Area-Wide Effect on Oribatid Mites (Acari) Following Application of Lindane for Protection of Lodgepole Pine from Bark Beetle Attack

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ABSTRACT The effect of lindane on numbers and community structure of oribatid mites was studied in northern Montana. Trees on five 0.4-ha plots were sprayed with lindane at 5.5 kg/ha (AI) in a simulated operational program to protect lodgepole pine from bark beetles. Sprayed plots and paired control plots were sampled before spray application and five times after treatment over a 2-yr period. Numbers of immature specimens changed more than numbers of adults. Both were significantly depressed 113 after treatment. Two superfamilies were significantly affected after spraying. Of five measures of community structure, none demonstrated an effect; however, the powers of the statistical tests were quite low. The importance of statistical power in studies of effect of pesticides on nontarget species is discussed. Because the observed effects were short-lived and of relatively small amplitude, we conclude it is unlikely there is a lasting effect on the oribatid community.

KEY WORDS Arachnida, community structure, nontarget insecticide effect, lindane

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LINDANE IS USED to protect pine trees from attack by bark beetles. The effect of such applications on nontarget species in the soil under the trees is a consideration in the registration by government regulatory agencies. The effect of lindane on forest soil arthropods, mites in particular, has been investigated in the eastern United States under eastern white pine, *Pinus strobus* L., and loblolly pine, *Pinus taeda* L. (Hastings et al. 1981, 1989), and in the western United States under *P. ponderosa* Lawson (Hoy 1980, Hoy & Shea 1981). Those studies were done at high rates of application, on plots of limited size, and over a relatively short period of time. A 10-yr study of the effect of high application rates was conducted by Hoy (1990).

Oribatid mites are an important functional component of the fauna of pine forest floor. They participate in recycling of nutrients trapped in leaf litter. Furthermore, oribatids lend themselves to nontarget impact studies because they are abundant, relatively easy to identify, and represented by many species. Presumably, the many species occupy a range of decomposer niches.

Under certain circumstances, such as in campgrounds or scenic areas, protection of selected trees in a large stand is desired by land managers. Where only a portion of the trees needs to be protected, a low application rate on an area basis may be adequate. This study was designed to determine the effect on oribatid mites, if any, after such an application. How is the oribatid fauna in the entire stand of trees affected? Are there particularly vulnerable segments of the decomposer community, and are there measurable effects on the structure as a whole?

Materials and Methods

Simulated operational application of lindane was done in stands of lodgepole pine, *Pinus contorta* Dougl., which ranged in size from 20 to 40 cm dbh (diameter breast height). Plots were located in northern Montana at ≈1,500 m elevation, ≈110 km east of the continental divide, and 90 km south of the Canadian–U.S. border. These study sites have short growing seasons and moderate amounts of precipitation.

Five pairs of square plots, each plot 0.4 ha in size, all within a 10-km radius, were randomly assigned one of two treatments: one untreated and one sprayed plot per pair. Each plot within a pair was separated by not less than 100 m. Each sprayed plot received 2.2 kg (4.88 lb) (AI) of lindane more or less equally divided among 100 trees scattered haphazardly throughout the plot. Approximately one-third of the trees in any given plot were sprayed, resulting in a "patchy" spray distribution.

Table 1. Mean numbers of oribatid mites per plot with 12 subsamples per plot, arranged by sampling time, taxa, and treatment

Time, d	Taxa	Control, $\bar{x}$ ± SE	Treatment, $\bar{x}$ ± SE	P
−1	Total specimens	78.8 ± 40.7	120.0 ± 52.3	0.20
	Immatures	0.4 ± 0.6	0.8 ± 1.3	0.75
+7	Total specimens	153.6 ± 77.6	142.6 ± 49.3	0.77
	Immatures	30.9 ± 95.4	24.5 ± 10.4	0.095
+29	Total specimens	250.0 ± 272.4	198.6 ± 82.9	0.84
	Immatures	91.5 ± 171.3	35.5 ± 27.4	0.44
+113	Total specimens	333.4 ± 75.8	172.4 ± 41.7	0.027
	Immatures	199.4 ± 143.0	53.1 ± 22.0	0.019
+364	Total specimens	174.9 ± 53.6	177.6 ± 47.1	0.91[a]
	Immatures	63.3 ± 53.3	34.0 ± 20.2	0.24
+730	Total specimens	188.1 ± 89.7	237.1 ± 117.6	0.56
	Immatures	57.2 ± 41.7	49.8 ± 25.7	0.91

Statistical significances are based on paired t tests with exact P values stated.

[a] The power of this test was 97%. All other tests that failed to reach the 0.05 significance level ($P < 0.05$) had power of less than 80%.

The spray was applied June 6–8, 1987 using a hydraulic sprayer. A 0.5% solution of lindane (20% emulsifiable concentrate) was applied to the bole from ground line to a height of 10 m until runoff, per label instructions (Ortho Isotox Lindane Spray No. 200, EPA Reg. No. 239-2363AA Chevron Corp., Richmond, CA.).

Residue Analysis. Lindane residues were determined with a Hewlett Packard 5890A gas chromatograph equipped with a 63 Ni electron capture detector and fitted for split–splitless capillary column operation. The analytical column was a fused-silica capillary column (25 m, 0.32 mm inside diameter) coated with a 0.5-µl film of methyl silicone. Gas chromatographic operating parameters, inlet, column and detector temperatures, and column flow rate were optimized before sample processing. The response variable (milligram lindane per gram substrate) was automatically quantified with a Hewlett Packard 3392A integrator by comparison with external standards.

Sampling Design. Samples were taken 1 d before and 7, 29, 113, 364, and 730 d after application of lindane. Within each plot, 12 subsamples (six random points on each of two random transects) were taken each time. Each subsample was a soil core (5 cm diameter, 5 cm deep). The first two and last two sampling times were in early June, the third time was in early July, and the fourth time was in early October.

Arthropod Extraction. Arthropods were extracted by inverting soil cores for 72 h in six modified, 20-unit Merchant-Crossley Tullgren-type (Norton 1985) extractors at 35°C above the samples and approximately 20°C below.

Specimen Identification. Specimens were sorted from debris without knowledge of the plot treatment. Identification was based on voucher specimens from the study area determined to species by R. A. Norton. All identification was done in alcohol under 50× magnification. The members of the genus *Suctobelbella* could not be reliably identified to species. Superfamily designations are based on Marshall et al. (1987).

Statistical Analysis. Paired comparison t tests, following transformation of counts to log (n + 1) for each sampling time, were the primary criteria for determining the effect of lindane (Kesselman & Rogan 1978, Little & Hills 1978). Results are presented as back-transformed means of counts, with exact P values stated for each comparison. All analyses used two-tailed tests.

The power of the test for detecting an 80% reduction was high (>90%) for a given coefficent of variation at the 5% significance level with five replicates.

Increases or decreases in total numbers of oribatids, immature specimens, and specimens by superfamilies were considered. Also, the structure of the oribatid community as a whole was analyzed with four commonly used measures: species number per plot, the modified Simpson's index (Peet 1974), an index of association (Hurlbert 1978), and Kendall's tau (Ghent 1963). Number of superfamlies per plot was also determined.

Results

The effect of simulated operational application of lindane on the oribatid mite fauna was assessed by changes in numbers of the oribatids as a whole, changes in numbers of specific taxonomic groups, and changes in five measures of species diversity.

Effect on Oribatids in General. The total number of oribatid mites (adults plus immature specimens) was significantly lower in sprayed plots at 113 d after spraying (Table 1). Likewise, the total number of immature specimens was significantly lower at that time (Table 1).

Pretreatment sampling 1 d before spraying indicated that populations within the sprayed plot were slightly higher, but not significantly different from, the paired control plot (Table 1); there-

Table 2. Species in control and treatment plots in Flathead County, Mont.

Superfamily	Genus and species
Palaeacaroidea	*Palaeacarus* cf. *appalachicus*
Brachychthonioidea	*Brachychthonius* sp.
	Liochthonius sp.
	Sellnickochthonius sp.
Phthiracaroidea	*Atropacarus striculus* (C. L. Koch)
	Phthiracarus sp.
	Protoribotritia sp.
Nothroidea	*Nothrus palustris* (C. L. Koch)
	Nothrus sp. nr. *terminalis*
	Camisia biuris (C. L. Koch)
	Heminothrus longisetosus Willmann
	Platynothrus "peltifer"
	Neonothrus humicolus Forsslund
	Trhypochthonius tectorum (Berlese)
	Malaconothrus mollisetosus Hammer
	Trimalaconothrus novus (Sellnick)
Nanhermannioidea	*Nanhermannia* sp.
Hermannielloidea	*Hermanniella* sp.
Gymnodamaeoidea	*Gymnodamaeus* sp.
Damaeoidea	*Dyobelba* sp.
	Epidamaeus sp. A
	Epidamaeus sp. B
Cepheoidea	*Cephus* cf. *latus*
	Eupterotegaeus rhamphosus Higgins & Woolley
Eremaeoidea	*Eremaeus* sp.
Gustavioidea	*Liacarus cidarus* Woolley
	Ceratoppia bipilus (Hermann)
	Cerotoppia sp.
	Pyroppia sp. nr. *lanceolata*
Carabodoidea	*Carabodes* sp.
	Tectocephus velatus (Michael)
Oppioidea	*Oppia* sp.
	Oppiella clavigera (Hammer)
	O. nova (Oudemans)
	O. sp. nr. *nova*
	Quadroppia quadicarinata (Michael)
	Allosuctobelba sp.
	Rhynchobelba sp.
	Suctobelbella sp. A
	Suctobelbella sp. B
	Suctobelbella sp. C
	Suctobelbella sp. D
Hydrozetoidea	*Limnozetes canadensis* Hammer
Oribatelloidea	*Oribatella* sp.
	Anachipteria sp.
	Parachipteria sp.
Ceratozetoidea	*Dentizetes rudentiger* Hammer
	Propelops sp.
Oripodoidea	*Oribatula* sp.
	Scheloribates sp. A
	Scheloribates sp. B
	Zygoribatula bulanovae Kulijew
Galumnoidea	*Protokalumna* sp.
	Pilogalumna sp.

See methods section for exact locations of plots.

fore, any posttreatment differences are in the context of control plots that could mask a decrease (or falsely emphasize an increase.)

Effect on Specific Taxonomic Groups. Numerous species and superfamilies were found in all plots. The fifty-four species in 18 superfamilies are shown in Table 2. The superfamilies and their abundances during the six sampling times over 2 yr are shown in Table 3. To avoid confounding natural abundances and treatment effects, data from control plots only are presented as temporal and geographic baselines. Only adult specimens were included in Table 2 because of the difficulty of identifying immature specimens. Adult abundances at 1 d before treatment were lower than for the other early June samples (7, 364, and 730 d after treatment). Furthermore, the numbers of immature specimens were very low at that time (Table 1).

Eleven superfamilies were common enough to average >10 specimens per plot per sampling time, and only those superfamilies were analyzed for treatment effect. Overall, abundances were greatest at 113 d after treatment; i.e., early October.

Two of the 11 common superfamilies were represented by only one species, whereas the others were represented by two to nine species (Table 2). The dominant superfamily, the Oppiodea, was given prominence by *Oppiella nova* (Oudemans) and an excess of members of *Suctobelbella*. Representatives of the Carabodoidea were also very common; especially common was *Tectocephus velatus* (Michaels). The third most common superfamily was the Nothroidea, largely represented by *Platynothrus "peltifer."*

Except for the three particularly abundant species mentioned above, grouping of species into superfamilies was necessary to provide numbers large enough for meaningful analysis. For any one sampling time, the aggregated nature of oribatids, the apportionment of specimens within a superfamily, and the difficulty of identifying oppioid specimens became important factors in the decision to combine species within superfamilies before analysis.

The P values for compared unsprayed versus sprayed plot means are presented in Table 4. The direction of the difference is indicated by a plus (+) where the treatment mean was larger than the control and a minus (−) where smaller. For 8 of 11 pretreatment superfamilies, the plots that were to be sprayed had the greater mean ($P = 0.05$). Three superfamilies were at or below the conventional 5% level of significance; the Gustavioidea at pretreatment, the Oripodoidea at 7 d after treatment, and the Nothroidea at 113 d after treatment.

Effect on Community Structure. The five statistics of community structure are presented in Table 5. The first three allow paired comparisons of sprayed and unsprayed plots; therefore, the probability of significance of observed differences can be calculated. The fourth and fifth statistics (association and rank correlation of species abundances, which are statistics of comparison) are presented only as an estimate of variation among the five pairs of plots.

The relatively low numbers of specimens during pretreatment sampling are reflected in the species per plot and superfamilies per plot for that time. Both statistics exhibit little difference

Table 3. Pooled oribatid mites collected in control plots only, arranged by superfamily and sampling time

Superfamily	Sampling time, d + treatment					
	−1	+7	+29	+113	+364	+730
Palaeacaroidea	0	0	2	3	0	1
Brachychthonioidea	3	2	3	6	1	4
Phthiracaroidea	13	37	47	33	26	15
Nothroidea	68	74	79	95	49	29
Nanhermannioidea	2	6	19	5	11	11
Hermanniellioidea	1	0	1	2	0	1
Gymnodamaeoidea	1	5	0	0	0	0
Damaeoidea	14	19	30	35	43	9
Cepheoidea	21	27	16	24	6	16
Eremaeoidae	20	25	34	10	24	29
Gustavioidea	11	10	12	18	18	21
Carabodoidea	58	112	87	85	76	109
Oppioidea	133	254	296	317	236	261
Hydrozetoidea	1	0	0	0	0	0
Oribatelloidea	6	17	19	5	11	11
Ceratozetoidea	3	2	10	8	6	6
Oripodoidea	46	68	85	50	58	108
Galumnoidea	0	0	4	0	5	0

The 11 superfamilies that averaged >10 specimens per plot per sampling time are in boldfaced type.

between treatment and control plots at any time throughout the study.

Heterogeneity, as measured by the modified Simpson index, is sensitive to changes in the numbers of the more common species. The maximum value is 1, representing an even distribution of all species; 0 represents a monotypic population. All means are between 0.59 and 0.78, indicating a generally even distribution regardless of time or treatment. Except for 29 and 730 d after treatment, the *P* value for the difference between means is 0.35, or higher than the difference would be by chance alone.

The species association and rank correlation statistics are alternatives to the Simpson index. They show no response to spray treatment. Species association means were very nearly the same at all sampling times and had high standard deviations in all cases. Rank correlation means varied more among periods than species association and most often had high standard deviations.

Oribatid Abundances Versus Residue Amounts at Subsample Sites. Oribatid counts in soil cores immediately adjacent to cores analyzed for residue varied greatly, as did the amount of lindane residue. Following log transformation of both data sets, no significant pattern of effect could be demonstrated.

Discussion

Effects on Groups. Oribatid mites were most abundant 113 d after treatment, particularly immature specimens, which may account for the clearest effects being found at that time. At no other time was there clear evidence of an effect, except that one of two affected superfamilies was reduced at 7 d after treatment. There was a significant reduction of oripodoid adults at 7 d after

Table 4. Exact *P* values for significance of differences of paired comparisons of the 11 most common superfamilies of oribatid mites, arranged by sampling times; + or − indicates increase or decrease in treatment plots compared with control plots

Superfamily	Sampling time, d + treatment					
	−1	+7	+29	+113	+364	+730
Phthiracaroidea	0.82−	0.95−	0.43−	0.56−	0.71−	0.67−
Nothroidea	0.25−	0.60−	0.11−	0.05−	0.52+	0.07−
Nanhermannioidea	0.37+	0.29+	0.54−	0.33−	0.41+	0.46+
Damaeoidea	0.78+	0.26+	0.11+	0.16+	0.61+	0.14+
Cepheoidea	0.48+	0.09−	0.34+	0.95−	0.64+	0.32+
Eremaeoidea	0.31+	0.28−	0.49+	0.78+	0.96+	0.25+
Gustavioidea	0.00+	0.57+	0.23−	0.52+	0.80+	0.75+
Carabodoidea	0.14+	0.57−	0.99−	0.07−[a]	0.95−	0.14−
Oppioidea	0.19+	0.26+	0.20+	0.66+	0.58+	0.23+
Oribatelloidea	0.42+	0.23−	0.49−	0.87+	0.90+	0.27+
Oripodoidea	0.15−	0.05−	0.29−	0.55−	0.60+	0.59−
Ratio[a]	8/3	4/7	4/7	5/6	9/2	7/4

The power of this test was 93%. All other tests that failed to reach the 0.05 significance level had power of less than 80%.
[a] Ratio of treatment plot means greater than control means/treatment plot means that are less than control means.

Table 5. Community structure statistics arranged according to sampling times and plot means or comparative indices of association

	Sampling time, d + treatment											
	−1		+7		+29		+113		+364		+730	
	C	T	C	T	C	T	C	T	C	T	C	T
Species per plot	13.6	13.0	17.4	18.8	20.8	16.8	18.6	19.6	18.6	21.6	16.8	19.6
	$P = 0.76$		$P = 0.65$		$P = 0.42$		$P = 0.69$		$P = 0.22$		$P = 0.44$	
	B = 0.008		B = 0.002		B = 0.064		B = 0.006		B = 0		B = 0.024	
Superfamily/plot	8.6	9.0	9.6	10.0	11.2	9.6	11.0	11.4	10.2	10.8	9.8	11.0
	$P = 0.62$		$P = 0.54$		$P = 0.41$		$P = 0.82$		$P = 0.59$		$P = 0.41$	
	B = 0		B = 0		B = 0		B = 0		B = 0		B = 0.001	
Modified Simpson index	0.69	0.65	0.73	0.61	0.78	0.61	0.72	0.70	0.76	0.70	0.73	0.59
	$P = 0.67$		$P = 0.38$		$P = 0.08$		$P = 0.82$		$P = 0.35$		$P = 0.08$	
	B = 0		B = 0.002		B = 0		B = 0		B = 0		B = 0	
Species association	0.55 ± 0.24		0.61 ± 0.11		0.60 ± 0.15		0.59 ± 0.19		0.63 ± 0.17		0.61 ± 0.22	
Rank correlation	0.30 ± 0.34		0.56 ± 0.08		0.47 ± 0.15		0.46 ± 0.13		0.54 ± 0.18		0.40 ± 0.22	

Exact probabilities of significance are immediately below means for paired comparisons, as are the beta statistics (B) (power = 1 − B). C, Control; T, treatment.

treatment and a significant reduction of nothroid adults at 113 d after treatment, both at the 0.05 level (Table 4). Also, gustavioid adults were very significantly more abundant in pretreatment unsprayed plots than in plots that were to be sprayed, a fact that underlines the importance of pretreatment sampling and devalues the post-treatment information for that superfamily.

When looking for an effect of pesticides on a nontarget species, the null hypothesis (that there is no difference following application) takes on more than its usual function as a straw man to be knocked down, because the acceptance of the null hypothesis can be either the result of no real effect or the masking of a real difference by a large variance. To avoid misinterpretation of accepted null hypotheses, tests must have "adequate power," or as stated by Toft & Shea (1985), ". . . the ability of a test to determine whether the null hypothesis is false." Specifically, adequate power reduces the probability of error of accepting that there is no difference when, in fact, an actual difference has been masked by a high variance. Power is gained by replication or some other device for reduction of the variance. Under the constraint of five replications in this study, intensive subsampling of each plot was used to maximize the efficiency (reduce the observed variance). The power of the tests were obtained by a computer program at the Pacific Southwest Forest and Range Experiment Station, Berkeley, Calif.

The power of a test depends not only on the variance but also on the difference between treatments that one expects to be able to recognize. In this study, the conventional standard of 5% level of significance was used. The study was planned with the assumption that there must be a reduction in the number of oribatids by 80% in spray plots to be biologically important. If one were to use a greater reduction as the threshold of biological importance, the power of the test would be greater in detecting an effect. Alternatively, if one assumed that only a 50% reduction is biologically important, the chance of detection (power of the test) would be less, and more comparisons would be in the inconclusive range. In the cases discussed below, where the null hypothesis has been accepted, the observed variance was low enough to provide a power of 80%.

The estimated power of the tests of total specimens and immatures was less than our criterion of 80% in all but one case, where the P value was more than the conventional 0.05 (Table 1). That one case was the total specimens at 364 d after treatment; the estimated power in that case was 97%. Specifically, there was a 97% chance of finding a difference in means of 80%, allowing one to conclude that for that case, there is good evidence of no effect at that time.

For our study, the effect of lindane, by taxonomic category, can be summarized as follows: total oribatids, immatures, and 2 of 18 superfamilies were significantly reduced ($p = 0.05$) during only one sampling time. Contrarywise, with one exception, the power of all tests was inadequate to conclude that there was no effect, given the criterion of an 80% reduction in population numbers.

This represents a marked difference between the results of our study and those by Hoy (1980), Hoy & Shea (1981), and Hastings et al (1989), in which applications of lindane were heavy, relatively uniformly applied throughout the experimental plots, and in which large effects were found.

In our study, the effect seems to be primarily on the immature specimens and possibly for only the season that lindane was applied. Demon & Eijsackers (1985) found juvenile terrestrial isopods more sensitive than adults to lindane. A similar phenomenon may explain the effect shown by immature oribatids in this study.

Community Structure. Inspired by the statement "... diversity is a system property likely to be a sensitive measure of ecosystem contamination" (Anonymous 1981), this study includes five measures of oribatid diversity in the face of lindane residue. Table 5 shows two measures of species richness, one of heterogeneity, and two of species association. There is no clear evidence that lindane affects community structure. Furthermore, for the first three statistics, the power of the tests was adequate for the conclusion that there is no effect. It may be worth noting that at two posttreatment sampling times, there was a difference in the modified Simpson index at the 0.08 level. Regarding the measures of association, no pattern of effect emerges, but the standard deviations are large in all but one case.

Dindal et al. (1975) found that oribatid species number (richness) and diversity increased following DDT application in an old field; however, the test was not replicated and had no pretreatment sampling. Norton & Sillman (1985), in a carefully structured experiment with pretreatment sampling, found dramatic decreases in species number and diversity following application of oily waste to replicated old-field plots. In light of these studies, as well as the results reported in Hoy (1980) and Hoy & Shea (1981) and the results reported here, it seems that oribatid diversity is a system property that may be sensitive to ecosystem contamination, but the direction of response is unpredictable. The initial abundances of "weed" (opportunistic or pioneer) species is the crucial factor; the investigator's choice of statistics is also important.

In this study, we attempted to find the range of effects of a "patchy" lindane application, both through time and across a clearly defined community. Some effects were observed and some effects were found unlikely to have occurred, based on the estimated power of statistical comparisons.

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