DIRECT, INDIRECT, AND RESIDUAL TOXICITIES OF INSECTICIDE SPRAYS TO WESTERN SPRUCE BUDWORM, *CHORISTONEURA OCCIDENTALIS* (LEPIDOPTERA: TORTRICIDAE)[1]

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Abstract

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The toxicities of acephate, aminocarb, carbaryl, chlorpyrifos, chlorpyrifos-methyl, methomyl, mexacarbate, permethrin, and trichlorfon to last instar western spruce budworm, *Choristoneura occidentalis* Freeman, were significantly altered by the presence of host-plant foliage. The pyrethroid permethrin was significantly more toxic when sprayed directly on fully exposed larvae than when first sprayed on foliage. However, all other toxicants were more toxic sprayed on foliage. A toxicologically-based method was used to assess the residual toxicities of the nine chemicals. Each chemical was applied to potted seedlings at its respective LD_{90}-foliage, then weathered for up to 7 days. Significant differences in toxicity were related to both insecticide and weathering interval. The toxicities of carbaryl and permethrin were least affected by weathering.

In insecticide research, laboratory bioassays are often used to define variables pertinent to toxicological effectiveness (e.g. Fisher and Menzies 1979). Such bioassays are predicated on the assumption that precise definitions of as many toxicological variables as possible will permit realistic forecasts of efficacy in field applications, or explain effects observed during field experiments.

We report here an investigation of the effects of two extrinsic toxicological variables—the presence of host-plant foliage, and postspray weathering—on the toxicities of nine insecticides to the western spruce budworm, *Choristoneura occidentalis* Freeman. An improved, realistic method for assessing residual toxicities in the laboratory is proposed.

Materials and Methods

Sixth instars were selected from a nondiapausing laboratory colony reared on artificial diet (Lyon *et al.* 1972). Only those weighing 30–70 mg were used, so that sufficient time remained for them to actively forage before pupation.

Sprays were applied with a stainless steel spray chamber (Robertson *et al.* 1979) as described by Robertson and Boelter (1979*a*). A volume equivalent to 9.3 L/ha was sprayed routinely. Spray targets were exposed to sprays for exactly 60 sec. Deposits were measured colorimetrically by using oil red dye in oil formulations and aniline blue in water formulations. Dosage was then calculated (g/ha). Insecticides selected for testing had been field-tested previously on western spruce budworm, or were regarded as candidates for field tests. These toxicants and formulations were:

Insecticide	Formulation	Solvent
Acephate	Orthene 75S	Water
Aminocarb	Matacil 1.4 EC	Diesel oil
Carbaryl	Sevin-4-oil	Diesel oil
Chlorpyrifos	Dursban-M3633 EC	Diesel oil
Chlorpyrifos-methyl	Dowco-214 M3196 EC	Diesel oil
Permethrin	FMC 33297 3.2 EC	Diesel oil
Methomyl	Lannate 1.8	Water
Mexacarbate	FS 15	TPM:kerosene (1:9)*
Trichlorfon	Dylox 1.5 L	Orchex 796

*TPM is tripropyleneglycol monomethyl ether.

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

Fresh solutions were prepared for each replication of each experiment on the basis of weight/volume concentrations of the active ingredient.

Intrinsic, direct, toxicity was tested by spraying groups of 10 larvae which had been anesthetized with CO_2. During spray application, the insects were held in 100×15-mm petri dishes lined with filter paper. For each replication, a minimum of 4 dosage levels of each insecticide were tested on at least 20 insects/dosage. Each test was replicated 3 times. A control group of 10 insects sprayed with solvent and dye was included in each replication. Immediately after spray application, larvae were transferred to 100×15-mm sterile plastic petri dishes lined with filter paper. They were fed artificial diet. Mortality was tallied after 7 days.

The effect of an extrinsic toxicological variable—host-plant foliage—was tested by spraying branchlets of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco. The branchlets were inserted into a pot of soil and thus held upright during spray application. To determine indirect toxicity, a minimum of five dosage levels were tested in each replication with each insecticide; at least 3 replications, each with 20 larvae, were performed with two branchlets per dosage. Immediately after spraying, each branchlet was inserted into a 15.2×40.6-cm nylon organdy cage. Ten larvae were placed on the foliage; the cage was then closed with rubber bands and suspended from a clothes line. Mortality counts were made after 4 days.

In experiments to define the effects of a second extrinsic variable—postspray weathering—each formulation was applied to potted 2-year-old Douglas-fir seedlings at a dosage approximately equal to its LD_{90} as determined by the sprayed-branchlet study. Control seedlings were sprayed with solvent and dye only. Four trees were sprayed for each weathering interval. These intervals were immediately postspray (0), and 1, 3, and 7 days thereafter. Weathering occurred outdoors in a patio area where environmental factors were not controlled. No rain fell during the weathering periods, but trees were watered daily at their bases. After the designated weathering interval, 25 insects were caged on the foliage of each seedling. Mortality was tallied after 5 days.

Data from the direct and indirect spray tests were analyzed by probit analysis (Russell *et al.* 1977). The dose–mortality regression lines for each chemical sprayed directly and indirectly were compared by likelihood ratio tests (Savin *et al.* 1977). The results of the postspray weathering, residual toxicity tests were analyzed by two-way analysis of variance and Scheffe's test of means (Guenther 1964).

Results

The dosage–response line for each insecticide applied directly to larvae differed from the line resulting from indirect application to Douglas-fir foliage (Table I). When the effects of direct application were compared with those of indirect application at LD_{50}, toxicity ratios from ca. 0.10 to ca. 9.0 were observed. Permethrin was the only chemical with a LD_{50} ratio of indirect to direct application of less than 1.0; for all others, the LD_{50} for direct application was higher than that for indirect application.

Such LD_{50} comparisons indicate relative differences between direct and indirect application, but they do not define the significance of the differences. This statistical information is provided by the likelihood ratio test. Each dosage–response line has two characteristics, the slope and the intercept. The likelihood ratio test for parallelism tests the hypothesis that the slopes of two or more lines are statistically equivalent. Application of this test to our data demonstrated that the direct, intrinsic response lines were parallel ($p > 0.05$) to the indirect, foliage treatment response lines in experiments with acephate, aminocarb, carbaryl, methomyl, and trichlorfon.

The slopes of indirect and direct application response lines for chlorpyrifos, chlorpyrifos-methyl, permethrin, and mexacarbate, however, were significantly different ($p < 0.05$).

The likelihood ratio test for equality tests the hypothesis that the slopes and intercepts of two or more lines are statistically equivalent. When this test was applied to each pair of direct and indirect lines, it was uniformly rejected ($p < 0.05$). Given the significant differences between direct and indirect application lines for each chemical, the decreasing order of toxicity varied. When intrinsic responses to each chemical, measured by direct application to sixth instars, were compared, the decreasing order of toxicity and relative toxicities at LD_{50} (LD_{50} least effective chemical ÷ LD_{50} chemical X) were: permethrin (277), aminocarb (55), mexacarbate (31), chlorpyrifos-methyl (27), chlorpyrifos (23), methomyl (5), trichlorfon (4), acephate (2), and carbaryl (1). Applied to foliage, the decreasing order of toxicity and relative toxicities at LD_{50} were: chlorpyrifos-methyl (62), mexacarbate (50), chlorpyrifos (45), aminocarb (30), methomyl and permethrin (11), acephate (6), trichlorfon (3), and carbaryl (1).

When an LD_{90} of each chemical, determined in the sprayed-branchlet bioassay, was applied to potted seedlings and insects were caged immediately after spray, means of per cent mortality ranged from 73 to 99% (Table II). This variation can be expected as a result of normal sampling error; it reflects the lack of precision notorious at the tails of dose–response lines (e.g. Copenhaver and Mielke 1977). ANOVA of the observed mortalities from all postspray weathering intervals indicated that significant differences were related to chemical ($F = 30.878$; d.f. = 3,210; $p < 0.05$), weathering interval ($F = 14.492$; d.f. = 9,120; $p < 0.05$), and interaction of these two factors ($F = 6.416$; d.f. = 27,120; $p < 0.05$). Control mortalities, not included in the ANOVA, were (mean percentage ± S.D., based on 4 trees, each caged with 25 insects): water—0±0; TPM:kerosene (1:9)—0±0; Orchex 796—1.0±2.0; and diesel oil—0.35±1.21.

The average toxicities of acephate and methomyl declined significantly ($p < 0.05$) with each weathering interval. The toxicity of chlorpyrifos decreased significantly from 0 to 1 day postspray, then remained at levels not significantly different from one another. The toxicity of trichlorfon declined significantly by the first day after spray, again between 1 and 3 days, but remained steady between 3 and 7 days. Permethrin showed no significant decline in toxicity between any of the weathering intervals.

The residual effects of aminocarb, carbaryl, chlorpyrifos-methyl, and mexacarbate were more variable than those of the other insecticides. With aminocarb, average per cent mortality at 1 day postspray was significantly higher than immediately after spray. Values at 1 and 3 days were not significantly different, while there was a significant decline between 3 and 7 days. The mortality observed with carbaryl decreased significantly by the first day after spray, but increased significantly between 1 and 3 days to a value comparable with that observed immediately after spray. Mortality remained steady between 3 and 7 days.

A significant decrease in mortality was observed between 0 and 1 day after spray with chlorpyrifos-methyl, followed by another significant decrease between 1 and 3 days. Between 3 and 7 days, however, mortality increased significantly. Finally, with mexacarbate, mortality increased significantly from 0 to 1 day postspray, but decreased significantly between 1 and 3 days, and was not significantly different when values at 3 and 7 days were compared.

Discussion

Identification of variables which affect toxicity is the primary function of laboratory bioassays in insecticide research. Such investigations generally begin with

Table I. Direct and indirect toxicity of insecticides to 6th instar western spruce budworm[a]

Spray target[a]	*N*	NC	C±S.E.	Beta±S.E.	LD_{50}[b]	95% CL[b]	LD_{90}[b]	95% CL[b]	Log L	Toxicity ratio[c]
					Acephate					
F	520	557	0.083±0.012	2.14±0.22	19.6	15.4–24.5	77.0	57.4–126	−413.6	8.9
I	386	40	0.035±0.027	1.94±0.24	175	119–231	770	581–1400	−202.3*	
					Aminocarb					
F	600	557	0.083±0.012	2.02±0.18	4.27	3.57–4.97	18.2	14.7–24.5	−457.5	1.8
I	320	30	0±0	2.71±0.35	7.70	5.81–9.10	23.1	18.9–28.7	−112.5*	
					Carbaryl					
F	740	557	0.088±0.012	2.30±0.20	126	91.0–168	462	343–770	−485.3	3.4
I	310	40	0.057±0.039	2.91±0.40	427	(224–602)[d]	1190	(805–3710)[d]	−179.8*	
					Chlorpyrifos					
F	600	557	0.081±0.011	1.71±0.18	2.80	0.392–5.04	15.4	8.40–126	−428.4	6.5
I	319	40	0.026±0.025	4.01±0.47	18.2	16.1–21.0	38.5	33.6–46.2	−116.9	
					Chlorpyrifos-methyl					
F	420	557	0.082±0.011	2.74±0.33	2.03	(0.980–2.73)[d]	6.02	(4.13–14.7)[d]	−393.8	7.9
I	447	50	0.075±0.021	4.35±0.51	16.1	(8.40–24.5)[d]	32.2	(21.7–143)[d]	−197.9	

Table I. *(Concluded)*

Spray target[a]	*N*	NC	C±S.E.	Beta±S.E.	LD_{50}[b]	95% CL[b]	LD_{90}[b]	95% CL[b]	Log L	Toxicity ratio[c]
					Methomyl					
F	630	557	0.082±0.012	1.71±0.20	11.2	7.70–16.8	61.6	32.9–252	−511.6	7.5
I	449	49	0.088±0.049	1.65±0.32	84.0	41.3–126	497	259–3920	−283.8*	
					Mexacarbate					
F	560	557	0.083±0.012	1.77±0.16	2.52	1.61–3.78	13.3	7.70–36.4	−445.8	5.6
I	400	30	0±0	3.82±0.31	14.0	9.80–21.0	29.4	19.6–91.0	−166.3	
					Permethrin					
F	480	557	0.083±0.012	2.44±0.27	11.2	9.10–13.3	37.8	30.8–49.0	−331.7	0.14
I	647	30	0.040±0.039	4.02±0.35	1.54	0.98–1.96	3.22	2.52–4.97	−247.9	
					Trichlorfon					
F	620	557	0.081±0.011	2.64±0.23	39.9	28.0–53.9	126	84.8–266	−469.4	2.6
I	369	50	0.017±0.017	3.16±0.33	105	35.0–161	273	175–1260	−156.5*	

[a]I = insects; F = foliage; N = number of insects treated with insecticide; NC = number treated with solvent only; C±S.E. = control mortality ± standard error; Beta±S.E. = slope ± standard error; 95% CL = 95% confidence limits; Log L = maximum log-likelihood function.
[b]Dosage expressed in g/ha.
[c]Toxicity ratio equals LD_{50} I ÷ LD_{50} F.
[d]90% CL. The value of g exceeded 0.50 at the 95% level.
*Indicates hypothesis of parallelism accepted, with $p > 0.05$.

Table II. Residual toxicity of insecticides to western spruce budworm

Insecticide	Dosage (g/ha)	Per cent mortality (mean±S.D.) after weathering interval (days)			
		0	1	3	7
Acephate	29.4	97.0±6.0	79.0±15.1	68.0±0.57	4.0±5.9
Aminocarb	10.5	73.0±25.0	88.0±5.7	91.0±6.8	76.0±17.0
Carbaryl	310.	96.0±8.0	80.0±22.9	93.0±6.0	93.0±9.5
Chlorpyrifos	15.4	96.0±8.0	59.0±25.2	55.0±26.6	64.0±18.2
Chlorpyrifos-methyl	0.840	99.0±2.0	72.0±6.5	64.0±40.5	84.0±11.3
Methomyl	35.7	91.0±6.0	61.0±21.8	39.0±22.5	0±0
Mexacarbate	11.2	80.0±5.7	92.0±3.3	74.0±16.5	76.0±18.2
Permethrin	31.5	83.0±15.1	83.0±10.0	78.0±19.7	86.0±12.4
Trichlorfon	175	98.0±4.0	68.0±11.8	19.0±14.0	26.0±30.7

first-level experiments to define the intrinsic toxicities of a number of chemicals to the target organism. This initial "screen" frequently results in the selection of the most toxic toxicants for further investigation (e.g., DeBarr and Nord 1978; Nigam 1970; Robertson *et al.* 1976). Once a chemical has been selected for more intensive investigation, other intrinsic factors, such as differential responses by different instars or life stages may be scrutinized (e.g., Granett and Retnakaran 1977; Robertson and Boelter 1979*a*).

Extrinsic variables affect response, but are factors outside the target organism. Environmental factors such as rainfall, for example, alter the effectiveness of some toxicants (Robertson and Boelter 1979*b*). Clearly, investigations of extrinsic variables must be realistic if their predictive value is to be high. Ultimately, laboratory data may be used to develop a simulation model to forecast efficacy. A simulation model has been developed for the boll weevil, *Anthonomus grandis* Boheman (Jones *et al.* 1977), but not for any major forest defoliator.

Host-plant foliage is an obvious extrinsic factor present during application of a toxicant in the field. Our data demonstrate that Douglas-fir foliage significantly alters the responses of last instar western spruce budworm to each of the nine toxicants tested. The nature of the alteration is clearly chemical-specific.

For some toxicants, the dosage–response line for direct application to insects parallels the line for indirect application via Douglas-fir foliage. Based on extensive pharmacological investigations of drug potency (e.g., Hardman *et al.* 1959; Kuperman *et al.* 1961), we interpret this observation to reflect qualitatively identical, but quantitatively different, biochemical modes of toxic action in direct and indirect applications. The position of the insect's intrinsic response line simply shifts while retaining the same slope. Thus, the modes of action of acephate, aminocarb, carbaryl, methomyl, or trichlorfon are presumed to be the same whether sprayed directly on insects or first sprayed on foliage. Significantly higher toxicities observed with indirect application may simply represent cumulative toxicity occurring during the insects' foraging.

For other toxicants, the presence of Douglas-fir foliage alters both the position and slope of the western spruce budworm's intrinsic response line. Thus, the toxicities of indirectly applied chlorpyrifos, chlorpyrifos-methyl, and mexacarbate were significantly greater than when applied directly to insects; in addition, the slopes of each pair of direct and indirect application lines were significantly different. Significant differences in position and slope may reflect alteration of the biochemical steps which result in toxicity. For example, metabolites of each chemical formed

in the foliage may be more toxic than the parent compound. Permethrin was the only chemical whose toxicity was significantly decreased; enzymatic degradation and formation of less toxic metabolites may have occurred in the foliage.

Besides host-plant foliage, another extrinsic variable relevant to field application of a chemical toxicant is the period of time over which lethal effectiveness is retained. To measure this residual toxicity, laboratory investigations may employ the following experimental design: Suppose that some target insects escape exposure to a toxicant when initially applied. At a later time, they contact the toxicant on host-plant foliage, or consume foliage containing the toxicant. What proportion will die? Does this proportion differ significantly from the proportion which succumb following exposure to the toxicant immediately after it is deposited on the foliage? Our investigation of residual toxicities represents an attempt to increase the comparative and predictive value of data gathered in such laboratory experiments.

Previous investigations of residual toxicity of insecticides to both western spruce budworm (Lyon and Robertson 1971) and Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough) (Robertson and Boelter 1979*b*) have used initial concentrations based not on toxicological response, but rather on recommended application rates. Yet, as described by Williams (1973), application rates released from spray aircraft may be far greater than deposit rates on target trees. Even if the application rate did equal the deposit rate, comparison of residual toxicity would be biased. For one chemical, a recommended application rate may be 10× the toxicant's LD_{90} to the target insect, while for another, the application rate may be only 2× the LD_{90}. Use of a uniform initial toxicological value as the dosage subjected to weathering permits realistic assessments and comparisons at the selected postspray intervals.

Finally, our investigation clearly demonstrates the potential liabilities of selecting one chemical over any other solely on the basis of a direct application bioassay. Germaine extrinsic factors should be assessed before such a choice is made.

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