Rhythms in Pheromone Production of the Male Boll Weevil

Abstract. *Male boll weevils,* Anthonomus grandis, *held in a light regimen of 16 hours of light and 8 hours of darkness released pheromone rhythmically during the 24 hours. The amount released during peaks was typically 20 times the amount released in valleys. The ratio of the two alcohol components of the pheromone also showed a daily rhythm. Under continuous light, both the release of pheromone and the ratio of the two alcohol components were arrhythmic. In darkness, pheromone release was diminished more than tenfold over the 20-day test period.*

The chemical structures of the four components of the sex pheromone [two terpene alcohols, (I) *cis*-2-isopropenyl-1-methylcyclobutaneethanol and (II) *cis*-3,3-dimethyl-$\Delta^{1,\beta}$-cyclohexaneethanol; and two terpene aldehydes, (III) *cis*-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde and (IV) *trans*-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde] of the boll weevil have been known since 1969 (*1*). Formulations of the synthetic sex pheromone grandlure have proved attractive in the field (*2*). This success, however, has actually delayed studies of the release of pheromone by the weevils. At the same time it has become increasingly clear that factors affecting release of pheromone are important to the effectiveness of all field-released, sexually sterile insects.

Although the release of pheromone by boll weevils is known to be affected by diet (*3*), age (*4*), chemosterilants (*5*), and bacterial contaminants (*6*), we now report what is, to our knowledge, the first attempt to show quantitatively, by gas-liquid chromatography (GLC), the relationship between pheromone release and photoperiodism in boll weevils. The photoperiodic response of pheromone release has been studied in the noctuid moth, *Trichoplusia ni* (*7*).

Attempts in our laboratory to find the anatomical site of pheromone release by dissection and analysis by GLC have been frustrated by the widely varying amounts of pheromone obtained from samples of insects. Since long-term rhythms in oxygen respiration had already been found in boll weevils held under a light cycle (*8*), it seemed likely that these and other short-term rhythms would dictate times of maximum release. Further, since mass-reared boll weevils are reared under continuous light, it was possible that the variations in the pheromone release of these weevils would be arrhythmic and therefore unpredictable.

Initially, frass was collected manually for 3 days at 3-hour intervals from 350 adult male weevils reared under a cycle of 16 hours of light and 8 hours of darkness (LD 16 : 8). There were 100-fold differences between high and low points in pheromone release. Subsequently, an automatic fraction collector was adapted to the collection of frass from 350 adult male weevils (*9*) at 3-hour intervals for 20 days after adult emergence. These weevils were held at 24°C, LD 16 : 8 [light (44 lux) on from 0400 to 2000 hours] and were fed freshly picked cotton squares (buds) daily just after the collection of frass at 1000 hours. One square was supplied for every five weevils. The cage volume was 1100 cm^3; the floor area was 133 cm^2. Each 3-hour sample of frass was weighed and extracted according to the method of McKibben *et al.* (*10*) in a micro-Soxhlet apparatus with 10 ml of pentane after the addition of standard (α-terpineol). The GLC analysis (*11*) allowed all four pheromone peaks to be quantified. The total amount of pheromone obtained for each 3-hour period was calculated by summing the amounts of the four pheromone components.

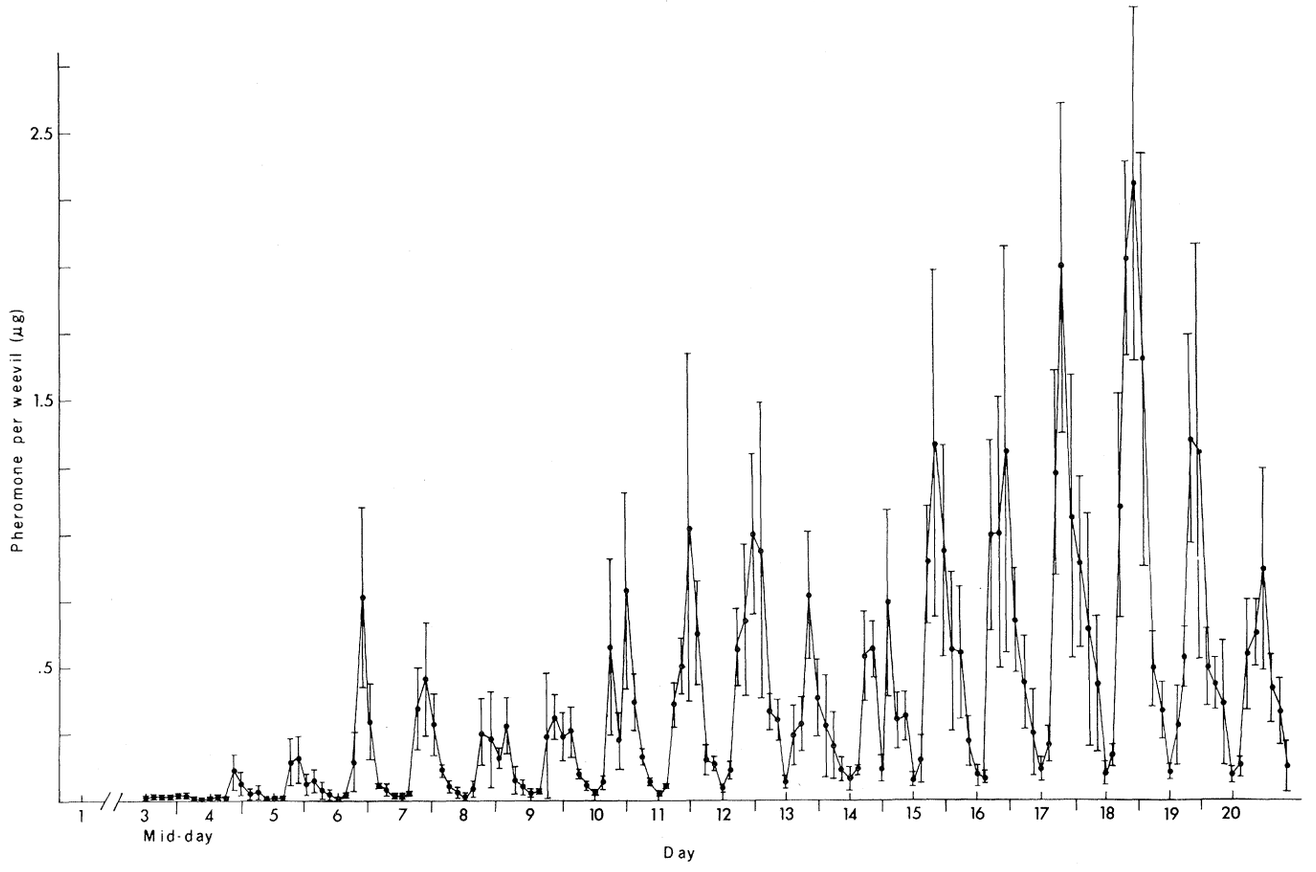

Fig. 1. Daily rhythm of pheromone production under LD 16 : 8. The initial point each day is that for the interval between 2200 and 0100 hours.

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Figure 1 shows the mean values for four replicates of pheromone determinations from four groups of 350 males sampled over a period of 5 months. The highest daily peak of pheromone usually occurred between 0700 and 1000 hours or between 1000 and 1300 hours. Examination of Fig. 1 suggests a long-term rhythm of 6 days with 24-hour amounts of pheromone higher on days 6, 12, and 18 than on preceding or succeeding days. Increased release of pheromone with age was evident (5.3 times as much pheromone was released in the second 10 days of adult life as in the first 10 days) (*12*). Day 18 was the day of highest release: approximately 1 μg of pheromone per hour per weevil was produced in the peak 3-hour period. The low point of release was consistently in the period between 2200 and 0100 hours.

Figure 2 shows the arrhythmic pattern of pheromone released when the weevils were held under constant light (44 lux). When the weevils were held in constant darkness (two replicates), only traces of pheromone were released (*13*). Thus, the LD cycle is apparently one cue on which the rhythm depends, and light stimulates pheromone release. Field observations show that locomotor activity of boll weevils is reduced by cool temperatures; pheromone release is probably mediated by temperature also. [Under continuous light (one replicate), two times as much pheromone was released as the average released under the LD cycle, although more pheromone was released by LD weevils during the first 10 days (*12*).]

Although the pheromone of the boll weevils is found in the feces, the amount released in a 3-hour period is not in direct proportion to the amount of feces produced. The amount of pheromone changes from one time period to the next. Thus, the graph of pheromone per gram of feces appears nearly identical to Fig. 1. Linear regression analyses of frass production versus pheromone content shows a significant ($P < .01$) negative correlation.

The ratio of the components of the pheromone that are terpene alcohols, I and II, was dynamic and had its own daily rhythm (Fig. 3). The ratio, I : II, was low when the amount of pheromone was high. No rhythm was evident in the I : II ratio when weevils were held under continuous light. Since the feces are a "slow-release formulation" (*14*) of pheromone, one can hypothesize that these changing ratios may be a signal to the searching female that the odor is fresh. This might prevent orientation of the female to abandoned frass rather than to the male.

Table 1. Ratio of amounts of alcohol II to aldehydes III and IV.

Time period	Replicate	
	1	2
2200–0100	1.50	0.14
0100–0400	1.67	1.47
0400–0700	1.26	3.21
0700–1000	1.83	1.93
1000–1300	2.29	3.41
1300–1600	2.70	2.95
1600–1900	0.47	2.82
1900–2200	0.90	1.59

The chemical structures of alcohol II and aldehydes II and IV suggest an oxidation-reduction step biosynthetically linking the alcohol to the aldehydes. However, the amounts of the aldehydes changed with respect to alcohol II. For purposes of comparison, data from two of the four replicates concerned in Fig. 1 were chosen for day 18 to show the changes given in Table 1.

During manual collection of feces, weevils in scotophase were observed to be quiescent; in photophase, weevils were active. Since locomotor activity, too, is rhythmic, weevils being reared for field-release programs should be ex-

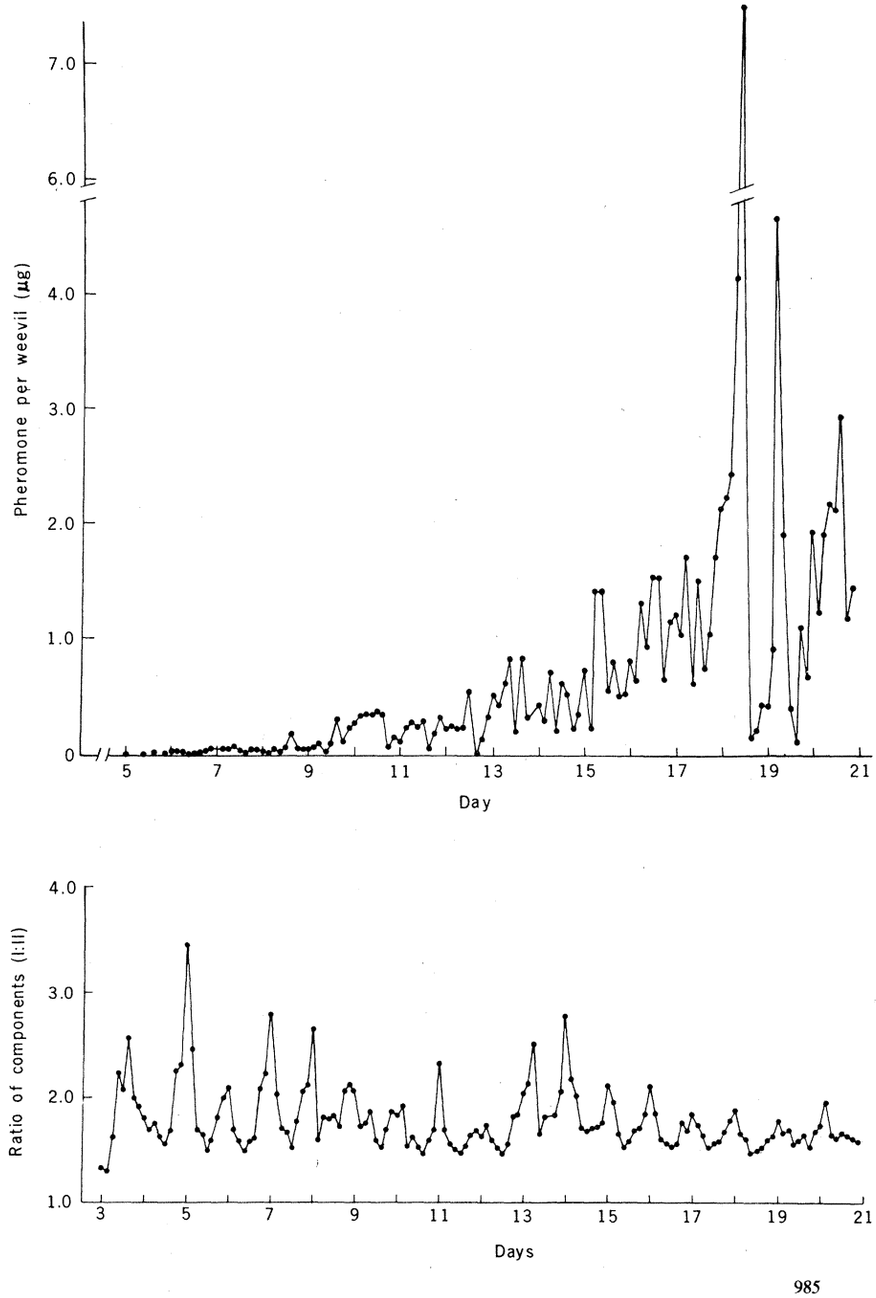

Fig. 2 (top). Daily pheromone production under continuous light (one replicate). Fig. 3 (bottom). Daily rhythm of ratio of pheromone component I to pheromone component II (average of the four replicates shown in Fig. 1).

posed not to continuous light but to a photoperiod that will ensure synchrony in locomotion and pheromone release with native weevils. Also, trap formulations of grandlure must have release rates equivalent to or greater than the peak release of pheromone by native males.

The role of light in pheromone release is consistent with field observations of boll weevil behavior. Artificial light could possibly be introduced into a field at night, as in an orchard, to alter rhythms of pheromone release and so to interfere with mating behavior of pest insects. The use of proper LD cycles in the insectary might also enhance the field performance of sexually sterile insects in the "sterile-male" approach to insect control.

We believe that chemical cues that play a part in insect attractants as related to sexual responses and host-parasite-predator behavior may, in general, be mediated by rhythms such as those observed in the specific case of the male boll weevil pheromone.

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References and Notes

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9. Gast ebony strain; weevils were mass-reared under the supervision of O. H. Lindig of the Boll Weevil Research Laboratory.
10. G. H. McKibben, W. L. McGovern, W. H. Cross, O. H. Lindig, *Environ. Entomol.* **5**, 81 (1976).
11. A stainless steel column (76 m by 0.76 mm inner diameter) coated with OV-17 was used at 160°C isothermally. The nitrogen flow was 8 ml/min.
12. The survival rate of male weevils rendered sexually sterile by irradiation is about 50 percent in 10 days. The survival rate in our study of the four replicates under the LD cycle was 84.4 percent in 20 days; under DD, survival for 20 days was 64.8 percent; under LL, survival for 20 days was 72 percent.
13. Over the 20-day test period, weevils held under the DD cycle produced 3.8 μg of pheromone per weevil, while those under the 16 : 8 light regimen produced 50.5 μg of pheromone per weevil.
14. Data of G. McKibben indicate a slow loss of pheromone from feces collected ½ hour after being excreted.
15. We thank B. Boyd for assistance in analyzing the samples and calculating the data.

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Measurement of Regional Substrate Utilization Rates by Emission Tomography

Abstract. *Emission tomography can be used to monitor, in vivo and regionally, the utilization of metabolic substrates labeled with positron-emitting radioisotopes produced by a cyclotron. The concept was validated by measuring brain glucose utilization with carbon-11-labeled glucose in rhesus monkeys.*

Present methods of studying organ metabolism in vivo have serious shortcomings. The appearance of linear accelerators and cyclotrons in the medical environment and the parallel development of rapid chemical techniques for incorporating their products into compounds have resulted in the availability of a large number of radioactively labeled substrates (*1*). When these labeled substrates are used with recently developed imaging systems employing the concept of positron emission tomography (*2*), the resulting data make it possible to calculate values for several parameters of physiological significance, using a mathematical model developed by Raichle *et al.* (*3*). This approach is analogous to quantitative autoradiography, but has the added advantage of allowing studies in vivo.

In this report we describe the basis for a low-risk, quantitative method of measuring glucose utilization regionally in vivo in the human brain. The method employs emission tomography to externally monitor the spatial distribution in brain of ^{11}C-labeled glucose, but is sufficiently general to be used with radioglucose or other radiolabeled pharmaceuticals in other organs. The data for our report were obtained on seven adult rhesus monkeys.

The monkeys were anesthetized with phencyclidine, paralyzed with gallamine, and passively ventilated with 100 percent oxygen. A peripheral artery was catheterized for measuring the specific activity of [^{11}C]glucose in blood. The monkeys were placed in an emission tomograph (*2*) and images of single transverse sections of brain tissue about 1.5 cm thick were obtained (Fig. 1). Because of the small size of the monkey brain relative to a resolution element of the imaging system (1.5 cm^3), data from several resolu-

Table 1. Measurements of the cerebral metabolic rate for glucose (Φ) and the cerebral blood volume (V_b) in seven adult rhesus monkeys by using quantitative emission tomography, [^{11}C]glucose, and [^{11}C]carboxyhemoglobin. Plasma glucose concentrations (C_b) were measured by standard enzymatic methods. Values in the last row are means ± standard deviations.

Φ (mg per 100 g min^{-1})	V_b (ml per 100 g)	C_b (mg per 100 ml)
4.51	4.5	65
4.59	4.1	78
4.90	3.9	83
4.93	4.0	86
6.80	4.4	132
5.08	4.2	80
5.67	4.2	115
5.21 ± 0.80	4.1 ± 0.2	96 ± 22

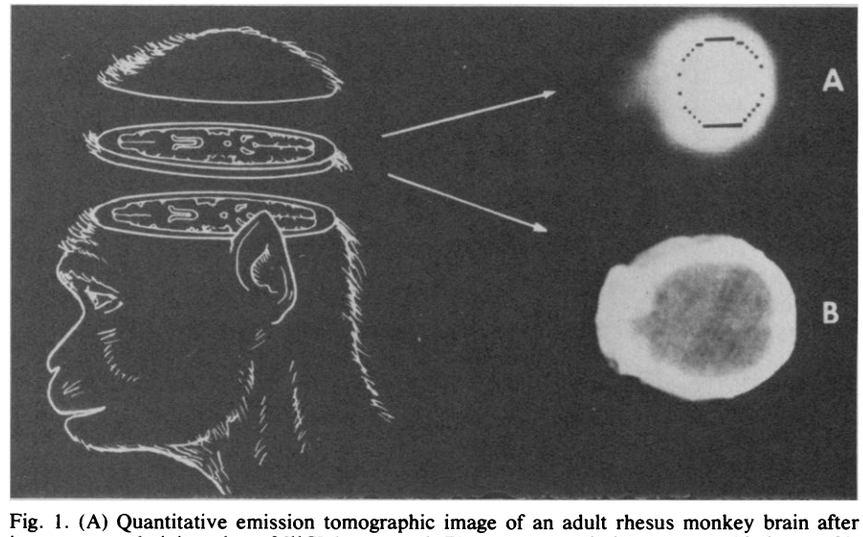

Fig. 1. (A) Quantitative emission tomographic image of an adult rhesus monkey brain after intravenous administration of [^{11}C]glucose and (B) x-ray transmission tomographic image (80-second scan with an 8-mm collimator on an EMI CT 5000 prototype body scanner) at the same level for comparison. The accompanying drawing shows the approximate anatomical orientation of the images.

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