THE UNIVERSITY OF MANITOBA

THE BIOLOGY OF CALLING BEHAVIOUR IN THE BERTHA ARMYWORM, *Mamestra configurata* WALKER (LEPIDOPTERA: NOCTUIDAE)

by

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ABSTRACT

Howlader, Md. Moksed Ali. Ph.D., The University of Manitoba, August 1985. The Biology of Calling Behaviour in the Bertha Armyworm, *Mamestra configurata* Walker (Lepidoptera: Noctuidae). Major Professor: George H. Gerber.

The biology of calling behaviour in *Mamestra configurata* Walker was investigated in virgin females in the laboratory. The moths were taken as pupae from a laboratory culture maintained on a synthetic diet. Unless stated otherwise, the pupae and moths were reared in environmental chambers at 20° ± 0.5°C, 60 ± 5% R.H., and a 16 h L : 8 h D photoperiod. The moths were observed during each scotophase under a red light of about 0.3 lux light intensity. The frequency of observations were from 10 sec to four h. There were 24 females/experiment or test.

The characteristics of calling behaviour were determined. The female had a definite calling posture: the ovipositor was extruded and curved downward at an angle of about 45°, the wings were raised above the abdomen in the form of a "V", and the antennae were directed posteriorly along the sides of the thorax. Calling had a discrete diel periodicity, occurring in the last half of the scotophase. Once calling was initiated, the females called almost continuously throughout the calling period; this pattern was characteristic of the continuous pattern of calling. The lights-off cue was the photoperiodic cue responsible for setting the timing of the diel periodicity of calling. The circadian rhythm of calling was endogenously based.

The effects of age, mating status, and ovarian maturation on calling behaviour were determined. Females initiated calling and mating during the second and third scotophases after emergence. Their ovaries also contained chorionated eggs for the first time at this age.

Oviposition was initiated during the third and fourth scotophases shortly after the termination of the first mating. Mated females resumed calling after a refractory period of two days. Mated females called for a shorter period during each scotophase than virgin females. The diel periodicity of calling was advanced and the duration of the daily calling period was increased with age until the seventh scotophase; thereafter, both remained relatively unchanged until the twelfth scotophase.

The effects of photoperiod and temperature on calling behaviour were determined. Photoperiod affected calling behaviour by changing the diel periodicity of calling and the length of the daily calling period. The females called earlier and the daily calling period was shorter if the scotophase was $\leq$ 10 h than if it is $>$ 10 h. Continuous constant temperature during the adult stage affected calling behaviour in four ways: age at first calling, diel periodicity of calling, length of the daily calling period, and percentage of females calling. Short-term temperature changes during the scotophase affected calling behaviour in three ways: diel periodicity of calling, length of the daily calling period, and percentage of females calling. The optimum temperature range for calling was, at least, 10°-25°C. The upper limit for calling was near 35°C and the threshold was $<$ 5°C.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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1 INTRODUCTION

1.1 Pest Status, Distribution, and Life History

The bertha armyworm, *Mamestra configurata* Walker, is one of the major pests in Canadian agriculture. It has occurred sporadically in outbreak numbers in Western Canada since 1921 (King 1928). Since 1940, damaging infestations of the insect, mainly on rapeseed (oil seed crops, *Brassica napus* L. and *B. campestris* L.) occurred on seven occasions (Turnock and Philip 1977). The most widespread outbreak occurred in 1971-1974 (McDonald 1972). During this outbreak, there was considerable crop loss despite insecticidal spraying (Turnock 1984). The other crops damaged by this insect are flax, sweet clover, alfalfa, cabbage, peas, beans, tomato, sugar beets, etc.

The insect is native to Canada. It occurs every year in Manitoba, Saskatchewan, Alberta, and British Columbia (Turnock and Philip 1977). The general distribution of the insect is from Mexico (Mexico City), north along the Cordillera to British Columbia and east through the Prairie Provinces of Canada to North Dakota (Kapatsa 1979).

It is bivoltine in California and Washington, as suggested by adult collection dates (Wylie and Bucher 1977). In the Prairie Provinces of Canada, it is univoltine; it completes one generation each year and only the diapausing pupal stage can survive the winter. The moths typically begin to emerge from the overwintering pupal stage early in June and are present in the field until early August. In most years, most moths emerge between mid-June and mid-July.

The moths are entirely noctural in habit (King 1928). They feed on flowers of various kinds, particularly on those of *Brassica* spp.

(Turnock 1984). In the laboratory, they initiate mating during the scotophase when they are 36 h or more old; they remain in copula for four h or more (Bucher and Bracken 1976). Individual moths mate several times in their lifetime. Oviposition also occurs in the scotophase, usually during the night following mating. The eggs are deposited on the leaves of the host plants. The leaves of lamb's-quarters (*Chenopodium album* L.) are the most suitable plant for oviposition. Under mass rearing conditions, 800-1000 eggs are laid per female. These are laid every night or so until the female dies. The eggs hatch in about a week.

The larvae pass through six instars. Larval development is completed in about three weeks at 25°C in the laboratory (Bucher and Bracken 1976). The mature larvae enter the soil to a depth of five to 15 cm, form a cell, and after a short prepupal period change to the pupal stage (Wylie and Bucher 1977). The pupae enter into diapause and remain in this condition until the next summer.

Crop damage is caused by the larvae. The larvae typically occur in large numbers after mid-July (Steck *et al.* 1984).

1.2 Pest Management of the Bertha Armyworm Using the Sex Pheromone

In the last several decades, the consequences of insecticide residues have stimulated the investigation of new methods of insect control in integrated pest management (IPM). The sex pheromones of Lepidoptera have been found to be an important tool in the IPM of these insects on several crops (Beroza 1976; Shorey 1977; Roelofs 1979). In most species, the pheromones are produced by females for communication

with the males for mating. For utility in IPM, several strategies have emerged with pheromones for manipulation and management of pest insect populations. Three strategies for which adequate techniques have been developed are: the use of pheromones in traps for the detection and survey of the pest, the use of pheromones in traps for the removal of males from the area before they mate with the females, and the use of pheromones to saturate the insects' environment to disrupt mating. Many field experiments with these techniques have demonstrated that they have real management potential.

Considering the life history, the bertha armyworm female sex pheromone may be best utilized for conducting surveys, for detecting possible outbreaks, and in assisting proper timing of control measures (Steck *et al.* 1984). Some progress already has been made in this direction. The multi-component pheromone system of the bertha armyworm has been identified (Underhill *et al.* 1977; Struble *et al.* 1984). Pheromone baited traps were found effective in capturing males in the field (Underhill *et al.* 1977; Steck *et al.* 1979; Turnock 1984). Also, the trapping of males has proved very useful in providing an index to the abundance of female moths and their egg-laying activities (Turnock 1984).

Before the pheromone can be used widely, it is essential to obtain a complete picture of the factors governing the biology of the female sex pheromone system and the responses of males to the pheromones of the females. Little information is available in this area (Struble *et al.* 1975; Chisholm *et al.* 1975). Females produce sex pheromone when three

days old; seven to nine day old females produce the maximum concentration. Males respond to the female sex pheromone when they are two days old. The males continue to respond to it until death. The females release pheromone ("calling") from five to six h after the beginning of the scotophase. However, the environmental factors, such as temperature and photoperiod, regulating the time of calling have not been investigated. Also, the calling pattern of individual females and the effects of age, ovarian maturation, and mating status on calling behaviour have not been studied.

1.3 The Aims of the Study

The present study was undertaken to obtain a comprehensive picture of the biology of calling behaviour of the bertha armyworm and of the factors that affect it.

The study is divided into eight sections. *Section 1* consists of an investigation of the sequence of nocturnal activities to determine the characteristics of calling posture, diel periodicity of calling, and calling pattern of virgin females. *Section 2* investigates the effects of age on calling behaviour of virgin females. *Section 3* determines the calling of mated females in relation to the first mating and subsequent oviposition. *Section 4* determines the lights-on/off cue(s) of the calling rhythm of virgin females. *Section 5* determines the endogenous regulation of the circadian rhythm of calling of virgin females. *Section 6* investigates the effects of photoperiod on calling behaviour of virgin females. *Section 7* evaluates the effect of temperature on

calling behaviour of virgin females. *Section 8* deals with the relationships between ovarian maturation and calling of virgin females.

The study is a part of the research programme which is being conducted by the Integrated Pest Control Section, Research Station, Agriculture Canada, Winnipeg, on the biology and ecology of the bertha armyworm. The information obtained from the study will be utilized in formulating a pest management system for this insect.

2 LITERATURE REVIEW

In some Lepidoptera, active release of sex pheromone by females is a necessary preliminary to the attraction of males for copulation (Jacobson *et al.* 1970; Jacobson 1974). Calling behaviour has been described in most detail in various families of moths. Therefore, the present review of the literature is restricted to moths. Since the bertha armyworm is a noctuid moth, particular attention will be given to moths of that family.

2.1 Calling Behaviour in Lepidoptera

2.1.1 Calling Posture

The term "calling" is used to describe the body posture of the females during pheromone release (Doane 1968). It was first used in reference to *Plodia* and *Ephestia* (Norris 1932). Now, it is widely used in the case of all females having a special, characteristic posture during pheromone release.

The posture mainly involves the protrusion of the ovipositor out of the tip of the abdomen to expose the pheromone glands and to release the sex pheromone. It may also involve movements of the wings, abdomen, or antennae. Calling behaviour has been described in many moths (Sanders 1969; Fatzinger and Asher 1971; Swier *et al.* 1976; Teal *et al.* 1978). There are differences in the calling postures among the different families studied.

In the Phycitidae, bending of the tip of the abdomen dorsally so that it projects between the wings of the female is the main

characteristic (Brady and Smithwick 1968; Fatzinger and Asher 1971). The wings are lowered until they touch the surface upon which the female rests. The female also vibrates the antennae and exhibits a rhythmic protrusion and retraction of the ovipositor.

In the Lymantriidae, the calling female rests with the head upwards on an upright surface, spreads the wings slightly, lowers the abdomen, and begins a rhythmic protrusion and partial retraction of the last segment of the abdomen (Doane 1968; Grant 1981).

In the Tortricidae, the females typically flex the ovipositor downwards at right angles to the main body axis, resulting in the abdomen assuming a characteristic "banana" configuration (Sanders 1969; Lawrence and Bartell 1972; Tamaki *et al.* 1976). In some species, the tip of the abdomen with the extruded pheromone glands is waved about while the females are calling.

In the Olethreutidae, the main characteristic is the lowering of the anterior part of the body so that it touches the substrate and raising of the posterior end by extension of the metathoracic legs (Castrovillo and Cardé 1979). In some species, the wings are slightly raised and the ovipositor is protruded and directed ventrally.

In the Noctuidae, the main component of the calling posture is the extension of the ovipositor to expose the pheromone gland (Kaae and Shorey 1972; Teal *et al.* 1978; Turgeon and McNeil 1982; West *et al.* 1984). The wings are elevated above the abdomen horizontally or at an angle of 45° so that the upper surface forms a "V". The protrusion of the ovipositor may be accompanied by fluttering of the wings (Swier *et al.* 1976). The ovipositor is often touched or dragged along the supporting surface.

2.1.2 Calling Pattern

In each species, calling has certain definite patterns (Sower *et al.* 1971a; Swier *et al.* 1977; Teal and Byers 1980; Turgeon and McNeil 1982). The patterns have been characterized by differences in the duration of calling at any one time within a calling period (bouts of calling), the frequency and distribution of the bouts, the non-calling periods between bouts, and by some other factors. The non-calling period may be a period of rest, flight, or some other activity.

In some species, the calling pattern has been termed "continuous". In the continuous pattern, the calling posture is maintained uninterrupted during most of the calling period. The females initially call for a short time with short bouts of high frequency before a sustained main bout (Sanders and Lucuik 1972; Teal and Byers 1980). The main bout then persists as long as the conditions for calling prevail.

In other species, the calling pattern has been termed "discontinuous". In the discontinuous pattern, the calling posture is frequently interrupted by periods of non-calling. The number of calling bouts per female during a calling period may reach 20 or more (Lawrence and Bartell 1972; Swier *et al.* 1977; Turgeon and McNeil 1982). The length of each bout may be from five min to two h. The long and short bouts are scattered irregularly throughout the calling period. The duration of the bouts, their frequency and distribution may vary considerably among different species and among individuals within a species.

2.1.3 Diel Periodicity

Female calling usually has a diel periodicity (Sower *et al.* 1970; Sanders and Lucuik 1972; Swier *et al.* 1977; Teal *et al.* 1978; Baker and Cardé 1979a; Castrovillo and Cardé 1979; Turgeon and McNeil 1982; Leibhold and Volney 1984). The onset of calling begins at a characteristic time of the 24 h cycle, reaches a peak, and then declines.

In the Noctuidae, calling occurs during the scotophase. In some species, it occurs shortly after the beginning of the first half of the scotophase (Teal *et al.* 1978; Byers *et al.* 1985) or later in the first half of the scotophase (Swier *et al.* 1977; Teal *et al.* 1978; West *et al.* 1984). In others, it is not initiated until the beginning of the last half of the scotophase or later in the last half of the scotophase (Sower *et al.* 1970; Teal *et al.* 1978; Turgeon and McNeil 1982).

Duration of calling may be variable in different species. In some species, the females may call for as much as 10 to 11 h in each 24 h cycle (Cardé *et al.* 1974; Gorsuch *et al.* 1975). In some other species, the females may call only a few hours in each cycle (Teal *et al.* 1978).

2.1.4 Photoperiodic Cue(s)

Lights-on/off cue(s) is the most important cue responsible for setting the timing of the female calling periodicity. The lights-on signal is the cue in most species of moths studied (Sower *et al.* 1971b; Sanders and Lucuik 1972; Cardé *et al.* 1975b; Baker and Cardé 1979a). In these species, calling is initiated a certain number of hours after the lights-on signal is received, and if the timing of the signal is changed the onset of calling is shifted by the same amount of time as the signal

is changed. The cue may be less critical in one species (Baker and Cardé 1979a). In this species, the magnitude of the shift in the timing of calling does not correspond to the magnitude of the change in the lights-on signal. The lights-off signal, rather than lights-on, probably is the critical cue governing the initiation of calling in other species of moths. This has been demonstrated only in one species (Cardé and Roelofs 1973).

2.1.5 Endogenous Circadian Rhythm

An endogenous circadian rhythm of calling is widespread in moths (Sower *et al.* 1970; Trainer 1970; Sanders and Lucuik 1972; Cardé and Roelofs 1973; Cardé *et al.* 1975b; Baker and Cardé 1979a; Castrovillo and Cardé 1979; Turgeon and McNeil 1982). In each species, the phase of the rhythm is reset each day, occurring at some specific time with relation to the lights-on/off cue(s). In the absence of the cue(s), the rhythm continues (free-runs) with the same periodicity. For example, in *Holomelina immaculata* (Reakirt), the female initiates calling between the second and fourth h of the scotophase (16:8 light:dark at 24°C), with more than 50% of the females calling by the third h of the scotophase (Cardé and Roelofs 1973). When females that have cycled through one calling period are maintained in continuous darkness at the same temperature, calling occurs at approximately the same time during each 24 h. Similarly, in the codling moth, *Laspeyresia pomonella* L., the female calling rhythm free runs in continuous photophase or continuous scotophase (Castrovillo and Cardé 1979). These examples clearly indicate that the female calling periodicity has an endogenous circadian basis.

2.2 Factors Affecting Calling Behaviour in Lepidoptera

In most species, the female does not necessarily call daily throughout its adult life. Instead, certain physiological factors within the females themselves and certain factors of the environment in which the females live control the occurrence and the timing of calling. The physiological factors include age, mating status, and stage of ovarian development. The environmental factors include temperature and photoperiod.

2.2.1 Age

Age may play an important role in determining the occurrence and timing of calling (Lawrence and Bartell 1972; Gorsuch *et al.* 1975; Swier *et al.* 1977; Kanno 1979; Turgeon and McNeil 1982; West *et al.* 1984). Some of the age-related changes observed are as follows: 1) The female normally initiates calling for the first time at a specific age. In some moths, calling occurs soon after emergence (Gorsuch *et al.* 1975; Sanders and Lucuik 1972; West *et al.* 1984). In others, it does not occur until the females are two to three days old (Lawrence and Bartell 1972; Kanno 1979; Swier *et al.* 1977; Turgeon and McNeil 1982). The age at which the females first call is relatively the same in most species (Lawrence and Bartell 1972; Sanders and Lucuik 1972; Gorsuch *et al.* 1975; West *et al.* 1984). In one species, however, there is considerable variation among the individuals (Turgeon and McNeil 1982). In this species, some females call when two days old, whereas the last females do not call until 10 days old. 2) The percentage of females calling each day may increase with age (Swier *et al.* 1977; Kanno 1979; Turgeon

and McNeil 1982). 3) The diel periodicity of calling may advance with age (Swier *et al.* 1977; Turgeon and McNeil 1982). The advance may be as much as 3.5 h in old females as compared with young females (Swier *et al.* 1977). 4) The number of daily calling bouts per female may increase or decrease with age (Lawrence and Bartell 1972; Swier *et al.* 1977; West *et al.* 1984; Turgeon and McNeil 1982). 5) The duration of the daily calling period may increase with age (Swier *et al.* 1977; Turgeon and McNeil 1982; West *et al.* 1984). The increase may be as much as two h in old females as compared with young females (Swier *et al.* 1977).

2.2.2 Mating Status

The females of multiple-mated species continue to produce and release pheromone after the first mating (Shorey *et al.* 1968a; Sanders and Lucuik 1972; Marks 1976; Swier *et al.* 1976). On the other hand, in species where the females mate only once, pheromone production diminishes rapidly after the first mating (Prez and Long 1964; Nagata *et al.* 1972).

Although multiple-mated females produce sex pheromone after mating, virgin females are always more attractive to males (Sanders and Lucuik 1972; Marks 1976; Swier *et al.* 1976). Males always mate with virgins in preference to mating with females that had already mated.

In some multiple-mated species, the females pass through a refractory period after mating (Shorey *et al.* 1968a; Sanders and Lucuik 1972). During this period, the females do not call, produce little or no pheromone and, therefore, are unattractive to the males (Sanders and Lucuik 1972). After a time, they resume calling and become attractive again.

2.2.3 Ovarian Maturation

The system for calling appears to mature and become operative at the same time as the eggs are maturing. Such a close correlation between the two events has been found in several of the species studied (Lawrence and Bartell 1972; Shorey *et al.* 1968a; Swier *et al.* 1976, 1977). For example in the black cutworm, *Agrotis ipsilon* (Hufnagel), the females initiate calling for the first time and the ovaries contain the first mature eggs on the first day after emergence. Calling reaches a maximum level on day four (Swier *et al.* 1977), the same day the ovaries contain the maximum number of mature eggs (Swier *et al.* 1976). Swier *et al.* (1977) suggested that calling at the time of maximum ovarian development ensures fertilization at peak reproductive maturity.

The females of the noctuid species studied by Shorey *et al.* (1968a) do not contain mature eggs and detectable sex pheromone in their glands until some time after emergence. They found that three reproductive processes (sex pheromone production, mating, and ovarian development) must all be matured before viable eggs can be laid. The sequence of maturation is: sex pheromone production is initiated first, mating occurs second, and maturation of eggs occurs third.

2.2.4 Temperature

Calling occurs within a certain temperature range which is characteristic for each species. Temperature normally affects the intensity of calling (Sower *et al.* 1971b; Sanders and Lucuik 1972; Cardé and Roelofs 1973; Cardé *et al.* 1975b; Gorsuch *et al.* 1975; Baker and

Cardé 1979a; Castrovillo and Cardé 1979; Turgeon and McNeil 1983; Haynes and Birch 1984b). In the laboratory, the effects of temperature were studied primarily in two types of experiments as follows. In one type, the females were raised continuously from emergence at different constant temperatures. In the other type, the females were shifted from one constant temperature to another constant temperature at some point after emergence. The observed changes in the calling behaviour are as follows. 1) The females may call at different ages at different temperatures. 2) The diel periodicity of calling may be advanced or delayed. 3) The duration of calling may be changed. 4) The calling posture may be changed. 5) Calling is decreased or inhibited by certain temperatures. 6) Calling is expressed optimally at certain temperatures.

The effects of continuous constant temperatures on calling behaviour were studied in only one species of Noctuidae, the armyworm *Pseudaletia unipuncta* (Haw.) (Turgeon and MacNeil 1983). In *P. unipuncta*, the females initiate calling for the first time at older ages at cool temperatures than at warm temperatures. The onset of calling each day occurs earlier at cool temperature than at warm temperatures. The duration of calling increases at warm temperatures.

In the shifted-temperature studies, when the females were shifted to temperatures lower than the rearing temperature, calling was modified in the following manner (Sower *et al.* 1971b; Sanders and Lucuik 1972; Cardé and Roelofs 1973; Cardé et al. 1975b; Gorsuch *et al.* 1975; Baker and Cardé 1979a; Castrovillo and Cardé 1979; Teal and Byers 1980; Haynes and Birch 1984b). The onset of calling each day usually occurs earlier

as the temperatures are lowered. The advance is as much as eight h for a shift of 8°C (Cardé *et al.* 1975b). However, females do not call if the temperature is too low. The temperatures at which calling is inhibited varies from 3.5°C (Sanders and Lucuik 1972) to 21°C (Gorsuch *et al.* 1975), depending on the species. The calling posture varies from calling to non-calling as the females are shifted to cooler temperatures (Cardé and Roelofs 1973; Teal and Byers 1980). The percentage of females calling and the duration of the calling period decreases as the temperatures are lowered (Sanders and Lucuik 1972; Baker and Cardé 1979a; Haynes and Birch 1984b).

On the other hand, in studies where females were shifted to temperatures higher than the rearing temperature, calling was modified as follows (Sanders and Lucuik 1972; Baker and Cardé 1979a; Haynes and Birch 1984b). The onset of calling each day occurs later as the temperatures are increased. The delay is more than an hour for a 5°C shift (Baker and Cardé 1979a). Calling is frequently interrupted and the females often spent more time in locomotion than calling at high temperatures (Sanders and Lucuik 1972; Cardé and Roelofs 1973). In addition, protrusion and retraction of ovipositor frequently is faster at high temperatures than at low temperatures (Cardé and Roelofs 1973). The percentage of females calling and duration of the calling period decreases as the temperatures are increased (Sanders and Lucuik 1972; Gorsuch *et al.* 1975; Haynes and Birch 1984b). However, these decreases occur when the shifts are made at temperatures unfavourable for the species studied (Haynes and Birch 1984b).

2.2.5 Photoperiod

The photoperiod at which the females are reared from emergence may influence the calling of the females. The changes observed were as follows. 1) The diel periodicity of calling may be changed. Under a short photophase (four to 12 h), the onset of calling each day was advanced in the spruce budworm, *Choristoneura fumiferana* (Clem.), (Sanders and Lucuik 1972), but delayed in the artichoke plume moth, *Platyptilia carduidactyla* (Riley) (Haynes and Birch 1984b). On the other hand, under a long photophase (14 to 18 h), the onset of calling was delayed in *C. fumiferana* (Sanders and Lucuik 1972), but advanced in *P. carduidactyla* (Haynes and Birch 1984b). 2) Calling may be disrupted by abnormally short (< 4 h) or long (> 18 h) photophases (Sanders and Lucuik 1972). The incidence of calling is low and calling is sporadic throughout the day. 3) Calling may be inhibited completely under a continuous photophase or scotophase.

Haynes and Birch (1984b) suggested that the effects of natural photoperiods on calling are not significant factors in those species where mating is confined to a short period during a season. However, they may have a major impact on the periodicity of mating of those species which are present in an area during the entire year and mate many times throughout the year.

2.3 Male Response Behaviour to Female Sex Pheromones in Lepidoptera

The manner in which the male utilizes the wind-borne pheromone released by a calling female to guide him to her is not fully understood (Shorey 1973; Farkas and Shorey 1974; Kennedy and Marsh 1974; Bartell

1977; Kennedy *et al.* 1981). The general understanding is that the wind carries the pheromone to the male at a distance by drawing out the odour cloud into a long plume from the source. The odour is received by specialized sensory sensilla of the antennae. It serves to release a hierarchic sequence of behavioural responses in the male to search for the female. They include: activation of the resting male, orientation of the male toward the source of the pheromone, and a series of short range responses in the presence of the female, which usually lead to copulation.

Activation of the resting male is the first recognizable step in the sequence of behavioural responses (Shorey 1964; Cardé and Hagaman 1983). The activation responses may include, in sequence, the moving of one or both antennae along the longitudinal axis of the body, the elevation of the body accompanied by a twitching of both antennae, the raising of the tarsi off the substrate, and the vibration of the wings first at low amplitude and finally at high amplitude. This behaviour is called chemokinesis.

The orientative behavioural responses result in the male's directional take off into the odour plume. Once he takes off, he flies upwind using optomotor, anemotaxis (wind-steering) behaviours. The optomotor reaction is the ability of males to respond to a pattern of stimuli moving across the visual field (Birch and Haynes 1982). The behavioural response to this reaction maintains the male's position relative to the changing environmental stimuli. The environmental stimuli are the ground patterns. While flying upwind, the males exhibit a zig-zag flight pattern (successive lateral reversals) that cuts across

the pheromone plume along an upwind trajectory (Marsh *et al.* 1978; Kennedy *et al.* 1981). The response is called reverse anemomenotaxis (meno-angled), because the male is oriented at an angle to the wind. The mechanism controlling the zig-zag pattern of flight is not clearly understood. Kennedy and Marsh (1974) suggested that it probably is a behavioural response to loss of the pheromone stimulus at the edge of the plume. The response tends to bring the male back into the plume again. However, Cardé (1984) suggested that the zig-zag flight pattern represents an internally generated flight pattern of the male, since the male shows the same zig-zag flight in homogeneous clouds of pheromone and in pheromone plumes. It probably is a course-setting manoeuver that facilitates upwind progress in the plume (Cardé 1984).

When the males are close to the females, either visual stimuli from the females or the pheromone alone may release a series of characteristic responses in the males before copulation occurs (Shorey 1964; Shorey and Gaston 1970; Farkas and Shorey 1974; Baker and Cardé 1979b; Den Otter and Klijnstra 1980; Teal *et al.* 1981; Haynes and Birch 1984a). The sequence involves the hovering of the male close to the abdomen of the female, touching the female's extruded ovipositor with one or both of the antennae, moving of the male to the side of the female, vibration of the wings of the male and female, extrusion of the genital claspers of the male, and curving of the tip of the abdomen of the male towards the tip of the abdomen of the female. The male may release a pheromone during courtship to induce her to settle and/or take up an acceptance posture (Grant 1981).

The response of the males to female sex pheromone has a circadian rhythm, which may be rigidly programmed. In nocturnal species, the

males begin mating activity at characteristic times of the night. This has been demonstrated by hourly light trap catches (Williams 1935; Hutchins 1940; Graham *et al.* 1964), by hourly pheromone trap catches (Saario *et al.* 1970; Minks and Noordink 1971; Mitchell 1973; Cardé *et al.* 1974; Rothschild and Minks 1974; Marks 1976; Swier *et al.* 1976), by pheromone studies in the laboratory (Shorey 1964; Baker and Cardé 1979a; Haynes and Birch 1984b), and by observing isolated males in the laboratory (Callahan 1958; Edwards 1962; Leppla 1976; Hsiao 1978; Leppla *et al.* 1979). In each species studied, the lights-on/off photoperiodic cues are responsible for setting the timing of the circadian rhythm of the male response (Edwards 1962; Shorey and Gaston 1965; Sanders and Lucuik 1975; Castrovillo and Cardé 1979; Leppla *et al.* 1979). Also, the rhythm is endogenously based in most species studied (Shorey and Gaston 1965; (Baker and Cardé 1979a; Castrovillo and Cardé 1979).

In each species for which pheromone response by males and pheromone release by females have been examined, the two phenomena correspond closely in timing (Sanders and Lucuik 1972; Truman 1973; Shorey 1974; Cardé *et al.* 1975b; Swier *et al.* 1976; Baker and Cardé 1979a; Haynes and Birch 1984b). The close synchrony increases the possibility that a male will encounter the airborne pheromone from a female and that attraction and copulation will occur (Truman 1973). It may be particularly important in closely related, sympatric species which share cross attractive pheromones (Kaae *et al.* 1973; Roelofs and Cardé 1974; Teal *et al.* 1978; Teal and Byers 1980; Byers *et al.* 1985).

Male response behaviour to female sex pheromone may be affected by several physiological, chemical, and environmental factors (Shorey 1974;

Bartell 1977). The factors include age, previous exposure to the pheromone, pheromone concentration, and temperature. Each of the factors has an optimum as far as the male response is concerned.

Age is an important factor which affects the male response to female sex pheromone, presumably reflecting the timing of sexual maturation (Shorey *et al.* 1968b; Bartell and Shorey 1969a; Trainer 1970; Den Otter and Klijnstra 1980; Turgeon *et al.* 1983). For example, the males of certain noctuid moths are capable of responding to the female sex pheromone after a maturation period of only one to two days (Shorey *et al.* 1968b). At this age, the spermatozoa move from the testes into the ductus ejaculatory duplex, from which position they can be transferred to the female during copulation. In *P. unipuncta*, the males respond for the first time when two days old; the number responding increases until the fifth day and then declines thereafter (Turgeon *et al.* 1983).

Previous exposure to pheromone may lead the male to sensory adaptation or habituation (Shorey 1974). In adapted males, the response threshold to further stimulation by pheromones is elevated. However, the elevation may only be transitory. For example, in the cabbage looper moth, *Trichoplusia ni* (Hübner), the males recover from sensory adaptation within 60 sec under experimental conditions (Payne *et al.* 1970). On the other hand, males may require several hours to recover from habituation effects. Adaptation or habituation seems to result from repeated or continuous stimulation from pheromones (Shorey and Gaston 1965; Bartell and Shorey 1969a; Lawrence and Bartell 1972; Bartell and Roelofs 1973; Kennedy *et al.* 1981).

The different pheromone components are responsible for releasing the male response hierarchy in species with multicomponent pheromone systems (Cardé *et al.* 1975a; Baker *et al.* 1976; Roelofs and Cardé 1977). There may be as many as four or more different chemicals which make up the pheromone in such systems. Some components elicit long-range responses and are called primary components. The others are responsible for releasing close-range responses and are called secondary components. For example, in *Grapholitha molesta* (Busck), two of the components are responsible for long-range orientation to the pheromone source, whereas a third component elicits close-range, courtship behaviour (Cardé *et al.* 1975a).

Pheromone concentration also may be involved in the release of the male response hierarchy (Bartell and Shorey 1969b; Bartell 1977; Farkas and Shorey 1974; Den Otter and Klijnstra 1980; Cardé and Hagaman 1983; Haynes and Birch 1984b). The initial step in the behavioural sequence requires the lowest concentration of pheromone while each successive step requires higher concentrations.

Temperature may have profound effects on male response. It affects the response in at least two ways: 1) temperatures below the threshold and above the upper limit inhibit the response, and 2) temperatures between these limits determine the timing of the response. For example, in the gypsy moth, *Porthetria dispar* L., the males never respond to the female sex pheromone at temperatures below 21°C (Collins and Potts 1932). They approach the source in increasing numbers as temperatures rise to 32°C. In *L. pomonella*, the males normally are captured in pheromone traps at sunset (Batiste *et al.* 1973). They are captured

several hours before sunset on comparatively cool days. In days when the afternoon is hot (28°C) and the evening is warm (21°C), moths are not captured until an hour after sunset (Castrovillo and Cardé 1979). On the other hand, male capture is advanced to about three h prior to sunset when the afternoon is warm (21°C) and the evening is cool (16°C). Similarly, the changes in temperatures that normally occur over a season may affect the timing of male response (Saario *et al.* 1970; Batiste *et al.* 1973; Cardé and Roelofs 1973; Comeau *et al.* 1976; Marks 1976; Liebhold and Volney 1984).

3 MATERIALS AND METHODS

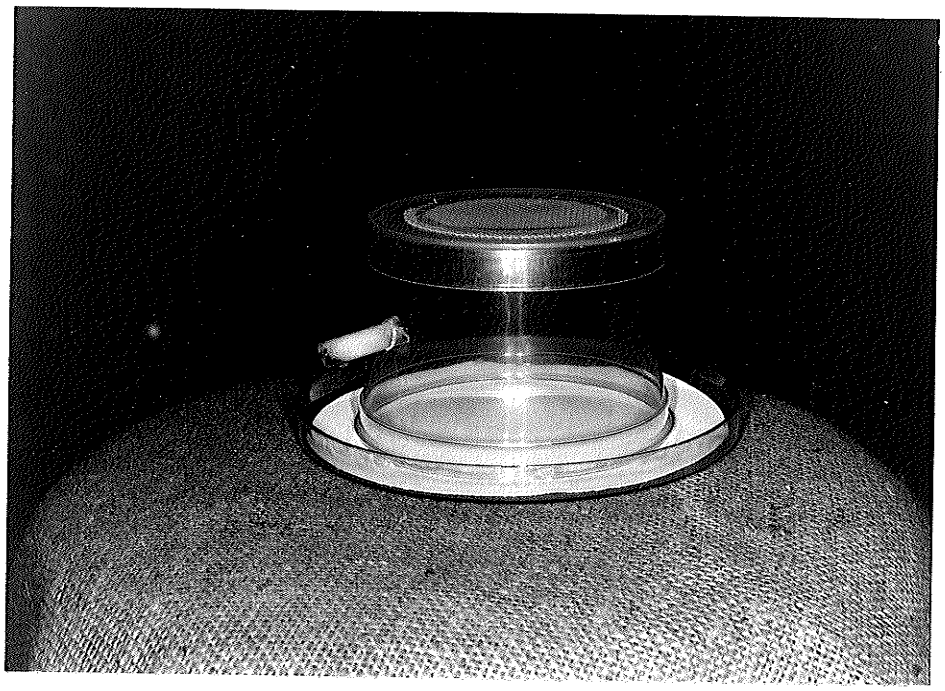
3.1 Experimental Moths

A continuous supply of adults of *M. configurata* were obtained from a stock colony maintained on the synthetic diet developed by Bucher and Bracken (1976). The rearing techniques from eggs to adults also were according to Bucher and Bracken (1976). The colony was initiated in 1981 from a general stock colony maintained at the Agriculture Canada Research Station, Winnipeg, for at least five generations.

The moths used in the experiments were collected from the stock colony as pupae. The pupae were sexed and placed in paper cartons which contained damp vermiculite and were covered with glass petri dishes. The sexes were incubated separately until the emergence of the moths in an environmental chamber at 20° ± 0.5°C, 60 ± 5% relative humidity, and a 16 h L : 8 h D photoperiod.

Until the experiments, the moths were maintained in the same environmental conditions as the pupae. They were housed individually in transparent plastic containers measuring 8 cm in height and 11.5 cm in diameter (Fig. 1). The tops of the containers were covered with gauze to permit the circulation of air, and the bottoms were placed on plastic dishes containing dry filter paper. The moths were fed a nutrient solution of a commercial beer and honey (1:3.41) (Bucher and Bracken 1976). The nutrient solution was replenished every second day. The filter papers at the bottom of the cages also were replaced every second day. The cages were changed twice a week.

Fig. 1. Cage used for observing calling behaviour in the bertha armyworm.



The photoperiod of the stock colony coincided with that in nature. The photophase and scotophase were reversed in the experiments; the photophase was from 1700 h to 0900 h and the scotophase was from 0900 h to 1700 h. The moths used in the experiments were transferred to the reverse photoperiod soon after pupation.

Newly-emerged moths were removed twice daily from the paper cartons. Moths that emerged during the photophase and scotophase were collected separately. Those moths that emerged during a photophase were collected at the end of the photophase, and those which emerged during a scotophase were collected at the beginning of the following photophase. In the experiments, observations on calling behaviour were initiated during the first complete scotophase after emergence or during a scotophase subsequent to the first one. Though the emergence pattern of the adults from the pupal stage is not known, it was assumed to be relatively uniform during the 24 h period. In the first case, the moths that emerged during the photophase and scotophase, therefore, were on average eight and 20 h old, respectively, at the beginning of the first complete scotophase. In the other cases, the moths that emerged during the scotophase were about 12 h older than those that emerged during the photophase.

Both photophase- and scotophase-emerged females were used in the experiments. In a particular experiment, either photophase- or scotophase-emerged females were used. This decision was taken in order to avoid possible variation due to the phase of emergence.

At the beginning of the experiments, the females in their cages were placed in an experimental chamber. Unless stated otherwise, the temperature, photoperiod, and relative humidity in the experimental

Fig. 2. Chamber (left) used to cover the window of the experimental chamber (right) while viewing calling females of the bertha armyworm.

chamber were the same as those in the rearing chamber for the pupae and adults. The light intensity at the centre of the chamber during the entire scotophase was approximately 0.3 lux under a red light (7.5 W GEC bulb). The viewing of the moths was accomplished through a window in the door of the chamber. The window was surrounded on three sides by a chamber made of light-proof cloth wrapped on a wooden frame to prevent the entry of light (Fig. 2).

3.2 Experimental Procedures

3.2.1 Characteristics of Calling Behaviour

The characteristics of calling behaviour were studied in virgin females during the fourth scotophase after emergence. The aims of this experiment were: 1) to characterize the calling posture of the females, 2) to determine the diel periodicity of calling, 3) to determine whether the calling pattern is continuous or discontinuous, and 4) to determine the sequence of events during calling. Only females that emerged during the photophase were used. They were transferred to the experimental chamber at the beginning of the third scotophase to expose them to the experimental conditions for 24 h before the experiment. Observations were made on 24 females.

The observations began at the start of the fourth scotophase and were made at 15- to 60-sec intervals until the females stopped calling in the following photophase. Records were kept on the nature of all activities of the females during the scotophase, the mean onset time of these activities (time after lights off), the mean onset calling time (time after lights off), the number of calling bouts, the duration of each calling bout, and the total time spent calling. The standard errors of the means were calculated for the data in this experiment and those of the other experiments in the thesis.

3.2.2 The Effects of Age on Calling Behaviour of Virgin Females

Age-related changes in calling behaviour were observed in virgin females for the first 12 scotophases after emergence. The objectives of this study were: 1) to determine the age at which the

females that emerged during the photophase and scotophase initiated calling for the first time, 2) to determine the changes in the onset of the daily calling time with age, and 3) to determine the changes in the duration of the daily calling period with age. For the first objective, females that emerged during the photophase or scotophase were investigated separately during the first to third scotophases after emergence. The investigations were made on the same females during each scotophase, and N was 24 for the photophase- and scotophase-emerged females. They were transferred to the experimental chamber at the beginning of the first scotophase. For the second and third objectives, females that emerged during the photophase were investigated for the first 12 scotophases after emergence and those that emerged during the scotophase were observed for the first five scotophases. The investigations were made on different females during each scotophase and N was 24 for each scotophase. The females were transferred to the experimental chamber at the beginning of the scotophase in which the observations were made.

The observations were made at 15-min intervals during each scotophase and continued until the last females stopped calling in the following photophase. Records were kept on the mean calling age at first calling, the mean onset calling time, and the mean duration of the calling period. In calculating the means, a female was considered calling 7.5 min earlier and later than the first and last observed calling. For the photophase-emerged females, the data for the mean onset calling time and duration of calling period were analyzed using t tests, one-way ANOVA, and Duncan's new multiple range tests. For the

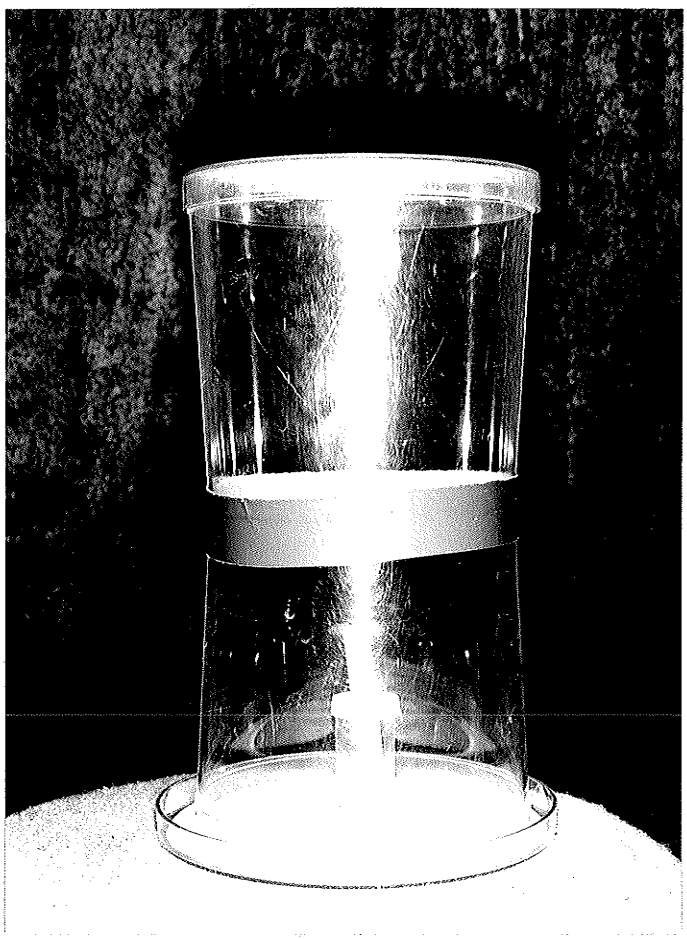
ANOVA, the data for the onset calling time were transformed using $x^{3.5}$ transformation and the data for the duration of the calling period were transformed using a log transformation so that the variance among means was stabilized. For the scotophase-emerged females, the data for mean onset calling time and duration of the calling period were analyzed using one-way ANOVA and Duncan's new multiple range tests. The data for mean onset calling time and duration of calling period from second to fifth scotophases for the photophase- and scotophase-emerged females were analyzed in pairs using t tests. Differences at the 5% level of confidence were considered significant in this experiment and other experiments in the thesis.

3.2.3 The Effect of Mating on Calling Behaviour of Females

Calling of females that mated only once after emergence was studied in relation to subsequent calling and oviposition until death. The objectives of the study were: 1) to determine the age and diel periodicity of the first mating, 2) to determine the length of the non-calling period between the first mating and the resumption of calling after mating, and 3) to determine the diel periodicity of calling and oviposition in mated females. Females and males, that emerged during the photophase and were the same age, were used. Observations were made on 24 pairs.

At the beginning of the first scotophase, the pairs were placed in clear plastic cages for mating (one pair/cage) and transferred to the experimental chamber. The mating cages were cylindrical, measuring 25 cm in height and 13.5 cm in diameter (Fig. 3). The tops were covered with gauze. Vials with the nutrient solution were provided as food.

Fig. 3. Cage used for observing mating in the bertha armyworm.



Following mating, the females were isolated from the males and placed in the calling observation cages (Fig. 1) for calling and oviposition. One female was placed in each cage. Each cage was provided with a sprig of lamb's-quarters (*C. album*) as an oviposition site. The plants were obtained daily from a culture grown in a greenhouse.

The observations were made at 15-min intervals during each scotophase after emergence and continued until the death of the last females. Records were kept on the time of the first mating, on the time mating was completed, on the mean duration of the non-calling period after mating, on the times calling and oviposition occurred during each scotophase following the first mating, on the percent females calling and ovipositing, on the mean number of fertilized and unfertilized eggs laid, on the percentage of the total oviposition that occurred daily, and on daily survival of the females. The eggs were counted daily under a binocular microscope using a grid (Bucher and Bracken 1976). In calculating the means, a female was considered calling, mating, or ovipositing 7.5 min earlier and later than the first and last observed calling, mating, or oviposition.

3.2.4 Photoperiodic Cue(s) of Calling

The photoperiodic cue(s) responsible for setting the diel periodicity of calling was investigated in virgin females during the fourth to sixth scotophases after emergence. The females were conditioned at a photoperiod of 16 h L : 8 h D for the first three scotophases, and then the lengths of the photophase and scotophase were changed by two to 10 h during the fourth to sixth scotophases in five tests as follows:

Test I: third scotophase, 16 h L : 8 h D; fourth scotophase, 6 h L : 18 h D; fifth scotophase, 6 h L : 18 h D; and sixth scotophase, 6 h L : 18 h D.

Test II: 16 h L : 8 h D; 10 h L : 14 h D; 10 h L : 14 h D; and 6 h L : 18 h D.

Test III: 16 h L : 8 h D; 14 h L : 10 h D; 10 h L : 14 h D; and 6 h L : 18 h D.

Test IV: 16 h L : 8 h D; 18 h L : 6 h D; 6 h L : 18 h D; and 6 h L : 18 h D.

Test V: 16 h L : 8 h D; 16 h L : 8 h D; 16 h L : 8 h D; and 16 h L : 8 h D (control).

Only females that emerged during the photophase were used. They were transferred to the experimental chamber at the beginning of the second scotophase. Observations were made on 24 females in each test.

The observations began at the start of the third scotophase and were made hourly during each scotophase. Records were kept on the mean onset calling time and percent females calling during each scotophase. In calculating the mean onset calling time, a female was considered calling 30 min earlier than the observed calling time. The data for each test were analyzed using one-way ANOVA and Duncan's new multiple range tests. The data for the third and fourth scotophases in Test IV were subjected to a t test. The data for the third scotophase in Tests I-V were analyzed using one-way ANOVA. The data for the fourth scotophase in Tests I-V were analyzed using the Kruskal-Wallis test.

3.2.5 Endogenous Circadian Rhythm of Calling

To determine whether the diel periodicity of calling is endogenously based, virgin females were kept in continuous darkness for 192 h. The females were conditioned at a photoperiod of 16 h L : 8 h D for the first three scotophases, and then at the beginning of the fourth scotophase, the photoperiod was changed to continuous darkness. Only females that emerged during the scotophase were used, and observations were made on a total of 37 females. They were transferred to the experimental chamber at the beginning of the second scotophase.

The observations began at the start of the third scotophase and were made hourly during that scotophase. During the period of continuous darkness, observations were made every two to four h during the times females were not calling and hourly during the times females were calling. The records kept were the same as in Section 3.2.4.

3.2.6 The Effects of Photoperiod on Calling Behaviour of Virgin Females

Effects of photoperiod on calling behaviour of virgin females were investigated during the first four scotophases after emergence. The aims of this investigation were to determine the effects of the length of the scotophase on the onset of calling and on the duration of the calling period. The photoperiods were 16 h L : 8 h D (control), 12 h L: 12 h D, and 8 h L : 16 h D. Only females that emerged during the scotophase were used. The investigations were made on the same females during each scotophase and N was 24 for each photoperiod. The newly-emerged females were transferred to the experimental chamber at the beginning of the first photophase after emergence.

The observations were made at hourly intervals during each scotophase and continued until the last female stopped calling in the following photophase. Records were kept on the mean onset calling time, the mean duration of the calling period, and percent females calling. The data for the means were analyzed using two-way ANOVA.

3.2.7 The Effects of Temperature on Calling Behaviour of Virgin Females

The effects of temperature on calling behaviour of virgin females were investigated in two experiments. In Experiment I, the females were reared continuously from emergence at six constant temperatures. In Experiment II, the females were reared at 20°C for the first two scotophases and then transferred to six different constant temperatures for one scotophase (third scotophase).

In Experiment I, the objectives of the investigation were: 1) to determine the age at which the females initiated calling for the first time at each temperature, 2) to determine the effects of temperature and age on the onset of calling and the duration of the calling period at each temperature, 3) to determine the percent females calling at each temperature, and 4) to determine the longevity of the females at each temperature. The temperatures were 10°, 15°, 20°, 25°, 30°, and 35°C. Only females that emerged during the photophase were used. They were transferred to the experimental chamber at the start of the first scotophase at each temperature. The investigations were made on 24 females at each temperature. For the first three objectives, the observations were made at 10-min intervals during the period from the beginning of the first scotophase to the end of the scotophase in which

the females called for the third consecutive scotophase. Records were kept on the mean age at first calling, the mean onset calling time, the mean duration of the calling period, and on the percent females calling during each scotophase. For the third objective, further observations were made at one-hour intervals during each scotophase after the third scotophase in which calling occurred. Records were kept on the percent females calling during each scotophase. For the fourth objective, the dead females were counted daily at the beginning and end of the scotophase. In calculating the mean longevity, a female was considered dead eight or four h earlier than the observed time if death occurred during the photophase or scotophase, respectively. In calculating the mean onset calling time and the mean duration of calling period, a female was considered calling five min earlier and later than the first and last observed calling. The data for the mean age of first calling and the mean longevity were analyzed using one-way ANOVA and Duncan's new multiple range tests. The data for the mean onset calling times and the mean duration of calling periods were analyzed using two-way ANOVA and Duncan's new multiple range tests (SAS 1982). The data for the incidence of females calling were subjected to chi-squared tests (2x2 contingency tables).

In Experiment II, the objectives of the investigation were: 1) to determine the mean onset calling time at each temperature, 2) to determine the duration of calling period at each temperature, and 3) to determine the percent females calling at each temperature. The temperatures were 5°, 10°, 15°, 20° (control), 25°, 30°, and 35°C. Only females that emerged during the photophase were used. They were

transferred to the experimental chamber at the beginning of the third scotophase. The investigations were made on 24 females at each temperature. The observations were made at 10-min intervals during the third scotophase and continued until the last female stopped calling. The records kept were the same as in Experiment I. The data for the means were analyzed using one-way ANOVA and Duncan's new multiple range tests.

3.2.8 The Relationship Between Ovarian Maturation and Calling of Virgin Females

Ovarian maturation was investigated in virgin females during the first six scotophases after emergence, or until death at four constant temperatures. The aims of the investigation were: 1) to determine the relationship between ovarian maturation and the initiation of calling, and 2) to determine the effects of high temperature (35°C) on ovarian maturation and calling. For the first objective, groups of virgin females were reared continuously at 10°, 20°, 30°, or 35°C for the first six scotophases after emergence. For ovarian maturation, one group of females was killed at the beginning and another group at the end of each scotophase at each temperature. For calling, the groups of females that were killed at the end of each scotophase were observed for calling during the scotophase preceding killing. Only females that emerged during the photophase were used at all temperatures, except 20°C. At 20°C, two groups were used, one emerging during the scotophase and one during the photophase. There were 24 females in each group at each temperature. For the second objective, calling was observed during

each scotophase until death in a group of 24 virgin females reared at 35°C. The dead females were collected and preserved to investigate ovarian maturation. Only females that emerged during the photophase were used. For both objectives, the females were transferred to the experimental chamber at the end of the photophase or scotophase in which they emerged. Observations on calling were made at five- to 10-min intervals during the last hour of each scotophase.

The abdomens of the killed and dead females were preserved in an aqueous solution of chloral hydrate, formaldehyde, and acetic acid (Weaver and Thomas 1956). The dissections were done under a binocular microscope. While dissecting, a solution of Sudan IV and 2% potassium hydroxide was used as an aid in distinguishing the ovaries from the fat body (Weaver and Thomas 1956). A solution of methylene blue in ethanol was used to differentiate the chorionated eggs from the unchorionated eggs in the ovaries (Shorey *et al.* 1968a).

Records were kept on the percent females containing chorionated eggs in the ovarioles and the lateral and common oviducts at the beginning and at the end of each scotophase and during their lifetime, on the percent females that called during each scotophase and during their lifetime, and on the percent females containing damaged ovaries.

4 RESULTS

4.1 Characteristics of Calling Behaviour

4.1.1 Calling Posture

The female moth usually initiated calling during the scotophase after a brief period of flight following a quiescent period (Fig. 4). During the quiescent period, the females rested on the sides of the cages and had the following posture: the head end was uppermost, the antennae were directed anteriorly or vertically, the abdomen was motionless with its ventral surface touching the cage wall, the wings were held above the abdomen in the form of an inverted "V", and the ovipositor was concealed within the last visible abdominal segment. The females returned to the sides of cages after the brief flight and again oriented themselves with the head uppermost. During calling (Fig. 5), the antennae were directed posteriorly and held close to the sides of the thorax. The ovipositor was extruded and curved downward at an angle of about 45°. The abdomen was bent so that the two ends were raised and the middle was lowered. The legs were extended so that the abdomen no longer touched the cage wall. The wings were elevated above the abdomen with the upper surface at an angle between horizontal and about 45° ("V"-shaped).

The calling posture varied in some females. The antennae were directed laterally in about 13% of the females. Also, about 13% of the females stretched their legs only partially so that the abdomen was almost touching the substrate.

Before the calling posture was taken up, movements of the ovipositor were noted. The ovipositor of most females was moved from

Fig. 4. Summary of the main activities of 24 virgin females of the bertha armyworm during the fourth scotophase under a 16 h L : 8 h D photoperiod and at 20°C. The numbers associated with the arrows are proportions of females performing the activities. The numbers in the boxes are the number of females performing the activities. The numbers on the left are mean onset times after lights off for the initiation of the activities.

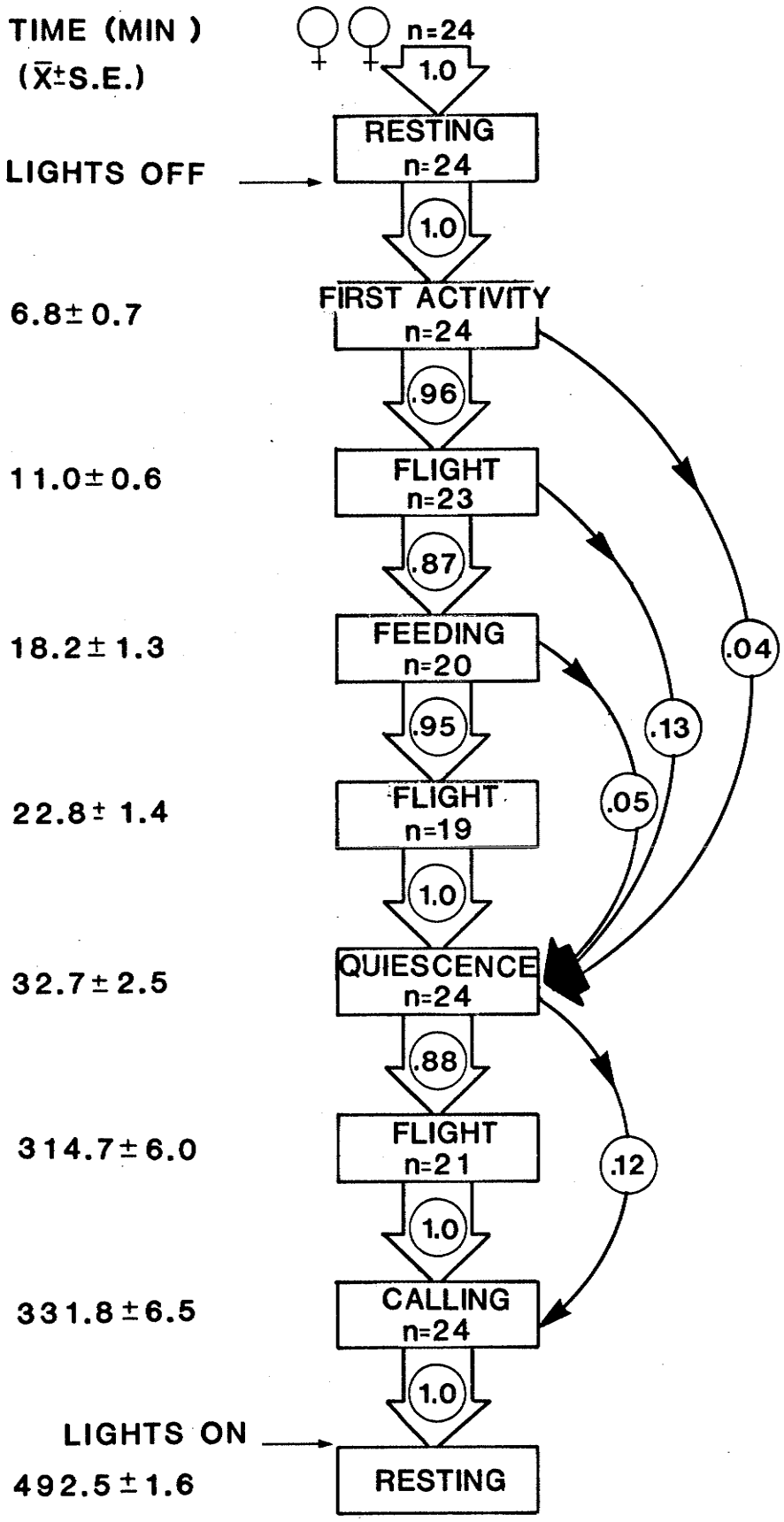
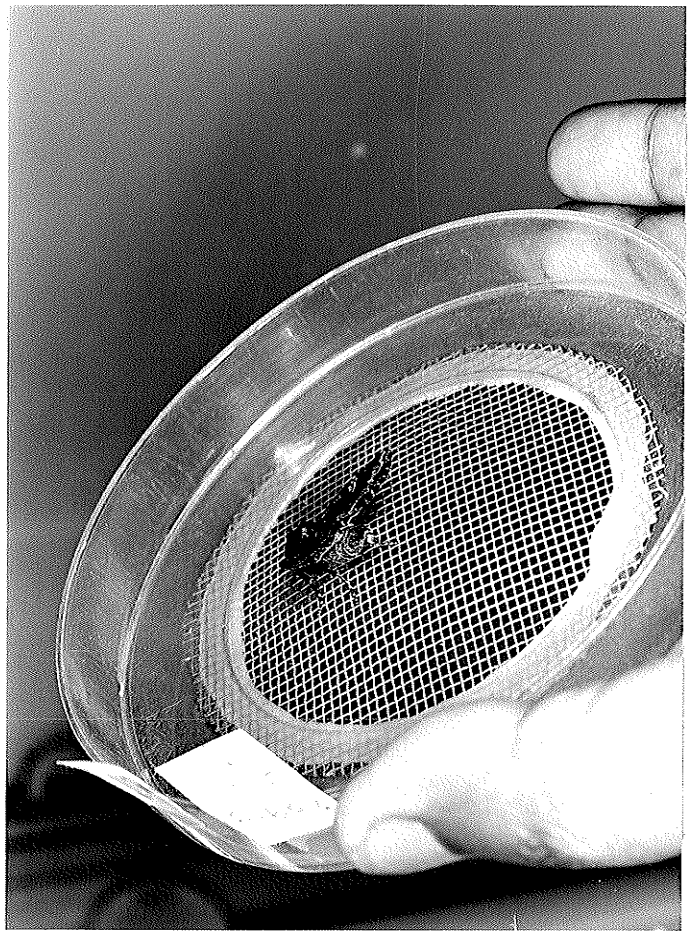

TIME (MIN)
(X̄±S.E.)
n=24
1.0
RESTING
n=24
LIGHTS OFF
1.0
6.8± 0.7
FIRST ACTIVITY
n=24
.96
11.0± 0.6
FLIGHT
n=23
.87
18.2± 1.3
FEEDING
n=20
.95
22.8± 1.4
FLIGHT
n=19
1.0
32.7± 2.5
QUIESCENCE
n=24
.88
314.7± 6.0
FLIGHT
n=21
1.0
331.8± 6.5
CALLING
n=24
1.0
LIGHTS ON
492.5± 1.6
RESTING
.04
.13
.05
.12

Fig. 5. Typical calling posture of a virgin female of the bertha armyworm.



side to side, or rotated in a circular fashion while remaining partially concealed within the last visible abdominal segment. This movement frequently was accompanied by rapid protrusion and retraction of the ovipositor. Occasionally, the abdomen with the protruded ovipositor was curved downward to touch or press against the substrate.

4.1.2 Diel Periodicity of Calling

The diel periodicity of calling and the sequence of main activities during the scotophase are illustrated in Fig. 4. The durations of the main activities were not always equal to the differences between successive mean onset times, because a small proportion of the females performed a number of incidental activities that appeared to be unrelated to calling behaviour and these latter activities, therefore, were not included in Fig. 4.

All females were resting when the lights were switched off. The females became active within 6.8 ± 0.7 min of lights-off (range, 2 - 14 min). At this time, a female raised her body slightly above the substrate, waved her antennae rapidly, and then began to vibrate the wings. The wings were vibrated on average for 4.0 ± 0.4 min (range, 1-7 min). The wing vibration activity was followed by flight. The females flew around the cages for 5.5 ± 0.8 min (range, 1-12 min) and finally settled on the food source. Feeding lasted for 4.5 ± 0.7 min (range, 1-12 min). After feeding, the females again flew around the cage, selected a suitable resting site, and entered into a long quiescent phase. Before the flight, most females were stationary for a few minutes and then vibrated the wings. The flight after feeding lasted 5.2 ± 0.9 min (range, 1-11 min). Most females (92%) selected the

sides of the cages for the resting site; none selected the feeding site.

While in the quiescent phase, the females remained completely stationary. The average duration of the quiescent period was 282.0 ± 5.3 min (range, 219-343 min). The quiescent period was followed by another period of flight. The females vibrated their wings and flew around the cages for 6.4 ± 2.1 min (range, 1 - 28 min) before they selected a suitable site for calling. Some females (13%) did not fly after the quiescent period. However, they stretched their legs to raise the abdomen above the substrate, waved the antennae keeping them directed forward or upward, and held the wings with the dorsal surface almost horizontal. This posture also was adopted by those that flew after the quiescent period. From this point, all females initiated calling in 8.0 ± 0.9 min (range, 2-20 min). The females usually selected the sides (83%) rather than the floor (13%) or the roof (4%) of the cages during the early part of the calling period. However, some females left the sides of the cage and moved to the roof or floor during the later part of the calling period. When the females called from the sides of the cages, the head was directed upward and the body remained parallel with the substrate. The head was close to the substrate and the body made an angle of about 45° with the substrate when they called from the roof.

The calling period was considered as the time between the first and last observed calling in the group of females. The period lasted for about 4.3 h (mean, 2.7 ± 0.1 h) and was extended for about 30 min into the following photophase. Individual females called on average for

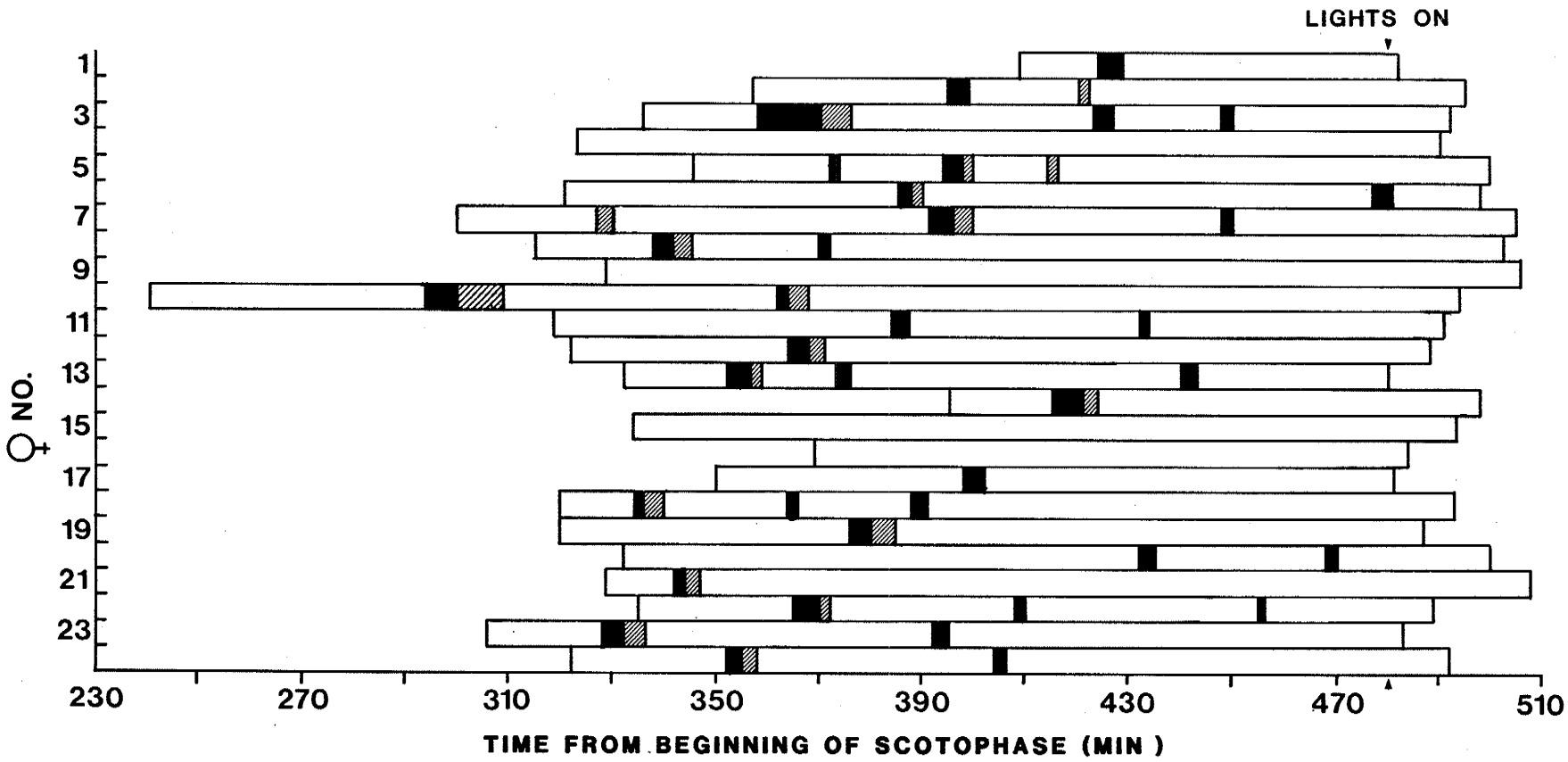
152.1 ± 7.1 min (range, 68-229 min). After the calling period, the females entered into a resting phase. The floor of the cages usually was selected as the resting place.

4.1.3 Calling Pattern

The calling pattern of individual females is shown in Fig. 6. In most females (83%), there was more than one bout of calling with short periods of interruption. The bout length was 51.6 ± 4.3 min (range, 13 - 161 min). The first bout usually was shorter (mean, 36.3 ± 5.0 min; range, 13-100 min) than the last bout (mean, 78.8 ± 8.3 min; range, 18-161 min) in individual females. In some females (17%), there was a single bout of calling; the bout length was 156.5 ± 13.9 min (range, 115 - 175 min). For all females, the mean number of bouts was 2.7 ± 0.2 (range, 1-4).

During the periods of interruption, the females flew around the cages and/or protruded and retracted the ovipositor a number of times (Fig. 6). The average duration of each interruption was 4.9 ± 0.6 min (range, 1-18 min).

Fig. 6. Calling patterns of 24 virgin females of the bertha armyworm during the fourth scotophase under a 16 h L : 8 h D photoperiod and at 20°C. White blocks indicate calling, black blocks indicate flight, and cross-hatched blocks indicate protrusion and retraction of the ovipositor.


LIGHTS ON
♀ NO.
1
3
5
7
9
11
13
15
17
19
21
23
230
270
310
350
390
430
470
510
TIME FROM BEGINNING OF SCOTOPHASE (MIN)

4.2 The Effects of Age on Calling Behaviour of Virgin Females

Newly-emerged females initiated calling for the first time during the second and third scotophases after emergence. In the females that emerged during the photophase, 38% of them called during the second scotophase and 100% called during the third scotophase. In the females that emerged during the scotophase, 100% of them called during the second scotophase. For the females that emerged during the photophase, the mean age of the first calling was 39.6 ± 4.3 h for those that initiated calling during the second scotophase and 62.4 ± 6.8 h for those that initiated calling during the third scotophase. For the females that emerged during the scotophase, the mean age of first calling was 50.9 ± 4.8 h.

The mean onset calling time differed significantly with age in the females that emerged during the photophase ($F=27.20$, d.f.=[9,230], $P < 0.05$; Fig. 7, Table 1) and the scotophase ($F=136.2$, d.f.=[3,92], $P < 0.05$; Table 2), the older the females the earlier calling occurred in the scotophase. The onset of calling was advanced on average by about 137 min during the period between the second and fourth scotophases for the females that emerged in the photophase and 109 min for the females that emerged in the scotophase. The mean onset calling times were not significantly different during the fourth and fifth scotophases among the females that emerged during the photophase and scotophase, and during the fifth and sixth and the sixth and seventh scotophases among those that emerged during the photophase. However, the onset of calling was advanced significantly during the period between the fourth and seventh scotophases (approximately 74 min) in

Fig. 7. Percent virgin females of the bertha armyworm calling during the first to twelfth scotophases under a 16 h L : 8 h D photoperiod and at 20°C. The numbers and arrows give mean times (min) for onset of calling after lights off. N=24 for each scotophase; females emerged during the photophase. The lights were switched on 480 min after lights off.

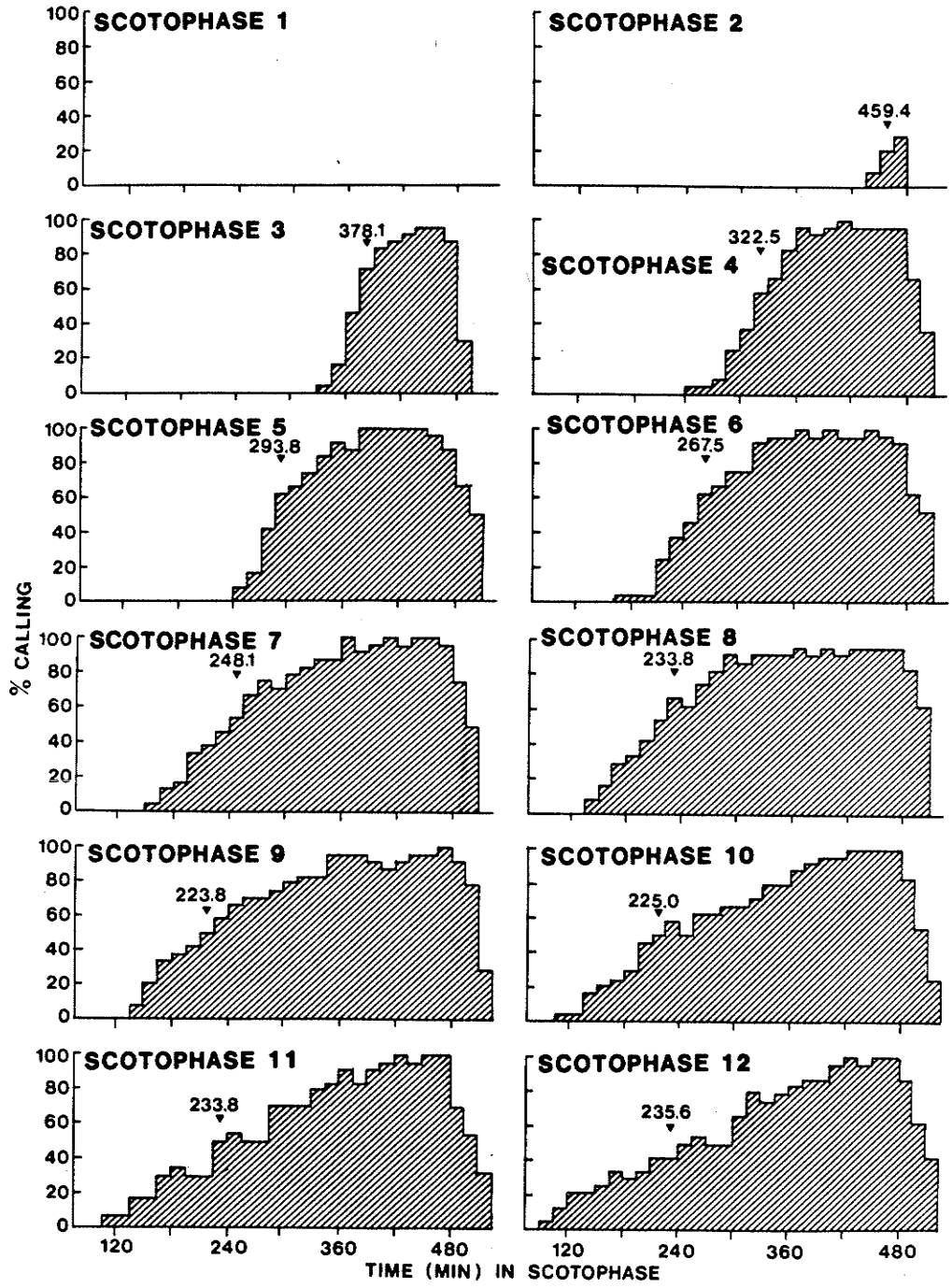

SCOTOPHASE 1
SCOTOPHASE 2
459.4
SCOTOPHASE 3
378.1
SCOTOPHASE 4
322.5
SCOTOPHASE 5
293.8
SCOTOPHASE 6
267.5
SCOTOPHASE 7
248.1
SCOTOPHASE 8
233.8
SCOTOPHASE 9
223.8
SCOTOPHASE 10
225.0
SCOTOPHASE 11
233.8
SCOTOPHASE 12
235.6
% CALLING
0
20
40
60
80
100
120
240
360
480
TIME (MIN) IN SCOTOPHASE

Table 1. Effect of age on the percent calling, the mean onset calling time, and the mean duration of the calling period of virgin females of the bertha armyworm that emerged during the photophase and reared under a 16 h L : 8 h D photoperiod and at 20°C. N=24 females/scotophase.

Age (scotophase)	% calling	Mean onset calling time[a] ($\bar{x}$ ± SE (min))		Mean duration of calling period[a] ($\bar{x}$ ± SE (min))	
1	0	-		-	
2	33	459.4 ± 4.4	f	28.1 ± 4.4	a
3	100	378.1 ± 4.8	e	112.5 ± 5.0	b
4	100	322.5 ± 6.6	d	180.6 ± 7.5	c
5	100	293.8 ± 6.3	cd	208.1 ± 6.6	cd
6	100	267.5 ± 10.6	cb	236.3 ± 10.5	de
7	100	248.1 ± 11.8	ab	258.1 ± 12.0	ef
8	100	233.8 ± 13.1	a	274.4 ± 13.3	f
9	100	223.8 ± 12.1	a	293.8 ± 13.3	f
10	100	225.0 ± 12.9	a	286.9 ± 12.4	f
11	100	233.8 ± 14.6	a	277.5 ± 14.8	f
12	100	235.6 ± 18.8	a	280.6 ± 18.9	f

[a]In each column, the means followed by the same letter are not significantly different (t test for second to third scotophases and Duncan's new multiple range test for third to 12th scotophases; $P>0.05$).

Table 2. Effect of age on the percent calling, the mean onset calling time, and the mean duration of the calling period of virgin females of the bertha armyworm that emerged during the scotophase and reared under a 16 h L : 8 h D photoperiod and at 20°C. N=24 females/scotophase.

Age (scotophase)	% calling	Mean onset calling time[a] ($\bar{x}$ ± SE (min))	Mean duration of calling period[a] ($\bar{x}$ ± SE (min))
1	0	-	-
2	100	415.0 ± 4.6 c	75.0 ± 5.2 a
3	100	340.6 ± 4.8 b	161.9 ± 4.7 b
4	100	306.3 ± 5.9 a	199.4 ± 5.5 c
5	100	282.5 ± 4.4 a	226.3 ± 4.4 c

[a]In each column, the means followed by the same letter are not significantly different (Duncan's new multiple range test, P>0.05).

those that emerged during the photophase. There were no significant differences among the mean onset calling times during the seventh to twelfth scotophases.

The females that emerged during the scotophase called significantly earlier in the second ($t=6.97$, $d.f.=30$, $P < 0.05$) and third ($t=5.69$, $d.f.=46$, $P < 0.05$) scotophases than those that emerged during the photophase (Tables 1 and 2). Calling occurred on average about 44 and 38 min earlier in the former groups than in the latter groups during the second and third scotophases, respectively. The mean onset calling times were not significantly different during the fourth and fifth scotophases in the scotophase- and photophase-emerged females.

The onset of calling was more synchronized among the younger females than among the older females (Fig. 7). The time between when there were no females calling and when about half the females were calling increased from about 45 min in the third scotophase to 135 min in the twelfth scotophase.

The mean duration of the calling period differed significantly with age in the females that emerged during the photophase ($F=24.93$, $d.f.=[9,230]$, $P < 0.05$; Table 1) and the scotophase ($F=172.90$, $d.f.=[3,92]$, $P < 0.05$; Table 2), the older the females the longer the calling period. From the second to the fourth scotophase, the mean length of the calling period increased on average by about 153 min in the females that emerged during the photophase and by about 124 min in those that emerged during the scotophase. The latter females spent on average about 47 and 49 min more time calling during the second and third scotophases, respectively, than the former; the differences were

significant (second scotophase, $t=6.37$, d.f.=30, $P < 0.05$; third scotophase, $t=6.54$, d.f.=46, $P < 0.05$). The mean duration of the calling period was not significantly different during the fourth and fifth scotophases in the photophase- and scotophase-emerged females, and during the fifth and sixth and sixth and seventh scotophases in the photophase-emerged females. However, the length of the calling period increased significantly between the fourth and seventh scotophases (approximately 78 min) in those that emerged during the photophase.

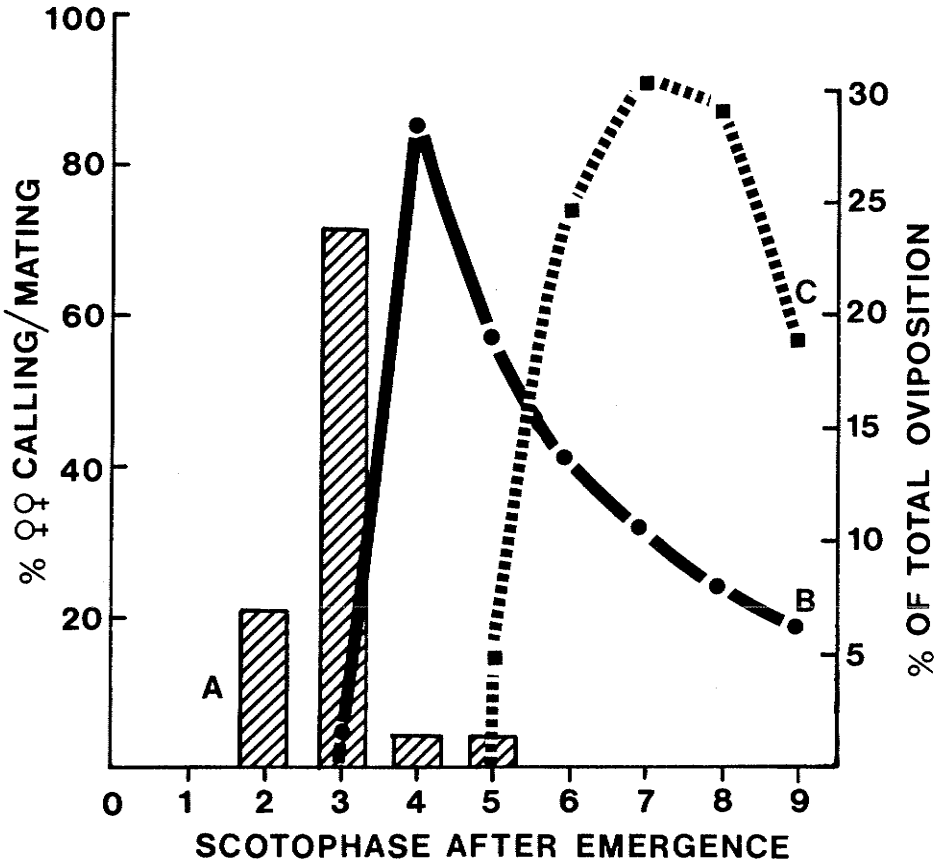
At all scotophases from the second to twelfth scotophases, the period when the highest percentage of females were calling occurred during the seventh and eighth h of the scotophase (Fig. 7). After the second scotophase, all females called during each scotophase. Calling was terminated within an hour after the lights were switched on in all instances.

4.3 The Effect of Mating on Calling Behaviour of the Females

Virgin females copulated for the first time during the second to fifth scotophases (Fig. 8). Most (71%) copulated during the third scotophase and the remainder during the second (21%) and fourth and fifth (8%) scotophases.

Copulation was initiated during the last three h of each scotophase and the first 30 min of the following photophase. The mean onset time for initiation of copulation was 7.3 ± 0.2 h of the scotophase. The mean duration of copulation was 17.0 ± 0.2 h, and the pairs separated soon after the lights were switched off in the scotophase following the initiation of copulation. About 61% of the pairs separated within the

Fig. 8. Percent females mating for the first time, percentage of the total oviposition (% of total number of eggs) of the mated females occurring during each scotophase, and percent mated females of the bertha armyworm calling during the first to ninth scotophases under a 16 h L : 8 h D photoperiod and at 20°C. A, bars represent percent females mating. B, curve represents percentage of total oviposition. C, curve represents percent mated females calling. N=24 females for mating and 23 females for oviposition and calling.


100
80
60
40
20
% ♀♀ CALLING/MATING
30
25
20
15
10
5
% OF TOTAL OVIPOSITION
A
B
C
0
1
2
3
4
5
6
7
8
9
SCOTOPHASE AFTER EMERGENCE

first 15 min of lights off, and the mean time for the separation was 17.9 ± 10.2 min.

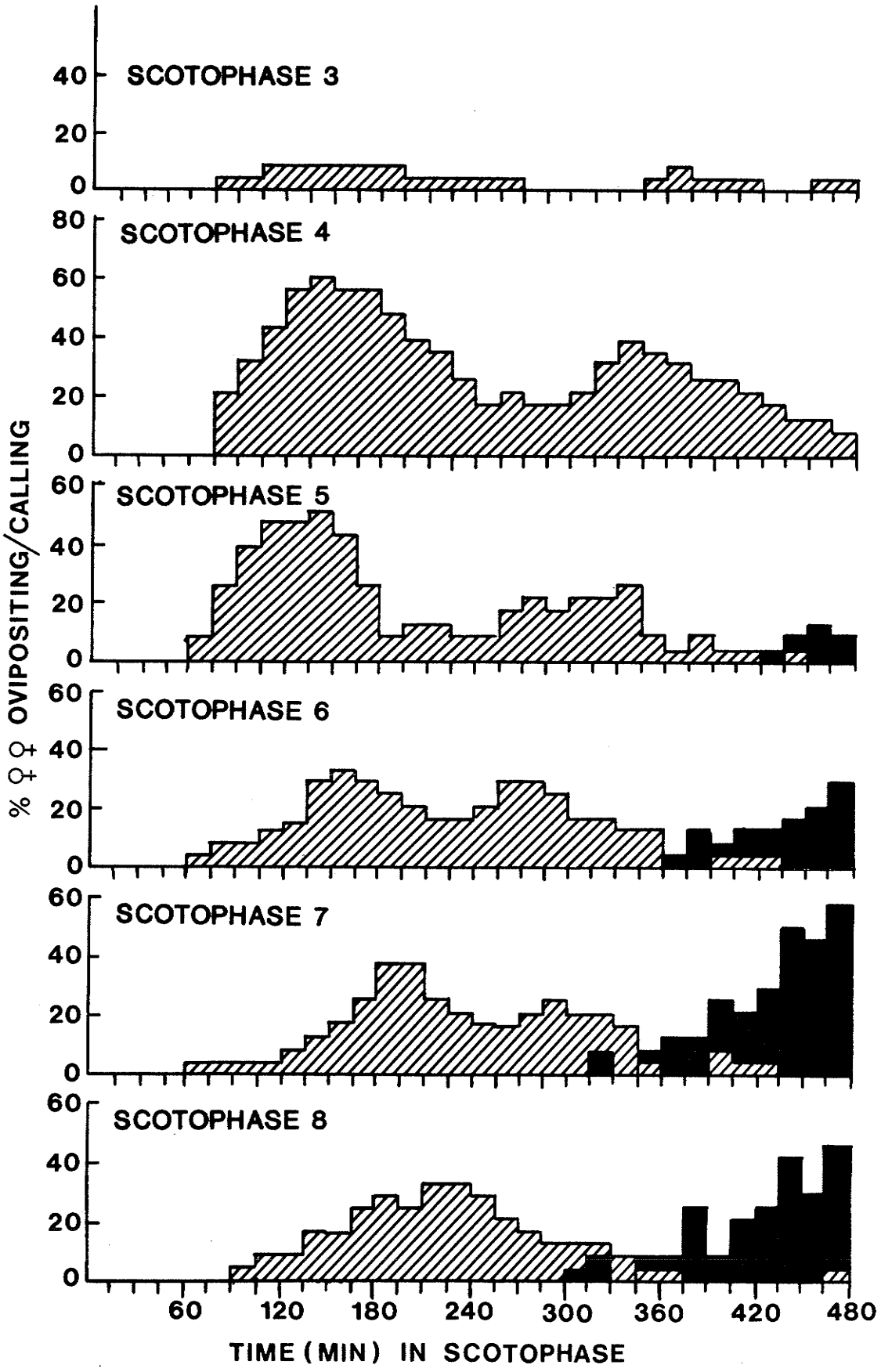
The mated females resumed calling one to four scotophases after the termination of the first mating. The mean duration of the non-calling period was 2.2 ± 0.2 days. The first resumption of calling occurred during the fifth scotophase after emergence (Fig 8). By the seventh scotophase, most mated females (91%) had called again.

All the females initiated oviposition for the first time during the same scotophase as the first mating was terminated, and most females oviposited every scotophase until death. The mean duration of the oviposition period was 9.5 ± 0.3 days (range, 7-13 days). The first eggs were laid during the third scotophase after emergence, and more eggs were laid during the fourth scotophase than any other scotophase (Figs. 8 and 9). Almost 75% of the eggs were laid by the end of the fifth day of the oviposition period (seventh scotophase). In each scotophase, oviposition was initiated during the second h and was almost completed by the end of the sixth h (Fig. 9).

The average number of eggs laid per female was 2143 ± 98.1. The average number of unfertilized eggs laid per female was 31 ± 4.5. One female laid only unfertilized eggs (800 eggs); it was presumed that she did not mate successfully and, consequently, the data from this female were not used in the calculation of the above means. Most of the unfertilized eggs (80%) were laid after the fifth day of the oviposition period.

Once calling was resumed after the first mating, the females oviposited and called during each scotophase (Fig. 9). In each female,

Fig. 9. Percent single-mated females of the bertha armyworm ovipositing and calling during the third to eighth scotophases under a 16 h L : 8 h D photoperiod and at 20°C. Cross-hatched bars denote oviposition. Black bars denote calling. N=23 females.


SCOTOPHASE 3
SCOTOPHASE 4
SCOTOPHASE 5
SCOTOPHASE 6
SCOTOPHASE 7
SCOTOPHASE 8
% ♀♀ OVIPOSITING/CALLING
TIME (MIN) IN SCOTOPHASE
0
20
40
60
80
120
180
240
300
360
420
480

calling was initiated after oviposition was completed. It was initiated during the last three h of the scotophase, and the length of the calling period increased from about one h during the fifth scotophase to three h during the eighth scotophase. There was a corresponding decrease in the length of the oviposition period. As in virgin females (Fig. 7), the number calling increased with time during each scotophase, and was highest during the last hour of the scotophase. After the eighth scotophase, the percentage of females calling and ovipositing decreased during each scotophase until death. The average length of the adult stage was 12.4 ± 0.3 days (range, 9-16 days).

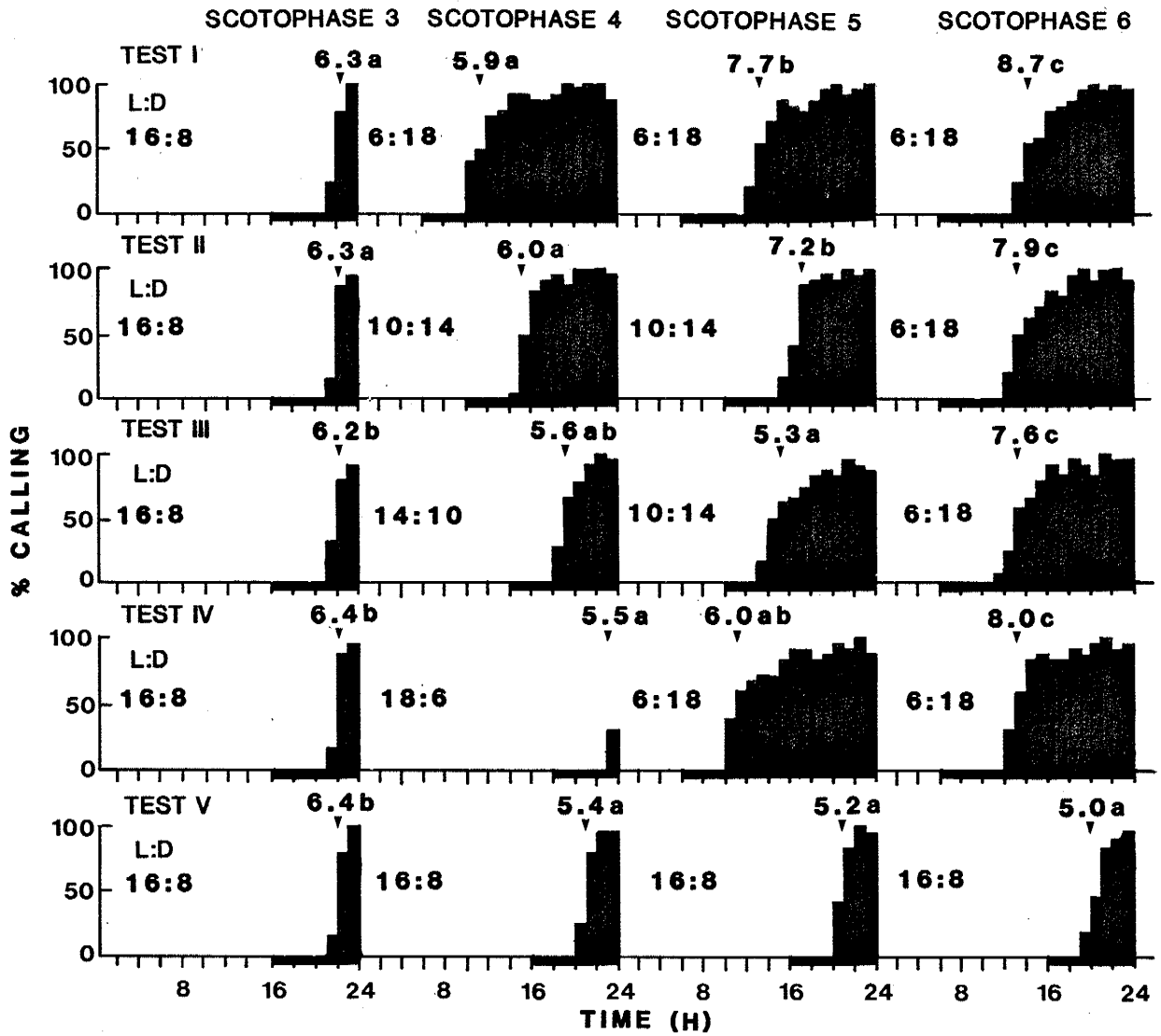
4.4 Photoperiodic Cue(s) of Calling

The mean times for the onset of calling after the lights were switched off were similar during the third and fourth scotophases of each of the five tests (Fig. 10). As in the control treatment (Test V), the mean times of the fourth scotophase were shorter than those of the third scotophase in the other four tests, but in only one test (Test IV) were the differences significant. Turning the lights off 10, six, and two h earlier (Tests I - III) during the fourth photophase than during the third photophase resulted in the females initiating calling about 10.4, 6.3, and 2.6 h earlier, respectively, during the fourth day. Similarly, turning the lights off two h later (Test IV) resulted in the females calling about 1.1 h later, although only 29% of the females called. Keeping the photoperiod the same (Test V) resulted in the females calling about one h earlier.

Among the tests, the mean times for the onset of calling were not significantly different during the third ($F=0.7$, d.f.=[4,116], $P > 0.05$) and fourth ($H'=349$, d.f.=4, $P > 0.05$) scotophase. There was significant heterogeneity of variance in the data for the onset of calling time during the fourth scotophase (Bartlett's test). This was due to some females initiating calling later in the scotophase than others in Tests I-III. In these data, no transformations were feasible. Therefore, the data for the fourth scotophase were subjected to the Kruskal-Wallis test.

During the fifth and sixth scotophases, the mean times for the onset of calling in the tests where the preceding scotophase was 14-18 h long were significantly longer than those in the tests where the preceding scotophase was six to 10 h long (Fig. 10). For the 14-h

Fig. 10. Percent virgin females of the bertha armyworm calling during the third to sixth scotophases at 20°C and different photoperiods. The numbers and arrows give mean times (h) for onset of calling after lights off. In each test, numbers followed by the same letter are not significantly different (Duncan's new multiple range tests for Tests I-V and t test for the third and fourth scotophases in Test IV, $P > 0.05$). The bars along the abscissa denote the scotophases. N=24 females/test.


SCOTOPHASE 3
SCOTOPHASE 4
SCOTOPHASE 5
SCOTOPHASE 6
TEST I
6.3a
5.9a
7.7b
8.7c
L:D
16:8
6:18
6:18
6:18
TEST II
6.3a
6.0a
7.2b
7.9c
L:D
16:8
10:14
10:14
6:18
TEST III
6.2b
5.6ab
5.3a
7.6c
L:D
16:8
14:10
10:14
6:18
TEST IV
6.4b
5.5a
6.0ab
8.0c
L:D
16:8
18:6
6:18
6:18
TEST V
6.4b
5.4a
5.2a
5.0a
L:D
16:8
16:8
16:8
16:8
100
50
0
% CALLING
8
16
24
TIME (H)

scotophase, calling was delayed about 1-2 h. For the 18-h scotophase, the delay was about 1.5-3 h. When the preceding scotophase was six to 10 h long, the mean times for the onset of calling were not significantly different from those of the third and fourth scotophases.

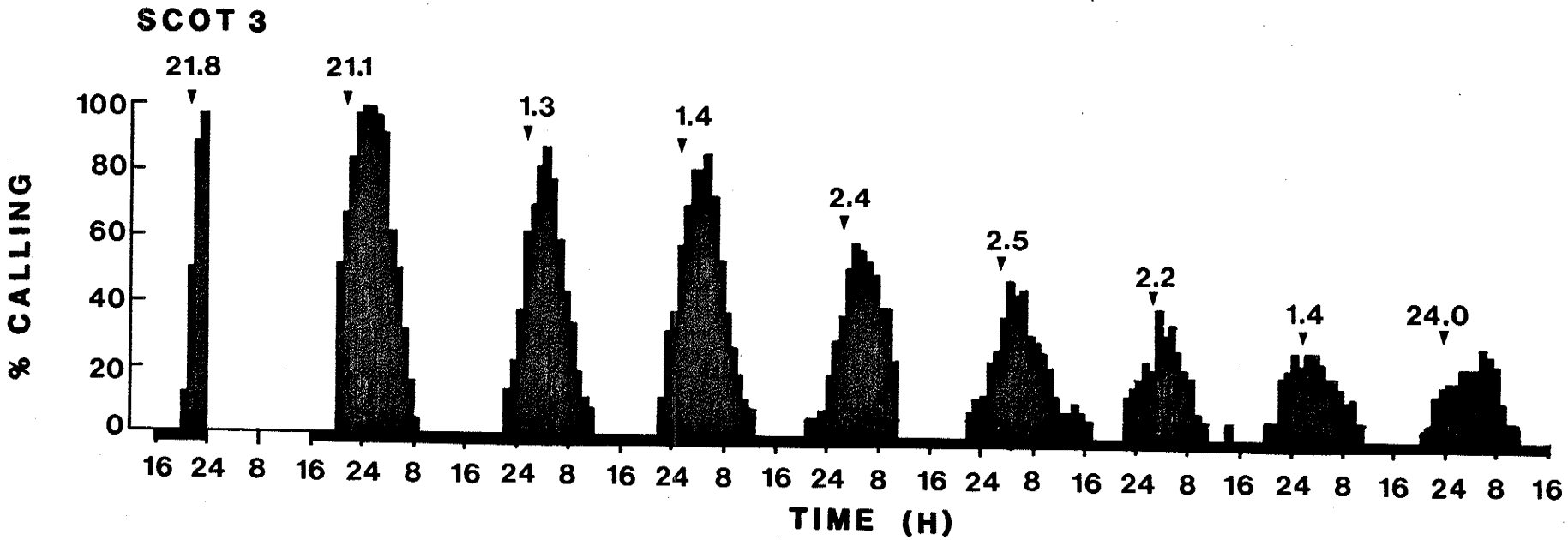
4.5 Endogenous Circadian Rhythm of Calling

During the 192-h period of continuous darkness, calling occurred at approximately 24-h intervals, but there was some degree of desynchronization (Fig. 11). During the first four cycles in the dark period, there was a shift in the time when calling occurred; the mean time for the onset of calling was about 5.3 h later during the fourth cycle as compared to the first cycle. The mean times for the onset of calling during the fourth to sixth cycles were similar. The onset of calling occurred one to 2.5 h earlier in the seventh and eighth cycles than in the preceding three cycles.

The length of the calling period during each of the cycles of the dark period was longer than that for the third scotophase (Fig. 11). The calling period was about three h long during the third scotophase and 13-18 h long during the cycles of the dark period. The calling period was terminated abruptly at the end of the third scotophase, but was terminated gradually in the cycles of the dark period.

The proportion of females calling decreased gradually during each of the cycles of the dark period (Fig. 11). It decreased from 100% calling in the first cycle to only 35% in the last cycle.

Fig. 11. Percent virgin females of the bertha armyworm calling at 20°C and during the third scotophase at a photoperiod of 16 h L : 8 h D, or during a 192-h period of continuous darkness beginning at the onset of the fourth scotophase. The numbers and arrows give the mean times (L) the 24-h cycle for onset of calling. The bars along the abscissa denote periods of darkness. N=37 females. Scot., scotophase.


SCOT 3
21.8
21.1
1.3
1.4
2.4
2.5
2.2
1.4
24.0
100
80
60
40
20
0
% CALLING
16 24 8 16 24 8 16 24 8 16 24 8 16 24 8 16 24 8 16 24 8 16 24 8 16 24 8 16
TIME (H)

4.6 The Effects of Photoperiod on Calling Behaviour of Virgin Females

The mean times for the onset of calling after lights off were significantly different in the three photoperiodic regimes tested ($F=139.4$, d.f.=[2,207], $P < 0.05$), the longer the length of the scotophase the later the onset of calling (Fig. 12, Table 3). On average, the onset of calling was about 1.9 h later under the 12-h scotophase than under the 8-h scotophase, and was about 4.7 h later under the 16-h scotophase than under the 8-h scotophase. The two-way analysis of variance indicated that the interaction between photoperiod and age was marginally significant ($F=2.69$, d.f.=[4,207], $P=0.04$) for onset of calling times. This likely was due to the advances in the onset calling times being larger during the second to third scotophases than during the third to fourth scotophases.

The mean duration of the calling period differed significantly with the length of the scotophase ($F=164.0$, d.f.=[2,207], $P < 0.05$), the longer the length of the scotophase the longer the time females spent calling (Table 3). On average, the calling period was about 2 h longer under the 12-h scotophase than under the 8-h scotophase and was about 3.2 h longer under the 16-h scotophase than under the 8-h scotophase. The two-way analysis of variance indicated that the interaction between photoperiod and age was marginally significant ($F=2.83$, d.f.=[4,207], $P=0.03$) for duration of calling periods. This likely was due to the increases in the duration of calling periods being larger during the second to third scotophases than during the third to fourth scotophases.

The mean onset calling times differed significantly with age ($F=505.6$, d.f.=[2,207], $P < 0.05$), the older the females the earlier

Fig. 12. Percent virgin females of the bertha armyworm calling during the second to fourth scotophases at 20°C and three different photoperiods from emergence. N=24 females/photoperiod. The numbers and arrows give mean times (h) for onset of calling after lights off. The black bars along the abscissa denote the scotophases.

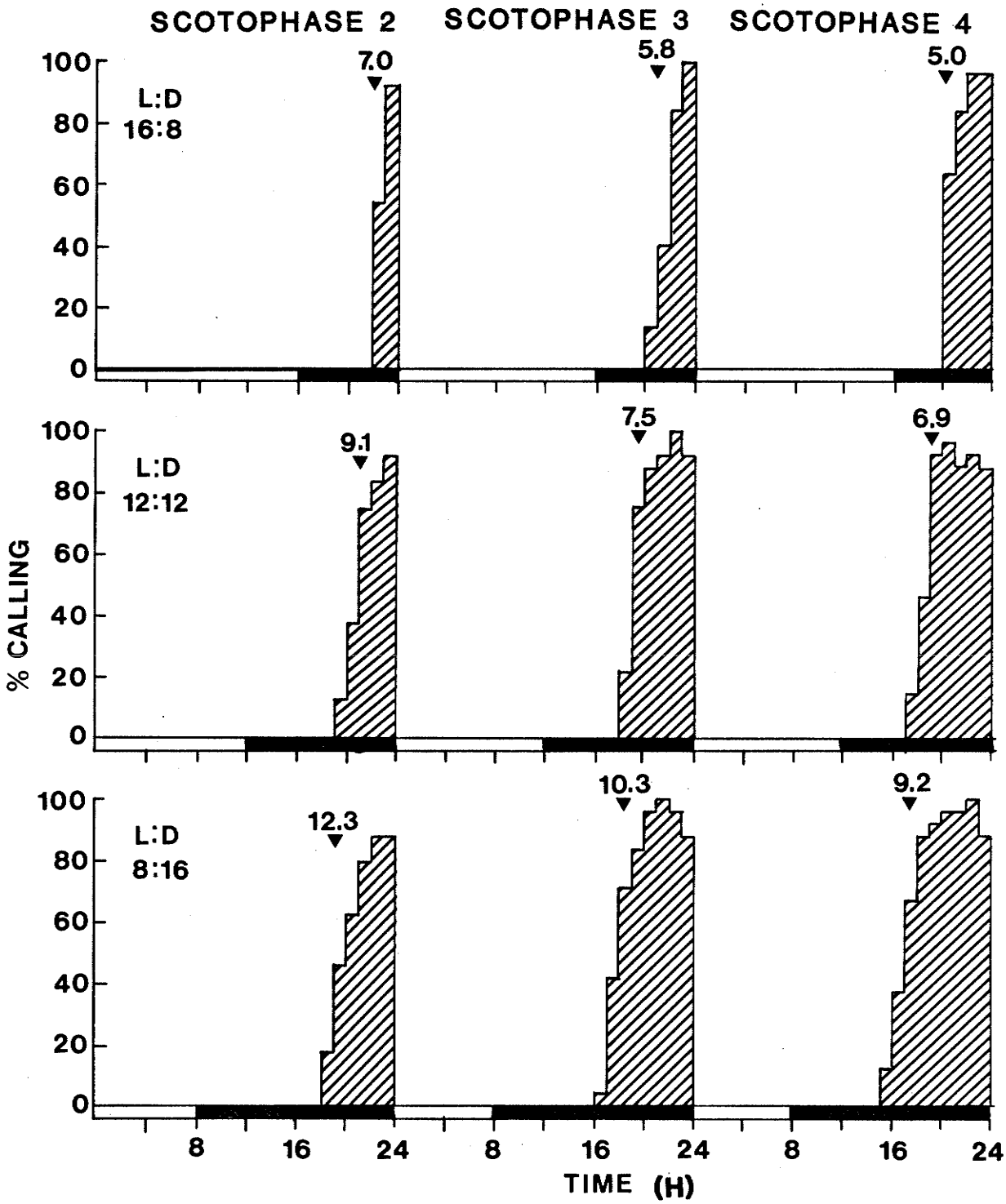

SCOTOPHASE 2
SCOTOPHASE 3
SCOTOPHASE 4
L:D
16:8
7.0
5.8
5.0
L:D
12:12
9.1
7.5
6.9
L:D
8:16
12.3
10.3
9.2
% CALLING
100
80
60
40
20
0
8
16
24
TIME (H)

Table 3. Effect of photoperiod on percent calling, mean onset calling time, and mean duration of the calling period of virgin females of the bertha armyworm during the second to fourth scotophases and at 20°C. N=24 females/photoperiod.

Age		Mean Onset Calling Time ($\bar{x}$ ± SE (hours))				Mean Duration of Calling Period ($\bar{x}$ ± SE (hours))			
Scoto-phase	% Calling	L:D = 16:8	L:D = 12:12	L:D = 8:16	Row Means	L:D = 16:8	L:D = 12:12	L:D = 8:16	Row Means
2	100	7.0 ± 0.1	9.1 ± 0.2	12.3 ± 0.3	9.5	1.5 ± 0.1	3.3 ± 0.2	4.1 ± 0.2	2.7
3	100	5.8 ± 0.1	7.5 ± 0.2	10.3 ± 0.2	7.9	2.7 ± 0.1	4.9 ± 0.1	6.1 ± 0.2	4.6
4	100	5.0 ± 0.2	6.9 ± 0.2	9.2 ± 0.2	7.0	3.5 ± 0.2	5.5 ± 0.2	7.2 ± 0.2	5.4
Column Means		5.9	7.8	10.6		2.6	4.6	5.8	

calling occurred in the scotophase (Table 3). On average, the onset of calling was advanced by about 1.6 h during the third scotophase than the second and by about 2.5 h during the fourth scotophase than the second scotophase.

The mean duration of the calling period differed significantly with age ($F=291.2$, d.f.=[2,207], $P < 0.05$); the older the females the longer the calling period (Table 3). On average, the calling period was about 1.9 h longer during the third scotophase than the second scotophase and 2.7 h longer during the fourth scotophase than the second scotophase.

4.7 The Effects of Temperature on Calling Behaviour of Virgin Females

Experiment I

The age at which the females called for the first time after emergence was significantly different at the six temperatures ($F=82.7$, d.f.=[5,124], $P < 0.05$) (Fig. 13, Table 4). The age at first calling was similar at 20° - 30°C, but the females were significantly older at the time of first calling at 10°, 15°, and 35°C. Most females initiated calling during the fifth and sixth scotophases at 10°C, third and fourth scotophases at 15°C, second and third scotophases at 20° - 30°C, and fourth and fifth scotophases at 35°C.

The results of the two-way analysis of variance showed that both temperature and age (scotophase of calling) significantly affected the time of initiation of calling and the duration of the calling period during each scotophase of the first three scotophases in which calling occurred ($P < 0.05$, Tables 5 and 6). The interactions between temperature and age also were significant for the onset calling times and the duration of the calling periods ($P < 0.05$). The effects of age on the onset calling times and the duration of the calling periods were greater than the effects of temperature, because the F values for age were 11-13 times greater than those for temperature. Since the interactions between temperature and age were significant and since the values of N were not the same in all of the treatments (Table 5), the results of Duncan's multiple range tests given below must be considered as tentative.

The mean onset calling times differed significantly among the six temperatures during the first three scotophases in which calling

Fig. 13. Percent virgin females of the bertha armyworm calling for the first time after emergence at six constant temperatures (10°-35°C) and under a 16 h L : 8 h D photoperiod. N=24 females/temperature.

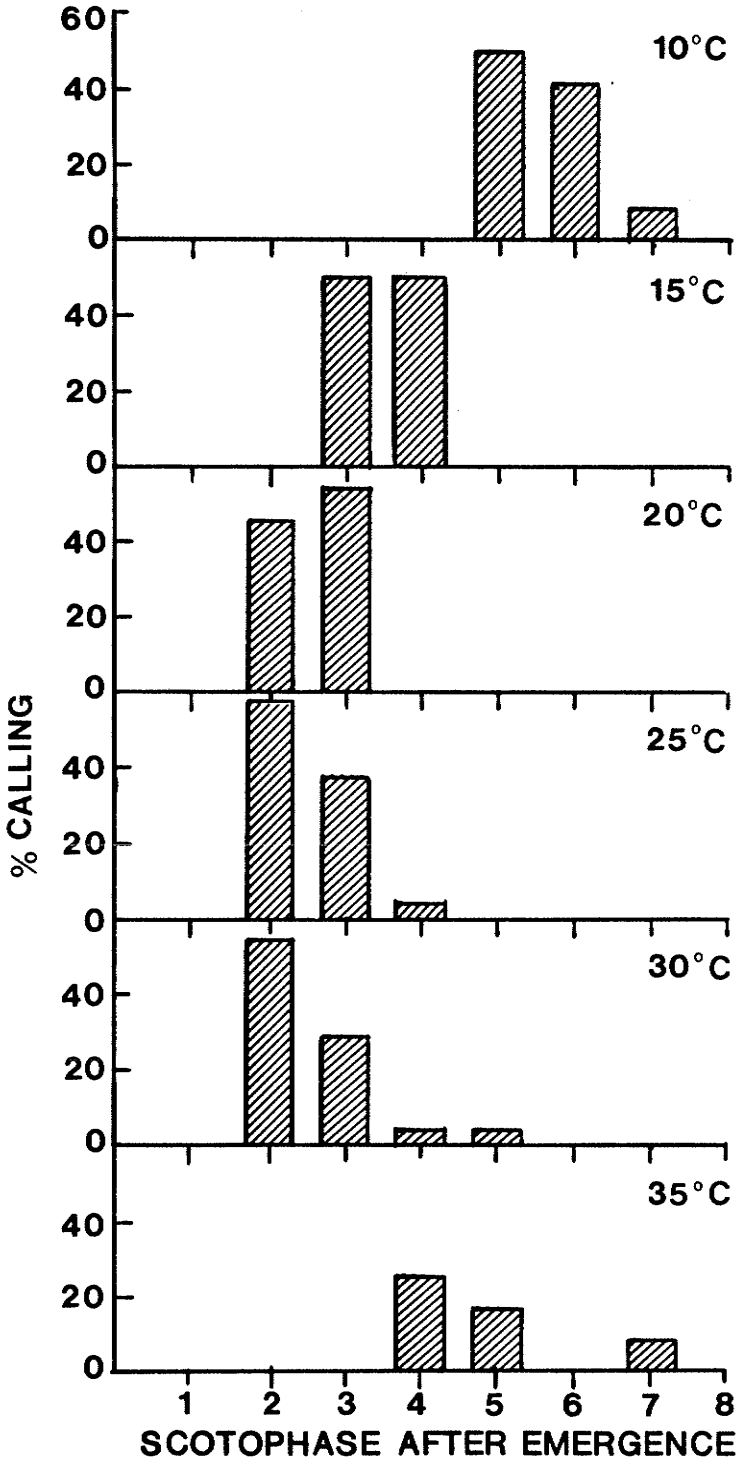

10°C
15°C
20°C
25°C
30°C
35°C
60
40
20
0
% CALLING
1
2
3
4
5
6
7
8
SCOTOPHASE AFTER EMERGENCE

Table 4. Effect of temperature on the percent females calling during their life time, the mean age at first calling, and mean longevity of virgin females of the bertha armyworm at six constant temperatures (10°-35°C) and a 16 h L : 8 h D photoperiod. N=24 females/temperature.

Temperature (°C)	% calling during life time[a]	Mean age at first calling[b] ($\bar{x}$ ± SE (hours))	Mean longevity[b] ($\bar{x}$ ± SE (days))
10	100 b	125.0 ± 3.2 d	30.0 ± 0.7 f
15	100 b	75.0 ± 2.5 b	26.5 ± 1.2 e
20	100 b	52.3 ± 2.4 a	20.8 ± 1.0 d
25	100 b	49.9 ± 2.8 a	11.2 ± 0.7 c
30	92 b	49.7 ± 4.6 a	8.3 ± 0.4 b
35	50 a	107.2 ± 7.7 c	5.4 ± 0.4 a

[a]The percentages followed by the same letter are not significantly different (chi-squared tests, 2x2 contingency table, $P > 0.05$).

[b]The means followed by the same letter are not significantly different (Duncan's new multiple range test, $P > 0.05$).

Table 5. Effect of temperature and age (scotophase of calling) on the mean onset calling time and the mean duration of calling period of virgin females of the bertha armyworm during the first three scotophases in which calling occurred at a 16 h L : 8 h D photoperiod. N=24 females/temperature.

Temp. (°C)	Mean Onset Calling Time[a] ($\bar{x}$ ± SE (min)) Age (Scotophase)						Mean Duration of Calling Period[a] ($\bar{x}$ ± SE (min)) Age (Scotophase)					
	2	3	4	5	6	7	2	3	4	5	6	7
10				425.0± 6.6 (12)	395.0± 8.6 (22)	385.4± 7.2 (24)				67.5± 6.3 (12)	100.0± 9.5 (22)	115.0± 7.1 (24)
15		430.8± 7.5 (12)	391.3± 6.0 (24)	360.1± 6.3 (24)				59.2± 7.7 (12)	103.8± 6.1 (24)	140.9± 6.3 (24)		
20	459.5± 3.7 (11)	383.8± 8.0 (24)	323.8± 8.5 (24)				29.1± 3.4 (11)	109.4± 7.2 (24)	175.4± 8.5 (24)			
25	427.1± 8.0 (14)	391.8± 9.2 (22)	362.7±11.9 (22)				60.7± 7.9 (14)	102.5± 8.3 (22)	131.8±10.5 (22)			
30	428.8± 6.6 (13)	417.6± 7.9 (19)	401.9±12.1 (13)				59.2± 6.1 (13)	74.2± 8.1 (19)	93.1±10.3 (13)			
35			426.7±11.7 (6)	405.0±13.4 (8)	407.0±18.5 (5)				58.3±11.7 (6)	72.9±11.8 (8)	76.0±20.1 (5)	

[a]The number of females that called are given in parentheses.

Table 6. F values from two-way analysis of variance to determine the effects of temperature and age (scotophase of calling) on the onset calling time and the duration of the calling period of virgin females of the bertha armyworm during the first three scotophases in which calling occurred and at six constant temperatures (10°-35°C) and a 16 h L : 8 h D photoperiod. N=24 females/temperature.

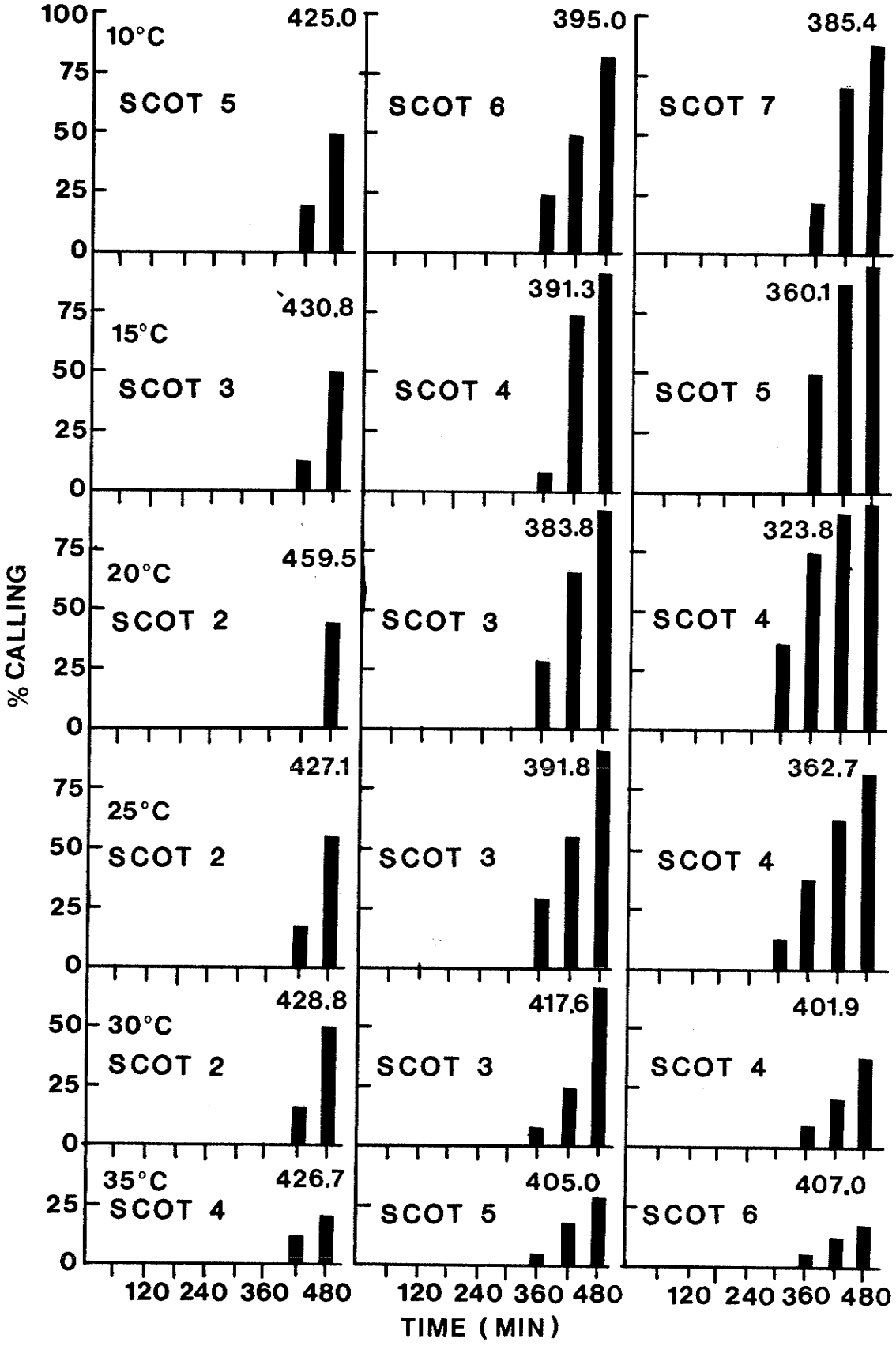
Source	df	Onset calling time	P	Duration of calling period	P
Temperature	5	3.53	<0.005	5.64	<0.0001
Age	2	44.97	<0.0001	62.37	<0.0001
Temperature x Age	10	4.43	<0.0001	5.27	<0.0001
Error	281				

occurred ($F=12.57$, d.f.=[17,281], $P < 0.05$, Table 5). The initiation of calling occurred significantly earlier at 15°-25°C than at 30° and 35°C. The differences at 10°-25°C were relatively small, and the only significant difference was between 10° and 20°C. However, the differences between 10° and 20°C were not consistent; the initiation of calling occurred earlier during the first scotophase at 10°C than at 20°C, but occurred later during the second and third scotophases at 10°C than at 20°C. There were no significant differences among 10°, 30° and 35°C.

The mean duration of the calling period differed significantly among the six temperatures during the first three scotophases in which calling occurred ($F=17.30$, d.f.=[17,281], $P < 0.05$, Table 5). The calling periods were significantly longer at 10°-25°C than at 30°C and 35°C. At 10°-25°C, there were no significant differences among 10°, 15°, and 25°C, but 20° C was significantly different from 10° and 25°C. However, the differences among 10°, 20°, and 25°C were not consistent; the calling period was shorter during the first scotophase at 20°C than at 10° and 25°C, but was longer during the second and third scotophases at 20°C than at 10° and 25°C. There were not significant differences between 30° and 35°C.

The mean onset calling times differed significantly with age (scotophase of calling) during the first three scotophases in which calling occurred ($F=44.97$, d.f.=[2,281], $P < 0.05$), the older the females the earlier calling occurred in the scotophase (Fig. 14, Table 5). The initiation of calling occurred significantly earlier during the second scotophase than the first scotophase and during the third scotophase than the second scotophase.

Fig. 14. Percent virgin females of the bertha armyworm calling each hour during the first three scotophases in which calling occurred at six constant temperatures (10°-35°C) and a 16 h L : 8 h D photoperiod. The numbers give mean times (min) for onset of calling after lights off. N=24 females/temperature. Scot., scotophase.


% CALLING
TIME (MIN)
10°C
SCOT 5
425.0
SCOT 6
395.0
SCOT 7
385.4
15°C
SCOT 3
430.8
SCOT 4
391.3
SCOT 5
360.1
20°C
SCOT 2
459.5
SCOT 3
383.8
SCOT 4
323.8
25°C
SCOT 2
427.1
SCOT 3
391.8
SCOT 4
362.7
30°C
SCOT 2
428.8
SCOT 3
417.6
SCOT 4
401.9
35°C
SCOT 4
426.7
SCOT 5
405.0
SCOT 6
407.0
0
25
50
75
100
120 240 360 480

The mean duration of the calling period was significantly different with age (scotophase of calling) during the first three scotophases in which calling occurred ($F=62.37$, d.f.=[2,281], $P < 0.05$), the older the females the longer the calling period (Table 5). The calling period was significantly longer during the second scotophase than the first scotophase and during the third scotophase than the second scotophase.

The differences among the onset calling times and the duration of the calling periods during the last two scotophases of calling at 10°C and all three scotophases at 30° and 35°C were relatively small as compared with the differences during the first two scotophases at 10°C and at all three scotophases at 15°-25°C (Table 5). This suggests that the effects of age (scotophase of calling) on the initiation and duration of calling were not as great during the former times and temperatures than during the latter times and temperatures and were inhibited partly at 10°, 30°, and 35°C. This may account for the significant interactions observed between temperature and age in the results of the two-way analysis of variance (Table 6).

The percentage of females that called during their lifetime was 100% at all temperatures, except 30° and 35°C (Table 4). At 30° and 35°C, 8 and 50% females did not call. The reduction in calling at 35°C was significant.

Once calling was initiated, 100% of the females called daily until death at 10°-20°C, but not at 25°-35°C. At 25°-35°C, there was a gradual decline in the proportion calling after the second scotophase of calling. At 25°C, the decline was from about 92% during the third scotophase to 71% during the eighth scotophase when the first female

died. At 30°C, the decline was from about 79% during the third scotophase to 33% during the seventh scotophase when the first female died. At 35°C, the decline was from about 33% during the fifth scotophase to about 4% during the seventh scotophase when only about 21% of the females were still alive.

The mean longevity of the females decreased significantly ($F=255.65$, d.f.=[5,138], $P < 0.05$) as the temperature increased from 10°-35°C (Table 4). The females survived on average 30.0 ± 0.7 days at 10°C and 5.4 ± 0.4 days at 35°C.

Experiment II

The mean onset calling times differed significantly with temperature during the third scotophase ($F=62.30$, d.f.=[6,136], $P < 0.05$), the lower the temperature the earlier calling occurred in the scotophase (Fig. 15, Table 7). Calling was initiated on average about 255 min earlier in the scotophase at 5°C than at 35°C. Calling was initiated on average about 177-28 min earlier at 5°-15°C than at 20°C and about 16-78 min later at 25°-35°C than at 20°C.

The mean duration of the calling period differed significantly with temperature during the third scotophase ($F=77.59$, d.f.=[6,136], $P < 0.05$), the lower the temperature the longer the calling period (Table 7). The calling period on average was about 259 min longer at 5°C than at 35°C. The calling period on average was about 173-25 min longer at 5°-15°C than at 20°C and about 28-86 min shorter at 25°-35°C than at 20°C.

Fig. 15. Percent virgin females of the bertha armyworm calling hourly during the third scotophase at seven constant temperatures (5°-35°C) and a 16 h L : 8 h D photoperiod. The numbers and arrows give mean times (min) for onset of calling after lights off. N=24 females/temperature.

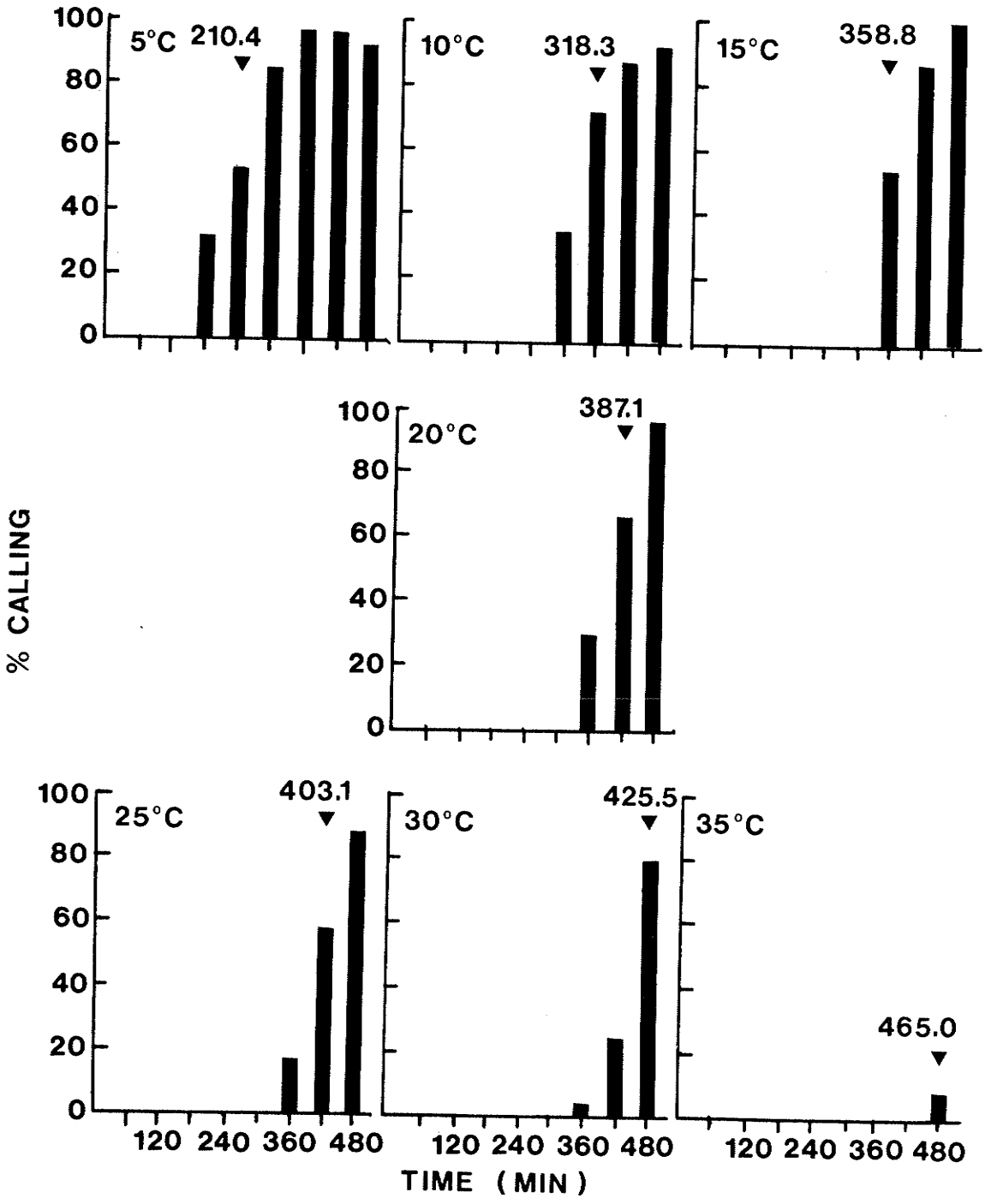

% CALLING
100
80
60
40
20
0
5°C
210.4
10°C
318.3
15°C
358.8
20°C
387.1
25°C
403.1
30°C
425.5
35°C
465.0
120 240 360 480
TIME (MIN)

Table 7. Effect of temperature on the percent calling, the mean onset calling time, and the mean duration of the calling period of virgin females of the bertha armyworm during the third scotophase at six constant temperatures and a 16 h L : 8 h D photoperiod. N=24 females/temperature.

Temperature °C	% calling[a]	Mean onset calling time[b] ($\bar{x}$ ± SE (min))	Mean duration of calling period[b] ($\bar{x}$ ± SE (min))
5	100 c	210.4 ± 11.7 a	285.8 ± 11.5 f
10	100 c	318.3 ± 11.3 b	174.2 ± 10.3 e
15	100 c	358.8 ± 7.2 c	137.1 ± 6.2 d
20	100 c	387.1 ± 8.3 d	112.5 ± 6.9 c
25	96 c	403.1 ± 8.7 de	84.9 ± 7.8 b
30	87 b	425.5 ± 7.3 ef	65.7 ± 6.6 ab
35	13 a	465.0 ± 5.8 f	26.7 ± 6.7 a

[a]The percentages followed by the same letter are not significantly different (chi-squared tests, 2x2 contingency table, $P > 0.05$).

[b]The means followed by the same letter are not significantly different (Duncan's new multiple range test, $P > 0.05$).

The percentage of females that called was 100%, except at 25°-35°C (Table 7). At 25°-35°C, 4 - 88% of the females did not call. At 35°C, the females did not call until near the end of the last hour of the scotophase (Fig. 15).

4.8 The Relationship Between Ovarian Maturation and Calling of Virgin Females

Chorionated eggs were produced at all temperatures from 10°-35°C after emergence from the pupal phase (Table 8). The oocytes were small in the youngest females examined (Fig. 16A). Oogenesis proceeded rapidly after emergence (Fig. 16B-C), and the first chorionated eggs were produced before the beginning of the second scotophase at 20° and 30°C (Fig. 16D, Table 8). At 10° and 35°C, the first chorionated eggs were produced by the end of the fourth and third scotophases, respectively, this being about 1.5 - 2.5 days later than at 20° and 30°C. Some of the chorionated eggs entered the lateral and common oviducts soon after they were produced (Fig. 16E-F, Table 8).

The time of first calling was synchronized closely with the appearance of the first chorionated eggs in the ovaries (Table 8). At 10° - 35°C in the females that emerged during the photophase, the first females that called did so during the scotophase in which many of the females contained chorionated eggs in the ovaries at the beginning of that scotophase. Also, the percentage of females that called during the first scotophase of calling was similar to the percentage of females with chorionated eggs in the ovaries at the beginning of that scotophase at each temperature from 10°-35°C. At 20°C in the females that emerged during the scotophase, many of the femles had chorionated eggs in the ovaries at the end of the first scotophase, but none called until the second scotophase. Therefore, it appears that if the females do not contain chorionated eggs in the ovaries at the beginning of a scotophase, the first calling will not be initiated during that particular scotophase, but will be delayed until the next scotophase.

Fig. 16. Ovarian maturation of virgin females of the bertha armyworm that emerged during the photophase and were reared at 20°C and a 16 h L: 8 h D photoperiod. Only the basal portions of the ovarioles are shown; the germaria and the remainder of the vitellaria have been removed.

(A) Ovary from a female killed at the beginning of the first scotophase after emergence, showing small oocytes in the ovariole (x18).

(B) Ovary from a female killed at the end of the first scotophase after emergence, showing an intermediate stage of egg development (x18).

(C) Ovary from a female killed at the beginning of the second scotophase after emergence, showing nearly fully-developed oocytes in the ovarioles (x18).

(D) Ovary from a female killed at the beginning of the second scotophase after emergence, showing chorionated eggs in the ovarioles (x18).

(E) Ovary from a female killed at the end of the second scotophase after emergence, showing chorionated eggs in the ovarioles and lateral oviducts (x18).

(F) Ovary from a female killed at the end of the second scotophase after emergence, showing chorionated eggs in the ovarioles and the lateral and common oviducts (x18).

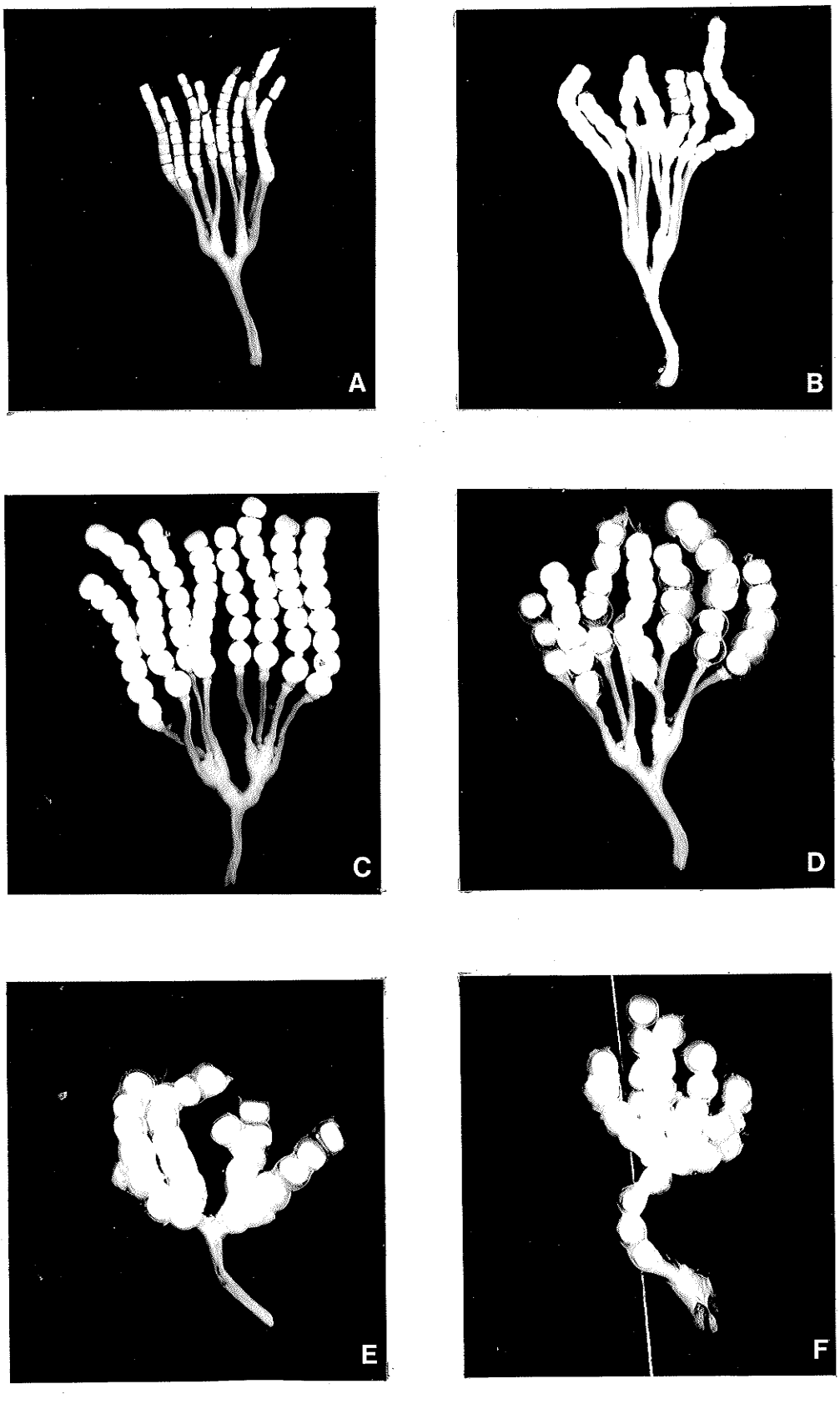

A
B
C
D
E
F

Table 8. Effect of temperature and age on percent virgin females with chorionated eggs in the ovarioles, lateral oviducts, and common oviducts and percent virgin females calling in the bertha armyworm that emerged during the photophase or scotophase and were reared at a 16 h L : 8 h D photoperiod. N=24 females/age-time group.

Age (scotophase)	Time[a]	% females with chorionated eggs[b]			% calling[b]
		ovarioles	lateral ducts	common ducts	
			10°C		
4	B	0	0	0	-
	E	8	0	0	0
5	B	54	38	33	-
	E	67	50	45	50
6	B	92	87	87	-
	E	100	100	100	92
			20°C		
1	B	0 (0)	0 (0)	0 (0)	-
	E	0 (50)	0 (0)	0 (0)	0 (0)
2	B	46 (100)	0 (46)	0 (38)	-
	E	92 (100)	17 (96)	9 (75)	42 (100)
3	B	100 (100)	100 (100)	100 (100)	-
	E	100 (100)	100 (100)	100 (100)	100 (100)
			30°C		
1	B	0	0	0	-
	E	0	0	0	0
2	B	63	0	0	-
	E	87	33	17	54
3	B	96	87	87	-
	E	96	96	96	96
			35°C		
3	B	0	0	0	-
	E	8	0	0	0
4	B	21	13	8	-
	E	25	17	17	21
5	B	38	38	33	-
	E	38	38	38	29

[a]The females were killed at the beginning of the scotophase (B) or at the end of the scotophase (E).

[b]The data for the scotophase-emerged females are given in the parentheses.

At 35°C, oogenesis and calling were adversely affected. In the group studied from emergence to death, only 42% of the females developed chorionated eggs and called. The females without chorionated eggs did not call.

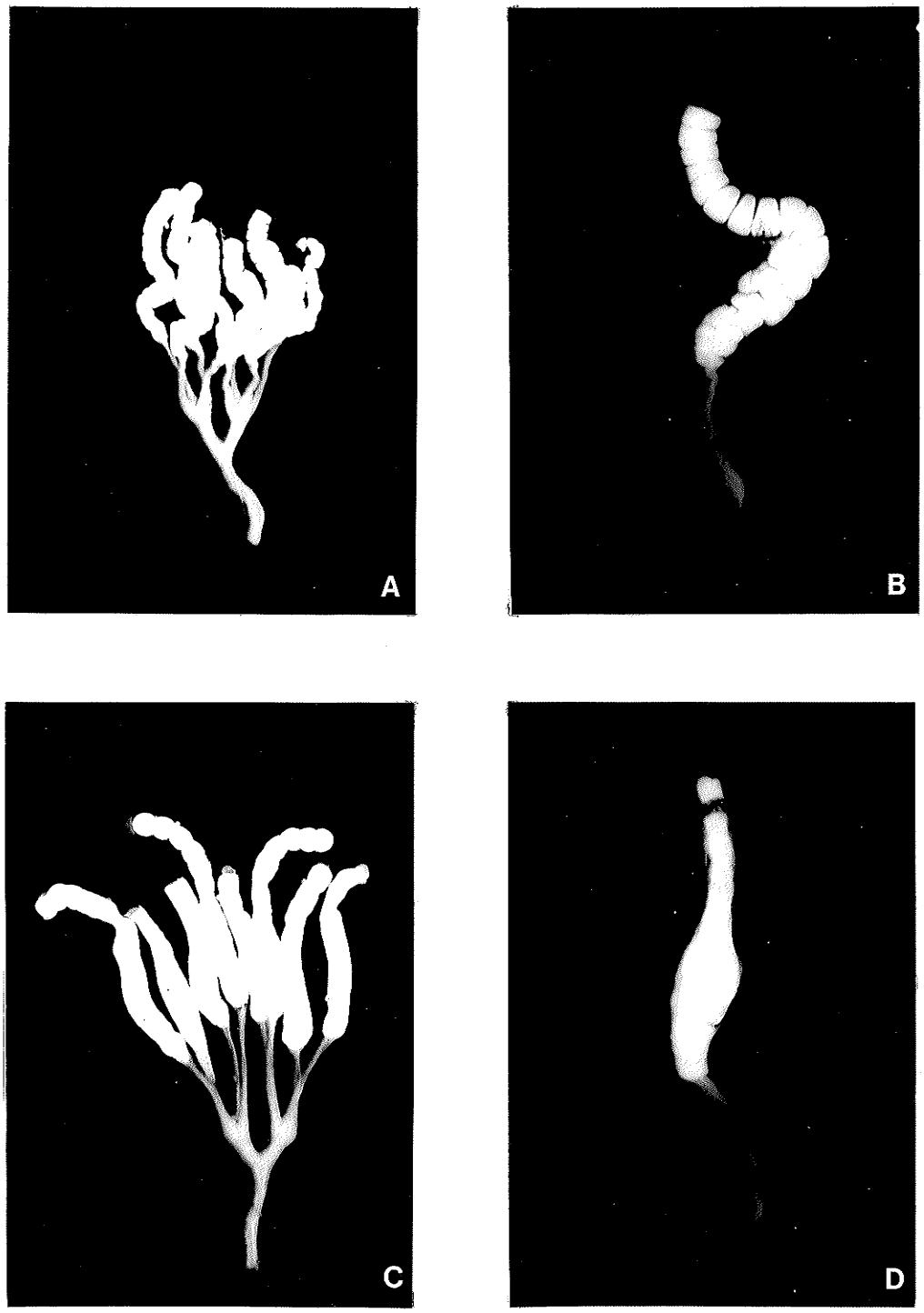
The oocytes of the females at 35°C showed signs of degeneration. In the females killed before and after the second and third scotophases, aggregations of misshapen oocytes were found in the basal region of the ovarioles of most ovaries (Fig. 17A-B). In those killed before and after the fourth and subsequent scotophases, the basal oocytes of most ovaries were fused together, making it difficult to identify individual oocytes (Fig. 17C-D). In females with chorionated eggs, the yolk of most eggs was shrunken and distorted. Also, the yolk of these eggs was a different colour; it was orange-brown as compared to cream colour in normal eggs. The number of chorionated eggs in the ovaries also was small.

The ovaries of the two females at 30°C without chorionated eggs during the third scotophase (Table 8) also had oocytes that were adversely affected, as at 35°C. Aggregations of the misshapen oocytes were found in the basal region of the ovarioles of these females. In the females with chorionated eggs, the yolk was shrunken, distorted, and orange-brown colour in most females (92%) after the beginning of the fourth scotophase. Although the chorionated eggs were not counted, there seemed to be fewer eggs at 30°C than at 10° and 20°C.

Fig. 17. Effect of high temperature (35°C) on ovarian maturation of virgin females of the bertha armyworm at a 16 h L : 8 h D photoperiod. Only the basal portions of the ovarioles are shown; the germaria and the remainder of the vitellaria have been removed.

(A-B) Ovary from a female killed at the end of the second scotophase after emergence, showing aggregations of degenerating oocytes in the ovarioles (A, an ovary (x18); B, an ovariole (x36)).

(C-D) Ovary from a female killed at the end of the fourth scotophase after emerence, showing fused oocytes in the ovarioles (C, an ovary (x18); D, an ovariole (x36)).


A
B
C
D

5.1 Characteristics of Calling Behaviour

The females showed a definite posture during calling. The extension of the ovipositor to expose the cuticle overlying the pheromone glands is the main characteristic of the calling posture, as in other Noctuidae (Kaae and Shorey 1972; Teal *et al.* 1978; Turgeon and McNeil 1982; West *et al.* 1984). The elevation of the wings above the abdomen in the form of a "V" also has been seen in some Noctuidae (Teal *et al.* 1978; Turgeon and McNeil 1982; West *et al.* 1984). However, such characteristics as lateral positioning of the antennae, the curving of the abdomen, and the flexion downward of the ovipositor at an angle of about 45° have not been observed in Noctuidae. The females of some Tortricidae, however, curve their extended ovipositor downward at an angle of about 45° (Sanders 1969; Lawrence and Bartell 1972).

Calling has a discrete diel periodicity. The period was restricted to the last half of the scotophase and the beginning of the following photophase. Chisholm *et al.* (1975) also found that calling occurred during the last half of the scotophase in *M. configurata*. In some other species of Noctuidae, calling also occurs during the last half of the scotophase (Sower *et al.* 1970; Teal *et al.* 1978; Turgeon and McNeil 1982). In other Noctuidae, the period occurs shortly after the beginning of the scotophase, during the middle of the scotophase (Teal *et al.* 1978; Byers *et al.* 1985), or during the last two-thirds of the scotophase (Swier *et al.* 1977; West *et al.* 1984). The observation that calling was terminated shortly after lights-on may be attributed to inhibition by lights, because the adults are nocturnal.

The calling pattern is more characteristic of the continuous pattern of calling than the discontinuous pattern. At the start of the calling period, there was a short period when the ovipositor was protruded and retracted rapidly. This phase was followed by a long period when the calling posture was maintained uninterrupted during most of the time. This calling pattern has been observed in three other species of Noctuidae (Teal and Byers 1980). In these species, there is an initial phase of calling lasting about 15 min, during which the ovipositor is partially to fully protruded for intervals of one to 10 min and retracted for three to 15 sec between the intervals. Following this, there is a sustained bout, during which the ovipositor remains fully extended until calling is terminated for the day. The mean length of the sustained bout varied from 47 to 84 min depending on age. However, the pattern is different in other Noctuidae (Sower *et al.* 1971a; Swier *et al.* 1977; Turgeon and McNeil 1982; West *et al.* 1984). In these Noctuidae, the pattern is discontinuous, because the number of interruptions during the long period is large and/or the length of each interruption is much longer than in *M. configurata*. For example, in *A. ipsilon*, the females have four or more bouts of calling per scotophase and the number may be as high as 21 per scotophase (Swier *et al.* 1977). Some bouts may be as long as 78 min; most bouts are one min or less. In *P. unipuncta*, the number of bouts may vary from four to 16 per female per scotophase depending on age (Turgeon and McNeil 1982). The average duration per bout varies from two to 30 min.

During most of the interruptions, the females flew around the cage. Kaae and Shorey (1972) suggested that the purpose of such flight was to

select better sites for calling. Swier *et al.* (1977) suggested that this flight may even bring females closer to males, resulting in attraction being made over shorter distances.

5.2 The Effects of Physiological Factors on Calling Behaviour

The physiological factors investigated in the present study were: age (Section 4.2), mating status (Section 4.3), photoperiodic cue(s) (Section 4.4), endogenous regulation (Section 4.5), and ovarian maturation (Section 4.8).

Virgin females initiated calling for the first time during the second and third scotophases after emergence at 20°-30°C. The differences in the initiation of calling between the scotophase- and photophase-emerged females can be attributed to age differences; the former were on average about 12 h older than the latter. These observations agree with those of Chisholm *et al.* (1975) and Struble *et al.* (1975), but not with Swailes *et al.* (1975). Chisholm *et al.* (1975) observed that most virgin females of *M. configurata* did not initiate mating for the first time until after the second scotophase after emergence. Struble *et al.* (1975) found that males of *M. configurata* did not respond sexually to the extracts from the abdominal tips of virgin females until the females were three days old. Swailes *et al.* (1975) reported that one-day old virgin females of *M. configurata* attracted males in traps in the field. There is no satisfactory explanation for the differences between the present results and those of Swailes *et al.* (1975), unless the females they used emerged during the photophase and were put in the traps at the beginning of the second scotophase. However, they did not give the time of emergence. The reason that Struble *et al.* (1975) did not observe males responding to extracts of two-day old females may have been due to the timing of the collection of the abdominal tips. If they did not collect abdominal tips at the end

of the second scotophase, the time when the first calling occurred in the present study, they may have missed the initiation of pheromone production. They did not specify precisely when the collections were made. Calling and pheromone production usually are synchronized in female moths (Jacobson 1972).

The synchrony in the initiation of calling for the first time after emergence has been observed in one other species of Noctuidae (West *et al.* 1984). As in *M. configurata*, most females of the potato stem borer, *Hydraecia micacea* Esper, initiated calling for the first time during a single scotophase (West *et al.* 1984). In the two other species of Noctuidae studied, the initiation of calling for the first time was not synchronous, because it was spread over a period of four to nine scotophases (Swier *et al.* 1977; Turgeon and McNeil 1982).

Two age-related changes occurred in the calling behaviour. The diel periodicity of calling was advanced and the length of the daily calling period was increased from the second to the seventh scotophase. These age-related changes in calling behaviour apparently took place at the same time as pheromone production is increased (Struble *et al.* 1975). They showed that pheromone production increased with age until the females were six days old, and that the high level of pheromone production was maintained until after the ninth day, after which it declined. A similar decline in calling was not observed in the present study.

The diel periodicity of calling was advanced significantly more among the scotophase-emerged females than among the photophase-emerged females during the second and third scotophases. These differences

likely were due to the former females being on average about 12 h older than the latter.

The two age-related changes in calling behaviour observed in *M. configurata* have been reported in a number of Noctuidae. The diel periodicity of calling was advanced and the length of the daily calling period was increased with age in *A. ipsilon* (Swier *et al.* 1977), three species of *Euxoa* (Teal and Byers 1980), *P. unipuncta* (Turgeon and McNeil 1982), and *H. micacea* (West *et al.* 1984). Swier *et al.* (1977) suggested that by calling earlier and for a longer period in the scotophase, older females increase their probability of mating by being the first to attract males. In *T. ni* (Sower *et al.* 1971a), the effects of age on calling behaviour were the reverse of those in *M. configurata*. The females of *T. ni* spent less time calling during each scotophase as they became older.

M. configurata is a multiple-mated species (Turnock, unpublished observations). The present study showed that following the first mating, the females passed through a refractory period that was about two days long. Calling was resumed after the refractory period, but the length of calling period during each scotophase was shorter than in virgin females. A refractory period after the first mating has been observed in *C. fumiferana* (Sanders and Lucuik 1972). Shorey *et al.* (1968a) found a refractory period for female sex pheromone production following the first mating in several species of Noctuidae, but they did not determine whether there also was a refractory period for calling.

The lights-off cue is the photoperiodic cue responsible for setting the timing of the diel periodicity of calling. The data for the third

and fourth scotophases of the photoperiodic cue experiment (Fig. 10) support this conclusion. In spite of the large differences in the length of the photophases preceding the fourth scotophase, the mean onset calling times were similar in both the third and fourth scotophases. Cardé and Roelofs (1973) concluded that the lights-off cue also was the photoperiodic cue in *H. immaculata*; females of this species responded similary to those of *M. configurata* to changes in the length of the photophase. The lights-on signal appears to be the cue in several other moths (Sower *et al.* 1971b; Sanders and Lucuik 1972; Cardé *et al.* 1975b; Baker and Cardé 1979a). In these species, the mean onset calling times were not similar in the scotophases following photophases in which the lengths of the photophase were changed.

The circadian rhythm of calling is endogenously based, as in other Lepidoptera (Sower *et al.* 1970; Trainer 1970; Sanders and Lucuik 1972; Cardé and Roelofs 1973; Cardé *et al.* 1975b; Baker and Cardé 1979a; Castrovillo and Cardé 1979; Turgeon and McNeil 1982). The fact that the females called at approximately 24-h intervals for eight days in continuous darkness after having called once during a normal photoperiod supports this conclusion. The only deviations from the normal circadian rhythm of calling seemed to be in the diel periodicity of calling and the length of the daily calling period. The age-related advancement in the diel periodicity of calling that normally occurs during the third to seventh scotophaes was not observed in continuous darkness. In fact, calling occurred later each day during this equivalent period in continuous darkness. This conclusion was based on the following assumption. Though calling was not studied in the scotophase-emerged

females after the fifth scotophase, it was assumed that their calling behaviour after the fifth scotophase would be similar to that of photophase-emerged females, because the calling behaviour of the two groups was similar in the fourth and fifth scotophases (Tables 1 and 2). The lengths of the daily calling periods in continuous darkness were about 2.5-6 times longer than those in a normal photoperiod; this probably was due to the absence of light.

The first chorionated eggs appeared in the ovaries at the end of the first scotophase in scotophase-emerged females and at the beginning of the second scotophase in photophase-emerged females at 20°-30°C. The females initiated calling and mated for the first time during the second scotophase. Most females contained chorionated eggs and had called and mated by the end of the third scotophase. Struble *et al.* (1975) detected the first sex pheromone in three-day old virgin females at 24°C, but, as pointed out earlier, he may have missed the initiation of pheromone production which likely occurred during the second scotophase. Thus, the maturation of the reproductive processes in young females of *M. configurata* are closely synchronized. This is consistent with what is found in other Noctuidae (Shorey *et al.* 1968a; Swier *et al.* 1976, 1977). In these other species, the first chorionated eggs appear in the ovaries and the other reproductive processes are initiated during the first few days of adult life.

5.3 The Effects of Environmental Factors on Calling Behaviour

The environmental factors investigated in the present study were: photoperiod (Sections 4.4 and 4.6) and temperature (Sections 4.7 and 4.8).

Photoperiod affected calling behaviour by changing the diel periodicity of calling. The data from the fourth to sixth scotophases of the photoperiodic-cue experiment (Fig. 10) and from the photoperiod experiment (Fig. 12, Table 3) support this conclusion. The data showed that the mean onset calling times were similar if the length of the preceding scotophase(s) was six to 10 h long, but the onset of calling was delayed by about one to five h if the preceding scotophase(s) was 12-16 h long. In addition, if the length of the scotophase was changed, the mean onset calling time also may be different in the next scotophase. Since the lights-off cue is responsible for setting the diel periodicity of calling in *M. configurata*, the effects of photoperiod apparently are to modify the response to this photoperiodic cue. The entrainment of the response to the photoperiodic cue, therefore, seems to occur in the adult stage and to take place in the scotophase preceding the one in which it is expressed. The females respond to differences in the length of the scotophase by calling earlier in the scotophase if the preceding scotophase(s) is $\leq$ 10 h long than if it is $>$ 10 h long. Similar changes in the diel periodicity of calling in response to different photoperiods were reported in another moth, *P. carduidactyla* (Haynes and Birch 1984b); the onset of calling occurred later in the scotophase when the length of the scotophase increased from eight to 16 h. However, *C. fumiferana*

responded differently to changes in the length of the scotophase (Sanders and Lucuik 1972). It called earlier when the length of the preceding scotophase was increased from eight to 20 h, but it initiated calling in the photophase and responded to the lights-on photoperiodic cue.

Temperature affected four aspects of calling behaviour (Experiment I): age at first calling, diel periodicity, length of the daily calling period, and percentage of females calling. The age at first calling was advanced with increases in temperature from 10°-20°C, but was delayed significantly at 35°C. The diel periodicity of calling was delayed and the length of the daily calling period was shorter during each scotophase at 30°-35°C as compared with 10°-25°C. The normal age effects on the diel periodicity of calling and the length of the daily calling period were observed at all temperatures, but the differences at 30°-35°C were small. The percentage of females calling during their lifetime was significantly lower at 35°C than at 10°-30°C. The percentage of females calling daily declined rapidly after the second scotophase of calling at 30°-35°C. Therefore, these data suggest that temperatures > 25°C adversely affect calling behaviour. The temperature effects on calling behaviour observed in *M. configurata* were similar to those observed in the only other noctuid studied (Turgeon and McNeil 1983).

Short-term temperature changes during the scotophase affected three aspects of calling behaviour (Experiment II): diel periodicity, length of the daily calling period, and percentage of females calling. The diel periodicity of calling was advanced or delayed as the females were

exposed to lower or higher temperatures. Likewise, the daily calling period was lengthened or shortened as the females were exposed to lower or higher temperatures. The advantage of calling earlier in the scotophase and for a longer period during the scotophase at cool temperatures may be to increase the probability of mating before the temperature drops below the threshold(s) for calling and mating. The percentage of females calling was significantly lower at high temperatures (30°-35°C) than at lower temperatures (5°-25°C). Similar changes in the diel periodicity of calling in response to short-term temperature changes were observed in other moths (Sower *et al.* 1971b; Sanders and Lucuik 1972; Cardé and Roelofs 1973; Cardé *et al.* 1975b; Gorsuch *et al.* 1975; Baker and Cardé 1979a; Castrovillo and Cardé 1979; Teal and Byers 1980; Haynes and Birch 1984b). Also, the length of the daily calling period varied similarly with temperature in other moths, except in two species (Castrovillo and Cardé 1979; Haynes and Birch 1984b). In these species, the length of the daily calling period did not vary significantly when the onset of calling was advanced or delayed.

Calling occurred at all temperatures tested from 5°-35°C. The data on diel periodicity of calling, length of the daily calling period, percent females calling daily and during their lifetime, longevity, and ovarian maturation suggest that 35°C is near the upper limit for calling. Since all females called at 5°C in the short-term temperature experiment, it appears that 5°C above the threshold. The optimum-temperature range for calling is, at least, 10°-25°C, because no aspect of calling behaviour was adversely affected at these

temperatures. However, additional work is needed to determine whether long-term exposure to temperatures < 10°C adversely affect calling and whether temperatures < 10°C should be included in the optimum range.

The mean daily minimum air temperatures are between 6° and 14°C during the flight period of *M. configurata* in Canada (Kendrew and Currie 1955; Wylie and Bucher 1977; Hare and Thomas 1979). The present investigations showed that short-term exposure to 5°C did not inhibit calling. Therefore, night time temperatures usually are suitable for calling of *M. configurata* in Canada. However, there is no information on whether the females release pheromone and the males respond to the pheromone and fly at temperatures as low as 5°C. Research, therefore, is needed to determine the threshold(s) for pheromone release by females, male response to pheromone, flight, and copulation.

High temperatures (30°-35°C) adversely affected oogenesis. The adverse effects were observed in females reared at 30°C for about three days and at 35°C for < two days. The maximum daily air temperatures may be > 30°C and the mean daily air temperatures may be > 25°C for several successive days during the flight period of *M. configurata*. It seems possible that under these weather conditions oogenesis is adversely affected and some eggs produced are not viable. In addition, if such weather conditions prevail for extended periods, large numbers of eggs might not be viable. Therefore, high temperature effects on developing eggs during the adult stage might be a significant mortality factor in *M. configurata*.

5.4 The Importance of the Circadian Calling Rhythm

In *M. configurata* the diel periodicity of calling is endogenously based and modified by physiological and environmental factors. The endogenous control mechanism in insects probably is precoded in a "pheromone-behaviour center" located in the central nervous system in a manner analogous to a computer program (Shorey 1974). Most of the time, calling behaviour is not expressed because of inhibitory messages arriving at the center from some other nervous system centers (e.g., the "feeding behaviour center" or "escape behaviour center"). The inhibitory messages communicate the state of the outside environment and of the internal physiology of the insect.

The circadian calling rhythm in the female and the circadian response rhythm to the pheromone in the male are synchronized in *M. configurata*. This conclusion is supported by the following observations. At 20°C and 16 h L : 8 h D, the males were active and only responded to the female sex pheromone during the last three h of the scotophase (Howlader, unpublished observations). This close synchrony between the female calling rhythm and the male response rhythm probably is one of the factors which ensures that mating occurs soon after sexual maturation in this species, as seems to be the case in the other species of Lepidoptera studied (Sanders and Lucuik 1972; Truman 1973; Shorey 1974; Cardé *et al.* 1975; Swier *et al.* 1976; Baker and Cardé 1979a; Haynes and Birch 1984b). However, additional research is required to determine whether the circadian response rhythm of males of *M. configurata* is modified by physiological and environmental factors, as is the circadian calling rhythm in the females.

Cardé and Baker (1984) have suggested that interspecific competition is an important selective force in the evolution of the diel periodicity of calling in insects. In some Lepidoptera, the diel periodicity of mating is the primary mechanism of reproductive isolation (Roelofs and Cardé 1974; Teal *et al.* 1978). These species usually are closely related, are co-occurring, and share either identical or similar pheromones so that they are cross-attractive (Kaae et al. 1973; Roelofs and Cardé 1974; Teal et al. 1978). Though both *M. configurata* and *M. curialis* (Smith), the only other species of *Mamestra* in North America, are found east of the Rocky Mountains, there is insufficient information on the latter species to determine whether the two species are sympatric. Also, it is not clear whether the diel periodicity of calling is an important reproductive isolation mechanism in these species, because the biology of *M. curialis* has not been studied.

Traps baited with the synthetic pheromone of *M. configurata* attract males of other species of Noctuidae in the field (Steck *et al.* 1984; Struble *et al.* 1984). However, the impact of these noctuids on the evolution of the diel periodicity of calling in *M. configurata* cannot be speculated on, because the diel periodicity of calling has not been studied in these species. Also, it has not been determined whether the natural pheromone of *M. configurata* is attractive to other Noctuidae.

5.5 Application in Integrated Pest Management

Sex pheromone trapping is a useful technique for monitoring populations of *M. configurata* (Steck *et al.* 1984). Turnock (1984) estimated patterns of egg-laying from the distribution of larval instars in field collections of *M. configurata*, and concluded that male moth captures in sex attractant traps provide a valid index to the abundance of female moths and their egg-laying activities. His estimations indicated that egg laying begins about seven days after the first male(s) is captured. The present studies showed that both sexes mated within the first three days and most females initiated oviposition within the first four days of emergence at 20°C, and that most females initiated calling and had mature eggs in the ovaries within the first six days at 10°-35°C. These observations confirm Turnock's estimations that adults become reproductively mature during the first week of emergence. This now permits pest managers to predict larval development in the field using male moth captures in sex attractant traps and degree-days requirements for development (Bailey 1976). Also, the time when larval surveys should be conducted may be determined more accurately than in the past.

6 CONCLUSIONS

The principal findings of the present studies are:

1. Female *M. configurata* has a characteristic calling posture. The key characteristics are: the ovipositor is extruded and curved downward at an angle of about 45°, the wings are raised above the abdomen in the form of a "V", and the antennae are directed posteriorly along the sides of the thorax.

2. Calling has a discrete diel periodicity. The period is restricted to the last half of the scotophase.

3. The lights-off cue is the photoperiodic cue responsible for setting the timing of the diel periodicity of calling.

4. The circadian rhythm of calling is endogenously based. Calling occurred at approximately 24-h intervals in continuous darkness.

5. The pattern of calling is characteristic of the continuous pattern. It consists of a short period at the beginning when the ovipositor is protruded and retracted rapidly and a long period when the calling posture is maintained uninterrupted for most of the time.

6. The age of virgin females affects calling behaviour in two ways. The diel periodicity of calling is advanced and the length of the daily calling period is increased with age.

7. The appearance of the first chorionated eggs in the ovaries is synchronized with the time of first calling.

8. The first mating is also closely synchronized with the time of first calling.

9. After the first mating, the mated females resume calling after a refractory period. The refractory period was two days at 20°C.

10. The daily calling period is shorter in mated females than in virgin females.

11. Oviposition is initiated shortly after the termination of the first mating.

12. At 20°C, most females had mated and oviposited within the first four days of emergence.

13. Photoperiod affects calling behaviour by changing the diel periodicity of calling and the length of the calling period. The females call earlier in the scotophase and the calling period is shorter if the scotophase is $\leq$ 10 h than if it is $>$ 10 h.

14. Continuous constant temperature during the adult stage affects calling behaviour in four ways: age at first calling, diel periodicity of calling, length of the daily calling period, and percentage of females calling.

15. Short-term temperature changes during the scotophase affects calling behaviour in three ways: diel periodicity of calling, length of the daily calling period, and percentage of females calling.

16. The optimum temperature range for calling is, at least, 10°-25°C.

17. The upper limit for calling is near 35°C and the threshold is < 5°C.

Monitoring populations of *M. configurata* with sex attractant traps is a useful tool in the IPM programme for this pest. The following information from the present studies may improve the monitoring programme. Both sexes mated within the first three days and most females initiated oviposition within the first four days of emergence at

20°C. Most females initiated calling and had mature eggs in the ovaries within the first six days at 10°-35°C. Pest managers now can predict larval development in the field using the data from sex attractant trap samples and degree-days requirements for development. Also, they may determine the timing of larval surveys in the fields more accurately than before.

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