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Title: ACOUSTIC AND OLFACTORY BEHAVIOR OF IPS PINI (SAY) (COLEOPTERA: SCOLYTIDAE) DURING HOST INVASION AND COLONIZATION

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J. A. Rudinsky

The anatomy of the male and female Ips pini head vertex and inner pronotal structure is compared, and the female's vertex-pronotal stridulatory apparatus is described. The male lacks this sound producing apparatus.

Female stridulation under different behavioral situations was described and compared. The situations were stress, attraction, in-gallery, and rivalry conditions. The following parameters were compared: the number of pulses per chirp, the number of interruptions (unusually large gaps between consecutive pulses), the chirp length excluding interruptions, and the pulse rate. The attraction stridulation produced by females attempting to enter a given type of attractive gallery was homogeneous, whether the gallery contained a male alone or a male plus 1-4 females. The chirps produced by females attempting to enter a hole containing a male plus three

females differed in the number of pulses per chirp and the pulse rate from the chirps produced during attempts to enter other types of attractive galleries. Chirps emitted contingent to stress, rivalry, in-gallery, and attraction situations differ. There was no significant difference between attraction and rivalry chirps, but all other comparisons showed significant differences in at least one of the parameters.

Olfactory studies reveal that the observed decrease in secondary attraction is significantly influenced by the relation of the number of females per male and the elapsed time since the female entered the gallery. That relation may involve a stop in production of the aggregative pheromone or the emission of an antiattractant by the male and/or female(s). In addition, the sex proportion of the attracted beetles was approximately 0.58, which is a 1.4:1 female to male sex ratio, and did not vary significantly between or within treatments. Lastly, early attack and colonization observations indicate that, in Oregon, nuptial chamber construction is completed within 1.4 days and the male accepts three females by 3.3 days.

Acoustic and Olfactory Behavior of Ips pini (Say) (Coleoptera: Scolytidae) during Host Invasion and Colonization

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Professor of Entomology
in charge of major

Redacted for privacy

Chairman of Department of Entomology

Redacted for privacy

Dean of Graduate School

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Typed by Mary Jo Stratton for James Archie Swaby

To my wife, Mary Ann, and to Him that makes

all things possible

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ACOUSTIC AND OLFACTORY BEHAVIOR OF IPS PINI (SAY) (COLEOPTERA: SCOLYTIDAE) DURING HOST INVASION AND COLONIZATION

I. INTRODUCTION

The genus Ips is represented by several species in the Pacific Northwest, but the most economically important species is the pine engraver, Ips pini (Say). This bark beetle attacks and breeds in cull logs, windfalls, dying trees, and logging or thinning slash. Periodically, populations attain such large numbers that healthy trees are attacked. Such outbreaks cause top-killing of larger trees and outright killing of young saplings or pole size trees.

F. P. Keen (1952), referring to the genus Ips, stated, "With the removal of mature forests, some authorities consider it likely that this group of bark beetles will outrank the Dendroctonus beetles in destructiveness to the second crop of pines." Probably all pine species within the range of Ips pini are attacked (Chamberlin, 1958), but in the Pacific Northwest, ponderosa pine, Pinus ponderosa Lawson, is the most economically valuable host (Sartwell, 1964). More than 60 percent of the ponderosa pine type forests in the Pacific Northwest have been cut over (Mowat, 1961) and the Ips problem is rapidly becoming a matter of concern.

Ips pini, like insects in general, relies upon a complex sensory system to assure their survival. They respond to visual, tactile, olfactory, acoustical and taste stimuli, but they are most sensitive to sound and scent (Evans, 1968). In comparing those stimuli, one discovers that visual cues can be patterned in a more complicated fashion than acoustic or olfactory cues and may be effective across as great or greater distances, but they are transmissible only during light or dark periods. Tactile stimuli are the most efficient at close range but they are restricted to direct contact transmission (Alexander, 1962).

Alexander (1962) defined a versatile communication system as one which not only effectively mediates a species diverse life situations, but also can be employed without confusion between species. Both long- and short-range functions must be served and it should be usable under a variety of different environmental conditions. Chemical signals have the widest range and their lingering nature imparts lasting information. However, the same signalling device and the same receptor cannot be utilized in transmission of different messages since precise patterning is impossible (Alexander, 1962; Dumortier, 1963; Wilson, 1971). Furthermore, the arrival of a chemical signal over a distance greater than a few centimeters is entirely dependent on the direction of the current transporting it (Dumortier, 1963). Acoustic signals, on the other hand, represent

the most complete and efficient information imparting mode: owing to their ability to diffuse, their resistance to disturbance, and their potential creative vocabulary made possible through diverse parameter variation (Alexander, 1962; Dumortier, 1963). Recently, the interaction of chemical and acoustic systems was shown to be important in Scolytidae (Rudinsky, 1968, 1969; Rudinsky and Michael, 1972; Rudinsky, Morgan, Libbey, and Michael, 1973). It was expected that a similar interaction may exist in polygamous bark beetles; therefore, Ips pini acoustic and olfactory behavior during host invasion and colonization was chosen for study.

Research Objectives

The study objectives were:

1. To comprehensively describe the female I. pini stridulatory apparatus and compare the body regions associated with those structures to the same areas on the male.
2. To describe and compare female I. pini stridulation under attraction, stress, and rivalry behavioral conditions.
3. To determine how the number of female I. pini per male in a gallery influences the sounds emitted by female I. pini attempting to enter that attractive gallery.
4. To determine how the number of female I. pini per male in a gallery influences the cessation of secondary attraction to

the gallery, including influences on the sex ratio of the beetles attracted.

5. To describe the sequence of events during I. pini invasion and early colonization of lodgepole pine.

II. LITERATURE REVIEW

Biology and Taxonomy

Hopping (1964) and Lanier (1972) have reviewed the taxonomic history of this species and its contemporary taxonomic description. Its distribution (summarized from Hopping, 1964; Wygant and Lara, 1967; Sartwell et al., 1971; Baker, 1972; Lanier, 1972) is throughout the North American pine and spruce forests from South Carolina to northern Canada and Alaska; south along the Pacific Coast east of the Canadian Coastal Range, Cascades and Sierra Nevada mountains to northern Mexico. The life history and habits of I. pini were recently described by Baker (1972), Lanier (1972), and Schmitz (1972). The species is holometabolous and has two to five generations a year, depending on the locality and flight season length. The adults overwinter under the bark, in the topsoil or duff. The flight season normally commences in April or May and continues to September. The male initiates the tree invasion by boring through the bark to the cambium where he constructs a nuptial chamber. Then one to eight females join the male and immediately after mating (which takes place in the nuptial chamber) they begin excavating egg galleries. The male extrudes the borings by way of the entrance or specially constructed air holes while the females each lay 30-60 eggs in niches along each

egg gallery wall. Insemination is repeated frequently during the egg deposition period. After egg deposition is completed the parent beetles often emerge and make a second or third attack in new hosts. Egg eclosion occurs in 4-12 days and the larvae make winding, frass packed galleries lateral to the egg galleries as they feed upon the phloem. Ten to 12 days are required to complete the three larval instars at which time a pupation chamber is constructed. New adults appear 2-12 days after pupation and these new adults may feed under the bark up to a month before emerging.

Chemical Communication

It has long been known that many bark beetle species are attracted to particular trees, which are then massively attacked. The contemporary belief, based on research of the past two decades, is that this attraction consists of primary attraction by the host tree and then secondary attraction by pheromones in the chemical communication systems of the beetles. Secondary attraction in animals has been extensively reviewed (Birch, 1974), and Borden and Stokkink (1971) compiled an annotated bibliography listing 175 articles on attraction in Scolytidae. Though the pioneer study on bark beetle attractants (Anderson, 1948) was done on Ips pini, later work has concentrated on other Ips species, and relatively little is known of I. pini pheromones.

Anderson found that massive I. pini attractions were directed towards logs infested by male I. pini and this attraction of males and females lasted for two to nine days. Lanier et al. (1972) conducted reciprocal field tests on attraction between I. pini populations from California, Idaho, and New York. Their study indicates geographic variation in pheromone systems.

Female I. pini landing on logs invaded by males probably orient by intensity discrimination towards attractive holes. Upon reaching the attractive site, the female searches in the frass until she finds the hole and attempts to enter. The male is normally situated near the entrance with his elytral declivity facing the female thus blocking her access. She pushes against his declivity and stridulates energetically until he moves down to the nuptial chamber thus permitting her to enter. The male normally accepts two or three females (Clemens, 1916; Anderson, 1948; Thomas, 1961; Sartwell, 1964; Sartwell et al., 1971; Barr, 1969; Schenk and Benjamin, 1969; Schmitz, 1972).

Acoustic Communication

Ips, like most scolytids, possesses the most common insect sound producing method, stridulation, or the rubbing together of two body surfaces to produce sound. Recently, reviews on sound communication in animals (Busnel, 1963), arthropod acoustical communication (Alexander, 1967), and insect sonic communication

(Haskell, 1961) have appeared. In reviewing sound production in the family Scolytidae, Barr (1969) found that past studies emphasized general descriptions of sound producing structures, with little attention to the role of sound in scolytid behavior.

Barr (1969) found stridulatory organs on females belonging to 16 of the 33 North American Ips species. Two species had a gula-prosternal type and the remaining 14 possessed a vertex-pronotal type. D. L. Wood (1961) found I. pini "reported as I. oregonis (Eichoff)" to have the vertex-pronotal type.

Normally the component which vibrates is termed the "pars stridens" and the excitatory part the "plectrum." However, since both elements of the I. pini apparatus appear functionally capable of vibrating, the portion on the head vertex is termed the pars stridens and the pronotal component the plectrum (Barr, 1969). Stridulation is accomplished through short head movements which rub the pars stridens against the plectrum (Wilkinson et al., 1967; Barr, 1969).

It has been demonstrated, through surgical removal of the pars stridens, that in Ips confusus female stridulation is required to naturally gain admittance to the gallery (Wilkinson et al., 1967; Barr, 1969). However, little work has been done to analyze the sound emitted by bark beetles under different behavioral situations. Such studies have been conducted on Dendroctonus pseudotsugae Hopk., D. brevicomis LeC., D. ponderosae Hopk., and D. frontalis Dz

(Michael and Rudinsky, 1972; Rudinsky and Michael, 1973, 1974) but species of Ips, including I. pini, have not been studied.

III. METHODS AND MATERIALS

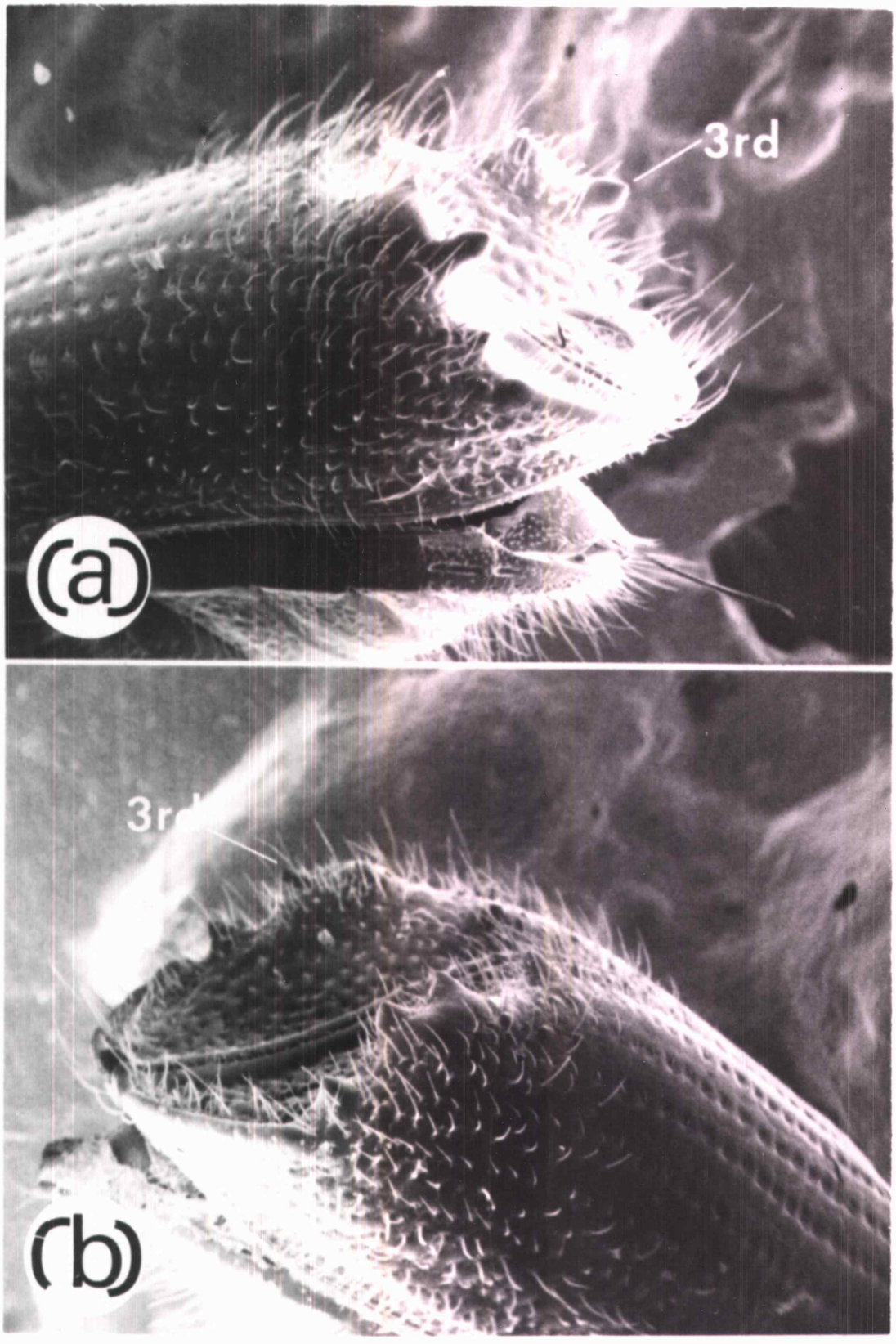
Morphology Studies

Ten I. pini males and 10 females were randomly selected and examined to determine stridulatory apparatus presence or absence. These beetles emerged from lodgepole pine (Pinus contorta Douglas) logs procured from the Pringle Falls Experimental Forest near La Pine, Oregon. They were sexed using the third declivital spine procedure which Rust (1935) and Sartwell (1964) have described (Fig. 1).

Since male I. pini lacks a vertex-pronotal stridulatory apparatus (D. L. Wood, 1961; Barr, 1969), only general descriptions of the male head vertex and inner pronotal surface were made. Detailed morphological examination was restricted to females. A Filar model 424C 10X micrometer eyepiece and a Spencer binocular stereomicroscope were utilized to measure maximum head width (gena to gena) and thickness (dorsal to ventral), in addition to maximum pronotal length (anterior to posterior), width and thickness. Then the pars stridens and plectrum were surgically removed.

The samples were mounted on microscope slides using Hoyer's mounting media. Pars stridens and plectrum maximum length, maximum width, number of ridges and distance between ridges were measured using the eyepiece micrometer and a Bausch and Lomb phase contrast microscope. That procedure is similar to that used by

Figure 1. Scanning electron micrographs depicting: (a) the male elytral declivity with its elongated, bent, blunt, third spine (X50) and (b) the female declivity with its short, straight, pointed, third spine (X50). 3rd = third declivital spine.


3rd
(a)
(b)

Michael and Rudinsky (1972) and Rudinsky and Michael (1973). In measuring the distance between plectral ridges, nine measurements were taken (three anterior, three medial, and three posterior) and a mean calculated. Since the maximum magnification of the phase contrast microscope (1940X) did not enlarge the pars stridens ridges enough to enable individual distance measurements, the number of ridges per eyepiece unit were counted. Three observations were taken (one anterior, one medial, and one posterior) and the mean number and distance between ridges were calculated. Further calculations were performed to determine the range, mean, standard deviation, and coefficient of variation characteristic to the female measurements.

Next, scanning electron micrographs depicting male and female head vertices and inner pronotal surfaces plus close-ups of the female pars stridens and plectrum were taken. To accomplish this, an electron microtechnician fixed the specimens in five percent gluteraldehyde Sorenson's phosphate buffer solution (pH 7.2 and 0.15 M) for about one hour. After distilled water rinsings, they were dehydrated by serial emersion through 30, 50, 70, 85, and 100 percent acetone followed by 30, 50, 70, 85, and 100 percent trichlorotrifluoroethane rinsings. They were then deposited in a freon 13 filled Bomar critical point dryer and brought to 900 psi at 38^{o}C. Maintaining the 38^{o}C temperature, the pressure was gradually reduced at

50 psi intervals down to 0 psi. The critical point for this system was 526 psi at 38°C. Then conductive silver paint was employed to mount the samples on 15 mm x 15 mm aluminum specimen mounts. A Varian model VE-10 vacuum evaporator was utilized to apply a 100 Å coating of 60-40 gold-palladium alloy. The rotary technique at 1 x 10^{-5} torr was employed. Finally an International Scientific Instruments Mini-Sem MSM-2 scanning electron microscope with a 15 Kv-100 μA beam was implemented to take the micrographs. Polaroid type 55 film was used.

Acoustic Communication Studies

Live beetles used during the acoustic studies emerged from naturally infested lodgepole pine logs obtained near La Pine, Oregon in the Pringle Falls Experimental Forest. The logs were transported to the Oregon State University Forest Insect Laboratory and stored at 4°C. When beetles were desired, the stored logs were moved to a greenhouse and brought to emergence temperatures. The emerging beetles were separated by sex and stored at 4°C until needed. All reported experiments were conducted using unfed virgin beetles which had emerged less than two days prior to testing.

Preliminary studies were conducted to determine, under laboratory conditions: (1) the best artificially drilled attack hole size, direction, angle and depth; (2) the time period required for the male to

construct his nuptial chamber, move to the attack hole entrance and become selectively receptive to attracted females; and (3) how much time must elapse between consecutive female introductions to permit the female to interact with the male, migrate down the tunnel and commence egg gallery construction thus liberating the male to interact with the next female. Those tests consisted of: (1) utilizing different size bits to drill artificial attack holes at various angles, directions, and depths into the bark of 15.2 cm diameter lodgepole pine logs; (2) introducing virgin, unfed males into the holes then removing the bark to examine their mining or introducing virgin, unfed females to the males after varied time spans; and (3) testing different time periods between female introduction. In this manner the procedure used below was established.

The first acoustically investigated behavior situations were female attempts to enter attractive galleries with a male alone or a male plus 1-4 female(s). A fresh lodgepole pine log 91.4 cm long, 15.2 cm thick was employed. Five artificial attack holes 5.1 cm apart were drilled down to the cambium at approximately a 45° angle towards the former tree top using a 3/32" bit. Then one I. pini was introduced into each hole and a fine meshed, flat, wire screen was affixed over the hole. After one day the screen was replaced by a fine meshed, convex, wire screen which permitted the male to remove the frass. Once the male had been excavating three days at 28°C, acoustic tests at 28°C were begun.

The five artificial attack holes were prepared for recording attracted female stridulation during entrance attempts. After removing the screens, clear V-shaped plastic walkways approximately 5 cm long and 1.5 cm deep were positioned on a previously planed area around each hole such that the narrow end opened out over the hole. Next, the microphone was adjusted over the first hole and a female was taken from 4°C storage and transferred to the walkway using soft forceps. Care was taken to minimize handling. Once she began moving, careful observations were made to make sure she was undisturbed (not seeking to escape).

Once an undisturbed female was heading towards the hole, the plastic walkway was slowly removed. When she reached the hole and commenced attempts to dig into the gallery, a two minute tape recording was made. A Hewlett-Packard model 15119A condensor microphone, a low-noise preamplifier with band width set at 300 Hz to 100 kHz, and an Ampex model Fr-1300 tape recorder operated in the frequency modulated mode at 60 in/sec were used. The frequency response for this system is within $\pm$ 3 db between 300 Hz and 30 kHz and within $\pm$ 10 db at 40 kHz (Rudinsky and Michael, 1972).

Once the recording was completed, a convex screen was secured over the hole. After three hours had elapsed since the first female's attempt to gain access to hole one, a second female's stridulation during attempts to enter each hole was recorded. This was repeated

until the stridulation emitted during five different female entrance attempts per gallery had been recorded.

The remaining two behavioral situations tested were stress and rivalry. Rivalry was induced by placing four males into separate artificial attack holes as described above. After three days, they were removed and a female was allowed to enter each hole. Immediately thereafter, a second female was forcibly introduced to each hole and a flat, fine meshed, wire screen secured over the hole. Once two females were in a hole, a two minute recording was made. After the sounds coming from the four holes had been recorded, a third female was forced into each hole and another two minute recording per gallery was made.

Finally, stress stridulation was recorded with a female held by the abdomen between the thumb and forefinger about 1 cm from the microphone. The head was freely moveable. Seven females were used and a two minute recording of each made.

The attraction stridulations were analyzed first. To select the chirps[1] to be statistically analyzed, the first minute of stridulation produced by five females subjected to a particular attraction situation was stratified into two 30 second intervals. Then ten random numbers

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[1] Following Broughton (1963), a "Chirp" is the "shortest unitary rhythm element of a sound emission that can be distinguished as such by the unaided ear."

between 0-30 and ten between 30-60 were generated. Each number was treated as that many seconds from the beginning of a female's stridulation. Two samples were taken from each time span (0-30 and 30-60 sec) for all five females recorded per attraction situation. The original recording was played back to locate where a given female's stridulation under a particular attraction situation started. The tape was then played back at 60 in/sec until the allotted time span for the sample was reached. Reducing the playback speed to 30 in/sec, the next chirp forward was isolated.

Once a sample chirp was isolated it was transcribed at 30 in/sec onto an Ampex PR-10-2 recorder at 7 1/2 in/sec. The chirp was played back again using the original recording and isolated on a Tektronix model 5103N storage oscilloscope. The stored image was utilized to count the number of pulses per chirp, the number of interruptions per chirp (unusually long gaps between consecutive pulses), to measure the interruption lengths, the chirp length including and excluding the interruption lengths, and the pulse rate calculated using the chirp length including and excluding the interruption lengths. The playback and oscilloscope sweep speeds varied depending on the chirp length but all measurements were converted to true time. In a similar manner, ten stress chirps, ten rivalry chirps, and ten in-gallery chirps were randomly isolated and measured except the first minute was not stratified. "In-gallery chirps" refers to chirps

occasionally produced when there was a male and more than one female per attractive gallery. The beetles were concealed within the gallery so their behavior could not be observed. The chirps may be associated with some later phase of attraction or with rivalry between two females in an egg gallery.

To statistically analyze the chirps emitted under different attraction situations, t-tests, analysis of variance, and Duncan's multiple range test were employed. T-tests were used to compare the acoustic parameters characterizing chirps produced contingent to stress, rivalry, in-gallery and attraction situations. Tabular summaries were developed.

Stress, rivalry, in-gallery, and various attraction chirp oscillograms were made through original tape playback into the storage oscilloscope. The storage mode was utilized to isolate the desired chirp and to coordinate the photographic procedure then it was deleted. The desired chirp was played back and at the appropriate time a single oscioloscope sweep was initiated while the shutter on a polaroid camera was opened. The playback speed, shutter speed, and oscilloscope sweep speed varied depending on the chirp length, but the chirp length was converted to true time. Lastly, audiospectrographs were made with a Kay Electric Company model 6061A Sona-Graph. Major frequency bandwidths characteristic to attraction, rivalry, stress and in-gallery chirps were deemed from the audiospectrographs.

Olfactory Communication Studies

Laboratory Studies

The first study investigated the influence that the number of females per male had on female attraction. To accomplish this the previously described methods were used to drill 50 artificial attack holes per log in five logs and to introduce one male per hole. After three days at 28°C, females were introduced three hours apart to each hole. The same number of females were placed in each hole on a log, thus each log was a male-female situation type. The five combinations were male plus one female, male plus two females, male plus three females, male plus four females, and male plus five females. Once three hours had elapsed since the last female introductions, frass was separately collected from each situation. Ninety-five percent ethyl alcohol was used to moisten the frass which was then stored in vials at 4°C until needed.

A plexiglass, olfactory walkway 35 cm long with a recessed, screened opening to house a glass vial of the test compound was employed to observe female responses to the various frass samples, as described by Jantz and Rudinsky (1965). Two additional controls were tested; namely, 95 percent ethyl alcohol alone and with artificially obtained fresh lodgepole pine borings. Twenty females were individually positioned in the screened track of the walkway and their

response (at 28°C) to the test substance were observed. A typical attracted female moved more slowly as she approached the screened opening and began vigorous searching efforts characterized by rapid antennal movement and side to side body movements. The maximum arrestment response was similar to that described for Dendroctonus pseudotsugae by Rudinsky and Michael (1972), i.e., the female stopped over the opening and excitedly attempted to penetrate the screen (this digging corresponds to the female's activity during attempts to enter a frass-filled gallery excavated by a male). Klinotaxis or repeated circling as described by D. L. Wood et al. (1966) also characterizes an arrested female's behavior. Once 20 females had been tested, the sample was replaced by a fresh sample of the same treatment type frass and 20 more females were tested. This was repeated once more to obtain a 60 female total per frass type. The results were statistically analyzed using analysis of variance.

Following basically the same procedure, female response to these situations were tested: male alone, male plus one female, male plus four females, and male plus five females. However, frass from each treatment was collected and tested at different elapsed times since the last female introductions. Care was taken to remove all frass from each artificial attack hole each time. Again, analysis of variance was used to statistically analyze the results.

Field Studies

The field studies were conducted in a 100-year-old lodgepole pine-ponderosa pine mixed forest approximately 1.5 miles north and 0.5 miles east of the Pringle Falls Experimental Forest Research Station near La Pine, Oregon (Fig. 2a, b). Experiments were conducted between July 31 and August 5, 1974, during the first generation flight. Three investigations were undertaken. In the first study, natural beetle attraction towards artificially established male-female gallery situations was tested. The following situations were tested: (1) male alone, (2) female alone, (3) male plus one female, (4) male plus four females, (5) male plus six females, and (6) fresh lodgepole pine logs. For each treatment, beetles were introduced into two 15.2 cm diameter, 91.4 cm long lodgepole pine logs using the methods previously described. Beetles were introduced so that when testing began the elapsed time since the last beetle (male or female) introductions was the same in all situations. The logs were set vertically in fine meshed, wire screened cages (47 x 61 x 86 cm) (Fig. 2c). After securely sealing the cages to plywood platforms, they were randomly positioned approximately 15 m apart around the clear-cut so they would be 50 percent shaded throughout the day (Fig. 3a).

At 15 minute intervals, the number of beetles alighting on each cage was recorded and the beetles collected. The total number

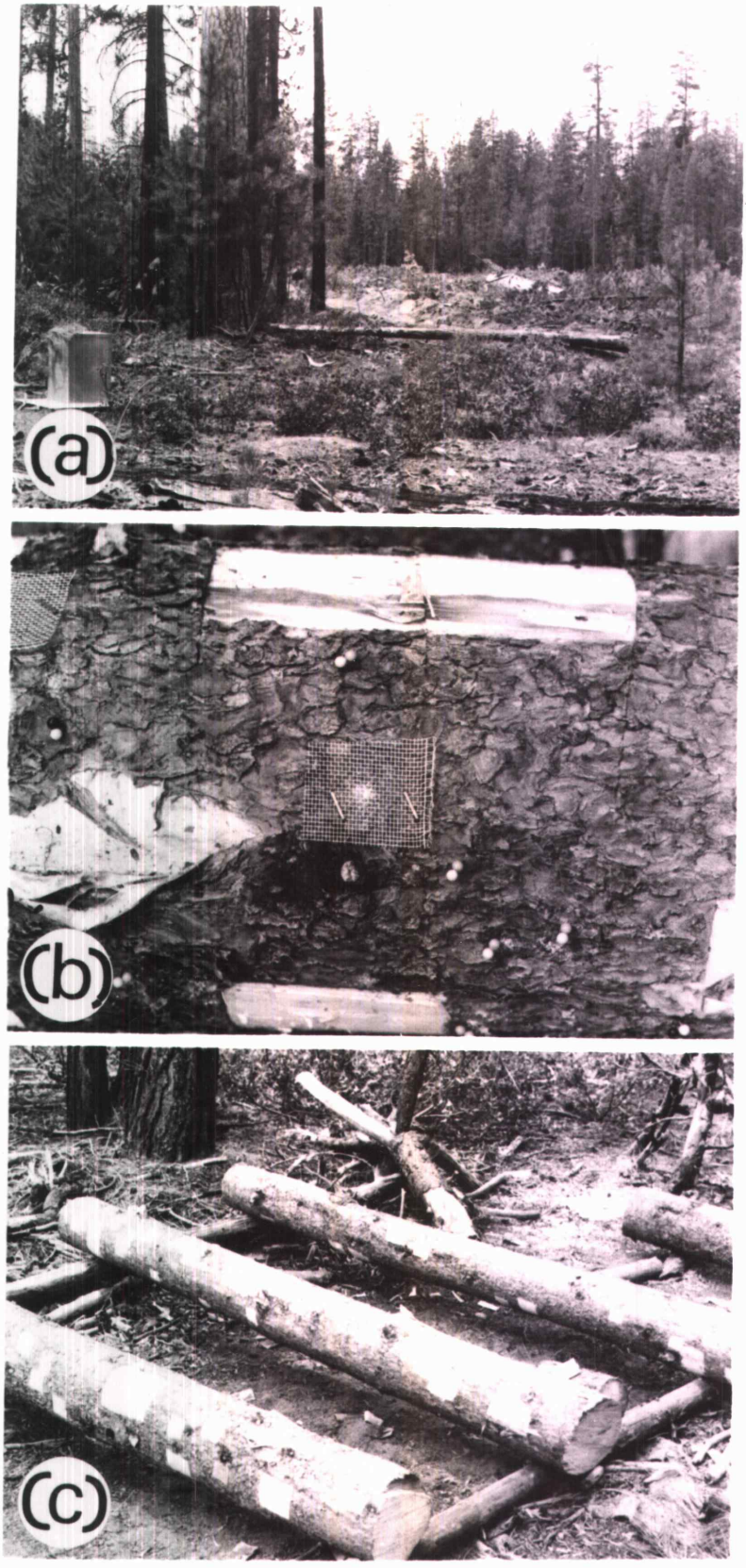
Figure 2. The first two photographs (a, b) depict the field study site and the the third (c), a wire cage containing a test situation log.


(a)


(b)


(c)

Figure 3. These pictures show (a) the typical positions of the attraction experiment cages and (b, c) the artificial male Ips pini introductions (screened hole), color pin coding of new attacks and natural attack excisions characterizing the colonization study.


(a)
(b)
(c)

alighting per hour per cage was recorded and two separate alcohol collections made; one in the morning and one in the afternoon.

While the first study was proceeding, the fresh lodgepole pine logs to be used in experiment two were subjected to natural I. pini attack for 1, 2, and 3 days. After three days, experiment one was terminated and testing of the second begun. Two logs for each beetle attack exposure period were set vertically in cages randomly positioned approximately 15 m apart around the clear-cut along with the experiment one male alone, female alone, and fresh lodgepole pine log cages. The counting and collecting procedure remained the same over the next three days.

Finally, a further field study was conducted. During experiments one and two, several fresh lodgepole pine logs were subjected to I. pini attack. Attack was induced by artificially introducing male I. pini beetles into each log and screening the holes. Then new natural attacks were color coded, using colored pins, as to their initiation time. After certain time periods had elapsed, 5 cm square bark samples around attack holes were removed (Fig. 3b, c). The male position, female number and position, egg gallery number, and nuptial chamber dimensions were recorded. Twenty samples were taken at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 days after the initial attack thus making a total of 140 samples. During these field studies a Foxboro hygro-thermograph was stationed at the field site.

After the six day test period had terminated, the naturally infested logs used in experiment two and the beetle collections were transported to the laboratory. There the bark was removed from the naturally attacked logs to establish the number of galleries and classify each gallery according to their male-female situation. The collected beetles were sexed and sex proportions determined. Results of experiments one and two were statistically analyzed using analysis of variance and Duncan's multiple range test. The experiment three bark removal data were analyzed using graphic interpretation.

IV. RESULTS AND DISCUSSION

Morphology Studies

Evidence from the morphology studies supports and supplements the work of D. L. Wood (1961), Wilkinson (1962), Wilkinson et al. (1967) and Barr (1969). Female I. pini possesses a vertex-pronotal type stridulatory apparatus but the male lacks such structures. The female's pars stridens is on the median vertex of the head (Fig. 4a, c, e) while the corresponding area on the male's head contains an elongated trough (Fig. 4b, d, f). Males also lack a plectrum, which is an oval, convex, flexible, thin plate suspended under a cavity on the inner, anterior, dorsal surface of the female's pronotum (Fig. 4g, h).

The plectrum and pars stridens contain a series of transverse ridges. The pars stridens ridges are spaced at approximately 0.3 μ intervals, uniformly rounded, and unbranched except towards the anterior and posterior extremities (Fig. 5). The plectral ridges are spaced at approximately 4 μ intervals, rounded and branched (Fig. 6). Table 1 summarizes the range, mean, standard deviation, and coefficient of variation for characteristics of the pars stridens, plectrum, head, and pronotum.

Figure 4. Scanning electron micrographs depicting: (a) the female Ips pini head with its vertex oriented pars stridens (X50), (b) the male Ips pini head (X50), (c) a vertex view showing the female pars stridens (note landmark I) (X200), (d) a vertex view of the male head which lacks the pars stridens (note landmark II) (X200), (e) an overall view of the female ridged pars stridens (X1000), (f) a male head close-up demonstrating the absence of the pars stridens (X1000), (g) the dorsal, anterior underside of a female Ips pini pronotum incorporating the plectrum (note landmark III) (X200), and (h) the dorsal, anterior underside of the male Ips pini pronotum which lacks a plectrum (X200). ant = anterior; O = occipital area; ps = pars stridens; vertical line = 0.01 mm.

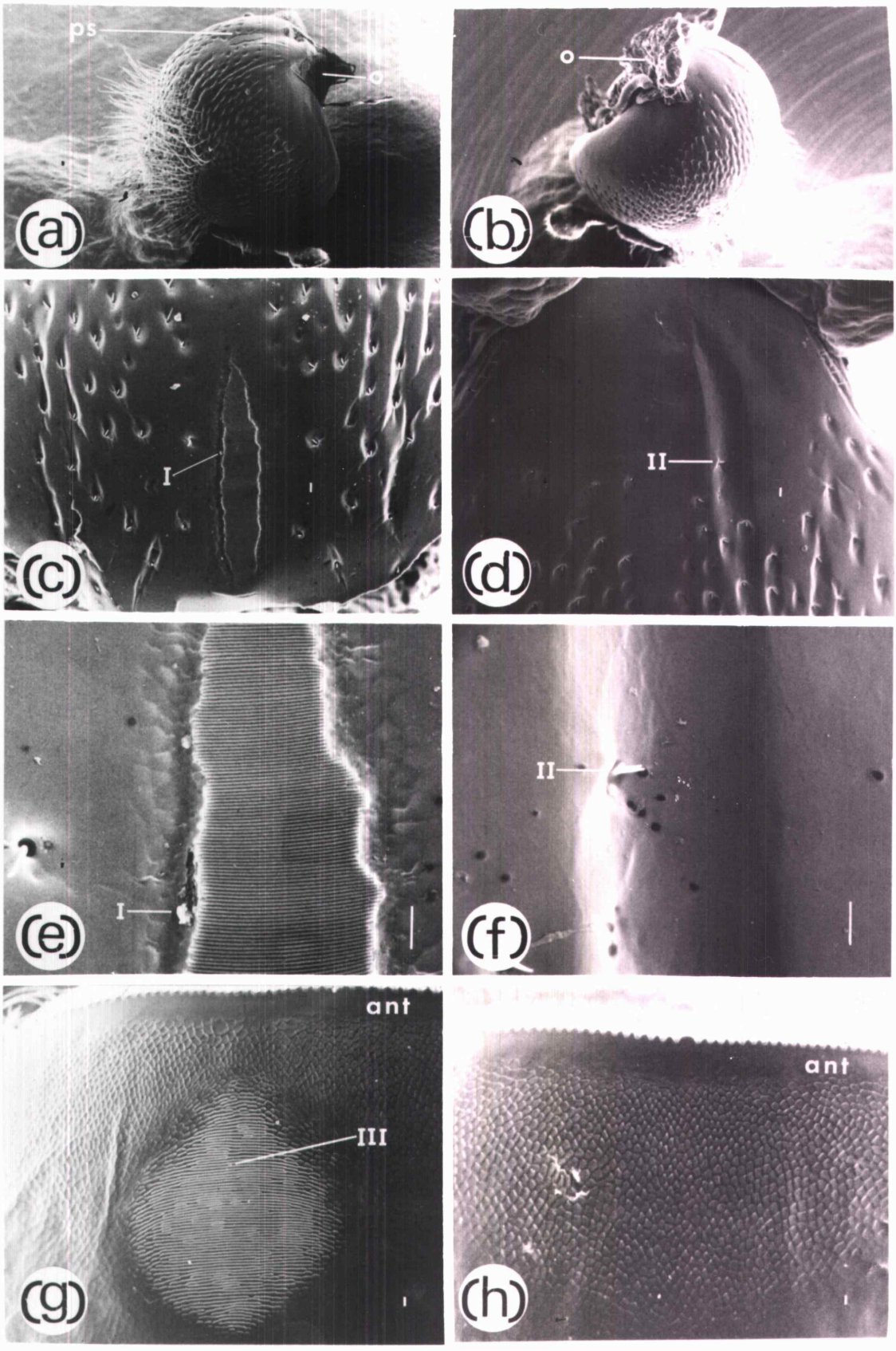

ps
o
(a)
o
(b)
I
(c)
II
(d)
I
(e)
II
(f)
ant
III
(g)
ant
(h)

Figure 5. Scanning electron micrographs showing: (a) the anterior portion of the pars stridens with ridge branching and fusion (X5000), (b) the medial portion of the pars stridens where the ridges are uniform (X5000), and (c) the posterior portion of the pars stridens with ridge branching and fusion (X5000).
ant = anterior; pos = posterior; vertical line = 1.0 μ.

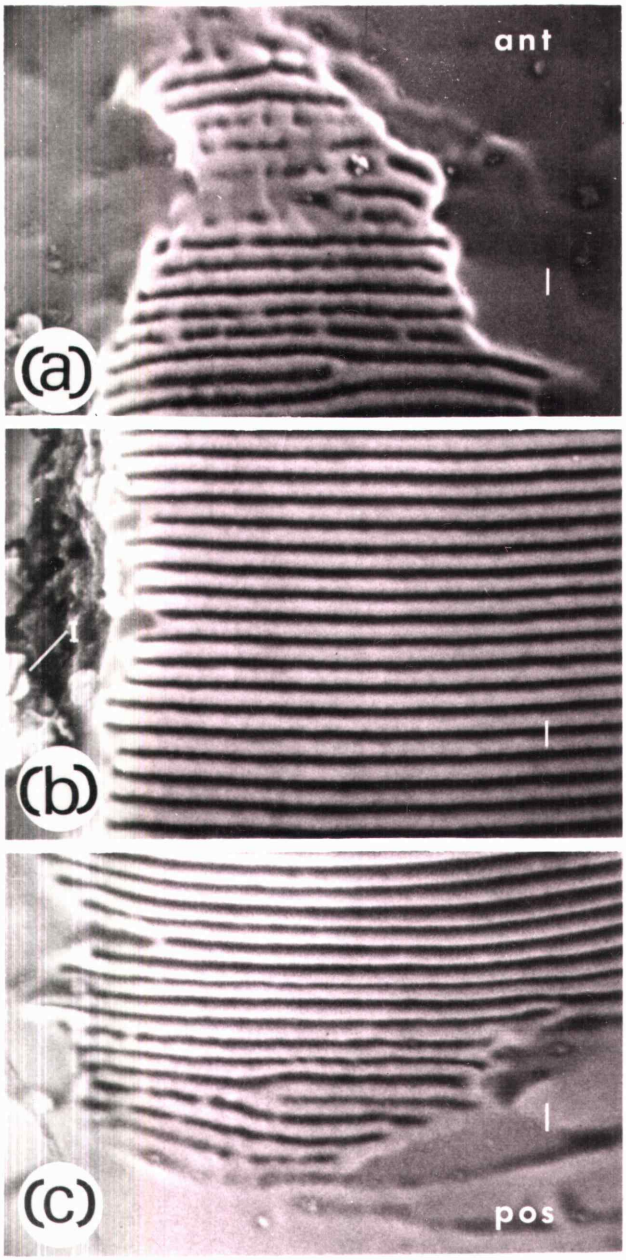

ant
(a)
(b)
(c)
pos

Figure 6. Scanning electron micrographs showing: (a) the anterior-medial region of the plectrum (X700), (b) the medial-lateral region of the plectrum (X1000), (c) the medial portion of the plectrum with its typical branched ridges (X1000), and (d) the posterior portion of the plectrum (X700).
ant = anterior margin of pronotum; pos = posterior; vertical line = 0.01 mm.

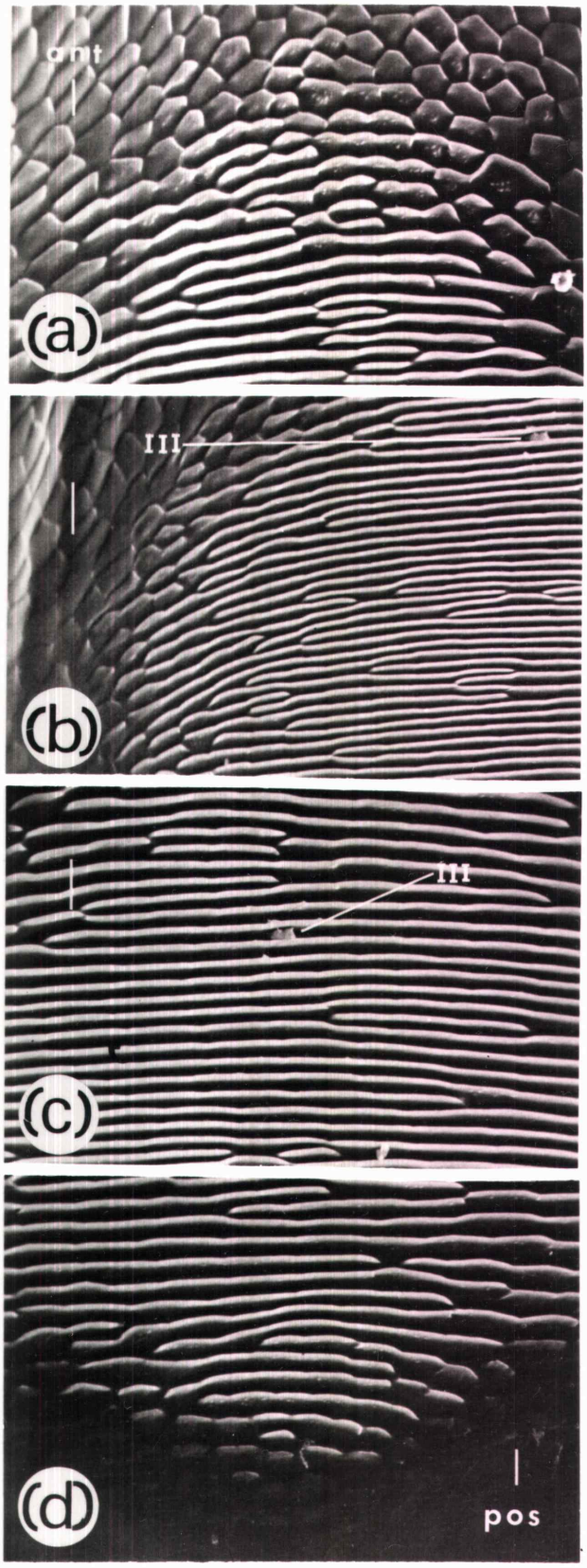

ant
(a)
III
(b)
III
(c)
(d)
pos

Table 1. Morphology of the Stridulatory Apparatus of Female Ips pini.

Morphological Structure	Measurement (mm)	Range	Mean	Standard Deviation	Coefficient of Variation (%)
Prothorax*	Max. width	1.46-1.66	1.53	± 0.061	4.00
	Max. length	1.55-1.85	1.72	± 0.104	6.09
	Max. thickness	1.21-1.37	1.28	± 0.065	5.07
Plectrum*	Max. width	0.22-0.34	0.27	± 0.032	11.77
	Max. length	0.29-0.37	0.34	± 0.029	8.46
	No. ridges	80.00-96.00	87.2	± 7.642	8.76
	Distance between ridges	0.004	0.004		
	Ratio of no. ridges to plectrum length (no. ridges/mm)	235.29-318.52	260.82	± 22.433	8.60
Cephalic*	Max. width	1.02-1.16	1.08	± 0.038	3.54
	Max. thickness	0.99-1.11	1.07	± 0.040	3.77
Pars stridens*	Max. width	0.04-0.07	0.054	± 0.001	17.89
	Length	0.22-0.31	0.358	± 0.034	13.15
	No. ridges	904.54-1022.83	992.909	± 46.136	4.65
	Distance between ridges	0.0002-0.0003	0.00025	± 0.000053	21.08
	Ratio of no. ridges to pars stridens length (ridges/mm)	3,131.38-4,774.25	3,915.99	±597.14	15.25

*n = 10

Acoustic Communication Studies

The mean chirp lengths including interruptions did not differ significantly from the mean chirp lengths excluding interruptions within the stridulation produced during rivalry, stress, in-gallery, and various attraction situations. Also the mean pulse rate calculated using chirp lengths including interruption lengths did not differ significantly from the mean pulse rate calculated using chirp lengths excluding interruptions. Therefore, further analysis was conducted using the number of pulses per chirp, number of interruptions per chirp, chirp length excluding interruptions, and pulse rate calculated using chirp lengths excluding interruptions.

Table 2 lists the range and mean for acoustic parameters characterizing chirps emitted by females under different attractive situations. The situations (treatments) are: (1) male alone, (2) male plus one female, (3) male plus two females, (4) male plus three females, and (5) male plus four females. Analysis of variance (Appendix II, Tables 10-13) indicates the number of females per male significantly ($P < 0.01$) influenced the number of pulses per chirp and pulse rate. Also the same two parameters differed significantly ($P < 0.05$) between the chirps recorded the first 30 and the second 30 seconds. However, these trend indications do not delineate which attraction situation(s) or time(s) contribute to the significance. For

Table 2. Female Ips pini Stridulation Properties Typifying Chirps Emitted under Different Attraction Situations.

Situation (females/male occupying hole)	No. of Pulses/Chirp		No. of Interruptions		Chirp Length Excluding Interruptions (true time: sec.)		Pulse Rate Excluding Interruptions (true time: pulses/sec.)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
0	17-272	129.4	0-2	0.45	0.025-0.159	0.081	371.8-4560.0	1765.84
1	20-272	87.8	0-3	0.50	0.030-0.238	0.107	241.0-1857.1	849.90
2	22-244	114.0	0-3	0.50	0.012-0.495	0.115	355.6-3000.0	1291.97
3	47-376	192.6	0-2	0.50	0.019-0.326	0.117	434.0-2848.5	1823.75
4	15-265	99.7	0-3	0.75	0.043-0.280	0.111	261.5-1879.4	874.55

n = 20

that reason, Duncan's multiple range test was employed to determine which attraction situation(s) influenced the number of pulses per chirp and pulse rate.

Table 3 illustrates the multiple range test results. These tests reveal that the number of pulses per chirp (a), produced by females subjected to situation four, significantly differ ($P < 0.05$) from those characterizing the stridulation associated with the other situations which group together. The pulse rate analysis (b) was more difficult to interpret. The pulse rate associated with female stridulation during attempts to enter treatments one and four holes differs significantly ($P < 0.05$) from that of chirps emitted subject to situation two and five entrance attempts. Thus there are two distinct groups: situations one and four and situations two and five. The situation three chirps may group with numbers one and four or with treatments two and five. However, the magnitude of the differences suggests that situation three chirps are closer to situations two and five. I believe the reason for this complexity lies in the chirp lengths. Those lengths do not differ significantly but when they are divided into the number of pulses per chirp in order to calculate the pulse rate, the differences in pulse rate are significant.

To determine which treatment time period differences were significantly contributing to the time effect on the number of pulses per chirp and the pulse rate, t-tests were conducted. The mean

Table 3. Duncan's Multiple Range Test of Influences of Female Number per Male on Pulses per Chirp and Pulse Rate.

Treatment No.	Situation Description	Pulses/Chirp (mean)	Pulse Rate (mean)
4	Male plus three females	192.6	1823.75
1	Male alone	129.4	1765.80
3	Male plus two females	114.0	1291.97
5	Male plus four females	99.7	874.55
2	Male plus one female	87.7	849.90

n = 20

number of pulses per chirp and the mean pulse rate for the 0-30 sec and 30-60 sec time periods were compared within each attraction situation. The only significant difference ($P < 0.05$) was between the mean number of pulses per chirp produced under situation one.

Duncan's multiple range test was employed to determine, within a given time period, which treatment(s) contributed to the significant differences in the number of pulses per chirp and the pulse rate (Appendix I, Tables 7-8). For the 0-30 sec period, the mean number of pulses per chirp delineated two distinct groupings: those being (1) situations 2, 3, and 5, and (2) treatment four. The situation one chirps may group with either, but the magnitude of differences indicates it is closer to the first group. The 30-60 sec results showed the same arrangement.

Turning to the pulse rate, the 0-30 sec time period attraction chirps showed four groups. Situation one and four comprise one group while treatments 2, 3, and 5 are separate groups. These same four groups were circumscribed in the 30-60 sec period. Thus, the differences between attraction chirp parameters did not change when the situations were compared within the two time periods.

Further t-tests were utilized to investigate the differences between the pooled mean number of pulses per chirp (pooled within each time period for all situations) and the pooled mean pulse rate compared between the two periods. Both parameters are significantly

different ($P < 0.05$). This explains the analysis of variance results.

Next, acoustic parameters characterizing chirps produced by females under attraction, stress, rivalry, and in-gallery situations were compared. T-tests reveal differences between chirp types. Those results and the data are listed in Table 4.

The measured parameters do not differ significantly between attraction and rivalry chirps, but the mean pulse rate differs significantly ($P < 0.05$) between stress and attraction chirps. Furthermore, the mean number of interruptions, mean chirp length, and mean pulse rate of attraction chirps significantly differ ($P < 0.01$) from those of in-gallery chirps. Similarly, the mean number of interruptions, mean chirp length and mean pulse rate of stress and rivalry situation chirps significantly differ ($P < 0.05$) from those of in-gallery chirps. The mean pulse rate and mean number of pulses per chirp significantly differ ($P < 0.05$) between chirps produced contingent to stress and rivalry conditions, and finally, the mean number of pulses per chirp differ significantly ($P < 0.01$) between stress and in-gallery chirps.

One may wonder how stress and attraction chirp mean pulse rates can differ significantly when their mean chirp lengths and mean number of pulses per chirp (used to calculate the pulse rate) do not. Table 4 shows that, while the difference is not significant, the mean number of pulses per chirp is larger and mean chirp length shorter in

Table 4. Comparative Acoustic Parameters Characteristic to Female Ips pini Stridulations under Attraction, Stress, Rivalry, and an Undefined Behavioral Situation

Situation	Parameters							
	No. of Pulses/Chirp		No. of Interruptions		Chirp Length Excluding Interruptions (true time: sec.)		Pulse Rate Excluding Interruptions (true time: pulses/sec.)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Attraction[a]	15-376	124.7	0-3	0.54[e]	0.012-0.495	0.106[h]	241.0-4560.0	1321.20[c,h]
Stress[b]	103-216	153.6[d,f]	0-4	0.90[g]	0.058-0.012	0.081[h]	1621.2-2190.5	1890.36[c,d,h]
Rivalry[b]	22-268	83.1[d]	0-6	0.70[g]	0.017-0.189	0.075[h]	314.3-2440.7	1205.11[d,h]
In-gallery[b]	29-145	85.5[f]	0-9	3.70[e,g]	0.058-0.495	0.319[h]	69.0-1379.2	374.92[h]

[a] n = 100

[b] n = 10

[c] There is a significant difference between attraction and stress chirps for this parameter at $P < 0.05$.

[d] There is a significant difference between stress and rivalry chirps for these parameters at $P < 0.05$.

[e] There is a significant difference between attraction and in-gallery chirps for this parameter at $P < 0.01$.

[f] There is a significant difference between stress and in-gallery chirps for this parameter at $P < 0.01$.

[g] There is a significant difference between in-gallery and both stress and rivalry chirps for this parameter at $P < 0.05$.

[h] There is a significant difference between in-gallery chirps and the other situations for this parameter at $P < 0.01$.

stress chirps than in attraction chirps. These two factors combine to yield significantly different mean pulse rates. Typical chirps emitted contingent to stress, rivalry, in-gallery, and various attraction situations are presented in Figure 7.

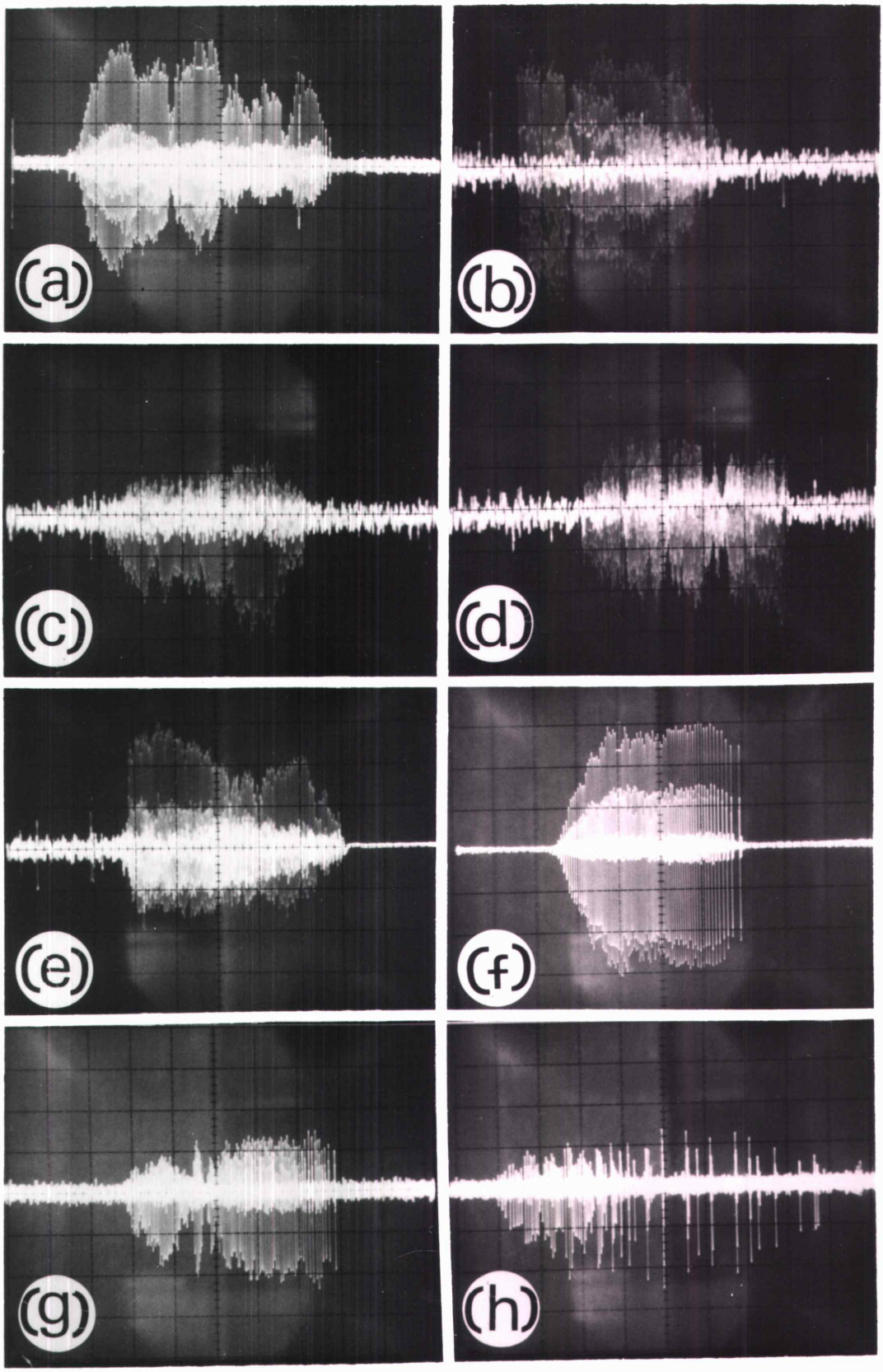
Audiospectrographs (Fig. 8) reveal that the major attraction chirp frequency band range is from < 1.0 to 25 kHz with less intense bands above 25 kHz. The major stress chirp frequency band range is < 1.0 to 27 kHz with less intense bands above 27 kHz, and rivalry chirps have intense frequency bands between 1.0 and 18 kHz with less intense bands above and below that range. Lastly, the in-gallery chirps display intense frequency bands between < 1.0 and 23 kHz with less intense bands reaching 26 kHz.

Olfactory Communication Studies

Laboratory Studies

The analysis of variance (Appendix III, Table 14) on the results from the investigation of female response to frass collected from various male-female combinations three hours after the last female introductions shows that the number of females per established male does not significantly influence the number of females attracted (Fig. 9a). These tests also reveal that females do not respond to 95 percent ethyl alcohol alone or combined with artificially obtained lodgepole pine borings.

Figure 7. Oscillograms depicting typical Ips pini chirps. Attraction chirps emitted during attempts to enter holes containing: (a) a male alone (0.016 sec/div), (b) a male plus one female (0.025 sec/div), (c) a male plus two females (0.025 sec/div), (d) a male plus three females (0.025 sec/div), and (e) a male plus four females (0.025 sec/div). (f) Rivalry (0.016 sec/div), (g) stress (0.016 sec/div), and (h) in-gallery (0.031 sec/div) chirps are also presented.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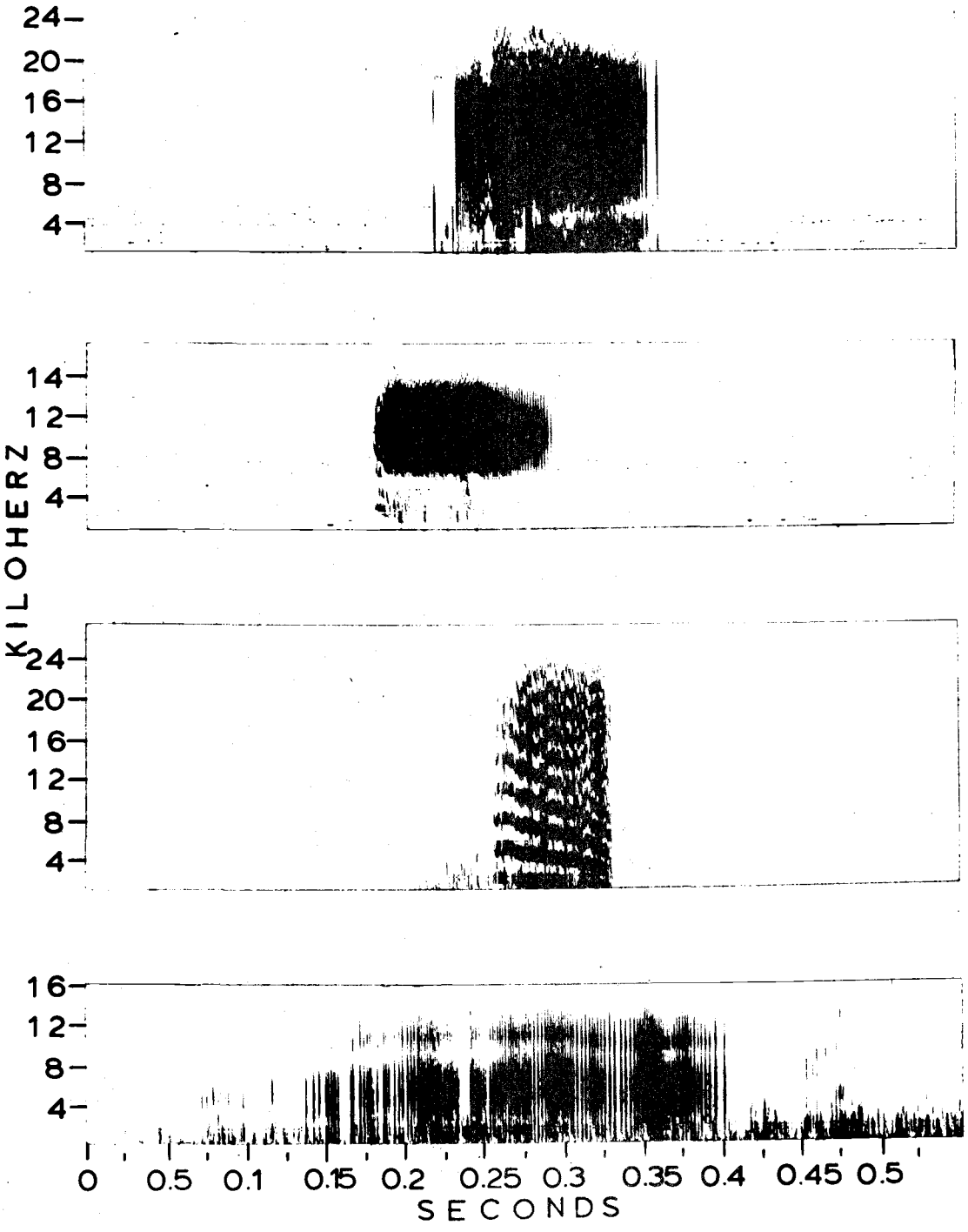

Figure 8. Audiospectrographs of female Ips pini chirps emitted under: (a) attraction, (b) rivalry, (c) stress, and (d) in-gallery situations.

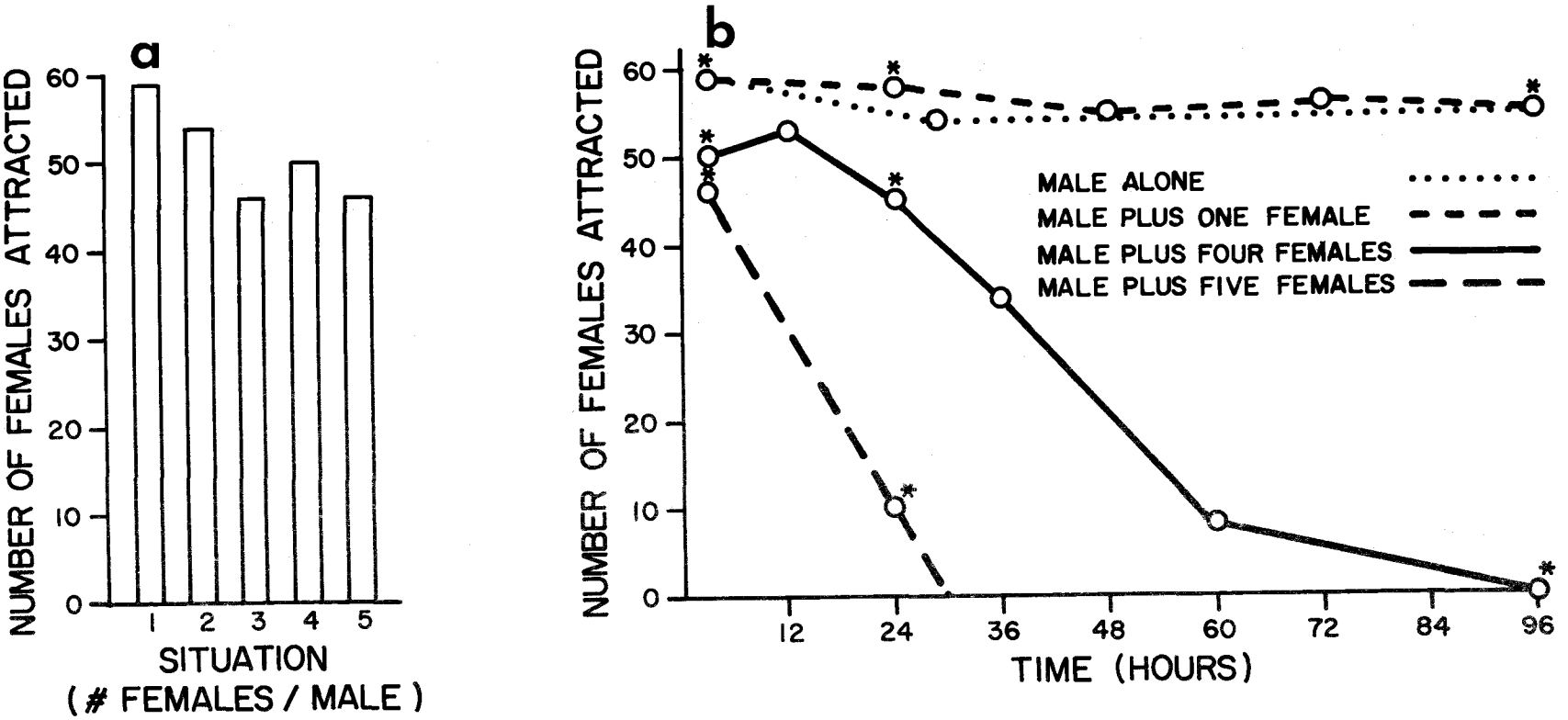

Figure 9. Laboratory studies on female Ips pini attraction to (a) frass produced by various male-female combinations collected three hours after the last female introductions and (b) frass produced by various male-female combinations collected at different time intervals since the last female introductions. Note: graph (a) does not include the artificially obtained lodgepole pine borings plus 95% ethyl alcohol or the 95% ethyl alcohol alone controls which did not attract females. * = observation points subjected to analysis of variance.

The next experiment tested the influence that the number of females per established male had on the number of females attracted when frass collections were taken at different time spans since the last female introductions. Numerous time intervals were tested but statistical analysis was restricted to frass collected 3, 24, and 96 hours after the last female introductions to these situations: male plus 1, 4, and 5 females. Those observations and others are presented in Figure 9b.

Analysis of variance (Appendix III, Table 15) shows that the number of females per established male and the elapsed time since the last female introductions significantly ($P < 0.01$) influence the number of females attracted. Also, the time-female number per male interaction significantly influences ($P < 0.01$) the number of females attracted. Therefore, the elapsed time since the last female enters the gallery and the number of females occupying the hole with the male interact to significantly regulate female attraction to the attack. As Figure 9b illustrates, the number of females attracted over time decreases more rapidly with increasing numbers of females per male.

Field Studies

The controls (fresh lodgepole pine logs and female alone logs) for the first field study which monitored *I. pini* response to artificial

attraction situations, did not attract I. pini beetles; therefore, statistical analysis was restricted to treatments 1, 3, 4, and 5 results. Analysis of variance (Appendix IV, Table 16) indicates that the number of females per established male, the number of females per male-day interaction, collection time and day-collection time interaction significantly ($P < 0.05$) influence the number of beetles attracted.

Natural beetle flight fluctuations throughout the day account for the collection time and day-collection time interaction (Fig. 10a, b). The number of females per established male and the number of females per male-day interaction significances support the laboratory results (Fig. 11a, b). The Duncan's multiple range test results (Appendix I, Table 9) show that, on day one, all situations were attractively separate groups but by day two, treatments four and five were attractively the same. The other situations remained separated through day three.

Next, the sex proportion data analysis of variance (Appendix IV, Table 17) shows that the sex proportion of the beetles attracted to the different artificial situations did not vary significantly. Furthermore, the sex proportion of the beetles attracted in the morning did not differ from that of the beetles attracted in the afternoon for any given situation and the pooled sex proportion for a treatment on a certain day did not differ from that on any other day. Finally, the sex proportions pooled over the three days for the different situations are

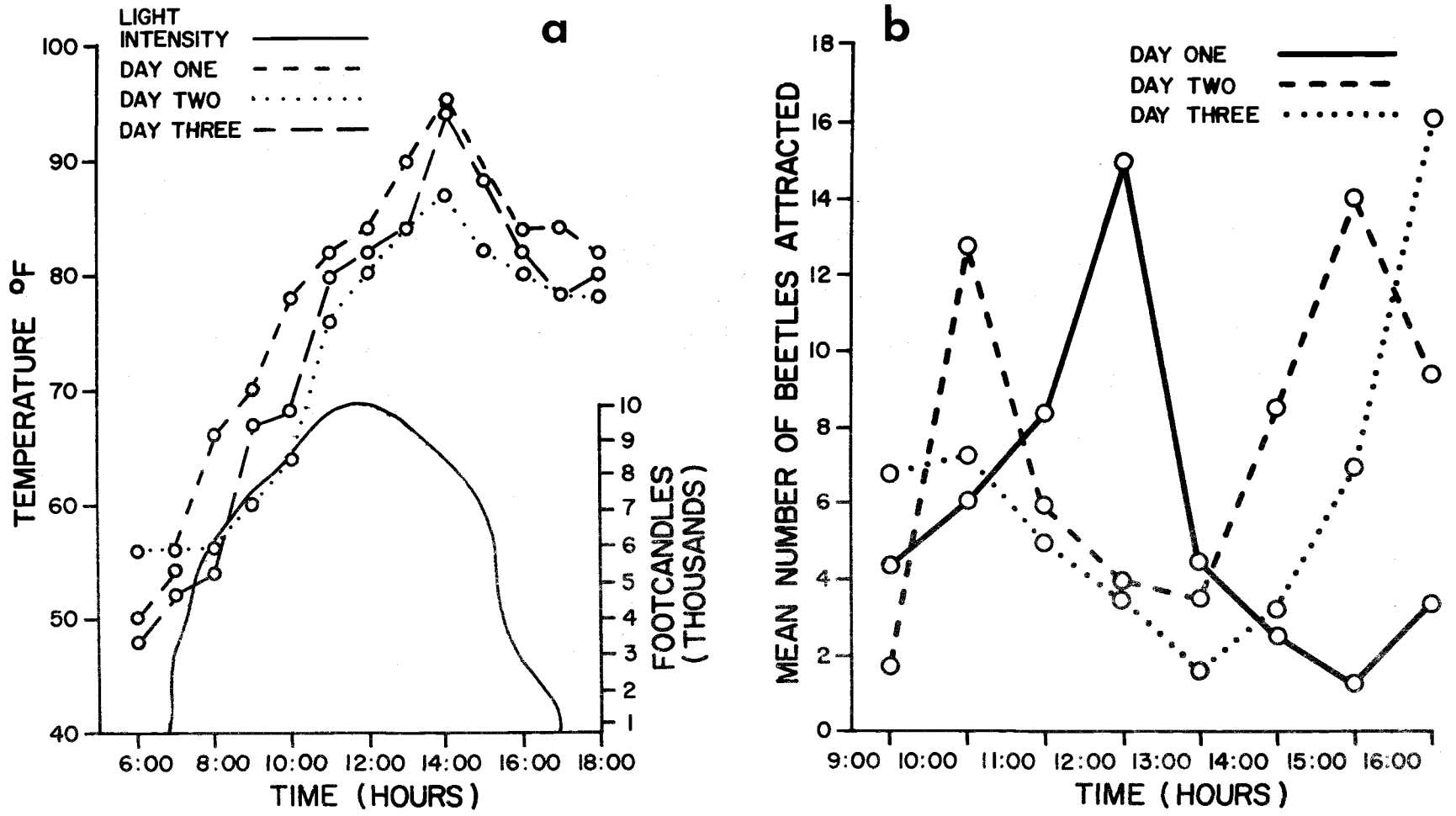

Figure 10. Field study results on artificial attraction situations showing: (a) temperature curves for each day and light intensity* versus time and (b) the mean number of beetles attracted per time period over three days versus time.
*The light intensity curve is from Rudinsky and Schneider (1969).

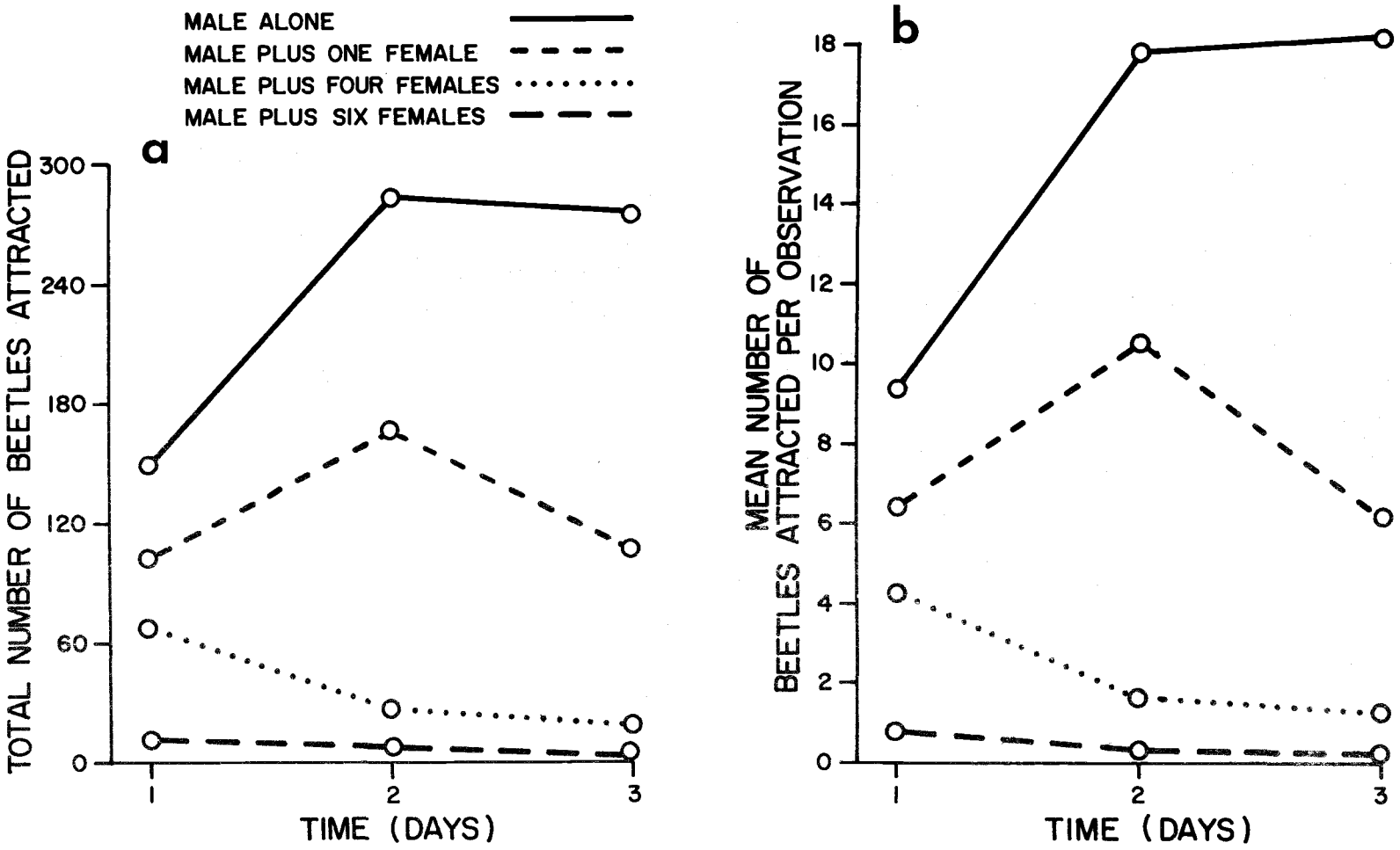

Figure 11. Field study results on artificial attraction situations showing: (a) the total number of beetles attracted versus time and (b) the mean number of beetles attracted per observation versus time. The female alone and fresh lodgepole pine log controls did not attract Ips pini beetles.

not significantly different. The mean pooled sex proportion is 0.56 which is a 1.25:1 female to male sex ratio.

The analysis of variance on the results from the field study testing beetle response to natural attraction situations (Appendix IV, Table 18) indicates that the beetle attack exposure period, day, exposure period-day interaction, collection time, and day-collection time interaction significantly ($P < 0.05$) influence the number of beetles attracted. Beetle flight fluctuations throughout the day and changes in beetle flight between days, particularly on day six which was cloudy and cooler (Fig. 12a, b), are seen to be the factors instigating the day, collection time and day-collection time interaction significances. The significant effects that the beetle attack exposure period and exposure period-day interaction have on the attracted beetle density verify the laboratory and artificial field study results. However, since these are naturally infested log attraction treatments, the exact male-female attraction situations are not known. One would assume that the one day exposed logs would attract more beetles per unit time and remain attractive over a longer period of time than the two day exposed logs which would attract more and longer than the three day exposed logs since the males in the one day exposed logs had less time to attract and interact with females than did the males in the two day exposed logs which had less time than the males in the three day exposed logs. But, as Figure 13a, b shows, the three day exposed logs attracted more beetles than the two day exposed logs.

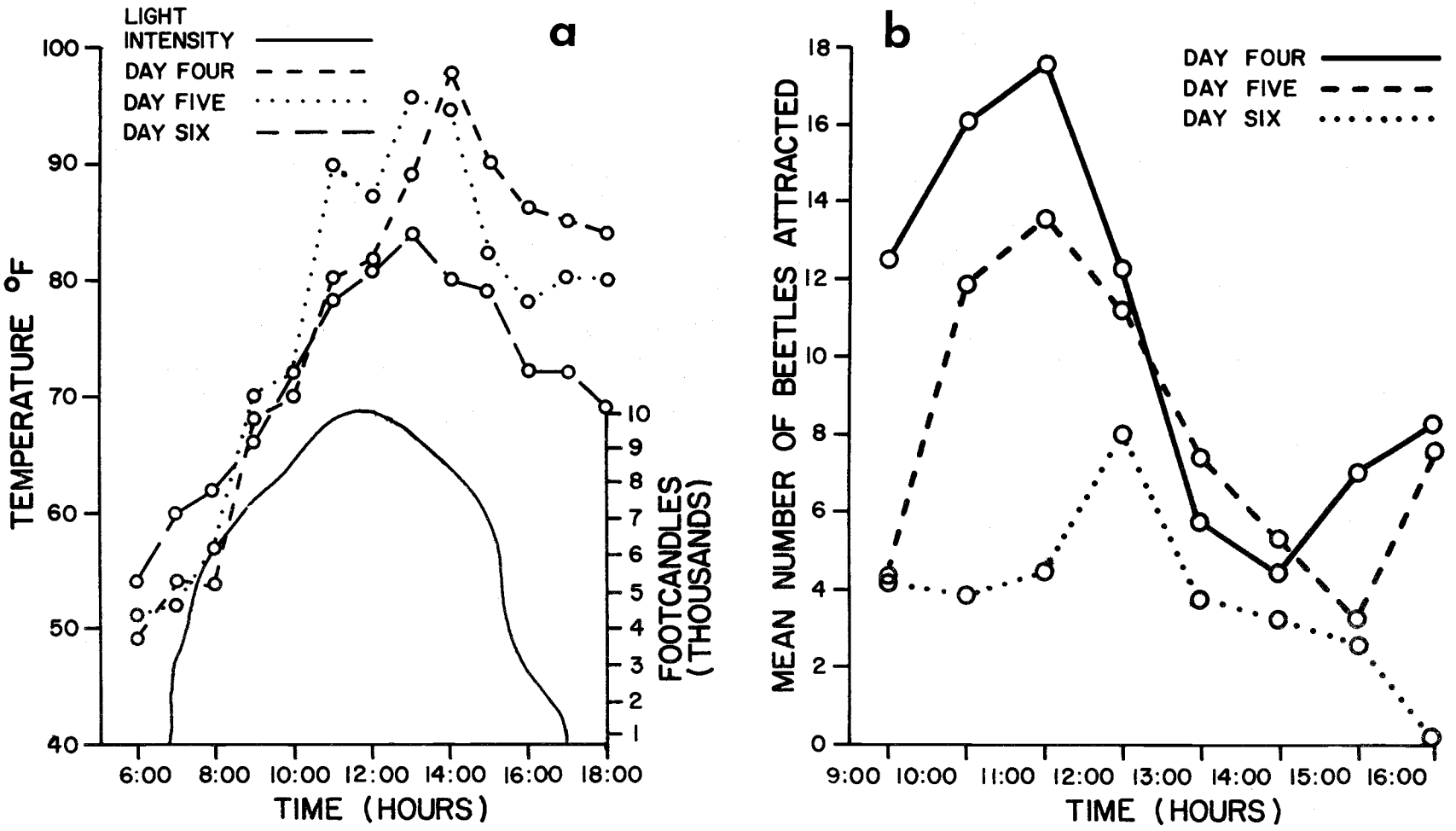

Figure 12. Field study results on one artificial and three natural attraction situations showing: (a) temperature curves for each day and light intensity* versus time and (b) the mean number of beetles attracted per time period over three days versus time.
*The light intensity curve is from Rudinsky and Schneider (1969).

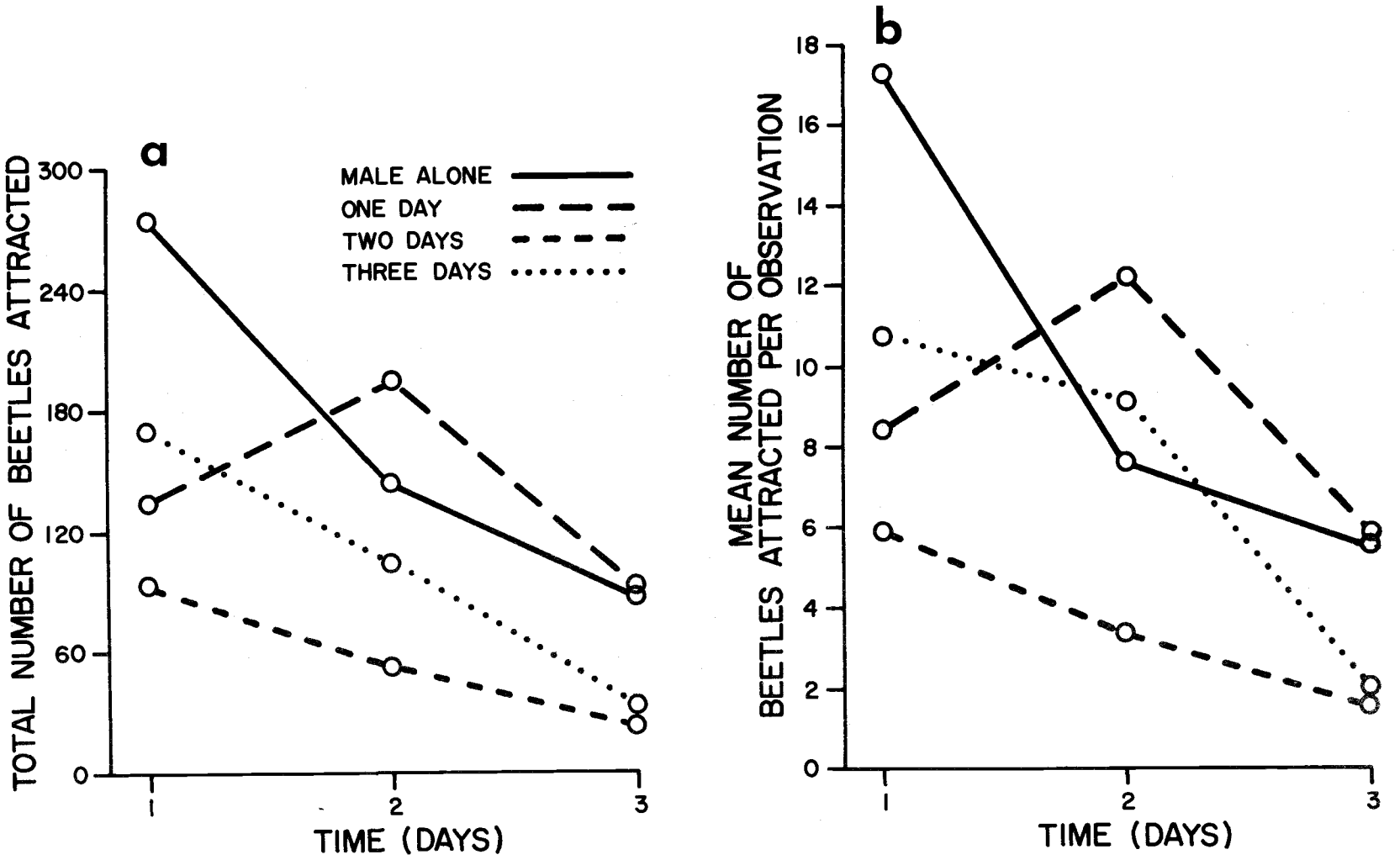

Figure 13. Field study results on one artificial and three natural attraction situations showing: (a) the total number of beetles attracted versus time and (b) the mean number of beetles attracted per observation versus time. The female alone and fresh lodgepole pine log controls did not attract Ips pini beetles.

One would reason that the three day exposed logs had more attractive potential during the test period than did the two day exposed logs. To investigate that logic, the bark on the 1, 2, and 3 day exposed logs was removed. Each gallery was typed according to the number of females present and the types were summarized. Then, using the mean number of beetles attracted per observation to the artificial field attraction situations over three days (Fig. 11b), an arbitrary attraction weighting was assigned each gallery type (Appendix V, Table 20). The summarized types and mean attraction potentials are listed in Table 5. This is an arbitrary weighting and conclusions drawn from it should be considered with that in mind.

Using the calculated mean attraction potentials, one can see that the one day exposed logs had the highest; therefore, they should have attracted more beetles per unit time and remained attractive over a longer period of time than either the three or two day exposed logs. Likewise, since the three day exposed logs had a greater attraction potential than the two day exposed logs, the three day exposed logs should have attracted more beetles and remained attractive longer than the two day exposed logs. That is what occurred (Fig. 13a, b). Referring to Figure 13 a, b, it should be mentioned that the male alone situations were the same male alone logs used during the three day study which tested attraction to artificial situations. Subsequently, those situations were becoming too old by days five and six and attraction to them decreased.

Table 5. Situation Summary and Associated Attraction Potential Means.

Ips pini Beetle Attack Exposure Period	Situation						Mean* Attraction Potential
	Male Alone	Male plus One Female	Male plus Two Females	Male plus Three Females	Male plus Four Females	Male plus Five Females	
One day	87	92	43	11	2	0	2296.6
Two days	66	49	26	10	11	1	1575.5
Three days	51	88	48	48	18	5	1955.7

*See Appendix IV, Table 20. This is an aribtrary weighting and conclusions drawn from it should be viewed with that in mind.

Analysis of variance of the sex proportion data (Appendix IV, Table 19) shows that the sex proportion of the beetles attracted to the natural situations did not significantly vary. Similarly, the sex proportion of the beetles attracted in the morning did not differ significantly from that of the beetles attracted in the afternoon for any given situation and the pooled sex proportion for a treatment on a certain day did not differ from that on any other day. Finally, the sex proportions pooled over the three days for the different situations are not significantly different. The mean pooled sex proportion is 0.61 which is a 1.55:1 female to male sex ratio.

Table 6 summarizes the early attack and colonization bark removal study results. By 1.6 days the number of females per male began leveling off and by three days it stabilized at approximately 3.3 females per gallery. As one would expect, the third day also saw the egg gallery number per attack stabilizing at 3.5 egg galleries. Finally, by 1.4 days the nuptial chamber size reached a consistent 4.5 x 5.9 mm dimensionality (Fig. 14 a-c).

Table 6. Summary of the Results of the Bark Removal Study Characterizing 20 Galleries Examined at Various Times since the Initial Ips pini Attack.

Days since Attack	Nuptial Chamber Dimensions (mm)				No. Egg Galleries		No. Females	
	Length		Width					
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
0.5	0.0-7.0	4.2	0.0-6.0	2.5	0-2	0.3	0-1	0.5
1.0	4.0-8.0	6.1	3.0-7.0	4.6	0-3	1.6	0-3	1.4
1.5	4.0-8.0	5.9	3.0-6.0	4.7	1-4	2.6	0-5	2.6
2.0	5.0-9.0	6.2	3.0-7.0	4.7	1-6	3.4	1-5	3.2
2.5	5.0-8.0	5.9	4.0-6.0	4.6	1-6	3.1	1-6	3.1
3.0	4.0-7.0	5.8	4.0-6.0	4.4	2-5	3.3	1-5	2.9
3.5	4.0-7.0	5.6	4.0-6.0	4.3	2-5	3.7	2-5	3.7

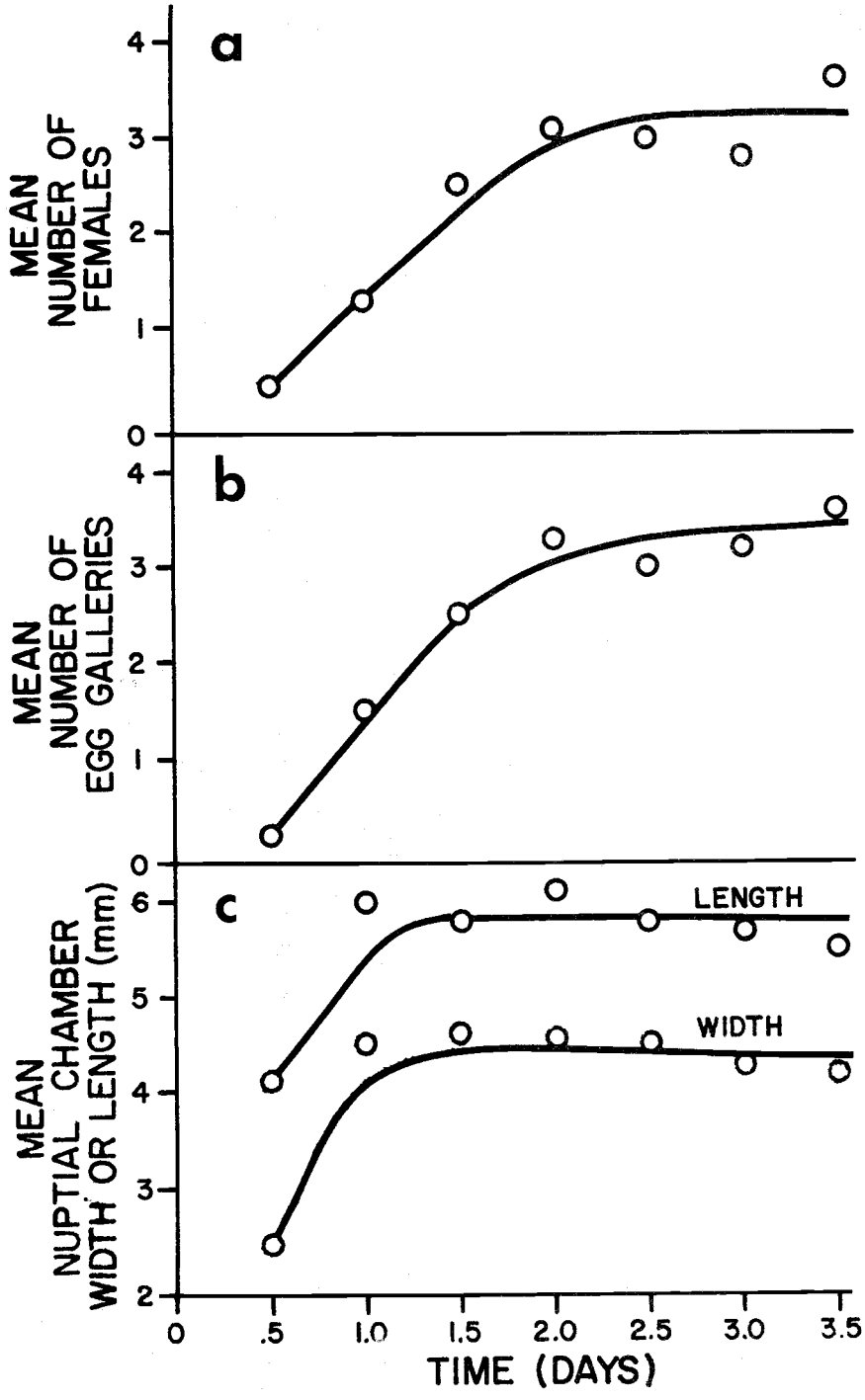

Figure 14. Attack and early colonization graphic analysis depicting: (a) the mean number of females per attack, (b) the mean number of egg galleries per attack, and (c) the mean nuptial chamber width and length versus time since attack initiation.

V. CONCLUSIONS

The finding of D. L. Wood (1961) and Barr (1969) that the female Ips pini possesses a vertex-pronotal stridulatory apparatus which the male lacks was confirmed. The male head area, corresponding to the female area housing the finely ridged pars stridens, has an elongate trough. No trace of a ridged plectrum similar to that of the female is found on the male pronotum.

While the time at which the attraction chirps are sampled does not influence the results, the stridulation produced by females subjected to various attraction situations differs. The attraction stridulation produced by females attempting to enter a given type of attractive gallery was homogeneous, whether the gallery contained a male alone or a male plus 1-4 females. The chirps produced by females attempting to enter a hole containing a male plus three females differed in the number of pulses per chirp and the pulse rate from the chirps produced during attempts to enter other types of attractive galleries. Chirps emitted contingent to stress, rivalry, attraction, and in-gallery situations differ with respect to the acoustic parameters measured. The measured parameters do not differ significantly between attraction and rivalry chirps, but the mean pulse rate differs significantly ($P < 0.05$) between stress and attraction chirps. Furthermore, with the attracted female chirps, the mean number of

interruptions, mean chirp length, and mean pulse rate significantly differ ($P < 0.01$) from those parameters of in-gallery chirps. Similarly, with stress and rivalry chirps, the mean number of interruptions, mean chirp length and mean pulse rate significantly differ ($P < 0.05$) from those of in-gallery chirps. The mean pulse rate and mean number of pulses per chirp significantly differ ($P < 0.05$) between chirps produced contingent to stress and rivalry conditions, and finally the mean number of pulses per chirp differs significantly ($P < 0.01$) between stress and in-gallery chirps.

Laboratory and field olfactory studies reveal that the observed decrease in secondary attraction is significantly influenced by the relation of the number of females per male and the elapsed time since the last female entered the gallery. That relation may involve a stop in production of the aggregating pheromone or the emission of an antiattractant by the male and/or female(s). Whatever regulatory mechanism affects the secondary attraction, it appears suitable for controlling attack density and egg deposition at a level most beneficial to brood survival and development. Furthermore, the sex proportion of the attracted beetles does not significantly vary between attraction treatments or observation periods and is about 0.58 which is a 1.4:1 female to male sex ratio. Lastly, early attack and colonization observations, under the biotic and abiotic factors present during the examination period, reveal that in Oregon the male Ips pini beetles

complete nuptial chamber construction within 1.4 days (33.6 hours), which supports Schmitz's (1972) findings, and the male accepts three females by 3.3 days (79.2 hours).

These studies indicate the need for further behavioral investigations into secondary attraction cessation in Ips pini and other polygamous bark beetles. Such studies will increase our understanding of the complex behavioral system enabling these beetles to successfully invade and colonize a host. From that knowledge (identification of an antiattractant for example) research will eventually isolate weak points in the behavioral system which can be utilized to control these pests.

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APPENDICES

APPENDIX I

DUNCAN'S MULTIPLE RANGE TESTS

Table 7. Duncan's Multiple Range Tests of Time Period (0-30 sec. or 30-60 sec.) and Treatment Influences on the Mean Number of Pulses per Attraction Chirp.

Treatment no.	Situation Description	Pulses/Chirp (mean)
	0-30 sec.	
5	Male plus four females	108.9
2	Male plus one female	110.2
3	Male plus two females	137.2
1	Male alone	158.8
4	Male plus three females	220.2
	30-60 sec.	
2	Male plus one female	65.4
5	Male plus four females	90.5
3	Male plus two females	90.8
1	Male alone	100.0
4	Male plus three females	164.9

Table 8. Duncan's Multiple Range Tests of Time Period (0-30 sec. or 30-60 sec.) and Treatment Influences on Attraction Chirp Mean Pulse Rates.

Treatment no.	Situation Description	Pulses/Chirp (mean)
	0-30 sec.	
5	Male plus four females	918.9
2	Male plus one female	1006.9
3	Male plus two females	1566.3
1	Male alone	1924.5
4	Male plus three females	1974.2
	30-60 sec.	
2	Male plus one female	692.9
5	Male plus four females	830.2
3	Male plus two females	1017.6
1	Male alone	1607.2
4	Male plus three females	1673.2

Table 9. Duncan's Multiple Range Tests of Female Number per Male Influences on Ips pini Attraction under Field Conditions.

Treatment no.	Situation Description	No. Beetles Attracted/ Observation (mean)
	First Day	
1	Male alone	9.3
3	Male plus one female	6.4
4	Male plus four females	4.2
5	Male plus six females	0.8
	Second Day	
1	Male alone	17.6
3	Male plus one female	10.4
4	Male plus four females	1.6
5	Male plus six females	0.3
	Third Day	
1	Male alone	17.9
3	Male plus one female	6.1
4	Male plus four females	1.2
5	Male plus six females	0.1

APPENDIX II

ATTRACTION CHIRP ANALYSIS OF VARIANCE TABLES

Anova Table 10. Pulses Per Chirp.

	Source of Variation	df	SS	MS	F_s
A	No. females/male in hole	4	134536.4	33634.10	5.77 **
B	Time period	1	50041.7	50041.70	8.59 **
A x B	No. females x time period	4	5028.8	1257.19	0.22 ns
	Error	90	524559.7	5328.83	
	Total	99	714201.6		

** $P < 0.01$ ns = not significant

Anova Table 11. Square Root Transformed Number of Interruptions.

	Source of Variation	df	SS	MS	F_s
A	No. females/male in hole	4	0.623952	0.155988	0.43 ns
B	Time Period	1	0.000093	0.000093	0.00 ns
A x B	No. females x time period	4	1.134244	0.283561	0.78 ns
	Error	90	32.582880	0.362032	
	Total	99	34.341169		

Anova Table 12. Chirp Length.

	Source of Variation	df	SS	MS	F_s
A	No. females/male in hole	4	0.0173996	0.00434996	1.02 ns
B	Time period	1	0.0000303	0.00003025	0.01 ns
A x B	No. females x time period	4	0.0275516	0.00688795	1.62 ns
	Error	90	0.0383058	0.00042562	
	Total	99	0.0832873		

Anova Table 13. Pulse Rate.

	Source of Variation	df	SS	MS	F_s
A	No. females/male in hole	4	17454520.0	4363630.0	7.44 **
B	Time period	1	2463580.0	2463580.0	4.20 *
A x B	No. females x time period	4	529816.0	132454.0	0.23 ns
	Error	90	52806510.0	586739.0	
	Total	99	73254426.0		

*$P < 0.05$ **$P < 0.01$

APPENDIX III

ANALYSIS OF VARIANCE ON LABORATORY, OLFACTORY STUDIES

Anova Table 14. Laboratory, Fixed Time, Female Ips pini Attraction Studies.

	Source of Variation	df	SS	MS	F_s
A	No. females/male in hole	4	40.13332	10.33333	2.21 ns
	Error	10	46.66670	4.66667	
	Total	14	86.80002		

Anova Table 15. Laboratory, Variable Time, Female Ips pini Attraction Studies.

	Source of Variation	df	SS	MS	F_s
A	No. females/male in hole	2	744.2960	387.1480	58.07 **
B	Times	2	560.2960	280.1480	42.02 **
A x B	No. females x times	4	338.3704	84.5926	12.69 **
	Error	18	120.0006	6.6667	
	Total	26	1762.9630		

** $P < 0.01$

APPENDIX IV

ANALYSIS OF VARIANCE ON FIELD, OLFACTORY STUDIES

Anova Table 16. Field Studies on Ips pini Beetle Attraction to Artificial Situations.

	Source of Variation	df	SS	MS	F_s
A	No. females/male	3	6081.9300	2027.3100	41.99 **
B	Day	2	171.1354	85.5667	1.77 ns
A x B	No. females x day	6	875.2380	145.8730	3.02 *
C	Collection times	7	810.7470	115.8210	2.40 *
A x C	No. females x times	21	1155.6930	55.0330	1.14 ns
B x C	Day x times	14	1933.1200	138.0800	2.86 **
A x B x C	No. females x day x times	42	2101.5120	50.0360	1.04 ns
	Error	96	4635.5040	48.2865	
	Total	191	17764.8794		

*P < 0.05 **P < 0.01

Anova Table 17. Field Studies on the Sex Proportion of the Ips pini Beetles Attracted to Artificial Situations.

	Source of Variation	df	SS	MS	F_s
A	No. females/male	2	0.18558	0.09279	0.84 ns
B	Day	2	0.06996	0.03498	0.32 ns
A x B	No. females/male x day	4	0.13852	0.03463	0.31 ns
C	Collection times	1	0.05812	0.05812	0.53 ns
A x C	No. females/male x times	2	0.01386	0.00693	0.06 ns
B x C	Day x times	2	0.00118	0.00059	0.01 ns
A x B x C	No. females x day x times	4	0.23956	0.05989	0.54 ns
	Error	18	1.98522	0.11029	
	Total	35	2.69200		

Anova Table 18. Field Studies on Ips pini Beetle Attraction to Natural Situations.

	Source of Variation	df	SS	MS	F_s
A	Beetle attack exposure period	3	1283.0580	427.6860	13.02 **
B	Day	2	1531.3440	765.6720	23.32 **
A x B	Exposure period x day	6	699.4920	115.5820	3.61 **
C	Collection times	7	1599.1640	228.4520	6.96 **
A x C	Exposure period x times	21	763.3185	36.3485	1.11 ns
B x C	Day x times	14	831.8226	59.4159	1.81 *
A x B x C	Exposure period x day x times	42	1489.0092	35.4526	1.08 ns
	Error	96	3152.4960	32.8380	
	Total	191	11349.7043		

*$P < 0.05$ **$P < 0.01$

Anova Table 19. Field Studies on the Sex Proportion of the Ips pini Beetles Attracted to Natural Situations.

	Source of Variation	df	SS	MS	F_s
A	Beetle attack exposure period	3	0.05257	0.01752	0.97 ns
B	Day	2	0.01677	0.00838	0.46 ns
A x B	Exposure period x day	6	0.17277	0.02879	1.60 ns
C	Collection times	1	0.02088	0.02088	1.16 ns
C x A	Exposure period x time	2	0.00069	0.00035	0.02 ns
C x B	Day x times	3	0.06305	0.02102	1.16 ns
A x B x C	Exposure period x day x times	6	0.11191	0.01865	1.03 ns
	Error	24	0.43317	0.01805	
	Total	47	0.87181		

APPENDIX V

ATTRACTION POTENTIAL WEIGHTING SCHEME

Table 20. Natural, Field, Attraction Situation Weighting Scheme.

Day	Male Alone	Male Plus One Female	Male Plus Two Females	Male Plus Three Females	Male Plus Four Females	Male Plus Five Females
One	9.3	6.4	5.6	5.0	4.2	0.8
Two	17.6	10.4	7.1	4.9	1.6	0.3
Three	17.9	6.1	4.2	3.1	1.2	0.1

See field studies on artificial situations Figure 11b. That data were used to obtain this weighting format and thus is arbitrary with no statistical basis and conclusions deemed from it must be viewed with that in mind.