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Environmental Regulation of the Periodicities of Female Calling and Male Pheromone Response and Courtship Behaviors in the Codling Moth (Laspeyresia pomonella L.)

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ENVIRONMENTAL REGULATION OF THE PERIODICITIES OF FEMALE CALLING AND MALE PHEROMONE RESPONSE AND COURTSHIP BEHAVIORS IN THE CODLING MOTH (*LASPEYRESIA POMONELLA* L.)

By

Paul Joseph Castrovillo

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ABSTRACT

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OF FEMALE CALLING AND MALE PHEROMONE RESPONSE
AND COURTSHIP BEHAVIORS IN THE CODLING MOTH
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Codling moth female calling and male pheromone responsiveness were studied in the laboratory. Both occurred during scotophase (23°C; L:D 16:8). Under continuous photophase or scotophase females continued to call with a circadian periodicity. Male response rhythmicity was maintained under continuous photophase. Decreasing temperature (23° to 16°C) resulted in calling termination (decrease during scotophase) or shifting of calling into photophase (decrease prior to scotophase).

Males orienting to a platform with a pheromone source spent a significantly greater amount of time at a quadrant containing a visual cue than at any other quadrant or the source. Thirty-three successful codling moth matings were videotaped and analyzed to elucidate the behavioral events occurring during courtship.

Field experiments indicated that male pheromone trap catch was unaffected by trap location within or between trees, and that a non-sticky trap catch may be comparable to a sticky one. Males observed approaching a trap were used to assess efficiency.

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INTRODUCTION

The codling moth, Laspeyresia pomonella L., is a cosmopolitan lepidopterous pest of apples, pears, walnuts and related crops. Although it has been under investigation for many years in the hopes of finding keys to a control program with a reduced reliance on insecticides biological and behavioral information, especially that concerned with courtship and mating, is very incomplete.

Borden (1931) reported that male codling moths find females in flight and follow them to foliage where copulation occurs: "That the females do attract the males by their quick movement and fluttering wings has repeatedly been observed." Recently this has proven to be quite incorrect. Proverbs (1965) demonstrated that males were attracted to caged living females, and Howell and Thorp (1972) determined that virgin moths were much more attractive than mated ones. Butt and Hathaway (1966) found that an extract of female codling moths could be used to lure males to a trap. Males exposed to this extract in the laboratory "performed a circling dance and attempted to copulate with other males (both dead and alive), empty pupal cases and pieces of corrugated cardboard."

Butt et al. (1968) screened 35 aldehydes and nitriles for codling moth attractiveness in the field, and 95 aldehydes and nitriles were tested in a laboratory bioassay in which positive responses were defined as "extending their claspers, vibrating their wings, spinning in a circle and (or) attempting to copulate." Few positive results were

obtained. On the basis of combined data from gas chromatographic tests, several chemical reactions, electroantennogram screening of a number of compounds and field-trapping Roelofs et al. (1971) proposed (E,E)-8,10-dodecadien-1-ol as the identity of a sex pheromone of the codling moth. Failing to locate this chemical in the insect McDonough et al. (1972) reported evidence that the codling moth possessed multiple sex pheromones with the major component being (Z,E)-2,6-7-methyl-3-propyl-decadien-1-ol. Using a computerized search of mass spectra obtained from GC effluent of female extract Beroza et al. (1974) demonstrated that a doubly unsaturated 12-carbon alcohol with retention time identical to that of (E,E)-8,10-dodecadien-1-ol was present in the codling moth. McDonough and Moffitt (1974), retracting the identification reported by McDonough et al. (1972), stated that they had found (E,E)-8,10-dodecadien-1-ol, by physical data and ozonolysis, to be the authentic natural sex pheromone. Buser and Arn (1975), using quadrupole mass fragmentography and high resolution gas chromatography, found approximately 3.5 ng of pheromone to be present per female.

Shorey (1970) theorized that in moths the sex pheromone was a single species or group-specific chemical attractant. Although it is now known that most Lepidoptera utilize multiple component pheromone systems, blends of related compounds in discrete ratios (Roelofs and Cardé 1977 and Tamaki 1977), no evidence of this pertaining to the codling moth has yet been found. Roelofs et al. (1972) conducted a field-trapping experiment to test the effect of various mixtures of the 3 geometrical isomers ((Z,E), (Z,Z) and (E,Z)) with (E,E)-8,10-dodecadien-1-ol. Results indicated that pure (E,E) was a potent male attractant and that the other isomers were unattractive alone and (at least in the ratios

tested) diminished trap catch when mixed with (E,E). In other field tests (E,E)-8,10-dodecadien-1-ol acetate and undecanol inhibited the attraction of males to females in traps (Hathaway et al. 1974), and traps baited with synthetic pheromone had decreased attractancy when the bait included the following (E,E)-8,10-dodecadien-1-ol derivatives: acetate, proprionate, ethyl ether, propyl ether, isopropyl ether and sec-butyl ether (George et al. 1975). Arn et al. (1974) screened 33 compounds, 11 to 14 carbon, singly unsaturated alcohols and acetates and found that the greatest catch reduction in traps baited with 1 mg of synthetic pheromone occurred with 5 mg of (Z)-8-dodecenyl acetate. Others which gave significantly lower catch were generally 12 or 13 carbon acetates with a (Z) double bond.

The natural pheromone is produced by the female codling moth in glandular tissue located dorsally on the ovipositor (Barnes et al. 1966). It is held within the abdomen, between the eighth and ninth segments except when she is calling, at which time the ovipositor is extended ventrally at a 90^{o} angle to the axis of the body (Fluri et al. 1974).

Males have been reported to approach calling females in a "typical buzzing dance:" walking in a rather straight line with buzzing wings, lifting the abdomen, and spreading their valves in the direction of the female. "As soon as a male touched a female copulation followed immediately (Fluri et al. 1974)." Apparently stimulation by the sex pheromone plays an important role in the mating process because Fluri et al. (1974) found that removal of both male antennae almost completely prevented mating of caged moths, while removal of 1 male antennae or both female antennae did not. On the basis of another caged moth mating

experiment Gehring and Madsen (1963) reported that probably no visual cues were necessary during codling moth courtship when males and females were in close proximity because mating was as successful in constant darkness as under a photocycle with both scotophase and photophase. Conversely, Hutt and White (1977) observed in the laboratory under close confinement the number of matings occurring with blind, antennectomized, blind and antennectomized and unaltered males and concluded that response to visual cues did play a part in mating.

Using a timing pheromone trap in California Batiste (1970) collected male codling moths from before noon to 4 A.M. (Pacific Standard Time). Under normal weather conditions, however, it is considered to be a crepuscular and nocturnal insect. The literature holds many measurements of codling moth activity time determined by field flight observations (Borden 1931 and Putnam 1963), bait trap (Worthley 1932 and Parrot and Collins 1934), UV light trap (Batiste et al. 1973a) and pheromone trap catches (Wong et al. 1971, Batiste et al. 1973a and 1973b and Mani et al. 1974) and all reported that on an average day moth flight and trap catch were concentrated at twilight, usually within the range of 30-60 minutes before to 30-60 minutes after sunset.

Apparently environmental conditions can modify this flight period. Batiste et al. (1973a) determined that in California codling moths in flight during spring and early summer were most active prior to sunset, while those flying later in the season were captured in greater numbers after sunset, and they attributed this to differences in early evening temperatures. This same paper also reported that male flight was limited below 13^{o} and above $27^{o}C$. Previously Batiste (1970) had indicated that last trap catch appeared to be correlated with temperature

decrease to 16°C. Worthley (1932) found during bait trapping that codling moth flight did occur below 16°C, but increased with increasing temperature. A series of observations on male flight in the orchard and attraction to pheromone traps (Mani et al. 1974) uncovered evidence that the effect of decreases and increases in temperature on male activity may have relied on the time the temperature changes occurred, the previous weather conditions and what other environmental factors, such as cloud cover, wind speed and precipitation, were altered. Their work also indicated that during the evening codling moths may exhibit 2 different types of flight activity: a period when moths (sex was not determined) are flying but little or no attraction to traps occurs and a period of flight when males are captured in great numbers. The mode of flight occurring at any time appears also to be a result of the combined effects of recent and current weather trends.

The ability of males to orient to pheromone has presented itself as a tool for use in monitoring and possibly controlling codling moth populations. Traps have been baited with virgin females (Howell 1974), female extract (Butt and Hathaway 1966) and synthetic pheromone (Butt et al. 1974). For the latter, when dispensed on a rubber septum, in the field, a dosage of approximately 1.5 mg with a release rate of 1.25 ug per hour appears to be optimal (Maitlen 1976).

Several trap designs have been tested on the basis of ability to catch, cost to produce, ease of maintenance and durability in the field. These have included open wing traps, 1 quart cylinder traps and BAB (cylinder with bottom half of opening closed) traps (Butt et al. 1974); ice cream carton, milk carton and wing traps (Howell 1972); Pherotrap 1C and Batiste traps (Westigard and Graves 1976); and BAB and aluminum

funnel above aluminum pie plate traps (Batiste and Joos 1972). All of them relied on a sticky surface to capture the moths.

A number of codling moth control programs have been initiated based on utilization of the pheromone in monitoring traps. Moth catch was used as a criterion for determining when insecticide sprays should be applied (Hudson and Johnson 1971, Madsen and Davis 1971, Madsen and Vakenti 1973 and Myburgh et al. 1974). These programs have successfully allowed a reduction in sprays with the resultant level of control acceptable. Other projects have attempted to keep populations under control directly by mass-trapping using pheromone baited traps (Proverbs et al. 1975 and MacLellan 1976) and these have been less successful. Mating disruption from atmospheric permeation with pheromone has been tested with promising results. During a one-year field trial Audemard et al. (1977) maintained orchard damage at a commercially acceptable level, but the amount of pheromone employed (0.55 g/ha/day) was fairly high. Cardé et al. (in press) demonstrated complete disruption of male attraction to synthetic pheromone and caged virgin females and mating of tethered females when pheromone was dispersed from hollow capillary fibers.

It has been suggested that in low level populations traps effectively compete with native female moths, but as population size increases to moderate or high numbers of individuals the sex attractant trap catches do not accurately reflect the increase and therefore cannot be used to estimate the population size or control it (Howell 1974 and Mani and Wildbolz 1975). In Michigan, where the codling moth is partially bivoltine, Riedl et al. (1976) observed that moth catch anticipated emergence of adults from pupae in banded trees and oviposition

during the spring flight, but lagged behind emergence from bands and closely followed oviposition during the second generation.

At present the limit for successful usage of the codling moth sex pheromone in applied entomological situations is as trap bait for monitoring population levels. However, an increased understanding of the behavior of the insect, especially its courtship, response to pheromone and traps and the mediating effects of weather on those responses could conceivably lead to greater success in control programs, due to more effective usage of the pheromone and more complete interpretation of the results.

This thesis research deals with several aspects of codling moth mating activity. Observations were made on the periodicities of female calling and male anemotaxis in response to pheromone stimulation and the effects of some extrinsic factors on these behaviors. The sequence of events occurring during codling moth courtship in the laboratory is reported, as well as the results from 3 field experiments concerned with male orientation to pheromone traps.

MATERIALS AND METHODS

Insect Culture

A culture of codling moths was maintained continuously in a growth chamber at the Pesticide Research Center, Michigan State University on small, green thinning apples obtained from a commercial supplier throughout the duration of the research. Stock insects were obtained from infested apples collected in an abandoned East Lansing apple orchard during September 1975, and small introductions of new material were made in the summer of 1976, summer of 1977 and fall of 1977. In the chamber temperature was maintained at 23 ± 1°C and the light cycle was 16 hours of photophase (1500 lux) followed by 8 hours of scotophase. Relative humidity was not held constant but generally remained about 75%.

The steps in the insect rearing were: mating of adults, collection of ova, infestation of apples with larvae and harvesting and sexing of pupae. Adults were placed in a 30 x 24 x 15 cm mating box, constructed of wooden top, bottom and sides and a plexiglas^{R} back and front. The back panel was darkened with a piece of black paper and the front panel was transparent. A wooden dowel attached to one side of the box served as a holder for a roll of waxed paper which was used to line the inside surface of the clear front panel. Fertilized female moths in the box readily oviposited on the waxed paper which was removed and replaced every 1 to 3 days. The sheet of paper containing the ova was placed in a 3.8 liter glass jar, sealed with parafilm and held in the growth chamber until larval emergence.

Ferro and Harwood (1973) reported that it may be possible for more than one codling moth larva to survive to maturity within a single apple, but one larva per apple is the normal situation (Howard 1887 and Geier 1967). Therefore it was necessary to devise a system which would result in little or no multiple larval entry of an apple to insure maximum survival. The method used relied on infesting each apple by hand. A newly emerged first instar larva was removed with a camel's hair brush from a jar containing ova. A very narrow slit (ca. 2 mm wide and 2.5 cm long) was cut in an apple and the larva placed in that slit. Then the apple was placed in a 3.8 liter glass jar and more infested apples were added until the jar was 3/4 full (ca. 50 apples per jar). Most larvae entered the apples at the slit in which they were placed.

To provide a substrate for cocoon formation of mature larvae that would allow easy pupal collection, paper and acetate "sandwiches," in some ways similar to a collection device used by Glenn (1922), were constructed. These consisted of an inner sheet of rippled cardboard, 2 thin sheets of clear acetate, one flat against each side of the cardboard, and a sheet of construction paper on the outside of each acetate sheet, all held together by 2 paper clips (and all measuring 15 x 4 cm).

Two sandwiches were placed in each jar of infested apples, a lid with a brass screen panel was screwed on the jar and they were placed on a shelf in the growth chamber to allow the larvae to develop. When ready to pupate, a majority of the larvae found the dark, sheltered area between the ripples of the cardboard a suitable site in which to spin up. Approximately 25 days after infestation the sandwiches were removed from the jars and pupae were collected. This was easily done by removing the rippled cardboard sheet with cocoons from the sandwich, opening

each cocoon with a curved steel teasing needle and removing the pupae with forceps. When cocoons contained larvae or prepupae they were left in place to allow development to continue. After all pupae were collected the rippled cardboard was returned to the sandwich and the sandwiches put back into the jars to allow more slowly developing larvae to pupate. Pupae were harvested a second time 1 week after initial collection and sometimes once more a week later, then the sandwiches were removed, the spent apples discarded and jars washed for re-use. Generally it could be expected that during the first collection 50% of the larvae introduced would be available as pupae and later collections would yield approximately 10% more of the population.

Once the pupae were harvested they were sexed (Petersen 1965). Males were placed in 295 ml clear plastic cups and these put into a 63 x 63 x 46 cm screened holding cage for emergence. Female pupae were placed in similar plastic cups, sealed loosely with parafilm and allowed to emerge. The virgin adults were collected each day and removed to other sealed plastic cups for holding. This was done to minimize the amount of pheromone released into the air of the growth chamber because only one chamber was available for rearing (both male and female adults had to be kept there), and it is possible that a high ambient concentration of pheromone might depress the responsiveness of males during behavioral bioassays (Shorey and Gaston 1964). Periodically adults were placed in the mating box to continue the culture and others were held in the growth chamber until used for laboratory or field experiments.

Pheromone Samples

Synthetic L. pomonella pheromone, (E,E)-8,10-dodecadien-1-ol (Roelofs et al. 1971) was obtained from Zoecon Corporation. GLC analysis on

a Carbowax 20M column indicated > 99% purity, with no evidence of the geometrical isomers. Samples for bioassay were prepared by dissolving 200 mg of the attractant in 2 ml of petroleum ether and preparing serial dilutions in decade steps. All treatments (both on filter paper rectangles and rubber septa) were dispensed in 10 µl of petroleum ether.

Female Calling Experiments

During experiments concerned with female calling periodicity each test insect (a virgin female moth) was held in a clear 35 ml plastic cup sealed with a circular piece of parafilm. Moths were always placed in the cups at least 1 hour before the first observation took place. Viewing during scotophase was accomplished with the aid of a small flashlight fitted with a Kodak Wratten® Filter #19. The filter eliminates light below 6100 Å, and Norris et al. (1969) reported that codling moths are insensitive to light greater than 6000 Å in wavelength.

Four plastic humidity chambers were constructed for use in the experiment dealing with possible effects of differing relative humidity on codling moth calling. Each chamber was fashioned from a 31 x 23 x 10 cm clear plastic box and 15 of the plastic cups described above. Three rows of five 24 cm diameter holes were cut into the lid of the box, an equal-sized hole was cut in the bottom of each cup and the cups were glued onto the box lid. An 18 x 27 cm rectangle of aluminum screen was glued against the inside surface of the lid. Three different 1.5 liter solutions of potassium hydroxide capable of producing relative humidities of 25, 50 and 75% (Solomon 1951) were prepared and during the test one solution was poured into each of three plastic boxes. The fourth box contained 1.5 liters of water (100% RH). A single virgin female was placed in each cup of the chamber and a circular piece of

parafilm on top and the aluminum screen below held her there. When the lid was positioned on a solution-containing box the desired RH was produced within the cups holding the females.

A. Periodicity of female calling under a 16 hour photophase and 8 hour scotophase regime

Six groups of 20 virgin females (aged 0-1 (group I), 1-2 (II), 2-3 (III), 3-4 (IV), 4-5 (V) and 5-6 (VI) days old) were set up singly in clear plastic cups and held under light and temperature conditions of L:D 16:8 and 23 $\pm$ 1^{o}C. The moths were observed 19 times throughout a 24 hour period. Each cup was numbered and a tally kept of which moths were calling during each observation. Behaviorally, a moth was designated as calling if it was possible to see the ovipositor protruding from the abdomen and directed ventrally at a 90^{o} angle to the body. In most cases this was made very apparent by a characteristic stance employed by many calling females: anterior of the insect low to the substrate; the posterior raised by extension of the metathoracic legs, and sometimes slight raising of the wings (Figure 1). This procedure was replicated 3 times.

B. Periodicity of female calling under continuous photophase

Six groups of 20 virgin females, as described above, were set up under conditions of L:D 16:8 and 23 $\pm$ 1^{o}C and observed for calling 17 times during a 24 hour period. Only those calling in the oldest 5 groups were recorded. At the end of the first observation period the moths in group VI were replaced by 20 new 0-1 day old ones (the new group I), each group designation was increased by one because of increased age, and observations were again carried out on the oldest 5 groups for 24 hours. This procedure was repeated on the third day and

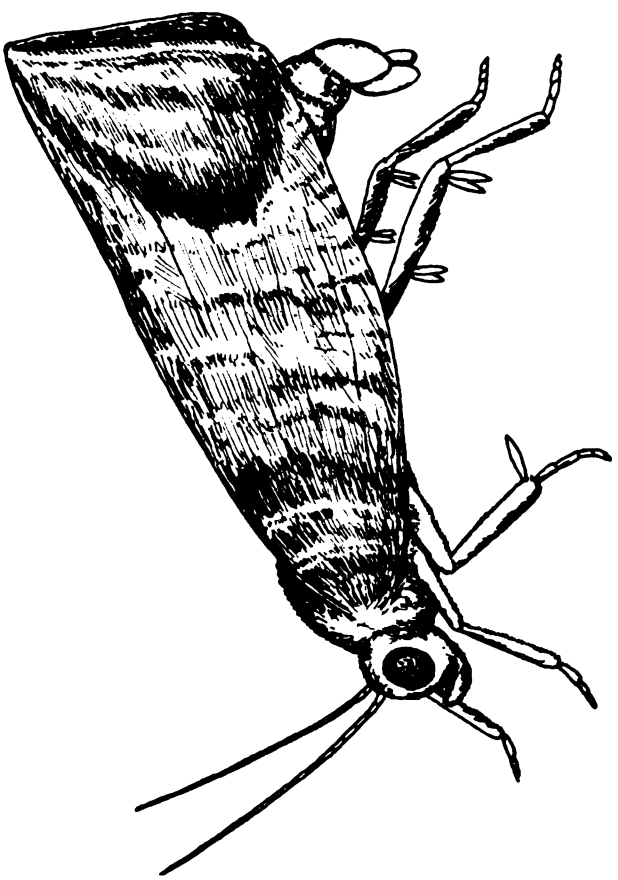

Figure 1. Calling stance of female codling moth.

on the fourth day group VI moths were removed, with the remaining moths once more being observed for 24 hours. Temperature was held constant during the 4 day period, but on the third and fourth days the insects were subjected to 48 hours of continuous photophase.

C. Periodicity of female calling under continuous scotophase

The above procedure with 6 groups of 20 virgin females was carried out again: group VI once more being replaced on the second and third days and discarded on the fourth. Temperature was maintained at 23 $\pm$ 1°C and during the first day L:D was 16:8, but the scotophase beginning on the second night was extended for the following 63 hours until termination of observations.

D. Effects of a decrease in temperature upon periodicity of calling

Three similar experiments were undertaken to demonstrate the effect of a temperature drop at different times during the day and night on calling. In the first, 5 groups of 20 virgin females (groups I-V: aged 0-5 days old) were set up and observed for calling 10 times throughout a 24 hour period, a new group I was added and V removed, and observations again made over the next 24 hours. The L:D 16:8 photocycle was maintained. Three hours after initiation of scotophase on the second day ambient temperature was lowered from 23° to 16°C. The drop occurred over a 2 hour period with the form presented in Figure 2. Following this the moths were kept at 16°C until termination of the observations.

The second experiment was identical to the first except that the temperature drop was begun 1 hour after the onset of scotophase. The third experiment extended over a 3 day period and employed 6 groups of virgin females as in the circadian periodicity demonstrations, group VI being discarded twice and replaced once with a new group I. The

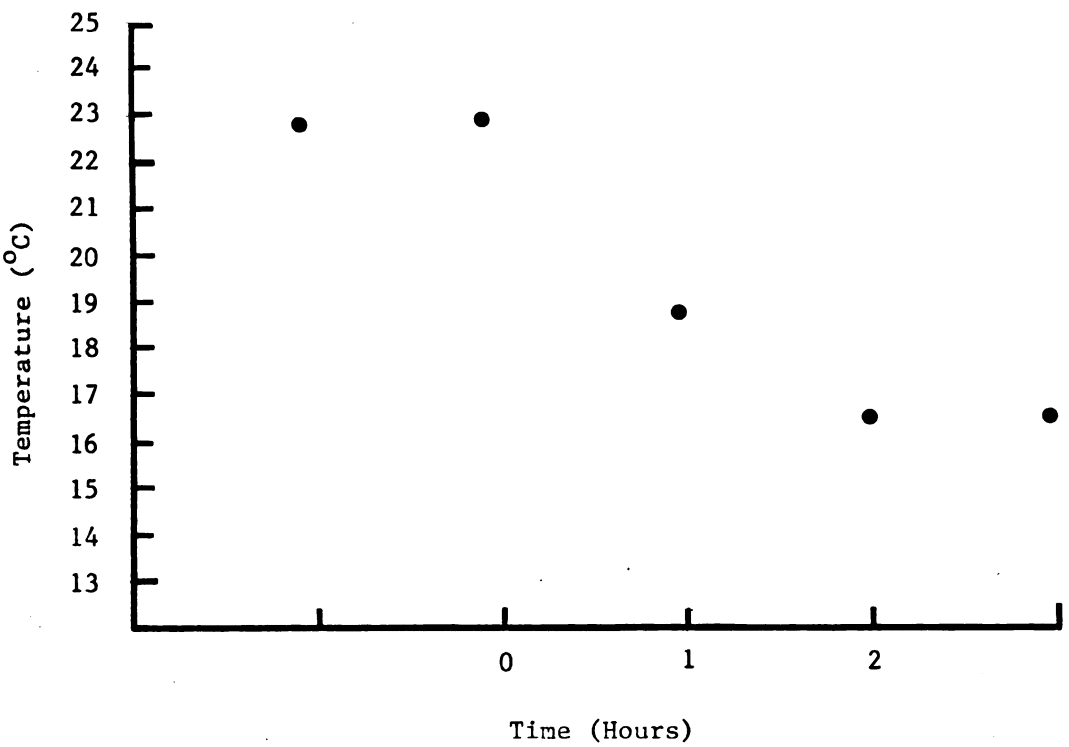

Figure 2. Form of 2 hour decrease in temperature occurring during experiments concerned with the effects of decrease in temperature on codling moth behavioral periodicity. Lowering begins at time 0 and is completed by time 2.

temperature drop this time occurred 3 hours prior to the beginning of scotophase on the second day. Therefore, in addition to observations being carried out under 30 hours of normal rearing chamber conditions, the insects were also held at 16°C and observed for 42 hours.

E. Effects of relative humidity on the periodicity of female calling

For each of the 4 RH's to be tested 15 virgin female moths were placed in a humidity chamber lid, one per cup: five aged 1-2, five 2-3 and five 3-4 days old. One lid was placed on each solution-containing chamber at least 4 hours prior to the start of scotophase and the number of females calling at each RH was tallied at 2 times before and 3 times during scotophase. The next day all of the lids were removed and the oldest 5 moths in each lid replaced with 5 moths 1-2 days old. Lids were randomly reassigned (i.e. females were not necessarily returned to the humidity level experienced during the previous night) to the boxes and placed back on them before scotophase in preparation for a new series of observations. This procedure was replicated 7 times.

Male Pheromone Response Experiments

Laboratory male behavioral bioassays of responses to synthetic pheromone were conducted in an orientation tube olfactometer (Figure 3) located in a bioassay chamber at the Pesticide Research Center. The olfactometer was similar to that described by Sower et al. (1973). An in-house air line passed air through an activated charcoal filter and a flowmeter to a glass manifold and then to the orientation tubes. Each glass orientation tube was 1 m long and 2 cm in diameter. The upwind end was formed into a 24/40 ground glass joint and a fine mesh brass screen plug was placed within the upwind end of each tube.

The orientation tubes were suspended above a 125 x 65 x 20 cm box

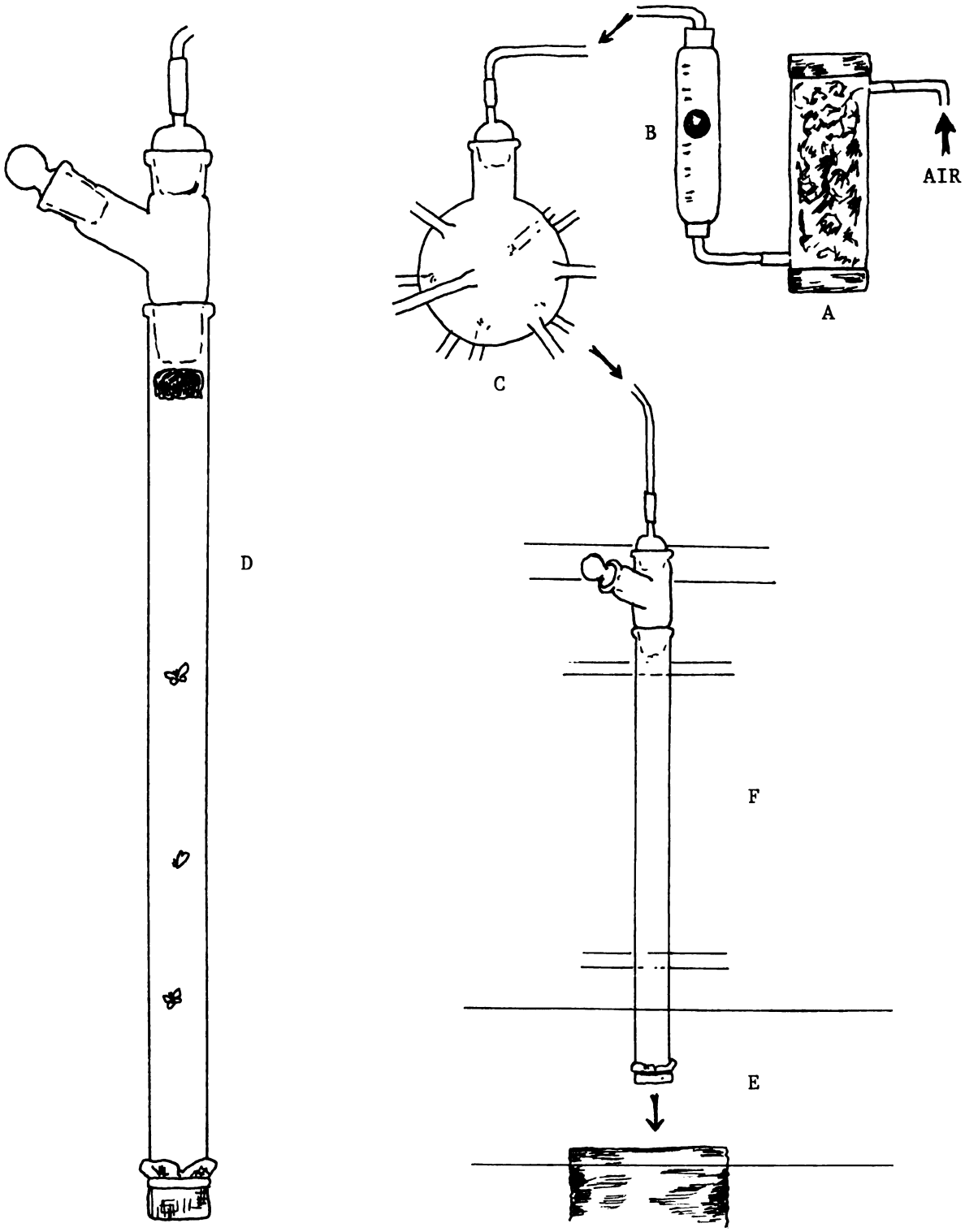

Figure 3. Orientation tube olfactometer used for male codling moth pheromone response bioassays. A: activated charcoal filter; B: flowmeter; C: manifold; D: orientation tube; E: exhaust hood; F: light box.

with a top surface of translucent white plastic. Six 7.5 watt incandescent lights connected to a rheostat were located inside the box. A 105° connecting tube interfaced with the upwind end of each orientation tube. The pheromone sample was introduced via the 105° tube into the airstream, and when no sample was present the third arm was closed with a ground glass stopper. A 65 x 25 x 12 cm plexiglas® hood over the downwind end of the orientation tubes was connected to an exhaust line venting pheromone-contaminated air from the bioassay chamber.

In conducting male bioassays the following procedure was employed. Male moths were removed from the holding cage, care being taken to utilize males from all parts of the cage equally, and placed in the orientation tubes. A 7 x 7 cm piece of cheesecloth was placed over the downwind end of each tube and held in place with a rubber band. The tubes were set up on the olfactometer and connected with the air line at least 1 hour but less than 12 hours prior to the bioassay to allow the moths to acclimate to test conditions. Air flow and exhaust were begun between 30 and 60 minutes prior to testing.

The first tube was observed for 30 seconds and (depending on the experiment) all moth activity was described onto a tape recorder or key behavioral responses were tallied. Then a pheromone sample was introduced into the airstream by replacing the ground glass stopper in the 105° connecting tube with a cork stopper and a steel clip holding a folded 2.5 x 1.25 cm piece of Whatman #1 filter paper containing the test stimulus. For 30 seconds moth behaviors were again observed and described or tallied. The sample was then removed from the tube, the stopper replaced and the observational procedure repeated with all of the remaining orientation tubes.

Following testing of the last tube the olfactometer was allowed to exhaust for a minute or two, then the air flow was stopped and within 12 hours the moths were returned to the holding cage. To remove any chemicals that may have adhered to the glass between use the orientation tubes, connecting tubes and glass stoppers were rinsed with acetone. All cheesecloth and pheromone samples on filter paper used during testing were discarded. No moths were ever bioassayed more than once during a 24 hour period. All bioassays were conducted with an air flow of 0.81 m/sec. During scotophase tests the light intensity beneath the tubes was 0.5 lux and during photophase testing light intensity was 1500 lux.

A. Periodicity of male response under a 16 hour photophase and 8 hour scotophase regime

Male bioassays were carried out using the orientation tube olfactometer as described above. Responses to a 100 ng sample of synthetic pheromone were measured at 2 hour intervals under conditions of L:D 16:8 and 23 $\pm$ 1^{o}C. Three replicates were performed at each test time, a replicate consisting of 8 orientation tubes each containing 3 male moths. Behaviors were described onto tape and transcribed.

B. Periodicity of male response under continuous photophase

Orientation tube olfactometer bioassays were conducted at 9 times during a 24 hour period for 2 consecutive days (23 $\pm$ 1^{o}C; L:D 16:8). On the third day the holding cages to which the test males had been returned were moved to the bioassay room and maintained under continuous photophase for 56 hours of constant light. During this period the moths were bioassayed on 2 days at the 9 test times. Two replicates were run at each time, 10 tubes per replicate and 3 male moths per tube. The

pheromone sample was 100 ng of synthetic pheromone. Upwind orientation, defined as walking upwind in the tube (with or without wing fanning) was the key behavioral response tallied.

C. Effects of a decrease in temperature upon periodicity of pheromone response

Males were bioassayed in the olfactometer at 5 times between 4 hours prior to and 3 hours following the initiation of scotophase on 3 consecutive days. During the first day room temperature was maintained at 23 $\pm$ 1°C. Three hours before scotophase on the second day the temperature was decreased from 23° to 16°C over a 2 hour period (Figure 2). During the remainder of the experiment all moths were held and bioassayed at 16°C. Two replicates were carried out at each time with 10 tubes per replicate and 3 moths per tube. The stimulus was 100 ng of synthetic pheromone and the response scored was number orienting upwind.

D. Effects of a decrease in temperature and decreases in light intensity upon periodicity of pheromone response

Two olfactometer bioassay replicates, each with 10 tubes and 3 moths per tube, were carried out 2 hours prior to the onset of scotophase under the following conditions: temperature of 23°C and light intensity of 1500 lux; 23°C and 150 lux; 23°C and 15 lux; 23°C and 1.5 lux; temperature decreased to 16°C and 1500 lux; 16°C and 150 lux; 16°C and 15 lux; and 16°C and 1.5 lux. During any tests in which the temperature and/or light level was lower than normal rearing chamber conditions (23°C and 1500 lux), the change in test conditions was initiated 1 hour prior to bioassay. Decrease in light intensity was immediate, but decrease in temperature followed the form presented in Figure 4. Number of moths walking upwind to a 100 ng sample of synthetic pheromone was tallied.

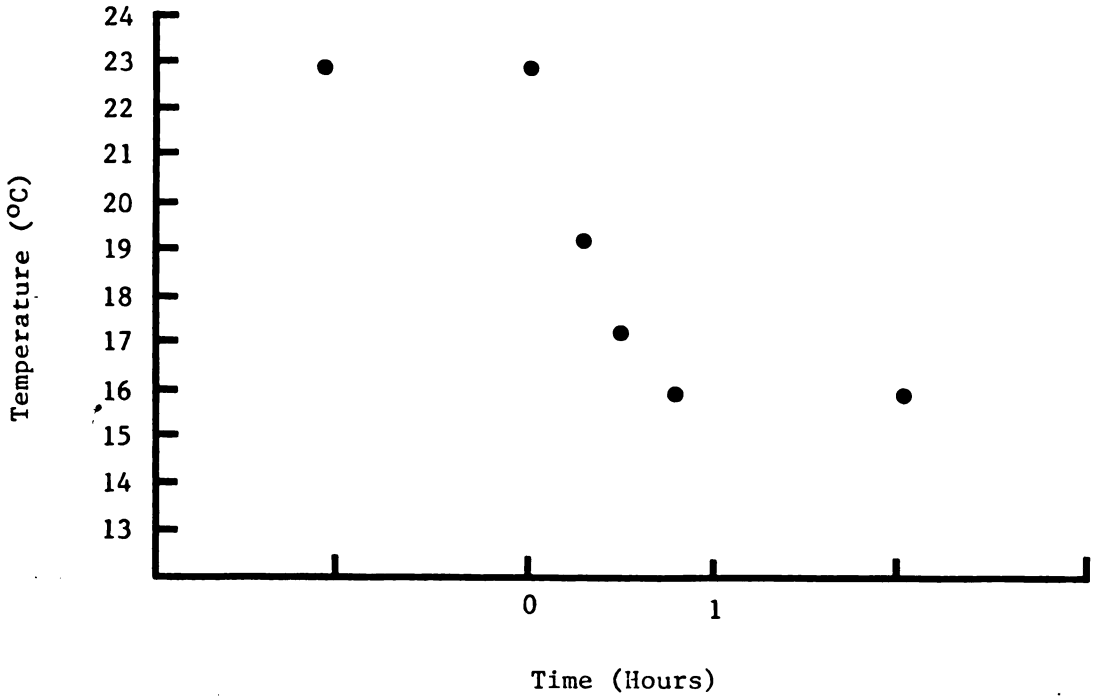

Figure 4. Form of 1 hour decrease in temperature occurring during experiment concerned with the effects of decrease in temperature and decreases in light intensity on male codling moth behavioral periodicity. Lowering begins at time 0 and is completed by time 1.

E. Effect of pheromone dosage on male response

Six concentrations of synthetic pheromone spanning 8 orders of magnitude were tested for male responses using the orientation tube olfactometer. All bioassays were conducted between 45 and 90 minutes into scotophase. At each replicate all 6 treatments were tested, 3 tubes per treatment with 4 male moths per tube. Shortly before the bioassays were to begin the 6 pheromone and filter paper samples were prepared and each one stored in a glass test tube. At bioassay time each sample was picked randomly. A verbal description of all male behaviors was recorded on tape. The experiment was replicated 9 times.

Codling Moth Courtship Studies

Laboratory observations of male orientation to a pheromone source and a visual cue and the videorecording of codling moth courtship were conducted in a wind tunnel (Figure 5) housed in a bioassay chamber at the Pesticide Research Center. The tunnel, 2.4 x 1.2 x 0.8 m, was constructed of an aluminum frame and clear plexiglas® and had the observational side divided into 3 movable panels which acted as sliding doors for access to the tunnel interior. The downwind and upwind ends were closed by aluminum screening to prevent the escape of insects in the tunnel.

Air flow was generated by a 0.7 m diameter fan connected to a rheostat for regulation of fan speed. The air was passed through a series of baffles to produce a nearly laminar flow and an exhaust line removed pheromone-contaminated air from the chamber. White and green striped canvas was stretched beneath the transparent wind tunnel floor. Light was supplied by four 7.5 watt incandescent lights suspended above the wind tunnel and connected to a rheostat. The light was diffused by a

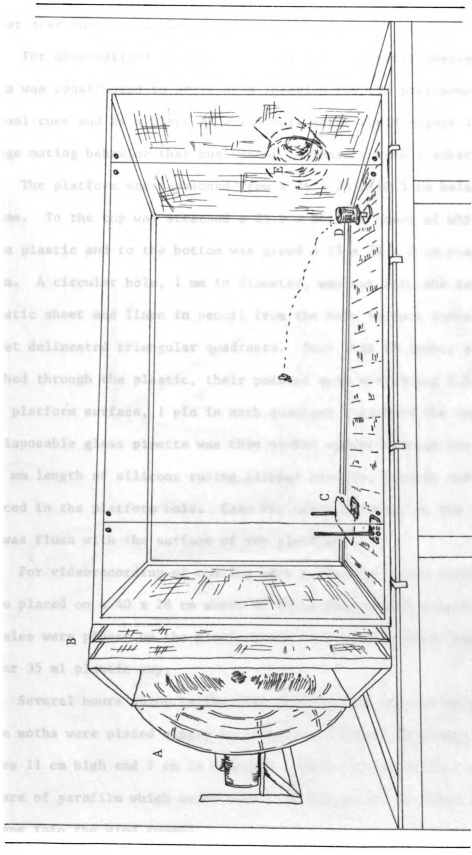

Figure 5. Wind tunnel used for codling moth courtship observations. A: fan; B: baffles; C: platform containing female; D: male release cage; E: exhaust vent.

sheet of translucent white plastic below the bulbs and a white cotton sheet over the top of the tunnel.

For observations of male orientation to synthetic pheromone a platform was constructed to serve as a location for the pheromone source and visual cues and as a place at which male moths could engage in any close range mating behavior that must occur in contact with a substrate (Figure 6). The platform was fashioned from a 24 x 23 x 10.5 cm balsa wood frame. To the top was attached a 23.5 x 24.5 cm sheet of white polystyrene plastic and to the bottom was glued a 15 x 24 x 2 cm piece of styrofoam. A circular hole, 1 mm in diameter, was cut into the center of the plastic sheet and lines in pencil from the hole to each corner of the sheet delineated triangular quadrants. Four size #0 insect pins were pushed through the plastic, their pointed ends projecting 1.5 mm above the platform surface, 1 pin in each quadrant 2 cm from the center hole. A disposable glass pipette was then pushed upward through the styrofoam, a 5 mm length of silicone tubing slipped over the pipette and its tip placed in the platform hole. Care was taken to position the tip so that it was flush with the surface of the platform.

For videorecording of codling moth courtship living virgin females were placed on a 40 x 28 cm sheet of white polystyrene plastic. Eight females were placed on the platform and each held in place beneath a clear 35 ml plastic cup.

Several hours prior to the time observations were to be undertaken male moths were placed singly in cylindrical copper screening release cages 11 cm high and 7 cm in diameter. Each cage was sealed with a square of parafilm which could easily be lifted off to allow the moth's escape into the wind tunnel.

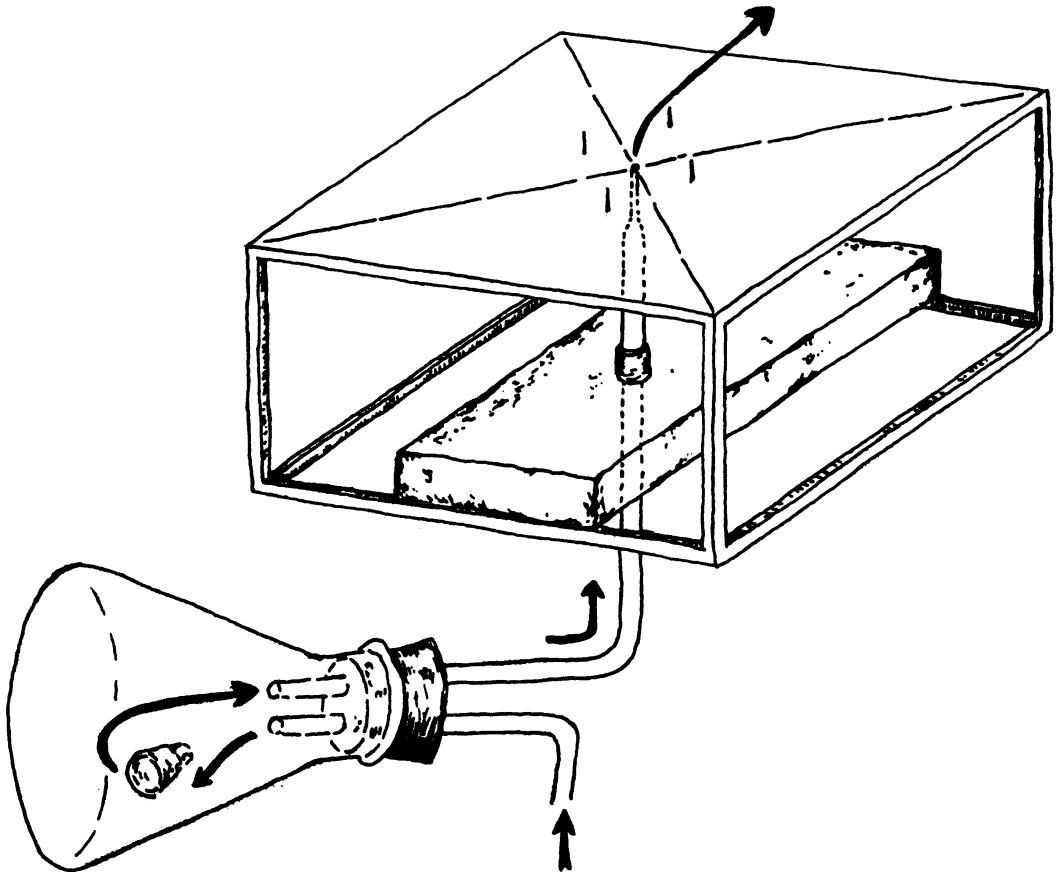

Figure 6. Orientation platform used for observations on the effects of visual cue presence and placement on male codling moth behavior.

A. Effects of visual cue presence and placement on the orientation of male codling moths

Air flow and exhaust of the wind tunnel were begun. The orientation platform was set up inside the tunnel 51 cm from the upwind end and 25 cm above the tunnel floor and supported by ring stands on each side. A rubber septum baited with 0.1 mg of (E,E)-8,10-dodecadien-1-ol was placed inside a 125 ml Erlenmeyer flask. The flask was plugged with a silicone stopper containing 2 pieces of glass tubing: one was connected to the pipette on the platform; the other was connected to an in-house air line. Air passing from the line carried pheromone out of the flask, through the pipette and out the hole of the platform where it was transported downwind in the tunnel.

For a visual cue a dead female codling moth rinsed several times in acetone was placed on the orientation platform and held in position by impaling it on one of the insect pins projecting from the surface. The female was placed in only one quadrant at a time and was always positioned with its head pointing upwind.

A male was induced to fly upwind in the tunnel to the platform by opening the downwind sliding door and placing a caged moth in front of the exhaust and removing the parafilm. Not all males exhibited anemotaxis but when one did leave the cage and began an upwind-oriented flight the sliding door was closed and the observer moved to the upwind end of the tunnel to describe on tape male behaviors at the platform for 2 minutes. Following that the male was observed for a measurement of the duration of his orientation to the platform. At the termination of each observation, if possible, the male was removed from the wind tunnel so as not to interfere with later tests.

Data was collected from 85 male orientations of 2 minutes or greater duration: 17 observations occurred with no visual cue present and 17 with the cue located in each quadrant. Between each observation the female was removed or randomly moved to a different quadrant. It was determined that the dead females were unattractive to the males without the synthetic pheromone present: when no air was passed through the flask males could not be induced to fly upwind to the platform; when males had reached the females with the aid of pheromone and the flask air flow was stopped the males left the platform very shortly without reorientation. Dead females were used several times, but discarded each time they were placed in the downwind quadrant, where they may have picked up pheromone from the plume passing over them.

Following conclusion of all observations air flow to the flask was ended and the wind tunnel was exhausted for approximately 15 minutes. Males were returned to a holding cage within 12 hours and to remove any pheromone that may have adhered to them all release cages were rinsed with acetone.

Observations occurred at 23^{o}C and 0.4 lux, a pheromone source air flow of 0.4 ml/sec and wind tunnel air speed of 0.68 m/sec. No moths were released for observation more than once during a 24 hour period and most were tested only once. All observations occurred during the 8 hours in which the moths were previously held in scotophase.

B. Laboratory courtship sequence of the codling moth

Air flow and exhaust of the wind tunnel were initiated. A platform holding 8 virgin females (several platforms were set up with females in preparation for these observations) was placed in the tunnel on the ring stands 51 cm from the upwind end and 25 cm above the floor. Cups were

removed from motionless females. When any females began calling males were released into the wind tunnel as described above until one was induced to fly upwind to the female. The behaviors of both insects were recorded using a Sony Videocorder and Videocamera AVC-3450, from when the male first moved within approximately 30 mm of the female until several seconds after copulation was complete or, in the case of an unsuccessful mating attempt, until the sequence was terminated by the male or female. After all of the females that appeared willing to call on the platform were mated the platform was replaced with another containing 8 new females.

Observations were conducted at a temperature of 20°C. The light level at the platform was 200 lux (near the lower light level limit of the Videocamera). A black cloth was draped above the downwind end of the tunnel, producing a light level of 100 lux: this decreased the likelihood of males immediately flying up to a bright ceiling upon release. All observations were made during the 8 hours corresponding with scotophase in the rearing room, where the moths were previously held.

Trapping Experiments

Pherocon 1C insect traps from Zoecon Corporation were used in the trapping experiments concerned with effect of trap placement on male catch and comparison of capture potential for a sticky (Pherocon) and non-sticky (Granett-type) pheromone trap. The Pherocon trap (widely used in pheromone trapping research) consisted of 2 pieces of cardboard 28 x 22.6 cm, folded in a roof-like fashion, with the bottom sheet of cardboard folded upward and the top sheet suspended above the bottom one, folded downward (Figure 7). A "Y"-shaped length of steel wire served as a handle for hanging the trap and also passed through openings in both

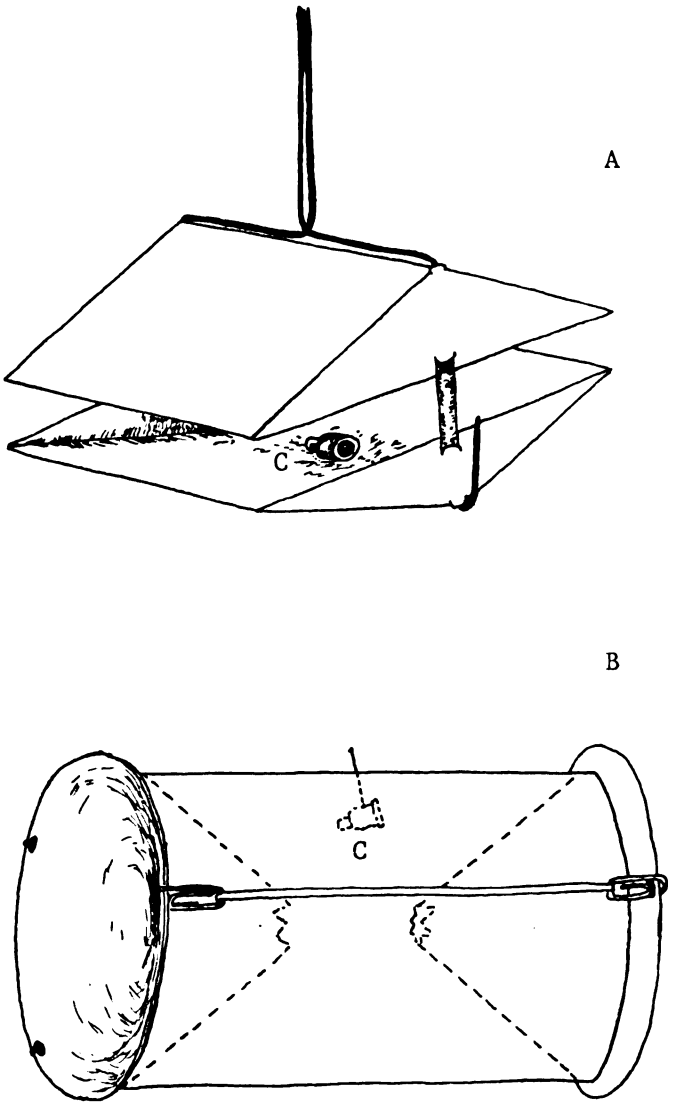

Figure 7. Pheromone traps. A: Pherocon 1C: B: Granett-type; C: pheromone-baited rubber septum.

sides of each piece of cardboard to hold the top and bottom in position and allow access to the interior of the trap. The inside bottom trap surface was coated with a very sticky glue. For trapping a pheromone-baited rubber septum was placed in the center of the sticky surface and male moths attracted to the pheromone entered the trap and became stuck in the glue.

During observations of male codling moth approach to a pheromone-baited trap, a Pherocon trap constructed as described above was used, but the sticky-surfaced bottom was replaced by a non-sticky upturned top piece. Males were enabled to enter the trap to approach the baited septum without being captured.

The Granett-type traps (Granett 1973) were made from a white polystyrene plastic cylinder 25 cm high and 19 cm in diameter and 2 plastic funnel-shaped end pieces projecting into the cylinder (Figure 7). A baited rubber septum was suspended inside the trap from the roof of the cylinder by a size #3 insect pin pushed through the plastic and into the rubber. A 4 x 2 x 0.4 cm block of plastic impregnated with Vapona was placed within the trap to kill moths entering it.

A. Effect of trap placement on trap catch

Pherocon 1C traps were placed in apple trees of an abandoned orchard located in Cascade, Kent County, Michigan. Three blocks were set up within the orchard with 3 trap locations in each block: a trap near the tip of a branch in the crown (the outside of the tree), a trap in the center of the crown (the inside of the tree) and a trap suspended from a length of twine extending from tree to tree between 2 rows (in the open between trees). All traps were hung approximately 2 m above the ground and were separated by at least 20 m. Each was baited with

1 mg of synthetic pheromone on a rubber septum. The traps were checked and captured males were counted, removed and discarded 9 times throughout the test period, with the septa and sticky bottoms being replaced once. Each time the traps were checked they were rotated to a new position within the block.

B. Comparison of catch in a sticky trap and a non-sticky trap

Four areas within 3 abandoned apple orchards located close together near the Clinical Sciences Center of Michigan State University were chosen as test blocks for this experiment. Within each block 2 Pherocon 1C traps and 2 Granett-type traps were placed in apple trees, at least 20 m apart and approximately 1.5 m above the ground. Each trap was baited with 1 mg of synthetic pheromone on a rubber septum. They were checked 6 times during which captured moths were counted and removed, and all 4 traps within each block were moved to another part of that block.

C. Observations of male behavior at a Pherocon trap

A non-sticky Pherocon 1C trap was hung approximately 1.6 m high on a branch of an apple tree in an insecticide-free experimental orchard maintained at Fennville, Allegan County, Michigan. The trap was set up and a rubber septum baited with 1 mg of synthetic pheromone placed within it 1 to 2 hours before sunset. Male codling moths approaching the trap were observed and a verbal description of their behaviors recorded and transcribed.

RESULTS

Female Calling Experiments

A. Periodicity of female calling under a 16 hour photophase and 8 hour scotophase regime

Figure 8 depicts the results of observations on the mixed age (0-6 days old) collection of virgin females throughout the 24 hour day. Under constant temperature a high degree of calling behavior was initiated with the onset of scotophase, continued throughout the dark period and was terminated at the beginning of photophase. Analysis of this behavior for each of the 6 age groups composing the total reveals that for moths 1-6 days old no significant differences apparently exist in number of females calling, $\bar{x}$ calling time (the point at which half of the calling-hours exhibited for the day was reached) or $\bar{x}$ number of hours called per female (Figures 9 and 10 and Table 1). The youngest moth group, aged 0-1 day old, however, did call less than the other groups. Fewer 0-1 day old females called than those that were 1-6 days old, and the young ones that did, engaged in it for a shorter length of time. Also, as measured by $\bar{x}$ calling time, the majority of calling of 0-1 day old moths took place earlier in the evening.

B. Periodicity of female calling under continuous photophase

Observations in the previous experiment demonstrated a diel periodicity of female calling behavior apparently associated with the beginning and end of scotophase. Data from this experiment and the next illustrate that this rhythm has an endogenous basis and therefore is circadian.

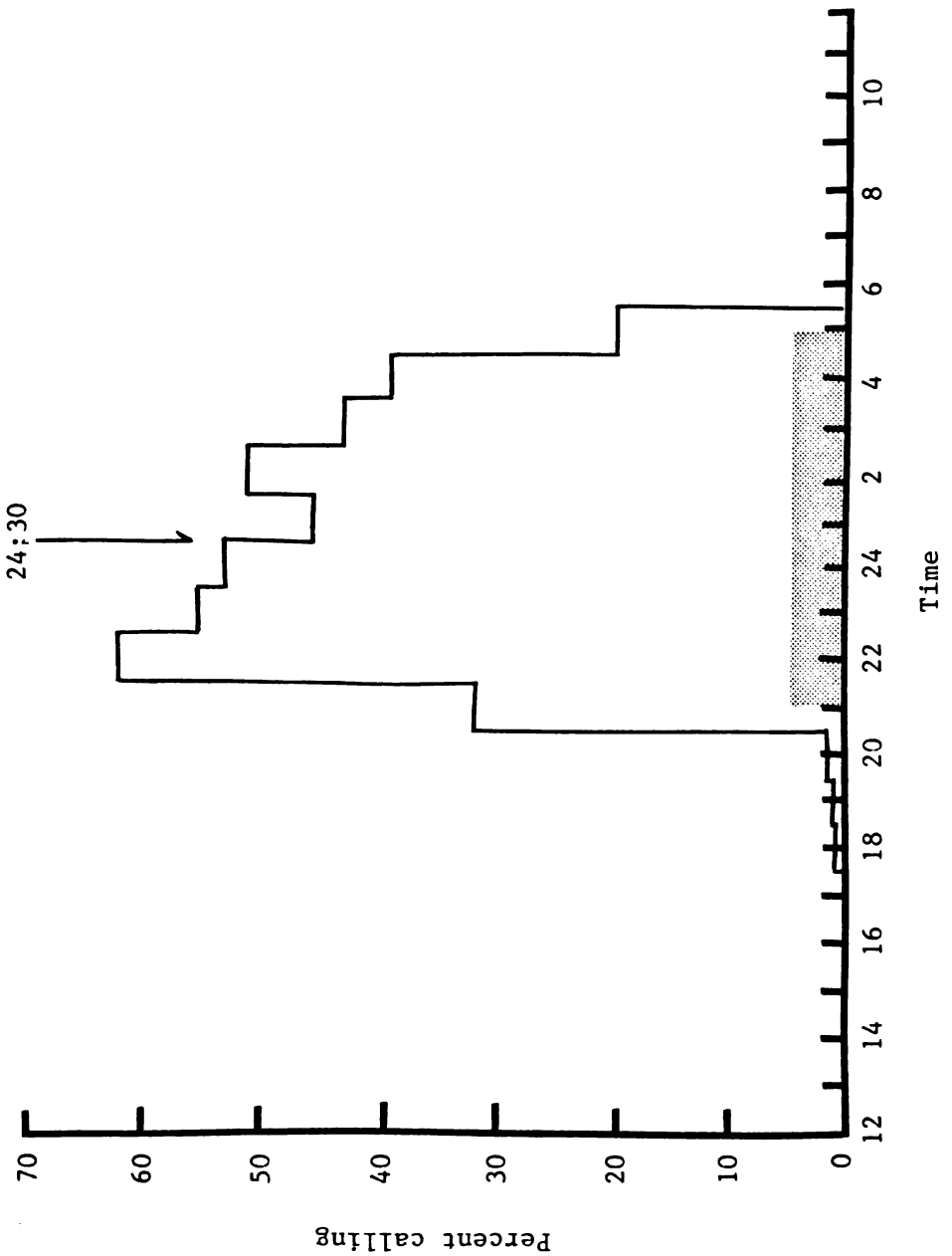

Figure 8. Percent calling of female codling moths aged 0 to 6 days old throughout 24 hours. Temperature = 23°C. Shading denotes scotophase. Arrow represents $\bar{x}$ calling time. N = 360.

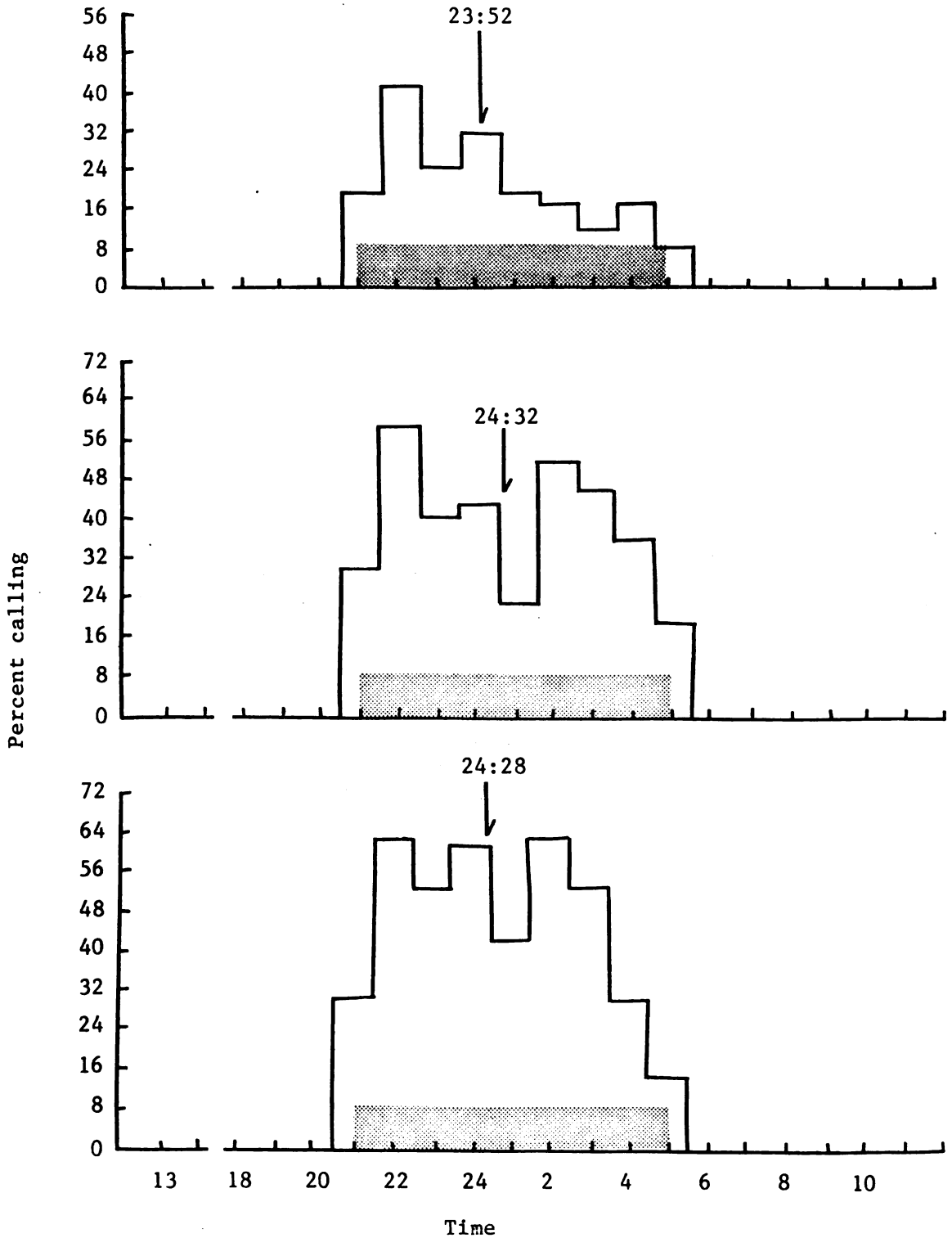

Figure 9. Percent calling of female codling moths throughout 24 hours. Temperature = 23°C. Shading denotes scotophase. Arrows represent $\bar{x}$ calling times. Moth age: A = 0-1 day old, B = 1-2 days old, C = 2-3 days old. N = 60.

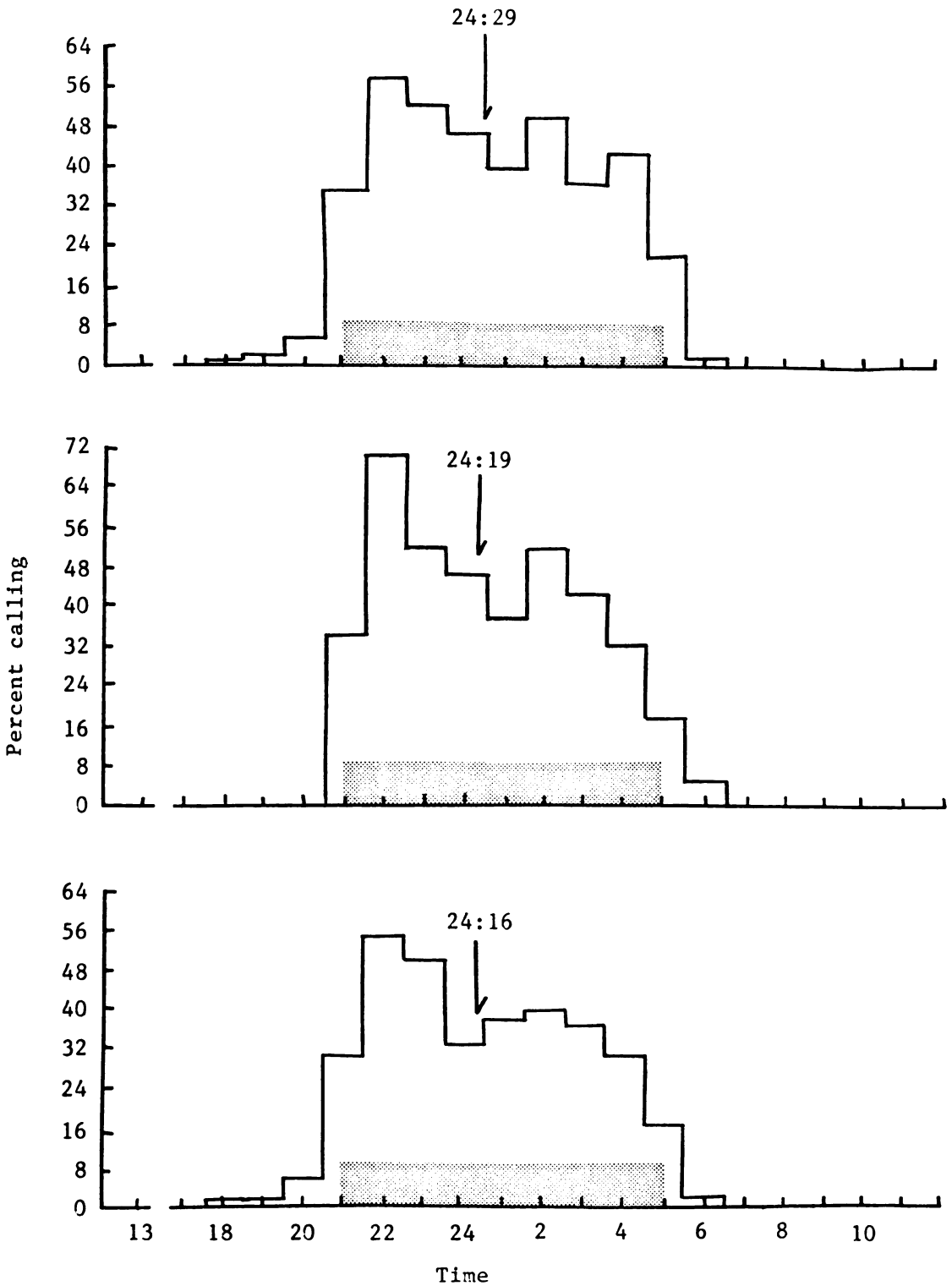

Figure 10. Percent calling of female codling moths throughout 24 hours. Temperature = 23°C. Shading denotes scotophase. Arrows represent $\bar{x}$ calling times. Moth age: D = 3-4 days old, E = 4-5 days old, F = 5-6 days old. N = 60.

Table 1. Effect of age on calling behavior of female codling moths.

Age of adults (days)	% observed calling[1,2]	$\bar{x}$ calling time (hour:minute)[3,4]	$\bar{x}$ hours called per female (all moths) ($\pm$ S.D.)[2,3]	$\bar{x}$ hours called per female (only calling females) ($\pm$ S.D.)[3]
0-1	61a	23:52a	2.0a $\pm$ (2.6)	3.3a $\pm$ (2.5)
1-2	85b	24:32b	3.9b $\pm$ (2.8)	4.6b $\pm$ (2.4)
2-3	87b	24:28b	4.5b $\pm$ (3.0)	5.3b $\pm$ (2.6)
3-4	80b	24:29b	4.4b $\pm$ (3.2)	5.4b $\pm$ (2.6)
4-5	78b	24:19b	3.8b $\pm$ (3.3)	4.9b $\pm$ (3.0)
5-6	73b	24:16b	3.8b $\pm$ (3.4)	5.1b $\pm$ (2.9)

[1] Values within columns followed by same letter not significantly different (χ^2, P = .05)

[2] N = 60

[3] Values within columns followed by same letter not significantly different (ANOVA, P = .05; means separated using Student-Newman-Keuls multiple range test)

[4] N = 60 for calculation of $\bar{x}$ calling time; N = 36 for test of significance: $\bar{x}$ calling time was designated on a numerical scale from 00:01 (12:01 A.M.) to 24:00 (12:00 midnight); there is no numerical representation for "did not call," so these moths had to be eliminated from the $\bar{x}$ calling time comparison; all age groups contained at least 36 calling females so in each comparison (to keep sample sizes equal) the first 36 values were compared and any remaining values discarded.

Figure 11 indicates that when scotophase was absent over a 2 day interval most of the females called during the 8 hours at which scotophase had been occurring: during days 1 and 2, 99.3% of the calling-hours occurred within 8 hours; during days 3 and 4, 87.8% occurred within 8 hours. The increase from a low level of calling to maximum was sharp when the lights-off cue was present: from less than 8% to greater than 60% calling in 2 hours; while with no cue the change was more gradual: less than 8% to approximately 50% taking 5 hours. A similar pattern was apparent at calling termination. When a lights-on cue was available calling ended abruptly; when photophase was continuous termination was more gradual.

Under continuous photophase $\bar{x}$ calling time was significantly shifted approximately 2.5 hours later into the evening than with a photocycle of L:D 16:8 (Table 2). This resulted from maximum calling as well as calling termination occurring later in the night on days 3 and 4 than the first two. It should be noted, however, that the $\bar{x}$ calling time shift occurred because of a change in the shape of the calling curve, rather than an increase in the length of the period. This is indicated by the fact that the new $\bar{x}$ calling time, from the first day with no scotophase, was maintained approximately 24 hours later on the following day.

C. Periodicity of female calling under continuous scotophase

Under conditions of continuous scotophase (Figure 12) calling occurred with a periodicity very similar to that observed with the L:D 16:8 photocycle. As with continuous photophase transition from no calling to maximum calling and vice versa was more gradual than when lights-on and lights-off cues were present: with the cues, 0% to greater than

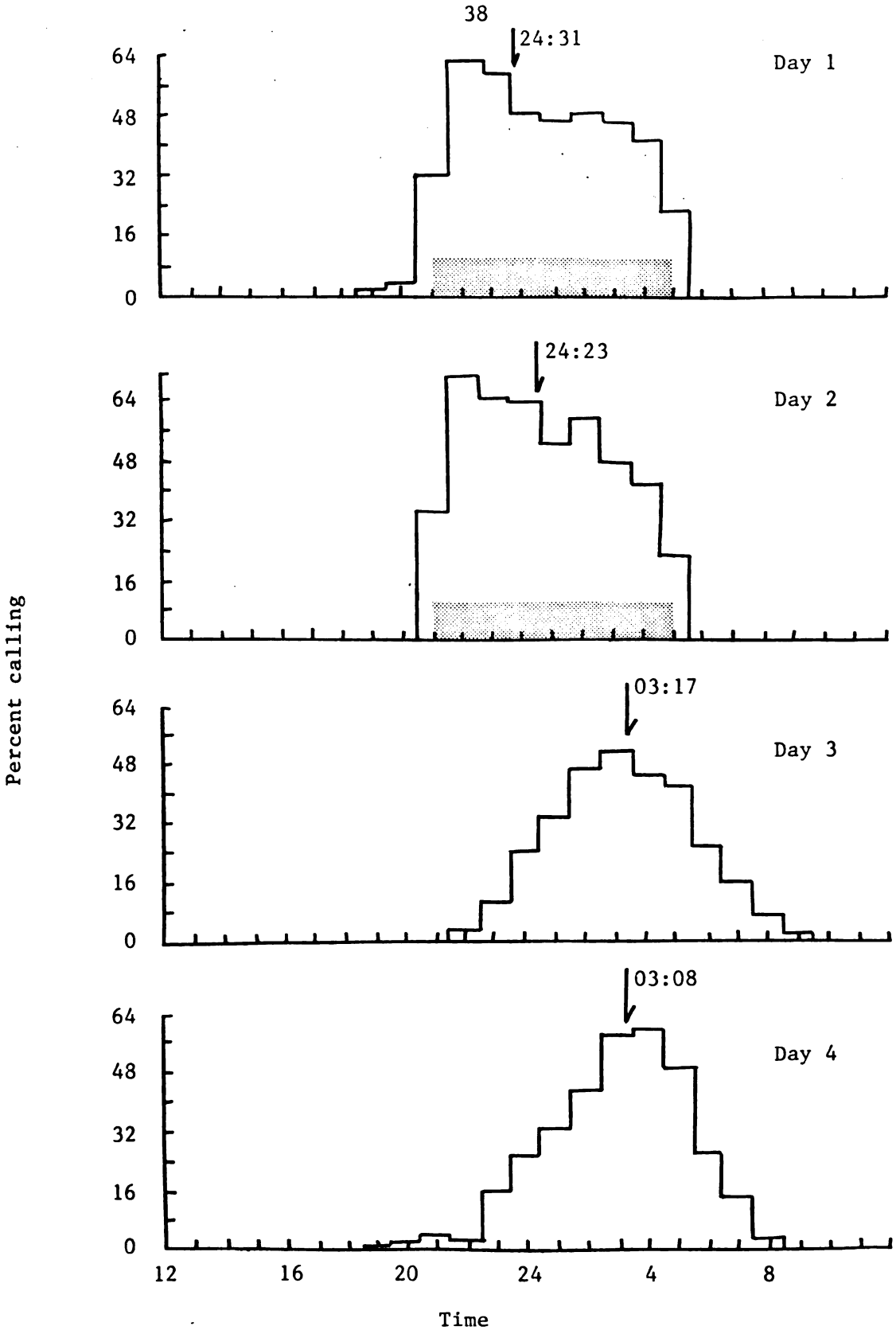

Figure 11. Percent calling of female codling moths under extended photophase. Temperature = 23°C. Shading denotes scotophase. Arrows represent $\bar{x}$ calling times. N = 100.

Table 2. Mean calling times of female codling moths under extended photophase and extended scotophase. Temperature = 23°C.

Day	Extended photophase $\bar{x}$ calling time (hour:minute)[1]	Extended scotophase $\bar{x}$ calling time (hour:minute)[1]
1	24:31a	24:27a
2	24:23a	01:34a
3	03:17b	24:23a
4	03:08b	23:29a

[1] Values within columns followed by same letter not significantly different (ANOVA, P = .05; means compared using Student-Newman-Keuls multiple range test)
N = 100 for calculation of $\bar{x}$ calling time; N = 50 for test of significance. Each group contained at least 50 calling females, so to discard non-calling females and keep sample sizes equal comparisons were made using only the first 50 $\bar{x}$ calling time values in each group.

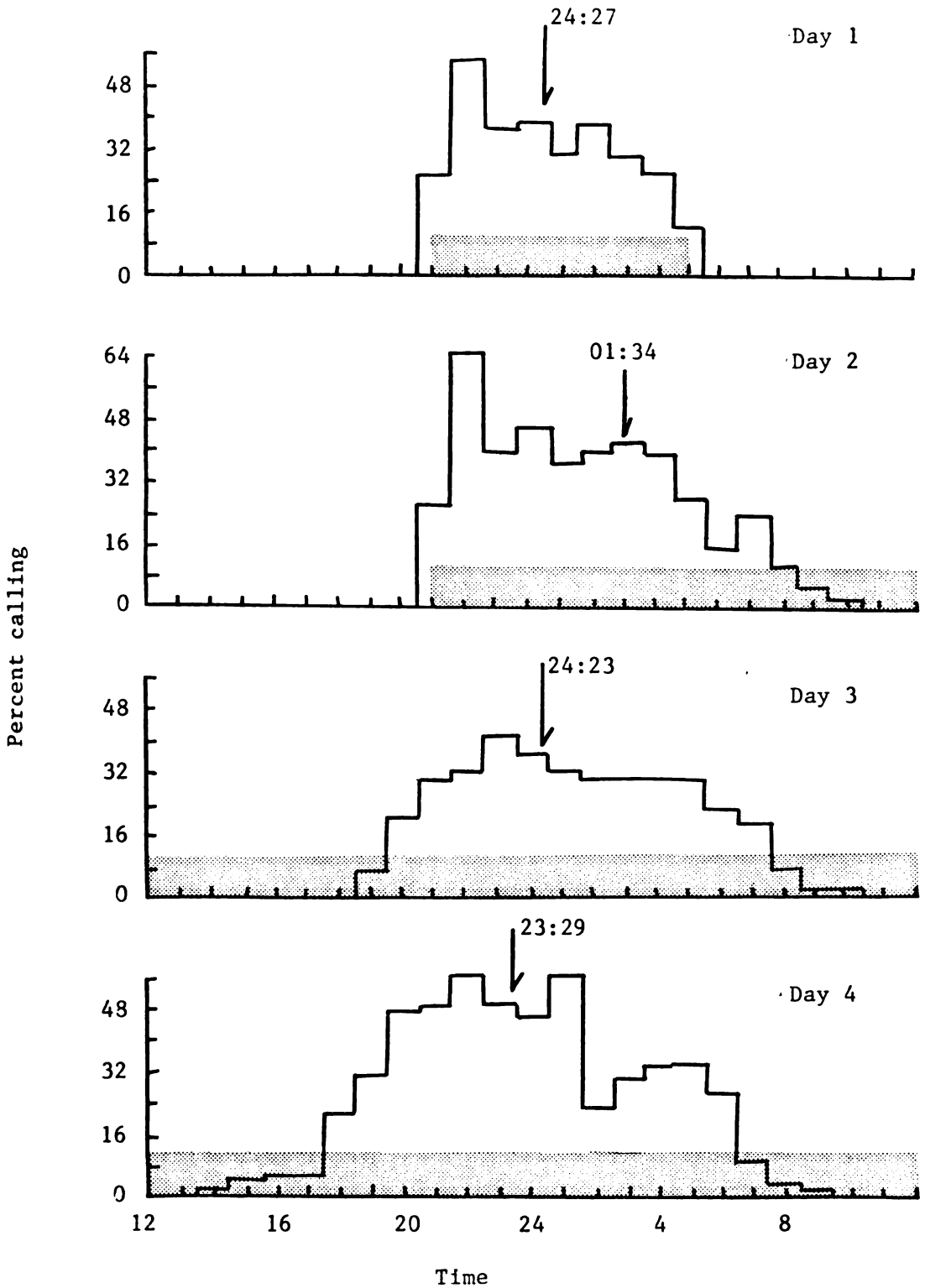

Figure 12. Percent calling of female codling moths under extended scotophase. Temperature = 23°C. Shadind denotes scotophase. Arrows represent $\bar{x}$ calling times. N = 100.

48% calling in 2 hours; without cues 0% to 48% calling in 5 to 6 hours. Under these conditions, nevertheless, in addition to calling being extended several hours beyond the time photophase had previously begun, initiation of calling occurred several hours prior to subjective lights-off. Thus, although the $\bar{x}$ calling time was not altered significantly (Table 2), the overall duration of calling was increased: on day 1, 100% of calling occurred within 8 hours; on day 4, 94% of calling occurred within 12 hours.

D. Effects of a decrease in temperature upon periodicity of calling

The daily pattern of calling behavior observed under a constant ambient temperature of 23°C was modified in the presence of both a temperature drop and an extended temperature of 16°C. As shown in Figure 13, a 7 degree decrease in temperature during scotophase resulted in complete termination of calling, no matter whether the drop occurred during the middle or very beginning of the dark cycle. Conversely, the same 7 degree change 3 hours prior to the onset of scotophase induced a very high degree of calling in the moths during a time when this behavior was very low at 23°C (Figure 14). With a pre-scotophase temperature drop much of the calling was discontinued shortly with lights-off: 91% of calling occurred during darkness at 23°C, while only 42% took place during darkness at 16°C.

On the day following the temperature drop moths maintained at 16°C showed a significant shift in the $\bar{x}$ calling time, approximately 5 hours earlier than the $\bar{x}$ calling time of moths at 23°C (Table 3). Few females called during scotophase compared to a high level of calling during the latter part of photophase.

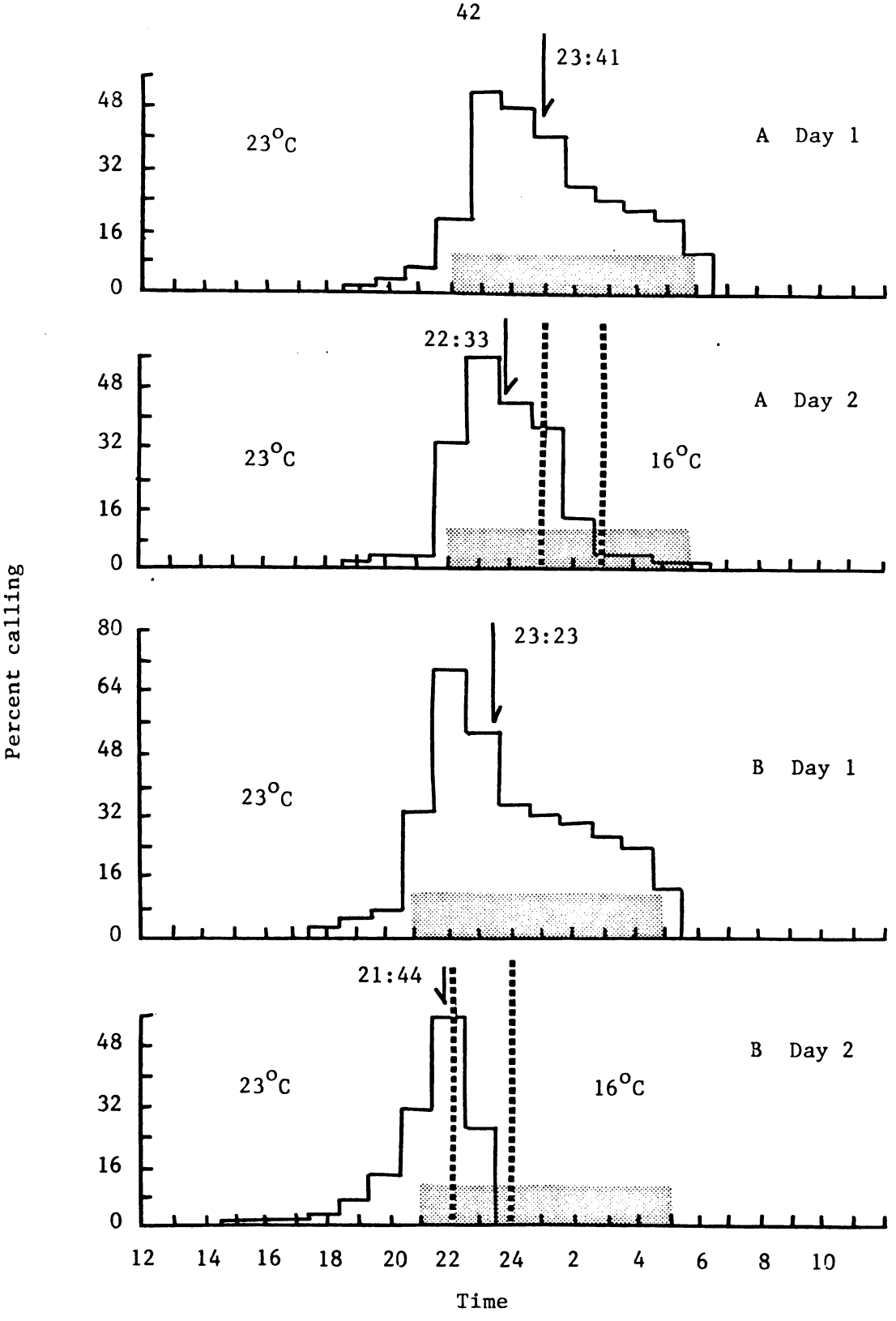

Figure 13. Effect of decrease in temperature on female codling moth calling behavior. A: temperature decrease 3 hours into scotophase; B: temperature decrease 1 hour into scotophase. Broken line represents decrease in temperature as in Figure 2. Shading denotes scotophase. Arrows represent $\bar{x}$ calling times. N = 100.

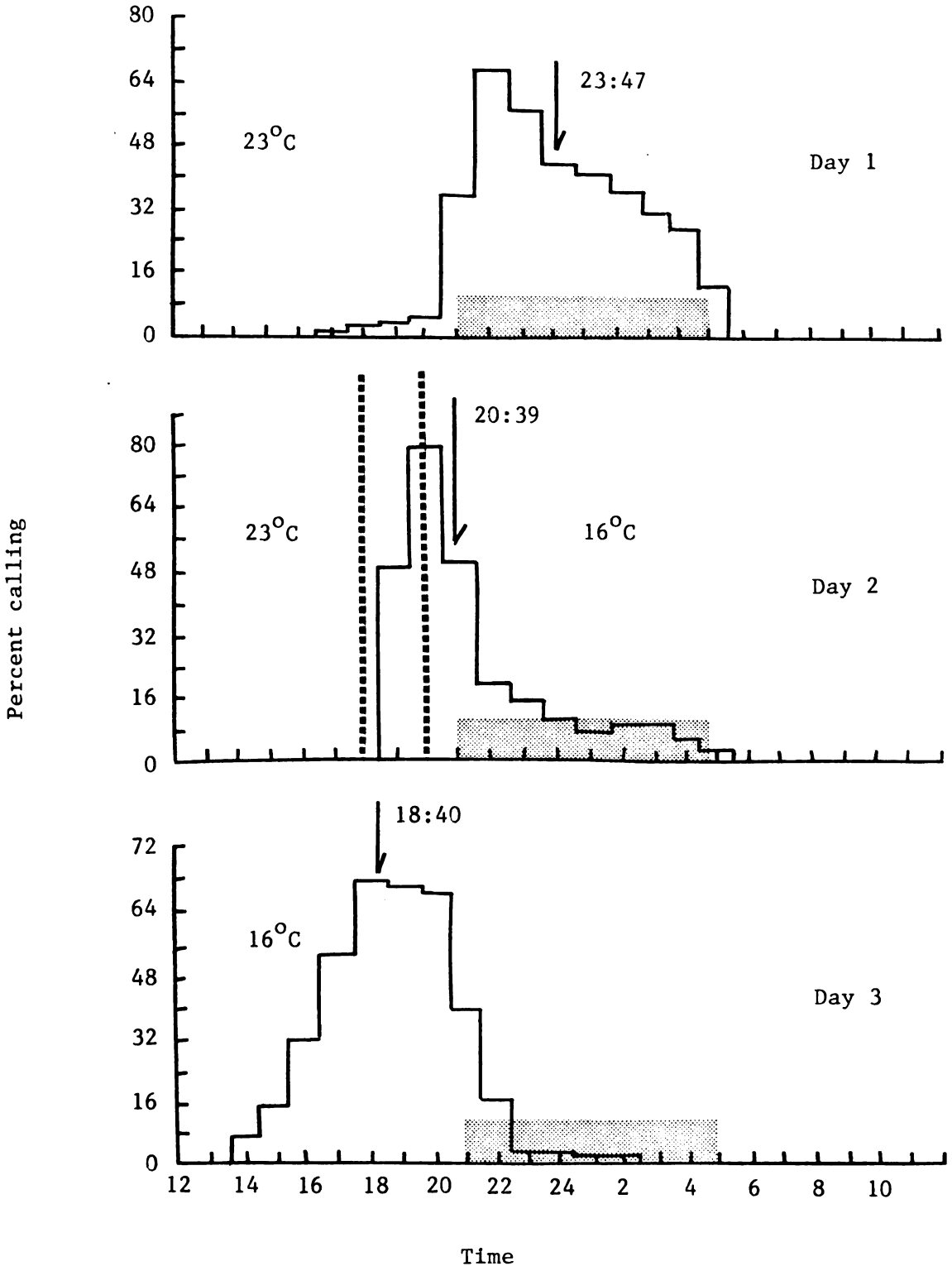

Figure 14. Effect of decrease in temperature on female codling moth calling behavior. Broken line represents decrease in temperature as in Figure 2. Shading denotes scotophase. Arrows represent $\bar{x}$ calling times. N = 100.

Table 3. Effect of decrease in temperature on $\bar{x}$ calling time of female codling moths.

Day	Temperature decrease 3 hours into scotophase: $\bar{x}$ calling time[1]	Temperature decrease 1 hour into scotophase: $\bar{x}$ calling time[1]	Temperature decrease 3 hours prior to scotophase: $\bar{x}$ calling time[1]
1	23:41a	23:23a	23:47a
2	22:33a	21:44a	20:39b
3	--	--	18:40b

[1] Values within columns followed by same letter not significantly different (ANOVA, P = .05; means separated using Student-Newman-Keuls multiple range test); N = 100 for calculation of $\bar{x}$ calling time; N = 50 for test of significance. Each group contained at least 50 calling females, so to discard non-calling females and keep sample sizes equal comparisons were made using only the first 50 $\bar{x}$ calling time values in each group.

E. Effects of relative humidity on the periodicity of female calling

The data from this experiment tabulated in Tables 4 and 5 indicate very little discernible effect on the degree of calling between groups at each test time or the $\bar{x}$ calling time of each group as a result of differing relative humidities.

Male Pheromone Response Experiments

A. Periodicity of male response under a 16 hour photophase and 8 hour scotophase regime

During this bioassay series behaviors exhibited by males in the tubes both prior to and following exposure to synthetic pheromone were described. The purposes were elucidation of the types of behavior elicited by pheromone stimulation, as well as possible determination of a key behavior usuable as a criterion for response in future bioassays.

States of behavior observed were: 1) motionlessness; 2) antennal raising: motionless insects rested with antennae either pressed laterally against the body or erect, perpendicular to the substrate upon which they were sitting; addition of pheromone to the airstream sometimes elicited raising of antennae that had been held against the body; 3) wing fanning: the males' tarsi were in contact with the tube, but wings were beating rapidly; for these bioassays activity was only considered wing fanning if it had a duration of 2 or more seconds; 4) upwind orientation: walking upwind in the tube, with or without wing fanning; 5) non-anemotactic movement: either hopping, walking or wing beating in bursts of less than 2 seconds, usually with little movement in the tube or alternating, apparently undirected, upwind and downwind movement; and 6) copulatory attempts: sometimes occurring when a male fanning and walking upwind contacted or passed within a few millimeters of another male; the

Table 4. Effect of relative humidity on degree of female codling moth calling at 5 intervals. Temperature = 23°C. Scotophase began at 21:00. Photophase began at 05:00.

Time	% females calling at relative humidity of:[1] 25%	50%	75%	100%
18:00	0.0a	1.0a	0.0a	0.0a
20:00	4.8a	12.3a	8.6a	8.6a
22:00	66.7a,b	78.1a,b	81.0a	62.8b
24:00	55.2a	57.1a	73.3a	52.4a
02:00	41.0a	47.6a	56.2a	47.6a

[1] Values within rows followed by same letter not significantly different (ANOVA, P = .05; means compared using Student-Newman-Keuls multiple range test); Values transformed to arcsin $\sqrt{p}$ for analysis; untransformed values reported in Table 4. N = 105

Table 5. Effect of relative humidity on $\bar{x}$ calling time of female codling moths. Temperature = 23°C. Scotophase began at 21:00. Photophase began at 05:00.

% relative humidity	$\bar{x}$ calling time (hour:minute)[1]
25	23:27
50	23:14
75	23:33
100	23:33

[1] No significant difference between any values (ANOVA, P = .05; means compared using Student-Newman-Keuls multiple range test). N = 105

approaching male curved his abdomen laterally and forward while opening his valves and then probed the abdomen of the second male.

Figure 15 depicts the changes in the degree of 4 of the most common classes of activity throughout a 24 hour day. All values represent a measure of the behavior during the 30 seconds when pheromone was present in the airstream, with corrections made for pre-pheromone background activity according to the formula:

$$\% \text{ response} = 100 \times \left(\frac{\text{no. active when pheromone present} - \text{no. active during background}}{N - \text{no. active during background}} \right)$$

Of the 4 behaviors non-anemotactic movement remained at the lowest level throughout the 24 hours. Wing fanning, upwind orientation and fanning with simultaneous upwind movement increased markedly with the onset of scotophase and were greatly reduced after initiation of photophase. Binomial confidence intervals (95%) for the measurement of wing fanning and upwind orientation overlap at the same times indicating no significant difference in thresholds for these behaviors with 100 ng of pheromone. Because a key behavioral response of the male in the field is upwind anemotactic flight, upwind orientation in the tube was selected as the key behavior for bioassays.

Diel periodicity of male pheromone response is represented in Figure 16. Under constant temperature upwind orientation elicited by pheromone was most evident during the dark part of the photocycle ($\bar{x}$ response time occurring close to the middle of scotophase). Spontaneous non-anemotactic movement of males observed during the 30 second background activity measurement period (prior to introduction of pheromone into the airstream) also showed a similar trend (Figure 17).

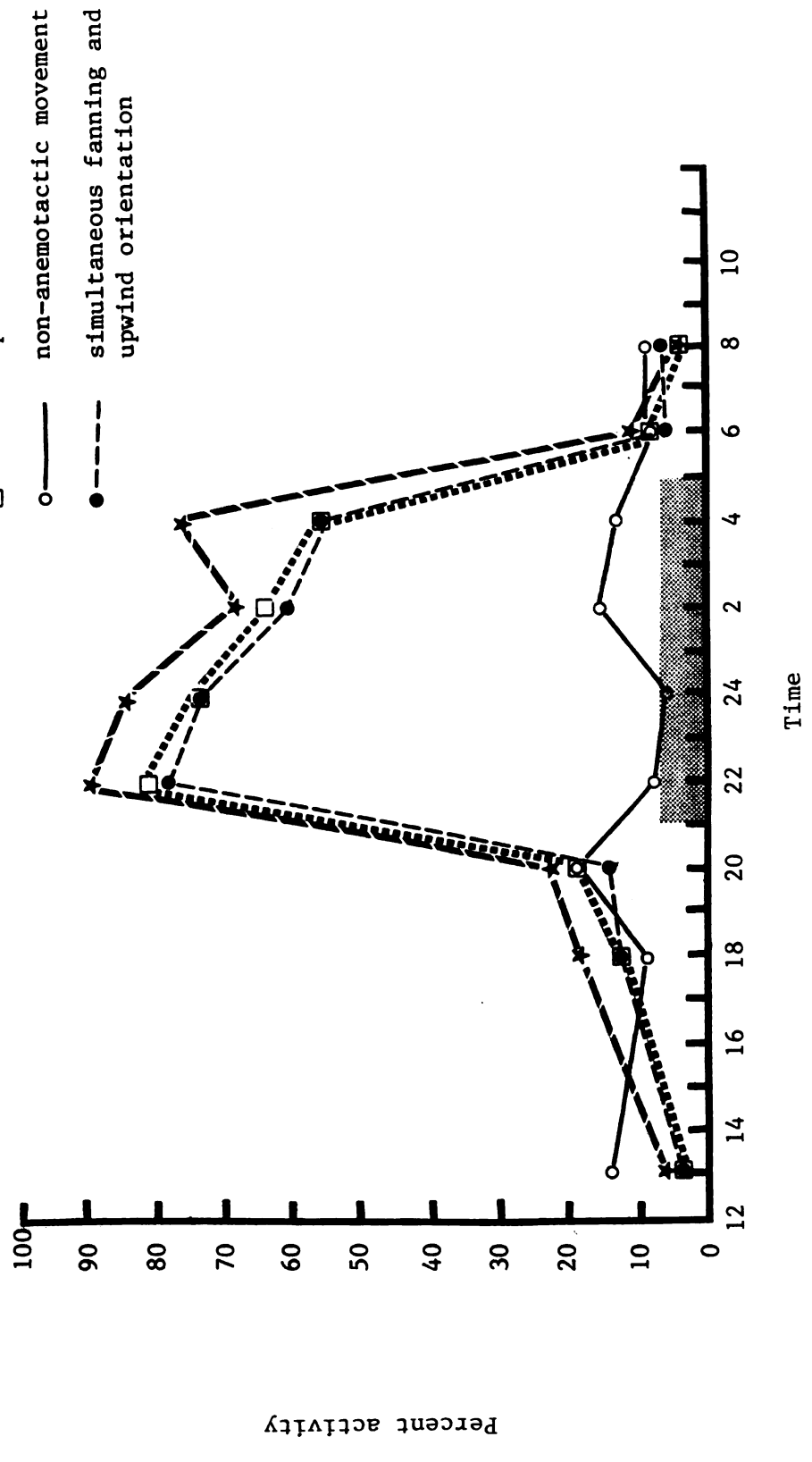

Figure 15. Male codling moth behaviors elicited by 100 ng of synthetic pheromone during 24 hours. Observations were 30 seconds in duration with pheromone present and were corrected for activity occurring during 30 seconds prior to pheromone exposure. Temperature = 23°C. Shading denotes scotophase. N = 60.

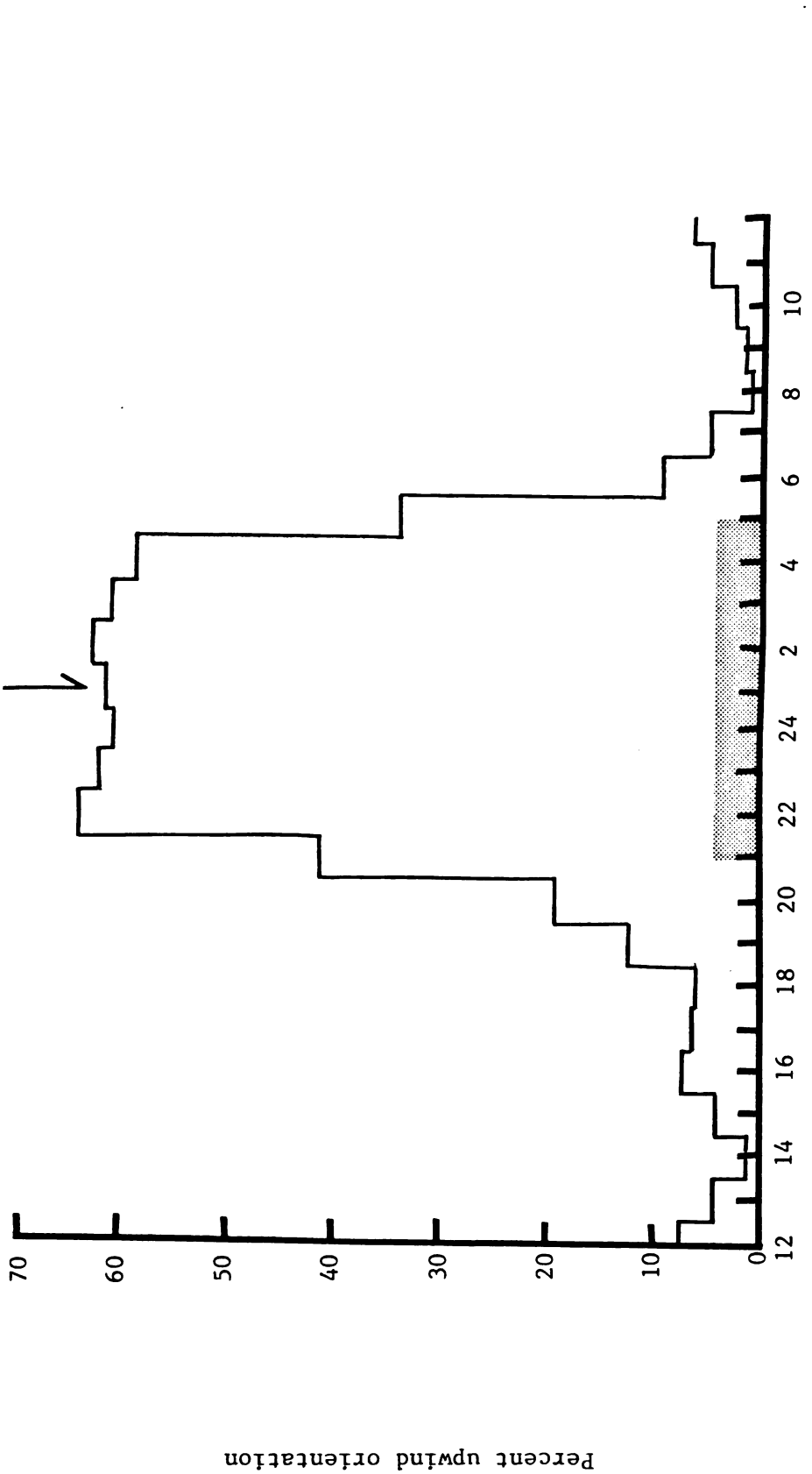

Figure 16. Male codling moth upwind orientation elicited by 30 seconds of exposure to 100 ng of synthetic pheromone during 24 hours. Temperature = 23°C. Shading denotes scotophase. Arrow represents $\bar{x}$ response time. N = 72.

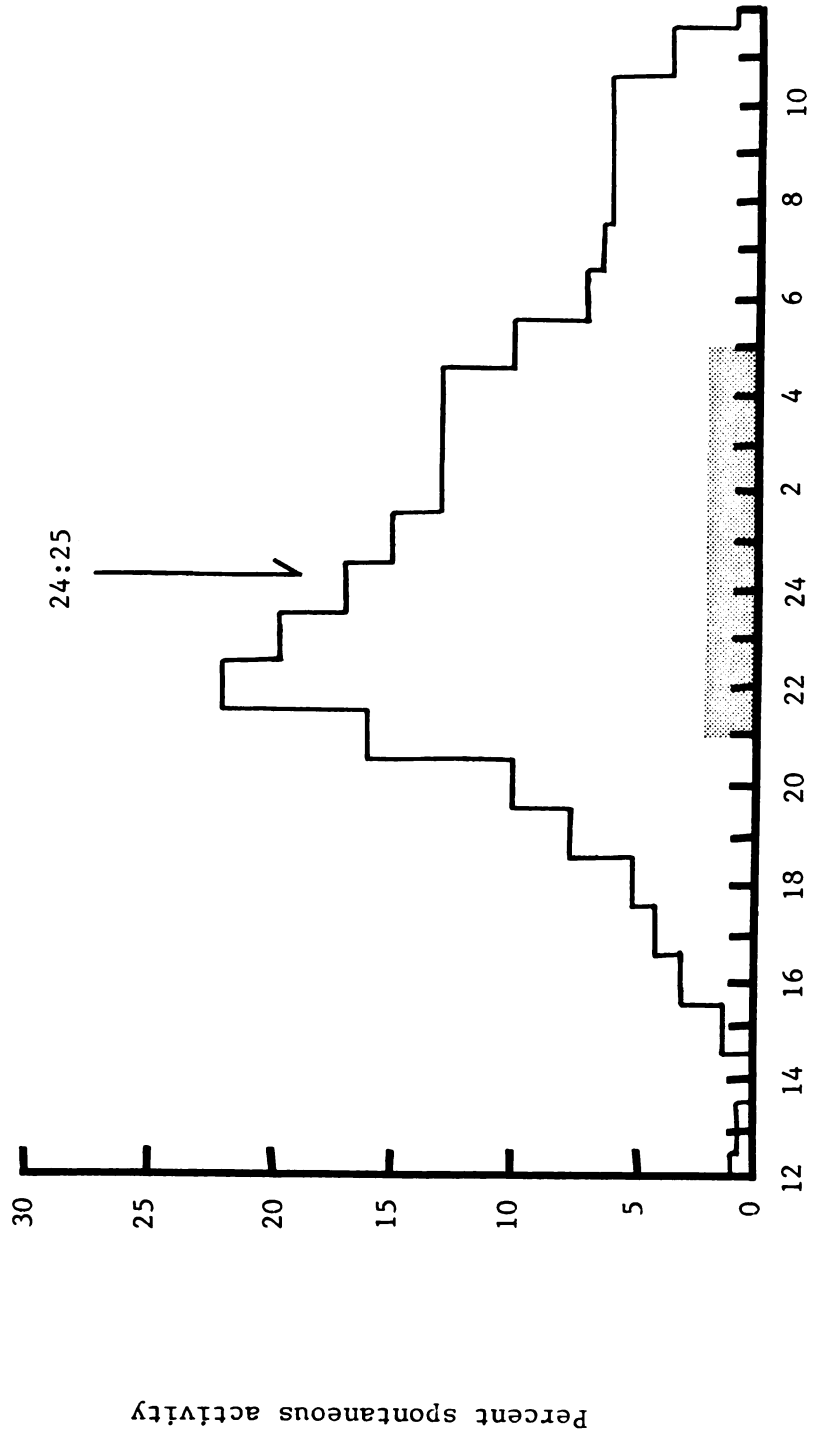

Figure 17. Male codling moth spontaneous non-anemotactic activity (hopping, walking and wing-beating) during 24 hours. Observations occurring for 30 seconds with no pheromone present. Temperature = 23°C. Shading denotes scotophase. Arrow represents $\bar{x}$ activity time. N = 72.

B. Periodicity of male response under continuous photophase

Male bioassays under 2 days with L:D 16:8 and 2 days with no scotophase (Figure 18 and Table 6) indicate that pheromone responsiveness occurred with a periodicity close to 24 hours, both in the presence and absence of lights-on and lights-off cues. When an abrupt light/dark cue was available, the number of upwind orientations elicited by pheromone changed greatly over a short period of time: 16% to 80% in 2 hours. During continuous photophase the degree of upwind orientation continued to rise and fall, although the change was more gradual: 16% to 60% in 8 hours.

C. Effects of a decrease in temperature upon periodicity of pheromone response

When male codling moths experienced a decrease in ambient temperature of 7 degrees (from 23° to 16°C) over a 2 hour period beginning 3 hours prior to the start of scotophase the number of pheromone-elicited upwind orientations appeared to be unaltered during the remainder of photophase; but the degree of upwind movement exhibited during darkness, which was very high at 23°C, was extremely depressed at 16°C (Figure 19). On the following day, with the moths still maintained at 16°C, little upwind orientation in response to pheromone was observed. With this decrease in temperature the $\bar{x}$ time of response was shifted approximately 2.5 hours earlier into the day. Unlike the shift in $\bar{x}$ calling time of the females during a decrease in temperature (Table 3), the shift in male upwind orientation was a result of the extremely lowered response level during scotophase, rather than an increase in response during photophase.

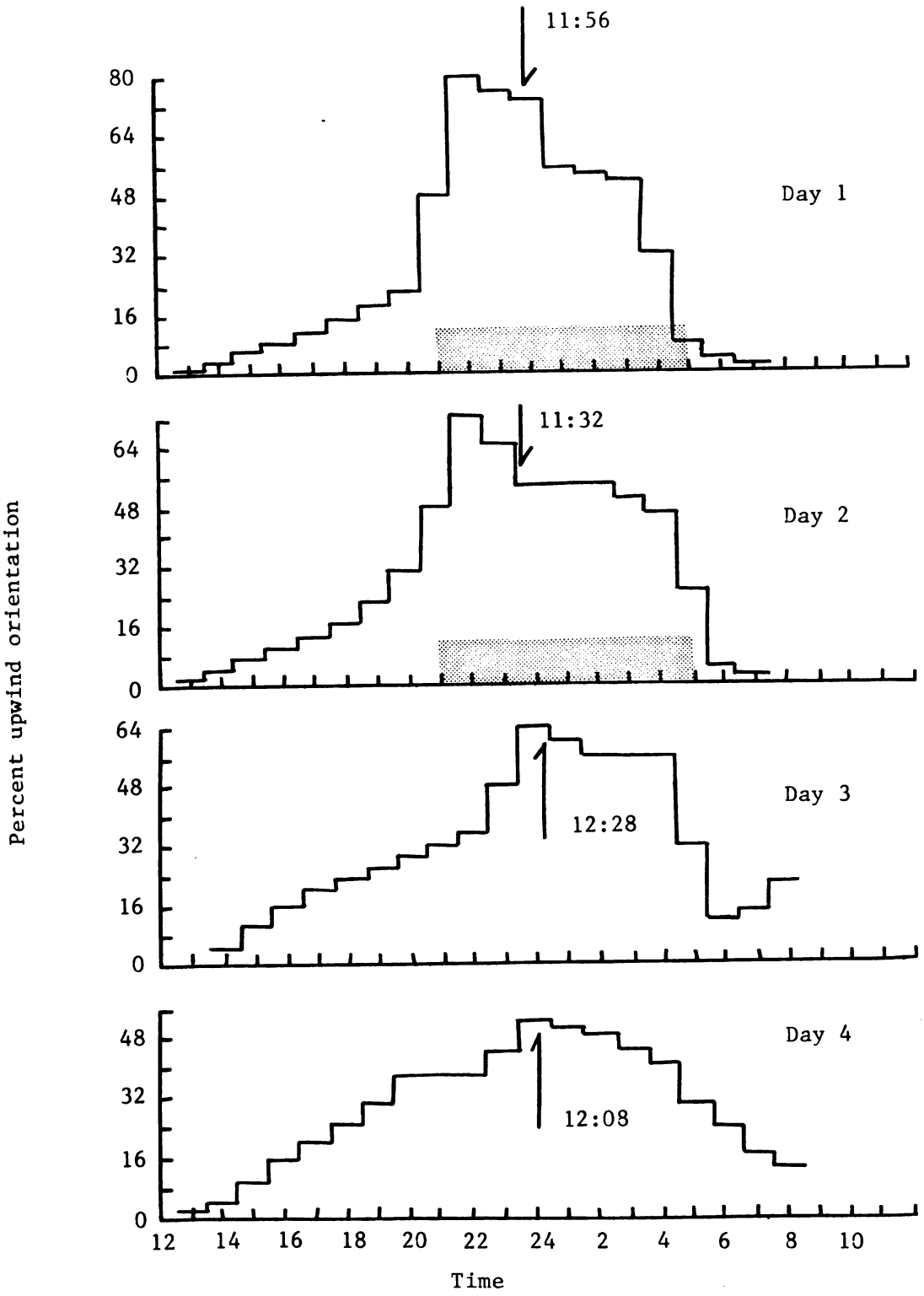

Figure 18. Male codling moth upwind orientation elicited by 100 ng of synthetic pheromone under extended photophase. Temperature = 23°C. Shading denotes scotophase. Arrows represent $\bar{x}$ response times. N = 60.

Table 6. Mean response times of male codling moths to 100 ng of synthetic pheromone under extended photophase.

Day[1]	$\bar{x}$ response time (hour:minute)[2]
1	11:56
2	11:32
3	12:28
4	12:08

[1] Normal photocycle on day 1 and day 2; continuous photophase on day 3 and day 4

[2] No significant difference between means (ANOVA, P = .05; means compared using Student-Newman-Keuls multiple range test). N = 60

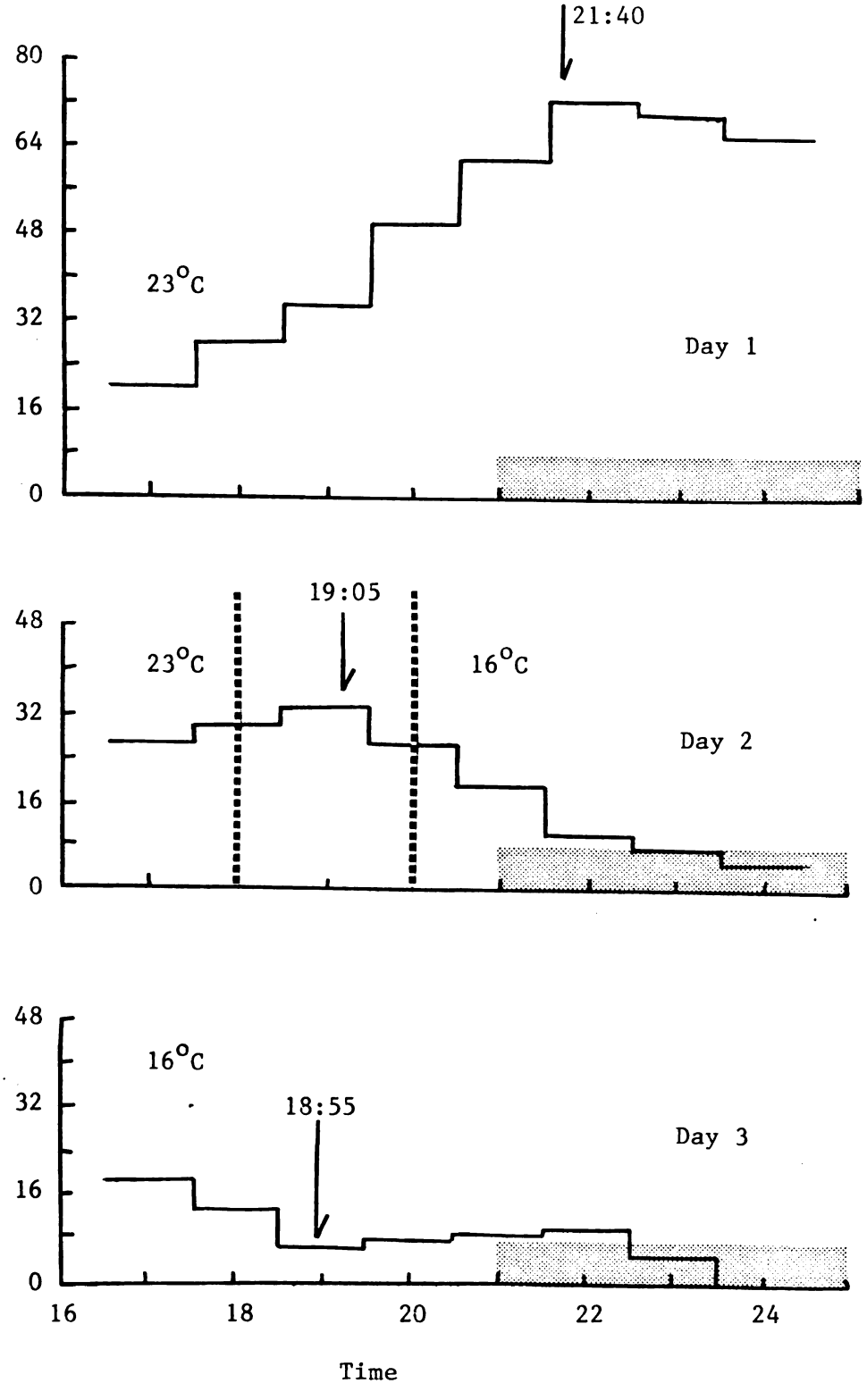

Figure 19. Effect of a decrease in temperature on male pheromone responsiveness. Broken line represents period of temperature decrease as in Figure 4. Shading denotes scotophase. Arrows represent $\bar{x}$ response times. N = 60.

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D. Effects of a decrease in temperature and decreases in light intensity upon periodicity of pheromone response

Table 7 presents the data obtained from testing pheromone response level at one time, 2 hours prior to the start of scotophase, for moths subjected to various temperature and light conditions. At 23°C, a light intensity decrease from 1500 to 150 lux was sufficient to increase significantly the percentage of males orienting upwind to pheromone. Further decreases in light intensity did not increase upwind movement beyond that effected by the 90% reduction. A 7 degree temperature decrease coupled with lowered light intensities gave less clear results: a significant decrease in activity occurred at 15 lux, while there appeared to be no differences between upwind orientation at 1500, 150 and 1.5 lux. When comparisons were made between number of moths moving upwind under equal light intensities but different temperatures few differences were found between those held at 23°C and those experiencing a decrease in temperature to 16°C (Table 8). The only effect was at 15 lux, where a significant decrease was observed.

E. Effect of pheromone dosage on male response

The male behaviors described on tape and transcribed were used to measure pheromone response by tallying those orienting upwind to each sample. Figure 20 indicates that from a dosage of 10^{-5} to 10^{-3} µg of synthetic pheromone little upwind orientation was observed, whereas, between 10^{-3} and 10^{-1} µg the number of males responding increased. Of the samples tested in this experiment 10^{-1} and 10^{0} µg produced the greatest upwind orientation, with no significant difference between the two on the basis of 95% binomial confidence intervals. An increase to 10^{2} µg greatly reduced upwind orientation.

Table 7. Effect of decreases in light intensity on % of male codling moth upwind orientation elicited by 100 ng of synthetic pheromone. Temperature and light decreased 3 hours prior to scotophase. Upwind orientation measured 2 hours prior to scotophase.

Temperature conditions	$\bar{x}$ % upwind orientation at light levels[1] 1500 lux	150 lux	15 lux	1.5 lux
23°C	41a	58b	76b	66b
16°C	50a	56a	28b	50a

[1] Values within rows followed by same letter not significantly different (χ^2, P = .05). N = 60

Table 8. Effect of decrease in temperature on % male codling moth upwind orientation elicited by 100 ng of synthetic pheromone. Temperature and light decreased 3 hours prior to scotophase. Upwind orientation measured 2 hours prior to scotophase.

Temperature conditions	$\bar{x}$ % upwind orientation at light levels[1] 1500 lux	150 lux	15 lux	1.5 lux
23°C	41a	58a	76a	66a
16°C	50a	56a	28b	50a

[1] Values within columns followed by same letter not significantly different (χ^2, P = .05). N = 60

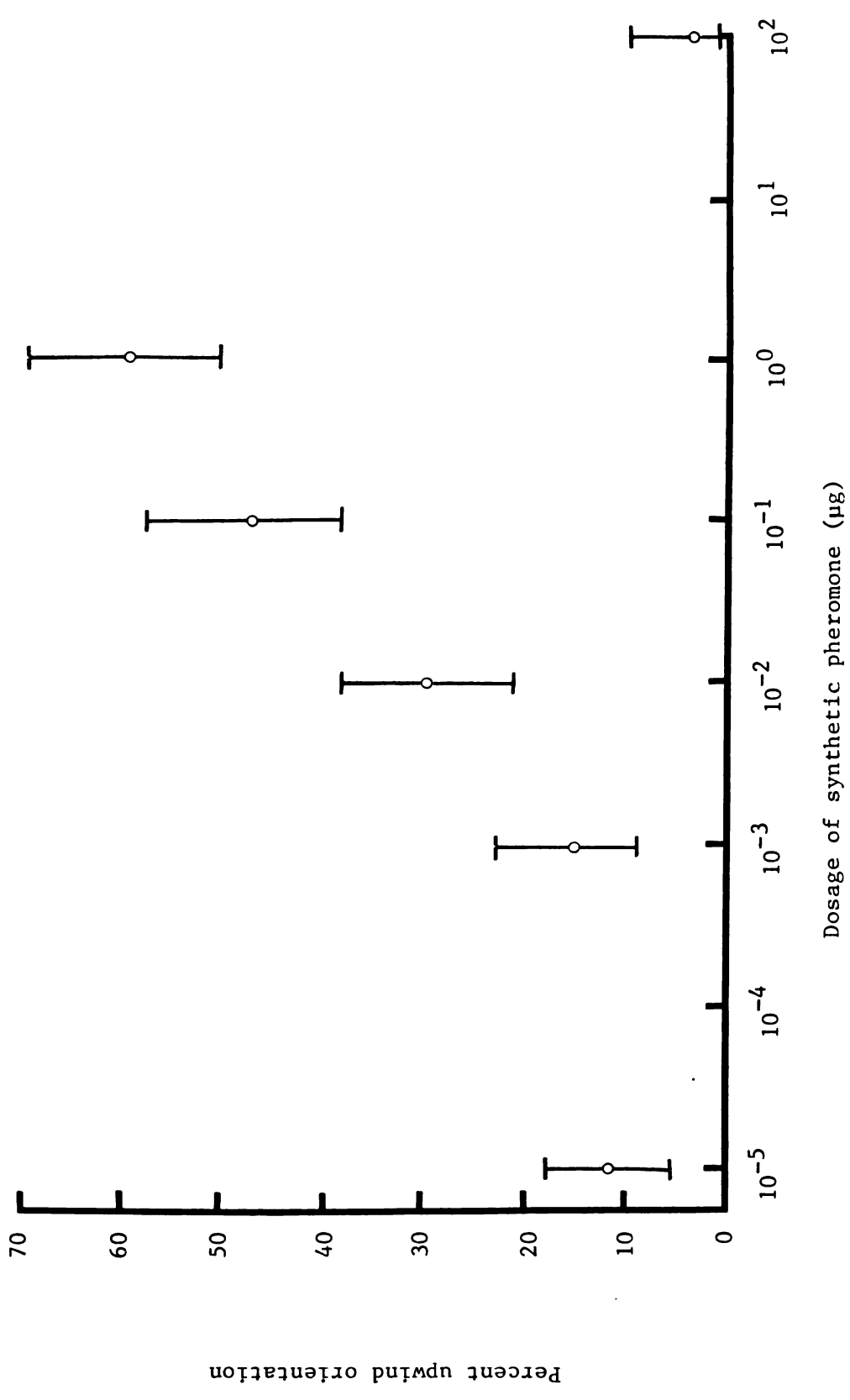

Figure 20. Percent male codling moth upwind orientation elicited by designated dosages of synthetic pheromone 1 hour into scotophase. Response values are bracketed by 95% binomial confidence intervals. Temperature = 23°C. N = 108.

Codling Moth Courtship Studies

A. Effects of visual cue presence and placement on the orientation of male codling moths

Male codling moths induced to fly upwind to the orientation platform engaged in the following patterns of behavior: hovering slightly downwind of the edge, often casting from side to side or forward and backward; hovering above the platform and dropping to its surface to touch it; and landing upon the platform to walk about (usually accompanied by wing fanning) and attempted copulation at the hole of the pheromone source or, when present, with a dead female. In almost every case each male exhibited all of the behaviors described and was seen, during one orientation to the platform, to re-orient to the source and/or the female several times. Orientation time near the platform was defined as the time from when the male first reached the downwind edge of the platform to when the male moved upwind of it, to the wind tunnel ceiling or side for 5 seconds, or downwind without returning upwind within 5 seconds. If a male sat motionless on the platform for 60 seconds this was also considered termination (and orientation duration was measured to the time at which motionlessness began).

Mean total orientation length and $\bar{x}$ time in contact with the platform (momentarily touching it, walking and fanning and attempting copulation) during the first 120 seconds of orientation, reported in Table 9, appeared to be unaffected by the presence or absence of a dead female visual cue. Location of the cue, when available, also had no effect on the amount of time spent at the platform.

However, as shown in Table 10, the placement of a visual cue did affect the amount of time a male spent at each quadrant. In all cases a

Table 9. Effect of visual cue placement on $\bar{x}$ orientation length and $\bar{x}$ duration of contact with platform exhibited by male codling moths.

Location of visual cue in relation to source[1]	$\bar{x}$ orientation length (± S.D.) (seconds)[2]	$\bar{x}$ time in contact with platform[2,3] (± S.D.) (seconds)
no cue present	440.9 ± (308.1)	43.9 ± (15.2)
upwind	475.2 ± (402.6)	48.3 ± (14.5)
to right	526.2 ± (371.6)	50.7 ± (17.1)
downwind	410.2 ± (234.1)	50.4 ± (21.2)
to left	417.4 ± (188.2)	39.2 ± (17.6)

[1] Visual cue 2 cm from source
[2] No significant difference between means within each column (ANOVA, P = .05; means compared using Student-Newman-Keuls multiple range test)
[3] During first 120 seconds of orientation

Table 10. Effect of visual cue placement on orientation of male codling moths.

$\bar{x}$ % of time on platform[1]	Location of visual cue in relation to source[2]				
	no cue present	upwind	to right	downwind	to left
at source	14.6a,b	5.8a	8.3a	9.3a	9.7a
on upwind quadrant[3]	17.0a,b	53.6c	16.0a,b	15.6a,b	13.7a
on crosswind (right) quadrant[3]	10.4a	7.9a	43.1c	9.5a	9.4a
on downwind quadrant[3]	34.2c	21.5b	23.1a,b	52.9c	27.0b,c
on crosswind (left) quadrant[3]	23.8b	11.2a	9.5a	12.7a,b	40.2c
attempting copulation with visual cue	-	43.3c	37.5b	30.3b	23.9a,b

[1] All observations were 120 seconds in duration

[2] Visual cue located 2 cm from source
Values in columns followed by same letter not significantly different (ANOVA, P = .05; data transformed to arcsin $\sqrt{p}$ for analysis; untransformed values reported in Table 10; means separated using Student-Newman-Keuls multiple range test). N = 17

[3] Includes time in contact with visual cue (copulatory attempts)

significantly greater amount of time was passed on the quadrant containing the dead female than any other quadrant or at the source. It appears, however, that all of the time a male spent on the quadrant containing the visual cue was not passed attempting to copulate with the female. In most cases the $\bar{x}$ time on the quadrant was significantly less than the $\bar{x}$ time in contact with the visual cue.

B. Laboratory courtship sequence of the codling moth

Thirty-three successful codling moth matings occurring in the wind tunnel and recorded on videotape were analyzed using stop-action and slow playback. This analysis was used to generate a flow diagram (Figure 21) depicting the observed steps leading to copulation (A similar study was undertaken with the Oriental fruit moth, Grapholitha molesta Busck; T. Baker personal communication). On a very basic level, codling moth courtship can be described as a process in which the male flies upwind to a female, lands upon the substrate on which she is sitting, walks (fanning simultaneously) to her and sexual coupling quickly follows. Table 11 presents data from these observations indicating that while calling and resting on a horizontal surface the females assumed no particular direction of body orientation. Similarly, the males, once they landed on the substrate following upwind flight, approached the female from all directions with equal frequency.

All of the males observed within 30 mm of a female (the approximate distance at which videorecording was begun) fanned their wings vigorously while walking. Initial contact was always made by the male, touching his head to the female. Table 12 reports significantly fewer first contacts at the posterior of the female compared with any other part of her body. Following this the male curved his abdomen laterally and forward

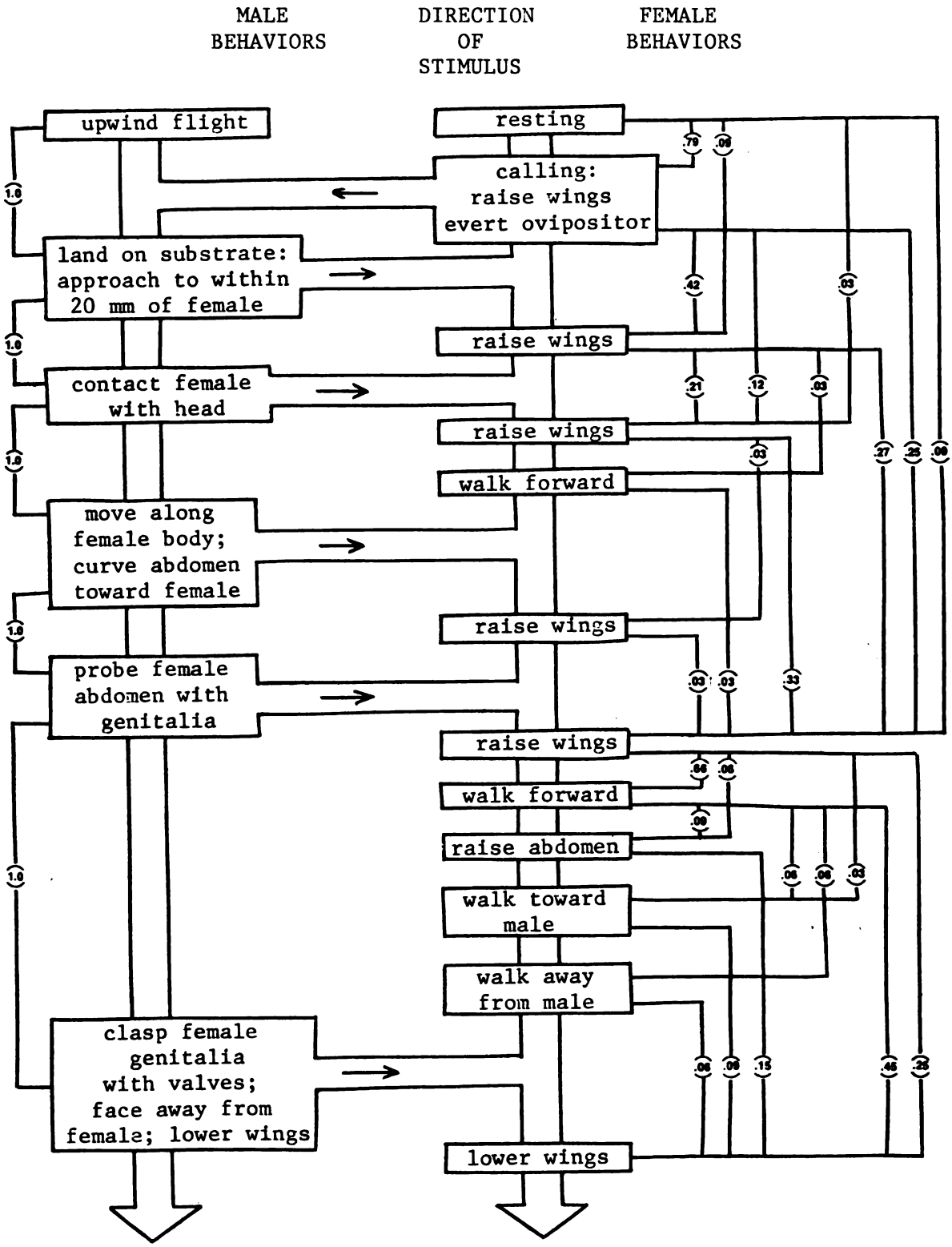

Figure 21. Sequence of behavioral events leading to successful mating of the codling moth developed from analyses of 33 matings. Direction of sequence is top to bottom. Pathways connecting boxes denote proportion of total sample moving from one state to the next.

Table 11. Behavioral events in codling moth mating sequence. Orientation of male and female codling moth at the beginning of courtship.

Orientation of moth	% females facing[1]	% males approaching[1]
upwind	30.3	30.3
crosswind (right)	33.3	24.2
downwind	24.2	24.2
crosswind (left)	12.1	21.2

[1] No significant difference between any values within columns (χ^2, P = .05). N = 33

Table 12. Behavioral events in codling moth mating sequence. Effect of initial male contact on subsequent movement of female.

Female behavior	% females exhibiting behavior after initial head contact by male[1]			
	female head	female abdominal tip	female left side	female right side
did not move forward	3.0a	0.0a	18.2a	9.0a
moved forward after being pushed by male	3.0a	3.0a	15.2a	24.2a
moved forward in response to lateral contact by male	15.2b	0.0a	0.0b	9.0a
% of total contacts[2]	21.1a	3.0b	33.3a	42.2a

[1] Values within columns followed by same letter not significantly different (χ^2, P = .05)
[2] Values in row followed by same letter not significantly different (χ^2, P = .05). N = 33

then walked along the side of the female, with his head still in contact with her, while he moved into a position enabling him to probe her abdomen with his. Wing fanning occurred during approach, initial male-female contact and abdomen curving and movement along the female, but as the male began abdominal probing he held his wings at roughly a 45^{o} angle to the horizontal and vibrated them. When the female genitalia were grasped by the male valves, wing vibration ceased and his wings were folded roof-like above him as he turned to face 180^{o} away from the female.

During male approach there was some variation as to when his valves were first opened. Of 20 observations 8 males opened them only after his head contacted the female. Two opened them intermittently during the approach and 10 held them open during the entire time they were within 30 mm of the female.

Of the 33 matings observed 30 began with the females holding their wings tightly against their bodies. As the males approached 53.5% of the females partially raised their wings, resulting in greater exposure of the abdomen and genitalia. An analysis of percent females raising wings in response to males approaching upwind, anterior to and lateral to the females (Table 13) suggests no differences between the stimuli.

Females walked forward to a small degree (3%) directly after initial male head contact, and to a very large degree (66%) as the male probed her abdomen with his. Male abdominal contact usually was directed laterally to the female due to his position beside her, but as he moved posteriorly his probing became more anteriorly directed. It appeared as if some females were induced to move forward in response to the lateral contact from the male head or abdomen, whereas other females only did so when apparently being pushed by the male during abdominal probing. Table

Table 13. Behavioral events in codling moth mating sequence. Female wing-raising during male approach.

Apparent stimulus	Number females exposed to stimulus	% females raising wings[1]
male approach	30	53.5
male passing near or in front of head	7	57.6
male passing upwind of female	11	54.5
male approaching female side	18	44.4

[1] No significant difference between values (χ^2, P = .05)

12 shows a significantly greater number of females responding to lateral contact when the first contact occurred between the male and female head than when it occurred anywhere else. From observations of 20 matings the $\bar{x}$ distance walked by a female during courtship was 17.6 mm (S.D. = 16.4), approximately twice the body length of a codling moth.

In most cases the male lowered his wings following coupling and the female lowered hers on top of his after which the moths remained motionless. Mean duration of the sequence from when the male first moved to within 30 mm of the female until motionlessness after wing-lowering was 4.7 sec (S.D. = 2.4).

Trapping Experiments

A. Effect of trap placement on trap catch

Data from this experiment is presented in Table 14. Total catch was 429 male codling moths with no significant catch difference appearing on the basis of trap placement within the orchard.

B. Comparison of catch in a sticky trap and a non-sticky trap

No significant difference was found between catch in the Pherocon trap and catch in the Granett-type trap using ANOVA (P = .05) and the Student-Newman-Keuls multiple range test for comparison of the means. Total number of males captured was 343 with $\bar{x}$ number per replicate caught in the Granett trap being 4.0 (S.D. = 5.5) and $\bar{x}$ number per replicate caught in the Pherocon trap being 11.5 (S.D. = 19.1).

C. Observations of male behavior at a Pherocon trap

Fifty-seven male approaches were described. Codling moth behavior at the trap included: fluttering outside and inside the trap (males appeared to be attempting to hover or fly slowly towards the trap, but variations in wind speed and direction almost continually caused erratic

Table 14. Effect of trap placement on male catch.

Location of trap	$\bar{x}$ number of males per trap per replicate (± S.D.)[1]	% of total catch[2]
outside edge of crown	6.5 ± (6.8)	41.0
center of crown	5.5 ± (5.8)	34.5
between trees	3.9 ± (3.2)	24.5

[1] No significant difference between means (ANOVA, P = .05; means compared using Student-Newman-Keuls multiple range test)
[2] Total catch = 429 males

movements), walking outside and inside the trap, almost always accompanied by wing fanning and copulatory attempts directed at the rubber septum (male curving abdomen, opening valves and probing septum with abdominal tip). Due to the low light level during codling moth flight (which usually occurred in the 30 minutes following sunset) and the males' erratic and rapid flight most observations could not be initiated until the moth was within 30 cm of the trap.

Of 57 males approaching 44 of them entered the trap. Thirty-four of these landed, walked and fanned on the inside bottom surface. Nine attempted copulation with the septum. Ten only fluttered within the trap, but due to the erratic flight of the codling moth almost all of them momentarily contacted the inside several times.

Outside the trap 8 of the 57 walked and fanned on the outer surface. Table 15 presents the $\bar{x}$ times spent inside and outside the trap fluttering, walking and fanning and attempting to copulate with the septum.

Table 15. Mean times of male codling moth orientation to a non-sticky Pherocon trap baited with 1 mg of synthetic pheromone.

Location	$\bar{x}$ time (± S.D.) (seconds) Total	Fluttering	Walking and fanning	Attempting copulation with septum
inside trap	13.2 ± (9.1)	6.1 ± (6.8)	10.8 ± (7.0)	2.0 ± (0.9)
outside trap	25.5 ± (23.4)	24.8 ± (22.6)	4.6 ± (3.2)	-

DISCUSSION

Laboratory Experiments: Periodicity and Courtship

Skirkevicius and Tatjanskaite (1975) reported that under laboratory conditions with 8 hours of scotophase at 20 $\pm$ 1°C female codling moths called only in darkness. The present study confirmed that most calling is confined to that period, however, Figure 10 indicates that occasionally females can be seen calling prior to and shortly following scotophase. Skirkevicius and Tatjanskaite (1975) also found that although the pheromone gland is fully developed prior to emergence from the pupa, the greatest amount of calling (62%) occurred on the third day of adult life. Day 3 yielded the largest percentage of female calling during the present observations (87%), but the degree of calling was statistically indistinguishable from that of the second day as well as the fourth to sixth days.

Female and male codling moths maintained at 23°C under 16 hours of photophase and 8 hours of scotophase exhibited diel periodicities for calling and pheromone-elicited upwind orientation respectively. Throughout the day at each observation time the level of activity for each sex was similar: i.e., initiation of calling and a dramatic increase in pheromone-stimulated upwind anemotaxis coincided with the onset of scotophase, a high degree of each behavior was found throughout the dark period and termination of calling and a sharp decrease in response to pheromone occurred with the beginning of photophase.

Moths initially held under an L:D cycle and then transferred to

aperiodic light conditions continued to call (females) and move upwind in response to pheromone (males) during a time period approximating the 8 hours in which the insects previously had experienced scotophase and repeated this with roughly a 24 hour regularity. However, without lights-on and lights-off cues the times of initiation and termination did shift.

When female codling moths experienced a decrease in temperature from 23° to 16°C during scotophase calling was terminated within 2 hours. When the temperature decrease occurred 3 hours prior to scotophase calling began within 1 hour and ended with the onset of scotophase, and when females were maintained at constant 16°C $\bar{x}$ calling time was shifted 5 hours earlier than $\bar{x}$ calling time of females held at 23°C. Results with males were less clear and the decrease in temperature which prominently shifted female calling period into the light appeared generally to depress male upwind orientation to a very low level.

Most organisms in nature exhibit a diel periodicity in activity which can be maintained in the laboratory under light and dark conditions similar to the photocycle in nature. In the absence of a photocycle, under continuous light or continuous darkness, many of these organisms maintain their behavioral rhythm on the basis of control by an "internal clock" or endogenous oscillation. When the rhythm continues under aperiodic conditions, or "free runs," the natural periodicity (τ) is often found to be slightly less or slightly more than 24 hours in length. A 24 hour periodicity controlled by an endogenous oscillation is termed a circadian rhythm. Prolonged maintenance under D:D or L:L will result in a gradual shifting of the behavior out of phase from the normal L:D cycle. If an organism with an internal clock is subjected to an

environmental photocycle not drastically dissimilar in length from τ its endogenous period will become the same as the exogenous one.

A double-oscillator model proposed by Pittendrigh and Bruce (1959) to explain the entraining mechanism presently receives the greatest amount of experimental support. The essential feature of the model is that an "A-oscillator," termed the "driver," is an internal pacemaker sensitive to cues (Zeitgebers) provided by the light cycle and a "B-oscillator," termed the "rhythm," controls expression of the behavioral event and is unaffected by light cues, but is coupled to the driver which sets it. Changes in photocycle, shifting of the Zeitgeber, instantly resets the driver, but modification of the rhythm is a more gradual process and must occur over several oscillatory cycles.

Data from the codling moth experiments dealing with calling under continuous scotophase and photophase and upwind orientation under continuous photophase indicate that the periodicities of both sexes of this insect are governed by a circadian rhythm. Holloway and Smith (1976) measuring the locomotor activity of the adult lesser cornstalk borer, Elasmopalpus lignosellus (Zeller), under continuous scotophase found it to recur with a 22 hour period. Each day peak locomotion occurred 2 hours earlier than the previous day. In both male and female codling moths τ appears to be close to 24 hours because no discernible shift was evidenced in $\bar{x}$ calling time or $\bar{x}$ pheromone response time under aperiodic conditions. The abrupt transition from no calling and little upwind orientation to a high degree of both and vice versa when lights-off and lights-on cues were present indicates that one or both of those cues probably acts as a Zeitgeber.

Endogenous oscillators are temperature-compensated. Generally the

Q_{10} value (a measure of the increase in τ with a decrease in temperature: τ at $(t-10)^{o}/\tau$ at t^{o}) of organisms studied is slightly greater than 1.0. This indicates that for a wide range of biological temperatures τ changes very little. But it has been demonstrated that temperature changes can modify behaviors which under constant temperature rely on photocycle entrainment. Most demonstrations of this have utilized Lepidoptera (Cardé and Roelofs 1973, Sower et al. 1971 and Cardé et al. 1975) and indicated that decreases in temperature can shift female calling and male response to pheromone earlier into the day. Pittendrigh (1954), measuring the periodicity of Drosophila pupal emergence, found that following a decrease in temperature only the first peak of pupal emergence was affected, and that if the temperature was returned to that experienced previously the system reverted back to its original periodicity. No detailed explanation for the effects of temperature alterations has been proposed, but it is believed that the B-oscillator may be the one sensitive to temperature pulses and cycles (Saunders 1976).

Data from timing pheromone trap catches of male codling moths (Batiste et al. 1973a) indicated that males fly regularly at sunset, but that temperature may modify flight in that catches are greater earlier in the day during cool days than warm ones. Field flight observations by Borden (1931) and Fluri et al. (1974) purportedly lend evidence to this, though it is unclear as to whether or not they were in fact observing males in the orchard. Male and female codling moths appear almost indistinguishable externally, even at rest, and neither author reports determination of the sex of the moths in flight. Field observations of periodicity are further complicated by reports (Mani et al. 1974) that codling moths may exhibit 2 daily flight periods - one in which males

are captured in pheromone traps in great numbers and an earlier flight in which few or no captures occur.

Observations by Mani et al. (1974) suggest that temperature at time of flight may not necessarily be the only modifying factor. The temperature several hours prior to sunset may play a role as well as present ambient temperature. Increases and decreases in temperature and sudden cloud cover may be responsible for early or late codling moth flights. During a hot (28°C) afternoon and a warm (21°C) evening no pheromone trap catch occurred prior to sunset, but during the hour following sunset a large number of moths were caught. With a warm (21°C) afternoon and a cool (16°C) evening the largest catch again occurred during the hour following sunset, but the remainder of the males were caught during the 3 hours prior to sunset. Cloud cover and mild rain during a warm afternoon and evening appeared to have no effect on trap catch period: it still was maximal during the hour after sunset. Cloud cover combined with a decrease in temperature (imminent storm conditions) resulted in the majority of moths being caught 2.5-1.5 hours prior to sunset, and very few after sunset.

Clearly a drawback to field observations is the inability to separate the effects of several different variables on the behavior of a system. In the last observation of Mani et al. (1974) described above it is unclear whether the dramatic shift in male flight was caused by low afternoon temperature (20°C), a decrease in temperature (early afternoon temperature was not specified), decrease in light level due to clouds, any combination of the above or yet additional factors. In the laboratory, when temperature was decreased to 16°C the low number of males orienting upwind in the olfactometer would lend little support to the

hypothesis that low temperature or temperature decrease is the modifying influence. The fact that decreasing light intensity from 1500 to 150 lux in the laboratory yielded a significant increase in degree of anemotaxis may indicate that the degree of cloud cover could play a significant role. Mani et al. (1974) also noted that when the temperature rose to 15°C after a "long period of bad weather," moths were caught in the traps, whereas, when the temperature dropped to 15°C after "nice warm weather" moths were never caught. Thus, the effect of temperature on codling moth mating periodicity may be complex, accounting for male decrease in activity with a decrease in temperature from 23° to 16°C.

Another perplexing aspect of codling moth periodicity is the contrast between the very similar calling period of females and pheromone response period of males at 23°C and at 16°C the very apparent shift of calling into photophase with no shift exhibited by males under the same conditions. Working with Antheraea pernyi Guerin-Meneville Truman (1973) reported that pupae reared at 12°C produced adult females that called and adult males that exhibited flight 1 hour into scotophase when held at 25°C. This was in contrast to adults held at 25°C after emerging from pupae also maintained at 25°C, which called and flew 5 hours into scotophase. He concluded that periodicity entrainment relied on conditions experienced during the pupal stage and was unaffected by temperature changes occurring during adult life. Cardé et al. (1975), however, demonstrating that female calling and male pheromone response of Argyrotaenia velutinana Walker could be immediately shifted by a change in temperature, argued that temperature conditions experienced by the adult play a critical role in periodicity of behavior. Female codling moths clearly appear to fit the model proposed by Cardé et al. (1975), but males may not.

At 23°C 4 hours prior to scotophase calling and upwind orientation are at low levels (< 30%), and at 2 hours prior to scotophase calling is still below 30%, while upwind orientation is approximately at 30%. One hour into scotophase calling and upwind orientation are exhibited by greater than 60% of the moths. When the temperature is decreased to 16°C 3 hours before lights-off calling increases to > 70% during the remainder of photophase, but pheromone responsiveness remains at 30%. Both behaviors are terminated with scotophase. At 16°C it may be energetically too expensive for an increase in male activity to occur, but calling probably requires less metabolic energy than upwind flight. Therefore, when the temperature is lowered to 16°C, it might be advantageous reproductively and energetically inexpensive enough for an increased number of females to call during photophase, thereby increasing the chances of attracting those males which are still capable of upwind flight and mating. On the basis of the observations by Mani et al. (1974) and the results of the present experiments, it appears that bioassays under several temperature and light regimes, as well as tests of moths reared under several sets of environmental conditions, may be needed before the roles of ambient temperature and temperature changes on mating periodicity can be resolved.

Very few detailed studies of moth sexual behavior, especially courtship, have been undertaken. In one such study, however, Grant and Brady (1975) described the behavioral sequences which occurred during courtship of the Indian meal moth, Plodia interpunctella (Hübner), and the almond moth, Cadra cautella (Walker). They reported that in both species several steps of alternating male and female behavior led to copulation. Basically a male was attracted to the pheromone of a calling female, he

approached and released an aphrodisiac which enabled him to move into a position with his head beneath the female's head, she remained stationary and turned up her abdomen in an acceptance posture and copulation quickly followed.

Similarities between a number of the steps during Indian meal moth and almond moth courtship indicated a possibility of interspecific courtship taking place, but successful cross-mating was not found to occur (Grant et al. 1975) and this was attributed in part to differences in direction of male approach, response of female to initial male contact being different between the species and the possible existence of species-specific male aphrodisiacs.

As indicated in Figure 21, during successful mating of the codling moth, it appeared that a number of different behavioral pathways were followed by females. The most commonly observed sequence consisted of resting, calling, raising wings at male approach, raising wings higher and walking forward during abdominal probing, then lowering wings after sexual coupling. The path followed by the smallest number of females was resting (no calling visible), wing-raising at contact with male head, raising wings higher as male moved along female body, raising wings and walking towards male during abdominal probing and wing-lowering following initiation of copulation. Other combinations of the behavioral events designated in the diagram occurred and the degree to which each was seen can be calculated by tracing the path from each behavioral state to the next (the path designates the percentage of moths moving from one state to the one connected to it).

Certain behavioral modes ("raise wings" and "walk forward") appear more than once in Figure 21. There are 2 reasons for this: 1) some of

the females engaged in an activity in response to a particular stimulus (i.e., 42% of the females raised wings at male approach) while others exhibited that same behavior only in response to a different cue (12% did not raise wings for the first time until male head contact); 2) also, some females responded to more than one stimulus with the same behavior (42% of the females raised their wings at male approach, 21% then raised them higher during male head contact and 3% appeared to elevate them a little more as the male began moving posteriorly along the female body while curving his abdomen toward her).

Analyzing the movement from each behavioral event to the next in a manner similar to that employed by Grant and Brady (1975) it can be interpreted that each behavior undertaken by the male is a releaser for eliciting the activity exhibited next in the female sequence. However, some or many events may not appear in a single female's repertoire: 3% of the females exhibit all but 3 of the behavioral states, while 9% of the females pass through only 3 of the behavioral states (resting to wing-raising in response to abdominal probing, followed by wing-lowering after the male grasps the female genitalia with his valves). Therefore, since a large amount of variation appears in which behaviors a particular female undertakes, and little or no variation is observed in the male sequence, female calling and male contact with the female may be the only releasers of the male behaviors. No matter whether the female exhibits only calling during the time the male approaches, makes head contact, moves along her body, curves his abdomen and begins abdominal probing, or she engages in calling and 3 series of wing-raising, the male behavioral sequence remains the same.

Hutt and White (1977) reported on the basis of laboratory experiments with blinded, antennectomized, blinded and antennectomized and

unaltered male codling moths that vision apparently plays a part in successful mating. The present study supports this hypothesis with evidence that, although the presence of a visual cue does not affect the amount of time spent in orientation, it does significantly affect where the moth orients. A male will spend a greater amount of time walking and fanning near and attempting to copulate with a visual cue than it will at a pheromone source to which it initially oriented during its upwind flight. Shorey and Gaston (1970) found that when dried Trichoplusia ni (Hübner) females or black paper silhouette models were positioned 2 cm away from a pheromone source the presence of a visual cue, as in the codling moth, did not influence the frequency of orientation, but did influence both the frequency and direction of copulatory attempts.

Field Experiments: Male Orientation to Pheromone Traps

Borden (1931) and Fluri et al. (1974) reported that during the evening male codling moths were found to swarm in the upper parts of trees. Mani et al. (1974) observed cases of codling moth orientation to conspicuous silhouettes of non-host as well as host plants. As reported earlier, though, none of the authors provide evidence for positive identification of the sex in flight. Pheromone-activated gypsy moth males, Lymantria dispar L., "investigated" vertical silhouettes such as tree trunks and it was demonstrated that trap placement near large vertical silhouettes (trees 0.5 m in diameter) could significantly increase trap catches over those hung on small trees (3-8 cm in diameter (Cardé et al. 1977).

The results presented in Table 14 indicate that an orientation to large objects exhibited by some codling moths does not affect male attraction to traps. No significant difference was discernible between

male catches within the tree crown, at the branch tips or at traps suspended in the open between trees. Therefore (at least at the population level present during this study) codling moth pheromone trapping appears to be unaffected by trap placement.

Presently all pheromone traps commonly used for codling moth monitoring rely on a sticky trap inner surface to catch the moths. This design has proven sufficient for the most part, but nevertheless, has certain characteristics which make it less than ideal. Moths entering the trap must come into direct contact with the glue to be captured. Those that do contact the glue are not always retained because moth scales from previously captured insects can accumulate and reduce the glue's stickiness. The capture potential of a trap is dependent upon the surface area of the glue, and at high population levels or over a long period of time in a lesser infestation it is possible for that surface to become overloaded with moths with each moth causing a progressive reduction in trapping ability. Sticky traps can be messy during normal servicing, and those insects removed are often in very poor condition and unsuitable for subsequent inspection or study. One of the greatest values of the pheromone trap supposedly lies in its specificity of capture. Personnel with little taxonomic background can responsibly monitor pest insects because the bait in the trap will only attract the species of interest. However, it is very uncommon for a sticky pheromone trap, especially hung within foliage, to not collect a large assemblage of various insects that have accidentally come into contact with the glue.

The Granett-type trap (Granett 1973) alleviates a number of these problems. Since access to the trap occurs only at a small port of entry at each end, though all unwanted insects are not kept out, the number is

reduced considerably because orientation must be more precise or persistent than with traps having larger openings. Vapona fumes kill the entering moths, therefore, effective capture potential is a function of trap volume, rather than surface area, and dead insects or moth scales do nothing to decrease trap efficiency (the trap used in this test could hold several thousand dead codling moths without overloading). During insect count and removal and bait replacement there is no glue present to get on the hands or clothes of the trap technician, and the dead specimens are usually in the condition in which they entered the trap.

Results from the comparison of Pherocon trap catch and Granett trap catch during this study yield no significant difference between the two, even though of 343 moths caught 74% were in the Pherocon and 26% in the Granett traps (much variation between replicates accounting for the lack of significance). Duration of trapping was 28 days and throughout this period the same Pherocon traps were used. Replacement of the sticky traps may have increased catch somewhat (Westigard and Graves 1976), but policy during other monitoring projects was typically to replace traps at 1 to 2 month intervals (Myburgh et al. 1974 and Proverbs et al. 1975). The Granett trap model used in this test was developed for the much larger gypsy moth and could conceivably be improved for capture of codling moths and other small Lepidoptera. Further testing of Granett-type traps with modifications would seem valuable in an attempt to develop a more efficient non-sticky pheromone trap.

Reports of trap "efficiency" in the past have been developed from comparisons of trap catch. Though trap catch is valuable, a more direct measurement may be obtained from observations of males approaching a trap in the orchard. During this study, of 57 males observed at a

non-sticky Pherocon trap 34 of these entered the trap and walked and fanned inside. If the assumption could be made that in a sticky trap each one of these would have been caught ($\bar{x}$ time spent walking and fanning was 10.8 sec) an efficiency of 59.6% results. Ten others fluttered within the trap ($\bar{x}$ time = 6.1 sec) but due to the erratic nature of codling moth flight almost all of them momentarily contacted the inside surface several times. This could possibly raise efficiency to an upper level of 77.2%. Extrapolation of data in a non-sticky trap to that of a sticky one may not be valid, but since a sticky trap would continually lose effectiveness with age and each time an insect was caught, true efficiency is probably a dynamic value of which any measure would be an approximation for limited conditions.

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