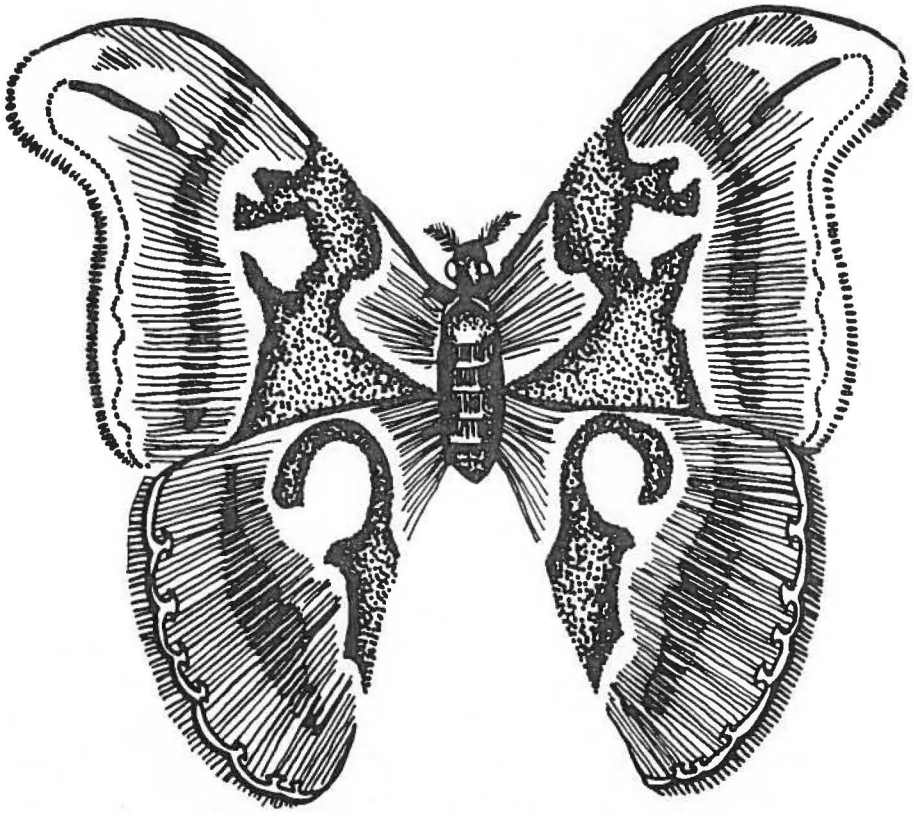
VOL. 10 1976

R. Brush

ISSN 0076-3810



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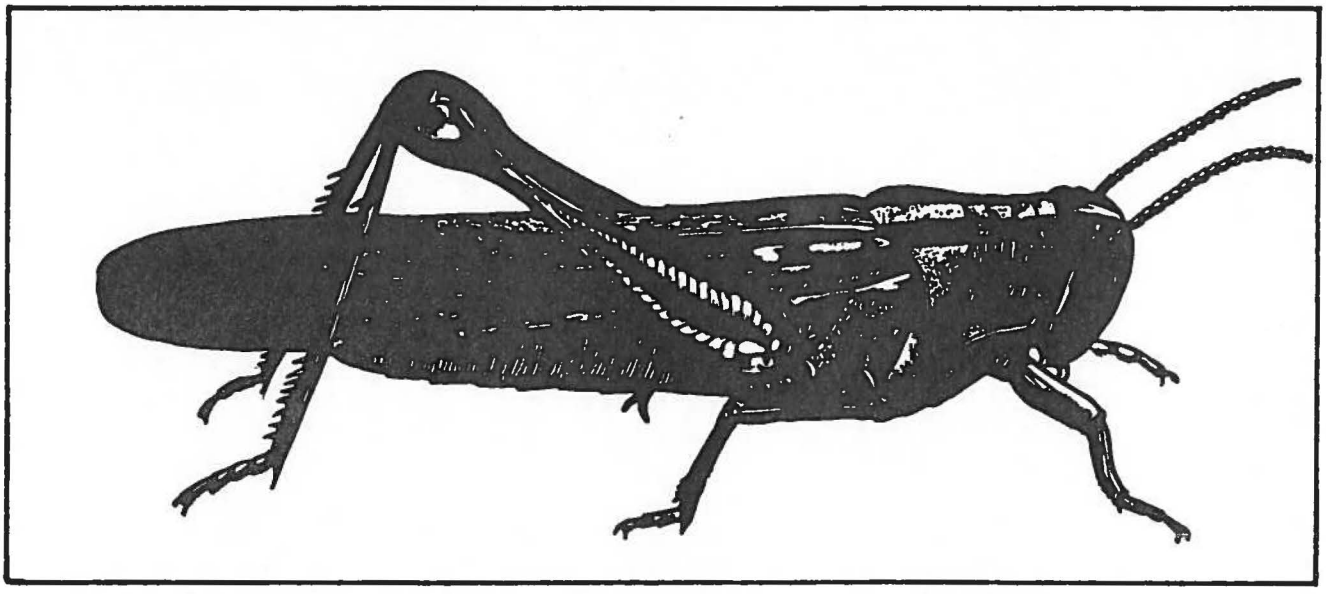

ISSN 0076-3810

THE MANITOBA ENTOMOLOGIST

VOLUME 10

1976

An official publication of the Entomological Society of Manitoba.

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Vol. 10 *1976*

THE MANITOBA ENTOMOLOGIST

An official publication of the Entomological Society of Manitoba, an organization to foster the advancement, exchange and dissemination of entomological knowledge

CONTENTS

WEIGHT GAIN IN CALVES IN RESPONSE TO CONTROL OF CATTLE GRUBS WITH INSECTICIDES

D. L. SMITH

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Winnipeg, Manitoba, Canada, R3C 0P8

ABSTRACT: In tests with systemic insecticides for the control of cattle grubs, *Hypoderma* spp., in calves, there was no significant difference in average daily weight gain between treated and untreated animals.

INTRODUCTION

The control of cattle grubs, *Hypoderma* spp., has long been promoted by those associated with livestock production and most of the promotion has been based on the heavy economic losses attributed to cattle grubs. Losses in the form of damaged hides and meat resulting from the presence of grubs in animals at the time of slaughter are well documented (Meat Packers Council of Canada, 1968, 1971). Rich (1970), however, pointed out that present cattle marketing practices prohibit the return of either the cost of treatment, or profit accruing in the abattoir, to the producer who controls cattle grubs. The effect on weight gains, of gadding caused by ovipositing flies is undocumented, while weight gain data relating to the presence of grubs in cattle, provides no conclusive proof that grub-free cattle outgain grub-infested cattle. Campbell *et al.*, (1973) found that treated calves gained an average of 12 pounds more than untreated calves over a 100 day feeding period. Collins & Dewhirst (1971) also found that it was economically advantageous to control grubs in calves. On the other hand, Cox *et al.*, (1967 a,b), Raun and French (1961), Rogoff and Kohler (1960), Jones and Matsushima (1959) and Knapp *et al.*, (1958) found no significant differences in weight gains between treated and untreated calves. Other experiments have produced variable results (Khan & Lawson, 1965). The lack of conclusive evidence indicating positive weight gains could explain why many cattle producers remain reluctant to take advantage of the excellent control methods now available. Of all agricultural pests, none can be controlled as easily, effectively and cheaply as cattle grubs.

The purpose of the following tests was to determine the effect of cattle grubs on weight gains of calves under average wintering management conditions in Manitoba.

METHODS AND MATERIALS

A total of 621 calves, 292 treated and 329 untreated, were used in the tests which were carried out at 19 different locations in Manitoba from 1960 to 1972. The experiments were conducted on calves being weighed under the Federal-Provincial Record of Performance Program. As the animals were weighed in the fall, every second animal was treated with a systemic insecticide and the weight and sex of each animal recorded. At sixteen locations, a ready-to-use pour-on formulation of trichlorfon 8% was applied at the rate of ½ oz per 100 pound body weight. At three locations a mixture of 64 fl oz crufomate 25% in 20 gal water was applied as a high pressure (400 psi) spray at the rate of ½ gallon per calf. At the end of the test period the following spring, the calves were weighed and the number of grubs per animal determined by palpation along the back and sides between the shoulder and tailhead. During the wintering period, the calves were fed a growing ration of domestic hay and grain, free choice, and although management varied from location to location, the treated and untreated animals at each test location received the same level of management. The calves in all cases were overwintered out-of-doors in loose housing shelters. Average mean temperatures in Manitoba during the overwintering months are, November -4.4°C, December -13.7°C, January -18.3°C, February -15.7°C, March -8.1°C and April 3.3°C.

RESULTS AND DISCUSSION

Of the 19 tests carried out, nine showed gains in favour of the treated animals and nine showed gains in favour of the untreated check animals. In one test there was no difference in the average daily gain between the treated and untreated animals (Table 1).

With the exception of tests 4 and 17, where relatively high grub infestations were present in the check animals, there was no consistent relationship between weight gains and the number of grubs in the check animals. In fact, the weight gains recorded in tests 8 and 9, where the check animals carried no grubs, were just as variable as weight gains recorded in the remainder of the tests.

When the data were pooled and analysed using the standard students T-test of significance for a quality of means for two independent samples of unequal size as described by Snedecor and Cockrane (1971), there was no significant difference ($P>0.01$) between the average daily gain of the treated and untreated animals.

ACKNOWLEDGEMENTS

Many thanks are due to Mr. W. T. Henderson and Mr. H. Kernested, Livestock Specialists, Manitoba Department of Agriculture for arranging for the livestock producers who cooperated in these tests and to Mr. B. Ward, Resource Economist, Manitoba Department of Agriculture for assistance with the statistical analysis of the data collected.

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(Received 10 January, 1977)

Table 1. Weight gain in calves in response to control of cattle grubs *Hypoderma* spp. with insecticides.

Test	No. of calves	Insecticide	Mean no. grubs per calf	% Control	Mean weight (lb)			Days on test	Mean daily gain (lb)
					Start	Finish	Gain		
1	22	trichlorfon	0.00	100	443	772	329	170	1.93
	22	—	3.86	—	438	786	348	170	2.05
2	10	trichlorfon	0.10	96.9	418	710	292	186	1.57
	12	—	3.33	—	444	740	296	186	1.59
3	16	trichlorfon	0.00	100	451	623	172	170	1.01
	17	—	5.29	—	477	652	175	170	1.03
4	7	trichlorfon	0.00	100	452	657	205	143	1.43
	9	—	36.55	—	413	586	173	143	1.21
5	24	trichlorfon	0.08	99.3	503	677	174	165	1.05
	24	—	12.21	—	458	641	183	165	1.11
6	26	trichlorfon	0.00	100	406	631	225	127	1.77
	37	—	0.97	—	360	577	217	127	1.71
7	7	trichlorfon	0.00		503	907	404	161	2.51
	9	—	0.00		507	936	429	161	2.66
8	6	trichlorfon	0.00		405	706	301	168	1.79
	6	—	0.00		428	697	269	168	1.60
9	22	trichlorfon	0.09	97.1	449	713	264	160	1.65
	25	—	3.16	—	424	700	276	160	1.72

continued next page

Table 1. (Continued)

Test	No. of calves	Insecticide	Mean no. grubs per calf	% Control	Mean weight (lb)			Days on test	Mean daily gain (lb)
					Start	Finish	Gain		
10	29	trichlorfon	0.48	95.8	617	811	194	165	1.17
	26	—	11.65	—	580	758	178	165	1.08
11	13	trichlorfon	0.53	89.0	418	634	216	168	1.29
	22	—	4.82	—	377	561	184	168	1.09
12	12	trichlorfon	0.50	95.1	401	657	256	166	1.54
	17	—	10.35	—	384	624	240	166	1.45
13	11	trichlorfon	0.00	100	432	684	252	167	1.51
	15	—	0.26	—	421	679	258	167	1.54
14	20	trichlorfon	0.10	98.7	462	667	205	165	1.24
	22	—	7.73	—	389	584	195	165	1.18
15	16	trichlorfon	0.56	72.5	474	719	245	167	1.47
	22	—	2.04	—	420	685	265	167	1.59
16	16	trichlorfon	0.00	100	433	844	411	169	2.43
	19	—	1.26	—	385	739	354	169	2.09
17	10	crufomate	1.60	92.7	440	627	187	182	1.03
	7	—	22.00	—	409	583	174	182	0.96
18	8	crufomate	2.70	82.3	522	858	336	189	1.78
	7	—	15.30	—	556	891	335	189	1.77
19	17	crufomate	2.80	77.6	482	752	270	188	1.44
	11	—	12.50	—	435	714	279	188	1.48

EFFECTS OF AN AERIAL APPLICATION OF THE SYNTHETIC PYRETHROID PERMETHRIN ON A FOREST STREAM

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ABSTRACT: Permethrin (NRDC-143) applied at the rate of 70 g ai/ha by airplane to a forest stream had little effect on caged or native fish and did not significantly affect aquatic insect populations. Dramatic but short-lived increases in the drift of aquatic insects as well as heavy knockdown of terrestrial insects and spiders occurred after treatment.

INTRODUCTION

For many years large scale insecticide spraying has been conducted over Eastern Canadian forest regions to control damage caused by the spruce budworm, *Choristoneura fumiferana* (Clemens). Over the years, many insecticides have been tested for their suitability for controlling this pest species with minimum disruption to non-target organisms of forest habitats. Recently, interest has been focused on newly developed synthetic pyrethroids as promising new tools for spruce budworm control. These compounds are structurally similar to the natural pyrethrins which have been used as insecticides for years, but are more suitable for use in forest pest control because they are more stable, more toxic to insects and less hazardous to mammals and birds than natural pyrethrins (Abernathy and Casida, 1973, Nishizawa, 1971). Unfortunately, in laboratory bioassays, synthetic pyrethroids have been reported to be highly toxic to fish (Mauck *et al.*, 1976). They also pose a hazard to aquatic insects due to their potent insecticidal properties.

In 1976, preliminary studies were carried out on the effects of the synthetic pyrethroid permethrin (NRDC-143) on aquatic organisms when applied under field conditions. The purpose of these studies was to determine if permethrin presents too great a hazard to aquatic ecosystems to be considered for use in forest pest control operations. This is a report on the impact of aerially applied permethrin on a forest stream.

SITE DESCRIPTION AND METHODS

The study was carried out on the grounds of the Petawawa Forest Experimental Station near Chalk River, Ontario. A stretch of Youngs Creek about 75 m wide and 5 km long between Race Horse Road and Meridian Road was treated with permethrin at the rate of 70 g active ingredient per hectare (ai/ha). Youngs Creek is a small forest stream originating from Youngs Lake about 6 km above the treated portion and entering the Ottawa River about 8 km downstream from the treated section. The creek flows at a moderate pace through an open valley approximately 50 to 100 m wide. The creek itself varies from 3 to 10 m in width and 30 cm to 1.5 m in depth. The creek bed is primarily fine sand which is covered with silt and aquatic vegetation, primarily wild-celery, *Vallisneria americana* (Mich.). In some sections Alders, *Alnus spp.*, and grasses grow along the banks but there is no overhead forest canopy. Maunsell Creek, a similar but smaller creek flowing into Youngs Creek 1.25 km upstream from the treatment area, was used as the untreated control for the study.

The insecticide, in No. 2 fuel oil, was applied to the creek between 1935 and 1945 hours on 2 June by a Cessna 185 fitted with a Micronair® spray emission system. At the time of application the air temperature was 20°C, the relative humidity 37% and the mean wind speed less than 1 m/s. Automate B dye was added to the spray formulation to facilitate measurement of insecticide deposit. Lines of four deposit samplers consisting of paired aluminum pans 13 by 17 cm were placed on the shore across the creek bed at each end and the middle of the treated section of the steam. Insecticide deposit on the aluminum pans was determined colorimetrically (Haliburton *et al.*, 1975).

Prior to treatment of the creek, native fish (cyprinids and mudminnows) were trapped and placed in a cage at the downstream end of the treated stretch. These were checked twice daily for mortality. Two minnow traps baited with bread set nearby were also checked twice daily and their catch recorded and released. A control group of fish was caged in Maunsell Creek.

Drifting aquatic invertebrates were sampled in Youngs Creek by placing a drift net in the creek for thirty minute periods each morning and evening. The net sampled a 46 cm (18 in) wide portion of the creek's flow from surface to bottom, including the surface film. Additional one hour drift net sets were made immediately before and after the insecticide application to document immediate effects on aquatic invertebrates and sample terrestrial arthropods knocked into the creek. Drifting aquatic and terrestrial organisms were also captured in a blocking seine set across the entire width of the creek. The seine was emptied each morning and evening.

Populations of bottom fauna in the treatment and control streams were sampled by taking a series of 0.093 m^2 (foot square) Surber samples (Surber, 1936). Two stations were sampled in the treatment stream: Station 1 was located on a gravel bottom at the downstream end of the treated section while Station 2 was situated along a silt covered, sand bottomed stretch of stream near the centre of the treated area. The control station on Maunsell Creek was located on a silt covered, gravel bottomed portion of streambed. Four samples were taken from each station on each sampling occasion and preserved in formaldehyde. Organisms were later separated from the substrate in the laboratory with the aid of a "bubbler" (Kingsbury and Beveridge, 1977).

RESULTS

Only 19.2% of the emitted spray reached the surface of the creek. Deposit rate of permethrin was 13.4 g ai/ha. This relatively light deposit was probably due to loss of spray to the atmosphere through evaporation, as the treatment was applied under warm, dry conditions.

Shortly after treatment began, fish were observed to be feeding on distressed insects floating down the surface of the creek. The morning after treatment, two minnows (Cyprinidae) were observed swimming belly-up in distress in front of the blocking seine set across the creek at the downstream end of the treatment area. No other affected fish were seen after this time. No mortality occurred up to four days after treatment among caged cyprinids and mudminnows (Umbridae). Northern redbelly dace, *Chrosomus eos* Cope, common shiner, *Notropis cornutus* (Mitchill), brook stickleback, *Culaea inconstans* (Kirkland), and central mudminnow, *Umbra limi* (Kirtland), catches from the creek fluctuated considerably before and after treatment but revealed no apparent effect on fish populations.

Prior to treatment, blackfly larvae (Diptera: Simuliidae) and midge larvae (Diptera: Chironomidae) were virtually the only aquatic insects found to be drifting in the creek. Immediately following treatment, a large variety of aquatic insects were represented in the drift (Table 1). Drift net catches remained high the morning after treatment, dropped somewhat by that evening and then fell to very low levels. Numbers of fish caught in drift nets before and after treatment were similar and only a small increase occurred in the catch of aquatic invertebrates other than insects. Blocking seine catches revealed greater numbers of aquatic insects drifting during the evening and night than during the day, with a large insecticide induced increase in drift superimposed on this diurnal periodicity but not disrupting it (Table 2). Particularly large catches of springtails (Collembola), mayfly nymphs (Ephemeroptera: Heptageniidae), water scavenger beetle larvae (Coleoptera: Hydrophilidae), midge larvae and pupae, water boatmen (Hemiptera: Corixidae), predaceous diving beetles (Coleoptera: Dytiscidae) and caddisfly larvae (Trichoptera) occurred the morning after treatment. A second large catch of mayfly nymphs occurred over the second dark period after treatment, but apart from this, aquatic insects caught in the blocking seine fell to low numbers while remaining fairly diverse in variety. Relatively large numbers of brook sticklebacks were captured in the blocking seine between two and four days after treatment, but all appeared healthy. The small drift net and blocking seine catches, three and four days after treatment, were a reflection of a 30 cm drop in the water level of the creek over the period drift was being sampled.

Table 1. Aquatic insects, other aquatic invertebrates and fish caught in 30 min drift net sets in Youngs Creek, 31 May to 6 June, 1976.

	Number of days before or after treatment													
	-2 pm	-1 am	-1 pm	-0 am	-0 pm	Immediately* Before	Immediately* After	+1 am	+1 pm	+2 am	+2 pm	+3 am	+3 pm	+4 am
Collembola	-	-	-	-	-	-	14	1	1	1	-	-	-	-
Ephemeroptera: Baetidae	-	-	1	1	-	-	15	-	1	-	-	-	-	-
Heptageniidae	-	-	-	-	-	-	-	8	1	-	-	-	-	-
Odonata	-	-	-	-	-	-	-	2	1	1	-	-	-	-
Plecoptera	-	-	-	-	-	-	8	2	-	-	-	-	-	-
Hemiptera: Corixidae	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Hebridae	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Coleoptera: Haliplidae	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Dytiscidae	-	-	-	-	-	-	3	-	-	-	-	-	-	-
Gyrinidae	-	-	-	-	-	-	5	1	-	-	-	-	-	-
Hydrophilidae	-	-	-	-	-	-	2	4	1	2	1	-	-	-
Elmidae	-	-	-	-	-	-	1	3	1	-	-	-	-	-
Trichoptera: Hydroptilidae	-	-	-	-	-	-	12	-	-	-	-	-	-	-
Unknown	2	-	-	-	-	-	2	3	1	-	-	-	-	-
Diptera: Culicidae	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Chironomidae	12	2	6	6	1	14	78	60	32	6	7	2	1	3
Simuliidae	60	84	20	19	17	31	196	54	43	10	5	1	-	1
Unknown	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Total aquatic insects	74	86	26	26	19	45	342	138	83	20	13	3	1	4
Oligochaeta	-	-	-	-	-	1	-	-	-	-	2	-	-	-
Hirundinea	-	-	-	-	-	1	-	1	-	-	-	-	-	-
Amphipoda	-	-	-	-	-	-	1	-	2	-	-	-	-	-
Acari	-	-	-	-	-	-	-	1	6	1	-	-	-	-
Total other aquatic invertebrates	-	-	-	-	-	2	1	2	8	1	2	-	-	-
Fish	2	2	-	2	1	1	-	2	-	2	-	2	1	-

*1 hour sets

Table 2. Aquatic insects, other aquatic invertebrates and fish removed from a blocking seine set across Youngs Creek, 31 May to 6 June, 1976.

	Number of days before or after treatment											
	-2 pm	-1 am	-1 pm	-0 am	-0 pm	+1 am	+1 pm	+2 am	+2 pm	+3 am	+3 pm	+4 am
Collembola	-	-	-	-	-	5872*	28*	6	8	-	-	-
Ephemeroptera: Ephemeridae	-	-	-	-	-	23	3	1	-	-	-	-
Heptageniidae	-	-	-	-	-	638	3	331	-	11	1	1
Baetidae	-	-	-	5	-	17	2	2	-	1	1	1
Odonata: Aeshnidae	-	-	-	-	-	2	-	-	-	-	-	-
Cordulegastridae	-	-	-	-	-	1	-	-	-	-	-	-
Libellulidae	-	-	-	-	-	1	-	-	-	-	-	3
Agrionidae	-	-	-	-	-	15*	-	1	-	1	-	1
Coenagrionidae	-	-	-	-	-	-	-	-	-	-	-	1
Hemiptera: Corixidae	-	-	-	-	-	316*	16	5	-	-	-	-
Notonectidae	-	-	1	-	-	-	-	-	-	-	-	-
Nepidae	-	-	-	-	-	2	1	-	-	-	-	-
Gerridae	-	-	-	-	1	38	11	3	-	-	-	-
Hebridae	-	-	-	-	-	3	7*	-	1	-	-	-
Coleoptera: Haliplidae	-	-	-	-	-	3	-	-	-	-	-	-
Dytiscidae (larvae)	-	-	-	-	-	-	16*	-	1	-	-	-
Dytiscidae (adults)	-	-	-	-	-	258*	58	-	3	-	-	1
Gyrinidae	-	-	-	-	-	18	7	5	1	-	-	-
Hydrophilidae (larvae)	-	-	1	-	1	415*	68	15	7	-	1	-
Hydrophilidae (adults)	-	-	-	-	-	7	1	-	-	-	-	-
Elmidae	-	1	1	-	-	-	-	-	1	1	-	1
Unknown	-	-	-	-	-	3	-	-	-	-	-	-

continued next page

Table 2. (Continued)

	Number of days before or after treatment											
	-2	-1	-1	-0	-0	+1	+1	+2	+2	+3	+3	+4
Trichoptera: larvae	-	2	-	1	2	244*	7	10	2	4	1	4
pupae	1	-	-	-	-	2	-	2	-	-	-	-
Diptera: Tipulidae	-	-	1	-	-	-	1	-	-	-	-	-
Chironomidae (larvae)	16	28	13	61	44	328*	14*	54	4	40	7	57
Chironomidae (pupae)	-	-	-	-	-	96*	-	-	-	-	-	-
Simuliidae	363	286	19	454	37	5	-	4	4	1	-	1
Unknown	1	-	-	-	2	-	6*	-	-	-	-	-
Total aquatic insects	381	317	36	521	87	8323*	228*	440	31	59	11	71
Nematoda	-	1	-	2	-	2	-	1	-	-	1	2
Oligochaeta	-	1	-	-	-	-	-	-	-	1	-	2
Hirudinea	-	1	1	-	-	-	-	1	-	-	-	-
Acari	-	-	-	-	-	192*	24	1	-	-	-	-
Gastropoda	8	2	22	12	-	55	19	58	7	75	9	3
Total other aquatic invertebrates	8	5	23	14	-	249*	43	61	7	76	10	7
Fish	1	1	1	-	-	5	1	1	11	19	2	16

*Includes numbers extrapolated from subsamples.

There were no significant differences in populations of aquatic insects taken in Surber samples from Youngs Creek and Maunsell Creek during the treatment period (Fig. 1). The areas sampled yielded limited populations of aquatic insects made up primarily of midge and other dipterous larvae. The few mayfly, dragonfly and stonefly nymphs, and caddisfly and coleopterous larvae taken, revealed no adverse effects of the treatment. No effects were noticeable on populations of other bottom organisms sampled. Substantial numbers of fish eggs were found in several bottom samples from both creeks before treatment. The presence of large numbers of fish fry with and without yolk sacs following treatment indicated that egg development and hatching was not noticeably affected by the treatment.

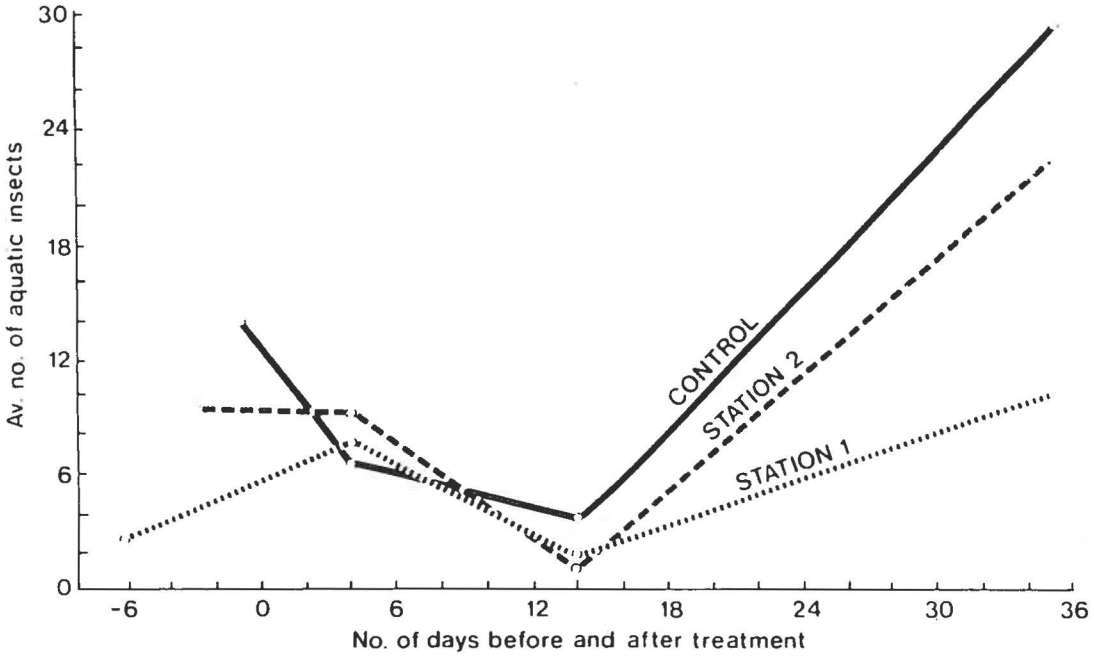

Figure 1. Aquatic insects present in bottom samples from Youngs and Maunsell Creeks, 27 May to 7 July, 1976.

Terrestrial insects and spiders knocked down onto the creek's surface were present in large numbers in drift net and blocking seine samples taken after treatment of the creek (Tables 3 and 4). The most pronounced effects were observed on adult midges and mosquitoes, hymenopterans, planthoppers and lepidopterous larvae. Knockdown was still apparent up to three days after treatment among some groups of dipterans, beetles, hymenopterans, lepidopterous larvae, caddisflies and spiders. Some of the knocked down terrestrial arthropods may have recovered eventually from their exposure to the insecticide after drifting on the creek's surface for a while.

CONCLUSIONS

The measured deposit of 13.4 g permethrin/ha on Youngs Creek had minimal effects on caged or native fish. Disturbance of aquatic insects was apparent from the increase in the number and variety of individuals present in the drift, but did not result in changes in population of bottom fauna, that were significantly different from changes in the untreated control stream. The knockdown of terrestrial insects and spiders into the creek was very heavy and relatively long-lasting despite the small area actually treated with insecticide.

Table 3. Terrestrial arthropods caught in 30 min drift net sets in Youngs Creek, 31 May to 6 June, 1976.

	Number of days before or after treatment													
	-2 pm	-1 am	-1 pm	-0 am	-0 pm	Immediately* Before	Immediately* After	+1 am	+1 pm	+2 am	+2 pm	+3 am	+3 pm	+4 am
Thysanoptera	-	-	-	-	-	-	4	-	-	-	-	-	-	-
Hemiptera: Nabidae	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Tingidae	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Lygaeidae	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Coriscidae	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Unknown	-	-	-	-	1	-	-	-	-	-	-	1	-	-
Homoptera: Cicadellidae	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Delphacidae	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Aphididae	-	-	-	-	-	-	2	1	-	-	-	-	-	-
Fulgoroidea	-	-	-	-	-	-	14	-	-	-	-	-	-	-
Coleoptera: Cicindelidae	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Carabidae	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Staphylinidae	-	-	-	-	-	-	8	1	3	-	-	-	-	-
Elateridae	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Curculionidae	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Unknown	-	-	-	-	-	-	5	-	1	1	-	-	-	-
Trichoptera (adults)	-	-	-	-	-	-	14	2	-	-	-	-	-	-
Lepidoptera (larvae)	-	-	-	-	-	-	20	7	5	2	-	1	-	-
Diptera: Tipulidae	-	-	-	-	-	-	5	-	-	-	-	-	-	-
Culicidae	-	-	-	-	-	-	4	-	-	-	-	-	-	
Chironomidae	-	-	2	1	-	-	125	19	6	10	1	4	-	3
Simuliidae	6	2	8	3	1	2	17	2	6	7	1	1	-	-
Bibionidae	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Unknown	-	-	-	-	-	-	71	5	7	3	1	7	3	-
Hymenoptera: Braconidae	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ichneumonidae	-	-	-	-	-	-	3	-	-	-	-	-	-	-
Formicidae	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Unknown	-	-	-	-	-	-	8	-	1	1	-	-	1	-
Arachnida	-	-	-	-	-	-	7	2	1	2	-	-	-	-
Total	6	2	10	4	2	2	325	39	30	26	3	14	4	3

*1 hour sets.

Table 4. Terrestrial arthropods removed from a blocking seine set across Youngs Creek, 31 May to 6 June, 1976.

	Number of days before or after treatment											
	-2 pm	-1 am	-1 pm	-0 am	-0 pm	+1 am	+1 pm	+2 am	+2 pm	+3 am	+3 pm	+4 am
Ephemeroptera	-	-	1	-	-	18	2	2	-	-	-	-
Odonata	-	-	-	-	-	-	-	1	1	-	-	-
Plecoptera	-	-	-	-	-	-	6	-	-	-	-	-
Thysanoptera	-	-	-	-	-	3	1	-	-	-	-	-
Hemiptera	-	-	-	-	-	102*	2	-	-	-	-	-
Homoptera: Fulgoroidea	-	-	-	-	-	1124*	-	1	1	-	-	-
Aphidoidea	-	-	-	-	-	397*	2	-	3	-	-	-
Unknown	-	-	-	-	-	5	21	-	-	2	-	-
Coleoptera: Staphylinidae	-	-	-	-	1	131*	17	4	4	-	-	-
Elateridae	-	-	1	-	-	105*	2	2	3	1	-	-
Coccinellidae	-	-	1	1	-	17	31	5	-	2	-	-
Chrysomelidae	-	-	-	-	-	-	-	-	1	-	1	1
Curculionidae	-	-	-	-	-	97*	1	1	-	-	-	-
Unknown	2	5	2	-	-	389*	76	20	13	2	-	1
Trichoptera	2	-	-	-	-	449*	54	27	4	5	2	1
Lepidoptera (larvae)	2	-	-	-	-	1023*	106*	2	33	1	3	-
(adults)	-	-	-	-	-	2	-	1	-	-	-	-
Diptera: Tipulidae	-	-	-	-	-	2	-	2	1	-	-	-
Chironomodae + Culicidae	-	-	-	1	1	12789*	196*	129	97	11	-	1
Simuliidae	-	-	-	-	1	42	362*	12	70	-	3	2
Unknown	-	1	-	-	-	1	456*	54	61	3	9	2
Hymenoptera: Formicidae	1	1	2	-	1	96	16	2	35	-	14	-
Unknown	3	-	1	-	-	1561*	79*	5	19	1	-	2
Unknown Insecta	-	-	-	-	-	12	-	7	1	1	4	-
Arachnida	-	-	-	-	-	651*	29*	10	3	1	1	-
Totals	10	7	8	2	4	18980*	1459*	287	350	30	37	10

* Includes numbers extrapolated from subsamples.

In conclusion, permethrin had no significant impact on the aquatic system treated, but its potential hazard to aquatic ecosystems requires further study before it can be used in forest pest control programs.

ACKNOWLEDGEMENTS

Support for this work was provided by Chipman Chemicals Ltd. and FMC Canada Ltd. Personnel of the Chemical Control Research Institute's Control Methods Research section assisted in the insecticide application and deposit assessment. D. Meisner, D. O'Gorman and R. Ostiguy provided technical assistance in the field and laboratory. The help of J. A. Armstrong, C. H. Buckner and O. N. Morris in reviewing the manuscript and of B. B. McLeod in drawing the figure is acknowledged with thanks.

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(Received 21 January, 1977)

FIELD APPLICATION OF THE MERMITHID NEMATODE, *ROMANOMERMIS CULICIVORAX* ROSS AND SMITH, FOR THE CONTROL OF MOSQUITOES, *AEDES* SPP., IN SPRING IN MANITOBA

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ABSTRACT: Temperature was the primary factor limiting infection by the mermithid nematode, *Romanomermis culicivorax* Ross and Smith, in mosquito larvae which develop in snow-melt pools. When preparasitic nematodes were applied to pools containing mosquito larvae, only about 20% of *Aedes canadensis* (Theobald) and *A. pionips* Dyar were infected at maximum temperatures of 5 - 10° C. No infection occurred at similar temperatures in larvae of *A. dorsalis* (Meigen) or *A. spencerii* Theobald. A few larvae of *A. communis* (De Geer) were infected at 0-5° C. Eight to 20% of the larvae of *A. vexans* Meigen) when appeared after an early spring rain were infected when preparasites were introduced into the pool. Larvae of *Mochlonyx velutinus* (Ruthe) and *Dixa* sp. and nymphs of Ephemeroptera were not infected. Survival of *R. culicivorax* during parasitic development was dependent upon host species and degree of superparasitism. Melanized nematodes were discovered in larvae of *A. vexans* and *A. canadensis,* and nematodes that had survived the winter and infected larvae of *A. communis* were all melanized. Mosquito larvae were apparently killed by high levels of multiple parasitism. *R. culicivorax* cannot be recommended at this time for the control of spring *Aedes* mosquitoes in Manitoba because of the low levels of infection, and the number of applications and high number of preparasites required to obtain infection.

INTRODUCTION

The mermithid nematode, *Romanomermis culicivorax* Ross and Smith, has a life cycle similar to that of most other aquatic mermithids. The infective stage, or preparasite, hatches from the egg when flooded by water. It then may locate and penetrate into the haemocoele of a host mosquito larva. After completion of its parasitic development, it emerges, still as a juvenile, killing its host. The postparasitic juvenile sinks to the bottom of the pool where it moults to an adult, mates and completes the life cycle.

The use of *R. culicivorax* for mosquito control has been investigated in Louisiana (Petersen and Willis, 1972; Petersen *et al.,* 1973), California (Petersen *et al.,* 1972) and in Taiwan (Mitchell *et al.,* 1974). The results are promising but the suitability of this mermithid for control of spring *Aedes* in temperate North America is unknown. Little information is available on either winter survival of *R. culicivorax* in northern latitudes or the susceptibility of many spring *Aedes* to *R. culicivorax.* It has been shown that both activity and infectivity of *R. culicivorax* preparasites were reduced at temperatures below 20° C (Galloway and Brust, 1977; Kurihara, 1976; Mitchell *et al.,* 1974; Petersen and Willis, 1971). The results of the first attempt to use *R. culicivorax* to infect *Aedes* spp. in snow-melt pools are presented in this paper.

MATERIALS AND METHODS

Preparasites of *R. culicivorax* were obtained from a laboratory culture maintained at 25° ± 0.5° C. Culture pans containing embryonated eggs were flooded at room temperature (20° - 25° C) with chlorine-free tap water 24 h before preparasites were to be introduced into field pools. Total numbers of preparasites were estimated by counting the number of preparasites in no less than five 1-ml aliquot subsamples 12 h after flooding. Approximately 12 h later,

preparasites were placed in containers in field pools for up to one hour to allow them to acclimate to the temperatures of the pools. They were then poured uniformly over each pool surface at rates of 20,000 (Site A) and 50,000 (Site B)/m^2.

At Site B, only pools containing >75 first and/or second instar mosquito larvae per dip of an 850 ml ladle, 135 mm in diameter, were chosen for treatment. Larval populations were estimated at the time of preparasite application from a series of at least 25 random dips. A minimum of 50 mosquito larvae were collected at random from each pool, where possible, at least twice weekly following treatment, and were taken to the laboratory where they were identified, classified to instar, and dissected to determine whether or not parasitic stages of *R. culicivorax* were present. Larvae were stored at 3°C to prevent moulting until they could be dissected.

Two sites were chosen as representative of habitats characteristically producing a generation of snow-melt *Aedes*.

Site A

This site was a vacant lot located in Winnipeg at the corner of Shore Street and Bison Drive. The area was dominated by numerous grass species with a few mixed forbs. Pools, formed from snow-melt in vehicle ruts or deep tire tracks, provided excellent breeding sites for *A. dorsalis* (Meigen) and *A. spencerii* Theobald.

Three pools were selected for treatment at this site in 1974. Two of the pools contained an average of 5-10 larvae/dip in a ratio of 10 *A. dorsalis* to 1 *A. spencerii*. These pools had estimated surface areas and average depths of 4.5 and 1700 m^2, and 10 and 7 cm, respectively. The third pool, which had a surface area of 3.6 m^2 and an average depth of 12 cm, contained 20-30 *A. dorsalis* larvae/dip. First and second instar larvae predominated in all pools, though some (<5%) third instar larvae were present.

The pools were treated with 20,000 *R. culicivorax* preparasites/m^2 on 14 May, 1974 and samples of mosquito larvae were taken 12, 24, 48 and 72 hours thereafter. No samples were taken after 72 hours, because heavy rains flooded the entire site and the pools became confluent. Maximum and minimum temperatures were recorded for the pools each day until sampling was discontinued.

Site B

This site was a typical woodland habitat 50 miles east of Winnipeg near the west boundary of the Sandilands Provincial Forest and 0.4 miles south of Highway No. 1 on Fire Road No. 19. Pools in this area were studied previously by the junior author, and were known to be free of nematode parasites of mosquitoes. Most of the pools, which were small (0.6 - 2.8 m^2), steep-sided and relatively deep (9 - 23 cm), were scattered over an area approximately 100m by 50m, adjacent to Fire Road No. 19. Pool bottoms were covered by sphagnum, litter and various grass and sedge species. A dense overhead canopy of conifers including cedar, *Thuja occidentalis* L., balsam fir, *Abies balsamea* (L.) and white spruce, *Picea glauca* (Molnch), provided shade which stabilized pool temperatures in the spring. Frost persisted in the pool bottoms during field trials in April and water temperatures were near freezing.

Each pool at Site B was randomly assigned a number from 1 - 60. From these pools, seven were selected for treatment in 1975 and 1976. As noted, only pools which contained >75 first and/or second instar mosquito larvae/dip were chosen. The designation, physical characteristics and dates of treatment for these pools are presented in Table 1. Pools 3, 30, 50, and 6 were treated in April when first and second instar *A. communis* (De Geer) and *A. churchillensis* Ellis and Brust[1] were present. These two species were the first to appear and hatched at mean daily temperatures

[1]Ellis and Brust (1973) defined *A. communis* and *A. churchillensis* as sibling species. As noted by these authors, both species were present at Site B in varying proportions in different pools. No attempt was made to separate these species in the present samples, with the understanding that all further references to *A. communis* include both *A. communis* and *A. churchillensis*.

of 0° - 3°C. Numerous other mosquito species hatched from late April to the end of May as daily temperatures increased. Pool 3, selected for a second time, and pools 18 and 38 were treated in May (Table 1) and contained first to fourth instar larvae of *A. pionips* Dyar, *A. punctor* (Kirby), *A. diantaeus* Howard, Dyar and Knab, *A. canadensis* (Theobald), *A. cinereus* Meigen and *A. communis* in varying proportions. Larvae of *Mochlonyx velutinus* (Ruthe) (Chaoboridae) and a *Dixa* sp. (Dixidae) were also present in these latter pools and were examined for infection.

Table 1. Characteristics and dates of treatment for pools at Site B where preparasites of *Romanomermis culicivorax* were applied at 50,000/m².

Pool Number	Surface area (m²)	Mean depth (cm)	Date of treatment
3	0.7	23	30 April 1975
30	2.8	20	30 April 1975
50	1.8	12	30 April 1975
18	3.3	21	12 May 1975
3	0.7	19	23 May 1975
38	0.6	9	23 May 1975
51	2.7	20	26 May 1975
6	1.5	12	20 April 1976

Pool 51 was a roadside pool at Site B and differed from the other pools in the area by its lack of forest cover. Heavy rainfall on 23 May, 1975, raised both the water level and temperature in pool 51 and resulted in a hatch of *A. vexans* (Meigen) larvae. This pool was treated with 50,000 *R. culicivorax* preparasites/m² three days later and mosquito larvae were examined for infection as for other pools. Mayfly nymphs (probably *Paraleptophlebia* sp.) from this pool were also examined for possible infection.

Continuous records of temperatures in the pools were taken from the time of each nematode treatment until the cessation of sampling using either a Webster or Taylor temperature recorder. The temperature probes were placed 5-8 cm from the pool bottom. Salinity (mg/l) levels were recorded for all pools at least once a week using a 451 Model 33 S-C-T metre (Yellow Springs Instrument Co.).

All pools which contained infected larvae in 1975, as determined from laboratory dissections, were sampled in the spring of 1976 for evidence of winter survival of *R. culicivorax*. At least 50 larvae from each pool were taken to the laboratory and dissected. Larval samples were collected twice weekly from the first appearance of *A. communis* larvae until the pools went dry. As a result of a dry spring in 1976, samples were taken only from 28 April until 15 May.

RESULTS

Site A

Two hundred *A. dorsalis* and *A. spencerii* larvae from each of two of the pools used and 400 *A. dorsalis* larvae from the third pool were dissected. No infected larvae were found, though both species were susceptible to infection in the laboratory. Although the pools were shallow and exposed to direct sunlight, temperatures remained low. Mean daily maxima for the three pools were 12 - 15°C and mean daily minima were 1 - 4°C.

Site B

Temperature data for pools 30, 18, 38 and 51 are shown in Table 2. Brown and Platzer (1977) found that *R. culicivorax* preparasites remained infective (I_{50}) in the laboratory for only 48 hours

Table 2. Maximum, minimum and mean temperatures in pools 30, 18, 38 and 51 at Site B in 1975.

Date	Temperature (°C) Maximum	Minimum	Mean	Date	Temperature (°C) Maximum	Minimum	Mean
Pool 30				Pool 38			
30 April	3.0	1.0	2.0	23 May	9.0	5.0	7.0
1 May	3.0	1.0	2.0	24	9.0	5.0	7.0
2	3.0	1.0	2.0	25	7.5	5.0	6.5
3	5.0	4.0	4.5	26	7.0	6.0	6.5
4	5.0	3.0	4.0	27	7.0	6.0	6.5
5	8.5	1.5	5.0	28	7.0	5.0	6.0
6	7.0	3.0	5.0	29	8.5	6.5	7.5
Pool 18				Pool 51			
12 May	7.0	6.0	6.5	26 May	15.0	13.0	14.0
13	8.0	7.0	7.5	27	15.0	10.5	12.5
14	8.0	7.0	7.5	28	15.0	10.0	12.5
15	8.0	5.0	6.5	29	14.0	11.0	12.5
16	10.0	7.0	8.5	30	13.0	9.5	11.5
17	9.5	8.5	9.0	31	13.0	8.0	10.5
18	12.0	9.0	10.5	1 June	15.0	9.0	12.0

at 12° C. Temperature data is, therefore, given only for the first seven days following preparasite application (Table 2). Temperatures in each of these pools were for the most part, representative of those in all pools treated on the indicated dates (Table 1). Salinity levels did not exceed 0.3 mg/l and thus would not be expected to interfere with the infectivity of *R. culicivorax* preparasites (Platzer and Brown, 1976).

In dissections of over 500 first to third instar *A. communis* larvae from each of pools 3, 30 and 50 in 1975 and pool 6 in 1976, only one second instar larvae was found to be infected and it contained a single nematode. Temperatures in these pools did not exceed 10° C during the period of expected preparasite activity.

Of the three pools treated 12 - 23 May, 1975 (Table 1), only pool 18 contained significant numbers of infected larvae. Infected larvae were present in pool 18, 48 hours after treatment even though recorded temperatures in the pool did not exceed 8° C during that time (Table 2). Small numbers of third and fourth instar larvae of *A. pionips, A. diantaeus* and *A. communis* were also present in pool 18, but none were found to be infected in either the 2- or 4- day post-treatment samples (Table 3). It was thus evident that only first and second instar larvae of *A. canadensis* and *A. pionips* were susceptible to infection. Since preparasites were infective for a relatively short period of time, only larvae which were susceptible to infection during this time contained nematodes. The development of this cohort of infected larvae is shown in Table 3. First instar larvae sampled from 8 - 28 days after treatment were not infected.

Levels of infection in pool 18, as determined by numbers of nematodes per infected host, peaked four days after treatment and significantly decreased in subsequent samples. This decrease was apparently caused by mortality of heavily infected mosquito larvae (i.e. larvae containing ≥ 8 nematodes) as indicated by the changes in the frequency distribution of the parasite (Table 4). There was no sample in which the frequency distribution of the data followed a Poisson distribution. Finally, postparasitic juveniles were observed emerging from their hosts on 17 June, 36 days after treatment.

Samples taken from pools 3 and 38 treated 23 May, 1975, indicated insignificant levels of infection. Seven *A. canadensis* larvae from pool 3 contained a single nematode each. Only one larva of *A. cinereus* from pool 38 was found to contain a nematode.

Table 3. Distribution of infection of *Aedes canadensis* and *Aedes pionips* larvae by *Romanomermis*

Days after trmt.	No. of first instar	% infection (No. infected)	Mean no./host (Mean no./infected host)	No. of second instar	% infection (No. infected)	Mean no./host (Mean no./infected host)	No. of third instar
2	15	20.0 (3)	0.26 (1.30)	172	25.6 (44)	0.45 (1.75)	9
4	12	25.0 (3)	0.25 (1.00)	184	33.2 (61)	1.01 (3.03)	4
8	2	0.0	0.00	35	40.0 (14)	0.60 (1.50)	105
10	3	0.0	0.00	22	22.7 (5)	0.23 (1.00)	128
21	0	-	-	4	0.0	0.00	5
28	2	0.0	0.00	2	0.0	0.00	5
36	0	-	-	0	-	-	16

Table 4. Frequency distributions of infection by *Romanomermis culicivorax* in *Aedes canadensis* and *Aedes pionips* larvae from pool 18, Site B, 14 May to 17 June, 1975.

No. of nematodes/ host	Days after treatment						
	2	4	8	10	21	28	36
0	153	136	119	113	55	21	15
1	33	35	28	42	6	2	6
2	7	15	7	8	0	0	0
3	1	1	3	1	0		0
4	3	2	2	0	0		1
5	2	1	2	1	0		1
6	0	3	0	1	0		
7	1	0	1	0	0		
8		3	0	1	0		
9		2	0		0		
10		0	1		1		
11		0	1				
12		0	0				
13		0	0				
14		1	0				
19		0	1				
30		1	0				

Pool 51 differed from the other treated pools at Site B by being more exposed than the others to direct sunlight. Consequently, temperatures were more favourable to preparasite activity (Table 2). Levels of infection in *A. vexans* larvae are given in Table 5. The low incidence of

culicivorax preparasites applied at 50,000 m/^{2} in pool 18, Site B, from 14 May to 17 June, 1975.

% infection (No. infected)	Mean no./host (Mean no./infected host)	No. of fourth instar	% infection (No. infected)	Mean no./host (Mean no./infected host)	Total no. of larvae	% of total infected (Total no. infected)	Mean no./host (Mean no./infected host)
0.0	0.00	4	0.0	0.00	200	23.5 (47)	0.40 (1.70)
0.0	0.00	0	-	-	200	32.0 (64)	0.93 (2.92)
30.5 (32)	0.91 (2.97)	23	0.0	0.00	165	27.9 (46)	0.70 (2.52)
39.1 (50)	0.61 (1.54)	7	0.0	0.00	160	34.4 (55)	0.49 (1.43)
0.0	0.00	39	18.0 (7)	0.36 (2.00)	48	14.6 (7)	0.29 (2.00)
20.0 (1)	0.20 (1.00)	2	50.0 (1)	0.50 (1.00)	11	18.2 (2)	0.18 (1.00)
18.8 (3)	0.19 (1.00)	7	71.4 (5)	1.71 (2.40)	23	34.8 (8)	0.65 (1.88)

multiple parasitism confirmed the relative resistance of *A. vexans* to infection by *R. culicivorax* reported by Petersen and Willis (1976). A few nematodes were completely melanized by a host reaction (Table 5). Third and fourth instar larvae and pupae of *A. communis, A. cinereus, A. pionips, A. punctor, A. canadensis* and *A. fitchii* Felt and Young were not infected though present in the pool when preparasites were introduced.

Several hundred larvae of *M. velutinus* and a *Dixa* sp. and nymphs of a mayfly were dissected for evidence of infection, but none was found.

Three second instar larvae each containing one nematode, were found in dissections of *A. communis* larvae collected from pool 18 in 1976, indicating that *R. culicivorax* was capable of surviving the winter in a natural mosquito breeding site in Manitoba. However, all the nematodes were completely melanized and killed at an early stage of development. There was no evidence of winter survival of *R. culicivorax* in either pool 3 or 38 following larval dissections in 1976.

Table 5. Levels of infection by *Romanomermis culicivorax* in *Aedes vexans* from pool 51, Site B, 28 May to 9 June, 1975.

Days after treatment	Total number mosquito larvae examined	*A. vexans*			
		Number of larvae	% infection	Mean number of nematodes/ infected host	Number of nematodes melanized
2	125	109	8.3	1.00	2
4	100	93	18.3	1.12	1
11	100	90	12.2	1.09	0
14	69	49	20.4	1.10	1

DISCUSSION

According to the results presented in this study, *R. culicivorax* is not an effective biological control agent for spring *Aedes* in Manitoba. Numerous factors contribute to its unsuitability.

First, as suggested by the results of laboratory experiments (Brown and Platzer, 1977; Galloway and Brust, 1977), temperatures encountered in snow-melt pools were below the threshold of infectivity for preparasites of *R. culicivorax*. Pool temperatures at both Site A and Site B rarely exceeded 10°C when the *Aedes* larvae were most susceptible to infection. Consequently, few larvae in the pools were infected. The highest incidence of infection was in pool 18 where 34.8% of the larvae were infected (Table 3) despite low temperatures (Table 2). Because of the exposed nature of the pool and because temperatures were recorded from a single probe, 5 - 8 cm from the pool bottom, it is probable that marked variations in temperature, not detected in this study, occurred within the pool as described by Haufe (1957) in similar pools near Churchill, Manitoba. Preparasites could have been more active in the marginal zones of the pool where, during periods of maximum solar heating, temperatures would be higher than in the rest of the pool. Infection of larvae in these areas of the pool would be further enhanced by the behavioural trait of early instars in congregating along the edges.

Second, preparasites which are directly applied to spring mosquito breeding sites do not remain infective long enough to be able to control all species of mosquitoes that may be present. For example, at site B, in 1975, there was a series of at least seven *Aedes* spp. which hatched sequentially over an eight week period. The maximum life expectancy of *R. culicivorax* preparasites under the recorded temperatures in these pools would be 6 - 8 days (Brown and Platzer, 1977). Therefore, numerous preparasite applications would be necessary before all mosquito species could possibly be exposed to infection. The recent application technique developed by Petersen and Willis (1976) for the control of flood-water mosquitoes in Louisiana may provide an alternative in overcoming the problem of prolonged hatching of *Aedes* spp. in snow-melt pools in Manitoba. They placed cultures of *R. culicivorax* containing embryonated eggs in the bottoms of known mosquito breeding sites. When these sites were flooded, preparasites hatched synchronously with the mosquito larvae. This technique may be particularly advantageous in Manitoba since it appeared that *R. culicivorax* was capable of surviving the winter at a latitude of about 50°N. However, other mermithid species in North America which normally infect spring *Aedes* and spend the winter as embryonated eggs in the pool bottom only infect the earliest hatching species of mosquitoes (Galloway and Brust, 1976; Welch, 1960). Observations on the overwintering population of *R. culicivorax* at Site B suggest that a similar relationship existed as only *A. communis* larvae were infected in the spring. Further field observations are necessary to confirm this possibility.

Third, the application rate of preparasites necessary to achieve a 30% infection under the most favourable conditions in the tests appears high. Present mass production, storage and application methods make widespread treatment at 50,000 preparasites/m^2 impractical for the thousands of acres of mosquito breeding sites treated in Manitoba each spring.

Consequently, *R. culicivorax* cannot be recommended at this time for control of *Aedes* in snow-melt pools. The superior effectiveness, low cost and ease of storage and handling of chemical insecticides make then the preferred control method. The high degree of host specificity (Ignoffo *et al.*, 1973; Ignoffo *et al.*, 1974) and the possibility of environmental adaptation by *R. culicivorax* to temperate climates make it an attractive alternative worthy of further investigation.

ACKNOWLEDGEMENTS

Appreciation is expressed to Jack Harlos, Eileen Adams and Gwen Muirhead for assistance during the course of this study. This research was supported by a National Research Council Scholarship to the senior author and by National Research Council grant A2545 to the junior author.

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(Received February 15, 1977)

CHRYSOPID PREDATION ON THE BERTHA ARMYWORM[1]

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ABSTRACT: Larvae of the common green lacewing, *Chrysopa carnea* Stephen, were found attacking larvae of the bertha armyworm, *Mamestra configurata* Walker, in field plots of rape, *Brassica* spp. artificially infested with high populations of the prey at Glenlea, Manitoba, in 1976. Laboratory studies on prey consumption indicated that the chrysopid density of 10/m² found in the field could account for most of the reduction in the density of the bertha armyworm population that was recorded while the host was passing through the first three instars that are susceptible to attack. Chrysopid predation is thus a mortality factor which can reduce populations of young bertha armyworms under some conditions.

INTRODUCTION

In 1976, we found the chrysopid predator, *Chrysopa carnea* Stephens = *ploribunda* Fitch and *californica* Coquillett, in field plots of rape, *Brassica* spp., at Glenlea, Manitoba, that had been artificially infested with young larvae of the bertha armyworm, *Mamestra configurata* Walker. As the density of chrysopids appeared to us to be high and as we observed chrysopid larvae feeding on bertha armyworm (ba) larvae in the field plots, we surmised that the predators were partly responsible for the rapid decline that occurred in numbers of young ba larvae. Therefore, we made some laboratory observations on the biology of the chrysopid larvae, especially to determine if they could utilize ba as an exclusive host throughout life and how many they would destroy under this condition. Our findings are reported in this paper.

Though the plots had been artificially infested to provide field-conditioned, fifth instar ba larvae for use in damage assessment tests on the rape crop, they also provided us with an opportunity to make some observations on the accuracy of sampling methods for determining the density of a population and its spatial distribution. It was during this sampling that we recorded chrysopid predation in conjunction with a rapid drop in host density.

METHODS

The artificial infestations of ba were established in three 6 x 10 m plots of blooming rape isolated from adjacent rape in the fields by a 2-m wide barrier of bare soil; in one plot an electric fence was erected to prevent maturing ba larvae from leaving. Cups of artificial diet (Bucher and Bracken, 1976), containing first instar larvae were placed on the ground throughout the plots to give an initial mean density of 800 larvae/m². Larval density was estimated at intervals of 2-3 days by destructively sampling 2-4 samples of 0.5 m². Plants in the samples were uprooted and larvae on the plants or on and in the soil were counted. The number of larvae per m² was expressed as percent survival based on the initial density. The mean number of chrysopid larvae and eggs per m² was recorded. A total of 26 m² was sampled or about 14% of the infested area.

The biology and food consumption of *C. carnea* were studied by individually rearing 18 field-collected larvae, and 36 eggs obtained from field-collected adults; 25 additional field-collected larvae were used for miscellaneous observations. Young larvae were reared in cotton-plugged shell vials and large larvae in petri dishes. Prior to hatching, each chrysopid egg was placed in a vial with embryonated ba eggs and a fresh piece of rape leaf as food for hatching prey. Fresh rape leaves and ba larvae of appropriate size were supplied every 1 or 2 days and the number and stage of prey destroyed in the previous interval was determined. Each chrysopid was provided with a slight excess of prey until it pupated. Larvae of the ba were reared on artificial diet until required.

[1]Contribution No. 789, Agriculture Canada, Research Station, Winnipeg, Manitoba

RESULTS

First instar ba larvae climbed the stems and established on the lower leaves of rape plants. During the first 3 instars there was little lateral dispersion of larvae away from the plants on which they had originally established. Therefore, the number of larvae on some plants was high whereas adjacent plants might have few larvae. This clumped distribution resulted in high variation between replicate samples of 0.5 m^2. In instars 4 to 6, some lateral dispersion of the population resulted in less variability between replicate samples. Consequently, population density could not be measured with accuracy unless many more samples were taken than we were able to do in these tests. Larval sampling in young natural populations has indicated less clumping than we found in our artificial infestations (W. Turnock, personal communication). Therefore, the clumping of our young larvae resulted in part from the method of liberating them from diet cups. Although individual estimates of prey density at a given time were inaccurate, density declined as a negative exponential function of time. A curve (Fig. 1) was the best fit of the observed data and has the equation $\log_e Y = 4.85 - 0.134 X$, where Y is % survival and X is days after original introduction of larvae. We believe this curve is a reasonable approximation of survival in the field plots.

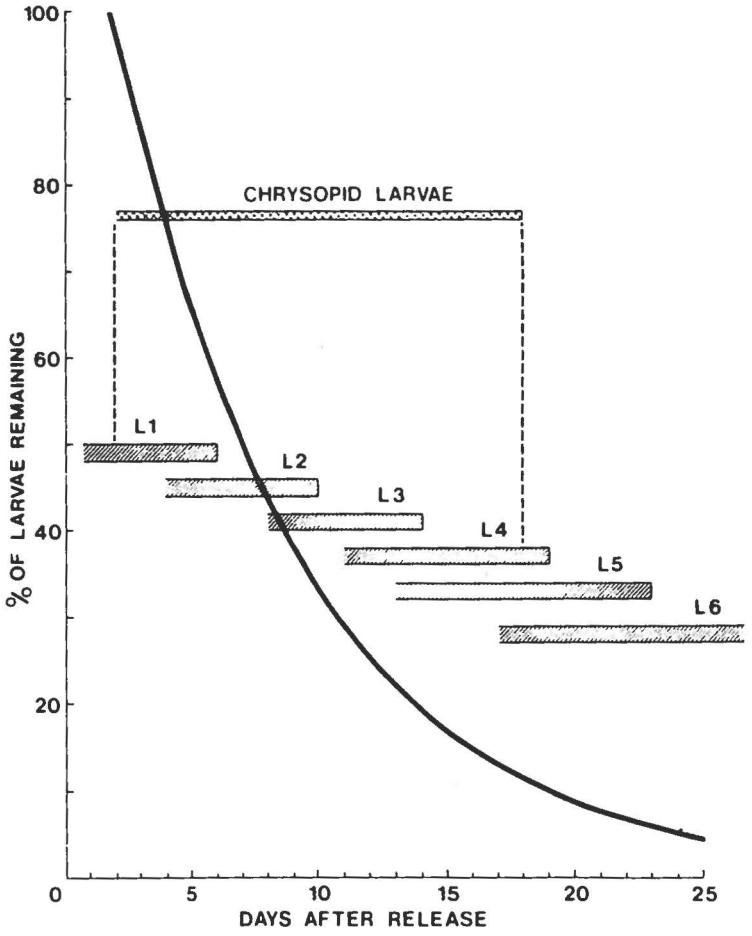

Figure 1. Exponential decrease of bertha armyworm populations in rape plots artificially infested with 800 first instar larvae per m^2. Bars L1 to L6 indicate the principal host stages present in proportion to time and survival. The chrysopid bar indicates a mean population of 10 ± 6 predacious larvae per m^2 during the period.

On the other hand, the population of chrysopids was relatively constant for 18 days, at which time most ba larvae had molted to the fifth instar. The mean and standard deviation was 28 ± 12 eggs and 10 ± 6 larvae per m^2; most chrysopid larvae were in the second and third instars, but as tiny first instar larvae are difficult to find, probably many were missed.

The times spent by immature chrysopids in various stages are given in Table 1, for rearing at 25°C, 60% RH, and a light/dark cycle of 16/8 h with illumination of 200 ft candles. Total time from egg to adult was 26.1 days; about half of which was spent as a feeding larva. At 20°C developmental time was 1.34 times the 25°C value, which places developmental zero at approximately 5.3°C. After forming a cocoon the L3 larva or prepupa spends about 3 days (Table 1) before molting to the pupal stage. When molting occurs, the L3 larval skin is packed into one end of the cocoon where it is visible as a dark silhouette. The pupa emerges from the cocoon by cutting a cap off from the opposite end of the cocoon with its heavy mandibles, and crawls 5-6 cm away from the cocoon before anchoring its feet. The adult leaves behind a very thin transparent pupal skin when it emerges, usually within an hour.

Table 1. Time for development and host consumption for immature chrysopids.

Predator stage	Number of insects N.	Days at 25°C Mean	SD	Hosts destroyed Mean	SD	Host stage
Egg	36	4.0	0.1			
L1	39	4.0	0.4	50	5	Egg, L1
L2	37	3.8	0.6	18	5	L2 equivalents
L3	44	5.5	1.4	60	24	L2 equivalents
Cocoon PP	48	3.3	0.8			
Cocoon P	48	5.5	1.2			

Time, egg-adult 26.1 days
Larval time 13.3 days

Measurements of immature stages based on at least 30 individuals are shown in Table 2. The 3 larval stadia are readily separated by body size and especially by the width of the head capsule, where there is no overlap between stadia. The feeding pincers, which are hollow tubes formed by the mandibles and maxillae, project in front of the head for about the same distance as the head capsule width. Maximum body width is a rough measure of the age of the larva within an instar; immediately after the molt it is equal to or slightly less than head capsule width, but at the end of the instar is 1.8 - 2.0 times this value.

After moving to within a short distance of the prey, larvae attack by a rapid lunge and grab action of the pincers. If the pincers are embedded in the prey, the larva begins to suck up its fluids while it is still alive. If the lunge is unsuccessful, the larva backs away and frequently seeks another host. Prey appear to be found by random movement of the predator, which appears to be oblivious of those that are more than 1 cm away. L1 chrysopids attack ba eggs and first instar larvae. L2 chrysopids attack eggs, L1, L2, and rarely L3 hosts. L3 chrysopids attack these stages and rarely small L4 hosts. The distance between the points of the curved pincers, when they are fully opened, appears to be one factor that limits the maximum size of the host that can be successfully attacked, for both pincers must be firmly embedded in a large larva for an attack to succeed. The arc described by the closing pincers of an L3 chrysopid is so large that it is difficult for the predator to sink both pincers into a ba egg, which is laid in a closely appressed plaque on a flat surface; thus we have rarely seen a chrysopid larva making a successful attack on a ba egg with only one pincer embedded in it.

During life, chrysopid larvae destroyed a mean of 144 ±15 prey, 50 of which were killed as eggs or L1 by the first instar predator. As L2 and L3 chrysopids eat a wider range of larger prey, it is

Table 2. Measurements of immature chrysopids (mm)

Stage	Length	Width	Head capsule Mean	SD
Egg	0.9 - 1.1	0.4 - 0.5		
L1	1.5 - 3.0	0.3 - 0.7	0.40	.02
L2	3.0 - 5.0	0.5 - 1.1	0.60	.05
L3	6.0 - 10.0	0.9 - 2.0	1.0	.1
Cocoon	5.5 - 7.0	4.5 - 5.5		

more meaningful to convert the actual prey eaten by them into L2 prey equivalents (Table 1). Previous measurement had shown the mean weights of ba larvae at the mid point of the instar to be 0.35 mg for L1, 1.28 mg for L2, and 5.10 mg for L3. Thus, actual consumption of L1 and L3 hosts was converted to L2 equivalents by multiplying by 0.273 and 3.98, respectively (Table 1).

On this basis, a chrysopid destroyed 78 L2 prey equivalents during the second and third instars or 4.7 per day during instar 2 and 10.9 per day during instar 3. On a daily basis, the L3 chrysopid destroyed 2.3 times as much host biomass as the L2, which in turn destroyed 1.4 times the host biomass as the L1.

Table 1 indicates that prey consumption is more variable for L3 chrysopids than for earlier instars. This results in part from some L3 remaining in the instar for 2 days longer than average and consuming a proportionally greater number of hosts

DISCUSSION

Although chrysopid larvae are commonly thought of as predators of aphids, they will attack a variety of prey when opportunity permits. They are cannibalistic and in our tests would attack another chrysopid larva even though bertha armyworm larvae were available. One mature larva was even found feeding on another that had partially completed a cocoon.

Though our tests showed that L1 chrysopids would complete the instar on a diet of ba eggs or L1, it seems likely that in our field plots they lived largely on aphids that are more readily attacked. An incipient infestation of aphids, present when the plots were set up, rapidly disappeared. Chrysopid larvae were still numerous when the bertha armyworm larvae were in instars 4-6, which are virtually immune from attack. However, at this time, small larvae of the diamondback moth, *Plutella xylostella* (Linneaus) were present and probably served as hosts.

From our observations we can estimate the number of bertha armyworm larvae removed from field plots by chrysopids. Such estimates vary depending on the accompanying assumptions.

If we assume that a chrysopid larva can destroy 144 ba during its life, a population of 10/m^2 could destroy 1,440 ba or considerably more than were originally released in the plots.

If we assume that only L2 and L3 chrysopids attack ba larvae in the field, each could destroy 78 L2 bertha armyworm equivalents while completing its development. At a density of 10/m^2, the estimated host loss is greater than actually occurred.

If we assume that a L2 chrysopid is present on day 4 (Fig. 1) when the ba population consisted of L1 and L2, we would expect it to eat 18 L2 before molting in 3.8 days. The resulting L3 chrysopid is expected to eat 10.9 x 2.2 L2 hosts in the following 2.2 days, during which the L2 stage of the host was commonly present, for a total of 24. During the last 3.3 days of its feeding period it would eat 36 L2 equivalents or 9 L3 hosts, because the latter instar would be commonly present in the plots. Thus, each chrysopid larva could destroy 51 ba larvae. Ten chrysopids per m^2 would deplete the host population from 800/m^2 to 290/m^2 or to 36% of the original density. As the curve (Fig. 1) shows survival of 24% on day 13, we conclude that chrysopids were partly responsible for the initial steep drop in host population density that occurred in our plots.

In 1973, at the close of the last outbreak of bertha armyworm on rape, chrysopid eggs and larvae were commonly observed in infested fields of rape. Wylie and Bucher (1977) suggested that chrysopids might prey on the ba larvae although actual predation was not observed at that time.

The impact of chrysopid predation on larval density of the bertha armyworm in rape fields would depend on the relative densities of the predator and host, on the synchronization of L2 and L3 chrysopids with the young susceptible stadia of the host, and on the availability of other more-preferred hosts. Although chrysopid parasites are known (Clancy 1946) we did not find parasites or diseases in field-collected larvae. Only 1 of 79 larvae under tests died from physiological causes, a case of incomplete casting of the L2 larval skin around one of the spiracles; 5 larvae escaped during rearing, 2 newly-hatched larvae were eaten by L3 bertha armyworms, and 10 were eaten by other chrysopid larvae when not reared individually. It appears that cannibalism would limit chrysopid density more than any other factor, especially if other hosts were not readily available.

We conclude that chrysopids can reduce high densities of young bertha armyworms to subeconomic densities before the host reaches the damaging fifth and sixth instars. Economic reduction of *Heliothis* larvae in cotton was obtained with releases of 10,000 - 100,000 chrysopid larvae per acre (Ridgway and Kinzer, 1974), which is in the same density range (2.5-25 per m^2) as we found naturally.

C. carnea overwinters as an adult (Sheldon and MacLeod, 1971) and we have no knowledge of its ability to survive the prairie winter. It is quite possible that densities of economic significance are attained in Manitoba only when adults are blown in from the south and have time to complete one generation of multiplication before ba populations are attacked.

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(Received 7 March, 1977)

EFFECTS OF FEEDING BY ADULTS OF THE RED TURNIP BEETLE, *ENTOMOSCELIS AMERICANA* BROWN [COLEOPTERA: CHRYSOMELIDAE], DURING LATE JULY AND AUGUST ON THE YIELD OF RAPESEED [CRUCIFERAE][1]

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ABSTRACT: Effects of feeding by adults of the red turnip beetle, *Entomoscelis americana* Brown, during late July and August on the yield of rapeseed were measured in 1975 and 1976 in small plots containing either the Midas cultivar of *Brassica napus* L. or the Torch cultivar of *B. campestris* L. For both cultivars, the yield of rapeseed in a treatment containing beetles (100/0.84 m^2) was not significantly different from that in a treatment containing no beetles. The data indicate that feeding by adults of the red turnip beetle during late July and August does not cause economic crop loss in rape and that control measures against the beetle are not required to protect yields at that time of the year.

INTRODUCTION

Adults of the red turnip beetle, *Entomoscelis americana* Brown, are a pest of rape crops in the Prairie Provinces and British Columbia (Gerber, 1974, 1975). The beetles emerge during the first 3 weeks of June and feed for 2-3 weeks on rape and cruciferous weeds. If they invade new rape fields at this time, they can cause sufficient damage to warrant the application of control measures. At the end of June, they enter the soil to aestivate for about a month. The adults re-emerge in late July and early August, migrate to new rape fields, and feed on the flowers, pods, leaves and stems of rape and cruciferous weeds. Though they are conspicuous and growers are concerned when they occur in large numbers, there is no information available on whether the damage caused to rape during the post-aestivation period is of economic significance. This paper deals with experiments designed to determine whether the adults cause significant yield loss to rape during late July and August.

MATERIALS AND METHODS

Yield losses were determined in 3 experiments conducted at Glenlea, Manitoba, in 1975 and 1976 (Tables 1 and 2). Each experiment was set up in a plot of rape which measured about 15 x 40 m and contained either Midas cultivar of *Brassica napus* L. or Torch cultivar of *B. campestris* L. In each experiment, 2 treatments (cages containing 100 beetles and cages containing no beetles) arranged in a randomized block design were used. Each treatment replicate consisted of an area of rape about 0.84 m^2. Seven replicates were used in 1975 and 10 in 1976.

The cages (Fig. 1), which were similar to the roll-up field cages of Nicholls (1970), measured 122 cm high x 91.4 x 91.4 cm and were made of 32-mesh Saran screen with a 5-cm aluminum strip attached to the bottom edge. In each experiment, the cages were arranged so that they were about 2.5 m apart with rape plants growing between cages.

The red turnip beetles used in the experiments were collected in June, before aestivation, in growers' fields between Roblin and Grandview, Manitoba, and kept in cages out-of-doors at Winnipeg until the time of the experiments. While in these cages, they were provided with plants of Zephyr cultivar of *B. napus,* which had not yet flowered. As the beetles emerged from aestivation in late July and August, they were put into the cages erected in the experimental plots

[1]Contribution No. 790, Agriculture Canada, Research Station, Winnipeg, Manitoba.

and allowed to feed until harvest. Though the beetles in the cages could not be counted at harvest, there were some present in all of them and there was no indication that the mortality rate was different from that in nature. The plants in each treatment replicate were harvested at about the same stage of ripeness as that which would occur in growers' crops at the time of swathing. Not all of the treatment replicates were set up the same day and not all of them were harvested the same day. The pertinent data for each experiment are presented in Table 1.

Table 1. Rape cultivars, initiation dates, plant growth stages* at the beginning of the experiments, and duration of the experiments.

Cultivar	Initiation dates	Plant growth-stage	Duration of experiments (days)
Midas	15-17 August 1975	4.0-4.2; flowering completed; seeds in some pods a dark green	13
Midas	27 July 1976	3.1-3.3; flowers; some pods starting to fill	27-28
Torch	22 July 1976	3.2-3.4; flowers; some pods with large seeds	18-22

*After Berkenkamp (1973)

The density of 100 adults/cage was selected, because it is much higher than that normally encountered in growers' fields during late July and August (Gerber, unpublished observations). In natural infestations, densities usually average less than 1/m^2, although local concentrations may exceed this level.

The rape plants were examined periodically to determine the types of damage that the beetles caused to the plants. At the end of each experiment, the rapeseed from each treatment was harvested and weighed to determine yield.

RESULTS AND DISCUSSION

Since the seed yields, expressed both in terms of g/0.84 m^2 and g/plant, in the treatment containing 100 beetles were not significantly different from those in the treatment containing no beetles (Table 2), it is clear that, in at least the cultivars tested, red turnip beetles do not cause significant yield loss to rape during late July and August (Table 2). These results were unexpected, because the beetles damaged most of the rape plants in the cages. It is obvious, therefore, that either the damage was not sufficiently severe to reduce the yield of rapeseed, or the rape plants were able to compensate for seed loss in damaged and destroyed pods. Observations made during the course of the experiments suggest that the former might account for the results in 1975 and the latter for the results in 1976.

In 1976, the plants of both cultivars were in the flowering growth-stage when the beetles were added to the cages (Table 1). Soon after they were put in the cages, most beetles climbed the plants and fed on the buds, flowers and small pods at the terminal end of each inflorescence (Figs. 2 and 3). Though a large number of these terminal buds, flowers and small pods were destroyed, the impact on yield was not as great as expected, because some of the terminal flowers normally abort and some of the last-formed pods remain small and contain few seeds. At the same time, other beetles fed on leaves and the outer covering of large pods, petioles and stems (Fig. 4). In some instances, holes were made in the sides of pods, or the terminal end of pods were consumed. After flowering was completed, the beetles continued to feed until harvest on leaves, pods, petioles and stems. The removal of the outer covering from pods caused some pods to dry

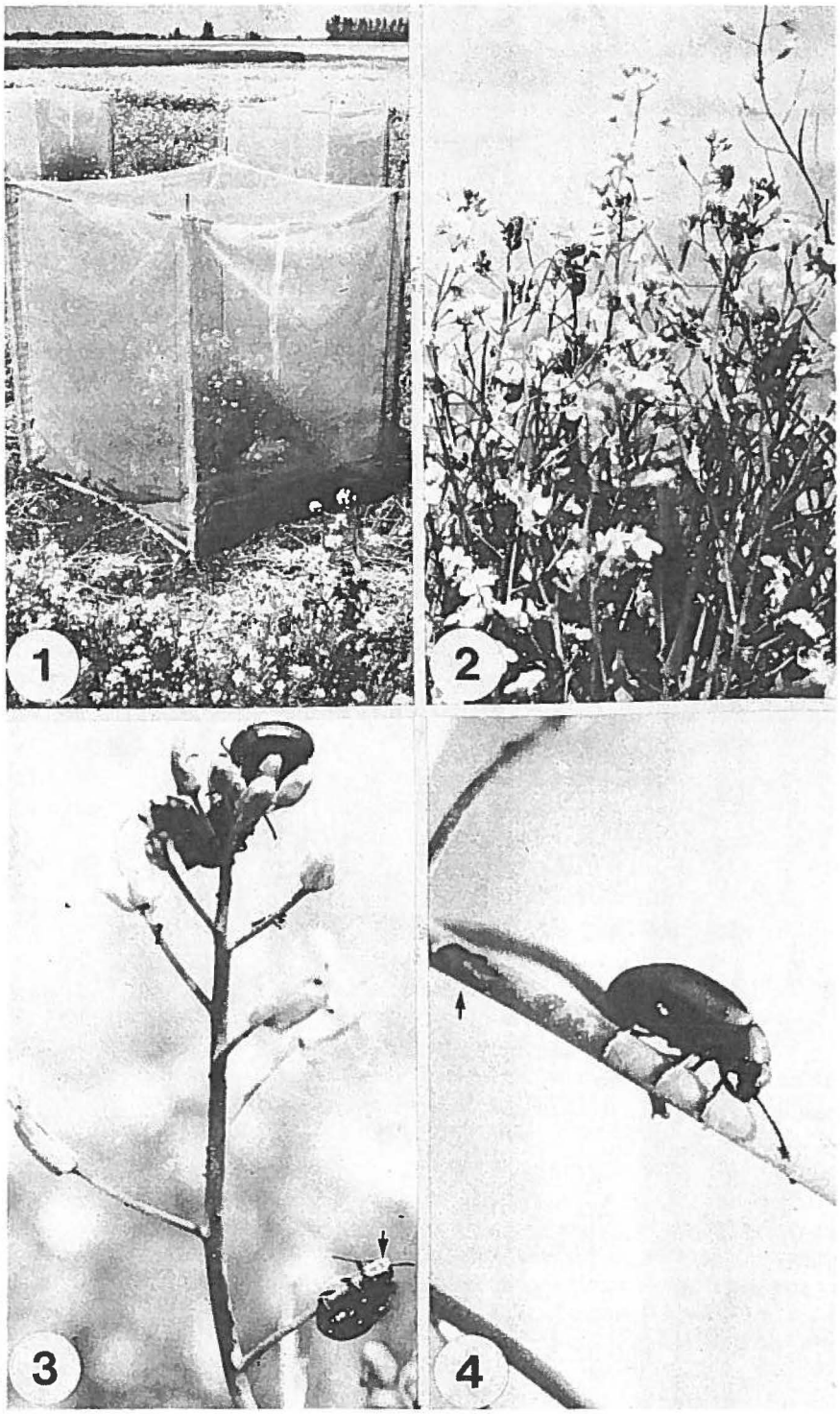

Figure 1. Roll-up type of field cage used in experiments.

Figure 2. Red turnip beetles feeding on rape in a field cage.

Figure 3. Red turnip beetles feeding on buds, flowers and a small pod (arrow) in a rape inflorescence.

Figure 4. Red turnip beetle feeding on outer covering of a rape stem and a damaged area on the stem (arrow).

Table 2. Numbers of plants and yields of rapeseed from small, caged plots (0.84 m²) infested with red turnip beetle adults or containing no beetles.

Year	Cultivar	Test	Mean number plants/0.84m² (±S.D.)	Mean yield (g/0.84 m² ± S.D.)	Mean yield (g/plant ± S.D.)
1975	Midas	Cage + 100 beetles	46.6 ± 9.9 a*	121.3 ± 9.7 a*	2.71 ± 0.59 a*
		Cage only	50.3 ± 18.7 a	116.4 ± 21.9 a	2.56 ± 0.85 a
1976	Midas	Cage + 100 beetles	165.2 ± 39.1 b	59.8 ± 23.6 b	0.35 ± 0.08 b
		Cage only	198.0 ± 34.1 b	80.1 ± 40.0 b	0.39 ± 0.15 b
1976	Torch	Cage + 100 beetles	289.8 ± 57.6 c	64.5 ± 13.6 c	0.23 ± 0.06 c
		Cage only	306.7 ± 45.4 c	74.5 ± 16.4 c	0.24 ± 0.04 c

* The means, for each category of data for each cultivar, followed by the same letter are not significantly different ($P > 0.05$; Students t test for unpaired data).

out prematurely and probably resulted in fewer and(or) smaller seeds being produced. Late germinating plants, which were still small and green at harvest, had all pods consumed, but this type of plant normally would not contribute significantly to yield. The seed loss in damaged and destroyed pods probably was compensated for in both damaged and undamaged plants by additional branching and flowering and(or) by more and(or) larger seeds being produced in undamaged pods.

In 1975, the plants had completed flowering at the time the experiment was initiated (Table 1). The beetles seemed to feed almost exclusively on leaves and the outer covering of pods, petioles and stems. Pods with holes and dried-out pods were not observed. The absence of yield loss apparently was related to the short feeding period and the small amount of tissue loss.

The plant growth-stages used in these experiments were typical of those normally encountered in growers' fields in the Prairie Provinces and British Columbia during late July and August. Also, the behaviour of the red turnip beetles in the cages appeared to be normal, because the types of damage seen in the cages were the same as those observed in growers' fields containing this insect at that time of the year. Since the adult density used in these experiments was much higher than those usually found in growers' fields, the present data indicate that in most, if not in all cases, control measures against red turnip beetles in rape fields during late July and August are not needed to protect yields.

ACKNOWLEDGEMENTS

I am grateful to Mr. J. Walkof for his technical assistance and to Mr. R. W. Sims for his assistance with the photographs.

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(Received 10 March, 1977)

OBSERVATIONS ON THE BIOLOGY OF *NEOPROCIPHILUS ACERIS* (Monell) [HOMOPTERA:APHIDIDAE] IN MANITOBA

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ABSTRACT: The aphid, *Neoprociphilus aceris* (Monell), produces both sexuparae and overwintering nymphs in Manitoba, but because the primary host, *Acer saccharum* Marshall, is not available, it maintains itself continuously on a secondary host, *Smilax lasioneura* Hook.

INTRODUCTION

The aphid, *Neoprociphilus aceris* (Monell), is indigenous to North America, and has been recorded in the United States from Maine to Florida and west to Utah (Smith and Graham, 1967). It alternates between in primary host, sugar maple, *Acer saccharum* Marshall, and species of *Smilax* as secondary hosts (Smith and Graham, 1967). Smith (1974) included this species of aphid in his keys to the genera of Pemphigini in North America. Smith and Graham (1967) summarized all available data on the life history and synonymy of *N. aceris*, and described the various forms. Most of their observations were based on their studies of this species in North Carolina.

The earliest known collections of *N. aceris* in Manitoba were made by Miss A. Bergen from *Smilax lasioneura* Hook near Sanford, on 2 September 1964 and 5 September 1967. In Manitoba, there are only two native species of maple, *Acer negundo* L. and *Acer spicatum* Lam., neither of which are probable primary hosts for *N. aceris*. Smith and Graham (1967) reported that *N. aceris* could remain on or near *Smilax* for the entire year, overwintering as nymphs in debris. When the early collections were made in Manitoba, it was not known whether the colonies resulted from *N. aceris* living the entire year on *Smilax*, or from temporary colonies produced by winged migrants carried into Manitoba on southerly winds. These colonies would not be expected to survive Manitoba winters. The senior author has recently made observations which show that *N. aceris* overwinters in the nymphal stage in Manitoba, and uses certain unusual behavioural activities for dispersal.

OBSERVATIONS

On 24 May 1975, near Lockport, Manitoba, large numbers of nymphs of *N. aceris* were noted crawling over wood chips in an old sawdust pile in a small clearing surrounded mostly by undisturbed vegetation, including many trees of the oak, *Quercus macrocarpa* Michx. There was no explanation at that time for this phenomenon and no host plants were visible. On 15 August 1975, winged adults (sexuparae) of *N. aceris* were found on numerous small climbing plants which were identified as *S. lasioneura*. Apterous alienicola, which produced overwintering nymphs, were also present.

The nymphs formed clusters, sometimes of more than one hundred individuals in broken acorns or curled oak leaves, on the ground. It was observed that if the clusters were broken and the aphids scattered, they very soon reformed into a closely knit mass. When some of these clusters were brought into the laboratory in the early spring or late fall, the aphids lived for about four weeks without feeding. Further observations showed that about the time the leaves of *Smilax* appeared in the spring the clusters of nymphs broke up. Some of the aphids moved over the ground apparently in search of host plants, while others were observed to climb objects such as fence posts or tree trunks. Some of these latter aphids were dislodged by high winds, and because of the waxy filaments on their abdomen were airborne for some distance.

The small area near Lockport was intermittently under observation by the senior author from early spring 1975, to late spring 1977. From these observations it was concluded that *N. aceris* is continuously present in Manitoba, that there are sexuparae which do not find a primary host, that the species is maintained all year on *S. lasioneura,* and that over-wintering nymphs are able to survive extremely cold winter temperatures through the formation of hibernating clusters. In the spring, the overwintering nymphs leave the clusters and find their way to new leaves of *Smilax*. The spring dispersal is accomplished either by searching along the ground, or by climbing high objects from which they may become transported by air currents. The nymphs do not feed for a period of about eight months when there are no green leaves on the *Smilax* plants.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Clyde F. Smith, North Carolina State University at Raleigh, for identifications of the different forms of *N. aceris.*

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(Received 6 May, 1977)

EFFECT OF DIFFERENT SEX RATIOS ON THE OVIPOSITION AND FERTILITY OF THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST)[1]

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ABSTRACT: The oviposition rate and fertility (female) of the red flour beetle, *Tribolium castaneum* (Herbst), reared at 27.5°C and 70% relative humidity, were measured for different sex ratios and densities between the 4th and 12th days after exposure. The ratios and densities were 5 ♀:1♂, 1 ♀:5 ♂, 3 ♀:3 ♂, and 1 ♀:1 ♂. Oviposition was highest for 1 ♀:1 ♂, equally reduced for 1 ♀:5 ♂ and 3 ♀:3 ♂ and least for the ratio 5 ♀:1 ♂. Fertility remained about 90% for all ratios.

INTRODUCTION

In many experiments designed to determine the oviposition of beetles, a sex ratio of unity has been used (Howe, 1950, 1962; Lefkovitch, 1962; Smith, 1962). The resultant oviposition was affected by such variables as genetic background of the male and female, moisture, temperature and food. Nevertheless, no reports have been found in the literature that show the effect of unequal sex ratios on the oviposition of beetles. Therefore, the present study was conducted to determine the effect of the sex ratio on the rate of oviposition and fertility of the red flour beetle, *Tribolium castaneum* (Herbst).

MATERIALS AND METHODS

The insects used in the experiment were obtained from a culture of *T. castaneum* reared continuously for 6 years, in the laboratory, at the Agriculture Canada Research Station in Winnipeg. The insects were reared on a standard culture medium of white flour plus dried brewer's yeast (19:1 ratio by weight) conditioned at 27.5°C ± 0.5°C and 70 ± 5% relative humidity. In the experiments to assess oviposition and fertility, the medium was passed through a 40-mesh screen (40 meshes per lineal cm, i.e. 100 meshes per lineal in) before and after conditioning to remove lumps of flour, and to facilitate the recovery of eggs. All experiments were done in a controlled environment at 27.5 ± 0.5°C and 70 ± 5% relative humidity.

The beetles used in the experiment were reared from eggs that were less than 24 h old. The sex of the beetles was determined in the pupal stage by examining the ventral-posterior region of the abdomen for genital lobes (Halstead, 1963). Male and female pupae were placed in separate jars to complete development. Four days after emergence, virgin adults were placed in vials (7.0 cm high and 3.0 cm wide) that contained 3.0 cm^3 (8 g) of moisture-conditioned medium. There were three different sex ratios with two densities of a 1:1 ratio: A - 5 ♀:1 ♂; B - 1 ♀:5 ♂; C - 3 ♀:3 ♂; D - 1 ♀:1 ♂, each replicated 10 times.

The beetles were allowed to lay eggs for 4 days; this 4-day period will be referred to as the 1st oviposition period (OP_1). At the end of this period, the beetles were sieved from each vial with a 20-mesh screen, and immediately placed in a vial with fresh medium for another 4-day period referred to as the 2nd oviposition period (OP_2). After the beetles were removed, the flour from each vial was sieved through a 24-mesh screen to remove the eggs which were counted under a binocular microscope (magnification 12x). The eggs and flour were returned to the original vial and incubated for 13 days. The number of larvae that emerged was used as a measure of egg

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fertility. After the incubation period, the vials were placed in a refrigerator at 5°C for 24 h to immobilize the larvae. Then the flour in each vial was sieved and the larvae counted. Immobilization prevented the larvae from crawling through the screen openings during sieving. In a preliminary test, over 99% of the larvae were removed by this method.

RESULTS

There was no difference in oviposition between the different sex ratios in OP_1, but in OP_2 the oviposition was greatest in D, and greater in B or C than in A (Table 1). In the total oviposition period of 8 days, the mean number of eggs laid per female was not significantly different between A and C or between B and C but was significantly different between A and B and between D and A, B or C.

Table 1. Mean number and range of eggs per female of *Tribolium castaneum* for different sex ratios and densities of adults during each of 2 oviposition periods

Sex Ratio (♀ : ♂)	OP_1 Mean	OP_1 Range	OP_2 Mean	OP_2 Range	$(OP_1 + OP_2)$ Mean	$(OP_1 + OP_2)$ Range	$\frac{OP_2}{OP_1}$ %
A(5:1)	15.2a	9.8-18.2	32.6	29.6-36.2	47.8a	41.6-53.4	114
B(1:5)	12.8a	0.0-22.0	42.0a	19.0-54.0	54.8b	32.0-76.0	228
C(3:3)	16.0a	12.3-20.0	38.2a	31.6-45.0	54.4ab	44.7-61.7	139
D(1:1)	16.2a	13.0-25.0	57.1	49.0-66.0	73.3	64.0-79.0	252

aMeans not significantly different (P = 0.05).
bMeans not significantly different (P = 0.05).

Table 2. Percentage hatch of *Tribolium castaneum* eggs for different sex ratios and densities of adults, and for each of 2 oviposition periods

Sex Ratio (♀ : ♂)	OP_1	OP_2	$OP_1 + OP_2$
A(5:1)	89.0 ± 2.2[1]	87.3 ± 1.6	87.8 ± 1.3
B(1:5)	82.8 ± 6.5	86.7 ± 3.2	85.8 ± 2.9
C(3:3)	87.7 ± 2.9	90.9 ± 1.7	90.0 ± 1.5
D(1:1)	92.6 ± 4.0	86.7 ± 2.8	88.0 ± 2.4

[1]Mean ± 95% confidence limits.

DISCUSSION

The highest oviposition rate was in D, the low density of the 1:1 ratio. The lower oviposition rates in the other sex ratios may have been caused by physiological or behavioural factors. For example, according to Vardell and Brower (1976), unmated females lay fewer eggs than do mated females. These authors reported that unmated females of *T. castaneum* laid about 2 eggs/day in the 2nd week after emergence and about 4.5 eggs/day in the third week, but none of the eggs hatched. In the present experiment, there was no significant difference in oviposition rate or in fertility of eggs among the different sex ratios or densities in OP_1 which suggests that all of the females had mated in all the ratios. There were significant differences in oviposition during OP_2. Decreases in the oviposition, in this case, were likely due to causes other than failure to mate since the decreases were not accompanied by decreases in fertility of eggs. Fertility remained about 87% in the different sex ratios and densities (Table 2). These values agree closely with those of Howe (1962).

One of the factors from the standpoint of the individual female which may have affected behaviour was crowding by other beetles. Since the lowest oviposition was recorded in sex ratio A, it appeared that crowding of a female by other females had a greater effect on oviposition than crowding by approximately equal numbers of males and females (C), or by males (B) (Table 1). A comparison of the oviposition for B and C, with that for D indicated that oviposition was decreased to a similar degree by a 3-fold increase in population when the additional beetles were either equal numbers of males and females or males only.

In the present experiments, it was unlikely that eggs laid in the flour were destroyed by cannibalism. Sonleitner (1961) showed that the rate of cannibalism was 0.02 eggs per female-day for females and 0.001 eggs per female-day for males when 32 females and 32 males were placed on 8 g of flour containing 200 eggs. It should be noted that the density used by Sonleitner was higher than that used in the present experiments.

Generally, for all females, oviposition was greater in OP_2 than in OP_1. This also agrees with the observations of Howe (1962). In the present experiment, the difference in oviposition between OP_1 and OP_2 was affected by the number of females in a vial. With sex ratio A, oviposition was 114% greater in OP_2 than in OP_1; with fewer females per vial the differences were greater - i.e. 139, 228 and 252% for C, B and D, respectively. The difference in oviposition between ratios B and D appears to have been caused by the different proportion of males (Table 1). When ratios A and B were compared separately with ratio D, it was clear that a sex ratio with an excess of females reduced egg production to a greater extent than a sex ratio with an excess of males (Tables 1).

To determine whether there was compensation in oviposition in OP_2 relative to that in OP_1, a comparison of correlation coefficients (r) was made. The correlation coefficients were not significant for any of the sex ratios or densities ($P>0.05$).

Changes in oviposition in response to an increase in the total number of adults, particularly if the increase was mainly females, would probably enable the beetles to adjust their population growth to the food and space available. The lower the proportion of females, the higher the oviposition would be and correspondingly, the rate of population growth. As the proportion of females increased, the rate of population growth would decrease. This relationship between the population density of females and the oviposition rate would be important if a pesticide was used that killed a higher proportion of females than males. The population would return to its original density more quickly than if the proportion of males killed was equal to, or greater than, the proportion of females killed.

ACKNOWLEDGEMENTS

We wish to thank Mr. M.G. Bickis for statistical advice on the analysis of the results.

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(Received 4 May, 1977)

INFLUENCE OF POPULATION DENSITY ON THE RESPONSE OF THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST), TO METHYL BROMIDE[1]

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ABSTRACT: The response of the red flour beetle, *Tribolium castaneum* (Herbst), to methyl bromide was the same at densities of 20 and 40 beetles per cage during fumigation and post-fumigation periods. The two groups of beetles were equally susceptible to methyl bromide.

INTRODUCTION

The influence of crowding on the survival of populations of insects subjected to insecticidal treatment was examined by Sun (1947), who showed that a greater proportion of granary weevils, *Sitophilus granarius* (L.), survived a concentration of 30 mg/ l of carbon disulphide at a density of 300 weevils per 30 g of wheat than at 50 weevils per 30 g of wheat. Morrison (1943) however, showed that for any concentration of nicotine sulphate in water, the fruit fly, *Drosophila melanogaster* (Meigen), was more susceptible at a density of 150 flies per container than at 15 flies per container. Stultz (1939) had previously suggested that crowding did not affect the susceptibility of the fruit fly to nicotine sulphate sprays. In view of this difference of opinion, McLeod (1944) re-examined this question and provided additional data to confirm the results of Morrison (1943).

In the bio-assay of the red flour beetle, *Tribolium castaneum* (Herbst), an insecticidal secretion, produced under circumstances such as crowding, may influence the relationship between population density and fumigant susceptibility (Gough, 1939; Loconti and Roth, 1953; Palm, 1946).

In experimental work to establish the levels of susceptibility of a species to a fumigant, the number of insects used may differ from one treatment to another in spite of the intentions of the experimenter. Tests in which 20 insects per treatment were desired may have had some treatments with as many as 25 or 30 insects. Barker (1967, 1970, and 1975) used 25, 20 to 25, and 10 beetles per treatment per replicate. The use of large number of insects in a bio-assay experiment is desirable because reliability of estimates are improved, but only as a function of the square root of the sample size (Spurr and Bonini, 1967) though there is a limit imposed by the logistics of the experiment and, as mentioned above, by the effects of crowding.

It was considered appropriate, therefore, to compare the tolerance of the red flour beetle to methyl bromide at a higher number of insects (40) per container with that of a moderate number (20) per container, especially in view of the production of a toxic secretion by this species.

MATERIALS AND METHODS

The strain of red flour beetle used has been reared at this laboratory for many years. Beetles, 5 to 13 days old, were sieved from a culture medium which consisted of unenriched flour and ground brewer's yeast in a ratio of 95:5 by weight. Twenty beetles (20-group) were placed into each of 44 test tubes, 8.5 cm long and 1.3 cm in internal diameter, and 40 (40-group) into each of another 44 test tubes of the same size. A strip of filter paper, 0.5 x 7.0 cm, was placed in each of the 88 test tubes to provide an additional crawling surface for the beetles and thus reduce the density of the beetles at the bottom of the test tubes.

[1]Contribution No. 806 from the Research Station, Agriculture Canada, Winnipeg.

Two tubes, one with 20 beetles and one with 40 beetles, were placed in each of 44 glass jars, 8.0 cm long and 5.0 cm in diameter. A jar with the two test tubes was placed in each of 44 sealers each with a volume of 1.735 l. The sealers were placed, at random, into 11 groups of 4. One group of 4 sealers was not treated; the others received methyl bromide in graded dosages from 5.23 to 9.16 mg per l of air. There were 10 dosages, and each dosage was replicated 4 times. The gas was measured into the sealers with a gas burette. The exposure period was 5 hours. This system was similar to that used by Barker (1967).

After fumigation, the insects were placed on 8 g of clean culture medium in vials 7 cm long and 3 cm in diameter. A snap-on cap with a hole covered with brass mesh confined the insects to the vials. The vials with the insects were then placed in incubators at 30° C and about 75% RH for 7 days. The numbers of live and dead insects were then recorded.

A logit analysis was performed on the data (Ashton, 1972) and a test was done for parallelism. The replicates of a dosage were not pooled so that the full range of variability in the results could be considered in the analysis for parallelism. For a dosage where there was a response in some replicates and not in others, and the number of insects treated per batch was about 20, a response of 0.25 insects was used instead of a zero response. Similarly, at the highest dosage where all insects were killed in some replicates and not in others, a response of N - 0.25 was used instead of N. When 40 insects were treated in each batch, responses of zero and N insects killed were substituted by 0.12 and N - 0.12. This technique was simpler than, but equivalent to, using 1/2n or 1 - 1/2n as suggested by Ashton (1972).

The percent accuracy of the experiments was determined by dividing the 95% confidence interval bands by the LC_{50}s and then multiplying the results by 100.

RESULTS AND DISCUSSION

In the 20-group, no insects were killed in 4 out of 8 batches of insects at the two lowest dosages of methyl bromide, while all were killed at the highest dosage. At the second-highest dosage, all insects were killed in 2 out of 4 batches.

In the 40-group there was one batch of insects at the lowest dosage in which no insects were killed. Survival at the highest dosage in the 40-group was slightly greater than in the 20-group, contrary to what had been expected, and in spite of the fact that the slope for the 20-group was less steep than in the 40-group. All insects survived in the 8 control batches. The results of the analyses of each group of beetles are listed in Table 1.

Table 1. The effect of two densities of insects during the treatment and post-treatment periods on the parameters of the response of the red flour beetle to methyl bromide.

Parameters	Insects per vial	
	20	40
Slope	22.52	25.44
Intercept	-19.09	-21.46
LC_{50} - C.I.[a] mg/l	6.89	6.88
LC_{50} mg/l	7.04	6.97
LC_{50} + C.I.[a] mg/l	7.19	7.06
LC_{95} - C.I.[a] mg/l	8.98	8.83
LC_{95} mg/l	9.51	9.10
LC_{95} + C.I.[a] mg/l	10.07	9.38
Logit Chi-square	35.48	45.51
Heterogeneity	1.04	1.19
N	36	40

[a] C.I. = Approximate 95% Confidence Interval.

At the LC_{50} there was a difference of 0.07 mg methyl bromide per l between the two groups. There was, however, considerable overlap between the approximate 95% confidence interval bands of the two groups.

There was a difference of 0.41 mg methyl bromide per l between the two groups at the LC_{95} level though there was some overlap of the 95% confidence interval bands between the 20- and 40groups (Table 1). When the ranges covered by the 95% confidence interval bands were considered, it was found that at the LC_{50} and LC_{95} the bands of the 20-group were twice as large as for the 40-group. At the LC_{50}, the range was 0.30 mg/ l for the 20-group and 0.16 mg/ l for the 40-group. The range of the 95% confidence interval of the LC_{50} for the 20-group amounted to 4.2% of the magnitude of the LC_{50}. Thus the requirement that the accuracy of the LC_{50} be within 5% of its true value was satisfied, and the use of fewer insects per group would not have been advisable.

For the 40-group, the 95% confidence interval band amounted to 2.2% of the LC_{50}, enough to satisfy the requirement that the LC_{50} be determined to within 5%. The approximate 95% confidence intervals were assumed to be reliable because the heterogeneity factors of both groups were close to unity.

The over-all chi-square was calculated for the two groups and it was found that the residual heterogeneity (Table 2) was not significant at the 5% level and the test for parallelism could be applied. The lines were parallel and the ratio of the tolerances to methyl bromide could then be expressed as the potency ratio which was 1.0081 (Table 3). The two groups therefore showed a similar response to treatment with methyl bromide.

Table 2. Test for parallelism of the regression lines describing the effect of two population densities on the response of the red flour beetle to methyl bromide.

Source of variation	Sums of squares	d.f.	Mean square
Parallelism of regressions	1.6376	1	1.6376
Residual heterogeneity	80.9931	72	1.1249
Total	82.6307	73	1.1319

Table 3. Pooled slope, new intercepts, and the ratio of tolerances of red flour beetles at two population densities, to methyl bromide.

Pooled slope	24.526
Standard error of pooled slope	1.058
New intercept for the 20-beetle group	-20.780
New intercept for the 40-beetle group	-20.694
Ratio of tolerances to methyl bromide (potency ratio)	1.008
Standard error of the ratio of tolerances	0.011

During the course of comparisons of the relative susceptibilities of various strains of the red flour beetle to methyl bromide, it was found that there were consistent tolerance ratios of about 1.1 between two strains (Barker, unpublished data), far more than the ratios found between the 20- and 40-groups. Thus, differences between strains cannot be attributed to different densities of beetles during fumigation and post-fumigation periods.

This work has shown that the numbers of red flour beetles used during treatment and post-treatment periods do not influence the outcome of the assays as long as there are sufficient beetles for proper testing of an adequate range of dosages, not less than 20 nor more than 40 beetles per batch, and 4 replicates at each dosage, the minimum used in this experiment. The use of 40 beetles per batch did not lead to deleterious effects due to high beetle density.

ACKNOWLEDGEMENTS

I wish to thank Mr. D. Kurtz for his diligent assistance in collecting the data. I appreciate the fruitful discussions on statistics with Mr. M. Bickis, and I thank L.B. Smith, W. Romanow, and F.L. Watters for their comments on the manuscript.

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(Received 7 June, 1977)

EFFECTS OF THE FUNGICIDE DITHANE® M-45 ON COLONIES OF HONEY BEES, *APIS MELLIFERA* LINNEAUS

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ABSTRACT: Colonies of honey bees, *Apis mellifera* Linnaeus, were monitored at two sites to assess the impact of an aerial application of the fungicide Dithane® M-45 to nearby fields. Mortality of adult bees commenced shortly after the fungicide application and reached its peak on the day of treatment. Activity increased after treatment resulting in an increase in pollen collecting. Hive weights increased slightly after 3 days. Queens and brood were not affected and honey production at the end of the season was estimated as normal.

INTRODUCTION

The impact of pesticides upon non-target organisms is of prime interest and concern to resource managers across Canada. The effects of pesticide applications upon honey bees. *Apis mellifera* Linneaus, and wild pollinators have been studied in both agricultural and forestry situations (Hocking, 1950; Anderson and Atkins, 1968; Kevin, 1975).

Colonies of honeybees being monitored to assess the impact of a chemical insecticide applied to forest plantations were exposed to and affected by an agricultural spray. The fungicide, Dithane® M-45 was applied as a prophylactic treatment against potato blight and was applied several days prior to the plantation trials.

MATERIALS AND METHODS

Sixteen two-pound packages of bees were set up and maintained at headquarters apiary and then moved to the experimental site in mid-August. The colonies were checked for queens and brood prior to moving and again after the hives were relocated. Eight colonies were placed in a pine plantation (Site "B") and 8 in an open site (Site "A") about 3 miles northeast of Site "B" and adjacent to a potato field. The bees were left for several days to adjust to their new surroundings and the hives were then fitted with the following equipment to measure various activities; an electronic counter attached to the hive entrance to record flight activity to and from the hives; an O.A.C.* pollen trap placed on the bottom board and collecting approximately 60% of the pollen bees brought into the hive; a dead bee trap attached to the front of each colony to collect the dead bees as they are ejected from the hive; and a scale to record colony weight gain or loss.

Daily hive monitoring commenced on August 25 and the potato crop adjacent to Site "A" was treated on August 28 with Dithane® M-45 at the emitted dosage rate of 2.242 kg ai/ha (2 lbs ai/acre) applied from a Piper Pawnee 234 aircraft. General weather data was recorded from the experimental area at the time when hives were checked.

RESULTS

Adult bee mortality was observed at the hives (Site A) shortly after the fungicide treatment had been completed. Mortality of the field force bees reached its peak by the evening of the day of treatment (Table 1). Hives closest to the treated field (Site A) registered a higher average adult

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Table 1. Activity of honeybees, *Apis mellifera*, at two locations before and after the application of the fungicide Dithane® M-45 near Barrie, Ontario. August 26 - September 3, 1977

Date and day relative to the treatment	Site A				Site B				Remarks
	Activity (bee trips)	Adult bee mortality	Pollen collected (gms)	Hive weight (kg)	Activity (bee trips)	Adult bee mortality	Pollen collected (gms)	Hive weight (kg)	
Aug. 26 (1-2)	25984	5	13.8	16.3	13312	6	15.7	13.6	
Aug. 27 (-1)	11392	1	0.7		10240	5	9.9		Showers all day, 18°C
Aug. 28 (0)	78720	165	45.6		50048	143	30.1		Sunny, 20°C
Aug. 29 (+1)	108544	99	44.9		53504	64	33.4		Sunny, 21°C
Aug. 30 (+2)	87680	62	65.0	17.2	50944	69	42.8	14.5	Sunny, 23°C
Aug. 31 (+3)	66560	10	21.4		33920	20	13.5		Cloudy, light winds
Sept. 1 (+4)	63616	9	28.2		43904	18	22.0		Showers, 15°C
Sept. 2 (+5)	24702	19	0.0	19.9	6016	26	0.0	15.4	Showers, 15°C
Sept. 3 (+6)	56192	7	29.3		37248	6	20.1		Cloudy, 15°C

bee mortality per colony than that recorded at the distant site (Site B). Mortality declined steadily at Site A after treatment (T) until T-day + 3 when average hive mortality reached near pre-treatment levels. Mortality at Site B also declined the day after treatment, remained at the level on T-day + 2, and then closely followed the decline recorded at Site A for the remainder of the monitoring period.

Bee activity, monitored at the hive entrance, showed a rapid increase on the day of treatment at both locations. This increase continued at Site A on T-day + 1 but declined on T-day + 2 in spite of warm sunny weather. The trend continued on T-day + 3 when light winds and cloudy weather were recorded (Table 1). Activity at Site B continued at treatment-day levels until T-day + 3. Cloudy conditions and rain showers were recorded on T-day + 3 and + 5. Activity remained about the same at Site A on T-day + 4, then decreased sharply on T-day + 5 and recovered on T-day + 6. Activity increased slightly at Site B on T-day + 4 and then paralleled that recorded at Site A on T-day + 5 and + 6.

The increased activity recorded at both sites on treatment day resulted in an increase in the amount of pollen collected. This increase continued through to T-day + 2 then decreased noticeably until T-day + 5, apparently because adverse weather conditions prevailed in the test area (Table 1).

Brood and queens in each colony at both sites were checked before, during and after the experimental period. No mortality amongst the queens was recorded and no reduction in brood production was observed.

All colonies were returned to the headquarters apiary following the experimental period. Examination of the hives from both sites in early September indicated all colonies to be healthy and the honey crop compared favorably with colonies which had not been subjected to pesticide treatments.

CONCLUSIONS

Dithane® M-45 applied as an aerial treatment at the rate of 2.242 kg ai/ha caused immediate mortality of foraging honey bees. The maximum impact occurred on the day of treatment and direct effects lasted approximately 5 days. The overall mortality to the field force was light. Neither queens nor brood were affected. Fluctuations in the amounts of pollen collected appeared to be related to weather factors rather than the pesticide. No overall hive weight loss was recorded and the hives closest to the treated fields recorded the greatest increase in weight following the treatment.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. K. Mortensen who assisted in the collection of field data.

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(Received 27 June, 1977)

NORMAL FLUCTUATIONS IN TOLERANCE TO METHYL BROMIDE OF THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST)[1]

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ABSTRACT: The response of the red flour beetle, *Tribolium castaneum* (Herbst), to methyl bromide varied from test to test, and the 95% confidence interval bands often did not overlap. Observed differences between the highest and lowest LC_{50} determinations, were not significant.

INTRODUCTION

In discussions of insect tolerance to insecticides, comparisons have been made between tolerant and susceptible strains (Bond, 1973; Yust *et al.*, 1951; Lichwardt, 1956) and between selected strains and their susceptible ancestors (Lindgren and Dickinson, 1945; Bruce and Decker, 1950; Decker and Bruce, 1952; Eddy *et al.*, 1955).

Sometimes there are reports of small differences in tolerance to a fumigant between various strains of a species; in these cases the tolerances may or may not be considered different. For example, although Lindgren and Vincent (1965) showed that the LC_{50} of 12 strains of the red flour beetle, *Tribolium castaneum* (Herbst), treated with methyl bromide during 4 hour exposure periods, varied from 8.9 to 9.6 mg/l, a range of 0.7 mg/l, they did not consider this to show a difference in tolerance. Yet, for *T. confusum* Du Val, a difference of 0.4 mg/l was considered, by the same authors, to be sufficient to indicate a difference in tolerance between strains.

In some studies there has been an effort to establish the normal variation of a susceptible strain by graphic means (Monro and Upitis, 1956; Whitney and Harein, 1959; Monro *et al.*, 1961). Ellis (1965) listed the LC_{50}s of a strain of *Sitophilus granarius* (L.), treated with ethylene dibromide, and found that they ranged from 2.2 to 3.3 mg/l, for 5 h exposure periods. Similarly, Sun (1960) mentioned that the concentration of aldrin required to kill 50% of house flies by precision spraying varied from 0.0072 to 0.014 per cent. These ranges of variation in LC_{50}s (or LD_{50}s) are important in tests where the differences between tolerant and susceptible strains are small, particularly in instances where slopes of the probit lines are very steep.

It is the purpose of this paper to document the normal range of variation of the LC_{50} of a standard strain of the red flour beetle, treated with methyl bromide, and to establish whether or not the extreme values found differ statistically from each other.

MATERIALS AND METHODS

The strain of red flour beetles used has been reared at this laboratory for more than 5 years. Adults, 5 to 13 days old, were sieved from a culture medium which consisted of unenriched flour and ground brewer's yeast in a ratio of 95:5 by weight. Approximately twenty insects were placed in each of 28 test tubes, 8.5 cm long and 1.3 cm in internal diameter. A strip of filter paper, 0.5 x 7.0 cm, was placed in each of the 28 test tubes to provide an additional crawling surface for the beetles and thus reduce the density of the beetles at the bottoms of the test tubes.

Each test tube was placed in a small glass jar, 8.0 cm long and 5.0 cm in diameter, so that the tube would be kept upright. A jar, with its test tube, was placed in each of 28 sealers, each with a volume of 1.735 l. The 28 sealers were arranged at random into 7 groups of 4. One group of 4 sealers was not treated; methyl bromide was added to the others at dosages ranging from 6.06 to

[1]Contribution No. 808. Agriculture Canada, Research Station, Winnipeg, Manitoba.

8.49 mg per 1. There were 6 dosages and each dosage was replicated 4 times. The gas was measured into the sealers with a gas burette and the volumes were later transformed into weights of gas (Parkes and Mellor, 1940). The exposure period was 5 hours. This method was similar to that of Barker (1976).

After fumigation, the insects were placed on 8 g of culture medium in vials that were 7 cm long and 3 cm in diameter. A snap-on cap with a hole covered with brass mesh confined the insects to the vials. The vials with the insects were then placed in incubators at 30° C and about 75% RH for 7 days. The number of live and dead insects were then recorded.

The entire experiment was repeated on 14 occasions between April and July 1976 with insects of the same strain and age interval, but from different culture jars and successive generations.

A logit analysis was performed on the data obtained (Ashton, 1972). The replicates of a dosage were not pooled so that the full range of variability in the data could be considered during analyses for parallelism, if needed. A test for parallelism was performed on the data from the two experiments which had extreme values.

RESULTS AND DISCUSSION

The LC_{50}s obtained are shown in Figure 1 and ranged from a minimum of 6.77, in test 11, to a maximum of 7.25 mg of methyl bromide per 1, in test 8. The mean LC_{50} was 6.97 mg/1.

The approximate 95% confidence interval bands ranged from 0.25, in test 9 and others, to 0.38 mg/1 in test 10, and constituted 5% or less of the magnitude of the corresponding LC_{50}s, except in test 10 where the confidence interval bands amounted to 5.5% of the LC_{50}.

The heterogeneity factors were close to unity and ranged from 0.88, in test 14, to 1.28 in test 7, so that the 95% confidence intervals can be assumed to be reliable.

The confidence intervals, though reliable and small in proportion to the LC50s, did not overlap in all cases (Fig. 1). For example, the 95% confidence interval band of test 8 did not overlap the 95% confidence bands of tests 1, 3, 4, 7, 11, 12, 13, and 14.

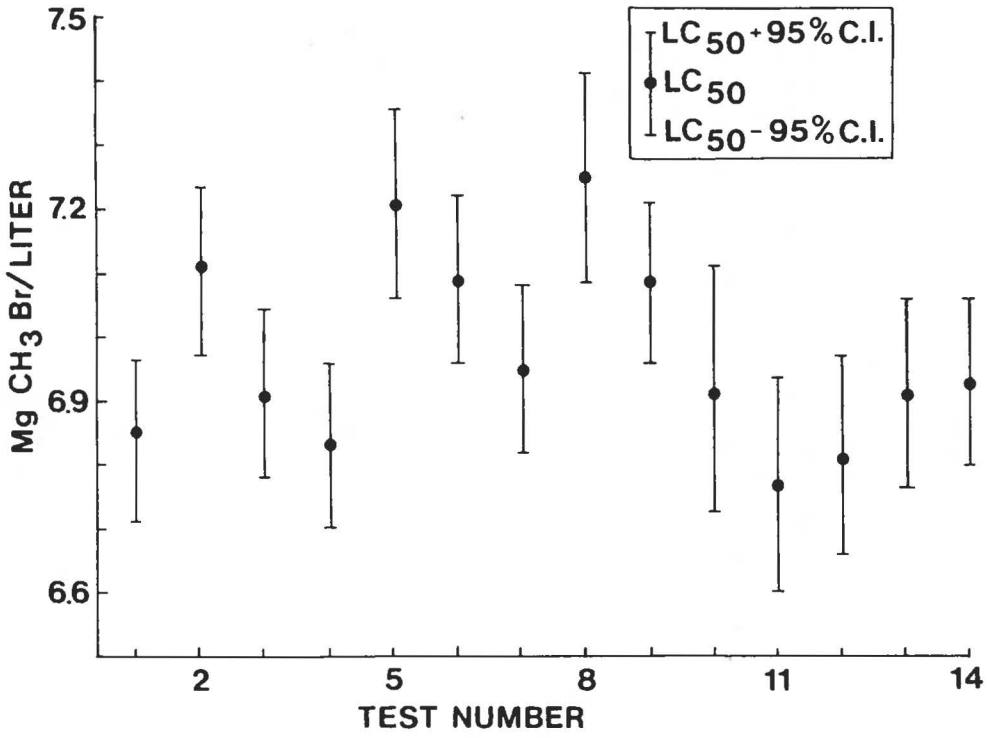

Figure 1. The LC_{50}s and 95% confidence intervals, determined in 14 successive tests, for the red flour beetle, *Tribolium castaneum,* treated with methyl bromide.

A test for parallelism was performed on the most widely separated sets of data (tests 8 and 11). There was no conflict with the hypothesis of parallelism between the logit lines obtained (Table 1). The potency ratio between these lines was 1.08 with a standard error of 0.017. In comparison, Sun (1960) showed that the highest LD_{50} for house flies treated with aldrin solutions was twice the magnitude of the lowest LD_{50}, a potency ratio of approximately 2. In *S. granarius,* treated with ethylene dibromide, the relative potency of the two extreme LC_{50}s was 1.5 (Ellis, 1965).

Table 1. Chi-square test for parallelism between tests 8 and 11 on the response of the red flour beetle, *Tribolium castaneum*, to methyl bromide.

Source of variation	Sum of squares	d.f.	Mean squares
Parallelism of regressions	0.000669	1	0.000669
Residual heterogeneity	35.812756	42	0.8526
Total	35.813424	43	0.8328

The potency ratio of 1.08 was so small that though there was a difference between the two extreme LC_{50}s, and the 95% confidence bands did not overlap, it could be assumed that the two populations tested were not different. To confirm this opinion, a studentized range test (Snedecor and Cochran, 1974) was performed on the extreme LC_{50} values (tests 8 and 11) and it was shown that the data were not distinguishable in these experiments.

The slopes of the $\log_{10}$-concentration logit-mortality lines were very steep and varied from 19.2 to 34.5. Steep slopes imply that the population tested is highly homogeneous in its response to the toxicant and that under field conditions an underestimation of dosages would result in survival of most of the population treated (Hoskins and Gordon, 1956).

In routine tests of the susceptibility of field-collected strains of the red flour beetle small differences are often found between strains, and because of the fluctuations of the log-concentration logit-mortality lines it is more difficult to establish whether or not one strain really is more tolerant than another. Consequently, the incipient stages of the development of resistance of a strain may be missed..

Difficulties could arise in the use of the discriminant dosage technique (Anon., 1975). If the strain being examined happened to show an unusually low tolerance at the time of the test, and all the insects were killed, it might be accepted as a normal strain when, in fact, it should have been regarded as tolerant.

This series of tests has shown that the LC_{50}s and 95% confidence interval bands determined for successive generations of the red flour beetle do not necessarily coincide or overlap even though statistical differences between data for different generations may not be demonstrable.

ACKNOWLEDGEMENTS

I wish to thank Mr. D. Kurtz for his technical assistance. I appreciate the fruitful discussions on statistics with M. Bickis, and thank J.T. Mills and F.L. Watters for their comments on the manuscript.

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(Received 28 June, 1977)

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(Received 16 November, 1977)

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