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Factors Relating to Diapause in the Alfalfa Weevil Parasite *Bathyplectes curculionis* (Thomson)

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FACTORS RELATING TO DIAPAUSE IN THE ALFALFA WEEVIL PARASITE *BATHYPLECTES CURCULIONIS* (THOMSON)

by

David S. Parrish

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David S. Parrish

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ABSTRACT

Factors Relating to Diapause in the Alfalfa Weevil Parasite Bathyplectes curculionis (Thomson)

by

David S. Parrish, Doctor of Philosophy

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Major Professor: Dr. Donald W. Davis
Department: Biology

Biological and ecological factors regulating diapause in the ichneumonid parasite of the alfalfa weevil, Bathyplectes curculionis (Thomson), were studied. In most experiments, both parasites and weevil larvae were maintained in the environmental conditions under study from the emergence of the adult parasite until the evaluation of results.

The factors either preventing or inducing diapause in B. curculionis were found to be an interaction of environmental factors. A long scotophase with cool temperatures prevented diapause. The percentage of nondiapausing parasites increased as the scotophase was increased to 15 hours. Over 15 hours of scotophase, fewer nondiapausing parasites were produced. The optimum temperature during scotophase was 7.2° C for maximum nondiapausing. The temperature during the nine-hour photophase had to be raised. The greatest percentage of nondiapausing individuals occurred when the temperature was raised to 25.0° C. No effects of relative humidity

were observed between 50-80 percent. Relative humidity held at near saturation produced high mortality, while below 20 percent increased the amount of diapause to over 58 percent. A 15-hour scotophase at 7.2° C, a nine-hour photophase at 25.0° C, and relative humidity between 50-80 percent, consistently more than 95 percent of the parasites did not enter diapause. At either a long photophase or failure to alternate temperatures, essentially 100 percent diapaused.

Continuous photophase or continuous scotophase, either with or without alternating the temperature cycle, produced 100 percent diapause.

Part, but not all, of the regulation of diapause was determined by the effects of the photoperiod plus temperature cycle on the adult parasite just prior to oviposition. The percentage of diapause in the offspring increased markedly when the adults were transferred directly from a diapausing regime, when compared to those undergoing a five-day period with ideal conditions prior to oviposition. In both cases oviposition was under the best temperature-light cycle.

No relationship to diapause was observed between offspring from diapausing and nondiapausing parents. No study was made related to parasites from different geographical areas.

The incidence of diapause did not vary based on the instar of the host. The results were conclusive with the first three instars, but not with the fourth, due to low acceptance by the parasite.

The tendency to produce diapausing offspring increased as the female parent grew older. The number of eggs produced in a given

period of time decreased markedly after the first 10 days. Very little oviposition occurred after 15 days, although some individuals survived for more than 20 days. Female parasites which were not exposed to weevil larvae lived longer than ovipositioning females.

Temperatures in excess of 30.0^{o} C for longer than 36 hours produced mortality in both diapausing and nondiapausing Bathyplectes larvae within cocoons.

(114 pages)

INTRODUCTION

The alfalfa weevil, Hypera postica (Gyllenhal) (Coleoptera: Curculionidae), has been an important pest of alfalfa in the United States for 70 years. It was first found in the United States in 1904 near Salt Lake City, Utah, and according to Titus (1909, 1910), was of European origin. From this initial introduction, it was dispersed throughout western USA. Concurrent with this wide dispersal and establishment of H. postica, damage levels were reached, which made it the foremost pest of alfalfa in the West (Cothran, 1966).

The alfalfa weevil was first reported in eastern USA from alfalfa fields in Maryland by Poos and Bissell (1953). The origin of this introduction is not known. Dispersal was very rapid according to Cothran (1966), and in a short period of time it became a major pest of alfalfa throughout eastern United States. Cothran (1972) reported that with the discovery of H. postica in Florida and Minnesota in 1970 the alfalfa weevil had been detected in all 48 conterminous states.

Since 1963 it has been considered the most important pest of alfalfa in the United States (USDA, 1968, 1972). The economic importance of the weevil is reflected in the large number of publications dealing with its biology and control (Cothran, 1966, 1968).

As is usually the case, a newly-introduced foreign pest is not accompanied by the introduction of its natural enemies (Puttler et al., 1961). Efforts at biological control of the weevil were initiated in 1911 when natural enemies were imported into the U. S. from Europe. W. F. Fiske, H. S. Smith, and W. R. Thompson worked extensively on collecting, rearing, and screening potential parasites of the alfalfa weevil. Bathyplectes curculionis (Thomson) (Hymenoptera: Icheumonidae) became established in Utah as a result of these importations (Chamberlin, 1924, 1926; Clausen, 1956). Since that time this parasite has been colonized elsewhere in western United States. Successful establishment has occurred more recently in eastern United States (Puttler et al., 1961; Brunson and Coles, 1968).

B. curculionis is an endoparasite of alfalfa weevil larvae. It has been the most important and successful parasite species introduced for control of the alfalfa weevil. Numerous literature citations reflect its abilities to become established and to alter the number of weevil larvae in the field (Chamberlin, 1926; Coles and Puttler, 1963; Armbrust et al., 1967; and Wilson et al., 1969).

This parasite has one complete generation and a partial second generation per year. The adult parasites emerge in the spring from over-wintering cocoons and oviposit in alfalfa weevil larvae. The full-grown parasite larva spins its own cocoon within that of the weevil. The parasitized weevil larvae usually are killed after they have spun their cocoons.

In the Great Basin region of western United States the overwintering B. curculionis larvae begin pupating during the latter half of March. Adult parasites begin emerging in the northern Utah area by late April or early May with the peak generally during the second week of May (Hamlin et. al., 1949). Davis (1970) reported that in Cache Valley during most years few alfalfa weevil larvae appear before the first week of May. Weevil larvae are most numerous during late May and early June but some are present into August. Only the small or partial second generation of parasites occurs after the middle of June. Consequently, a more effective parasite with a complete second and/or a later generation would be advantageous.

If the factors that induce diapause in B. curculionis were known, it might be possible to increase the parasite populations during the entire period of weevil activity. There are only scattered references on the factors regulating diapause in parasitic Hymenoptera. The major objectives of this work were to study the factors, both biological and ecological, which contribute to the induction of diapause in B. curculionis.

REVIEW OF LITERATURE

Chamberlin (1925) reported that Bathyplectes curculionis was first described by Thomson in 1887, who assigned it to the genus Canidia. Ashmead, in 1900, changed the generic name to Canidiella. In 1909, Schmiedeknecht reverted to the use of Canidia. Finally, in 1914, Viereck found Canidia to be isogenotypic with Bathyplectes Forester, which gave the latter name preference.

B. curculionis (Thomson), an ichneumonid parasite, was first imported from Europe into the United States in 1911. From 1911 to 1914 approximately 1,500 adult parasites were collected and shipped to Utah and released along with 11 other species of parasites. These were the results of a search in Europe for natural enemies of the alfalfa weevil for biological control purposes. Of these 12 species only B. curculionis became established (Chamberlin, 1924, 1926; Clausen, 1956). Chamberlin (1926) stated that by 1916 B. curculionis parasitized weevil larvae from fields 10 miles from the first release site. Dispersal and establishment over a 50-mile radius were reported by 1918 and 230 miles by 1920. According to Clausen (1956) B. curculionis was colonized in Colorado by 1918, Nevada by 1921, California by 1933, and Oregon by 1934.

With the discovery and dispersal of the Egyptian alfalfa weevil in Arizona and the finding of the alfalfa weevil on the East Coast, there was a renewed interest in new parasite importations. In 1941 B. curculionis was released near Yuma, Arizona, on the Egyptian alfalfa

their cocoons within a short time period. Host weevil larvae still present in the field are attacked by these new adults. Parasites of this type are produced in the light textured, light brown cocoons that give rise to new adults, thus resulting in a second generation in the same season. Parasites developing from resultant adults are all diapausing forms. These individuals, plus most of the individuals from the first generation, produce diapausing cocoons. These cocoons are darker and more heavily constructed. This type of cocoon persists through the growing season, then overwinters. It contains late instar larvae which do not pupate until the following spring (Chamberlin, 1926; Newton, 1933; Hamlin et al., 1949; Brunson and Coles, 1968). Wilson and Armbrust (1970) found in Indiana that 39 percent of the first generation cocoons of B. curculionis were nondiapausing.

In the western United States the overwintering larvae of B. curculionis begin pupating during the latter half of March, with the pupal stage mostly present by the middle of April. The emergence of adult parasites reaches its peak about the middle of May (Hamlin et al., 1949).

Brunson and Coles (1968) reported that in the eastern United States the first brood of parasites begins approximately four weeks before the peak abundance of the alfalfa weevil larvae, while the second brood begins about one to two weeks after the peak abundance of the host larvae.

Diapause in insects

Diapause is a widespread form of dormancy among insects and is characterized by many features (Beck, 1968; Lees, 1968; Danilevsky et al., 1970).

The initiation and maintenance of diapause often cover large temporal intervals in the phenological calendar of an insect species. Diapause may encompass part of a season or it may cover several seasons.

Several factors that change regularly during the season may serve as instruments for diapause initiation or termination. These include nutrition, host availability, temperature, and humidity. However, the major factor or signal is day length. This signal is not subject to chance fluctuations and is the initial reason for seasonal climatic variations. In general, the diapause characteristics of a species, such as incidence of diapause, critical photoperiod and diapause intensity, are under polygenic control (Danilevsky, 1965). Diapause induction may occur at any particular developmental stage, depending on the insect, although usually in only one stage of a particular species.

Photoperiod is well established as having a major role in diapause induction in insects. Danilevsky et al., (1970) states that photoperiod undoubtedly plays the main part in diapause induction and all other factors are supplemental. Photoperiod induction of diapause has been widely investigated in many insects. These include studies of responses to photoperiod, adaptive nature of photoperiodic response, and

physiology of photoperiodism (Lees, 1955, 1968, 1972; deWilde, 1962; Beck, 1968; Danilevsky et al., 1970; Mansingh, 1971).

Tauber and Tauber (1973a) have categorized studies to define the effects of day length on diapause induction into three types based on insect responses. In the first type it is not important for photoperiods to change, but the only significant factor is for them to be longer or shorter than a critical photoperiod. A second type includes insects that respond to a change in day length that crosses a critical photoperiod. The third type of species responds to changes in day length that do not need to cross any critical photoperiod.

Although light is considered the major environmental factor controlling the induction of diapause, in some insects temperature can act as the primary diapause-inducing factor (Hogen, 1960; Missonnier, 1963). Thermoperiod in other insects can serve as the primary diapause-inducing factor (Menaker and Gross, 1965; Saunders, 1973). Temperature and thermoperiod may also have roles of varying importance in modifying the effects of photoperiod (Hughes, 1968).

In many species high temperatures and long photoperiods tend to act in concert, as do low temperatures and short photoperiods (Beck, 1963; Lees, 1968; Thurston, 1972).

DeWilde (1952), Danilevsky (1965), Beck (1968), and Lees (1968) have summarized the literature covering the role of temperature in the induction of diapause.

Induction of diapause in relation to nutrition and host availability have been reported several times in the literature. Either

nutrition or host condition may modify the primary effects of photoperiod and thereby change the incidence of diapause (Parr and Hussey, 1966; Saunders, 1966; McMullen, 1967; Saunders et al., 1970; Vinogradova and Zinojeva, 1972). Host species is an important controlling factor of diapause in the hymenopteran parasite, Nasonia vitripennis (Saunders et al., 1970). Tauber and Tauber (1973a, 1973b) found both food and photoperiod to be major factors controlling the facultative, reproductive diapause in green lacewings. The effect of dehydration and water absorption on diapause induction have been reported by Andrewartha (1952), Lees (1955), and Denlinger (1972). Stross (1969) reported that crowding had an influence on diapause induction.

Danilevsky (1965) stated that the diapause characteristics of a species such as incidence of diapause, critical photoperiod and diapause intensity, are under polygenic control. Many studies have been conducted and indicate that selection in the laboratory can produce both diapause and nondiapause strains.

Natural populations, even from a single area, contain some reservoir of intrinsic variability which affect insect responses to photoperiods. This store of variability has been used in a number of experiments in modifying the diapause characteristics of a particular strain of insects (Harvey, 1957; Barry et al., 1966; House, 1967; Maslennikora, 1968). This underlies the ability of the species to adapt to various latitudes and localities. In addition, the sexes may differ in their diapause responses (Earle and Newsom, 1964; Danilevsky, 1965; Rabb, 1969; Ring, 1971; Denlinger, 1972).

Tauber and Tauber (1973a, 1973b) showed that both critical photoperiods and the intensity of diapause (diapause duration) vary with populations from different areas. Danilevsky (1965) conducted a series of hybridizing experiments with geographical strains of various species of Lepidoptera. Danilevsky et al. (1970) summarized an investigation dealing with research concerning the inheritance of photoperiodism and illustrated how it undoubtedly plays the main part in diapause induction in insects.

The nature of diapause in the aculeate Hymenoptera is, in general, poorly understood. However, studies have been conducted on diapause induction in the suborder Symphyta and a few parasitic wasps. Clausen (1940) stated that diapause in the parasitic Hymenoptera is adaptive in that it delays development until the host reaches a certain stage at which time it is presumably most suitable for the nutritional requirements of the parasite.

Flanders (1944) reported that the occurrence of diapause in any individual is determined by the environmental conditions (either internal or external to its mother) to which that individual is exposed. Schneiderman and Horwitz (1958) and Saunders (1965) illustrated that larval diapause in the parasitic wasp, Nasonia (Mormoniella) vitripennis, is induced by environmental factors affecting the maternal generation.

Fisher (1971) reported that diapause in parasitoids was induced by photoperiodic response experienced either directly or indirectly through the maternal generation, and light and temperature are very

important in terminating the diapause of endoparasites. In addition, he stated that synchronization of the life cycle of host and parasitoid is achieved by either independent reactions of both species to light and temperature or by a developmental response of the parasite to a hormonal stimulus of the host.

Temperature is as important as photoperiod in diapause induction in some parasitic wasps. Schneiderman and Horwitz (1958) showed that by exposing females of N. vitripennis to low temperatures (10.0° C) during oogenesis the progeny entered diapause in the last larval instar. This also demonstrates maternally-controlled diapause.

Saunders (1973) raised females of N. vitripennis from the egg stage in total absence of light but subjected to daily temperature cycles and found that the wasps were able to distinguish a "short-day" thermoperiod (less than 13 hours at 23.0° C per day) and produce diapausing or developing progeny accordingly.

Ryan (1965) stated that the incidence of diapause in the larvae of the parasitic wasp Coeloides brunneri Vier (Braconidae) depended upon the photoperiod to which the parent females were exposed. Diapause induction caused by photoperiodic control has been demonstrated or inferred for the following families of Hymenoptera: Tenthredinidae (Danilevsky, 1961), Diprionidae (Sullivan and Wallace, 1965, 1967, 1968; King and Benjamin, 1965; Philogene, 1971; Philogene and Benjamin, 1971), Trichogrammatidae (Danilevsky, 1961; Saunders, 1965, 1966), Ichneumonidae (Claret, 1973).

Saunders (1966) and Saunders et al. (1970) demonstrated that variables such as diet, size, host shortage and host species, have affected incidence of diapause induction in the parasitic wasp Nasonia vitripennis. In most cases, however, the composition of the insect's diet proved to be either without an effect or to exert only a minor modifying influence on the insect's photoperiodic response.

Very little has been reported relating to factors inducing diapause in the family Ichneumonidae. This general dearth of information includes the genus Bathyplectes. Many have reported on the occurrence of diapause in B. curculionis (Chamberlin, 1926; Newton, 1933; Hamlin et al., 1949; Brunson and Coles, 1968). Most authors, when referring to B. curculionis, state that this parasite has a second or partial second generation (Michelbacher, 1940; Hamlin et al., 1949; Miller, 1970b). The one exception is Horn (1968) who stated that in New York B. curculionis is a single-brooded species.

Demonstrating a partial second generation suggests that some factor or factors are influencing some individuals not to diapause. Miller (1970a) reported that in Massachusetts most emergences of nondiapausing B. curculionis occurred before June 5. After July 5, all B. curculionis larvae diapaused. From work in northern Georgia it was shown that after April 15, 90 percent or more of the B. curculionis entered diapause (Miller et al., 1972). From personal observation in northern Utah, adults of the partial second generation occur in greatest numbers during the first half of July.

MATERIALS AND METHODS

This investigation required the synchronization of a parasite and its host over a period of time. Consequently, a continual supply of alfalfa weevil larvae plus an adequate properly-timed source of adult parasites was needed. An efficient method of insuring parasitism and rearing parasitized larvae had to be developed.

Source of parasite-free larvae

Newly-matured alfalfa weevils were collected from the northern Utah fields in July and August during each year of the investigation. They were brought to the laboratory and stored in one-gallon cardboard cartons (Sealright Co. Inc.). The adult alfalfa weevils were supplied with bouquets of alfalfa and wrinkled paper towels for hiding places. The cartons were stored in a walk-in cooler maintained at a constant temperature of 4.0^{o} C without regulated photoperiod. Very little feeding took place under these conditions.

The adult weevils were removed from storage, starting at 60 days, to establish laboratory cultures. Cultures were maintained (Figure 1) in plexiglass cages. Each unit consisted of four cylinders 27 cm high and 8.9 cm in diameter. Each cylinder contained a 25-dram vial stoppered with plastic foam and holding a bouquet of alfalfa. The top was covered by a screen lid. Approximately 50 adult alfalfa weevils were kept in each cylinder. Cultures were held in a walk-in growth chamber programmed for eight hours of light and 16 hours of darkness. The temperature was constant at 22.2^{o} C with the relative

humidity ranging between 50 and 80 percent. The alfalfa bouquets were replaced with fresh alfalfa every three days. Alfalfa grown in the greenhouse was used to insure its being free from alfalfa weevil eggs and larvae. The old stems were taken from the vials, stripped of remaining foliage and weevil eggs removed. Two methods were employed in removing the eggs. The usual method was to split the stem with a razor blade, then remove the eggs with a moist camel hair brush, and place them on moist filter papers in 9 cm-diameter Petri dishes. However, when many stems were involved, they were cut into smaller pieces and placed in approximately 600 ml of water. The eggs were extracted using short (5-10 sec) periods of grinding at the low range of a two-speed Waring blender. Pulverized plant material and eggs were washed with cold water through a series of 9-, 14-, and 60-mesh screens (Tyler Standard Screen Scale equivalent to 10, 16, 60 U. S. Series) to separate eggs from the stems. Following the first time through the screens, the plant material was returned to the blender and the process repeated until all the stems had been completely split. At this time the eggs were concentrated on the 60-mesh screen along with finely macerated plant material. The fine plant material and eggs were then washed into the 60 ml beaker and stirred in a circular motion. Weevil eggs remained on the bottom of the beaker with the other material suspended in the water. When the swirling motion stopped, the plant material was vacuumed off. Water was then added to the beaker and its contents poured over a piece of 15.0 cm (Whatman #1) filter paper. The filter paper was placed in a

Buchner funnel mounted in a 1000 ml filtering flask. The water was drawn through the paper and the eggs concentrated on the paper. The filter paper containing the eggs was incubated in 14 x 2.5 cm plastic Petri dishes at 22.2° C. Hatching occurred in 10 ± 2 days, with the majority hatching on the tenth day.

The newly-hatched first instars were placed in 4.5 x 2.5 cm small plastic cups (Bird Plastics Co.) with a piece of parachute cloth under the snap-typed top to reduce condensation. Larvae were supplied with alfalfa bud or meristematic tissue, which was renewed every other day and fresh plant material furnished. Following 5-7 the larvae were usually transferred to 14 cm plastic Petri dishes with alfalfa bouquets. The bouquets were placed in 8 ml corsage vials (Aquapic Co.). The rearing of the larvae was maintained at a temperature of 22.2° C with a photophase of eight hours. Using these conditions, sufficient numbers of alfalfa weevil eggs and larvae were available throughout the study.

Sources of adult parasites

Adult parasites were obtained from field-collected alfalfa weevil larvae during spring and summer. These larvae were reared on fresh-cut alfalfa in the laboratory in one-gallon Sealright cartons covered with screened lids. These cartons were cleaned periodically and food was added as necessary until adult weevils or parasite cocoons were formed and recovered.

Diapausing Bathyplectes curculionis cocoons were kept at 23.0-25.0° C and 16 hours photophase for two months, then stored in a

walk-in refrigerator at 4.0° C for at least three months. The cocoons were then removed as needed and placed in a growth chamber at the temperature and photoperiod scheduled to be used later with the adult parasites.

The emergence cages consisted of plastic 25-dram vials with the bottoms removed and replaced by parachute cloth. Cocoons were placed in the vials, on top of wire mesh resting on a water-filled plastic carton within a one-gallon glass jar. Humidity was provided by the evaporation from water in the carton. Upon emergence, the parasites were transferred to one-pint cartons. These cartons had holes in the sides with plugs of cotton moistened with honey to serve as food for the parasites. The parasites were used in the experiments after a 24-hour period had passed, thus allowing time for mating.

Parasitization and rearing

Parasitization cages were designed to obtain a combination of isolation and parasitization while rearing fairly large numbers of alfalfa weevil larvae. Each unit had four separate plexiglass cylinders 8.9 cm in diameter (Figure 2). A lid was made by gluing plastic screen to a plexiglass ring. A bouquet of alfalfa was placed inside each cylinder with the stems passing through a hole in the bottom into a vial of water. Fresh alfalfa was supplied as dictated by larval feeding. When alfalfa was changed, all larvae were transferred to a freshly-cleaned unit. All alfalfa used in the units was grown in the greenhouse and was free from weevil larvae. These units allowed for observation of both the alfalfa weevil larvae development

and parasite activity. With four cylinders within one unit, it was convenient to conduct replicated experiments. These units were easy to handle and required little space.

Most of the experiments were performed in the laboratory in four growth chambers (Percival Model I 30 BL, Percival Co.) (Figure 3). Since the chambers were accurate to $\pm 2.0^{o}$ C, all temperatures in this study should be considered as having a $\pm 2.0^{o}$ C range.

The relative humidity for all experiments, except those dealing specifically with controlled himidity, were maintained between 50 to 80 percent, through the use of anhydrous calcium sulfate. It was placed in each chamber daily prior to the start of the cool dark photoperiod. During the warmer photoperiods the calcium sulfate was dehydrated in a vacuum oven.

The alfalfa weevil larvae, of the desired instar, were removed from the Petri dishes and isolated in the rearing/parasitization cylinders. Individual larvae were transferred with a wet camel hair brush (0 or 00) and placed directly on plant buds or leaves. In the case of small instars, the small amount of moisture served as a medium for transport besides helping hold the larvae on the plant substrate. In most laboratory experiments, 25 larvae were distributed evenly on the plants in each cylinder. The instar, conditions of the particular growth chamber, and date were recorded on each cylinder. A 12-24 hour period was allowed for the larvae to adjust. Substantial mortality occurred when larvae did not have time to burrow into the bud or meristem before being exposed to parasites.

Figure 1. Unit used for oviposition and rearing of adult alfalfa weevil.

Figure 2. Parasitization and rearing unit for alfalfa weevil larvae.

Figure 3. Growth chamber containing parasitization and rearing units.

Figure 1

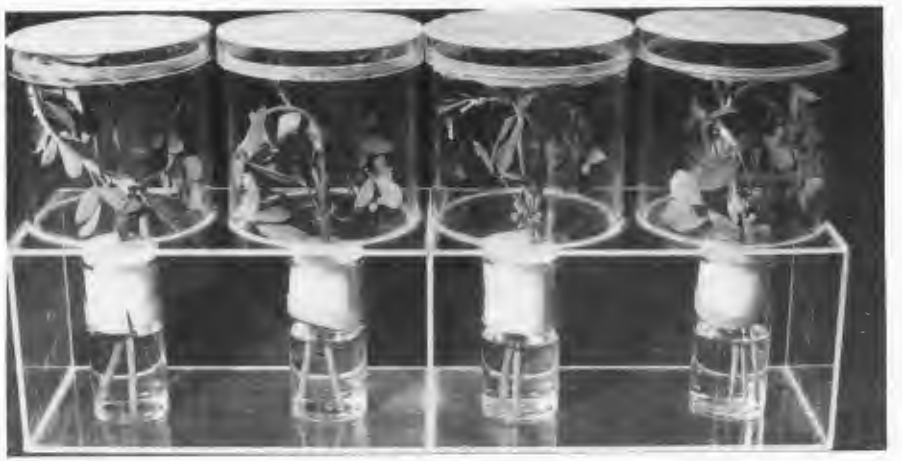

Figure 2

Figure 3

Adult B. curculionis were taken from the holding chambers with an aspirator. Only the most active females were chosen, and in most cases, one female was used in each cylinder. In all experiments, except the maternal-induced studies dealing with the effects of differing emergence, the female parasite was allowed to emerge under the same conditions in which it would be working later. The data concerning each female parasite were recorded on the cylinder. Each parasite was allowed access to the host larvae for 24 hours, after which it was removed and usually discarded.

In all of the investigations except the fecundity work and field experiments testing the effect of host age, 25 larvae were used in each of eight replications. These were divided and conducted as two series of four replicates each.

Except in the three preliminary investigations, B. curculionis cocoons were handled identically. The cocoons were allowed to remain under the same conditions they developed in up to and including the 45th day. If the parasite had not emerged, the remaining cocoons were transferred to optimum conditions until the 75th day. Following this date any remaining cocoons were dissected. All cocoons which contained dead adult parasites were considered nondiapausing and the others were counted as diapausing.

The effects of environmental factors on Bathyplectes curculionis diapause

Weather data from previous years were studied to determine the approximate environmental conditions under which nondiapausing

parasites occur in the field. The daylight hours occurring at these periods were also calculated. From this information it was decided that in three preliminary investigations the growth chambers should be programmed to resemble field conditions. Four growth chambers were programmed to operate at 21.0° C and eight hours photophase, 21.1° C and 16 hours photophase, 26.7° C and eight hours photophase, and 26.7° C and 16 hours photophase. This was followed by two series of experiments using 14 hours photophase and 10 hours scotophase. One sequenced used 21.1° C daytime temperatures with night temperatures ranging from 10.0° C to 29.4° C and nighttime temperatures held constant at 10.0° C.

For each of these preliminary experiments two separate series were run. Generally they were divided equally between second and third instars with 25 host larvae and one female parasite per cylinder. The developmental times, mortality, and percentages of nondiapausing parasites for each experiment were recorded.

The later experiments were based on the findings of the preliminary experiments. It was evident that the percentage of nondiapausing parasites increased when the scotophase was accompanied by cool temperatures. The basic experimental design was to maintain some constant environmental conditions while varying others. Six types of combinations were used. All experiments within one type were run simultaneously and compared statistically to controls kept at 25.0° C, 12 hours photoperiod, and a relative humidity of 50-80 percent.

1. A series of experiments used 25.0° C daytime and 7.2° C

nighttime temperatures, with a relative humidity of 50-80 percent. The photoperiod was varied. The scotophase hours were 4, 6, 8, 10, 12, 14, 16, 17, 19, and 21.

2. The photophase was kept at 9 hours at 25.0° C, and 50-80 percent RH (relative humidity). The nighttime temperatures were set at 5.0° C intervals between 1.6 and 15.6° C.

3. The photophase was kept at 9 hours, 25.0° C, and 50-80 percent RH. The duration of the 7.2° C temperatures was varied from 4 to 17 hours and centered on the scotophase.

4. Nine hours at 25.0° C and 15 hours at 7.2° C at 50-80 percent RH was maintained in either 24 hours photophase or 24 hours scotophase. The 24-hour scotophase experiments were conducted simultaneously with one at optimum temperatures and photoperiods to gauge the progress of the dark experiment. Twenty-five mixed second and third instar weevil larvae were housed in each of four, aluminum foil covered cartons (12.5 cm in diameter and 14.7 cm high). The cartons had parachute cloth tops. Number 10 cans (15.5 cm in diameter and 17.0 cm high) were painted entirely black and placed over the cartons which rested on black felt cloth on wooden risers. This allowed air to circulate. This apparatus was checked for total darkness prior to the experiment by placing Kodak Tri-x-pan film (Eastman Kodak Co.) in the carton then exposing the entire setup to growth chamber conditions. The larvae were changed and fresh bouquets of alfalfa added by the use of Wratten Series 1A Safelight filter (Eastman Kodak Co.) in a dark room. The female parasites were allowed to emerge, mate and work in the dark.

5. The temperature and photoperiod of these investigations were kept at 25.0° C during photophase and 7.2° C during the 15-hour scotophase. A high relative humidity above (80 percent) and a low relative humidity below (20 percent) were compared to the standard of 50-80 percent.

6. The interaction of thermoperiod and photoperiod was tested. The hours of photoperiod were reversed. A temperature of 25.0° C was maintained during 15 hours of scotophase and 7.2° C for the nine hours of photophase.

Influence of alfalfa weevil host on diapause

Experiments were conducted to determine if the age or larval instar of the host had any significant effect on the induction of diapause in the parasites. These studies were performed after optimum conditons for nondiapausing had been discovered. All experiments were done in chambers programmed at 25.0° C during days and 7.2° C nights with 15 hours scotophase.

Each instar was parasitized separately and the percentage of diapausing and nondiapausing was determined. One hundred larvae of each instar were separated in units of 25 and placed in the parasitization/rearing cylinders. Two replications per instar were conducted on two separate occasions; thus two hundred larvae from each instar were used. Each unit was exposed to two female parasites for 12 hours. The length of time for the parasites, attacking the different instars, to become adults and the percentage of nondiapausing parasites were recorded.

Effort was made to correlate laboratory data with field data. Two female parasites and 25 larvae of each instar were placed on potted plants in the field within separate 30-cm square cages and 38 cm high (Figure 4). This process was repeated weekly from April 25 to July 11, 1973, with two replications per week per instar used. The parasites were allowed to emerge and mate at the experimental site. The potted plants were watered weekly and new plants from the greenhouse were used for each new experiment. When weevil pupation occurred, all cocoons were placed in one-pint cartons covered by parachute cloth, then left in the field cages. The temperature was monitored daily with a maximum-minimum thermometer (Taylor Instrument, Sybron Corp.) and weekly by a hydrothermograph (Weather Measure Corp.).

Maternally-inducted or
inherited diapause

Seven growth chambers with a 12-hour photophase were used at the following temperatures: 10.0, 15.6, 21.1, 23.9, 26.7, 29.4, and 32.2^{o} C. A large number of B. curculionis cocoons was put in emergence cages, described previously.

In one test, the adult parasites, upon emergence, were allowed to remain in that particular chamber for 24 hours to feed and insure mating. They were then transferred to the optimum conditons for nondiapausing studies (25.0^{o} C at nine hours photophase and 7.2^{o} C at 15 hours scotophase). In the other investigations, emergence, mating and oviposition were performed under the same conditions and

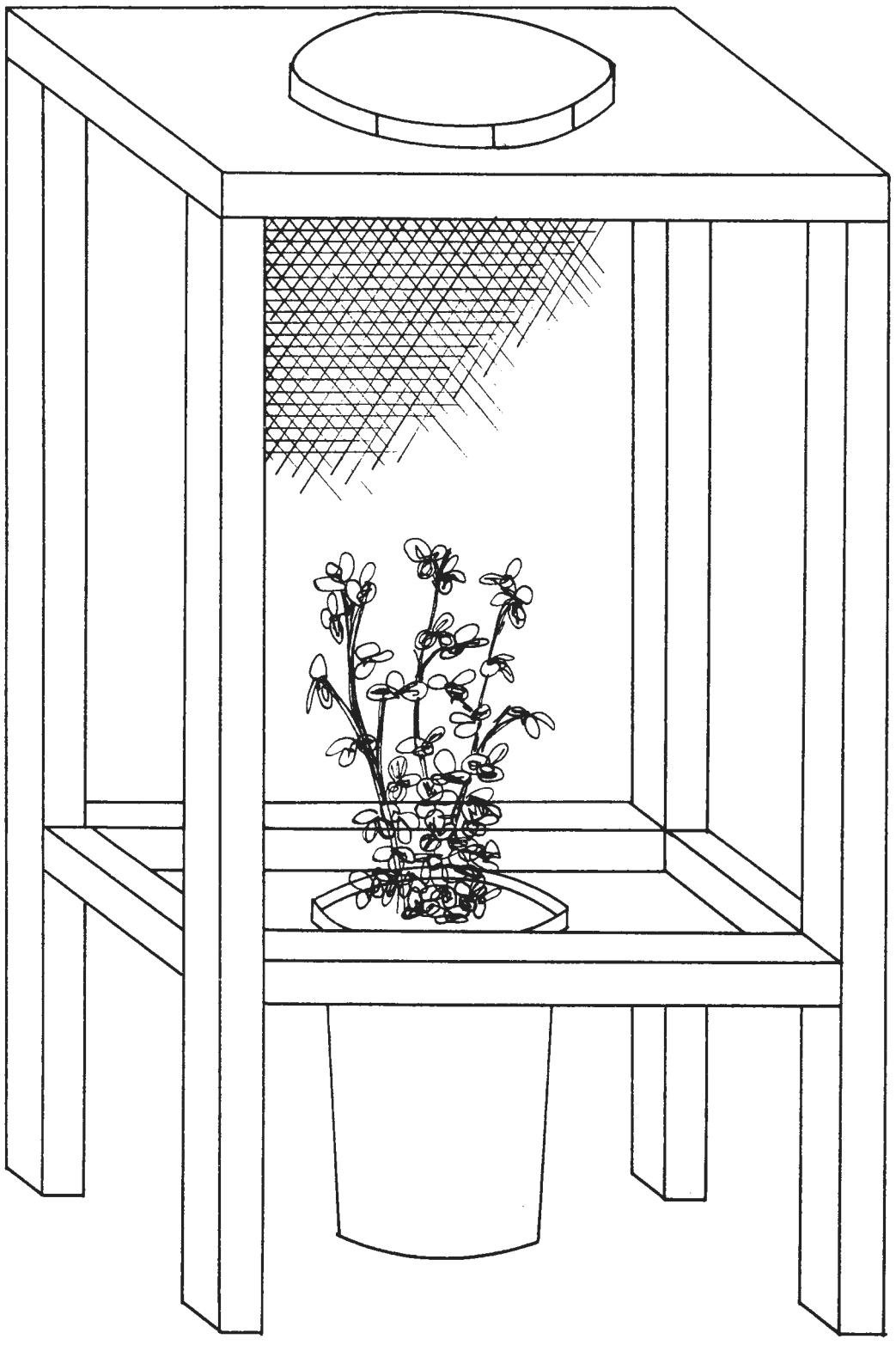

Figure 4. Cage used for parasitization and rearing of alfalfa weevil larvae in the field.

left for 24 hours before transferring the parasitized weevil larvae to the optimum conditions. In both experiments, eight randomly-selected female parasites from each temperature were selected, and one parasitization/rearing unit with four cylinders was used at each temperature. Each cylinder contained 25 larvae. All parasitization/rearing units were then placed in a growth chamber with nine hour photophase at 25.0° C and 15 hours scotophase at 7.2° C with a relative humidity of 50-80 percent. Two replicates of each temperature and each type of experiment were performed at the same time but in separate chambers.

For comparison, parasitism was allowed to occur at 25.0° C with a 15-hour dark photoperiod but no cool temperatures. On day 6 through 30 the cool nighttime temperature 7.2° C for 15 hours was applied to the parasitized larvae.

To test for any genetic effects on the percent of diapause, a basic four-way cross was performed. This was executed under the optimum conditions for nondiapausing to occur, 25.0° C days and 7.2° C nights with 15 hours scotophase. The following matings were used: nondiapausing males x diapausing females; nondiapausing females x diapausing males; diapausing males x diapausing females; nondiapausing males x nondiapausing females. A female parasite from each combination was introduced to 25 larvae. There were eight replications of each combination.

Diapause related to parasite age

A series of tests was conducted to determine any changes in diapause related to age of B. curculionis adults. Any changes in percentage of nondiapausing progeny were noted. Ten female parasites were picked randomly from emergence cages using 25.0° C day and 7.2° C temperature night and a 15 hour scotophase. The parasitism occurred under these same conditions. The parasites were introduced to a series of 10 second instar larvae for 12 hour periods. This was repeated at five-day intervals using the same 10 females until the parasites perished. It was felt than 10 hosts per week would be more realistic than the customary 25 larvae, considering the duration of the study. Each female parasite was fed sugar water when not with the weevil larvae.

Analysis of data

The statistical analysis of the data in most of the preliminary studies (with the exception of the constant temperature and different photoperiod experiment) was by analysis of variance using the F test of significance. An LSD test was used to measure differences among means in experiments with two or more treatments. A 2 x 2 factorial test was run on the data from the constant temperature and different photoperiod experiment.

The data from the combinations of environmental conditions were analyzed by the F test, follwed by an LSD test. The interaction test between photoperiod and thermoperiod was analyzed by the "t" test.

Statistical analysis of the data on the host instar parasitization, from both field and laboratory studies of host age influence, was by the analysis of variance and the F test. It was followed by an LSD test to determine significance. The data from the nondiapause studies were analyzed by analysis of covariance, followed by an LSD test. The data from the parasitized vs unparasitized studies were analyzed by the "t" test.

Data from the maternally-induced studies were usually analyzed by an LSD test. Exceptions were the test of comparisons between oviposition and nonoviposition in different treatments, and the changes in environment after oviposition, which were analyzed by a "t" test.

The longevity portion of the fecundity study was analyzed by a "t" test. Data from the nondiapausing portion were analyzed by factorial experiments showing analysis of variance, followed by an LSD test.

The developmental data (egg-adult) for the nondiapausing parasites throughout the research were analyzed by an LSD test.

All analyses were made following consultation with Dr. Donald V. Sisson.

RESULTS

Investigations of Environmental Effects on the Induction of Diapause

The early studies were designed to get a general idea of which combinations of environmental factors are associated with nondiapausing B. curculionis larvae. The basis for the investigation was an analysis of nondiapausing larvae in field populations.

Constant temperature and different photoperiod

The occurrence of nondiapausing larvae together with the developmental times and sex ratios under two different photoperiods are given in Table 1. The mean number of nondiapausing parasites at the different temperatures and corresponding lengths of photophase differed significantly at the 5 percent level from each of the other means, as determined by a 2 x 2 factorial test. The greater numbers of nondiapausing larvae were associated with the short daylengths. The long daylength, combined with warmer temperatures, produced all diapausing individuals. The developmental time from egg to emerging adult parasites did not differ significantly within the same temperature but was different between temperatures, 21.0° C and 26.7° C. There was a fairly high incidence of nondiapausing larvae with the combination of 26.7° C and eight hours of photophase.

Table 1. Effects of two constant temperatures at two different photoperiods on the prevention of diapause in the progeny of B. curculionis

Temperature °C	Hours of photophase	No. of larvae per replicate	Mean percentage of parasitism 1/	Nondiapausing progeny		
				Percent of individuals 1/2/3	Mean egg adult developmental time 4/ in days	Sex ratio (F:M)
21.1	8	25	24.5	12.2b	65.2a	2.1
21.1	16	25	19.5	10.3c	64.0a	1:1
26.7	8	25	18.0	28.3a	58.0b	1.1:1
26.7	16	25	27.5	0.0d	-	-

1/ Mean of eight replicates.
2/ Means followed by the same letter are not significantly different at the 5 percent level as determined by a 2 x 2 factorial test.
3/ Based on number of parasite cocoons.
4/ Mean followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.

Different low temperature during
10 hours scotophase

The effects of 14 hours of photophase at 21.0° C and 10 hours of scotphase at different low temperatures are summarized in Table 2. There was no significant difference in percentage of nondiapausing larvae between 7.2° C and 10.0° C. The other temperatures, both higher and lower, produced extremely low numbers of nondiapausing progeny which did not differ significantly from each other.

The developmental time at 4.4° C was significantly longer than all other temperatures except 10.0° C. There were no significant differences between 7.2, 10.0, 12.8, 15.6, 18.3, and 21.1° C.

High temperatures during
14 hours of photophase

The effects of 14 hours of photophase at different temperatures and 10 hours of scotophase at 10.0° C were tested in this experiment. The mean percentage of nondiapausing parasites between the range of temperatures from 21.1° C to 26.7° C did not differ significantly (Table 3). The only other temperature showing any nondiapausing individuals was 18.3° C. No significant difference existed between 18.3° C and 21.1° C.

Combinations of Environmental Conditions

Following the preliminary work which showed that an interrelationship of temperatures and photoperiod was involved in preventing diapause in B. curculionis, a series of more precise experiments was

Table 2. Effects of 14 hours photophase at 21.1° C and 10 hours scotophase with different selected temperatures on nondiapausing progeny of B. curculionis

Temperature °C during scotophase	No. of larvae per replicate	Mean percentage of parasitism 1/	Percent of Individuals 2/3/	Nondiapausing progeny: Mean egg-adult developmental time in days 2/	Nondiapausing progeny: Sex Ratio (F:M)
1.7	25	10.0	0.0b	-	-
4.4	25	11.5	4.4b	63.0a	1 female only
7.2	25	16.5	18.2a	58.0b	1.5:1
10.0	25	26.1	15.4a	59.4ab	1.5:1
12.8	25	15.0	3.3b	57.0b	1 male only
15.6	25	29.5	2.7b	58.0b	1 male only
18.3	25	19.0	1.2b	58.1b	1:1
21.1	25	24.5	2.0b	57.0b	1:5:1

1/ Mean of eight replicates.
2/ Means followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.
3/ Based on number of parasite cocoons.

Table 3. Effects of 14 hours photophase with varied temperatures and 10 hours scotophase at 10.0° C on the prevention of diapausing progeny of B. curculionis

Temperature °C at 14 hours	No. of larvae per replicate	Mean percentage of parasitism 1/	Percent of Individuals 1/2/3/	Nondiapausing progeny	
				Mean egg-adult developmental time in days 2/	Sex ratio (F:M)
10.0	25	20.5	0.0b	-	-
12.8	25	15.0	0.0b	-	-
15.6	25	16.5	0.0b	-	-
18.3	25	30.5	4.9b	55.0a	2:1
21.1	25	20.5	12.2ab	53.0a	1.5:1
23.9	25	16.0	25.0a	45.0b	1:1
26.7	25	22.5	20.0a	44.0b	1.3:1
29.4	25	25.5	0.0b	-	-

1/ Mean of eight replicates.
2/ Means followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.
3/ Based on number of parasite cocoons.

conducted. The experiments centered around those temperatures and photoperiods showing the greatest percentage of nondiapausing individuals in the preliminary phase, and also included some work with relative humidity.

Length of scotophase at 7.2° C

The mean percentage of nondiapausing larvae resulting from varying the length of scotophase (scotophase at 7.2° C and photophase at 25.0° C) is shown in Table 4 and Figure 5. These tests compared effects of various scotophase lengths to a control with a continuous temperature of 25.0° C and 12 hours photophase. The percentage of nondiapausing larvae was the greatest at 15 hours scotophase, yielding consistently over 95 percent. This was higher than any other scotophase interval, even those of 14 and 16 hours. At periods less than eight hours or greater than 19 hours, all individuals went into diapause. The percentage of nondiapausing larvae at 14, 16, and 17 hours of scotophase were not significantly different from each other. There were many nondiapausing larvae at the 12 hours of scotophase but the remaining scotophase regimes yielded low percentages.

The developmental time from egg to adult increased progressively from the eight hour scotophase experiment to 19 hours, probably due to fewer hours of warmer temperatures. The developmental time at 17 and 19 hours of scotophase were not significantly different from each other; however, 16, 15 and 14 hours were distinct.

The female to male sex ratio had an overall average of 1.75:1.

Table 4. Effects of various lengths of scotophase at 7.2° C on the prevention of diapausing progeny of *B. curculionis*

Hours of scotophase at 7.2° C 1/	No. of larvae per replicate	Mean percentage of parasitism 2/	Percent of individuals 2/3/4/	Nondiapausing progeny: Mean egg-adult developmental time in days 3/	Nondiapausing progeny: Sex ratio (F:M)
4	25	26.5	0.0e	-	-
6	25	28.5	0.0e	-	-
8	25	20.0	5.0e	38.0de	1:1
10	25	27.0	18.5de	43.4de	4:1
12	25	24.5	48.9cd	45.1de	1.6:1
14	25	28.5	66.6bc	47.2d	.9:1
15	25	31.0	96.7a	53.5c	1.2:1
16	25	25.5	80.4b	58.2b	1.2:1
17	25	29.5	54.2bc	66.0a	3.1:1
19	25	21.5	9.3e	70.0a	1:1
21	25	29.5	0.0e	-	-
Control 25° C at 24 hrs 12PP	25	19.8	0.0e	-	-

1/ Photophase was at 25° C.
2/ Mean of eight replicates.
3/ Means followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.
4/ Based on number of parasite cocoons.

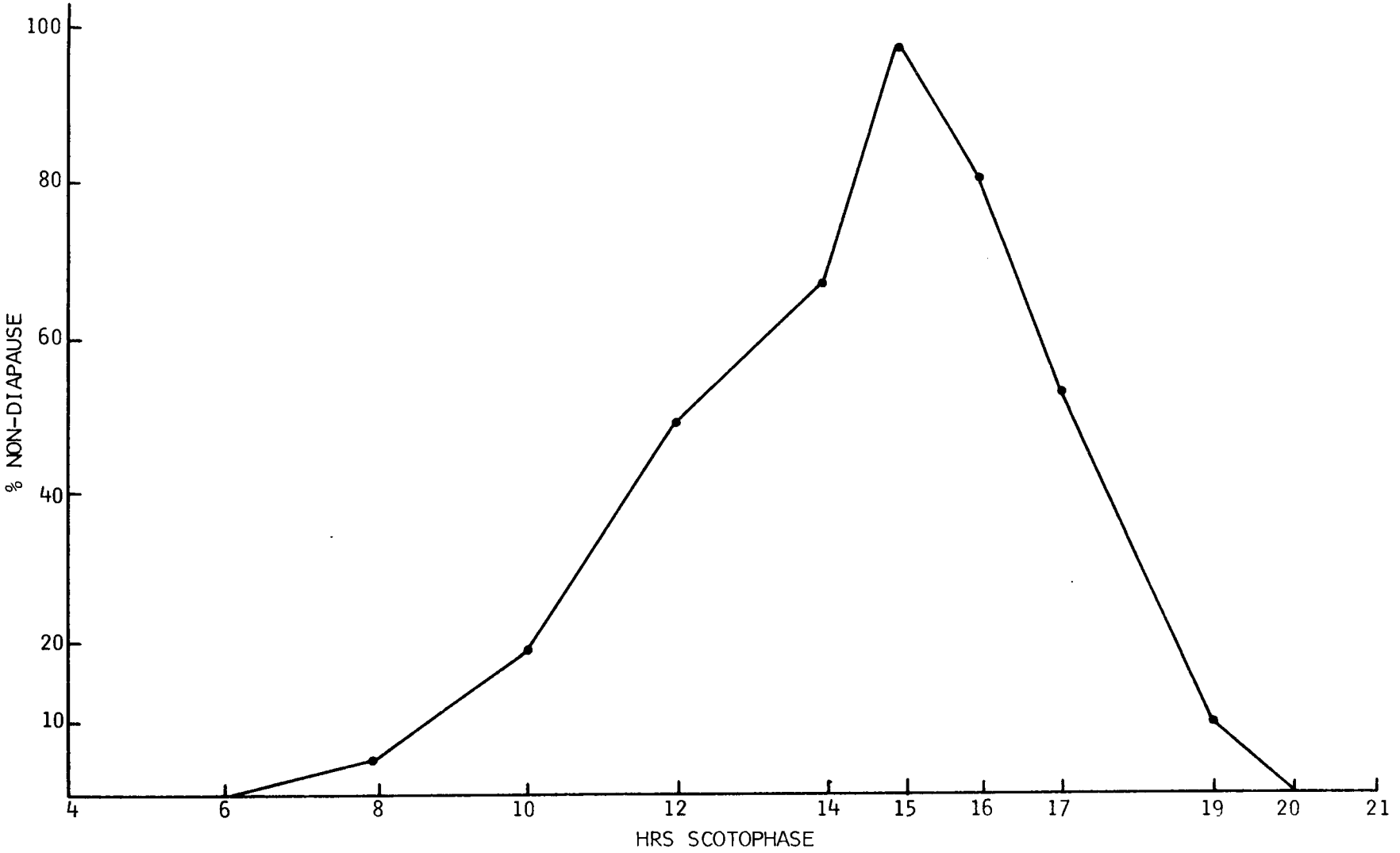

Figure 5. Percent of nondiapausing B. curculionis larvae resulting from 7.2° C during various hours of scotophase with the remaining photophase hours at 25.0° C.

Various low temperatures at
15 hours scotophase

The temperatures were then manipulated during the 15 hour scotophase, but held at 25.0° C during the photophase. The percentage of nondiapausing progeny, the developmental periods and the sex ratio induced by the different low temperatures are summarized in Table 5 and Figure 6.

Both 7.2° C and 4.4° C resulted in more than 70 percent nondiapausing parasites, with 7.2° C producing nonsignificantly more than 4.4° C. Fairly large numbers of nondiapausing individuals occurred at 1.7 and 10.0° C, but there was a significant difference from the 7.2 and 4.4° C.

The temperatures of 7.2° C and 4.4° C resulted in egg to adult developmental times that were not significantly different. There was a tendency for the higher temperatures to have more rapid developmental rates.

The sex ratio for this experiment was 1.1:1 female to male. A parasitism mean of 26 percent occurred in this experiment.

Various lengths of cool temperatures
centered during a 15 hour scotophase

The photoperiod was kept constant at nine hours. Centered within the scotophase were various lengths of time when the temperature was lowered from 25.0° C to 7° C. The results are summarized in Table 6 and Figure 7. The greatest numbers of nondiapausing parasites were at 15 hours (96.5 percent) and 16 hours (91.8 percent).

Table 5. Effects of various low temperatures during 15 hours scotophase on the prevention of diapausing progeny of B. curculionis

Scotophase temperature °C 1/	No. of larvae per replicate	Mean percentage of parasitism 2/	Percent of individuals 2/3/4/	Nondiapausing progeny: Mean egg-adult developmental time in days 3/	Nondiapausing progeny: Sex ratio (F:M)
1.7	25	24.5	40.8b	47.5d	1.2:1
4.4	25	30.5	75.4a	50.5b	1.6:1
7.2	25	29.5	94.9a	51.5ab	1.1:1
10.0	25	20.0	52.5b	49.3cd	.6:1
12.8	25	50.5	2.0c	55.0a	1:1
15.6	25	19.0	0c	-	-
Control 25° C at 24 hrs 12 PP	25	26.2	0c	-	-

1/ Photophase for the duration of nine hours at 25° C.
2/ Mean of eight replicates.
3/ Means followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.
4/ Based on number of parasite cocoons.

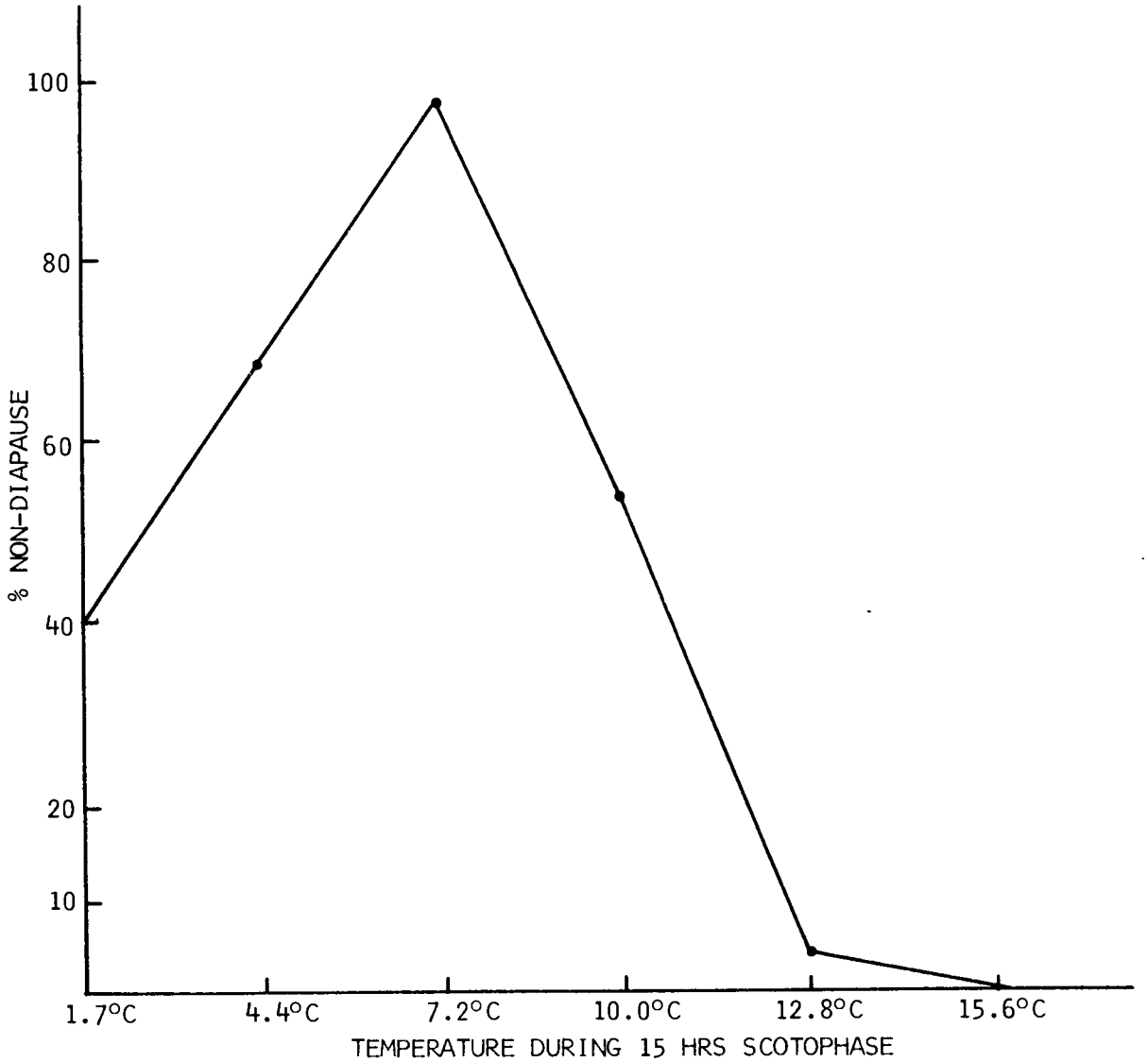

Figure 6. Percent of nondiapausing B. curculionis larvae resulting from low temperature for the duration of 15 hours scotophase and the photophase at 25.0° C.

Table 6. Effects of various hourly periods of cool temperature at a photoperiod of L:D 9 hours: 15 hours on the prevention of diapausing progeny

Temperatures °C				Nondiapausing progeny			
Daytime	Nighttime	Hours of cool temperature	Mean percentage of parasitism 1/	Percent of individuals 1/2/3/ 5%	1%	Mean egg-adult developmental time in days 2/	Sex ratio (F:M)
Control							
25.0	25.0	15	27.0	0f		-	-
25.0	7.2	4	24.0	0f		-	-
25.0	7.2	6	31.0	0f		-	-
25.0	7.2	8	23.5	8.7f		41.5f	1:1
25.0	7.2	9	31.0	12.9ef		41.5f	.9:1
25.0	7.2	10	29.5	20.3de		45.4e	1.5:1
25.0	7.2	12	25.5	54.9c		48.0d	2:1
25.0	7.2	15	28.5	96.5a	NS	52.0c	1.6:1
25.0	7.2	16	24.5	91.8b	NS	53.0b	1.3:1
25.0	7.2	17	20.0	42.5d		56.0a	2:1

1/ Mean of eight replicates.
2/ Means followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.
NS=Not significantly different from the other mean at the 1 percent level.
3/ Based on number of parasite cocoons.

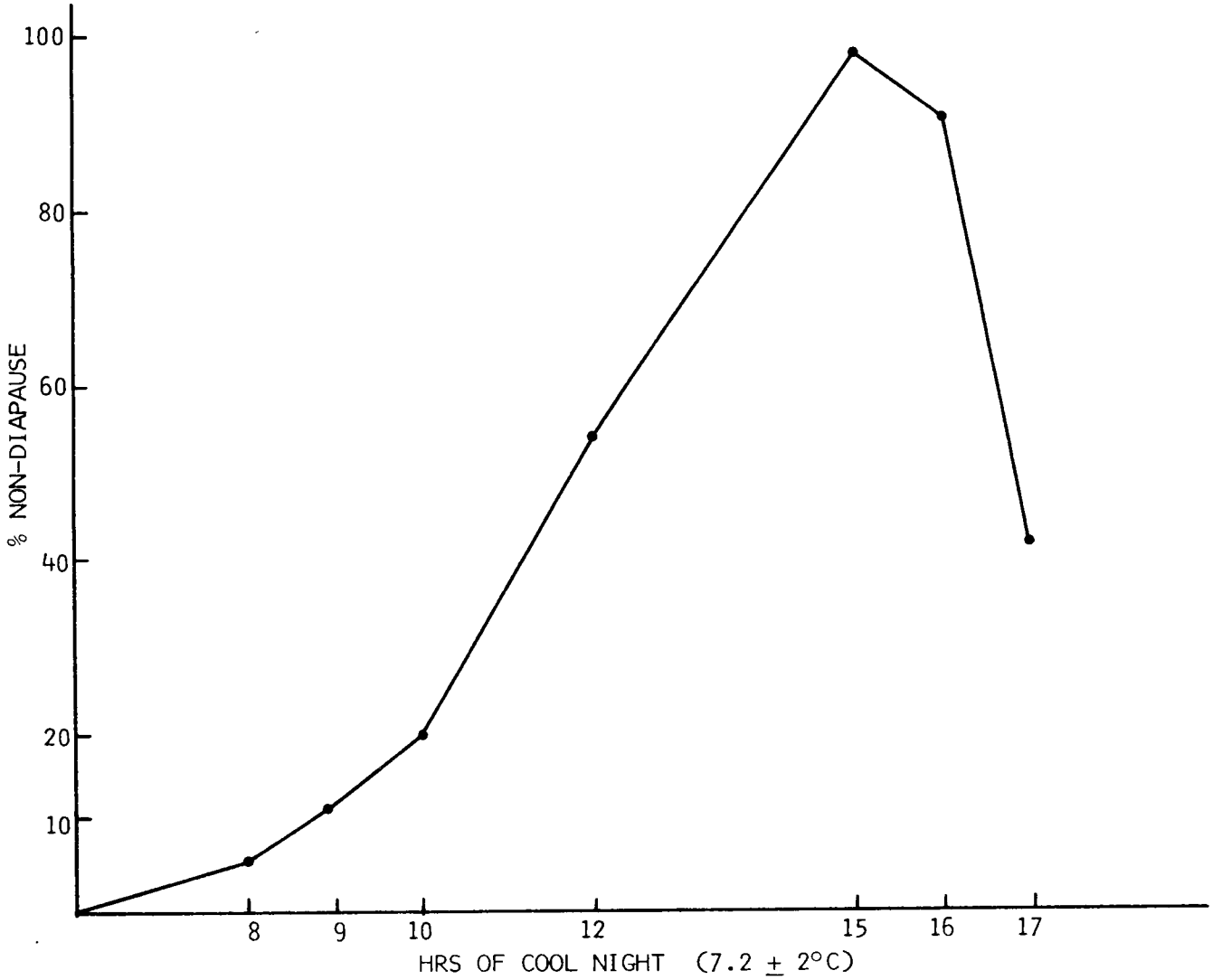

Figure 7. Percent of nondiapausing larvae resulting from varying the number of hours of cool temperature centered during a fixed 15 hour scotophase regime, with the remaining time kept at 25.0° C.

At the 5 percent level, there was a significant difference between nondiapausing progeny in these temperatures but not at the 1 percent level. There were substantially higher percentages of nondiapausing larvae at 12 hours than at 17 hours. They were different from each other at both the 1 percent and 5 percent levels.

The developmental time of nondiapausing larvae differed significantly between all the hourly conditions tested except the eight and nine hourly cycles. The longer the cool cycle, the slower the development.

The mean percentage of parasitism for this combined experiment was 24.6 percent. The sex ratio for the nondiapausing progeny was 1.5:1 females to males.

Continuous scotophase and photophase with the temperature cycle

The results from total scotophase or photophase are recorded in Table 7. During these periods the temperature was 25.0^{o} C for nine hours and 7.0^{o} C for 15 hours. There were no nondiapausing progeny produced in total darkness. Seven cocoons produced nondiapausing generations in total light, which was significantly different from that of the control where there was a nine hour photophase.

The developmental time under total light was significantly faster than the control.

The sex ratio was 2.5:1, female to male, under total light.

Table 7. Effect of both total scotophase or photophase under optimum temperature conditions on the prevention of diapausing B. curculionis progeny

Photoperiod (all 9 hrs at 25.0° C and 15 hrs at 7.2° C)	No. of larvae per replicate	Nondiapausing progeny			
		Mean percentage of parasitism 1/	Percent of individuals 1/2/3/	Mean egg-adult developmental time in days 3/	Sex ratio (F:M)
24 Hours of scotophase	25	32.0	0c	-	-
24 Hours of photophase	25	27.5	10.9b	36.4b	2.5:1
Control L:D 9 hrs:15 hrs	25	28.5	96.5a	52.0a	1.6:1

1/ Mean of eight replicates.
2/ Based on number of parasite cocoons.
3/ Means followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.

Interaction of photoperiod and thermoperiod

The interaction of photoperiod and thermoperiod was checked in an experiment under optimum conditons for nondiapausing (25.0° C during nine hours of photophase and 7.2° C during 15 hours of scotophase). This was compared to parasites reared under the same temperature regime with the photoperiod interchanged (15 hours photophase and nine hours scotophase). The results are shown in Table 8. From 58 cocoons in the reversed cycle, only seven larvae did not diapause, or 12 percent. This was significantly different from the mean of 96.5 percent which occurred with 15 hours of scotophase.

Effects of relative humidity

This investigation was performed under temperatures and photoperiod conditions which had resulted in maximum numbers of nondiapausing larvae. The rates of nondiapause are given in Table 9. When the relative humidity continually exceeded 80 percent, only one larva did not diapause out of 75 cocoons. Statistically, this percentage was not significant when compared to the 42.4 percent mean resulting from humidity conditions below 20 percent. However, both of these percentages were significantly different from the 97 percent of nondiapausing larvae resulting from the normal range of humidity, 50-80 percent.

In each test, the duration for development from egg to adult was not affected.

Table 8. Effects of the interaction of photoperiod and thermoperiod on the prevention of diapausing *B. curculionis*

Photophase: Scotophase 1/	No. of larvae per replicate	Percentage of nondiapausing progeny 2/
15:9	25	12**
9:15	25	96**

1/ Temperature cycle of 9 hrs at 25.0° C centered on the photophase, and 15 hrs at 7.2° C centered on the scotophase.
2/ Mean of eight replicates.
** Significantly different from the other mean at the 1 percent level as determined by a "t" test.

Table 9. Effect of relative humidity on occurrence of nondiapausing larvae of B. curculionis

Range of percent R. H. 1/	No. of larvae per replicate	Mean percentage of parasitism 2/	Nondiapausing progeny		
			Percent of individuals 2/3/4	Mean egg-adult developmental time in days 3/	Sex ratio (F:M)
80-100	25	37.5	1.3b	59.0a	1 female
50- 80	25	30.0	97.0a	54.0a	1.2:1
below 20	25	32.5	42.4b	59.3a	.8:1

1/ All experiments were operated with conditions of 25.0° C at nine hours photophase and 7.2°C at 15 hours scotophase.
2/ Mean of eight replicates.
3/ Means followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.
4/ Based on number of parasite cocoons.

A sex ratio of 1:1 female to male was recorded from a parasitism percentage of 32.2 percent.

It was noted that upon conclusion of the test dealing with excessively high relative humidity all cocoons had a leathery appearance. Upon dissection of these cocoons it was discovered that the majority of the larvae were dead. On the other hand, the majority of larvae within the cocoons reared under low relative humidities were still viable.

Influence of Host Age

A series of tests were conducted to determine if the age of the larval instar of the host had any significant effect on the induction of nondiapause in B. curculionis larvae. These experiments were performed at the known optimum environmental conditions for highest incidence of nondiapausing larvae.

Comparisons of different instars

The rate of parasitism by B. curculionis of the four instars of the alfalfa weevil H. postica, with the mean number of survivors (hosts and parasites) at the termination of the experiment, is shown in Table 10. The first, second, and third instars differed significantly from the fourth instar at the one percent level. There was no significant difference among the means for the first three instars.

The percent of nondiapausing progeny for each instar is also given in Table 10. Since the rate of parasitism of the fourth instar

Table 10. Effect of age of host on rate of parasitism and occurrence of nondiapausing larvae of *B. curculionis*

Instar 1/	No. of larvae per instar per replicate 2/	Mean no. of survivors (host and parasites) per replicate at termination of experiment	Mean percent of parasitism 3/4/	Percent of nondiapausing progeny 3/5/
1st	25	11.2	64.5a	89.7a
2nd	25	17.4	67.5a	91.8a
3rd	25	18.1	66.5a	87.9a
4th	25	23.2	10.5b	76.2b

1/ Reared in 25.0° C at nine hours photophase and 7.2° C at 15 hours scotophase.
2/ Two female parasites per replicate.
3/ Mean of eight replicates.
4/ Means followed by the same letter are not significantly different at the 1 percent level as determined by a LSD test.
5/ Means followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.

larvae was so low, an analysis of covariance test was performed in addition to the LSD test. This allowed for the small numbers in the fourth instar and indicated that there was a significant difference between the rate of nondiapausing progeny of the four different instars. There was no difference shown by the LSD test between the means of the first, second and third instars but there was a difference between them and the fourth instar.

The sex ratio for this experiment was 1.3:1, and the mean percentage of parasitism was 52.5.

The developmental time for all four alfalfa weevil instars, from the beginning of each instar to pupation and from egg to adult of the nondiapausing parasite,is summarized in Table 11. The developmental time at 25.0^{o} C for the nine hours photophase and 7.2^{o} C for the 15 hours scotophase showed no difference between the parasitized and unparasitized first or second instar larvae. However, the mean for developmental times for the parasitized third and fourth instar larvae was significantly longer than their unparasitized counterparts.

Each host instar differed significantly from all other instars when comparative developmental periods of the parasite within that particular instar (egg to adult) were reviewed.

Cage field studies

The four instars of H. postica were exposed to B. curculionis in field cages. The rates of parasitism are reported in Table 12. The means are from the number of survivors (hosts and parasites) at the

Table 11. Developmental times for parasitized and unparasitized host instars and the period from egg to adult of the parasite.

	Mean no. of days from inception of instar to cocoon 2/		Mean no. of days from parasitism (egg-laid) to adult parasite 2/3/
Instar 1/	Unparasitized	Parasitized	
1st	26.0	26.0	50a
2nd	19.6	19.9 NS	46b
3rd	12.4	14.6 **	41d
4th	7.2	12.8 **	43c

1/ Reared in 25.0° C at nine hours photophase and 7.2° C at 15 hours scotophase.
2/ Mean for 200 larvae.
3/ Means followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.
NS=Not significantly different from other mean at the 5 percent level as determined by a "t" test.
** Significantly different from other mean at the 1 percent level as determined by a "t" test.

Table 12. Rate of parasitism of the four larval instars of H. postica within field cages

Instar	No. of larvae per instar per replicate 1/	Mean no. of survivors (host and parasites) per replicate at termination of experiment 2/	Mean percent of parasitism 2/3/4
1st	25	8.0	8.5a
2nd	25	12.4	11.3a
3rd	25	17.2	11.6a
4th	25	20.1	4.8b

1/ Two parasites per replicate.
2/ Means of 12 replicates; two replicates per instar per week for six weeks (period when nondiapausing occurred).
3/ Means followed by the same letter are not significantly different at the 1 percent level as determined by a LSD test.
4/ Based on number of survivors (hosts and parasites).

termination of the experiment. In each test 25 host larvae were exposed to two female parasites. Two replicates per instar were studied each week for the 12-week period. Nondiapausing progeny occurred during only six of these weeks.

Percent parasitism in the first, second and third instars differed significantly from the fourth at the one percent level. On the other hand, there were no significant differences between the mean parasitism rates in the first three instars.

The mean percent of nondiapausing larvae for each instar is shown in Table 13. An analysis of covariance test was performed on these means, compensating for the small percentage of parasitism representing the fourth instar. With this test the means differed significantly. The LSD test did not show differences between the means of the first three instars. The mean of nondiapausing progeny for the fourth instar differed significantly from the other three larval instars.

Each instar differed significantly in length of time taken for the parasite within that particular instar to develop from egg to adult.

The sex ratio of nondiapausing females to males was 1.8:1, and the mean percent of parasitism was 9.1 percent.

The data from the field experiment were analyzed and adapted for use in the computer. A multiple linear regression equation was applied. The intent was to find if a correlation existed between a particular instar, the temperature at which it was subjected and

Table 13. Effect of host age on nondiapausing B. curculionis progeny in field conditions

Instar	No. of larvae per instar per replicate 1/	Mean percent of parasitism per replicate 2/	Nondiapausing progeny	
			Percent of individuals 2/3/	Mean egg-adult developmental time in days 3/
1st	25	8.5	70.9a	51.0a
2nd	25	11.3	74.3a	48.2b
3rd	25	11.6	70.9a	42.6d
4th	25	4.8	34.7b	46.3c

1/ Two parasites per replicate.
2/ Means of 12 replicates; two replicates per instar per week for six weeks (period when nondiapausing occurred).
3/ Means followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.

the occurrence of nondiapausing progeny. It was obvious that no correlation existed between the temperatures and the amount of nondiapausing larvae in any of the four instars. The multiple correlation coefficient was low (r = .67).

Maternally-Induced Diapause Studies

Seven different temperatures were programmed for these experiments. They were: 10.0, 15.6, 21.1, 23.9, 26.7, 29.4 and 32.2^{o} C all with a photophase of 12 hours. Cocoons were placed in each of the temperatures. Adults were allowed to emerge and mate. Half of the females were kept in the emergence environment during oviposition while half oviposited under optimum conditions for nondiapausing progeny to occur.

Emergence, feeding and mating but not oviposition

The effect of having parasitism occurring in optimum conditions for nondiapausing progeny to occur was tested in this experiment. Parasites were kept at different temperatures until 24 hours after emergence. They were then transferred to 25.0^{o} C for nine hours photophase and 7.2^{o} C for 15 hours scotophase to parasitize weevil larvae. The parasitized larvae were reared under these latter conditions.

The mean percentage of nondiapausing larvae is summarized in Table 14. The temperatures of 23.9^{o} C and 26.7^{o} C both resulted in a high percentage of nondiapausing larvae. Nondiapausing larvae,

Table 14. Effect of various temperatures on the prevention of diapausing progeny with the emergence and mating of the parent B. curculionis occurring in these conditions, but parasitism occurred under optimum conditions

Temperature °C of chambers (12 hr photo-period)	No. of larvae per replicate	Mean percent of parasitism 1/	Nondiapausing progeny		
			Percent of individuals 1/2/3/	Mean egg-adult developmental time in days 2/	Sex ratio (F:M)
10.0	25	18.5	0	-	-
15.6	25	22.5	8.9bc	51.8a	.33:1
21.1	25	21.0	16.7b	52.3a	1.3:1
23.9	25	21.0	61.9a	56.1a	1.2:1
26.7	25	19.0	81.6a	53.1a	2.1:1
29.4	25	25.0	3.9bcd	56.6a	2:1
32.2	(no emergence of adult parasites)		-	-	-

1/ Mean of eight replicates.
2/ Means followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.
3/ Based on the number of parasite cocoons.

in very low percentages, resulted at 15.6, 21.1 and 29.4° C; however, there were no significant difference within themselves. No adult parasites emerged from cocoons at 32.2° C, which upon dissection contained either dead larvae or prepupae, but never adults.

There were no significant differences between the means development time for any of the temperatures. All larvae which did not diapause developed from egg to adult in about 55 days.

The ratio of female to male was 1.4:1, with a percentage of parasitism for the entire investigation of 21.3 percent.

Emergence, feeding, mating and oviposition

Instead of having parasitism take place under ideal conditions as in the previous experiment, the newly-parasitized weevil larvae were held at the various temperatures for 24 hours. They were then transferred to the optimum conditions of 25.0° C and 7.2° C with nine hours photophase and 15 hours scotophase; a summary of the results is given in Table 15. Extremely low numbers of nondiapausing parasites occurred, and these only at 21.1, 23.9 and 26.7° C. The means were not significantly different. The developmental time for the nondiapausing larvae was longer at 21.1° C than at 23.9 and 26.7° C. There were no differences in developmental time of parasites between 23.9 and 26.7° C.

The female to male ratio was 1.4:1, with a mean percent of parasitism of 29.0 percent.

Table 15. Effect of various temperatures on the prevention of diapausing progeny with the emergence, mating and parasitizing of the parent B. curculionis occurring in these conditions

Temperature ^{o} C of chambers (12 hr photo-period)	No. of larvae per replicate	Mean percent of parasitism 1/	Nondiapausing progeny		
			Percent of individuals 1/2/3/	Mean egg-adult developmental time in days 2/	Sex ratio (F:M)
10.0	25	24.5	0	-	-
15.6	25	30.5	0	-	-
21.1	25	30.0	6.7a	52.5a	1:1
23.9	25	20.0	10.0a	41.0b	.3:1
26.7	25	50.5	7.9a	45.0b	3:1
29.4	25	19.0	0	-	-
32.0	(no emergence of adult parasites)				

1/ Mean of eight replicates.
2/ Means followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.
3/ Based on number of parasite cocoons.

Comparison of nonoviposition vs oviposition under different emergence conditions

The results from the previous two experiments were compared and presented in Table 16. In both experiments, the parasitized larvae were reared under identical conditions following oviposition (25.0° C at nine hours photophase and 7.2° C at 15 hours scotophase). The percent of nondiapausing larvae for each temperature under the conditions of nonoviposition differed significantly from the mean of that same temperature under the oviposition situation. The percentage of nondiapausing progeny in all temperatures where oviposition transpired in that temperature was extremely low in comparison to that in conditions where optimum nondiapausing conditions were used for parasitism. Upon dissection following termination of the experiment, over 80 percent of the cocoons from the conditions where oviposition occurred within that temperature contained living larvae. The remaining cocoons contained dead larvae.

Change in temperature after oviposition

A series of experiments was conducted using adults which had been reared and mated under the long scotophase regime without the cool temperature. After oviposition the weevil larvae were kept at 25.0° C for five days, still under long scotophase. On day six through 30 the cool nighttime temperatures of 7.2° C were resumed.

Table 16. Comparison of temperature effects on nondiapause from parents treated up to oviposition with those treated through oviposition

Temperature ^{o} C for adults (12 hr photoperiod)	Mean percent of nondiapausing larvae	
	Temperatures during development only 1/2/	Temperatures during development and oviposition 1/2/
10.0	0.0	0.0
15.6	8.9 **	0.0
21.1	16.7 **	6.7
23.9	61.9 **	10.0
26.7	81.6 **	7.9
29.4	3.9 **	0.0
32.0 (no emergence of adults)	-	-

1/ Following the adult treatment the weevil larvae were kept at 25.0^{o} C at nine hours photophase and 7.2^{o} C at 15 hour scotophase.
2/ Mean of eight replicates.
** Significantly different from other means at the 1 percent level determined by a "t" test.

Summarized in Table 17 are the results of this test and the results from a test performed using optimum nondiapausing conditions. Forty-six percent did not diapause, compared to 96.7 percent which were started out on the cool dark phase. The differences were highly significant. The developmental times also varied significantly with those reared under warmer conditions developing more rapidly.

Genetic influence

All rearing was under optimum conditions for nondiapausing progeny. Twenty-five host larvae were exposed to each mated female parasite. A total of eight replications for each genetic cross was performed. The percent of nondiapausing larvae per genetic cross is summarized in Table 18. In order to analyze this experiment which involved a set of independent comparisons, the data were statistically analyzed with a 2 x 2 factorial approach. There was no significant difference between any of the means.

The mean developmental period for the crosses involving nondiapausing females were not significantly different at the 5 percent level as determined by the LSD test. However, they were significantly different from the crosses involving nondiapausing males and diapausing females. The diapausing male and female cross differed significantly from all other crosses in amount of developmental time.

The mean percentage of parasitism for the entire experiment was 35.8 percent. A sex ratio of females to males was 1.3:1.

Table 17. Effect of cool temperature after oviposition on diapause in B. curculionis

Treatment 1/	Percent of nondiapausing larvae 2/	Mean egg-adult developmental time in days
Days 1-45 15 hrs dark at 7.0° C plus 9 hrs light at 25.0° C (day #1 = egg laid)	96.7 **	53.5 **
Days 1-5 constant 25.0° C, days 6-30 dark hrs at 7.0°C (day #1 = egg laid)	46.0 **	36.6 **

1/ Photophase 9 hrs, scotophase 15 hrs.
2/ Mean of eight replicates.
** Significantly different at the 1 percent level determined by a "t" test.

Table 18. Effect of genetic lines on the prevention of diapausing B. curculionis progeny

Mating pairs 1/	No. of larvae per replicate	Mean percent of parasitism 2/	Nondiapausing progeny		
			Percent of individuals 2/3/	Mean egg-adult developmental time in days 3/	Sex ratio (F:M)
diap. male x nondiap. female	25	32.0	90.6a	47.5a	1.2:1
nondiap. male x diap. female	25	34.0	91.2a	45.0b	1.6:1
nondiap. male x nondiap. female	25	41.5	90.4a	46.5a	1.2:1
diap. male x diap. female	25	35.5	91.5a	43.7c	1.1:1

1/ Reared in 25.0° C at nine hours photophase and 7.2° C at 15 hours scotophase.
2/ Mean of eight replicates.
3/ Means followed by the same letter are not significantly different at the 5 percent level as determined by a 2 x 2 factorial test.

Age Related to Nondiapause

There was a possibility that the age of the adult parasite was related to the proportion of offspring which did not enter diapause.

Ten supposedly-mated female parasites were chosen at random and each was placed with 10 second instar weevil larvae. Each five days the parasites were transferred to new weevil larvae. This process was continued until the female died. Each female was allowed 24 hours after emergence to feed and mate before starting the series. The number of successive five-day intervals, the number and percent of nondiapausing larvae from these cocoons, and the longevity of each female was recorded. A female parasite of the same age was maintained under the same environmental conditions, without being used to parasitize. These parasites were not transferred at five-day intervals. The results are shown in Table 19. Of the ten experimental females, #9 did not parasitize any weevils but lived for 20 days. Two females lived only two successive five-day periods. Three parasites lived for three exposures and the remaining survived over the duration of four periods. Two of the parasites had fewer cases of parasitism during the first five-day exposure than the second, while seven had fewer during the second period. Only one of these, #4, parasitized fewer weevils on the second exposure than the third test. Two of the parasites, #2 and #8, parasitized fewer weevils from the third exposure than from the fourth.

Table 19. Effects of adult parasite age on fecundity and on percent of nondiapausing B. curculionis progeny 1/

Female parasite #	Replication at 5-day intervals	No. of B. cur. cocoons	No. of nondia-pausing	Percent of nondia-pausing	Adult female longevity in days 2/	
					Active parasite	Control
1	1	6	5	83.3	15	20
	2	8	4	50.0		
	3	2	1	50.0		
2	1	8	8	100.0	18	17
	2	4	3	75.0		
	3	0	0	-		
	4	2	1	50.0		
3	1	4	2	50.0	7	19
	2	3	2	66.6		
4	1	8	7	87.5	15	18
	2	4	1	25.0		
	3	5	2	40.0		
5	1	8	7	87.5	18	19
	2	7	6	85.7		
	3	0	0	-		
	4	0	0	-		
6	1	6	6	100.0	11	17
	2	5	4	80.0		

Table 19. Continued

Female parasite #	Replication at 5-day intervals	No. of *B. cur.* cocoons	No. of nondia-pausing	Percent of nondia-pausing	Adult female longevity in days 2/	
					Active parasite	Control
7	1	8	7	87.5	20	25
	2	6	3	50.0		
	3	0	0	-		
	4	0	0	-		
8	1	3	3	100.0	21	20
	2	6	4	66.6		
	3	0	0	-		
	4	1	0	-		
9	1	0	0	-	22	17
	2	0	0	-		
	3	0	0	-		
	4	0	0	-		
10	1	8	7	87.5	15	19
	2	1	1	100.0		
	3	0	0	-	16.1**	19.1**

1/ Adult parasites emerged, mated and parasitized in 25.0° C at nine hours photophase and 7.2° C at 15 hours scotophase.
2/ Females which were never used to parasitize.
** Significantly different from the other mean as determined by a "t" test.

Even though female #9 parasitized no weevils, it was decided to use her in the statistical analysis. During the course of this research many females were found to impose no parasitism. In all likelihood this situation also occurs in local field populations. The nine viable B. curculionis females which had successful oviposition, averaged 2.67 successive five-day exposures. The mean days of longevity for all the female parasites was 16.1, as compared to 19.1 days in the controls, which was probably due to less handling and no oviposition. Through the use of a "t" test, these two means were shown to be significantly different at the one percent level.

Factorial experiments for the purpose of showing analysis of variance between different females and their numbers of nondiapausing progeny over successive exposures were performed. The results, comparing the first two successive exposures, are summarized in Table 20. A 2 x 10 factorial was performed comparing females #3 and #6 who lived for only two trials of parasitism. The results were compared to those of the first two trials of the remaining eight females. Significance was shown to exist between the different females and the results of nondiapausing progeny over two successive exposures.

An LSD test, comparing the means of nondiapausing larvae resulting from the eight females over the first two parasitism trials, indicated that females #8 and #9 differed significantly from the others, and from each other.

Table 20. Effects of two successive sessions of parasitism at 5-day intervals on the number of nondiapausing B. curculionis progeny

Female parasite 1/	No. of cocoons per replicate 2/	Nondiapausing progeny	
		No. of larva per replicate 3/	Percent of nondiapausing larvae per replicate
1	6	5	83.3
	8	4[a]	50.0
2	8	8	100.0
	4	3[a]	75.0
3	4	2 **	50.0
	3	2	66.6
4	8	7	87.5
	4	1[a]	25.0
5	8	7	87.5
	7	6[a]	85.7
6	6	6 **	100.0
	5	4	80.0
7	8	7	87.5
	6	3[a]	50.0
8	3	3	100.0
	6	4b	66.6
9	0	0	-
	0	0[c]	-
10	8	7	87.5
	1	1[a]	100.0

1/ All work performed in 25.0° C for nine hours photophase and 7.2° C for 15 hours scotophase.
2/ Based on 10 host larvae per replicate.
3/ Replications followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.
** Females that were omitted from LSD test because their results were shown to be significantly different from others by a 2 x 10 factorial.

The results for females who were exposed to larvae for three successive times of parasitism are summarized in Table 21. A 3 x 8 factorial was performed on this data. The resulting numbers of nondiapausing larvae from females #1, #4 and #10, those which lived for three trials, were compared to the females who lived to parasitize more than three successive times. There was significance between the different females and their resulting nondiapausing progeny over three successive trials. An LSD test indicated that there was no significance between any females and their number of nondiapausing progeny except #9, which had no parasitism.

All of the female parasites that survived to be subjected to host larvae for the fourth time were exposed to 40 larvae total. The effects are listed in Table 22. The significances between these five parasites and the resulting number of nondiapausing larvae were tested by a 4 x 5 factorial. Female #9 produced no cocoons and there was significant difference between her results and the other four females. Through a LSD test this same fact existed. All four of the surviving females were shown to have no significant difference between their numbers of nondiapausing progeny; however, all differed significantly from #9.

Table 21. Effects of three successive sessions of parasitism at 5-day intervals on the number of nondiapausing B. curculionis progeny

Female parasite 1/	No. of cocoons per replicate 2/	Nondiapausing progeny	
		No. of larvae per replicate 3/	Percent of nondiapausing larvae per replicate
1	6	5	83.3
	8	4**	50.0
	2	1	50.0
2	8	8	100.0
	4	3a	75.0
	0	0	-
4	8	7	87.5
	4	1**	25.0
	5	2	40.0
5	8	7	87.5
	7	6a	85.7
	0	0	-
7	8	7	87.5
	6	3	50.0
	0	0	-
8	3	3	100.0
	6	4a	66.6
	0	0	-
9	0	0	-
	0	0b	-
10	8	7	87.5
	1	1**	100.0
	0	0	-

1/ All work performed in 25.0° C for nine hours photophase and 7.2° C for 15 hours scotophase.
2/ Based on 10 host larvae per replicate.
3/ Replications followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.
** Females that were omitted from LSD test because their results were shown to be significantly different from others by a 2 x 10 factorial.

Table 22. Effects of four successive sessions of parasitism at 5-day intervals on the number of nondiapausing B. curculionis progeny

Female parasite 1/	No. of cocoons per replicate 2/	Nondiapausing progeny	
		No. of larvae per replicate 3/	Percent of nondiapausing larvae per replicate
2	8	8	100.0
	4	3	75.0
	0	0a	-
	2	1	50.0
5	8	7	87.5
	7	6	85.7
	0	0a	-
	0	0	-
7	8	7	87.5
	6	3	50.0
	0	0a	-
	0	0	-
8	3	3	100.0
	6	4	66.0
	0	0a	-
	1	0	-
9	0	0	-
	0	0	-
	0	0b**	-
	0	0	-

1/ All work performed in 25.0° C for nine hours photophase and 7.2° C for 15 hours scotophase.
2/ Based on 10 host larvae per replicate.
3/ Replications followed by the same letter are not significantly different at the 5 percent level as determined by a LSD test.
** Female which was omitted from LSD test because its results were shown to be significantly different from others by a 4 x 5 factorial.

DISCUSSION

Studies Investigating the Effect of Environmental Factors on the Induction of Diapause

From the very inception of this research, it was hoped that when the factors influencing diapause were understood, whether environmental or genetic, a reversal of these factors would yield a nondiapausing generation. The basic environmental conditions most successful in the laboratory rearing of the host insect, H. postica, resulted only in diapausing B. curculionis larvae. Literature, however, indicated that field populations of B. curculionis during the spring months produced varying numbers of nondiapausing progeny. Later in the season as conditions changed, with longer day lengths and warmer temperatures, all individuals entered diapause. Miller (1970a) indicated that in Massachusetts, nearly all emergences of nondiapausing B. curculionis occurred before June 5. After July 5 all larvae diapaused. Again in northern Georgia it was shown that after April 15, 90 percent of the B. curculionis entered diapause (Miller et al., 1972). In northern Utah, Hamlin et al. (1949) reported that the transforming (nondiapausing) individuals had virtually disappeared within two weeks after the first cutting. Since the first cutting generally had been completed by the tenth of June, it would indicate very few nondiapausing larvae after the first of July. It was also noted

during the preliminary phases of this study that 10-20 percent of the early field collected Bathyplectes larvae brought into the laboratory for rearing were nondiapausing. This pattern occurred until mid-June, after which the percentage dropped sharply.

Temperature, photoperiod and relative humidity data were gathered from dates approximating periods of nondiapausing B. curculionis larvae in the field. Based on this information the initial investigations were begun. Fairly cool and rather long nights with warm but not hot days were indicated from these field dates. When constant high temperatures and 12-hour photoperiods were used in the rearing, all of the progeny diapaused. This parallels the findings of Lees (1959) who showed, when working with the aphid Megoura viciac Buck., that constant temperature of this sort may considerably modify or even abolish the insect reaction to photoperiod, hence,completely changing patterns.

The preliminary investigations were designed to study fluctuating daily cycles. The first such test (Table 1) indicated that cooler temperatures within moderately long periods of scotophase resulted in larger numbers of progeny which did not enter diapause. Higher temperatures,with long periods of light, resulted in 100 percent diapause.

Ten hours of scotophase associated with temperatures below 21.0^{o} C caused an increase in nondiapausing parasite larvae (Table 2). The results were clear cut. Temperatures between 7.2-10.0^{o} C yielded more nondiapausing progeny than those below or above this range.

An optimum daytime temperature for nondiapausing progeny to be produced was arrived at by using 10 hours of dark with temperatures of 10.0° C followed by a photophase at temperatures above 10.0° C. Temperatures up to 29.4° C were used. Most nondiapausing parasites occurred in the range of 21.0-27.0° C (Table 3).

Throughout the series of preliminary studies the mean parasitism was only 20.4 percent based on initial host numbers. Weevil mortality was high. This mortality could be explained largely by the effect of parasites. The oviposition of the adult parasites in conjunction with the ratio of parasites to host larvae may have influenced the survival and even the behavior of the host larvae. On many occasions dead weevil larvae were observed in plant terminals or leaf axils. Their death could be from multiple stings or wounds. Duodu and Davis (1974b) indicated this was most definitely a major cause of mortality. Confinement is not natural in the field so mortality could have resulted from abnormally high ratio of parasites to host in the experimental units. It could also have been associated with the behavioral characteristics of the host larvae in the presence of the parasite. Many host larvae were knocked off the plants through probing by the adult parasites. This forced the weevils to cope with situations not suitable for their successful development. High mortality was also caused by direct parasitism. The host mortality caused premature death or prevention of parasite cocoon formation. This would prevent the parasite from spinning its cocoon. Regardless of which factor or factors caused the

mortality, it is very important to note that this relationship is not identical to field situations.

The inability to detect parasitized larvae by sight prolonged the study and made it more difficult. Many experiments were carried through to a point of host pupation only to discover that the weevil larvae were free from parasites. At first, it was thought that the female parasites had not mated, but later observations showed that mating was not necessary to accomplish parasitism. It was noticed that some female parasites were less aggressive, not seeking out host larvae to parasitize. This was likely one reason for the lack of parasitized larvae in many experiments.

The developmental time from egg to adult for the parasites within the preliminary tests varied greatly, due to the wide range of both temperature and photoperiods. The basic pattern was that parasites reared under cooler temperatures for longer time periods took longer to develop. The sex ratio of females to males in the initial tests was 1.1:1.

The duration of scotophase is definitely one of the interacting determinates of nondiapause in B. curculionis. In order for diapause to be prevented there appears to be a relationship to critical photoperiod, nearly nine hours, modified by cool temperatures during the scotophase. The degree to which diapause is prevented appears to depend primarily on the length of cool scotophase hours. When experiments were operated under lengthy cool scotophase, probably one of the following occurred to prevent a diapausing

generation: the 15 hours scotophase was too short in relation to the actual critical photoperiod required by the species to diapause, or the day length hours did not cross the critical photoperiod because of the length of scotophase. The control experiment with a constant 25.0° C for 12 hours photophase modified the photoperiod and crossed the critical photoperiod. Under control conditions the insect's reactions to photoperiods were possibly abolished and total diapause resulted. Such an effect was seen in the case of the vetch aphid, where short-day photoperiod did not induce the production of oviparous females when the ambient temperatures were high, greater than 20.0° C (Lees, 1959).

From this particular experiment the mean time for development (egg to adult) increased proportionately with the length of the cool scotophase. The temperatures, rather than the day length, appeared to regulate the developmental rate.

Different low temperatures during a 15 hour scotophase

The 15 hour scotophase for maximum production of nondiapausing parasites was clear cut; therefore, a large proportion of the remaining work used nine hour photophase with 15 hours darkness.

Low temperatures were maintained for the entire scotophase, then raised to 25.0° C during the photophase in the studies shown in Table 5 and Figure 6. During the 15 hours scotophase, both the temperatures of 4.4° C and 7.2° C consistently were in excess of

70 percent of the B. curculionis progeny not entering diapause. At the temperatures of 1.67° C and 10.0° C, there were over 40 percent nondiapausing offspring. Significantly more parasites did not diapause at 4.4° C and 7.2° C than at 1.67° C or 10.0° C, which in turn had fewer diapausing individuals than higher temperatures. The parasites did not develop at temperatures below 1.67° C. There were more nondiapausing individuals at 7.2° C than at 4.4° C, although not significantly, in addition to more rapid development of the parasites; therefore, 7.2° C was selected for most of the remaining experiments.

Nondiapausing resulted in this experiment from an interaction of low temperatures and 15 hours of scotophase. Since all larvae diapaused near the warm end of the scale but not at lower temperatures, the 15 hours of scotophase alone could not be the single inducing factor preventing diapause. Goryshin (1964) studied the effects of combined thermoperiod and photoperiods on the induction of diapause in three lepidopterous species: species: the sorrel dagger moth, Acronycta rumicis Schiff; the satin mother, Leucoma salicis L.; and the cabbage butterfly, Pieris brassicae L. He concluded that low scotophase temperatures tended to increase the incidence of diapause and high scotophase temperature would suppress diapause in the insects studied. Also scotophase temperatures had little influence when the daylength was in excess of 18 hours. The results with B. curculionis were the reverse from those reported by Goryshin.

Some other factor or factors are involved in B. curculionis. Beck (1968) indicated that different levels of fluctuating temperatures are never as precise as a photoperiodic rhythm; it nevertheless forms a thermoperiodic rhythm and on some occasions combined effects with those of photoperiod. Results from an interaction (Table 8) between photoperiod and thermoperiod indicated that temperature and/or thermoperiod together with photoperiod (long scotophase) have a combined effect on nondiapause in the B. curculionis. These results were attained by a reversal of photoperiod lengths. The length of scotophase of 15 hours was operated during 25.0° C and the 7.2° C temperature for nine hours of photophase. A total of 12 percent of the larvae did not diapause. It was fairly clear that photoperiodism alone was not responsible for nondiapause. Should the results have been 100 percent diapause, then either thermoperiod or temperature could have been eliminated as causative determinant of nondiapause in B. curculionis.

Different lengths of temperatures centered during a 15 hour scotophase

Having established the nine hour light:15 hour dark photoperiodic cycle as optimum for nondiapausing parasites, with alternating 7.2 and 25.0° C temperatures, the next question was how closely must the temperature and light cycles be synchronized. What is the critical point in the degree of cool temperatures needed for lowest percent

incidence of diapause? This was accomplished by operating all experiments at 7.2° C minimum and 25.0° C maximum temperatures with a photoperiod of 9L:15D. The length of time temperatures were held at 7.2° C was varied, but they were centered during the scotophase. The percentage of diapause was greatest at the shorter hours of cool temperatures (Table 6 and Figure 7). Nondiapausing progeny first appeared at eight hours of cool temperatures and increased progressively to 96.5 percent at 15 hours. At 16 hours this decreased to 91.8 percent or 4.7 percent below that of 15 hours. At the 17 hour mark it declined to 42.5 percent from the maximum in total nondiapausing larvae. The critical point in amount of cool thermoperiod at 15 hours scotophase lay between 15 and 16 hours of cool thermoperiod or almost identical to the scotophase. Beck (1968) stated that photoperiodic effects may be intensified when the low temperature phase occurs during the scotophase, making it probable that the greatest incidence of nondiapausing would correspond to the scotophase. This happens because of the intensification of photoperiod effects.

The mean for developmental time and the overall range for the parasite egg to emerge into adults increased proportionately as the degree of cool thermoperiod increased.

Continuous illumination and
continuous darkness

The temperature cycle was maintained at optimum as determined by previous experiments; however, the B. curculionis were kept in either

continuous light or continuous dark. With constant illumination and optimum thermoperiod, only 10.9 percent of the parasite larvae did not diapause (Table 7). This was small in comparison with results from the optimum thermoperiod combined with photoperiod of nearly 100 percent nondiapausing. These results were different from those of Danilevsky (1961) with the cabbage worm. In this species there was no diapause at any temperature when the insects were reared under constant illumination. An 89.1 percent of diapause occurred with B. curculionis.

Under continuous darkness there was a 100 percent incidence of diapause (Table 7). These results with B. curculionis agree with Danilevsky (1961). He stated that in continuous darkness and lower temperatures, a high incidence of diapause occurred in the cabbage worm. In contrast, however, he reported that higher temperature resulted in only nondiapausing progeny. Saunders (1973) found the same effect with female wasp parasites (Nasonia vitripennis) raised from the egg stage in total darkness but subjected to daily temperature cycle (13.0^{o} to 23.0^{o} C). These insects were able to distinguish a short-day thermoperiod and produced diapausing progeny.

The length of developmental time for parasites to emerge as nondiapausing adults was 15.6 days shorter under total illumination when compared to optimum conditions. This illustrated the effect that scotophase hours had on the duration of development.

Interaction of photoperiod and thermoperiod

It was not clear if photoperiod and thermoperiod had combined effects or if one was masking the other. A reversal of photoperiod lengths should partially clarify this point. The 15 hours of cool temperatures and nine hours of warm were maintained, but the light period was 15 hours. Twelve percent nondiapausing larvae occurred at 15 hours photophase (Table 8); therefore, photoperiod could not be the sole interacting determinant in preventing diapause in B. curculionis. If no larvae had bypassed diapause, photoperiod could be considered the only determinant. Both temperature and thermoperiod had a role in modifying the photoperiodic effects. There is the possibility that in the laboratory under these conditions, temperature and thermoperiod influences nondiapause by possibly shifting the critical photoperiod, thus evading diapause.

Effects of relative humidity

The photoperiod and temperature cycles were kept at the level considered to be optimum for nondiapausing B. curculionis, but the relative humidity was kept at three different levels. At or near saturated relative humidity only one larva emerged (Table 9). The remaining cocoons were found to have a thick, almost leathery appearance on the outer cover. It was determined through dissection that 84 percent of the cocoons contained nonviable parasites, usually larvae or pupae. However, some cocoons contained adults which had

died just before emerging. Those remaining, even though alive, definitely were unhealthy and distorted in appearance. Within all dissected cocoons there was excessive moisture. The cause of death among these stages was not certain, though these larvae were programmed to bypass diapause. There is a possibility that excessive moisture was incorporated within the cocoon upon formation; or those nondiapausing larvae which had formed adults were unable to escape through the leathery cocoon. Most individuals appeared to be nondiapausing parasites which failed to develop properly. It should be pointed out that prolonged conditions of high relative humidity were not similar to actual field conditions.

With relative humidity below 20 percent, 42.2 percent of one parasites did not diapause and emerged as normal adults (Table 9). This differed significantly from the 50-80 percent RH range where 97 percent did not diapause. Upon dissection, the majority of the cocoons, except those from which adults emerged, were found to contain viable larvae. They appeared to be in a true state of diapause, but the reasons are not known. It could be that the relative humidity might modify the effects of thermoperiod and photoperiod, thus inducing diapause. It could have modified the insect response to either or both photoperiod or thermoperiod. Relative humidity around 20 percent or less is common during the summer in Utah. Following the first cutting, B. curculionis larvae are exposed to excessively low relative humidity ranges. Under Utah

conditions, relative humidity probably has some determinate influence on the absence of a large second generation. The degree of influence would depend on the timing of the cutting in relation to the progress of the Bathyplectes population. This condition is possibly less of a factor in more humid areas or areas with earlier growth seasons. Wilson and Armbrust (1970) found 39 percent of the first generation pupae were nondiapausing and that these individuals accounted for a second population peak.

Influence of Host Age

Tests were performed to determine what effect, if any, larval instar of the host had on the induction of diapause. These were conducted at the levels considered optimum for nondiapausing B. curculionis.

Comparisons of different instars

The rates of parasitism (Table 10) show that the fourth instar weevil larvae were parasitized far less frequently than the first three. There was no significant difference shown at the five percent level in the percentage of parasitism among the other three larval instars. The results show that the first three instars were approximately equally susceptible to the parasite, the fourth instar was not as preferred or resisted attack. Both Miller (1970b) and Duodu and Davis (1974b) reported similar results.

Survival following parasitism of the H. postica instars was poorest in the youngest instars and best with the fourth. It

appeared that the first instar larvae died more easily from parasite action. This mortality included both oviposition by the parasite and being dislodged from their feeding places.

The percentage of parasitism by B. curculionis showed that the first three H. postica instar hosts did not differ at the 1 percent level in the LSD analysis (Table 10). To test the influence that any instar might have had in inducing nondiapausing progeny, the nondiapausing mean from each instar was used in an analysis of covariance. This test was used rather than the analysis of variance method which uses the F test. The covariance test would indicate if there was significance among means and at the same time would allow for the small percent of parasitism in the fourth instars. Results showed a significant difference at the 5 percent level between the percent of nondiapausing progeny resulting from each of the four instars. Using these results, an LSD test showed no significant different in percent of nondiapausing progeny between the first three instars. Nondiapausing larvae occurred as readily from any one of the first three host instar sources as from another. No substantial influence as a result of the larval age could be attributed to the amount of nondiapause resulting from the first three instars. The probability of the fourth instar alfalfa weevil having any direct affect on nondiapause is still not certain. Even though the analysis of covariance test was designed to compensate for the small percentage of parasitism, it is not certain whether this test was successful. If this statistical test was successful

in its purpose of allowing for the small percent of parasitism, then the fourth instar has influence on nondiapause in the parasite. In some reported cases both nutrition and host conditions can alter the primary effect of photoperiod, and thereby change the incidence of diapause. Saunders et al. (1970) illustrated that the situation of the host had an effect on the induction of diapause in the parasite wasp Nasonia vitripennis. This may be a determinant when considering fourth instar weevil larvae and the parasite B. curculionis. Further investigations must be done on this point in order to draw a valid conclusion.

The length of time for the parasite to develop from egg to emerged adult parasite differed significantly between all four instar larval hosts at the 5 percent level (Table 11). It took longer for a parasite from a first instar weevil larva to develop than it did the other instars and longer for the second than from the last two. The fourth instar took a little longer than the third, however. The parasite probably modified the physiology of its host, enabling itself time to develop before host pupation. A similar case is known from Heliothis zea (Boddie) and the wasp Macrophlitis croceipes (Cresson) (Lewis, 1970).

Under temperatures that fluctuated through a daily cycle, the number of days from the start of an instar to the cocoon showed no difference at the 1 percent level between parasitized and unparasitized in the first and second instars. Third and fourth unparasitized larvae developed faster than the parasitized larvae (Table 11).

Duodu and Davis (1974a) found similar results under constant photoperiods and thermoperiods as shown under these fluctuating conditions. The two sets of results paralleled one another.

Field studies

Similar data concerning development was obtained in the field studies to those in the laboratory (Table 12). The percentage of parasitism and mean number of survivors in the field were, however, considerably lower. Two parasites were used per cage. The weevil larvae appeared to lack aggressiveness in attacking alfalfa. First and second instars that fell to the cage floor were repeatedly stung, causing a premature death. Those host larvae not stung repeatedly generally did not seek out the plant and eventually perished.

An analysis test of covariance performed on the percent of nondiapausing larvae from each host instar showed (Table 13) significant differences between instars. Using this test, the effect of the fourth instar variable was tested separately from the effect of the other three instars. These results were then analyzed by LSD test. The LSD test showed the fourth instar to be significantly different from the other three instars at the 5 percent level when considering percent of nondiapausing progeny. The developmental time paralleled the pattern shown in the laboratory experiments. The parasites having developed in fourth instars took longer than those from third. The developmental time for all field larvae was longer than those of laboratory, probably due to some cooler

daytime temperatures. Results indicated that in the field no influence of host age for the first three host instars could be detected in the amount or frequency with which the parasite larva did not enter diapause.

The weather conditions in the field were studied with the intent of predicting conditions best related to nondiapause. Because of the many dependent variables in relation to the small number of observations no correlations could be substantiated.

The ratio of nondiapausing females to males for the laboratory and field were 1.3 and 1.4 females, respectively, to one male.

Maternally-Induced Diapause Studies

To study the parental effects on progeny diapause, four separate types of experiments were performed.

Effects of temperatures on adult parasites

Prior to oviposition, the adult parasites were kept under 10 different temperatures and a 12 hour photoperiod during emergence and mating. Parasitization, however, occurred under optimum nondiapausing conditions. The results appear in Table 14. The highest percentage of nondiapausing B. curculionis occurred at 26.7^{o} C, dropping off rapidly with both warmer and cooler temperatures. The differences in the incidence of nondiapausing larvae between 23.9 and 26.7^{o} C were not significant; however, the remaining five

temperatures yielded few nondiapausing larvae. All progeny diapaused from females which emerged and mated at 10.0° C. No adult parasites emerged for future parasitism work from 32.2° C. The effect of heat on B. curculionis larvae was reported first by Hamlin et al. (1949). They reported a goodly portion of the B. curculionis larvae succumbed to stubble field heat following first cutting. This study verified the fact that a high temperature heat is devastating to the larvae within the cocoon. The heat factor is undoubtedly a cause of high B. curculionis mortality in the field.

The results from experiments where the 10 emergence temperatures were extended into the oviposition period appear in Table 15. Extraordinarily low percentages of nondiapausing parasites occurred at 21.1, 23.9 and 26.7° C. Table 16 presents statistical comparison of nondiapausing parasites from both temperatures during development and temperatures during development plus oviposition. At each temperature the incidence of nondiapause resulting from oviposition in conditions of 25.0° C at nine hours photophase and 7.2° C at 15 hours scotophase was significantly less when the experimental conditions were carried through the oviposition period.

It appears that the exposure of the female parent to varying temperatures has an impact on diapause in their progeny. The effects were greater when the parents are exposed through the oviposition period. The relative importance of environmental conditions on the offspring and on the parent female is not certain. The small percent of nondiapause which occurred when oviposition took place

outside favorable conditions is not understood. There is the definite possibility that genetic variability relating to diapause could have occurred, but it was not clear from these experiments. Geispitz (1968) reported that this could happen even within populations derived from single females. Within some of the original females the sensitive stage could have occurred a generation earlier, or a generation between determination and manifestation. The experiment testing genetic effects or the influence heredity has on nondiapause somewhat discounts this possibility (Table 18). More study is needed on maternal influence on nondiapause in B. curculionis.

There was no difference between the sex ratio in these two tests. They both resulted in 1.4:1 female to male.

To investigate the total lack of emerging adults from the 32.2° C conditions, a new batch of cocoons from cold storage were placed in 32.2° C. After each 24 hours of exposure, five cocoons were removed at random and placed in 26.7° C. The results showed that up to and including the fourth day, normal adult B. curculionis emerged. From that day on, none emerged. Upon dissection, dead prepupae were found.

Effects of cool temperature after oviposition on diapause

The experiment was conducted using adults which had been reared and mated under the long scotophase without cool temperature. Parasitized larvae were kept five days at 25.0° C. On days six

through 30 a cool night temperature of 7.2° C were used in the regular cycle. The results from this comparative suty are shown in Table 17. The length of scotophase and cool temperature were both important in nondiapause induction. When the parasites were not exposed to cool temperature until the sixth day there was a decline in the incidence of nondiapause by 51 percent. Maternal sensitivity to environmental conditions before oviposition is involved but not understood at this point. A greater effect occurred when the parents were exposed during oviposition. It appeared that a portion of the female parents were induced to program nondiapausing progeny because of photoperiod, especially the length of scotophase. The question remains unanswered regarding the influence that genetic variability may have had on a particular series resulting from one female, as some diapaused occurred.

The mean length of times for development between larvae resulting from a test of optimum nondiapausing conditions and ones from a test of cool temperatures after five days were significantly different. With less cool temperature the latter experiment mean of development time was 16.9 days less than the mean for the optimum conditions.

Genetic influence

Four-way genetic crosses were conducted under optimum conditions for the incidence of nondiapause. No significant difference was found between the mean percent incidence of nondiapause from any of the genetic crosses (Table 18). The probability of nondiapausing

larvae related to an inheritance factor was not shown. It appears that the induction of diapause originating from grandparents is also unlikely. All crosses showed essentially the same in number of nondiapause. The small percentage of nondiapausing larvae which occurred sporadically could not be explained by maternal influence in the experiments.

There was a significant difference in length of developmental time between those crosses that involved nondiapausing female parents and those which involved diapausing females. The reason is not known. The significance of this finding is questionable as to its importance in this study.

Age Related to Nondiapause

This test was conducted to investigate the influence of parental age on the incidence of nondiapausing progeny. The longevity of female B. curculionis was also studied. The results for each female parasite tested is shown in Table 19. The number of successive five-day intervals of parasitism, number of cocoons per replicate, number of nondiapausing larvae, percent incidence of nondiapause, and the longevity of ovipositing parasites were compared to a control of nonovipositing parasites. There was a significantly greater longevity of the nonovipositing parasites. A few days following the end of oviposition a female parasite would die. She either spent her energy through constant searching and working, or having depleted her own egg supply, then died.

The control females lived an average of three days longer. A total of 70 percent of the female parasites lived to parasitize during the third time period. On three occasions, they were dead by the end of the first 24 hours of the third exposure. Forty percent lived into the fourth time period, but almost no parasitism occurred. There were fewer parasitized larvae per replicate as the number of exposures increased. They dropped sharply after the second exposure.

The incidence of nondiapausing B. curculionis larvae from the five-day intervals was analyzed and compared on the basis of 2, 3 or 4 trials. The results are shown in Tables 20, 21, 22. The incidence of nondiapause between females living through only two exposures was significantly different from those living to parasitize additional times. These results were analyzed by a 2 x 10 factorial test. The incidence of nondiapausing for the first two trials comparing the eight females which lived longer was compared using a LSD test at the 5 percent level. It indicated that females #8 and #9 differed significantly from the others and also from each other. The results of those with only three exposures differed significantly from the three exposures of those who lived longer. An LSD test showed that no difference occurred in the significance between the results of any female and their number of nondiapausing progeny during these number of trials, with the exception of #9, which had no parasitism. Five parasites lived to parasitize for the fourth time. The resulting number of nondiapausing progeny

was tested by a 4 x 5 factorial. The results of female #9 was significantly different from the remaining four. Through a LSD test at the 5 percent level there also existed a significant difference between the results of the four parasites which had nondiapausing offspring and #9.

In general, fewer nondiapausing larvae occurred as the number of successive trials increased and the female grew older (Table 19). Saunders (1962) found similar results when working with female Nasonia vitripennis. Simmonds (1946, 1948) also showed that old females of Spalangia drosophilae, a pteromalid parasite, produced a greater proportion of diapausing larvae in their offspring than young females. The results of my investigation appear to correlate with these findings. However, a few females, such as #8, show an opposite trend,producing more diapausing larvae at the beginning of her life.

CONCLUSIONS

Biological and ecological factors relating to the induction of diapause in the icheumonid parasite Bathyplectes curculionis (Thomson) were studied. The effects of photoperiod, temperature, relative humidity, host age, maternal influence, and parental age of the parasite were measured. For most of the experiments, the adult parasites emerged from cocoons, parasitism took place and the parasitized weevil larvae were kept under conditions discussed. Several conclusions can be drawn from the information compiled during this investigation. First, in the laboratory, scotophase periods under 10 hours produce large numbers of diapausing B. curculionis larvae. Second, as the duration of scotophase is increased up to 15 hours, the incidence of nondiapause increases. Scotophase periods over 15 hours scotophase result in fewer nondiapausing larvae. At 21 hours of scotophase 100 percent of the larvae will diapause. Third, low temperatures during the scotophase will induce nondiapausing progeny. The cool range of most importance is between 4.4 and 10.0^{o} C, with highest incidence at 7.2^{o} C. The degree of nondiapause in B. curculionis larvae is dependent primarily on the length of cool scotophase hours. Fourth, the prevention of diapausing B. curculionis larvae is caused by interaction of a long scotophase during which there are cool temperatures. The optimum conditions are 25.0^{o} C for nine hours scotophase and 7.2^{o} C for 15 hours scotophase. Fifth, constant illumination under optimum

thermoperiodic conditions result in few nondiapausing larvae. Continuous scotophase under optimum thermoperiod will generally yield all diapausing larvae. Sixth, a range of 50-80 percent RH maintained the 25.0° C for nine hours photophase and 7.2° C for 15 hours scotophase consistently induce over 95 percent of the B. curculionis to bypass diapause. At or near saturated conditions, the excess moisture is detrimental to larvae within the cocoons. Nondiapause is difficult to evaluate when there is high relative humidity because of excessive mortality. Relative humidity below 20 percent resulted in over 58 percent diapausing B. curculionis larvae. Seventh, there is no significant difference in the rate of parasitism between the first three instars, but there is a significant difference the fourth instar and the other three. Eighth, there is no difference between the first three weevil instars and their influence on the percent of nondiapausing parasite larvae. Results from the fourth instars were inconclusive. Ninth, the conditions under which the adult parasites are kept have a definite effect on the diapause of their offspring. The impact is greater if these conditions are maintained through oviposition. Tenth, when cocoons from cold storage are placed in temperatures above 29.4° C for more than brief periods of time, there is high mortality. Ninety-six hours at 32.0° C was completely lethal. Eleventh, when no cool thermoperiod is given parasites during the first five days of their life (day one being oviposition), the incidence of diapause will increase. Twelfth, inheritance has no apparent effect on the

amount of nondiapause in the progeny of B. curculionis originating from northern Utah. No studies involved individuals from other areas. The evidence that the induction of diapause originating from a grandparent influence is also nonexistent. Thirteenth, fewer nondiapausing larvae occur as the adult female parent grows older. The longer an adult female lives, the fewer eggs she lays. Fourteenth, a correlation exists between the length of developmental time, from egg to adult, and the length of cool scotophase hours during a daily regime. Temperature rather than the day length appears to regulate the developmental rate. With less cool scotophase time, the mean days for development is less.

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