Reprinted from the
JOURNAL OF ECONOMIC ENTOMOLOGY, VOL. 75, NO. 2, APRIL 1982

Laboratory Bioassays for Selecting Candidate Insecticides and Application Rates for Field Tests on the Western Spruce Budworm[1,2]

MICHAEL I. HAVERTY AND JACQUELINE L. ROBERTSON

Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, Berkeley, California 94701

ABSTRACT

J. Econ. Entomol. 75: 179–182 (1982)

Derivation of a series of multiplication factors for suggesting field application rates of insecticides to control *Choristoneura occidentalis* Freeman based on laboratory data is described. The seven chemicals (acephate, carbaryl, fenitrothion, methomyl, permethrin, sulprofos, and thiodicarb) on which these factors are based had been field tested previously against *C. occidentalis* so that an estimated minimum effective dose to cause 90% mortality in the field (MED_{90}) was available for each. The first laboratory bioassay estimated the effective dose resulting in 90% mortality (ED_{90}) for each chemical by conventional procedures in which the doses tested resulted in 5 to 99% mortality when sprays were applied to larvae on foliage. In the second bioassay, each ED_{90} was reestimated by testing doses predicted from the first bioassay to result in 75 to 98% mortality. Finally, the rainfastness of each chemical applied at the ED_{90} dose was determined to assess the degree to which rainfall affects efficacy. The ratio of the MED_{90} to the precisely estimated ED_{90} for each insecticide was then determined. With future field tests of rainfast chemicals, conversion factors of 4, 8, and 12 seem appropriate. Increasing the factors to 5, 10, and 15 should suffice to assure comparable effects for chemicals adversely affected by rain.

For many years, laboratory bioassays of insecticides being developed for use on forest defoliators were limited to topical and spray application experiments designed to determine dose-response relationships (Lyon et al. 1972, Robertson et al. 1975). Based on these simplistic bioassays, the relative toxicity of new chemicals compared with those tested both in the laboratory and in the field were determined. A loose extrapolation was then made to suggest field application rates for the new chemical.

To improve the process of selecting chemical insecticides with the best potential for suppressing the western spruce budworm, *Choristoneura occidentalis* Freeman, a series of bioassays and ranking criteria that use a multiphase experimental approach was done (Robertson and Haverty 1981). Although this investigation demonstrated the benefits of using expanded choice criteria, it did not address the selection of field application rates for two reasons. First the conventional design of relevant experiments did not permit precise estimation of the effective dose that would result in 90% mortality (ED_{90}) in the laboratory. Second, a generalized relationship between laboratory ED_{90} and field application rates had not been described.

Reported here is the derivation of a series of multiplication factors for suggesting field application rates of insecticides based on laboratory data. These factors were derived by means of a comparison between extant field test data and laboratory ED_{90} values estimated by a new experimental protocol. In this protocol, dose-response lines were first estimated by a conventional design (e.g., Finney 1971) when each insecticide was applied to foliage on which 6th instars were present. A new design was then used to refine the estimated ED_{90} for each formulation. Finally, the reestimated ED_{90} was tested for rainfastness in a deluge simulation. We assessed the relationship between laboratory ED_{90} and the minimum effective dose necessary to obtain 90% mortality in the field, and we derived a series of multiplication factors for both rainfast and nonrainfast chemicals to suggest application rates to be tested in future field experiments with new candidate insecticides.

Materials and Methods

Insects and Foliage

Insects used in all bioassays were recently molted, sixth-stage larvae, weighing from 20 to 60 mg each. Larvae were selected from the 87th to 88th generation of a nondiapausing laboratory colony reared as described by Robertson (1979). Foliage of Douglas-fir, *Pseudotsuga menziesii* [Mirb.] Franco, was collected every 2 to 3 weeks from small trees in a plantation on the Russell Evergreen Tree Farm, University of California, Lafayette, and stored in plastic bags at 5°C.

Twenty-four hours before treatment, small branchlets (15 to 25 cm in length and composed of ca. 50% of the current year's foliage) were cut from the larger branches. Ten larvae were selected at random and placed on each branchlet. Each branchlet with the insects was held in a Randall cage, and the branchlet stem was placed in water.

Insecticides and Treatment Procedures

Seven insecticide formulations were used: acephate (Orthene 75S®), sulprofos (Bolstar 6E®)

[1]Lepidoptera: Tortricidae.

[2]This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and or Federal agencies before they can be recommended. Received for publication 23 January 1981.

thiodicarb (UC51762 4F®) carbaryl (Sevin-4-oil®), fenitrothion (Sumithion 8E®), methomyl (Lannate 1.8®), and permethrin (Pounce 3.2E®). All were formulated in water, except carbaryl, which was formulated in diesel oil. A new stock solution of each toxicant was formulated on the basis of wt/vol of AI, then serially diluted to the desired concentrations for each replication. To assess spray deposit, 1% (wt/vol) oil red dye was added to the carbaryl formulation and 1% (wt/vol) aniline blue was added to the other formulations.

Sprays were applied to insects on foliage with the Moellman spray chamber (Robertson et al. 1979). A delivery volume of 0.25 ml (0.125 ml for carbaryl) was expelled through the nozzle at 704 g/cm², spray targets were exposed for 60 sec, and average volume of spray deposit was equivalent to 9.35 liters/ha (4.68 liters/ha for carbaryl).

Immediately before spray, each branchlet was removed from the Randall cage and the stem was held so that it was perpendicular to the plane of the spray table. A funnel of filter paper was placed beneath the foliage to catch insects that fell during spray application. Immediately after spray application, the branchlet was removed from the spray chamber, fallen larvae were replaced on the foliage, the branchlet was placed back within the Randall cage, and the stem again was placed in water.

Determination of Conventional Dose-Response Lines

After initial dose-fixing experiments to bracket concentrations resulting in mortalities of 5 to 99%, eight final concentrations of each formulation were tested. Each dose-response determination was replicated three times. All formulations were tested in a given replication before the next replication began. For each replication, two branchlets were sprayed simultaneously with one concentration. The order in which the insecticide formulations were applied in a given replication was randomized. For each insecticide formulation, test solutions were applied in order of increasing concentration after application of the appropriate water or diesel oil control.

Larvae fed on the treated foliage for 7 days, and mortality was assessed at the end of this period. Results from both branchlets were combined so that each data point of each replication represented the response of 20 insects. Data were analyzed with the probit option of POLO (Russell et al. 1977) to determine the dose-response line and estimates of dose that would cause a given level of mortality.

Refinement of ED_{90} Estimate

ED_{90} values can be estimated from the dose-response lines described earlier. Considerable variation, however, is associated with the extremes of a dose-response line, such as ED_{10} or ED_{90} (Copenhaver and Mielke 1977). Further testing is necessary to refine the ED_{90} estimate to recommend application rates for field testing.

This bioassay was done in a manner similar to that described for the dose-response determination. The age class of insects, the insecticide formulations, and the spray application and treatment procedures were all the same. One of five application rates of the insecticide formulations was applied to two branchlets in each of nine replications. The five concentrations tested were those that would theoretically produce 75, 85, 90, 95, and 98% mortality as predicted by the line. The refined estimate of ED_{90} was determined by subjecting these data to log-probit regression. This refined estimate of the ED_{90} was then evaluated for rainfastness.

Rainfastness

To assess rainfastness, the ED_{90} of each formulation was applied to four branchlets in a randomized complete block design; this procedure was replicated eight times. Branchlets were sprayed with insects present, as previously described. Immediately after spraying, insects were removed from the branchlet and were held temporarily in petri dishes. The sprayed branchlets were allowed to dry for 2 h. Two of the branchlets were chosen at random and subjected to 1.27-cm-equivalent rainfall over a 5-min period with the rain chamber described by Robertson and Boelter (1979); the other two branchlets were not subjected to rainfall. One hour later, the insects were replaced on their original branchlet. Each branchlet was then held in a Randall cage, and mortality was assessed as described earlier.

Contingency table analysis (Steel and Torrie 1960) was used to determine whether rainfall washes off a significant amount of each insecticide to reduce western spruce budworm mortality caused by each formulation at the ED_{90} level, or whether differences in mortality between formulations subjected to rainfall were statistically significant ($\alpha = 0.05$).

Results

Conventional Dose-Response Lines

Conventional dose-response lines and estimates of the ED_{90} were determined for each of the seven formulations (Table 1). For methomyl, the value of g exceeded the conservative safety limit of 0.50; 95% confidence limits for the initial ED_{90}, therefore, were not estimated. The relative toxicities at ED_{90} of the seven insecticide formulations were (index of relative toxicity = ED_{90} for least toxic formulation, carbaryl, divided by the ED_{90} for formulation x): thiodicarb, 28.3; methomyl, 25.6; permethrin, 15.7; acephate, 8.3; sulprofos, 7.6; fenitrothion, 2.6; and carbaryl, 1.0.

Refined ED_{90} Estimates

Bracketing the doses tested between the estimated ED_{75} and ED_{98} provided a more reliable estimate of the dose that will give 90% mortality compared with values obtained with the conventional design (Table 1). The confidence limits for the refined ED_{90} were always calculated and were generally much narrower. Relative toxicities, on the basis of the refined ED_{90} values, were: permethrin, 26.4; thiodicarb, 10.4;

Table 1.—Toxicity (ED_{90}) of seven insecticide formulations to 6th-instar western spruce budworm feeding on branchlets of Douglas-fir in two experiments[a,b]

Insecticide	Expt 1: conventional design					Expt 2: refined design				
	n	NC	Slope (SE)	ED_{90}[c]	95% CL	*n*	NC	Slope (SE)	ED_{90}[c]	95% CL
Acephate	489	39	1.93 (0.16)	74.8	31.5–2,562.1	887	90	3.19 (0.37)	65.7	56.4–81.5
Sulprofos	446	40	2.76 (0.27)	81.4	61.5–123.9	858	88	3.76 (0.49)	74.1	63.4–90.5
Thiodicarb	470	39	1.98 (0.23)	22.0	15.4–39.9	865	91	1.96 (0.27)	24.1	18.4–38.5
Carbaryl	469	40	1.36 (0.13)	622.5	320.4–2,249.1	877	87	2.56 (0.36)	250.7	208.3–334.7
Fenitrothion	476	39	1.82 (0.24)	236.1	135.1–927.7	885	86	1.96 (0.26)	128.2	100.3–191.7
Methomyl	481	40	4.02 (1.16)	24.3	—[d]	866	89	3.27 (0.50)	33.7	29.3–44.4
Permethrin	466	39	0.82 (0.10)	39.7	12.5–361.5	866	90	1.14 (0.16)	9.5	5.8–22.9

[a] In the first experiment, the eight dosages selected resulted in mortality ranging from 5 to 99%. In the second, a refined estimate of the ED_{90} was derived from dosages that resulted in mortality of 75 to 98%.
[b] *n*, Number of insects treated; NC, number of controls; SE, standard error of slope; 95% CL, 95% confidence limit for ED_{90}.
[c] Dosage expressed as g/ha; estimate was made by using the probit model.
[d] Confidence limits were not computed because the *g* statistic exceeded 0.5.

methomyl, 7.4; acephate, 3.8; sulprofos, 3.4; fenitrothion, 2.0; and carbaryl, 1.0.

Rainfastness

The rainfastness experiments validated the reestimated ED_{90}'s; the percent mortalities resulting from the estimated ED_{90} of all seven insecticide formulations not subjected to rainfall (Table 2) were not significantly different from one another ($\chi^2 = 11.1$ with 6 df), nor were any of them significantly different from 90%. Mortality resulting from the ED_{90} of acephate, thiodicarb, fenitrothion, and methomyl was significantly reduced by application of 1.27-cm-equivalent rainfall (Table 2). The difference among mortalities resulting from ED_{90} dosages subjected to rainfall was highly significant ($\chi^2 = 79.3$ with 6 df).

Multiplication Factors for Field Application Rates

All insecticides tested in this study currently are commercially available. Each is a registered product and has been field tested by aerial application or simulated aerial application against western spruce budworm. The simplest relationship between laboratory ED_{90} and minimum effective dosage that will cause 90% mortality in the field (MED_{90}) is the ratio MED_{90}/ED_{90}. For all insecticide formulations tested, except fenitrothion, this ratio fell between 4.46 and 11.91 (Table 3).

In field tests, three application rates are usually tested. If one of the rates selected fails to provide 90% mortality, a minimum effective dose (i.e., the next highest dosage) can be selected. The ratios of MED_{90} to ED_{90} in this study suggest that multiplication factors 4, 8, and 12 times the laboratory ED_{90} are likely to predict an effective application rate for candidate insecticides in a field test. For insecticide formulations which are adversely affected by rainfall, such as acephate, thiodicarb, fenitrothion, and methomyl, increasing the multiplication factors slightly should account for lack of rainfastness. Multiplication of ED_{90}'s by 5, 10, and 15 should suffice.

Discussion

Previously, we developed a method to select chemicals with the best potential for controlling western spruce budworm (Robertson and Haverty 1981). We used a series of bioassays to develop toxicological ranking citeria. This method necessarily emphasized comparisons among insecticides. Five insecticides (permethrin, sulprofos, fenitrothion, carbaryl in the Sevin-4-oil formulation, and thiodicarb) were tested in both the present and the previous investigation. In the previous study, dose-response lines were obtained by spraying the insecticides directly onto larvae in petri dishes; in the present

Table 2.—Effects of 1.27-cm simulated rainfall on toxicity of seven insecticides applied at the estimated ED_{90} to 6th-instar western spruce budworm feeding on Douglas-fir branchlets

Insecticide	Dosage (g/ha)	% Mortality[a,b]		Yates'-corrected χ^2[c]
		No rain	Rain	
Acephate	66.2	86.3 (13.6)	43.1 (26.0)	63.3
Sulprofos	73.9	92.5 (11.6)	87.5 (19.3)	1.7
Thiodicarb	24.6	90.6 (20.8)	68.1 (32.9)	23.5
Carbaryl	251.0	83.1 (14.4)	73.5 (21.9)	3.7
Fenitrothion	127.8	86.0 (17.0)	67.5 (22.5)	13.7
Methomyl	33.9	85.4 (13.0)	64.3 (20.9)	17.9
Permethrin	9.4	82.5 (17.9)	73.5 (20.8)	3.2

[a] Mean percent mortality (SD). Means are based on mortality resulting from eight replications of 20 insects each.
[b] Mortality from insecticides not subjected to rainfall was not significantly different ($\chi^2 = 11.1$, 6 df). Mortality from insecticides subjected to rainfall was significantly different ($\chi^2 = 79.3$, 6 df); acephate and sulprofos contributed most to the χ^2 value.
[c] Value for comparison of no rainfall and rainfall for each chemical. For $\alpha = 0.05$, critical $\chi^2 = 3.84$, with 1 df.

Table 3.—Ratios of minimum effective dosage for 90% control of western spruce budworm in the field (MED_{90}) to the laboratory ED_{90} from which general multiplication factors are derived

Insecticide	MED_{90} (g/ha)	Lab ED_{90} (g/ha)	MED_{90}/ED_{90}
Acephate[a]	560[b]	66.2	8.45
Sulprophos	350[c]	73.9	4.74
Thiodicarb[a]	209[c]	24.6	8.50
Carbaryl	1,120[d]	251.0	4.46
Fenitrothion[a]	420[e]	127.8	3.28
Methomyl[a]	280[f]	33.9	8.25
Permethrin	112[d]	9.4	11.91

[a] Not rainfast.
[b] From Markin (1979).
[c] From Richmond (1981).
[d] From Markin and Johnson (1982).
[e] From Morris et al. (1974).
[f] From Markin and Johnson (1979).

study, insects were on foliage when sprayed. Our previous ranking of the five chemicals was generally confirmed by the similarity in relative toxicity values. Thiodicarb, however, appeared to be more toxic in the present study. Reasons for this chemical's enhanced toxicity could not be specifically determined but might be related to combined effects of contact and feeding toxicity in the present study.

Because our investigation emphasized prediction rather than comparison, we used a bioassay procedure innately more natural than direct-spray application to fully exposed larvae. Spray application to larvae on foliage subjected the insects to contact with the spray droplets, contact with spray residues on foliage, and ingestion of toxicant when foliage was consumed. We developed a two-phase estimation procedure to calculate a precise estimate of the ED_{90} and, thereby, to further improve prediction. The conventional estimation procedure uses data encompassing a response range of 5 to 99%. After first estimating this type of line, we focused attention on the upper portion of the response where 75 to 98% mortality occurred. The predictive value of this procedure was confirmed in the rainfastness experiments.

The ED_{90}s from a laboratory bioassay cannot be used directly as recommended field rates. Often, as much as 80% of spray aerially applied in the field is not deposited at tree level (Williams 1973). Likewise, spray is deposited throughout the forest canopy, not just on single branches. Previously, laboratory ED_{90} estimates were arbitrarily multiplied by 3 to obtain a field dosage (Williams 1973). In general, the connection between laboratory estimates and field application rates has been tenuous at best. Since our multiplication factors are based on all available extant field data, they should provide a sound scientific basis for future experiments. The adoption of our protocol should reduce the number of aerial application field tests that do not result in the identification of an effective application rate for new candidate insecticides.

REFERENCES CITED

Copenhaver, T. W., and P. W. Mielke. 1977. Quantit analysis: a quantal assay refinement. Biometrics 33: 175–186.

Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge University Press, Cambridge. 333 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: 928–930.

Markin, G. P. 1979. Effects of acephate sprayed on western spruce budworm in Idaho, 1976. Ibid. 72: 414–415.

Markin, G. P., and D. Johnson. 1979. Western spruce budworm control with Lannate, 1978. Insecticide Acaricide Tests 4: 176.

1982. Western spruce budworm aerial field test, 1979. Ibid. (in press)

Morris, O. N., J. A. Armstrong, G. M. Howse, and J. C. Cunningham. 1974. A 2-year study of virus-chemical insecticide combination in the integrated control of the spruce budworm, *Choristoneura fumiferana* (Tortricidae:Lepidoptera). Can. Entomol. 106: 813–824.

Richmond, C. E. 1981. Simulating aerial sprays for the western spruce budworm (*Lepidoptera:Tortricidae*). Evaluation of technique and two candidate insecticides. Can. Entomol. 113: 819–825.

Robertson, J. L. 1979. Rearing the western spruce budworm. U.S. Dep. Agric., Washington, D.C. 18 pp.

Robertson, J. L., and L. M. Boelter. 1979. Toxicity of insecticides to Douglas-fir tussock moth. *Orgyia pseudotsugata* (Lepidoptera: Lymantriidae). II. Residual toxicity and rainfastness. Can. Entomol. 111: 1161–1175.

Robertson, J. L., and M. I. Haverty. 1981. Multiphase laboratory bioassays for the selection of chemicals to be field-tested on the western spruce budworm, *Choristoneura occidentalis* Freeman. J. Econ. Entomol. 74: 148–153.

Robertson, J. L., R. L. Lyon, T. L. Andrew, 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: 192–196.

Russell, R. M., J. L. Robertson, and N. E. Savin. 1977. POLO: a new computer program for probit analysis. Bull. Entomol. Soc. Am. 23: 209–213.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York. 481 pp.

Williams, C. B. 1973. Field tests of four insecticides against the Douglas-fir tussock moth in Oregon, pp. 77–83. Perm. Assoc. Comm. Proc., West. For. Conservation Assoc., Portland, Oreg. 182 pp.