Multiphase Laboratory Bioassays to Select Chemicals for Field-Testing on the Western Spruce Budworm[1,2,3,4]

JACQUELINE L. ROBERTSON AND MICHAEL I. HAVERTY
Pacific Southwest Forest and Range Experiment Station, USDA Forest Service, Berkeley, Calif. 94701

Reprinted from the
JOURNAL OF ECONOMIC ENTOMOLOGY

Multiphase Laboratory Bioassays to Select Chemicals for Field-Testing on the Western Spruce Budworm[1,2,3,4]

JACQUELINE L. ROBERTSON AND MICHAEL I. HAVERTY

Pacific Southwest Forest and Range Experiment Station, USDA Forest Service, Berkeley, Calif. 94701

ABSTRACT

J. Econ. Entomol. 74: 148–153 (1981)

A multiphase bioassay of 10 insecticide formulations was conducted with last instars of *Choristoneura occidentalis* Freeman. Based on assessments of intrinsic toxicity (spray and feeding), residual activity, and rainfastness, the probable decreasing order of efficacy of the formulations is: BAY SIR 8514 (*N*-([trifluoromethoxy) phenyl]carbamoyl)-2-chlorobenzamide), carbaryl (Sevin XLR formulation), thiodicarb, sulprofos, permethrin, carbaryl (Sevin-4-oil formulation), fenitrothion, malathion, phosmet, and phosmet with 2% rhoplex.

Laboratory investigations of various aspects of the toxicities of insecticides to both the western spruce budworm, *Choristoneura occidentalis* Freeman, and the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough), have clearly demonstrated that selecting one chemical over another on the basis of one type of bioassay may result in an incorrect selection. In addition to intrinsic toxicity, germane extrinsic factors that may affect efficacy should be considered before chemicals are recommended for small or large-scale field testing (Robertson and Rappaport 1979).

This study was conducted to select chemicals with the best potential for controlling *C. occidentalis*. Using a multiphase bioassay approach, we rated 10 chemical formulations in order of probable effectiveness under field conditions. The specific bioassays were: contact toxicity—formulations were sprayed directly on larvae; feeding toxicity—technical quality insecticides incorporated into artificial diet were fed to larvae; residual toxicity—formulations were aged for 7 to 14 days after application to potted Douglas-fir, *Pseudotsugata menziesii* (Mirbel) Franco, seedlings; and rainfastness—each formulation was subjected to 1.27-cm of simulated rainfall after application to Douglas-fir seedlings.

Materials and Methods

Insects and Trees

Insects used in all experiments were recently molted 6th stage larvae weighing from 50–100 mg. These were selected from the 79th and 80th generations of a nondiapausing laboratory colony reared as described by Robertson (1979). On each test day, rearing containers were selected at random for each bioassay.

Two-year-old bare root Douglas-fir seedlings were obtained from the USDA Forest Service Humboldt Nursery in December 1978. They were repotted in Berkeley, Calif., and held in an open patio until used in residual activity and rainfastness experiments.

Insecticides and Treatment Procedures

Ten insecticide formulations were tested. In the spray, residual, and rainfastness tests, these were: carbaryl—Sevin-4-oil® and Sevin XLR®; permethrin—Pounce® 3.2; phosmet—Imidan® 1E and Imidan® 1E with 2% rhoplex, BAY SIR 8514 25 WP (*N*-([trifluoromethoxy)phenyl]carbamoyl)-2-chlorobonzamide); sulprofos—Bolstar® 6E; malathion—Cythion® 5E; fenitrothion—Sumithion® 8E with 2% rhoplex; and thiodicarb—UC 51762 4F. All were formulated in water except Sevin-4-oil, which was formulated in diesel oil. In feeding tests, technical quality insecticides were dissolved in acetone (carbaryl, permethrin, phosmet, sulprofos, and fenitrothion), acetone:water (1:1) (malathion), or dimethyl formamide (BAY SIR 8514). The thiodicarb 4F formulation, however, was diluted with acetone:water (1:1) because technical material was not available.

A stock solution of each toxicant was formulated on the basis of wt/vol AI, then serially diluted to the desired concentrations. One % oil red dye was added to oil formulations and 1% aniline blue added to water formulations; these dyes were used for colormetric assessments of spray deposits in the spray, residual toxicity, and rainfastness experiments. In the feeding toxicity experiment, 1% methylene blue dye was added to each formulation to permit visual assurance of thorough mixing of toxicants in aliquots of reliquified artificial diet. A new stock solution was prepared for each replication of each experiment. The 1st replication for each chemical in a given experiment was completed before the 2nd replication was begun.

Sprays were applied to insects or seedlings with the Moellman spray chamber (Robertson et al. 1979). Parameters of application were a 0.25-ml delivery volume expelled through the nozzle at 704 g/cm^2, 60-sec exposure of spray targets, and an average spray deposit volume of 9.35 liters/ha (1 gal/acre). For deposit assessment, a filter paper disc, 9 cm diam, was placed in each treatment dish containing insects to be sprayed; a petri dish cover containing the filter paper disc was placed at the base of each seedling to be sprayed. The amount of dye on each disc was determined colorimetrically (Rayner 1956) following elution with distilled water (aniline blue deposits) or reagent grade toluene (oil red deposits). The insecticide dosage was then calculated in g/ha from the dye deposit.

Contact and Feeding Toxicity

The selection of dosages or concentrations for each experiment was made from preliminary exposures of insects to a range of logarithmic dilutions for each chem-

[1] Lepidoptera: Tortricidae.

[2] Work leading to this publication was funded, in part, by a USDA Forest Service-sponsored program entitled Canada/United States Spruce Budworms Program.

[3] Received for publication Apr. 16, 1980.

[4] This paper does not recommend the chemicals used, nor does it imply that the uses discussed here have been registered by any agency.

0022-0493/81/0201-4806$02.00/0

ical. Each dosage or concentration was sprayed or fed to 10 insects. Eight dosages, encompassing mortalities from 5 to 99%, were then selected for each insecticide. Once the 8 dosages or concentrations were selected, the full experiment was completed by at least 3 replications, each performed on a different day. For each replication, 20 insects/dosage or concentration were treated. A control group was included with each replication for every chemical. For contact toxicity experiments, the dosage actually deposited was calculated for each group of insects treated. Mortality data were analyzed with the dosage deposited rather than the dosage applied.

For feeding toxicity assessments, toxicants were added to reliquified artificial diet (Robertson 1978). A 10-ml aliquot of liquid diet was placed in each 29.6 ml plastic jelly cup. Two hundred μl of the toxicant solution were added to the diet with a Micropettor®, then the diet+toxicant mixture was stirred thoroughly with a small spatula. A new Micropettor capillary tube and spatula were used for each chemical; the teflon Micropettor plunger was washed with clean solvent to prevent cross-contamination between chemicals. After the diet had hardened, the jelly cup was cracked and removed, and the diet aliquot was placed in a 100×20-mm sterile plastic petri dish lined with filter paper.

Mortality was tallied 7 days after treatment except in experiments with the molt inhibitor BAY SIR 8514. For this candidate, it was necessary to delay observations until the pupal-adult molt or until mortality was evident in larvae or pupae. During the posttreatment observation period, sprayed larvae were provided with artificial diet. In the feeding tests, the 10-cc diet aliquot provided enough food for 7 days, or until pupation. Dose-mortality data based on dosage deposited or concentration in the diet were analyzed with computer program POLO (Russell et al. 1977) using the probit model. The toxicities of the 2 formulations of either carbaryl or phosmet were statistically compared by likelihood ratio tests (Savin et al. 1977) at the $\alpha = 0.05$ level.

Residual Toxicity

Two concentrations, those sufficient to provide the LD_{50} and LD_{90} determined from the contact toxicity bioassay, were tested for each chemical. Each chemical+dosage was applied to a group of Douglas-fir seedlings which were then aged in greenhouse, at ca. 20°C, for either 0, (within 1 h after spray), 7, or 14 days. A grid pattern was devised to divide the counterspace in the greenhouses into 25.4-cm squares. Each seedling was then randomly placed on the grid.

A randomized complete-block design was used, with all treatment combinations replicated in each of 5 consecutive weeks (blocks). The order in which the 10 formulations and 2 controls (oil and water) were applied was randomized. Six trees were randomly assigned to each insecticide-application rate or control; 2 of the 6 trees were then randomly assigned to each postspray aging interval within each treatment group. Within each formulation group, the trees receiving the LD_{50} were sprayed first, the LD_{90} last. This arrangement minimized necessary cleaning of the spray chamber since cleaning only had to be done between formulations and not between treatments.

Insects were caged on seedlings after the appropriate aging interval. Cages made of cotton voile, ca. 46 cm high and 15 cm wide, were placed on each seedling, then cinched around the stem with a rubber band. After 10 insects had been placed within, the top of the cage was cinched shut with a rubber band.

With the conventional insecticides, percent mortality was recorded after 5 days. In the BAY SIR 8514 treatments, larvae were allowed to completely consume the foliage of the treated trees, then they were transferred to artificial diet for the duration of their development to the adult stage, or until mortality was evident in larvae or pupae. Percent mortality data (x) were subjected to a 2-way analysis of variance after transformation by arcsin $\sqrt{x}$. Significant differences between the transformed treatments were tested with Scheffe's test of means (Guenther 1964) at the $\alpha = 0.05$ level.

Rainfastness

The same application rates for each chemical were used in this bioassay as were used in the residual toxicity bioassay. The design used for this experiment was a randomized complete-block. The experiment was performed in 5 blocks, 1/week for 5 consecutive weeks. In each block, 4 trees were randomly assigned to each insecticide treatment; 2 received rainfall and 2 received no rainfall. The order in which the 10 formulations and 2 control treatments were applied was randomized in each block. For the treatments within each formulation, those receiving an LD_{50} were sprayed first, followed by those receiving an LD_{90}. Trees receiving rainfall were sprayed with insecticide, allowed to dry for 2 h, then subjected to simulated rainfall equivalent to 1.27 cm (Robertson and Boelter 1979). One h after application of rainfall, 10 insects were caged on each tree. Trees not receiving rainfall were sprayed with insecticide and allowed to dry for 3 h; 10 insects were then caged on each tree (see Residual Toxicity).

Percent mortality was recorded after 5 days for conventional toxicants, or until adult emergence for BAY SIR 8514. Percent mortality data (x) were subjected to a 2-way analysis of variance after transformation by arcsin $\sqrt{x}$. Significant differences between transformed means for rainfall vs. no rainfall within the same insecticide treatment, and between transformed means of all treatments and their respective controls were tested with Scheffe's test of means at the $\alpha = 0.05$ level.

Rating System for Recommendations

A scoring system for the 3 primary efficacy factors quantified in our experiments was developed to assess performance. These 3 factors were intrinsic toxicity, residual toxicity, and rainfastness. We considered intrinsic toxicity to be composed of equal contributions from contact (spray) and feeding components. Scores for spray performance and performance when ingested were, therefore, one-half the point values assigned to either the residual toxicity or rainfastness results. One of 4 point values was assigned each treatment for each of the 4 experiments (Table 1). Insecticide formulations were rated from high probability of success to low probability of success by summing the point value for each of the 4 experiments and rating them from high to low.

Table 1.—Criteria for scoring toxicological effectiveness.

Score (points)	Intrinsic Toxicity: Spray Toxicity	Intrinsic Toxicity: Feeding Toxicity	Residual Toxicity	Rainfastness
High (100)	LD_{50} and LD_{90} < 7 g/ha (50)	LC_{50} 1–10 ppm (50)	≥90% mortality at LD_{90} and ≥50% mortality at LD_{50}; <10% decrease in mortality at both LD_{50} and LD_{90} application rates by 14 days postspray.	No significant difference with application of 1.27 cm rainfall with both LD_{50} and LD_{90} application rates.
Medium (70)	LD_{50} > 7 g/ha, < 70 g/ha; LD_{90} between 70–700 g/ha (35)	LC_{50} 11–20 ppm (35)	≥90% mortality at LD_{90} and ≥50% mortality at LD_{50}; <10% decrease in mortality at both LD_{50} and LD_{90} by 7 days postspray, but >10% decrease by 14 days	Significant decrease with 1.27-cm rainfall at either application rate; decrease <59% of mortality observed with no rainfall.
Low (30)	LD_{50} > 70, < 700 g/ha	LC_{50} 21–40 ppm	>90% mortality at LD_{90} or >50% at LD_{50}, >10% decrease in mortality at either application rate after 7 days.	Significant decrease with 1.27-cm rainfall at either application rate; decrease 60% of mortality observed with no rainfall.
None (0)	LD_{50} > 700 g/ha	LC_{50} > 40 ppm	<90% mortality at LD_{90} and <50% mortality at LD_{50} application rates.	Significant decreases with 1.27-cm rainfall at both application rates.

We realize that our criteria for scoring are arbitrarily established but represent what we consider to be the important components in laboratory evaluation of insecticides. Other investigators may find it more useful to use different criteria or distribute the points differently.

Results

Contact Toxicity

The relative contact toxicity at LD_{50} (Table 2, A) of the 10 formulations was (index of relative contact toxicity = LD_{50} for the least toxic formulation, carbaryl in the Sevin-XLR formulation, divided by the LD_{50} for formulation x): permethrin, 184; BAY SIR 8514, 5.2; sulprofos, 4.7; fenitrothion with 2% rhoplex, 4.6; carbaryl in the Sevin-4-oil formulation, 3.7; phosmet, 3.1; malathion, 1.9; phosmet with 2% rhoplex, 1.6; thiodicarb, 1.3; and carbaryl in the Sevin-XLR formulation, 1.0.

Because of the wide variation in slopes exhibited by the formulations, the relative toxicity at LD_{90} differed from that at LD_{50}. The order of relative contact toxicity at LD_{90} was: permethrin, 208; sulprofos, 6.2; fenitrothion with 2% rhoplex, 5.3; phosmet, 3.8; BAY SIR 8514, 2.3; carbaryl in the Sevin-4-oil formulation, 1.9;

Table 2.—Toxicity of 10 insecticides formulations to 6th instar western spruce budworm.[a]

Insecticide	N	NC	C±SE	Slope±SE	LD_{50}	95% CL	LD_{90}	95% CL
A. Contact Toxicity[b]								
Permethrin	619	80	0±0	3.74±0.28	1.3	0.97–1.8	2.7	1.9–6.6
BAY SIR 8514[c]	480	60	0.096±0.036	1.76±0.28	45.9	34.6–58.0	246	181–387
Sulprofos	480	60	0±0	5.11±0.42	50.4	43.0–58.0	89.8	75.6–121
Fenitrothion	479	60	0±0	4.07±0.35	51.9	43.0–60.5	107	87.0–155
Carbaryl (Sevin-4-oil)	630	80	0.012±0.012	1.95±0.22	64.7	49.2–78.2	294	225–452
Phosmet	480	60	0.016±0.015	4.47±0.38	77.1	69.8–83.9	149	135–1000
Malathion	619	81	0±0	3.17±0.26	124	107–140	314	274–377
Phosmet (with rhoplex)	479	60	0.037±0.019	3.60±0.36	148	133–163	336	290–412
Thiodicarb	630	80	0±0	2.70±0.25	178	145–244	530	346–1270
Carbaryl (Sevin XLR)	639	80	0.013±0.34	3.40±0.34	239	214–266	564	464–802
B. Feeding Toxicity[d]								
BAY SIR 8514[c]	639	80	0.237±0.047	1.32±0.15	1.1	7.3–17.3	10.6	7.3–17.3
Permethrin	640	80	0±0	3.22±0.21	3.4	2.9–3.9	8.5	7.2–10.7
Sulprofos	641	81	0.022±0.015	3.82±0.29	5.3	4.7–5.8	11.4	10.3–12.8
Fenitrothion	320	80	0±0	7.10±0.74	7.9	7.3–8.4	11.9	11.1–13.2
Malathion	459	80	0±0	4.52±0.33	30.8	25.9–36.5	59.2	48.7–77.6
Carbaryl	637	80	0.010±0.010	2.58±0.21	35.6	17.2–52.2	112	77.7–205
Thiodicarb	480	60	0±0	3.82±0.33	67.4	61.0–73.4	146	131–168
Phosmet	620	80	0±0	4.58±0.32	67.9	51.5–83.1	129	104–190

[a] Column headings are: N = number of insects treated; NC = number of controls; C±SE = estimated natural mortality ± SE; Slope ± SE = slope ± SE; 95% CL = 95% confidence limits.
[b] Dosage expressed in g/ha.
[c] Mortality tallied at adult eclosion or until mortality was evident in larvae or pupae. With all other chemicals, mortality was tallied after 7 days.
[d] Lethal concentration in ppm.

malathion, 1.8; phosmet with 2% rhoplex, 1.7; thiodicarb, 1.1; and carbaryl in the Sevin-XLR formulation, 1.0.

We compared the response lines for phosmet and phosmet with 2% rhoplex by using likelihood ratio tests and found that they were not equal but parallel. This means the insects responded to the phosmet formulations similarly, but with different threshold dosages for a response. When the 2 formulations of carbaryl (Sevin-4-oil and Sevin XLR) were compared we found the response was totally different, i.e., not even parallel.

In decreasing order, scores for spray toxicity were: permethrin—50 points; BAY SIR 8514, sulprofos, carbaryl in the Sevin-4-oil formulation, and fenitrothion—35 points; carbaryl in the Sevin XLR formulation, thiodicarb, malathion, phosmet, and phosmet with 2% rhoplex—15 points.

Toxicity of Insecticides by Feeding

The toxicities of the insecticides by feeding also demonstrated shifts in relative effectiveness at LC_{50} and LC_{90} because of variation in slopes (Table 2, B). At LC_{50}, the order of relative effectiveness (index of relative feeding toxicity = LC_{50} of the least active chemical ÷ LC_{50} of chemical x): BAY SIR 8514, 61.7; permethrin, 20.0; sulprofos, 12.8; fenitrothion, 8.6; malathion, 2.2; carbaryl, 1.9; thiodicarb, 1.0; and phosmet, 1.0. At LC_{90}, the relative order of effectiveness was: permethrin, 17.2; BAY SIR 8514, 13.8; sulprofos, 12.8; fenitrothion, 12.3; malathion, 2.4; carbaryl, 1.3; phosmet, 1.1; and thiodicarb, 1.0.

In decreasing order, scores for feeding toxicity were BAY SIR 8514, sulprofos, permethrin, and fenitrothion—50 points; carbaryl (both formulations) and malathion—15 points; thiodicarb, phosmet and phosmet with 2% rhoplex—0 points.

Residual Toxicity

The significant differences ($P < 0.05$) we observed in percent mortality depended on postspray aging period ($F = 59.10$; df = 2 and 582), chemical treatment ($F = 77.09$; df = 21 and 582), but not interaction of these experimental factors ($F = 2.78$; dg = 42 and 582) (Table 3).

Within the 0-time aging interval, each insecticide treatment resulted in significantly greater ($P < 0.05$) mortality than its comparable control group except permethrin applied at 1.6 g/ha and Imidan at 73.6 g/ha. The order of increasing effectiveness at the LD_{50} application rate (deviation from expected 50% mortality in parentheses) was: phosmet (−22), permethrin (−18), phosmet with 2% rhoplex (−13), fenitrothion with 2% rhoplex (+15), sulprofos (+17), carbaryl in the Sevin XLR formulation (+20), malathion (+22), carbaryl in the Sevin-4-oil formulation (+26), BAY SIR 8514 (+44), thiodicarb (+48). At the LD_{90} application rate, observed mean mortalities ranged from 52 to 99.5%. The increasing order of effectiveness at this application rate (deviation from expected 90% mortality in parentheses) was: phosmet with 2% rhoplex (−38), phosmet (−37), permethrin (−27), malathion (−9), sulprofos (−5), fenitrothion with 2% rhoplex (−2), carbaryl as Sevin-4-oil (+5), carbaryl as Sevin XLR (+6.7), BAY SIR 8514 (+9), thiodicarb (+9.5).

Table 3.—Residual toxicity on insecticide sprays on trees to 6th instar western spruce budworm (N = 8 to 10 trees, each with 10 insects caged at designated postspray interval).

Formulation	Dosage g/ha	Dosage (oz/acre)	Expected 0-time LD level(%)	Postspray weathering interval (days) 0	7	14
				Percent mortality (mean+SD)		
Water (control)	0	0	0	2.0±4.2	2.0±4.2	3.3±5.0
Diesel oil (control)	0	0	0	2.5±4.2	5.0±7.1	3.0±4.8
Carbaryl	68.7	0.98	50	76.0±15.8	42.0±19.3	46.0±22.7
(Sevin-4-oil)	294.2	4.2	90	96.0±9.7	84.0±22.7	70.0±27.4
Permethrin	1.6	0.023	50	32.0±20.4	16.0±11.7	9.1±8.8
	3.2	0.046	90	63.0±27.1	31.0±27.8	18.0±17.5**
Thiodicarb	173.0	2.47	50	98.0±3.5	98.0±4.2	98.0±4.2
	532.4	7.60	90	99.5±1.6	95.0±9.7	99.0±3.2
BAY SIR 8514	56.0	0.80	50	94.4±17.7	100±0	100±0
	252.2	3.60	90	99.0±3.2	100±0	100±0
Fenitrothion with 2% rhoplex	53.2	0.76	50	65.0±25.5	27.0±21.7	20.0±21.6
	106.5	1.52	90	88.0±9.2	41.8±20.0*	35.0±25.9**
Phosmet with 2% rhoplex	151.3	2.16	50	37.0±18.3	46.7±24.2	21.0±13.7
	340.5	4.86	90	52.0±16.2	55.0±14.3	39.3±26.2
Carbaryl (Sevin XLR)	239.6	3.42	50	70.0±24.5	59.0±26.0	64.0±21.7
	567.5	8.10	90	96.7±7.1	93.0±12.5	86.0±17.1
Malathion	127.5	1.82	50	72.2±12.0	20.0±14.9	11.3±8.3**
	318.8	4.55	90	81.0±16.0	39.2±21.1*	36.0±24.1
Sulprofos	50.4	0.72	50	67.0±27.9	54.0±15.1	22.0±17.5**
	88.3	1.26	90	85.0±15.1	61.1±34.4	64.4±13.3
Phosmet	73.6	1.05	50	28.0±26.6	20.0±12.5	5.6±7.3
	147.1	2.10	90	53.0±26.3	25.0±17.8	31.0±21.3

* Statistically significant difference between mortality observed after 7 days postspray weathering and mortality immediately postspray.
** Statistically significant difference between mortality observed after 14 days postspray weathering and mortality immediately postspray.

At the 7-day postspray aging interval, several treatments did not differ significantly ($P < 0.05$) from their control (water). These are: permethrin applied at 1.6 and 3.2 g/ha, malathion at 127.5 g/ha, and phosmet at 73.6 and 147.1 g/ha. At the 14-day postspray aging interval, the mortality observed with treatment of permethrin applied at 1.5 and 3.2 g/ha, fenitrothion with 2% rhoplex at 53.2 and 106.5 g/ha, phosmet with 2% rhoplex at 151.3 and 340.5 g/ha, malathion at 127.5 and 318.8 g/ha, sulprofos at 50.4 g/ha, and phosmet at 73.6 and 147.1 g/ha did not differ significantly ($P < 0.05$) from the control (water) treatment. Carbaryl, as Sevin-4-oil applied at 68.7 g/ha, did not differ significantly ($P < 0.05$) from the diesel oil control.

When each treatment was compared at successive postspray weathering intervals, only 2 significant decreases were noted ($P < 0.05$). Mortality observed 7 days after application of fenitrothion at 106.5 g/ha or malathion at 318.8 g/ha was significantly less than that observed immediately postspray. When mortality immediately postspray was compared to that observed after 14 days, the effects of permethrin applied at 3.2 g/ha, fenitrothion with 2% rhoplex at 106.5 g/ha, or malathion at 318.8 g/ha was significantly less than that observed immediately postspray. When mortality 7 days postspray was compared to that observed at 14 days, the effects of permethrin applied at 3.2 g/ha, fenitrothion with 2% rhoplex at 106.5 g/ha, malathion at 127.5 g/ha, and sulprofos at 50.4 g/ha had decreased significantly ($P < 0.05$).

Scores for residual toxicity were: BAY SIR 8514, carbaryl as Sevin XLR, and thiodicarb—100 points; sulprofos, carbaryl as Sevin-4-oil, fenitrothion and malathion—30 points; permethrin, phosmet, and phosmet with 2% rhoplex—0 points.

Rainfastness

Significant differences ($P < 0.05$) in means of percent mortality were related to rainfall application ($F = 207.69$; df = 1 and 389), chemical treatment ($F = 65.17$; df = 21 and 389), and interaction of these 2 factors ($F = 5.02$; df = 21 and 389) (Table 4).

Within the group subjected at 1.27-cm rainfall, the mortality observed with some treatments did not differ significantly from that observed with application of the control solution. These treatments were: permethrin applied at 1.6 g/ha, fenitrothion with 2% rhoplex at 53.2 g/ha, phosmet with 2% rhoplex at 151.3 g/ha, malathion at 127.5 and 318.8 g/ha, and phosmet at 73.6 and 147.1 g/ha. In all instances within the group not receiving simulated rainfall, insecticide application resulted in mean percent mortality significantly greater than that of the appropriate control ($P < 0.05$).

When the effects of rainfall application on each insecticide treatment were compared to the effects of no rainfall, significant decreases in mortality due to the rainfall were observed with carbaryl (Sevin-4-oil) applied at 68.7 g/ha, permethrin at 3.2 g/ha, fenitrothion with 2% rhoplex at 53.2 g/ha, phosmet with 2% rhoplex at 151.3 and 340.5 g/ha, malathion at 127.5 and 318.8 g/ha, and phosmet at 147.1 g/ha.

Scores for rainfastness were: BAY SIR 8514, carbaryl in the Sevin XLR formulation, thiodicarb, and sulprofos—100 points; permethrin and carbaryl in the Sevin-4-oil formulation—70 points; fenitrothion and phosmet—30 points; malathion and phosmet with 2% rhoplex—0 points.

Discussion

Our investigation demonstrates the potential for error in recommending field experiments solely on the basis of the results of a single type of laboratory bioassay. Were we to make our recommendations from just one of the 4 assessments in the present investigation, the candidates recommended might vary drastically. For example, permethrin was outstanding in its direct contact toxicity, while thiodicarb and carbaryl in the Sevin XLR formulation were poor. Were contact effectiveness used as our only criterion, thiodicarb and the carbaryl Sevin XLR formulation would not be considered for further testing. If residual toxicity were the single criterion, thiodicarb and carbaryl in the Sevin XLR formulation would appear outstanding and permethrin might not be considered.

In general, we conclude that the experimental approach used in this study provides sufficiently broad toxicological profiles of the candidate materials to make scientifically sound recommendations. Completion of a multiphase bioassay before any field testing would improve the basis for field testing at minimum cost.

Using our scoring system, the overall decreasing order of the 10 formulations was BAY SIR 8514—285 points; carbaryl in the Sevin XLR formulation—230 points; thiodicarb and sulprofos—215 points; permethrin—170 points; carbaryl in the Sevin-4-oil formulation—150 points; fenitrothion—145 points; malathion—60 points; phosmet and phosmet with 2% rhoplex—15 points. The molt inhibitor BAY SIR 8514 demonstrated the best combination of contact, feeding, and residual toxicity. Its residual toxicity and rainfastness were outstanding and direct toxicity moderate to high. Of the 10 candidates BAY SIR 8514 appears to merit highest priority for field testing. Carbaryl, the active ingredient of both the Sevin-4-oil and Sevin XLR formulations, is registered for control of the western spruce budworm as the Sevin-4-oil formulation. Both carbaryl formulations are low to moderate in direct contact and feeding toxicity. Sevin XLR was outstanding in residual toxicity and rainfastness, but Sevin-4-oil was low to moderate. The Sevin XLR formulation, applied in water, appears to be a good choice for field testing. Thiodicarb and sulprofos are also likely choices for field testing. Thiodicarb was minimally toxic by direct contact and ingestion but outstanding in residual toxicity and rainfastness. Sulprofos was moderately to highly toxic by contact and ingestion, outstanding in rainfastness, but low in residual toxicity. Permethrin is highly toxic by contact and ingestion; however, it appears to be inactivated by Douglas-fir foliage (Robertson and Rappaport 1979).

Field dosages may have to be raised to compensate for the poor residual toxicity and rainfastness observed in our experiments. Fenitrothion was moderately to highly toxic in spray and feeding bioassays, while its residual toxicity and rainfastness were low. Malathion had low direct toxicity and residual toxicity, and no apparent rainfastness. The 2 lowest rated formulations,

Table 4.—Effects of rainfall application on observed toxicity of insecticides to 6th instar western spruce budworm (N = 8 to 10 trees, each with 10 insects.

Formulation	Dosage		Percent mortality (mean±SD)	
	(g/ha)	(oz/acre)	No rainfall	1.27-cm rainfall
Water-control	0	0	4.0±5.2	2.5±4.3
Diesel oil-control	0	0	4.5±6.0	2.5±4.3
Carbaryl (Sevin-4-oil)	68.7	0.98	75.5±17.1	32.0±18.1*
	294.2	4.20	98.0±4.2	87.0±13.4
Permethrin	1.6	0.023	27.0±9.5	15.0±14.3
	3.2	0.046	69.0±23.8	32.0±28.2*
Thiodicarb	173.0	2.47	95.0±7.1	78.0±22.0
	532.4	7.60	99.0±3.2	92.0±12.3
BAY SIR 8514	56.0	0.80	100±0	80.0±20.8
	252.2	3.60	99.0±3.2	99.0±3.2
Fenitrothion with 2% rhoplex	53.2	0.76	57.0±23.1	21.0±16.6*
	106.5	1.52	76.7±18.0	50.0±29.4
Phosmet with 2% rhoplex	151.3	2.16	45.0±27.2	10.0±12.5*
	340.5	4.86	58.0±11.4	25.0±17.8*
Carbaryl (Sevin XLR)	239.6	3.42	80.0±22.1	69.0±16.0
	567.5	8.10	95.0±7.1	87.0±14.2
Malathion	127.5	1.82	69.0±24.2	11.0±9.9*
	318.8	4.55	85.0±12.7	3.0±13.2*
Sulprofos	50.4	0.72	64.4±20.7	54.0±27.2
	88.3	1.26	92.0±6.3	67.0±17.7
Phosmet	73.6	1.05	23.0±9.5	12.0±16.2
	147.1	2.10	39.0±23.3	9.0±12.9*

* Statistically significant difference between rainfall and no rainfall group.

phosmet and phosmet with 2% rhoplex, demonstrated low spray and poor feeding toxicities. Their residual toxicities and rainfastness were poor. Phosmet in either formulation appears to be an extremely poor candidate for future testing against the western spruce budworm.

We recognize that the results from the bioassays we have described are not the only criteria to be considered in evaluating insecticides for field testing on western spruce budworm populations. Certainly, the effects of candidate materials on environmental and human safety will heavily influence the selection of an insecticide. Unless there is a substantial body of safety data already available, environmental problems can often be evaluated only after field testing. However, it may be possible to eliminate an insecticide from consideration from laboratory bioassays if its environmental profile is undesirable. The economics of insecticide production change. This, coupled with dependence of cost on the amount of insecticide required to accomplish the desired level of control, makes cost another variable to be considered. However, these economic and environmental considerations were beyond the scope of this investigation.

Previous laboratory investigations (e.g., Lyon et al. 1972, Robertson et al. 1975) were limited to topical or spray applications which were too simplistic to provide more than a means of ranking chemicals at a specific level of effect. Multiple bioassays such as those which we describe here should provide sufficient data for entomologists to select the most promising insecticides for future field experiments and to estimate the application rates which would be necessary for the desired level of control.

Acknowledgment

Samples of the formulations tested were supplied by American Cyanamid Co., FMC Corp., Mobay Chemical Corp., Stauffer Chemical Co., and Union Corp.

REFERENCES CITED

Guenther, W. C. 1964. Analysis of Variance. Prentice-Hall, New York. 199 pp.

Lyon, R. L., S. J. Brown, and J. L. Robertson. 1972. Contact toxicity of sixteen insecticides applied to forest tent caterpillars reared on artificial diet. J. Econ. Entomol. 65: 928–30.

Rayner, A. C. 1956. Colorimetric estimation of dye insecticide spray deposit using a paper sampling surface. Can. Entomol. 88: 279.

Robertson, J. L. 1978. Feeding tests of insecticides to western spruce budworm. Insecticide Acaricide Tests. 3: 145.

1979. Rearing the western spruce budworm. USDA Misc. Publ. Canada/United States Spruce Budworms Program: 18 pp.

Robertson, J. L., and L. M. Boelter. 1979. Toxicity of insecticides to Douglas-fir tussock moth *Orgyia pseudotsugata* (Lepidoptera Lymantriidae): II. Residual activity and rainfastness. Can. Entomol. 111: 1161–75.

Robertson, J. L., and N. G. Rappaport. 1979. Direct, indirect, and residual toxicities of insecticides sprays to western spruce budworm *Choristoneura occidentalis* (Lepidoptera:Tortricidae). Ibid. 111: 1219–16.

Robertson, J. L., R. L. Lyon, R. L. Andrews, E. E. Moellman, and M. Page. 1979. Moellman spray chamber: versatile research tool. U. S. For. Serv. Res. Note PSW 337: 6 pp.

Robertson, J. L., R. L. Lyon, and M. Page. 1975. Toxicity of selected insecticides to pests of western hemlock. J. Econ. Entomol. 68: 192–6.

Russell, R. M., J. L. Robertson, and N. E. Savin. 1977. POLO: a new computer program for probit analysis. Bull. Entomol. Soc. Am. 23: 209–13.

Savin, N. E., J. L. Robertson, and R. M. Russell. 1977. A critical evaluation of bioassay in insecticide research: likelihood ratio tests of dose-mortality regression. Ibid. 23: 257–66.