

Experimental aerial application of a juvenile
hormone analog against the eastern hemlock
looper Lambdina fiscellaria fiscellaria (Guen.)
in Anticosti Island in July 1973

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Abstract

An insect growth regulator Altosid^R (also called ZR-515; Zoecon Corp., Calif., U.S.A.) was tested against the hemlock-looper (Lambdina fiscellaria fiscellaria) by aerial application from a helicopter in Anticosti Island, Quebec during July, 1975. Greenhouse pre-testing indicated the material was resistant to UV radiation and wash-off.

In 3 test blocks (10 acres each), the material was applied at dosages of 0.25, 1 and 3 oz in 2 gallons of spray mixture per acre. Comparison of pupal and moth counts in check and spray plots showed significant population reduction.

No effect on small mammals, birds or aquatic fauna was seen.

Résumé

Un régulateur de croissance connu sous le nom commercial de "Altosid" (ou ZR-515) et fabriqué par la compagnie Zoecon, Californie, U.S.A. a été utilisé par un arrosage expérimental, effectué par hélicoptère, à l'île d'Anticosti en Juillet 1973 comme essai de lutte contre l'arpenteuse de la pruche (Lambdina fiscellaria fiscellaria). Des travaux effectués en laboratoire et en serre ont démontré la résistance du matériel expérimental aux radiations ultraviolettes et au délavage. Le produit expérimental a été appliqué dans trois parcelles, de dix acres chacune, au taux de 0.25, 1 et 3 onces, diluées dans deux gallons de'eau à l'acre. Une étude comparative des populations de chrysalides et papillons dans les parcelles traitées et la parcelle témoins mit en évidence une diminution significative du niveau des populations. Aucun effet n'a été décelé chez les petits rongeurs, les oiseaux ou la faune aquatique.

I. Introduction

Growth and development in insects has been shown to be under the control of at least 3 hormones. The characteristic moulting process that permits growth has been well studied and is central to the understanding of the endocrine regulation of metamorphosis. Typically, the larva of a lepidopteran after emerging from an egg undergoes a series of moults and goes through a pupal stage prior to becoming an adult. The brain secretes the prothoracotropic hormone (also called ecdysiotropin or brain hormone) which stimulates the prothoracic gland to secrete the second hormone, ecdysone (also called moulting hormone) which in turn initiates the moulting process. The outcome of the moult depends on the titre of a third hormone, juvenile hormone, in the insect. This hormone is secreted by a pair of small glands, the corpora allata, situated under the brain. If a high titre of juvenile hormone is present when moulting occurs then another larval stage results. The titre of juvenile hormone is extremely low during the larval-pupal moult. When the pupa moults into an adult, there is no juvenile hormone in the system. In the adult, the corpora allata resume secretion of juvenile hormone which is necessary for ovarian de-

velopment. Some insects overwinter as adults during which period the corpora allata remain inactive and as a result the ovaries are not developed. At the onset of spring, these glands secrete juvenile hormone which in turn initiates reproductive maturity.

Application of juvenile hormone to a stage where the endogenous titre of the hormone is either low or absent induces abnormal differentiation leading to mortality.

Soon after the structure of juvenile hormone was elucidated it was reported that certain synthetic analogues of juvenile hormone were more potent than the natural hormone. Following this, to date, over 200 analogues have been synthesized in various laboratories around the world. Some of these compounds are more active, more stable and simpler to synthesize than the natural hormone.

Although juvenile hormone is not species specific, it acts at a particular developmental stage and only those insects that are in the last larval stage will be affected. This affords a certain amount of development isolation of the pest-species and hence the likelihood of effects on useful species such as predators and pollinators is much reduced or minimal.

Many of the juvenile hormone analogs vary in their

degree of activity from species to species. While this does not assure specificity it nevertheless narrows the spectrum of activity.

The discovery, structural elucidation, chemical nature and physiological properties of juvenile hormone have been extensively reviewed in recent years (Novak, 1965; Roller and Dahm, 1968; Berkoff 1969; Williams 1969; Highnam, 1970; Wigglesworth 1970; Slama, 1971; Menn and Beroza, 1972; Wyatt, 1972; Doane, 1973; Slama, et al 1974; Burdette, 1973).

Laboratory investigations leading to a field experiment on the morphogenetic control of the eastern hemlock looper, Lambdina fiscellaria fiscellaria are presented below.

II. Laboratory Studies

Several analogs of juvenile hormone were tested against 3 species of forest insects: the forest tent caterpillar, (Malacosoma disstria); the eastern hemlock looper, (Lambdina fiscellaria fiscellaria); and the spruce budworm (Choristoneura fumiferana). Topical treatment of the last larval instar resulted in abnormal pupation; such pupae retained some larval characteristics. The larval-pupal mosaic was unable to complete its metamorphosis. This morphogenetic effect was used as the criterion for assaying the activity of the different hormone analogs.

The compounds tested for morphogenetic activity are listed in Table 1. Of the compounds tested, ZR-515 (or Altosid) was the most active and of the insects used, the eastern hemlock looper was the most sensitive. The effect on this insect is shown in Table 2. As is the case with other insect species, the fifth instars showed more deformities. It is not unusual to find that at higher concentrations the effect was less pronounced. The mechanism behind the optimum concentration effect was studied on the spruce budworm (Retnakaran 1973). Massive doses of hormone appears to prolong the larval stage without inducing any obvious deformities.

It was found that the insect was more sensitive to the analog during a specific period in the last larval stadium and the effect was apparent with low dosages. When 1 μ g of active ingredient was applied to each larva, it was found that the sensitive period or "activity window" extended from day 6 to day 10 of the last larval stadium (Table 3). Ingestion of the material had similar effects.

Field-simulation studies were conducted in the greenhouse using 2 feet high balsam-fir trees (Abies balsamea) in pots. Using a 0.2% w/v solution of Rhodamine-B^R (Dupont) it was determined that 0.2 ml solution sprayed from a height of 1 meter covered an

area of 1000 cm^2 . This volume and area corresponded to 2 gallons per acre (GPA). The details of the spray system were obtained from Mr. W. Haliburton, Chemical Control Research Institute, Ottawa. A 0.2 ml solution of the Rhodamine-B was drawn into a disposable plastic tip of a 200 μl Eppendorf pipette. The plastic tip alone containing the solution was placed on the intake end of a nozzle (Micro IIa-19/32). Air was passed through the nozzle with the aid of an air pump set at a pressure of 40 mm Hg to spray the solution. The spray deposit obtained on a card resembled that of an aircraft spray and the area covered was approximately that of the potted tree used in the experiment.

Two different formulations were used in the field simulation studies. In one, the technical material was dissolved in kerosene which had a phytotoxic effect on the balsam fir. Therefore, further tests with this mixture were abandoned. In the second formulation an emulsifiable concentrate was mixed with water. Inasmuch as the population in the field is not synchronous, a mixture of 10 fourth instars and 10 fifth instars were placed on each sprayed tree. The trees were then covered with a plexiglass container and the behaviour of the larvae observed. The results (Table 4) indicate that 2.5 mg. of active ingredient in 0.2 ml of water per tree gave good results. This dosage is equivalent to 0.3 oz. of active

ingredient in 2 gallons of spray mixture per acre. It was therefore decided that under actual field conditions ten times this dosage (3 oz. active ingredient in 2 gal of spray mixture per acre) would probably be the best dosage to use. The 10X recommendation is a rule of thumb used by the Chemical Control Research Institute in many of their operations.

It was essential to determine how the analog ZR-515 would be affected by environmental conditions. The degree of persistence of the material on foliage, degradation by ultraviolet radiation in the sunlight, and leaching from foliage by rain had to be tested. For these tests spruce budworm was used as the experimental animal. This insect is less sensitive to the analog and therefore higher concentrations had to be used.

As described earlier, potted balsam-fir trees were sprayed with 200 μ l of a 0.1, 1.0, or 10% emulsion of ZR-515 in water. Twenty, last instar larvae (6th instars in the case of the spruce budworm) were placed on each tree 15 days after the spray. The results indicate that the material is persistent in the foliage (Table 5). When the material was suspended in tap water in a beaker and left on the laboratory bench for 15 days it lost all its activity. The material being terpenoid and relatively simple in structure was possibly degraded by microorganisms in the water.

The effect of UV on ZR-515 was studied by exposing treated trees to short wave UV using a Mineralight (Fisher Scientific) for various lengths of time. Sixth instar larvae were placed on the trees 15 days after spray. The results indicate that no marked breakdown of the material occurred (Table 6).

The effect of spraying water at the rate of 11.5 l/min for 5 sec for various number of days on treated trees was studied. The larvae were placed on the trees 15 days after the hormone treatment during which time the trees were subjected to water spraying. The results indicate that there was no serious leaching (Table 7). It is hypothesized that the ZR-515 being a terpenoid material was probably adsorbed by the cuticular waxes on the coniferous needles and thereby protected from the water.

The laboratory tests indicated that ZR-515 would be an excellent candidate to be tested against the eastern hemlock looper.

It was earlier found that the activity of ZR-512 (Table 1) was similar to that of ZR-515. In addition, independent studies by Drs. Shepherd and Sahota at the Pacific Forest Research Centre at Victoria, B.C. on the western hemlock looper (Lambdina fiscellaria lugubrosa) have shown that ZR-512 was very active (Personal communication). Gomez et al (1973) reported that 10 ppb of ZR-512

induced early metamorphosis in the barnacle causing loss of ability to attach themselves to any substratum. ZR-515 on the other hand had no such effect. It was therefore felt that the latter compound would probably have negligible side effects on other crustacea and hence was chosen over ZR-512 to test in the field.

III. Choice of site for field testing
ZR-515 on the eastern hemlock
loopers

Three potential areas were considered for a field test: British Columbia; Newfoundland; and Anticosti Island in Quebec. Anticosti Island was best suited because of the infestation being relatively recent, an excellent background study had been conducted by the Laurentian Forest Research personnel, and the willingness of the then owners of the island (Consolidated Bathurst Company) to provide us with all the facilities for a test area and field laboratory.

Anticosti is a large, low lying island in the Gulf of St. Lawrence. The island is 140 miles long by a maximum of 35 miles wide and covers an area of 3,100 square miles. A part of Duplessis County of the Province of Quebec, it is separated by 20 miles from the north shore of the Gulf by the Jacques Cartier Strait. Some 45 miles separates the south shore of the island from the nearest point of the Gaspé coast. Although maximum elevation is 1,025 feet above sea level, most of the island is below 500 feet in elevation.

The Indians called the island "NOTISKUAN", meaning "where bear are hunted". The first record of the island was made when Jacques Cartier, who discovered it August 15, 1534, gave it the name of "Ile de l'Assumption". The name "Anticosti" had come into use by 1660 and it was

suggested that the name is derived from the Spanish "anti" (before) and "costa" (coast).

In 1680, the island was granted to the explorer Louis Jolliet by Louis XIV, King of France. It was held as a seigniory by Jolliet and his heirs throughout the French period. In 1763 it was annexed to Newfoundland, but in 1774 it was returned to Canada. It was again to be part of Newfoundland from 1808 to 1825. Records of the island's ownership indicate that numerous land transactions occurred during the nineteenth century.

In 1895, the island was sold for \$125,000, to Henri Menier, a wealthy French chocolate manufacturer and entered a period of prosperity. Menier expanded the village of Baie Ste. Claire and founded the village of Port Menier, pouring out money to develop the resources of the island in the form of wood-cutting, farming, commercial lobster fishing, etc. In 1926, a group of pulp and paper companies - later to become Canada Power and Paper Corporation - became interested in Anticosti and formed a company known as the Anticosti Corporation. Consolidated Paper Corporation, formed in 1931, was a successor company to Canada Power and Paper. Its woodlands operations on the island were suspended at the beginning of the '30's, resumed in 1946, and discontinued at the end of 1971. The island population grew from 250 around the time Menier purchased

Anticosti to some 500 in 1903. Seasonal fluctuations ran as high as 4000 when periods of active lumber operations were initiated by pulp and paper company but the base population remained near 500 until 1968 when it declined to about 240 in 1972.

Because hundreds of ships were wrecked on Anticosti during the eighteenth and nineteenth centuries, the island had a reputation as "the graveyard of the Gulf." The Canadian Government began building lighthouses to help prevent such disasters.

Since there are no large predators on the island the white-tailed deer, imported from the Riviere du Loup region by Henri Menier, have multiplied to make the island a hunter's paradise. The rivers, especially the Jupiter, offer some of the best Atlantic salmon fishing in the world. Quebec red trout are found in most of the numerous rivers and lakes. Sea trout, which enter the coastal streams, are also found. Anticosti Island is a haven for migrating birds. Species such as the Canada goose and the bald eagle nest undisturbed here, as do numerous species of seafowl. The flora on Anticosti is of a subarctic type; its characteristics determined by the northerly latitude (50°) and the island's low elevation in an area of cold marine currents. The calcareous nature of the soil also had its effect on the type of flora. The dominant tree species

are black and white spruce, balsam fir and white birch. The aspect of the vegetation has been greatly modified, the broad-leaved species in particular, by the proliferation of the introduced Virginia white-tailed deer. Their heavy browsing has favored the growth of conifers.

The forest resources consist of an estimated 9.3 million cunits of spruce and balsam, suitable mainly for pulpwood. In the absence of severe forest fires, an annual allowable cut of 120,000 cunits is considered feasible in perpetuity.

The outbreak of eastern hemlock looper in Anticosti that began in 1971 required extensive aerial sprayings by the Quebec Department of Lands and Forests of some 425,000 acres in 1972 and 10,300 acres in 1973. A pocket infestation near Lac Larouche was made available to us (Fig. 9a). An area near Port Menier airstrip and located about three miles from the experimental area served as the control (Fig. 1).

IV. Biology of the eastern hemlock looper on Anticosti Island.

The biology of the eastern hemlock looper, Lambdina
fiscellaria fiscellaria (Guen.) was previously studied
by various authors, the most recent being that of Otvos
et al (1971) in Newfoundland. In the course of an
aerial spraying operation of mature balsam fir stands
on Anticosti Island in 1972 a study of the looper de-
velopment was carried out for timing of insecticide
application and assessment of results. The following
description of the life history and habits of the insect
is based on field observations and material obtained
from periodical sampling of balsam fir trees throughout
the season and from laboratory reared material. The
various stages of the life history are shown in Fig. 2.

LIFE HISTORY AND HABITS

Adults. The first adults occurred in the field
August 26 and were seen until the second week of October.
The adult moth has a wing expanse of 32 mm and colour
varies from creamy-yellow to greyish-brown. The fore-
wings have two greyish zigzag lines. Hindwings have
only one transverse line which is continuous with the
outermost line of the frontwing. Both pairs of wings
have a distinct dark colored dot. From a total of 1,040
adults obtained from field-collected pupae, 45.3 per cent
were males and 55.7 per cent females. Field emergence of

the males preceded that of the females by about 5 days. During warm sunny days with low wing velocities, adult males flew actively and were seen feeding on nectar and pollen of wild plants in bloom. Females are rarely seen flying and remained on tree trunks. On cool and cloudy days, moths exhibited little activity. Egg laying was obtained from five-day old adults in oviposition cages. Males and females lived about two weeks in the cages.

Eggs. Eggs are laid singly but sometimes in groups of two to three, mostly in September, on moss and lichens growing on tree trunk and branches, in bark crevices, stumps and logs. Freshly laid eggs are pale green in colour, but change to copper-brown in about two weeks. They are oval-shaped and measure approximately 0.9 mm. The insect overwinters in the egg stage. Sampling of eggs and first instar larvae in June showed that the hatching period extended from about June 20 to the second week of July but mostly occurred during the latter part of June.

Larva. Head-capsule measurements of individually reared larvae and field collected specimens indicate that the looper has four larval instars. Average head widths for each instar of field collected larvae fell within four groups given in Table 8.

The body of the first instar looper is dark grey with black circular bands which gives a distinct

annulated appearance. In older larvae the ground colour of the body varies from greyish-yellow to greyish-brown and there are irregular dark longitudinal lines extending the full body length. Full grown larvae average 32 mm long.

The first and second instar larvae fed on the current year's foliage of balsam fir for a period of approximately three weeks. The initial feeding damage to new foliage is light and is characterized by reddening of the needle tips; later, all the damaged new foliage turns reddish. Third and fourth instar larvae attack mostly old foliage, usually consuming only part of the base or side of the needle. Partly eaten needles turn brown and damage becomes obvious about mid-July.

Larvae were seen in the field from June 20 to the first week of September. Larval development requires about 50 days and overlapping of all four instars occurred in the field during the third week of July (Fig. 3).

Pupa. Prepupae were observed on July 30 and first pupae were collected on August 3. Most larvae were in the pupal stage three weeks later. Pupae are dark brown in colour and average 14 mm in length. Bark crevices, lichens on branches and tree trunks, loose bark on decaying stumps are preferred sites for pupation. The

duration of pupal period for both males and females, determined by 85 individuals reared at room temperature, ranged from 14 to 19 days, averaging 16 days for both sexes.

As mentioned earlier, the ZR-515 compound is probably adsorbed by the cuticular waxes on the needles and retains its activity for about 3 weeks. This persistence is essential for the success of applied sprays since the population is not synchronized at any given time during the larval life history of the insect. It was felt that application of the material at the time when 50% of the larvae reach the last (fourth) larval instar would have the best effect. The last instar would be subjected to the effect of the analog immediately and most of the earlier instars would pass through the sensitive last instar stage on the foliage on which the analog was earlier shown to persist.

V. Plot description and location
on Anticosti Island.

The plots were selected on the basis of (i) accessibility (ii) reasonable population density (20-40 first instar larvae/branch tip) (iii) new infestation (first year) and (iv) absence of fungal pathogens (principally Entomophthora spp.), and parasites.

The presence of a new infestation was detected by egg-survey during fall of 1972. Three plots, A, B, and C near Lac Larouche and a control plot near the Port Menier airstrip was chosen. Consolidated-Bathurst Company made available at no cost, a home which we used as a place of residence as well as the field laboratory. An adjacent field was used as a helicopter landing site (Fig. 2).

The details of the three plots are shown in Fig. 4. Each plot covered an area of 10 acres with a buffer zone between them and had a trail leading to the middle of the plot where a clearing was made. Thirty trees in groups of three were selected and tagged as shown in Fig. 5. One of the three trees was the sample tree from which pre and post-spray samples of larvae were taken. Another was the burlap-trap tree from which the pupae were sampled for evaluating the post-spray population. The third tree was used for taking foliage samples to detect

possible accidental drift of Fenitrothion^R. (The Provincial Government was spraying approximately 10,000 acres in the west end of the island with Fenitrothion^R and whether or not any of the sprayed insecticide drifted into the plot area had to be monitored prior to our field experiment.)

VI. Fenitrothion detection system in the plots

During the last week in June, using a CL-25 aircraft based in Bonaventure, Gaspé, extensive spraying of Fenitrothion^R was undertaken by the Quebec Department of Lands and Forests. In order to prevent accidental spraying of the hormone plots special markers were set up. In addition to the natural landmarks (the three lakes around the plot) balloons were set up to the north and south of the hormone plots (Fig. 2).

Any possible drift of the insecticide was monitored by doing a foliage-wash analysis by gas liquid chromatography (GLC) and a field bioassay. Foliage samples of 100 g from each marked tree were packed in mason jars containing 200 ml of pesticide-grade benzene and sent to Dr. Yule at the Chemical Control Research Institute in Ottawa for analysis. There were two Fenitrothion^R sprays at Anticosti and the monitoring was done 24 hr after each spray. The results of the GLC analysis is shown in Table 9. Dr. Yule concluded by comparing the data with the studies conducted at CCRI that the amount of insecticide detected was too low to have any apparent effect on the insect. The insecticide found probably was from drifts of 1972 spray

operations.

Bioassays using foliage from the marked trees and 2nd instar larvae were conducted in the field laboratory. The larvae did not suffer any ill effects. It was therefore concluded that the plots were not affected in any way by the Fenitrothion^R spray operations in the adjacent areas.

VII. Pre-spray sampling

Pre-spray sampling was first done on June 28, when the larvae were mostly in the first and second instar stage. At this stage they feed on the new foliage and are concentrated on branch tips (Fig. 10d). Branch tips (18") were collected from mid-crown of the balsam fir trees using a pole clipper equipped with a basket (Fig. 11a). Substantial numbers of larvae were detected in all the plots. Two branch tips were collected from each sample tree and all the larvae counted (Table 10).

Larvae were collected at different times and sent to Dr. Smirnoff at Laurentian Forest Research Centre, Ste. Foy, Quebec to determine the incidence of Entomophthora. On June 22, 1800 first instar larvae were sent to Ste. Foy and no fungus could be detected. A second sample of a mixture of first and second instars (1,500 in all) were sent for Entomophthora detection; again no fungus could be detected. On July 9 fungus was detected in the plots. Dead insects were found hanging on the lower branches. Some of them were collected and sent to the Insect Pathology Research Institute for identification and subsequently Drs. MacLeod and Tyrrell confirmed that Entomophthora egressa was present.

Eight days of rain and warm weather, from July 2 to July 9 probably helped disseminate the fungus (Table 11). First and second instar larvae were particularly susceptible to the infection. The incidence of the fungus was about 11% but it subsequently declined. A record of mortality due to Entomophthora was maintained in all further studies.

It became evident that a second pre-spray sampling prior to spraying was warranted. Most of the larvae were now in the 3rd and 4th instars and were observed to be actively moving about all over the trees (Fig. 9a). Branch-tip sampling was therefore a poor indicator of the population in the plots and so a beating method developed by Dr. Otvos, Newfoundland Research Centre, was used. A sheet was placed under a 30-40 ft. tree and the branches on one side with a beating pole. The larvae that fell on the sheet were counted. Using this method, seven trees were sampled from each of the plots A, B, and C and six at the control plot for sampling. The results are shown in Table 12.

Since spraying was to be performed when 50% of the population reached the ultimate larval instar, the larval-population distribution was periodically monitored. The sampling on July 17 indicated that

approximately 50% of the population had reached the last larval instar (Table 13).

VIII. Spray Operation

The spray operation was carried out between 7:00 and 9:00 a.m. on July 19 using a Bell G-5 helicopter (Yvon Fournier Ltd., Cap-de-la-Madeleine, Quebec) equipped with a boom and nozzle system. The pilot, Mr. Paul Goulet, calibrated the spray system at the Port Menier airstrip to deliver 2 GPA. The helicopter was to fly 30 ft. above the tree tops at a speed of 35 mph and make 10 passes over each plot, the swath width being 70 feet to cover each 10 acre plot.

The hormone analog, ZR-515 was available in an emulsion formulation (5 lbs active ingredient per gallon of emulsifiable concentrate). The spray mixture was prepared in three separate 45 gallon drums for the three plots at volumes of 30 gallons per plot. Plot A was sprayed at the rate of 3 oz/2 U.S. gallons/acre; plot B, 1 oz/2 U.S. gallons/acre and plot C, 0.25 oz/2 U.S. gallons/acre. Rhodamine B (20% solution, Dupont, Toronto) was added at the rate of 10 ml/U.S. gallon.

A telethermometer was set up at the plot area with two thermistor probes, one at tree top and the other at the middle to monitor the onset of inversion. The ideal condition for spraying was

determined by the following formula:

$$\text{S.R. (stability ratio)} = \frac{T_2 - T_1 \times 10^5}{\bar{U}^2}$$

where T_2 is the top temperature (at tree top level), T_1 is the bottom temperature (at the middle of the tree) and \bar{U} is the wind speed. A positive reading indicates stable conditions for spraying (inversion) whereas a negative reading indicates unstable or lapsed condition (Information provided by Dr. J. Armstrong, Chemical Control Research Institute, Ottawa). As it turned out it was a cloudy day and the relative humidity was between 95-100% throughout the spray period. No clearcut inversion was observed (Table 14). The condition and timing of spray is summarized in Table 15.

A stream selected for studying the effect of ZR-515 on aquatic fauna (Fig. 1 and Fig. 10c) was sprayed at the rate of 3 oz/acre.

The deposition of the spray in the plot was evaluated using the system developed by Mr. Randall (Chemical Control Research Institute, Ottawa). Each spray plate consisted of a folder made of two aluminum plates. One plate had a krome-kote card and the other had two glass slides, each with a surface area of 37.5 cm^2 (Fig. 11b). Immediately

before spraying, a 100 ml sample of the spray mixture containing Rhodamine-B was removed from the aircraft spray tank and was used for preparing a standard curve for determining gallons per acre. Five μ l of this mixture was diluted to 5 ml in absolute ethanol and used as the basal solution. Seven dilutions of the basal dilution ranging from 1:1 to 1:7 was made in ethanol as before. The optical density of the various dilutions was read at 540 nm (absorption peak of Rhodamine-B) in a spectrophotometer and plotted against the volume of spray solution converted to gallons per acre. The conversion was accomplished by taking the original volume of the spray mixture used in O.D. determination (μ l) and the area of the 2 glass slides ($37.5 \times 2 = 75.0 \text{ cm}^2$) and converting the $\mu\text{l}/75 \text{ cm}^2$ to U.S. gallons/acre. The standard curves for plots A, B and the stream are shown in Fig. 6, 7, and Table 16; many cards on plot C were missed by the spray and therefore no data is presented for this plot.

The spray deposit on the two slides from each spray plate was washed with 5 ml of absolute ethanol, filtered through a sintered glass filter and the O.D. of the solution was read at 540 nm. From the appropriate standard curve the GPA

corresponding to the O.D. was read. The average deposit was 0.13 GPA in plot A, 0.15 GPA in the stream and 0.22 GPA in plot B. This was far less than what was anticipated. The details for plot A stream, and plot B are summarized in Tables 17, 18 and 19.

IX. Post-spray Evaluation

The effects of the spraying were assessed by four methods:

(1) Post-spray collections of larvae were reared in the field laboratory to determine whether or not morphogenetic deformities occurred; (2) the population decline was assessed by collecting the pupae using burlap traps; (3) by surveying the adult moths; (4) by collecting eggs oviposited on lichens on trees.

Post-spray larval assessment:

About 100 larvae were collected from each plot (A, B, and C) 1, 5, and 10 days after spraying and reared on untreated foliage in the field laboratory. Morphogenetic deformities were noted as the characteristic hormone-syndrome became apparent (Table 20). Only those larvae that were marginally affected could be collected since those that were more severely affected fell off the trees (Fig. 8). The purpose of this assessment was merely to establish the hormonal effect. The degree of deformities progressively increased indicating a hormonal effect.

Post-spray pupal assessment:

The population decline was assessed by counting

the number of pupae in the burlap traps. Each trap consisted of 3 feet long by 1 foot wide bands of burlap, wrapped around the balsam-fir tree trunks. The larvae crawled down the tree and pupated in the burlap trap (Fig. 9b). This method was originally described by Otvos (1973). Fifteen traps were set in each treatment plot and the airport-control had 10 traps. The pupae were reared and examined for Entomophthora as well as parasites. The results are summarized in Table 21. To determine whether pupation might have occurred predominantly in the tree crowns as a consequence of altered behavior following hormone treatment, five trees from each plot as well as control were felled and the crowns examined for pupae. The results indicate that very few larvae pupated in the crown (Table 22).

Moth Survey

Adult moths were surveyed by two methods: using pherotraps with three virgin females/trap (Fig. 9c) and counting the moths flushed from trees after beating the trunk with an axe. These samplings were carried out from August 17 to September 10. The results are summarized in Table 23 and they agree with the results obtained from burlap traps.

It was observed that many moths in the treated plots, especially plot A, had crumpled wings and remained on tree trunks obviously unable to fly (Fig. 9d). Many of these had defective genitalia.

Egg Survey

Eggs of the hemlock looper in the treatment and control plots were examined by collecting lichen clumps from branches and trunks of balsam fir trees. The eggs were placed in cold storage and were examined after three months for viability. The results are summarized in Table 24.

Moth populations in all sprayed blocks collapsed completely a week after beginning of emergence and at this time adult emergence was at its peak in the control plot.

The results of our post-spray analyses indicate that the desired reduction in population was obtained. Some defoliation was expected in the sprayed blocks and examination of the sample trees showed damage to the new shoots and an aerial survey indicated moderate to severe defoliation throughout the test blocks. As discussed earlier the foliage protection from an operational spray of an insect growth regulator occurs the succeeding year.

X. Studies of Birds, Mammals and Aquatic Fauna
in an Area Treated with Juvenile Hormone
Analogue

Three plots (A, B, and C) were selected for the monitoring of bird and small mammal populations. Resident breeding bird and small mammal populations census plots were established on these plots. A control plot was located well outside of the treatment area. A small stream running through the research area was also sprayed in order to determine possible side effects to aquatic fauna.

Bird populations were recorded on the entire plot area (10 acres for the treatment plots, 20 acres for the control plot) using techniques described by Buckner 1965 and consisted of traversing the plots along lines 132 feet apart and recording all singing males and sighted birds on a plot map. Results are expressed as number of birds (singing males = a breeding pair, + all sighted birds) per 100 acres.

Small mammal populations were monitored on three consecutive nights approximately six weeks after treatment. Standard snap-back traps were set

at 10 yard intervals along a 90 yard line. Five traps set at 1 yard intervals across the centre line provided a total of 50 traps or 150 trap nights. All animals taken were identified, sexed, age determined and examined for the presence of external parasites. Each specimen was dissected and the breeding condition and general health recorded.

Bottom fauna populations in the sprayed stream were monitored by taking series of five square foot Surber samples before and after spraying at two stations. Station 1 was situated in a moderate-flowing riffle area about 100 yards downstream from the small lake which was the stream's source and Station 2 was located in a slightly faster flowing and shallower riffle about 250 yards further downstream. Organisms were picked from the samples taken at Station 1 while still alive whereas samples from Station 2 were preserved whole and picked later in the laboratory. The organisms from each sample were identified to Class or Order and counted. Two Surber samplers set in the current at the foot of riffle areas were used to sample drifting organisms for three days following spraying of the stream. Some incidental observations of fish and frog populations were also made.

Birds

The breeding and foraging territories of some species had started to break down for the season by the time population census was taken. However several species inhabiting various ecological areas were still defending breeding territories. Species inhabiting the upper crown area of the forest and those nesting and foraging in open areas are the most likely to come into direct contact with material applied aerially and were watched very carefully during the actual spray and for several hours thereafter. Those species which inhabited the lower crown and forest floor area do not readily come into direct contact with the spray but could be affected by it through contaminated foods.

Populations of winter wren, American robin, hermit thrush and Swainson's thrush which inhabit the lower crown and forest floor areas remained fairly constant throughout the census period as did those species foraging in the upper crown areas. Decline of populations of the black-throated green warbler on treatment plot C (Table 28) probably does not reflect an impact of the treatment as populations of other resident upper crown feeders were not affected (Tables 25-28). Fox sparrows

and slate-colored juncos inhabiting the fringe and open areas were also unaffected.

Comparison of populations of birds on the control plot (Table 25) and the treatment plots (Table 26, 27, and 28) indicate that the applications of chemical had no impact upon resident breeding birds in the treatment areas.

Small Mammals

Only one species of small mammal indigenous to the island, the deer mouse, Peromyscus maniculatus anticostiensis Moulthrop was trapped. Very low population levels were recorded on all plots and only 7 animals in all were trapped, 2 males and 5 females. No juvenile or sub-adult animals were recovered and no specimens were trapped on the control plot. Dissection of the animals revealed good fat deposits and the general health was also good. None of the females were pregnant and none contained placental scars indicating the recent birth of a litter (Table 29). The low populations encountered no doubt reflect a normal low point in the population cycle rather than a result of the application of the hormone analog.

Aquatic Fauna

Prespray Surber samples showed that Stations 1 and 2 in the sprayed stream differed significantly

in the composition and abundance of their benthic invertebrate population. The small lake just upstream from Station 1 served as a source of plankton, microorganisms and organic debris which supported significantly larger numbers of almost all groups of organisms at Station 1 than were present at Station 2. The input of organic matter into Station 1 was directly responsible for the large populations of current filtering caddisfly larvae (Trichoptera, Fam. Hydropsychidae) and fingernail clams (Mollusca, Fam. Sphaeriidae) at this station. These two groups of organisms strain organic matter from the current by means of silk nets and gills respectively and in so doing reduce the amount of food available to organisms further downstream. Outflow from the lake also carried weak swimming water mites (Arachnida, Order Acari) and scuds (Crustacea, Order Amphipoda) to Station 1 and relatively large populations were present there whereas these two groups were almost completely absent from Station 2.

Bottom fauna populations at both Station 1 and Station 2 showed no significant changes in composition or numbers following spraying (Table 30). The groups showing the greatest differences in abundance before and after spraying were those which showed relatively

large variation in numbers between individual square foot samples as reflected in the standard deviations calculated for each group.

Drift net samples revealed no significant increase in the number of drifting aquatic or terrestrial organisms after spraying (Table 31). The number of threespine sticklebacks, (Gasterosteus aculeatus (L.) and ninespine sticklebacks, Pungitius pungitius (L.) caught in the drift nets increased slightly after spraying, but all the fish caught were alive and healthy when removed from the net except for some captured in the samples of 24-hour or longer duration. Several schools of banded killifish, Fundulus diaphanus (Le Sueur) and some small American eels, Anguilla rostrata (Le Sueur) were observed to have been unaffected by the spray. Frog populations along the banks of the stream also appeared normal after spraying.

The data obtained from monitoring resident breeding bird and small mammal populations and aquatic fauna from a treated stream indicate that under the conditions of application no adverse side effects were suffered by these components of the environment.

XI. Conclusion and Recommendations

This experiment conducted at Anticosti Island is perhaps one of the earliest attempts to test a juvenile hormone analog against a forest insect pest in the field. The mode of action of the hormone analog is not fully understood but it has been suggested that many of the analogs resemble the breakdown product of the natural hormone. Perhaps, at least in part, they block the degradation process of the natural hormone by inhibiting the enzymes involved. The structure-activity relationship is another area which needs classification. The reason why an analog is active in one species and not in another, even some closely related species has not been resolved. Juvenile-hormone research is still in its infancy and many properties have yet to be studied. The future looks promising and perhaps new analogs that are not only more active but also specific will be developed.

Most of the analogs have only carbon-carbon, carbon-hydrogen, and carbon-oxygen bonds. Almost all of them have a terpenoid structure. The simplicity of the structure indicates the ease in which the material can be broken down by micro-

organisms and this has been fully corroborated in degradation studies on ZR-515.

The hormone-analog has to be applied when the insect is in the last larval instar. By this time much of the damage has been done. In addition, the analog tends to prolong the larval stadium, thereby increasing the potential damage. It is apparent that any foliage protection afforded will not be realized until the succeeding year of application.

The toxicological studies done by many groups on ZR-515 have shown that it is perhaps one of the safest materials available for insect control. It has no apparent adverse effects on birds, mammals, fish, and most invertebrates other than insects. Thus, control of insects using hormone analogs would be aesthetically satisfactory in environmental terms.

The success of our small scale experiment warrants further testing in the field. It is important to select early infestations so that the trees can withstand the current year's damage. It is also necessary to select large enough plots so that infiltration from adjacent areas does not mask the results of the experiment. The blocks

should at least have an area of 200 acres. While the use of a helicopter equipped with boom and nozzle gives good coverage, in order to make the system more realistic for later use on large areas, micronair system on fixed wing aircraft with application rates of not more than 1 gallon per acre should be used.

The timing of the spray is an important part of the operation as described earlier. Simple methods to detect the optimum time for spraying should be developed.

Newer analogs that are available should be tested in the lab and greenhouse and made available for field testing.

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References

- Berkoff, C. E. (1969). The Chemistry and Biochemistry of Insect Hormones. Q. Rev. (The Chemical Society) 23: 372-391.
- Buckner, C. H. and Turnock, W. J. (1965). Avian Predation on the Larch Sawfly, Pristiphora erichsonii (Hartig) (Hymenoptera: Tenthredinidae) Ecology 45: 223-236.
- Burdette, W. J. (1974). Invertebrate endocrinology and hormonal heterophyly. Springer-Verlag, New York.
- Doane, W. W. (1973). Role of hormone in insect development. In Developmental Systems: Insects (Edited by Counce, S. J. and Waddington, C. H.), Vol. II, pp. 291-497. Academic Press, New York.
- Gomez, E. D., Faulkner, D. J., Newman, W. A., and Ireland, C. (1973). Juvenile hormone mimics: Effects on Cirriped crustacean metamorphosis. Science 179: 813-814.
- Highnam, K. C. (1970). Mode of action of arthropod steroid and other hormones. Adv. Steroid Biochem. Pharmacol. 1: 1-50.
- Menn, J. J. and Beroza, M. (1972). Insect juvenile hormones - Chemistry and Action, Academic Press, New York.

- Novak, V. J. A. (1965). Insect hormones. Methuen, London.
- Otvos, I. S. (1974). A collecting method for pupae of Lambdina fiscellaria fiscellaria (Guen.) (Lepidoptera: Geometridae). Can. Ent. 106: 329-331.
- Otvos, I. S., Clark, R. C., and Clarke, L. J. (1971). The hemlock looper in Newfoundland: The outbreak 1966 to 1971; and aerial spraying, 1958 and 1969. Newfoundland Forest Research Centre, St. John's, Newfoundland Information Report N-X-68.
- Retnakaran, A. (1973). Hormonal induction of supernumerary instars in the spruce budworm, Choristoneura fumiferana (Lepidoptera: Tortricidae) Can. Ent. 105: 459-461.
- Roller, H. and Dahm, K. H. (1968). The chemistry and biology of juvenile hormone. Rec. Progr. Hormone Res. 24: 651-680.
- Slama, K. (1971). Insect juvenile hormone analogs. A Rev. Biochem. 40: 1079-1102.
- Slama, K., Romanuk, M., and Sorm, F. (1974). Insect hormones and bioanalogues. Springer-Verlag Wien, New York.
- Wigglesworth, V. B. (1970). Insect Hormones. Freeman, San Francisco.

Williams, C. M. (1969). Juvenile hormone insecticides.

Acad. Naz. Lincei, Roma 128: 79-87.

Wyatt, G. R. (1972). Insect Hormone. In Biochemical

action of hormones (Edited by Litwack G.),

Vol. II, pp. 385-490. Academic Press, New

York.

Table 1 Compounds tested for morphogenetic activity against some forest insects.

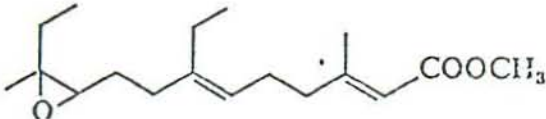

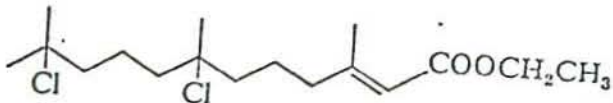
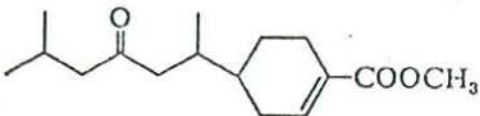
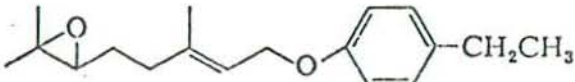
Formula and chemical name	Referred to in text as, and provided by
<p>1.</p>  <p>Methyl 10,11-epoxy-7-ethyl,3,11-dimethyl-2,6-tridecadienoate (eight isomers)</p>	Ayerst compound (Ayerst)
<p>2.</p>  <p>1-methoxy-3,7,11-trimethyl-2-cis/trans, 6 trans,10-dodecatriene</p>	FME (Eco-Control)
<p>3.</p>  <p>Ethyl 7,11-dichloro-3,7,11-trimethyl-2-trans dodecanoate (Ethyl dichloro farnesoate)</p>	Dichloro analog (Eco-Control)
<p>4.</p>  <p>1-methoxycarbonyl-4(1,5-dimethyl-3-oxohexyl)-cyclohex-1-ene</p>	Juvabione (Eco-Control)
<p>5.</p>  <p>(3'-ethylphenoxy)-3,7-dimethyl-6,7-epoxyoct-2-ene (Epoxidized aromatic ether of geraniol)</p>	Stauffer compound (Stauffer)

Table 1 Compounds tested for morphogenetic activity
against some forest insects. (cont'd)

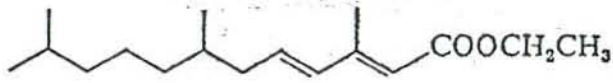

Formula and chemical name	Referred to in text as, and provided by
<p>6.</p>  <p>Ethyl 3,7,11-trimethyldodeca-2,4-dienoate</p>	<p>ZR-512(Zoecon)</p>
<p>7.</p>  <p>Isopropyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate</p>	<p>ZR-515(Zoecon)</p>

Table 2 Effect of ZR-515 on the eastern hemlock looper. Different concentrations of the technical material in acetone was topically applied at the rate of 1 μ l/4th or 5th instar larva. Twenty larvae were used for each treatment.

Stage treated	Treatment	% larval mortality*	% Normal adults	% Deformed
A) 4th instar larvae	Control (1 μ l acetone)	90	5	5**
	5 μ g/ μ l	50	0	50
	10 "	65	5	30
	50 "	85	0	15
	100 "	80	0	20
	200 "	85	0	15
B) 5th instar larvae	Control (1 μ l acetone)	40	50	10**
	5 μ g/ μ l	55	0	45
	10 "	10	0	90
	50 "	45	0	55
	100 "	45	0	55
	200 "	50	0	50

* These larvae died without manifesting any apparent deformities.

** Deformed insects in the control were later shown to be due to accidental exposure to vapours of the analog.

Table 3 "Activity window" during the last larval instar of the eastern hemlock looper to ZR-515. Three different levels of the analog in acetone were applied topically to the last larval instar beginning from day 1 to day 11 of the last larval stadium. The % deformities observed in each treatment of 100 larvae is indicated.

Day of last larval stadium	Control (1 μ l acetone/larva)	Treatment (μ g/ μ l/larva)		
		1	10	100
1	0	12	52	73
2	2	9	70	97
3	0	42	83	99
4	0	23	94	76
5	1	22	72	90
6	2	41	68	95
7	1	55	89	91
8	0	61	87	56
9	0	75	82	95
10	3	62	74	89
11	0	21	31	62

Table 5 Persistence of ZR-515 applied to potted balsam-fir trees.

Treatment (200 µl/trees)	No. sixth instars	Average days to pupation	% Deformed	% Normal Adults
Control	20	9	0	65
0.1%	20	-	75	0
1.0%	20	-	65	0
10.0%	20	-	80	0

The larvae were transferred to the trees 15 days after the trees were sprayed with the hormone analog.

Table 6 Effect of short wavelength U.V.
(240-260 nm) light on ZR-515 (1%)
sprayed on trees.

Treatment	No. sixth instars	Average days to pupation	% Deformed	% Normal Adults
Control	20	10	0	56
ZR-515	20	29	90	0
60 min UV/day	10	9	0	20
ZR-515 + 10 min UV/day	30	-	80	0
ZR-515 + 30 min UV/day	30	-	93	0
ZR-515 + 60 min UV/day	30	-	87	0

Table 7 Effect of a water spray (5 sec. at 11.5 l/min)
on trees treated with ZR-515 (1%).

Treatment	No. sixth instars	Average days to pupation	% Deformed	% Normal Adults
Control	20	10	0	50
ZR-515	20	-	85	0
ZR-515 + Water every 3rd day	20	-	90	0
ZR-515 + Water spray every 2nd day	20	-	87	0
ZR-515 + Water spray every day	20	-	60	0

Table 8 Head-capsule measurements of L. fiscellaria
fiscellaria larvae collected in the field on
Anticosti Island.

	1 Instar	2 Instar	3 Instar	4 Instar
No. measured	79	90	155	576
Range	0.40 - 0.50	0.65 - 0.80	1.0 - 1.35	1.40 - 2.20
Mean width in mm	0.45	0.73	1.20	1.90

Table 9 Fenitrothion residue as indicated by
GLC analysis of benzene-washings of
foliage from plots.

Foliage Samples		Fenitrothion (ppm)
Spray I	Plot A	0.025
	B	Trace
	C	0.015
Spray II	Plot A	0.020
	B	0.033
	C	0.020

Table 10 Pre-spray sample of eastern hemlock
 looper from plots A, B and C. The
 larvae were in the first and second
 instar stages.

PLOT A				PLOT B				PLOT C			
Tree #	Sample		Tot.	Tree #	Sample		Tot.	Tree #	Sample		Tot.
	1	2			1	2			1	2	
1	41	36	77	1	41	54	95	1	83	57	140
2	25	58	83	2	26	9	35	2	48	20	68
3	65	18	83	3	84	62	146	3	16	56	72
4	16	3	19	4	18	11	29	4	11	19	30
5	21	11	33	5	63	43	106	5	11	12	23
6	7	13	20	6	24	22	46	6	0	4	4
7	4	3	7	7	41	25	66	7	3	8	11
8	29	42	71	8	32	20	52	8	1	3	4
9	43	27	70	9	17	11	28	9	7	5	12
10	60	49	109	10	25	18	43	10	5	10	15
$\bar{x} = 28.55$				$\bar{x} = 32.3$				$\bar{x} = 18.95$			

Table 11 Weather conditions in June and July of 1973
at Port Menier, Anticosti Island.

Date	Temp. Max.-Min. (°F)	Rainfall (inches)	Date	Temp. Max.-Min. (°F)	Rainfall (inches)	Date	Temp. Max.-Min. (°F)	Rainfall (inches)
June 1	57 - 44	0.33	June 22	73 - 56	0	July 13	64 - 56	0.04
2	- -	-	23	60 - 52	0.50	14	64 - 55	0.13
3	- -	-	24	66 - 52	0.13	15	66 - 55	0
4	59 - 39	0.02	25	71 - 46	0	16	64 - 54	0.03
5	55 - 31	0	27	73 - 48	0	17	71 - 55	0
6	59 - 32	0	27	73 - 59	0	18	73 - 50	0
7	71 - 50	0.01	28	71 - 62	0.16	19	72 - 59	0
8	70 - 50	0	29	66 - 55	0	20	68 - 58	0.15
9	- -	-	30	78 - 56	0	21	69 - 50	0
10	- -	-	July 1	76 - 62	0	22	70 - 46	0
11	70 - 45	0	2	77 - 62	0.11	23	64 - 43	0.02
12	63 - 32	0	3	75 - 61	0.17	24	69 - 52	0.11
13	62 - 41	0.10	4	75 - 64	0.14	25	70 - 53	0
14	58 - 49	0.40	5	69 - 63	0.85	26	76 - 56	0
15	57 - 42	0.09	6	70 - 61	0.54	27	77 - 57	0
16	52 - 41	0	7	77 - 59	0.09	28	76 - 63	0.40
17	60 - 33	0	8	69 - 56	0.03	29	70 - 67	0.16
18	65 - 36	0	9	67 - 61	0.01	30	70 - 60	0.13
19	65 - 40	0	10	68 - 56	0	31	72 - 60	0
20	74 - 50	0	11	67 - 58	0.09			
21	69 - 59	0.19	12	63 - 54	0.54			

Table 12 Pre-spray beating samples

Sample No.	Number of larvae/tree	
	Plots A,B,C	Control Plot
1	540	132
2	304	288
3	1089	180
4	396	207
5	873	216
6	208	225
7	1224	-
\bar{x}	662	208

Table 13 Larval-population distribution
in the plot area.

Sampling date	Larvae collected	% Insect development (instars)			
		1	2	3	4
July 8	208	3.0	28.0	68.0	1.0
July 12	108	1.3	2.4	85.4	10.9
July 17	59	0	0	45.7	54.3

Table 14 Telethermometer readings for detecting
the onset of inversion on July 19, 1973.

Time	Temperature (°F)		
	Top (T_2)	Bottom (T_1)	Δ ($T_2 - T_1$)
7:00 A.M.	60.2	60.4	-0.2
7:20	62.2	62.0	+0.2
7:40	62.6	63.0	-0.4
8:00	64.2	65.0	-0.8
8:20	62.5	62.5	0

Table 15 Spray time and conditions

Plot	amount sprayed (oz./a.i./acre)		passes	wind (mph)	Sky cover	R.H. (%)
C	0.25	7:05-7:25	10	3	over- cast	97
B	1.00	8:15-8:40	10	2	light fog	100
C	3.00	8:50-9:10	10	0	light fog	100

Table 16 Optical density of progressive dilutions of spray mixtures used for conversion of slide area (75 cm²) to gallons/acre.

Tube No.	Plot	Volume Solution "A" (μl)	O.D.	G.P.A.
blank	-	-	0.000	0.000
1	A	5.000	0.407	1.425
2	A	2.500	0.203	0.713
3	A	1.250	0.102	0.356
4	A	0.625	0.052	0.178
5	A	0.312	0.026	0.089
6	A	0.156	0.008	0.044
7	A	0.078	0.003	0.022
8	A	0.039	-	0.011
blank	-	-	0.000	0.000
1	B	5.000	0.315	1.425
2	B	2.500	0.165	0.713
3	B	1.250	0.089	0.356
4	B	.625	0.052	0.178
5	B	.312	0.031	0.089
6	B	.156	0.015	0.044
7	B	.078	0.009	0.022

Table 17 Spray plate analysis from Plot A.

Spray plate no.	Total volume of wash	Average O.D.	Volume of (μl) Spray on Plate	G.P.A.
A1x	5 ml	-		
A1y	"	0.350	0.351	0.100
A2x	"	.047	.456	0.130
A2y	"	.071	.765	0.218
A3x	"	.060	.614	0.175
A3y	"	.025	.253	0.072
A4x	"	.060	.614	.175
A4y	"	.032	.315	.090
A5x	"	.033	.315	.090
A5y	"	.025	.253	.072
A6x	"	.057	.589	.168
A6y	"	.028	.291	.083
A7x	"	.051	.548	.156
A7y	"	.023	.218	.062
A8x	"	.066	.667	.190
A8y	"	.060	.614	.175
A9x	"	.111	1.134	.323
A9y	"	.033	.315	.090
A10x	"	.024	.246	.070
A10y	"	.031	.312	.089
A11x	"	.023	.245	.070
A11y	"	.018	.179	.051
A12x	"	.022	.200	.057
A12y	"	.018	.179	.051
A13x	"	.019	.179	.051
A13y	"	.007	.084	.024
A14x	"	.129	1.316	.375
A14y	"	.039	.393	.112
A15x	"	.105	1.071	.305
A15y	"	.041	.418	.119
Σ		-	0.4559	0.1296

Table 18 Spray plate analysis from stream

Spray plate no.	Total volume of wash	Average O.D.	Volume (μl) of spray on plate	G.P.A.
LF 166	5 ml	.036	.358	.102
LF 167	"	.026	.316	.090
LF 168	"	.027	.316	.090
LF 1699	"	.040	.404	.115
LF 170	"	.036	.358	.102
LF 171	"	.026	.316	.090
LF 172	"	.025	.249	.071
LF 173	"	.026	.253	.072
LF 174	"	.063	.621	.177
LF 175	"	.045	.456	.130
LF 176	"	.025	.249	.071
LF 177	"	.033	.386	.110
LF 178	"	.086	.871	.248
LF 179	"	.068	.695	.198
LF 180	"	.043	.421	.120
LF 181	"	.063	.621	.177
LF 182	"	.086	.871	.248
LF 183	"	.163	1.997	.569
LF 184	"	.045	.456	.130
LF 185	"	.039	.404	.115
\bar{x}	"	-	0.5309	0.1513

Table 19 Spray plate analysis from Plot B.

Spray plate no.	Total volume of wash	Average O.D.	Volume (μ l) of Spray on plate	G.P.A.
B1X	5 ml	.079	.856	.244
B1y	"	.060	.702	.200
B2x	"	.059	.695	.198
B2y	"	.029	.348	.099
B3x	"	.044	.505	.144
B3y	"	.050	.579	.165
B4x	"	.096	1.106	.315
B4y	"	.074	.856	.244
B5x	"	.059	.695	.198
B5y	"	.081	.926	.264
B6x	"	.089	1.046	.298
B6y	"	.137	1.924	.548
B7x	"	.049	.576	.164
B7y	"	.088	1.043	.297
B8x	"	.052	.590	.168
B8y	"	.059	.695	.198
B9x	"	.079	.913	.260
B9y	"	.093	1.060	.302
B10x	"	.047	.562	.160
B10y	"	.058	.692	.197
B11x	"	.053	.597	.170
B11y	"	.060	.702	.200
B12x	"	.039	.460	.131
B12y	"	.044	.516	.147
B13x	"	.034	.404	.115
B13y	"	.031	.358	.102
B14x	"	.049	.576	.164
B14y	"	.078	.849	.242
B15x	"	.089	1.046	.298
B15y	"	.088	1.043	.297
\bar{x}	"	-	0.7640	0.2176

Table 20 Post-spray larval assessment
Deformities in laboratory reared
larvae taken from sprayed plots.

Days after spray	No. of larvae collected	Plot	% Deformities
1	92	A	16.3
	80	B	12.5
	90	C	8.89
5	100	A	13.0
	100	B	14.0
	100	C	10.0
10	102	A	28.43
	24	B	29.17
	92	C	11.96


Table 21 Post-spray pupal sampling

Plot	Pupae/burlap trap	Entomo- phthora	Parasitized	Total healthy pupae	Healthy pupae/ tree	
Control (Airport)	1984/10	226	139	1619	161.9	1
A (3 oz/2 gal/acre)	86/15	10	4	72	4.8	
B (1 oz/2 gal/acre)	156/15	18	3	135	9.0	
C (0.25 oz/2 gal/acre)	593/15	72	30	491	32.7	2

Table 22 Pupal population in cut down trees.

Plot	Trees cut	Pupae	Entomo- phthora	Parasitized	Healthy	Healthy/ tree
Control	5	94	21	28	45	9.0
A	5	54	2	9	43	8.6
B	5	23	1	0	22	4.4
C	5	27	1	2	24	4.8

Table 23 Moths found in trapping and beating.



Plot	Moths found	
	in traps (per trap - \bar{x} of 5)	in beatings (no. per tree - \bar{x} of 25)
Control	36.4	4.80
A	5.4	0.55
B	4.6	0.65
C	4.2	0.85

Table 24 Egg population survey

Plots	Total Eggs/Tree	Viable	Non-viable	% Viable
Control	22.9	10.6	12.3	46.3
A	9.7	1.4	8.3	14.4
B	9.4	2.2	7.2	23.4
C	11.7	1.1	10.6	9.4

Table 25

Small forest bird populations on control plot

Anticosti Island, Quebec

July 16-24, 1973

Family	Species	Pre-spray				Ave. No. of Birds Per Day	Post-spray						Ave. No. of Birds Per Day
		Day					Day						
		-3	-2	-1	0		0	+1	+2	+3	+4	+5	
CORVIDAE	Canada Jay	0	0	0	0	0.0	0	10	5	0	0	0	2.5
	Common Raven	10	0	0	0	2.5	0	0	0	0	0	0	0.0
PARIDAE	Boreal Chickadee	10	10	10	15	11.3	15	30	10	0	0	40	15.8
	Black-capped chickadee	20	0	0	0	5.0	0	0	0	0	0	0	0.0
TROGLODYTIDAE	Winter Wren	0	5	0	15	5.0	15	30	30	45	0	20	23.3
TURDIDAE	American Robin	15	20	20	20	18.8	20	20	20	15	15	10	16.6
	Hermit Thrush	25	0	0	5	7.5	5	20	25	25	10	15	16.6
	Swainson's Thrush	10	50	25	25	27.5	25	35	30	15	20	10	22.5
PARULIDAE	Bay-breasted Warbler	0	10	10	0	5.0	0	30	30	20	10	20	18.3
	Black-throated Green Warbler	15	20	0	10	11.3	10	10	20	20	0	20	13.3
	Black-poll Warbler	40	100	30	40	52.5	40	50	60	30	30	50	43.3
	Magnolia Warbler	10	10	0	0	5.0	0	0	0	0	0	0	0.0
	Tennessee Warbler	0	0	0	0	0.0	0	0	10	10	10	10	6.6
	Myrtle Warbler	0	10	0	0	2.5	0	0	0	0	0	0	0.0
	Wilson's Warbler	10	0	10	20	10.0	20	0	0	0	0	0	3.3
FRINGILLIDAE	American Goldfinch	0	0	0	10	2.5	10	0	0	0	0	0	1.6
	Fox Sparrow	75	40	50	50	53.8	50	30	50	30	40	30	38.3
	Purple Finch	5	0	0	10	3.8	10	10	0	10	5	0	5.8
	Slate-colored Junco	0	0	0	35	8.8	35	50	40	25	40	10	33.3
	White-throated Sparrow	20	50	40	60	42.5	60	20	25	60	35	40	40.0
	Swamp Sparrow	0	0	0	0	0.0	0	10	0	0	0	0	1.6
	White-winged Crossbill	0	0	0	0	0.0	0	0	0	0	0	0	1.6
TOTALS		265	325	195	315	24.6	315	355	355	305	215	285	24.3

Table 26

Small forest bird populations, on treatment Plot A

Anticosti Island, Quebec

July 16-24, 1973.

Family	Species	Pre Spray				Ave. No. of Birds Per Day	Post Spray					Ave. No. of Birds Per Day
		Day					Day					
		-3	-2	-1	0		0	+1	+2	+3	+5	
HIRUNDINIDAE	Tree Swallow	0	0	0	10	2.5	0	10	0	0	0	2.0
CORVIDAE	Canada Jay	0	0	20	30	12.5	0	0	20	20	10	10.0
PARIDAE	Boreal Chickadee	0	20	0	0	5.0	20	0	20	0	0	8.0
TROGLODYTIDAE	Winter Wren	0	0	0	20	5.0	20	20	0	20	0	12.0
TURDIDAE	American Robin	60	10	20	30	30.0	0	20	30	30	20	20.0
	Hermit Thrush	0	0	30	50	20.0	80	60	40	40	70	58.0
	Swainson's Thrush	0	120	70	70	65.0	50	50	40	10	0	30.0
PARULIDAE	Bay-breasted Warbler	0	0	40	0	10.0	0	20	0	20	20	12.0
	Black and White Warbler	0	0	0	20	5.0	0	0	0	0	0	0.0
	Black-throated Green Warbler	30	80	80	20	52.5	80	40	40	60	10	46.0
	Black-poll Warbler	60	0	0	0	15.0	0	0	0	0	0	0.0
	Wilson's Warbler	40	0	0	0	10.0	0	0	0	0	0	0.0
	Yellowthroat	20	0	0	0	5.0	0	0	0	0	0	0.0
FRINGILLIDAE	Evening Grosbeak	0	0	20	0	5.0	0	0	0	10	0	2.0
	Fox Sparrow	0	0	20	40	15.0	40	20	40	20	0	24.0
	Purple Finch	0	0	0	20	5.0	10	0	10	0	0	4.0
	Slate-colored Junco	0	60	0	40	25.0	40	10	10	10	30	20.0
	White-throated Sparrow	20	40	30	60	37.5	0	40	60	10	20	26.0
	TOTALS	230	330	330	410	41.0	330	300	300	260	180	32.6

Table 27

Small forest bird populations on treatment Plot B

Anticosti Island, Quebec

July 18-24, 1973

Family	Species	Pre Spray		Ave. No. of Birds Per Day	Post Spray					Ave. No. of Birds Per Day
		Day			Day					
		-1	0		0	+1	+2	+3	+5	
CORVIDAE	Canada Jay	20	10	15.0	0	0	0	0	0	0.0
PARIDAE	Boreal Chickadee	20	0	10.0	20	30	20	40	20	26.5
TROGLODYTIDAE	Winter Wren	0	20	10.0	20	40	60	20	0	28.0
TURDIDAE	Hermit Thrush	40	20	30.0	20	100	20	40	40	44.0
	Swainson's Thrush	70	70	70.0	80	60	40	80	40	60.0
VIREONIDAE	Warbling Vireo	80	0	40.0	0	0	0	0	10	2.0
PARULIDAE	Bay-breasted Warbler	20	0	10.0	0	0	0	0	0	0.0
	Black-throated Green Warbler	20	20	20.0	40	0	20	20	20	20.0
	Wilson's Warbler	20	0	10.0	0	0	0	0	0	0.0
	Magnolia Warbler	0	0	0.0	0	0	0	20	0	4.0
	Black-poll Warbler	0	0	0.0	40	0	0	0	0	8.0
	Myrtle Warbler	0	0	0.0	0	0	0	0	20	4.0
	Nashville Warbler	0	0	0.0	20	0	0	0	0	4.0
	Yellowthroat	0	0	0.0	20	0	0	0	0	4.0
FRINGILLIDAE	Fox Sparrow	0	20	10.0	20	40	40	20	40	32.0
	Slate-colored Junco	20	20	20.0	20	0	60	20	20	24.0
	White-throated Sparrow	20	40	30.0	60	60	40	20	20	40.0
	Evening Grosbeak	0	0	0.0	0	0	10	0	0	2.0
	Purple Finch	0	0	0.0	0	20	0	0	0	4.0
	TOTALS	330	220	30.3	360	350	310	280	230	34.7

Table 28

Small forest bird populations on treatment Plot C

Anticosti Island, Quebec

July 18-24, 1973

Family	Species	Pre Spray		Ave. No. of Birds Per Day	Post Spray					Ave. No. of Birds Per Day
		Day			Day					
		-1	0		0	+1	+2	+3	+5	
TETRAONIDAE	Ruffed Grouse	0	0	0.0	0	0	20	0	0	4.0
PICIDAE	Yellow-bellied Sapsucker	0	20	10.0	20	0	20	0	0	8.0
CORVIDAE	Canada Jay	0	0	0.0	0	0	0	40	0	8.0
PARIDAE	Boreal Chickadee	0	30	15.0	0	0	20	0	40	12.0
TURDIDAE	American Robin	20	20	20.0	0	20	20	30	0	14.0
	Hermit Thrush	30	0	15.0	20	40	80	40	0	36.0
	Swainson's Thrush	30	60	45.0	20	60	20	20	60	36.0
VIREONIDAE	Warbling Vireo	0	0	0.0	0	60	10	20	0	18.0
PARULIDAE	Bay-breasted Warbler	0	0	0.0	20	20	20	20	0	16.0
	Tennessee Warbler	10	0	5.0	20	0,0	40	60	0	24.0
	Black-throated Green Warbler	120	40	80.0	80	20	20	0	0	24.0
	Black-poll Warbler	0	20	10.0	0	0	0	0	0	0.0
	Nashville Warbler	40	0	20.0	0	0	0	0	0	0.0
FRINGILLIDAE C	Fox Sparrow	20	60	40.0	30	20	40	0	0	18.0
	Purple Finch	0	20	10.0	20	0	40	20	0	16.0
	Slate-colored Junco	40	20	30.0	0	20	0	0	0	4.0
	White-throated Sparrow	100	80	90.0	140	80	20	70	40	62.0
	White-crowned Sparrow.	0	0	00.0	0	0	0	20	0	4.0
TOTALS		410	370	41.3	370	340	370	340	140	37.6

Table 29

Small mammal populations on treatment and control plots

Anticosti Island, Quebec

September 19-21, 1973

PLOT NO.	MALES				FEMALES							
	Juv.	Sub Adults	Adults	Total	Juv.	Sub Adults	Adults				Total	Total Animals
							Pregnant	Pregnant with Placental Scars	Placental Scars Only	Not Pregnant		
Control	0	0	0	0	0	0	0	0	0	0	0	0
Treatment Plot A	0	0	2	2	0	0	0	0	0	3	3	5
Treatment Plot B	0	0	0	0	0	0	0	0	0	1	1	1
Treatment Plot C	0	0	0	0	0	0	0	0	0	1	1	1
TOTALS	0	0	2	2	0	0	0	0	0	5	5	7

Table 30

Mean numbers and standard deviations of organisms/sq. ft. collected by Surber sampler in a small stream before and after exposure to an aerially applied juvenile hormone analogue spray.

Anticosti Island, Quebec

July 17-24, 1973.

	STATION 1		STATION 2	
	Pre Spray	Post Spray	Pre Spray	Post Spray
Trichoptera	59.2 ± 40.5	116.2 ± 96.1	9.8 ± 4.2	9.0 ± 3.4
Ephemeroptera	70.4 ± 30.0	46.4 ± 32.2	29.0 ± 9.0	33.8 ± 17.3
Coleoptera	26.2 ± 12.7	73.4 ± 49.2	29.8 ± 16.9	17.4 ± 4.4
Diptera	36.2 ± 20.5	19.6 ± 7.8	23.0 ± 7.7	18.8 ± 16.8
Turbellaria	2.2 ± 1.8	0.8 ± 1.0	0.8 ± 0.5	1.2 ± 0.8
Oligochaeta	3.0 ± 0.9	2.6 ± 1.7	1.0 ± 0.8	0.2 ± 0.4
Hirudinea	2.8 ± 1.8	5.2 ± 3.2	1.8 ± 2.5	1.6 ± 1.9
Amphipoda	23.4 ± 11.9	27.6 ± 27.5	1.8 ± 1.3	2.4 ± 1.9
Hydracarina	7.6 ± 4.1	11.6 ± 5.5	0.5 ± 0.9	—
Mollusca	331.8 ± 275.0	208.6 ± 57.4	94.0 ± 70.1	70.0 ± 44.5
Total	562.8 ± 307.2	512.0 ± 137.4	191.2 ± 82.9	154.4 ± 55.7

Table 31

No. of organisms / hr. collected in drift nets before and after spraying of a small stream with juvenile hormone analogue.

Anticosti Island, Quebec
July 19-22, 1973.

	Drift Net 1							Drift Net 2				
Hours before or after spraying sampling period begun	-2	-1	0	+1	+2	+10	+34	-2	-1	0	+1	+2
Duration of sampling period (hrs.)	1	1	1	1	4	24	37	1	1	1	1	4
Trichoptera	2	4	2	3	2.0	1.4	0.9	4	4	4	1	1.0
Ephemeroptera	13	9	5	5	9.0	1.9	0.9	14	16	18	9	10.8
Coleoptera	-	1	2	-	0.2	0.3	0.2	2	-	1	1	-
Diptera	4	1	-	1	1.5	2.5	3.2	4	3	2	3	2.5
Amphipoda	-	-	-	-	0.2	1.3	3.6	-	-	1	-	-
Pisces	3	2	1	10	9.8	6.5	2.0	-	1	5	1	-
Other aquatic organisms	-	1	1	-	0.2	0.4	0.1	1	-	-	1	-
Terrestrial organisms *	9	25	38	28	38.5	6.1	8.3	14	16	34	16	9.2
Total	31	43	49	47	61.4	20.4	19.2	39	40	65	32	23.5

* Primarily adult Ephemeroptera and Diptera

Fig. 8. The effect of ZR-515 on eastern hemlock looper larvae. Various degrees of morphogenetic deformities are shown. These deformed, larval-pupal mosaics were collected from plot A.

Fig. 9. Stages in the life history of the eastern hemlock looper (collected in the field).

- a) Fourth instar larvae feeding on balsam fir needles. The spray operation was carried out when the larvae were at this stage of development.
- b) Pupal population was estimated by collecting pupae in burlap traps. The trap is exposed to show some of the pupae.
- c) Sampling of adult males was done with pheromone traps. Each trap had three virgin females as a source for the sex attractant.
- d) Many adult moths emerged with crippled wings, a delayed effect of the hormone analog. The moth in the middle is normal.

Fig. 10. Topographic situations encountered in the field operation.

- a) An aerial view of the location of the treatment plots between the three lakes, the largest being Lac Larouche. The logging road runs between the plots.
- b) An aerial view near the Vaureal River to show balsam trees dead as a result of hemlock looper infestations over a few years.
- c) A stream at the southern end of the treatment plots that was sprayed with the hormone analog to study the effects on fresh water fauna.
- d) Tree tops in the treatment area newly defoliated by the hemlock looper.

Fig. 11. Aspects of operational procedures and bases used during the field experiment.

- a) Some of the early sampling was done with pole clippers equipped with collection baskets.
- b) The spray deposit was monitored using spray plates. Each plate has a kromekote card on one side to show the droplet size and spatial distribution. The other side had two glass slide washings of which were spectrophotometrically analysed for spray deposit.
- c) The base camp and field laboratory were located at Port-Menier.
- d) The spraying was done using a Bell G-5 helicopter.

FIG. 1 EASTERN HEMLOCK LOOPER INFESTATION AREA
SELECTED FOR SPRAYING ZR-515

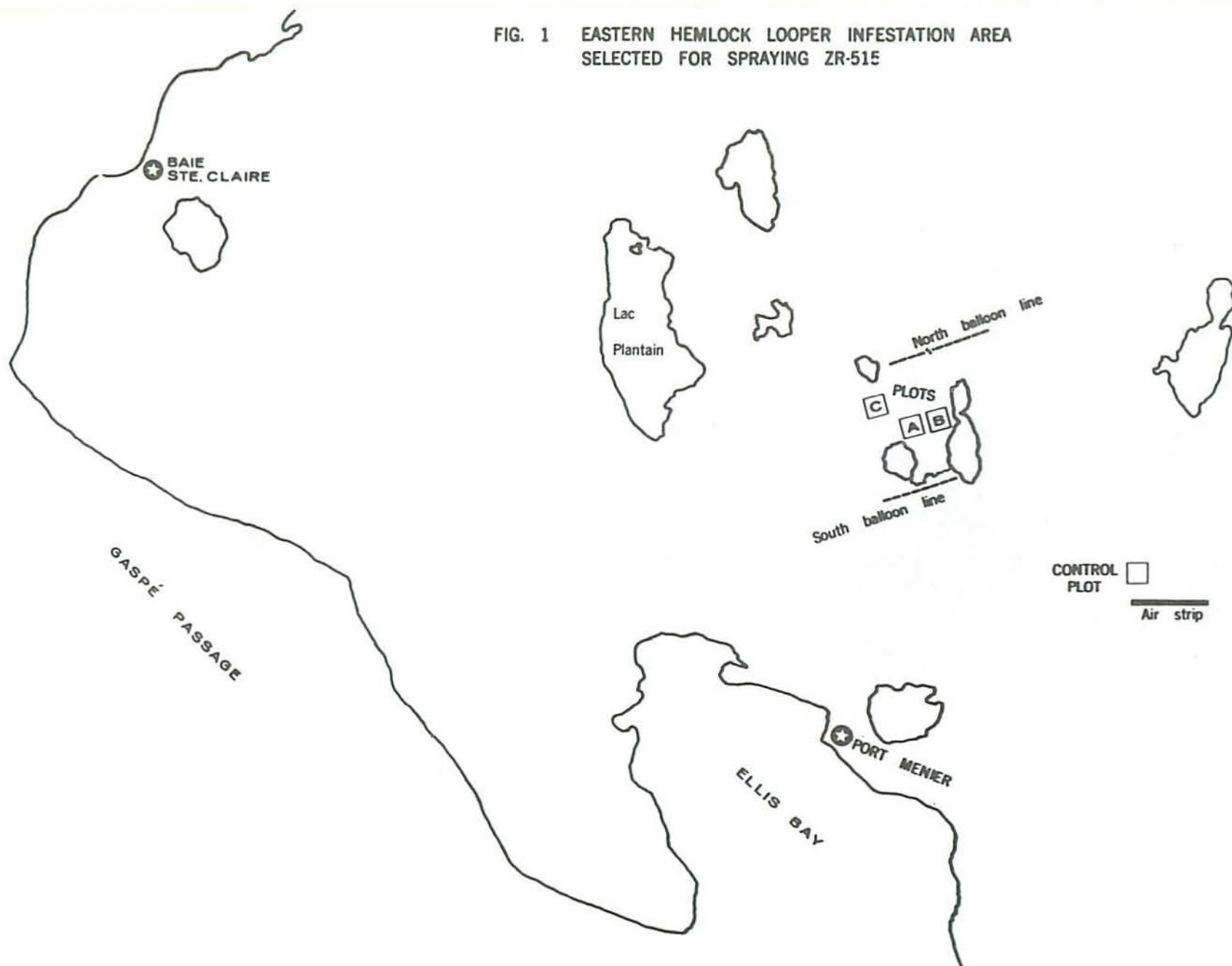


FIG. 2 STAGES IN THE LIFE HISTORY OF THE EASTERN HEMLOCK
LOOPER, *Lambdina fiscellaria fiscellaria*

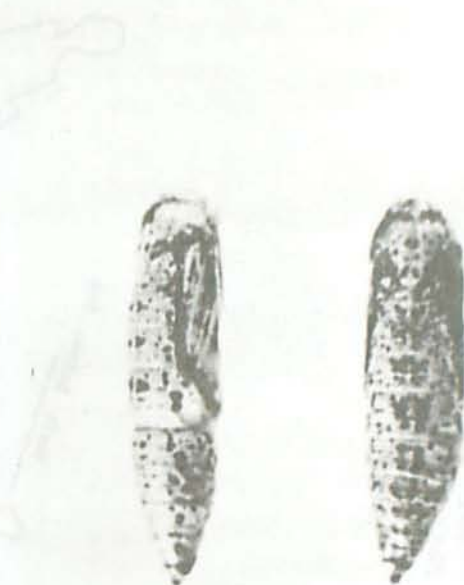
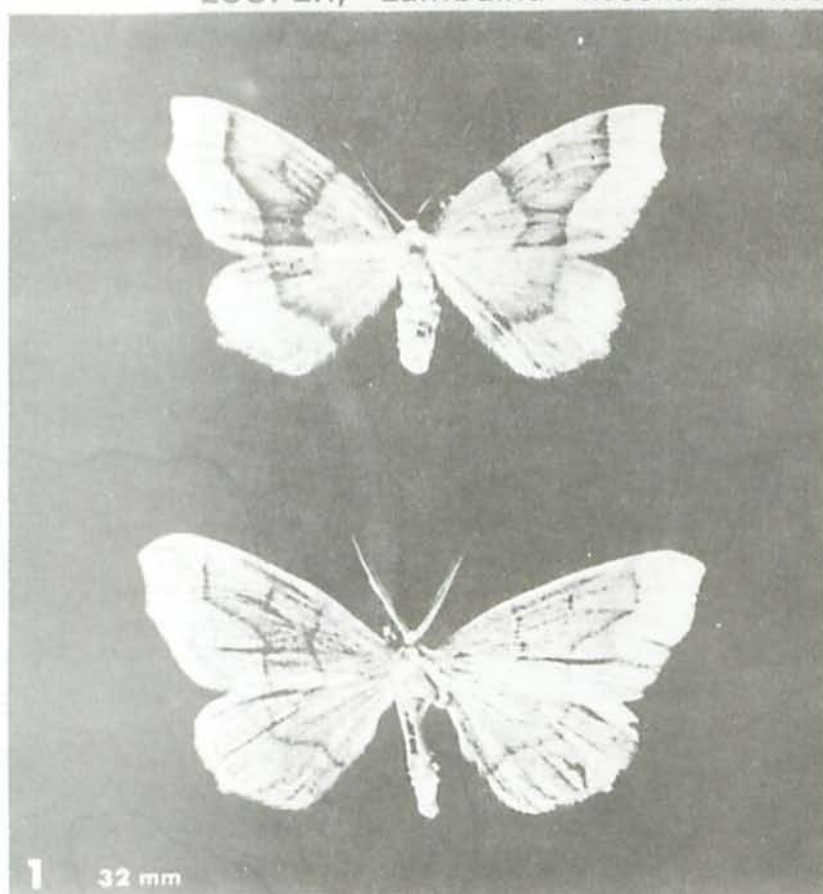


FIG. 3 LARVAL POPULATION OF THE EASTERN HEMLOCK LOOPER DURING THE SUMMER OF 1972

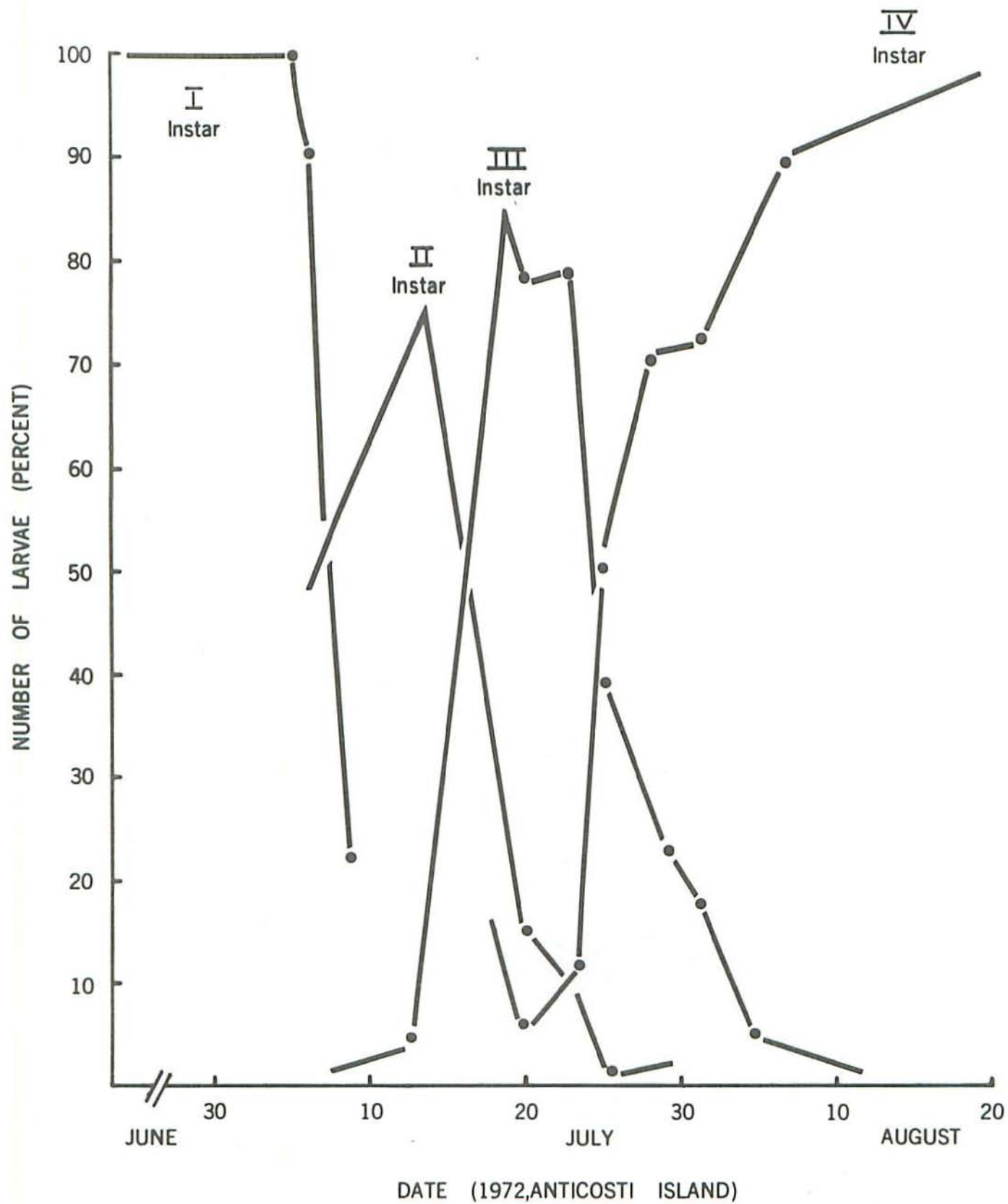


FIG. 4 LAYOUT OF PLOTS A,B, AND C AT ANTICOSTI ISLAND

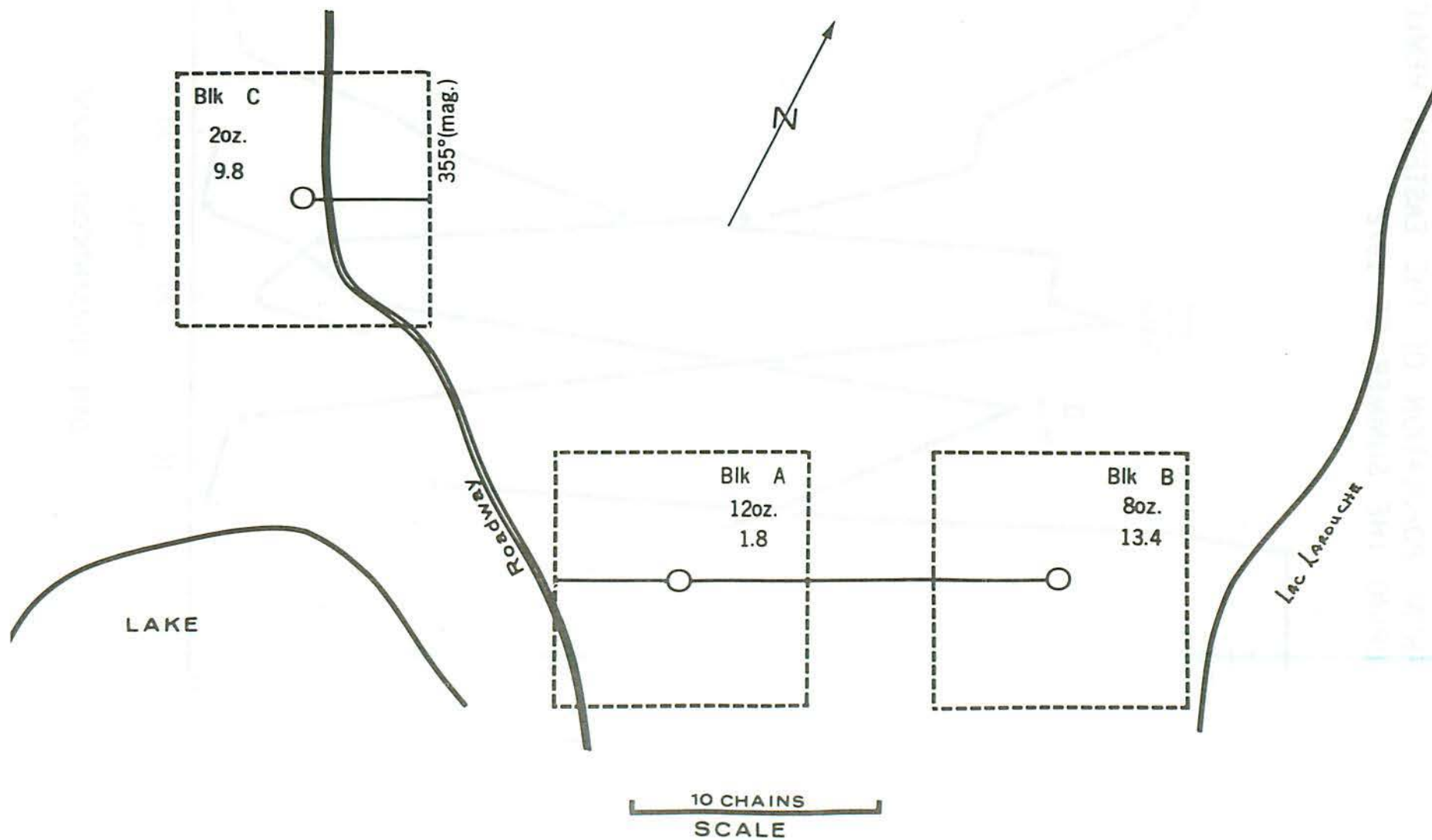


FIG. 5 DETAILS OF PLOT MARKING

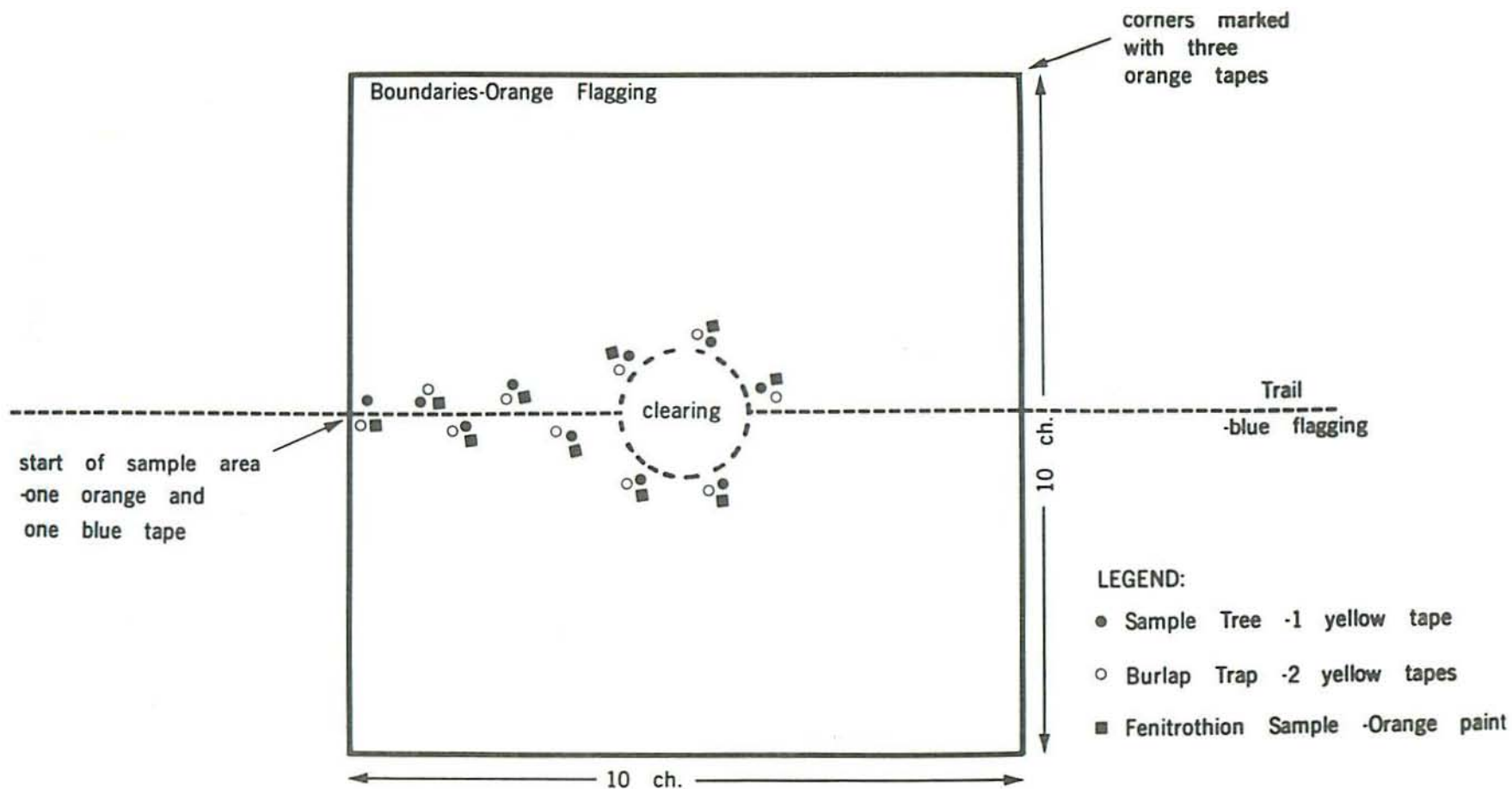


FIG. 6 STANDARD CURVE FOR PLOT A AND STREAM
(ZR-515; 3 OZ./2 GAL./ACRE)

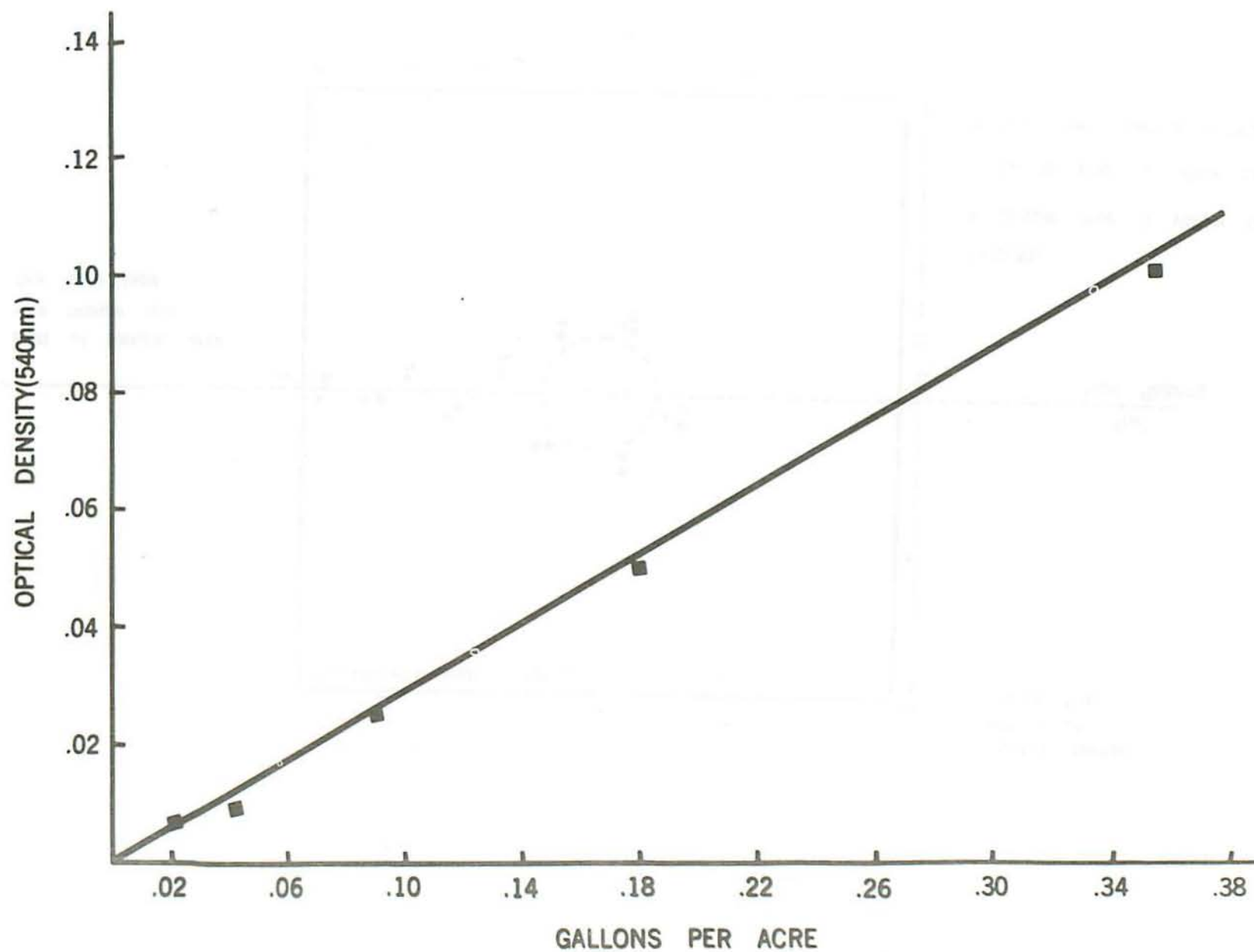
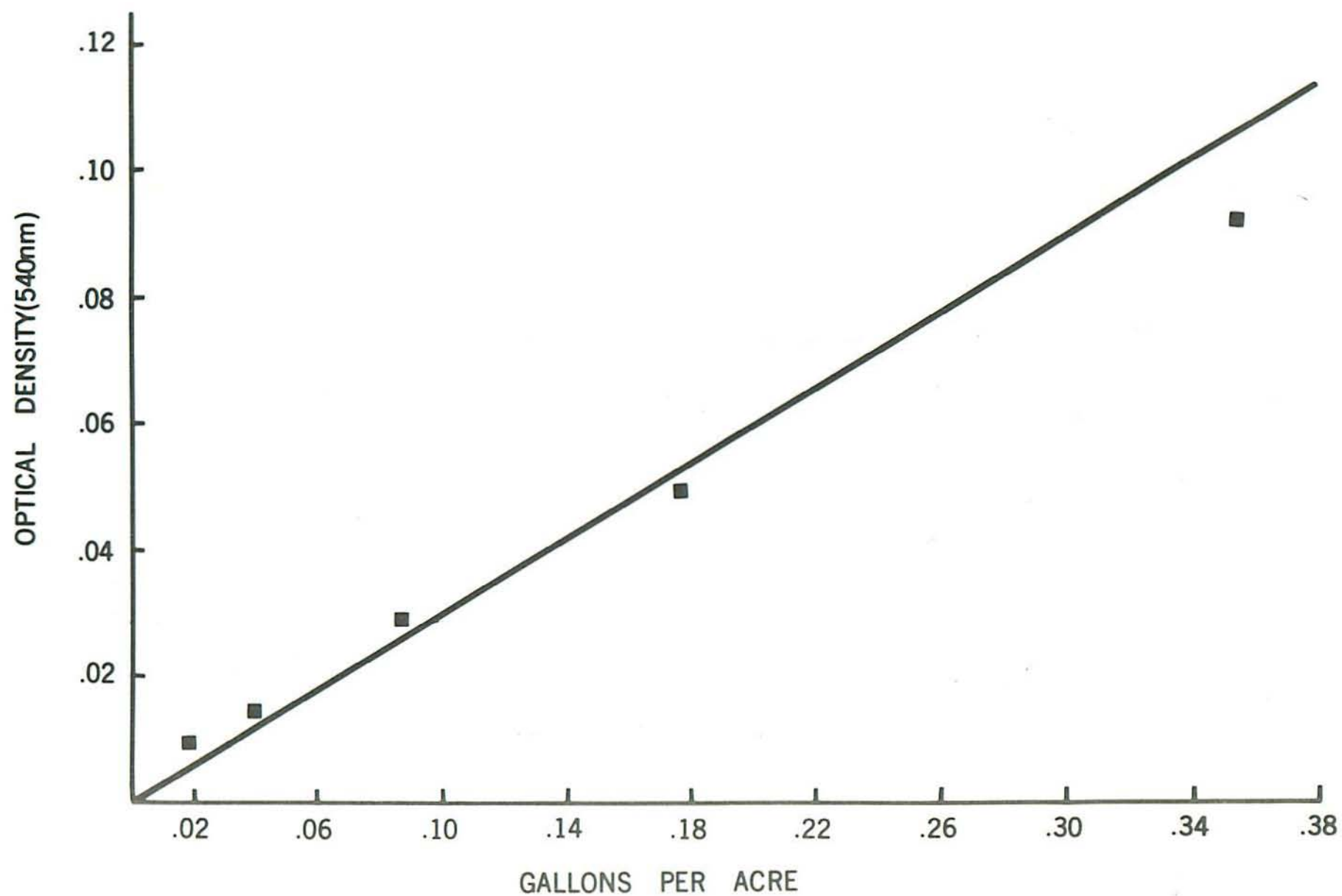


FIG. 7 STANDARD CURVE FOR PLOT B (ZR-515; 1 OZ./2 GAL./ACRE)



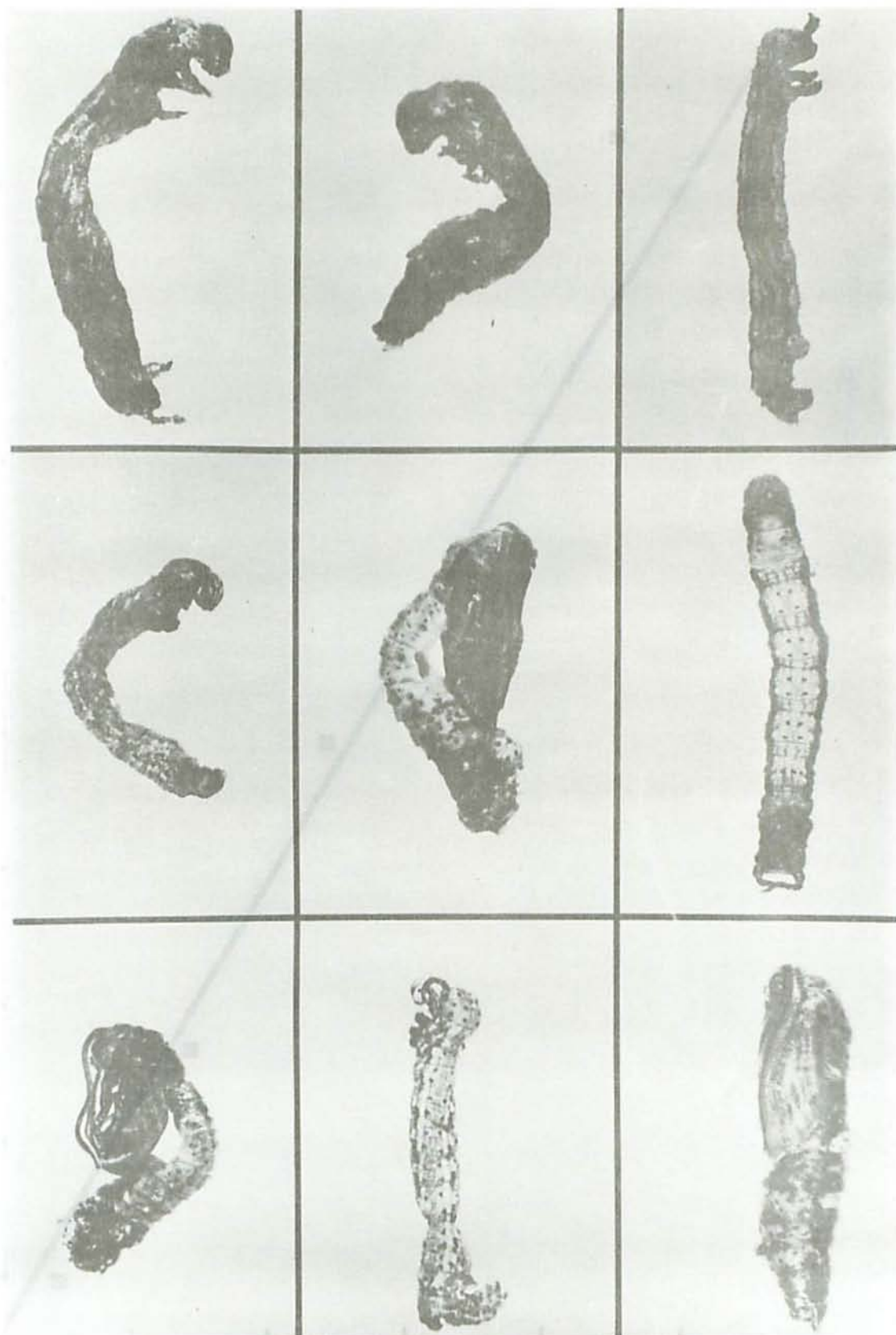


Fig. 8. The effect of ZR-515 on eastern hemlock looper larvae. Various degrees of morphogenetic deformities are shown. These deformed larval-pupal mosaics were collected from plot A.

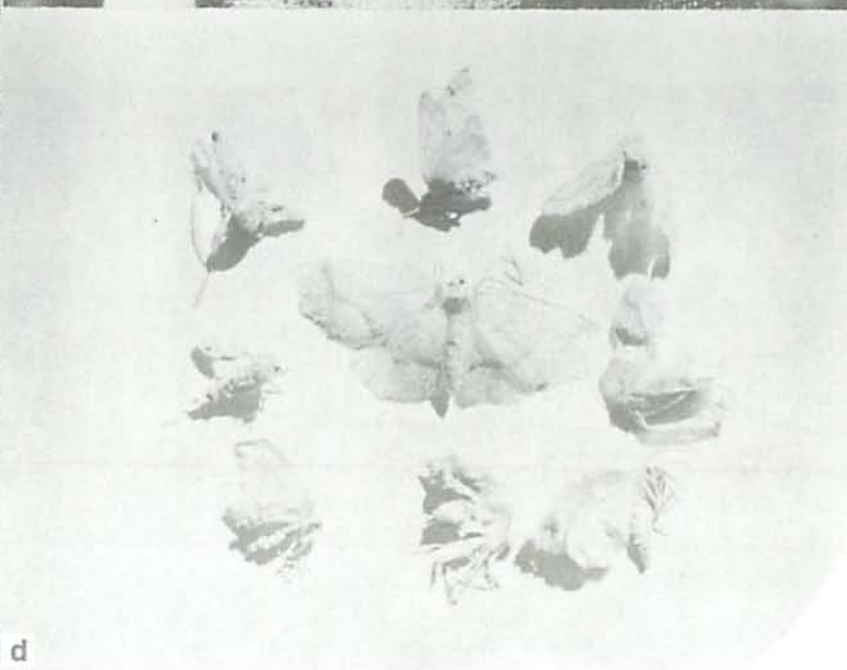
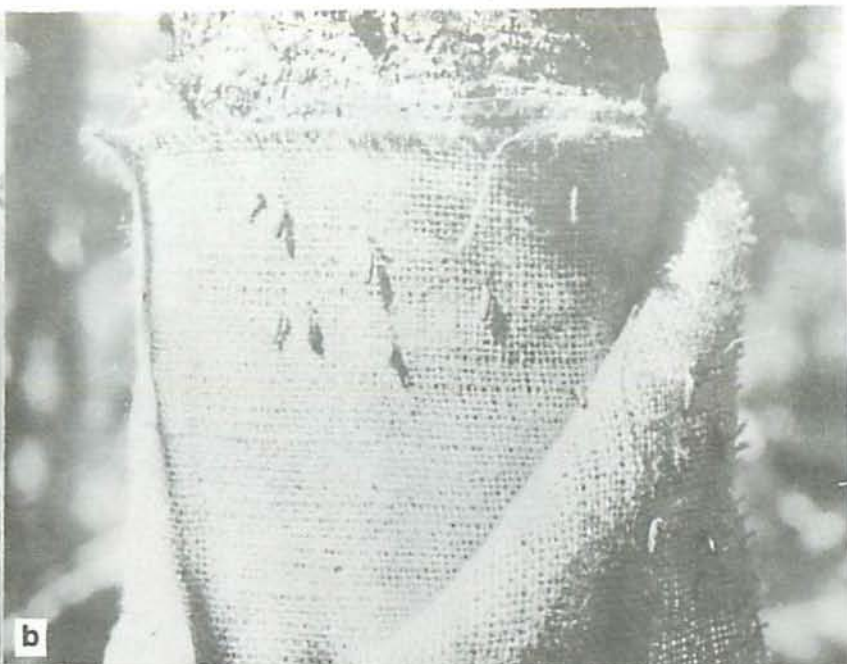
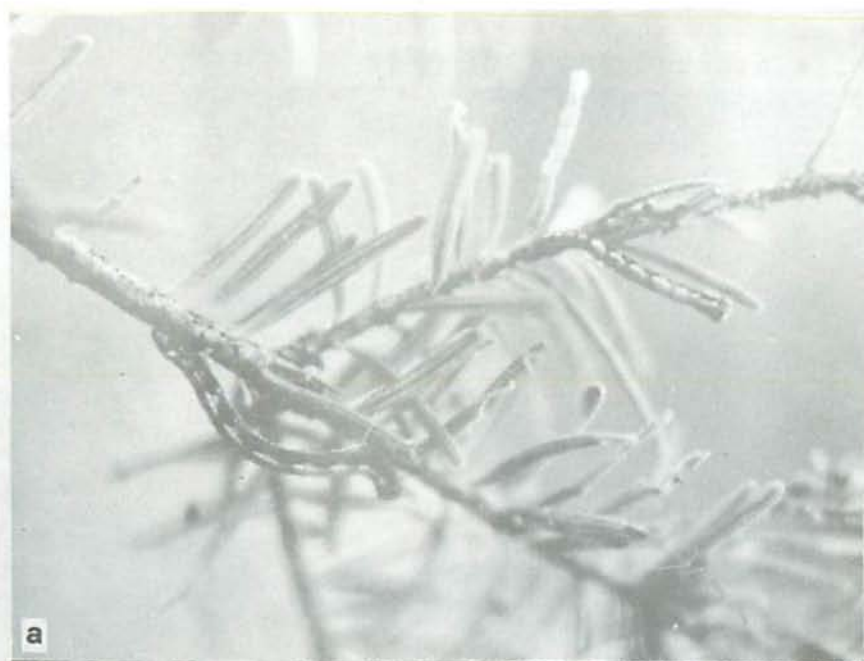


Fig. 9. Stages in the life history of the Eastern hemlock
looper collected in the field.

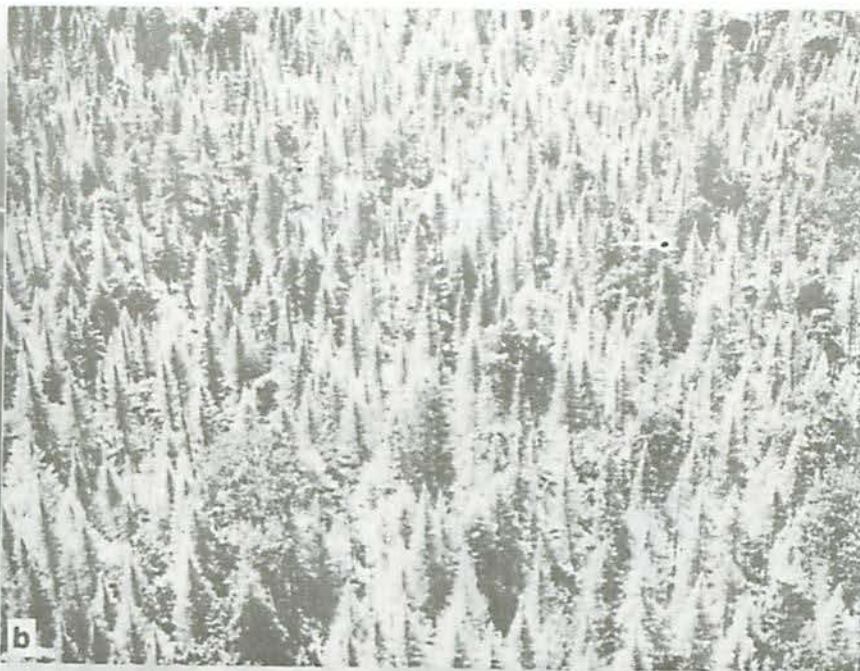


Fig. 10. Topographic situations encountered in the field operation.



Fig. 11. Aspects of operational procedures and bases used during the field experiment.