5-1976

Ecological Management of the Alfalfa Leafcutter Bee, Megachile pacifica (Panzer), with Emphasis on Diapause Induction

Ron M. Bitner
Utah State University

UtahStateUniversity
MERRILL-CAZIER LIBRARY

ECOLOGICAL MANAGEMENT OF THE ALFALFA LEAFCUTTER BEE,

MEGACHILE PACIFICA (PANZER), WITH

EMPHASIS ON DIAPAUSE INDUCTION

by

Ron M. Bitner

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Biology

Approved:

_____   _____
Major Professor   Committee Member

_____   _____
Committee Member   Committee Member

_____   _____
Committee Member   Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

1976

ACKNOWLEDGMENTS

I am grateful to my major professor, Dr. Ting H. Hsiao for his advice and critical review of this dissertation. My thanks are also due to the other members of my dissertation committee, Dr. Frank Parker and Dr. George Bohart (retired) of the USDA Bee Biology and Systematics Laboratory; Dr. Ivan G. Palmblad, Associate Professor of Biology; and Dr. Raymond T. Sanders, Professor of Biology, for their valueable suggestions.

I wish to acknowledge the USDA for providing supporting funds in 1971 and Utah State University's Man and the Environment Program for providing funds in 1972-1973.

I am greatly indebted to my parents whose support and encouragement throughout my years of schooling has been greatly appreciated.

Lastly, I must thank my wife, Nikki, for her help in the preparation and typing of the manuscript and for being keenly interested in the work that I do.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Ecological Management of the Alfalfa Leafcutter Bee,

Megachile pacifica (Panzer), with

Emphasis on Diapause Induction

by

Ron M. Bitner, Doctor of Philosophy

Utah State University, 1976

Major Professor: Dr. Ting H. Hsiao
Department: Biology

The effects of photoperiod and temperature on diapause induction in the alfalfa leafcutter bee, Megachile pacifica (Panzer) (=M. rotundata Auct.), were studied during the summers of 1972, 1973 and 1974. The influence of photoperiod and temperature on mortality, rate of development and incidence of diapause was measured during the developmental stages of this insect. The aim of this research was to assess the potential for manipulation of the number of generations of this bee per season so as to develop a practical and ecologically-sound method of management.

Eggs, larvae, pupae and adults of M. pacifica were subjected to regimens of temperatures of 5, 10, 14, 16.5, 20, 21, 25, 26.5 and $30^{\circ}C$ and photoperiods of 0, 8 and 16 hours of light. Experiments conducted during 1972 and 1973 involved treating eggs and larvae directly to determine whether diapause was induced in the immature stages. No difference was found between the test and control groups. Experiments of 1973 and 1974 involving treatment of either pupae or adults were designed to determine if inducement of diapause was maternal. Treated adults were released, their progeny were collected and reared to check for percent

pupation. These experiments on the adults failed to show any difference

between the experimental and control groups in percentage of diapause.

Treatment of the pupal stage was conducted in 1974 by subjecting

stages from the dark pupa to pre-emerged adult to a temperature of $10^{\circ}C$

for 3 hours daily for 8 consecutive days. This low-temperature treatment

proved to be most effective in inducing diapause, resulting in 96 percent

diapause as compared to 60 percent for the control group.

The findings indicate that diapause in M. pacifica is maternally

induced and that the possibility of developing a practical method for

producing either univoltine or bivoltine generations per season is pro-

mising. Recommendations are given for better management of M. pacifica.

(74 pages)

INTRODUCTION

Megachile pacifica (Panzer) (= rotundata Auct.) is a member of the
large cosmopolitan leafcutting bee genus Megachile Latreille. It belongs
to the subgenus Eutricharaea which is wholly Old World in origin although
many of the species have been spread to the New World in recent times.
M. pacifica is native to Eurasia and was confirmed in the United States
in the Washington metropolitan area in 1947 (Krombein, 1948). It has
extended its range westward and has been reported in ten states from
coast to coast. Since 1962, M. pacifica has been fostered in southern
Alberta (Robertson, 1966).

Alfalfa seed growers have been aware of the potential value of this
bee as a pollinator since the early 1960's. Each female has the poten-
tial to trip enough flowers to produce up to 1.07 pounds of alfalfa seed
(Klostermeyer and Gerber, 1969). Leafcutter bees are responsible for
substantial yields in areas where alfalfa seed could not previously be
grown economically. In eastern Washington, without either this bee or
the alkali bee (Nomia melanderia Ckll.), seed yields are apt to be from
100-300 pounds per acre (Gerber and Akre, 1969). In the same region,
seed yields have been obtained up to 2,000 pounds per acre when M. paci-
fica was used as the pollinator. Throughout the 1960's and 1970's,
alfalfa seed growers of Utah, Idaho, Oregon, Washington, Montana, South
Dakota, California and Alberta have been following recommendations of
various experiment stations in using the leafcutter bee (Bohart and
Knowlton, 1964; Hobbs, 1973; Johansen et al., 1969; Waters, 1969).

In 1972, 181,600 acres of alfalfa seed were harvested in the western United States with an average per acre yield of 480 pounds (USDA 1973 Agriculture Statistics). The total value of this bee can be calculated by multiplying the number of acres by the number of bees per acre and the cost of each bee. At a current market price of two cents each, the total value of the bee in the Western United States is $8,716,800 (181,600 acres x 2,400 bees per acre x 2 cents). Including supporting industries involved in supplying nesting materials, parasite control, etc., plus the value of bees exported from North America, the figure jumps to $40,000,000. However, these figures do not allow for the natural re-production of the bees from year to year in the growers' fields. If totals are added, one finds that value of the western area of the alfalfa seed industry is well over $170,000,000 (Phil Torchio, personal communication). From these figures, one can readily see the economic impact this one species of bee has on the alfalfa seed industry and on the agri-economic welfare of entire communities.

The advantages of using this bee for pollination have been reviewed by Bohart (1972), the most important one being its manageability. Significant population increases can be obtained by providing simple shelters and nesting sites and by using suitable management practices. Overwintering bees can be incubated so that their emergence coincides with alfalfa bloom, thus synchronizing the pollination processes.

During its life history, M. pacifica overwinters as a fifth (final) instar larva. In most areas it generally has one generation per year with a partial second generation occurring during July and August. Currently, there is much discussion as to the importance of the second generation of M. pacifica. Where the bee is used only for seed set, and

propagation is not the important factor, emergence of the second-generation bees may actually destroy many of the diapausing larvae that are ahead of them in tunnels. Second-generation females will also remove larvae from other tunnels to prepare a place to nest. The emergence of second-generation bees requires the grower to leave the nesting boards in the field longer and thus exposes diapausing larvae to longer periods of predation and parasitism. It also exposes another generation of adults to the dangers of insecticide applications and early cold weather in the fall.

In 1974, in the Boise valley region of central Idaho, a major alfalfa seed producing area, the emergence of second-generation bees was believed to be one of the largest ever, with estimates from growers of 70 to 90 percent second-generation emergence. The second generation of bees in this area did not begin emerging until August. In many areas, defoliation and harvesting of the seed crop had already begun, resulting in an insufficient bloom for the bees to rebuild their populations to the size they were before emergence. Krunic (1972) reports that a majority of second-generation bees are lost in Canada due to the short growing season.

On the other hand, persons propagating M. pacifica for sale to seed growers would have an advantage if there were a large percentage of the bees emerging into a second generation and perhaps even a third. If sufficient bloom were provided, the second-generation females would produce large numbers of progeny and increase overall population size.

Currently, the emergence of a second generation of bees is not manageable, but if the factors of diapause were known, it should be possible to control the production of univoltine or bivoltine bees. For

the aculeate Hymenoptera in general, the triggering mechanism of diapause

is poorly known. Information gained through a study of M. pacifica should

increase understanding of diapause in wasps and bees.

The objective of this research is to determine the effect of photo-

period and temperature on the induction of diapause in different life

stages of M. pacifica so that a practical method may be developed to

produce univoltine or bivoltine generations as desired.

REVIEW OF LITERATURE

Diapause Induction in Insects

Diapause is a widespread form of dormancy among insects. It is characterized by a number of features, including various degrees of arrested growth and development (Chapman, 1969; Beck, 1968; Lees, 1968; Danilevsky et al., 1970). Reproduction and development processes can occur only at favorable times in insects. Diapause is an adaptive mechanism which synchronizes these processes to enhance survival during unfavorable periods within the rhythmic seasonal cycles. Unfavorable conditions include such factors as temperature and moisture extremes or recurrent absence of food.

Initiation and maintenance of diapause as well as postdiapause reproductive development, often cover large time intervals in the phenological calendar of an insect species; in some species diapause may encompass part of a season, or it may span several seasons.

Various factors that regularly change during a season may serve as the signals for diapause initiation. They include light, humidity, temperature, and the need for synchronization with the host life cycle. The most reliable signal is the seasonal change in day length, which during the year is not subject to chance fluctuations and is the initial reason for seasonal climatic variation. All of these signals are regulators of the seasonal cycle. In general, the diapause characteristics of a species, such as incidence of diapause, critical photoperiod, and diapause intensity, are under polygenic control (Danilevsky, 1965).

For insects as a whole, diapause induction may occur at any developmental stage, but for a particular species it can usually be induced in only one stage.

Induction by photoperiodism and temperature

Photoperiodism has been well established as being essential to diapause induction in insects. Danilevsky, et al. (1970) state that photoperiod undoubtedly plays the main role in diapause induction and that all other factors are supplemental. The various aspects of photoperiodic induction, including types of responses to photoperiod, adaptive nature of photoperiodic responses, and physiology of photoperiodism have been well investigated and discussed (Lees, 1955, 1968, 1972; deWilde, 1962; Danilevsky, 1965; Beck, 1968; Danilevsky et al. 1970; Mansingh, 1971).

Tauber and Tauber (1973) have devised three general categories to define the effect of changes in day length on diapause induction. The first includes species for which a gradient of increasing or decreasing day length is not important. The only significant factor being the occurrence of a day length that is sufficiently long or short in relation to a critical photoperiod to induce diapause. A second group of insects responds to a change in day length that crosses a critical photoperiod, such as the long day/short day effect. Finally, some species seem to respond to changes in day lengths that do not cross a critical photoperiod.

Tauber and Tauber (1970), in their studies with <u>Chrysopa</u> <u>carnea</u> Stephens, were the first to demonstrate experimentally that an insect can enter diapause in response to the direction of change in day length that does not encroach on the critical photoperiod. They found that

the decreasing day lengths of early and midsummer did not induce diapause, but that diapause occurred in response to decreasing day length at the end of August when there is a large daily change in day length and the day length is near, but has not crossed, the critical photoperiod.

Most of the photoperiod investigations to date have been done in the laboratories. Although it is often difficult to design experiments to test photoperiod induction under field conditions, field studies would have more unique significance to the insect being studied.

Although light is considered to be the major environmental factor that controls the induction of diapause in insects, photoperiodic reactions are generally only expressed within definite temperature limits. In many species, high temperatures and long photoperiods tend to act in concert, as do low temperatures and short photoperiods (Beck, 1965; Lees, 1968; Thurston, 1972). In some insects, temperatures (or thermoperiod) can act as the primary diapause-inducing factor in the laboratory (Scheiderman and Horowitz, 1958; Hogan, 1960; Saunders, 1973).

Induction in relation to nutrition

There are several reports on the effects of nutrition on diapause induction and of its influence on photoperiodic effects. Morris (1967) reported that food was at least 1 of 4 important factors influencing diapause in the fall webworm, Hyphantria cunea Drury. Increasing maturity of the food plant has been shown to induce diapause in the Colorado potato beetle, Leptinotarsa decemlineata (Say) (deWilde, Bongers, and Schooneveld, 1969) and a collembolan (Wallace, 1968). Tauber and Tauber (1973, 1973b) found both photoperiod and food to be major factors regulating the facultative reproductive diapause in the green lacewing, Chrysopa carnea, Mohave

strain. Host species is an important controlling factor of diapause in the hymenopteran parasite, <u>Nasonia vitripennis</u> (Walker) (Saunders, Sutton, and Jarvis, 1970). Thus, it appears that for induction of diapause in insects, nutrition may modify photoperiod, act as one of the co-determinants, or play the major role.

Induction and genetic variability

Natural populations, even from a single area, contain a considerable reservoir of intrinsic variability with regard to responses to factors involved in diapause induction. This store of variability has been used in a number of selection experiments, the object of which has usually been to modify the diapause characteristics of a particular strain of insects (Waloff, 1949; Harvey, 1957; Barry, et al., 1966; House, 1967; Maslennikova, 1968).

Genetic variability within field populations underlies the ability of a species to adapt to various localities. When insects spread to new localities, there is an inevitable elimination of individuals which do not adapt to new conditions. Often the significant factor is the critical threshold of their photoperiodic response.

As indicated earlier, several strains of diapausing or non-diapausing species of insects have been selected for study in the laboratory. The kind of genetic differences which characterize natural geographical populations has recently been researched in a few groups of insects. Danilevsky (1965) has conducted an extensive series of hybridizing experiments with the geographical strains of various species of Lepidoptera. A continuous gradient of gene frequencies for critical photoperiod was found from north to south within the area of distribution. Tauber and Tauber (1972, 1973a,

1973b) have shown for C. carnea that both the critical photoperiod and the intensity of diapause (diapause duration) vary with geographical location. Adaptive differentiation apparently has been occurring in the European corn borer in the northern U. S. (Beck, 1961). Danilevsky, et al. (1970) have summarized the research concerning the inheritance of photoperiodism in insects.

Induction in the Hymenoptera

The nature of diapause in the Hymenoptera in general is very poorly understood, especially for the Aculeata (or stinging species) to which M. pacifica belongs. Studies on diapause induction have used the broad-waisted wasp species or parasitic wasps. To date, there is only one piece of literature on diapause induction in the Aculeata (Johansen and Eves, 1973).

Photoperiodic control of diapause induction has been demonstrated or reasonably inferred for the following families of wasps: Tenthred-inidae (Danilevsky, 1961; Saringer, 1966), Diprionidae (Sullivan and Wallace, 1965, 1967, 1968; Philogene and Benjamin, 1971); Braconidae (Ryan, 1965; Danilevsky, 1961); Trichogrammatidae (Danilevsky, 1961); and Pteromalidae (Schneiderman and Horowitz, 1968; Danilevsky, 1961; Saunders, 1965, 1966).

Temperature has been shown to be as important as photoperiod in diapause induction in the parasitic wasps, Nasonia vitripennis. Ex-periments subjecting the egg stage to daily temperature cycles between 13 and $23^{\circ}C$ in total absence of light show the wasps are able to distin-guish a "short-day" thermoperiod (less than 13 hours at $23^{\circ}C$ per day) from a "long-day" thermoperiod (more than 13 hours at $23^{\circ}C$ per day) and produce diapausing or developing progeny accordingly.

More than likely, in most species of insects, temperature is cor-
related with photoperiod to induce diapause. Dietary factors such as
host size, host availability and host species affect the incidence of
diapause induction in N. vitripennis (Saunders, 1966, 1970).

Intrinsic factors are important in all species, but the extent of
influence by extrinsic factors is often hard to determine. Sullivan and
Wallace (1968), working with natural populations of the European pine
sawfly larva, Neodiprion sertifer (Geoffroy), obtained from localities
within the same latitude in Ontario, found considerable differences be-
tween populations in their response to photoperiods including non-diapause.
In addition, within a single population, the incidence of non-diapause
varies inversely with larval rearing temperatures. In N. vitripennis,
the critical day length is shorter in a strain from Woods Hole (42°N),
than in a strain from Cambridge, England (52°N) (Saunders, 1966a).

Genetic manipulation was used by Maslennikova (1968). Working with
a Leningrad population of the chalcid, Pteromalus puparum (L.), he was
able to decrease the critical day length by more than 2 hr by means of
selection of non-diapausing individuals during 4 generations.

Seasonal History and Biology of
Megachile pacifica (Panzer)

Life history

Much work has been done on the leafcutter bee's life cycle (Osgood, 1964; Tirgari, 1963; Stephen and Torchio, 1961; Szabo, 1969; Kukovica, 1966; Eves, 1973). This bee is proterandrous. Depending on the locality and climatic conditions from year to year, males begin to emerge the last week of May and females a week later. Shortly after emergence, mating occurs and within 2 or 3 days the female begins to nest, always in pre-existing tunnels, not excavating its own nesting tunnel as do some other megachilids. A thimble-shaped cell is formed with pieces of leaves which she cuts, carries, and manipulates with her mandibles, chewing the edges of the leaf pieces and then pushing them with her mandibles against the walls of the nesting tunnel.

She provisions the cell with nectar and pollen, lays an egg, and closes the cell with a round piece of leaf. On the average, a female builds from 4 to 7 cells in a nesting tunnel, plugs it with many leafcuttings and then repeats her labors in other tunnels. During warm and dry weather, a female can complete an average of 1 cell per day. Since she lives from 4 to 6 weeks, she can produce 30 to 40 offspring. Klostermeyer and Gerber (1969) monitored the nesting activity of M. pacifica with an event recorder. Female bees took an average of 2 hr and 27 min to construct a cell, requiring 15 leaf-collecting trips and 17 provisioning trips. Oviposition averaged 0.8 min.

The single egg is laid on the outer surface of each pollen mass, appearing to float on the surface of a film of liquid that covers the

food mass. In laboratory rearing at 17°C and 55 percent relative humidity, the average number of days required for each stage was as follows: egg and first instar--2; second and third instar--1.8; fourth instar--1.5; growing fifth instar--2.6; full-grown to completion of cocoon--3.7; total--11.6 (Tirgari, 1963). Second through fifth instar larvae can most accurately be distinguished morphologically by progressive changes in their mandibles. Many of the eggs laid during June will emerge as a new generation of bees during July and August. The progeny of this generation develop to the nonfeeding larval stage, enter diapause, and overwinter (Gerber and Akre, 1969). A third generation of the leafcutter bee has been reported in Nevada (Arnett, personnel communication). Presently unexplained is the factor (or factors) responsible for inducing diapause in the leafcutter bee.

The sex ratio of M. pacifica is variable and may depend to a certain degree on the depth and diameter of the nesting tunnel. Tunnels have been found from which either all males or all females have emerged; however, a sex ratio of 2 to 3 males per female is most common (Tirgari, 1963; Osgood, 1964; Stephen and Osgood, 1965; Waters, 1970). Tunnels deep enough for only 1 cell generally are provided with male eggs. Almost invariably, the first of several cells in a nesting tunnel contains females; the rest contain males. In this species, as in most Hymenoptera, females develop from fertilized eggs and males from unfertilized eggs. Gerber and Klostermeyer (1970) concluded that egg fertilization is not a random event nor the end result of a stereotyped sequence of behavior, but is an entirely voluntary act of the female parent during the oviposition process.

Seasonal cycle

In the Pacific Northwest, M. pacifica has 2 adult emergence periods
each year. Generally from 10 to 40 percent of the progeny of the first-
generation females will emerge the same season as second- or summer-
generation adults.

Johansen and Eves (1973) state that diapause in M. pacifica is only
partially facultative since their research indicated that neither induction
nor termination is under the exclusive control of environmental conditions.
They believe that induction is controlled by an endogenous system since
the first offspring of first-generation females always contains the greatest
number of second-generation individuals. They also noted that in a consid-
erable number of leafcutter bee rearing studies all cells in a given nest
tunnel tended to contain either second-generation or diapause progeny.
Similar results were given by Krunic (1972). Further, Krunic said that
M. pacifica is a bivoltine species and reported a morphological difference
in the wing venation between adults that emerged in the spring and those
that emerged during August as second-generation bees.

For 40 to 50 years, M. pacifica has been in the United States. This
may be a long enough period of time in which to attribute the variability of
numbers of second-generation bees to selective factors. But the fact that
the highest proportion of second-generation bees comes from the earliest
nesting females seems to indicate that a changing environmental factor
(or factors) is responsible for the decrease in numbers as the season pro-
gresses. Inheritance is not necessarily involved when tunnels contain
progeny from a single female which will emerge either as a second genera-
tion or remain in diapause. Micro-variations in temperatures between
nesting tunnels and within tunnels (J. Undurraga, personal communication)

could account for the differences in percentages of second generation among bees coming from different holes. Intrinsic factors are important, but extrinsic factors cannot be ignored as being less important. Especially, photoperiod and temperature effects on the different life stages should be considered. (That is the purpose of the present study.)

The leafcutter bee is not an ideal insect to study. There is only 1 complete generation to work with each year and the period of nesting activity lasts for only 4 to 6 weeks. There is only about a 3-week period during which the percentage of diapause is very low; experiments conducted before or after this period seem to have little effect, with the percentage of diapause generally being high (Krunic, 1972; Johansen and Eves, 1973). However, M. pacifica is an economically important insect, and the problem of managing the bee in order to control the number of generations per year is likewise important.

MATERIALS AND METHODS

Two types of experiments were conducted to determine the stage most sensitive to differing photoperiods and temperatures for diapause induction. One type measured photoperiod and temperature effects on immature stages (eggs and larvae) of the leafcutter bee. The other type was conducted on the pupal and adult stages, with offspring observed for diapause. Data on development, mortality, nesting and a morphological check for polyvoltinism are contained in a third section. General materials and methods that apply to all sections are presented in this section.

Greenhouse Design

A greenhouse was used during April, May and June of 1972 and 1973 to increase the length of the season to work with M. pacifica. The greenhouse section of 6.10 x 7.62 m was located in the USDA Bee Biology and Systematics Laboratory, Logan, Utah. Average daily temperature was maintained at $21 \pm 2^{o}C$. The following plants, potted or in bouquets, provided pollen and nectar to the bees: alfalfa (Medicago sativa L.), sweetclover (Melilotus spp.), goatsrue (Galega officinalis L.) and Phacelia tanaceteifolia (Benth).

The bees used in the greenhouse were purchased as diapausing larvae in loose cells from Dority Bee Boards, Inc., Nyssa, Oregon. The cells received from Dority in 1972 had originally come from northwestern Canada. In 1973, the cells purchased were from the Nyssa area.

Since $\underline{M}$. <u>pacifica</u> has existed in the United States and Canada for only 40 years, and because the population has been under management the past 20 years, genetic variability between various populations may be minimal although unknown. Commercial activities have moved the leafcutter bee to many locations resulting in population intermixing.

The loose cells were incubated at $30^{\circ}C$ in the laboratory and the adults were released into the greenhouse. The largest adult population was reached in June with about 125 females and 200 to 300 males both years.

In 1972, the nesting blocks were sections of lumber 150 x 74 mm which had been drilled with holes 6 mm in diameter and 62 mm in depth, regularly spaced at a density of about 110 holes per 100 cm^2. Sections of paper straws, 5 mm in diameter, were inserted into the holes and trimmed flush with the block surface. Four of these nesting blocks were set on the west wall. In 1973, grooved plastic mega-boards were cut into blocks 150 x 57 mm. Holes were regularly spaced at a density of 100 holes per 100 cm^2. Paper straws 5 mm in diameter were again inserted into the holes and cut flush with the surface. Four of these blocks were placed on the west wall, as in 1972.

Field Design

Because of the availability of bloom, larger populations of bees can be maintained in the field. Bees were released in 2 locations in the summers of 1972 and 1973 and in 3 locations the summer of 1974. They were released either the last week in June or the first week in July each year, with 1 population released in August, 1973.

The largest population was maintained in an open-faced barn in North Logan, Utah, for the 3 summers. The barn was surrounded by several other

old wooden buildings. Major pollen sources used by the bees included alfalfa, sweetclover and goatsrue.

A smaller population was maintained for the 3 summers in cages over alfalfa grown on Utah State University's Greenville Farm located just north of the campus. In 1974, an additional population was kept on the USDA Poisonous Plant Research Laboratory grounds, which are adjacent and to the south of the Greenville Farm. Bloom availability was the same as described for the bees in North Logan. All temperature records were taken from the weather station located on the Greenville Farm. Temperatures were correlated with pupation rates throughout the nesting season.

The bees released in the field were provided by the USDA Crops Research Laboratory in Logan. They were from populations maintained each year at 3 different locations in northern Utah. The bees were incubated at 30°C in the laboratory until the first males emerged, then all cells were taken to the field for completion of emergence.

Five mega-boards (300 x 150 x 57 mm) with straws inserted into the holes were used as nesting blocks at the North Logan site. They were set in the rear of the barn and were never exposed to direct sunlight. Wooden boards of the size used in the greenhouse were used in the cages. Two blocks were used in each of 6 cages. They were placed in the northwest corners of the cages with the blocks facing east. Two mega-boards (600 x 300 x 114 mm) with straws inserted were used at the Poisonous Plant Research Laboratory site. These boards were set in a wooden shelter facing east. Female bees were counted at different times throughout the season at all 3 field sites.

Rearing Techniques

Filled straws were removed from the blocks and replaced with empty ones daily in 1972 and 1973 and every 5 days in 1974. Depending on straw length, there were from 4 to 10 cells in a plugged straw, but some straws contained fewer cells. A female bee is capable of producing at least 1 cell per day (Klostermeyer, 1969); thus, if there were 5 cells in a straw, the innermost cell would be at least 5 days old and the outer most cell would be 1 day old. On days of inclement weather the bees suspended foraging activities.

Three methods were used for rearing the eggs and larvae. In the first, filled straws were split open with a razor blade and the individual cells removed. Capping leaf pieces were taken from the cells. Open cells with exposed eggs or larvae were placed into individual styrofoam plastic cells which had been placed in 60 x 20 mm petri dishes (Lab-Tek No. 4036). There were 15 plastic cells in each petri dish. Bee cells were labeled as eggs, one-day-old, two-day-old, or three-day-old or as a particular larval stage. First, second and third instar larvae were grouped together because of difficulty in distinguishing a particular larval stage. In the second method, the individual bee cells were placed into size 0 gelatin capsules, which were labeled and mounted on 305 x 405 mm cardboard sheets with 2-sided adhesive tape. In the third method, the filled straws were placed, without removing the cells, into plastic vials with perforated lids. Two sizes of vials were used: 85 x 48 mm ID and 108 x 44 mm ID. The vials were labeled with the date the straws were removed from the field, incubating temperature and photoperiod.

The petri dishes and plastic vials were placed in 340 x 265 x 85 mm plastic boxes maintained at ca. 70 percent relative humidity by a saturated NaCl solution. They were then placed in rearing chambers with appropriate photoperiods and temperatures for each experiment. If mold developed, the saturated salt solution was removed. The gelatin capsules mounted on the cardboard were placed directly into the rearing chambers.

In the first rearing method, eggs placed in the petri dishes were checked daily until the larvae hatched, after which they were checked every other day. Mortality and rate of development were recorded. After cocoons were spun, they were removed and the larvae checked every other day for pupation. For the other 2 rearing methods, only mortality and pupation were checked. Pupation indicated termination of diapause.

The leafcutter bee has a type of diapause that generally requires temperatures of 5 to 15°C before diapause is terminated. However, many individuals will break diapause within several months without low-temperature treatment. In the field, emergence of second-generation adults requires a minimum of 23 days from cell construction (Johansen and Eves, 1973). The pupal stage lasts from 4 to 5 days, so that bees in the field either pupate or enter diapause in a minimum of 18 to 20 days. In the laboratory, from egg to first pupation requires 32 days when reared at 20°C and 17 days at 26.5°C. For this study, pupation rates were considered only for a 2-week time period after the initial pupation began.

A technique which had been developed by the Bee Biology and Systematics Laboratory was used in 1974 to check for pupation and mortality in the bee cells. Bee cells still in the straw were x-rayed at a medical clinic. Larvae, pupae, parasites and uneaten provisions showed very

clearly on the x-ray plates and were counted. This method greatly reduced the time normally required by opening each cell. Treated cells were retained to be released the following spring for observation of any abnormalities that may have been caused by the irradiation of x-rays.

Laboratory Design

Temperatures of 5, 10, 14, 16.5, 20, room temperature (20±2), 25, 26.5 and 30°C and photoperiods of 0, 8, and 16 hr were used in various combinations for rearing of eggs, larvae, pupae and adults to determine their effects on diapause induction. A shorthand notation (for example, 8:20) for photoperiod and temperature is used throughout this paper.

Several types of rearing chambers were used. The 5°C temperature was provided by a walk-in cool room maintained in the Bee Biology and Systematics Laboratory. The room was kept dark except when it was entered. The temperature cabinet set at 8:14 was a Biotron. It was difficult to maintain at a constant temperature and fluctuated ±2°C.

The cabinet set at 8:20 was a Model 805 Precision Scientific incubator. A small room was kept at 16:25. The 8:25 chamber consisted of a modified 20-gal garbage can set in the small room. The chamber was lightproof with a light source of 1 Westinghouse F8T5/CN fluorescent bulb. Air was circulated through the chamber by a fan and 2 air ducts. All other 8- and 16-hr photoperiods and temperatures were provided by Percival Growth Chambers. The 0-hr photoperiods were accomplished by placing the progeny in the petri dishes into lightproof gal containers which were, in turn, set into appropriate cabinets. The eggs in these containers were exposed to light for a 10-min period every other day, when they were checked for mortality and development.

PROCEDURES AND RESULTS

Experiments with Immature Stages to Induce Diapause

Effects of photoperiod and temperature
on eggs and larvae of greenhouse bees

In 1972, eggs and larvae were treated from July 7 to 17. Four combinations of photoperiod and temperature (8:20, 16:20, 8:30 and 16:30) were used to study effects on diapause induction. Each treatment employed from 75 to 100 eggs and 25 to 130 larvae. Table 1 summarizes data on developmental rate, mortality and incidence of diapause. With the exception of the 8:20 treatments, the mortality was low. The developmental rate was similar to that reported in the literature (Tirgari, 1963; Kukovica, 1966). The substantially higher mortality in the 8:20 treatments as compared with the 16:20 treatments resulted from a greater day-night temperature fluctuation of the incubator, which was actually 2 to $3^{\circ}C$ lower than its designation of $20^{\circ}C$. The high incidence of diapause exhibited in these treatments was probably due to the lower temperature alone which delayed development, rather than to the photoperiod variation. In the other 3 treatments, no significant differences are shown in the incidence of diapause for either the egg or larval stages used (Table 1).

In 1973, the same experiment was conducted with 4 other combinations of photoperiod and temperature: 8:14, 16:16.5, 8:26.5 and 16:26.5. The eggs and larvae were treated from May 14 through July 6. Data on development rate, mortality and diapause are given in Table 2. No data for the 8:14 treatment are included in the table because over 80 percent mortality

Table 1. Effects of photoperiod and temperature on eggs and larvae[1] of greenhouse bees in 1972

Date of treatment	Photoperiod(hr) temperature(^{o}C)	Stage used	No. treated	% mortality	Duration from egg to pupa (days) Range	Mean	No. pupated	% diapause
July 14	8:20	egg	82	36.6	-	-	0	100.0
14	8:20	larva	25	31.4			1	92.9
17	16:20	egg	102	5.9	21-33	26.8	62	35.4
14	16:20	larva	129	8.5			75	36.4
14	8:30	egg	91	4.4	10-17	13.3	64	26.4
12	8:30	larva	102	7.8			51	45.7
14	16:30	egg	73	8.2	11-16	13.2	49	26.9
11	16:30	larva	76	6.6			48	32.4

[1]First through fifth instar larvae.

Table 2. Effects of photoperiod and temperature on eggs and larvae[1]/ of greenhouse bees in 1973

Date of treatment	Photoperiod(hr) temperature(°C)	Stage used	No. treated	% mortality	Duration from egg to pupa (days)		No. pupated	% diapause
					Range	Mean		
May 24-31	16:16.5	egg	44	22.7	135	135.0	15	55.9
		larva	6	16.7			2	60.0
June 18-25		egg	37	29.7			0	100.0
May 14	8:26.5	egg	11	0.0	21	21.0	1	90.9
June 1-15		egg	6	16.7	14	14.0	1	80.0
		larva	11	27.3			6	25.0
16-30		egg	27	33.3	20	20.0	3	83.3
		larva	36	16.7			2	93.3
July 3		egg	5	40.0	22	22.0	1	67.7
		larva	32	3.1			2	93.5
May 17	16:26.5	egg	26	11.5			0	100.0
June 1-15		egg	18	11.1	18-21	19.5	4	75.0
		larva	23	13.0			1	95.0
16-30		egg	9	22.2	17	17.0	2	71.4
July 3-6		egg	12	41.7			0	100.0

[1] First through fifth instar larvae.

23

occurred during rearing. On the whole, mortality was higher in 1973 than in 1972 (Table 1). In comparing the incidence of diapause among the 3 treatments (Table 2), no significant differences are noted although the overall percentage of diapause in 1973 is much higher than that of 1972.

Effects of photoperiod and temperature on eggs of field bees

Only eggs were treated in this series of experiments. In 1972, from July 26 to August 10, 9 photoperiod and temperature combinations were tested: 0:20, 8:20, 16:20, 0:25, 8:25, 16:25, 0:30, 8:30 and 16:30. Data on developmental rate, mortality and diapause are given in Table 3. The results show an overall low mortality but an extremely high incidence of diapause. This is in contrast to the greenhouse bees, which had a much lower incidence of diapause (Table 1).

In 1973, from July 10 through 13, eggs were treated with 4 other combinations of photoperiod and temperature: 8:14, 16:16.5, 8:26.5 and 16:26.5. Sixty eggs were treated under each combination. The results showed 100 percent mortality for the 8:14 treatment and 95 percent for the 16:16.5 treatment. Data for the other two combinations are shown in Table 4. One interesting aspect is the low incidence of diapause for both groups, as compared to the same treatments on the greenhouse bees (Table 2) which showed a significantly higher incidence of diapause even though the latter populations were the earlier-emerging bees. The significance of this difference will be elaborated in a subsequent section.

Table 3. Effects of photoperiod and temperature on eggs of field bees in 1972

Date of treat-ment	Photoperiod: temperature (hr:^{o}C)	No. treated	% mortal-ity	Duration from egg to pupa (days) Range	Mean	No. pupated	% dia-pause
July-Aug. 28 - 5	0:20	64	0.0	32-34	33.0	3	95.3
27 - 4	8:20	108	3.7	42	42.0	1	99.0
28 - 1	16:20	60	3.3	25-40	32.5	3	94.9
29 - 9	8:25	90	1.1	24	24.0	1	98.9
26 - 5	16:25	74	1.4	19-28	23.5	4	94.5
29 - 10	0:30	56	1.8	14	14.0	6	89.1
27 - 4	8:30	68	5.9	16	16.0	8	87.5
31 - 9	16:30	70	1.4	13-14	13.5	3	95.7

Table 4. Effects of photoperiod and temperature on eggs of field bees in 1973

Date of treat-ment	Photoperiod: temperature (hr:^{o}C)	No. treated	% mortal-ity	Duration from egg to pupa (days) Range	Mean	No. pupated	% dia-pause
July 10-13	8:26.5	60	6.7	17-20	18.8	49	12.5
10-13	16:26.5	60	1.7	17-20	18.5	48	18.6

Experiments with Pupal and Adult Stages to Induce Diapause

Effects of photoperiod on adults
of a greenhouse population

In 1972, adult female bees from a greenhouse population were subjected to a short-day photoperiod (8 hr of light) to determine the maternal effect of diapause induction. The experiment began on July 20 and continued for 3 weeks. Three nesting blocks were selected for the study. The bees ordinarily stopped nesting activities and remained at their nests from 2000 hr daily. At 2015 hr each day 1 block containing approximately 60 females was selected for treatment and was put into a dark chamber. At 1215 hr the next day it was removed and placed in its original position. Thus, females from this block were exposed to only 8 hr of light per day. The chamber was always in the greenhouse so that it was exposed to the same temperatures as the other 2 nesting blocks which were used as controls. The females in the control blocks received the normal daily photoperiod of about 15 hr.

To determine if females were changing nesting blocks during the day, each block was examined every night and the number of females recorded. If the numbers remained nearly constant, it was assumed that the females were nesting in the same blocks. Eggs and larvae obtained from all females were reared in the laboratory at $30^{\circ}C$ to determine incidence of diapause.

The percentage of diapause for the 2 treatments is given in Table 5. The number of female bees per nesting block remained nearly constant throughout the study period. The number of females in the experimental group which received the short-day treatment ranged from 52 to 63. The number of bees in the control group ranged from 55 to 93. The wider range in the control population was due to the addition of more females to the greenhouse.

Table 5. Effects of photoperiod on adults in 1972; progeny reared at 30°C

Treatment	Date of straw removal	No. progeny treated	% mortality	No. pupated	% diapause
Experimental (8 hr photoperiod)	July 24-31	182	8.2	29	82.6
	Aug. 1-16	132	12.1	3	97.4
Control (15 hr photoperiod)	July 24-31	249	11.6	33	85.0
	Aug. 1-16	80	1.3	1	99.1

Since the incidence of diapause was high for both the experimental and the control group, it would seem that the shortening of the photoperiod has no effect on the females. However, it should be kept in mind that the time of treatment of the bees was the latter part of July and early August and it is possible that the bees had already passed the critical period of sensitivity to the photoperiodic response.

Effects of combining photoperiod and low temperature on adults

To determine the combined effect of photoperiod and low temperature on adults, bees were released at the North Logan farm site from July 2 through 7, 1973, and from June 24 through July 3, 1974. Two experiments were conducted in 1973. In the first experiment, beginning July 10 and continuing for 8 consecutive nights, 1 of the nesting blocks (Treatment I)

was removed from the field station at 2100 hr, after nesting activity had ceased. It was placed in the Biotron at 14°C for 12 hr, and then returned to the field the following morning at 0900 hr. The Biotron was not lighted, so the bees in Treatment I received less than 12 hr of light. A second experiment was conducted for 2 days (July 24 and 25). Before the commencement of activity, 1 of the remaining untreated bee boards (Treatment II) was brought into the laboratory, placed at 14°C for 1/2 hr, then at 5°C for 3 hr, and then returned to the field. The treatment was repeated once. Straws were brought in daily and placed in a 16:26.5 chamber.

In 1974, after nesting was established, 1 block containing females was brought into the laboratory in the mornings and placed at 5°C for 3 hr, then returned to the field. This was done for 3 consecutive mornings beginning July 9. Filled straws were removed every 5 days and placed in a 16:26.5 chamber. Some straws were left in the field.

Table 6 lists data for low-temperature treatment on adult females in 1973 for 5-day periods for which the data had been pooled. For the 1974 season, data on the incidence of diapause from the low temperature-treated adults and the non-treated control are given in Table 7.

In comparing incidence of diapause between the treatments for 1973 and 1974, the most outstanding feature was that after the third week in July the percentage increases very rapidly; the exception being with the 5°C-treated females in 1973 in which the incidence of diapause was the lowest of any treatment for the last week of July. After July 31 the percentage of diapause was again high, as with all other treatments. As stated earlier, treatment of the 5°C females in 1973 occurred on July 24 and 25. It is interesting to note the dramatic drop in percentage of

Table 6. Effects of low-temperature treatment on adults in 1973

Treatment	Date of straw removal		No. progeny treated	% mortality	No. pupated	% diapause
I[1]	July	10	421	7.6	207	46.8
		15	427	7.5	180	54.4
		20	412	8.3	191	49.5
		25	399	9.0	163	55.1
		31	348	34.8	78	65.6
	Aug	5	174	39.1	12	88.7
		10	29	3.5	1	96.4
II[2]	July	25	49	12.2	29	32.6
		31	102	15.7	72	16.3
	Aug	5	245	9.8	43	80.5
		10	47	25.5	1	97.1
III[3]	July	15	162	9.9	120	17.8
		20	65	3.1	54	14.3
		25	142	19.0	63	45.2
		31	89	19.1	18	75.0
	Aug	5	165	17.0	17	87.6
IV[4]	July	10	399	11.8	233	33.8
		15	460	18.3	259	31.1
		20	276	26.5	133	34.5
		25	66	40.9	28	28.2
		31	77	26.0	29	49.1
	Aug	5	91	26.4	7	89.5
		10	13	0.0	0	100.0

[1] Females treated w/14°C nights (July 10-17, progeny reared at 26.5°C.

[2] Females treated w/5°C nights (July 24-25), progeny reared at 26.5°C.

[3] Females untreated, progeny reared at 26.5°C.

[4] Females untreated, progeny reared under field conditions (range 7.2°C to 35.0°C, average 22.0°C).

Table 7. Effects of low-temperature treatment on adults in 1974; adults
exposed to 5°C, 3 hr a day for 3 consecutive days

Treatment	Date of straw removal		No. progeny treated	% mortality	No. pupated	% diapause
Experimental	July	10	441	7.9	290	28.6
(offspring		15	197	23.9	85	46.9
reared at		20	96	8.3	32	63.6
26.5°C)		25	110	24.5	29	76.6
		31	140	11.4	6	95.1
	Aug	5	44	34.1	2	93.1
Experimental	July	15	79	31.6	31	42.6
(offspring		20	44	2.3	10	76.7
reared at		25	No Sample			
field temp.)		31	31	19.4	3	88.0
	Aug	5	23	13.0	0	100.0
Control	July	5	940	9.9	455	46.3
(offspring		10	195	6.7	132	27.3
reared at		15	188	13.3	74	54.6
26.5°C)		20	90	11.1	28	62.7
		25	437	7.3	105	74.1
		31	320	20.3	34	86.7
	Aug	5	232	23.3	0	100.0
Control	July	10	591	33.2	292	26.1
(offspring		15	112	27.7	51	37.0
reared at		20	47	31.9	16	50.0
field temp.)		25	211	38.4	55	57.7
		31	154	19.5	7	94.3
	Aug	5	77	11.7	2	97.1

diapause for the larvae collected that following week. A low-temperature treatment on females late in the season may temporarily reverse the diapause process and indicates a need for further investigation.

Effects of nighttime temperature
manipulation on adults

The purpose of this study was to investigate the influence of varying nighttime temperatures on the incidence of diapause. Bees were incubated during the last week of July in 1973, and emerging adults were released into 6 field cages at the Greenville Farm (see Materials and Methods Section for detail). Within a day after their release, some of the blocks in which the bees were nesting were removed and placed at different nighttime temperatures; others were left in the field. From 30 to 90 females nested in the blocks in each of the cages. From August 10 through 26, blocks from 2 of the cages were removed nightly at 2100 hr, placed in a temperature cabinet maintained at $21^{o}C$, and returned by 0900 hr the following day to the cages. This treatment provided a 12-hr night of constant $21^{o}C$ for 16 days. In another cage, a block was removed each night for 2 nights (August 13 and 14), placed at $5^{o}C$, and returned the following morning. This treatment provided a 12-hr night of constant $5^{o}C$ for 2 days. In the remaining 3 cages, the blocks were left untreated.

Straws were removed daily, labeled with cage designation, inserted into plastic vials, and placed into a cabinet set at $26.5^{o}C$. Table 8 shows that there was no pupation for all treatments and controls. Average nighttime temperature in the field from August 10 to 26 was $12.5^{o}C$. Thus, even in the experiment where the females were kept at a temperature $8.5^{o}C$ higher than the average ambient temperature, the progeny entered into diapause. The low-temperature treatment did not produce the initial

increase in pupation as in the case described in the previous section. The fact that the females treated in this experiment were newly emerged and not 3 wk old before the 5°C treatment may have been the cause of the difference.

Table 8. Effects of nighttime temperature treatments on adults in 1973; progeny reared at 26.5°C

Treatment	Date of treatment	No. progeny treated	% mortality	No. pupated	% diapause
Experimental (12 hr darkness at 21°C, 16 days)	August 10-26	102	31.4	0	100.0
Experimental (12 hr darkness at 5°C, 2 days)	August 13-14	97	27.8	0	100.0
Control (average 12.5°C)	August 10-26	129	25.6	0	100.0

Effects of low temperature on pupae

The pupal stage was not treated until the summer of 1974 because of the known susceptibility of the pupae of other bee species to low temperatures during development (Torchio, personal communication). Having had no success in inducing diapause with the previously described treatments, it was decided to treat the pupal stage of M. pacifica.

In 1974, cells from the northern Utah stock were incubated the second week of June. Beginning June 24, when males were beginning to emerge as adults and most females were either in the dark pupa state or were newly-

formed adults ready to emerge from the cell in 2 to 3 days, a group of 15,000 cells was removed daily for 8 days, placed at 10°C for 3 hr (from 1400 to 1700 hr), and then returned to the incubation chamber. Emerging adult bees were released each day in a shelter on the USDA Poisonous Plant Research Laboratory grounds. Releasing of adults ended when emergence ceased on July 8. Approximately 70 treated female bees nested in the boards.

A group of untreated bees from the same stock was released at the barn in North Logan. These were used as a control group in which to check percentage of diapause. Filled straws were removed every 5 days.

After emergence was complete, 100 cells were sampled to check for pupal mortality. There were 30 emerged adults, 25 cells containing Tetrasticus sp. (parasites), 6 dead larvae, 30 dead pupae and 9 dead adults.

Table 9 gives the diapause percentages for the offspring of the females subjected to low temperatures as pupae. The diapause percentages for the control groups are those given in Table 7. Incidence of diapause was extremely high, even at the beginning of the season, for the offspring of the treated pupae reared in the laboratory as well as for those reared in the field.

Table 9. Effects of low-temperature treatment on pupae in 1974; pupae
          exposed to 10°C 3 hr per day for 8 consecutive days then re-
          leased as adults in the field; control is the same as given in
          Table 7

Treatment	Date of straw removal	No. progeny treated	% mortality	No. pupated	% diapause
Experimental	July 5	392	11.5	13	96.2
(offspring	10	384	11.2	48	85.9
reared at	15	378	6.3	11	96.9
26.5°C)	20	321	6.2	23	92.4
	25	782	9.5	10	98.6
	31	293	6.1	1	99.6
	Aug 5	180	8.3	0	100.0
Experimental	July 15	238	10.9	24	88.7
(offspring	20	123	17.9	0	100.0
reared at	25	291	58.8	6	95.0
field temp)	31	99	37.5	0	100.0

Development, Mortality, Polyvoltinism and Nesting Data

Development

In the course of this study, records were maintained on the development of eggs and larvae under various regimens of temperature and photoperiod for both the greenhouse and field populations. The data presented in Tables 1 through 4 were recorded only for the duration of development from hatching to pupation for the purpose of determining the incidence of diapause. However, this index was not used by several previous workers, who commonly determined developmental rates from egg development through spinning of the cocoon. To make a direct comparison of the current data with that of previous workers, Table 10 was prepared to show the developmental rate of eggs under various photoperiod and temperature treatments of populations from greenhouse and field sources.

Scheffe's mean comparison test (Edwards, 1967) was used to determine statistical differences between treatment means. The treatment at $16.5^{\circ}C$ and the treatments at $20^{\circ}C$ differed from each other and all other treatments at the 1 percent level. This may be accounted for in part by the larger variations from the means at the lower temperatures. At 26.5 and $30^{\circ}C$ there was no difference between developmental times for all treatments regardless of photoperiod or population.

Development time as recorded may vary a day or more because of the difficulty in determining the exact age of the eggs before treatment. Therefore, data in this study may vary from that of other studies. Tirgari (1963) demonstrated that as temperature increased, egg and larval development were accelerated. At $31^{\circ}C$ and 55 percent relative humidity, development time from egg to cocoon was 9.8 days. Kukovica (1966) reported that cocoon spinning began when the larvae were 8 to 9 days old

Table 10. Development rates for eggs at the various temperatures and photoperiods

Population	Photoperiod(hr) temperature(^{o}C)	No. treated	Duration from egg to cocoon spinning (days) Mean ($\pm$ SE)
Greenhouse	16:16.5	81	36.0** $\pm$ 7.1
Field	8:20	108	21.6** $\pm$ 4.1
Greenhouse	16:20	102	15.0** $\pm$ 2.6
Field	16:20	60	18.1** $\pm$ 1.1
Greenhouse	8:26.5	49	10.4 $\pm$ 1.2
Greenhouse	16:26.5	65	10.7 $\pm$ 1.8
Greenhouse	8:30	91	8.7 $\pm$ 2.1
Field	8:30	56	9.8 $\pm$ 1.2
Greenhouse	16:30	73	8.5 $\pm$ 1.8
Field	16:30	70	9.2 $\pm$ 0.4

**Treatments significantly different from other means at the 1% level as determined by Scheffe's mean comparison test.

at $32^{\circ}C$ and 60 percent relative humidity. In this study, the mean number of days from egg to cocoon spinning at $30^{\circ}C$, regardless of photoperiod, was from 8.5 to 9.2 days.

The present study indicates that the lower range of development is at $16.5^{\circ}C$. At $14^{\circ}C$, mortality was 100 percent for the egg stage and 55.6 percent for the larval stage. At $16.5^{\circ}C$, the mortality was 22.7 and 29.7 percent for the eggs from the greenhouse (Table 2), 16.7 percent for the larvae (Table 2) and 95 percent for the eggs from the field. The eggs from the greenhouse may have been more acclimated for the low-temperature treatment because the average daily fluctuation in greenhouse temperature was much less than that of the field. Stephen and Osgood (1965) found that below $19^{\circ}C$, the overwintering larval stage remained for 2 years without further development and subsequently died.

Mortality factors

Progeny mortality due to developmental conditions was recorded for the various temperatures and photoperiods and was shown in Tables 1 through 9.

Mortality in these experiments was caused by low temperature, desiccation, parasitism and predation. In 1972 and 1973, the mortality factors were more closely observed in experiments in which the progeny were placed into individual rearing cells. At the 14 and $16.5^{\circ}C$ rearing temperatures, mortality was very high. This was attributed to the fact that the lower threshold temperature for development is around $16.5^{\circ}C$.

Disregarding those experiments with the low temperatures, the overall mortality for the progeny from the greenhouse was 17.7 percent for the eggs and 11.1 percent for the larvae. The overall mortality for eggs removed from the field in 1972 and 1973 was 2.5 percent. This difference

in mortality between progeny from the field and progeny from the greenhouse was due to desiccation. The pollen masses made in the greenhouse appeared to lack the necessary nectar content to prevent them from drying out, re- sulting in the collapse of the egg or early instar larvae. This problem did not occur with the pollen masses in the field. The possibility that the variety of the pollen and nectar sources in the field was greater than that found in the greenhouse may have accounted for part of this difference.

Leafcutter bees that are placed in large alfalfa fields are largely restricted to alfalfa as the source of pollen and nectar (much as the bees in the greenhouse were restricted to 2 or 3 plants). If the grower were to make a wider variety of plants available to the bee, it might help to reduce some of the unexplained mortality of the egg and early instar that currently results when bees are raised in the large acreages of alfalfa.

The fact that the pupae are susceptible to cooler temperatures, as shown in the low-temperature treatments (30 percent mortality) indicates a need for further investigation. Not only would this help reduce the mortality during these treatments, but it would also have practical ap- plication where the bees are incubated at a constant temperature (ca. $30^{\circ}C$) so as to be synchronized with alfalfa bloom. When males first begin to emerge, the bee-boards are removed from the incubators and placed in the field. Thus, the female bees, which are 2 to 3 days behind the males in development, are subjected to nighttime temperatures of $10^{\circ}C$ or lower while they are still in the pupal stage. If there is mortality occurring here, the current incubation practices need to be changed. Possible changes might include the fluctuation of incubation temperatures (daily from 18 to $30^{\circ}C$) so that the bees are not subjected to such a temperature difference when they are removed from a constant $30^{\circ}C$ temperature and placed directly into the field.

Parasitism and predation were also important in the overall mortality figures for the leafcutter bee during this study. As stated in the Introduction, parasites and predators cause high mortality in bee-boards that are left in the grower's fields. These boards have to be left in the field during the summer so that the second generation of bees may hatch. Mortality data of progeny reared in the field were compared with data on progeny reared under laboratory conditions. Laboratory progeny were not exposed for as long a period of time to parasites and predators. The results illustrated in Figure 1 are pooled from all rearing between July 10 and August 5, 1974, with 6974 cells examined. Egg and larval mortality are nearly the same for those progeny reared in the laboratory and those reared in the field. Mortality due to parasitism and predation was nearly 5 times higher in the field than in the laboratory. The chief parasite was the eulophid wasp, Tetrasticus megachilidis Burks. The main predators were the clerid beetle, Trichodes ornatus Say, and the meloid beetle, Nemognatha lurida Le Conte. T. megachilids parasitized up to 22 percent of the bee larvae in the field as compared to only 2 percent in the laboratory. The results definitely show that parasitism and predation can be minimized by removing the bee-boards from the field. In this regard, the rearing of a single generation of bees would be advantageous.

Polyvoltinism

Krunic (1972) contended that M. pacifica is a polyvoltine bee and that there is a difference in wing venation between bees that emerge in the first- and second-generation. Krunic found that first-generation individuals had 80 percent of what he designated as type A wings, 11 percent type B, and 9 percent type C; second-generation bees possessed 19 percent type A, 19 percent type B, and 62 percent type C wing venation.

Egg and Larval Mortality Due to Causes
other than Parasites and Predators

DATE OF STRAW REMOVAL: JULY 10-AUGUST 5

▨ PROGENY REARED IN THE LABORATORY

▤ PROGENY REARED IN THE FIELD

Egg and Larval Mortality Due to Parasites and Predators

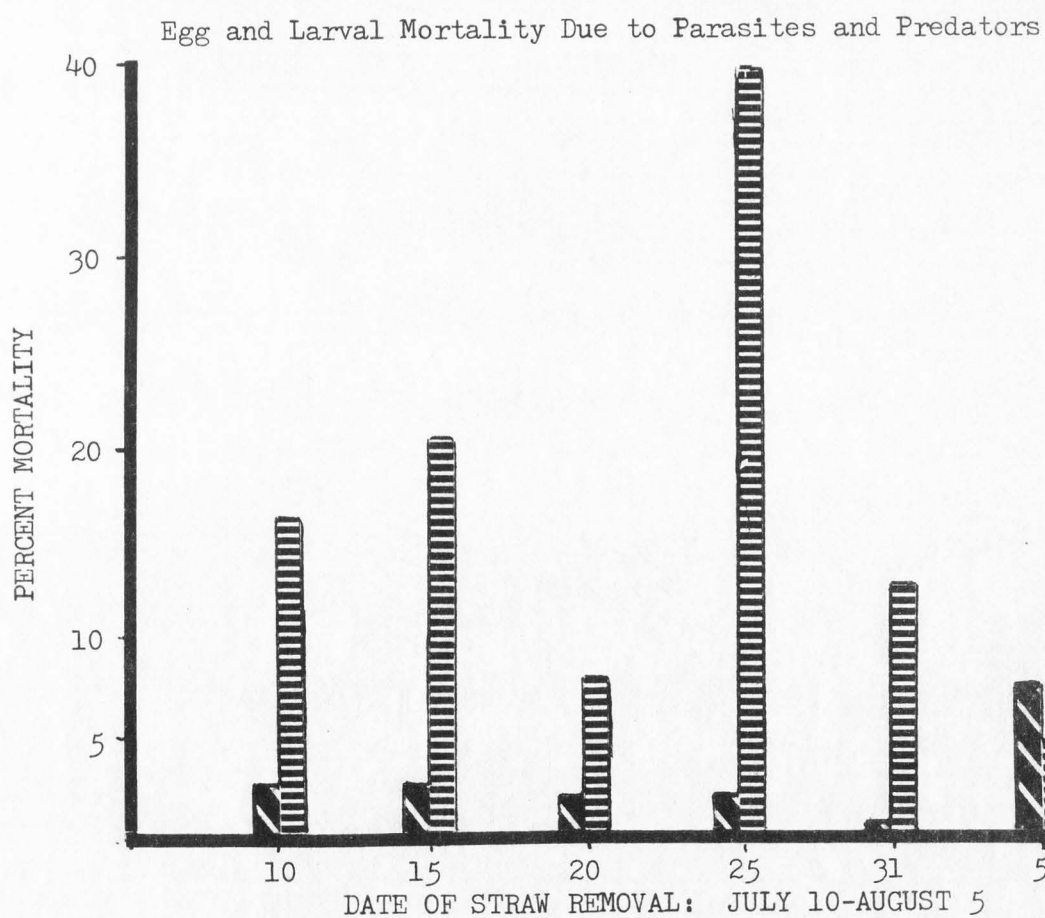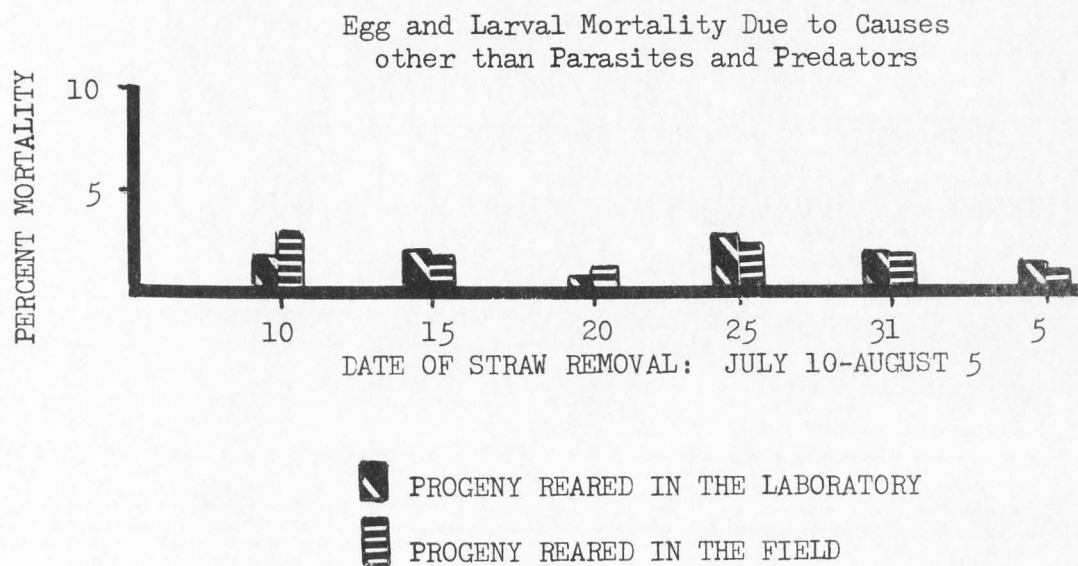DATE OF STRAW REMOVAL: JULY 10-AUGUST 5

Figure 1.   Progeny mortality in 1974.

(The different wing types were based on the relationship between the radio-medial cross-vein to the second-cubital cross-vein.)

In 1972, 200 second-generation bees from the present study were checked. Both sexes had approximately 12 percent with type C wing venation as compared to the Canadian bees which had 62 percent type C. From this count, it was concluded that the contention that M. pacifica is polyvoltine and that the different races of the bees can be distinguished on the basis of wing venation does not hold true, at least for bees from northern Utah which have a partial second generation. Furthermore, wing venation in the Hymenoptera is often quite variable even among the same species and may not be a good morphological character on which to base a voltine comparison.

Nesting data

Nesting activity for M. pacifica in the past has been recorded for 3 or 4 individual bees in the field (Klostermeyer and Gerber, 1969; Klostermeyer, Mech and Rosmussen, 1973) and under greenhouse conditions (Tirgari, 1963; Kukovica, 1966). Klostermeyer, et al (1973) used an electronic recording balance and found that females made on the average of 1 cell per day, with 1 female bee making up to 3 cells per day, depending on weather conditions and availability of pollen sources.

In the current studies, determination of the number of cells made per day by a female was calculated indirectly using large numbers of females. By recording the total number of females nesting in the sample blocks (nighttime inspection at various intervals during the season with the aid of a light) and dividing this number into the number of eggs removed from the nests over a 5-day period, the average number of cells laid per day was calculated. The results given in Table 11 agree closely

with those of previous workers. The females in these studies made from 1/2 to 1 cell per day in the field.

Undurraga (1975) produced a life table for M. pacifica. He found that the highest number of cells produced in a day did not begin until 3 weeks after the initial release of bees and then declined as the season progressed. This differed from the present study in that the number of cells made throughout the nesting period in Utah did not vary more than 1/2 cell per day (Table 11). Again, weather conditions and availability of bloom could account for the differences between these 2 studies. It is significant to note that according to the present study nesting efficiency concerning number of cells made per day was as high at the end of the season as at the beginning. This has important implications for the management of the bee and will be discussed further in the section on ecological management.

Table 11. Nesting data for the low-temperature-treated adults in 1973
and 1974

Date of straw removal	No. females	No. straws	No. cells	Cells/ straw	Cells/ female/ 5 days	Cells/ female/ day
			1973			
July 15	204	162	849	5.2	4.2	.8
20	185	134	635	4.7	3.4	.7
25	185	92	355	3.6	1.9	.4
31	150	161	610	3.8	4.1	.8
Aug 5	105	120	520	4.3	5.0	1.0
10	90	114	437	3.8	4.9	1.0
15	70	45	189	4.2	2.7	.5
20	45	43	180	4.2	4.0	.8
Total		871	3775	4.3		
			1974			
July 10	79	79	441	5.6	5.6	1.1
15	71	44	197	4.5	2.8	.6
20	44	20	96	4.8	2.2	.4
25	25	35	110	3.1	4.4	.9
31	26	31	140	4.5	5.4	1.1
Aug 5	21	14	44	3.1	2.1	.4
10	8	12	31	2.6	3.9	.8
Total		235	1059	4.5		

DISCUSSION

Experiments with Immature Stages to Induce Diapause

Eggs and larvae from the greenhouse failed to show any differences between treatments except at 8:20 where pupation did not occur or was very low (Table 1). This high percentage of diapause is attributed more to the length of time required for development rather than to a difference between the 8- and 16-hr photoperiods. The single larva that pupated did not do so until approximately 105 days after the beginning of the treatment. In 1973, the incidence of diapause was high with bees in the greenhouse for the whole season (Table 2). Nye, (personal communication) based on his work with M. pacifica in an adjacent green-house section, had an overall 85 percent diapause for the summer of 1973. The high rate of diapause may have been due to the low tempera-tures on the early emerging adults. These temperatures are later shown to be an important factor in inducing diapause. Overall, progeny taken from the greenhouse for treatments were too few to make many valid state-ments about the experiments conducted with them.

Only eggs were removed from the field for treatment. In 1972, there was no difference between treatments with all diapause rates being high (Table 3). The treatments were not begun until July 27. As was found with later experiments, the only time diapause rates were low was during the first 2 weeks of July. In 1973 the eggs treated between July 10 and 13 had a low percentage of diapause, with no difference between treatments (Table 4).

The original idea of using the greenhouse was to extend the time with which to work with M. pacifica. But, because nesting activities

were so much less efficient in the greenhouse the time spent may not be
worth while. It was much easier to obtain a larger number of progeny
for treatments from the field even though the nesting season was shorter
than that maintained in the greenhouse. There was also a difference
between percentage of diapause from progeny of the greenhouse and of the
field even during the same time periods, which is not explainable
at this time.

Results from these experiments indicate that photoperiod and temp-
erature changes do not induce the immature stages of M. pacifica to
enter diapause. This has direct implications for growers and will be
discussed further under the management section.

Experiments with Pupal and Adult Stages to Induce Diapause

These experiments involved treating either pupae or adults, releas-
ing the adults, then collecting and rearing the progeny to check for
pupation.

In 1972 the experiment to determine the effect of photoperiod on
adults showed little difference between the experimental group and the
control group in percentage of diapause (Table 5). The percentage was
high for both groups. In 1973 and 1974, adult females were treated
with low temperatures 1 week after being released in the field during
which time nesting had been established. Low-temperature treatment
varied from 5 to 14°C (Tables 6 and 7). Progeny were reared under field
and laboratory conditions. Progeny from untreated females in 1973 and
1974 were also reared in the field and in the laboratory. Treatments
did not differ from controls but a trend was evident that in the early
part of the nesting period, the incidence of diapause was low and grad-
ually increased as the season progressed. The one exception to this was

with the 5°C treated females in 1973. This treatment did not begin until July 24 and the incidence of diapause for the progeny collected the following week was the lowest of all other groups of treated and untreated adults.

Because of the previous failure to induce diapause by treatment of the egg, larval or adult stage, the pupal stage was treated with low temperature in 1974. The dark pupal stage through the adult stage of the bee which had not emerged from the cell were treated 3 hr a day at 10°C for 8 consecutive days. These bees were then released as adults in the field and the progeny were collected and reared (in both the laboratory and the field) to check for pupation (Table 9).

Table 12 shows the statistical analysis done on all experimental and control groups for 1974 (Table 7 and 9). An analysis of covariance on a completely randomized design as outlined in Steel and Torre (1960) was used. Percentages of pupating bees in each group (control, low-temperature-treated adults, low-temperature-treated pupae) were analyzed with the date of progeny removal from the nest (this being equal to the number of eggs laid the previous 5-day period) being the covariant. Using this analysis, the high correlation of percentage of pupation and date of progeny removal could be accounted for: i.e., the later the date of progeny removal, the lower the percentage of pupation (or the higher the percentage of diapause). A t-test was used to test for significance between groups. Incidence of diapause between progeny reared in the field (average daily temperature, 22.1°C) and progeny reared in the laboratory at 26.5°C was also analyzed but showed no significant differences. This further substantiates the findings that exposure of the progeny to different photoperiods and rearing temperatures will not alter the incidence of diapause. The most fruitful findings of all treatments was the low-temperature treatment of the pupae. These were significantly different at the 10 percent level

Table 12. Analysis of covariance on treatments and control in 1974 (data from Tables 7 and 9)

Treatment	Date of progeny removal mean difference	Percent pupation mean difference	Variance	$T=\frac{\%\text{pupation}}{\sqrt{V}}$ [3]
Control F.T.[1] vs Control L.T.[2]	3.88	9.70	295.03	.57
Low-temp-treated adults F.T. vs low-temp-treated adults L.T.	.07	-4.72	294.53	- .28
Low-temp-treated pupae F.T. vs low-temp-treated pupae L.T.	2.46	4.15	294.79	.24
Control L.T. vs low-temp-treated adults L.T.	-5.14	-1.72	295.70	- .10
Control L.T. vs low-temp-treated pupae L.T.	0.00	31.14	294.53	1.81*
Control F.T. vs low-temp-treated adults F.T.	-1.83	12.71	294.68	.74
Control F.T. vs low-temp-treated pupae F.T.	9.17	36.69	294.56	2.14**

[1]F.T. = progeny reared under field temperatures (range 5.5 to 35.6 C; avg. daily temp. 22.1°C).

[2]L.T. = progeny reared under laboratory temperatures (26.5°C).

[3]Degrees of freedom = 28

*Significantly different at the 10% level.

**Significantly different at the 5% level.

from the control group progeny reared under the same laboratory conditions at 26.5°C. The low-temperature-treated pupae reared under field conditions was significantly different at the 5 percent level from the control group progeny reared under the same conditions.

Figure 2 compares the percentage of diapause and date of straw removal (or progeny removal) for the low-temperature-treated dark pupae, the low-temperature-treated adults and control adults taken from 1974 data. Progeny from each group were combined, disregarding rearing temperature, since there was no significant difference between rearing temperatures (Table 12). Overall percentage of diapause for the control group was 59.8 percent; for the low-temperature-treated adults, 55.4 percent; and the low-temperature-treated pupae, 95.7 percent.

The 1974 data clearly indicates that low-temperature treatment during the late pupal and early adult stages induces diapause in the offspring. On the basis of this finding, the incidence of diapause in the progeny of the adults from the control groups between July 10 through August 5, 1973 and 1974 was compared. In a separate diagram the field temperature data of these 2 years were also compared to assess if the nighttime field temperatures might influence the seasonal trend of diapause. The threshold temperature of 10°C was used as the base line because this was the treatment temperature on the pupae. The nighttime field temperatures were taken from the weather station located on the Greenville Farm. The daily minimum temperature was used to calculate the accumulated number of degree-days above 10°C for the 14-day period after release of adults. Figure 3 illustrates the percentage of diapause of progeny at the different dates of straw removal as well as the accumulated field nighttime temperatures during this period. The 1974 adults were released 1 week
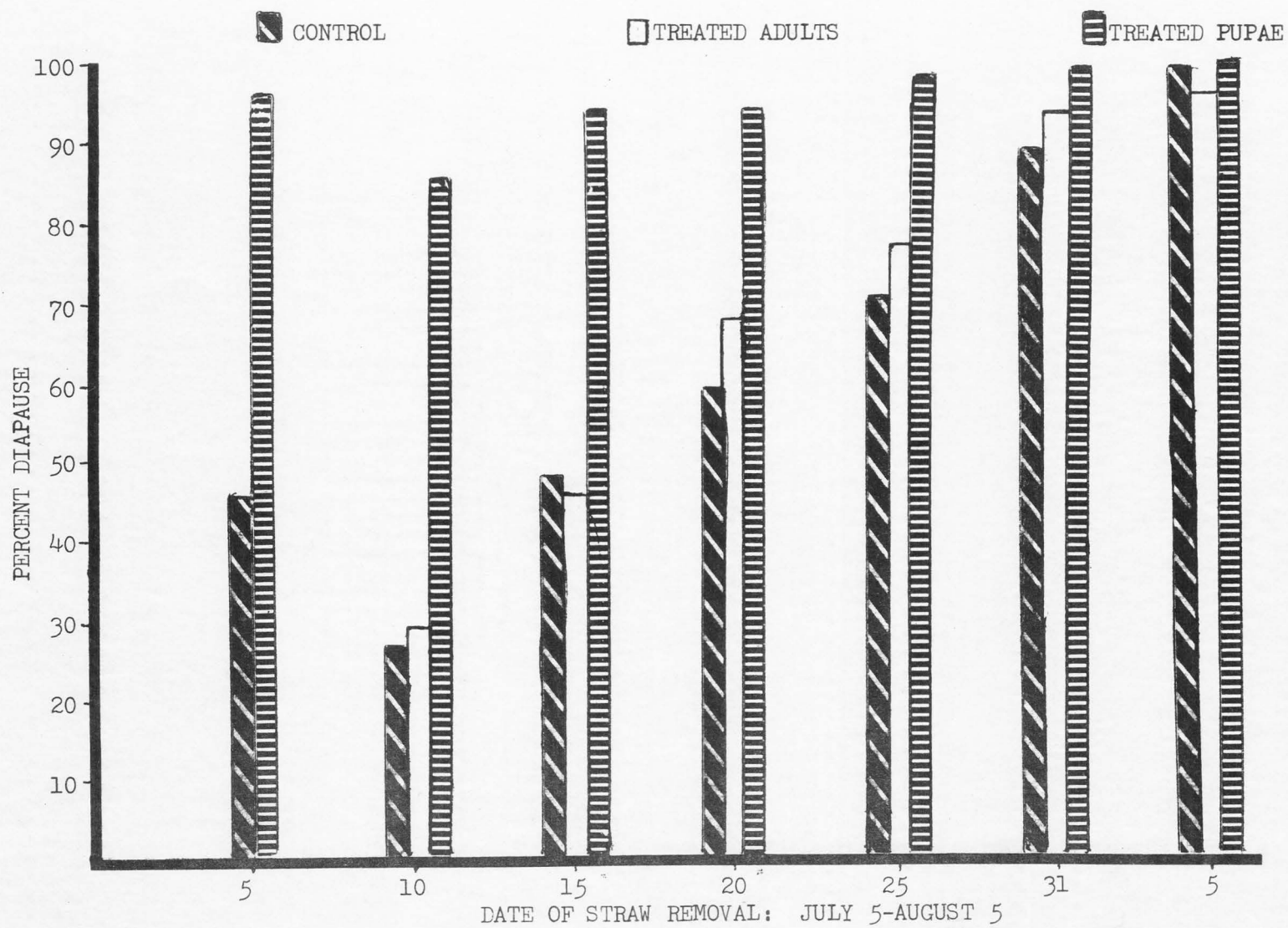
Figure 2: Comparison of percentage of diapause of progeny from low-temperature-treated pupae, low-temperature-treated and untreated adults in 1974.
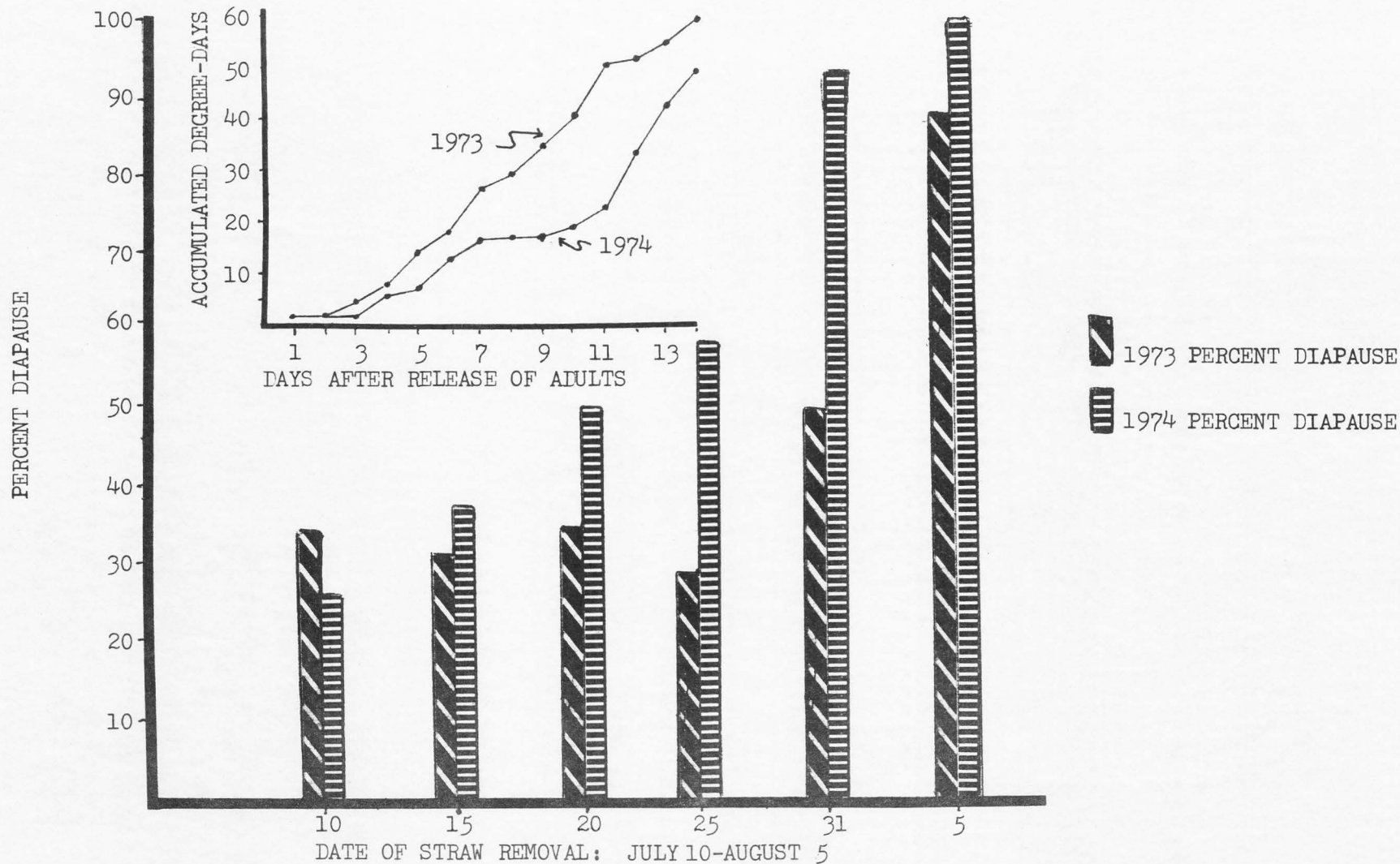
Figure 3. Percentage of diapause vs. accumulated degree-days above 10°C for progeny of untreated females (1973, 1974) reared in the field.

earlier than the 1973 adults. Percentage of diapause increased more rapidly and the overall percentage was higher for the 1974 population than the 1973 population. The accumulated degrees above 10°C for the nighttime temperatures was higher in 1973 (60°) than in 1974 (50°). Another significant and possibly more important factor is the number of nights the temperature was 10°C or below during the 14-day period after adults were released. In 1973 there was only 1 night, and in 1974 there were 4 nights during which the temperature was 10°C or below. If the early-emerging adults are susceptible to low temperatures, as are the pupae, in inducing diapause, the cool nighttime temperatures in 1974 might explain the higher percentage of diapause for the 1974 population.

Figure 3 also illustrates clearly the population trend of low incidence of diapause early in the nesting season and then increases as the season progresses. By the end of the second week following adult release, nearly all progeny reared enter diapause. This phenomenon has also been reported by several other workers. Thus, Krunic (1972) found in Canada that the only time pupation rates are going to be high is the first few weeks after the emergence of the first-generation adults. Johansen and Eves (1973) in their studies in Washington also found that the greatest number of second-generation bees were obtained from cells made between June 15 to July 15. This trend of diapause appears to correlate with a species characteristic of the leafcutter bee. It can influence the outcome of diapause induction experiments if late-season bees were used.

As already mentioned in the literature review sections, factors inducing diapause in Aculeate Hymenoptera are not known. The findings of this study provide the first experimental evidence that low-temperature treatment of pre-emerged females can induce diapause in the offspring of the

leafcutter bee. However, such maternal influence of diapause has been reported among the parasitic Hymenoptera. Ryan (1965) showed that short-day photoperiods acting on adult female Coeloides brunneri Vier induced diapause in the larvae. Saunders (1966) found that short-day photoperiods acting on adult Nasonia vitripennis females induced diapause in their offspring in the fourth instar larvae. The only experiments which show that temperature alone acting on adults can induce diapause in the offspring were conducted by Schneiderman and Horowitz (1958). They exposed pupal and adult stages of Nasonia vitripennis to low temperatures (10°). The following stages were treated and the percentage of diapause in the offspring is shown as follows: red-brown eyed pupae, 10.1 percent; black head and thorax pupae, 21.4 percent; all black pupae, 38.5 percent; newly emerged adults, 100 percent; one-day-old adults, 86.8 percent; two-day-old adults, 100 percent. In their studies, the low-temperature treatment affected the adults for only a few days, with mostly diapausing offspring being produced at first and then followed non-diapausing offspring. This situation may be similar with M. pacifica in that the all-black pupae and newly emerged adults are the most susceptible to low temperature.

What are the areas requiring further investigation as to the nature of diapause induction in M. pacifica? First, there is the problem of the susceptibility of pupae to low temperatures. The mortality of low-temperature-treated pupae for this experiment was 30 percent. This may possibly be prevented or lowered by decreasing the number of days of low-temperature treatment, the length of time of treatment, or by acclimatizing the pupae to a gradual increase of cold temperatures instead of subjecting them directly to the coldest temperature. Also, the exact age of the low-temperature-susceptible stage can be determined by removing the pupae and adults from

the cells so they can be identified and treated. Then the incidence of diapause of their offspring is recorded. Conceivably, certain of the stages may be less vulnerable to low temperatures than others.

It is difficult to determine at this time, but perhaps even a 30 percent loss of pupae may result in a larger population increase than a mortality of 70 to 90 percent in the second-generation bee due to un-available nectar and pollen in the field.

In the current studies bees were treated either at low temperature early in the season or at warm temperature late in the season. Two other possibilities involving reversal of treatment conditions have not been investigated. Is it possible that by providing warm temperatures to the pupae and young adults during the spring incubation that there would be a 90 to 100 percent second generation? Likewise, would it be possible to low-temperature treat the developing larvae late in the season, thus preventing them from entering diapause and allowing the bees to emerge into a third generation? Further studies into these areas would provide additional insight on the mechanisms of diapause induction in the leafcutter bee.

Ecological Management of Megachile pacifica

Several findings of this research have direct implications for the grower attempting to manage alfalfa leafcutter bee populations. Firstly, it was shown that the egg and larval stages can not be induced to enter diapause by changing photoperiod or temperature. They are programmed from birth to either pupate or enter diapause. Thus, it would not be ad-vantageous to the grower to remove his bee-boards from the field as soon as nesting is completed and place them into cold storage in hopes of pre-venting a second-generation development, since those bees destined to

pupate will do so and will eventually die during the cold-storage period.

The production of a single generation of M. pacifica now seems possible by low-temperature treatment of the pupal and early adult stage. The rearing of a single-generation leafcutter bee has several advantages. When a second-generation bee is present, mortality can be considerable due to the hatching of second-generation bees which kill or injure diapausing larvae while the new adults are attempting to emerge. Emerging second-generation adults are then often faced with a shortage of bloom for nectar and pollen and a short period of favorable weather to nest. With only a single generation, the grower can remove his bee-boards from the field as soon as the first-generation bee is through nesting. Placing the bee-boards into cold storage earlier will prevent the loss of bees due to parasitism and predation, which can be considerable as shown in these studies. An increase in mortality due to a longer period of storage may occur (Frank Parker, personal communication) and should be taken into account. Once the bees are removed from the field the grower will also be able to spray his field for control of the seed-destroying insects without fear of killing his bees.

If we consider that second-generation bees are lost because of un-availability of bloom or low temperatures in late season, we can calculate a monetary loss on the emerging bees from the control group versus the low-temperature-treated pupal group. Taking the example from Table 7, in the control group 1,251 out of 3,109 larvae emerged. At a value of $.02 per bee, $25.02 worth of bees were lost. In the low-temperature-treated pupal group, 136 out of 3,175 larvae emerged (Table 9). At $.02 each, $2.72 worth of bees were lost. On a large scale such as the grower is confronted with, this would be very important when it comes time to replace those bees the following season.

If a grower wishes to increase his bees and the bees are in areas where plenty of bloom is available late in the summer, and other environmental conditions are favorable, the production of a second generation of bees would be advantageous. The factors leading to the production of non-diapausing bees were not specifically studied in this work, but some of the data indicates that merely providing warm temperatures to developing pupae later in the season is not enough to prevent diapause induction in their offspring. Other factors, such as photoperiod and dietary factors may also be involved. There was some indication that a low-temperature treatment in late season may reverse this process, (Table 6) but this needs further investigation. Another possibility with future studies would involve providing warm temperatures to developing pupae in the spring to demonstrate the possibility of a 90 to 100 percent emergence of second-generation bees.

In the current studies, nesting efficiency as defined by the number of cells made per day per female was shown to be nearly the same throughout the nesting season. In an alfalfa seed field at about the middle of August, the field is chemically defoliated to aid maturation. This results in a large reduction in the bloom available for bees to obtain pollen. Since the bees are just as efficient nesting at this time of the season as they are earlier, from the management point of view, it would be to the advantage of the grower to provide additional sources of bloom late in the summer if he wants his bee populations to increase.

Personal observations made during the summer of 1975 indicate that overall bee mortality may be less during the latter part of the season, due to the effect of lower temperatures on the egg and early instar stages. Those adult females nesting at that time produce the most viable progeny for the following year.

The grower is faced with many problems in his attempt to raise alfalfa seed. It is hoped that through these studies the problems he faces in providing adequate pollinators will be lessened.

SUMMARY AND CONCLUSIONS

The effects of photoperiod and temperature on diapause induction in the alfalfa leafcutter bee, Megachile pacifica (Panzer) were studied. The relationships of photoperiod and temperature on mortality, rate of development and percentage of diapause were measured for several life stages of this bee. Correlations between number of females nesting and number of offspring produced were also made. Efforts were made to find a practical method to control the number of generations of this economically important bee.

Eggs, larvae, pupae and adults were subjected to varying regimens of the following temperatures and photoperiods: 5, 10, 14, 16.5, 20, 25, 26.5 and $30^{o}C$ with 0, 8 and 16 hr of light. After treatments, the treated individuals or their progeny were reared and percentage of diapause was calculated approximately 2 weeks after initial pupation began.

The threshold of development was at $16.5^{o}C$. Optimal development from egg to pupa occurred at $30^{o}C$ regardless of photoperiod. The egg stage and early instar larvae had the highest mortality. Low temperatures during development and desiccation of the pollen mass appeared to be the main factors contributing to this mortality. Parasitism and predation also contributed considerably to the overall mortality of M. pacifica.

In 1972 and 1973, eggs and larvae were treated to determine if diapause was induced in the immature stages. Progeny were removed from a greenhouse population and a field population. Results from these experiments indicated that photoperiod and temperature exert no influence on the immature stages of M. pacifica to enter diapause.

Experiments were conducted from 1972 through 1974 by treating pupae and adults and checking the incidence of diapause in their progeny. In 1972, to determine the effect of photoperiod on adults, 50 to 60 females were subjected to an 8-hr photoperiod. A control group of females received the natural photoperiod of 15 hr of sunlight. Both groups were subjected to the same temperatures in the greenhouse. There was no difference between the experimental group and the control group in percentage of diapause.

In 1973 and 1974, adult females were treated with low temperature for 1 week after being released and established in the field. In 1973, 1 group of adults was treated over an 8-day period for 12 hr daily at 14°C. Later in the season, another group of adults was treated at 5°C for a 3-hr period daily for 3 days. Progeny of both groups were collected and reared at 16:26.5. Some individuals were left to develop under field conditions. There was no difference between treatment and control diapause percentages.

The pupal stage was treated with low temperature in 1974. Dark pupae through young adults (adults which had not yet left the leaf cell) were treated daily for 3 hr at 10°C for 8 days. The bees were then released as adults and their progeny gathered and reared either at 26.5°C or under field conditions. This experiment proved to be the most fruitful with an overall percentage of diapause of 95.7 as compared to the control of 59.8 percent. Mortality of pupae due to the low temperature was 30 percent.

On the basis of the findings of 1974, the incidence of diapause in the progeny of the adults from the control groups of 1973 and 1974 was compared to determine if the seasonal trend of diapause was related to the nighttime temperature in the field. The results showed that percentage of diapause was higher for the 1974 control population than for the 1973

control population. The accumulated degree-days above 10°C for the night-
time temperatures was higher for the 1973 (60°) than in 1974 (50°). In
1973 there was only 1 night below 10°C, while in 1974 there were 4 nights
of 10°C or below. Since low temperatures induce diapause in early-emerging
adults as well as in pupae, the cool nighttime temperature in 1974 would
explain the higher diapause percentage for the 1974 population.

Percentage of diapause was consistently the lowest during the first
2 weeks of July, and then it increased rapidly. Treatment of newly-emerged
adults late in the season (mid-August) with either low or warm nighttime
temperatures had no discernible effect. All progeny entered diapause.

Records of the number of females nesting and the number of offspring
produced indicated that M. pacifica females made 1/2 to 1 cell per day
throughout the nesting season.

Several results of this research have direct implications for the
grower maintaining alfalfa leafcutter bee populations. First of all,
the egg and larval stages cannot be induced to enter diapause by changing
photoperiods or temperatures. Thus, the grower will not prevent the
emergence of a second generation of bees by placing his bee-boards into
cold storage early. This only slows down development time; those larvae
destined to pupate will either do so or die.

Secondly, inducing a single generation of M. pacifica seems quite
possible. If future experiments prove this, the grower will be able to
remove his bee-boards as soon as the first generation of bees is through
nesting and the progeny are allowed to develop to the diapause stage.
This will be advantageous to the grower for several reasons: 1) there
will be no injury to diapausing larvae caused by emergence of second-
generation bees; 2) the overall bee population will not decline because

the second-generation adults would normally face a shortage of pollen and nectar sources or inclement weather; 3) there will be lower mortality because time of exposure to parasites and predators is lessened; 4) pesticides may be applied to seed fields without fear of killing bees.

Thirdly, nesting efficiency as defined by the number of cells made per day was shown in the current studies to be nearly the same throughout the nesting season. A grower needs to provide additional sources of bloom late in the summer if he wants to increase his bee population.

It is hoped that through these studies, better management techniques can be devised to provide an adequate supply of pollinators so that the many problems a grower faces in his attempts to raise alfalfa seed may be lessened.

LITERATURE CITED

Barry, B. D. and D. L. Adkisson. 1966. Certain aspects of the genetic factors involved in the control of the larval diapause of the pink bollworm. Ann. Entomol. Soc. Amer. 59:122-125.

Beck, S. D. and J. W. Apple. 1961. Effect of temperature and photoperiod on voltinism of geographical populations of the European corn borer, Pyrausta nubilalis. J. Econ. Entomol. 54:550-558.

Beck, S. D. 1968. Insect photoperiodism. Academic Press, New York, 288p.

Bohart, G. E. 1972. Management of wild bees for the pollination of crops. Ann. Rev. Entomol. 17:287-311.

Bohart, G. E. and G. F. Knowlton. 1964. Managing the alfalfa leafcutting bee for higher alfalfa seed yields. Utah State Univ. Ext. Serv. EL 104:1-7 (revised in 1967).

Chapman, R. F. 1969. The insects: structure and function. Amer. Elsevier Co. 819p.

Danilevsky, A. S. 1961. Photoperiodism and seasonal development of insects. English Ed., 1965. Olivar and Boyd. Edinburgh. 242p.

Danilevsky, A. S. 1965. Photoperiodism and seasonal development of insects (English translation). Oliver and Boyd Ltd., London. 282p.

Danilevsky, A. S., N. I. Goryshin and V. P. Tyshchenko. 1970. Biological rhythms in terrestrial arthropods. Ann. Rev. Entomol. 15:201-244.

Edwards, A. L. 1967. Statistical methods. Holt-Rinehart and Winston, Inc., New York. 462p.

Eves, J. S. 1970. Biology of Monodontomerus obscurus, a parasite of the leafcutting bee, Megachile rotundata. Melanderia 4:1-18.

Eves, J. S. 1973. Population dynamics of larvae of the alfalfa leafcutting bee, Megachile rotundata, in eastern Washington. Unpublished Ph. D. dissertation. Washington State Univ., Pullman, Washington. 58p.

Gerber, H. S. and R. D. akre. 1969. The external morphology of Megachile rotundata (F.) (Hymenoptera; Megachilidae). Melandaria 1:1-36.

Gerber, H. S. and E. C. Klostermeyer. 1970. Factors affecting the sex
ratio and nesting behavior of the alfalfa leafcutter bee. Wash.
Agr. Exp. Sta. Tech. Bull. 73. 11p.

Harvey, G. T. 1957. The occurrence and nature of diapause-free develop-
ment in the spruce budworm, Choristoneora fumiferana. Clem. Can. J.
Zool. 35:549-572.

Hobbs, G. A. 1973. Alfalfa leafcutter bees for pollinating alfalfa in
western Canada. Can. Dept. Agr. Publ. 1495. 30p.

Hogan, T. W. 1960. The onset and duration of diapause in eggs of Acheta
commodus (Walk.) (Orthoptera). Aust. J. Biol. Sci. 13:14-29.

House, H. L. 1967. The decreasing occurrence of diapause in the fly,
Pseudosarcophaga affinis through laboratory-reared generations.
Can. J. Zool. 45:149-153.

Johansen, C. A. and J. D. Eves. 1973. Effects of seasonal conditions on
emergence of the alfalfa leafcutting bee. Environ. Ent. 2(1):23-26.

Johansen, C. A., E. C. Klostermeyer, J. D. Gerber and J. Eves. 1969. Sug-
gestions for alfalfa leafcutter bee management. Washington State Univ.
Agr. Ext. Serv. EM 2775 (revised) 8p.

Klostermeyer, E. C. and H. S. Gerber, 1969. Nesting behavior of Megachile
rotundata monitored with an event recorder. Ann. Entomol. Soc. Amer.
62:1321-25.

Klostermeyer, E. C., S. J. Mech, Jr., and Wm. B. Rosmussen. 1973. Sex and
weight of Megachile rotundata (Hymenoptera Megachilidae) progeny asso-
ciated with provision weights. J. Kan. Entomol. Soc. 46(4):536-548.

Krombein, K. U. 1948. An adventive Megachile in Washington, D. C. Proc.
Entomol. Soc. Wash. 50(1):14.

Krunic, M. D. 1972. Voltinism in Megachile rotundata (Megachilidae:
Hymenoptera) in southern Alberta. Can. Entomol. 104:185-188.

Kukovica, I. 1966. A study of the reproductive capacity, foraging behav-
ior and environmental adaptability of the leafcutting bee, Megachile
rotundata, in southern Ontario. Unpublished M. S. thesis. Univ. of
Guelph, Ontario, Canada. 210p.

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

Lees, A. D. 1968. Photoperiodism in insects, p. 47-137. In A. C. Geise
(ed.) Photophysiology. Academic Press, New York.

Lees, A. D. 1972. The role of circadian rhythmicity in photoperiodic
indiction in animals. Int. Symp. Circadian Rhythmicity. Proc. 87-
110.

Mansingh, A. 1971. Physiological classification of dormancies in insects, Can. Entomol. 103:983-1009.

Maslennikova, V. A. 1968. The regulation of seasonal development in parasitic insects. pp. 129-152. In Photoperiodic adaptations in insects and acari. Danilevsky, A. S. Ed. Leningrad State Univ., Leningrad. (In Russian).

Morris, R. F. 1967. Factors inducing diapause in Hyphantria cunea. Can. Entomol. 99:522-528.

Osgood, C. E. 1964. Foraging and nesting behavior of the leafcutter bee, Megachile rotundata. Unpublished M. S. thesis, Oregon State Univ., Corvallis, Oregon. 129p.

Philogene, B. J. R. and D. M. Benjamin. 1971. Temperature and photoperiod effects on immature stages and adults of Neodiprion swainei (Hymen: Diprionidae). Can. Entomol. 103:1705-1715.

Robertson, D. R. 1966. Observations on the alfalfa leafcutter bee, Megachile rotundata, at Hodgson, Manitobia in 1966. Proc. Entomol. Manitobia. 22:33-37.

Ryan, R. B. 1965. Maternal influence on diapause in a parasitic insect, Coeloides brunneri Vier. (Hymenoptera: Braconidae). J. Insect Physiol. 11:1331-1336.

Saringer, G. 1966. Effect of photoperiod and temperature on the diapause of Athalia glabricollis Thomos (Tenthred. Hym.). Acta Phytopath. 1:139-144.

Saunders, D. S. 1965. Larval diapause of maternal origin: induction of diapause in Nasonia vitripennis. J. Exp. Biol. 42:495-508.

Saunders, D. S. 1966a. Larval diapause of maternal origin-II. The effect of photoperiod and temperature on Nasonia vitripennis. J. Insect Physiol. 12:569-581.

Saunders, D. S. 1966b. Larval diapause of maternal origin-III. The effect of host shortage on Nasonia vitripennis. J. Insect. Physiol. 12:889-908.

Saunders, D. S. 1970. The effect of host species on diapause induction in Nasonia vitripennis. J. Insect Physiol. 16:405-416.

Saunders, D. S. 1973. Thermoperiodic control of diapause in an insect: Theory of internal coincidence. Science 181:358-360.

Saunders, D. S., D. Sutton and R. A. Jarvis. 1970. The effects of host species on diapause induction in Nasonia vitripennis. J. Insect Physiol. 16:405-416.

Schneiderman, H. A. and J. Horowitz. 1958. The induction and termination of facultative diapause in the chalcid wasps _Mormoniella vitripennis_ (Walker) and _Tritneptis klugii_ (Ratzeburg). J. Exp. Biol. 35:520-551.

Steel, R. G. and J. H. Torre. 1960. Principles and procedures of statistics. McGraw Hill Book Co., Inc. New York. 481p.

Stephen, W. P. and C. E. Osgood. 1965. The induction of emergence in the leafcutter bee, _Megachile rotundata_, an important pollinator of alfalfa. J. Econ. Entomol. 58(2):284-286.

Stephen, W. P. and P. F. Torchio. 1961. Biological notes on the leafcutter bee, _Megachile_ (Eutricharea) _rotundata_. Pan-Pac. Entomol. 37:85-93.

Sullivan, C. R. and D. R. Wallace. 1965. Photoperiodism in the development of the European pine sawfly, _Neodiprion sertifer_ (Geoff.). Can. J. Zool. 43:233-245.

Sullivan, C. R. and D. R. Wallace. 1967. Interaction of temperature and photoperiod in the induction of prolonged diapause in _Neodiprion sertifer_. Can. Entomol. 99:834-850.

Sullivan, C. R. and D. R. Wallace. 1968. Variations in the photoperiodic response of _Neodiprion sertifer_. Can. J. Zool. 46:1082-1083.

Szabo, T. I. 1969. Use of the leafcutter bee _Megachile rotundata_ for greenhouse pollination. Unpublished M. S. thesis. Univ. of Guelph, Ontario, Canada. 84p.

Tauber, M. J. and C. A. Tauber. 1970. Photoperiodic induction and termination of diapause in an insect; response to changing day lengths. Science 167: 170.

Tauber, M. J. and C. A. Tauber. 1972. Geographic variation in critical photoperiod and in diapause intensity of _Chrysopa carnea_ (Neuroptera). J. Insect Physiol. 18:25-29.

Tauber, M. J. and C. A. Tauber. 1973a. Insect phenology; criteria for analyzing dormancy and for forecasting postdiapause development and reproduction in the field. Search Agr. 3(12):1-16.

Tauber, M. J. and C. A. Tauber. 1973b. Quantative response to day length during diapause in insects. Nature. 244:296-297.

Thurston, R. 1972. Induction of diapause in Tobacco hornworms from Kentucky and California by temperature and photoperiod. Environ. Entomol. 1(5):638-640.

Tirgari, S. 1963. The biology and management of the alfalfa leafcutter bee _Megachile rotundata_. Unpublished M. S. thesis. Utah State Univ., Logan, Utah. 130p.

Torchio, P. F. 1974. Biology and control of _Saypga pumila_, a parasite of the alfalfa leafcutting bee. Utah State Univ. Agr. Exp. Sta. Res. Rep. 16. 13p.

Undurraga, J. 1975. Factors affecting the increase of the alfalfa leafcutter bee (Megachile pacifica). p. 14-25. In Sixth Annual Northwest Legume Seed Growers Short Course. Oregon State Univ. Agr. Ext. Serv.

U. S. D. A. 1973. Agricultural Statistics. U. S. Government Printing Office, Washington. 639p.

Wallace, M. M. H. 1968. Ecology of Sminthurus viridis (Collembola). II. Diapause in the aestivating egg. Aust. J. Zool. 16:871-883.

Waloff, N. 1949. Observations on larvae of Ephestia elutella Hb. during diapause. Trans. Roy. Entomol. Soc. 100:147.

Waters, N. D. 1969. Alfalfa pollinators. Idaho Agr. Ext. Serv., Idaho Curr. Info. Ser. 106. 2p.

Wilde J. de. 1962. Photoperiodism in insects and mites. Ann. Rev. Entomol. 7:1-26.

Wilde, J. de, W. Bongers and H. Schoonveld. 1969. Effects of host plant age on phytophagus insects. Entomol. Exp. Appl. 12:714-720.

VITA

Ron M. Bitner

Candidate for the Degree of

Doctor of Philosophy

Dissertation: Ecological Management of the Alfalfa Leafcutter Bee
Megachile pacifica (Panzer), with Emphasis on Diapause
Induction

Major Field: Entomology

Biographical Information:

Personal Data: Born July 22, 1946, Weiser, Idaho; married Nikki Y.
Boyd, November 11, 1974; four children, Julie, Andy, Jamie, Amy.

Education: High school diploma from Midvale High School, Midvale,
Idaho, in 1964; attended the College of Idaho, Caldwell, Idaho,
and received the Bachelor of Science degree in Biology in 1968;
attended Purdue University, Lafayette, Indiana and received the
Master of Science degree in Entomology in 1970; completed require-
ments for the Doctor of Philosophy Degree in Biology at Utah State
University in 1976.

Employment: Employed by Pioneer Hi-bred International, Agri-Con
Division, Nampa, Idaho, as the Leafcutter Bee Project Manager
from October, 1974 to December, 1975.

Self-employed as a private consultant specializing in pollina-
tion of crops, January, 1976; Pollination Consulting Service,
Nampa, Idaho.