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RESISTANCE TO ORGANOCHLORINE INSECTICIDES IN THE TUBER FLEA BEETLE, *EPITRIX TUBERIS* GENT. (COLEOPTERA: CHRYSOMELIDAE), IN BRITISH COLUMBIA[1]

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ABSTRACT

Laboratory and field experiments showed that **Epitrix tuberis** Gent. had developed strains that were highly resistant to dieldrin and less so to DDT. Both adults and larvae were resistant to the cyclodiene insecticides. Strains resistant to cyclodienes were centered in the Salmon Arm and Vernon areas. Strains resistant to DDT had a wider range and were present as far north as Pavilion. All tuber flea beetles tested in the province were highly susceptible to diazinon and presumably to other organophosphorus compounds.

INTRODUCTION

In the southern interior of British Columbia the tuber flea beetle, *Epitrix tuberis* Gent., has been controlled effectively since 1953 by incorporating into the soil the cyclodiene organochlorine insecticides: aldrin, chlordane, dieldrin and heptachlor (Banham, 1960). These insecticides gained a ready acceptance and were widely used because one low-cost application gave broad-spectrum insecticidal effectiveness. In 1963, laboratory tests were conducted to determine the susceptibility of *E. tuberis* to dieldrin and DDT. Dieldrin was included because of the reported failure in 1960 of soil applications of the cyclodiene insecticides to control *E. tuberis* in Clackamas County, Oregon, (Morrison, 1962). DDT was included because it was used in British Columbia as a foliar treatment against this pest after 1948 following investigations by Finlayson and Neilson (1954); it remains an alternative to soil treatments with the cyclodienes.

The first suspicion that resistant *E. tuberis* were present in British Columbia came at harvest in 1964, in the Salmon River Valley. Six growers reported excessive larval tunneling damage in their potatoes in spite of the use of aldrin or dieldrin at recommended rates. This paper reports the initial laboratory experiments in 1963 and further tests in 1965. Data are reported also from a field experiment in the Salmon River Valley in 1965 to confirm the occurrence of resistance.

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MATERIALS AND METHODS

Laboratory Experiments

Larvae of this species were not used for the susceptibility tests because they are extremely small and difficult to rear. They are root and tuber feeders that desiccate rapidly on exposure. Field-collected second generation adults were used because of their hardiness, abundance, and ease of handling. The sex ratio is 1:1 (Neilson and Finlayson, 1953), but there are no external sex characteristics, and no attempt was made to determine differences in male and female susceptibility. Collections were made at the peak of emergence from nine major potato growing areas in 1963 and from eight areas in 1965 (Fig. 1.) The beetles were held at 4 to 10°C in screen-topped glass jars and provided with fresh, uncontaminated potato foliage. Prior to testing, beetles from each area were acclimatized in screened cages at 22°C. Active beetles were removed from the cages with an aspirator, anaesthetized with CO_2, and held temporarily in a 150 mm Büchner funnel under a continuous flow of the gas. Anaesthetized beetles were transferred with a brush or forceps to the exposure cages.

In 1963 impregnated papers from two sources were used: the Macdonald Test Kit and the W.H.O. Test Kit[2]. The Macdonald exposure cage consisted of a cardboard Dixie cup with a silk screen lid, a plastic ring, and an impregnated exposure paper that covered the sides and bottom of the cup. This exposed the beetles to contact with the impregnated paper on all but the top, screened surface of the cage. The concentrations of the impregnated papers used were: 0.0, 0.25, 0.5, 1.0, 2.0, and 4.0% DDT in Risella oil. The W.H.O. papers (W.H.O., 1960) were impregnated with: 0.0, 0.1, 0.2, 0.4, 0.8, 1.6, and 4.0% dieldrin in Risella oil. Each was fitted to the inside of a 40x100 mm cardboard tube with screened ends.

In 1965 the only exposure cage used was the W.H.O. Test Kit, a transparent plastic cage with screened ends. The impregnated papers used included W.H.O. dieldrin papers as described above and also W.H.O. DDT papers with concentrations of 0.0, 0.5, 1.0, 2.0, and 4.0% DDT in Risella oil. In addition, two series of papers prepared at the Vancouver Research Station were used: the first included concentrations of 0.0, 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0% dieldrin in a 1:1 mixture of Risella oil and trichloroethylene; the second included 0.0, 0.0625, 0.125, 0.25, 0.5 and 1.0% diazinon in a 1:1 mixture of acetone and corn oil. The papers were prepared by applying uniformly 2.0 ml of insecticide solution to a 12x15 cm sheet of Whatman No. 1 filter paper placed on a horizontal plane of points. After the more volatile solvents evaporated, each paper was attached to a cord by a paper clip and hung to dry for at least 24 hours before use.

The toxicities of laboratory - and W.H.O.-prepared dieldrin papers were found to be comparable when susceptible and resistant strains of beetles were exposed to each series.

Each replicate consisted of ten beetles per concentration of insecticide. Depending on the number of beetles available, the number of replicates per collection area varied from one to three in 1963 and from one to five in 1965. When there were not enough beetles from one location to complete a replication, those remaining were combined with beetles of similar susceptibilities from three or more areas.

The caged beetles were exposed to the insecticides in a cabinet at 22°C and 75% relative humidity. Exposure periods ranged from one to four hours. Knockdown, or inability to walk normally, was recorded at the end of the exposure. The beetles were then transferred to clean holding tubes containing fresh, uncontaminated

[2] The Macdonald Test Kit was supplied by Prof. F. O. Morrison, Dept. of Entomology and Plant Pathology, Macdonald College, Ste. Anne de Bellevue, P.Q.; the W.H.O. Test Kit by Dr. R. Pal, Division of Environmental Health, World Health Organization, Geneva.

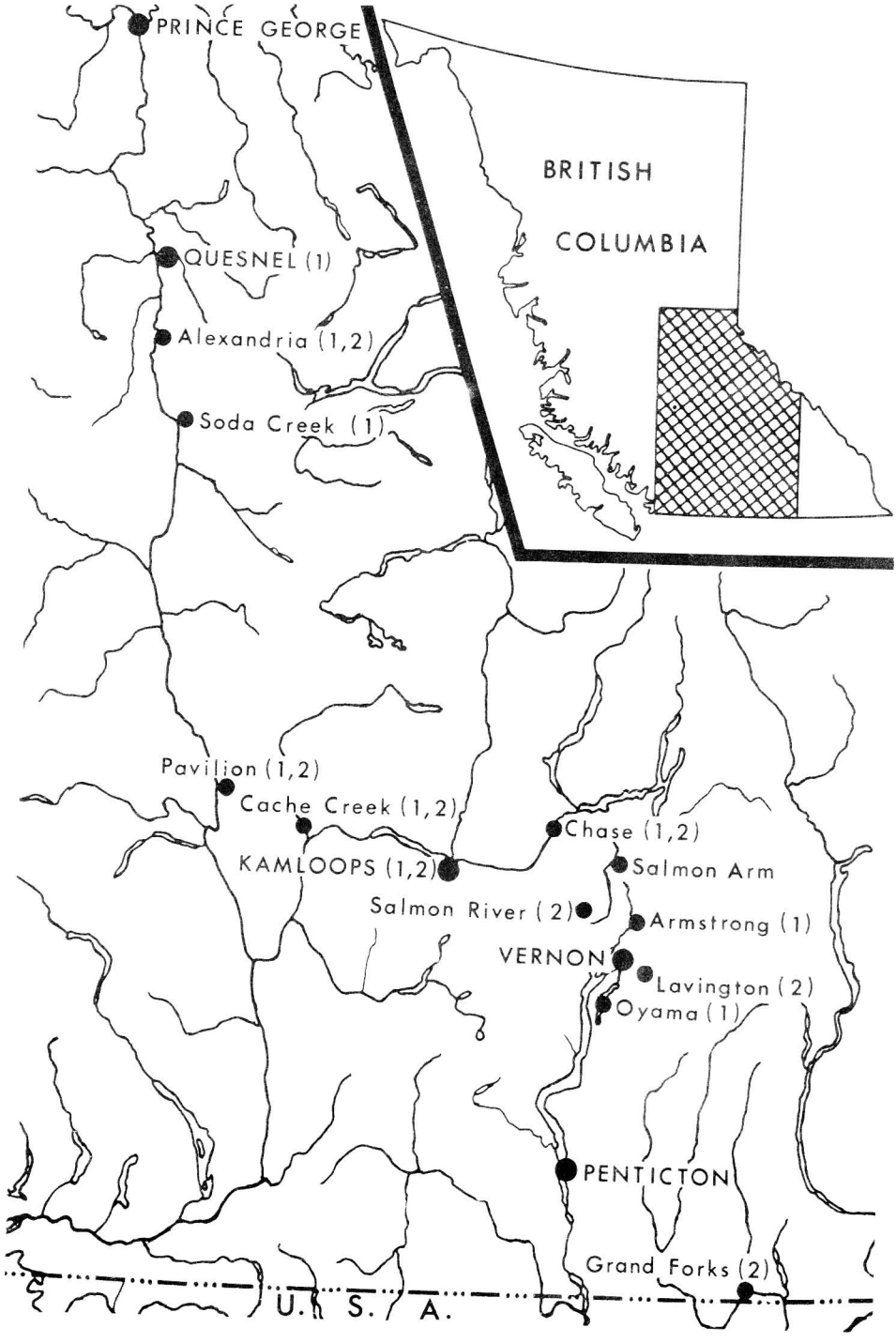

Fig. 1.—Potato growing areas in British Columbia where tuber flea beetles, **Epitrix tuberis** Gent., were collected: (1) 1963; (2) 1965; and (1,2) 1963 and 1965.

potato foliage and returned to the cabinet. Mortality counts were made at the end of a 24-hour recovery period. Beetles unable to walk were counted as dead.

In these tests, beetles from a given locality were considered to be resistant if the slope of the dosage-mortality curve was flat, or if a ten-fold increase in concentration resulted in less than a 20% increase in mortality. Populations showing an increase in mortality greater than 20% but less than 90% at this increased concentration, were defined as tolerant; those with increases greater than 90% were defined as susceptible. The data were averaged. Corrections for natural mortality were made using Abbott's formula (Abbott, 1925).

Field Experiments

In 1965 an experiment to compare aldrin-treated and untreated plots was set out in the Salmon River Valley (Fig. 1). The plots, approximately 24 yd² (22 m²), were replicated four times in randomized blocks. Aldrin 20% emulsifiable concentrate, was sprayed on the soil, at the recommended rate of 4 lb. toxicant acre (4.48 kg ha), prior to planting. The aldrin was incorporated into the soil to a depth of 3 to 4 inches (7.5 to 10 cm) by discing. Two samples of tubers were taken from the treated and untreated plots: the first, 84 days after planting, was to determine the damage inflicted by first generation larvae; the second, 147 days after planting, was to determine the seasonal damage by first and second generation larvae. To assess damage, a subsample of 25 tubers of a minimum diameter of 1.5 inches (4 cm) was selected from each plot sample. The tubers were peeled to a uniform depth and the number of larval tunnels recorded.

RESULTS

Laboratory Experiments

Knockdown and mortality counts of beetles exposed to DDT, with minor exceptions, were highest at the longest periods of exposure. For any given concentration and exposure, knockdown counts paralleled the mortality counts but at lower levels.

In 1963 (Table 1) there was little, if any, resistance to DDT at Chase, Quesnel, or Soda Creek. However, at Alexandria, Armstrong, Cache Creek, Kamloops and Pavilion the results indicated the first stages of resistance. There was little evidence of resistance to dieldrin.

TABLE 1.—Susceptibility to DDT and dieldrin of adult **E. tuberis** in British Columbia, 1963.

Location	Exposure (hr.)	Mortality (%)[1] at 24 hr. DDT (%)				
		0.25	0.5	1.0	2.0	4.0
Alexandria	2	10.0	30.0	10.0	30.0	60.0
Alexandria	4	20.0	50.0	50.0	90.0	100.0
Armstrong	4	50.0	50.0	90.0	80.0	80.0
Cache Creek	4	50.0	0.0	0.0	80.0	100.0
Chase	2	50.0	60.0	60.0	70.0	100.0
Kamloops	2	17.0	27.0	37.0	50.0	67.0
Kamloops	4	40.0	50.0	65.0	90.0	100.0
Pavilion	2	10.0	10.0	10.0	15.0	30.0
Quesnel	2	22.2	—	11.1	—	100.0
Soda Creek	2	20.0	40.0	30.0	100.0	100.0
Composite[2]	4	88.9	66.7	88.9	88.9	100.0
			Dieldrin (%)			
		0.1	0.2	0.4	0.8	1.6	4.0
Alexandria	4	—	60.0	80.0	100.0	100.0	100.0
Kamloops	1.5	10.0	30.0	60.0	70.0	90.0	100.0
Kamloops	2	0.0	75.0	62.5	87.5	100.0	100.0
Kamloops	4	20.0	70.0	100.0	100.0	100.0	100.0
Pavilion	1.5	3.0	13.0	37.0	80.0	97.0	100.0
Composite[2]	2	30.0	0.0	70.0	90.0	90.0	100.0

[1] Average corrected by Abbott's formula (1925) [2] Armstrong, Kamloops and Oyama.

By 1965 (Table 2) there was strong evidence of DDT-resistance at Lavington and Salmon River. Of the beetles from eight potato-growing areas, those from Lavington and Salmon River also exhibited a high resistance to dieldrin, probably approaching a homozygous-resistant population. Beetles from Cache Creek and possibly those from Pavilion showed less resistance, or a heterozygous population. Beetles from Alexandria, Chase, Grand Forks and Kamloops were still susceptible. The beetles with high DDT and dieldrin resistance, from Lavington and Salmon River, were highly susceptible to diazinon. A composite sample of beetles from Alexandria, Cache Creek, Chase, Kamloops and Pavilion were also equally susceptible.

Field Experiments

At Salmon River, tuber samples taken 84 and 147 days after planting

TABLE 2.—Susceptibility to DDT, dieldrin, and diazinon of adult **E. tuberis** in British Columbia, 1965.

Location	Exposure (hr.)			DDT (%) Mortality (%)[1] at 24 hr. 0.5	1.0	2.0	4.0
Lavington	1			0.0	10.0	10.0	10.0
Lavington	2			0.0	0.0	10.0	20.0
Salmon River	1			0.0	0.0	5.0	15.0
Salmon River	2			10.0	10.0	0.0	20.0
Composite[2]	1			0.0	23.5	0.0	58.8
Composite[2]	2			0.0	10.0	0.0	60.0
				Dieldrin (%)			
		0.125	0.25	0.5	1.0	2.0	4.0
Alexandria	1	59.5	81.1	97.3	86.5	100.0	100.0
Cache Creek	1	30.0	50.0	45.0	50.0	70.0	65.0
Chase	1	90.0	95.0	100.0	100.0	100.0	100.0
Grand Forks	1	73.9	100.0	100.0	100.0	100.0	100.0
Kamloops	1	70.0	80.0	90.0	100.0	100.0	100.0
Lavington	1	0.0	0.0	0.0	0.0	5.0	0.0
Pavilion	1	30.0	40.0	60.0	90.0	90.0	100.0
Salmon River	1	3.1	0.0	3.1	0.0	0.5	6.1
				Diazinon (%)			
			0.0625	0.125	0.25	0.5	1.0
Lavington	1		60.0	75.0	90.0	100.0	100.0
Salmon River	1		65.0	90.0	90.0	95.0	100.0
Composite[2]	1		80.0	90.0	90.0	90.0	100.0

[1] Average corrected by Abbott's formula (1925).
[2] Alexandria, Cache Creek, Kamloops and Pavilion.

from untreated and aldrin-treated plots showed little difference in the amount of larval feeding damage. This confirmed the laboratory evidence for cyclodiene resistance in *E. tuberis*. Average numbers and ranges of larval tunnels per tuber from aldrin-treated and untreated plots were as follows:

84 DAYS	Average	Range
aldrin-treated	56.6	11-209
untreated	57.7	9-139
147 DAYS		
aldrin-treated	229.1	27-484
untreated	187.6	7-536

DISCUSSION

It is difficult to determine the level of resistance in an insect species when the range of concentrations of the test insecticides is restricted by the availability of field-collected specimens. The level should be determined by direct comparison of the LD_{50} of the suspect strain with that of the normal susceptible strain (Brown, 1958).

From the results obtained in 1963, beetles from Pavilion were resistant to DDT while those from Alexandria, Armstrong, Cache Creek and Kamloops were tolerant. All the beetles from the nine locations sampled in 1963 were susceptible to dieldrin. Suspected resistance at Salmon River in

1964 was confirmed by laboratory tests in 1965. The same tests confirmed resistance at Lavington. The failure of a soil-incorporated application of aldrin in the field experiment showed that the larvae were resistant also. Soil treatments of aldrin or other cyclodiene insecticides normally prevent damage by killing the newly emerged 1st, 2nd and on occasion, 3rd instar larvae while they search in the soil for potato roots or tubers. It was demonstrated that these populations had cross-resistance to DDT, but not to diazinon, and presumably not to other organophosphorus compounds. Beetles from Cache Creek were highly tolerant to dieldrin, and the DDT - tolerance shown in 1963 by beetles from Alexandria, Cache Creek, Kamloops, and Pavilion was reflected in the low mortality counts of the composite sample after exposure to DDT in 1965.

In the interior of British Columbia the tuber flea beetle has developed resistance to DDT and dieldrin in areas where extensive use of soil-incorporated cyclodiene insecticides commenced in 1953 and 1954 superseding foliar applications of DDT. Use of cyclodiene insecticides, known to be persistent (Banham, 1961), resulted in accumulations of insecticidally active residues in the soil. Since *E. tuberis* is virtually host specific, the whole population at one location was continually exposed to broadcast or band applications of the current year plus the accumulated residues from previous years. This, coupled with the tendency of growers to shorten the sequence of crop rotation under conditions of concentrated production, subjected this species to increased selection pressure.

It has been shown (Varzandeh *et al.*, 1954) that development of resistance has no apparent effect on the biotic potential of *Musca domestica* L. Results of tuber damage assessments from field plots at Salmon River in 1965 clearly indicate that this applies as well to *E. tuberis*.

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