SOME ENVIRONMENTAL FACTORS AFFECTING DEVELOPMENT AND EMERGENCE OF THE DOUGLAS-FIR BEETLE, *DENDROCTONUS PSEUDOTSUGAE* HOPKINS (COLEOPTERA : *SCOLYTIDAE*), IN THE LABORATORY

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ROGER BAKER RYAN

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Redacted for privacy

Associate Professor of Entomology

In Charge of Major

Redacted for privacy

Chairman of Department

Redacted for privacy

Chairman of School Graduate Committee

Redacted for privacy

Dean of Graduate School

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INTRODUCTION

Why and How This Study Started

This paper is a result of a project headed by Dr. Julius A. Rudinsky, Associate Professor of Entomology at Oregon State College, to develop and perfect mass, laboratory rearing methods for forest insects. The Douglas-fir beetle, because of the re-emphasis of its importance as an enemy of the number one timber producing tree in the country by its recent outbreaks in the Pacific Northwest, was to be given top priority in a proposed insecticide screening program. The uncertainty of a supply of beetles at certain seasons of the year gave impetus to these studies on rearing methods. Two publications (41, 51), one on the effects of temperature upon the activity and the behavior of the beetle and the other a study of the development of the insect, were a result of the early investigations of this project. Rudinsky and Vité in side studies found that laboratory-reared broods of the Douglas-fir beetle failed to emerge from the logs in which they had been reared. In these studies after developing to the adult stage, the beetles were allowed to remain in the warm environment of the laboratory where after more than two months they still had not emerged[1]. As an inquiry into some of

1. Personal communication.

the possible reasons for this, the author launched this study on some of the environmental factors which affect the development and emergence of the Douglas-fir beetle.

Life Cycle Under Natural Conditions

The life cycle of the Douglas-fir beetle under natural conditions has been described many times (7, p. 7-9; 39, p. 2-3; 52, p. 6-8). Barring differences in attack periods due to climatic conditions, there is general agreement, so only a few salient points will be stressed here. The beetle has one generation per year. The broods pass the winter either as young adults, full-grown larvae, or pupae, however, predominately as adults. The young adults emerge in early spring and attack healthy or weakened Douglas-fir trees and establish broods. Shortly thereafter many of these beetles re-emerge, reattack another tree and establish a second brood, after which they die. Their first brood usually develops to the adult stage by mid-summer and remains under the bark through the rest of the summer and through the fall and winter, emerging in the early spring.

Those beetles that overwinter as larvae or pupae complete their development by mid-summer when they emerge from the tree and establish broods in another tree, remaining there through the fall and winter to re-emerge, reattack and establish a second brood in the early spring. Their first broods reach the late larval or pupal stage by late fall and overwinter in this stage.

The Problem and Possible Solutions

The problem, of course, is to determine the best and quickest method of obtaining large numbers of mature Douglas-fir beetles. The groundwork for laboratory rearing of this beetle, the logical means to this end, was laid by Vité and Rudinsky with their studies on the development of this insect (51, p. 156-157). Their method of rearing seemed to be a good one, but the failure of the adult beetles to emerge from the logs and establish broods, indicated that there is a complex, internal, regulatory mechanism controlling the maturation of the beetles. They state that after being stored outside for a portion of the winter the beetles would, however, emerge.

> "Emergence from logs brought into the greenhouse in November began after two weeks' exposure and continued over four weeks, with peak emergence at the end of the second week. The time of exposure necessary for emergence of the beetles at 22^{0} C. decreased in reverse ratio to the length of the insects' hibernation."

Obviously some process took place inside the beetle's body while the logs were stored outside that was not happening in the laboratory. The nature of these responses seem characteristic of diapausing insects. Although coined by Wheeler (55, p. 68) to apply to the period of rest between anatrepsis and katatrepsis in a developing embryo, the word diapause has gradually taken on a different connotation. It was first used to apply to the arrest of growth, whether in the egg or in the developing insect, by Henneguy (24, p. 424-427).

Andrewartha (3, p. 52-53) regarded development as having two distinct phases:

> "In considering diapause it is helpful to think of development in terms of its morphological aspect, morphogenesis, and its physiological aspect, which by analogy may be called physiogenesis. Diapause may then be considered (at least for ecological purposes) as a stage in physiogenesis which must be completed as a prerequisite for the resumption of morphogenesis. In the more complicated or intense diapause a whole chain of stages in physiogenesis may need to be completed before morphogenesis may be resumed. We shall therefore speak of the diapause stage, meaning that stage in the life cycle during which morphogenesis is more or less at a standstill; and we shall use diapause development to mean the physiological development, or physiogenesis, which goes on during the diapause stage in preparation for the active resumption of morphogenesis."

It is in this sense that these terms shall be used here. Therefore, when stored outside for part of the winter the Douglas-fir beetles were undergoing physiogenesis or diapause development which, as Andrewartha pointed out, must have been completed before morphogenesis could be resumed.

Lees (34, p. 63-64) has pointed out that the action of temperature on diapause development may be regarded as having two distinct optimums, a low temperature optimum where the process is initiated, and a higher temperature optimum where the final stages are completed. The more time, longer than a certain critical minimum, spent at the lower temperature the shorter would be the time required at the higher temperature. This seems quite analagous to the responses of the Douglas-fir beetle to longer periods of exposure to outside conditions as observed by Vité and Rudinsky. The certain critical minimum exposure to low temperature evidently required, was not satisfied in their laboratory rearings, but was satisfied when beetles were stored outside.

On the basis of Vité and Rudinsky's work, we might say then that the Douglas-fir beetle exhibits an imaginal diapause which probably is terminated by a sufficient cold rest period. As previously stated, however, in the brief summary of the life history of this insect under natural conditions, not only adults, but also larvae and pupae overwinter. These transform to the adult stage but do not go into a diapause state as adults. They emerge and attack that very same season without having to undergo a cold rest period. If they had an imaginal diapause they would have to undergo a winter before being ready to attack.

Is there any explanation for these apparently contradictory phenomena?

Possibly, under natural conditions, the insect does not go into a true diapause, but merely that cold weather of fall overtakes it and slows down morphogenesis before it has a chance to emerge; whereas, in the summer the weather is sufficiently warm to allow completion of morphogenesis, which results in emergence. The available evidence hardly favors this view. This hypothesis would not explain the inverse relationships found to exist between the length of cold rest and the length of the period subsequent to return to favorable temperatures that it took for emergence. Nor would it explain the failure of laboratory-reared beetles to undergo continuous development.

In some insects diapause is not realized in each generation. The onset of facultative diapause, as this is called, is influenced by environment and may be either induced or averted by proper manipulation of the various factors of the environment (34, p. 5). If this is the case, one would reason that under natural conditions the environment of late summer induces diapause whereas that of late spring or early summer does not. Also those conditions in the laboratory where the beetles were reared induced diapause. If this is true with the Douglas-fir beetle, the continuous rearing of the insect in the laboratory will be a simple task once the conditions which induce diapause are determined and are avoided in the rearing.

Another possibility exists to explain why laboratory-reared adults and overwintering adults of the wild population go into diapause while adults from overwintering larvae and pupae do not.

Perhaps the insect is truly univoltine with a strict diapause in each generation but that conditions which serve to terminate diapause are effective when applied either to the larval or to the adult stages. Overwintering larvae as well as adults after experiencing these conditions may then develop and emerge without interruption. Laboratory-reared insects, on the other hand, never having been exposed to these conditions would be forced into diapause once they reached the adult stage.

The processes involved in the arrest of growth and the subsequent resumption of morphogenesis are not entirely known. Excellent works by Andrewartha (3) and Lees (34) have summarized some of the latest knowledge on the subject, and works by Williams (62) and Van der Kloot (50) have aided materially in our understanding of the physiology of diapause. As Lees (34, p. 2) states, "It is now well known that both the arrest of growth and the accompanying metabolic adjustments are governed by the organs of internal secretion. These endocrine centers are in turn responsive to certain definite stimuli from the environment."

We shall now consider some environmental factors which have been known to act as stimuli to these endocrine centers. Photoperiod has proven to be a regulatory mechanism in the life cycle of some insects, principally insects which are continually exposed to the light such as external feeding Lepidoptera. Since they remain under the bark, probably photoperiod can be discounted in the case of the Douglas-fir beetle, for undoubtedly the bark acts as an efficient block for all visible light rays except possibly an infinitesimal amount seeping through entrance and ventilation holes.

In some insects the characteristics of the food injested induces diapause. Thus, when larvae of Pectinophora gossypiella Saund. are fed on green cotton bolls they develop without arrest; while those fed on ones containing ripe black seeds entered diapause (45). It is unlikely that the chemical composition of the inner bark on which the Douglas-fir beetle feeds is in any way, except in those affected by moisture content, correlated with a certain season of the year. Probably there is a progressive deterioration from the time of attack until that when the bark is unsuitable for beetle development. Beetles in trees attacked at any month of the year all show the same strictly univoltine characteristics.

Humidity of the air and moisture content of the bark would, on the other hand, be correlated to the season of the year. Since the general area where these studies were conducted receives most of its precipitation in the winter (49), the environment of the beetles, i.e. the inner bark of the logs, would tend to be more moist during the winter than in the summer. Could the more moist conditions during the winter, then, serve to terminate the diapause state? If so, this hypothesis would not explain the diapause in the laboratory-reared beetles since the logs were continually kept in quite a humid atmosphere. There are cases in the literature where apparently the addition of water has served to terminate diapause. However as pointed out by Lees (34, p. 74), "Many insect larvae which enter diapause with an increased proportion of dry matter restore the water balance in the post-diapause period by imbibing moisture from their surroundings. If these insects are denied contact water the resumption of growth may be delayed or even prevented indefinitely. However, their condition is now one of quiescence for they are soon reactivated if water is supplied. Because of this effect water has often been mistaken for the agency which terminates diapause." Church (14, p. 85-97) has shown this to be the case with Cephus cinctus.

The most likely environmental factor controlling the inception and termination of diapause is temperature. Lees, considering arthropods in general (34, p. 53), states, "....this factor is by far the most important environmental agency controlling termination of diapause...." The lack of low temperature would explain the failure of laboratory-reared beetles to emerge and also the reactions of the wild population.

It was, therefore, hypothesized that the Douglas-fir beetle is strictly univoltine in nature because of the low temperature requirements for diapause and, also, that a cold rest period would terminate the diapause if applied to the larval, pupal or adult stage.

Brief History of Rearing Bark Beetles

There is little information in the literature about mass rearing of bark beetles in the laboratory. Most of the references on the subject refer to "rearing" in one of two ways. In one it entails the mere collection of naturally infested material in the field and taking it into the laboratory where the insects are collected as they emerge. The other consists of the precise rearings and observations of only a relatively few insects in connection with life history and other studies.

Griswold (22), however, described a mass, laboratory rearing method for Scolytus multistriatus (Marsh.) and Hylurgopinus rufipes (Eich.) in connection with Dutch elm disease investigations. Elm wood was artificially infested in the laboratory where the beetles developed satisfactorily at a temperature of 78° F. and a humidity of 50 to 60 percent. A critical factor in the success of the rearing was the condition of the host wood, best results being realized in Scolytus rearing with wood seasoned ten or fifteen days but for Hylurgopinus no seasoning was required before beetle introduction. After developing in the rearing room adults would start to emerge in about five and eight weeks for Scolytus and Hylurgopinus, respectively. Obviously no diapause complicated the rearing of this insect.

MATERIALS AND METHODS

Obtaining Beetles

The beetles used in this study were from three sources. The greatest number were obtained from Oregon State College MacDonald Forest approximately five miles north of Corvallis, Oregon by cutting 10- to 15-inch Douglas-fir trees during early spring of 1956 and 1957. The trees were limbed and allowed to lay as they fell through the spring and summer months. Thus, they were exposed to and attacked by the overwintering beetles as they emerged in April and May of each year. Where the trees were exposed to the direct rays of the sun and after they had been attacked they were covered with branches to protect the developing broods from excessively high temperatures. In the fall the trees were bucked into approximately 3-foot sections, and stored in the insectary until used.

The other two sources of beetles were as follows. In 1956 sections of an infested windfall from the Mary's Peak area, 20 miles west of Corvallis, Oregon, were stored and used along with those from MacDonald Forest. In August 1957 one standing infested Douglas-fir from an area 10 miles west of Philomath, Oregon was used for experiments in that fall. The infested logs were sealed on both ends with paraffin to conserve the moisture within as much as possible.

As beetles were needed throughout the ensuing winter and spring the logs were brought into the laboratory and placed inside plastic-screened cages. After being in the 75° F. heat of the laboratory for periods of from 1 to 16 days the beetles would start to emerge from the logs. They collected on the sides and top of the cages where they were easily gathered either in a screw capped mason jar fitted with a screen cover or petri dishes.

Cages

The framework of the cages consisted of 2-inch by 2-inch Douglas-fir lumber nailed together, strengthened at the corners by screwing on "L" shaped corner braces. Sixteen mesh plastic screening was stretched around the outside of the four sides of the framework and tacked in place. This left the top and bottom open. A cover was made of the same 2-inch by 2-inch wood to the exact length and width dimensions of the cage and screening tacked over the top. The cover rested on top of the cage and was prevented from moving sideways by laths tacked to the inside of the top framework and which hung down so that half their width extended into the cage. These, therefore, held the top securely in position as well as prevented any beetles from getting between the cage and the top.

The bottoms of the cages were purposely left unscreened in order to facilitate caging and uncaging logs. If screening completely enclosed the cage except for the top it would necessitate lifting the logs up and over the side and setting them down into the cage. Whereas, with the bottom open it was a simple matter to roll the log in place and set the cage down over it. This was especially appreciated when handling the larger logs. The bottom of the cage was constructed so that it set perfectly flat. Strips of foam rubber about 1/2 inch by 1 inch were nailed to the bottom of the cages to compensate for the unevenness of the floor and greenhouse tables upon which the cages were placed. When set on greenhouse tables a sheet of heavy brown wrapping paper was placed underneath the cage to prevent beetles from escaping through the cracks between the boards.

The cages proved entirely satisfactory. Only one small improvement could be suggested to future users of cages of this design. At 75° F. the beetles regularly take flight. When many beetles emerge from a log in one day in a cage of this design it is difficult to prevent escape of a few beetles while collecting them. Those that did escape in this manner flew to the light and were easily caught. Cheesecloth sleeves were fastened in place in two cages to see if they would make collection easier, however, the beetles easily chewed through these and escaped. These were abandoned. Should one wish to raise these beetles in large numbers it would facilitate collection if the emergence cages were designed so that they were completely enclosed with dark material to keep out the light except for a glass collection jar. Since, through the normal range of temperatures the beetles are positively phototropic they would collect at the only light source, the collecting jar.

Obtaining Attack

The method of obtaining attack was similar to that employed by Vité and Rudinsky (51, p. 157). Beetles obtained from infested logs as described in the section "Obtaining Beetles" were simply placed in a cage with a 3-foot section of Douglas-fir which had been stored in a cool place after being cut no more than four months previously in the preliminary experiments and one month previously in the final experiments. Beetles could be stored in petri dishes at a temperature of 35° F. for several weeks before introduction into the logs without suffering any noticeable loss of fecundity.

The logs were sealed on one, both, or neither of the ends with paraffin. In the preliminary experiments both ends were sealed and the logs were placed in cages during beetle development, however, these logs seemed to dry out excessively resulting in a high larval mortality. In the final experiments the logs were sealed on one end only, and the other was placed in moist sand.

The rearing room at the entomology farm at Oregon State College had windows at one end only. To prevent uneven distribution of attack around the log the windows were sealed with heavy brown paper while beetles were being introduced. When they were introduced in the greenhouse there was some concentration of attack because of the proximity of the greenhouse room used to the head house. When a relatively few beetles were introduced attack was heaviest on the side away from the head house. Most of the beetles bored in within 24 hours.

Eliminating Parasites and Predators

The method of introducing adult beetles described in the previous section eliminated all parasites and predators of the beetle with the exception of various species of mites and nematodes.

A few experiments were conducted in which mites were cleaned off the beetles before introduction in order to evaluate their predacity. The beetles were held gently under a binocular dissecting microscope with a forceps while small mites were removed with a blunt dissecting needle dipped in alcohol. Large mites were removed with sharp pointed forceps. However, for most of the introductions it was felt that time spent manually cleaning mites from all beetles could be more profitably spent along other lines. No effort was made to introduce nematode-free beetles. Nematodes were observed under the elytra of the insects, a most difficult place for removal without inflicting injury to the beetles. Also, one or more endoparasitic nematode species were present, the difficulty of removing which is obvious.

Maintaining Desired Environmental Conditions

Temperature - From the time of introduction of adult beetles into a log until their progeny developed sufficiently so that simulated winter conditions could be imposed upon them, the logs were maintained at an average temperature of 75° F. with fluctuations usually not exceeding 5° F. in the preliminary experiments and 3° F. in the final.

Low temperatures were obtained by several series of walk-in coolers maintained by the Departments of Horticulture and Food and Dairy Technologies at Oregon State College. Also used were the walk-in cooler at the Forest Insect Laboratory and a temperature cabinet built by the Oregon State College Physical Plant. The latter was constructed 4 feet by 4 feet by 4 feet of 3/4 inch plywood and heated by four light bulbs in series with a thermostat, and in which was placed a small electric fan that ran constantly, circulating the air. The cabinet was placed in a walk-in cooler where its heating unit maintained its temperature 5° F. above the ambient temperature. A Foxboro hygrothermograph, checked by a hand-aspirated psychrometer, model HA-2 of The Friez Instrument Division of the Bendix Aviation Corporation and several mercury-filled glass thermometers of the Owens-Illinois Company, were used to check the temperature and humidity in the various coolers and in the rearing room. Temperature fluctuations of the coolers generally did not exceed 2° F.

Moisture and humidity - Maintaining the phloem region at a desired moisture level was attempted in several ways. First, logs as freshly cut as possible were used for raising the beetles. In the preliminary experiments both ends of the logs were sealed with paraffin flowed onto the ends while in a liquid state with a paint brush. The object of this was to prevent, as much as possible, any evaporation from the cut ends of the logs, and any moisture loss would then have to pass through the corky layers of the outer bark. In the final experiments only one end of each log was thus sealed and the other was placed in moist sand. Water was conducted upward, keeping the phloem moist. Finally, the logs were kept in a room maintained at a relative humidity of approximately 60 percent. It was felt that the humid environment would further prevent evaporational losses. The relative humidity was maintained by means of one or two electric fans blowing on a wall which was kept wet by the constant dripping of water. Water from a garden hose slowly trickled into a shallow wooden trough inclined at a slight angle to the horizontal. Along the bottom of the trough a series of small holes had been drilled about every 4 inches to allow the escape of the water. Thus, the water dripped evenly for the length of the wall. By manipulating the number of fans running and the opening of the window vents in the room a fairly constant humidity could be maintained.

Debarking Proceedure

After the various cold rest periods the logs were again placed at 75° F. to observe the emergence of the beetles. For at least 45 days in the preliminary experiment and 30 days in the final experiment the cages were examined daily and all emerged beetles were collected. Emergence data and other pertinent information were recorded on a 3-inch by 5-inch index card thumb tacked to each log. At the end of this period the logs were debarked. A single cut was made with hammer and chisel through the bark longitudinally and the bark carefully pried loose. All alive adult insects were counted.

Rearing Individual Beetles

In two experiments vials were employed to rear and observe the development of individual beetles similar to the method recommended by L. H. McMullen[1]. Each vial (Fig. 1) was filled about two-thirds full of inner phloem of Douglas-fir which had been ground in a Waring blender and wrung to a moist consistency in cheesecloth. The vial was stoppered with a plug of glass wool. This was found to be very suitable to observe the development of individual insects. When it was desired to place a pupa in a vial it was a simple matter to construct a pupal cell for it by means of a dissecting needle with the tip bent at about a 100 degree angle about 1/4 inch from the tip. This tool could be inserted through the mouth of the vial and could be used to pack the phloem in an area next to the glass. After the pupa was placed in, the upper part of the pupal cell was completed, leaving enough room for the adult to spread its wings. A strip of masking tape was placed about the neck of the vial on which the time and date of transformation to the adult stage were recorded.

Fig. 1. Vial used for individual rearings.

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1. Presented at the Seventh Annual Western Forest Insect Work Conference, December 1955, Spokane, Washington.

Determining Moisture Content

Frequently it was desirable to determine the moisture content of the inner bark of the logs or of the ground bark used for individual rearings. A small sample of the material was placed in a 2- or 3-dram vial and weighed on a chain-drop balance to the nearest .001 gram. The vial was then placed in a 90° F. oven and dried to a constant weight. By subtracting the weight of the vial before and after drying the sample the wet weight and dry weight of the samples were obtained. Moisture percent was then calculated according to the formula

$$\frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \times 100 = \text{Moisture Percent.}$$

Statistical Treatment Used in Evaluating the Effectiveness of the Various Cold Rest Treatments

Undoubtedly, the ideal way to evaluate the effectiveness of a cold rest treatment would have been to observe the insects until all had either terminated diapause and emerged from the bark or had died. But in these experiments practicality precluded any such effort. It was decided to express the effectiveness of the cold rest treatments in terms of the percentage of insects which emerged in a given period following treatment. In the preliminary experiments 45 days were used; in the final experiments 30 days were used.

It was more difficult to compare mathematically the rate of termination of diapause in the populations. For, at the end of the period, an equal percentage of the insects may have terminated diapause and emerged from the logs, and yet the rate at which the beetles emerged may have been quite different. In some cases there was no well defined peak emergence which could be used to compare all treatments. Had the insects been observed until all had emerged the mean day of emergence would have been an appropriate statistic to use. For some of the cold rest treatments, especially the most effective ones, the cumulative percentage of emergence when plotted against the number of days after the cold rest period on a log scale, approximated a straight line. Regression analysis in these cases could have been used. However, for many of the treatments this method of presentation seemed inappropriate since straight lines were obviously not formed. One reason was that since eggs are laid over a period of several weeks, all the beetles were not of the same age at the time of the cold rest treatments, and therefore the populations were not homogeneous in respect to their responsiveness to the cold rest.

It was decided that the day of median emergence, or the day by which 50 percent of the beetles had terminated diapause and emerged from the bark, was the best expression of the rate of diapause termination in the populations. But this statistic fails to express the scatter of the individuals, so for this a plot of the cumulative percent of emergence over the number of days after treatment must be referred to.

EXPERIMENTS AND RESULTS

The Nature of the Response to Normal Winter Temperatures

Logs were cut and naturally infested at the Oregon State College MacDonald Forest in the early spring of 1956. The infested logs remained in the forest throughout the spring and summer, and by late August all beetles were in the adult stage. The logs were then transferred to the insectary at the Oregon State College farm, and there left exposed to the outside temperatures throughout the winter (see APPENDIX, Table 8).

Starting December 7, 1956 a log was removed at approximately one-month intervals until emergence began on April 3, 1957. The logs were caged in the rearing room at a temperature of 75° F. Table 1 and Figure 2 show the emergence for the five logs used in this experiment.

Although the number of days from the first to the last insect emerging from any one log depends to a certain extent on the number of insects, it is shown in Figure 2 that this spread of emergence generally varied inversely with the duration of exposure to outside temperatures. Thus, emergence from the logs lasted for 28, 19, 12, 15 and 6 days. Evident from Table 1 and Figure 2 is the fact that the length of time necessary at 75° F. varied in the same inverse relationship with the exposure.

Table 1. Summary of emergence of wild beetles from logs caged at 75° F. following outside storage for different lengths of time.

Log no.	Stored outside until	Emergence - No. days after caging																																											Total No. emerged	Day of median Emergence
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43		
1	Dec. 7																5			12	12	8	4	7	9		10	6	1	4		2		1			1							1	83	22.0
2	Jan. 2											15			25[2]	18	5	23	14	4		4[1]						4[2]		1[1]															113	15.3
3	Jan. 31						2	3	5	20	28	22	28	14	2	2	3	3																											132	10.4
10	Feb. 28			1	21	52	76	28	19	12	6	7	4	3		5[1]		1																											235	5.5
16	Mar. 30	1	1	6	2	1	1																																						12	2.7

1. Represents the emergence of two days.

2. Represents the emergence of three days.

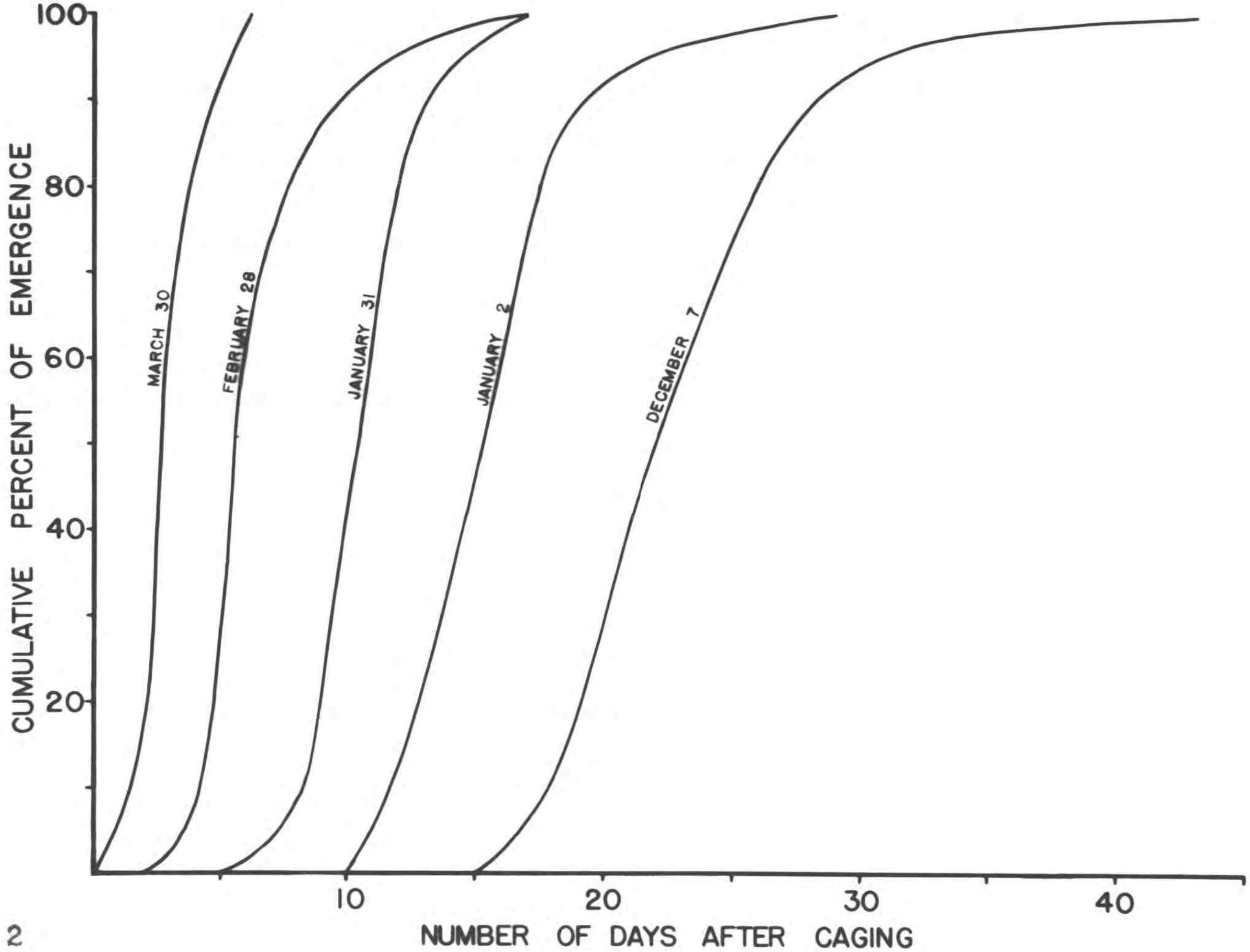

Fig. 2

Emergence of wild beetles from logs at 75° F. following outside storage for different lengths of time.

The Role of Temperature in the Termination of Diapause in Laboratory-Reared Beetles

From 20 to 40 beetles were introduced into each of a series of 45 logs in accordance with the method previously described. These logs were then kept in a rearing room maintained at 75° F. and a relative humidity of approximately 60 percent while the broods developed. The logs were previously sealed on one end with paraffin; the other was placed in moist sand while the broods developed. Periodic sampling of other logs maintained at the same temperature showed that most of the brood would be in the adult stage about 75 days after introduction of the parent beetles. After a brief maturation period, a cold rest was inflicted on the insects by transferring the logs to a walk-in cooler. After this they were returned to the 75° F. room and placed in cages so that the insects could be collected as they emerged.

Preliminary experiments (see APPENDIX) had indicated that a cold rest period plays an important role in the termination of diapause in the Douglas-fir beetle. Cold rest periods of 20, 50, and 90 days at temperatures of 33, 43, and 54° F. were applied to the beetles 90 and 110 days after the date the parent beetles were introduced into the logs. The 43° F. temperature was far more effective in terminating diapause than either the 33 or the 54° F. temperatures. At 43° F. both the 50- and 90-day cold rest periods satisfied the diapause requirements of most of the beetles, with the 90-day period causing slightly accelerated emergence compared to the 50-day period. The 20-day cold rest period, however, was relatively ineffective in causing beetle emergence during the 45 days subsequent to the cold rest. Identical cold rest treatments were applied to the different aged beetles to ascertain if the termination of diapause was influenced by the age of the beetles. Slight differences were noted, but because of small temperature fluctuations beyond control during the developmental and cold rest periods these differences could not be called significant. The results of these preliminary experiments are to be found in Table 9 and Figures 12 and 13 in the APPENDIX.

With this information as a basis, a new series of cold rest treatments were applied to the Douglas-fir beetle in order to establish more conclusively the beetle's response to various cold rest treatments. Each of the 45 logs in this new series received a different treatment. Beetles which had developed for 40, 80, and 120 days were exposed 20, 50, and 90 days to temperatures of 33, 39, 43, 49, and 55° F. The temperatures and duration of cold rest were the same as in the preliminary experiments with the addition of two extra temperatures, 38 and 48° F. In order to bring out more clearly the difference that the age of the beetles had on the effectiveness of the cold rest treatments as indicated in the preliminary experiments, 80- and 120-day-old beetles were used instead of 90- and 110-day-old beetles. Finally, to test the hypothesis that the cold rest would terminate the diapause in the immature stages as well as in the adults, 40-day-old beetles were used. According to Vité and Rudinsky (51, p. 161) the 40-day period at 75° F. would be just sufficient development for the first eggs laid to reach the adult stage and most of the brood would therefore be in the last larval or pupal stage.

As column 2 of Tables 2, 3 and 4 indicate only 20 beetles were introduced into each log for the first part of the series because of the scarcity of beetles at that time. It was felt that 20 per log, if mortality of the brood was not excessive, would yield a sufficient number of offspring to evaluate the effectiveness of the cold rest treatments. For the latter part of the series 40 beetles per log were introduced because there were more available and to utilize the logs more completely.

The results of this experiment are shown in Tables 2, 3, and 4 and in Figure 3. As indicated in Table 2 the broods in logs 76 through 90, which were mostly in the late larval and pupal stages at the time of cold rest, almost always failed to emerge during the 30-day period following the cold rest. Because of this, the data in Table 2 were omitted in Figure 3. Most of the adults which did emerge from logs 76 through 90 were believed to be the re-emerging parent beetles. Only in logs 89 and 90 at the higher cold rest temperatures for the 90-day cold rest are there indications of brood emergence. Under these conditions probably morphogenesis as well as diapause development could proceed sufficiently so that the beetles would mature during the subsequent 30 days at high temperature. It is this writer's belief that had the ensuing period at high temperature been extended, some of the other logs would have shown emergence also.

Table 2. Summary of emergence from logs to which cold rest periods were applied 40 days after introduction of parent beetles.

1	2	3	4	5																														6	7	8	9	10
				Emergence at 75° F. - Number of days after cold rest																																		
Log no.	No. beetles introduced	No. days cold rest	Temp. of cold rest °F.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total brood emerged	No. debarked	Total no. brood and unemerged old adults	Percent emerged by 30 days	Day of median emergence
76	20	20	33		1					1[1]																								0	84	84	0	>30
75	"	"	39		1[1]																													0	131	131	0	"
79	"	"	43											1[1]																				0	0	0	0	—
74	22	"	49		1[1]		1[1]																											0	239	239	0	>30
73	20	"	55		1[1]																													0	112	112	0	"
80	"	50	33																															0	89	89	0	"
81	"	"	39																				1[1]											0	258	258	0	"
83	"	"	43																															0	28	28	0	"
84	"	"	49																															0	58	58	0	"
85	"	"	55	1[1]																														0	156	156	0	"
86	"	90	33																															0	1	1	0	—
87	"	"	39															1[1]																0	80	80	0	>30
88	"	"	43																															0	35	35	0	"
89	"	"	49																										1	1	2	3		7	162	169	4.1	"
90	"	"	55																	2[1]						1[1]				1	1	2		4	117	121	3.3	"

1. Assumed to be a re-emerging parent.

Table 3. Summary of emergence from logs to which cold rest periods were applied 80 days after introduction of parent beetles.

1	2	3	4	5																														6	7	8	9	10
Log no.	No. beetles introduced	No. days cold rest	Temp. of cold rest °F.	Emergence at 75° F. - Number of days after cold rest																														Total brood emerged	No. debarked	Total no. brood and unemerged old adults	Percent emerged by 30 days	Day of median emergence
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30					
91	20	20	35																			1[1]												0	46	46	0	>30
92	"	"	39																															0	188	188	0	"
93	"	"	44																															0	134	134	0	"
94	"	"	50																															0	39	39	0	"
95	"	"	55															1[1]																0	140	140	0	"
96	"	50	35																															0	18	18	0	"
97	"	"	39												2	1	1	6	3	4	10	2	5	2	7	1			2	1	1		4	52	73	125	41.6	"
98	"	"	44									1[1]	1[1]					1	3	3	1	1												9	1	10	90.0	16.2
99	32	"	50	1[1]		1[1]							1		3		5	14	17	38	30	34	37	35	20	31	17	20	14	12	8	13	10	357	315	672	53.1	28.0
100	40	"	55				1[1]			1[1]			4		7	2	3	6	5	5	12	4	12	7	8	12	8	11	6	7	10	7	13	147	424	571	25.7	>30
101	"	90	33									2		5	7	10	8	7	5	13	12	6	7	9	14	7	8	5	11	13	6	11	5	171	473	644	26.6	"
102	"	"	39						1	7	5	17	27	41	50	40	29	34	20	23	6	13	12	8	7	5	7	4	2	3	3	3	7	374	29	403	92.8	13.4
103	"	"	43							3	2	4	6	16	40	33	54	76	54	64	46	42	28	28	10	9	12	7	2	3	4	1	3	547	20	567	96.5	16.1
104	"	"	49	1[1]					1	7	8	18	29	24	54	73	55	57	56	53	28	29	17	4	6	4	5	1	1	2	1	1	1	535	23	558	95.9	14.2
105	"	"	55		1	1		1	2		7	3	13	14	24	21	25	28	33	26	35	29	26	15	16	9	7	10	5	6	1	4		362	64	426	85.0	17.3

1. Assumed to be a re-emerging parent.

Table 4. Summary of emergence from logs to which cold rest periods were applied 120 days after introduction of parent beetles.

1	2	3	4	5																														6	7	8	9	10
				Emergence at 75° F. - Number of days after cold rest																																		
Log no.	No. beetles introduced	No. days cold rest	Temp. of cold rest ° F.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total brood emerged	No. debarked	Total no. brood and unemerged old adults	Percent emerged by 30 days	Day of median emergence
106	40	20	33			1[1]																		1[1]								1[1]		0	162	162	0	>30
107	"	"	39	1[1]																										1	1		3	5	413	418	1.2	"
108	"	"	42							1[1]																			3		1		1	5	389	394	1.3	"
109	"	"	50																															0	692	692	0	"
110	"	"	55							1[1]		1[1]																			2		1	3	744	747	0.4	"
111	"	50	33		1[1]				1[1]					1[1]			1[1]											1[1]						0	379	379	0	"
112	"	"	39				1[1]							1	1		5	1	3	4	2	3	5	4	2	2		3	1	1	1	3	2	44	71	115	38.3	"
113	"	"	42										3	6	4	19	27	21	28	28	19	23	19	10	12	5	2	2	5	3	1	3	7	247	42	289	85.5	17.5
114	"	"	49								1	1		4	3	5	5	10	5	10	15	13	10	12	11	8	18	11	7	5	5	4	4	167	212	379	44.1	>30
115	"	"	55										2	1			1		2	3	1	3		2	1	4	2	1		2	1	5	1	32	235	267	12.0	"
116	"	90	33										1	1				1	1		2		1		5	2	1	6	2	1				24	444	468	5.1	"
117	"	"	39	1[1]					1	2	6	16	31	47	61	74	70	55	33	25	16	10	5	3	1	2	1	1	3	2	1		4	470	2	472	99.6	13.1
118	"	"	42					1			3	7	14	25	36	49	60	44	42	21	20	10	5	3	5	2	1	1	1					350	2	352	99.4	13.8
119	"	"	49								1	2	4	11	8	21	20	16	23	16	18	2	5	4	5	1	1	1	1	1		1		162	7	169	95.9	15.1
120	"	"	55							1			2	1	1	5	1	6	6	3	6	3	3	3	3	1	1							46	9	55	83.6	17.4

1. Assumed to be a re-emerging parent.

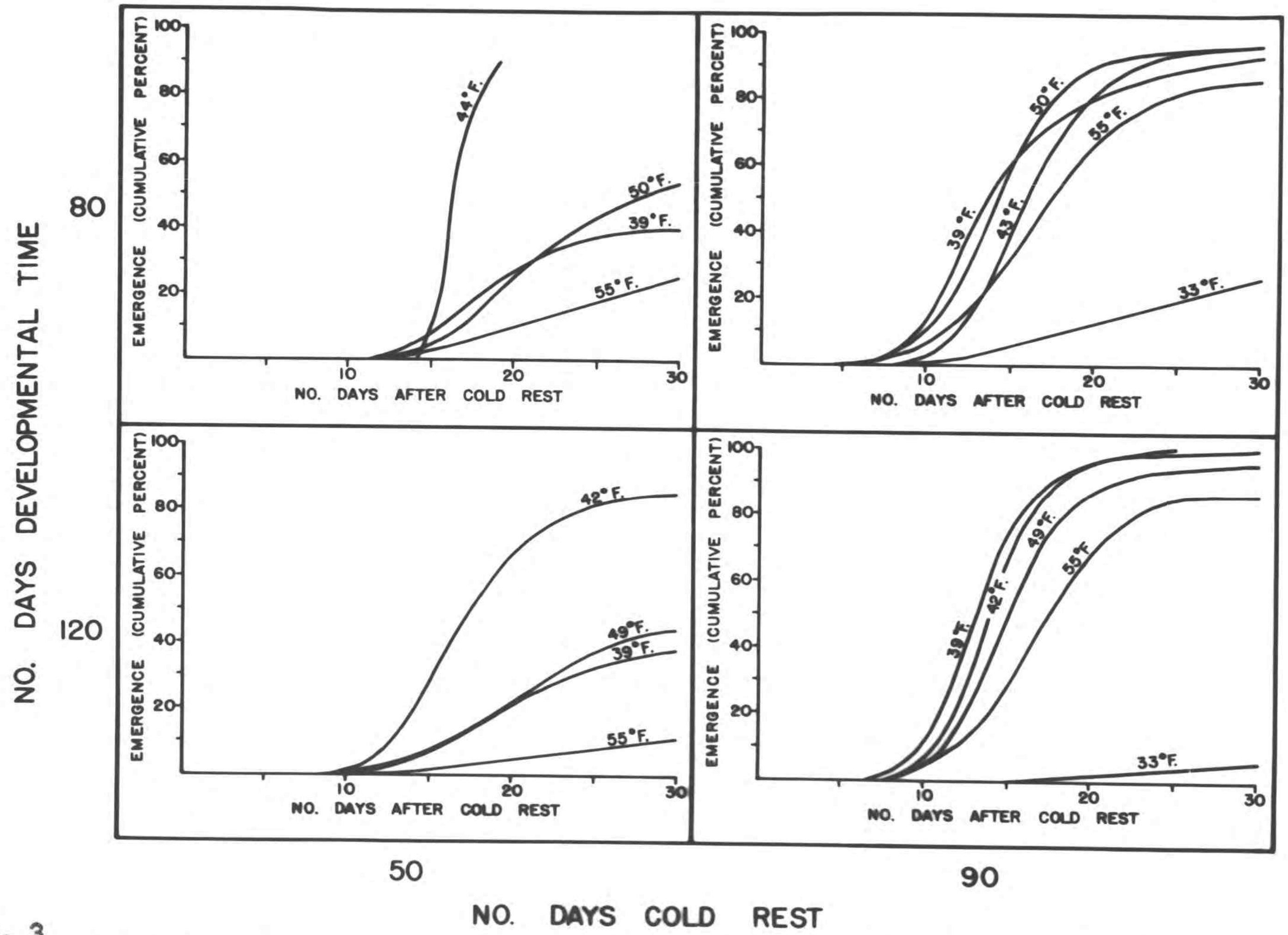

Fig. 3
Emergence of laboratory-reared beetles at 75° F. following various cold rest periods.

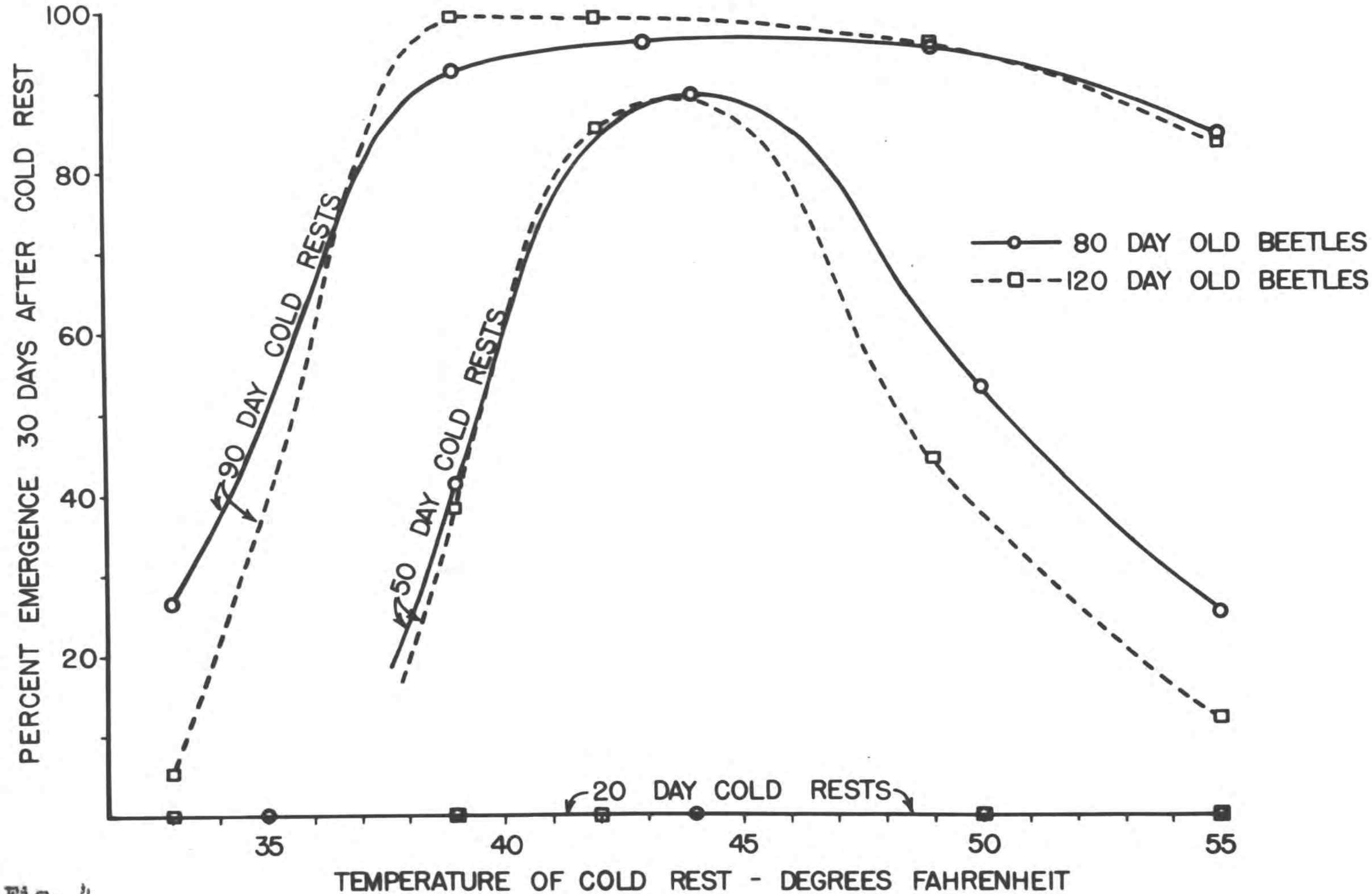

Fig. 4
Effectiveness of the various cold rest treatments as measured by the percent of emergence by the thirtieth day after treatment.

It is obvious from Tables 3 and 4 that the 20-day cold rests always caused virtually no emergence. Fifty days cold rest resulted in some emergence but a 120-day rest was the best of the three in this respect. The relative effectiveness of the various treatments as measured by the percent of emergence by the thirtieth day after treatment is presented in Figure 4. The two 50-day curves are positively skewed indicating a steeper threshold at the lower temperatures. The high points occur at approximately 44° F. The 90-day curves cover a much wider range of temperatures, are displaced higher than the 50-day curves, and are relatively flat-topped from 39 to 50° F. At the higher temperatures the two 50-day curves diverge, the lower one being the curve representing the 120-day-old beetles at the time of cold rest. At the lower temperatures the two 90-day curves also diverged. Again the curve representing the 120-day-old beetles at the time of cold rest was the lower one. These divergences were tested for significance by comparing logs 101 and 116, and 100 and 115 for the 90- and 50-day curves, respectively, and found significant at the .001 percent level (36, p. 407-408).

Another experiment was designed to see if diapause development could be arrested by transferring the beetles to a lower temperature. For this experiment from 18 to 27 beetles were introduced into each of six logs. All six logs received a 90-day cold rest period, three after the brood had developed for 80 days and three after developing 120 days. All logs were initially placed at a cold rest temperature of 43° F. for a portion of the 90 days and then transferred to 33° F. for the remainder. The three durations of exposure to the 43° F.

temperature, 30, 50, and 70 days, were applied to both the 80- and 120-day-old beetles. Thus, all treatments were essentially the same except for the duration of exposure to the various temperatures. Table 5 lists these treatments and the emergence of the brood upon return to 75° F. Little variation occurred in either the percent of the insects which emerged or the rate at which they emerged.

Re-emergence of Parent Beetles

Figure 5 shows, by sex, the beetles which emerged from 11 logs for a period of 48 days after introduction. The number of beetles of undetermined sex introduced into each log varied from 22 to 82, and totaled 468. The first day after introduction a total of 19 had not bored in and were removed. Therefore, only 449 beetles attacked these logs. During the 48-day period a total of 199 had re-emerged according to the pattern shown in the figure. These insects were sexed by dissection to observe the gonads. It is probable that more did re-emerge than were collected, for the cages were visited but once each day, and these insects re-entered the same log without having been collected.

It can be seen that the peak re-emergence period came about the twenty-fifth day with more males than females re-emerging before that time, and more females than males after that time. Although 48 days was as far as this study was continued, beetles continued to re-emerge slowly until the logs were transferred to the coolers and also subsequent to the cold rest periods as indicated in Tables 2, 3,

Table 5. Summary of emergence from logs to which 90-day cold rest periods consisting of different durations at 43 and 33° F. were applied.

1	2	3	4	5	6																														7	8	9	10	11
Log no.	No. beetles introduced	No. days developmental time	No. days at 43° F.	No. days at 33° F.	Emergence - No. days after cold rest																														Total emerged	Total debarked	Total	Percent emerged	Day of median emergence
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30					
67	27	80	30	60											1	3	9	13	18	25	12	25	16	11	4	6	2	1	1	1	5	1	1		155	8	163	95.1	16.8
68	23	"	50	40										4	3	15	21	24	14	17	20	8	5	5	8	1		1							146	1	147	99.3	14.5
69	18	"	70	20											2	3	14	14	21	13	8	8			1			1							85	1	86	98.8	14.4
70	19	120	30	60										2	1	10	22	13	9	9	2	12	7		3				1					1	92	3	95	96.8	14.1
71	20	"	50	40				1[1]					4	14	17	51	71	62	37	32	8	2	1	1		1				1	1				303	1	304	99.7	12.9
72	21	"	70	20									1	5	9	11	17	19	18	6	3	2		4	1	1	1			2	1	1	1		103	0	103	100	13.4

1. Assumed to be a re-emerging parent.

and 4 by the scattered emergence elsewhere than the main emergence period. Even when emergence of the brood seemed complete and the

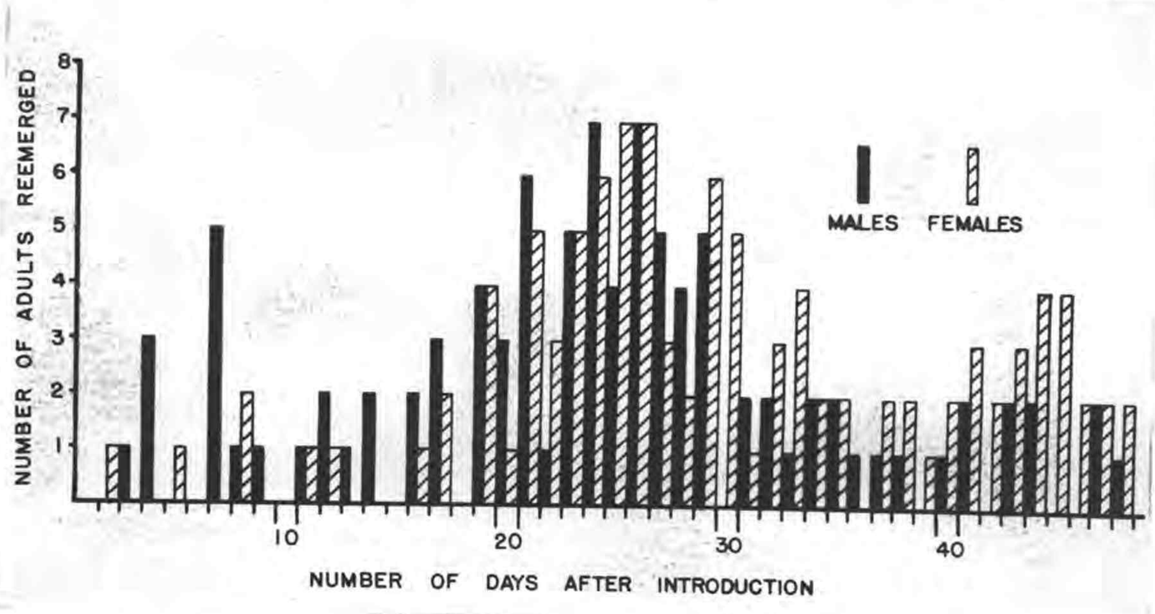

Figure 5. Re-emergence of parent beetles from eleven logs for a period of 48 days after introduction.

logs were finally debarked, frequently there remained a few beetles which had not emerged. It is known that at least a few of those remaining were parent beetles because the gallery patterns could be traced in the inner bark. However, where there was a large number of beetles introduced and good brood survival, the gallery patterns were obscure and it could not be ascertained whether those remaining were parents or brood.

Competition

By counting the number of attacks and measuring the size of the logs, the number of attacks per square foot of bark area was computed for 17 logs. The number of attacks ranged from 0.6 to 3.2 per square foot. An estimate of the amount of phloem utilized by the developing broods showed that percent utilization increased as the number of attacks increased until at 3.2 attacks per square foot it was estimated that 95 percent of the phloem had been utilized. If there are more than this number, it is probable that there will be excessive mortality due to competition.

Effect of Moisture on Survival

In the preliminary experiments the broods developed in logs kept at 75° F. and 20 to 40 percent relative humidity and which had been waxed on both ends. No further attempts were made to conserve or to supplement the original moisture in these logs. Mortality was quite high, survival being an average of 81.7 beetles for 18 logs. The average number introduced was 35. Probably the logs dried out at too rapid a rate to be a suitable environment for the beetles to develop. In the later experiments attempts were made to correct this rapid drying by setting one end of the log in moist sand. The relative humidity in the rearing room was also maintained at a higher percent, 60 to 70 as compared with 20 to 40 in the preliminary experiments.

Considerable variation was observed in the number of insects which survived in the logs treated in this manner. Survival per log ranged from 0 to 747 insects even though the number of insects introduced per log varied only between 20 and 40 and all logs received the same treatment while the broods were developing. The greatest portion of the mortality occurred in the larval stage. Those insects which successfully pupated remained, for the most part, healthy from that time until they either emerged from the bark or were exposed during the final examination.

The surviving insects in certain logs frequently were concentrated in one portion of the log, generally one side of the upper end; the insects in the other portions of the log were dead. Evidently in the latter case the moisture had been maintained at such a high level except in these areas where the insects were concentrated, that they were killed. Whenever the number of insects introduced into a log was high in relation to the size of the log or when the relatively few attacks were concentrated in one portion of the log, the conducting system of the phloem was effectively broken, and less moisture moved upward from the sand below.

There was a mortality relationship existing between the number of insects introduced and the number of brood surviving. As shown in Table 6, when a larger number of insects was introduced a higher number of insects survived. This number was far greater than would be expected considering only the number of insects introduced. Thus, roughly twice as many insects were introduced in the first part of the experiments as in the latter part, but, instead of twice as many brood surviving, there were 4.3 times as many.

Table 6. Relationship of number of insects introduced and number of brood surviving in logs set in moist sand.

No. of Logs	Av. No. Insects Introduced	Av. No. Brood Surviving
23	20.1	98.3
22	39.6	418.1

Another experiment was designed to confirm the hypothesis that excessive moisture associated with the small number of introduced beetles caused an excessively high brood mortality. Also a second objective was to ascertain the advisability of using moist sand and wax to maintain phloem moisture. Four adjacent sections were cut from a freshly felled Douglas-fir and designated A, B, C, and D. After logs A and B were coated on one end with wax, 25 and 8 pairs of beetles were introduced into the cages containing A and B, respectively. One day later these logs were placed wax end up in moist sand. The beetles were sexed according to the method of Chapman (12). Log C was waxed on both ends but D received no wax. Eight pairs of beetles were introduced into these two logs, but they were not placed in sand.

The same results obtained in Table 6 were obtained with the two logs placed in sand, survival being higher in the log where more beetles attacked, 36.7 percent in A compared with 4.3 percent in B (Table 7). Survival in the logs out of sand was 29.7 and 30.8 percent for the waxed and unwaxed logs, C and D, respectively. If the 95 percent confidence level is taken, B differed from each A, C, and D, while these latter did not differ significantly among themselves.

Table 7. Comparison of survival in logs under different treatments.

Log	Ends Waxed	Beetle Pairs Introduced	Percent Brood Surviving 72 Days After Introduction	Phloem Moisture (Percent of Oven Dry Weight-Average of Three Samples)
In sand:				
B	upper	8	4.3	179.4
A	"	25	36.7	157.4
Not in sand:				
C	both	8	29.7	131.5
D	neither	8	30.8	107.5

Not all of the females introduced into the cage with log A established broods. Therefore, not all of the phloem conducting system was disrupted, which resulted in a moisture level somewhat higher than was expected. On the basis of this experiment alone it is not possible to say whether the differences in survival between logs A, C, and D would have resulted if there had been complete utilization of the phloem in log A by the brood.

Morphological and Physiological Manifestations of Diapause

Beetles which have emerged from the bark are physiologically mature and are ready to attack other trees and establish broods. At this time they are capable of sustained flight for protracted periods (41, p. 263) and their gonads are fully mature. During the present experiments beetles removed from the bark while in diapause made no attempts to fly. Dissection of these beetles showed that the flight muscles were extremely underdeveloped. In addition, no eggs could be seen in the lateral oviducts of the females and the male seminal vesicles were underdeveloped when compared to those of mature individuals. Probably the development of the flight muscles and the maturation of the gonads proceed concomitantly after some stimulus, in this case adequate chilling, has set in motion the proper endocrine activity leading eventually to maturation and emergence of the beetles from the bark.

It was desirable therefore to trace the course of physiological maturation as evidenced by the development of the flight muscles and gonads. Insects were reared at 75° F. to the pupal stage in logs. The logs were debarked and each pupa was placed in a separate vial containing moist, ground phloem as described in the section "MATERIALS AND METHODS". These insects were examined twice daily and as each became an adult the time and date, considered from then on as time zero, were recorded on the vial. Some of the vials were kept at 75° F. until the insects were preserved. A few insects which were constantly at this warm temperature were preserved on days 5, 10, 20, 30, 40, and 60. Others were transferred to 43° F. for a 90-day period commencing on the tenth day, after which they were returned to 75° F. A few of these beetles experiencing the cold rest were preserved 2, 4, 6, 8, 10, and 12 days after being returned to 75° F. Thus, the age of each beetle was known within approximately 12 hours, and the beetles of known age formed two continuous series, one having been exposed to the cold and the other not. All beetles kept constantly at the 75° F. temperature had flight muscles and ovaries or testes similar to those in the left illustrations of Figures 6, 7, 8, and 9. The illustrations of the flight muscles have been confined to the longitudinal median dorsal, the oblique lateral dorsal and the tergosternal muscles, because these are the major ones (44, p. 228-233) and the others seemed always to be in a state of development comparable to those illustrated. Those beetles which underwent a cold rest when dissected through the tenth day after the cold rest period seemed in the same state of development as those which did not undergo a cold rest. However, on the twelfth day after cold rest one male was dissected which had flight muscles and testes intermediate between those illustrated in Figure 6, 7, and 9 signalling the resumption of growth in this individual following diapause. It is unfortunate that this experiment was not carried to perhaps the twentieth day after cold rest so that fully mature beetles could have been found.

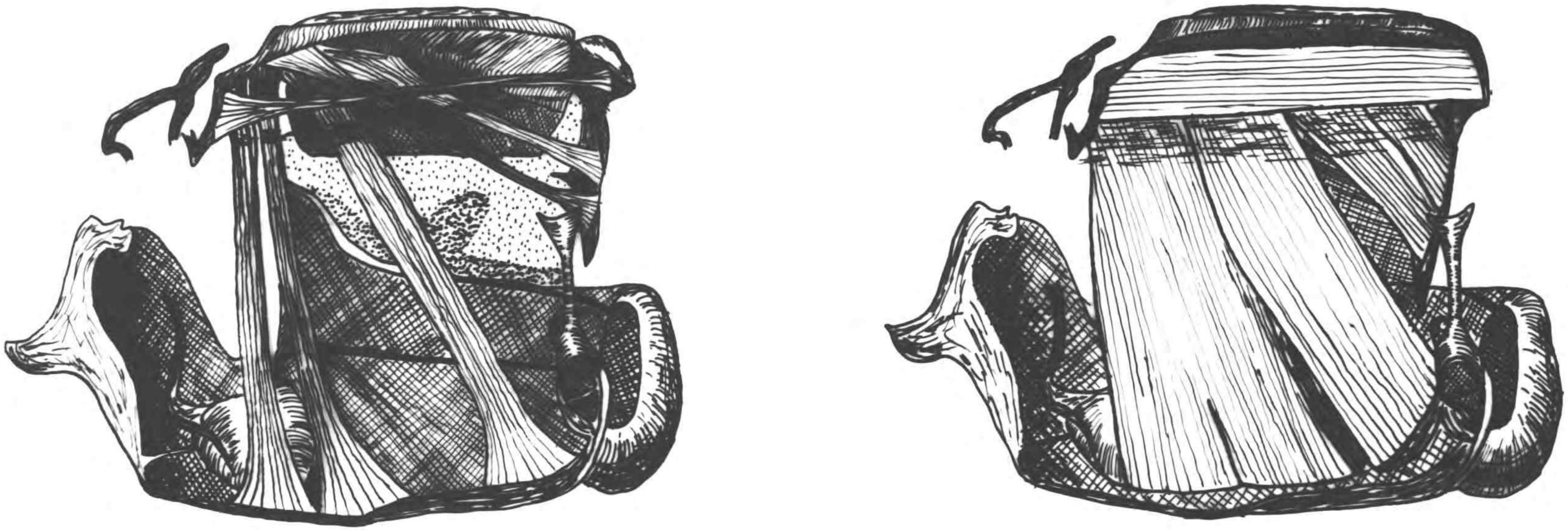

Fig. 6
Internal view of the right side of the thorax of two beetles showing the development of the main muscles of flight at two different times in the life cycle. Left: beetle in diapause. Right: mature beetle.

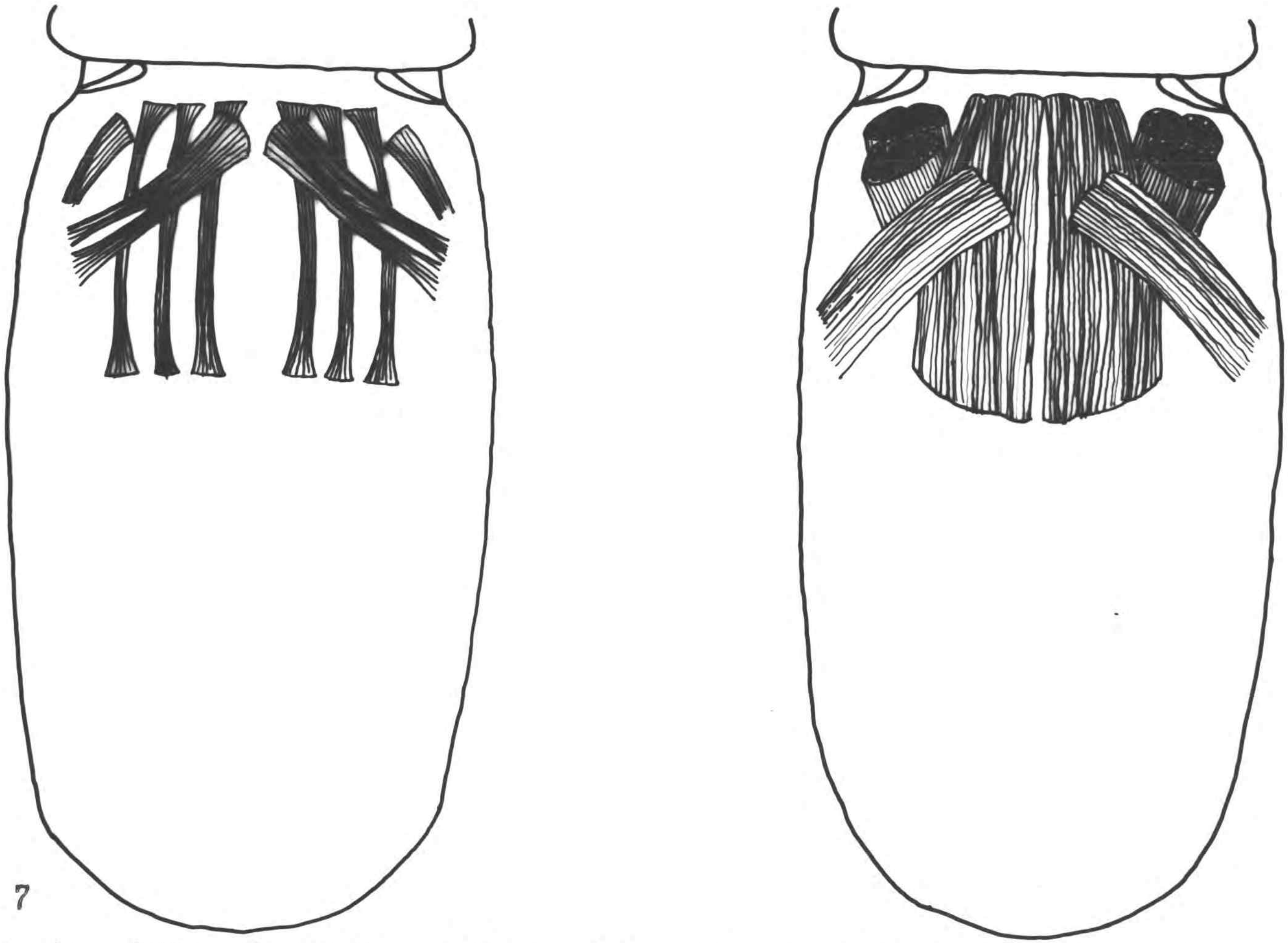

Fig. 7

Dorsal view of the main muscles of flight at two different times in the life cycle.
Left: beetle in diapause. Right: mature beetle.

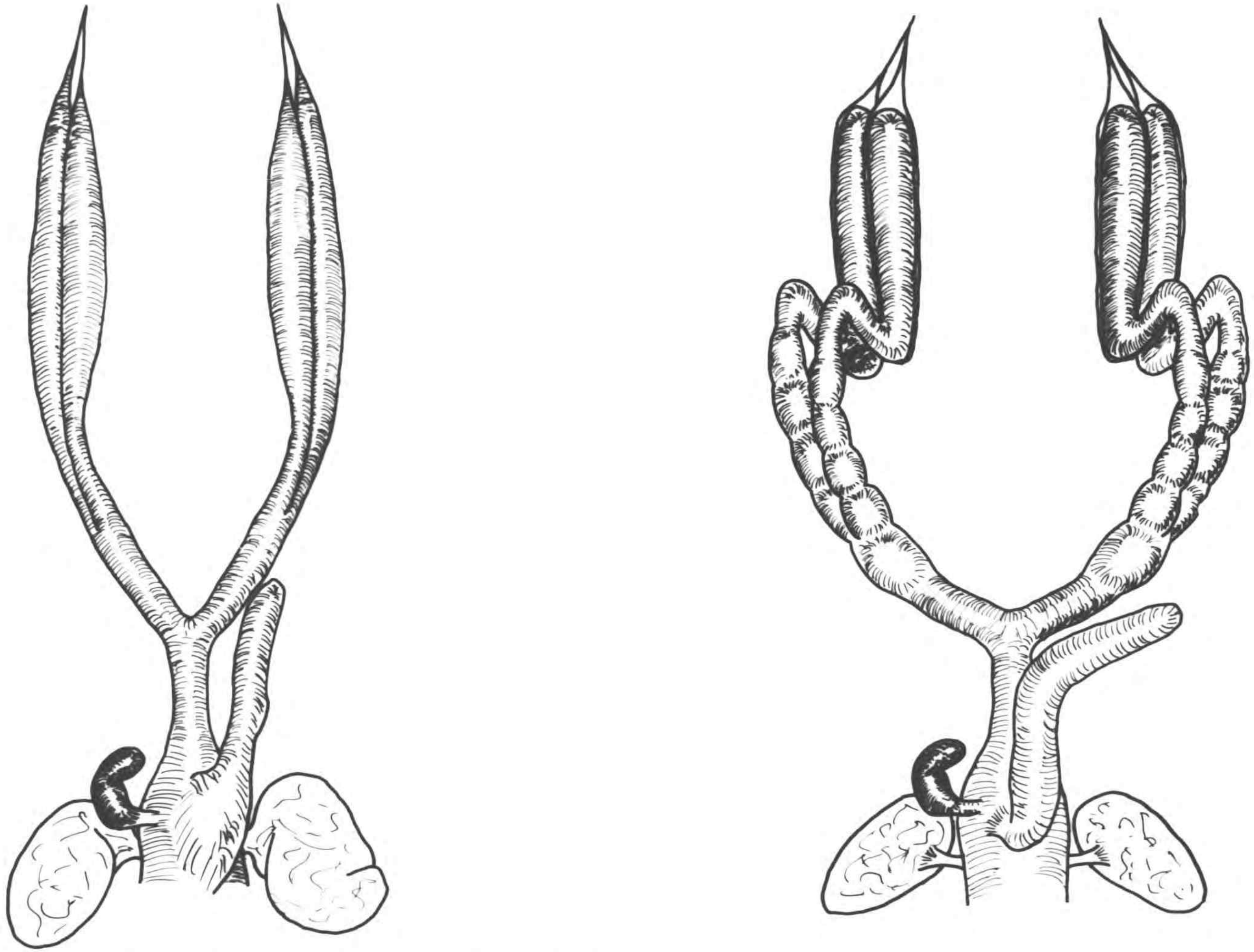

Fig. 8
Ovaries of two females showing the development at two different times in the life cycle.
Left: beetle in diapause. Right: mature beetle.

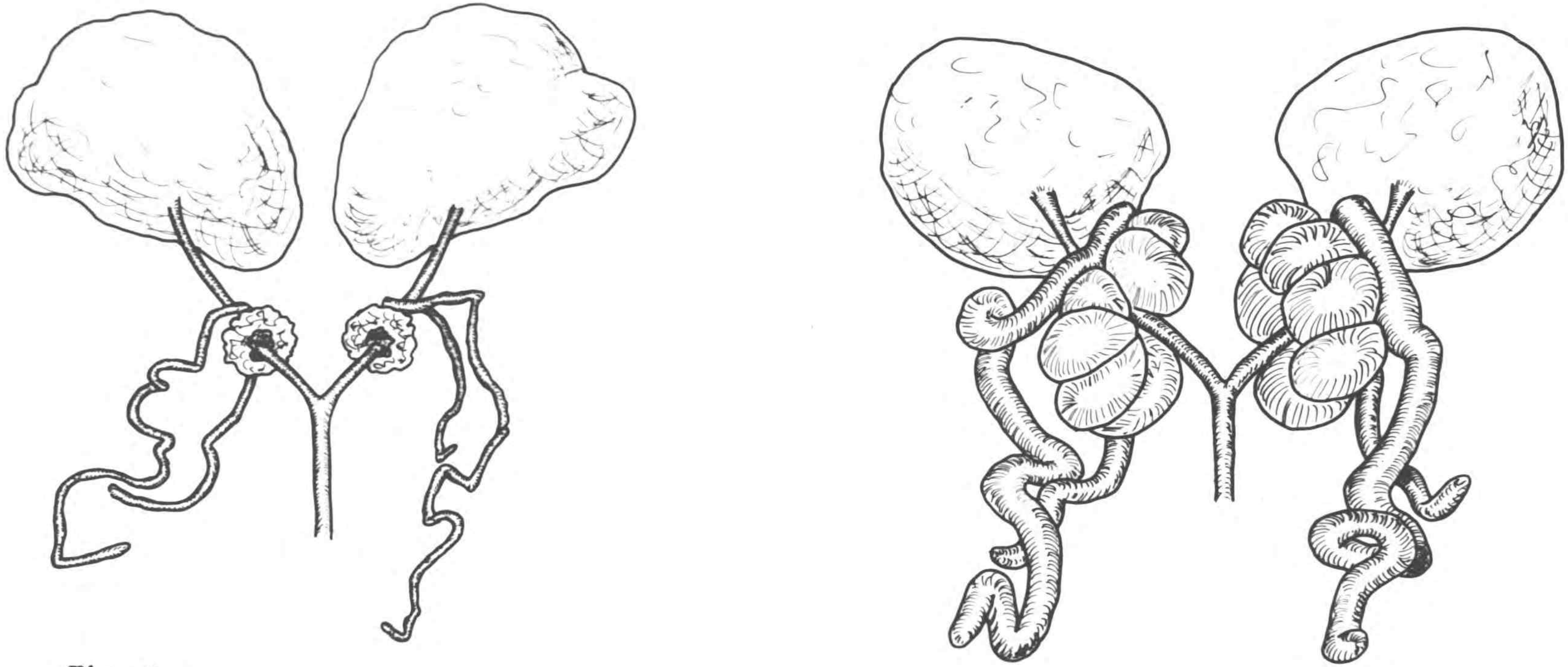

Fig. 9

Testes of two males showing the development at two different times in the life cycle.
Left: beetle in diapause. Right: mature beetle.

DISCUSSION

A period of cold rest is necessary before laboratory-reared Douglas-fir beetles will emerge in large numbers at any one time. This fact is well illustrated by Figure 10 in which emergence from a log that was not subjected to cold rest is compared to that of several logs that were subjected to cold rest. The numbers on each curve represent the number of days at 75° F. before cold rest, the numbers of days at the low temperature, and the temperature of cold rest, respectively. Thus, 120-90(42) represents a log in which the beetles developed at 75° F. for 120 days before undergoing a 90-day cold rest at 42° F.

With the experience gained to date the minimum time necessary for a complete laboratory generation may be estimated. In the experiments described in this paper the minimum time was 146 days. This was log number 98 receiving a cold rest of 50 days duration commencing 80 days after the introduction of the parents. The median

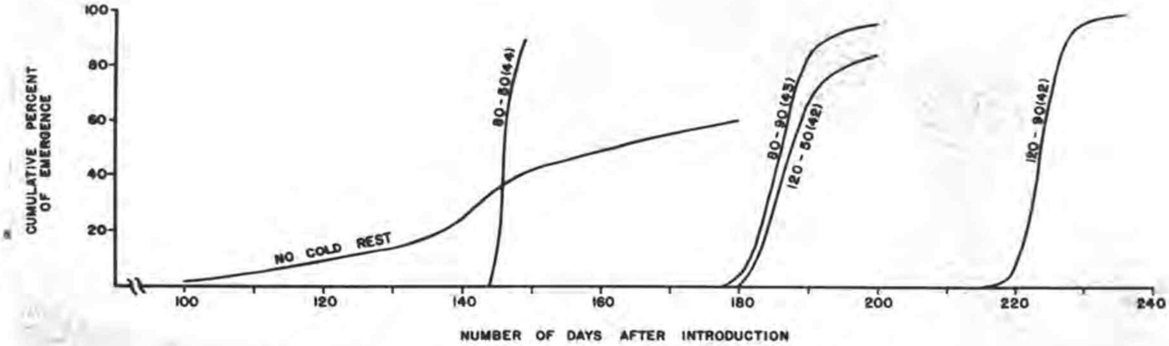

Fig. 10. Comparison of emergence of beetles from several logs after cold rest with those from one which received no cold rest.

emergence of the brood occurred on the sixteenth day after cold rest. However, these insects developed at 75° F. instead of at the optimum of 84-86° F. determined by Vité and Rudinsky (51, p. 161). According to them the brood will reach the adult stage approximately five days sooner at 84-86° F. than at 73-75° F. We may reasonably assume, therefore, that the cold rest period may be applied five days sooner or when the beetles have developed for 75 days. The total elapsed time would then be 141 days for a complete generation or at the rate of 2.6 laboratory generations per year.

It should be pointed out that the minimum developmental time and the minimum duration of the cold rest have been ascertained to lie only between the wide limits of 40 and 80, and 20 and 50 days, respectively. Therefore, with further experiments the 141 days necessary for one generation probably can be reduced still further to bring the total number of generations possible per year closer to three. This represents a marked increase over the one generation per year under natural conditions.

SUMMARY

1. Beetles were reared in 3-foot sections of freshly cut logs. The optimum number of beetles to introduce was approximately three pairs per square foot of bark area.
2. Provided there were more than approximately two attacks per square foot, survival did not differ significantly among logs with one end set in moist sand and logs not set in sand, either coated on the cut ends with paraffin or not. Survival was significantly reduced in logs set in moist sand if there was less than approximately two attacks per square foot.
3. The insects are univoltine under natural conditions, and low temperature plays an important role in the termination of diapause.
4. When reared in the laboratory under a constant temperature of 75° F. beetles went into an imaginal diapause. Emergence from the logs, therefore, was delayed and occurred over a period of several months.
5. By subjecting laboratory-reared beetles to various cold rest periods it was determined that a temperature of approximately 44° F. was the optimum for diapause development. Twenty days at this temperature was not enough to cause a significant percent of the brood to terminate diapause and emerge from the logs during a 30-day period following cold rest. Fifty days cold rest caused some of the brood to terminate diapause and emerge during the ensuing 30-day period, and 90 days caused almost all of them to do so.

6. Beetles must have developed at 75° F. for more than 40 days before cold rest is inflicted in order that significant emergence will take place during the 30-day period following cold rest. By the eightieth day at 75° F. development has proceeded sufficiently so that most of the beetles will terminate diapause during the 30-day period following the proper cold rest. By the 120th day of development there was a reduction of the effectiveness of some of the cold rest treatments when compared with treatments applied to the 80-day-old beetles, while others were not significantly different.
7. Diapausing adults were found to have underdeveloped flight muscles and gonads when compared with mature individuals.
8. The minimum time for one complete laboratory generation in these experiments was 146 days; but with further experimentation and proper manipulation of the temperature, it is felt that this may be reduced to approximately 130 days.

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APPENDIX

Preliminary Investigations

Before experimentation was started on a large scale, the probable temperatures and duration of cold rest that would suffice to terminate diapause in the Douglas-fir beetle were ascertained by performing a few simple experiments and by consulting existing weather bureau records. One of the first experiments performed was simply an attempt to rear beetles under a constant temperature of 75° F. following the method previously described. Eighty-two freshly emerged beetles were introduced into a fresh log and the brood was allowed to develop at a constant temperature of 75° F. Periodic sampling of the log revealed that about 75 days after introduction most of the brood was in the adult stage.

The cage containing the log was examined each day and all emerged beetles were collected. On the 137th day the log was cut into two equal portions. The bark of one portion was then watered thoroughly once each day, but the other received no water. Figure 11 shows the number of beetles which emerged each day. Until the

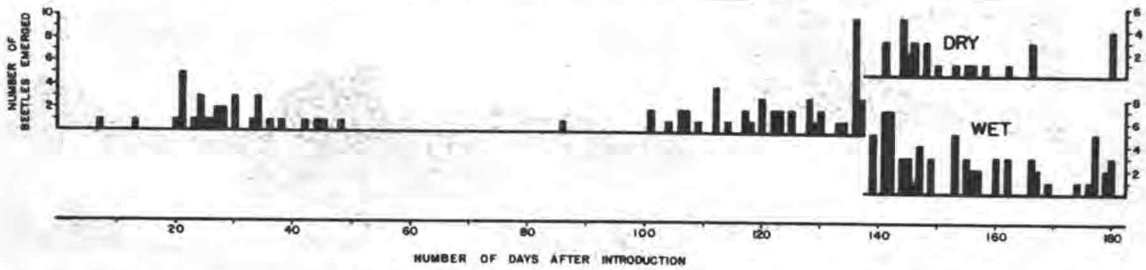

Figure 11. Emergence of beetles reared under a constant temperature of 75° F. The log was cut in half on the 137th day and one section was thereafter kept thoroughly moist.

fiftieth day the emerging beetles were, of course, the re-emerging adults seeking to establish their second broods in another log. Finally, on the 180th day both sections were debarked revealing 48 alive adults still unemerged in the watered section and 43 in the dry section.

Attempts were made to induce some of these beetles to attack fresh logs. On the 155th and 156th day 25 and 35 beetles were carefully removed from under the bark of the wet and dry sections of the log, respectively. These were placed in a cage with fresh logs. Four days later 18 from the wet portion and 17 from the dry portion had attacked. About three weeks after placing these beetles in the cages the logs were debarked. In each of the two logs there was only one egg gallery, each containing both male and female, where young larvae were present. In addition, in the log which had been attacked by beetles from the dry portion there was a gallery containing a single female and three eggs which apparently were unfertilized. (51, p. 166) The other beetles had attacked the logs but had made only very short galleries ranging from 1/4 to 2 inches in length. These beetles were evidently in diapause and not sexually mature. There are two alternatives for explaining the presence of the females which were sexually mature. One is that they were parent beetles which were ready to re-emerge and re-attack at the time they were debarked. By that time all the brood were very dark in color and indistinguishable from the parents. Also, the beetles in the original logs at the time of debarking had long since obliterated any hope of distinguishing parents from brood by tracing gallery patterns. Therefore, it would have been an easy matter to debark parent beetles inadvertently along with the brood. But Bedard[1] has shown that re-emerging beetles need no second fertilization to establish a second brood. If these sexually mature beetles were, in fact, the parents of the brood, the female that laid unfertilized eggs would not have needed to have a male to fertilize her. Therefore, the second explanation as to the presence of the sexually mature beetles seems more plausible. That is that these sexually mature beetles were offspring which had terminated diapause. Mr. Ken Wright[2] has shown that Douglas-fir beetles will eventually emerge as sexually mature beetles if maintained in a warm environment (70° - 75° F.) (personal communication). It is thought that these few insects represented a small percentage of insects which had ended diapause.

Temperature data were obtained for the fall and winter months (Table 8) for the general area where the logs were stored (49). Using them, an attempt was made to establish the probable temperature and duration of the necessary cold rest period that would cause the beetles to emerge. Since beetles had emerged from a log caged during

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2. Entomologist, Division of Forest Insect Research, Pacific Northwest Forest and Range Experiment Station, P. O. Box 4059, Portland 8, Oregon.

the first part of December, it was felt that the experimentation should be based primarily on the range of average temperatures encountered during the preceding months, or 41 to 51° F., and a cold rest period of two to three months.

Table 8. Temperatures for the months September 1956 through March 1957 as recorded at the Oregon State College Weather Station.

Month	Av. max.	Av. min.	Av.
September	76.5	47.1	61.8
October	61.2	40.8	51.0
November	50.5	32.7	41.6
December	45.0	33.8	39.4
January	37.6	25.8	31.7
February	49.3	34.5	41.9
March	53.1	39.5	46.3

Role of Temperature in the Termination of Diapause: Preliminary Experiments

Various numbers of beetles ranging from 14 to 110 were introduced into a series of 18 logs. These were kept in a rearing room maintained at 75° F. while the brood developed. No attempt was made to control the relative humidity of the room or moisture level of the logs other than the initial sealing of both ends of the logs with paraffin immediately after felling and bucking.

As previously stated most of the brood was found to be in the adult stage 75 days after introduction. Approximately 15 additional days were allowed for feeding and maturation of the young adults and also to make certain that all beetles would be in the adult stage.

Then a cold rest period of approximately 20, 50, or 90 days at a temperature of 33, 43, or 54° F. was imposed on each of nine of the logs. Twenty days later similar conditions of cold rest were imposed on the remaining nine logs. The actual number of days developmental time or cold rest in some cases varied from the 90 or 110 and the 20, 50, or 90 days, respectively, for which the design of the experiment called, but in only one case was there as much as a three day variance. This variance is shown in Table 9 but because of difficulty of presentation in the graph has been disregarded in Figure 12. Columns four and five of Table 9 show the cold rest treatment which was applied to each log and column six the daily emergence at 75° F. after the cold rest treatment.

After 45 days had elapsed, the logs were debarked and all remaining insects were counted. The percentages of beetles which had emerged prior to that time were plotted in Figure 12. Lines connect treatments which were similar except for the temperature of cold rest. The light and heavy lines represent beetles which had developed 90 and 110 days and the interrupted, dashed, and solid lines represent the 20-, 40-, and 90-day treatments, respectively. Unfortunately the conditions under which the beetles developed appeared to be too dry, for four logs not represented at the 54° F. temperature had no insects surviving. However, it is felt that enough survived in the rest of the logs to give a good indication of the effectiveness of the various treatments in terminating diapause. The three temperatures appear to straddle the optimum cold rest temperature with 43° F. being much more effective that either 33 or 54° F. At the 43° F. temperature both the 50- and 90-day treatments seem adequate for most of the beetles to terminate the diapause, whereas the 20-day treatment appears not long enough. Slight differences can be seen between the effectiveness of the cold rest treatments applied to the 90-day-old beetles when compared with those applied to the 110-day-old beetles. However, these differences may have been due to experimental error, since due to reasons beyond control the environment could not always be maintained at the desired temperature.

The emergence patterns for the 50- and 90-day cold rest treatments at 43° F. can be seen in Figure 13. Again the light and heavy lines represent the 90- and 110-day-old beetles and the dashed and solid lines the 50- and 90-day cold rest treatments, respectively. The longer cold rest period definitely was more effective in bringing about a faster emergence since the two curves of the 90-day treatment are distinctly closer to the origin than the 50-day curves.

Table 9. Summary of emergence after the various cold rest treatments in the preliminary experiments.

1	2	3	4	5	6																																														7	8	9	10	11
Log no.	No. beetles introduced	No. days developmental time	No. days cold rest	Temp. of cold rest	Emergence at 75° F. - Number of days after cold rest																																														Total emerged	No. debarked	Total no. insects	Percent emerged by 45 days	Day of median emergence
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45					
11	50	93	20	33		2		1					3										1																											7	133	140	5.0	>45
43	18	90	"	43											1					1														1		1														4	22	26	15.4	"
36	30	"	"	54								1																1																						2	45	47	4.3	"
9	70	88	48	33																							1																1							2	196	198	1.0	"
40	44	91	50	43													1		3	5	6	15	8		18	6		4	7		3	1	1	5		5			1	2	2		1		1			1		96	18	114	84.1	21.6
15	26	90	91	33					1						1				1																	1									1		1			6	87	93	6.5	>45
39	40	"	90	43								1	1	4	1	7	2	5	5	3	4	3	4		2	2																								44	0	44	100	14.1
42	18	110	20	33																																														0	103	103	0	>45
44	18	"	"	43	1																												1																	2	83	85	3.5	"
27	22	111	50	33	1					1		3	1																		8[1]				1								1	2		2		2		22	148	170	13.5	"
34	26	110	"	43													1	5	6	1	7	7	16	1	8	5	10	5	3		7	3	1	2	1	1	1					1								92	2	94	97.9	20.2
48	14	112	"	54													1				1						3	2	5	1	2	3	2	3	1	1	7	3	2		2	7	4	2	3	4	2	4	2	67	106	173	38.7	>45
28	29	111	91	33				2				1	1				3	3	2	3	7			1	2	1	1		1		5	1	3	1	2	1	2	1	2	2	2	2	2	1					4	59	54	113	52.1	41.3
33	110	110	"	43									4	5	4	3	7	9	4	4	7	7	2	1	2	1	3	1			1		1			1														67	4	71	94.2	14.9

1. Represents the emergence of 14 days.

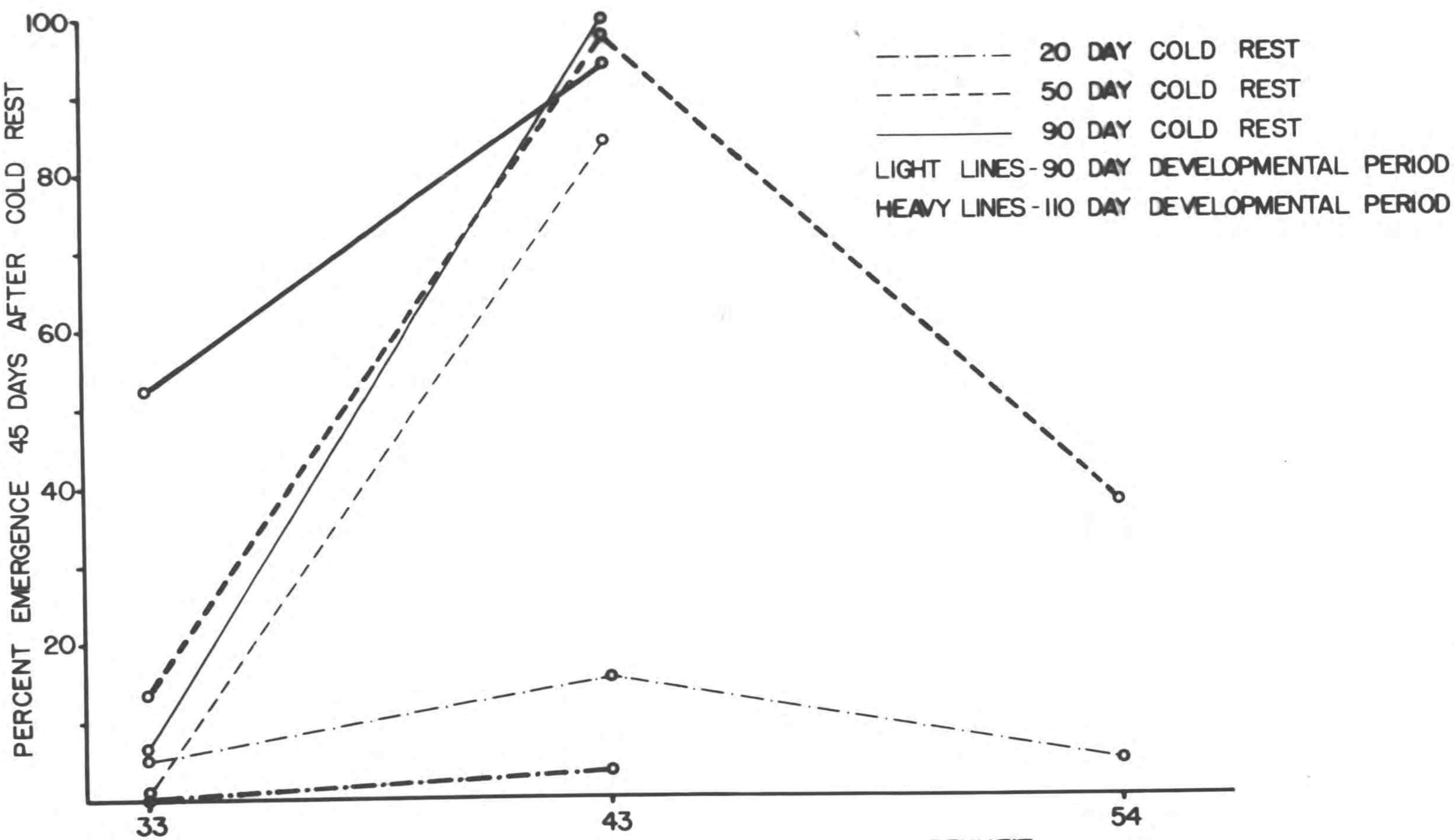

Fig. 12

Percent of emergence by 45 days after the various cold rest treatments in preliminary experiments.

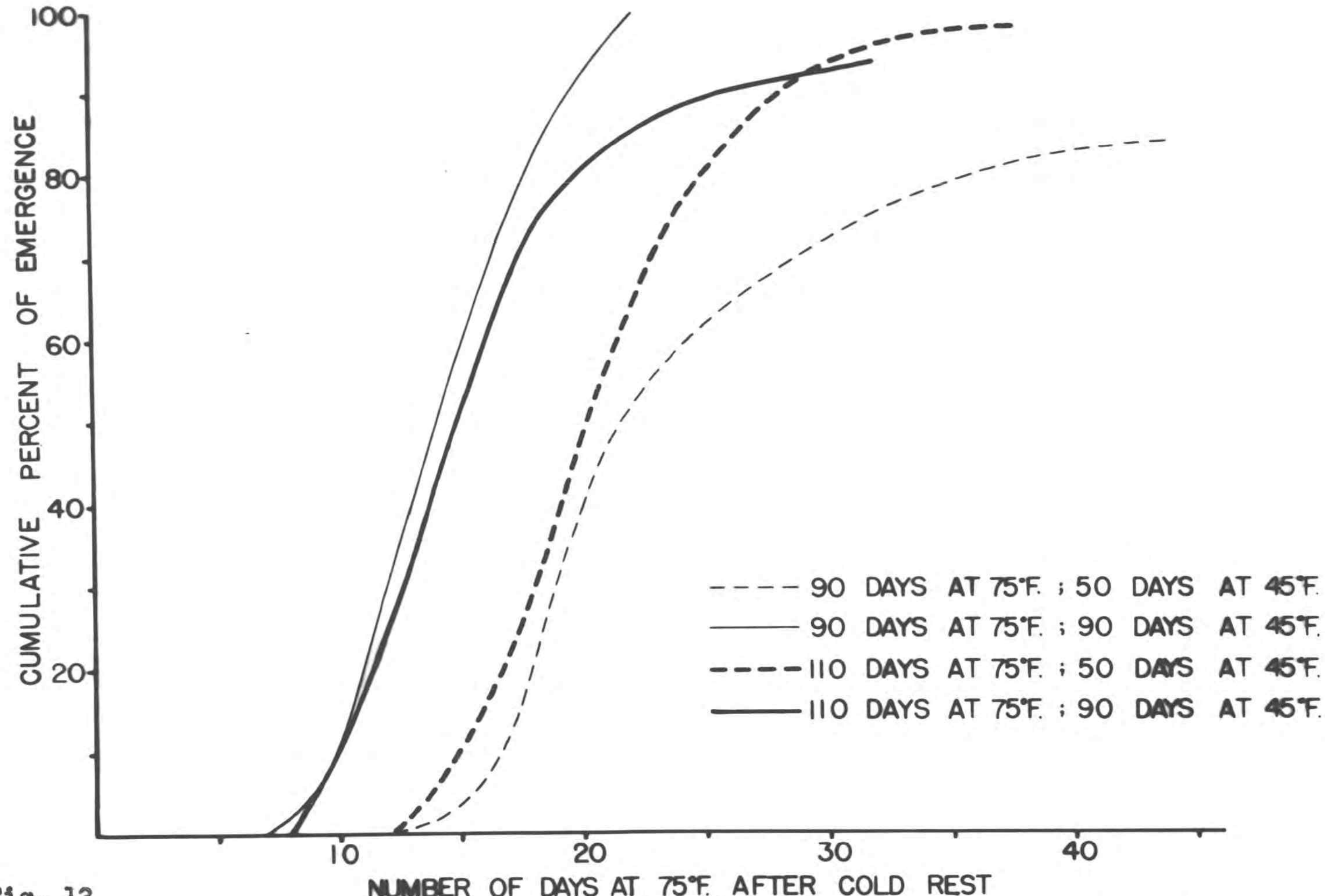

Fig. 13
Emergence patterns exhibited by the beetles in the preliminary experiments after the 50 and 90 day cold rest periods at 43^{0} F.