Evidence of DDT Resistance in Populations of Spruce Budworm, *Choristoneura fumiferana* (Clem.), From DDT-sprayed Areas of New Brunswick

By A. P. RANDALL[1]

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A series of laboratory toxicological experiments using various concentrations of oil formulated DDT solutions (AR-50/fuel oil (2:7 V/V)) was carried out on 5th and 6th instar spruce budworm larvae collected in the field from DDT-sprayed and untreated areas of New Brunswick, Canada, and Maine, U.S.A.

Results obtained in 1959, 1961, and 1962 with larvae collected from isolated, unsprayed areas in New Brunswick showed a consistent, straight log-dosage probit mortality curve. Larvae collected in 1962 and 1963 from infestation centres previously subjected to three, four, and five applications of non-consecutive large-scale aerial sprays of DDT showed a significant departure from the straight log-dosage probit curve previously obtained. The departure occurs as a change in the shape of the curve as well as a shift to the higher concentration range of DDT. The magnitude of change appears to be correlated with the number of sprays to which the population was exposed. Results obtained in 1962 and 1963, from untreated control and inter-spray areas, bounded by DDT-sprayed forest lands, showed a small but significant departure from the normal straight probit line of a susceptible population. These changes are indicative of a progressive development of DDT resistance in wild populations of spruce budworm.

Studies on the effect of the tolerance of spruce budworm larvae within instar classes to the action of DDT showed that the early phase of instar development immediately after moulting is more susceptible to the action of DDT, whereas the latter phase of instar development immediately prior to moulting is more tolerant to topical application of DDT than the average for the instar. This effect is evident in both susceptible and resistant populations.

The data interpretation assumes that a deviation from the straight line probit dosage – mortality curve is indicative of a difference in the DDT-susceptibility factor of the budworm population and that in the course of the tests, the amount of toxicant causing mortality was not proportional to the dosage.

Introduction

In 1944–45 a series of aerial spray experiments by Stewart (1949) established DDT as a field insecticide for use against the spruce budworm, *Choristoneura fumiferana* (Clem.), in Canada. Since then, DDT has been used extensively for forest insect control throughout the provinces, particularly British Columbia, Quebec, and New Brunswick.

Large-scale aerial applications of DDT have been carried out in New Brunswick yearly from 1952 to the present date, excepting 1959 (Webb *et al.* 1961; MacDonald 1963). Satisfactory control of the budworm was obtained with a single application of DDT in the initial years of the spray program but, by 1957, the need to respray certain areas became evident because of high larval or egg populations following one application of DDT. At approximately the same time it became evident that DDT was injurious to aquatic fauna, particularly aquatic insects, trout and salmon fry. This stimulated a search for new chemicals and further research into insecticide formulation, and application methods. Results of numerous laboratory and field tests indicated that the insecticidal efficiency of such promising, low hazard materials as sevin, malathion, korlan, DDD, dimethoate, phosphamidon, and a bacterial preparation, *Bacillus thuringiensis* (Berliner) did not equal that of DDT against the budworm larvae, thus necessitating the continued use of this insecticide for the control of the spruce budworm. Investigations conducted in 1959 and 1960 (Fettes 1960) in unsprayed

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areas in New Brunswick indicated that satisfactory control could be achieved at a dosage as low as ¼ lb. DDT per acre, and presented a lower degree of hazard to young salmon and trout. Should a higher dosage of DDT be required for budworm control, because of the development of DDT resistance, the subsequent increase in toxic hazard to fish and fish food organisms would eventually eliminate DDT as an acceptable insecticide. A laboratory study was established early in 1962, to determine whether a differential response to DDT existed between budworm populations from repeatedly treated and untreated areas. The results and conclusions of these experiments are presented in this paper.

Materials and Methods

Test Insecticide

Technical DDT was formulated in a dyed oil carrier (AR-50/fuel oil (2:7 V/V)), 0.5% Dupont oil red, to provide a stock solution containing 10% DDT (W/V). Aliquots of the stock solution were diluted with the dyed oil carrier to provide the various concentrations of DDT used in the tests (0.5, 2.0, 4.0, 6.0, 8.0, and 10.0% for the 1959, 1960 and 1962 series) and (0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0% for the 1963 series). Colorimetric calibrations were established for each concentration for purposes of spray tower adjustment and dosage calculation. The ratio of DDT/dye concentration provided a means of estimating the DDT concentration.

Test Insects

The test insects consisted of healthy spruce budworm larvae collected from balsam fir, *Abies balsamea* (L.) Mill. and white spruce, *Picea glauca* (Moench) Voss at various locations in the epidemic infestation areas of New Brunswick, Canada, and Maine, U.S.A. All larvae were sent by air express on the date of collection to the Ottawa laboratory, where the toxicity tests were carried out. Only 5th- and 6th-instar larvae of apparent health, and vigour, were selected for the tests. The larvae were separated into instar classes, placed on fresh balsam foliage, and held for 48 hours at rearing temperature (70° F. and 70% R.H.) prior to spray application. The year of collection, location, and history of insecticide treatment for each site was presented in Table I and shown in Figs. 1 and 2 for central New Brunswick and the Portage Lake area in Maine, U.S.A., respectively.

Experimental Technique

The experiments were conducted over a period of five years. Every effort was made to standardize shipment of larvae, preparation of the insecticide formulation, method of spray application and the rearing of the test insects. Where time and material were available, insect collections and experiments were duplicated to reduce variance due to uncertainty of larval treatment during transportation. All tests with controls were carried out using the maximum number of larvae available. In 1959, 1961, and 1962, each test constituted a total of 320–360 larvae (100 for controls, and 40 for each concentration treatment). In 1963 the number of concentrations of DDT used in the tests was increased from 6 to 11 to provide scalar dosages in order to differentiate more precisely the change in the slope of the dosage–mortality curve. Each test represents a total of 600–720 larvae.

The spray procedure was briefly as follows: A conveyor belt spray tower apparatus with a modified Potter's tower and nozzle assembly was used for spray application. With the exception of experiment A-8, which was sprayed at 1 G.P.A., the spray tower and conveyor belt were adjusted to deliver a constant volume rate of ½ gal. per acre of solution to each sampling unit which consisted

TABLE I

Date, location, and history of spruce budworm collection sites at the time of larvae collection

Year	Location	No. of years DDT application	Prior DDT spray history of area
1959	Scotch Lake, N.B.	0	Approx. 10 miles south and outside of 1958 DDT spray boundary
1961	Chipman, N.B.	0	Forest Biology non-spray check plot. Closest spray boundary approx. 10 miles south and southwest of this area
1962	University of New Brunswick, Fredericton, N.B.	0	1958 DDT spray boundary approx. 6–8 miles north of this area
1962	Boiestown, N.B.	3	Sprayed with DDT in 1957 (½ lb./acre); 1960 (¼ lb./acre); 1961 (¼ lb./acre)
1962	Ludlow, N.B.	4	Sprayed with DDT in 1956 (½ lb./acre); 1957 (½ lb./acre); 1960 (⅜ lb./acre); 1961 (¼ lb./acre)
1962	Cains River, N.B.	0	Approx. 5 mile strip of non-spray area bounded on the north by DDT spray zones (1956, 1957, 1960, 1961) and on the south by a one-year DDT spray zone (60)
1963	French Island, N.B.	0	Non-spray. Bounded on north by a DDT spray zone in 1961. Approx. ½ mile from spray boundary
1963	Boiestown, N.B.	3	Sprayed with DDT in 1957 (½ lb./acre); 1960 (¼ lb./acre); 1961 (¼ lb./acre)
1963	Ludlow, N.B.	5	Sprayed with DDT in 1956 (½ lb./acre); 1957 (½ lb./acre); 1960 (⅜ lb./acre); 1961 (¼ lb./acre); 1962 (¼ lb./acre)
1963	Rocky Brook, N.B.	0–5	Adjacent to Miramichi River near Ludlow, N.B.
1963	Dungarvon River, N.B.	3–4	Sprayed with DDT in 1956 (½ lb./acre); 1957 (½ lb./acre); 1960 (⅜ lb./acre); 1961 (¼ lb./acre)
1963	Bartholemew Brook, N.B.	3–4	Sprayed with DDT in 1956 (½ lb./acre); 1957 (½ lb./acre); 1960 (⅜ lb./acre); 1961 (¼ lb./acre)
1963	Portage Lake, Maine, U.S.A.	2 × 2	Sprayed with DDT in 1958, 1960 (two yearly applications ½ lb./acre)

of the larvae and a filter paper sample. Larvae of similar instar age were anaesthetized with CO_2 and separated into six random groups of 10 larvae. Each group of larvae was arranged adjacent to a 9-cm. Whatman No. 1 filter paper on the sample card. The units were passed under the spray tower in lots of six cards making 60 larvae per treatment. Control groups of 60 to 120 larvae were established for each treatment as natural mortality checks in the test population. Immediately after treatment, the larvae were placed on clean balsam fir foliage in glass containers and removed to the observation room. Temperature and relative humidity were maintained at 70 ± 2° F. and 70% R.H. respectively, throughout the duration of the test. Mortality counts were taken daily over a period of five days. The effect of natural mortality was removed by use of Abbott's formula (Abbott 1925). Spray deposit density for each card was determined by colorimetric assessment of dye collected on the filter paper samples using a Klett–Summerson colorimeter. The results were calculated and expressed in terms of micrograms of DDT per square centimeter (11.2 μg. DDT/cm.^{2} = 1 lb. DDT/acre).

Results

Results of the dosage–mortality analysis on the series of tests are presented graphically in Figs. 3 to 8, inclusive. The corrected mortality data and calculated

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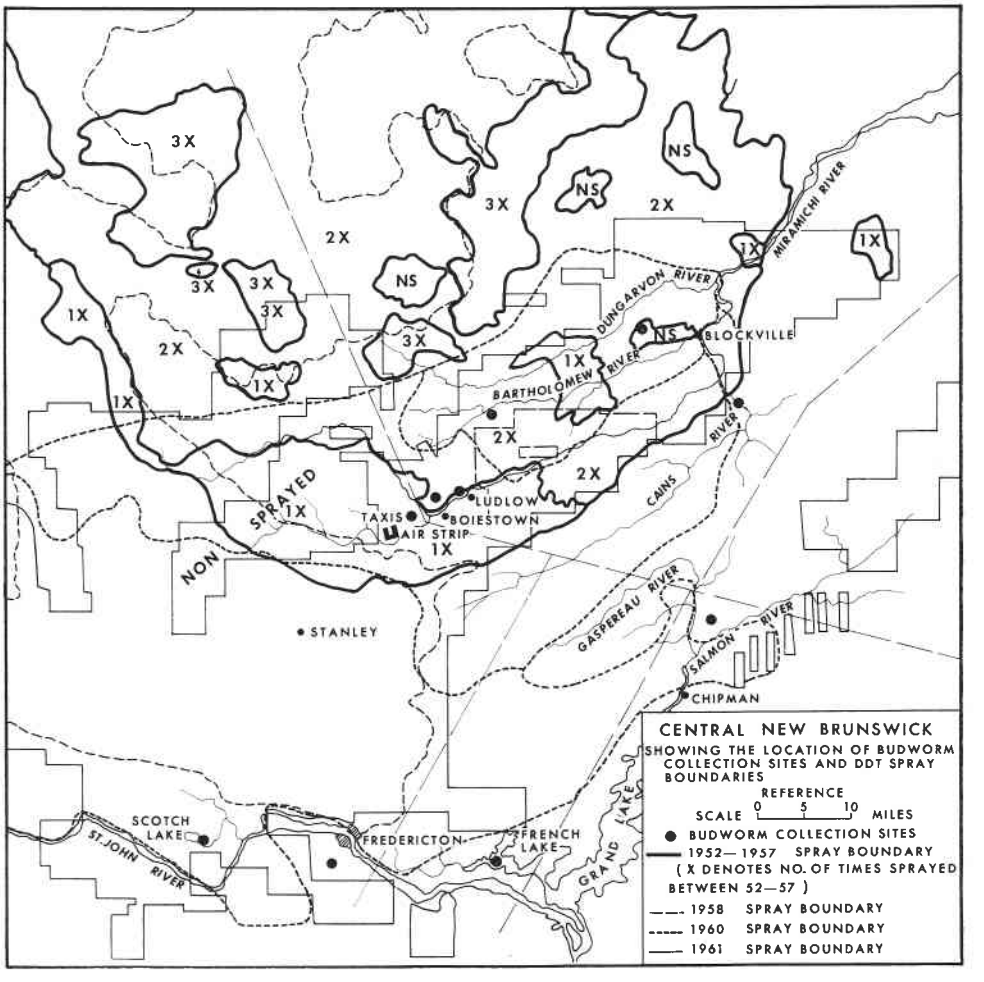

Fig. 1. Composite map of central New Brunswick showing location of budworm collection sites and DDT spray boundaries.

dosage expressed as μg. DDT/cm.^{2} were plotted on logarithmic probability paper.

The typical straight line dosage–mortality response for spruce budworm larvae collected from unsprayed areas in New Brunswick is shown in Fig. 3. The graphs represent data obtained in 1959 from the Scotch Lake area for 6th-instar larvae; in 1961, from the Chipman area for 5th- and mid-6th-instar larvae; and in 1962 from the University of New Brunswick Woodlot, Fredericton, for 5th-instar larvae. These results, when expressed as LC_{50}'s are in close agreement with earlier findings by Rayner and Hurtig (1953) and Hurtig and Rayner (1953). Their estimated 48 hour LC_{50} for topical applications of DDT droplets to individual 6th-instar larvae were in the order of 0.3 μg. DDT/insect with the expected true value to be between 0.1 and 0.6 μg. DDT/larva. Since the dorsal surface area of a full grown 6th-instar larva is in the order of ½ cm.^{2}, the estimated 48 hour LC_{50} for 6th- and mid-6th instar would be 0.5 and 0.9 μg. DDT. By comparison, the estimated LC_{50} values for 5th-instar larvae, taken from the 1961 and 1962 data, would be in the order of 0.3 and 0.4 μg. DDT per insect.

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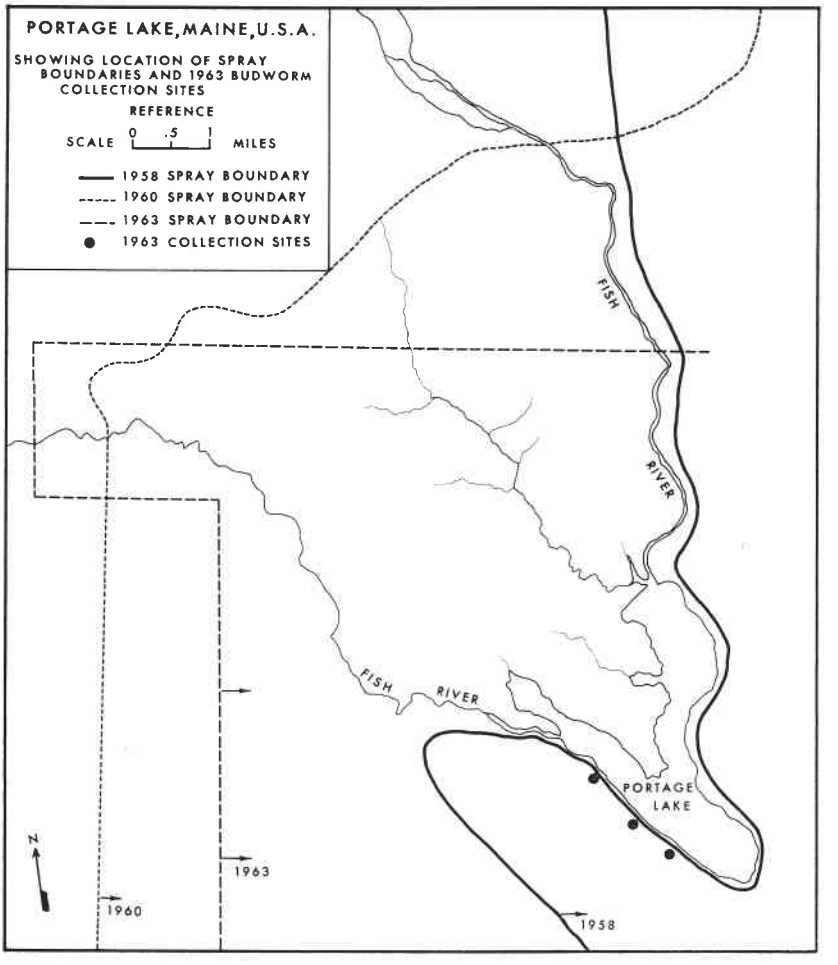

Fig. 2. Portage Lake area, Maine, U.S.A., showing location of budworm collection sites and DDT spray boundaries.

Dosage–mortality curves derived from the experiments using larvae collected from areas adjacent to boundary lines of previously DDT-sprayed forests are shown in Fig. 4. The Cains River area (A-2) is representative of a boundary area surrounded on three sides by forest land subjected to 1 to 3 years of DDT application; whereas the French Island site, Fig. 4 (D-2) is representative of an area bounded on the north by a single, recent application of DDT. In both graphs, a departure from the expected straight probit curve occurs at the higher dosage concentrations. The change is most pronounced at the 24-hour level, and may be indicative of the first stages of the development of DDT resistance.

Dosage–mortality curves obtained in 1962, and in 1963, using larvae collected from forested areas subjected to 3 years of non-consecutive DDT field sprays are presented in Fig. 5. The departure from the standard, straight probit curve is strongly evident in all of the graphs.

The results of the 1962 tests on larvae collected from the Boiestown area (Fig. 5, A-3, A-4 and A-5) show a marked departure in the shape, position, and slope of the dosage–mortality curve as compared to those in Figs. 3 and 4. The validity of these dosage–mortality curves was confirmed and further substantiated

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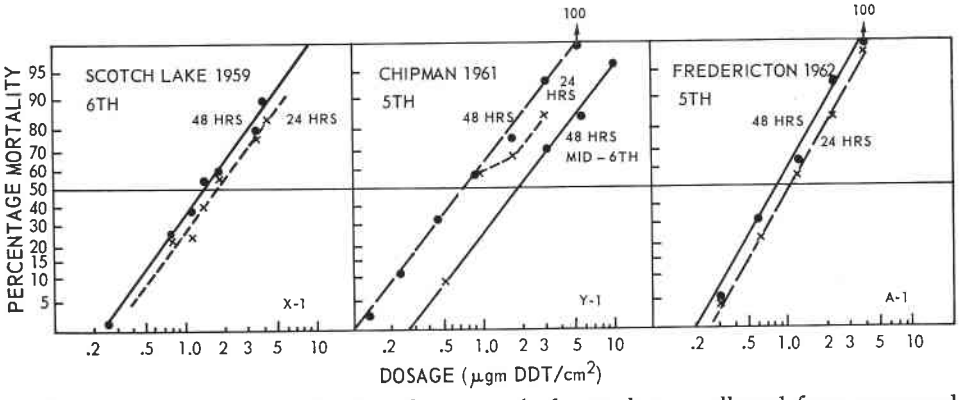

Fig. 3. Log-dosage mortality lines for spruce budworm larvae collected from unsprayed areas in New Brunswick: X-1, Scotch Lake 1959; Y-1, Chipman 1961; A-1, University of New Brunswick Woodlot, Fredericton 1962.

in 1963, using larvae collected from the identical 1962 collection site. The shape of the curve in the 1963 series of tests was strengthened by increasing the number of larvae per test from 400 to 600 and by providing additional dosage concentrations to cover more thoroughly the intermediate range of dosages used in the 1962 series. Although the 1963 series of graphs (Fig. 5, D-3, D-4 and D-5) are not identical with those obtained in 1962, they do, however, show a common trend in the response of the larvae to DDT. Similar response is also shown in toxicity data from larvae collected from other widely separated areas in New Brunswick that were subjected to 3 years of DDT field sprays. Results of DDT toxicity tests using larvae collected from the Dungarvon River area (D-7) and the Bartholomew junction (D-5) show a dosage–mortality curve similar to that obtained from the Boiestown area, which indicates an overall degree of resistance of the budworm larvae to higher levels of DDT.

Dosage–mortality curves obtained from larvae collected in 1962 in the Ludlow area subjected to 4 years of DDT spray history are shown in Fig. 6 (A-6, A-8) for 6th- and late 6th-instar larvae. With the exception of a decided shift in the position of the graph to the higher dosage level, a similar pattern of change occurs in the shape of the dosage–mortality curve as previously shown in Fig. 5. This pattern was also obtained in 1963 when larvae collected from the

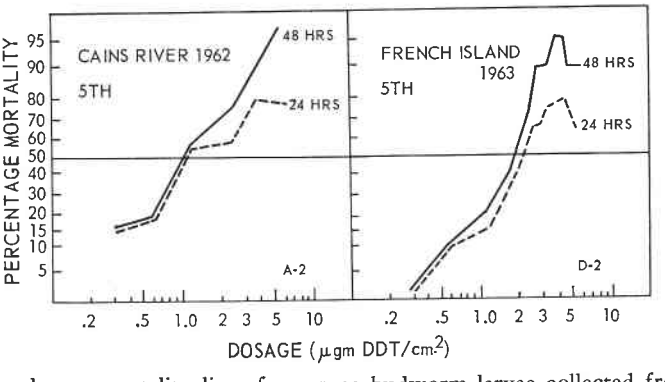

Fig. 4. Log-dosage mortality lines for spruce budworm larvae collected from unsprayed areas lying adjacent to DDT spray boundaries: A-2, Cains River 1962; D-2, French Island 1963.

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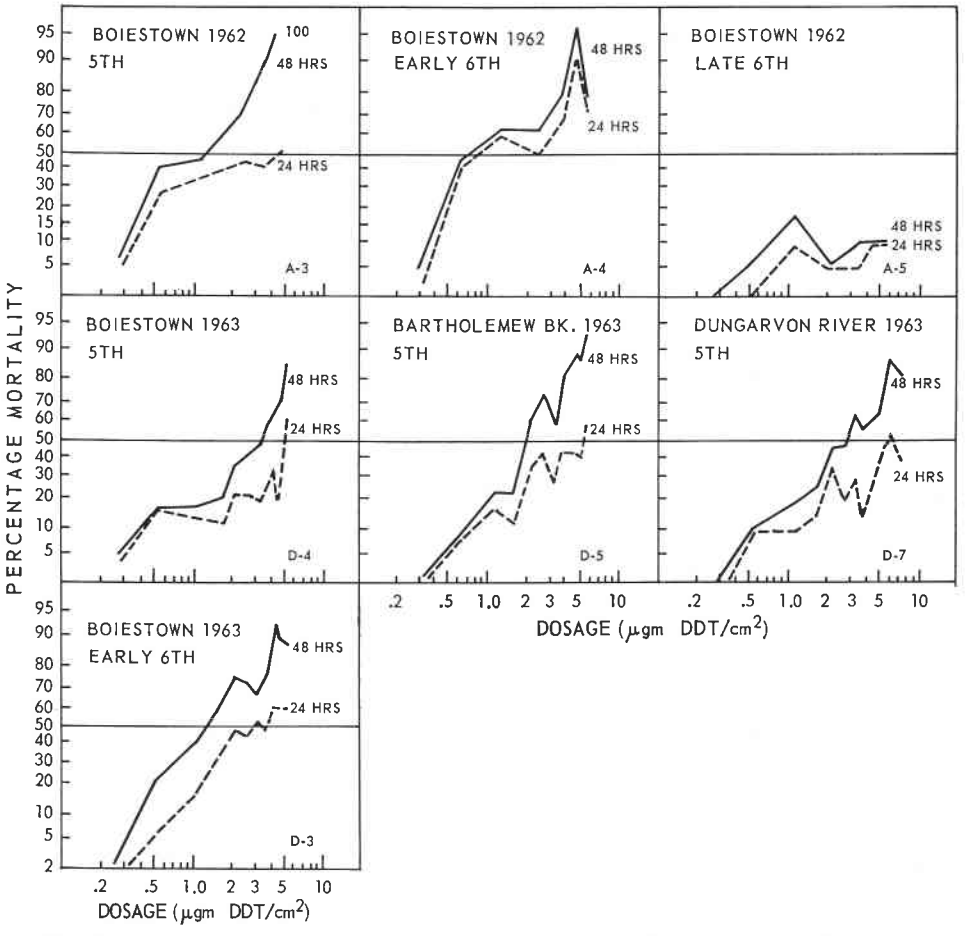

Fig. 5. Log-dosage mortality curves for spruce budworm larvae collected from forested areas subjected to three years of non-consecutive DDT spray application: A-3,4,5, Boiestown 1962; D-3,4, Boiestown 1963; D-5, Bartholemew Brook 1963; D-7, Dungarvon River 1963.

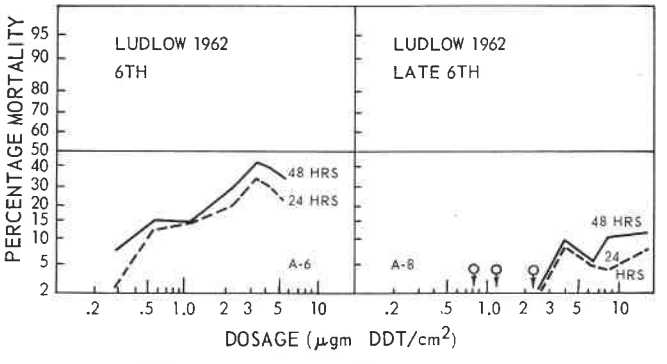

Fig. 6. Log-dosage mortality curves for spruce budworm larvae collected from forested areas subjected to four years of non-consecutive DDT spray application: A-6, A-8, Ludlow 1962.

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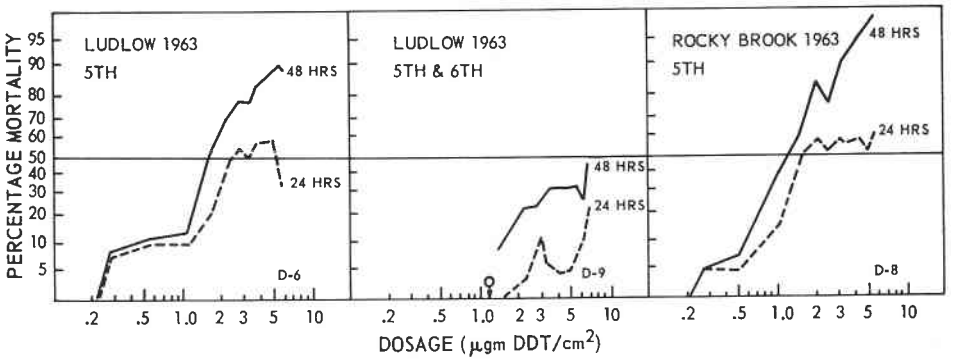

Fig. 7. Log-dosage mortality curves for spruce budworm larvae collected from forested areas subjected to five years of non-consecutive DDT spray application: D-6, D-9, Ludlow 1963; D-8, Rocky Brook 1963.

same location, but with an additional year of DDT field spray application, were subjected to the DDT toxicity tests as shown in Fig. 7 (D-6, D-9).

An exception to the development of DDT resistance occurred in the toxicity data from larvae collected from the Rocky Brook area which is located close to the Miramichi River, near Ludlow, but within the zone of 5 years of DDT application. The results, as shown in Fig. 7 (D-8) for 5th-instar larvae, resemble the dosage–mortality lines obtained from larvae from the Cains River area and those obtained from French Island (Fig. 4). This may be due to the fact that this particular collection site was located within the very narrow (¼ mile) untreated zone established along all major rivers by Forest Protection Ltd., to reduce the toxic effects of direct DDT sprays on aquatic fauna.

To determine the geographical extent of the development of DDT resistance in treated wild populations of spruce budworm, larvae were collected from a DDT-sprayed epidemic area in central Maine, U.S.A. The area is approximately 120 miles northwest of the Boiestown–Ludlow infestation of New Brunswick and has been subjected to two years of double applications of DDT aerial sprays (½ lb. DDT/½ gal. per acre). The dosage–mortality results for 6th- and late 6th-instar larvae shown in Fig. 8 compare with those obtained from the Boiestown–Ludlow areas. These results suggest a similar pattern of DDT resistance in wild budworm populations over the entire area studied.

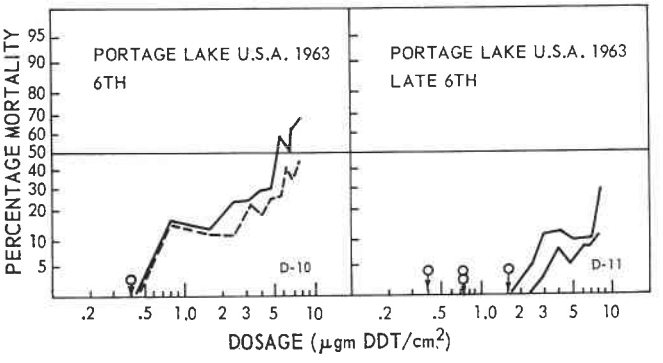

Fig. 8. Log-dosage mortality curves for spruce budworm larvae collected from forested areas subjected to two years of double application of non-consecutive DDT spray application: D-10, D-11, Portage Lake 1963.

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Discussion

Resistance to a specific insecticide is the consequence of selection pressure exerted by the presence of a particular toxic chemical in the environment, rather than the result of a build-up of tolerance in the individual to repeated exposure of sublethal amounts. Resistance in a population is a matter of degree and must be compared to a normal susceptibility level. The first indications of the emergence of a resistant strain of insects usually comes from observations in the field in areas of repeated treatment. General observations are imprecise and must be substantiated by carefully controlled laboratory experiments.

The establishment of a dosage–mortality probit regression curve permits the comparison of the response of insect populations with a repeated history of insecticide treatment to that of a normal population, assuming that the original probit line is representative of a susceptible population, and that the slope is indicative of the amount of variation within that population. Deviations from this standard slope will indicate incipient resistance which is evident at the higher dosage levels (Busvine 1957). As a larger proportion of the population becomes resistant, the displacement in the curve will occur at lower dosage levels until finally it may become stabilized at the level of a fully resistant strain. Populations comprising mixtures of normal resistant, intermediate and susceptible individuals will give irregular, flattash dose–mortality curves with several changes in slope as shown by the theoretical findings of Tsukamoto (1963) in his studies on the genetic analysis of insecticide resistance in the housefly (Fig. 9). When the per cent mortalities of a homogeneous population of insects are plotted against varying log-doses on graph paper, a sigmoid curve (Fig. 9,B) is formed which is the integral curve for the normal frequency distribution (Fig. 9,A) in a population. The per cent mortality curve (Fig. 9,B) can be transformed into a straight probit line (Fig. 9,C) by the use of probit tables or an appropriate equation. In a heterogeneous population (Fig. 9,D) comprising several sub-populations of different resistance levels, the per cent mortality curve (Fig. 9,E) does not give rise to a straight line (Fig. 9, F) even after probit transformation.

The establishment of the log-dosage probit mortality curve for spruce budworm larvae in 1959, 1961 and 1962, from widely separated untreated areas in New Brunswick, permits comparison of segments of budworm populations collected from the DDT-sprayed epidemic areas to the original standard probit lines as shown in Fig. 3. The consistent departure of the log-dosage mortality curves obtained in tests conducted on larvae from areas of repeated DDT applications (Figs. 4–7) are indicative of the development of DDT resistance. The validity of the shape of the curves obtained in the 1962 tests was further substantiated and confirmed by the use of increased numbers of scalar dosage concentrations and larvae.

The degree of resistance appears to be quite variable, with the magnitude of variability ranging from incipient resistance to 10 times that of a normal susceptible population. The variability may be partly due to collection or by the cross mixing of surviving populations. Thus the sample parameters may have contained isolated islands or pockets of susceptible, intermediate, or resistant type budworm individuals, or it may represent a genetic mixing of residual populations with introduced populations through moth flight or larval drift. The latter possibility appears to be quite probable since a resistance-like response occurred in populations from the Cain River and French Island areas. Both of these collection sites were in close proximity to DDT spray boundaries and in the path of the normal flight drift of moth populations. A pure popula-

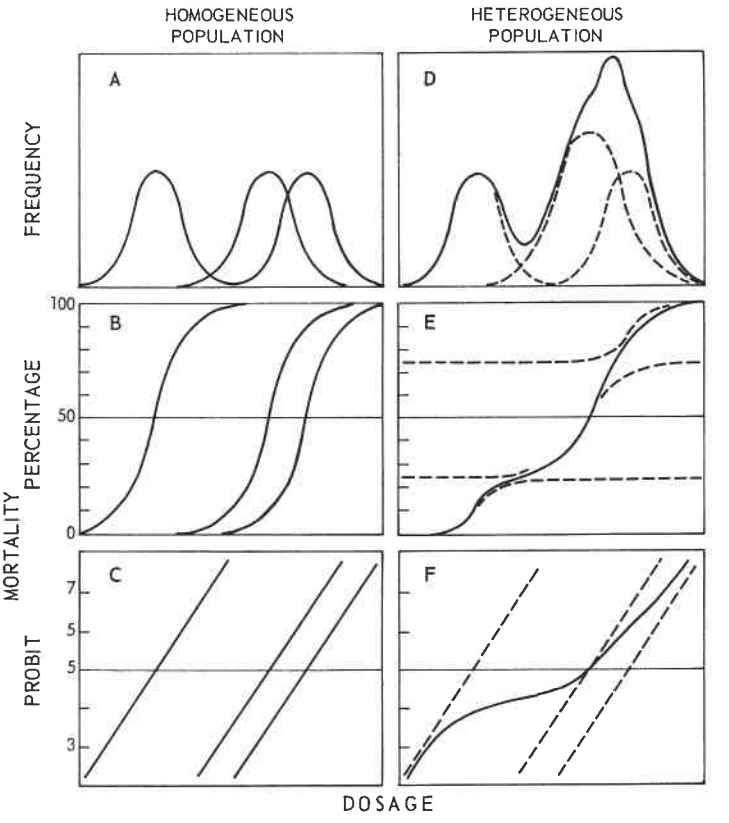

Fig. 9. Comparison of the dosage–mortality relation between homogeneous and heterogeneous populations (after M. Tsukamoto 1963).

tion of either susceptible or resistant budworm is not likely to occur but, rather, a heterozygous population between the extremes is more probable.

The shape of the log-dosage probit curves obtained in the 1962–63 series of toxicological tests for the Boiestown, Ludlow and Portage Lake areas, as shown in Figs. 5–8 is suggestive of Tsukamoto's synthesized plateau type log-dosage probit curves obtained in a monofactorial inheritance system where resistance is completely dominant. In such a system, a single distinctive plateau may be expected at the 25% mortality level. The development of two distinct plateaux in the dosage–mortality curves, as shown in Fig. 7 for the Ludlow area, is strongly suggestive of incomplete dominance or a population of insects in which a considerable degree of crossing between susceptible, hybrid and resistant individuals has occurred. The curves obtained closely resemble the dosage–mortality curves obtained by Coker (1958) in his studies on inheritance of DDT resistance of mosquitoes in which case the backcross progeny gave a two-stepped curve. This was maintained in subsequent backcrosses with selection and it was concluded that the inheritance was monofactorial. In the Ludlow area where the selection pressure would be considerable due to repeated DDT sprays, a decrease in the proportion of susceptibles would be expected from the crossing of hybrid and resistant individuals.

The variation in the position and slope of the dosage–mortality curves for larvae of similar instar size and apparent history of DDT treatment was initially

TABLE II
Age classification of larvae used in the DDT resistance tests

Exp. code 1962	Total no. larvae	Percentage of larvae by instar class															Category
		0 hr.		24 hr.			48 hr.			96 hr.			144 hr.				
		5th	6th	5th	6th	P	5th	6th	P	5th	6th	P	5th	6th	P		
1962																	
A-1	330	100		100			55	45		9	91		4	96			mid 5th
A-2	320	100		85	15		63	27		22	78		7	93			mid 5th
A-3	320	100		81	20		63	27		17	83		5	95			mid 5th
A-4	320		100		100			100			98	2		87	13		early 6th
A-5	317		100		94	6		55	45		37	63		21	79		late 6th
A-6	320		100		99	1		95	5		80	20		36	44		mid 6th
A-8	320	0	100		96	4		79	21		44	56		27	73		late 6th
1963																	
D-2	600	100		47	53		18	82		13	87		2	98			late mid 5th
D-3	600		100		100			100			100			87	13		early 6th
D-4	600	100		70	30		39	61		7	93		4	96			mid 5th
D-5	598	100		70	30		36	64		9	91		1	99			mid 5th
D-6	600	100		74	26		50	50		17	83		1	99			mid 5th
D-7	599	100		85	15		46	54		33	67		5	95			early mid 5th
D-8	596	100		76	24		53	47		24	76		3	97			mid 5th
D-10	600		100		94	6		91	9		83	17		62	38		mid 6th
D-11	600		100		99	1		87	13		71	29		39	61		late 6th

unclear. However, Busvine (1960) stated, "A given concentration/mortality probit line should be characteristic of a particular species and insecticide under specific conditions. Should a change in the environment or physiological state of the insect occur, then the position of the regression line may alter," and from this the following explanation may be drawn. It is strikingly evident in the difference in LD_{50} values for DDT between the various larval instars of the spruce budworm (LD_{50} for 2nd-instar larvae, 0.003 μg. DDT/larva and an LD_{50} for 6th-instar at 0.9 μg. DDT/larva). The same effect apparently exists within instar classes.

To determine what effect changes in physiological age within instar classes might have on budworm mortality, the survivors of each of the tests were checked daily and the interval time between the next moult or pupation recorded. The results are presented as a daily percentage of surviving larvae by instar class at the time of checking (Table II). It is apparent from this table that the time interval between the date of spray application and the next moult is not the same for all the tests. For purposes of rapid identification, the surviving larvae from each test were grouped into three categories, i.e. early, mid, or late instar, according to the development rate of the survivors on a daily basis. Larvae that readily moulted to a succeeding instar or pupated were classified as being late instar at the time of spray application, and conversely, a slow change or no change in larval instar class within a five-day period would be classified as being early instar.

It is apparent that in field collections of larvae used for laboratory toxicity tests, it is possible to have a population of one instar age group separated in time and physiological age with an apparently similar instar group. Subjection of field collections of such larvae to toxicity tests can result in a considerable difference in larval mortality, as shown in Fig. 10. The difference is most

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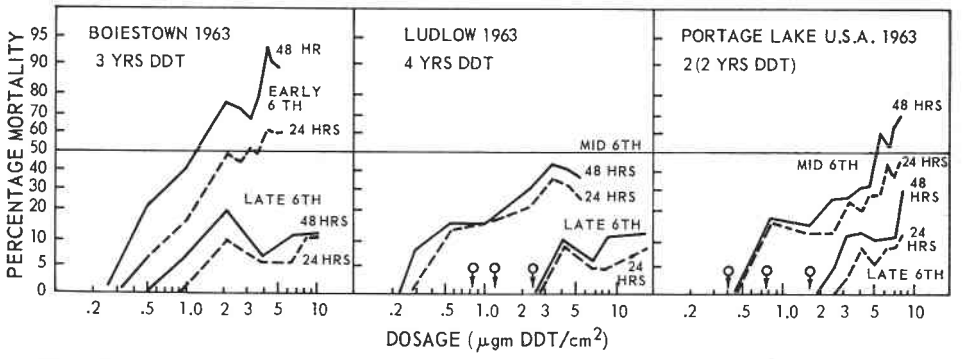

Fig. 10. Log-dosage mortality curves for 6th-instar spruce budworm larvae showing intra-instar response to increasing dosages of DDT.

pronounced in the final instar class (6th instar) as shown by the position of the various log-dosage mortality curves for 6th-instar larvae collected from areas of similar spray history. It is also interesting to note that the curves for mid and late 6th-instar larvae from the Ludlow and Portage Lake areas are very similar in shape and position, yet these collection areas are separated by approximately 120 miles of forest. They do, however, have the same cumulative total of DDT spray but the intervals between sprays are different.

The consistent departure from linearity in the slope of the log-dosage mortality curves obtained in the tests is positive evidence of DDT resistance in wild populations of spruce budworm. The geographic extent of this resistance may be expected in other areas subjected to repeated DDT sprays. The degree of resistance, however, may be quite variable, ranging from incipient resistance to as high as 10 times that of a normal, susceptible strain. In a resistant population (Busvine 1959) portions of the regression line will change shape according to the degree of acquired resistance in the population until 100% resistance occurs. When both resistance and the effect of intra-instar tolerance to an insecticide occurs as in the testing of wild populations of insects, it becomes imperative to separate the various effects in order to determine the degree of resistance.

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Ecology of Species of *Bombus* Latr. (Hymenoptera: Apidae) in Southern Alberta. III. Subgenus *Cullumanobombus* Vogt[1]

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The subgenus *Cullumanobombus* is represented by *Bombus rufocinctus* in the Nearctic region. In southern Alberta, its distribution is confined to the wooded areas. There, it is adaptable in its nest-selecting habits, and establishes nests mostly in June. It made 11.2 ± 1.4 cells in the first brood. In each cell, one egg was laid in the first brood, 4.2 ± 1.2 eggs in the second and third, and 8.2 ± 3.9 in the fourth and later broods.

Queens required about 22 days to rear workers. The second and third broods were usually workers and the fourth and later broods usually males or queens. The workers in a colony varied little in size. The dominant color pattern was *B. rufocinctus* s. str. This species is a prolific producer of wax.

The queens mated in the morning and hibernated in the afternoon, about an inch deep in the soil.

Many queens invaded other nests of the same species. The natural enemies were the three species of *Psithyrus* indigenous to the area, the big-headed fly, *Physocephala texana*, and ants.

Introduction

Although *Bombus* (*Cullumanobombus*) *rufocinctus* Cress. is a common species of bumblebee in North America, little has been written about its biology. Putnam (1866) found a colony in Bridport, Vt., that was later identified from the specimens taken from it as that of *B. rufocinctus* by Franklin (1913). Putnam noted that the honey pots were sealed and that the species was savage in disposition. Medler (1957) noted that the maximum number of workers found in a nest in Wisconsin was 110 and the maximum number of males and queens 87. Medler (1960) obtained 19 colonies in artificial domiciles, but recorded no data on biology other than to note that *B. rufocinctus* was a late-season species and a

[1]Contribution from the Entomology Section.