertheless, Schaubert *et al.*'s (1997) hypothesis has not been tested experimentally. The objective of our study was to test Schaubert *et al.*'s (1997) hypothesis that a light rainfall would alter exposure and resultant demography of voles compared to sites without rainfall. We predicted a greater chemical-induced mortality in a "rain" treatment than in dry treatments and in controls.

## **Materials and Methods**

### *Study Site and Enclosures*

The research site is located at the Hyslop Agronomy Farm of Oregon State University, approximately 10 km north of Corvallis, Oregon (123°12'W, 44°38'N). Twenty-four 0.2-ha enclosures have been constructed at the research site. Each enclosure is 45 x 45 m and is constructed of galvanized sheet metal approximately 90 cm above ground and buried 90 cm deep to prevent escape or entry by burrowing animals. Each enclosure was planted with a mixture of pasture grasses

composed of fawn tall fescue (*Festuca arundinacea*), Linn perennial ryegrass (*Lolium perenne*), perennial tetraploid ryegrass (*Lolium perenne*), annual ryegrass (*Lolium multiflorum*), and Potomac orchardgrass (*Dactylis glomerata*), which is similar to the natural habitat of gray-tailed voles. The coverage of grasses in all enclosures was 95-100%. A 1-m strip along the inside of each fence was kept bare by mowing to minimize small mammal activity near the fence. Average annual precipitation was 108 cm, most of which falls in the winter and spring. Eighty-one, large-size Sherman live traps were placed in each enclosure in a 9 x 9 array with 5-m trap spacing. We randomly chose 16 enclosures for this study.

#### *Study Species and Test Chemical*

We used gray-tailed voles as our model species in this study. Gray-tailed voles are a common small mammal species of grasslands and agricultural areas in the Willamette Valley of western Oregon. Breeding occurs from March through November, adult female gray-tailed voles are territorial, adult males have large home ranges that overlap those of several females, and dispersal occurs primarily among young males (Wolff *et al.* 1994). We used Guthion<sup>®</sup> 2S (*O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-(4*H*)-yl)methyl] phosphorodithioate) as our test chemical. This compound has been identified by the USEPA Office of Pesticide Programs as causing avian die-offs in the field that were not predicted by their QM risk assessment (Grue *et al.* 1983).

### *Establishment of Experimental Populations and Chemical Application*

In early June, five adult male and five adult female wild-caught gray-tailed voles were released into each of the 16 enclosures to start the experiment. The 16 enclosures were randomly assigned to four treatments: dry control (sprayed only a volume of water equivalent to Guthion<sup>®</sup> 2S-water mixture); wet control (sprayed a volume of water equivalent to Guthion<sup>®</sup> 2S-water mixture, then sprinkled with water equivalent to 0.25 cm of rainfall); dry treatment (Guthion<sup>®</sup> 2S in liquid formulation at 2.44 kg [AI]/ha); wet treatment (Guthion<sup>®</sup> 2S in liquid formulation at 2.44 kg [AI]/ha, then sprinkled with water equivalent to 0.25 cm of rainfall within 24 hours after the Guthion<sup>®</sup> 2S application). We used four replicates for each treatment group. We predicted that negative population-level effects of Guthion<sup>®</sup> 2S on voles in wet treatment enclosures would be greater than in dry treatment enclosures. On 4 August 1999, liquid Guthion<sup>®</sup> 2S was applied in the early morning (beginning at 0600 h) by a licensed applicator using a small tractor and trailer tank with a 7.6-m long spray boom. The boom was set at a height of 60 cm, approximately 20 cm above the top of the vegetation. The speed of the tractor and the pressure of the trailer tank were calibrated before the spray to deliver the desired amount of liquid mixture or water within the enclosures. Water was sprayed first in the controls, and then the Guthion<sup>®</sup> 2S was sprayed in the treatment grids to avoid Guthion<sup>®</sup> 2S contamination in the control grids. A lack of wind in early morning prevented chemical drift to the control enclosures. Spray tank contents were sampled before spraying the first enclosure and resampled after

spraying the last enclosures. Tank sample analysis confirmed our application rate of 2.44 kg/ha. We simulated a 0.25-cm rainfall with an irrigation sprinkler system. Twenty-five sprinklers were arranged in a  $5 \times 5$  array, with about 8 m between sprinklers in each "rain" enclosure. We mounted screens on the sprinklers along the four sides of enclosures to avoid sprinkling water into neighboring enclosures. Sprinklers rotated 360 degrees during the irrigation. We measured the amount of rainfall in 10 rain gauges randomly located in each enclosure. The sprinklers ran for 10-15 minutes until approximately 0.25 cm of rainfall was recorded in the gauges in each enclosure.

#### *Trapping Procedures for Gray-tailed Voles*

Voles were trapped in the enclosures for 4 consecutive days (trap period) at 2-week intervals from early June through the end of September 1999 using mark-recapture procedures (Edge *et al.* 1996). Traps were baited with oats and sunflower seeds, set just before sunset and checked once a day at sunrise. All captured animals were ear-tagged for identification, and data on mass, age, sex, reproductive condition, and trap location were recorded for each capture. Voles with holes or rips in their right ears were assumed to have lost an ear-tag and were retagged with a new tag. Previous tag numbers were identified by similarities in sex, weight and trap location between previous captures and the newly tagged animal. Females were considered in reproductive condition if they were lactating (large nipples and white mammary tissue surrounding the nipples) or pregnant (obviously swollen

abdomen). Field personnel were trained for a 2-week period in accordance to an approved quality control plan.

### *Population Parameters and Statistical Analyses*

We used capture-recapture methodology to estimate survival rates and population sizes of gray-tailed voles (Cormack 1964; Jolly 1965; Seber 1965; Rexstad and Burnham 1992). Sex-specific survival rates were estimated using derivations of Cormack-Jolly-Seber's method with programs RELEASE (Burnham *et al.* 1987) and SURGE (Pradel and Lebreton 1991). The best models for male and female survival probabilities were identified using Akaike's Information Criterion (AIC). We measured recruitment by the number of newly tagged voles captured in an enclosure per adult female captured in the same enclosure 4 weeks (two trap periods) earlier. The time lag allowed recruits to reach trappable size. We used multivariate repeated measures ANOVA to test for differences in population size, population growth rate, juvenile recruitment, and proportion of adult females in reproductive condition among treatments over time, and for detecting time by treatment interactions. In this study, we applied the contaminant part way through the study, so the treatment-time interaction was the primary effect of interest (Paine and Paine 1996). When a main effect or an interaction was detected, we conducted a two-way ANOVA for each trap period. Natural variation in demographic variables in our previous experiments has always been high. To

enhance the power of statistical analyses, we set  $\alpha = 0.1$  to detect biologically meaningful results (Schauber and Edge 1999).

## Results

### *Population Size*

Vole populations fluctuated around 10 to 15 animals/enclosure throughout the period of study, with an exception of a mean of 32 animals/enclosure in wet treatment enclosures (Fig. 4.1). Population sizes differed over time ( $F_{7,6} = 3.52$ ,  $P = 0.07$ ). We also detected a time by treatment interaction ( $F_{7,6} = 6.80$ ,  $P = 0.02$ ). In the trap period just prior to implementation of treatments, we detected a difference between “rain” and “nonrain” enclosures ( $P < 0.05$ ). Guthion<sup>®</sup> 2S and nonguthion enclosures differed in population sizes during trap period 5 just after the chemical application ( $P < 0.05$ ). We found a rain-chemical interaction only during trap period 7 ( $P < 0.05$ ); vole population sizes in wet control enclosures increased more rapidly than the populations in dry control enclosure after Guthion<sup>®</sup> 2S applications (Fig. 4.1). Vole populations in dry treatment enclosures declined shortly after Guthion<sup>®</sup> 2S application; however, vole populations in wet treatment enclosures increased slightly (Fig. 4.1). Our simulated rainfall appeared to enhance vole populations and may have mitigated the adverse effects of Guthion<sup>®</sup> 2S on vole populations in the wet treatment.

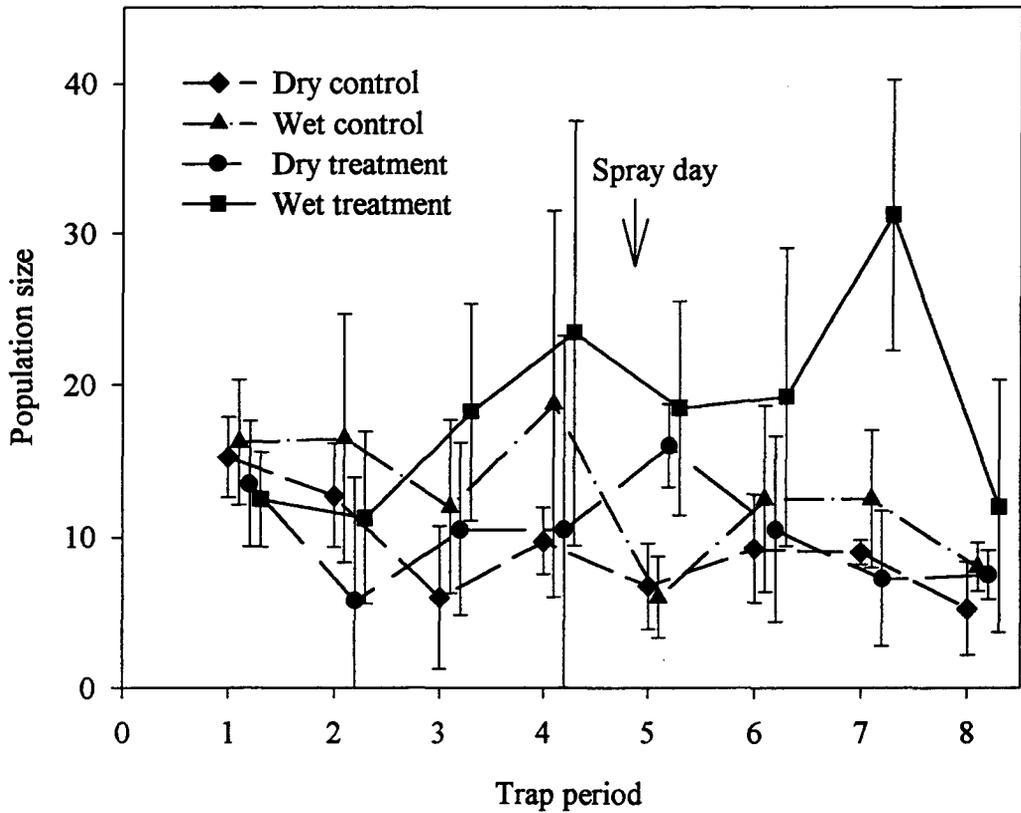


Figure 4.1. Mean population size of gray-tailed voles in dry-control, wet-control, dry-treatment, and wet-treatment enclosures at Hyslop Agronomy Farm, Benton County, Oregon, June-September 1999. Vertical lines are one standard deviation.

### *Population Growth Rate*

Population growth rates of gray-tailed voles fluctuated substantially throughout the study, ranging from  $-1.42$  to  $1$  (Fig. 4.2). Population growth rates did not differ over time ( $F_{6,7} = 2.28$ ,  $P = 0.1532$ ), but we detected a time by treatment interaction ( $F_{6,7} = 8.45$ ,  $P = 0.01$ ). In the period just prior to treatment implementation (trap periods 3-4), population growth rates neither differed between Guthion® 2S and nonguthion enclosures ( $P > 0.1$ ) nor between “rain” and “nonrain” enclosures ( $P > 0.1$ ). Population growth rates in dry-treatment enclosures declined after Guthion® 2S application, while population growth rates in control enclosures increased (Fig. 4.2). During trap periods 5-6, population growth rates were lower in Guthion® 2S enclosures than in nonguthion enclosures ( $P < 0.04$ ). Population growth rates in wet-treatment enclosures tended to be greater than in dry treatment enclosures during trap periods 5-6, but not significantly (Fig. 4.2), while control enclosures had greater population growth rates than Guthion® 2S enclosures (Fig. 4.2). Therefore, application of Guthion® 2S negatively affected vole population growth rates, and the light rainfall may have reduced these effects.

### *Reproductive Activity and Recruitment*

The mean proportion of adult female voles in reproductive condition ranged from  $0.0$  to  $0.89$ , and fluctuated around  $0.5$  throughout the study in all enclosures. The proportion of adult female voles in reproductive condition did not differ over

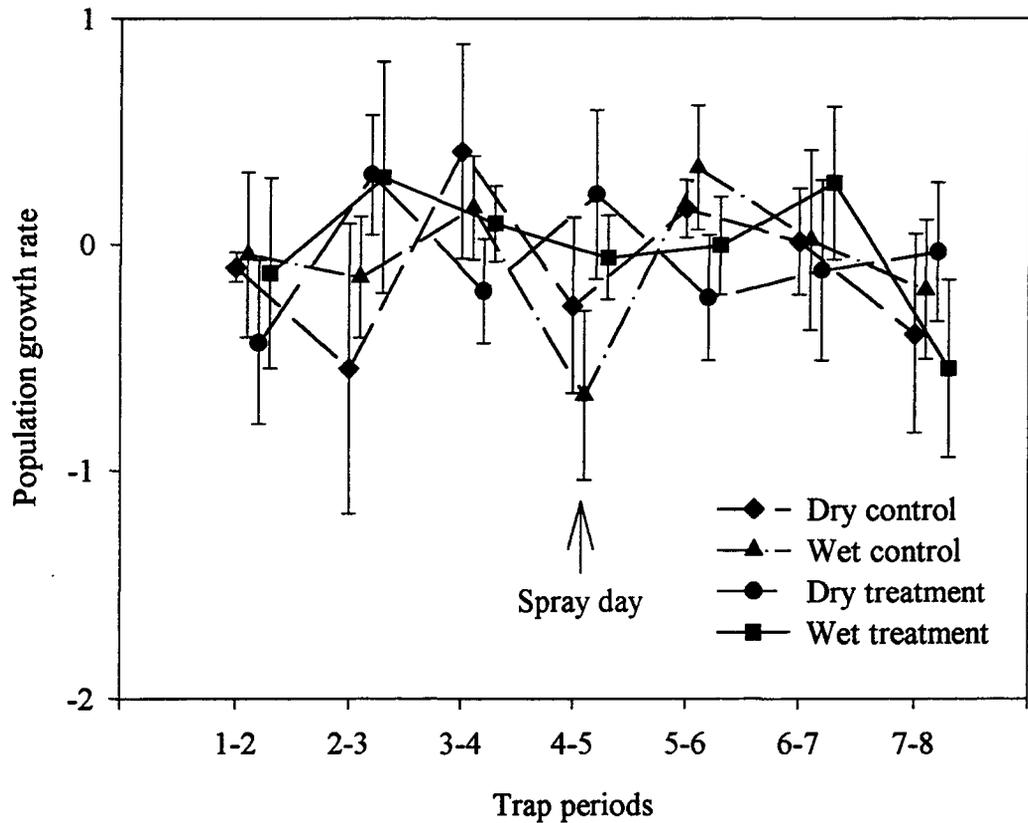


Figure 4.2. Mean population growth rate of gray-tailed voles in dry-control, wet-control, dry-treatment, and wet-treatment enclosures at Hyslop Agronomy Farm, Benton County, Oregon, June-September 1999. Vertical lines are one standard deviation.

time ( $F_{7,6} = 1.132$ ,  $P = 0.448$ ), but we detected a time by dry treatment interaction ( $F_{7,6} = 6.898$ ,  $P = 0.02$ ). However, no differences in proportion of adult females in reproducing conditions between treatment groups were found in all post-treatment trap periods ( $P > 0.1$ ) in two-way ANOVAs. Juvenile recruitment in all enclosures gradually declined over time ( $F_{5,8} = 4.07$ ,  $P = 0.04$ ), and we detected a time by treatment interaction ( $F_{5,8} = 5.841$ ,  $P = 0.02$ , Fig. 4.3). However, changes and levels of juvenile recruitment were not consistent in the post-treatment periods (Fig. 4.3), and effects of neither treatment were obvious. Therefore, neither treatment appeared to alter reproductive activity or recruitment.

### *Survival Rate*

In preliminary analyses, the survival probabilities of vole populations in each replicate of control or treatment groups could be modeled with the same best model, and survival estimates after replicates were combined generally were consistent with our preliminary findings. Our best model for male voles suggested that male survival rates were different over time and among treatments. The survival rate of male voles in dry treatments declined after Guthion<sup>®</sup> 2S application by 0.06 and continued to decline throughout the rest of study (Fig. 4.4). Male voles in wet treatment enclosures had survival rates similar to the males in the wet controls between trap periods 3 and 4 just prior to the treatment implementation. After treatment implementation, male survival in wet treatments did not decline as male survival in dry treatments did. In contrast, male survival rates increased to

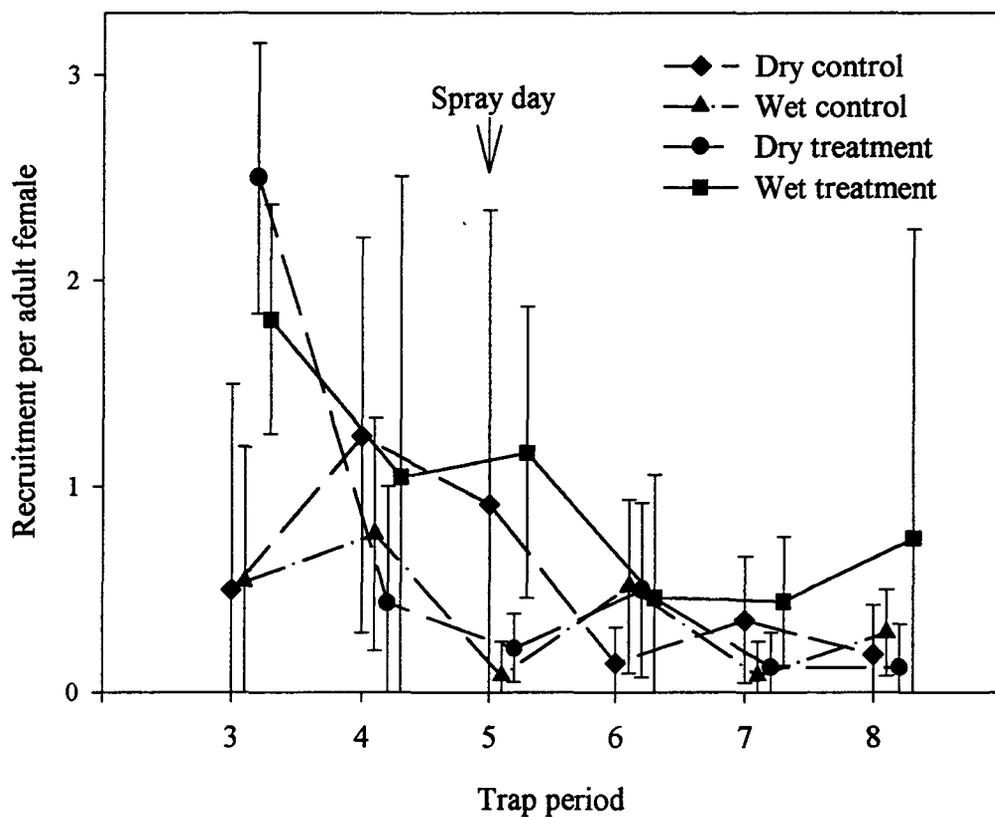


Figure 4.3. Mean number of recruits per adult female gray-tailed vole in dry-control, wet-control, dry-treatment, and wet-treatment enclosures at Hyslop Agronomy Farm, Benton County, Oregon, June-September 1999. Vertical lines are one standard deviation.

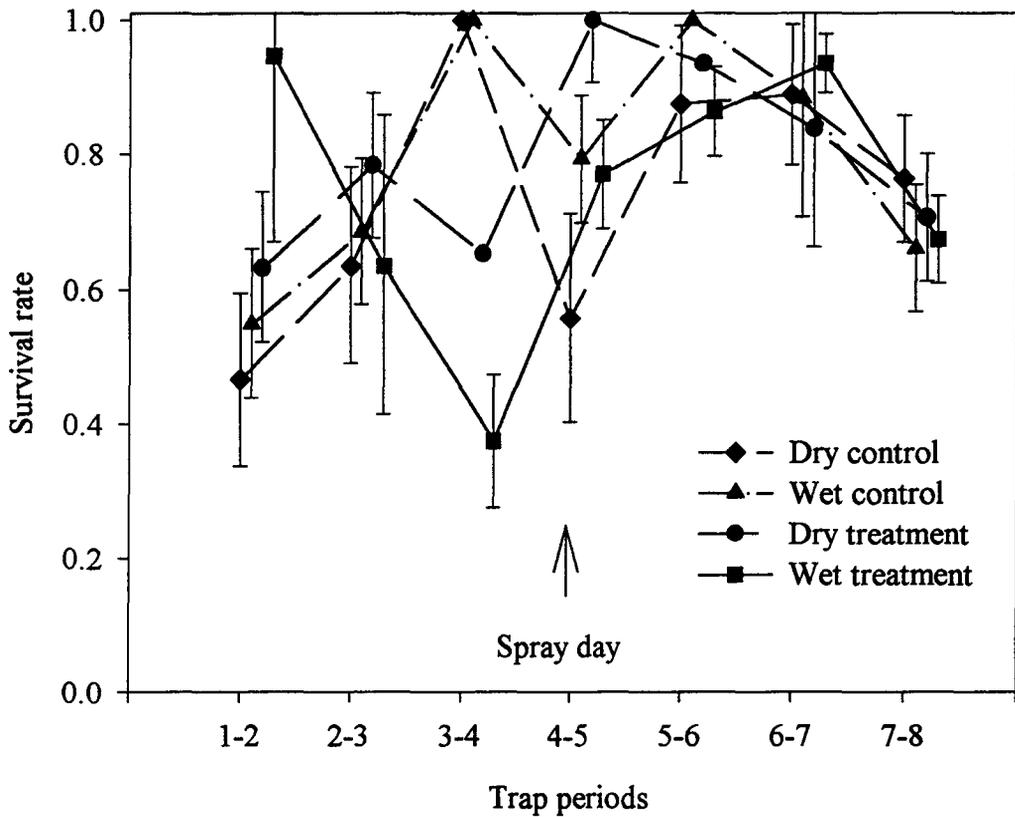


Figure 4.4. Survival rate of male gray-tailed voles in dry-control, wet-control, dry-treatment, and wet-treatment enclosures at Hyslop Agronomy Farm, Benton County, Oregon, June-September 1999. Vertical lines are one standard deviation.

0.86 in wet treatments, while male survival in wet-control enclosures increased to 1.00 in trap periods 5-6. Our best model for female survival had survival rates equal among all treatment groups throughout the experiment.

## Discussion

Our results did not support Schauber *et al.*'s (1997) hypothesis and our prediction of greater adverse effects on demographic parameters of gray-tailed voles in wet treatment enclosures than in dry treatment enclosures. However, our results indicated adverse effects of Guthion® 2S on vole population sizes, population growth rates, and male vole survival rates, especially in dry conditions. Edge *et al.* (1996) and Schauber *et al.* (1997) detected significant reductions in population sizes, population growth rates, and survivals of male gray-tailed voles exposed to  $\geq 3.11$  kg/ha of Guthion® 2S, which was greater than the application rate in this study (2.44 kg/ha). Their results confirmed the prediction of the QM for Guthion® 2S at these application rates. However, Wang *et al.* (1999a) did not detect measurable responses of gray-tailed voles to Guthion® 2S applied at 1.55 kg/ha in the same research facility; a rate at which the QM predicted a risk to gray-tailed voles. Therefore, the QM appears to be conservative in predicting risks to this herbivorous small mammal.

We detected a difference in the survival responses between male and female voles. Male vole survival rates declined after the chemical application in the dry treatment, while female survival did not. Male gray-tailed voles have larger home

ranges than female voles do. Male vole home ranges usually overlap the home ranges of 3-4 female voles (Wolff *et al.* 1994). In addition, male capture probabilities (0.89-0.96) were consistently higher and significantly different from female capture probability (0.74-0.83) (Edge *et al.* 1996). During the growing season, female voles in reproductive condition may spend more time in burrows nursing newborns. These differences in the activity patterns between sexes may have resulted in greater exposure of males than females that could differentially affect survival.

The pattern of adverse effects of Guthion<sup>®</sup> 2S was difficult to detect because of large fluctuations in demographic parameters. Population sizes of gray-tailed voles in this study were below the average from previous studies (Edge *et al.* 1996; Schauber *et al.* 1997; Wang *et al.* 1999*a, b*). Mean maximum population size was about 32 animals, about one third of the maximum mean sizes of the populations in 1997 and 1998 (Wang *et al.* 1999*a, b*). Mean population growth rates fluctuated from -0.5 to 0.5 between trap periods (Fig. 4.2). Low population growth rates and oscillation-like changes in all enclosures may have masked the divergence of control and treatment populations. Small populations usually have greater demographic stochasticity (Goodman 1987), which could result in greater variation in population growth rates among all enclosures. This increased variation would in turn affect the power of experiments like ours. Schauber *et al.* (1997) suggested that the differences between their study and that of Edge *et al.* (1996) in detected demographic responses of voles to Guthion<sup>®</sup> 2S at similar application rates

might be due to greater variation of vole population sizes at the time of spraying in Edge *et al.*'s (1996) study. Further, we compared the mean vole population sizes at the spraying time between Edge *et al.* (1996), Peterson (1996), Schauber *et al.* (1997), Wang *et al.* (1999a), and this study. The studies detecting negative effects of Guthion<sup>®</sup> 2S at similar application rates on vole demography had population sizes of 30-50 voles/enclosure and small within treatment variation—population sizes greater than that of this study. Stochastic population growth rate,  $r_t$ , is more sensitive to perturbations in small populations than in large populations because  $r_t$  is inversely related to population size. Thus, the impact of chemicals on stochastic population growth of a small population would be greater than on that of a large population, and the adverse effect of chemicals would be more difficult to detect because of increased variation among replicates. The QM method does not incorporate this type of demographic response of wildlife populations into the assessment.

We detected interactions of the effects of rainfall and Guthion<sup>®</sup> 2S on population size and population growth rate. However, the interactions took a different form than we expected. We predicted greater reductions of population parameters in the wet-treatment enclosures, based on Schauber *et al.*'s (1997) hypothesis that water dripping from contaminated plants may provide an alternative route of exposure to Guthion<sup>®</sup> 2S and put voles at greater risk. Our results suggested that population growth rates and male vole survival in the wet control and treatment enclosures tended to be greater than in dry treatment enclosures

(Figure 4.2 and 4.4). This increased survival may be the result of improved habitat conditions in wet enclosures. Most *Microtus* species are distributed in mesic or wet habitats (Getz 1985). Moisture conditions are a very important habitat factor influencing local distribution of *Microtus* (Getz 1985). From mid-July through August, the weather at our research site was very dry. It is possible that simulating rainfall improved the survival rate of male voles and increased population size by improving vole habitats. Weather conditions were also found to be a factor influencing the population dynamics of small mammals. Pinter (1988) found that population dynamics of *Microtus montanus* were inversely related to precipitation during May in northwestern Wyoming. However, Leirs *et al.* (1996) found that rodent outbreaks in Tanzania, were preceded by abundant rainfall. Alternatively, the intensity of “rain” may have washed most of the product to the soil, reducing the exposure of voles through ingestion. An acute sublethal exposure of mammals to OP insecticides can cause pronounced, but short-lived, hypothermia, reducing body temperatures and impair thermoregulation in mammals (Grue *et al.* 1997). Our population-level results suggest that rain did not make voles more susceptible to hypothermia.

In conclusion, our study suggests that the QM is robust to the assumption that rainfall does not increase exposure of voles to Guthion<sup>®</sup> 2S in grasslands. However, the interaction between rainfall and Guthion<sup>®</sup> 2S application resulted in a deviation from the predicted risk. This study indicated that intrinsic status of a population is an important factor in ecological risk assessment of agrichemicals to

wildlife. The current QM protocol does not account for the demographic status of wildlife populations. The results of this study suggest that future studies should include demographic and environmental stochasticities in risk assessment, especially for long-term assessments.

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## Chapter 5

### Demographic Uncertainty in Ecological Risk Assessments

Guiming Wang, W. Daniel Edge, and Jerry O. Wolff

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Demographic Uncertainty in Ecological Risk Assessments

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## Abstract

We built a Ricker's model incorporating demographic stochasticity to simulate the effects of demographic uncertainty on responses of gray-tailed vole (*Microtus canicaudus*) populations to pesticide applications. We constructed models with mark-recapture data collected from populations in 1998 and 1999. We ran 30 simulations of a single pesticide application for small (~ 30 voles), medium (~ 50 voles), and large (~ 100 voles) population sizes for 1998 data. Significantly less uncertainty in detecting pesticide effects was exhibited at large population sizes. Fifty percent of simulations for small or medium population sizes suggested no differences between control and treatments. Due to population fluctuations resulting from demographic stochasticity and small population sizes, we detected no significant differences in the simulations using 1999 data. Population sizes may affect the recovery ability of vole populations following pesticide-induced mortality. Vole population-size declines were significant for pesticide applications at large population sizes, but greater uncertainty existed in the simulations of low and medium population sizes. Our results suggested that the Quotient Method (QM), an ecological risk assessment model, should differentiate the short-term risk of a chemical to small and large populations. Our results also suggested that the QM could not predict or may underestimate the long-term extinction risk of rare or endangered wildlife species from contamination by pesticides.

*Key words:* Demography; *Microtus canicaudus*; Pesticides; Population dynamics; Simulation; Stochasticity

## Introduction

The U.S. Environmental Protection Agency (USEPA) assesses ecological risk of agrichemicals to wildlife using the Quotient Method (QM) (Urban and Cook, 1986). The QM divides toxicity ( $LC_{50}$  or  $LD_{50}$ ) of a chemical by its expected environmental concentration (EEC). EEC is estimated as a direct function of application rate of agrichemicals on the basis of a nomogram derived from a database of residues measured on crops (Hoerger and Kenaga 1972). Toxicity is based on laboratory studies using surrogate species. Although intuitively simple, the QM does not incorporate several intrinsic and extrinsic factors, which may affect the results of ecological risk assessment of agrichemicals (Tiebout and Brugger 1995). One factor not incorporated into the QM is the demography of wildlife populations prior to chemical applications. Both theoretical and empirical studies demonstrate the importance of variation or uncertainty of demographic parameters in population dynamics and its implication for conservation biology (Goodman 1987; Lande and Orzack 1988; Lande 1993; Sæther et al. 1998). Demographic and environmental stochasticities are the main source of variation in population dynamics. Demographic stochasticity is a significant factor in small populations, while environmental stochasticity is important to both small and large populations (Lande 1993). These two types of uncertainties play a primary role in population viability analysis. Ginzburg et al. (1982) recognized the importance of incorporating demographic and environmental uncertainties of population growth into ecological risk assessment. However, in practice, the main difficulty in

incorporating these uncertainties into risk assessments is the estimation of variances in population growth because of the scarcity of data. Stochastic population models and Monte Carlo simulations are two approaches for studying stochasticities in population growth rates (Burgman et al. 1993; Lande 1993). We use these two approaches to evaluate the uncertainty of predictions of the QM.

Density-dependent regulation of population dynamics has been argued for several decades. During our studies of gray-tailed vole responses to pesticide applications, population growth trajectories of voles in experimental enclosures were sigmoid (Edge et al. 1996; Schauber et al. 1997; Wang et al. 1999a, b). This pattern suggests that population growth of voles in enclosures may be described by a nonlinear sigmoid-type equation with population density as an explanatory variable of population growth rate. Under density-dependent regulation, an increase in mortality in a population can be compensated by a subsequent increase in reproduction or immigration (Sibly 1996). This may be especially true at low population densities when population growth rates are close to the maximum growth rate,  $r_m$ . This relationship implies that the compensation ability following pesticide-induced mortality may be weaker at higher population densities than at lower densities. On the other hand, population growth rates exhibit greater demographic stochasticity in small populations than in large populations. Thus, we predict that demographic responses of voles to pesticides will be less easily detected at small population sizes than at large population sizes. Our objective was to assess the role of demographic stochasticity in detecting the response of voles to

pesticides using mark-recapture data collected in enclosures in 1998 and 1999. We wanted to determine if population declines of voles during post-treatment periods were more pronounced for large population than for small populations.

## **Materials and Methods**

### *Study Site and Enclosures*

Our field research site was located at the Hyslop Agronomy Farm of Oregon State University, approximately 10 km north of Corvallis, Oregon (123°12'W, 44°38'N). Average annual precipitation was 108 cm. Twenty-four 0.2-ha enclosures have been constructed at the site. Each enclosure is 45 x 45 m and is constructed of galvanized sheet metal approximately 90 cm above ground and buried 90 cm deep to prevent escape or entry by burrowing animals. Each enclosure was planted with a mixture of pasture grasses composed of fawn tall fescue (*Festuca arundinacea*), Linn perennial ryegrass (*Lolium perenne*), perennial tetraploid ryegrass (*Lolium perenne*), annual ryegrass (*Lolium multiflorum*), and Potomac orchardgrass (*Dactylis glomerata*), which is similar to the natural habitat of gray-tailed voles. The coverage of grasses in all enclosures was 95-100%. We kept a 1-m strip along the inside of each fence bare by mowing to minimize small mammal activity near the fence. Eighty-one, large-size Sherman live traps were placed in each enclosure in a 9 x 9 array with 5 m between traps. We randomly

chose three enclosures in 1998 and four enclosures in 1999 for our controls populations.

### *Establishment of Experimental Populations*

In mid-May, 10 adult male and 10 adult female wild-caught gray-tailed voles were introduced into each of three control enclosures to start the experiment in 1998. In early June 1999, five adult male and five adult female wild-caught gray-tailed voles were released into each of four enclosures to begin the experiment. During the application of pesticides in 1998 and 1999, we sprayed pesticides in early morning (about 0600h) when the air was calm decreasing chemical drift into control enclosures.

### *Trapping Procedures for Gray-tailed Voles*

Voles were trapped in the enclosures for 4 consecutive days (trap period) at 2-week intervals from mid-May through mid-September 1998 and from early June to the end of September 1999, using mark-recapture procedures (Edge et al. 1996). Traps were baited with oats and sunflower seeds, set just before sunset and checked once a day at sunrise. All captured animals were ear-tagged for identification, and data on mass, age, sex, reproductive condition, and trap location were recorded for each capture.

### *Population Parameters*

We used mark-recapture methodology to estimate survival rates and population sizes of gray-tailed voles (Cormack 1964; Jolly 1965; Seber 1965; Rexstad and Burnham 1992). We used the Jackknife model to estimate population sizes (Manning et al. 1995). Sex-specific survival rates were estimated using derivations of Cormack-Jolly-Seber's method with programs RELEASE (Burnham et al. 1987) and SURGE (Pradel and Lebreton 1991). We used Akaike Information Criterion (AIC) (Akaike 1973) to identify the best model of survival probability of voles.

### *Model of Gray-Tailed Vole Populations*

Vole population dynamics were modeled with the Ricker model,  $N_{t+1} = N_t \exp(r_0 - bN_t)$ , where  $N_{t+1}$  is the population size at time  $t+1$ ,  $N_t$  is the population sizes at time  $t$ ,  $r_0$  is the mean growth rate, and  $b$  is the intensity of effect of population size on population growth rate (Ricker 1975). The parameters  $r_0$  and  $b$  were estimated by regressing  $\ln(N_{t+1}/N_t)$  on  $N_t$  where  $\ln(N_{t+1}/N_t) = r_0 - bN_t$  (Burgman et al. 1992). The interval between  $t + 1$  and  $t$  in this study represented two weeks. Only one population each year met our criteria of  $r^2 > 0.5$  in the regressions.

### *Simulation of Demographic Stochasticity*

Because we only monitored vole populations for one growing season, from May or June to September, we disregarded environmental stochasticity, even though environmental stochasticity is important to long-term stochastic population growth rates. Weather conditions at the study site were fairly stable during the summers of 1998 and 1999, and therefore, we assumed that environmental stochasticity was unimportant for our data. We estimated the stochastic population growth rate as  $r_t = r_0 + (\sigma_d/N_t)s - bN_t$ , where  $\sigma_d$  represents demographic stochasticity,  $N_t$  is the population size at time  $t$ ,  $s$  is a random variable of Gaussian distribution, which has a mean of zero and standard deviation of one. The parameter  $\sigma_d$  was estimated with a random birth-death process (Barlett 1960). We approximated the variance of mean population growth rate with the sum of variances caused by random recruitment and survival of nonreproductive individuals in each time period, using the formula  $\sigma_d^2 = p(1-p)\lambda^2 + p(1-p)$ , where  $p$  is the survival probability of voles,  $\lambda$  is the Poisson process parameter, which models the birth process. The term  $p(1-p)\lambda^2$  is the variance in recruitment (Kokko and Ebenhard 1996). The term  $p(1-p)$  is the variance of a binomial distribution (Karlin 1966). In a sense,  $\sigma_d^2$  is the variance of population growth rate of a theoretical population, which only has one individual (Goodman 1987). We used a mean litter size of five (Wolff et al. 1994) to approximate  $\lambda$ . The variance in survival of newborns was accounted for by  $p(1-p)$  in the formula above. We assumed an age- and sex-independent survival rate of 0.9, which is close to the average two-week survival

rate of male and female voles in our previous studies (Wang et al. 1999a, b). The expected population growth rate,  $m$ , was approximated by the first-order derivative of a probability generating function of a single-type branching process (Arthreya and Ney 1972), that is,  $m = p\lambda$ ,  $r = \ln(m) = \ln(p\lambda)$ . We computed the coefficient of variation (CV) of population growth rates as  $\sigma_d/r$ . The accuracy of  $\sigma_d$  was verified by the median CV of population growth rates among control enclosures in the first three trap periods for the 1998 and 1999 experiments. In the first three trap periods when population sizes were small ( $< 30$  voles), variation in population growth rate among replicate populations has a substantial component of demographic stochasticity. Our estimate of demographic stochasticity,  $\sigma_d$ , using the branching process was 1.3829, and the CV of  $r$  was 0.9194. The median CV of population growth rates pooled from the first three trap periods of both experiments was 0.8976. Therefore, we used  $\sigma_d = 1.3829$  for populations both years. Each simulated population started at the initial size of the population that had the best estimate of  $r_0$  and  $b$  in the regressions of  $\ln(N_{t+1}/N_t)$  on  $N_t$ . We computed the stochastic growth rate,  $r_t$ , at each trap period  $t$  for each simulated population. The population size at each trap period was determined by  $N_{t+1} = N_t \exp(r_t)$ .

### *Simulating the Effects of Pesticides on Voles*

We simulated effects of a pesticide on population sizes by imposing 20% mortality on populations at the beginning of a trap period when a pesticide was applied. The mortality induced by a single application in the second trap period

after the application was 10%. The effect of a pesticide application only lasted two trap periods. In contrast, control populations were not subjected to extra mortality induced by pesticides in our simulations. In previous studies, organophosphorus pesticides produced effects on gray-tailed vole population sizes for one or two trap periods (Edge et al. 1996; Schauber et al. 1997). In the simulation of the 1998 experimental populations, a single pesticide application was simulated at the third, fourth, and seventh trap period. Population sizes during the three periods were approximately 30, 50, and 100 voles, respectively. In one simulation of a single pesticide application during each of the three application periods, 15 replicate populations were simulated with our model using a random term of demographic stochasticity for each treatment group and control. Each replicate population had nine trap periods. Mean population sizes and 95% confidence intervals (CI) were computed for the 15 replicates for each trap period. Fisher's Least Significant Difference was used to test the post-treatment difference in population sizes between controls and treatments in each simulation. We ran 30 simulations for each of the three application periods of 1998 populations. Failures to detect significant differences in post-treatment population sizes were tallied in the 30 simulations for each of the three application periods. The effects of demographic stochasticity on the response of population size to pesticide applications were evaluated by the frequency of failing to detect significant differences in the 30 simulations. In the simulation of the 1999 experimental populations, a single pesticide application was simulated during the second or fifth trap period. We did

not simulate multiple applications for any of our populations. We predicted that failure frequency would be greater when pesticides were applied at small population sizes than at large population size because of greater demographic stochasticity in small populations.

## Results

### *Population Models*

Population growth rate ( $\ln[N_{t+1}/N_t]$ ) was inversely related to population size,  $N_t$ , for 1998 ( $r^2 = 0.762$ ,  $P < 0.01$ ) and 1999 ( $r^2 = 0.635$ ,  $P < 0.07$ ) (Fig. 5.1). Mean population growth rate,  $r_0$ , and effect intensity of population size,  $b$ , were 0.6043 and 0.0045 in 1998, and 1.4533 and 0.1886 in 1999, respectively. The expected population growth rate,  $r$ , estimated with a single-type branching process, was 1.50, close to  $r_0$  of 1999.

We used the deterministic version of the Ricker model,  $N_{t+1} = N_t \exp(r_0 - bN_t)$  to represent the two chosen populations (Fig. 5.2). The model closely approximated the population trajectory in 1998 except for period 7, which was above the predicted value (Fig. 5.2). However, observed values of the 1999 population fluctuated around the predicted values (Fig. 5.2). Thus, we concluded the Ricker model adequately represents both the magnitude and trend of vole population dynamics.

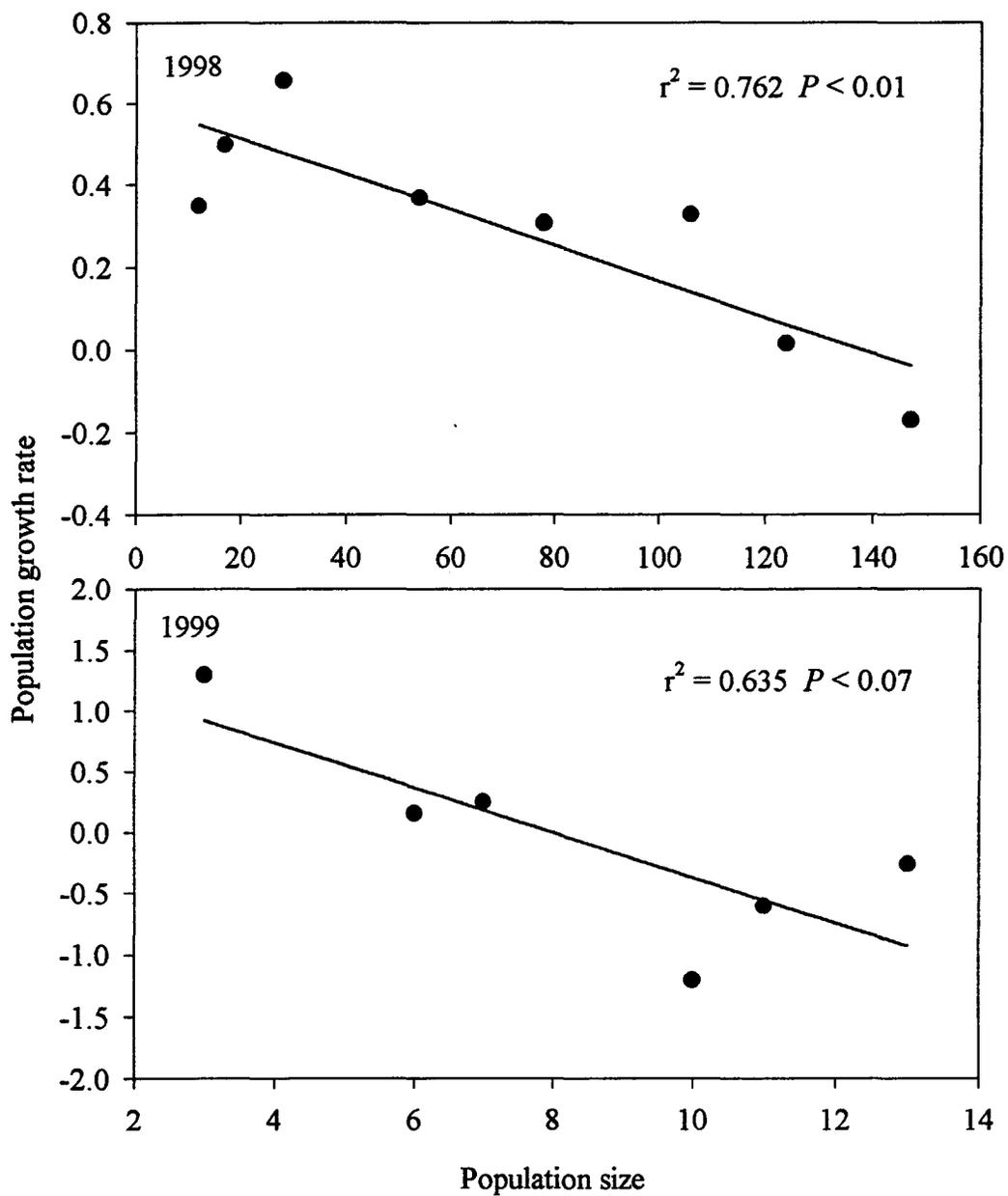


Figure 5.1. Linear regression of population growth rates on population sizes for gray-tailed voles in control enclosures in 1998 (top) and 1999 (bottom) at Hyslop Agronomy Farm, Benton County, Oregon.

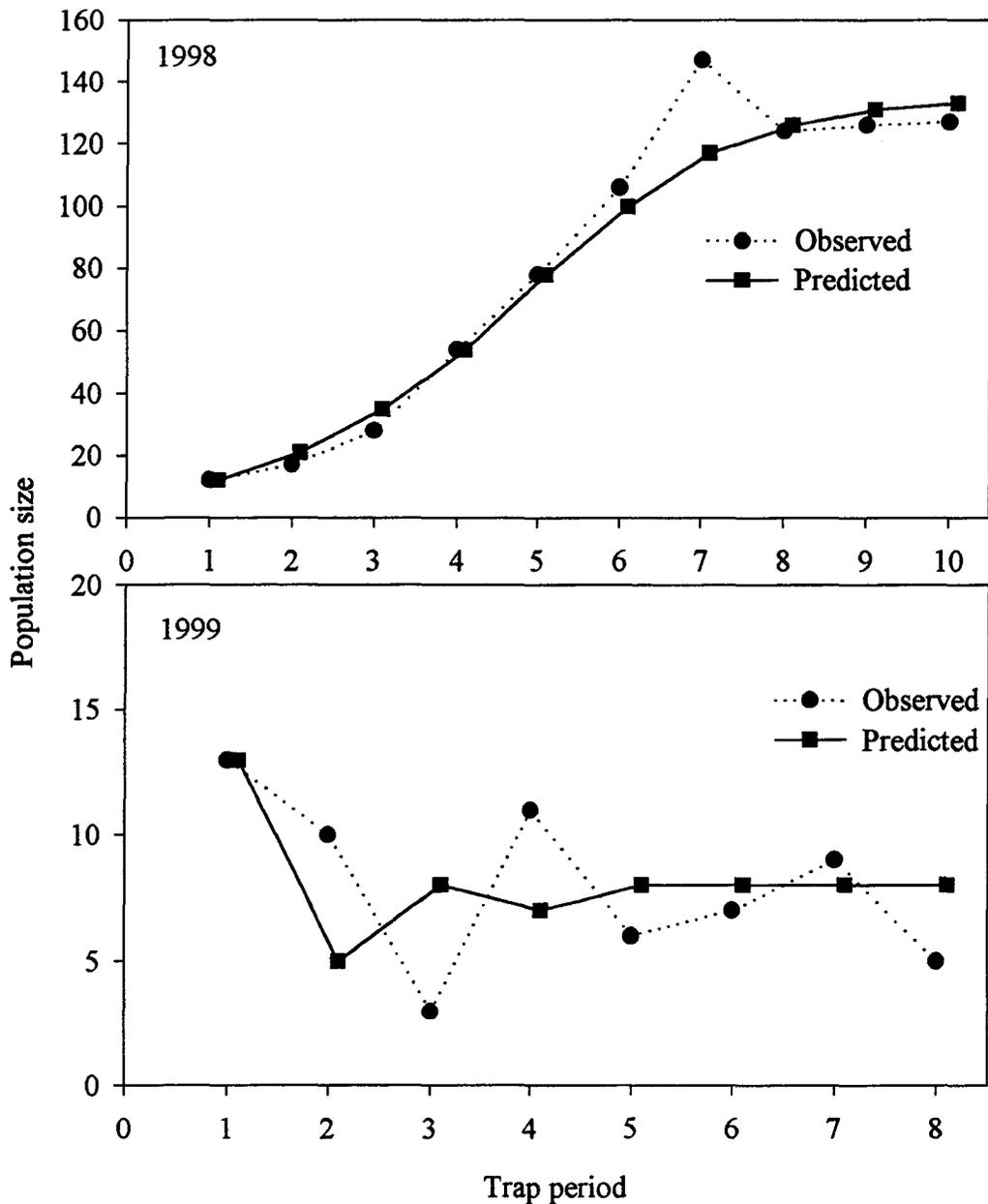


Figure 5.2. Predicted and observed population trajectories of gray-tailed voles at Hyslop Agronomy Farm, Benton County, Oregon in 1998 (top) and 1999 (bottom). Predicted trajectories were obtained from the Ricker model (see methods).

*Simulation of Pesticide Effects*

In 30 simulations of pesticide applications during the third trap period at small population sizes, 16 simulations (53 %) failed to detect differences in population sizes between controls and treatments ( $P > 0.05$ ). Significant differences were detected, but delayed one or two periods later than the period when the pesticide application was simulated in the other 16 simulations (Fig. 5.3). Similarly, in 30 simulations of applications simulated at the fourth period for medium population sizes, 13 simulations (43%) failed to detect significant differences in population sizes after application. Differences in population sizes were detected, but delayed one period after treatments in the other 17 simulations (Fig. 5.3). However, only two of 30 simulations (7%) did not detect differences when applications were simulated during the seventh trap period when populations were large. No delays in the differences occurred when applications were simulated at large population sizes (Fig. 5.3). Thus, the chance of failing to detect significant differences in population sizes between treatments and control were greater when pesticides were applied at lower population sizes.

Populations fluctuated throughout the experiment during 1999 (Fig. 5.2). Because population sizes did not substantially increase with time during 1999, we did not conduct multiple simulations for each pesticide application period. In the two simulations for 1999 experimental populations, no differences in population size were detected among treatments and controls for early or late pesticide applications.

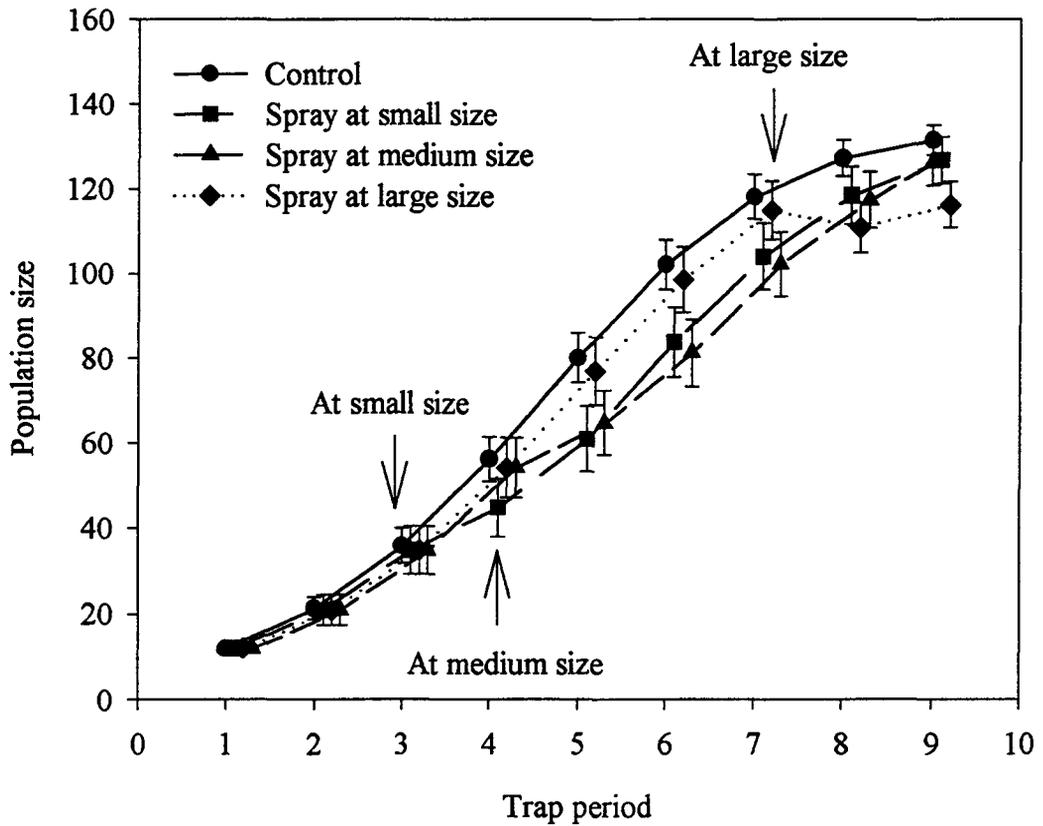


Figure 5.3. Mean population sizes of simulated control and treatment populations of gray-tailed voles. Pesticide applications were simulated for small, medium and large population sizes. Vertical lines are 95% confidence intervals.

## Discussion

We found that the responses of vole populations to the pesticide application were affected by demographic stochasticity and depended on the population size when the pesticide was applied (Fig. 5.3). Our simulations for single pesticide applications at 30 or 50 voles resulted in greater uncertainty in comparisons between control and treatment populations. About 50% of simulations for applications at smaller population sizes failed to detect significant differences even though vole mortality was 20% and 10% during the two periods after the treatment, respectively. Furthermore, significant differences that were detected were delayed one or two periods. However, when a single pesticide application was simulated at large population sizes (about 100 animals), significant differences between controls and treatments were detected immediately in 93% of the simulations. Our simulation models incorporated both demographic stochasticity and a density effect term. Demographic stochasticity was inversely related to population size; smaller populations had greater variation in population growth rates. On the other hand, at lower population sizes, population growth suffered less reduction from density effects. The mean population growth rate,  $r_0$ , of voles in 1999 when all populations were small was over twice that of voles in 1998, and the effect intensity of vole population size,  $b$ , in 1999 was greater than that of populations in 1998. Thus, density effects reducing the population growth rate,  $r_t$ , were less severe for small populations. Because small populations can grow at a rate closer to  $r_0$ , demographic stochasticity can be compensated to some extent by more rapid

growth (Burgman et al. 1992). When population sizes are over 100 animals, demographic stochasticity becomes ignorable (Lande and Orzack 1988). Additionally, population growth rates at large population sizes experience greater dampening from density effects. A mortality of 20% in large populations cannot result in increased population growth rates to the same degree as it does at smaller population sizes. The more rapid growth of small-size populations may compensate for the pesticide-induced mortality and delay differences between treatments and controls one or two periods. These results suggest that the response of vole populations to the pesticide application were density-dependent.

Our simulations suggested that the prediction of ecological risk of pesticides by the QM has greater uncertainty when demographic stochasticity and density effects are not considered in a nontarget population. The results of field trials may deviate from the QM's prediction. A 20% chemical-induced mortality is equivalent to a quotient of 0.4, which predicts an ecological risk for that species and chemical, no matter how large the exposed population is. However, 50% of our simulations for the pesticide application at small population sizes failed to detect treatment effects. In addition, our simulations were of short-term responses after the pesticide application, corresponding to our field study. In the long run, extinction probabilities and persistence time are mainly determined by initial population sizes and variances of population growth rate (Goodman 1987). Small populations with large variances in population growth rate are more likely to go extinct and would be more vulnerable to pesticide exposure than large populations.

Burgman et al. (1992) found that population persistence time decreased with increasing variance in the growth rates of the white-toothed shrew (*Crocidura russula*). McCarthy et al. (1994) found that the probabilities of extinction were inversely related to initial population sizes of the helmeted honeyeater (*Lichenostomus melanops cassidix*). Pesticide-induced mortalities can further reduce population sizes of small, isolated populations, and in turn increases the probabilities of extinction over the long term. The QM may not predict or may underestimate long-term ecological risk of a pesticide to small populations such as rare or endangered wildlife species unless demographic stochasticity of small populations is considered. Further studies using stochastic modeling and Monte Carlo simulation or population viability analysis (PVA) are needed to improve ecological risk assessments of pesticides to wildlife.

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## Chapter 6

### Summary

My dissertation involves validation of three assumptions of the Quotient Method (QM). The QM has been extensively used in ecological risk assessment of pesticides to wildlife by the United States Environmental Protection Agency. The QM is a semi-quantitative assessment, based on data from laboratory hazard tests of pesticides on surrogate species, and from a database of pesticide residues on crops. Recent regulatory revisions do not require field tests in the first and second tier screens of new pesticides. The revision raises concerns about untested assumptions of the QM, which may undermine the confidence in the use of the QM.

From 1997 to 1999, I conducted three separate but related field experiments to test three assumptions of the QM, regarding behaviors of animals to avoid exposure to chemicals, relationships between foraging behaviors of wildlife and risk from different formulations of a pesticide, and weather conditions shortly after a pesticide application. Last, I constructed a stochastic population model to simulate the effects of demographic uncertainty on the prediction of the QM. Herein, I summarize the main results of the four parts of my dissertation.

## Experiment 1

I used gray-tailed voles (*Microtus canicaudus*) as an experimental model species to test the QM assumption that nontarget wildlife do not move out of a contaminated area to avoid exposure to potentially harmful agricultural chemicals. In May 1997, I placed voles into 12, 0.2-ha enclosures planted with a mixture of pasture grasses. In late July, I applied 1.5 kg/ha of the insecticide Guthion<sup>®</sup> 2S (azinphos-methyl) in three treatments; full spray (all of the habitat sprayed with Guthion<sup>®</sup> 2S), half-spray (one-half of the habitat sprayed with Guthion<sup>®</sup> 2S and one half with water), and a control (all habitat sprayed with water). Five replicates were used for the half-spray and control, and two replicates for the full-spray. I radio-tracked 44 females and three males before and after the spray treatment. None of the 47 animals moved out of their established home ranges after treatment and no animals moved from the contaminated to uncontaminated areas. Additionally, no biologically meaningful differences occurred in home range size, mean maximum distance moved, or average distance between two successive radio locations. Reproducing adult voles were relatively sedentary and did not leave their established home ranges in response to insecticide exposure. These results suggest that small mammals are not likely to reduce exposure by moving from the contaminated area, supporting the QM assumption that exposure to small mammals is a function of the spray application. However, behavioral responses such as contamination avoidance may be specific to the chemical, species, and habitat.

## **Experiment 2**

I used gray-tailed voles and northern bobwhite quail (*Colinus virginianus*) as experimental model species to test whether birds and small mammals respond differently to equivalent concentrations of a pesticide applied in granular and flowable formulations. In mid-May 1998, I placed voles into 15, 0.2-ha enclosures planted with a mixture of pasture grasses. In mid-July, I placed quail into the same enclosures with the voles. In late July, I applied the organophosphorus insecticide diazinon in five treatments; a control (all habitats sprayed with water), liquid formulation of diazinon at 0.55 kg/ha, liquid formulation of diazinon at 1.11 kg/ha, broadcast of granular diazinon at 1.11 kg/ha, and broadcast of granular diazinon at 2.22 kg/ha. The diazinon treatment in liquid and granular formulations did not depress population size or growth rate, or survival of voles. I found a significant difference in the survival rate of quail between the controls and treatments; granular diazinon caused a measurable decline of quail survival, while the liquid application at an equivalent rate did not significantly affect quail survival. The results suggest that ground-feeding birds are more susceptible to granular insecticides than flowable applications, but voles were not susceptible to either formulation at the application rate used in this study.

## **Experiment 3**

I used gray-tailed voles as an experimental model species to field test the QM assumption that the expected environmental concentration is estimated

immediately after a pesticide application, by simulating a 0.25-cm rainfall with an irrigation system shortly after a pesticide application. In June 1999, I placed voles into 16, 0.2-ha enclosures planted with a mixture of pasture grasses. In early August, I applied 2.44 kg/ha of the insecticide Guthion<sup>®</sup> 2S (azinphos-methyl) in four treatments; a dry control, “rain” or wet control, dry treatment (sprayed with Guthion<sup>®</sup> 2S but no “rain”), and wet treatment (sprayed with Guthion<sup>®</sup> 2S and “rain” within 24 hours). I used four replicates for each treatment. Survival probabilities of male voles in dry control enclosures declined throughout the rest of study after the treatment, while survival probabilities in other treatments indicated a short-term increase. Rainfall improved male survival in the “rain” enclosures and may have mitigated the adverse effects of Guthion<sup>®</sup> 2S in the wet-treatment enclosures. I detected significant interactions between treatment and time on population sizes and population growth rates of voles ( $P < 0.05$ ) in repeated measures ANOVA. The results indicate that the Guthion<sup>®</sup> 2S treatment depressed population size or growth rate in the dry treatment. However, rainfall may have reduced the risk of voles to Guthion<sup>®</sup> 2S by improving habitats or washing away Guthion<sup>®</sup> 2S residues. My study suggests that the QM is robust to the assumption that rainfall does not increase exposure of voles to Guthion<sup>®</sup> 2S in grasslands. However, the interaction between rainfall and Guthion<sup>®</sup> 2S application resulted in a deviation from the predicted risk.

## **Model Simulation**

I built a Ricker's model with a demographic stochasticity term to simulate the effects of demographic uncertainty on responses of gray-tailed vole populations to pesticide applications. Population models of gray-tailed voles were constructed with data from the mark-recapture studies in 1998 and 1999. I simulated a single pesticide application 30 times each for small, medium, and large population sizes for 1998 populations. Significantly less uncertainty in treatment effects was exhibited at large population sizes. However, 50% of simulations for small or medium population sizes failed to detect differences between control and treatments. Because of population fluctuations resulting from demographic stochasticity and smaller population sizes, no significant differences were detected in the simulations of 1999 vole populations. Population sizes may affect the recovery ability of vole population growth following pesticide-induced mortality. Vole population sizes declined significantly when applications occurred at large population sizes. Greater uncertainty existed in the results of simulations at small and medium population sizes. My results suggested that the QM did not differentiate the short-term risk of a chemical to small and large populations. My results also suggested that the QM could not predict or may underestimate the long-term extinction risk of rare or endangered wildlife species from contamination by pesticides.

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