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Optimum Host Age and Stage for Development of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) Reared on the Rice Weevil (Coleoptera: Curculionidae) in Wheat

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ABSTRACT: Suitability of different ages of the rice weevil *Sitophilus oryzae* (L.) in wheat for the development of the parasitoid wasp *Anisopteromalus calandrae* (Howard) was examined. Infested wheat kernels were isolated using x-ray techniques. Groups of five infested kernels of wheat were exposed to individual *A. calandrae* females 16–18, 18–20, or 20–22 days following rice weevil oviposition, and kernels were dissected at 2-day intervals during the next 4–14 days. The oldest of these 3 rice weevil age groups was predominantly teneral pupae, and there was a significant difference in the tunnel widths between the younger 2 larval age groups. In this no-choice test, older rice weevils were paralyzed and parasitized more often than younger ones. All *A. calandrae* paralyzed and parasitized at least some hosts, suggesting that variation in behavior among females was not the primary factor in the difference between the rate at which these three age groups were parasitized. Some previous studies have found that *A. calandrae* parasitized larvae more often than pupae. These observations suggest that the most frequently parasitized stage in a continuous population at low density is not necessarily the optimum stage for supporting parasitoid production in high density infestations.

Age of hosts available to parasitoids can be an important factor in biological control. The pteromalid wasp *Anisopteromalus calandrae* (Howard) parasitizes larvae and pupae of several economically important pests that develop inside grain kernels, including the rice weevil *Sitophilus oryzae* (L.), the maize weevil *Sitophilus zeamais* Motschulsky, and the lesser grain borer *Rhyzopertha dominica* (Fabricius) (Throne, 1986; Brower et al., 1996). The parasitoid female locates and oviposits on a host inside of a grain kernel. The larvae are solitary ectoparasites, but, like their host, are hidden from view inside the grain.

Recent studies have demonstrated that augmentative release of hymenopteran parasitoids can suppress pest populations in stored whole grain (Brower et al., 1996; Flinn et al., 1996; Schöller et al., 1997). Moreover, this biological control is compatible with the practice of suppressing pest population growth by reducing grain temperature using aeration (Flinn, 1998; Reed and Harner, 1998). These observations suggest that mass rearing and release of *A. calandrae* might have practical application.

Previous studies have examined which host stages are most often parasitized by *A. calandrae*. These studies have tended to emphasize continuous host populations, such as natural infestations or laboratory colonies not reared as discreet cohorts (Smith, 1993; Ahmed, 1996). However, differences between the frequency of parasitization of different host stages involve both elements of host-finding and suitabil-

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ity of the host to sustain optimal parasite development. In heavily infested laboratory colonies, host finding may be less relevant. Also, in laboratory colonies reared for production of parasitoids, it may be desirable to produce hosts of a narrow age range, thereby limiting the choices of host stage.

The present study was conducted to determine optimal parasitoid and host rearing procedures for an insectary. The objectives were to determine the highest infestation rate of rice weevils attainable within a narrow age range, and to examine host suitability of different ages of rice weevils for *A. calandrae* by eliminating host finding as a factor.

Materials and Methods

INSECTS AND X-RAY PROCEDURES: Rice weevil hosts were from a long-established colony from Manhattan, KS. *A. calandrae* were from an organophosphate-susceptible laboratory strain originally established in Savannah, GA, and currently maintained in Manhattan, KS. Rice weevil adults and larvae were held at 27°C. Rice weevil adults (500 adults per 500 ml of wheat) were allowed to oviposit on hard red winter wheat that had a moisture content of 13%, and then sieved from the grain after 48 hr. This procedure was repeated several times in succession to obtain groups differing in age by 48 hr. To obtain infested kernels, wheat was attached to a cellulose sheet with double-sided tape and x-rayed using the materials and procedures described by Throne (1994), except an exposure time of 3 min was used. The resulting x-ray was used as a template to select infested kernels from the cellulose sheets. While on cellulose sheets or in glass tubes, the wheat was held in chambers containing saturated sodium chloride solution to maintain a relative humidity of 75% (Winston and Bates, 1960). The x-ray procedure was performed 12–14 days after the beginning of the oviposition period.

TUNNEL SIZE OBSERVATIONS: To compare tunnel size and age, we used the procedure described above to select infested wheat kernels from three age ranges. These kernels were attached to cellulose sheets, and kernels from all 3 age groups were x-rayed on the same day. This provided x-ray images of wheat containing weevil immatures aged 17, 19, or 21 days from the beginning of oviposition. A hand lens (Measuring Magnifier, Bausch and Lomb, Rochester, New York) was used to measure the diameter of tunnels in these kernels on the x-ray film, and to identify the stage of the immature rice weevils. Tunnels were measured as close as possible to the midpoint of the weevil. Three x-ray sheets (1869 kernels) were selected at random and examined to determine the proportion of wheat kernels infested with rice weevils.

EFFICACY OF PARASITIZATION: Infested kernels from the three age ranges were isolated from a second cohort as described above. Sixty kernels were randomly selected and set aside. The remaining kernels were placed in closed 13 × 100 mm glass tubes, 5 kernels per tube. There were 20 such tubes for each age. Rice weevil immatures were exposed to a mated *A. calandrae* female 17, 19, or 21 days after the beginning of the rice weevil oviposition period. *A. calandrae* females were approximately 48 hours old. The females had access to a food source (honey) and males prior to this experiment. A single *A. calandrae* was placed in a 13 × 100 mm glass tube containing 5 kernels of wheat, and left for 24 hours. All *A. calandrae* females were alive and healthy at the end of the oviposition period.

Each replicate was randomly assigned a dissection date prior to parasitization. Kernels from 4 tubes were dissected 4, 6, 8, 10, 12 , and 14 days after the beginning of

Table 1. Tunnel widths and distribution of developmental stages among immature rice weevil larvae.

Age at time of parasitoid exposure	Width	Cohort 1 (tunnel width measurements)			Cohort 2 (parasitization)		
		Larvae	Prepupae	Pupae	Larvae	Prepupae	Pupae
16–18 days	1.1 ± 0.1a	10	0	0	20	0	0
18–20	1.4 ± 0.1b	8	2	0	19	0	1
20–22	1.5 ± 0.1b	0	5	5	6	9	5

Mean ± SD, means with different letters are significantly different at $\alpha = 0.05$.

parasitization. In addition, 60 infested non-parasitized wheat kernels were dissected at the same time that the parasites were added to the tubes. During this and many other dissections of larvae not exposed to parasitoids, we found that all rice weevil larva, prepupae, and pupae we dissected reacted by crawling or moving from side to side when touched with forceps. We therefore recorded when we found rice weevil larvae and pupae did not respond to probing after being dissected out of the wheat kernel.

STATISTICS: Statistical comparisons were made using SAS 6.12 (SAS Institute Inc., 1989). Tunnel widths were compared using PROC ANOVA, and differences among means were testing using the Tukey-Kramer procedure (Sokal and Rohlf, 1995). Categorical comparisons of frequency of stages, parasitization and responsiveness were made between age groups by using PROC FREQ to perform Fisher's Exact Test.

Results

Pilot tests were performed to determine the effects of the duration of oviposition period on the grain on percent weevil infestation. When females were given 48 hr to oviposit the infestation rates were 15, 21, and 32%, but when rice weevil adults were given only 24 hr to oviposit the infestation rate was less than 1%. Forty-eight-hour age groups were used for subsequent experiments.

Tunnel widths were measured for a portion of the larvae used to examine the effects of oviposition time. Stage frequency data were collected for both that cohort and the three age groups used to examine host suitability (Table 1). The mean tunnel width of 16–18 day post oviposition rice weevils was significantly smaller than those of 18–20 or 20–22 days (Table 1). The 20–22 day rice weevils were all prepupae or pupae in the cohort used for tunnel width measurements. Likewise, most of the 20–22 day weevils in the cohort used to examine parasitization were pupa or prepupae. In contrast, all 16–18 day rice weevils and most of those 18–20 day insects were larvae.

To compare percent parasitization between age ranges, age groups were initially examined separately. No correlation was found between the proportion of rice weevil larvae parasitized and the time of dissection (Fisher's Exact Test, $P = 0.316$, 0.275, and 0.056, respectively, for cohorts 1, 2, and 3; data not shown). The data for all dates were then pooled and parasitization state was examined by age group. When analyzed this way, there was a significant association between age group and parasitization for all comparisons. Rice weevils that were 20–22 days at time of exposure to *A. calandrae* were successfully parasitized more often than those exposed at younger ages (Fisher's exact test, $P < 0.001$ for both comparisons) (Table 2). Rice weevils that were 18–20 days old when exposed to *A. calandrae* also were success-

Table 2. Proportion of rice weevil immatures parasitized by *Anisopteromalus calandrae*.

Age at time of parasitoid exposure	Total infested kernels	Non-responsive	Parasitized	Stung (non-responsive + parasitized)	Attack rate (stung-only / total)	Success rate (parasitized / stung)
16–18 days	119	28	52	80	67%	65%
18–20	120	18	77	95	79	81
20–22	120	6	110	116	97	95

fully parasitized significantly more often than rice weevils that were 16–18 days old at time of exposure (Fisher's Exact Test, $P = 0.002$).

The number of rice weevils that were apparently paralyzed but not parasitized varied between the age groups. Within age groups, there was no correlation between dissection date and the rice weevils' response to stimulus (Fisher's Exact Test, $P = 0.186$, 0.358, and 0.667 and for rice weevils aged, respectively, 16–18, 18–20, and 20–22 days at time of exposure; data not shown). Examining the difference within each cohort between the proportion of unparasitized rice weevil immatures that were responsive before exposure to parasitoids (i.e., day 0) and after, significantly more unparasitized rice weevil immatures dissected after exposure to parasites failed to respond to stimulus (Fisher's Exact Test, $P < 0.001$ for rice weevils aged 16–18 and 18–20 days at time of parasitoid exposure, and $P = 0.03$ for 20–22 day old rice weevils) (Table 2). There were no significant differences between cohorts in the proportion of unparasitized larvae dissected from days 4–14 that failed to respond to stimulus. The majority of the unparasitized rice weevils failed to respond to stimulus following exposure to parasitoids at 20–22 days of age, whereas the converse is true for larvae exposed to *A. calandrae* at 16–18 or 18–20 days of age. However, too few larvae from cohort 20–22 day old rice weevils escaped parasitism to allow significant comparisons with the other 2 age groups.

The age of the host did not seem to affect the developmental time of *A. calandrae* larvae (Fig. 1). For all three cohorts, *A. calandrae* pupae were first seen 8 days after parasite oviposition, and *A. calandrae* adults were first seen from the wheat kernels 14 days post parasitization. All *A. calandrae* females attacked at least one host (Fig. 2), and all parasitoid females successfully parasitized at least 1 host (data not shown). Among rice weevils aged 16–18 and 18–20 days at time of parasitization, the frequency distribution of parasitized larvae was not significantly different from that predicted by the null hypothesis that females were equally likely to parasitize 0, 1, 2, 3, 4 or all 5 of the larvae available to them (Fisher's Exact Test, $P = 0.062$ and 0.544, respectively, for larvae aged 16–18 and 18–20 days at time of exposure to *A. calandrae*, data not shown). Most *A. calandrae* exposed to 20–22 day hosts parasitized all 5 of the available hosts.

Discussion

In this study, in which host suitability was studied independently of host-finding, we found that *A. calandrae* is more successful at parasitizing rice weevil in wheat as prepupae or teneral pupae rather than as last instar larvae. Rather than examining success of parasitism among hosts of uniform age, previous studies examining the stage and age most frequently parasitized by *A. calandrae* and have yielded varied

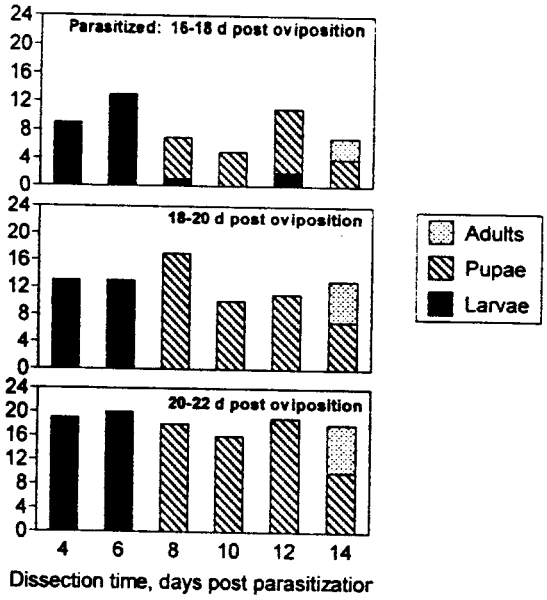

Fig. 1. Count of *Anisopteromalus calandrae* larvae, pupae, and adults dissected from kernels at various times following parasitization.

results. A previous laboratory study of *A. calandrae* parasitizing maize weevils in corn with a stable age distribution found that larvae were parasitized more often than pupae, both on an absolute basis and as a proportion of available host stages (Smith, 1993). However, in earlier laboratory studies in which *A. calandrae* chose between different host ages and species dissected from unspecified grains and placed in gelatin capsules, rice weevil pupae were parasitized nearly as often as old larvae (Chatterji, 1955). In that same study, lesser grain borer pupae were parasitized only 25% as often as old lesser grain borer larvae. However, both lesser grain borer larvae and pupae were lightly parasitized in that experiment, in which *A. calandrae* had simultaneous access to both rice weevil and lesser grain borer larvae and pupae. In more recent field observations of wheat stored in Saudi Arabia, much higher rates of parasitism of lesser grain borers by *A. calandrae* were observed (Ahmed, 1996). In that study, prepupae and pupae comprised the majority of hosts parasitized for all

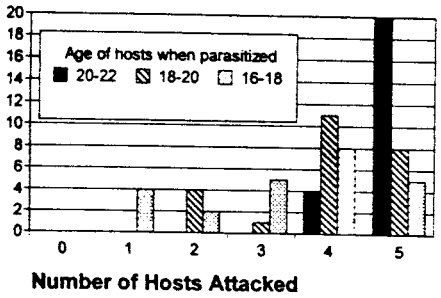

Fig. 2. Proportion of available hosts attacked by each *Anisopteromalus calandrae* female over the 24 hr parasitoid exposure period.

months except December and January, when no lesser grain borers were parasitized by *A. calandrae*. Others have noted that there are few data on the actual cues used by *A. calandrae* to locate its host (Sharifi, 1972; Smith, 1993), but that, at low host density, locating as many acceptable hosts as possible is probably more important to reproductive fitness than finding optimal hosts (Smith, 1993). Taken together, these observations suggest that which stage is most often parasitized by *A. calandrae* depends on an interaction of several factors, including host species, substrate, and density. The current study indicates that, given very high rates of infestation of wheat by rice weevils, prepupae and teneral pupae are better than other stages for optimizing the yield of *A. calandrae* progeny.

Hymenopteran parasitoids generally sting their hosts to immobilize them and reduce host immune defenses before ovipositing. We found rice weevil larvae, prepupae and pupae that were not previously exposed to parasitoids always responded when touched with forceps. When we dissected rice weevils that had been exposed to *A. calandrae*, we found that some rice weevils that did not have immature *A. calandrae* on them did not respond to probing with forceps. We inferred that these rice weevils had been stung but not successfully parasitized. Thus we use the term "attacked" to include both parasitized rice weevils and those apparently stung but not parasitized. The apparently-paralyzed larvae appeared identical to normal larvae, except for lack of movement. A few paralyzed pupae were found, but no adults. Many hymenopteran parasitoids prevent their host from molting as a result of stinging. For instance, *Habrobracon* (*Bracon*) *hebetor* (Say) (Hymenoptera: Pteromalidae) is known to paralyze pyralid larvae (Brower, 1995), which then remain suitable for oviposition or adult feeding for weeks (Hagstrum, 1983). *A. calandrae* developed from last instar larvae to adults at the same rate regardless of host age. The lack of melanization and healthy appearance of the paralyzed larvae suggest paralysis without parasitization, as described in *H. hebetor*. However, data from the present study do not preclude the possibility that differences between the age groups in the proportion of paralyzed hosts that were parasitized are due to differences in survival between 0 and 4 days post oviposition rather than to differences in rates of oviposition following parasitization.

One question concerning the difference in success of *A. calandrae* in paralyzing and parasitizing the three age groups is whether the variation is in part due to the behavior of the females; i.e., in our no-choice trials, did some of the females given younger hosts simply fail to attack any of hosts available to them? We tested this possibility by examining the number of available hosts attacked by each *A. calandrae* female as frequency distributions. If the lower attack rate on rice weevil larvae were due to variation in the propensity of *A. calandrae* females to attack younger hosts, then one would expect a more nearly bimodal distribution in the attack rate among the 16–18 and 18–20 day old hosts and some incidents of no hosts attacked in these younger cohorts. Clearly, this is not the case.

There was a significant difference in size between the 16–18 and the 18–20 day old rice weevils, and there was a significant difference between the frequencies of developmental stages (larvae, prepupae, and pupae) between 18–20 and 20–22 day old rice weevils. The tunnel diameter of the 18–20 day old cohort in the present study was within the range of 1.20 to 1.48 mm reported for 4th instar rice weevils in wheat in a previous study (Sharifi and Mills, 1971). However, the tunnel size of the 16–18 day old cohort was intermediate between that range and the 0.70 to 0.97 mm range reported for 3d instar rice weevils in wheat in that study. Tunnel sizes have usually

not been reported for prepupae and pupae because these stages are readily identifiable and because there is less correspondence between body size and tunnel size following the excavation of a pupal chamber (Sharifi and Mills, 1971). The tunnel widths we observed are distinctly smaller than those observed associated with maize weevils in corn (Smith, 1993) or wheat (Sharifi and Mills, 1971). It has been generalized that fitness of *A. calandrae* and other ectoparasitoids attacking internally-feeding grain and seed pests increases with size of host. It therefore would be interesting to know whether prepupae and teneral pupae would support *A. calandrae* development better than other stages if different host species infesting grains with larger kernels are examined.

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Three-Dimensional Orientation of Male *Cadra cautella* (Lepidoptera: Pyralidae) Flying to Calling Females in a Windless Environment

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ABSTRACT A male *Cadra cautella* (Walker) flying in a windless environment first reacts behaviorally to the sex pheromone plume of a calling female from a distance of ≈40 cm. Its angular velocity (turning rate in degrees per second) increases and, if it approaches within ≈20 cm, its velocity decreases gradually until it lands near the female. The orientation pattern differs from orientation in wind primarily in the fraction of turns with net movement away from the female, initially ≈0.5. This fraction decreases to ≈0.2 if the male approaches within ≈10 cm. Previously developed computer models suggest that such behavior is less efficient for finding a pheromone source than pheromone-stimulated optomotor anemotaxis but more efficient than random searching. Thus, it can be expected that pheromone traps are less effective at sampling remote populations of insects in a warehouse than in a field, but are helpful in pinpointing nearby infestations.

KEY WORDS stored product moths, searching behavior, sex pheromone, communication

AN INSECT IN SEARCH OF a mate quickly modifies its flight behavior when it detects sex pheromone. Information it perceives through olfaction, vision, and mechanoreception triggers changes in velocity and angular velocity (degrees per second) that direct its motion toward the pheromone source. The motion is strongly affected by the concentration, shape, and structure of the pheromone plume (for example, Cardé and Hagaman 1979, Payne et al., 1986, Vickers and Baker 1992, Charlton et al. 1993), all of which are modulated by dynamic changes in wind turbulence, velocity, and direction (for example, Gibson and Brady 1985, Willis and Arbas 1991, Liu and Haynes 1993). The mechanisms of searching behavior are now understood sufficiently to enable some control of the insect's turning frequency and speed in a wind tunnel by presenting it with short, precisely timed pheromone pulses (Mafra-Neto and Cardé 1994, Vickers and Baker 1994).

Much of the research on pheromone-stimulated searching behavior has focused on the typical case where the insect is flying in wind; nevertheless, the mechanisms by which insects find pheromone sources in the absence of wind are of practical and ethological concern. Pheromone traps are commonly used in warehouses to pinpoint infestations of stored product insects. Experimental modifications of trap locations, sizes, shapes, and emission sources have enabled improvements in trap utility (Burkholder and Ma 1985, Vick et al. 1990), but further improvements would accrue from a better understanding of searching behavior in these environments.

Literature concerning orientation without wind is scattered through many different research areas. A large group of studies deals with orientation to resources (foraging) without directional cues. Historically, orientation without directional cues was described as orthokinesis, klinokinesis, or klinotaxis. In recent years, strict definitions of these terms have been difficult to maintain as more examples have been described (for example, Bell and Tobin 1982). Stinner and Bachelor (1993) reviewed the literature on foraging and derived a search model that has applicability to mate-seeking behavior either with or without wind. In their model, search near a resource is a spiral with a high angular velocity and low velocity. A gradual transition to low angular velocity and higher velocity occurs away from the resource.

Other studies consider orientation without wind as a case of reduced orientational information. Bell and Tobin (1982) described the general case. Baker and Kuenen (1982), Kuenen and Baker (1982), and Baker et al. (1984) analyzed the behavior of male *Grapholita molesta* (Busk) under different wind conditions (steady, ceasing, zero wind), with and without pheromone. David and Kennedy (1987) compared flight by *Lymantria dispar* (L.) with and without wind and pheromone. Willis and Cardé (1990) analyzed *L. dispar* flights at several wind speeds including zero. In all these studies, the angles between zigzags (turn magnitudes) were

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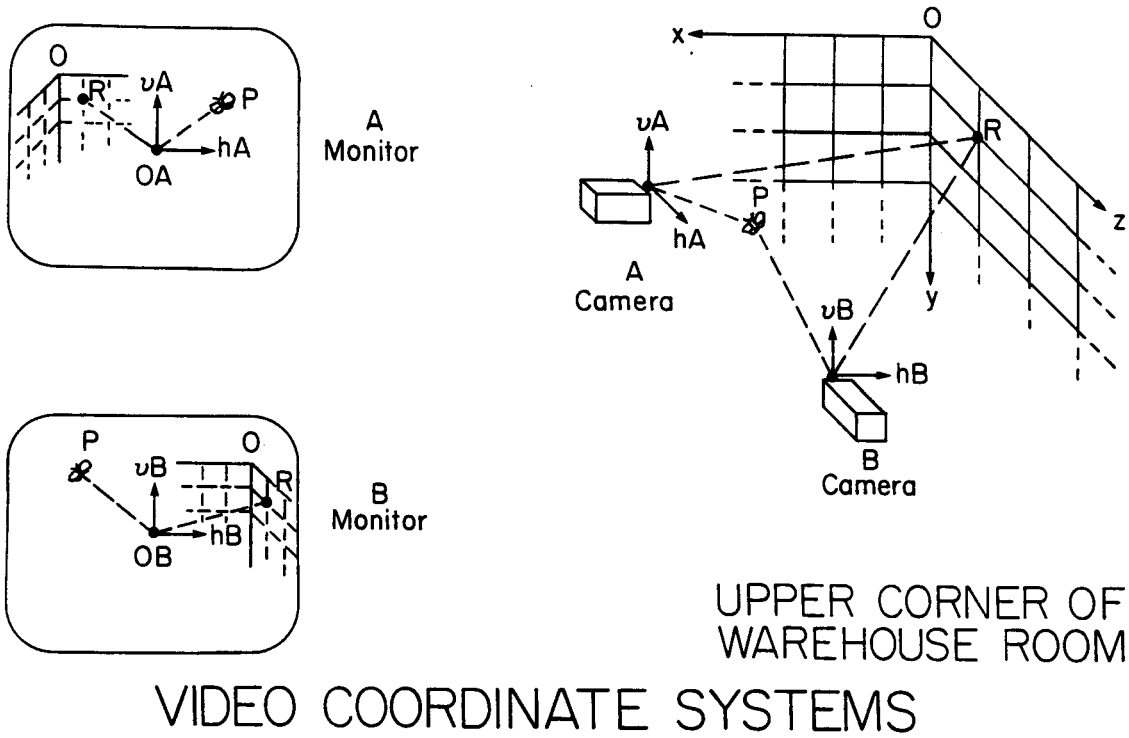

Fig. 1. Coordinate systems used for position, P, of a moth flying in a warehouse room and the positions of the moth on the monitors of cameras A and B recording the flight. O, OA, and OB are, respectively, the origins in the room, A-camera, and B-camera coordinate systems. R is a fixed reference point on 1 wall. In the room coordinate system, y is the vertical axis; x and z are horizontal axes. In the monitor coordinate systems, νA and νB are the vertical axes, and hA and hB are the horizontal axes for the A and B monitors, respectively.

greater without than with wind. Velocity decreased as windspeed decreased, but at zero windspeed it increased. Turning frequency was unaffected by changes in windspeed. The fraction of turns downwind (that is, turn bias away from female) increased when wind decreased to zero. On average, moths could orient without wind to pheromone sources but their efficiency was reduced.

Several studies have examined mate location by stored product moths in a warehouse environment (Hagstrum and Davis 1980, 1982; Mankin et al. 1983). The instantaneous wind velocity in a warehouse is not necessarily zero even if the building is airtight and the temperature is uniform. However, orientational cues are much reduced compared with those in the field and in wind-tunnel experiments cited above. The warehouse studies focused on coarse-scale flight patterns (movement among 1.4-m cubes), but it was observed that flights by *Cadra cautella* (Walker) and *Plodia interpunctella* (Hübner) males became area restricted (that is, net movement decreased) near a pheromone source. For male *C. cautella*, area-restricted flight began at distances <60 cm from the female. This distance decreased in a pheromone-permeated atmosphere.

To gain more insight into the mechanisms of orientation to pheromone in a windless environment, and to characterize male *C. cautella* area-restricted flight precisely, we conducted a detailed, 3-D video analysis of searching behavior with and without calling females in a warehouse room.

Materials and Methods

Arena. Flights were observed inside an interior room (5.9 by 5.9 by 5.9-m) of a warehouse described previously in Hagstrum and Davis (1980) and Mankin et al. (1983). The north and east walls of the upper northeast corner had been gridded in lines 10-cm apart (Fig. 1). Light was provided by sixteen 40-W fluorescent bulbs paired at 8 evenly spaced locations on the ceiling. The translucent covers beneath the light fixtures were covered by Plexiglas barriers. Wind currents were negligible during tests because of the arena's interior location, remote from sources of wind drafts or temperature differentials. Periodic checks with a hot-wire anemometer (ThermoSystems model 1610-12, St. Paul, MN) typically found velocities of 2–5 cm/s, the same order of magnitude as the thermal currents generated by the hot-wire itself. Temperatures during the tests were 24–28°C.

Two surveillance cameras (Panasonic model 1050A, Matsushita Electric, New York) were con-

nected to separate time-lapse videotape recorders and observed from monitors (Panasonic model 8030 and TR195B, respectively). They were pointed perpendicular to each other and toward the ceiling at the northeast corner of the room. Specifically, camera A faced east, 440 cm from the east wall, 10 cm below the ceiling, and 60 cm from the north wall (XA, YA, ZA = 440, 10, 60 cm in room coordinates). Camera B faced north from (XB, YB, ZB = 60, 10, 440 cm). Digital timers (0.1-s resolution) were set near the walls in both fields of view to enable synchronization of the frames recorded from the 2 cameras. The total space in the combined fields of view was approximately the same volume contained in the 1.4-m cubes used in an earlier study of *C. cautella* searching behavior in the entire warehouse room (Hagstrum and Davis 1980).

Insects. *C. cautella* was reared and handled as described in Hagstrum and Davis (1980) (a photoperiod of 14:10 [L:D] h, with observations beginning at the start of scotophase at 1400 hours). Unmated adults were collected in the morning of the previous day and placed in separate vials. A female, when present, was strapped before testing on the ceiling inside a petri dish at FEMREFPT (female reference point = 50, 0, 50 cm) in the room coordinate system. The dish was removed just before males were released in the center of the floor. If a male ceased flying, another was released, up to a total of 4. The place where a female settled and called, FEMCALLPT, was usually within ≈10 cm. The reference origin for calculation of distances and angles was FEMCALLPT when a female was present, and FEMREFPT otherwise.

Flight Analysis. Moth flights in the field of view of both cameras were digitized from the monitor screen with a digitizer (Numonics model 1220, Lansdale, PA) and converted to 3-D room coordinates by subroutines (Mankin et al. 1987) operating on a microcomputer (Digital Equipment model PDP 11/23, Maynard, MA). The recordings were replayed and digitized, frame by frame. The digitizer sent the moth's coordinates to a printer or a datalogger (Campbell Model CR20, Logan, UT), which stored them on a digital cassette recorder. The data on the cassette recorder were transmitted to the microcomputer for analysis.

A customized program containing ≈80 user-written and 50 vendor-supplied (DEC-graphics) subroutines calculated the moth's room coordinates from the digitized monitor images. The functions done by the software included file retrieval from the digitizer station, timing synchronization between files recorded from different cameras, translation of files into binary format, 3-D reconstruction of the flight from the monitor coordinates, and 3-D motion analyses of individual flights. Part of the retrieval program was written in assembly language. The remainder of the programming was in FORTRAN IV.

The calculation of 3-D position from 2 cameras is described elsewhere (for example, Rogers and Adams 1976, and Longuet-Higgins 1981). The algorithm used in this report is given in *Appendix 1.*

Calibration and Error Reduction. Several procedures were instituted to reduce digitization and synchronization errors. Digitizer errors were minimized by averaging 6 consecutive frames (1/60-s intervals) to construct data sets of average x, y, and z coordinates in 0.1-s intervals (*Appendix 1*). To determine the precision of the digitizer-microcomputer system, the screen coordinates of 4 points (grid intersections) on the east wall were digitized and stored at the beginning of each flight file. The positions of 3 points were predicted from calculations based on the 4th point. These were compared with the actual positions in 2 separate recordings. The discrepancy between the predicted and actual position of a grid intersection increased with its distance from the reference point up to 4 cm for points at the edge of the screen. To reduce calibration error, the orientation reference chosen for each flight was the measured intersection closest to the moth initial position.

Statistical Analysis. Nineteen flights of individual males and 8 flights of males that found females were stored as SAS data files (SAS Institute 1985) of average x, y, and z coordinates in 0.1-s intervals. The distance between male and female, velocity, and angular velocity were calculated by the algorithm described in *Appendix 2.* Turn bias was calculated by subtracting the distance between male and female from that in the previous interval, setting the bias for distances <0 to 0 and >0 to 1, and averaging over each cm distance from the female.

Equations were fit to mean velocity, angular velocity, and turn bias as a function of distance from female (DISTFEM) using PROC REG and PROC NLIN procedures (SAS Institute 1985). One-tailed *t*-tests were applied to the standard errors of the predicted values to find the distances at which slopes were significantly different from zero and the distances at which motion parameters were significantly affected by a calling female.

For comparisons with other studies of searching behavior in wind, a second data set was constructed by approximating each flight as a series of straight tracks between turns >45°. Turn magnitude (TurnMag) was calculated as the angle between 2 consecutive straight tracks. An estimate of track angle (TrkAngle) was calculated as the supplement of the heading (Akers 1989); that is, the angle between the straight track and the line pointing to the reference origin. Note, however, that the actual track angle (Marsh et al. 1978) is undefined in zero wind. Length of straight track was calculated as the distance between 2 consecutive turns. Interturn interval was the time between 2 consecutive turns. The interturn intervals, lengths, track angles, and turn magnitudes were compared at dif-

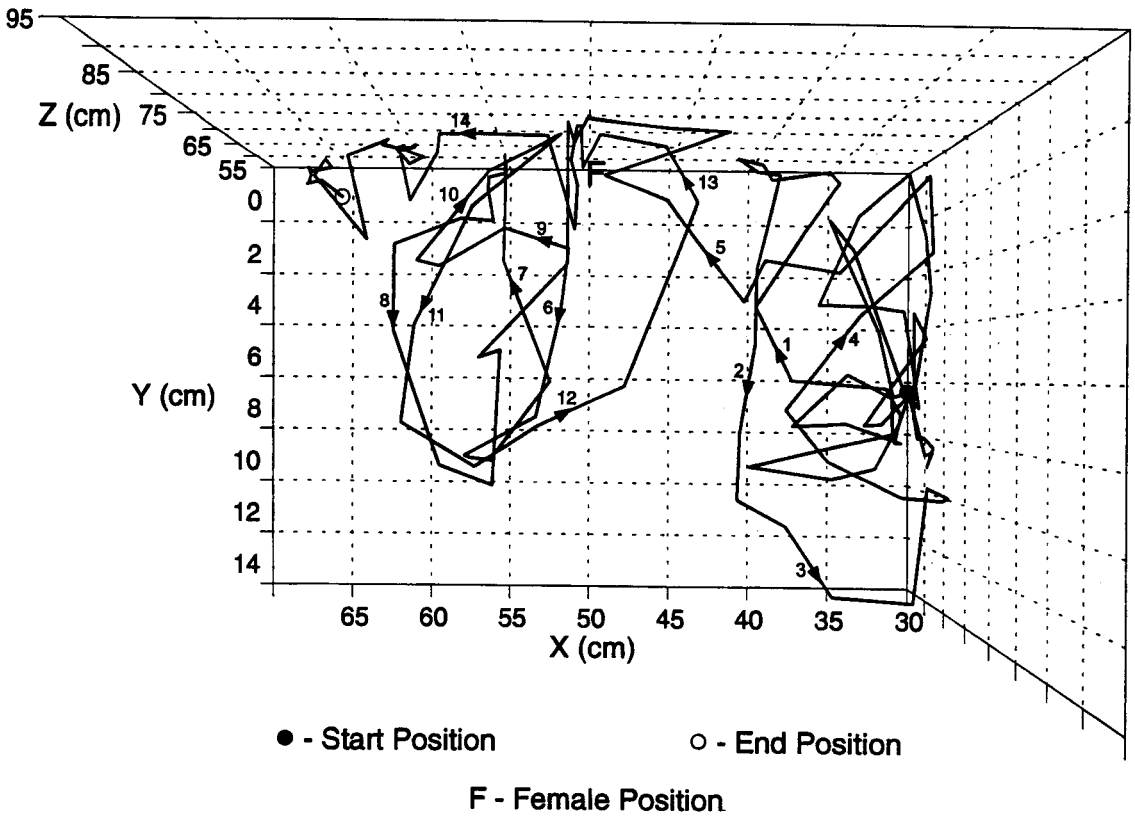

Fig. 2. Example of a typical flight of a male *C. cautella* to a calling female. Closed circle indicates entry into field of view. Open circle indicates last point before male touched ceiling, after which it bounced closer, landed, walked to female and mated. Solid lines connect consecutive positions at 0.1-s intervals. Arrows indicate direction of flight, and the numbers by each arrow indicate the sequence of movements.

ferent distances from the reference origin by use of the Duncan–Waller test (SAS Institute 1985).

Results

The flights in Figs. 2 and 3 show typical differences between male *C. cautella* searching behavior with or without a calling female present. The figures were constructed by plotting position at 0.1-s intervals, with solid lines connecting consecutive positions. Dotted lines in Fig. 3 denote distance from the ceiling. For clarity, distance from the ceiling is not shown in Fig. 2, but arrows are added to show flight direction. A number by each arrow denotes the time sequence of movement. Males fly faster and turn less frequently without a calling female. These differences appear in the figures as longer distances and shallower angles between line vertices. Males loop back less frequently into a previously explored area without a calling female. This difference appears in Fig. 2 as a difference in the total length of observable path. All the males bounced on the ceiling, landed away from the female, and walked the remaining distance. The male in Fig. 2 landed on the ceiling (at 65, 0, 58 cm) ≈20 cm away from the female and walking the remaining distance to her.

The differences appearing in Figs. 2 and 3 are reflected in average flight behavior at different distances from female (Fig. 4). Mean angular velocity increased from 500 to 1,000°/s, and mean velocity decreased from 40 to 5 cm/s as a male approached the calling female. The vertical and horizontal components of velocity did not differ significantly. The proportion of turns away from the female decreased from 0.5 to 0.2 as the male approached the female.

The regressions of angular velocity, velocity, and turn bias were nonlinear on distance from female, when the female was present, but linear without the female present (Table 1; Fig. 4). The slopes of the regressions for turn bias, velocity, and angular velocity with the female present were significantly different from zero ($P < 0.05$) at distances from female of <16, 50, and 37 cm, respectively. The slope of mean velocity, but not angular velocity or turn bias, was significantly different from zero ($P < 0.05$) when no female was present.

For flight near a calling female, the distances at which turn bias, velocity, and angular velocity be-

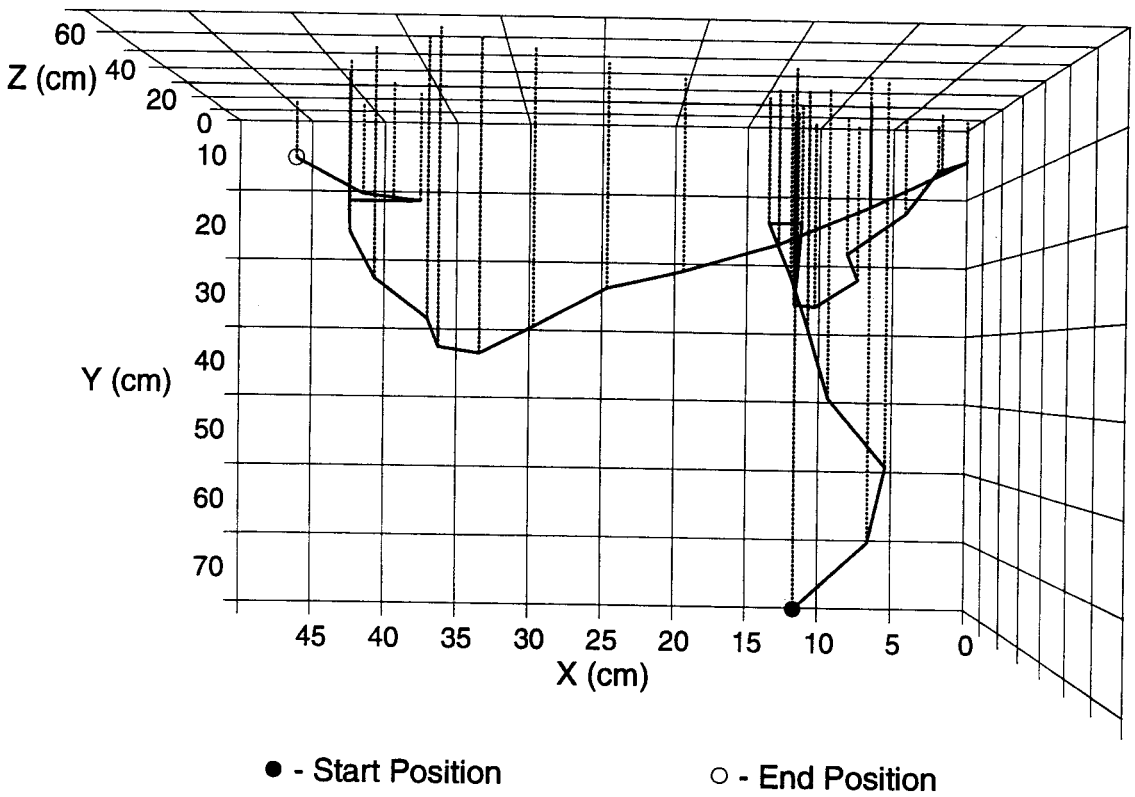

Fig. 3. Example of a typical flight of a male *C. cautella* without a female present. Closed circle indicates entry into field of view. Open circle indicates exit from field of view. Solid lines connect consecutive positions at 0.1-s intervals. Dotted lines indicate distance from ceiling.

came significantly different ($P < 0.05$) from those of males without females present were 12, 21, and 44 cm, respectively. The following behaviors can thus be zoned as follows: open flight (DISTFEM > 44 cm), a zone where neither velocity nor angular velocity differs significantly from that when no female is present; conditioned flight (21 < DISTFEM < 44 cm), a zone where 1 but not both differ from those when no female is present; and restricted flight (DISTFEM < 21 cm), a zone where both differ from those when no female is present.

The reduced data set containing only turns >45° yielded trends in interturn interval, turn magnitude, track angle, and turn length (Table 2) that were consistent with the regressions in Fig. 4. Interturn interval is significantly smaller and turn magnitude is significantly greater in the restricted than in the open zone, as expected from the increased angular velocity. Track angle is inversely proportional to turn magnitude and is significantly smaller in the restricted than in the open zone. Turn length, like velocity, is smaller in the restricted than in the open zone. Interturn interval, turn length, and track angle in the open zone are the same as when no female is present, which suggests that the female is not detected in the open zone.

Discussion

Similarities Among Search Behavior Patterns. Search behavior in still air conforms to rules that may have considerable generality—whatever the nature of the orienting stimulus, the resource being sought, the sex of the searcher, or the mode of locomotion (Akers 1989, Bell 1990, Stinner and Bachelor 1993). Typically, the angular velocity of a searching insect increases, its velocity decreases, and its turn bias away from an attractant source decreases as it approaches the source. Stinner and Bachelor (1993) developed a 2-dimensional model of similar behavior, "stochastic spiral search," and compared its efficiency in finding a resource with that of a "restricted random" pattern in which turning angle is normally distributed. Their computer simulations suggested that a spiral search pattern is more efficient than a random pattern. In this case, the pheromone source is found less efficiently when no wind is present to polarize the direction of turns, but the increased turning angle and decreased velocity result in an increase in time spent near the point of detection. Arrestment near the female increases the likelihood of location.

Comparison with Orientation in Wind. Without wind, the moth lacks the cues to polarize prop-

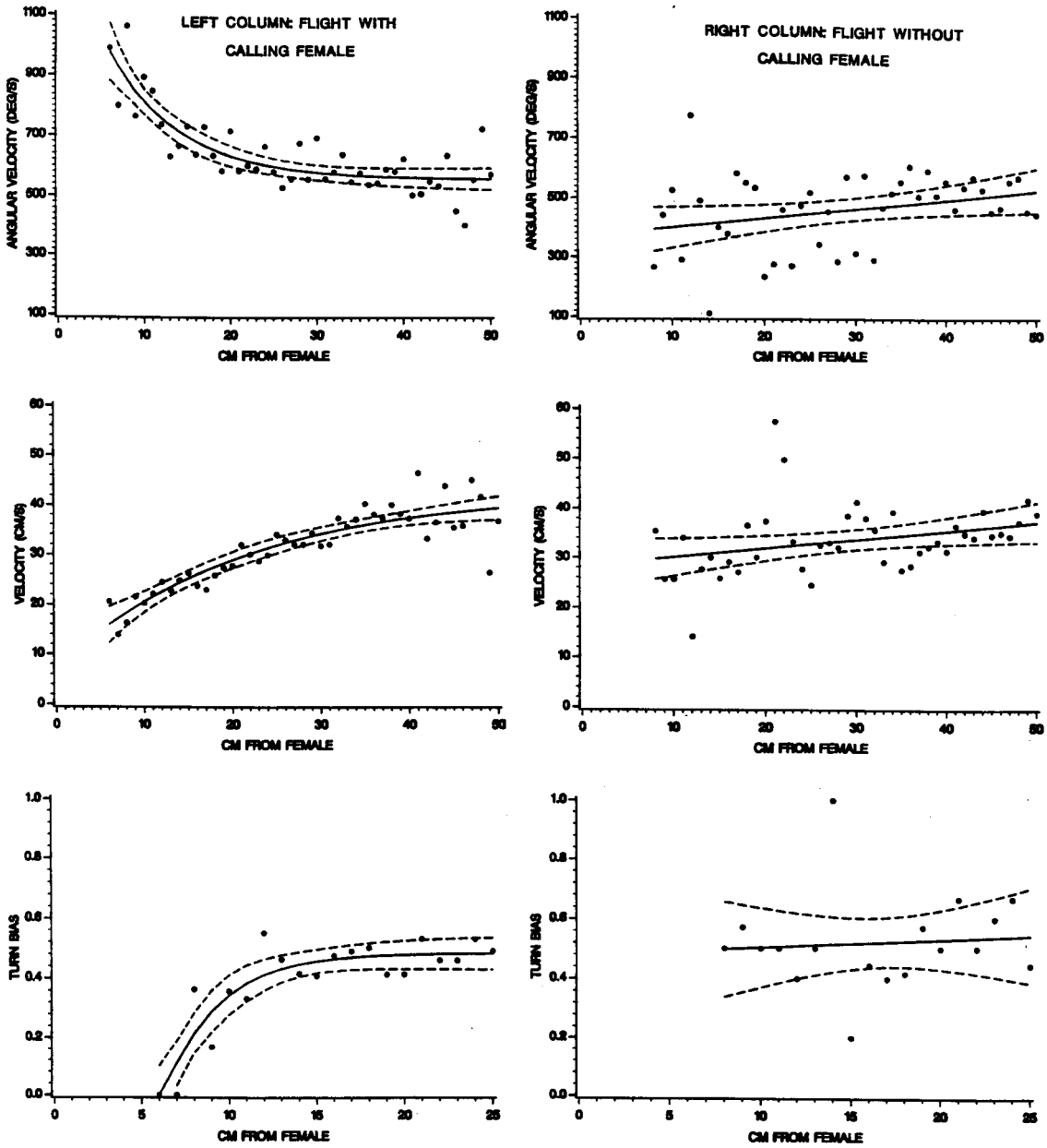

Fig. 4. Differences in male *C. cautella* searching flight behavior with (left column) and without (right column) calling female: angular velocity (mean rate of deviation from the direction in the previous 0.1-s interval); velocity (mean rate of travel during preceding 0.1-s interval); turn bias (mean fraction of turns with male more distant from female than in preceding interval; and cm from female (= DISTFEM) is distance from calling point if female is present, or from reference point if no female is present.

erly its turns or zigzags toward the female (Baker et al. 1984), but otherwise its behavior is similar to that of males detecting pheromone in wind. The decrease in track length from 11 to 6 cm (Table 2) is similar to the decrease from 14 to 8 cm reported by Marsh et al. (1978) for *P. interpunctella* males flying to a sex pheromone source in wind.

It is interesting that the 0.32- and 0.4-s differences between turns in the restricted and open zones correspond with measurements by Mafra-Neto and Cardé (1994) of flights by male *C. cautella* exposed in wind to pheromone plumes of 2 different structures. In their study, a 0.29-s interturn interval after exposure to slow pulses of pher-

Table 1. Motion parameters ± SEM for equations describing male *C. cautella* mean angular velocity, velocity, and turn bias away from female in relation to DISTFEM (= distance from FEMCALLPT [calling female present] or FEMREFPT [male without calling female])

Motion	Regression parameter						
	Male without calling female: motion = A + B * DISTFEM			Male with calling female: motion = A * $e^{-B*DISTFEM}$ ± C			
	A (Intercept)	B (Slope)	r^2	A	B	C	r^2
Angular velocity	367.9 ± 47.7	3.1 ± 1.5	0.00039	882.09 ± 202.6	0.122 ± 0.028	554.8 ± 19.32	0.707
Velocity	28.3 ± 2.6	0.18 ± 0.083	0.036	−35.68 ± 2.79	0.0471 ± 0.015	42.83 ± 3.49	0.799
Turn bias	0.47 ± 0.13	0.0027 ± 0.0075	0.030	−3.77 ± 2.16	0.330 ± 0.087	0.48 ± 0.026	0.807

omone increased to 0.4 s after fast pulses—the same as that measured in this study in the open zone (and for males with no female present). A possible explanation for this correspondence is based on another finding of Mafra-Neto and Cardé (1994) that pheromone-induced counterturns can be suppressed if the tempo of pheromone pulses exceeds a certain frequency. If the trigger to counterturn on detection of a pheromone pulse is suppressed, the resultant rate of counterturning may be reduced to about the same level as the rate when no pheromone is present.

Angular velocities, like turning rates, are similar to those reported in wind (for example, 450–490°/s in Charlton et al. [1993]). One parameter, mean track angle, was greater than the mean of 60° without wind obtained by Baker et al. (1984).

Three-Dimensional Versus 2-D Analysis. The original descriptions of moth searching behavior mechanisms were based on 2-D analysis of flights in wind tunnels (Traynier 1968, Kennedy and Marsh et al. 1978), and 2-D analyses remain in common use (for example, Cardé and Charlton 1983, Baker 1989, and Quartey and Coaker (1993). However, 3-D techniques have been used in some recent wind-tunnel studies (for example, Witzgall and Arn 1990, Riley et al. 1990, Aluja et al. 1989) and previous studies of insects confined in small volumes (Shinn and Long 1986, Okubo et al. 1981, Wyss and Pollak 1981, and Buelthoff et al. 1980). 3-D analysis is more complicated and time-consuming than 2-D analysis, although the availability of modern digital signal-processing techniques makes it easier now than a decade ago. The distances over which flights can be analyzed typically is more restricted in 3-D than in 2-D cases because the insect must be in the fields of view of both cameras. However, it is the only way to obtain precise measures of velocity vectors and positions of behavioral transitions when the moth position is unconstrained. A 2-D analysis is satisfactory in a wind tunnel arena because the tunnel constrains motion in 2 of the 3 directions.

Applications to Use of Pheromone Traps. An important question in the understanding of competition between females and artificial pheromone sources is the distance over which communication occurs. A communication distance for male and female *C. cautella* can be estimated from the regressions in Table 1. Angular velocity began to increase when the male passed to within 44 cm of the female. The velocity began to decrease within 21 cm, and the turn bias away from the female began to decrease within 12 cm. Each of these changes began at a different distance from the female and may have been initiated by different pheromone concentrations, considering that the pheromone concentration usually decreases with distance from the calling female. It should be noted, however, that the instantaneous pheromone distribution in a warehouse can and often does vary considerably from the mean (see discussion below on plume shape and intermittency).

The communication distances estimated here compare well with the estimate by Hagstrum and Davis (1980) that *C. cautella* male–female communication distances were <60 cm. They also compare well with the distance of 0.4–2.5 m pre-

Table 2. Mean ± SEM for interturn interval, length of track, track angle (TrkAngle), and turn magnitude (TurnMag) for male *C. cautella* flying in specified zones (d = DISTFEM in cm [*Appendix 2*]) in search of calling female

Interturn	Unit	Search zone		
		Restricted (d < 21)	Conditioned (21 ≤ d < 44)	Open[a] (44 ≤ d)
Interval	s	0.32 ± 0.009b	0.33 ± 0.009b	0.40 ± 0.02a
Length	cm	6.36 ± 0.30c	8.73 ± 0.35b	11.37 ± 0.69a
TrkAngle	°	81.22 ± 2.27b	89.42 ± 2.08ab	91.88 ± 4.46a
TurnMag	°	119.63 ± 2.39a	115.89 ± 2.09a	101.13 ± 3.98b

Means in a row followed by the same letter are not significantly different ($P < 0.05$; Waller–Duncan test [SAS Institute 1985]).
[a] Parameter magnitudes in this zone are not significantly different from those when no female is present (Waller–Duncan test [SAS Institute 1985]).

dicted for trapping *P. interpunctella* in Mankin et al. (1980) and the 30-cm distance where *G. molesta* males began shortening their zigzags in still air (Baker et al. 1984). The distance of restricted search (<21 cm) compares well with the distance from which walking female bark beetles find a pheromone source in still air (Akers 1989).

A related question concerns the shape and intermittency of the pheromone plume emitted by either a female or a pheromone trap in a warehouse. The plume emitted by the female was not controlled precisely as in Mafra-Neto and Cardé (1994), but comparison of interturn interval and track angles in the 2 studies suggests a relatively close fit between flights by male *C. cautella* in the observation arena and by males exposed to 0.17-s pulses, 1.45 s apart. When pulses were rapid, track angles were smaller and interturn intervals were longer than those in this study. Interturn intervals were much smaller than in this study when the plume was continuous. Such a result is to be expected because the instantaneous concentration of pheromone near the female varies considerably (Mankin et al. 1980, Murlis et al. 1992). Small-scale, low-energy turbulent eddies, some of them generated by the male's own wingbeats, reshape the emitted plume into a series of small discontinuous puffs. The behavior of the male in Fig. 2, for example, suggests that a puff was encountered near (30, 5, 55 cm), and then a 2nd puff near (55, 8, 70 cm).

The short communication distance of ≈40 cm suggests that pheromone traps in a warehouse work best to pinpoint local infestations rather than to attract insects from long distances (Vick et al. 1990). Emitting pheromone from a trap at a high release rate may be counterproductive in a windless environment because it may trigger restricted searching behavior too far from the trap, keeping the insect from approaching closer to the actual source. Further improvements in pheromone trap designs for stored-product insects are more likely to result from efforts to make them more visually attractive to the insects or from forced laminar air flow.

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Appendix 1

Algorithm for Calculation of XYZ Flight Coordinates. The 3-D coordinates were reconstructed from the corresponding images on 2 video monitors by specifying a reference point, R, in all 3 coordinate systems and the cameras' positions in room coordinates. An example reference point R (XR, YR, ZR) is the intersection of the first 2 grid lines on the northeast wall: (0, 10, 10 cm). In Fig. 1, the origins of the monitor coordinate systems, OA and OB, are shown at the centers of the respective monitor screens, (HCA, VCA) and (HCB, VCB). The male's position, P (X, Y, Z) in the room coordinate system, is (HA, VA) in A-monitor coordinates and (HB, VB) in B-monitor coordinates.

The H and V coordinates were smoothed as 0.1-s averages of 6 consecutive frames. That is,

$$HA_{t=0.1s} = \left(\sum_{i=1}^{6} HA_i\right)\Big/6 \quad \text{and}$$

$$HA_{t=0.2s} = \left(\sum_{i=7}^{12} HA_i\right)\Big/6,$$

where i is the number of frames from the beginning of analysis.

Equations relating the XYZ coordinates to the smoothed H and V coordinates of the A and B monitors are listed below. The algorithm relates the area of an object on the monitor to its silhouette perpendicular to the camera axis. The area of the object on the monitor is inversely proportional to its distance from the camera. The moth's position, P (X, Y, Z), also can be determined by other referents, for example, by the positions of 6 noncoplanar reference points specified in room and monitor coordinates (Rogers and Adams 1976).

The proportional relationships between real XYZ coordinates and the camera coordinates are described by the equations:

$$VA' = (VA - VCA)/(VRA - VCA) = [(Y - YA)/(X - XA)] \cdot [(XR - XA)/(YR - YA)], \quad (1)$$

$$HA' = (HA - HCA)/(HRA - HCA) = [(Z - ZA)/(X - XA)] \cdot [(XR - XA)/(ZR - ZA)], \quad (2)$$

$$VB' = (VB - VCB)/(VRB - VCB) = [(Y - YB)/(Z - ZB)] \cdot [(ZR - ZB)/(YR - YB)], \quad (3)$$

$$HB' = (HB - HCB)/(HRB - HCB) = [(X - XB)/(Z - ZB)] \cdot [(ZR - ZB)/(XR - XB)], \quad (4)$$

with the horizontal and vertical coordinates from cameras A and B defined as in Fig. 1. Using the definitions

$$YRA = YR - YA, \quad YRB = YR - YB,$$
$$XRA = XR - XA, \quad XRB = XR - XB,$$
$$ZRA = ZR - ZA, \quad \text{and} \quad ZRB = ZR - ZB,$$

equations 1–4 have solutions:

$$X = [YB - YA - (VB'/HB')(YRB/XRB)XB + VA'(YRA/XRA)XA] \div [VA'(YRA/XRA) - (VB'/HB')(YRB/XRB)], \quad (5)$$

$$Y = [HB'(YRA/XRA)YB - VB'(YRB/XRB)YA + VA'VB'(YRA/XRA) \cdot (YRB/XRB)(XA - XB)] \div [VA'HB'(YRA/XRA) - VB'(YRB/XRB)], \quad (6)$$

$$Z = [YA - YB - (VA'/HA')(YRA/ZRA)ZA + VB'(YRB/ZRB)ZB] \div [VB'(YRB/ZRB) - (VA'/HA')(YRA/ZRA)]. \quad (7)$$

Appendix 2

Flight Motion Parameter Algorithm. X, Y, and Z are 3-D coordinates relative to the origin, O (Fig. 1), in centimeters. RX, RY, and RZ are coordinates relative to the reference origin. TIME is the time from beginning of analysis in 0.1-s increments. LAGn(variable) = magnitude of variable at *n*th previous valid time before before TIME. The algorithm discarded intervals where the moth moved less than 1 body length (Tourtellot et al. 1991). At each otherwise valid interval, the algorithm calculated:

Variable	Unit	Definition
DISTFEM	cm	Distance between position of flying male and calling female
TDIFF	s	TIME − LAG1(TIME)
VEL	cm s^{-1}	Velocity (distance between current and previous position/TDIFF)
VX, VY, VZ		X, Y, and Z velocity components
ANG	°	Angle between line connecting positions at LAG2(TIME) and LAG1(TIME) with line connecting positions at LAG1(TIME) and TIME
ANGV	$°s^{-1}$	Angular Velocity (ANG/TDIFF)
ANGACC	$°s^{-2}$	Angular Acceleration (ANGV − LAG(ANGV))/TDIFF

Note: Flights are presorted, starting at TIME = 0.1. STIM = 'F' (female present) or 'N' (none present). Major variables are in **bold**.

```
data allpts;  /* data file  */
infile 'c:\ecorient\ecorient.prn';
retain oldtime oldx oldy oldz oldxdif oldydif oldzdif
  olddis oldangv oldang count olddfem ang;
keep flt stim time x y z rx ry rz distfem vx vy vz
  vel ang angv angacc;
input stim $ flt $ TIME y x z ry rx rz;
DISTFEM = (rx*rx + ry*ry + rz*rz)**.50;
if time = 0.1 then do; /* initial time in each flight */
  count = 1; oldtime = time; oldx = x; oldy = y;
  oldz = z; olddfem = .; vel = .; angv = .; angacc
  = .; ang = .; absang = .; oldang = .; end;
else do;  /* all other times */
  oldang = ang; xdif = (x - oldx); ydif = (y -
  oldy); zdif = (z - oldz); VX = abs(xdif); VY =
  abs(ydif); VZ = abs(zdif); TDIFF = time -
  oldtime;
  DISMAL = (xdif*xdif + ydif*ydif + zdif*zdif)
    **0.5; if dismal > 0.5;
  VEL = dismal/tdiff; VX = vx/tdiff; VY =
    vy/tdiff; VZ = vx/tdiff;
  if count = 1 then do;
    count = 2; oldtime = time; oldx = x; oldy =
    y; oldz = z; olddfem = distfem; oldxdif = xdif;
    oldydif = ydif; oldzdif = zdif; olddis = dismal;
    angv = .; angacc = .; ang = .; oldang = .; end;
  else do;
    xx = xdif*oldxdif; yy = ydif*oldydif; zz =
    zdif*oldzdif; div = dismal*olddis; cosang =
    (xx + yy + zz)/div;
  if cosang >= 1 or cosang <= -1 then angl = 0;
  else  angl = arcos(cosang);
  absang = angl*(180/3.1415927);
  ANGV = absang/tdiff;
  if distfem > olddfem then ANG = -absang;
  else  ANG = absang;
  if count = 2 then do;
    count = 3; oldtime = time; oldx = x; oldy =
    y; oldz = z; oldxdif = xdif; oldydif = ydif;
```

```
    oldzdif = zdif; olddis = dismal; oldangv =
    angv; olddfem = distfem; angacc = .; end;
else do; count = count + 1;
    ANGACC = (angv - oldangv)/(time -
    oldtime); oldtime = time; oldx = x; oldy =
    y; oldz = z; oldxdif = xdif; oldydif = ydif;
    oldzdif = zdif; olddis = dismal; oldangv =
    angv; olddfem = distfem; end; end; end;
```