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Electronic fraction collector used for insect sampling in the photoperiod-induced diel emergence of bark beetles

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ABSTRACT. An electronic timer and fraction collector consisting of CMOS integrated circuits is described. It converts 50- or 60-Hz AC to real-time pulses in programmable whole-number increments (1, 10 or 60 s) from 3 to 16659, producing timing periods from 3 s to more than 11 days. The fraction collector contains a leaf-switch feedback circuit that automatically adjusts to various gear motor speeds and sample tube spacings so that proper positioning results. Hourly collections by the device of the bark beetles *Ips typographus* L. and *Pityogenes chalcographus* L. (Scolytidae) emerging from logs of Norway spruce, *Picea abies*, indicated that both species emerged with a diel periodicity. A unimodal emergence peak for both sexes of both species occurred at midday in LD 20:4 at a constant 25°C and 80% r.h.

Key words. Fraction collector, sampling, timer, bark beetle, insect emergence, *Ips typographus, Pityogenes chalcographus,* Scolytidae, diel periodicity.

Introduction

Fraction collectors are commonly used in biochemistry and physiology for sequentially collecting 'fractions' of chromatographic effluents. In principle, they can also be used in certain behavioural investigations, e.g. for detecting rhythms of insect emergence and defecation. They are expensive, however, costing hundreds of dollars.

Cameron & Borden (1967) using 2-h manual collections described a diel periodicity of emergence for the bark beetle *Ips paraconfusus* Lanier (from California), but they could not separate the effects of temperature and photoperiod (or humidity) on the emergence pattern because these parameters were uncontrolled.

The inexpensive fraction collector and timer described here was designed in order to determine the effect of photoperiod on the emergence of the sexes of *Pityogenes chalcographus* L. and *Ips typographus* L. by automatically collecting the beetles hourly as they emerged from brood logs of Norway spruce, *Picea abies* (L.) Karst, under constant temperature and humidity. The fraction collector can be used in any situation where precisely timed automatic collections in real-time are required, and is inexpensive, reliable, and relatively simple to build.

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Methods and Results

Fraction collector

The schematic circuit of the electronic timer portion of the fraction collector is shown in Fig. 1, and the gear motor control circuit in Fig. 3. For 50-Hz AC, the circuit shown in Fig. 2 must be inserted in Fig. 1 instead of that shown for IC 3, IC 1B, IC 4A and 4B. These circuits are shown to operate

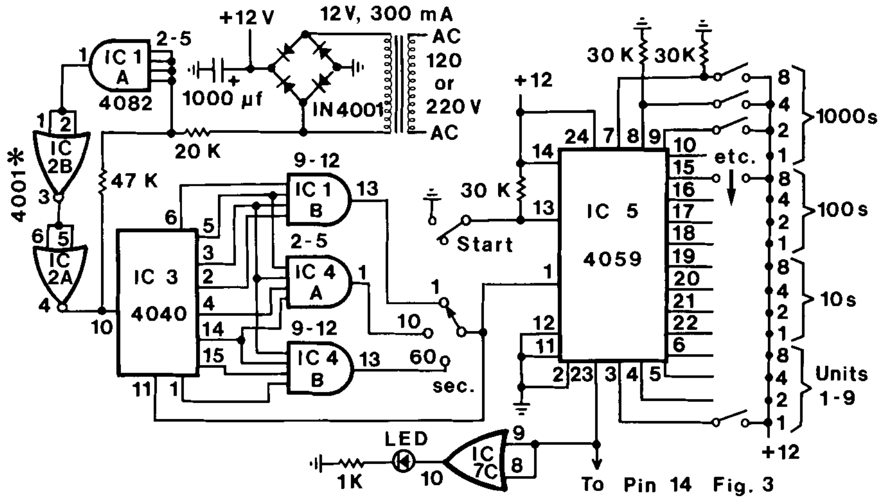

FIG. 1. Schematic circuit of the timer section of the fraction collector (60-Hz AC). The cycle time is set by (1) the three-position rotary switch at 1, 10 or 60s and by (2) programming IC 5 by means of DIP switches at the left of the figure as indicated. For example, a cycle time of 631s is obtained by setting the rotary switch at the 1s place and closing the first DIP switch in the 'Units' section plus the first and second switches in the '10s' section (1 + 2) and the second and third switches in the '100s' section (2 + 4) to yield 1 + 30 + 600 = 631s. All switches are each connected to ground through a 30 kΩ resistor as indicated for the last two switches in the '1000s' section. Timing is initiated by first connecting the Start switch to ground for at least three clock pulses and then opening the switch at the desired starting time. ICs 1, 2, and 4 have +12 V (DC) applied to pin 14 and ground to pin 7, while IC 3 has +12 at pin 16 and ground at pin 8. *Pins 8, 9, 12, and 13 of IC 2 are connected to ground. All resistors are 1/4 W. CMOS integrated circuits should not be inserted into their sockets until all connections have been made.

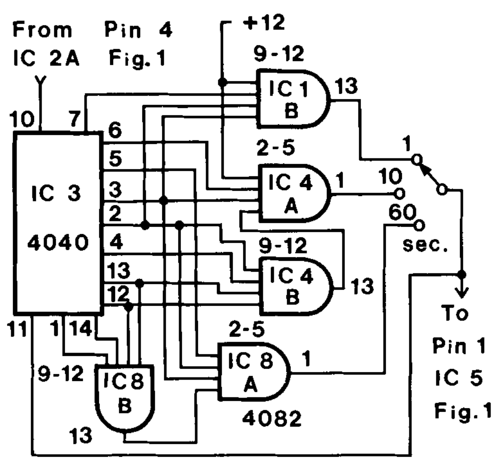

FIG. 2. Schematic circuit of the 50-Hz AC divider section to be inserted in Fig. 1 (for use in Europe) instead of the corresponding American (60-Hz) circuit consisting of IC 3, IC 1B, and IC 4A and B. The same power connections are used as in Fig. 1; in addition IC 8 requires +12 V at pin 14 and ground at pin 7.

at 12 V (DC), although any voltage between 5 and 15 V can be used with an appropriate relay (Fig. 3).

In Fig. 3, a normally open leaf-switch with a roller is used to sense the position of test-tubes on the fraction collector and so must be placed as shown in Fig. 4. The npn transistor, Q1, was a 2N2222 or similar and the 12-V relay (SPST) must have contracts rated at 120–220 V. Fig. 4 shows the general plan for constructing the fraction collector; many variations are possible, however, depending on the needs and skill of the builder. The leaf-switch feedback will accommodate many different collector dimensions and gear motor speeds. Gear motors of 2 rpm down to about 0.25 rpm will work; slower speeds are more appropriate for larger numbers of tubes and larger discs. For proper operation it is critical that the distance between the axle and all test-tubes, i.e. the radius, is approximately equal, otherwise the leaf-switch will not function consistently. Spacing between tubes around the disc

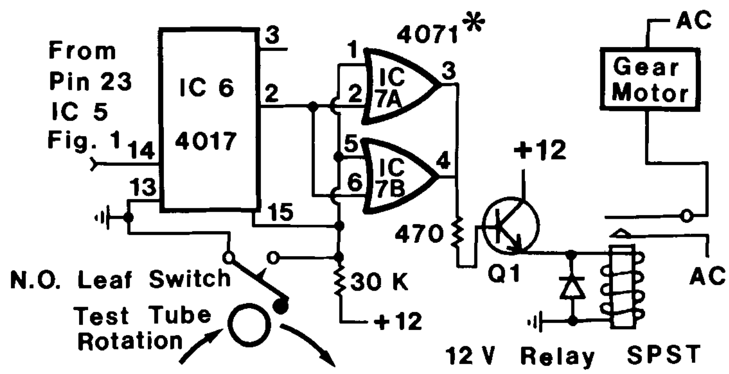

FIG. 3. Schematic circuit of the test-tube position sensor and motor turn-on interval control section of the fraction collector that will accomodate various gear motors of <2 rpm. *Pins 7, 12 and 13 of IC 7 and pin 8 of IC 6 are connected to ground while pin 16 of IC 6 and pin 14 of IC 7 are connected to +12 V (DC).

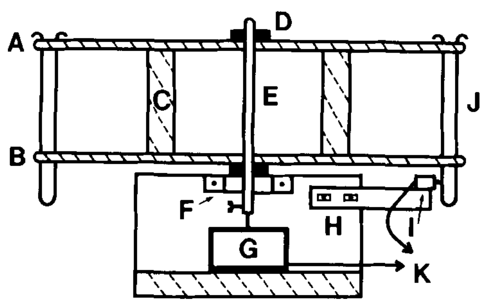

FIG. 4. Diagram of fraction collector in cross-sectional side view. Two discs (A and B) are drilled appropriately around the circumference to hold test-tubes, separated by two support blocks (C), and held in place by two rings with set screws (D) above and below. The test-tube-holding discs are rotated by an axle (E) in bearing (F) attached to a stand, and the axle is fitted with the shaft of the gear motor (G). An adjustable block (H) is attached to the normally open leaf-switch (I) which detects the position of test tubes (J), and wires from the gear motor and leaf-switch (K) connect to the control unit (Fig. 3).

periphery does not have to be precise because of the feedback circuit.

Bark beetle emergence

P.chalcographus and *I.typographus* were obtained from laboratory cultures, maintained on Norway spruce logs, originally from the province of Värmland, Sweden. About seventy-five unsexed *P.chalcographus* were allowed to bore freely for 24 h on each of two spruce logs (28× 7 cm diam.) on 16 March 1982. A third log (28× 10 cm diam.) had thirteen holes drilled through the outer bark, and each hole had a male *I.typographus* inserted. After 24h, nineteen females were released, and soon joined the males in their nuptial chambers.

The logs were then placed inside a clear plastic emergence box (22× 16× 32 cm) painted first black and then white externally except for one side 'window' (7.5 × 16 cm high) under which a plastic funnel was placed beneath a hole in the floor of the box to collect beetles that were attracted to the light. A plastic tube from the funnel directed the beetles to a test-tube in the fraction collector. A thin film of sebaceous oil around the top inside wall of the tubes prevented beetles from escaping. Illumination inside the box was 2100 lx near the window and <100 lx at the back.

The emergence box and fraction collector were placed inside a 12.6-m^3 environmental chamber (Karl Weiss, Giessen, Germany) which regulated the temperature at 25 ± 0.2° C and humidity at 80 ± 5% r.h. Air from the chamber was drawn through the box with a suction system at about 11 l/min. The photoperiod was 20 h light : 4 h dark, with the photophase beginning at 01.00 hours local time. Beetles were caught hourly by the fraction collector from 10 to 31 April 1982, and the mean time of emergence was calculated for each sex.

The emergence of both sexes of *I.typographus* and *P.chalcographus* exhibited unimodal peaks occurring at approximately midday (Fig. 5). The mean time of emergence for males and females of *I.typographus* was 11.28 h local time ±48 min (±95% CI) and 11.58 h ±47 min, respectively (not significantly different, $P>0.1$, t-test). For male and female *P.chalcographus*, mean times were at 10.57 h ±43 min and 10.48 h ±17 min (difference also NS).

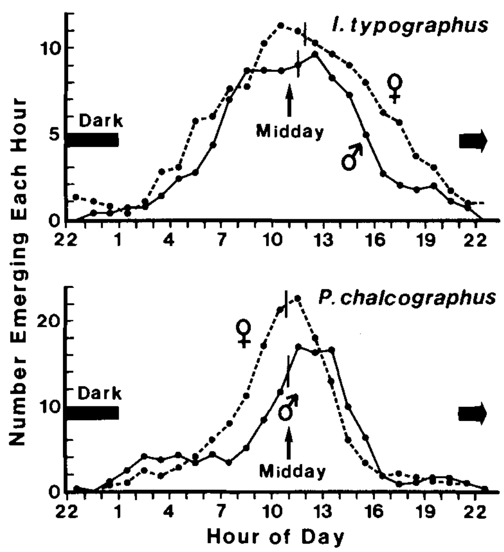

FIG. 5. Effect of photoperiod on the time of emergence of *Ips typographus* and *Pityogenes chalcographus* from separate spruce logs contained in the same plastic emergence box held at constant 25°C and 80% r.h. (10–31 April 1982). The vertical bars on each curve designate the mean emergence time. Points are the result of 3-h rolling averages. Sample data obtained from the fraction collector.

The sex ratio (♂:♀) of emergence for *I.typographus* during the period 10–31 April was 1:1.35, and did not differ significantly ($P>0.1$, chi-square) over the three successive periods 10–15, 16–22 and 23–31 April. The sex ratio for *P.chalcographus* was 1:1.18 during the same period, and similarly did not significantly differ over the three successive periods. The mean time of emergence for both sexes of both species occurred approximately at midday on all three successive periods above.

Discussion

Fraction collector operation

The AC sinusoidal waveform from the 110–120V 60-Hz or 220–240V 50-Hz power outlets is 'squared' by ICs 1A, 2A and 2B to provide a real-time base. These timing pulses are applied to pin 10 of the binary counter/divider, IC 3, which can count up to 2^{12} (or 4096) before recycling. Any number up to 4096 can be obtained by coupling one or more of the twelve pin outputs representing 2^0 to 2^{11} to a multi-input AND gate (IC 1B and IC 4). When all outputs selected go 'high', the AND gate output then goes 'high' and by connecting its output to the reset of IC 3 (pin 11) the counter is instantly reset to begin the timing cycle again. For example, to obtain a 1-s pulse the 60-Hz is multiplied by 60 ($2^2+2^3+2^4+2^5$; pins 6, 5, 3 and 2) as shown in Fig. 1 for IC 1B. A three-position rotary switch is used to select the appropriate timing pulse of 1, 10 or 60 s.

The brief pulse from the AND gate resets IC 3 to zero so all its outputs go 'low', but the pulse is long enough to 'clock' IC 5 one count via pin 1. IC 5 is a programmable divide-by-N counter (Jameco Electronics, Belmont, California) which in Fig. 1 has been connected in the divide-by-10 mode (other modes are possible, see *COS/MOS Integrated Circuits* 1980, RCA Corp. 688p.) with an output pulse (at pin 23) equal to one cycle of the clock-input signal. In other words, the LED will turn on for 1, 10 or 60 s depending on the rotary switch setting.

The time between LED turn-on is programmed by means of the 16 DIP switches (on-off) all connected to +12V and each connected to ground through a 30-kΩ resistor and to the respective 'jam inputs' (pins 3, 4, 5, 6, etc., as shown in Fig. 1). Any time period in whole increments between 3 and 16659 multiplied by 1, 10 or 60 s can be obtained. The 'units' section can only be programmed from 1 to 9 while the '10s', '100s' and '1000s' can be programmed from 1 to 15 so the highest programmable number is 9 + 150 + 1500 + 15000 = 16659 (over 11.5 days at the 60-s position). However, a timing pulse less than three counts cannot be programmed (< 3 s).

The timing period can be initiated when

desired by connecting the Start switch (from pin 13, IC 5) to ground for at least three clock pulses (3, 30, or 180 s) and then switching at the appropriate starting time. This function is important when one wants to have the fraction collector rotate at a specific time such as on the hour.

The output pulse from IC 5 is coupled to the 'clock' input of IC 6 (Fig. 3) so that pin 3 which is 'high' now goes 'low' and pin 2 goes high and causes the OR gates (doubled for more output drive) to go high. This biases the transistor Q1 to conduct DC and turns on the relay conducting AC to the gear motor and rotates the fraction collector. As the test-tube moves away from the leaf-switch (Fig. 3), the contacts of the switch open in the gap between tubes causing the other inputs to the OR gates to go high thus maintaining power to the motor, but pin 15 of IC 6 also goes high which resets the IC so that pin 2 now goes low and pin 3 becomes high again (the initial condition).

The motor continues to turn, however, until the next test-tube closes the leaf-switch and a 'low' condition at all OR gate inputs results. The 'low' OR gate output then stops the transistor/relay, and the collector ceases to rotate until the next timing pulse from the circuit in Fig. 1. The leaf-switch position must be adjusted by set screws at 'H' in Fig. 4 to facilitate proper contact with the test-tubes. The type of gear motor and the spacing between adjacent test-tubes does not need to be precisely determined because of this feedback sensing circuit.

The multiple-output electronic timer designed by Byers (1981) can also be used as a supplementary timer for the fraction collector. This is done by connecting the output of the 4082 AND gate (IC 11A) in Fig. 1 of Byers (1981) to pin 1 of IC 5 in Fig. 1 here. The four inputs of the AND gate (IC 11A) must then all be connected to an output of either IC 7 or IC 8 to obtain pulses of every 10 min or h, respectively, for deriving periods of up to 16659 h (over a year) in whole number increments.

If the fraction collector circuitry is 'false triggered' by spurious high-voltage transients on the power-line, sometimes caused by inductive motors on the same line, the use of a power-line filter commonly used for home computers (R. L. Drake Co., Miamisburg, Ohio, or similar) allows stable operation.

Bark beetle emergence

The decline in emergence that begins at midday indicates that the beetles are anticipating the onset of darkness, since no environmental parameters were changing during this time (Fig. 5). This is evidence for a light-cycle-induced periodicity of emergence which may be controlled by a circadian rhythm. The influence of temperature and other conditions in nature may alter this basic pattern, however. For example, flight activity during the spring 'swarming' in Sweden is often limited to the warmest part of the day, so emergence peaks in nature may be more compressed than in Fig. 5.

There was no evidence to indicate that the sexes of each species emerged differently, but in *I.paraconfusus* in California, temperature appears to influence the emergence of each sex differently (Cameron & Borden, 1967). In this species a unimodal peak emergence for both sexes occurs near the middle of the day at temperatures below 22°C, but at intermediate temperatures (22–26°C) males have a unimodal peak just before midday while females exhibit a bimodal peak, one coinciding with the male's and a later peak in the afternoon (16.00–18.00 hours). The peaks of emergence of males and females appeared to diverge at temperatures above 26°C, with males emerging before noon and females in the afternoon (but both avoiding midday high temperatures).

Although light intensity, humidity, and especially temperature were not controlled, Cameron & Borden argued that the gradual decline in emergence during favourable temperatures which preceded dusk indicated that the emergence might be under the control of some other factor, such as response to an external key (e.g. light intensity) or a circadian rhythm.

They also hypothesized that the bimodal flight response to pheromone that occurs in the morning and afternoon (Vité & Gara, 1962; Gara & Vité, 1962; Gara, 1963), apparently temperature dependent, was a result in part of the emergence patterns. Furthermore, they stated 'the single peak of flight activity observed by Gara & Vité (1962) under cool spring field conditions could be a reflection of the single emergence peak' at temperatures below 22° C. However, this may be over-emphasizing the influence of emergence on the time of catch on traps releasing pheromone, since the effect of temperature and other factors on flight activity and their ability to respond may be more significant.

In the case of *I.typographus* and *P.chalcographus,* the temperatures favourable for flight response to pheromone usually occur during midday and the subsequent few hours (Annila, 1969), so emergence rhythms have probably evolved to coincide with this time. In California, where midday temperatures on the bark are likely to be lethal, this has probably selected for beetles which do not emerge during high temperature (usually at midday) and which make use of internal (circadian) timing to avoid emerging near the end of the light period.

Thus all three species apparently emerge at the time of day most favourable for survival, when the temperature is optimal for flight and possibly with sufficient time to locate breeding areas. They apparently avoid emerging just before dusk since they would be forced to spend the night and the next morning exposed to the risk of predation and other hazards. Further work is needed to determine if the diel periodicities observed are the result of circadian rhythms.

Acknowledgments

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