

## NOVEL FRACTION COLLECTOR FOR STUDYING THE OVIPOSITION RHYTHM IN THE TURNIP MOTH\*

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**Abstract**—Turnip moths, *Agrotis segetum* (Schiff.) (Lepidoptera: Noctuidae), were held in a transparent plastic box with a window opening to a rotating paper drum upon which the females could lay eggs. A novel fraction collector, consisting of a standard 24-hr wall timer and simple electronic circuit, served to rotate the paper in hourly increments. The entire apparatus was housed in an environmental chamber on a 16 hr light : 8 hr dark photoperiod at constant 23.7°C and 55% r.h. Under these conditions a circadian rhythm of egg laying was indicated with a mean activity time 0.2 hr before dark and standard deviation of 2.1 hr. The relative egg productions during the second to seventh day of oviposition peaked on the third day. Differences in the circadian rhythms of mating and oviposition are considered in terms of ecological fitness.

**Key words**—*Agrotis segetum*, turnip moth, Noctuidae, circadian rhythm, oviposition, fraction collector, timer.

### Introduction

In many studies of chronobiology it is advantageous to observe the various circadian rhythms with electronic monitors or collectors. One useful device for observing insect rhythms such as defecation, domicile construction, oviposition, feeding and emergence is the multi, test-tube holding fraction collector. While fraction collectors are commercially available they are quite expensive, especially if several are required. Byers (1) described an inexpensive fraction collector, consisting of seven integrated circuits, that can collect fractions of seconds, minutes or hours with feedback positioning of the test tubes. In many cases, however, there is no need for such a sophisticated device that can provide very brief or precise timing periods. A more simple device that would produce periods of 15, 30 or 60 min is often all that is needed to properly evaluate many kinds of rhythms.

The electronic fraction collector described by

Byers (1) was modified in the present study by replacing the more complicated timer circuits with a standard 24-hr wall timer and a simple circuit. The collector disk also was modified to collect eggs deposited by female moths so that oviposition rhythms could be studied under controlled conditions.

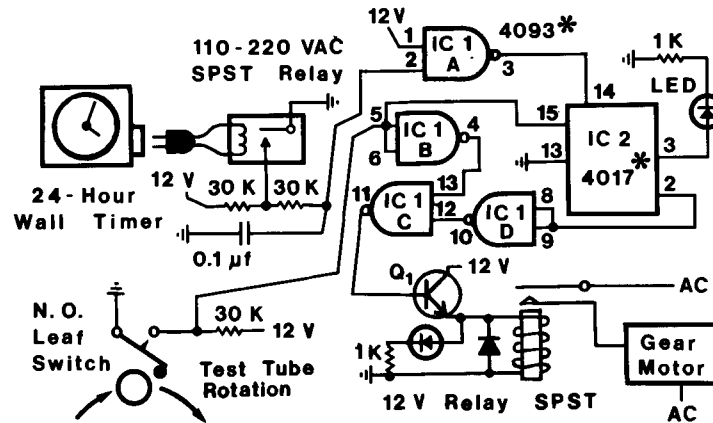
### Methods

#### *Fraction collector*

The schematic circuit of the electronic fraction collector is shown in Figure 1. Although the circuit is shown to operate at 12 V (DC), any voltage between 5 and 15 V can be used with an appropriate relay. The npn transistor, Q<sub>1</sub>, was a 2N2222 or similar and the 12-V relay must have contacts rated at 120–220 V. The AC operated relay can be any type but must conform to the house voltage since it is actuated by the 24-hr wall timer. A normally open leaf-switch with a

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**Figure 1.** Schematic circuit of the fraction collector timer (with the 24-hr wall timer), test-tube position sensor and motor turn-on interval control. Integrated circuits (IC) 1 and 2 have +12 V (DC) applied to pins 14 and 16, respectively, and ground at pins 7 and 8, respectively. All resistors are 1/4 W. CMOS integrated circuits should not be inserted into their sockets until all connections have been made.

roller is used to sense the position of test-tubes on the fraction collector.

The general plan of the fraction collector disk, test-tube positioning, gear motor and leaf-switch can be found in [Figure 4 in Ref. (1)]. The leaf-switch feedback circuit will accommodate many different collector dimensions and gear motor speeds. Gear motors of 2 rpm down to about 0.25 rpm will work, but slower speeds are better for larger numbers of tubes and larger disks. For proper operation it is crucial that the distances between the disk axle and the ring(s) of test-tubes be equal, otherwise the leaf-switch will not function consistently. However, spacing between tubes around the disk periphery does not have to be precise because of the feedback circuit.

#### *Fraction collector operation*

A 24-hr wall timer that plugs into an AC outlet is used to actuate an AC relay that connects pin 2 of IC1A to ground. This causes the CMOS-4093 NAND Schmitt trigger to go "high" at pin 3 and "clocks" the 5-stage Johnson decade counter (CMOS-4017) which causes its pin 3 to go "low" (turning off the LED) and pin 2 to go high. Both inputs of IC1D (pins 8 and 9) thus go high causing output pin 10 and input pin 12 to be high. Assuming the leaf-switch is closed when in

contact with a test-tube during the non-rotating period (1 hr in this case), then input pins 5 and 6 of IC1B and the reset pin 15 of IC2 are connected to ground. This allows the counter (IC2) to advance and also causes output pin 4 of IC1B to be high. Thus, inputs 13 and 12 of IC1C are high when the wall timer actuates the relay and this causes output pin 11 to go high and bias the npn transistor into conducting current and turning on the 12 V relay and second LED and also switches AC power to the gear motor.

As the motor turns the fraction collector, the test-tube moves away from the leaf-switch which opens and allows the inputs 5 and 6 of IC1B to go high as well as pin 15 of IC2 which resets the counter (pin 2 goes low and pin 3 goes high). Power to the motor continues, however, because the inputs to IC1C are both low causing pin 11 to remain high. Shortly thereafter, the next test-tube rotates into contact with the leaf-switch and pins 5, 6 and 15 go low which causes the inputs of IC1C both to go high and turns-off the gear motor (pin 11 goes low). The counter is reset and ready to advance upon the next relay pulse from the wall timer, approximately 1 hr later.

The fraction collector with 24 test-tubes was modified to collect moth eggs by encircling strip-chart recorder paper around the collector disk

head such that a vertical paper tube was constructed. The recorder paper, trimmed to 13 cm width, was taped to the two plexiglass disks of the collector head (45 cm diam.). Thus, discrete areas of the paper tube (about 5.8 cm strips) could be exposed sequentially to egg-laying females in hourly increments (controlled by the test-tube sensing circuit and wall timer).

#### *Oviposition rhythm*

*Agrotis segetum* were obtained from a laboratory culture based on insects originating from southern Sweden and Denmark. Larvae were reared on artificial diet (2) using potato instead of pea beans. The culture was maintained on a L:D 17:7 hr photoperiod at about 23–26°C.

Insects were separated by sex as pupae and placed in containers with 1–3 adults that were fed 5% honey solution for 1–2 days. Eight females and four males were then allowed to mate in a 5l bottle with paper towelling for two days inside a walk-in environmental chamber under L:D 16:8 hr photoperiod (about 2000 lx) with scotophase beginning at 2200 hr and constant 23.7°C and 55% r.h. Sucrose solution (5%) in sponge pads was available to moths during this time and for the remainder of the experiment.

Eggs were observed on the paper towelling beginning on the second day (July 18, 1985). The moths then were transferred to a plexiglass holding box in the form of an isocetes triangular wedge that was truncated on the apex (sides 9 × 16 cm, back 9 × 21 cm, and front 9 cm high × 4 cm wide). The front apex was open and appressed to within 1–2 mm of the rotating paper drum/fraction collector so that females could deposit eggs that would clear the sides of the box during rotation but not allow moths to escape. Eggs were scrapped off the paper and counted each day during times when no eggs were deposited (0700–1300).

#### Results and Discussion

The L:D regime was repeated for four days (Figure 2) with maximum egg production on the third day of oviposition. The existence of a circadian rhythm is indicated by the peak in egg

laying on the fifth cycle under constant illumination (Figure 2). The summary of egg laying during the four days of photoperiod is shown in Figure 3 with the mean time of laying, 2148, just preceding the onset of darkness.

Eggs deposited by the noctuid *A. segetum* were collected efficiently by the paper drum because the females normally glue their eggs, singly or in small groups, to host plants. The egg numbers collected represent a relative output since they were deposited throughout the inside of the holding box. Crevices and surface irregularities are often the preferred ovipositional sites in Lepidoptera (3), but initially all surfaces were smooth until eggs were deposited. The build-up of eggs on the plexiglass surfaces could have influenced the relative rates of oviposition on the paper drum. This is because the previously deposited eggs could have acted as physical stimuli inducing oviposition or, on the other hand, possibly as chemical deterrents that reduce oviposition to avoid competition. It is well known that moths are receptive to short-range olfactory stimuli from their host plants, and several noctuid species are influenced by specific host-plant compounds (4). Furthermore, there is evidence that long-term oviposition behaviour in some Lepidoptera is influenced by contact with host plants (5). In my experiments host plants were not present so it is not known what effect they might have on the oviposition rhythm.

The continued presence of males with the females also could have influenced the oviposition rhythm or egg output. In the noctuid *Earias fabia* Stoll, continuous male presence increased the egg output of the female (6). It is also possible that males could disrupt the oviposition behaviour with their repeated attempts to mate. Finally, the sucrose solution probably had an effect on, at least, egg output since Krishna *et al.* (6) found that *E. fabia* females produced many more eggs when fed sugar solutions. Female noctuid moths in nature are most certainly able to increase their egg output by feeding on nectar from flowers.

Löfstedt *et al.* (7) found that virgin *A. segetum* females had a "calling rhythm" of pheromone release which had a mean activity time of  $3.9 \pm 0.9$  hr ( $\pm$  s.d.) after scotophase began on a

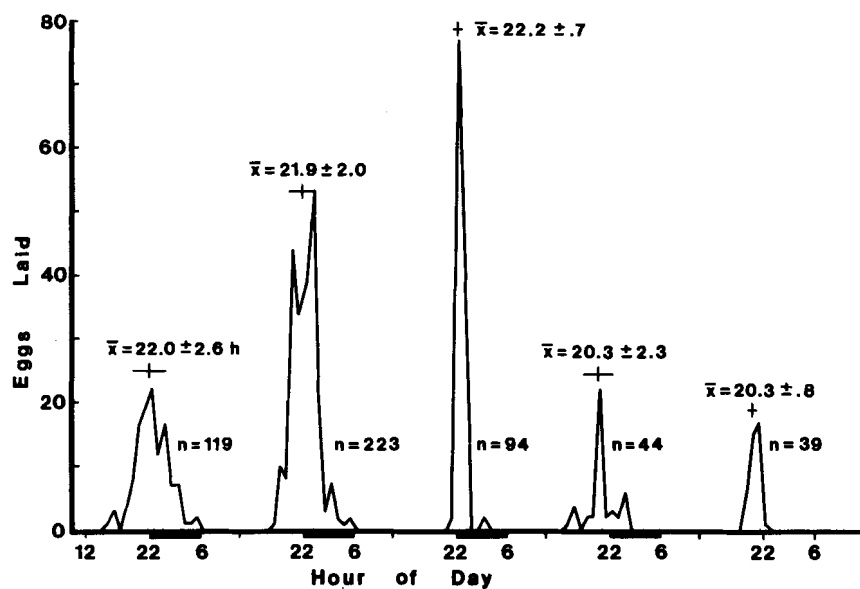


Figure 2. Eggs laid per hour on paper sections by 8 female *Agrotis segetum* beginning on the third night with 4 males. Moths were held in a small plastic chamber with a 4 cm wide  $\times$  9 cm high window appressed to a rotating paper cylinder inside an environmental chamber at constant 23.7°C and 55% r.h. and a scotophase of 8 hr as indicated. Average mean times ( $\bar{x}$ ) of egg laying  $\pm$  standard deviation ( $\pm$ s.d.) are shown with total eggs per day given by  $n$ .

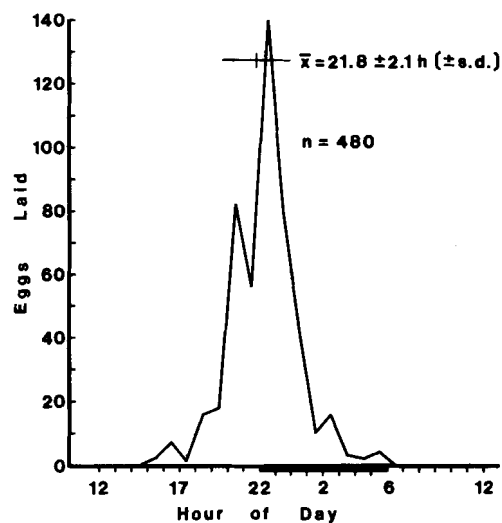


Figure 3. Total eggs laid per hour by *Agrotis segetum* over a 4 day period on a L:D cycle of 16:8 hr. Average mean time ( $\bar{x}$ ) of egg laying  $\pm$  standard deviation ( $\pm$ s.d.) and total eggs laid ( $n$ ); conditions as in Figure 2.

17L : 7D photoperiod. This rhythm appears to be different from the oviposition rhythm found in the present study which had a mean activity time 0.2 hr before scotophase on a similar photoperiod of 16L : 8D. The zeitgebers for these two rhythms may be different since it is probable that the oviposition rhythm must be timed by lights-on (16 hr earlier) while the calling rhythm may be timed by lights-off. The zeitgeber for the oviposition rhythm would probably not be at lights-off as this would require a phase angle of about 357°. The oviposition and calling rhythms of *A. segetum* are similar to those of another noctuid, *Leucania separata* on a 16L : 8D cycle (8). These rhythms may be independent (i.e. different oscillators) for it is known that the circadian rhythms of eclosion and activity in *Drosophila* are functionally distinct (9).

The adaptive significance of a certain phase angle between the LD zeitgeber and the circadian acrophase in a particular activity can often be generally understood. Oviposition of the diurnal grape root borer moth, *Vitacea polistiformis*, begins from 0800 to 0900 in the morning and continues only during the daylight. Furthermore, its mating and calling rhythms are also during the afternoon (10). This is not surprising as the moth uses aposematic coloration to mimic wasps and undoubtedly derives reproductive benefits by increasing its visual apparency. On the other hand, noctuids are drab and camouflaged to escape detection during the day while nocturnal activities increase their fitness.

One of the basic tenets of biology is that a

more simple theory (one clock) is preferred over more complex ones (two clocks) if they equally explain the observation. Evolutionary theory also presumes that a point can be reached where the costs of increasing mechanistic complexity are greater than the benefits on reproductive fitness and thus there would be no selection pressure to evolve further complexity. However, there are several reasons why oviposition and calling (mating) rhythms in *A. segetum* and other noctuids may be temporally distinct. The most obvious is that these different activities are often mutually exclusive; but calling and then oviposition are done at different times in the life cycle so in theory they could have the same rhythm. Feeding and oviposition activity would most effectively be done at dusk or early evening when some light remained to help in food-plant location (both flowers and larval hosts). Also, in terms of optimal foraging and energy consumption it would be best to undertake feeding and oviposition during the warmest part of the crepuscular/nocturnal period when energy requirements to maintain flight temperatures are least [noctuids thermoregulate by vibrating the thoracic wing muscles (11)]. Calling activity could be done later in the night when light is not essential and when colder temperatures have little effect on energy consumption because the females must remain sedentary and wait for males. On the other hand, males at this time must expend maximal energy when flying to calling females; but then they have little choice and besides they do not have to lay eggs [cf. (12)].

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