THE EFFECT OF COLD TEMPERATURES ON THE LENGTH OF DIAPAUSE OF THE SPRUCE BUDWORM (*CHORISTONEURA FUMIFERANA* (CLEM.))

by

DAVID GENE FELLIN

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1960

APPROVED:

[redacted]

Associate Professor of Entomology

In Charge of Major

[redacted]

Chairman of Department of Entomology

[redacted]

Chairman of School Graduate Committee

[redacted]

Dean of Graduate School

Date thesis is presented May 5, 1960

Typed by Clara Homyer

ACKNOWLEDGMENTS

This study was made possible by the Intermountain Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, Ogden, Utah, and was conducted under Line Project FS-2-17-1-INT. Assistance during the planning, organization, and execution of the study came from both Forest Service personnel and faculty members of Oregon State College. I would like to express my appreciation particularly to:

Dr. Paul O. Ritcher, and Dr. Julius A. Rudinsky, Head and Associate Professor, respectively, Department of Entomology, Oregon State College, for criticism and suggestions in planning and for permission to use this study for a thesis.

Dr. Lyle D. Calvin, Professor of Statistics, and Statistician, Agricultural Experiment Station, Oregon State College, for assistance and criticism in statistical procedures.

Personnel of the Intermountain Forest and Range Experiment Station, namely:

Mr. Reed W. Bailey, Director, for making available Station facilities, equipment and material.

Mr. Donald E. Parker, Chief, Division of

Forest Insect Research, for helpful suggestions during the preparation of a plan of work.

Mr. Philip C. Johnson and Mr. Tom T. Terrell, Leader and Entomologist, respectively, Missoula (Montana) Forest Insect Laboratory, for help during all phases of the study, particularly in planning and organization, and preparing the photographs.

Mr. Robert E. Denton, Entomologist, Missoula Forest Insect Laboratory, for reviewing the manuscript.

Mr. Jack Parsell, District Forest Ranger (now retired) Sula Ranger District, Bitterroot National Forest, Hamilton, Montana, for providing lodging and meal accommodations for field crews and for reserving a suitable area for a field laboratory.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

THE EFFECT OF COLD TEMPERATURES ON THE LENGTH OF DIAPAUSE OF THE SPRUCE BUDWORM (*CHORISTONEURA FUMIFERANA* (CLEM.))

INTRODUCTION

A current outbreak of the spruce budworm, *Choristoneura fumiferana* (Clem.), which covered 406,000 acres when first reported in central Montana in 1948, has subsequently covered approximately 6 million acres of timberland in western Montana, northern Idaho, and Yellowstone National Park, Wyoming. More than 2 million acres of budworm-infested Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) (Franco)) were treated by aerial application of DDT insecticide to control this native insect during the years 1952, 1953, 1955, 1956, 1957, 1958, and 1959. It is still epidemic on 3.5 million acres in western Montana.

During the years of budworm control in western Montana between 1952 and 1959, plans were made in the fall for control operations to be carried out the following summer. In planning control projects several factors are considered, any one of which can affect the size and location of units tentatively proposed for control. Before these factors are considered, however, units supporting the greatest budworm populations usually are given the highest priority for control consideration.

It would be desirable, therefore, in control planning, if information on budworm population densities, which is not available until April or May, would be available in late fall or early winter. That it is not always easy to make an accurate estimate of spruce budworm populations is attested by Dowden and Carolin (16, p.775) who state that, "Probably no research problem dealing with the spruce budworm is more difficult than that of determining the number of budworms present in an area."

EVENTS LEADING TO THE PROBLEM

Each year Intermountain Forest and Range Experiment Station crews conduct ground surveys to record current year's defoliation to be compared with that of the previous year (27, p.1). This type of survey, based on host tree damage, serves as an index of budworm population trends from year to year. This index is not, however, a definite indication of future populations.

Since these damage surveys are made in August and September of each year, they measure defoliation that occurred during the preceding May, June and July. This defoliation--the basis of the budworm population index--is the result of the feeding of a generation of insects no longer existent.

While damage surveys are being made, the budworm

population which will begin feeding on foliage the following summer is lying dormant as inactive second stage larvae within tiny hibernacula concealed beneath bark flakes on host trees. Terrell (34, p.1) states, "The lack of a direct relationship between needle damage and the inactive budworm population leaves estimates of the following year's population drawn from summer survey data somewhat questionable; and because applied control will be directed against this emerging brood, positive information regarding its abundance is important."

During the 8 to 10 months following damage surveys, budworm larvae are subjected to daily fluctuations of air temperatures. Overwintering larvae nearly always endure sustained periods of near- or below-freezing air temperatures during the winter months. It is known that at this time certain physiological changes take place within the insect which will assure its continued development when temperatures moderate in the spring. Although overwintering second stage larvae are often referred to as "hibernating larvae" or "larvae in hibernation", Harvey (19, p.7) shows that second stage spruce budworm larvae undergoing these physiological changes are in a state of true diapause as distinguished from a hibernation or quiescence. He is supported by recent definitions of a true diapause summarized by

Prebble (32, p.296) as follows:

> "The term 'diapause' coined by Wheeler to designate a temporary immobility between the dorsal migration of the embryo and its return to the ventral surface of the egg, and extended by Henneguy to include resting periods during any stage in the life cycle, is by Shelford considered to be synonymous with true dormancy, and is defined as '... a condition in which no further activity or progress can be induced until certain physiological changes of a physico-chemical character have taken place.'"

When larvae emerge from their hibernacula in spring, they travel to branch tips and begin feeding on new foliage or they mine old needles or new buds until new foliage appears. Larvae feed on current foliage during June and July and pupate among needles of the host tree. Emerging moths mate and females begin laying eggs on needles of host trees soon thereafter. In this region after eggs hatch late in July or early in August, most first stage larvae wander about until they find locations under bark flakes on host tree boles where they can conceal themselves. Here they spin hibernacula and molt to second stage larvae. A second stage larva in its hibernaculum is shown in Figure 1.

Late fall months seem to be an ideal time to study overwintering budworm populations since most second stage larvae are dormant within hibernacula. However, these hibernacula are so small and so concealed that to

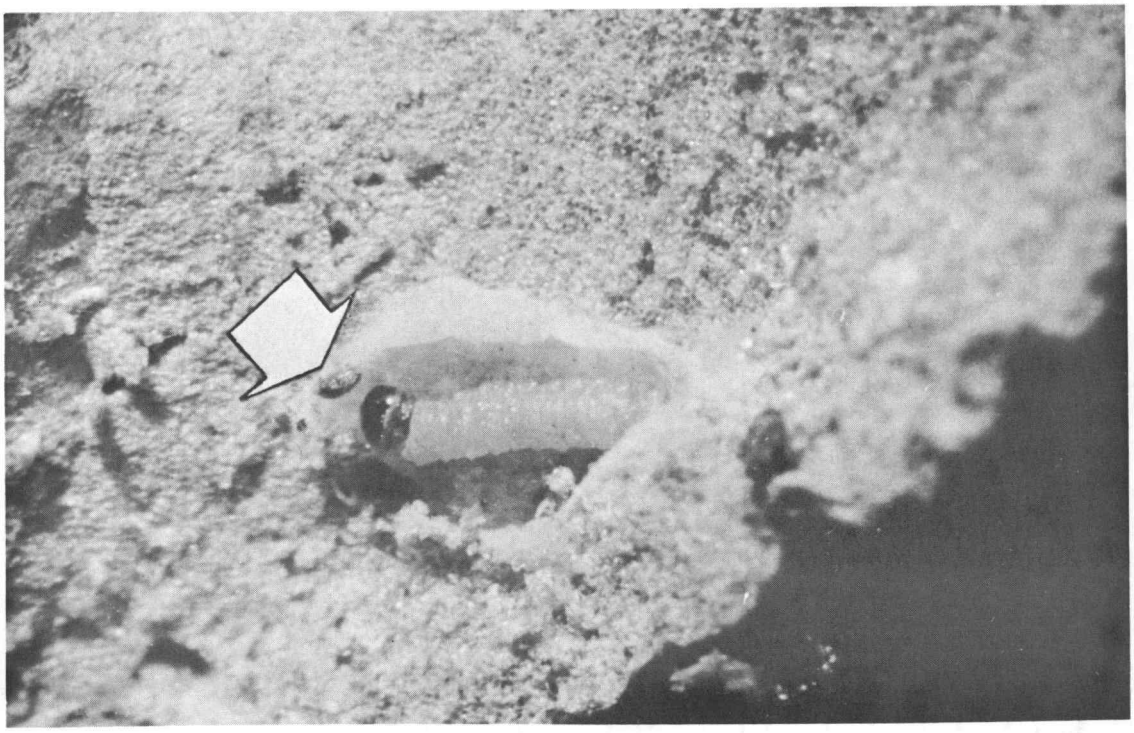

Figure 1. Second-instar spruce budworm larvae in hibernaculum (26X). Note shed head-capsule (arrow) of first-instar larvae.

accurately count them by direct examination is impracticable.

Techniques have been developed to estimate overwintering spruce budworm populations. In Oregon in 1949 Lindsten and Wright (26, p.29) collected crown and bole samples from Douglas-fir to determine favored overwintering sites of second-instar larvae in hibernacula. They collected smooth- and rough-bark sections from the tree bole, large branches, small branches, small twigs, and needles. Results showed that rough limbs and crevassed bark of larger branches and main bole were preferred overwintering sites. Carolin (6, p. 15-21) also found a considerable portion of the population overwintering on the bark of larger branches and on tree trunks, and Whiteside et. al., (40, p. 33) used rough-bark limbs as a sampling unit. More recent investigators in Oregon (5, p. 6) found that limb section samples give a more reliable estimate of budworm populations in hibernation than do bole section samples.

In the northern Rocky Mountains, according to Denton (12, p. 69) the greatest number of larvae, at least in heavy infestations, overwinter on rough bark surfaces of tree boles at about breast height. Terrell (36, p. 2-3) indicates that a more representative sample of overwintering budworm populations can be obtained by

using as a sampling unit a section cut from the tree bole approximately 3 feet below the junction of smooth and rough bark. This sampling unit described by Terrell is presently being used in spruce budworm population sampling in western Montana.

After bole section samples are cut from the tree they are removed to the laboratory, placed in rearing cartons and the overwintering larvae forced to emerge. Several different types of rearing cartons have been tried and used (41, p. 2) but the most efficient seems to be five-gallon cylindrical cardboard ice cream cartons (11, p. 3). Glass vials are inserted into one end of these sealed rearing cartons. By keeping the rearing room at approximately 70°F. and continuously lighted, photopositive larvae will emerge and crawl into the vials. Emerged larvae are counted daily. By comparing larval emergency from samples collected in units proposed for control, it can be determined if sufficient budworm populations exist to justify control that was recommended on the basis of defoliation surveys.

Final entomological justification of all 7 spruce budworm control projects in western Montana, northern Idaho and Yellowstone National Park in Montana and Wyoming between 1952 and 1959, 1954 excepted, was based on results of forced emergency of overwintering spruce

budworm larvae (14, p. 3) (15, p. 2) (28, p. 3) (34, p. 6) (35, p. 2).

Overwintering spruce budworm population data collected late in winter also can be used to predict damage that can be expected to occur in Douglas-fir forests the following summer. Carolin (6, p. 16) working in Oregon and Denton (9, p. 6-7) (11, p. 5-8) (13, p. 4-5) working in Idaho were able to establish relationships between the number of larvae overwintering on host trees and the degree of infestation the following summer. In 1953 Denton (14, p. 3) was able to successfully predict the degree of damage in a spruce budworm control unit on the basis of overwintering spruce budworm population data.

Since diapause requirements of overwintering spruce budworm larvae are fulfilled during the winter, investigators (10, p. 2) have been cautious not to attempt to force emergence of overwintering larvae too early in winter. Studies in Oregon (40, p. 33-35) show that sample material can be collected in November before roads become blocked by snow, stored at accessible locations outdoors, and brought indoors to force larvae to emerge in midwinter.

In western Montana, too, many roads are still blocked by snow in late spring. Consequently spring collections of budworm-infested samples are difficult to

make. Sampling points often must be planned for accessibility rather than for ideal sampling distribution (36, p. 5).

An experiment was conducted in 1956 and 1957 at the Missoula Forest Insect Laboratory to compare larval emergence from sample bole sections collected in fall and stored, with that from spring-cut sections. In the fall of 1956 Terrell (36, p. 5-6) collected 210 bole sections from sample points in 5 proposed spruce budworm control units in western Montana. The bole sections were stored in burlap sacks in a rather tightly-packed pile on a ground-level concrete floor in a field insectary at Missoula, Montana.

Early in March, 1957, these bole sections were taken to a greenhouse and placed in rearing cartons to induce the overwintering larvae to emerge. Also in early March, fresh bole sections were cut at the fall collection points that were accessible. These bole sections were also placed in rearing cartons in the greenhouse to induce larval emergence. Comparative data on larval emergence between the fall-cut and stored to spring-cut bole sections is shown in the following tabulation:

Unit	No. of Samples	Larvae per square foot: Fall-cut and Stored Samples	Larvae per square foot: Spring-cut Samples
1	9	8.7	42.1
2	2	9.7	41.2
3	7	14.4	74.7
4	3	2.6	9.6
5	4	9.3	43.5

Larval emergence from fall-cut and stored samples was an average of only 21.4 per cent as great as larval emergence from spring-cut samples. Results of this experiment indicated that diapause requirements of approximately 79.6 per cent of overwintering larvae were not satisfied by these conditions of storage at the field insectary.

The present study was planned to determine whether artificial temperature conditioning could force hibernating second-instar spruce budworm to emerge in fall or early winter and whether this population would be a reliable estimate of larval populations the following summer. It would be advantageous if this particular phase of spruce budworm population sampling could be done in fall. In addition to the ease of getting sample bole sections out of the woods while roads are still passable, information on relative population estimates from areas considered for control would be available months sooner.

LITERATURE REVIEW

Only a small part of the voluminous research work on the spruce budworm has been directed toward investigating the diapause requirements which must be satisfied before second stage larvae will continue their development. This research work usually has involved rearing successive generations of the insect throughout the year. Objectives usually have been achieved by subjecting second-instar budworm to various treatments of time and temperature in an attempt to shorten the diapause.

The present study is concerned with the possibilities of shortening the diapause period as a prerequisite to sampling overwintering spruce budworm populations. Literature on diapause studies of the spruce budworm with this objective is sparse, although a few authors have presented methods, techniques and ideas concerning general studies of forced emergence. Concerning spruce budworm populations on balsam fir in Ontario, Fettes (17, p. 131) states,

> "The technique for forcing hibernating larvae from foliage samples, utilizing the photopositive reaction of the larvae, have been used with some success, but there is no certainty of the fraction of the larvae recovered. Furthermore, the artificial conditions set up in the laboratory or insectary may be detrimental to the larvae and cause abnormal mortality. The techniques also assume that the majority of the larvae hibernate in the foliage or on small twigs."

Harvey (19, p. 1) induced second stage spruce budworm larvae to resume development following a 15-30 week period of cold storage at a near-freezing temperature. He felt that the resumed development was evidence that the cold storage period satisfied diapause requirements of the larvae. He concluded from his experiments that with only minor exceptions spruce budworm reared under laboratory conditions with approximately natural photoperiods do require a period of cold storage treatment of at least 10 weeks at 32°F. He found that 14 weeks seemed to be the effective storage time if the photoperiod was lengthened to 24 hours.

Bergold (1, p. 17-23) found that second stage spruce budworm larvae develop successfully following a period of artificial cold storage. He stored second-instar budworm in hibernacula for one week at 6°C., followed by 4-6 months at 1°C. The larvae become active after being exposed to 22°C., and 60-80 per cent relative humidity. In this way he was able to successfully rear 57 per cent of the stock.

Cole (8, p. 2-5) explored the effect of cold temperature treatment on terminating diapause of second stage spruce budworm larvae in southern Idaho. His work was done with larvae in hibernacula on 15-inch-long bole sections (billets) of Douglas-fir. Cole describes his

techniques as follows:

> "All logs (billets) were first stored for 1 week at a temperature range of 32°to 40°F. After this period 4 billets each were stored under the following temperature-time conditions:
>
> | -25°F. | 32°-40°F. |
> |---|---|
> | 8 hours | 7 days |
> | 24 hours | 14 days |
> | 7 days | 25 days |
>
> At the end of each period the billets were removed to a constant temperature room where the average daily cumulative temperature was 33.45 degrees."

Cole summarizes his results by stating, "Diapause of the overwintering larvae was broken by subjecting the larvae to (1) 32° to 40°F. for 1 week, (2) -25°F. for 8 hours, and (3) 70°F. until emergence."

Miller (31, p. 416-422) has thoroughly described two methods of assessing second-instar budworm populations in hibernacula. In the first method he collected foliage and bark samples and clipped them into small sections. The samples were placed in paper bags in a controlled-temperature room, first at 42°F. for 5 days, then at 32°F. for approximately 26 weeks, and again at 42°F. for 5 days. Then the bags were placed in a rearing room at 72°-76°F. and approximately 70 per cent relative humidity, well sprayed with water daily for 3 days, dried on the fourth day, and placed in an emergence cage. The second method

differs from the first in that samples were collected in spring after larvae had overwintered under natural conditions, and in that the sampling unit was a whole branch. In both methods Miller adjusted the population by the percentage emergence figure obtained either from controlled experiments conducted simultaneously or from dissection of hibernacula. The percentage emergence of larvae from hibernacula ranged from 59 to 85 per cent in Method I and from 78 to 83 per cent in Method II.

Miller concluded that if a choice were available Method II would be the better technique because the population overwintered under natural conditions and no special facilities were needed to recover larvae from hibernation.

Techniques of these research workers investigating spruce budworm diapause requirements have been helpful in planning the present study. However, none of the methods described is wholly applicable to reach present objectives. Cole's work lacks a percentage emergence figure by which the population can be adjusted. The others--Bergold, Harvey and Miller--present data on percentage of larval emergence but the periods of cold storage described by them are too long to be useful in sampling overwintering spruce budworm populations in the northern Rocky Mountain region. In this Region

information on overwintering budworm populations is available by the middle of April since sampling usually begins the first of that month. If, for example, Method I of Miller were used in this Region, results would be available no sooner than with present methods because cold temperature treatments could not begin until the last week in September. If the period of cold storage could be shortened, Method I of Miller would be suitable to use in estimating overwintering budworm populations in the Northern Rocky Mountains.

OBJECTIVES

The immediate objectives of this study were to determine (1) whether artificial temperatures for varying lengths of time would induce second-instar spruce budworm to emerge from hibernacula, (2) what fraction of the larval population would emerge with varying periods of artificial temperature conditioning, (3) what temperature and length of exposure would be required to cause maximum percentages of larvae to emerge, and (4) what length of exposure to natural outdoor fluctuating temperatures would be required to cause maximum percentages of larvae to emerge.

METHODS

Two experiments were performed during this study to estimate percentages of larval emergence following various cold storage treatments. The first experiment was conducted to determine percentages of larval emergence from hibernacula established on natural spinning sites--Douglas-fir bole sections. From previous experience it was expected that difficulties in examining bole sections for larvae established in hibernacula might render inaccurate the expression of larval emergence as percentages. Therefore in the second experiment percentages of larval emergence were determined from larvae emerging from hibernacula established on artificial spinning sites--gauze webbing in Petri dishes. Although artificial, a very accurate assessment could be made of the number of larvae established in hibernacula and the number of larvae emerging following cold storage. Field and laboratory methods are discussed in detail below.

FIELD

To obtain a quantity of second stage larvae established in hibernacula, almost 10,000 sixth stage larvae and pupae of the preceding generation were collected and daily brought to a field laboratory in the

Bitterroot National Forest five miles north of Sula, Montana.

The most intensive collecting of larvae and pupae was done in areas where fecundity of female moths was expected to be highest. Moth fecundity was important because the more eggs obtained per moth reared, the more second stage larvae would be available for the experiment. Studies made in Ontario (2, p. 448) indicate that fecundity of female adults is lowest in areas where larval starvation occurred because of insufficient new foliage. Collections were restricted, therefore, to areas where intensity of larval feeding on new growth was low to moderate. Techniques developed by Buchanan (4, p. 1) in Colorado were used to make collections.

At the field laboratory, larvae and pupae were separated from foliage and placed in one of four cages each enclosing a young Douglas-fir about 5 feet high (Figure 2). Egg masses deposited by ensuing gravid female moths were removed from cages each day, but early in the morning while moths were inactive.

A laboratory technique developed by Stehr (33, p. 424) in Canada was used for rearing second stage larvae that would be established in hibernacula on artificial sites. His technique was modified for use under field conditions. As egg-bearing Douglas-fir

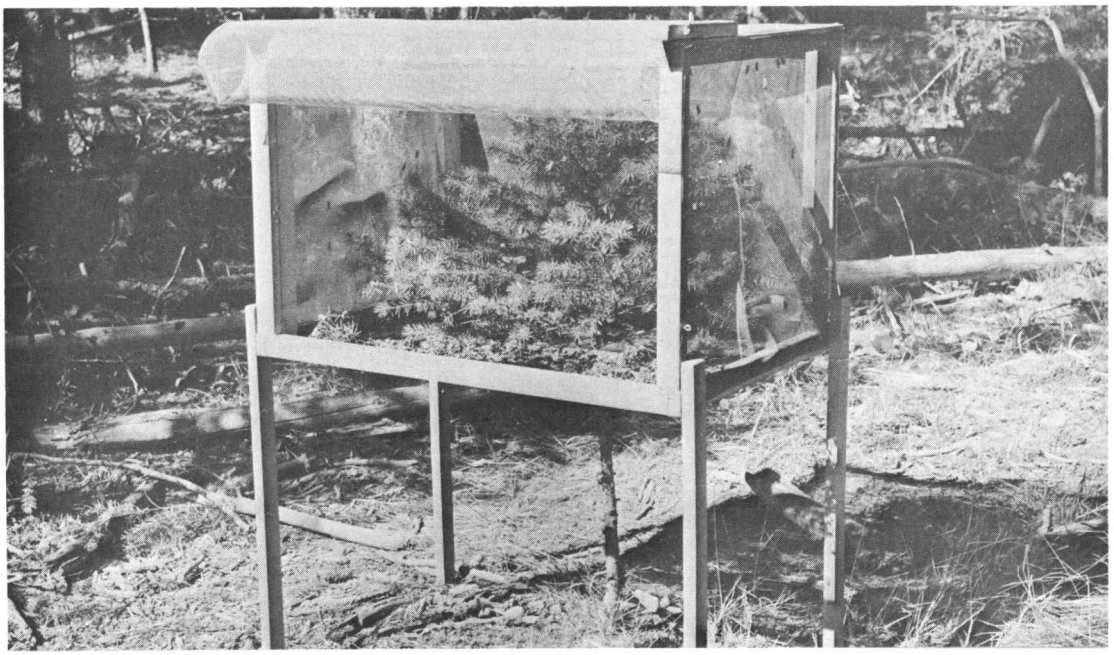

Figure 2. Cage enclosing young Douglas-fir. Note spruce budworm moths clinging to screen and tree foliage.

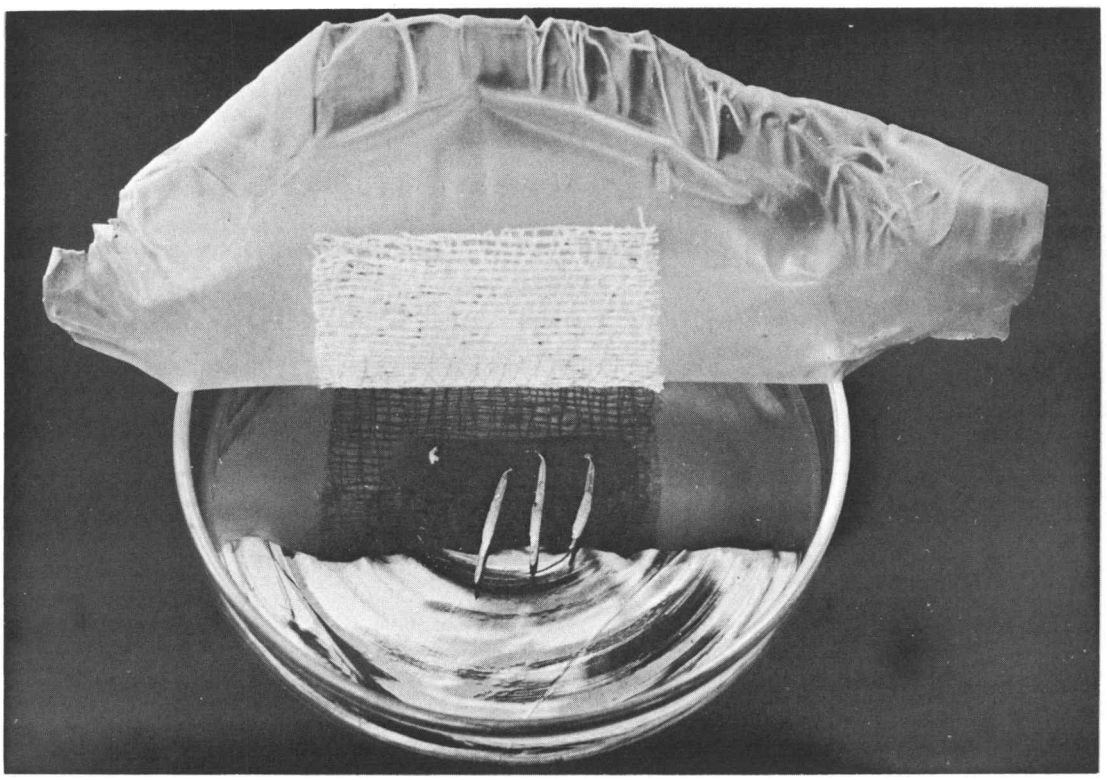

Figure 3. Petri dish with egg-bearing needles cemented to the bottom. Second-instar spruce budworm larvae in hibernaculae appear as dark flecks in the gauze on the parafilm cover.

needles were taken from cages they were cemented to the inside of the bottom half of a Petri dish, over which was placed a parafilm covering to which previously had been attached a double layer of 2- x 2-inch ordinary gauze (Figure 3). This in turn was covered by the other one-half of the dish. The equivalent egg output of one female was used per dish. Each dish was placed in an envelope, which had holes punched in the under side, in such a way that the gauze-parafilm was next to the holes. The envelopes, containing the dishes, were placed horizontally on wooden racks which were constructed so that there was a vertical distance of approximately 3 inches between envelopes. The racks were placed in one of two large cartons from which all light was excluded except from below. The cartons were attached to spruce trees about five feet above the ground (Figure 4). Photopositive first stage larvae hatching from egg clusters directly above the gauze were attracted to it by light entering from below and reached it by dropping (38, p. 57). Approximately 320 Petri dishes containing second stage larvae in hibernacula were stored in this manner at the field laboratory until late October.

One-half of the total number of eggs gathered were treated as above while the remaining one-half were placed on rough-barked trees for hatching. Fourteen small trees

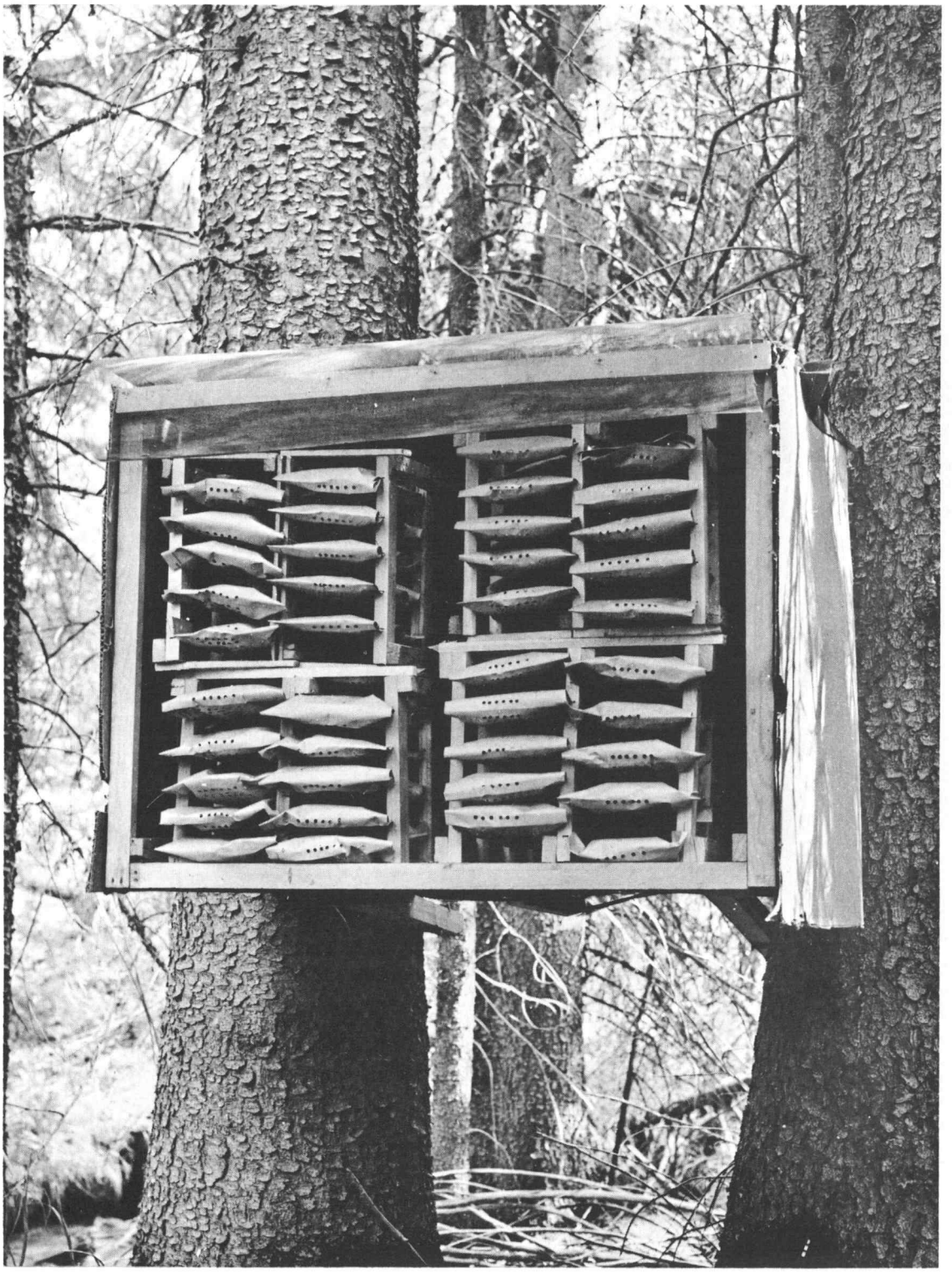

Figure 4. Storage carton attached to spruce trees five feet above the ground. Front panel removed to show wooden racks supporting envelopes containing Petri dishes.

4-5" in diameter, were selected which together had a total of 84 linear feet of the lower bole with rough-barked surface as a suitable habitat for overwintering larvae. Fir needles bearing the equivalent egg output of at least 2 female moths were fastened to each 6-inch section of bole. Possible losses from wandering first stage larvae were minimized by placing egg-bearing needles close to eventual hibernating sites under bark scales (Figure 5). Some authors (16, p. 774) found that first stage larvae wander considerably before spinning their hibernacula, but others (23, p. 223) feel that if desirable places are available larvae do not wander far before hibernating. Trees were banded with tanglefoot, both above and below the rough-barked area selected, to discourage apterous predators from feeding on unhatched eggs. The 168 six-inch bole sections were stored *in situ* until late October when the trees were felled and sectioned.

During field collections and rearing, insect mortality which could have been caused by direct handling was avoided whenever possible. Sixth stage larvae were collected from trees and placed in rearing cages by handling foliage enclosing them. Forceps were used to extricate pupae from foliage and webbing enclosing them and to place them in rearing cages. During transfer,

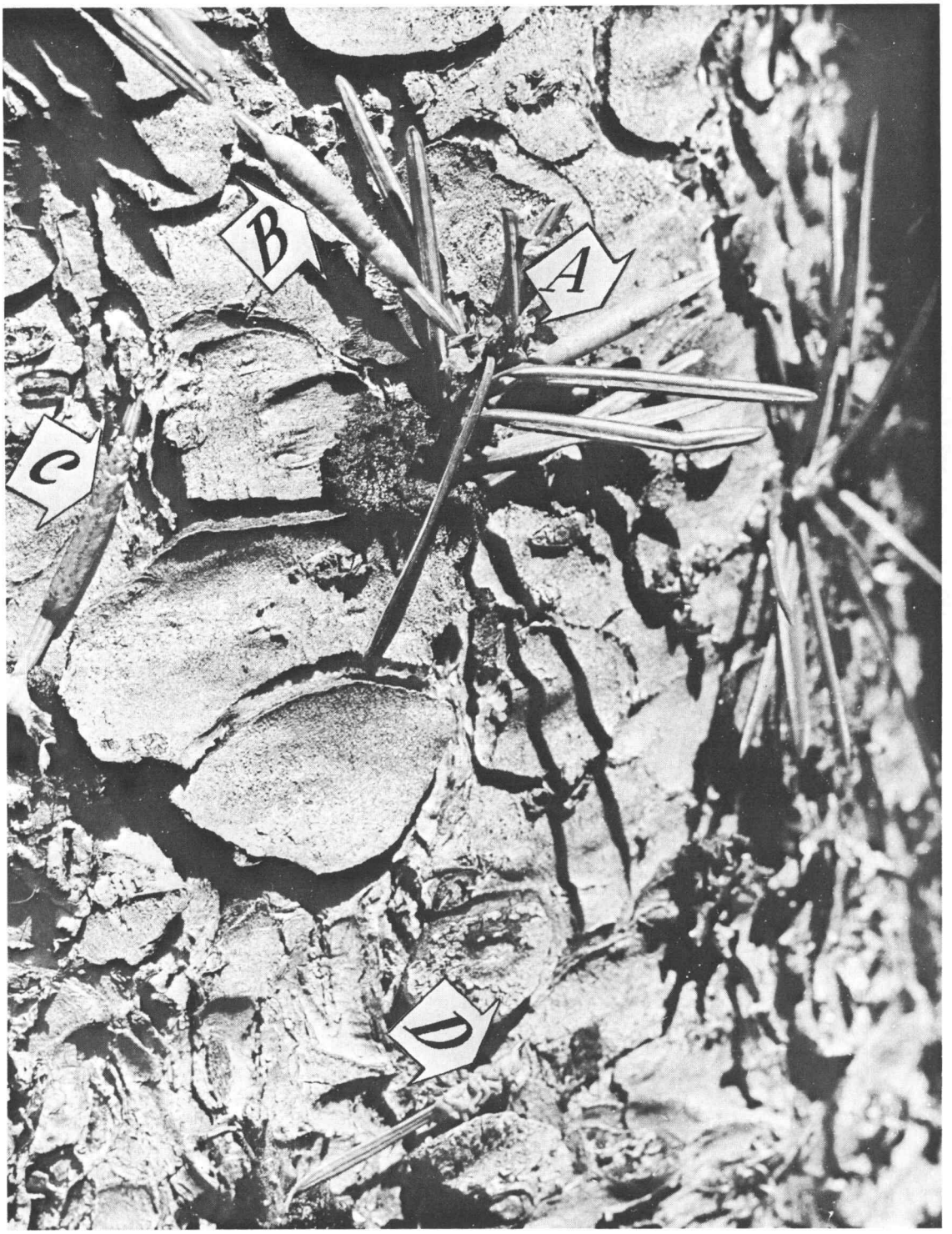

Figure 5. Needles bearing spruce budworm egg masses fastened to Douglas-fir bole (2.5X): A & B, new egg masses; C, 5-day-old egg mass about 24 hours before hatching with black head capsule of first-instar larvae visible through transparent chorion; D, eggs hatching.

pupae were grasped with forceps by their cremaster. Forceps were also used in handling egg-bearing needles.

LABORATORY

To determine the effect of cold temperatures on the length of diapause of the spruce budworm the following cold storage treatments were chosen: artificial cold storage at constant temperatures of -4°F. (-20°C.), 10°F. (-12°C.), and 32°F. (0°C.); and natural cold storage at fluctuating outdoor temperatures at a field insectary. Why these particular treatments were chosen is explained in the following paragraph.

Insects vary in their thermal requirements for diapause development (24, p. 54-55). Many insects from temperate climates with moderately severe winters have been found to respond most readily to temperatures within the general range 0°C. to 12°C. There are exceptions. For example, diapause development in the tent caterpillar, Malacosoma disstria (Hbn.) falls off below the thermal optimum (2°C.) but remains appreciable at -5°C. Further, in the sawfly, Gilpinia polytoma (Hartig), diapause can be completed at -10°C., while a temperature of 10°C. is too high for satisfactory diapause development. Since temperature requirement for diapause development in second-instar spruce budworm probably is not too

different from that of other forest insects of temperate climates described above, the cold temperature treatments mentioned in the preceding paragraph were chosen.

Introduction to Cold Storage

Near the end of October at the field laboratory, the Petri dishes were taken from the storage cartons and numbered consecutively from 1 to 320. The six-inch bole sections were numbered from 1 to 168 beginning with the lower-most section of tree #1 and ending with the uppermost of tree #14. At the same time, ends of each bole section were sealed with paraffin to retard drying. The entire group of bole sections and Petri dishes was then taken from the field laboratory and removed to a field insectary at Missoula, Montana, where they were randomized into 4 groups of 42 and 80, respectively.

The transfer of bole sections and Petri dishes from the field laboratory to the field insectary was made as efficiently as possible to keep abrupt temperature changes to a minimum. Experiments with early-instar spruce budworm in Connecticut (3, p. 5-6) indicate that sudden changes in temperature have a much more adverse effect on larvae than gradual changes.

At midday on October 23 placement of bole sections and Petri dishes in their respective cold storage units

was begun. The temperature at the insectary at this time was 41°F. Forty-two bole sections and 80 Petri dishes were placed directly into 2 household refrigerators at 32°F. Eighty-four bole sections and 160 dishes were placed in a cold temperature cabinet at 34°F. To prevent larvae from suddenly being exposed to rapid temperature changes, temperature of the cabinet was gradually lowered to -4 F. as follows:

Date	Hour	Cabinet Temperature Lowered From	No. Hours At Lowered Temperature	Cumulative Hours During Temperature Lowering
Oct. 23	6:00 p.m.	34°F. to 28°F.	5	5
Oct. 24	6:00 p.m.	28°F. to 20°F.	24	29
Oct. 25	9:00 a.m.	20°F. to 15°F.	15	44
Oct. 26	3:00 p.m.	15°F. to 4°F.	30	74
Oct. 27	8:00 a.m.	4°F. to -4°F.	17	91

While the cabinet temperature was at 15°F. on October 25, 42 sections and 80 dishes were removed and placed in a locker in a cold storage locker plant where temperature was maintained at 10°F. Material was transferred from the cabinet to the locker plant in a 5-gallon insulated container which was pre-cooled to 15°F. The 42 sections and 80 dishes remaining in the cold temperature cabinet were further cooled to -4°F. Forty-two sections and 80 dishes remained at the field insectary where they were to be exposed to daily fluctuations in temperature. By midday on October 27, all bole sections and Petri

dishes were in their respective cold storage units.

Period of Cold Storage

During the period of cold storage, bole sections and Petri dishes were arranged in their respective cold storage units in such a way that air circulation among them would be most efficient. Log bole sections were stored vertically end to end. Dishes in envelopes were stored in flat layers with 1-inch square wooden sticks separating each layer. A recording thermograph was used to maintain a continuous record of temperatures in the cold temperature cabinet, in the locker at the cold storage plant, and at the field insectary. Space did not permit the use of a recording thermograph in the household refrigerators. However, two maximum-minimum thermometers were placed in each of the two refrigerators and temperatures were checked daily. This technique did not supply a continuous temperature record but it did enable temperature fluctuations to be detected and corrected.

Twenty-seven days after the experiment began, the valve controlling the refrigerant in the cold temperature cabinet became defective. Before it could be repaired, temperature in the tank warmed from -4°F., to 20°F. Repairs were made immediately and seven hours later the

temperature had returned to -4°F. However, all larvae in the first group of 4 Petri dishes taken from the cabinet following this mishap were dead. Four more dishes were removed and in these, too, all larvae were dead. Death of these larvae indicated the detrimental effect abrupt temperature changes may have on second stage spruce budworm larvae in hibernacula. At this point cold storage treatment in this cold temperature cabinet was discontinued.

Removal from Cold Storage

At midday on October 31, and every four days thereafter for a period of 80 days, four dishes and two 6-inch bole sections were removed from each cold storage unit and from the field insectary to the rearing room. As much as possible, temperature and relative humidity in the rearing room were maintained at 70-80°F. and from 60 to 80 per cent, respectively. Material from the two refrigerators at 32°F. and from the field insectary was taken directly to the rearing room. Material from both the cold temperature cabinet at -4°F. and the cold storage locker at 10°F., was brought to rearing room temperature gradually by placing it in a 5-gallon insulated container.

Every 4 days, 2 bole sections and 4 Petri dishes from both the cold storage locker and the cold temperature cabinet were placed in the insulated container which was then placed in the rearing room. As the temperature within the insulated container warmed to rearing room temperature, it was measured with two 28-guage copper-constantan thermocouples connected to a "Queen" portable potentiometer. It took approximately seven hours for temperature within the container to warm to rearing room temperature. When the two temperatures were in equilibrium, the Petri dishes and bole sections were removed from the container.

Petri dishes and bole sections then were handled as follows: Petri dishes were taken from the envelopes and placed on glass shelves illuminated from below by continuous light but shielded from all other light (Figure 6). As larvae emerged from hibernacula they were attracted to the light and dropped to the bottom of the dishes.

Each six-inch bole section was placed in a 5-gallon cylindrical paperboard ice cream carton. A 22 x 100 mm. pyrex shell vial was placed into a reinforced

Figure 6. Petri dishes on glass shelves illuminated from below by continuous light.

hole in the lid of each carton (Figure 7). A small stick about the size of a wooden match was tapped into the end of each bole section in such a way that it extended out into the vial when the lid was placed on the carton. Cartons then were placed on shelves in the rearing room (Figure 8). Newly-emerged second-instar budworm, being strongly photopositive (39, p. 169), were attracted by continuous room light and crawled out onto the sticks and into the vials.

Recording and Adjusting Emergence

Two days after being placed on one of the glass shelves, each dish was removed for approximately 10 minutes while hibernacula established in the gauze were thoroughly examined for living and dead larvae.

During this examination it was noted that nearly all dishes contained some dead and desiccated second stage larvae on gauze outside of their hibernacula. This was of interest since Harvey (18, p. 1) found similar occurrences when examining laboratory-reared material following cold storage at 32°F. He noticed that larvae would leave the hibernacula soon after the second molt. According to Harvey (20, p. 554-556), these individuals may correctly be called "non diapause" insects and their

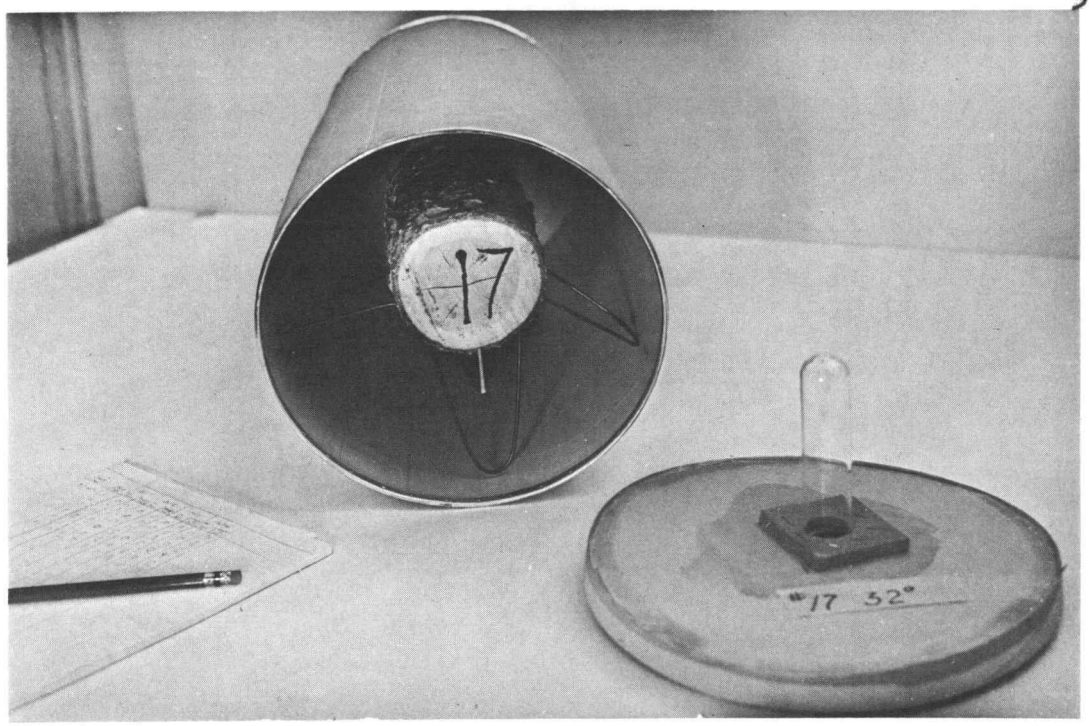

Figure 7. Cylindrical carton with 6-inch Douglas-fir bole section, cover, and pyrex shell vial.

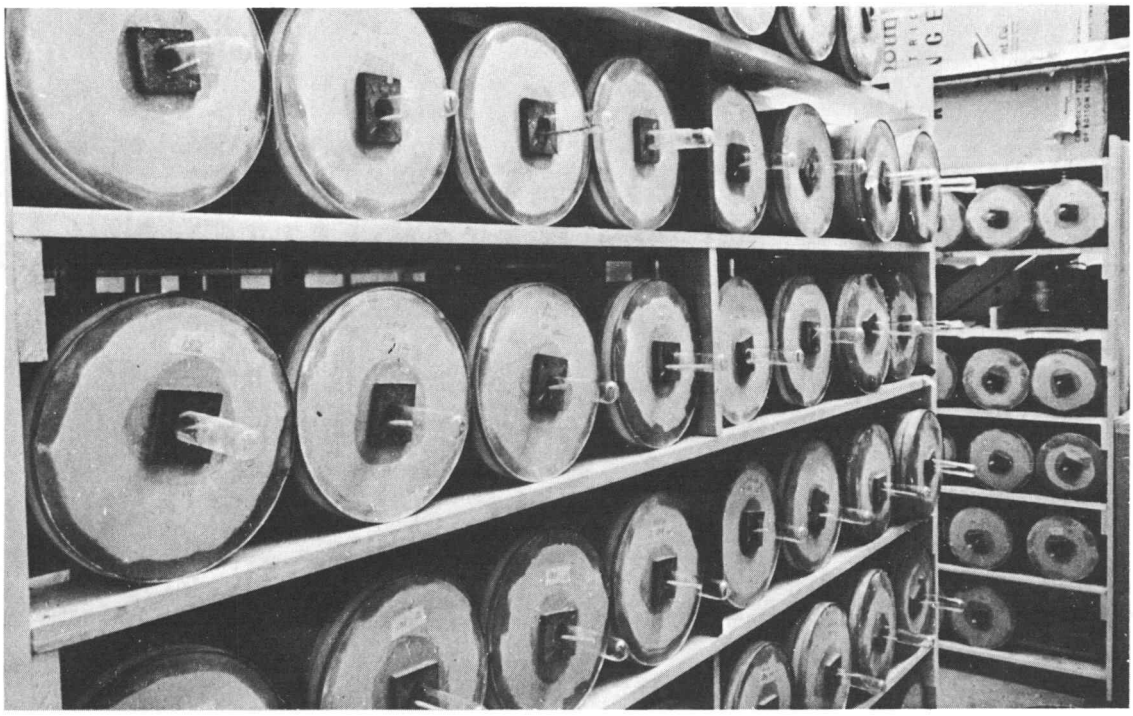

Figure 8. Cylindrical cartons on shelves in rearing room. Emerging larvae crawl into the vials on sticks protruding from Douglas-fir bole sections within the cartons.

development referred to as "diapause free." At least in laboratory-reared stocks apparently 3-4 per cent of the larvae from each generation require no period of arrested growth.

In examining hibernacula, each was gently touched with the tip of a soft camel's hair brush. Live larvae would react by instantaneously withdrawing the part of their body nearest the stimulus. For each dish the living, dead, and total number of larvae was recorded, and the percentage living was calculated. An average percentage of larvae alive in the Petri dishes following cold storage was then computed for each of the 4 treatments.

Soon after the dishes were placed on the shelves, larvae began to emerge from their hibernacula in the gauze in the dishes. As they emerged they were counted daily. When six days passed with no larval emergence in a particular dish, that dish was removed from the shelf. Total larval emergence in each dish was expressed as a percentage of total number of larvae alive in hibernacula in that dish two days after it was taken from cold storage. An average percentage of larval emergence was then computed for each 4 dishes taken from each cold storage treatment every 4 days.

Larvae emerging from hibernacula on each 6-inch bole section were also counted each day. When larval emergence ceased, each bole section was thoroughly examined with a high-magnification reading glass for hibernacula containing unemerged larvae. This was done in order to express larval emergence from bole sections on a percentage basis. Since each six-inch bole section presumably supported unequal numbers of larvae in hibernaculae, larval emergence expressed in whole numbers as the number of larvae per section or per square foot would have been meaningless.

Because of technique difficulties in examining bole sections for unemerged larvae, discussed in detail on pages 48-51, it is doubtful if examiners recovered all larvae. However, since bole sections were all nearly the same size it was felt that by examining each section for an equal period of time, a proportionate number of hibernacula would be recovered from each one. Any obviously larger or smaller than average sections were examined for a longer or shorter time, respectively.

There is also doubt that all of the unemerged larvae in hibernacula found during the examination of each bole section were alive when that section was removed from cold storage several weeks prior.

Therefore, the number of unemerged larvae found during the examination was adjusted by a reduction corresponding to the average percentage of larvae found dead in hibernacula in the Petri dishes following similar cold storage treatments. The total larval emergence from hibernacula on each bole section was then expressed as a percentage of the adjusted number of larvae alive following cold storage. An average percentage of larval emergence was computed for each 2 bole sections taken from each cold storage treatment every 4 days.

RESULTS

Percentages of larval emergence from Petri dishes (Figure 9) were dissimilar to percentages of larval emergence from bole sections (Figure 10). Because of these discrepancies in percentages of larval emergence, results of each method are presented separately in the first two sections below. The third section below is concerned with larval mortality during cold storage.

LARVAL EMERGENCE IN PETRI DISHES

Percentages of second stage spruce budworm larvae emerging from hibernaculae spun in gauze in Petri dishes following various periods and intensities of cold storage are presented graphically in Figure 9.

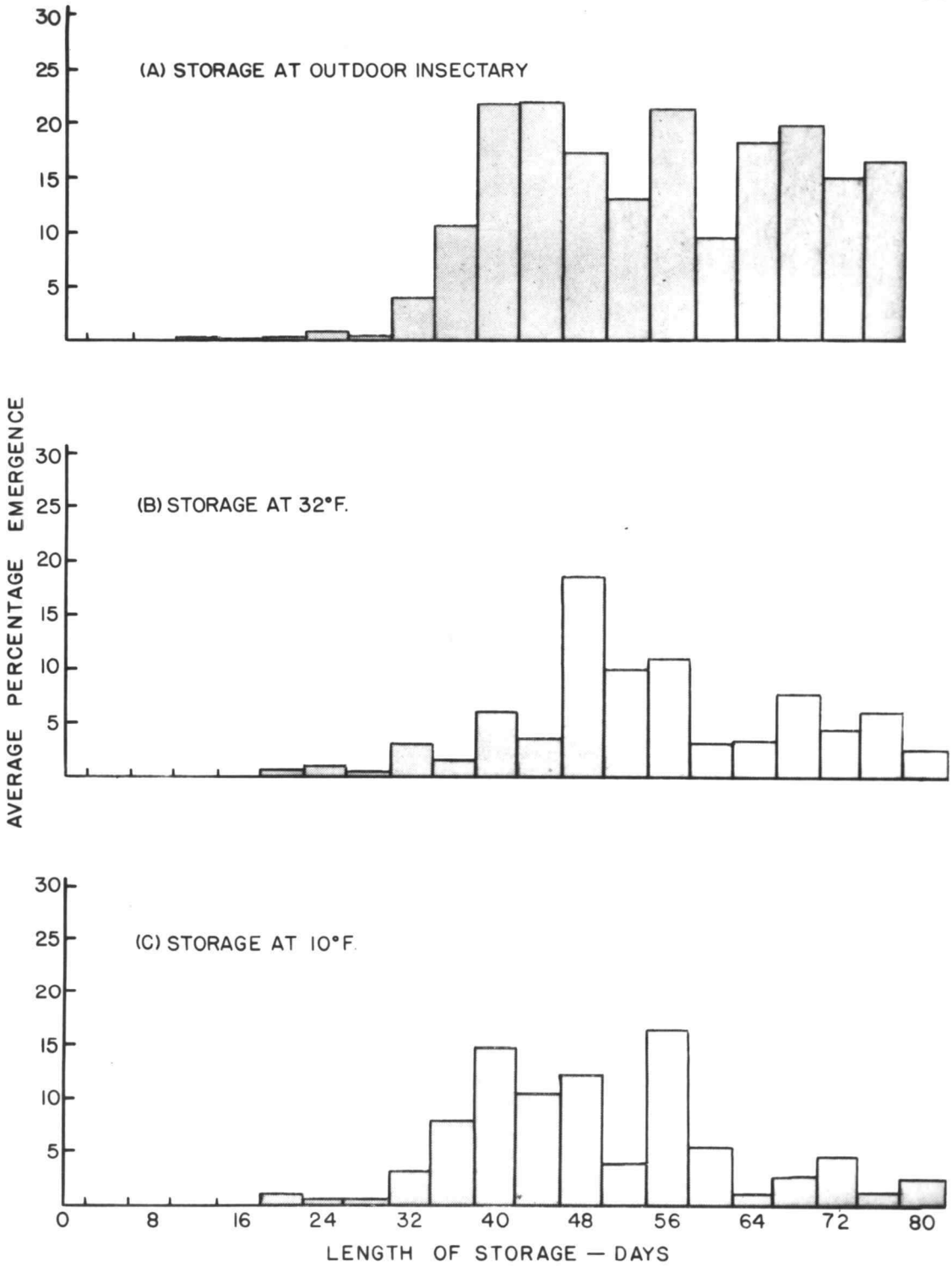

Figure 9. Comparison of the effect of length of cold storage on the emergence of overwintering second-instar spruce budworm larvae, from hibernaculae established in gauze in Petri dishes, after (A) natural cold storage at fluctuating temperatures in an outdoor insectary, and (B & C) artificial cold storage at constant temperatures of 32°F., and 10°F., respectively. Average percentage emergence for each 4-day period of cold storage was computed from the percentage of larval emergence from 4 Petri dishes.

These same data are presented in detail in tabular form in Tables 2, 3 and 4. Length of time larvae were in cold storage at the outdoor insectary, expressed in Figure 9(A) in terms of "calendar days" is also expressed in terms of "degree days below 42°F." in Table 1.

Regardless of the cold storage treatment, artificial constant temperatures or natural fluctuating temperatures, there was no appreciable larval emergence until larvae were subjected to (1) 32°F. or 10°F. for at least 32 days (Figure 9 (B&C)), or (2) natural outdoor fluctuating temperatures for at least 32 calendar days (Figure 9(A) or for 202 degree-days below 42°F. (Table 1).

The highest percentage of larval emergence following any one of the 3 treatments was approximately 22 per cent. It occurred following cold storage at natural fluctuating temperatures at the outdoor insectary for 40-44 calendar days. (Figure 9(A). This peak emergence decreased moderately to a low of about 18 per cent after the cold storage period was extended to 76 days. Insufficient larvae established in hibernacula forced this treatment to be terminated at 76 instead of 80 days.

Following cold storage treatments at artificial constant temperatures of 32°F. or 10°F. for 48 and 40 days, respectively, approximately 15 per cent of the

Table 1

Accumulated degree days below 42°F. during a period of natural cold storage at fluctuating temperatures at the outdoor insectary.[1/]

Date	No. of Calendar Days of Cold Storage	Degree Days Below 42°F.	Accumulated Degree Days Below 42°F.
Sept. 18-Oct. 24 (period prior to cold storage)			46
October 25	0	0	46
28	4	0	46
November 1	8	3	49
5	12	27	76
9	16	28	104
13	20	13	117
17	24	21	138
21	28	50	188
25	32	14	202
29	36	24	226
December 3	40	22	248
7[2/]	44	32	280

1/ Accumulated degree days were calculated by adding the differences obtained when subtracting the mean temperature for each day from 42°F. If the difference was negative it was considered zero. Mean temperatures were computed using U.S. Weather Bureau techniques, i.e., ½ of the sum of the maximum and minimum temperatures.

2/ Accumulated degree days were not computed beyond this period of cold storage since the highest percentage of larval emergence occurred following 40-44 calendar days or 248-280 degree days of cold storage.

larvae emerged after being exposed to rearing room temperature of 70°F. This was the peak of larval emergence with the 32°F. treatment but a second peak, slightly higher than the first, occurred with the 10°F. treatment following cold storage for 56 days (Figure 9 (B & C)). Following the peaks, percentage of larval emergence decreased rather sharply, in both the 32°F. and the 10°F. treatments, to less than 5 per cent at the end of the cold storage period.

The 15 per cent larval emergence following cold storage at 32°F., for 48 days, discussed in the paragraph above, is considerably less than Harvey (21, p. 1205) produced. He induced almost 70 per cent of the larvae to emerge after cold storage at 32°F. for 6 weeks followed by a 24-hour photoperiod for 55 days in the rearing room. Difference between his results and results of this study probably is due at least in part to length of photoperiod. The effects of photoperiod length are described in detail on pages 45-47.

LARVAL EMERGENCE FROM BOLE SECTIONS

Percentages of second stage spruce budworm larvae emerging from hibernacula spun beneath bark flakes on Douglas-fir bole sections following various periods and

intensities of cold storage are presented graphically in Figure 10. These same data are presented in detail in tabular form in Tables 5, 6, and 7.

Figure 10 purposely was prepared identical in style to Figure 9 so percentages of larval emergence from bole sections could be compared with percentages of larval emergence from the Petri dishes for equal periods and intensities of cold storage.

Several things indicating an inconclusiveness of the results presented in Figure 10 are pointed out as follows: (1) absence of any larval emergence following any period of cold storage at 10°F., (2) erratic distributions of larval emergences following cold storage at outdoor temperatures and at 32°F., (3) absence of any emergence following cold storage at 32°F. for 48-64 days, and (4) dissimilarity in percentages of larval emergence between Figures 9 and 10.

Difficulties of the technique of assessing spruce budworm populations on bole sections, which are in part responsible for discrepancies in percentages of larval emergence between the two methods, are discussed in detail on pages 48-51.

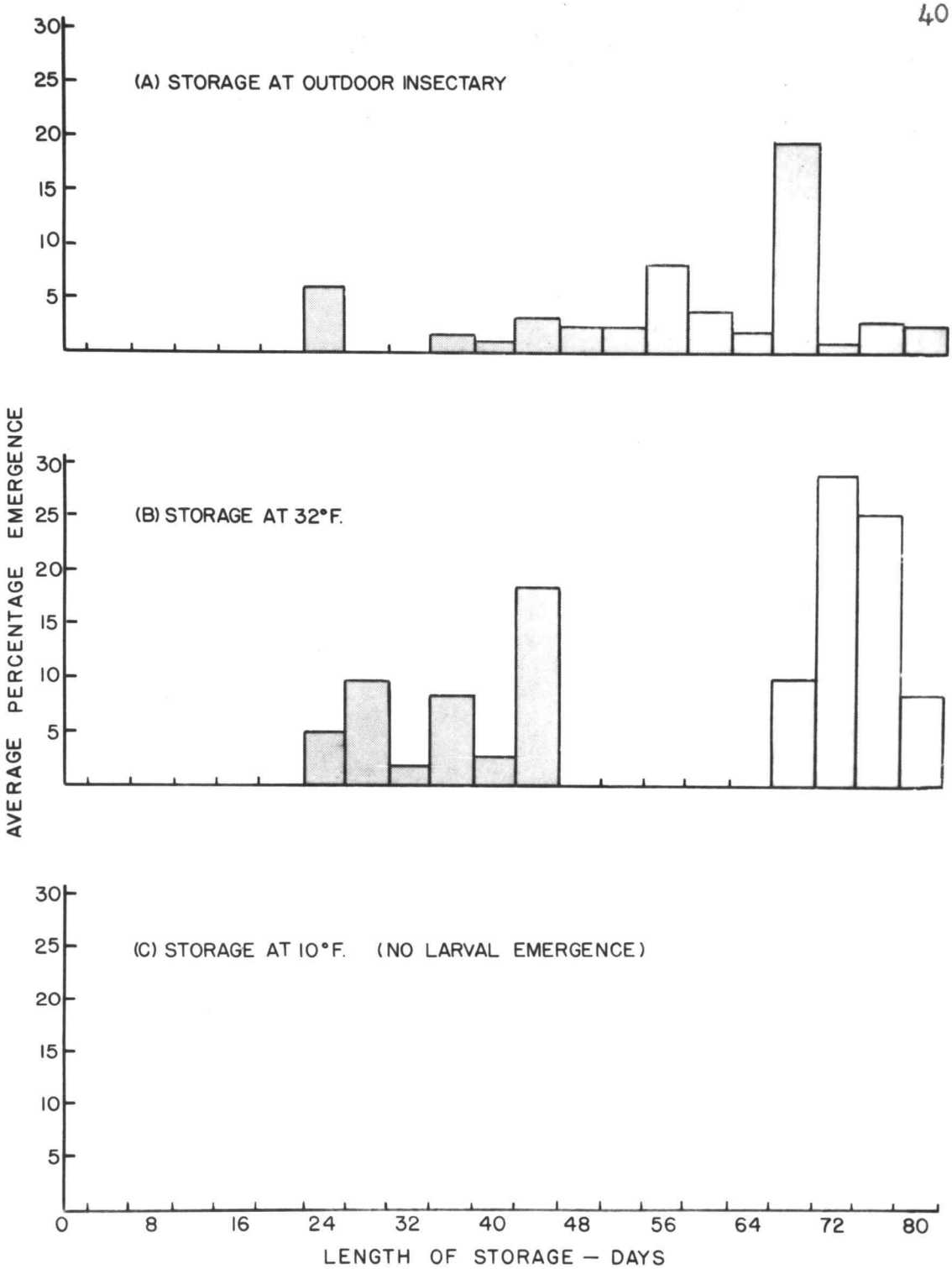

Figure 10. Comparison of the effect of length of cold storage on the emergence of overwintering second-instar spruce budworm larvae, from hibernaculae established beneath the bark flakes on Douglas-fir bole sections, after (A) natural cold storage at fluctuating temperatures in an outdoor insectary, and (B & C) artificial cold storage at constant temperatures of 32°F., and 10°F., respectively. Average percentage emergence for each 4-day period of cold storage was computed from the percentage of larval emergence from 2 bole sections.

LARVAL MORTALITY DURING COLD STORAGE

On pages 30-34 a method is described whereby larval mortality attributable to cold storage treatments was determined. Average percentages of larvae alive in Petri dishes following cold storage treatments were as follows: 89.8 per cent of those which had been in cold storage at the field insectary, 84.7 per cent of those in cold storage at 32°F. and 70.7 per cent of those in cold storage at 10°F. Until the mechanical failure of the cold temperature cabinet occurred (explained on page 26), approximately 65 per cent of the larvae were alive in their hibernacula in this cold storage treatment of -4°F.

Survival percentages listed in the previous paragraph indicate a higher percentage of larval mortality with lower cold storage temperatures. Percentage of larval mortality following cold storage at 32°F., however, was considerably higher than that found by others. Brown (3, p. 6) found that a temperature of 31°F. for 68 hours had practically no killing effect on second-instar spruce budworm. Harvey (19, p. 6) found mortality of larvae in hibernacula stored at 32°F. for 30 or 35 weeks in the laboratory to be negligible.

DISCUSSION

Three points were brought out in the results of this study which require further elaboration or explanation. They are: (1) differences in percentages of larval emergence between Petri dishes and bole sections, (2) differences between percentages of larval emergence following cold storage at artificial constant temperatures and percentages following cold storage at natural fluctuating temperatures and (3) the technique of expressing period of cold storage as accumulated degree days below 42°F. These three points are discussed in detail below.

DISCREPANCIES IN LARVAL EMERGENCE BETWEEN PETRI DISHES AND BOLE SECTIONS

Larval emergence from hibernacula in gauze in Petri dishes (Figure 9) was consistently greater than larval emergence from hibernacula spun beneath bark flakes on bole sections (Figure 10). Several factors could be responsible for higher percentages of larval emergence from Petri dishes for comparable periods of cold storage treatment. The factors are discussed and evaluated below.

1. Relative humidity in the rearing room. In the past some investigators felt that larval emergence was influenced by the amount of moisture in bole sections, twigs, and/or foliage on which first-instar spruce budworm had spun hibernacula. In most cases it was the drying of this material that caused most concern. Some researchers attained high humidities in rearing cartons by moistening material prior to placing it in cartons for larval emergence. In New Haven, Connecticut, in January and February of 1950, Dowden and Carolin (3, p. 9-10) found that regardless of the type of rearing carton used, very few, if any, larvae emerged unless a supply of moisture was provided. They sprayed foliage lightly with water and lined rearing cartons with paraffin paper. They did not, however, experiment with the type of rearing carton used during the present study--a 5-gallon cylindrical ice cream carton. Also in their studies they used twigs which probably dry much faster than do tree bole sections.

Denton (11, p. 3) in Idaho, lined 5-gallon ice cream cartons with moisture-proof paper to keep evaporation and bark drying at a minimum. Larval emergence was greater from these lined 5-gallon containers than from that obtained previously from unlined, rectangular, cardboard boxes. However, he did not

compare larval emergence between lined and unlined 5-gallon cartons. An unlined 5-gallon ice cream carton is much more moisture-proof than an unlined, ordinary cardboard box.

Later work during the winter of 1951-1952 at Portland, Oregon (41, p. 7) showed that excessive humidity occasionally was encountered with the 5-gallon type of rearing carton. At times mold formed on the logs and excessive humidity was sometimes indicated when moisture would accumulate in emergence vials.

Miller, (31, p. 422) working with balsam fir, found that if foliage was too wet before it was placed in emergence cages fungus growth soon developed and samples had to be discarded. However, he also found that mortality of larvae in hibernacula would increase if foliage was too dry. Foliage, as well as twigs, undoubtedly dries much faster than does bark on tree bole sections.

Other work has shown that excessive moisture could be more important in retarding larval emergence than excessively dry conditions. Laboratory observations (39, p. 169) show that saturated air depresses the locomotor activity of all instars of spruce budworm.

Because of experience of others no attempt was made during this study to regulate relative humidity within cartons. Rather, an attempt was made to

regulate relative humidity in the rearing room at approximately 60-80 per cent during the period when larvae were emerging. There was no way to completely control humidity so some daily fluctuations were unavoidable. Exceptionally high and low humidities were avoided as much as possible by opening and closing outside windows, by the use of circulating fans and by placing open-topped containers of water on steam radiators.

Rearing room humidity did not appear to be detrimental to larval emergence from hibernacula in Petri dishes. An occasional dish was taken from the shelf, after it had been there for several days following cold storage, and examined for larvae still alive but unemerged. One such dish which had been on the shelf since December 6, was removed and examined on December 23. Of 154 larvae in the dish, 147 were still active and moved readily when hibernacula enclosing them were disturbed.

Relative humidity in the rearing room during the period of larval emergence probably was not responsible for the low incidence of larval emergence from hibernacula on bole sections.

2. Photoperiod. Length of photoperiod is one of several factors influencing both onset and termination of

diapause in insects (24, p. 13,65). Harvey (19, p. 6) found length of photoperiod affected the percentage of spruce budworm larval emergence after various periods of cold storage. He describes the effect of photoperiod on larval emergence from Petri dishes as follows:

> "After six weeks storage (at 32°F.) the physiological processes of diapause which limit further growth and development have been completed in only a small proportion of the insects - those emerging within the initial period. Under most conditions the remainder of the insects cannot proceed with their development and eventually die. If, however, these insects which cannot emerge are subjected to continuous light they do emerge, after a short time and continue their development. In other words the continuous light has somewhat the same effect at this stage as further cold treatment. After 14 weeks storage there is little of this effect left, as might be expected, since by this time the cold treatment has been long enough to make most of the insects capable of resuming their development."

Harvey found that exposure to any photoperiod, even continuous light, failed to produce appreciable emergence after storage for less than four-six weeks. Even after six weeks storage at least ten days of continuous light were needed to effect emergence of larvae which would not emerge in more normal photoperiods.

During the present study, Petri dishes were removed from shelves four or five days after the initial emergence period. There was no possibility, therefore,

for insects not emerging during the initial emergence period to emerge later after further exposure to continuous light.

Length of photoperiod apparently was not responsible for the higher percentage of emergence of larvae from hibernacula in Petri dishes.

3. Light striking hibernacula. Light intensity, as well as length of photoperiod, probably has some influence on behavior of second stage spruce budworm larvae in hibernacula.

A limited experiment at the Missoula Forest Insect Laboratory has shown that the amount of light entering the emergence carton or container may affect incidence of larval emergence. Four 18-inch bole sections were cut from each of four budworm-infested trees and randomized into four groups. The eight sections in group one and two each were placed in one of eight five-gallon rearing cartons such as those used during this study (Figure 7). The four sections from group three were placed in a galvanized iron can with a flat lid to which was attached a one-half pint jar. The four sections from group four were placed in a similar galvanized iron can but having a cone-shaped lid. A bank of lights continuously illuminated the vials on the rearing cartons and cans. Average number of larvae emerging from each

of the four groups was as follows:

Group	Larvae per sq.ft.
1	44.6
2	70.4
3	30.4
4	71.5

It is interesting to note that more than twice as many larvae emerged from the galvanized can with the cone-shaped lid (group 4) than from the galvanized can with the flat lid (group 3). Evidently light striking the sides of the cone-shaped lid was projected inward causing greater illumination within the can. Larval emergence from group 4 also was much higher than average emergence from groups 1 and 2. Results of this experiment are by no means conclusive but they indicate that intensity of light striking bole sections may have some influence on the emergence of second stage larvae from hibernacula.

4. Examination of hibernating sites. Gauze in Petri dishes and rough-barked surfaces of tree bole sections both were used as strata on which first stage budworm larvae could spin hibernacula. The former was used because larval populations in gauze were easy to evaluate and the latter because tree bark is the natural stratum on which hibernacula are spun. It was simple to determine the number of hibernacula spun in the gauze,

and the number of dead and living second stage larvae in hibernacula following cold storage. But, direct examination of bole sections to recover larvae in hibernacula, in order to express larval emergence from bole sections on a percentage basis, was attended with certain difficulties.

The technique is tedious, time-consuming, and at times inaccurate. Hibernacula are so small and so concealed that to find them all would seem to be nearly impossible. Nevertheless the technique has been used by other workers. In a study to determine some of the causes of mortality of overwintering spruce budworm larvae, Jaynes and Speers (23, p. 223) minutely examined, twig by twig, with a binocular microscope, an entire 6-foot balsam tree and expressed their results as percentages of the total population found.

During the preseht study, too, results were expressed as percentages of the total population found. However, an adjustment had to be made in this total population recovered since it is doubtful that this entire population was alive following cold storage.

It was impossible to examine bole sections immediately following cold storage and before emergence began, as was done with the Petri dishes, because all bark flakes had to be removed in searching for concealed

hibernacula. If this would have been done prior to emergence, the sample obviously would have been destroyed. So of necessity the examination was made when larval emergence had stopped. An assumption was made that percentages of larvae still living following the cold storage period were the same as percentages of larvae alive in the Petri dishes following similar cold storage treatment. On this assumption larval populations found on bole sections were adjusted by a percentage corresponding to the percentage of larval survival in the Petri dishes. In other words, suppose for example that during the examination 25 larvae in hibernacula were found on a six-inch bole section that had been in cold storage at 32°F. This number would have been reduced by 15.3 per cent, which was the average percentage of mortality that occurred to larvae in Petri dishes after cold storage at 32°F. The number of larvae assumed to have been alive on this particular bole section after cold storage would have been 21.

Errors involved in accurately expressing larval populations on bole sections in terms of percentages were admittedly high. These errors--the physical difficulties of the technique in examining bole sections for tiny hibernacula, and assumptions that had to be made in order to adjust the larval population recovered--

were no doubt largely responsible for erratic percentages of larval emergence that were realized from the bole section method.

5. Sample size. Variances of sample means are determined partly by sample sizes (25, p. 37); i.e., the smaller the sample size, as a rule the larger the variance between sample means. Sample mean variances probably were higher from bole sections than from Petri dishes since for each period of cold storage average percentage of larval emergence was computed from only two bole sections but from four Petri dishes. Bole section sample size was unavoidably restricted to two by limited cold storage space.

In addition to the smaller sample size, larval populations on bole sections were much lower than those established in gauze in Petri dishes. Of approximately 200 first stage larvae successfully hatching from eggs on each six-inch bole section, an average of less than 20 established themselves in hibernacula. An average of 87 larvae were established in hibernacula in Petri dishes. If larval populations on bole sections would have been higher, the accuracy of the attempt to express larval populations on bole sections would no doubt have been correspondingly higher.

Smaller sample size and low larval populations probably accounted in part for low and erratic percentages of larval emergence from bole sections.

In summary then, discrepancies in larval emergence between Petri dishes and bole sections are believed attributable to three causes: (1) inaccuracy in examining larval hibernating sites by direct examination of bole sections, (2) smaller sample size in the bole section method, and (3) low larval populations established on bole sections. Not considered to be responsible for the discrepancies are: (1) relative humidity in the rearing room, (2) length of photoperiod, and (3) intensity of light striking hibernacula. There is no explanation for the complete absence of larval emergence from bole sections following cold storage for any length of time at 10°F., or for 48-64 days at 32°F.

CONSTANT VS. FLUCTUATING COLD STORAGE TEMPERATURES

Larval emergence from hibernacula following cold storage at fluctuating temperatures was consistently greater than larval emergence from hibernacula following cold storage at constant temperatures. This discrepancy merits some discussion.

Literature contains numerous references concerning relative effects of constant and fluctuating

temperatures on various life processes of insects and related arthropods. Most of the work has been concerned with the effect of these types of temperatures on the rate of insect development.

Validity of work done with constant temperatures is often questioned from the ecological point of view, since temperatures in natural environments usually are not constant (7, p. 53). Although there is not general agreement about the advantage of fluctuating or constant temperatures in insect development, there are instances of more beneficial effects of fluctuating temperatures on development of insects that normally undergo fluctuating temperatures in nature.

Results of some investigations on rate of development of the spruce budworm, which normally is subjected to widely fluctuating temperatures during most of its life cycle, indicate fluctuating temperatures to be more beneficial than constant temperatures. Harvey (20, p. 552) found average incubation period of spruce budworm eggs to be more than twice as long, in terms of Developmental Units with constant temperatures, than did McGugan (29, p. 440) with natural fluctuating conditions. Miller (31, p. 418-422) obtained a higher percentage of larval emergence from a treatment in which

larvae had overwintered under natural conditions than from a treatment where larvae had been subjected to artificial overwintering conditions of cold storage at a constant temperature of 32°F.

Results of the present study also indicate that diapause requirements of more second stage larvae in hibernacula are satisfied by natural outdoor fluctuating temperatures than by artificial constant temperatures. For equal periods of cold storage the average percentage of larval emergence following cold storage at fluctuating outdoor temperatures (Figure 9(A)) was consistently higher than average percentages of larval emergence following cold storage at constant temperatures of either 32°F. (Figure 9(B)) or 10°F. (Figure 9(C)).

The previous comparisons and citations are presented to: (1) support results of the present study--that cold storage under natural field conditions was more efficient in satisfying diapause requirements of overwintering larvae than was cold storage under artificial conditions, and (2) review other instances where effects of fluctuating temperatures apparently have been more beneficial than constant temperatures on phases of development of the spruce budworm.

COLD STORAGE EXPRESSED AS DEGREE DAYS BELOW 42°F.

It did not seem entirely meaningful to express periods of cold storage at the outdoor insectary in terms of calendar days of cold storage since warm days probably did not contribute to diapause requirements of overwintering larvae. Therefore the period of cold storage at the outdoor insectary, represented as the abscissa of Figure 9(A), was transformed into degree days of cold storage below 42°F., and presented in Table 1. The following discussion is presented as an explanation of why this particular technique of expressing cold storage was used.

Many investigators have expressed the rate of development of plants and animals on the basis of so many developmental units, developmental degrees or degree days, above some specific temperature often referred to as a threshhold temperature or threshhold of development. In 1894 Merriam (30, p. 212) in describing geographical distribution of animals and plants in North America assumed a temperature of 6°C. (43°F.) to represent the onset of physiological activity in spring. He described the northward distribution of terrestrial animals and plants to be restricted by the sum of positive temperatures above 43°F. during the season of growth and

reproduction.

Specifically with the spruce budworm, McGugan (29, p. 439-440) and Harvey (20, p. 552) both used Developmental Units or degree-hours above 42°F. in expressing periods of incubation of spruce budworm eggs. Wagg (37, p. 10), studying environmental factors affecting spruce budworm growth in Oregon, used Merriam's principle of heat accumulation. He calculated degree days by subtracting the threshhold of development (42°F.) for grand fir from average daily temperatures. Degree days were then added to give accumulated degree days. Hanson (22, p. 2) states, "In the absence of radiant heat, an air temperature of 42°F. has been determined as the threshhold for commencement of movement of second stage larvae." He refers to spruce budworm larvae.

It seems logical that if spruce budworm larvae are activated by air temperatures above 42°F. and if environmental factors affecting their rate of development can be expressed as an accumulation of degree days above this temperature that the same principle could be applied to their period of inactivity below 42°F. In other words, the period of cold storage which satisfies diapause requirements of second-instar budworm in hibernacula could be expressed in terms of accumulated

degree days below 42°F.

Cool days in spring and early summer retard budworm development and are accounted for when development is expressed as an accumulation of degree days above 42°F. It seems reasonable to expect, therefore, that warm days in late fall and early winter would not contribute to diapause requirements during a period when cool temperatures are apparently desirable. If this is true, the ineffectiveness of warm days during this period would be accounted for if a period of cold storage were expressed as degree days below 42°F.

In summary, work of others with spruce budworm activity above 42°F., and the reasoning that principles of heat accumulation could be applied in accumulation of cold, led to the technique used in the present study--that of expressing periods of cold storage at outdoor fluctuating temperatures on the basis of accumulated degree days below 42°F.

CONCLUSIONS

1. Cold storage conditioning at either artificial constant temperatures or natural fluctuating temperatures will satisfy diapause requirements of some second-instar spruce budworm overwintering in gauze in Petri dishes and enable them to emerge in fall or early winter, i.e.:

A. Artificial cold storage of second-instar spruce budworm at constant temperatures of 10°F., or 32°F., for 40 or 48 days, respectively, will enable approximately 15 per cent of the larvae to emerge when placed in a rearing room at 70°F.

B. Cold storage under natural conditions of fluctuating outdoor temperatures at a field insectary for approximately 40-44 calendar days or for 248-280 degree days below 42°F. will enable approximately 22 per cent of the larvae to emerge when placed in a rearing room at 70°F.

C. Of A and B above, the latter is considered the most satisfactory cold storage treatment to satisfy diapause requirements of larvae overwintering in Petri dishes. This outdoor cold storage treatment is considered advantageous over an artificial constant temperature cold storage treatment because: (1) diapause requirements of a greater percentage of larvae

are satisfied, (2) larvae in cold storage are subjected to conditions very similar to those which they normally undergo in nature, (3) there is a lower incidence of larval mortality during the period of cold storage, and (4) no special cold storage facilities are required.

2. Cold storage conditioning at either artificial constant temperatures or natural fluctuating temperature will satisfy diapause requirements of some second-instar spruce budworm established in hibernacula on Douglas-fir bole sections and enable them to emerge in fall or early winter. However the attempt to express larval emergence from bole sections as a percentage of total larval populations established on the sections was considered to be unsuccessful. The attempt failed primarily because of (1) the inability to accurately assess larval populations by direct examination of the bole sections and (2) low larval populations established in hibernacula on the bole sections.

3. Percentages of larval emergence from hibernacula on Douglas-fir bole sections, the natural stratum, were not comparable with percentages from hibernacula in gauze in Petri dishes, the artificial stratum, where accurate determinations were made of percentages of larval emergence; and since bole sections must be

used in the field application of this research, no recommendations can be made or procedures given for fall sampling of overwintering spruce budworm populations in the northern Rocky Mountain region.

BIBLIOGRAPHY

1. Bergold, G. H. The polyhedral disease of the spruce budworm, Choristoneura fumiferana (Clem.) (Lepidoptera: Tortricidae) Canadian Journal of Zoology 29(1): 17-23. 1951.

2. Blais, J. R. Effects of the destruction of the current year's foliage of Balsam fir on the fecundity and habits of flight of the spruce budworm. The Canadian Entomologist 85(12):446-448. 1953.

3. Brown, R. C. (comp.). First quarterly report for the calendar year 1950. April 14, 1950. 15 p. (Unpublished report)(U.S. Dept. of Agric., Bureau of Entomology and Plant Quarantine, Forest Insect Laboratory, New Haven, Conn.)

4. Buchanan, W. D. Spruce budworm report 1946. February 19, 1947. 5 p. (Unpublished report)(U.S. Dept. of Agric., Bureau of Entomology and Plant Quarantine, Forest Insect Laboratory, Fort Collins, Colorado)

5. Buckhorn, W. J., P. W. Orr, and C. B. Williams, Jr. Report on forced emergence of overwintering spruce budworm larvae on units within the proposed 1958 Oregon control project. May, 1958. n.p. (Unpublished report)(U.S. Dept. of Agric., Forest Service, Pacific Northwest Forest and Range Experiment Station, Portland, Oregon)(Processed)

6. Carolin, Valentine M. Jr. Studies of the biological control of the spruce budworm in Oregon - 1950. November 20, 1950. 25 p. (Special report (Unpublished))(U.S. Dept. of Agric., Bureau of Entomology and Plant Quarantine, Forest Insect Laboratory, New Haven, Conn.)

7. Chapman, R. N. Animal ecology with especial reference to insects. New York, McGraw-Hill, 1931. 464 p.

8. Cole, Walter E. Spruce budworm population-damage study. 1957. 14 p. (Progress report)(U.S. Dept. of Agric., Forest Service, Intermountain Forest and Range Experiment Station, Boise Research Center, Boise, Idaho)(Processed)

9. Denton, Robert E. Investigations of the spruce budworm and its control: Some applications of the results of forced emergence of hibernacula. May 7, 1951. 8 p. (Progress report, season of 1950)(U.S. Dept. of Agric., Bureau of Entomology and Plant Quarantine, Forest Insect Laboratory, Coeur d' Alene, Idaho)

10. _____ Spruce budworm conference in Union, Oregon, June 9, 1951. July 25, 1951. 4 p. (Unpublished memorandum to the files)(U.S. Dept. of Agric., Bureau of Entomology and Plant Quarantine, Forest Insect Laboratory, Coeur d' Alene, Idaho)

11. _____ Studies of methods of improving the accuracy and general efficiency of spruce budworm surveys: 1951 progress report. April, 1952. 9 p. (Unpublished report)(U.S. Dept. of Agric., Bureau of Entomology and Plant Quarantine, Forest Insect Laboratory, Coeur d' Alene, Idaho) (Processed)

12. _____ The spruce budworm problem in the Northern Rocky Mountains. Northwest Science 27(2):67-72. 1953.

13. _____ Studies of methods of improving the accuracy and general efficiency of spruce budworm surveys: 1952 progress report. April 2, 1953. 7 p. (Unpublished report)(U.S. Dept. of Agric., Bureau of Entomology and Plant Quarantine, Forest Insect Laboratory, Coeur d' Alene, Idaho)(Processed)

14. _____ Entomological aspects of the 1953 spruce budworm control projects in the Northern Rocky Mountains. Progress report. 1954. 8 p. (Unpublished report)(U.S. Dept. of Agric., Bureau of Entomology and Plant Quarantine, Forest Insect Laboratory, Coeur d' Alene, Idaho)(Processed)

15. Dodge, H. R., T. T. Terrell and P. C. Johnson. Entomological phases of the 1955 Montana spruce budworm control project. November, 1956. 16 p. (U.S. Dept. of Agric. Forest Service. Intermountain Forest and Range Experiment Station, Ogden, Utah. Misc. pub. no. 9)

16. Dowden, P.B. and V.M. Carolin. Natural control factors affecting the spruce budworm in the Adirondacks during 1946-48. Journal of Economic Entomology 43(6):774-783. 1950.

17. Fettes, J.J. Investigations of sampling techniques for population studies of the spruce budworm on balsam fir in Ontario. Ph.D. thesis. Toronto, Ontario, Canada, University of Toronto, 1951. 212 numb. leaves.

18. Harvey, G.T. Absence of diapause in rearings of the spruce budworm. Canada Department of Agriculture Forest Biology Division Bi-monthly Progress Report 10(4):1-4. 1954.

19. _____ Studies of the diapause requirements of the spruce budworm. Paper read at the annual meeting of the Entomological Society of Canada, Lethbridge, Alberta, Oct., 1957.

20. _____ The occurrence and nature of diapause-free development in the spruce budworm, Choristoneura fumiferana (Clem.)(Lepidoptera:Tortricidae). Canadian Journal of Zoology 35:549:572. 1957.

21. _____ A relationship between photoperiod and cold-storage treatment in the spruce budworm. Science 128(3333):1205-1206. 1958.

22. Henson, W.R. Influence of meteorological factors on behaviour of the spruce budworm. Canada Department of Agriculture Forest Biology Division Bi-monthly Progress Report 4(6):1-4. 1958.

23. Jaynes, H.A. and C.F. Speers. Biological and ecological studies of the spruce budworm. Journal of Economic Entomology 42(2):221-225. 1949.

24. Lees, A.D. The physiology of diapause in arthropods. London, Cambridge University Press, 1955. 151 p.

25. Li, J.C.R. Introduction to statistical inference. Michigan, Edwards Brothers, 1957. 546 p.

26. Lindsten, A. and Kenneth H. Wright. Report on the 1949 western Oregon spruce budworm control project. March 30, 1951. 71 p. (Unpublished report)(U.S. Dept. of Agric., Forest Service, Pacific Northwest Forest and Range Experiment Station, Portland, Oregon)(Processed)

27. McComb, David. Spruce budworm ground appraisal survey procedures. July, 1957. 4 p. (Unpublished report)(U.S. Dept. of Agric., Forest Service, Intermountain Forest and Range Experiment Station, Forest Insect Laboratory, Missoula, Montana) (Processed)

28. McComb, David and T. T. Terrell. Status of the spruce budworm outbreak in the northern Rocky Mountain region, 1956. February, 1957. 17 p. (Unpublished report)(U.S. Dept. of Agric., Forest Service, Intermountain Forest and Range Experiment Station, Forest Insect Laboratory, Missoula, Montana) (Processed)

29. McGugan, B.M. Needle mining habits and larval instars of the spruce budworm. The Canadian Entomologist 86(10):439-454. 1954.

30. Merriam, C.H. The geographic distribution of animals and plants in North America. In: U.S. Dept. of Agriculture. The Yearbook of Agriculture 1894. p. 203-214.

31. Miller, C.A. The measurement of spruce budworm populations and mortality during the first and second larval instars. Canadian Journal of Zoology 36(3):409-422. 1958.

32. Prebble, M.L. The diapause and related phenomena in Gilpinia polytoma. Factors influencing the inception of diapause. Canadian Journal of Research 19(10):295-322. 1941.

33. Stehr. G. A laboratory method for rearing the spruce budworm, Choristoneura fumiferana (Clem.). (Lepidoptera: Tortricidae). The Canadian Entomologist 86:423-428. 1954.

34. Terrell, Tom T. Spruce budworm population densities in the Northern Rocky Mountain Region. June 6, 1957. 22 p. (Unpublished report)(U.S. Dept. of Agric., Forest Service, Intermountain Forest and Range Experiment Station, Forest Insect Laboratory, Missoula, Montana)(Processed)

35. _____ Survey of overwintering spruce budworm larval population Helena National Forest and Yellowstone National Park 1958. September, 1958. 3 p. (Unpublished report)(U.S. Dept. of Agric., Forest Service, Intermountain Forest and Range Experiment Station, Forest Insect Laboratory, Missoula, Montana) (Processed)

36. _____ Sampling populations of overwintering spruce budworm in the northern Rocky Mountain Region. March, 1959, 8 p. (U.S. Dept. of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station, Ogden, Utah. Research Note no. 61)

37. Wagg, J.W. Bruce. Environmental factors affecting spruce budworm growth. Corvallis, Oregon, Forest Lands Research Center, 1958. 27 p. (Research Bulletin No. 11)

38. Wellington, W.G. The light reactions of the spruce budworm. The Canadian Entomologist 80:56-82. 1948.

39. Wellington, W.G. and W. R. Henson. Notes on the effects of physical factors on the spruce budworm, Choristoneura fumiferana (Clem.). The Canadian Entomologist 79:168-170. 1947.

40. Whiteside, John M., C.P. Wessela and L.M. Compton. Report of the 1955 Oregon Spruce budworm control project. April 1, 1956. 60 p. (Unpublished report) (U.S. Dept. of Agric., Forest Service, Pacific Northwest Forest and Range Experiment Station, Portland, Oregon)(Processed)

41. Wright, Kenneth H., A. Lindsten and R. E. Stevens. Results of experiments to improve techniques for sampling overwintering spruce budworm populations in Oregon and Washington. May 20, 1952. 8 p. (Progress report)(Oregon State Board of Forestry, Salem, Oregon)(Processed)

APPENDIX

Table 2.--Summary of second-instar spruce budworm emerging from hibernacula in Petri dishes following periods of cold storage at 32°F.

Petri Dish No.	No. of Days in Cold Storage	No. of Larvae Alive Following Cold Storage	No. of Larvae Emerging Following Cold Storage	Per Cent Emergence Col. 4 ÷ Col. 3	Average Per Cent Emergence
263		100	-	-	
239		100	-	-	
108		124	-	-	
122	4	101	-	-	-
171		75	-	-	
228		141	-	-	
273		61	-	-	
90	8	141	-	-	-
195		60	-	-	
155		78	-	-	
169		38	-	-	
47	12	48	-	-	
270		72	-	-	
236		132	-	-	
248		107	-	-	
267	16	75	-	-	
225		95	2	2.10	
259		103	-	-	
186		83	-	-	
165	20	75	-	-	0.53
308		165	1	0.60	
36		78	1	1.28	
77		64	1	1.56	0.86
177	24	89	-	-	
159		97	1	1.03	
262		132	1	0.75	
185		80	-	-	
232	28	88	-	-	0.45

Table 2 - Continued

Petri Dish No.	No. of Days in Cold Storage	No. of Larvae Alive Following Cold Storage	No. of Larvae Emerging Following Cold Storage	Per Cent Emergence Col. 4 ÷ Col. 3	Average Per Cent Emergence
120		36	1	2.77	
54		-	-	-	
224		65	3	4.61	
274	32	21	1	4.76	3.04
41		167	8	4.79	
304		93	-	-	
83		108	1	0.92	
142	36	84	1	1.19	1.73
80		77	6	7.79	
229		26	1	3.84	
257		114	3	2.63	
288	40	150	16	10.66	6.23
10		116	2	1.72	
180		12	-	-	
315		52	3	5.76	
226	44	69	5	7.24	3.68
272		103	6	5.82	
84		75	17	22.66	
200		75	19	25.33	
105	48	19	4	21.05	18.72
158		114	-	-	
183		86	10	11.62	
13		14	2	14.28	
298	52	136	19	13.97	9.97
237		18	1	5.55	
160		88	2	2.27	
314		46	13	28.26	
107	56	89	7	7.86	10.99

Table 2 - Continued

Petri Dish No.	No. of Days in Cold Storage	No. of Larvae Alive Following Cold Storage	No. of Larvae Emerging Following Cold Storage	Per Cent Emergence Col. 4 / Col. 3	Average Per Cent Emergence
213		97	6	6.18	
130		85	1	1.17	
91		3	-	-	
208	60	95	5	5.26	3.15
32		102	5	4.90	
55		72	2	2.27	
18		120	2	1.66	
110	64	78	3	3.84	3.29
61		105	8	7.61	
246		108	17	15.74	
9		90	-	-	
277	68	136	11	8.08	7.86
222		110	1	0.90	
50		98	5	5.10	
133		123	7	5.69	
255	72	116	6	5.17	4.22
252		127	5	3.93	
23		114	6	5.26	
7		69	1	1.44	
44	76	98	13	13.26	5.97
74		111	1	0.90	
250		99	3	3.03	
45		105	2	1.90	
143	80	68	3	4.41	2.56

Table 3.--Summary of second-instar spruce budworm emerging from hibernacula in Petri dishes following periods of cold storage at 10°F.

Petri Dish No.	No. of Days in Cold Storage	No. of Larvae Alive Following Cold Storage	No. of Larvae Emerging Following Cold Storage	Per Cent Emergence Col. 4 ÷ Col. 3	Average Per Cent Emergence
135		94	-	-	
254		64	-	-	
279		61	-	-	
214	4	98	-	-	-
34		59	-	-	
43		76	-	-	
215		28	-	-	
104	8	25	-	-	-
33		59	-	-	
317		112	-	-	
241		88	-	-	
287	12	112	-	-	-
306		47	-	-	
82		12	-	-	
65		74	-	-	
216	16	104	-	-	-
271		71	2	2.81	
99		102	-	-	
94		24	-	-	
150	20	69	1	1.44	1.06
81		66	1	1.51	
211		40	-	-	
48		95	-	-	
230	24	16	-	-	0.38
3		81	-	-	
194		72	1	1.38	
251		54	-	-	
261	28	127	-	-	0.35

Table 3 - Continued

Petri Dish No.	No. of Days in Cold Storage	No. of Larvae Alive Following Cold Storage	No. of Larvae Emerging Following Cold Storage	Per Cent Emergence Col. 4 ÷ Col. 3	Average Per Cent Emergence
64		33	4	12.12	
20		56	-	-	
240		75	1	1.33	
40	32	108	1	0.92	3.59
114		50	4	8.00	
203		107	3	2.80	
265		86	17	19.76	
201	36	112	3	2.67	8.31
168		45	1	2.22	
268		120	2	1.66	
75		83	14	16.86	
115	40	18	7	38.88	14.91
173		72	-	-	
109		88	26	29.54	
4		91	6	6.59	
59	44	32	2	6.25	10.60
95		91	7	7.69	
175		98	1	1.02	
198		116	38	32.75	
290	48	57	5	8.77	12.56
179		76	2	2.63	
234		83	6	7.22	
112		149	8	5.36	
35	52	62	-	-	3.80
96		92	14	15.21	
258		81	22	27.16	
121		82	17	20.73	
282	56	127	5	3.93	16.76

Table 3 - Continued

Petri Dish No.	No. of Days in Cold Storage	No. of Larvae Alive Following Cold Storage	No. of Larvae Emerging Following Cold Storage	Per Cent Emergence Col. 4 ÷ Col. 3	Average Per Cent Emergence
266		119	5	4.20	
307		89	8	8.98	
70		129	5	3.87	
123	60	69	4	5.79	5.71
102		83	-	-	
146		23	-	-	
291		107	1	0.93	
164	64	84	2	2.38	0.83
26		-	-	-	
139		66	-	-	
157		52	1	1.92	
86	68	76	7	9.21	2.78
38		103	3	2.91	
156		69	-	-	
278		22	3	13.63	
8	72	65	1	1.53	4.52
17		50	-	-	
231		137	1	0.72	
161		88	-	-	
299	76	80	2	2.50	0.81
312		102	3	2.94	
128		34	1	2.94	
302		54	2	3.70	
311	80	115	1	0.86	2.61

Table 4.--Summary of second-instar spruce budworm emerging from hibernacula in Petri dishes following periods of storage in insectary.

Petri Dish No.	No. of Days in Insectary	No. of Larvae Alive Following Period in Insectary	No. of Emerging Larvae Following Period in Insectary	Per Cent Emergence Col. 4 ÷ Col. 3	Average Per Cent Emergence
136		37	-	-	
113		101	-	-	
245		83	-	-	
79	4	64	-	-	-
256		46	-	-	
15		48	-	-	
87		43	-	-	
116	8	125	-	-	-
293		116	1	0.86	
319		66	-	-	
2		103	-	-	
289	12	115	-	-	0.22
318		1	-	-	
100		73	-	-	
71		153	1	0.65	
281	16	22	-	-	0.16
1		153	1	0.65	
172		117	-	-	
283		86	1	1.16	
78	20	86	-	-	0.45
140		70	-	-	
42		132	6	4.54	
125		127	-	-	
119	24	70	-	-	1.14
166		95	-	-	
153		117	1	0.85	
264		175	1	0.57	
167	28	94	1	1.06	0.62

Table 4 - Continued

Petri Dish No.	Days in Insectary	No. of Larvae Alive Following Period in Insectary	No. of Emerging Larvae Following Period in Insectary	Per Cent Emergence Col. 4 ÷ Col. 3	Average Per Cent Emergence
85		83	7	8.43	
118		62	-	-	
218		130	1	0.76	
6	32	114	9	7.89	4.27
210		99	6	6.06	
152		82	11	13.41	
276		113	23	20.35	
60	36	55	2	3.63	10.86
309		65	21	32.30	
52		59	10	16.94	
103		92	11	10.86	
134	40	32	9	28.12	22.06
97		82	10	12.19	
197		119	20	16.80	
5		135	32	23.70	
300	44	136	49	36.02	22.18
196		75	11	14.66	
187		153	36	23.52	
280		101	7	6.93	
111	48	61	17	27.86	18.24
190		189	21	11.11	
37		120	8	6.66	
227		119	35	29.41	
170	52	104	8	7.69	13.72
22		123	37	30.00	
106		9	1	11.11	
285		172	20	11.62	
101	56	100	33	33.00	21.43

Table 4 - Continued

Petri Dish No.	No. Days in Insectary	No. of Larvae Alive Following Period in Insectary	No. of Emerging Larvae Following Period in Insectary	Per Cent Emergence Col. 4 ÷ Col. 3	Average Per Cent Emergence
76		38	2	5.26	
154		86	6	6.97	
25		112	7	6.25	
305	60	111	22	19.81	9.57
98		5	1	20.00	
275		92	17	18.47	
62		52	14	26.92	
199	64	43	4	9.30	18.67
217		44	10	22.72	
93		22	7	31.81	
151		136	6	4.41	
11	68	110	24	21.81	20.19
144		73	6	8.21	
243		148	26	17.56	
202		110	14	12.72	
131	72	111	25	22.52	15.25
191		66	15	22.72	
219		91	16	17.58	
209		76	18	23.68	
138	76	69	3	4.34	17.08
178		78	14	17.94	
127		101	61	60.39	
72	80	105	16	15.23	31.85

Table 5.--Summary of second-instar spruce budworm emerging from hibernacula on Douglas-fir bole sections following periods of cold storage at 32°F.

Log No.	No. of Days in Cold Storage	No. of Larvae Found in Hibernacula on Bole Section	No. of Larvae Emerging Following Cold Storage	Per Cent Emergence Col. 4 ÷ Col. 3	Average Per Cent Emergence
82		12	-	-	
116	4	7	-	-	-
7		2	-	-	
102	8	47	-	-	-
150		31	-	-	
140	12	61	-	-	-
26		16	-	-	
5	16	20	-	-	-
17		4	-	-	
92	20	14	-	-	-
72		15	-	-	
2	24	9	1	10.0	5.0
13		23	2	8.3	
139	28	37	4	9.8	9.1
73		17	-	-	
144	32	35	1	2.8	1.4
157		63	8	11.3	
56	36	22	1	4.3	7.8
119		38	-	-	
149	40	21	1	4.50	2.3
31		16	6	27.3	
84	44	14	-	-	13.7

Table 5 - Continued

Log No.	No. of Days in Cold Storage	No. of Larvae Found in Hiber-nacula on Bole Section	No. of Larvae Emerging Following Cold Storage	Per Cent Emergence Col. 4 ÷ Col. 3	Average Per Cent Emergence
64		12	-	-	
61	48	7	-	-	-
6		15	-	-	
36	52	26	-	-	-
9		5	-	-	
94	56	14	-	-	-
128		12	-	-	
50	60	9	-	-	-
104		9	-	-	
122	64	8	-	-	-
117		4	-	-	
93	68	10	2	16.7	8.4
160		12	6	33.3	
57	72	36	3	7.7	20.5
159		14	4	22.2	
156	76	19	4	17.4	14.8
75		12	-	-	
80	80	34	4	10.5	5.3
27		5	-	-	
34	84	21	2	8.7	4.4

Table 6. -- Summary of second-instar spruce budworm emerging from hibernacula on Douglas-fir bole sections following periods of cold storage at 10°F.

Log No.	No. of Days in Cold Storage	No. of Larvae Found in Hibernacula on Bole Section	No. of Larvae Emerging Following Cold Storage	Per Cent Emergence Col. 4 ÷ Col. 3	Average Per Cent Emergence
98		15	-	-	
22	4	15	-	-	-
33		13	-	-	
135	8	77	-	-	-
41		11	-	-	
25	12	5	-	-	-
70		10	-	-	
38	16	21	-	-	-
28		5	-	-	
151	20	27	-	-	-
40		26	-	-	
12	24	30	-	-	-
115		15	-	-	
4	28	16	-	-	-
43		15	1		
11	32	28	-	-	-
89		32	-	-	
138	36	32	-	-	-
65		7	-	-	
45	40	4	-	-	-
96		11	-	-	
58	44	15	-	-	-

Table 6 - Continued

Log No.	No. of Days in Cold Storage	No. of Larvae Found in Hiber-nacula on Bole Section	No. of Larvae Emerging Following Cold Storage	Per Cent Emergence Col. 4 ÷ Col. 3	Average Per Cent Emergence
67		17	-	-	
18	48	12	-	-	-
116		11	-	-	
10	52	62	-	-	-
35		13	-	-	
23	56	3	-	-	-
127		3	-	-	
137	60	19	-	-	-
21		8	-	-	
155	64	17	-	-	-
112		16	-	-	
32	68	26	-	-	-
111		8	-	-	
121	72	12	-	-	-
79		27	-	-	
44	76	26	-	-	-
53		4	-	-	
129	80	9	-	-	-
141		21	-	-	
88	84	38	-	-	-

Table 7. -- Summary of second-instar spruce budworm emerging from hibernacula on Douglas-fir bole sections following periods of storage at an outdoor insectary.

Log No.	No. of Days in Cold Storage	No. of Larvae Found in Hibernacula on Bole Section	No. of Larvae Emerging Following Cold Storage	Per Cent Emergence Col. 4 ÷ Col. 3	Average Per Cent Emergence
71		38	-	-	
147	4	32	-	-	-
19		14	-	-	
106	8	8	-	-	-
16		13	-	-	
146	12	40	-	-	-
48		13	-	-	
126	16	31	-	-	-
167		10	-	-	
69	20	20	-	-	-
20		40	1	2.4	
145	24	46	5	9.8	6.1
133		5	-	-	
131	28	7	-	-	-
105		16	-	-	
118	32	26	-	-	-
8		7	-	-	
152	36	56	2	3.4	1.7
154		48	1	2.0	
29	40	34	-	-	1.00
142		22	-	-	
109	44	81	5	5.8	2.9

Table 7 - Continued

Log No.	No. of Days in Cold Storage	No. of Larvae Found in Hibernacula on Bole Section	No. of Larvae Emerging Following Cold Storage	Per Cent Emergence Col. 4 ÷ Col. 3	Average Per Cent Emergence
77		17	-	-	
39	48	26	1	3.7	1.9
114		25	1	3.8	
143	52	30	-	-	1.9
59		8	-	-	
3	56	12	2	14.3	7.2
100		14	1	6.7	
46	60	17	-	-	3.4
37		39	1	2.5	
52	64	6	-	-	1.3
30		29	6	21.6	
153	68	54	4	6.9	14.3
110		28	-	-	
108	72	5	-	-	-
49		20	1	4.8	
168	76	21	-	-	2.4
14		26	1	3.7	
76	80	11	-	-	1.9
107		10	-	-	
95	84	4	2	33.3	16.7