AN ABSTRACT OF THE THESIS OF

Eric M. Schauber for the degree of Master of Science in Wildlife Science, presented on September 28, 1994.

Title: Influence of Vegetation Structure and Food Habits on Effects of Guthion 2S® (Azinphos-methyl) on Small Mammals

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W. Daniel Edge

The Quotient Method (QM), a laboratory-based risk assessment methodology used by the Environmental Protection Agency to evaluate pesticides for registration and use, has not been thoroughly field-tested and its performance has not always been reliable. My objective was to determine if variation in vegetation structure or diet of exposed animals could result in adverse ecological effects that were not predicted by the QM. In April and early May 1993, I established populations of herbivorous gray-tailed voles (*Microtus canicaudus*) and omnivorous deer mice (*Peromyscus maniculatus*) in 24 0.2-ha enclosures planted with alfalfa (*Medicago sativa*). Alfalfa in 12 enclosures was mowed on 22 June to reduce vegetation height. Small mammal populations were monitored by live trapping from May through August 1993. On 14 July, an organophosphorus insecticide, azinphos-methyl, was applied at 0, 0.88, and 3.61 kg/ha. Insecticide residues were measured on canopy-level spray cards, soil samples, and alfalfa. I compared the observed residue concentrations with predictions based on the nomogram used to estimate exposure for QM risk assessments. I also compared QM predictions of risk with observed effects on population size and growth, survival, reproductive activity, recruitment, body growth, movements, and diet of the small mammals.

Much of the insecticide reached ground level in mowed enclosures, but dense alfalfa intercepted most of the spray in unmowed enclosures. The mean half-life of azinphos-methyl on alfalfa was 3.4 days and was not affected by mowing. Mean

residue concentrations on mowed alfalfa and the top 15 cm of unmowed alfalfa were underestimated by the QM exposure nomogram. Therefore, pesticides may pose greater risk to organisms inhabiting sparse vegetation or the tops of plants than predicted by the QM.

Treatment with azinphos-methyl at 3.61 kg/ha caused severe effects in both mowed and unmowed enclosures on population size and growth, survival, recruitment, and body growth of voles. Effects of azinphos-methyl on vole recruitment and body growth and on survival of female voles were greater in mowed than in unmowed enclosures. However, I did not find that population-level responses of voles to the chemical differed between mowing treatments. Most effects on voles were of short duration (<27 days) but vole densities in 3.61 kg/ha enclosures remained depressed ≥6 weeks after spraying. The 3.61 kg/ha application rate resulted in a 42% decrease in deer mouse densities in mowed enclosures during the week of spraying, but the insecticide had no adverse effects on deer mice in unmowed enclosures. In addition, the insecticide may have reduced recruitment of deer mice in mowed enclosures. Analysis of deer mouse feces indicated that consumption of arthropods increased in insecticide-treated enclosures just after spraying occurred. Survival, reproductive activity, body growth, and movements of deer mice were highly variable and not significantly affected by azinphos-methyl.

Mowing resulted in greater residue concentrations than predicted and, consequently, the insecticide adversely affected voles and deer mice in mowed enclosures at application rates characterized as low risk by the QM. However, food aversion or selective feeding on alfalfa tops may have resulted in similar exposure of voles to the 3.61 kg/ha treatment in mowed and unmowed enclosures. I did not find that insectivorous feeding behavior of deer mice made them more susceptible than predicted. Although residue concentrations on alfalfa did not follow predictions, the gross pattern of effects on small mammals was consistent with QM risk characterization. However, the QM may underestimate exposure and risk when pesticides are sprayed on sparse vegetation.

Influence of Vegetation Structure and Food Habits on Effects of Guthion 2S® (Azinphos-methyl) on Small Mammals

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TABLE OF CONTENTS

LIST OF FIGURES

INFLUENCE OF VEGETATION STRUCTURE AND FOOD HABITS ON EFFECTS OF GUTHION 2S® (AZINPHOS-METHYL) ON SMALL MAMMALS

INTRODUCTION

Variation among organisms in their susceptibility to pesticides may hinder assessment of ecological risks posed by these chemicals. Characteristics intrinsic to the organisms or heterogeneity in biotic and abiotic features of the environment may cause such variation. Before the U.S. Environmental Protection Agency (EPA) registers a pesticide for use, it is required under the Federal Insecticide, Fungicide, and Rodenticide Act of 1970 to evaluate the likelihood that applying the pesticide will cause unreasonable ecological damage. The EPA uses a Quotient Method (QM) to conduct preregistration ecological risk assessments (Urban and Cook 1986). The quotient of risk for a particular chemical and species of test organism is calculated by dividing the estimated exposure by the estimated hazard. For the QM, exposure is generally measured by the expected environmental concentration (EEC), which, for terrestrial animals, represents the estimated concentration in or on primary foods. EEC is estimated by a nomogram derived from a database of residues measured on crops (Urban and Cook 1986). Estimates calculated by this nomogram represent maximum expected residue concentrations (Hoerger and Kenaga 1972). Hazard in the QM denotes the chemical's potential to cause adverse effects. Although hazard may be estimated by a variety of ecotoxicological endpoints (e.g., reproduction, mutagenesis, growth, bioconcentration potential) on any target organism, LD_{50} or LC_{50} tests on standard laboratory animals are usually used. A risk quotient >1 means that expected exposure exceeds the estimated hazard and is interpreted to indicate unacceptable risk. Quotients considerably <1 indicate comparatively low risk. To account for error and differences among species, the EPA presumes risk at a quotient of 0.2 (EEC = 1/5 the LC_{50}; National Research Council 1983).

The QM allows a rapid semiquantitative measure of the potential risks of applying a particular chemical. However, assumptions underlying the QM have been

challenged after chemicals, for which quotient values indicated "low risk," were implicated in causing wildlife die-offs (Grue et al. 1983, Blus et al. 1989). Estimation of EEC incorporates the assumption that residue concentrations increase proportionately with application rate (i.e., doubling the application rate should double residue concentrations) and independently of habitat characteristics. Estimation of hazard by LD_{50} or LC_{50} tests on laboratory animals requires the assumptions that animals will not select or avoid contaminated foods and that indirect effects are negligible. These assumptions are largely untested. Bennett et al. (in press) found that the vegetation nomogram underestimated azinphos-methyl residues on alfalfa. Edge et al. (in press) observed responses of enclosed gray-tailed vole (*Microtus canicaudus*) populations to azinphos-methyl that corresponded with characterizations of risk by the QM. However, Bennett et al. (unpublished data) observed mortality of bobwhite quail (*Colinus virginianus*) chicks after exposure to azinphos-methyl at application rates characterized by the QM as posing low risk to this species. Carey (1993) and Bennett et al. (in press) suggested that interception of insecticide sprays by dense vegetation may reduce exposure of animals at ground level. Acute toxicity of insecticides differs among test species (Cholakis et al. 1981, Fleming and Grue 1981) but food habits may affect susceptibility independently of toxicity. Animals that prey on arthropods may receive greater doses of insecticides than herbivorous species by selectively feeding on intoxicated arthropods after spray (Morris 1970, Robel et al. 1972, Stehn et al. 1976). Insecticides may also affect insectivorous species indirectly by reducing the abundance of arthropod prey (Barrett and Darnell 1967). Such differential effects among species may alter the structure of ecological communities (Barrett and Darnell 1967, Morris 1970).

My objective was to test the assumptions (1) that the vegetation nomogram accurately predicts environmental concentrations of insecticides after application and (2) that responses of wild animals to insecticide exposure are unaffected by vegetation structure and the diets of the receptor species. I compared EEC with observed residue concentrations after applying azinphos-methyl to alfalfa. In addition, I compared QM risk characterizations with observed responses of populations (density, growth rate, and

recruitment) and individuals (survival, body growth, reproductive activity, and movements) of herbivorous gray-tailed voles (hereafter referred to as voles), and omnivorous deer mice (*Peromyscus maniculatus*) to application of the organophosphorus (OP) insecticide azinphos-methyl (O,O-dimethyl S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl] phosphorodithioate; trade name Guthion 2S®; Mobay Corporation, Agricultural Chemical Division, Kansas City, Missouri, USA) to mowed and unmowed alfalfa in 24 0.2-ha enclosures.

INFLUENCE OF VEGETATION STRUCTURE ON THE DISTRIBUTION AND PERSISTENCE OF AZINPHOS-METHYL RESIDUES ON ALFALFA

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ABSTRACT

We field-tested the vegetation nomogram used by the Environmental Protection Agency to estimate maximum expected concentrations of pesticide residues on plants when conducting preregistration risk assessments. This nomogram is based on a relatively small database of residue studies and does not account for vegetation structure. Our objectives were to test whether residue concentrations were conservatively predicted by the nomogram and to determine the influence of vegetation structure on residue distribution and degradation. We applied 0.0, 0.88, and 3.61 kg/ha of azinphos-methyl to 24 0.2-ha enclosures planted with alfalfa (*Medicago sativa*), 12 of which were mowed 3 weeks earlier to reduce vegetation height. Residue concentrations were measured on canopy-level spray cards, soil samples, and alfalfa on days 0, 2, 6, 14, and 28 after spraying. Deposition of azinphos-methyl on spray cards was generally lower than the application rate measured from spray tank samples. Residue concentrations increased proportionately with application rate. However, mean concentrations on mowed alfalfa and unmowed alfalfa tops were underestimated by the nomogram. Residue concentrations on the top 15 cm of unmowed alfalfa were more than four times greater than those on the bottom 15 cm, indicating that much of the insecticide was intercepted in the canopy of unmowed alfalfa. Residue concentrations on mowed alfalfa tended to be higher than on unmowed alfalfa tops. The half-life of azinphos-methyl ranged from 1.7 to 5.1 days. Mowing did not apparently affect residue persistence. Our results indicate that the vegetation nomogram may underestimate pesticide residue concentrations on alfalfa and that variation in vegetation structure may substantially influence exposure of herbivores to pesticides.

INTRODUCTION

Exposure of target and nontarget organisms to pesticides may be influenced by the structure of the vegetation they inhabit, hindering laboratory-based ecological risk assessment. The U.S. Environmental Protection Agency (EPA) uses a Quotient Method

(QM) to conduct preregistration ecological risk assessments (Urban and Cook 1986). In the QM, exposure of nontarget organisms is generally measured by the expected environmental concentration (EEC), which, for terrestrial animals, represents the estimated maximum concentration in or on primary foods. EEC is estimated by a nomogram derived from a database of residues measured on crops (Urban and Cook 1986, based on Hoerger and Kenaga 1972), and risk is characterized by the quotient EEC/dietary toxicity (Urban and Cook 1986).

The QM allows a rapid semi-quantitative measure of the potential risks of applying a particular chemical. However, assumptions underlying the vegetation nomogram used to estimate EEC may limit the reliability of this method of risk assessment. To facilitate extrapolation from the relatively small database of residue measurements, the nomogram incorporates the assumptions that residue concentrations increase proportionately with application rate and independently of vegetation structure. However, distribution of pesticide residues can be affected by the height and density of the vegetation onto which the chemicals are applied (Ebeling 1963, Bennett et al., in press). In addition, vegetation structure may influence the action of environmental factors, such as solar radiation, temperature, humidity, wind, and precipitation, that determine rates of pesticide degradation from foliage (Willis and McDowell 1987).

Our objective was to test assumptions of the vegetation nomogram by comparing EEC with observed concentrations of azinphos-methyl on mowed and unmowed alfalfa (*Medicago sativa*). In particular, we tested for effects of the mowing treatment, which reduced the height of alfalfa, on the vertical distribution and persistence of residues. We hypothesized that the initial availability of azinphos-methyl to ground-level herbivores would be greater in mowed enclosures, but that greater exposure to sunlight and precipitation in mowed enclosures would result in lower residue persistence.

METHODS

Study Area

We conducted our experiment at Oregon State University's Hyslop Agronomy Farm, approximately 10 km north of Corvallis, Oregon. The site was surrounded by agricultural fields of various crops and had an elevation of approximately 70 m, level topography, and well-drained silty-clay loam soil. We used 24 0.2-ha (45 x 45 m) enclosures constructed of galvanized sheet metal extending approximately 1 m above ground. Enclosures were designed to contain populations of small mammals for pesticide toxicology experiments (Edge et al., in press), but also reduced drift of pesticide sprays among enclosures. Alfalfa was planted in the enclosures in spring 1991, and was mowed to a height of approximately 8 cm on 4-5 May 1993.

Experimental Design

We applied the organophosphorus insecticide azinphos-methyl at three application rates (0.0, 0.88, or 3.61 kg/ha) to mowed and unmowed enclosures. The maximum registered application rate for azinphos-methyl on alfalfa is 0.84 kg/ha, and it may be applied up to four times per year. Four replicate enclosures were randomly assigned to each combination of mowing and azinphos-methyl treatments in a 3 x 2 factorial design.

Mowing

Alfalfa in 12 randomly-chosen enclosures was mowed on 22 June, 22 days before insecticide application. Alfalfa in the other 12 enclosures was left unmowed. A Robel pole was used to measure vegetation height in unmowed and mowed enclosures on 13 July. Measurements made by this method correlate strongly with the total

biomass of aboveground vegetation (Robel et al. 1970). The lowest visible point on a white pole was determined by an observer 4 m away at an eye level of 1 m. Measurements from the four cardinal directions were recorded for each of 10 randomly selected sites in each enclosure.

Insecticide Application

Azinphos-methyl was applied on 14 July (day 0) by a licensed applicator, using a four-wheel all-terrain vehicle and trailer tank with 7.6-m spray booms. Four mowed and four unmowed enclosures were sprayed with each of the 0.0, 0.88, and 3.61 kg/ha application rates; control (0.0 kg/ha) enclosures were sprayed with an equal volume of water. Enclosures were sprayed in order of increasing application rate. Spray booms were adjusted to apply the chemical from approximately 45 cm above the vegetation in both mowed and unmowed enclosures. Two samples were taken from the spray tank before spraying the first enclosure and two more were taken after spraying the last of the eight enclosures for each application rate. Tank samples were stored on ice in opaque glass containers and analyzed that day. For each application rate, we report the mean tank sample concentration as the actual application rate.

Approximately 0.08 cm of rain fell on day 0, beginning within 1 hour after azinphos-methyl was applied. Rain fell again during six of the first nine days after spraying, totalling 1.6 cm (Fig. 1).

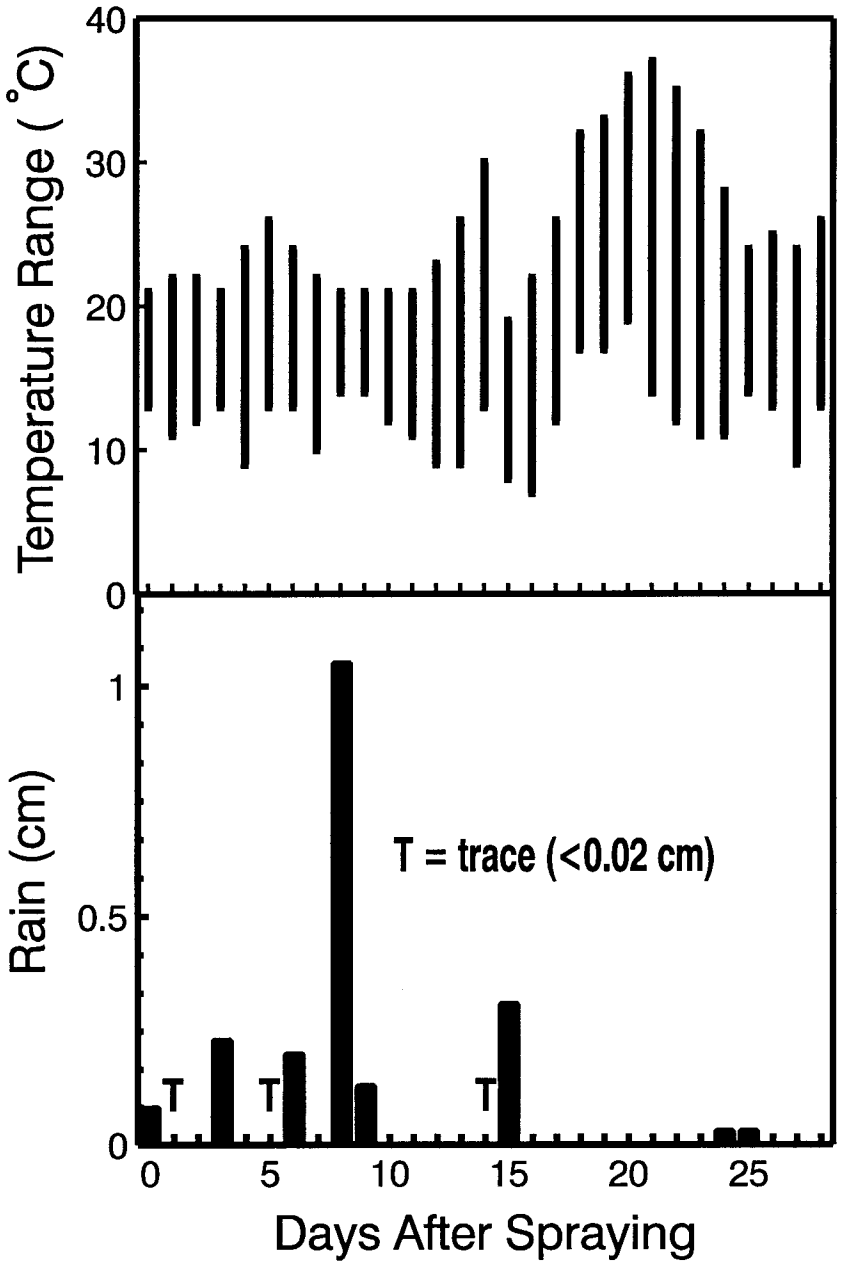

Figure 1. Daily temperature range and rainfall after application of azinphos-methyl at Hyslop Agronomy Farm, Benton County, Oregon, 14 July 1993.

Residue Sampling

We sampled residues on canopy-level spray cards, alfalfa, and soil on days 0, 2, 6, 14, and 28 after spraying to estimate the availability of azinphos-methyl to herbivores and to determine its persistence over time. Samples were collected, following the methods used by Bennett et al. (in press), at 10 random points in each enclosure on day 0 and at five of the 10 points thereafter. Entire alfalfa stems were collected from mowed enclosures and the top 15 cm and bottom 15 cm of stems were sampled in unmowed enclosures. Residue and spray tank samples were analyzed without clean-up using high-resolution gas chromatography.

Data Analysis

We used Statistical Analysis System (SAS Version 6.05; SAS Institute, Inc. 1990) for all data analyses. We analyzed the natural logarithm of residue values to normalize distributions and stabilize variances. Multivariate repeated-measures analysis of variance (RMANOVA) was used to test (1) whether residue concentrations for each sample type and mowing treatment differed among application rates and sampling dates, and (2) whether concentrations on each sample type and sampling data differed among mowing and application rate treatments. RMANOVA included Wilk's Lambda tests for day x application rate interactions (Rao 1973), which we used to determine whether decay curves were parallel among application rates, and polynomial contrasts, from which we inferred the shape of degradation curves (linear, quadratic, or cubic). Because day x application rate interactions were significant for both alfalfa sample types in both mowing treatments, we used least-squares regression to model separate decay curves for each application rate, sample type, and mowing treatment. We used these regression models to determine residue half-lives by setting the residue value to the natural log of 1/2 the back-transformed y-intercept residue level and solving for the number of days. Rain filled many soil sample petri dishes after day 2, possibly removing insecticide or soil. Therefore, we considered those data to be

unreliable and did not model decay curves for residues on soil samples. For each mowing treatment, we used least-squares regression to test whether untransformed residue concentrations on samples collected <1 hour after application increased with application rate at the slope predicted by the vegetation nomogram. Because variance increased with application rate for untransformed residue data, values for each application rate, mowing treatment, and sample type were weighted by the reciprocal of their variance.

RESULTS

Vegetation Structure

Mowing reduced the height of the alfalfa canopy from >1 m to approximately 8 cm. Mowed alfalfa rapidly regrew to approximately 30 cm in height by 13 July, when vegetation height was measured. We recorded 465 Robel pole measurements for mowed enclosures and 459 for unmowed enclosures. Despite the rapid regrowth of alfalfa, height of visual obstruction in mowed enclosures ($\bar{x}$ = 20 cm, SE = 0.7) was less than one-half that measured in unmowed enclosures ($\bar{x}$ = 60 cm, SE = 1.1; t = 27, $d.f.$ = 22, 1-sided $P < 0.0001$).

Insecticide Residues

We collected 1,962 samples between days 0 and 28 after spraying. On day 0, 240 spray cards, 240 soil samples, 120 mowed alfalfa stems, 120 unmowed alfalfa bottoms, and 120 unmowed alfalfa tops were collected from the 24 enclosures. On each sampling date thereafter, we collected 60 each of mowed alfalfa stems, unmowed bottoms, and unmowed tops. Despite efforts to keep sampling sites away from the path of the spraying apparatus, several soil samples were crushed by the spray vehicle or personnel. Therefore, 118, 96, 118, and 98 soil samples were collected on days 2, 6, 14, and 28, respectively. Azinphos-methyl was not detected in spray tank contents applied

to control (0.0 kg/ha) enclosures or on spray cards, soil samples, or alfalfa in control enclosures; thus, no detectable insecticide drifted among enclosures.

Residues on Day 0: Vertical and Horizontal Distribution

Residue concentrations on soil samples and unmowed alfalfa bottoms varied widely within each enclosure, resulting in mean coefficients of variation of natural log-transformed residue concentrations ranging from 21% to 766% (Table 1). Residues on alfalfa tops and unmowed alfalfa bottoms were less variable, with mean coefficients of variation <10%. In unmowed enclosures, azinphos-methyl concentrations on unobstructed materials (spray cards and alfalfa tops) were more than four times greater than those on soil and alfalfa bottom samples (Fig. 2), indicating that the dense alfalfa intercepted much of the pesticide spray before it reached ground level. However, initial deposition of azinphos-methyl was much greater in mowed than unmowed enclosures on alfalfa bottoms and soil (both $\underline{F} \geq 48$; $\underline{d.\ f.} = 1, 156$; $\underline{P} < 0.0001$) for both application rates. Mean concentrations on mowed alfalfa tended to be slightly greater than those on unmowed alfalfa tops (Fig. 2b).

Residues on Day 0: Relationships with Application Rate

The concentration of azinphos-methyl residues increased linearly with application rate for all sample types and mowing treatments except for spray cards and soil samples in mowed enclosures (Fig. 2a). Polynomial regression equations improved model fit over that of simple linear equations for residues on spray cards and soil samples in unmowed enclosures (both $\underline{t} \geq 2.2$, $\underline{d.\ f.} = 9$, $\underline{P} \leq 0.052$):

$$\text{spray card residue} = 0.0540 + 8.27(\underline{\text{rate}}) - 0.345(\underline{\text{rate}}^2);\ \underline{R}^2 > 0.99$$

$$\text{soil residue} = 0.0786 + 1.95(\underline{\text{rate}}) - 0.441(\underline{\text{rate}}^2);\ \underline{R}^2 = 0.60$$

The coefficient of the $\underline{\text{rate}}^2$ term was negative in both cases, indicating decreasing slope as application rate increased.

Application of 1 kg/ha should deposit 10 $\mu g/cm^2$ on an unobstructed flat surface. However, the slope of the linear relationship between azinphos-methyl residues on spray cards and application rate was lower than the expected slope of 10 for the mowed treatment ($t \geq 5.9$, d. f. = 10, 2-sided $P \leq 0.0001$) and 95% confidence intervals for mean residues did not include predicted values for the unmowed treatment (Fig. 2a). Thus, the amount of active ingredient deposited was less than the application rate determined by the concentration of the spray mixture. For both mowing treatments, the slopes of the relationships between day 0 residues on soil samples and application rate were much lower than expected for an unobstructed surface (Fig. 2a).

Residue concentrations on unobstructed alfalfa (mowed alfalfa and unmowed alfalfa tops; Fig. 2b) were underestimated by the vegetation nomogram (Urban and Cook 1986). The nomogram predicts that azinphos-methyl residues (Y) on alfalfa, expressed in ppm, should increase with application rate (X), expressed in kg/ha, as $Y = 52X$. The slope ($\pm$ SE) of this relationship was 60 ± 3.2 ($R^2 = 0.97$, $n = 12$) for unmowed alfalfa tops and 70 ± 3.2 ($R^2 = 0.98$, $n = 12$) for mowed alfalfa, both of which exceeded the expected slope of 52 (both $t \geq 2.3$, d. f. = 10, 2-sided $P \leq 0.041$). Conversely, concentrations on unmowed alfalfa bottoms (slope = 14 ± 1.9; $R^2 = 0.85$) were greatly overestimated by the nomogram ($t = 20$, d. f. = 10, 1-sided $P < 0.0001$; Fig. 2b). Mean residue concentrations on mowed alfalfa, unmowed tops, and unmowed bottoms in enclosures sprayed with 3.61 kg/ha were, respectively, 3.5, 4.2, and 3.6 times those in 0.88 kg/ha enclosures.

Table 1. Mean coefficient of variation (%) of natural-log-transformed azinphos-methyl concentrations on spray cards, soil, and alfalfa samples collected in each of 12 mowed and 12 unmowed enclosures within 1 hour after application of 0.88 and 3.61 kg/ha (n = 40 per treatment) at Hyslop Agronomy Farm, Benton County, Oregon, 14 July 1993.

	Application rate (kg/ha)			
	0.88		3.61	
	Unmowed	Mowed	Unmowed	Mowed
Spray cards	24%	18	10	12
Soil samples	419	766	312	39
Alfalfa	tops: 10 bottoms: 31	9	tops: 7 bottoms: 21	7

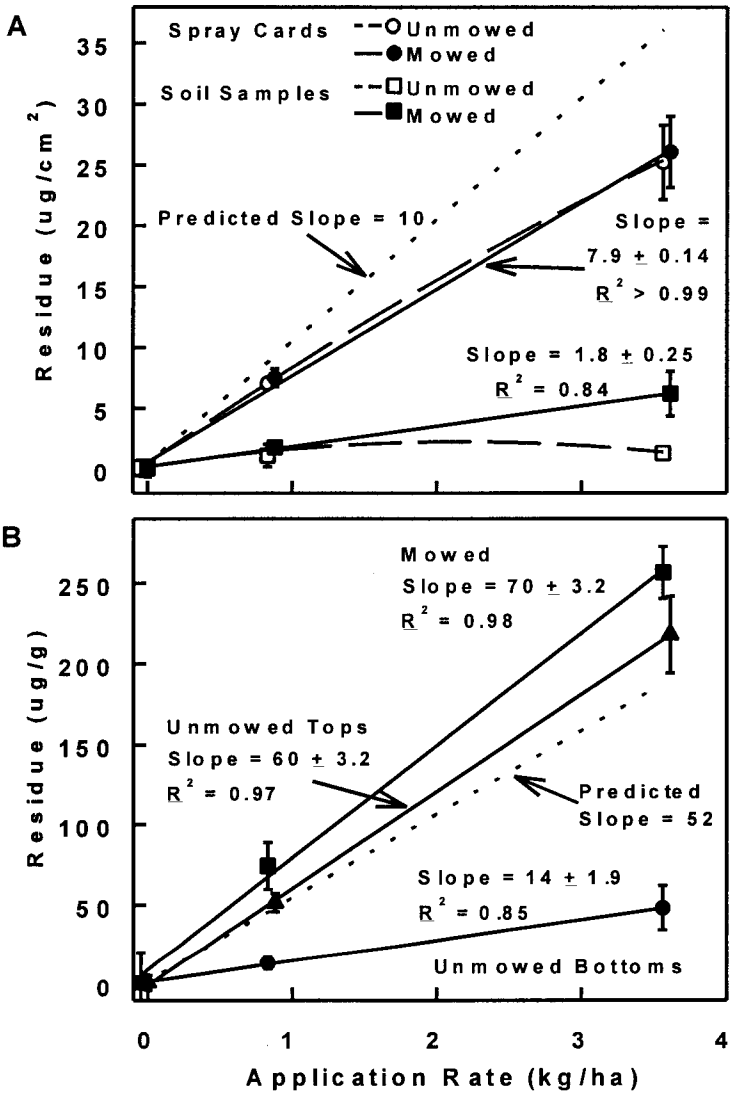

Figure 2. Residue concentrations (means and 95% confidence intervals) on (a) canopy-level spray cards and soil samples and (b) alfalfa samples collected <1 hour after application of azinphos-methyl at 0.0, 0.88, and 3.61 kg/ha on mowed and unmowed alfalfa at Hyslop Agronomy Farm, Benton County, Oregon, 14 July 1993.

Residue Degradation

Degradation curves for azinphos-methyl residues on alfalfa were best fit by cubic regression equations for all sample types, application rates, and mowing treatments (Fig. 3). Half-life estimates for residues on alfalfa ranged from 1.7 to 5.1 days and did not differ systematically among mowing or application rate treatments. Concentrations on mowed alfalfa remained higher than those on unmowed bottoms (all $\underline{F} \geq 9.7$; $\underline{d}.\ \underline{f}. = 1,76$; $\underline{P} \leq 0.003$) through day 28 postspray (Fig. 3b). Similarly, concentrations in enclosures sprayed with 3.61 kg/ha remained higher than 0.88 kg/ha (all $\underline{F} \geq 5.8$; $\underline{d}.\ \underline{f}. = 1,76$; $\underline{P} \leq 0.02$). Mean concentrations on alfalfa tops (Fig. 3a) differed between application rates at all sampling times (all $\underline{F} \geq 75$; $\underline{d}.\ \underline{f}. = 1,38$; $\underline{P} < 0.0001$) except day 28 postspray ($\underline{F} = 2.1$; $\underline{d}.\ \underline{f}. = 1, 38$; $\underline{P} = 0.15$).

DISCUSSION

Estimation of EEC by the vegetation nomogram (Urban and Cook 1986) used by the EPA in pesticide risk assessment requires the assumption that residue concentrations increase proportionately with application rate. Our results support this assumption. The 3.61 kg/ha rate was 4.1 times greater than 0.88 kg/ha, and ratios of mean residue concentrations for the two application rates ranged from 3.5 to 4.2. Although residues increased proportionately with application rate, EEC values calculated from the nomogram were lower than observed residue concentrations. Ideally, the vegetation nomogram predicts maximum expected residue concentrations on plant parts (Hoerger and Kenaga 1972). However, we measured mean azinphos-methyl concentrations on alfalfa tops and mowed alfalfa bottoms that were as much as 37% greater than nomogram predictions, even though spray card concentrations were below nominal application rates. Others have also reported mean residue levels on alfalfa exceeding nomogram predictions (Bennett et al., in press, and

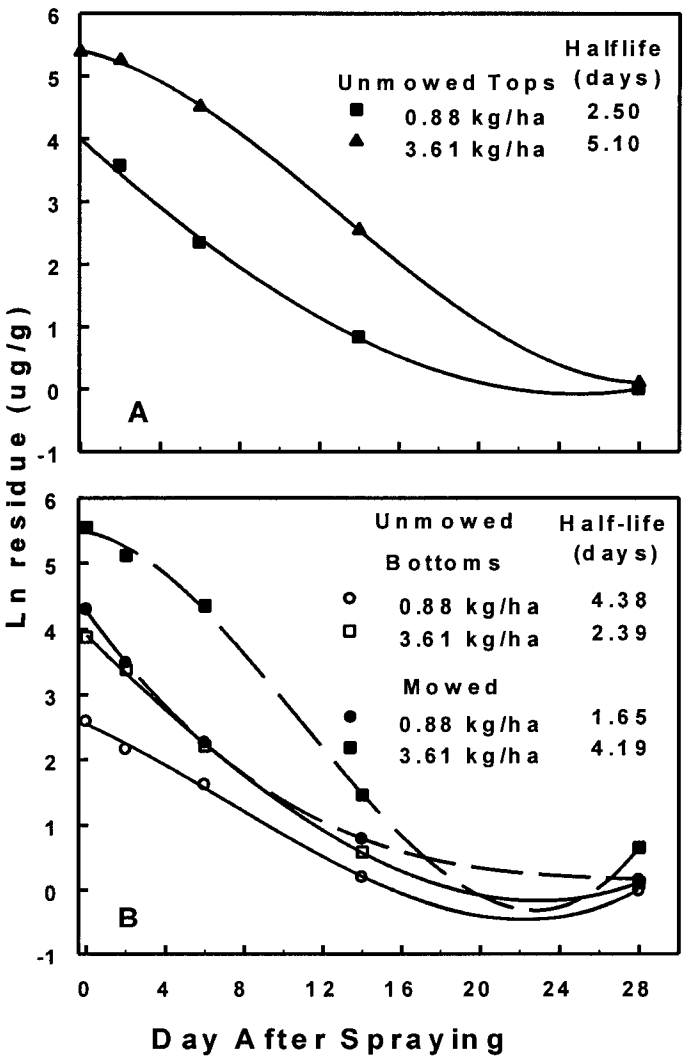

Figure 3. Temporal changes in mean concentration of azinphos-methyl on (a) top 15 cm of unmowed alfalfa and (b) mowed and bottom 15 cm of unmowed alfalfa after application of 0.88 and 3.61 kg/ha on 14 July 1993 at Hyslop Agronomy Farm, Benton County, Oregon.

references therein). Therefore, our results support modification of the vegetation nomogram used in risk assessments to estimate exposure through consumption of alfalfa. However, these results do not necessarily indicate that the nomogram underestimates residues on other crops.

Bennett et al. (in press) observed that much greater azinphos-methyl residues were deposited on alfalfa at canopy level than at ground level, and suggested that exposure of herbivores may be affected by vegetation structure. We found that deposition of azinphos-methyl at ground level was greatly affected by vegetation structure. In unmowed enclosures, where alfalfa height exceeded 1 m, mean residue concentrations on the bottom 15 cm of alfalfa were $\leq$25% of those in the canopy. Mowing reduced vegetation height, resulting in pesticide residue concentrations on alfalfa bottoms that equalled or exceeded values for unmowed alfalfa tops. Thus, dietary, dermal, and inhalation exposure of organisms at ground level may depend greatly on the structure of vegetation they inhabit. However, exposure of organisms feeding primarily in upper vegetation strata may be largely independent of vegetation structure. Vegetation structure is not accounted for in EPA risk assessments (National Research Council 1983), and therefore represents a potential source of significant variation in performance of such risk assessments.

Persistence of pesticide residues is affected by a variety of environmental conditions, including temperature, solar radiation, wind, humidity, and precipitation (Willis and McDowell 1987). We hypothesized that reduction of vegetation height by mowing would increase the penetration of sunlight and precipitation, resulting in more rapid degradation of azinphos-methyl in mowed enclosures. However, azinphos-methyl did not degrade more rapidly from mowed alfalfa than from unmowed alfalfa. Similarly, precipitation did not seem to affect degradation of residues. Although rain fell intermittently for several days after we applied the chemical, our estimates of residue half-life (1.7-5.1 days) were comparable to those reported by Bennett et al. (2.5-4.5 days; in press) for azinphos-methyl applied on alfalfa in 1992, when no rain fell for 7 days after spraying.

Relaible characterization of ecological risk requires accurate estimates of bioavailability and exposure. Our findings indicate that the vegetation nomogram (Urban and Cook 1986) used by the EPA in risk assessments can underestimate residues on alfalfa. Also, factors that are not accounted for by the nomogram, such as vegetation structure, precipitation, and feeding behavior, can strongly influence the exposure of target and nontarget organisms to pesticides. Incorporation of these factors, along with the best available residue data, into the estimation of exposure may be necessary for complete and accurate assessment of pesticide risks.

INSECTICIDE EFFECTS ON SMALL MAMMALS: INFLUENCE OF VEGETATION STRUCTURE AND DIET

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ABSTRACT

The Quotient Method (QM), a laboratory-based risk assessment methodology used by the Environmental Protection Agency to evaluate pesticides for registration and use, has not been thoroughly field-tested and its performance has not always been reliable. Our objective was to test whether differences in the diets of nontarget organisms or in vegetation structure could result in adverse ecological effects not predicted by the QM. In April and early May 1993, we established populations of herbivorous gray-tailed voles (*Microtus canicaudus*) and omnivorous deer mice (*Peromyscus maniculatus*) in 24 0.2-ha enclosures planted with alfalfa (*Medicago sativa*). We monitored small mammal populations by live trapping from May to August 1993. Alfalfa in twelve enclosures was mowed on 22 June to reduce vegetation height. On 14 July, we applied the organophosphorus insecticide azinphos-methyl at 0, 0.88, and 3.61 kg/ha. We compared predictions of risk with observed effects on population density and growth, survival, reproductive activity, recruitment, body growth, movements, and diets of the small mammals. The QM predicted that the 3.61 kg/ha rate posed high risk to gray-tailed voles but low risk to deer mice, and that 0.88 kg/ha posed low risk to both species.

Treatment with azinphos-methyl at 3.61 kg/ha reduced population density and growth, survival, recruitment, and body growth of voles in both mowed and unmowed enclosures. Survival of female voles exposed to 3.61 kg/ha was lower in mowed than in unmowed enclosures. The 0.88 kg/ha rate affected vole recruitment and body growth only in mowed enclosures. Most effects on voles were short-lived (<27 days) but vole densities in 3.61 kg/ha enclosures remained depressed >6 weeks after spraying. Azinphos-methyl did not cause detectable effects on reproductive activity of female voles, although the statistical power for detecting such effects was low ($1-\beta \leq .44$). We found no differences among application rates in movement distances of voles 2-5 days after spraying; thereafter, movements of males were greatest in 3.61 kg/ha enclosures. The 3.61 kg/ha application rate resulted in a 42% decrease in deer mouse densities in mowed enclosures within five days after spraying, but we could not detect any adverse

effects of the insecticide on deer mice in unmowed enclosures. Azinphos-methyl may have reduced deer mouse recruitment in mowed enclosures. Analysis of deer mouse feces indicated that consumption of arthropods just after spraying was greater in insecticide-treated enclosures than in controls. Reproductive activity, body growth, and movements of deer mice were highly variable and not significantly affected by azinphos-methyl.

Low vegetation height resulted in adverse insecticide effects on voles and deer mice at supposedly low-risk application rates. However, we did not find that insectivorous feeding behavior of deer mice made them more susceptible than predicted by the QM. Precipitation after spraying may have increased exposure of the mammals to azinphos-methyl, resulting in greater effects on voles than reported in a similar experiment. In general, the QM adequately predicted effects on the small mammals, but its performance may be affected by vegetation structure and precipitation.

INTRODUCTION

Both intrinsic and extrinsic factors may cause variation among organisms in their susceptibility to pesticides. Such variation may hinder assessment of the ecological risks these chemicals pose. Before the U. S. Environmental Protection Agency (EPA) registers a pesticide for use, it is required under the Federal Insecticide, Fungicide, and Rodenticide Act of 1970 to evaluate the likelihood that applying the pesticide will cause unreasonable ecological damage. The EPA uses a Quotient Method (QM) to conduct preregistration ecological risk assessments (Urban and Cook 1986). The quotient of risk for a particular chemical and species of test organism is calculated by dividing the estimated exposure by the estimated hazard. For the QM, exposure is generally measured by the expected environmental concentration (EEC), which, for terrestrial animals, represents the estimated concentration in or on primary foods. To calculate EEC, the EPA uses a nomogram derived from a database of residues measured on crops (Urban and Cook 1986, based on Hoerger and Kenaga 1972). Hazard in the QM denotes the chemical's potential to cause adverse effects. Although hazard may be

estimated by a variety of ecotoxicological endpoints (e.g., reproduction, mutagenesis, growth, bioconcentration potential) on any receptor organism, acute toxicity tests (LD_{50} or LC_{50}) on standard laboratory animals are usually used. A risk quotient >1 means that expected exposure exceeds the estimated hazard, which is interpreted to indicate high risk. Quotients considerably <1 indicate comparatively low risk. To account for error and differences among species, the EPA presumes risk at a quotient of 0.2 (EEC = 1/5 the LC_{50}; National Research Council 1983).

The QM allows a rapid semiquantitative measure of the potential risks of applying a particular chemical. However, assumptions underlying the QM have been challenged after chemicals approved for registration based on quotient values indicating "low risk" were implicated in causing wildlife die-offs (Grue et al. 1983, Blus et al. 1989). Estimation of EEC incorporates the assumption that pesticide residue concentrations increase proportionately with application rate (i.e., doubling the application rate should double residue concentrations). Estimation of hazard by LD_{50} or LC_{50} tests on laboratory animals requires the assumption that animals will not select or avoid contaminated foods and that indirect effects are negligible. These assumptions are largely untested. Carey (1993) and Bennett et al. (in press) suggested that interception of insecticide sprays by dense vegetation may reduce exposure of animals at ground level. Acute toxicity of insecticides differs among test species (Cholakis et al. 1981, Fleming and Grue 1981) but diet may affect susceptibility independently of toxicity. By selectively feeding on intoxicated arthropods after spray, insectivores may receive greater doses of insecticides than herbivores (Morris 1970, Robel et al. 1972, Stehn et al. 1976). Insecticides may also affect insectivores indirectly by reducing the abundance of arthropod prey (Barrett and Darnell 1967). Such differential effects among species may alter the structure of ecological communities (Barrett and Darnell 1967, Morris 1970).

Our objective was to determine if differences in vegetation structure, caused by mowing, or species-specific differences in diet alter the responses of populations (density, growth rate, and recruitment) and individuals (survival, body growth, reproductive activity, and movements) of gray-tailed voles (*Microtus canicaudus*) and

deer mice (*Peromyscus maniculatus*) to field application of the organophosphorus (OP) insecticide azinphos-methyl (O,O-dimethyl S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl) methyl] phosphorodithioate; trade name Guthion 2S®; Mobay Corporation, Agricultural Chemical Division, Kansas City, Missouri, USA). Small mammals are appropriate test animals because they are ubiquitous, common in agricultural areas, and vulnerable to contaminant exposure across soil, air, water, and vegetation media (Talmage and Walton 1991). Gray-tailed voles and deer mice are abundant in agricultural fields in the Willamette Valley, Oregon. Gray-tailed voles are primarily herbivorous (Verts and Carraway 1987, Edge et al., unpublished data), whereas deer mice feed on seeds, fruits, arthropods, and fungi (Wolff et al. 1985). Diets of deer mice may consist almost entirely of arthropods when arthropods are abundant (Jameson 1952). We used azinphos-methyl for this experiment because it has been implicated in wildlife kills (Grue et al. 1983, Durda et al. 1989) and because the QM classified it as posing low risk to mammals. Azinphos-methyl is more toxic to gray-tailed voles (LC_{50} = 297 ppm, LD_{50} = 32 mg/kg) than to deer mice (LC_{50} = 1,200 ppm, LD_{50} = 48 mg/kg; Meyers and Wolff 1994).

We hypothesized that azinphos-methyl would adversely affect population density and growth, survival, reproductive activity, recruitment, and body growth of small mammals, and that these effects would increase in magnitude with application rate. We hypothesized that movements of the mammals would either increase after spray, as individuals attempt to move to uncontaminated areas, or decrease because of pesticide-induced intoxication. We hypothesized that azinphos-methyl would have greater effects in enclosures in which vegetation density had been reduced by mowing. Finally, we hypothesized that the insectivorous deer mice in unmowed enclosures would be adversely affected at lower application rates than predicted by the QM.

METHODS

Experimental Design

We used a replicated, 3 x 2 factorial experiment to compare responses of small mammals to application of azinphos-methyl at 0.0, 0.88, and 3.61 kg/ha in mowed and unmowed field enclosures; 0.84 kg/ha is the label rate for azinphos-methyl for alfalfa. Four replicate enclosures were randomly assigned to each combination of mowing and azinphos-methyl treatments.

Study Area and Enclosures

We conducted our experiment at Oregon State University's Hyslop Agronomy Farm, approximately 10 km north of Corvallis, Oregon. The site was surrounded by agricultural fields of various crops and had an elevation of approximately 70 m, level topography, and well-drained, silty-clay loam soil. During this study, the experimental site received 33.9 cm of rain, 25.7 cm of which fell during April and May.

We used 24 0.2-ha (45 x 45 m) enclosures constructed of galvanized sheet metal extending approximately 1 m above ground and 0.6-1 m below. Corners and vertical supports were blocked with sheet-metal baffles to prevent escape by climbing rodents. Alfalfa (*Medicago sativa*) was planted in each enclosure in spring 1991. A variety of annual and perennial weeds were a minor component (<5% cover), although their prevalence varied among enclosures. The herbicides Paraquat® and Sencor® were applied to all enclosures in March 1993 to kill annual weeds. A 1-m strip along the inside of each fence was mowed frequently to minimize small mammal activity near the fence and to prevent contact with high concentrations of insecticide dripping down the fence after application. Alfalfa in all enclosures was mowed to a height of approximately 8 cm on 4-5 May 1993.

Study Animals

Populations of gray-tailed voles were established in the enclosures in April 1992 for a prior pesticide study (Edge et al., in press). Voles were trapped 6-8 April 1993 and redistributed among the enclosures to equalize populations at approximately 10 heterosexual pairs/enclosure and to minimize inbreeding. Each untagged vole was marked with a numbered aluminum ear tag before release. We released five pairs of wild-caught deer mice into each enclosure 8-14 May 1993. Deer mice were tagged when first captured in the enclosures. We provided each enclosure with five nest boxes, constructed from hollow concrete construction blocks (after King 1983), to minimize the potential for competitive exclusion of deer mice. Each nest box had two chambers with cotton batting as nest material and two entrance/exit holes too small for most adult gray-tailed voles.

Trapping

Each enclosure had 75 Sherman traps and 25 pitfall traps in a 10 x 10 array with 5 m between stations. Pitfall traps, 45 cm deep and 15 cm in diameter, were placed at all odd-number trap stations (e.g., 3-3, 5-7) and 8- x 9- x 23-cm Sherman live traps (model LFATG; H. B. Sherman Traps, Inc., Tallahassee, Florida) were placed at the remaining stations. Accumulation of rainwater prevented the use of most pitfall traps until early July. We trapped small mammals for four consecutive nights (four trap nights = one trap period) at two-week intervals from 18 May to 27 August 1993. Only one week separated the trap periods before and after we applied azinphos-methyl. Traps were set and baited with oats in the evening, then examined and closed the following morning.

Ear tag number, species, sex, reproductive condition, body mass, and trap station were recorded for each animal captured. We released each animal at its site of capture. We assumed that all newly tagged animals had been born in the enclosures and defined them as recruits. Body mass was measured to the nearest 1 g with Pesola®

spring scales. We weighed each animal at first capture each trap period. Animals captured on the first trap night of a trap period were reweighed if recaptured on the fourth trap night, providing us with a measure of mass change over three days. We defined voles with body mass ≥30 g and deer mice ≥18 g as adults. We considered females to be in reproductive condition if they were lactating or pregnant or, for voles, if they had widely open pubic symphyses. Because pregnancy in these species is obvious only within one week of parturition, we considered lactating females and those with wide pubic symphyses to have been pregnant during the previous trap period, if the animals were not also lactating at that time.

To reduce within-treatment variation in abundance and reduce interspecific competition for space, we kept vole densities at approximately 15 adults of each sex per enclosure by removing young and untagged voles each trap period until 30 June, two weeks before azinphos-methyl was applied. Enclosures with fewer than 15 voles of each sex were supplemented with voles removed from other enclosures. Between 30 June and 3 July, we supplemented 11 mowed enclosures with a total of 59 female and 46 male voles (2-10 females and 0-10 males/enclosure), which were removed from unmowed enclosures and grassy berms outside the enclosures. We added five pairs of deer mice to one enclosure, in which the population had gone extinct, on 12-13 June.

Mowing and Insecticide Application

On 22 June, alfalfa in 12 randomly chosen enclosures was mowed with a flail mower towed behind a tractor. Azinphos-methyl was applied on 14 July using a four-wheel all-terrain vehicle and trailer tank with 7.6-m spray booms. Four mowed and four unmowed enclosures were sprayed with each of the 0.0, 0.88, and 3.61 kg/ha application rates. Spray booms were adjusted to apply the chemical from approximately 45 cm above the vegetation in both mowed and unmowed enclosures. Spray tank contents were sampled before spraying the first enclosure and resampled after spraying the last of the eight enclosures for each application rate. We report actual application rates, determined by calculating the mean sample concentration for each

application rate. Approximately 0.08 cm of rain fell on 14 July, beginning within one hour after azinphos-methyl was applied. Rain fell again during six of the first nine days after spraying, totalling 1.6 cm.

Diet

We used microhistological examination of feces to quantify consumption of arthropods by deer mice. We collected feces from deer mice captured in nest boxes on the day before the beginning of each of five trap periods. For each sampling date, feces from each enclosure were combined in a glass vial containing 70% isopropyl alcohol. Subsamples from each vial were mounted on two slides and fifty fields of view on each slide were systematically examined through a 100X microscope. The presence or absence of invertebrate or plant tissue was recorded for each field. Plant and arthropod tissues were identified by referring to reference slides containing fecal material from animals that had been fed only plants or arthropods for several days. We did not identify plant or arthropod taxa. For the two slides from each enclosure and sampling date, we used the mean proportion of nonempty fields that contained arthropod material as an index to the prevalence of arthropods in the diets of deer mice. Because digestibility differs among food types, this method did not allow us to estimate the absolute proportion of the diet composed of arthropods. However, it provided a measure of the relative dietary prevalence of arthropods that we could examine for differences among treatments and sampling dates.

Population and Individual Measures

Values for population density and growth, survival rate, reproductive activity, recruitment, body growth, and movements were determined for each species, enclosure, and trap period of the study. We used program CAPTURE (Rexstad and Burnham 1992), under the closed population model incorporating heterogeneous capture probabilities among animals, to estimate densities of small mammal populations for

each trap period. We measured weekly growth rate for each population by dividing the change in ln(density + 1) between successive trap periods by the number of weeks between trap periods. We used the mean maximum distance moved (MMDM) calculated by program CAPTURE (White et al. 1978) as a relative index to the activity of small mammals; absolute estimates of activity were impossible to obtain using our methods. MMDM was calculated as the average, for each species and enclosure, of the maximum straight-line distances animals moved between capture locations within a trap period (Wilson and Anderson 1985).

We estimated sex-specific survival rates using derivations of the Cormack-Jolly-Seber mark-recapture methodology (Cormack 1964, Jolly 1965, Seber 1965). We used programs RELEASE (Burnham et al. 1987) and SURGE (Pradel and Lebreton 1991) for survival modelling. We adopted the modelling philosophy espoused by Burnham et al. (1987) and Lebreton et al. (1992), in which the goodness of fit of each model and the number of parameters required is evaluated. Good models are those that fit the data well, have small numbers of parameters, and reflect what is already known about the species. Specific hypotheses can be tested by comparing goodness of fit of competing models. We used the following approach: (1) populations within enclosures were each modelled separately with an emphasis on sex-specific differences; (2) because most replicates within a treatment had the same best-fit model, those data were combined (Burnham et al. 1987:250); and (3) treatment effects on survival were explicitly tested by comparing relative fit among models. The most parsimonious models were identified using Akaike's Information Criterion (Akaike 1973, Lebreton et al. 1992:83-85). Model notation follows that of Lebreton et al. (1992). This approach has been used and is further explained by Paradis et al. (1993) in estimating sex- and age-specific survival rates of the Mediterranean vole (*M. duodecimcostatus*) in Europe. After the initial analyses on gray-tailed voles, the data for the two sexes were modelled separately to reduce the complexity of the problem, and because all initial analyses revealed sex-specific survival and capture probabilities.

We measured recruitment by (1) the proportion of animals captured composed of recruits, and (2) the number of recruits captured in an enclosure per adult female captured in the same enclosure three to four weeks (two trap periods) earlier. The time lag allowed recruits to reach trappable size. We measured reproductive activity by the proportion of adult females in reproductive condition. We measured body growth rates by the average percent change in body mass (100 x change in mass/initial mass) of males between the first and fourth trap nights of each trap period. We did not analyze body growth of females because of confounding effects associated with pregnancy and lactation.

Statistical Analysis

We used Statistical Analysis System (SAS Version 6.05; SAS Institute, Inc. 1990) to conduct all data analyses. To increase statistical power, we used $\alpha = .1$. We excluded animals from all analyses for the trap period when they were introduced into an enclosure. Because the number of untagged animals we captured during a trap period depended on the length of time since the preceding trap period, we adjusted measures of recruitment for each trap period by the number of weeks since the preceding trap period. We analyzed the arcsine of the square-root of proportions to normalize distributions and stabilize variances. Means and 90% confidence intervals for proportions are back-transformed.

We used multivariate repeated-measures analysis of variance to test whether population and individual measures differed among treatments during any trap period of the study. Significant mowing x application rate interactions provided evidence that azinphos-methyl had different effects in mowed and unmowed enclosures. We used Tukey's Studentized range test to test for pair-wise differences between treatment means when significant application rate effects or mowing x application rate interactions were detected by the analysis of variance.

We used a univariate analysis, treating mowing and application rate as whole-plot factors and time as a split-plot factor (Huynh and Feldt 1970), to test for

effects of treatment and time on MMDM and body growth. We MMDM movements separately for males and females and used the mean number of captures per animal in each enclosure and trap period as a covariate. Population density was also included as a covariate because home-range size, and therefore movement distance, of small mammals is known to be strongly density-dependent (Wolff 1985, Erlinge et al. 1990). To adjust for size-dependent rates of body growth, we included as a covariate the average initial body mass measurement, for each enclosure and trap period, of animals included in analysis of body growth. Because of missing values, we only analyzed body growth for four trap periods between 8 July and 13 August. We present covariate-adjusted means, F-values, and P-values for treatment effects on MMDM and body growth.

We used a general linear model procedure (SAS Institute, Inc. 1990:891-996) to test for effects of and interactions between mowing and application rate treatments on the prevalence of arthropod material in deer mouse feces collected on 15 July, the day after azinphos-methyl was applied. Missing values prevented analysis of dietary data from more than one trap period.

We estimated power ($1-\beta$) for detecting the observed effect sizes for insecticide application and mowing x application rate interactions on population density and growth, reproductive activity, and recruitment for each postspraying trap period or trap-period interval. The noncentrality parameter ϕ and degrees of freedom were calculated for each test for insecticide effects and interactions (Neter and Wasserman 1974:584), and power for $\alpha = .1$ was determined graphically for each ϕ, using a graph drawn from published power values (Tiku 1972). We present estimated power for tests for which we failed to reject the null hypotheses of no treatment effects and no interactions.

RESULTS

Vegetation Structure and Quality

Mowing reduced the height of the alfalfa canopy from >1 m to approximately 8 cm. Mowed alfalfa rapidly regrew to approximately 30 cm in height by 14 July, when azinphos-methyl was applied. In addition to reducing canopy height, mowing resulted in qualitative changes in the alfalfa crop. Unmowed alfalfa became dry and woody with little new growth, especially at ground level, by August. However, mowed alfalfa produced shoots that remained green and succulent through the end of our experiment.

Small Mammals

In approximately 77,000 trap nights between 18 May and 30 August 1993, 3,460 gray-tailed voles were captured 15,765 times and 402 deer mice were captured 3,976 times.

Gray-tailed voles

Population density and growth—Mean ($\pm$ SE) density of voles was 43 $\pm$ 3 voles/enclosure (range = 0-170). Mowing resulted in lower growth rates (F = 6.9; d. f. = 1, 18; P = .017; Fig. 1b) and densities (F = 18; d. f. =1, 18; P = .0005; Fig. 1a) of vole populations. During the interval between trap periods 2 and 3, when enclosures were mowed, mean ($\pm$ SE) density of voles decreased from 32 $\pm$ 3.4 to 17 $\pm$ 2.8 voles/mowed enclosure and increased from 29 $\pm$ 3.4 to 31 $\pm$ 2.1 voles/unmowed enclosure. Because we added voles to mowed enclosures, mean ($\pm$ SE) densities of voles were similar in mowed (36 $\pm$ 3.6) and unmowed (35 $\pm$ 3.3) enclosures during the last trap period (4) before azinphos-methyl application.

We detected an interaction between effects of mowing and azinphos-methyl on growth of vole populations during the trap-period interval (4 to 5) when we applied azinphos-methyl ($F = 4.3$; d. f. = 2, 18; $P = .031$; Fig. 1a) and on vole densities during trap period 5, which began two days after application ($F = 2.7$; d. f. = 2, 18; $P = .098$; Fig. 1b). However, the interaction between treatments did not take the expected form. Mean population growth rates and postspray densities decreased with increasing application rate, as expected, but were higher in mowed than unmowed enclosures treated with 0.88 and 3.61 kg/ha and lower in mowed than unmowed 0 kg/ha enclosures. No interaction between the effects of mowing and insecticide on densities or growth rates of vole populations was evident for any other trap period or trap-period interval (all $F \leq 1.2$; d. f. = 2, 18; $P \geq .32$; $1\text{-}\beta \leq .34$). Growth rates of vole populations (Fig. 1b) between trap periods 5 and 6 (2-15 days after spraying) differed among insecticide treatments ($F = 27$; d. f. = 2, 18; $P < .0001$); growth rates were lower in enclosures sprayed with 3.61 kg/ha than in enclosures sprayed with 0 or 0.88 kg/ha (Tukey, $P < .05$), but were similar between 0.88 and 0.0 kg/ha treatments (Tukey, $P > .1$). Growth rates of vole populations did not differ among insecticide treatments after trap period 6 (all $F \leq 0.61$; d. f. = 2, 18; $P \geq .56$; $1\text{-}\beta \leq .22$). However, vole densities (Fig. 1a) differed among application rates during trap periods 6, 7, and 8 (all $F \geq 7.3$; d. f. = 2, 18; $P \leq .005$). Mean vole densities in enclosures treated with 3.61 kg/ha remained less than half those treated with 0.0 or 0.88 kg/ha for the last three trap periods (Tukey, all $P < .05$). During trap period 6, vole densities were slightly lower in enclosures treated with 0.88 kg/ha than in control enclosures (Tukey, $P < .1$). Growth of vole populations was more rapid in mowed than in unmowed enclosures during the intervals from trap period 6 to 7 and 7 to 8 (both $F \geq 3.8$; d. f. = 1, 18; $P \leq .067$), resulting in higher densities in mowed enclosures during trap period 8 ($F = 6.7$; d. f. = 1, 18; $P = .018$).

Survival rates—Survival estimates for female voles were consistently higher (+0.02-0.08) than estimates for males during all preliminary analyses for single enclosures, and survival estimates after replicates were combined generally were consistent with the preliminary findings. Male capture probabilities (0.84-0.96) were

consistently higher and significantly different from female capture probabilities (0.54-0.83) during all preliminary analyses for single enclosures. Most models indicated that capture probabilities did not vary with time for males but female capture probabilities in mowed enclosures were lower for the first 2-5 weeks than for the rest of the experiment.

Our best model of male survival rates revealed both mowing and insecticide related decreases in survival (Fig. 2a). Male survival rates in all mowed enclosures were less than half of survival rates in unmowed enclosures the trap-period interval (2 to 3) when mowing occurred. Survival rates were substantially lower in both mowed and unmowed 3.61 kg/ha enclosures and somewhat lower in mowed 0.88 kg/ha enclosures than other enclosures for two trap-period intervals (5 to 6 and 6 to 7) immediately after the insecticide application before recovering towards prespray levels. This model incorporated capture probabilities that were equal among all groups and constant over time.

Our best model of female survival rates incorporated a mowing response in capture probabilities and both mowing and pesticide responses in survival rates. Capture probabilities of female voles in mowed enclosures (0.62 ± 0.04) were lower for two or three trap periods after mowing than capture probabilities during other periods and for unmowed groups (0.81 ± 0.03). Survival rates of female voles were constant and equal for all groups during the intervals from trap period 2 to 3 and 3 to 4, and then declined in the 3.61 kg/ha enclosures for two trap-period intervals (5 to 6 and 6 to 7) following application of the pesticide (Fig. 2b). The pesticide response was more pronounced in mowed enclosures than in unmowed enclosures during the second trap-period interval (6 to 7) following application of the pesticide.

Reproductive activity—The proportion of adult female voles in reproductive condition averaged 0.77 (range = 0.13-1) and did not differ among azinphos-methyl treatments (all $\underline{F} \leq 2.1$; $\underline{d}.\ \underline{f}. = 2, 18$; $\underline{P} \geq .15$; $1\text{-}\beta \leq .37$) or manifest treatment interactions (all $\underline{F} \leq 1.5$; $\underline{d}.\ \underline{f}. = 2, 18$; $\underline{P} \geq .26$; $1\text{-}\beta \leq .44$) during any trap period. Female reproductive activity was lower in mowed than in unmowed enclosures during trap period 4, the second trap period after mowing ($\underline{F} = 11$; $\underline{d}.\ \underline{f}. = 1, 18$;

$P = .0042$) and higher in mowed enclosures during trap period 8 ($F = 6.9$; d. f. = 1, 18; $P = .017$).

Recruitment—The mean weekly proportion of captured vole composed of recruits was 0.16 (range = 0-0.62; Fig. 3a). The proportion of recruits was lower in mowed than in unmowed enclosures during trap period 4 ($F = 3.3$; d. f. = 1, 18; $P = .087$). The proportion of vole recruits did not differ significantly among treatments during trap period 5, just after azinphos-methyl application (all $P \geq .1$, $1\text{-}\beta \leq .49$), but differed among application rates during trap period 6 ($F = 5.1$; d. f. = 2, 18; $P = .017$): it was lower in enclosures treated with 3.61 kg/ha than in those sprayed with 0.0 or 0.88 kg/ha (Tukey, $P < .05$). Recruits constituted a higher proportion of captured voles in mowed than in unmowed enclosures during trap period 8 ($F = 5.1$; d. f. = 1, 18; $P = .038$). We detected no interaction between mowing and insecticide effects on the proportion of recruits during any trap period (all $F \leq 2.0$; d. f. = 2, 18; $1\text{-}\beta \leq 0.49$).

The mean weekly number of vole recruits/adult female was 0.59 (range = 0-3.7; Fig. 3b). Fewer recruits/adult female were captured in mowed than in unmowed enclosures during trap period 3, just after mowing ($F = 3.9$; d. f. = 1, 18; $P = .063$). We found evidence of an interaction between the effects of mowing and azinphos-methyl application on vole recruits/adult female during the first ($F = 5.1$; d. f. = 2, 18; $P = .017$) and second trap periods (5 and 6) after spraying ($F = 2.9$; d. f. = 2, 18; $P = .082$). During trap period 5 the number of recruits/adult female was greater in mowed than in unmowed enclosures for all application rates (Tukey, $P < .05$). During trap period 6, mean recruits/adult female decreased with increasing application rate and tended to be lower in mowed than in unmowed enclosures for 0.0 and 0.88 kg/ha treatments. For both trap periods 5 and 6, the difference between mowing treatments was greatest in enclosures treated with 0.88 kg/ha.

Body growth—Application of azinphos-methyl had effects on vole body growth (Fig. 4) that differed among trap periods ($F = 4.1$; d. f. = 6, 53; $P = .002$) and between mowing treatments ($F = 7.8$; d. f. = 2, 17; $P = .004$). From 2 to 5 days after insecticide application (trap period 5), male voles lost mass in all enclosures

sprayed with 3.61 kg/ha and in mowed enclosures sprayed with 0.88 kg/ha, whereas mean change in mass was positive in control enclosures and unmowed enclosures treated with 0.88 kg/ha. Initial mass was a highly significant covariate for this analysis ($F = 30$; d. f. = 1, 53; $P < .0001$).

Movements—Movements of male voles differed among insecticide treatments ($F = 3.2$; d. f. = 2, 16; $P = .066$) and tended to decrease over time ($F = 2.4$; d. f. = 6, 106; $P = .030$; Fig. 5). After azinphos-methyl was applied (trap periods 5-8), male voles tended to move farther in enclosures treated with 3.61 kg/ha than in 0 or 0.88 kg/ha enclosures. Effects of the insecticide did not differ between mowing treatments ($F = 0.49$; d. f. = 2, 16; $P = .62$). Movements of female voles were not significantly affected by azinphos-methyl ($F = 1.5$; d. f. = 12, 106; $P = .12$). However, movements of female voles were reduced in mowed enclosures during the trap period (3) just after mowing, resulting in a mowing x trap period interaction ($F = 2.4$; d. f. = 6, 106; $P = .033$). Capture frequency was a highly significant covariate for both sexes ($F \geq 9.3$; d. f. = 1, 106; $P \leq .003$)

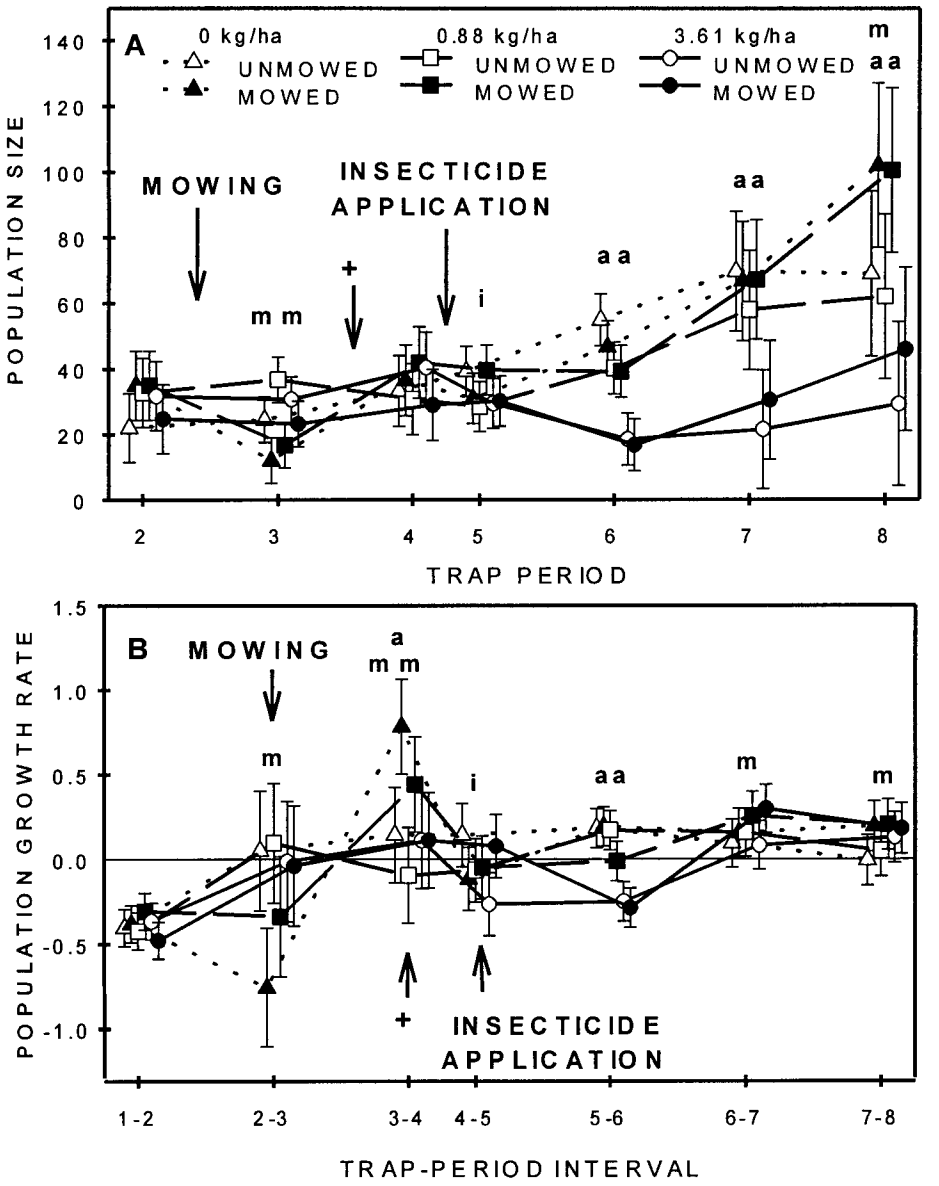

Figure 4. Responses (means and 90% confidence intervals) of (a) density and (b) growth rate of enclosed populations of gray-tailed voles to mowing and application of azinphos-methyl at Hyslop Agronomy Farm, Benton County, Oregon, 1993. Significant ($\underline{P} < .1$) differences among treatments are denoted by "a" for application rate treatments, "m" for mowing treatments, and "i" for treatment interactions. Double letters indicate a significance level of $\underline{P} < .01$. A "+" indicates addition of supplemental voles to mowed enclosures.

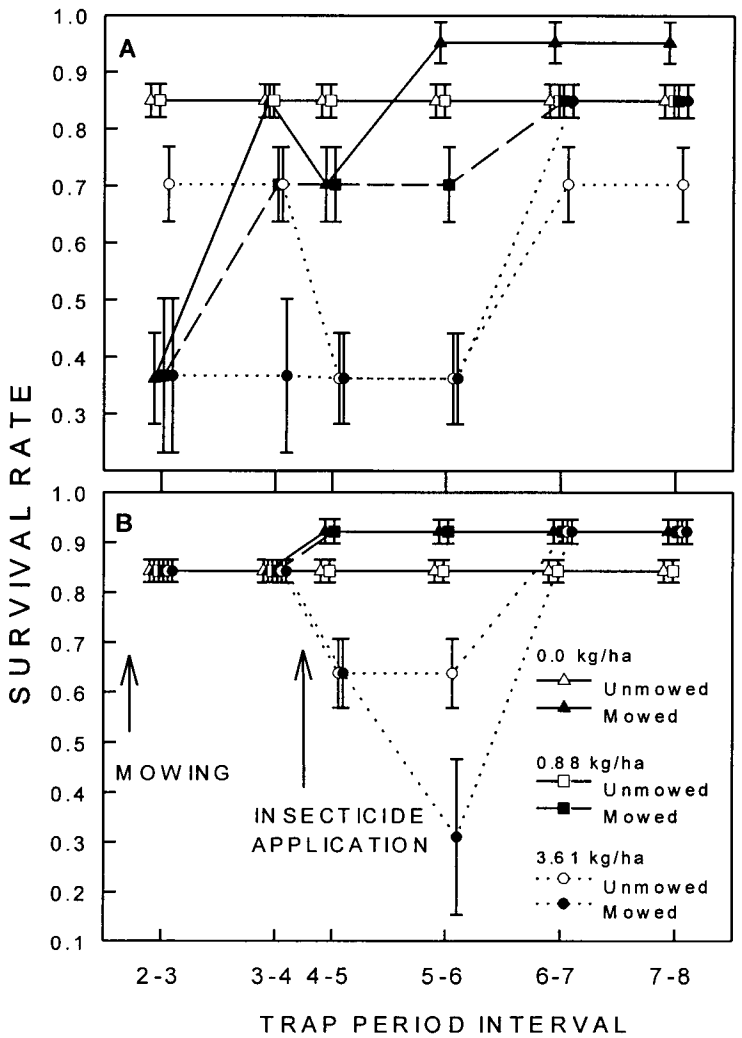

Figure 5. Gray-tailed vole survival probabilities and standard errors for (a) males and (b) females in mowed and unmowed enclosures by application rate and date, for 0.2-ha enclosures treated with azinphos-methyl at the Hyslop Agronomy Farm, Benton County, Oregon, 1993. A "+" indicates addition of supplemental voles to mowed enclosures.

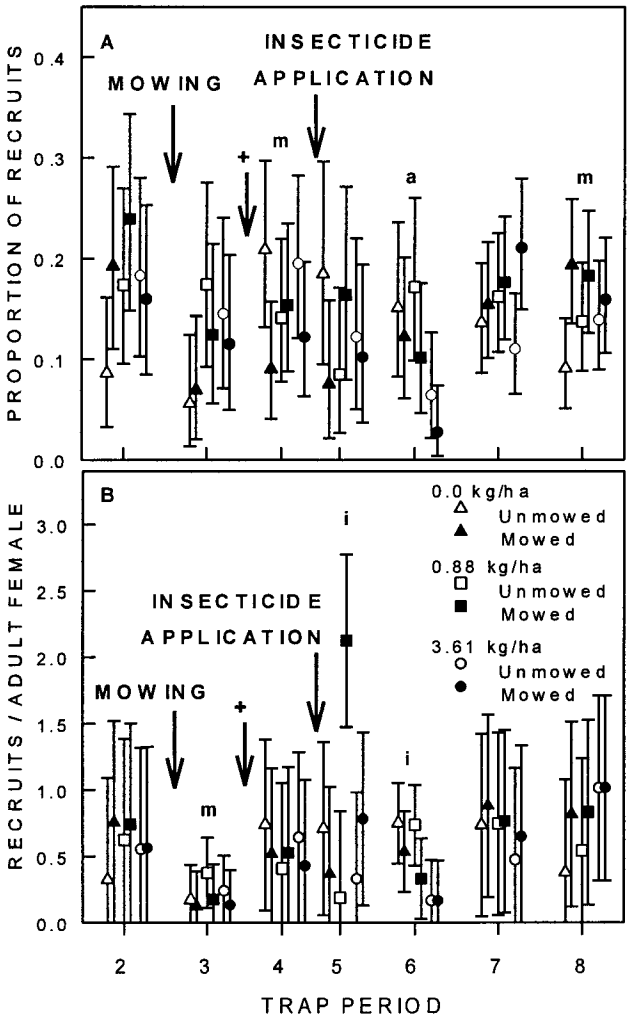

Figure 6. Recruitment of gray-tailed voles (means and 90% confidence intervals), measured by (a) proportion of captured voles composed of recruits and (b) recruits/adult female, in response to mowing and application of azinphos-methyl at Hyslop Agronomy Farm, Benton County, Oregon, 1993. Means are adjusted for duration of trap-period intervals. Significant ($\underline{P} < .1$) differences among treatments are denoted by "a" for application rate treatments, "m" for mowing treatments, and "i" for treatment interactions. Double letters indicate a significance level of $\underline{P} < .01$. A "+" indicates addition of supplemental voles to mowed enclosures.

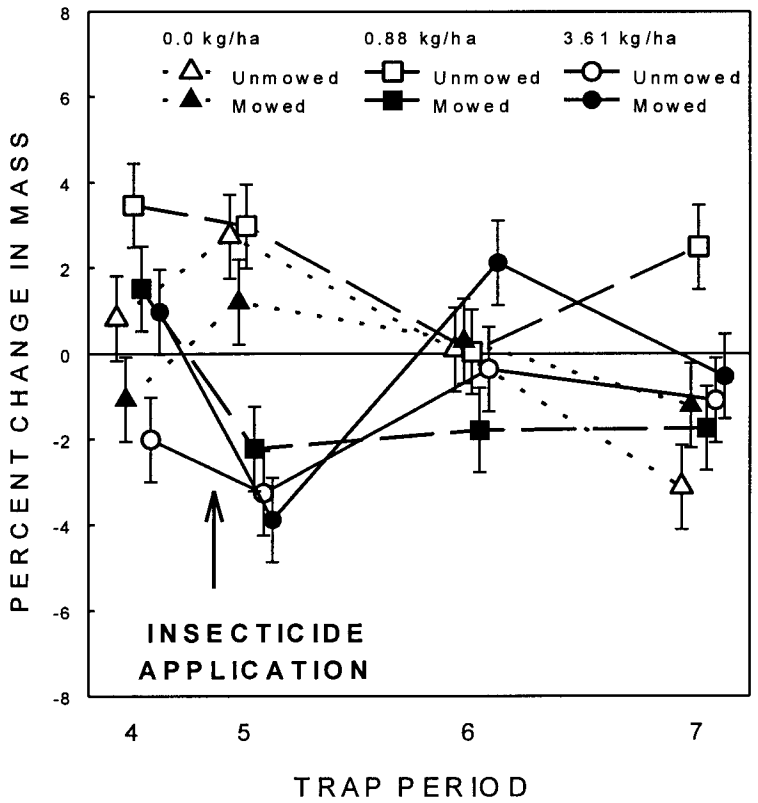

Figure 7. Changes in body mass (means and 90% confidence intervals) of gray-tailed voles in response to application of azinphos-methyl on 14 July 1993 in mowed and unmowed enclosures at Hyslop Agronomy Farm, Benton County, Oregon. Means are adjusted for initial mass.

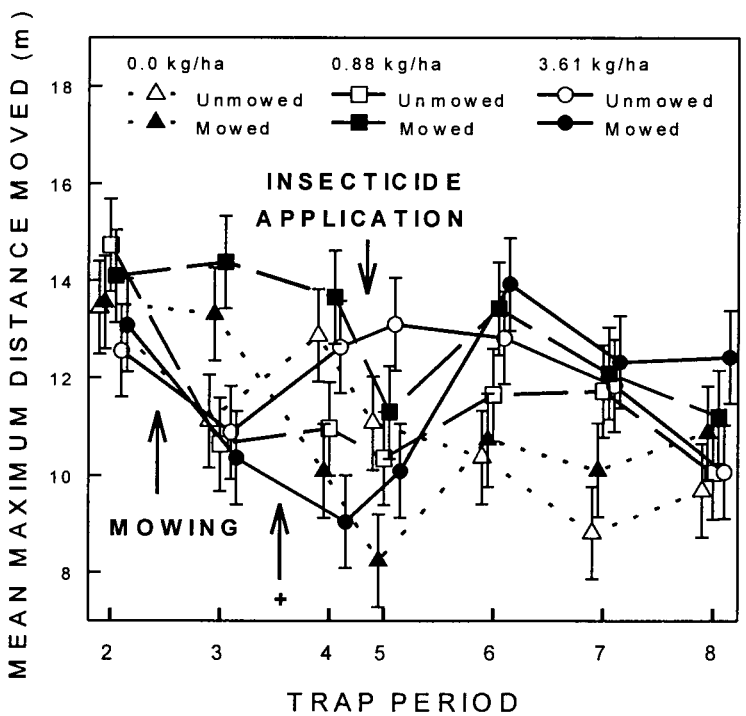

Figure 8. Maximum movement distances (means and 90% confidence intervals) of gray-tailed voles in response to application of azinphos-methyl on 14 July 1993 in mowed and unmowed enclosures at Hyslop Agronomy Farm, Benton County, Oregon. A "+" indicates addition of supplemental voles to mowed enclosures. Means are adjusted for capture frequency and population density.

Deer mice

Population density and growth—Populations of deer mice ranged from 0 to 30 mice/enclosure ($\bar{x}$ = 7, SE = 0.33; Fig. 6a). Mean population density in mowed enclosures sprayed with 3.61 kg/ha decreased 42% during the trap-period interval (4 to 5) when we applied azinphos-methyl, while populations in unmowed enclosures treated with the same application rate grew or did not change. Interactive effects of mowing and application rate on population growth were marginally significant for that trap-period interval (F = 2.6; d. f. = 2, 18; P = .106; Fig. 6b). However, we detected no effects of insecticide or treatment interactions on population density or growth during any other trap period or trap-period interval (all $F \leq 1.6$; d. f. = 2, 18; $P \geq .23$; $1\text{-}\beta \leq .40$). Deer mouse densities were lower in mowed than unmowed enclosures during trap periods 3, 5, 6, and 7 (all $F \geq 3.3$; d. f. = 1, 18; $P \leq .084$).

Survival rates—Preliminary analyses of individual treatment groups provided no evidence for sex-specific difference in survival rates or capture probabilities and best models were typically those in which estimates were constant over time. However, confidence intervals were very broad because few animals were represented in these analyses ($n < 25$ mice/sex). After we combined the six treatment groups into a single analysis, two competing best models emerged providing weak evidence for a mowing response in survival rates. One model provided for a constant survival rate that was not sex-specific (0.69 ± 0.06). The alternative model also set survival rates equal for both sexes, but rates in mowed enclosures (0.53 ± 0.21) were depressed for the intervals from trap period 3 to 4 and 4 to 5. Deer mice in unmowed enclosures had a constant survival rate (0.71 ± 0.06) that was equal to the survival rate in mowed enclosures for the last four trap-period intervals. Both models had capture probabilities that were equal for both sexes and constant over time (0.77 ± 0.07). Models incorporating pesticide-related effects fit our data poorly.

Reproductive activity—The average proportion of adult female deer mice in reproductive condition declined from 0.46 (range = 0-1) to 0.25 (range = 0-1) between trap periods 2 and 8 (14 June - 30 August). During trap period 8, no adult female deer mice in reproductive condition were captured in enclosures treated with 0.88 kg/ha, resulting in a significant difference among application rates for that trap period ($F = 3.2$; d. f. = 2, 18; $P = .067$). However, the proportion of reproductive female deer mice was not significantly affected by mowing (all $F \leq 0.95$; d. f. = 1, 18; $P \geq .34$; $1\text{-}\beta \leq .25$), insecticide application (all $F \leq 1.6$; d. f. = 2, 18; $P \geq .23$; $1\text{-}\beta \leq .67$), or an interaction between treatments (all $F \leq 2.2$; d. f. = 2, 18; $P \geq .14$; $1\text{-}\beta \leq .54$) during any other trap period.

Recruitment—Deer mouse recruitment varied greatly among weeks and treatments. The mean weekly proportion of captured deer mice that were recruits increased from 0.0011 (range = 0-0.6) before trap period 4 to 0.027 (range = 0-0.86) thereafter (Fig. 7). We detected an interaction between mowing and application rate on the proportion of recruits during trap period 5, the first postspraying trap period ($F = 2.9$; d. f. = 2, 18; $P = .083$); the proportion of recruits was higher in mowed than in unmowed control enclosures and lower in mowed than in unmowed enclosures treated with 0.88 and 3.61 kg/ha. The proportion of recruits was lower in mowed than in unmowed enclosures during trap period 6 ($F = 6.3$; d. f. = 1, 18; $P = .021$).

The mean weekly number of deer mouse recruits/adult female increased from 0.0089 (range = 0-0.3) before trap period 4 to 0.24 (range = 0-1) thereafter, and was not significantly affected by mowing (all $F \leq 2.1$; d. f. = 1, 18; $P \geq .17$; $1\text{-}\beta \leq .41$), insecticide application (all $F \leq 1.7$; d. f. = 2, 18; $P \geq .21$; $1\text{-}\beta \leq .40$), or an interaction between treatments (all $F \leq 2.5$; d. f. = 2, 18; $P \geq .11$; $1\text{-}\beta \leq .58$) during any trap period of the study.

Body growth—Because of missing values, we were only able to analyze body growth of deer mice in 13 enclosures (2/treatment, except 3 unmowed-3.61 kg/ha). Initial mass was a highly significant covariate ($F = 16$; d. f. = 1, 20;

P = .0008), but body growth did not differ among trap periods or treatments (all P ≥ .14).

Movements—We did not detect any effects of azinphos-methyl on movements of deer mice of either sex (both F ≤ 0.86; d. f. = 6, 106; P ≥ 0.59). MMDM of males tended to be higher in mowed than in unmowed enclosures (F = 5.0; d. f. = 1, 16; P = 0.039) and varied among trap periods (F = 2.4; d. f. = 6, 106; P = 0.030). For both male and female deer mice, density-adjusted MMDM was strongly associated with capture frequency (both F ≥ 43; d. f. = 1, 106; P ≤ 0.0001) but not with population density (both F ≤ 1.1; d. f. = 1, 106; P ≥ 0.29).

Diet—The prevalence of arthropod material in deer mouse feces was similar among application rate treatments before the insecticide was applied (Fig. 8). However, on the day after insecticide application, arthropod material was more prevalent in deer mouse feces collected from insecticide-treated enclosures than in those from control enclosures (F = 24; d. f. = 2, 6; P = .0014), with no difference between 0.88 and 3.61 kg/ha application rates (Tukey, P > 0.1). For each application rate, the incidence of arthropod material in deer mouse feces the day after spraying was greater in unmowed than in mowed enclosures (F = 16; d. f. = 1, 6; P = .0075). By two weeks after spraying, the incidence of arthropod material in deer mouse feces was similar among application rates.

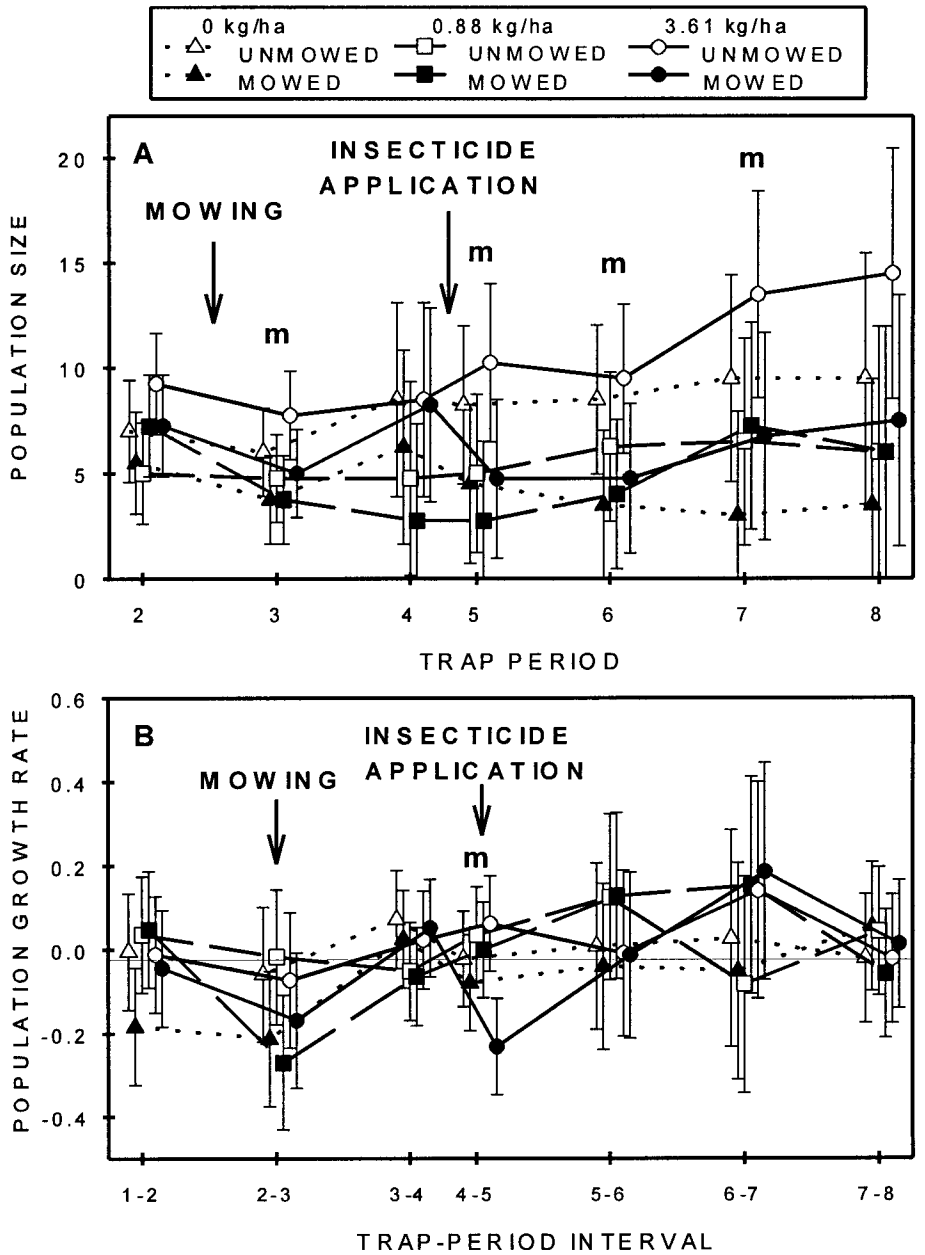

Figure 9. Responses (means and 90% confidence intervals) of (a) density and (b) growth rate of enclosed populations of deer mice to mowing and application of azinphos-methyl at Hyslop Agronomy Farm, Benton County, Oregon, 1993. Significant ($\underline{P} < .1$) differences among treatments are denoted by "a" for application rate treatments, "m" for mowing treatments, and "i" for treatment interactions. Double letters indicate a significance level of $\underline{P} < .01$.

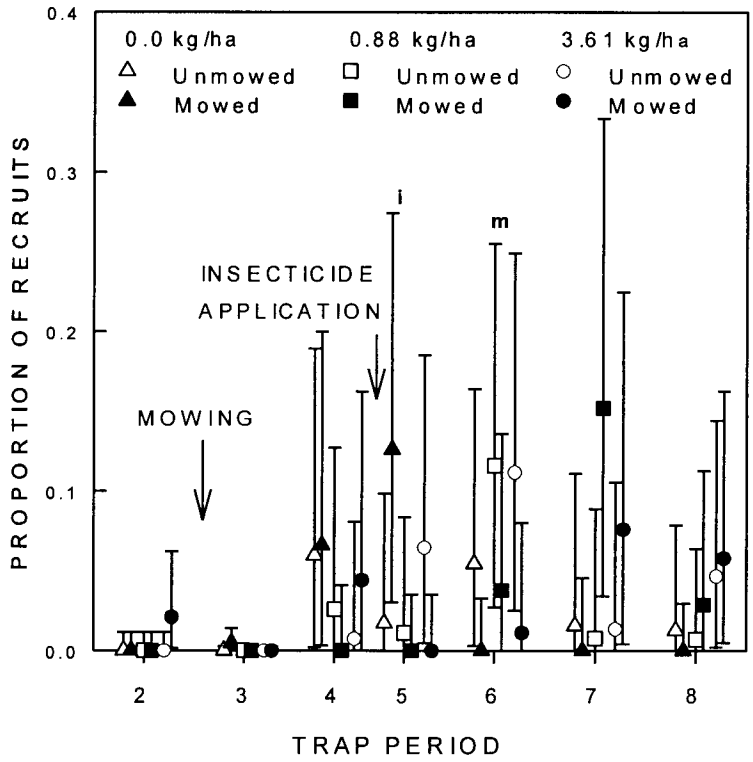

Figure 10. Proportion (means and 90% confidence intervals) of captured deer mice composed of recruits, in response to mowing and application of azinphos-methyl at Hyslop Agronomy Farm, Benton County, Oregon, 1993. Means are adjusted for duration of trap-period intervals. Significant ($\underline{P} < .1$) differences among treatments are denoted by "a" for application rate treatments, "m" for mowing treatments, and "i" for treatment interactions. Double letters indicate a significance level of $\underline{P} < .01$.

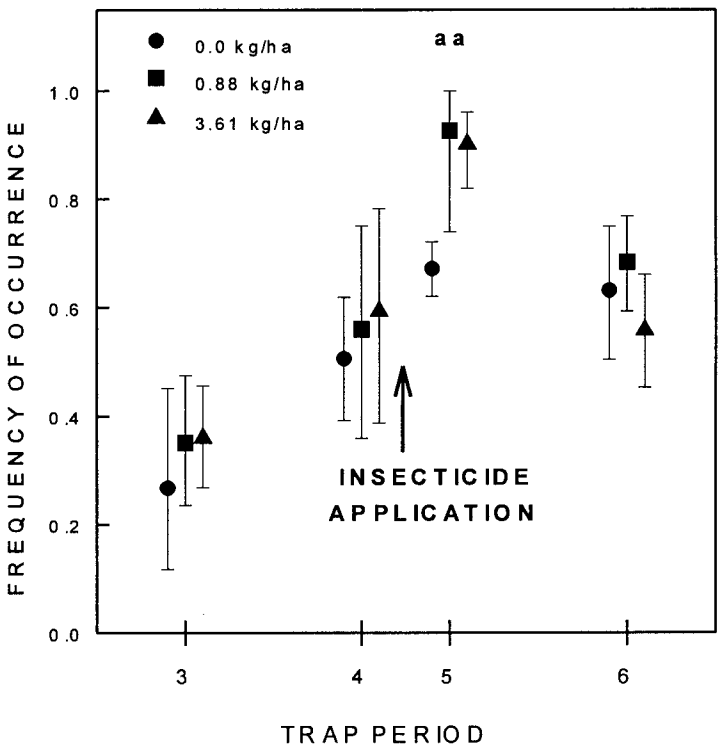

Figure 11. Incidence of arthropod material (means and 90% confidence intervals), determined by microhistological analysis, in the feces of deer mice in enclosed alfalfa plots before and after application of azinphos-methyl on 14 July 1993 at Hyslop Agronomy Farm, Benton County, Oregon. Significant ($\underline{P} < .1$) differences among application rate treatments are denoted by "a." Double letters indicate a significance level of $\underline{P} < .01$.

DISCUSSION

Insecticide Effects

The QM characterized 3.61 kg/ha of azinphos-methyl as "high risk" for gray-tailed voles but "low risk" for deer mice. Consistent with this characterization, 3.61 kg/ha of azinphos-methyl reduced recruitment, survival, and body growth of voles, resulting in vole densities <40% of controls. However, population growth rates and recruitment of deer mice in mowed enclosures were also reduced by the 3.61 kg/ha rate, which was predicted to pose low risk to deer mice. Reproductive activity was not significantly affected for voles or deer mice, even at 3.61 kg/ha, although power to detect such effects was low ($1-\beta \leq .44$). Movements of male voles were positively associated with insecticide application, but we found no insecticide effects on movements of females. All insecticide effects were manifested within 15 days after spraying. Effects on population growth, recruitment, and body growth were detectable for only one trap period, whereas differences in population densities and movements of male voles persisted until the end of the experiment, over six weeks after spraying.

These results provide evidence that the primary effect of azinphos-methyl was a short-term increase in mortality of small mammals, without long-term toxic effects. Survival rates of voles in enclosures treated with 3.61 kg/ha were reduced for only 15 days after application. Although vole densities in enclosures sprayed with 3.61 kg/ha remained approximately half of those that received lower pesticide treatments until the end of the study, population growth trajectories after 1 August were parallel for all application rates, indicating that azinphos-methyl did not have long-term effects. Azinphos-methyl is a relatively short-lived pesticide, and its half-life on alfalfa was less than five days (Chapter II, see also Bennett et al., in press). Symptoms of OP intoxication in mammals are also generally of short duration, with substantial recovery within 48 h after a single exposure (Pasquet et al. 1976, Montz and Kirkpatrick 1985b). Although reductions in survival, population growth, and

recruitment of voles were short-lived, vole populations treated with 3.61 kg/ha remained substantially smaller than controls for more than six weeks. This indicates that even a highly fecund species may not recover quickly from the short-term, acute effects of OP insecticides.

We could not detect any insecticide effects on the proportion of adult female small mammals that were pregnant or lactating. This measure of reproductive activity would have been affected by reductions in birth rate or neonatal survival. OP's can disrupt production of reproductive hormones (Civen et al. 1977, Rattner and Michael 1985) and thus may affect mating and parental behaviors (Alcock 1989; pp. 84-88), gamete production (Russell et al. 1987), and lactation (Barnes and Denz 1951) in mammals. Also, OP's can cross the placental barrier (Ackermann and Engst 1970) and are excreted in milk (Mosha et al. 1991), resulting in direct fetal and neonatal toxicity (Fish 1966, Budreau and Singh 1973, Short et al. 1980). Finally, physiological stress on female mammals, due to direct toxicity or feed aversion, can cause reproductive delay, embryo resorption, or stillbirth (Linder and Richmond 1990, Dost 1991). However, the potential of OP's to impact mammalian reproduction has not been confirmed by other field studies (Jackson 1952, Montz et al. 1984, Carey 1993).

If the reproductive activity of females was not affected, then reductions in recruitment of voles and deer mice probably resulted from increased juvenile mortality. Negative effects on the proportion of recruits indicate that azinphos-methyl caused greater mortality of juveniles than of adults. Young mammals tend to be more susceptible to OP's than adults because detoxifying mechanisms are not fully developed (Gagne and Brodeur 1972, Benke and Murphy 1975). Similar short-term reductions in recruitment have been observed for hispid cotton rats (*Sigmodon hispidus*; Barrett 1968) and meadow voles (*Microtus pennsylvanicus*; Barrett 1988) after exposure to the carbamate insecticide Sevin®. However, previous field studies using OP's have not demonstrated adverse effects on mammalian recruitment (Jackson 1952, Jett et al. 1986, Carey 1993, Edge et al., in press).

Body growth of male voles was negatively affected by azinphos-methyl for several days after application. Lower body growth could have resulted from direct physiological harm, anorexia, or avoidance of contaminated food. Unfortunately, our sampling methods did not allow us to determine which of these factors contributed to the observed effect. Ataxia and neuromotor dysfunction that characterize OP intoxication (O'Brien 1967; p. 56) may have reduced the foraging capability of intoxicated animals, thereby retarding growth. Feed aversion, but not anorexia, has been demonstrated in mammals exposed to pesticides (Linder and Richmond 1990). Gray-tailed voles can detect and avoid food contaminated with azinphos-methyl at concentrations $\leq$100 ppm (T. Manning and J. O. Wolff, unpublished data). Furthermore, in 10-day no-choice dietary LC_{50} tests of the toxicity of azinphos-methyl to gray-tailed voles, Meyers and Wolff (1994) observed that many of the voles that died after 5 days died of starvation, whereas deaths before day 5 probably resulted from acute poisoning. Thus, even when voles had no choice but to eat contaminated food or starve, they stopped eating to avoid exposure and starved to death in 5-10 days.

Movements of voles increased after spraying in enclosures treated with 3.61 kg/ha, even after accounting for changes in density. However, the effect was not apparent until two weeks after application and lasted for another four weeks. Thus, voles did not evidently become more sedentary due to intoxication or attempt to move out of the treated alfalfa plots immediately after spraying, when contamination was greatest. The late manifestation and long duration of the increase in movements of voles suggest that it reflects disruption of their social structure rather than a direct response to the chemical. Morris (1970) observed a similar long-term increase in movements of unenclosed meadow voles after application of the organochlorine insecticide endrin.

Our results contrast to those of the 1992 experiment by Carey (1993) and Edge et al. (in press) on the effects of azinphos-methyl on gray-tailed voles in the Hyslop Agronomy Farm enclosures. Although they applied the insecticide at rates up to 4.67 kg/ha, the highest application rate caused only a 24% reduction in vole

populations and did not significantly affect on recruitment, body growth, or movements. Differences between that experiment and ours that might have contributed to the dissimilarity of observed effects were (1) we controlled vole densities in 1993 and (2) more rain fell during summer in 1993 than in 1992. Vole densities at the time of spraying ranged from 14 to 120 voles/enclosure in 1992, but only from 14 to 61 in 1993. By keeping vole densities near 30/enclosure, we decreased within-treatment variation and, presumably, increased statistical power for detecting population-level effects. However, the difference in magnitude of observed effects is not explained by vole population control.

Wet weather may have contributed to the greater effects in 1993. Rain fell within an hour after we applied azinphos-methyl in 1993 and continued intermittently over the next several days, whereas no rain fell for seven days after spraying in 1992. Precipitation did not result in lower persistence of azinphos-methyl on alfalfa in 1993 (Chapter II) than in 1992 (Bennett et al., in press). OP insecticides can reduce body temperatures and impair thermoregulation in small mammals (Meeter and Wolthuis 1968, Coudray-Lucas et al. 1981, Montz and Kirkpatrick 1985a). Therefore, exposure to azinphos-methyl may have increased the susceptibility of voles to hypothermia. Alternatively, water dripping from the alfalfa canopy may have provided an alternate route of exposure to the chemical if voles ingested the chemical while grooming their pelage. Rodents do not seem to avoid grooming after contact with toxicants, and this trait is exploited by contact rodenticides, which are applied so that rodents acquire poison on their fur and ingest it as they groom (Gibson 1982, Davis 1983). This route of exposure is not accounted for by the QM, which only incorporates exposure through contaminated foods. Thus, rainfall soon after insecticide application may put nontarget mammals at greater risk than predicted if their exposure through grooming is substantial. Conversely, drier conditions may reduce the risk of adverse pesticide effects because of reduced pelage contamination or grooming activity. Further study is needed to evaluate the relationship between weather and susceptibility of nontarget animals to pesticides

Influence of Mowing on Insecticide Effects

Vegetation structure may influence the exposure of organisms to pesticide sprays by affecting the distribution and persistence of residues. However, vegetation structure is not accounted for by the QM and, therefore, may constitute an important source of unexplained variation in the performance of risk assessments. We hypothesized that azinphos-methyl would have greater adverse effects on small mammals in mowed than in unmowed enclosures. Residue concentrations at ground level were much higher in mowed than in unmowed enclosures (Chapter II), and the chemical caused greater effects in mowed enclosures on deer mouse population growth and recruitment, on vole recruitment and body growth, and on survival of female voles. However, responses of vole populations to azinphos-methyl application were almost identical for the two mowing treatments. Populations of deer mice were reduced by application of 3.61 kg/ha in mowed enclosures but not in unmowed enclosures. This application rate was predicted by the QM to pose low risk to deer mice. For voles, mowing seemed to increase effects of the 0.88 kg/ha application rate on body growth and recruitment/adult female. Again, the quotient of risk for this application rate indicated "low risk". Therefore, application of azinphos-methyl to areas of relatively short vegetation resulted in harmful effects not predicted by the QM.

Although azinphos-methyl was present at ground level at higher concentrations (Chapter II) and caused higher mortality of female voles in mowed than in unmowed enclosures, interactive effects between mowing and insecticide treatments were not manifested at the population level for voles. Statistical power for detecting observed mowing x application rate interactions was generally low ($\leq$ .34), but low power could have resulted from within-treatment variability or small effect sizes. The nearly identical responses of vole populations to the insecticide suggest that interactive effects were small or absent. Foraging behavior of the voles may have equalized their potential exposure to azinphos-methyl, and avoidance of contaminated vegetation may have limited their actual exposure. Gray-tailed voles

can access the tops of alfalfa by cutting down alfalfa stems and may climb into the alfalfa canopy (R. Bentley, personal communication). We have often found alfalfa stems cut near ground level and 5- to 10-cm lengths of alfalfa stored in burrows, Sherman traps, and under pitfall covers. Thus, the exposure of voles was probably not limited to the amount of chemical present at ground level, and may have been similar for both mowing treatments, even though much of the chemical was prevented from reaching ground level by unmowed alfalfa. However, gray-tailed voles can detect and avoid azinphos-methyl at dietary concentrations as low as 100 ppm (T. Manning and J. O. Wolff, unpublished data). In our field experiment, mean azinphos-methyl concentrations on mowed alfalfa and tops of unmowed alfalfa sprayed with 3.61 kg/ha exceeded 200 ppm (Chapter II). The uneven spatial distribution of residues (Chapter II) probably ensured that voles could find high-quality forage that was relatively uncontaminated.

No other investigators have compared the ecological effects of pesticides in areas of differing vegetation density. Field studies of pesticide effects on small mammals have generally been conducted in dense grasslands (e.g., Morris 1970) or forests (e.g., Giles 1970). Carey (1993) concluded that applications of azinphos-methyl up to 4.67 kg/ha did not affect reproduction, recruitment, body growth, or movements of gray-tailed voles in enclosed alfalfa plots, and attributed these negative results to interception of the insecticide spray by the dense alfalfa. This hypothesis was supported by residue concentrations that were lower at ground level than in the alfalfa canopy (Bennett et al., in press). However, high variability in vole population densities and a significance level of .05 resulted in low statistical power in tests for insecticide effects. Despite the lack of detectable effects on parameters measured by Carey (1993), survival rates and population densities of the voles were reduced by application rates $\geq$1.55 kg/ha (Edge et al., in press), in concordance with QM predictions.

Influence of Diet on Insecticide Effects

Although EEC is calculated for the primary foods of receptor organisms, the QM does not account for differential susceptibility as a result of differences in diet. We hypothesized that the insectivorous diet of deer mice would result in adverse effects in unmowed enclosures at lower application rates than predicted, because of feeding on contaminated arthropods or indirect effects from elimination of the arthropod prey base. Insectivorous small mammal species are known to increase their relative intake of arthropods after insecticide application (Stehn et al. 1976). We found that feces of deer mice contained more arthropod material after the insecticide was applied, indicating that the mice were feeding on dead or dying arthropods. However, we were unable to detect any effects of azinphos-methyl on deer mice in unmowed enclosures. Because deer mice have much greater physiological tolerance to azinphos-methyl (LC_{50} = 1,200 ppm; Meyers and Wolff 1994) than do gray-tailed voles, they may have been able to eat contaminated arthropods with little adverse effects. The prevalence of arthropods in diets of deer mice in insecticide-treated enclosures measured two weeks after spraying did not drop below that of controls, providing evidence that the insecticide treatment did not cause a long-term reduction in prey availability. Abundance of foliar and ground-dwelling arthropods in insecticide-treated enclosures had returned to control levels by two weeks after spraying (J. Miller, personal communication). Thus, insectivory did not increase the observed susceptibility of deer mice to insecticide exposure.

Mowing Effects

Mowing negatively affected both species of small mammals, at least initially. Populations of voles were impacted most, requiring us to transfer voles to mowed enclosures to maintain those populations. The reduction in recruitment and reproductive activity of voles that we observed during the second trap period after

mowing probably resulted from the transfer of voles: introduced females apparently stopped reproducing as they adjusted to their new surroundings. The more immediate (5-8 days after mowing) reduction in vole recruits/adult female is evidence that young animals were more susceptible to mowing effects than adults. By the end of the experiment, however, vole populations were larger and growing faster in mowed than in unmowed enclosures. We suggest that growing shoots of mowed alfalfa constituted more nutritious forage for voles than unmowed alfalfa, which was drier and woodier.

The initial adverse effects of mowing on small mammal populations could have resulted from increased activity and efficiency of predatory birds after vertical cover was removed, direct mortality caused by the mowing apparatus, or sudden removal of food. While mowing the border strips in the enclosures, we have noticed that gray-tailed voles may freeze in place rather than trying to escape the approaching machine. Therefore, voles may have been killed by the mowing apparatus. However, the enclosures were mowed during midday, a period of inactivity for deer mice and relatively low activity for gray-tailed voles. Therefore, the mower probably did not directly kill enough voles to account for the large effects on their populations. Mowing removed almost all the standing crop of alfalfa in mowed enclosures, possibly limiting forage availability. However, over-winter survivorship of gray-tailed voles is generally high and they sometimes breed in the fall, when there is no green vegetation above ground (J. Peterson, unpublished data). Gray-tailed voles are apparently able to survive and breed on a diet of alfalfa roots. Therefore, we suggest that food shortage is also an inadequate explanation of the adverse mowing effects on the small mammals.

The rapid and severe reduction of vole populations in mowed enclosures and the much less substantial effects on deer mice are consistent with the hypothesis that the primary effect of mowing was to increase the vulnerability of small mammals to avian predators. Because tall, dense vegetation presumably can impair the ability of raptors to locate and capture small mammals, mowing alfalfa to <8 cm in height probably increased predator efficiency. Gray-tailed voles would be expected to suffer

most from the removal of cover because they are more diurnal, larger, and less agile than deer mice (Kotler et al. 1988). The observed reductions in vole movements and capture probabilities of female voles may have been a behavioral response to greater exposure to predators. However, a great increase in predator activity would seem to be required to explain the dramatic vole population crashes that occurred within 5 days after mowing. Observation of diurnal raptor activity did not support this explanation (T. DeWilde and W. D. Edge, unpublished data). Owls, which were not observed because of their nocturnal habits, could have been largely responsible for the observed responses in vole populations. However, deer mice presumably are more susceptible to nocturnal than to diurnal predators and their populations might, therefore, be expected to be substantially affected. Predation seems to be the explanation most consistent with observed effects of mowing on small mammal populations, but it is not supported by observations of predator activity or success.

Edge et al. (unpublished data) found that mowing the enclosures at Hyslop Agronomy Farm in autumn 1992 reduced gray-tailed vole densities by approximately 50%. Recruitment was also reduced by mowing, and the proportion of animals attempting to disperse increased. No explanation for the adverse effects of mowing was provided. Other investigators that have examined responses of small mammals to removal of cover have also reported impacts on microtines and invoked predation as the most likely explanation (LoBue and Darnell 1959, Kotler et al. 1988).

CONCLUSIONS

The QM is a relatively simple, laboratory-based methodology used by the EPA to conduct ecological risk assessments. However, environmental and biological factors that are not accounted for by the QM may substantially influence the susceptibility of nontarget organisms and result in unpredicted adverse effects. I conducted a replicated, factorial experiment to test whether the distribution of residues and responses of small mammals after application of azinphos-methyl concorded with predictions by the QM, despite differences in vegetation structure and small mammal diets. Although residue concentrations increased proportionately with application rate, the nomogram used by the EPA to estimate exposure in QM risk assessments underestimated mean concentrations on unobstructed alfalfa by as much as 37%. Dense vegetation intercepted the insecticide spray before it reached ground level. Thus, the exposure of herbivores to insecticides may depend on the vegetation strata in which they feed. However, vegetation structure did not affect residue persistence. Although the vegetation nomogram underestimated pesticide residue concentrations on alfalfa, the gross pattern of pesticide effects was consistent with QM predictions: 3.61 kg/ha caused a long-term reduction in vole populations and the chemical had greater effects on voles than on deer mice. However, both species exhibited short-term adverse effects in mowed enclosures at application rates that were predicted to pose low risk. Effects on vole populations were similar in both mowing treatments, despite greater insecticide effects on recruitment, body growth, and survival of voles in mowed than in mowed enclosures. Behavioral characteristics of voles may have reduced the influence of mowing on insecticide effects. I observed greater effects than those reported by Edge et al. (in press) for the same vole species, insecticide, and experimental site, although we applied a lower concentration. Rainfall after spraying may have increased exposure to the toxicant or vulnerability to hypothermia during this experiment. The susceptibility of small mammals to azinphos-methyl was not apparently influenced by their diets. My results suggest that vegetation structure, precipitation, and behavioral responses can

affect the exposure of terrestrial, nontarget species to pesticide sprays and, consequently, influence the magnitude of adverse ecological effects. Incorporation of these factors into the Quotient Method may improve the ecological foundation and consequent performance of risk assessments.

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