ESTIMATION OF RATES AND TIMES OF APPLICATION FOR SELECTED INSECT GROWTH REGULATOR FORMULATIONS[1] APPLIED TO WESTERN SPRUCE BUDWORM[2]

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ABSTRACT

Four insect growth regulator formulations – BAY SIR 8514, UC 62644, methoprene:permethrin (9:1), and TH 6040:methoprene (9:1) – were tested in a series of bioassays designed to estimate their optimal time of application relative to a theoretical instar distribution of a population of western spruce budworm, *Choristoneura occidentalis* Freeman. Field application rates dependent on each formulation's rainfastness were calculated based on laboratory ED_{90} values. The 6th instar was the most susceptible stage to all formulations. The formulation with the greatest activity and broadest predicted optimal time of application was UC 62644. The TH 6040:methoprene mixture cannot be considered a viable candidate for field testing because of solubility problems around the ED_{90}.

Key Words: *Choristoneura occidentalis*, western spruce budworm, insect growth regulators, BAY SIR 8514, UC 62644, methoprene:permethrin (9:1), TH 6040:methoprene (9:1)

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

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Insect growth regulators (IGRs) of both the molt-inhibiting and juvenile hormone types have high potential for use in integrated pest management (IPM) systems (Metcalf 1980). As classes of chemicals, they are generally regarded as safe. Although many of each type have been tested in the laboratory on the western spruce budworm, *Choristoneura occidentalis* Freeman (*e.g.*, Gillette *et al.* 1978, Robertson and Kimball 1979), their actual use in an IPM strategy with this insect has been hampered by lack of commercial development of many of the most active compounds.

In this investigation, we tested IGR formulations in a series of laboratory bioassays designed to estimate the most appropriate time of application relative to an instar distribution of a population and to suggest field application rates, depending on rainfastness, which would result in 90% population mortality. Two of the IGRs are molt inhibitors which may eventually become available for forest use. The remaining two formulations are mixtures of commercially available products which demonstrated enhanced activity in other experiments.

METHODS AND MATERIALS

Instar Distribution: Second instar western spruce budworm from the 4th generation of a diapausing laboratory colony were selected at random as they emerged from their hibernacula. Each was placed in a 29.7-ml plastic jelly cup containing a 1 cm cube of artificial diet (Robertson 1979). The cup was closed with a tight-fitting paper lid. A total of 100 larvae were placed in containers on the same day. Each container was checked twice daily for the presence of a shed skin or headcapsule; the dates of each molt were recorded and the duration of each instar in days was determined. Once all individuals had undergone larval-pupal ecdysis (23% did not survive to the pupal stage), the numbers of each instar present at 1-day intervals following emergence from diapause were determined. To approximate a situation observed in field populations, we assumed that groups of larvae would emerge from diapause on each of 21 days following the appearance of the first group.[4] Cohorts of 100 larvae with the same frequency distribution as the group actually observed were therefore added to the population for each of 21 successive days; a theoretical instar frequency distribution was then calculated for this model as a whole. The resulting frequency distribution (Figure 1) was used to estimate the probability of each instar's presence at a given time after the first insect appeared.

Insects and Foliage in Bioassays: Recently-molted larvae estimated to have entered instars 3-6 less than 24 hours earlier were selected at random from the 87th and 88th generations of a nondiapausing laboratory colony reared as

[4]This procedure was suggested by Carroll B. Williams, Principal Research Entomologist, Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, Berkeley, California 94701.

described by Robertson (1979). Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) foliage was collected from small trees in a plantation on the Russell Reservation, University of California, Lafayette, California. Foliage was stored in plastic bags at 5C; fresh foliage was collected every 2-3 weeks. Twenty-four hours before treatment, branchlets 15-25 cm in length were cut from the larger branches. Each branchlet was positioned within a ventilated Randall cage so that its stem could be placed in water. Ten larvae of the same stage were placed on a branchlet, the cage was closed, and the larvae were left to feed for about 24 hours before spray application.

IGR Formulation: Four formulations were tested. These were UC 62644 25% WP, BAY SIR 8514 25% WP, a 9:1 mixture of TH 6040 25% WP and methoprene 5 lb/gal EC, and a 9:1 mixture of methoprene 5 lb/gal EC and permethrin 3.2 lb/gal EC. Stock solutions of all formulations were prepared in distilled water on the basis of weight per volume (w/v) of the active ingredients. Spray application concentrations were prepared by serial dilution of the concentrate. A fresh stock solution was made for each replication of an experiment. Aniline blue dye (1% w/v) was added to each formulation to permit spray deposit assessment.

Spray Application Procedure: Sprays were applied to insects on foliage with the Moellman spray chamber (Robertson *et al.* 1979). Parameters of spray application were: 0.25 ml delivery volume expelled through the nozzle at 704 g/cm^2, a 60-second exposure of branchlets to the spray, and an average spray deposit volume of 9.35 l/ha.

Immediately before spray, each branchlet was removed from the Randall cage. The stem was pressed into a hole cut in the plastic lid of a disposable food dish (150 ml) so that the branchlet foliage was relatively perpendicular to the plane of the spray table. A funnel of filter paper was positioned beneath the foliage to catch any insects falling during spray application. Immediately after spray, the branchlet was carefully removed from the

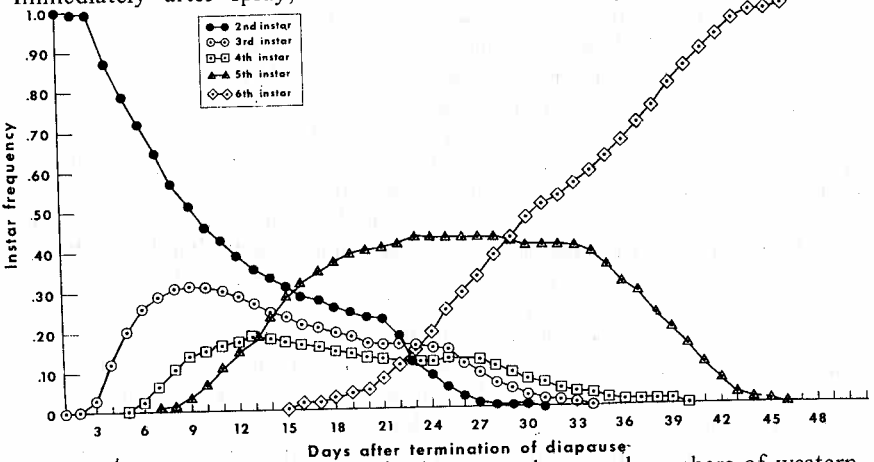

Fig. 1. – Instar frequency distribution assuming equal numbers of western spruce budworm emerge from diapause on each day for 21 days after appearance of the first group.

holder, the filter paper was removed, and the branchlet was placed again within the Randall cage. Fallen larvae were put back on the foliage, the cage was closed, and the stem again placed in water.

Instar Response Comparisons: Following initial experiments to bracket concentrations resulting in 5-90% mortality of 6th instars, eight final concentrations of each formulation were tested in full-scale experiments with instars 3-6. The 6th instar was used for dose selection because less time was required for lethal effects to be observed. With all other instars, the time required before all lethal effects could be discerned would have introduced possible intergeneration factors into the instar comparisons. The significance of generation-related responses to IGRs is unknown at present, but experiments with conventional insecticides (Savin *et al.* 1977) suggest that they might confound comparisons of instar response.

Three replications of each dose-response determination were performed with each instar. Within each replication, all instars were from the same generation (Robertson 1979). 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 formulation, solutions were sprayed in order of increasing concentration following application of distilled water alone to a control group.

Larvae were allowed to feed on treated foliage for 7 days; live larvae were then transferred to 100- x 15-mm sterile plastic petri dishes containing artificial diet. Pupae were transferred either from the Randall cage or the larger petri dish to 60- x 15-mm sterile plastic petri dishes lined with filter paper. Lethal effects were assessed for both larvae and pupae originating from each replication group. Survivors were those which emerged as morphologically normal adults. Total lethal effects for both branchlet groups which had been sprayed simultaneously were considered one data point; all points were used to estimate a dose-response line for an instar with the probit option of POLO (Russell *et al.* 1977). In each instance where the *t*-ratio of the slope indicated a significant regression, the line was reconstructed to determine the estimated proportion lethally affected, given a specified dose.

Estimation of Optimal Times of Application: The ED_{90} of the most susceptible instar was chosen as the theoretical application rate at day y after the first group in a population emerged from diapause. For each y, expected mortality was calculated as the sum, over all instars, of the proportion lethally affected by the dose while in $instar_X$, times the probability of being in $instar_X$. The optimal time of application was considered to be the days on which 90 ± 5% mortality would be expected.

Estimation of Field Application Rates: Field application rates were estimated from laboratory data as developed by Haverty and Robertson (1982). In brief, the procedure began with estimation of dose-response lines as already described for instar response comparisons. Next, each ED_{90} was reestimated by another dose-response bioassay designed to minimize the variation associated with the extreme upper end of the line. Finally, the new ED_{90} estimate was then multiplied by a set of factors dependent on whether or not the formulation was rainfast.

Because the 6th instar was the most susceptible to each of the four IGR formulations, the reestimation experiments were performed to estimate field application rates for this developmental stage. Five concentrations were tested for BAY SIR 8514, UC 62644, and methoprene:permethrin (9:1); concentrations for each chemical were predicted by the 6th instar response line to produce 75, 85, 90, 95, and 98% lethal effects. Procedures for selecting 6th instars, spray application, and postspray handling were the same as described previously for instar response comparisons. Each of the five concentrations of the formulations was applied to two branchlets in each of nine replications. The TH 6040:methoprene (9:1) mixture was not tested further in this reestimation or the subsequent test of rainfastness because the predicted 95 and 98% dosages exceeded the solubility limits of the wettable powder component and the 90% dosage was barely liquid.

Refined estimates of ED_{90}s for the three formulations were next tested for rainfastness. Each ED_{90} was applied to four branchlets in a randomized complete-block design; this procedure was replicated eight times. Branchlets were sprayed with 6th instars present as in the dose-response bioassays. Two hours after spray application, insects were removed from the branchlet and held temporarily in 100- x 15-mm petri dishes. Two branchlets, chosen at random, were then subjected to 1.27-cm equivalent rainfall with the chamber described by Robertson and Boelter (1979); the other two branchlets were not subjected to rainfall. One hour later, the 10 insects were placed again onto their original branchlet. Post-treatment holding conditions and assessment of lethal effects were the same as described for the dose-response bioassays.

Contingency table analysis (Steel and Torrie 1960) was used to determine whether rainfall significantly reduced IGR lethal effectiveness at the 90% level, and whether the observed effectiveness of formulations not exposed to rain differed from the expected 90% lethal effectiveness level.

RESULTS

Instar Response Comparisons: The 6th instar was the most susceptible to each of the four IGR formulations (Table 1). Significant regressions were obtained for instars 3-6 treated with the same nine concentrations of UC 62644 or methoprene:permethrin (9:1). Relative instar tolerance ratios at ED_{50} for methoprene:permethrin (9:1) ($i_{50} = ED_{50}$ instar$_X \div ED_{50}$ instar$_6$) were 3rd – 2.5; 4th – 2.4; 5th – 4.1; 6th – 1.0. At ED_{90}, i_{90} values also suggested a decrease in relative tolerance of the younger instars: 3rd – 1.3; 4th – 1.4; 5th – 1.8; 6th – 1.0. In treatments with UC 62644, i_{50} values were: 3rd – 1.4; 4th – 2.8; 5th – 4.5; 6th – 1.0. At ED_{90} the relative tolerance of 3rd and 4th instars increased substantially. Values of i_{90} were: 3rd – 23.5; 4th – 39.3; 5th – 4.6; 6th – 1.0.

BAY SIR 8514 had lethal effects only on 3rd and 6th instars; the *t*-ratios of the slope coefficients for 4th and 5th instars indicated that neither slope was significantly different from zero. Values of i_{50} and i_{90} for 3rd instars were 1.8 and 9.1, respectively. The mixture of TH 6040 and methoprene (9:1) produced lethal effects only on 6th instars. The *t*-ratios for the slope

Table 1. – Lethal effectiveness of four IGR formulations to instars 3 to 6 of the western spruce budworm.[a]

Formulation	Instar	N	NC	Slope±S.E.	ED_{50}	ED_{90}
					g/ha	
BAY SIR 8514	3	310	22	0.70±0.32	10.7	731.0
	4	337	16	–0.78±0.24[b]	–	–
	5	427	29	0.33±0.61[b]	–	–
	6	476	40	1.15±0.15	6.1	79.9
TH 6040:	3	309	22	–0.23±487[b]	–	–
methoprene	4	390	17	0.44±1.66[b]	–	–
(9:1)	5	406	28	0.30±0.23[b]	–	–
	6	460	30	1.00±0.17	43.3	824.4
Methoprene:	3	379	30	2.00±0.44	12.4	54.5
permethrin	4	426	22	1.86±0.38	11.9	58.0
(9:1)	5	429	29	2.21±0.39	19.9	75.9
	6	373	29	1.40±0.26	4.9	41.6
UC 62644	3	303	42	0.65±0.22	0.18	16.7
	4	363	27	0.68±0.26	0.37	27.9
	5	445	29	1.72±0.55	0.58	3.25
	6	496	39	1.78±0.21	0.13	0.71

[a]In column headings, N = number of test subjects, NC = number of controls, S.E. = standard error of slope, ED_{50}, ED_{90} = calculated doses for 50% and 90% lethal effect, respectively.

[b]Value of the *t*-ratio <1.96. The slope, therefore, was not significantly different from zero at the $\alpha = 0.05$ level.

coefficients of instars 3-5 indicated that none of their slopes was significantly different from zero.

Estimated Optimal Times of Application: For practical purposes, 3rd instars are usually considered the earliest possible spray target in field applications. Our instar frequency distribution (Figure 1) indicated that 2nd and 3rd instars would be present simultaneously. Our experimental method, however, was inappropriate for determining the responses of 2nd instars; at this stage, insects are mining needles (Furniss and Carolin 1977). Therefore, we arbitrarily assumed that 2nd and 3rd instar responses to each IGR are equivalent.

For UC 62644, the optimal time of spray application appeared to be 29 days or more after insects from the first cohort emerge from their hibernacula (Figure 2). At 29 days, about 40% of the population would be in the 5th instar, 40% of the population would be in the 6th instar, and the residual 20% would be in instars 2-4. Methoprene:permethrin (9:1), with less cumulative toxicity among the instars, would not achieve the desired effect unless applied 39 days or more after the first cohort emerged. At this time, only about

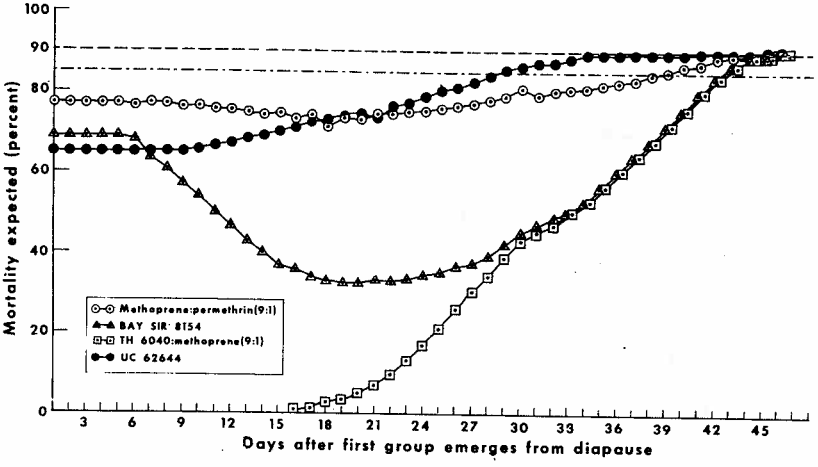

Fig. 2. – Cumulative percent mortality for insect growth regulator formulations over western spruce budworm instars 2-6.

the 6th instar. BAY SIR 8514 and TH 6040: Methoprene, though differing in relative toxicity to the larval stages, could not be applied until at least 44 days after the first population cohort emerged from diapause. At this time, only 6th instars would be present in the population.

Estimation of Field Application Rates: The doses estimated from the original 6th instar response lines as those producing 75, 85, 90, 95, and 98% mortality with BAY SIR 8514, methoprene:permethrin (9:1) and UC 62644 produced actual mortality which was lower than expected. Consequently, the reestimated ED_{90} values were higher (Table 2). The reliability of the new estimates was demonstrated in the rainfastness experiments (Table 3): the percent mortalities resulting from application of the new ED_{90}s of the three formulations not subjected to rainfall did not differ significantly from the expected 90% ($\chi^2 = 1.71$ with 1 d.f.). The formulations were rainfast, with no significant decrease in lethal effectiveness caused by 1.27-cm rainfall.

These results indicated that multiplication of the reestimated ED_{90}s by factors of 4, 8, and 12 was appropriate to obtain suggested field application rates (Table 2) for 90% lethal effect, one rate below that value, and one rate above. Of the formulations, UC 62644 appeared to be the most effective.

DISCUSSION

Previous estimates of optimal times of application for conventional chemical toxicants of IGRs relative to *Choristoneura* spp. instar distributions have been based on simplistic empirical comparisons of toxicity or relative effectiveness among the instars (*e.g.*, Granett and Retnakaran 1977, Robertson 1980, Robertson and Kimball 1981). The method demonstrated in this investigation provides a more definitive procedure which identified timespans over which desired levels of control are likely to occur. Use of an instar probability

Table 2. – Refined dosage estimates for nonconventional insecticides and their suggested application rates for 6th instar western spruce budworm.[a]

Formulation	N	NC	Slope±S.E.	ED_{90} (95% CL)	Suggested application rates
					(g/ha)
BAY SIR 8514	889	88	0.261±0.047	118 (78.9-219)	472, 944, 1416
Methoprene: permethrin (9:1)	894	99	0.161±0.037	84.6 (52.5-255)	338, 677, 1015
UC 62644	892	82	0.283±0.049	1.80 (1.05-8.46)	7.2, 14.4, 21.6

[a]In column headings, N = number of test subjects; NC = number of controls; Slope±S.E. = calculated slope ± standard error; ED_{90} (95% CL) = effective dose for 90% mortality (95% confidence limits).

Table 3. – Effects of 1.27-cm equivalent rainfall on lethal effectiveness of three IGR formulations applied at reestimated ED_{90} to 6th instar western spruce budworm feeding on Douglas-fir branchlets.

Formulation	Dosage[a]	Mean percent mortality[b]		Yates' corrected χ^{2}[c]
		No rain	Rain	
BAY SIR 8514	118	93.5 (11.1)	88.6 (11.4)	1.46
Methoprene: permethrin (9:1)	84.6	96.0 (6.5)	95.0 (10.3)	0.075
UC 62644	1.80	96.3 (7.2)	94.7 (7.9)	0.035

[a]Dosages expressed in g/ha.

[b]Means are based on lethal effects observed in eight replications with 8 to 10 insects per replication.

[c]Value for comparison of no rain and rain treatments. The critical value of χ^2 at the $\alpha = 0.05$ level is 3.84, with 1 df.

distribution in conjunction with instar dose-response data provides an effective use of toxicological relationships during population development.

Use of response lines for nondiapausing insects and a probability distribution for diapausing insects to forecast times of application should not be regarded as a source of error. A concurrent study with nondiapausing insects revealed an equivalent distribution of instars over time when the starting point used was the beginning of the 2nd instar (Robertson, unpublished data). Thus, diapausing and nondiapausing laboratory colonies demonstrate comparable developmental patterns aside from the presence of diapause. Likewise, their response to chemicals is comparable (Robertson 1980). However, our theoretical probability distribution would be improved by acquisition of data concerning the frequency distribution of the termination of diapause, hence the pattern in which cohorts are added to populations. The effects of environmental variables such as temperature on instar distribution and cohort emergence from diapause might be ascertained to more precisely match recommendations to a set of environmental conditions.

The bioassay method described here has more practical value than separate definitions of the various facets of lethal effectiveness, such as effect by contact or effect by ingestion. Although separate types of effects are of interest in basic toxicological studies, it is often difficult to define overall toxic or lethal effectiveness based on separate effects. Such an attempt was described by Robertson (1980). With the present method, insects are subjected to contact with sprays and spray deposits; they also ingest the IGR formulation when foliage is consumed. These combined exposures are more comparable to exposure in the field than contact or ingestion effects considered separately.

The two-phase estimation procedure used to calculate each ED_{90} on which the field application rate is in turn based provides more reliable esti-

mates than the usual single-phase method used in the instar comparison. Its predictive value was confirmed in the rainfastness experiments; the statistical basis for the method will be described in a subsequent report.[5] However, there are a number of situations in which the number of test subjects available (for example, collections from field populations) precludes use of the two-phase method. In these cases, a single phase estimate with doses resulting in 5-98% response but with most doses clustered between 75-98% response may suffice to obtain a slightly less precise suggested field rate.

The value of the conventional one-phase estimation process in comparisons of the instar responses described here has not been questioned. We assume that the position of the instar response lines relative to one another would remain the same even if the upper portion of each response line were reestimated. Examination of the validity of this assumption was beyond the scope of our present consideration, but may be undertaken in the future.

Our ultimate goal was selection of a series of application rates or each IGR formulation. The ED_{90}s of chemicals obtained in a laboratory bioassay cannot be used directly for a variety of reasons; they are usually extremely optimistic. Previously, laboratory ED_{90} estimates were arbitrarily multiplied by 3 to obtain a field application rate (Williams 1973). Given the combination of imprecise estimation procedures in the laboratory and the lack of availability of experimentally derived multiplication factors, expected and observed results in field applications have been highly divergent. Most laboratory work on the western spruce budworm leading to field testing has concerned conventional insecticides. As a result, the derivation of multiplication factors to relate laboratory ED_{90} to field MED_{90} (minimum effective dose for 90% control) is based wholly on these chemicals. Until field data on IGR formulations becomes available, it will be impossible to derive multiplication factors specifically for IGRs. In the interim, the factors for conventional insecticides must be assumed to pertain to the nonconventional chemicals as well.

Whether or not an IGR formulation will actually be field tested depends on industrial decisions. If these matters were decided only on the basis of scientific evidence, UC 62644 would appear to be the IGR of choice for testing on the western spruce budworm. An alternative, though less desirable from the predicted dosage required, might be either BAY SIR 8514 or the methoprene:permethrin mixture. The TH 6040:methoprene mixture cannot be considered a viable candidate because of solubility problems at the dosages required for 90% effect.

ACKNOWLEDGMENTS

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[5] J. L. Robertson, N. E. Savin, and M. I. Haverty. Experimental design and model fit in dose-mortality regression. Manuscript in preparation.

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