

**Biology and Rearing Methods of the  
New Guinea Sugarcane Weevil,  
*Rhabdoscelus obscurus***

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# Biology and Rearing Methods of the New Guinea Sugarcane Weevil, *Rhabdoscelus obscurus*<sup>1</sup>

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## INTRODUCTION

The New Guinea sugarcane weevil, *Rhabdoscelus obscurus* (Boisduval), belongs to the family Curculionidae and subfamily Calandrinae of the order Coleoptera. This weevil was first described as *Calandra obscura* from the specimens collected from New Ireland in the Pacific by Boisduval (1835). The weevil has been subsequently known as *Sphenophorus insularis* Boheman, 1859; *S. nidicollis* Kirsh, 1877; *S. promissus* Pascoe, 1885; *S. interruptocostatus* Schaufuss, 1885; and *Rhabdocnemis obscurus* (Boisduval) (Marshall, 1921, 1931; Zimmerman, 1941).

Since the generic name *Rhabdocnemis* Faust, 1894 was preoccupied by Pomel's genus of sponge, Marshall (1943) proposed the generic name *Rhabdoscelus* which was then accepted, and is now valid. The current name of the New Guinea sugarcane weevil thus becomes *Rhabdoscelus obscurus* (Boisduval) (Zimmerman, 1945).

According to Muir and Swezey (1916), and Timberlake (1927), the original habitat of this weevil was New Guinea and the adjoining islands. The weevil was probably introduced into Hawaii along with two varieties of cane, Cuban and Lahaina, brought from Tahiti by Edwards in 1854 (Baldwin, 1882; Swezey, 1954). The cane damaged by this weevil was first observed in Lahaina, Maui, as early as 1865 by Baldwin (Muir & Swezey, 1916).

Host-plant records of this weevil include: cabbage palm, *Sabal palmetto* (Walt.) Lodd. and Schult. f. and wine palm, *Caryota urens* L. (Terry, 1907); sago palm, *Metroxylon sagu* Rottb., and other palms (Muir & Swezey, 1916); pritchardia palm, *Pritchardia martii* (H. Wendl.); royal palm, *Roystonea elata* (Bart.); betel-nut palm, *Areca catechu* L.; traveller's tree, *Ravenula madagascariensis* J.F. Gmel.; solitaire palm, *Ptychosperma elegans* (R.BR.) Bl.; and Alexandra palm, *P. alexandrae* F. Muell. (Lepisme, 1947); and

<sup>1</sup> This technical bulletin is part of a thesis submitted by the senior author to the Graduate Division of the University of Hawaii in partial fulfillment of the requirements for the Master of Science degree.

coconut, *Cocos nucifera* L. (Swezey, 1954). Other nonpalm host-plant records include: banana, *Musa* spp. (Muir & Swezey, 1916); corn, *Zea mays* L. (Muir & Swezey, 1916; Zimmerman, 1941; and Lepisme, 1947); papaya, *Carica papaya* L. (Zimmerman, 1941); and bird-of-paradise, *Strelitzia reginae* Banks (Pemberton, 1951).

In 1910, the New Guinea tachinid fly, *Lixophaga sphenophori* (Ville-neuve), was introduced from Amboina and Ceram by Muir for the biological control of this weevil (Williams, 1931). According to Pemberton (1948) and Sweetman (1958), this tachinid parasite became established and has given successful control.

The general life cycle of *R. obscurus* has been reported by Koebele (1900), Terry (1907), and Muir & Swezey (1916). Eggs are laid on the internodes of the sugarcane (*Saccharum officinarum* L.) plant. When the eggs hatch, the larvae bore into the stalk and usually downward to the base. When fully grown, they pupate within the tunnels. The adults later emerge from the stalk through emergence holes made by the larvae prior to pupation.

Recently it has been reported that for unknown reasons *R. obscurus* has increased to such numbers that it has caused considerable damage to sugarcane in certain localities. This situation has become a direct concern to some plantations. Cooperative investigations are now being conducted by the Hawaiian Sugar Planters' Association, the State of Hawaii Department of Agriculture, and the Hawaii Agricultural Experiment Station, University of Hawaii. The present contribution, concerned with general biology and rearing methods of *R. obscurus*, constitutes one aspect of these investigations.

## GENERAL METHODS

The present investigation is concerned with the biology and rearing of *R. obscurus* on artificial and semi-artificial media. Methods used in investigations of this nature are somewhat diversified, since no single material or method would be applicable to all aspects. Therefore, only the general methods will be described here; others will be dealt with in the appropriate sections.

The temperature and relative humidity of the laboratory in which these studies were carried out were measured by a hygrothermograph. The relative humidity within the humidity jars in which the adults were kept was measured by Honeywell Humidity and Temperature Meter. These measurements are plotted on Fig. 1.

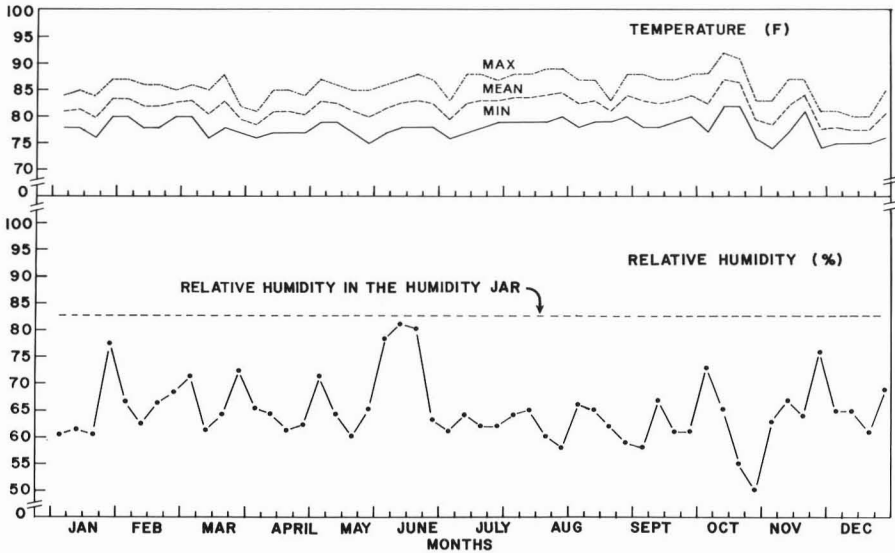


FIG. 1. Temperature and relative humidity data of the laboratory where all investigations were carried out. The relative humidity within the humidity jars is also indicated.

### Source of weevils

Field-collected weevils were utilized for establishing laboratory stock cultures. They were collected on Oahu from experimental plots of the Experiment Station, Hawaiian Sugar Planters' Association, at Honolulu and Kunia, and on Kauai, principally from the Grove Farm Plantation.

### Humidity jar for adults

This is a multipurpose jar (Fig. 2) designed for keeping adults in captivity and for holding pupal cocoons until adults emerge. Since a high humidity is of vital importance to the weevils, the jar was designed in such a way that satisfactory humidity and sanitary conditions could be maintained with a minimum of labor.

A 1-gallon jar with an opening about 12.0 cm in diameter was modified for use as the humidity jar. This jar was divided into two compartments by a layer of plaster of Paris about 1.5 cm thick situated about 10.0 cm above the bottom of the jar. The plaster of Paris was cast with a hole which made it possible to pour in or take out water from the bottom compartment.

After pouring water into the bottom compartment, the hole was plugged with a cork stopper. The water kept the layer of plaster of Paris contin-

uously damp, so that a uniformly high humidity was maintained in the upper compartment of the jar. To prevent microbial growth in the water in the jar, 25 ml of an 18 percent copper sulfate solution was added per liter of water. With this procedure, a relative humidity at 3.0 cm above the layer of plaster of Paris in this type of jar was found to be automatically maintained at  $80 \pm 5$  percent.

The method of placing the plaster of Paris septum in the jar needs explanation. The septum was made by pouring coarse sand into the jar to a level of about 8.5 cm from the bottom. An 8-dram shell vial, 2.5 cm in diameter wrapped in polyethylene sheet, was then placed vertically into the sand. Plaster of Paris mixed with water was poured over the sand to form a uniform layer about 1.5 cm in thickness and allowed to harden, then the vial was removed and the sand poured out.

Every 4 weeks, the jars were cleaned and sterilized with a 0.05 percent Clorox solution. All moribund and dead weevils found in the jars were removed, to prevent fungus infections commonly associated with moribund and dead weevils.

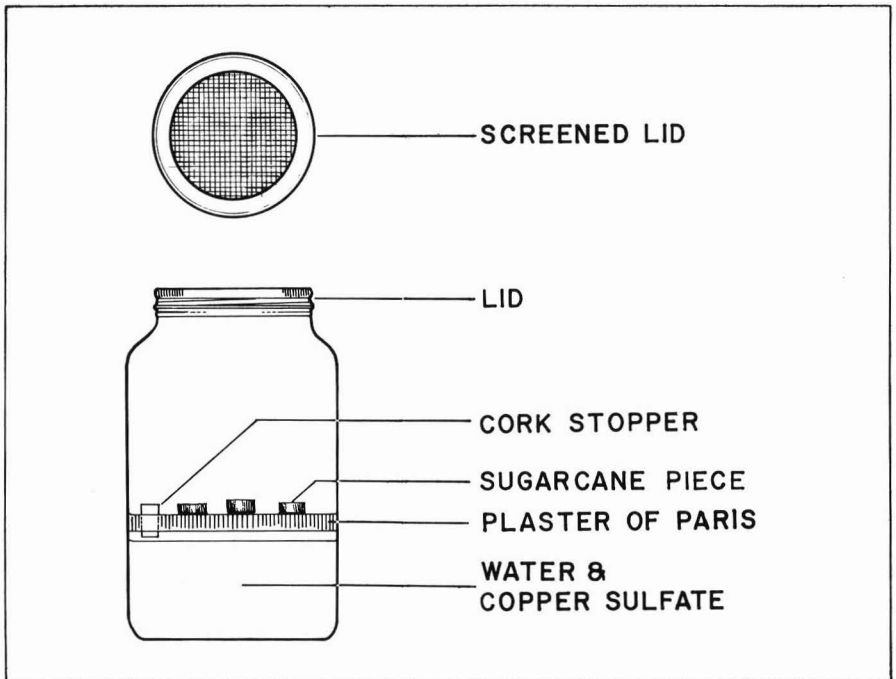


FIG. 2. Humidity jar in which the adults were kept captive.

### **Storage of sugarcane stalks**

Sugarcane stalks obtained from the field were first washed and cut into uniform pieces about 3.0 cm long. They were then rinsed in tap water, placed in plastic bags, and kept in the refrigerator. By this method, it was possible to store the cane for up to 2 months. However, the storage life of the cut stalks was later prolonged by wet refrigeration. Stalks were immersed in a 0.05 percent Clorox solution which was kept in the refrigerator. By changing the Clorox solution every month, the cut pieces may be stored for as long as 6 months in perfect condition. Sugarcane pieces stored by this method were first rinsed in tap water before using them. Such treatment was not found to be harmful to the weevils.

### **Feeding adult weevils**

Adult weevils were allowed to feed on two or three pieces of sugarcane stalks placed in each jar. The adults fed readily on the cut ends. An attempt was made to increase egg laying by placing enzymatic yeast hydrolysate, which contains a number of amino acids, on the cut ends of the sugarcane pieces. Since there was only a slight increase in eggs produced, this practice was not continued.

### **Collecting eggs**

A number of egg collecting methods were tried. The best method was to expose the cane pieces to the adults. After 2 to 3 days, the eggs were dissected out of the cut ends of the cane pieces.

### **Rearing of larvae**

To facilitate the study of the biology of the weevil, the larvae were reared on a shredded coconut-husk medium instead of sugarcane. The preparation of this medium is described in the section on studies of rearing methods, page 39. The rearing of larvae on other media is also discussed in that same section.

## **IMMATURE STAGES**

### **Incubation period and fertility**

Newly laid eggs of *R. obscurus* are smooth, translucent white, elongated, ellipsoidal, and slightly curved with rounded ends (Fig. 5A), and enclosed in a thick chorion. With age, the color changes from translucent white to opaque white. In this study, the length ranged from 1.3 to 1.7 mm,



TABLE I. Incubation period and egg fertility of *R. obscurus*, under laboratory conditions

Trial no.	Total no. of eggs per batch	Mean incubation period (days)	Fertility (percent)
1	18	4.9	50.0
2	24	5.2	66.7
3	12	4.9	75.0
4	32	4.3	50.0
5	86	4.6	36.0
6	145	5.2	41.4
7	74	4.0	27.0
8	45	4.3	44.4
9	34	4.5	55.9
10	121	4.6	51.2
11	19	4.7	36.8
12	22	4.3	63.6
13	38	4.5	63.2
14	16	4.8	37.5
15	35	4.8	65.7
16	54	5.3	40.7
17	62	4.0	38.7
18	24	4.8	33.3
19	82	4.3	48.8
20	74	4.3	48.6
Mean		4.6 ± 0.4	48.7 ± 12.9
Range		4.0 — 5.3	27.0 — 75.0

with a mean of  $1.45 \pm 0.12$  mm; and the width, 0.4 to 0.6 mm, with a mean of  $0.54 \pm 0.02$  mm.

An investigation was carried out in the laboratory to determine the incubation period of the eggs of *R. obscurus*. Fifty humidity jars were used, each containing 20 pairs of weevils and 2 pieces of cane for oviposition. Cane pieces were exposed to the adults for 24 hours and then they were removed and the eggs dissected out. Eggs obtained were sterilized by immersing them in an 18 percent copper sulfate solution for 3 to 5 minutes, washed with sterile distilled water (Nettle & Betz, 1966), and then placed on wet filter paper in petri dishes. Care was taken to keep the filter paper moist by adding sterile distilled water periodically. A total of 1107 eggs in 20 batches of eggs, ranging from 12 to 145 eggs per batch, were used in the study. The number of eggs that hatched on each day was recorded and from these data the incubation period and percent fertility were computed.

Temperature conditions during the experiment are shown in Fig. 1, page 9. Humidity conditions were approximately 90 to 100 percent because the filter paper in the petri dishes was kept moist.

Data, shown in Table 1, indicated that the incubation period ranged from 3 to 7 days with a mean of  $4.6 \pm 0.4$  days. Egg fertility ranged from 27.0 to 75.0 percent with a mean of  $48.7 \pm 12.9$  percent.

### Number of larval instars

Koebele (1900) reported that it was very difficult to ascertain the number of moults of the larvae. He estimated it at six. Terry (1907) admitted that he and Koebele failed to determine definitely the number of larval moults, because the feeding habits made this practically impossible (Muir & Swezey, 1916). Since then, no attempt has been made to determine the exact number of larval instars of *R. obscurus*.

An investigation on the number of larval instars was attempted as part of the present study, utilizing two methods. Both methods involved measurements of the width of head capsule. In one method, successive measurements and observations were made on the same series of larvae throughout their development. In the other, measurements were taken on the head capsules of larvae sampled in the field and in laboratory cultures. Instars were identified by the frequency distribution of the head-capsule measurements. In the present study, the former method is referred to as the "direct" method and the latter as the "indirect" method.

In the direct method, the larvae were reared on a shredded coconut-husk medium. Two newly hatched larvae were placed on top of the medium in each plastic box. The larvae thus inoculated were dissected out for head-capsule measurement, every 3 days during the first 2 weeks and once each week thereafter. All measurements were made by an ocular micrometer. A total of 60 larvae were used in this study, but only 37 of them completed development.

In the indirect method, a large series of field-collected and laboratory-reared larvae and prepupae were killed in KAAD mixture and preserved in 70 percent ethyl alcohol. Head-capsule measurements were made on the preserved samples, using an ocular micrometer.

It was evident from the results obtained by the direct method that there were 6 larval instars, excluding the prepupal stage. The mean width of the head capsule of 37 larvae was computed for each instar, with the consecutive geometric progression averaging 1.46 (Table 2).

Dyar (1890) observed the head width of 28 species of Lepidoptera and found that it followed a regular geometric progression in successive instars.

TABLE 2. Observed and theoretical width of the head capsule of the larval instars of *R. obscurus* ( $n = 37$ )

Instar	Observed width of head capsule (mm)	Geometric progression	Theoretical width of head capsule (mm)	$\chi^2$
1st	0.37	0.58/0.37 = 1.59	0.37	0
2nd	0.58	0.79/0.58 = 1.36	0.37 × 1.46 = 0.54	.003
3rd	0.79	1.17/0.79 = 1.47	0.54 × 1.46 = 0.79	0
4th	1.17	1.61/1.17 = 1.38	0.79 × 1.46 = 1.15	.0004
5th	1.61	2.52/1.61 = 1.56	1.15 × 1.46 = 1.68	.0029
6th	2.52	3.56/2.52 = 1.41	1.68 × 1.46 = 2.45	.002
Prepupa	3.56		2.45 × 1.46 = 3.58	.0001
Mean geometric progression = 1.46				.0084 ( $P > .99$ )

This finding has become known as Dyar's Law; it applies also to saw-fly larvae and Collembola (Imms, 1960). Since it has not been applied extensively to Coleoptera, the data from this investigation were subjected to the chi-square test to determine how well they conformed to Dyar's Law, using the method of Snedecor & Cochran (1967).

As shown in Table 2, the theoretical width of the head capsule was computed from the mean geometric progression of 1.46. The computed pooled chi-square value of .0084,  $d.f. = 6$ , was nonsignificant ( $P > 0.99$ ).

Figure 3 shows the straight-line relationship between larval instars including the prepupal age and width of the head capsule expressed in logarithms. With this relationship it would be possible to estimate the larval instars by measuring the width of the head capsule.

The data on head-capsule measurements obtained by the indirect method are presented in Fig. 4, page 16. The number of larval instars based on the frequency distribution of the head width is ambiguous, especially among the young larvae. Only 4 peaks, occurring at about 0.4, 1.9, 2.9, and 3.6 mm, are distinct. Excluding the prepupae, which were also measured, the results seem to show only 3 larval instars. However, close examination reveals 4 peaks between 0.3 and 1.6 mm. When these peaks are included, there would appear to be 6 larval instars. The close proximity of the peaks of the first 4 instars could be explained by the differential rate of development of the larvae. Growth at each moult from the 1st

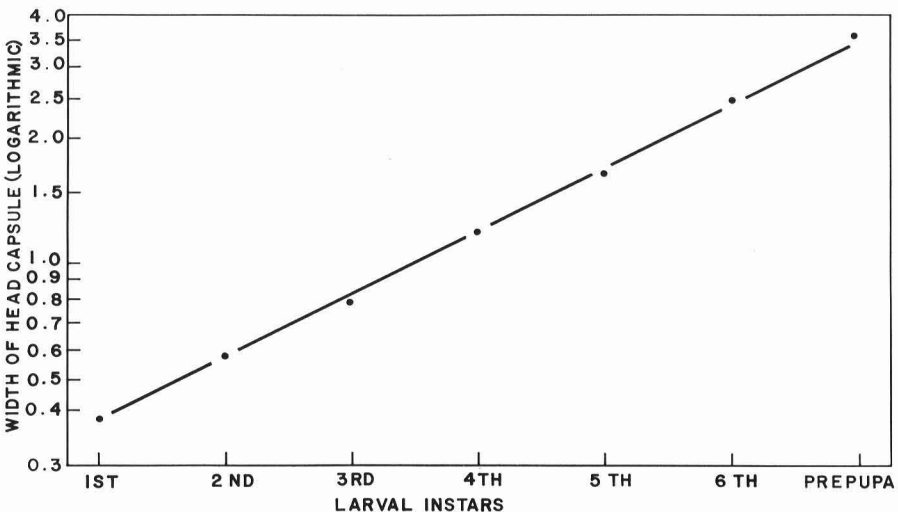


FIG. 3. The relationship between the width of the head capsule and the larval instars and prepupa of *R. obscurus*.

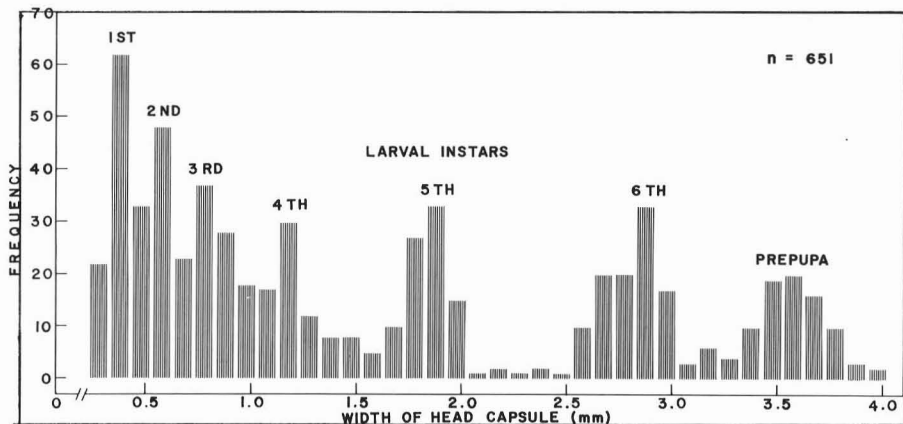


FIG. 4. Frequency distribution of the width of the head capsule of *R. obscurus* larvae and prepupae sampled in the field and laboratory cultures, according to the indirect method.

through the 4th instars occurs in small increments, while growth of the later instars occurs in large increments. Therefore, in the early instars, the peaks tend to be close together; in the later instars the peaks tend to be farther apart.

### Larvae and larval period

The larva of *R. obscurus* is typical of a calandrine larva (Fig. 5 B,C). The head is entirely exerted, rounded, highly sclerotized, with light brown epicranial areas and intense dark brown coloration on the extremities of the mouthparts. The mandibles are highly sclerotized. The cervical shield is sparingly sclerotized. The larva is apodous with the body somewhat cyphosomatic or crookneck-shaped, fleshy with two or more plicae on each segment on the dorsum. The mid-abdominal segments, especially the fifth, are usually much larger than either the thoracic or the caudal segments.

The body is white and transparent to such an extent that ingested food is visible. There are sparse short stiff setae on the whole body with the exception of the last few abdominal segments, which have longer stiff setae situated on the chalazae. The function of these setae is probably for movement within the tunnel made by the larvae.

Detailed descriptions of the larvae may be found in Riley (1888), Terry (1907), and Muir & Swezey (1916).

Earlier studies of the duration of the larval stage of *R. obscurus* were those of Koebele (1900) and Terry (1907). Koebele (1900) reported that

the larval period was about 7 weeks depending upon the condition of food, while Terry (1907) reported that it ranged from 76 to 91 days with an average of 81 days. The methods that they used and the conditions under which their experiments were conducted were not given. Thus it is not possible to compare the results of the present study with those of the past.

In the present study, the shredded coconut-husk medium was used. This soft medium made it possible to dissect out and observe the larvae without damage. Newly emerged larvae were placed on the medium and observed daily for moulting throughout the developmental period. By observing the moulted head capsule and the changes in the color of the head capsule, it was possible to determine the duration of each of the larval instars.

Data thus obtained were averaged for each larval stadium and for the prepupal, and pupal periods. See Table 3. The mean duration of the larval period was  $54.3 \pm 3.7$  days. The observed mean durations of the 1st through 6th larval instars were 3.0, 5.4, 6.9, 11.5, 13.7, and 13.8 days respectively.

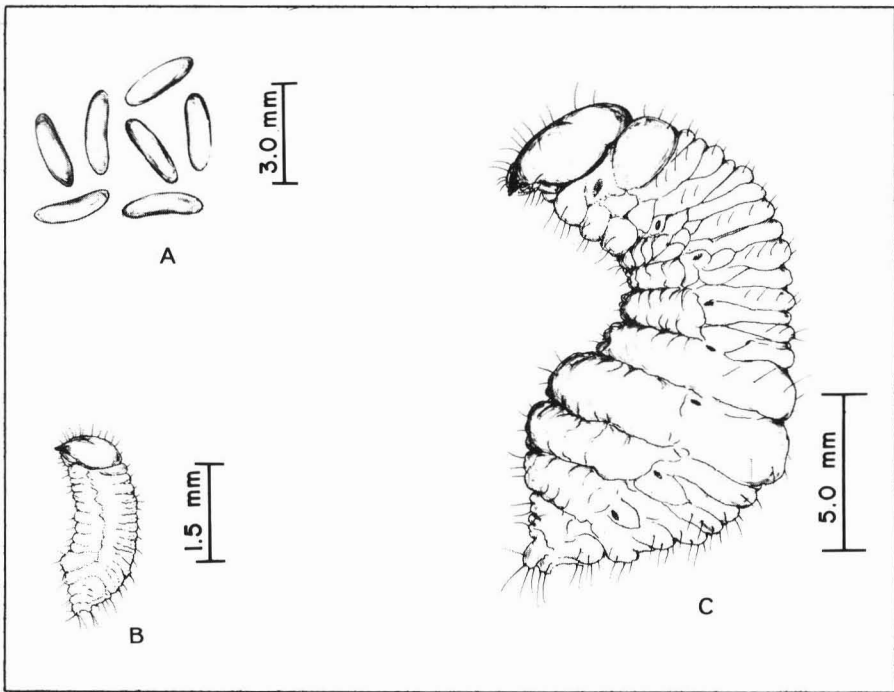


FIG. 5. Eggs and larvae of *R. obscurus*. A, Eggs; B, newly hatched larva; C, fourth instar larva.

TABLE 3. The duration of the various developmental stages of *R. obscurus* (rearing medium shredded coconut husk) ( $n = 37$ )

Stage of development	Duration (days)		Range
	Mean*	Cumulative	
Egg	4.6 ± 0.4	—	3- 7
Larva			
1st instar	3.0 ± 0.3	3.0	2- 4
2nd instar	5.4 ± 1.0	8.4	4- 6
3rd instar	6.9 ± 1.2	15.3	5- 9
4th instar	11.5 ± 1.9	26.8	9-15
5th instar	13.7 ± 2.3	40.5	10-19
6th instar	13.8 ± 3.7	54.3	10-18
Total	54.3 ± 3.7		45-61
Prepupa	7.2 ± 1.5	61.5	6- 9
Pupa	20.9 ± 2.8	82.4	17-25
Larva to pupa	79.3 ± 5.0	82.4	67-88

\* Mean ± standard deviation.

The relationship between the observed cumulative duration data and the corresponding developmental stage indicated that the duration increased linearly with each stage (Fig. 6). The greatest deviation from linearity occurred at the pupal stage. This deviation can probably be explained by a habit of the teneral adults. They do not leave the cocoon promptly after emerging from the pupal stage. Therefore, the actual duration of the pupal stage was actually shorter than the measured duration.

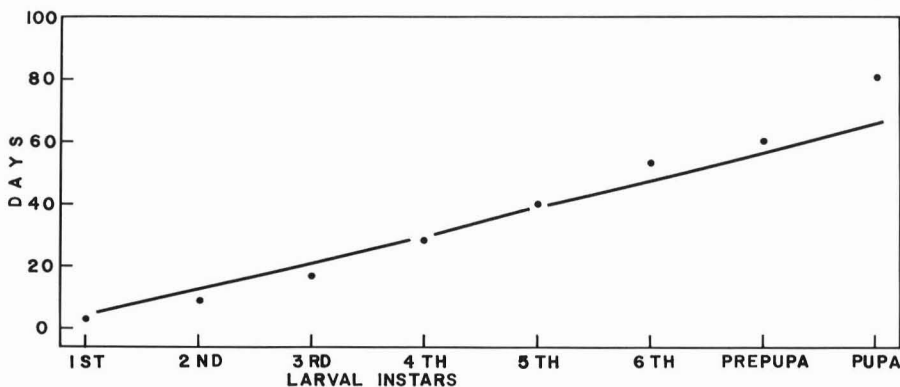


FIG. 6. Cumulative time and the respective developmental stages of *R. obscurus*.

### Larval mortality

During the course of these studies, it was observed that young larvae, especially the first and second instars, died from unknown causes. Inherent weakness of the larvae, unsuitable cane varieties, unfavorable environmental conditions, mechanical injuries by handling, cannibalism, or diseases might have been involved. These mortality factors, as well as others, might be responsible for the mortality observed under the laboratory and field conditions. Field examination of cane stalks infested by the borers in the field occasionally showed moribund and dead larvae.

Studies were carried out to obtain data on the extent of mortality among the young larvae. The eggs were first obtained by exposing pieces of cane to the adults in the humidity jars. After the eggs hatched, 2 young larvae were transferred to one cut end of each cane piece with a fine camel hair brush. The cane pieces were then placed in a plastic container matted with moist paper towels. Water was added periodically, to keep the towels wet. After 1 week, the cane pieces were cut open and the number of living larvae in each cane piece recorded. By this time all of the larvae were either in the 2nd or 3rd instars. There were 10 replications, each with 20 pieces of cane, in this test.

The data obtained from this investigation revealed that the survival of early instar larvae of *R. obscurus* ranged from 42.5 to 97.5 percent. The mean survival was  $60.3 \pm 16.9$  percent.

### Prepupa, pupa and pupation

After passing the 6th stadium, the full-grown larva transforms to a prepupa (Fig. 7A). Its general body shape differs from that of the 6th instar larva by the absence of the posterior enlargement. The body becomes shortened and assumes a more cylindrical shape than that of the 6th instar larva. The mean prepupal period was  $7.2 \pm 1.5$  days, with a range of 6 to 9 days.

Pupation usually takes place within the cocoon which is found in the tunnel made by the larva. It usually takes the prepupa 24 to 48 hours to transform into the yellowish white exarate pupa (Fig. 7B). The newly formed pupa is covered with a transparent membrane which is gradually replaced by another ensheathment. Pupation takes place within a spirally woven fibrous cocoon (Fig. 7C).

Terry (1907) observed that the head of the pupa in the cocoon was oriented downward within the vertically standing cane stalks. In the laboratory, it was noted that the orientation of the pupa varied with the containers. In an artificial medium placed in a shell vial, the position of



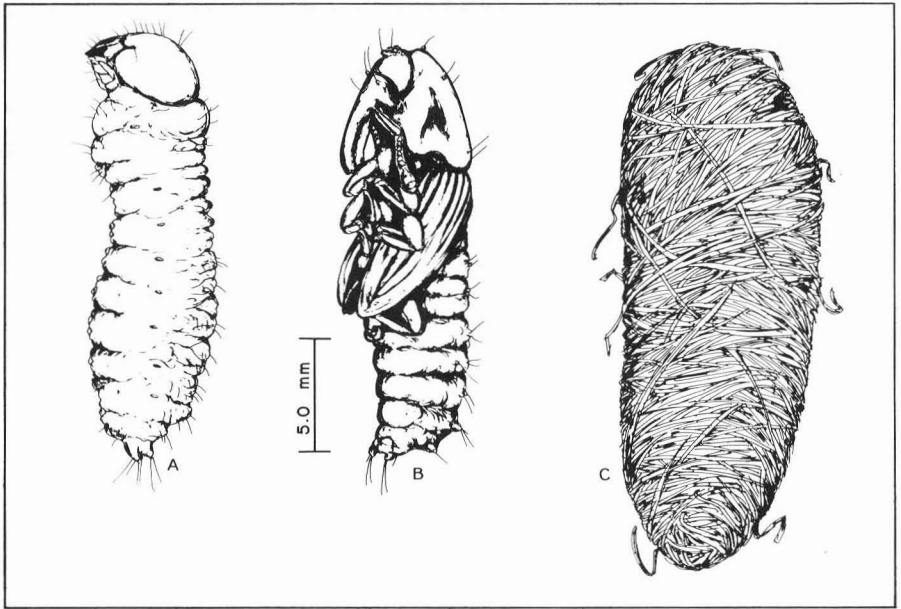


FIG. 7. The late developmental stages of *R. obscurus*. A, Prepupa; B, pupa; C, cocoon.

the pupa was vertical with the head oriented downward; when the medium was placed in square plastic containers, the position of the pupa was horizontal.

The cocoon is a mass of fibers spirally woven into an elongated oval structure with a cavity in which pupation takes place. The actual spinning of the cocoon was not observed, but it appeared that it was woven by the prepupa. Observations made through a transparent plastic box with rearing medium revealed that the prepupa tends to roll itself spirally by muscular movement. It sometimes moves back and forth in the tunnel. It is probably through these movements that the prepupa spins and shapes the fibers into a cocoon before pupation. It is not only the cane fibers that the prepupa uses to build the cocoon as reported by Terry (1907). The frass, masticated and left in the tunnel, is also utilized in making the cocoon.

The emergence of the adult from the cocoon is also incompletely known. When the adult emerges from the pupal stage, it cuts the ensheathed membrane by the movement of the appendages. However, the adult does not emerge from the cocoon immediately but remains inactive within it for a considerable period. Upon emergence from the cocoon, most of the adults are light in color while some are dark.

The actual duration of the pupal period of the weevil is obscure. The pupal period, the period between the end of the prepupal stage and the emergence of the adult from the pupa within the cocoon, was  $9 \pm 2$  days. The adult remains within the cocoon for an additional  $12 \pm 2$  days, which is referred to as the postpupal period. The mean apparent pupal period, including the pupal and the postpupal period, was  $20.9 \pm 2.8$  days, with a range of 17 to 25 days.

### ADULT STAGE

#### Description of weevil

The adult of *R. obscurus* is a weevil of medium size (Fig. 8). The length of the body is  $10.0 \pm 3.0$  mm, and the width,  $3.5 \pm 1.1$  mm. The oblong body with a protruding rostrum and well developed prothorax are typical of the curculionid subfamily Calandrinae. The anterior end of the rostrum bears the sclerotized mandibles. The dorsal body coloration is predominantly brown with a lighter shade of brown on the prothorax. The coloration of the elytra varies considerably, but generally has lateral and central dark brown patches. The elytra are well developed with longitudinal grooves or striae. The hind wings are membranous and strongly developed. The tarsal formula is 4:4:4.

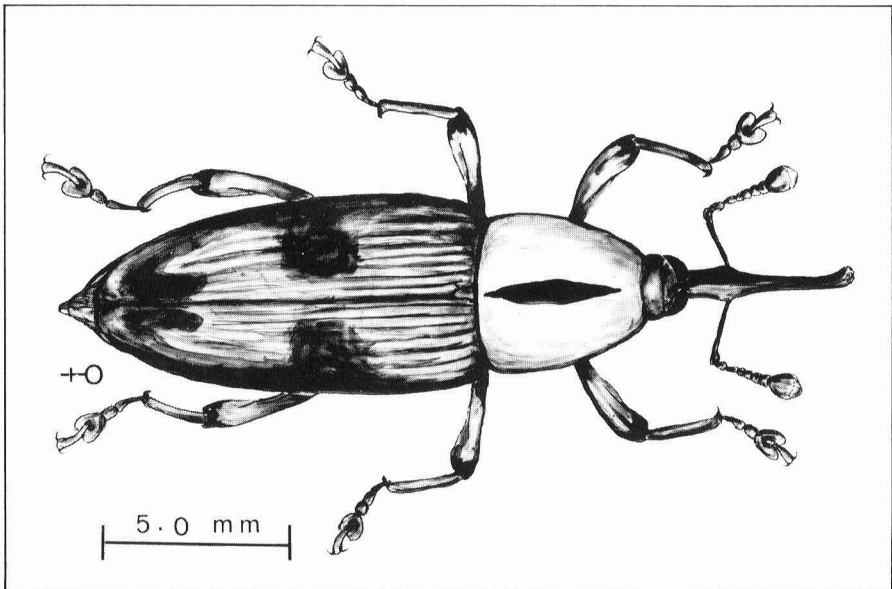


FIG. 8. Adult female of *R. obscurus*. (Group II, type A; see Fig. 19.)

A detailed description of *R. obscurus* was given by Boisduval (1835) as *Calandra obscura*, and Riley (1888) as *Sphenophorus obscurus* Boisd. The weevil was also described to a certain extent by Perkins (1899), Terry (1907), Muir & Swezey (1916), Dammerman (1919, 1929), Marshall (1931), Williams (1931), Zimmerman (1941), and Lepisme (1947). In these references the descriptions are given under either one or another of the generic names, *Calandra*, *Sphenophorus*, and *Rhabdocnemis*.

### Sexual dimorphism

In the Curculionidae there are comparatively few instances of close morphological similarity between sexes, and there should be no difficulty in distinguishing them in the adult stage. In some calandrine genera, the male may be distinguished either by a row of tubercles, or a crest of hairs on its dorsal surface, or by a dense fringe of ventrally located long hairs on the rostrum (Marshall, 1916). In the case of *R. obscurus*, two distinct

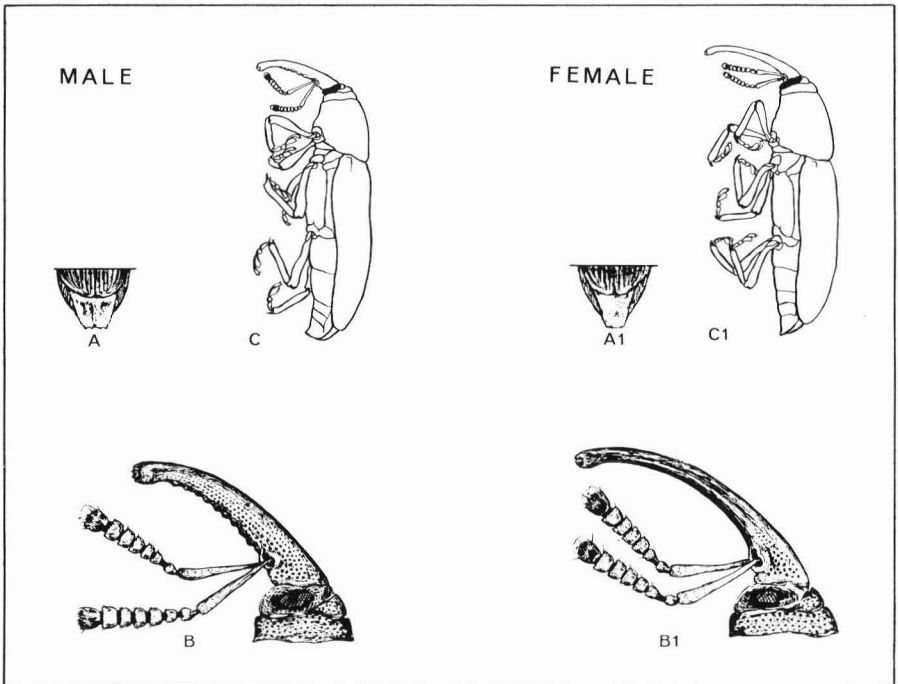


FIG. 9. The morphological characteristics of the sexes of *R. obscurus*. A, Male pygidium; A1, female pygidium; B, male rostrum; B1, female rostrum; C, male; C1, female.

morphological characteristics are present and they may be employed in distinguishing the sexes. As shown in Fig. 9 B,B1, the rostrum of the male is shorter, less curved, and more robust than that of the female. It is also ventrally serrated with a double row of highly sclerotized tubercles, varying in number from 5 to 8 pairs. In the female, the rostrum is finely tapered with no ventral serration.

Another morphological difference between the sexes may be found in the last abdominal tergite or the pygidium which usually protrudes slightly beyond the tip of the elytra in both sexes. In the male, the pygidium is blunt; in the female, it is somewhat pointed (Fig. 9 A,A1).

In the living adults, the male and female can be most readily distinguished by the shape and serration of the rostrum. The sexes cannot be recognized by the size alone for there is no significant difference in the overall body dimensions.

### Sex ratio

The sex ratio of *R. obscurus* obtained in the present investigation did not agree with the 7:3 ratio reported by Koebele (1899), or the 3:2 ratio reported by Terry (1907).

To determine the sex ratio of *R. obscurus*, 3241 weevils were collected in traps baited with split canes and were examined. In this collection there were 1859 males and 1382 females, or a male to female sex ratio of 4:3. This result was subjected to a chi-square test with the hypothesis that the sex ratio was 1:1. The computed chi-square value of 71.6 was not consistent with this hypothesis ( $P < 0.005$ ). The same test was again used to test the hypothesis of a 4:3 ratio. A chi-square value of 1.056, which was consistent with the 4:3 hypothesis, was obtained ( $P > 0.50$ ).

### Reproductive system

The earliest accounts on the reproduction of *R. obscurus* were those of Koebele (1899, 1900), and Girault (1914). Koebele (1899) observed that field-collected females when kept in the laboratory without males produced fertile eggs daily for 5 months. Girault (1914) also noted that some field-collected females laid fertile eggs, when isolated from males, for about 4 months. These field-collected females could have been predated and the sperm was stored in the spermatheca. It appears, therefore, that the sperm is retained and fertilization continues for long periods after mating.

To obtain a clear understanding of the reproductive system of *R. obscurus*, the structure of the reproductive organs was studied. Newly emerged as well as approximately 15-day-old males were dissected and

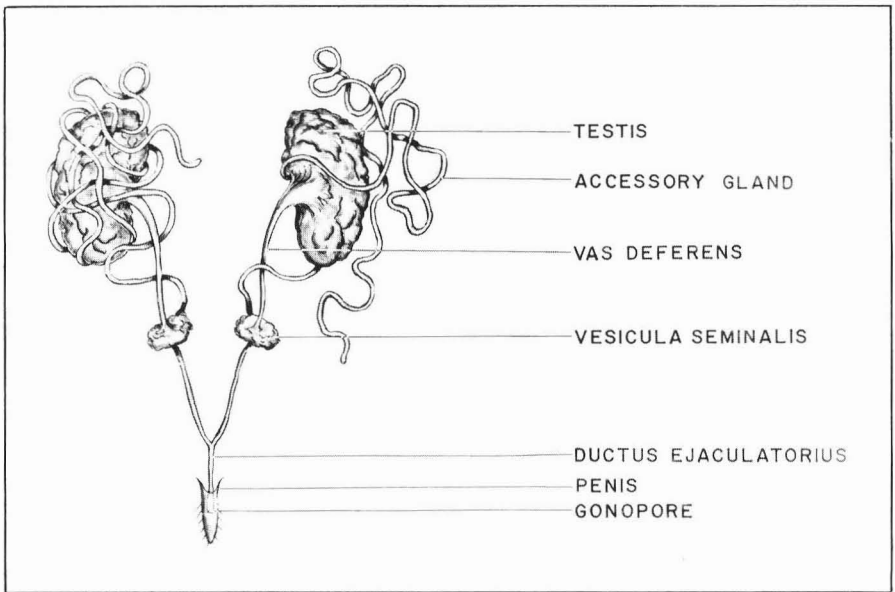


FIG. 10. Male reproductive system of *R. obscurus*.

studied. Both newly emerged and non-gravid females were also studied in the same manner.

The male reproductive system of *R. obscurus* (Fig. 10) consists of a pair of kidney-shaped *testes*, a pair of lateral ducts, or *vasa deferentia*, and a median ectodermal tube, or *ductus ejaculatorius*. There is an enlargement of a section of the *vas deferens* which is the seminal vesicle, or *vesicula seminalis*, in which the sperm is stored. Attached to the *vesicula seminalis* is the extremely long accessory gland which usually coils around the *testis*. The gonopore is situated on the *penis*. There was no distinctive difference in the reproductive system of the newly emerged and approximately 15-day-old males except the larger size of the *testes* in the sexually mature males.

The female reproductive system of *R. obscurus* is shown in Fig. 11. Ovaries were found to be generally asymmetric, i.e., the numbers of ovarioles on the left and right ovaries were not equal. In general, the number of ovarioles per ovary varied from 1 to 4.

The ovaries of *R. obscurus* are synovigenic, i.e., eggs are continuously produced, nourished, and laid. The ovarioles are of the panoistic type in which there are no special cells for egg nourishment. The ovarioles fuse at the pedicels to form an egg calyx which leads to a short lateral oviduct.

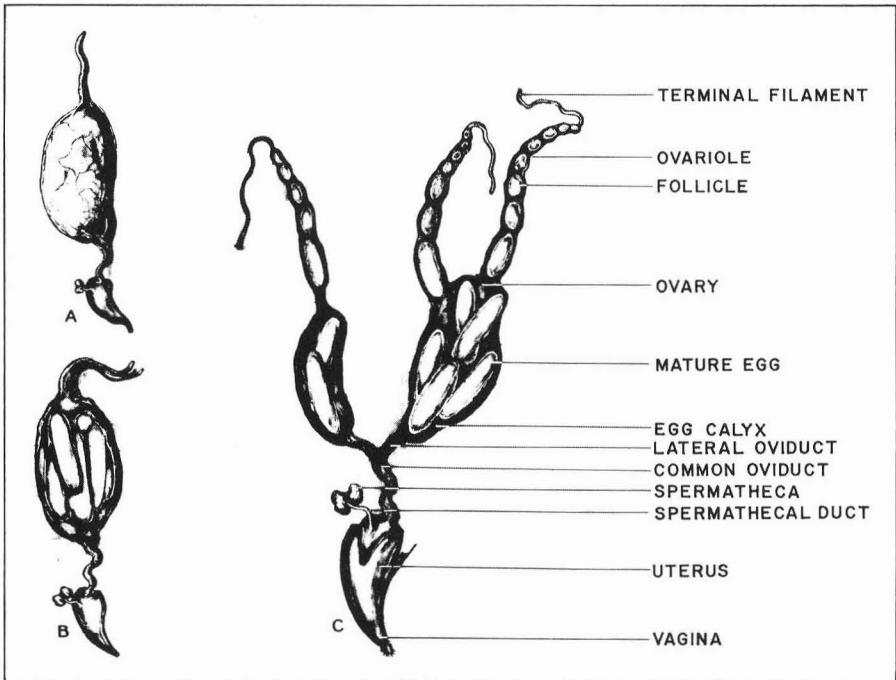


FIG. 11. Female reproductive system of *R. obscurus*: A, of a newly emerged female; B, of a partially gravid female; C, of a gravid female.

The left and right oviducts fuse to form the common oviduct, or *oviductus communis*. The common oviduct opens into the enlarged pouch, or *uterus*, which in turn opens into the *vagina*. The spermatheca, or *receptaculum seminis*, is a bilobed sac with a short spermathecal duct leading into the *uterus*.

The female reproductive system of *R. obscurus* is not completely developed at the emerging time. It consists of vestigial sac-like ovaries in which the lateral oviducts are enclosed in a thin membrane (Fig. 11A). After the female feeds for a period of time, the eggs in the ovaries develop; however, the lateral oviducts still remain fused (Fig. 11B). Gradually the left and right ovaries separate and the eggs may be seen in each ovariole (Fig. 11C).

### Longevity and fecundity

The longevity and fecundity of *R. obscurus* are not clearly known. Koebele (1899) speculated that in Hawaii the weevil was long-lived, and that the females collected and kept in the laboratory laid at least 200 eggs

per female. Koebele (1900) also reported that the longevity of the weevil was probably 10 to 12 months, and the number of eggs laid per female during this period was 400 to 600 eggs. Terry (1907) gave an account of this weevil but he determined neither the fecundity nor the longevity of the weevil. He simply repeated the statements made by Koebele. In North Queensland, Australia, Girault (1914), experimenting with 11 pairs of males and females, found that the longevity of the male ranged from 81 to 114 days, and that of the female, 69 to 135 days. The progeny, eggs and larvae, produced per female during 109 days was 22.

By marking adults of *R. obscurus* and later recovering them in traps using 30 split canes in bundles, Van Zwaluwenberg and Rosa (1940) found that at Kailua, Oahu, adult weevils survived in the field for more than 25 weeks or longer.

Attempts were made to carry out further investigations on the longevity and fecundity of *R. obscurus*. The ideal method would be the utilization of the newly emerged weevils and keeping records of their longevity and fecundity. However, this procedure was not feasible due to the long life cycle of the weevil and time limitations. Field-collected weevils of unknown ages were utilized in the present investigations.

The series of studies on longevity and fecundity were carried out with a duration of 198 and 490 days, respectively. In the first study, 10 pairs of weevils were selected at random. Each pair was kept in the humidity jar and fed on fresh cane pieces replaced weekly. Number of eggs and/or larvae in each cane piece was recorded. The study was continued for 198 days at which time there were no living weevils. The second study was carried out in a similar manner, again using field-collected weevils. In this

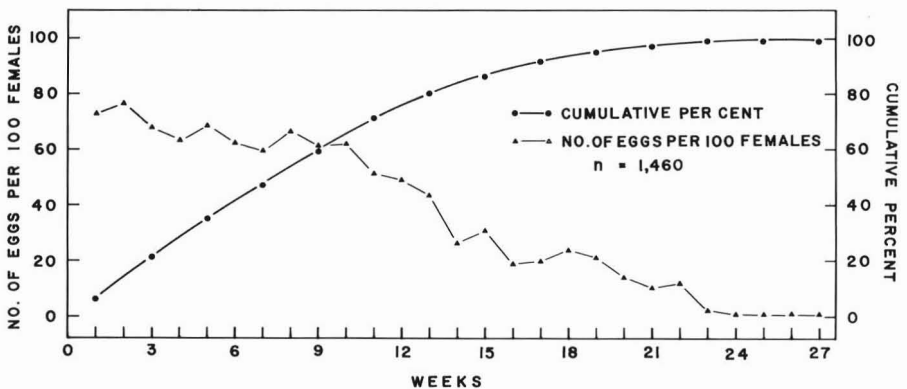


FIG. 12. Fecundity and cumulative percent of eggs laid by *R. obscurus*, 198-day study.

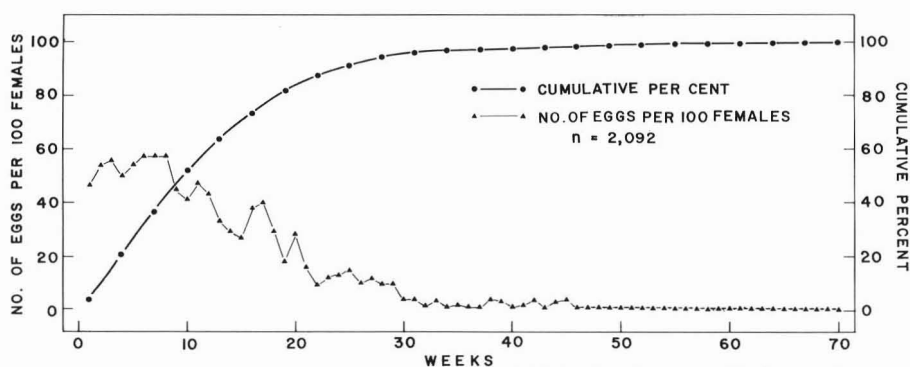


FIG. 13. Fecundity and cumulative percent of eggs laid by *R. obscurus*, 490-day study.

series 13 pairs of males and females were kept in each of the 10 humidity jars.

The results of the first experiment showed that the longevity of the male and female were not significantly different. The longevity of the male was  $167 \pm 7.0$  days, while that of the female was  $160 \pm 6.8$  days. The mean fecundity per female during the 160 days in which the weevils were kept in captivity was  $146 \pm 22$  eggs. These figures indicated that the mean fecundity per female per day was  $0.9 \pm 0.2$  eggs.

The data on the weekly trend in egg production in the first experiment, shown in Fig. 12, indicate that during the first 10 weeks the egg production rate remained approximately the same. Between 10 and 16 weeks the rate declined. Thereafter the trend in egg production was fairly constant. Figure 12 also shows the data on cumulative percent of eggs laid at weekly intervals for 27 weeks. It can be seen from this figure that 50 percent of eggs were laid in the first 7 weeks, and 90 percent in the first 16 weeks. After 16 weeks the percentage of eggs laid was very small.

The result of the second experiment, presented in Fig. 13, showed similar trends. The egg production rate remained fairly constant during the first 11 weeks, then declined slowly up to 21 weeks. From 21 weeks onward to 70 weeks, the egg production rate remained almost constant. Figure 13 also shows the data on the cumulative percent of eggs laid at weekly intervals for 70 weeks. It may be noted that 50 percent of eggs were laid during the first 10 weeks, and 90 percent during the first 23 weeks.

### Age structure

There is no precise and rapid method to ascertain the age of the adult insects. This problem becomes increasingly difficult in tropical areas where there is a great overlap in generations.



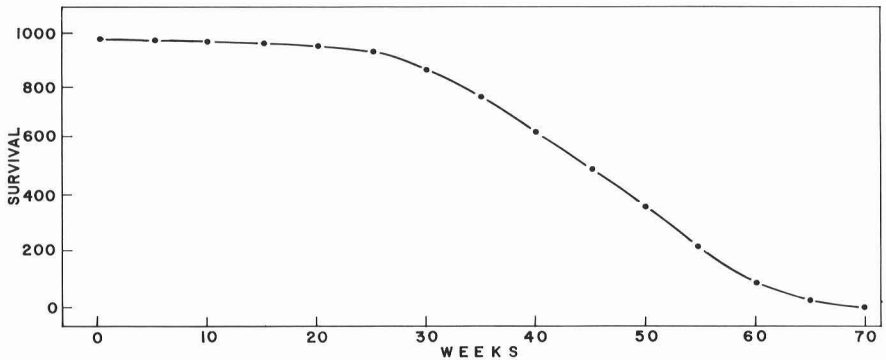


FIG. 14. Survival of field-collected adults of *R. obscurus* kept in captivity.

Data on age structure of the adults of *R. obscurus* were obtained from a survivorship curve, based on field-collected adults. The survivorship data were in turn obtained from the longevity studies presented previously. The estimated relative age of the individuals was, therefore, based on the number of weeks they survived after capture rather than after emergence from the pupal stage. It was assumed that the youngest field-sampled individuals lived the longest in captivity and the oldest, the shortest.

The survivorship curve obtained from the data on field-collected weevils is shown in Fig. 14. A rapid decline in survival occurred after 25 weeks. There was a 50 percent survival at about 45 weeks after capture.

The age structure of the sample population of weevils is shown in Fig. 15. Some of the pertinent features of this age structure are (i) a small proportion of young adults; (ii) a relatively small proportion of old adults;

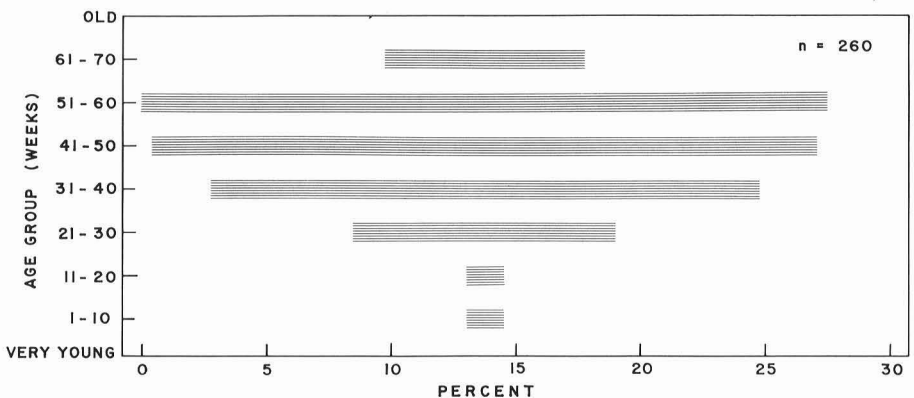


FIG. 15. Age structure of field-collected adults of *R. obscurus*.

and (iii) a high proportion of adults of intermediate ages. The small proportion of young individuals indicates a low recruitment rate. In other words, young adults were not being added to the population very rapidly. The predominance of adults of the intermediate ages, in spite of low recruitment rate, may be due to the longevity of the weevils and, possibly, to immigration. It is an unsatisfactory situation from an economic standpoint for it is these individuals that are reproductively most active.

### Variation in size

Considerable difference in the size of adult weevils was noted in field populations of *R. obscurus*. It is recognized that the food quality and quantity during the immature stages are among the factors that influence the size of the adults. Studies on size variation of the adults were made to ascertain size ranges in the natural populations. From such data the adequacy of artificial media may be evaluated by comparing the size of the laboratory reared adults and field sampled adults. Field samples of weevils were obtained and the body weight of each weevil and the width of the pronotum measured.

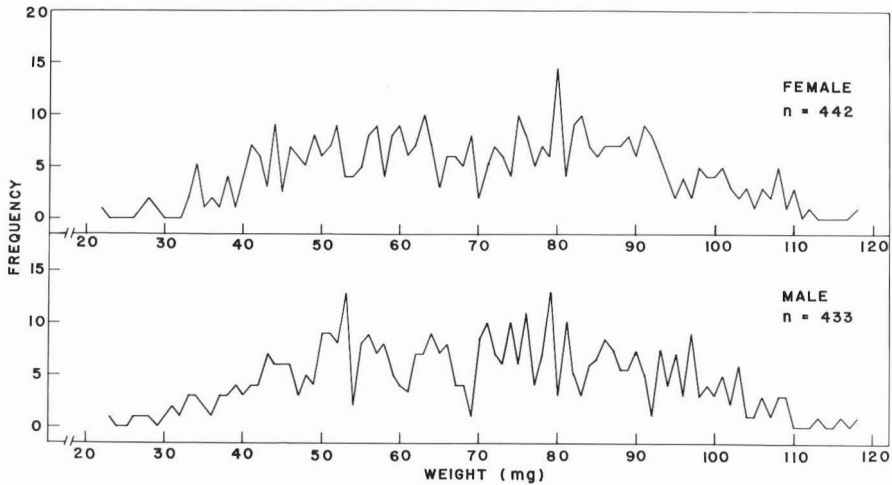


FIG. 16. Frequency distributions of the weight of the males and females of *R. obscurus*.

Data on size variation based on the body weight of the males and females indicated considerable variation among weevils collected in the field (Fig. 16). The body weight of the males varied from 23.2 to 118.1 mg. Mean body weight of 433 males was  $66.1 \pm 28.3$  mg. The body weight of the females varied from 21.3 to 118.2 mg. Mean body weight of 442 females

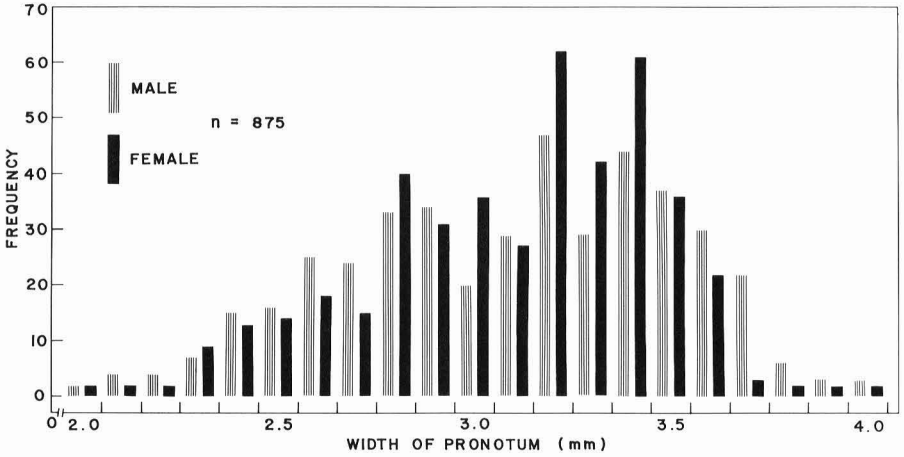


FIG. 17. Frequency distributions of the width of the pronotum of the males and females of *R. obscurus*.

was  $67.8 \pm 29.9$  mg. There was no significant difference in body weight between the sexes.

Size variation based on the width of the pronotum did not vary as greatly as that based on body weight. In both sexes the size ranged from 2.0 to 4.0 mm. Mean width of the pronotum of 875 males and females was  $3.1 \pm 0.4$  mm. The frequency distribution of the width of the pronotum, shown in Fig. 17, indicated that the distribution tended to be skewed towards the larger end of the size scale. The highest frequencies occurred at about 3.2 to 3.4 mm. The frequency distributions of the width of the

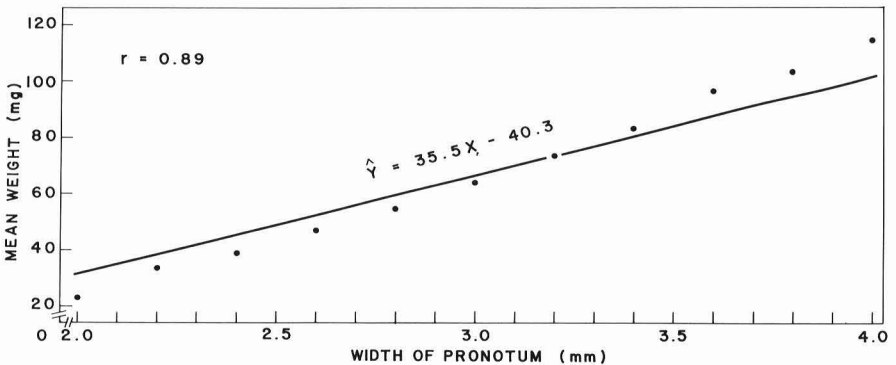


FIG. 18. The regression of body weight on width of the pronotum of *R. obscurus*.

pronotum for both males and for females were approximately equal.

The relationship between body weight and width of the pronotum is shown in Fig. 18. There was a high correlation ( $r = 0.89$ ). It is, therefore, possible to use either the body weight or the width of the pronotum as an index of the size of adult weevils. In field work, measuring the pronotum might be more practical than measuring weight.

### Variation in color patterns

Variation in color patterns of the adults of *R. obscurus* was first observed by Muir and Swezey (1916). They reported that the median dorsal marking on the pronotum and those on the elytra varied in size and shape considerably among specimens collected from different localities in the Pacific.

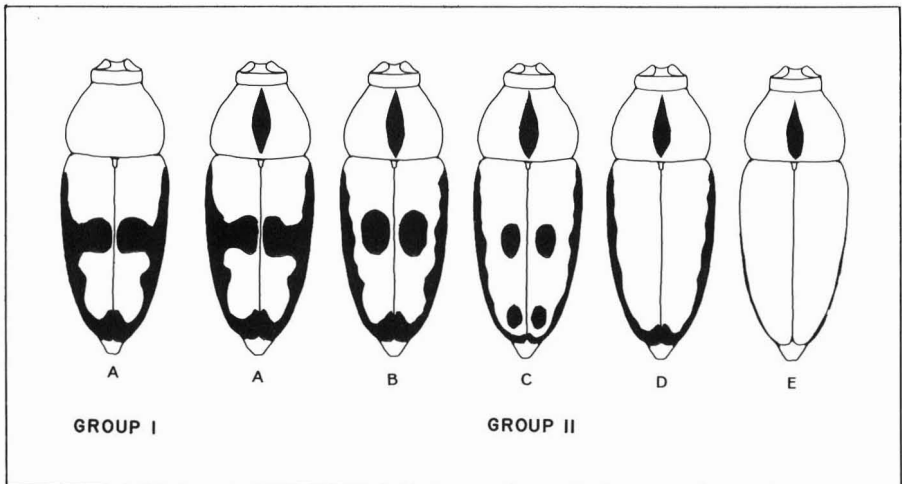


FIG. 19. Color variation among adults of *R. obscurus*. Group I contains only type A; Group II contains types A, B, C, D, and E.

In the present study, examination of adult weevils, collected from the islands of Kauai and Oahu, indicated that there were at least six color types which were separated into two groups (Fig. 19). In Group I the black median dorsal marking on the pronotum is absent; in Group II, it is present. The color types in Group II can be easily separated by the color markings on the elytra. In Type A the black markings on the elytra merge into those on the lateral margins; in Type B there are two distinct spots; in Type C there are four distinct spots; and in Types D and E there is no spot. Types D and E are similar; however, they can be distinguished by the presence

of a dark area at the tip of the elytra and on the lateral margins of the elytra in Type D. These dark areas are absent in Type E. These color variations were not found to be associated with sex. The genetics of these color variants were not within the scope of the present investigation.

To determine the frequency of occurrence of the different color types, a total of 2164 weevils were collected from Lihue, Kauai, and from Kunia, Oahu. Attempts were also made to collect weevils from different localities on Maui and Hawaii, but the numbers were not substantial enough to give any significant information.

TABLE 4. Occurrence of the different color groups and types of the adults of *R. obscurus* in populations sampled from the islands of Kauai and Oahu

Groups and types	Oahu		Kauai	
	Frequency	Percent	Frequency	Percent
Group I				
Type A	0	0	5	0.3
Group II				
Type A	176	41.0	459	26.5
Type B	142	33.1	808	46.6
Type C	18	4.2	77	4.4
Type D	87	20.3	372	21.4
Type E	6	1.4	14	0.8
Total	429	100.0	1,735	100.0

The data showing the frequency and percentage of each color type collected from Kauai and Oahu are shown in Table 4. It is evident that there were variations in color types in the weevil populations from different localities. On Oahu, the percent distribution of Group I, Type A; and of Group II; Types A, B, C, D, and E were 0, 41.0, 33.1, 4.2, 20.3, and 1.4, respectively; on Kauai, they were 0.3, 26.5, 46.6, 4.4, 21.4, and 0.8, respectively. From these data it may be seen that Type A of Group II is the dominant one on Oahu, and Type B of Group II, the dominant one on Kauai.

Muir & Swezey (1916) also reported the difference in color patterns on the elytra of weevils collected from Amboina, Ceram, Larat, New Guinea, Queensland, Fiji, and Hawaii. The factors associated with these color patterns of *R. obscurus* were not reported.

## BEHAVIOR

### General behavior

The adults of *R. obscurus* were secretive during the day. They were often seen between the loosened sheath of mature leaves and stalks. Other sheltering places where the adults might be found during the day were cracks in broken cane stalks, wounds in stalks made by rats, and debris of decaying organic matter on the ground in the cane fields.

The flight habit of the adults is inadequately known. Koebele (1900) reported that, "often the *Sphenophorus* beetle has been seen flying during the hottest part of the day, around mills in operation on these Islands; most numerous they were observed on Kauai at sunset until dusk, coming from a recently burned field across the road and setting down upon the

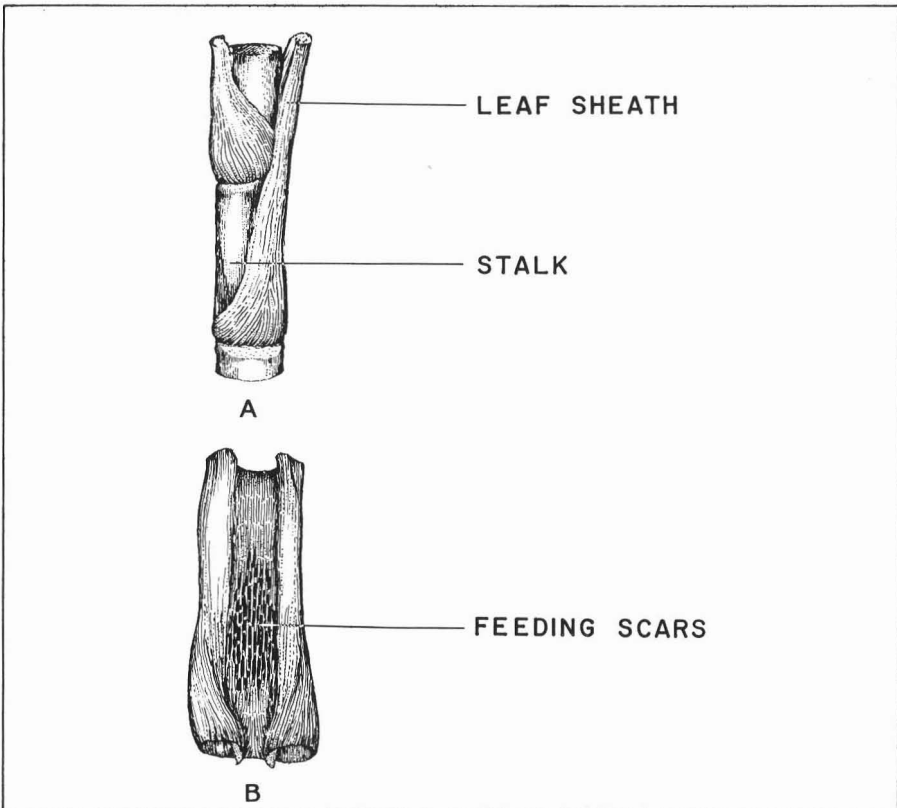


FIG. 20. Leaf sheath and stalk of sugarcane: A, stalk and loosened leaf sheath; B, inner surface of leaf sheath showing feeding scars made by the adult weevils.

young plants." In the present study the flight of the weevils during the day was not observed, perhaps because it occurs only infrequently and under certain conditions. The exact distance the weevil flies is not known. Van Zwaluwenberg & Rosa (1940) reported that marked weevils were found as far as 500 meters away from the point of release.

The feeding behavior of the adults has not been reported to any great extent in the literature. From general observations it is known that the adults feed on the cane stalks. In the present study, adults were observed to feed on the inner surfaces of the leaf sheaths as well as on cane stalks. The former feeding habit was also confirmed by field observations. The feeding scar on leaf sheath (Fig. 20) can be used effectively in sampling, for evaluation of infestation in the field. The adults are polyphagous; other than sugarcane, they were found to feed on coconut, *Cocos nucifera* L., pritchardia palm, *Pritchardia martii* (H. Wendl.), and in the laboratory, on the fruits of other plants such as apple, banana, grape, papaya, and pear.

Captive adults fed on the pith from the cut ends of cane pieces provided them in the humidity jar. After the pith was eaten, only bristlelike fibers remained.

### Circadian activity of adults

Detailed studies have shown that the activity of each of the biological components of the ecosystem is not constant (Reinberg & Ghata, 1964). For each component there are phases of high and low activity. Some of these phases occur so regularly that they are called rhythmical cycles of activity.

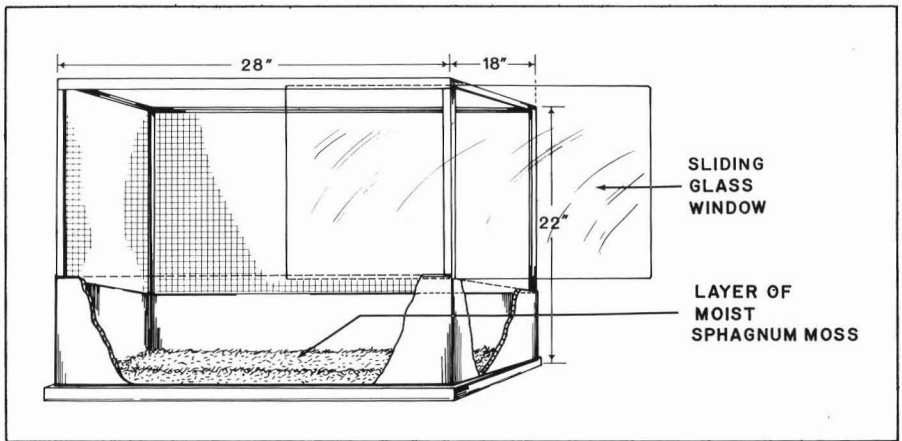


FIG. 21. Cage designed for studying the circadian activity of adult *R. obscurus*.

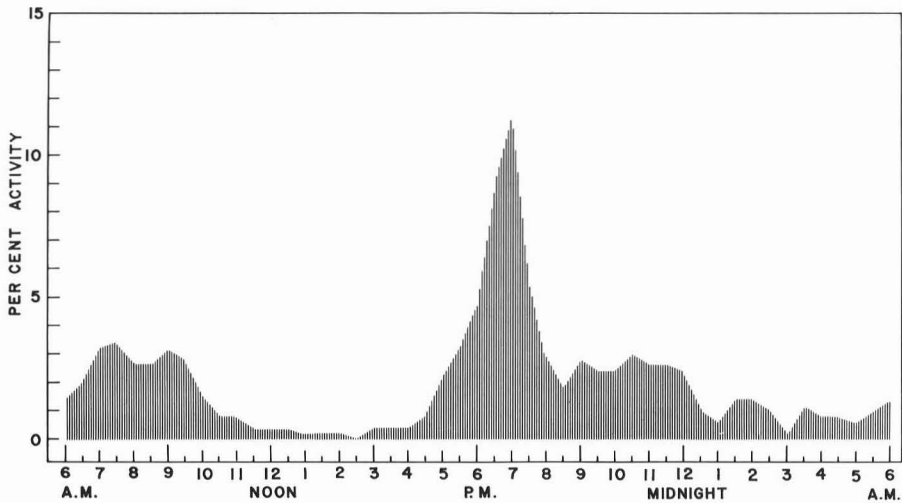


FIG. 22. Circadian rhythmic activity of *R. obscurus* under laboratory conditions.

There are several terminologies referring to this type of rhythmic activity which may be induced either by endogenous or exogenous factors. The terms "diurnal rhythm," "daily rhythm," "24-hour rhythm," or "nycthemeral rhythm" are misleading because they are concerned with phases that do not fit into a 24-hour cycle (Reinberg & Ghata, 1964). The expression "circadian rhythm," introduced by Halberg *et al.* (1959) is a more useful and universally accepted term.

In the present study, a screened cage,  $28 \times 22 \times 18$  inches, equipped with a sliding glass window, was constructed to study the circadian rhythm of the adults of *R. obscurus* (Fig. 21). Cane pieces were provided on the top of a layer of sphagnum moss 5.0 cm deep on the floor of the cage. The cage was placed near the windows for exposure to natural light. No attempts were made to control either the duration or the intensity of light. The temperature ranged from 77 to 88 F. The relative humidity in the cage was about 85 to 90 percent because of the moist sphagnum moss.

Two hundred weevils of both sexes were released in the cage. Three days after release, records were taken on their activity by counting the numbers of weevils that came out of the sphagnum moss and were seen flying, crawling, or mating on the sides of the cage. Records were taken at 30-minute intervals starting from 6:00 AM on the first day and continuing for 14 days. Because the general periodicity of activity for each 24-hour observation was similar, the data were pooled. Results are shown in Fig. 22. The percentage of activity is reported upon the frequency with which



active weevils were seen coming out of the sphagnum moss, not upon the total number of weevils released in the cage.

Results indicated a marked variation in the activity of the weevils during a 24-hour period. There were two peaks of activity: a small one between 6:00 and 10:00 AM, and a large one between 6:00 and 8:00 PM. Although the highest peak occurred at 7:00 PM, it was evident a small percentage of weevils were active almost throughout the night.

Although no studies were made to determine the exogenous factors that influence the rhythmic activity of the adults of *R. obscurus*, it appeared that temperature and humidity had no influence on the activity of the weevils. Field and laboratory observations appeared to indicate that light intensity might be an important factor. The role of the endogenous factors is not known.

### **Mating behavior**

Field observations on mating behavior of the weevils are not possible, because of the secretive and crepuscular habits of the adults. The present account on mating behavior of the weevils is based on observations made on weevils kept in the humidity jars. The activity of the weevils in these transparent jars can be observed with least disturbance.

Apparently there are three different steps involved in mating; chase, courtship, and sexual engagement. Chasing is usually performed by the male following the female actively, with raised rostrum and actively moving antennae. After a period of time, the female that is being chased becomes less active; then the male exhibits courtship behavior. In courting, the male vibrates rapidly either one or both forelegs and moves the antennae over the female. The male attempts to mount while still keeping the forelegs vibrating freely. The actual sexual engagement is accomplished upon successful mounting. The three consecutive steps involved in mating do not have to be repeated, consecutively all over again should there be a failure of sexual engagement. When sexual engagement is unsuccessful and the pair becomes separated, the male does not begin with the first step. He usually begins with the second step.

It was found that in mating the male chased the female from a few minutes to about 10 minutes, and he courted from a few minutes to at least 30 minutes. After mounting, the male remained in that position from few seconds to several hours and in many cases overnight. Though the weevils may remain in such a copulation-like position for several hours, there may be no actual sexual engagement. However, the duration between attempts and actual copulation may last from a few seconds to several minutes. The observations made in the study revealed that the male is polygamous, and

the female, polyandrous. Multiple mounting and perversion have also been observed in this weevil. Frequently the male remained mounted while the female was walking or feeding.

The mating activity of *R. obscurus* observed in the present study indicated that it was usually highest during the peak of the circadian activity of the adults. This is the period between 6:00 and 8:00 P.M. However, it appeared that some individuals mated during any time of the day.

### Oviposition behavior

According to Terry (1907), the eggs of *R. obscurus* are usually laid in the internodal area above the bud, or "eye," which is still loosely enclosed by the leaf sheath. The eggs are laid in cavities, approximately 3.0 mm deep, made by the female with the sharp mandibles on the tip of the rostrum. After making the cavity, the female turns around and oviposits a single egg in each cavity. The time taken for making the oviposition cavity was usually 3 to 7 minutes. The time taken for oviposition was variable, 4 to 6 minutes as observed by Koebele (1900) on many occasions, and on another only  $\frac{1}{2}$  minute. In the present study, the duration of the act of oviposition was from few minutes to as long as 18 minutes.

Not all eggs were laid in the vicinity of the internode; the eggs are also laid in other areas, occasionally they were deposited in the leaf sheaths and midribs of the leaf blades. The number of eggs laid in the various oviposition sites is not known. Koebele (1900) noted only 2 to 3 oviposition punctations per internode.

The female of *R. obscurus* does not appear to be selective as to where the eggs are laid. Besides laying eggs in cavities made for that purpose, she may lay eggs in feeding scars, Fig. 20 B, page 33 made by other females and males, in cracks in the stalks, in fresh tissues exposed by stalks broken by wind or other causes, and in wounds made by rats feeding on the cane stalks (Muir & Swezey, 1916).

Observations made on the females in captivity indicated that the female makes her own egg laying cavities and oviposits eggs in them, or she lays eggs in the egg laying cavities made by other females.

Newly emerged females usually did not lay eggs for a considerable length of time. The weevils probably required a long period for egg maturation and fertilization before egg-laying. It was also observed that in spite of having synovigenic ovaries, the females did not lay eggs continuously. Oviposition appeared to occur in irregular cycles and at irregular intervals.

The period between emergence and first egg deposition is referred to as the preoviposition period. The period in which egg deposition occurred is

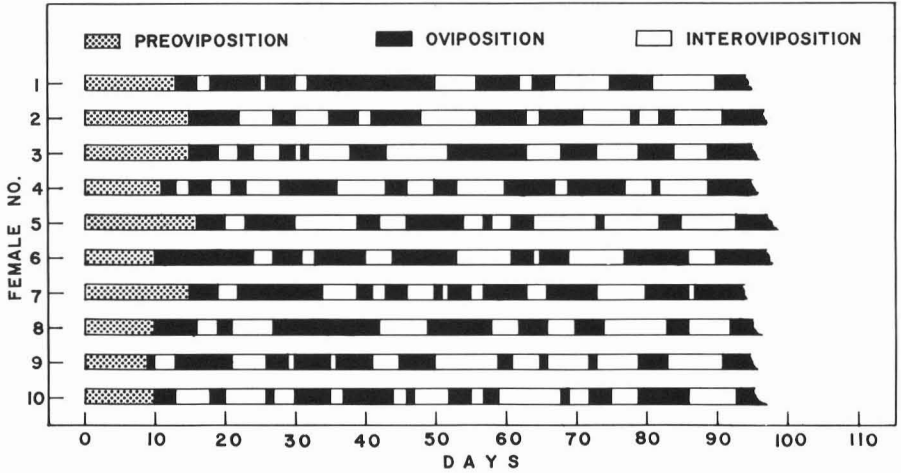


FIG. 23. Preoviposition, oviposition and interoviposition periods of 10 females of *R. obscurus*.

referred to as the oviposition period, and the interval between two oviposition periods, the interoviposition period.

To study the periodic oviposition behavior of *R. obscurus*, 10 newly emerged females, each with a male, were kept separately in the humidity jars for about 90 days. Each day a freshly cut cane piece was placed in each jar for 24 hours. The number of eggs laid in the cane piece was then recorded daily. Results of this study indicated that oviposition was not continuous (Fig. 23). There was an alternation of oviposition and non-oviposition periods. The time interval between these periods was not the same for each female.

### Larval behavior

Information on larval behavior was obtained by dissecting a long series of cane stalks infested with the various developmental stages of the weevils. Upon hatching, the larva begins to bore into the stalk tissues, tending to bore downward into the lower and older parts of the stalk. With the growth of the larva, the tunnels made become correspondingly larger. After reaching the 3rd instar, and thereafter, the larva cuts openings or windows in the rind. As the larva approaches maturity, the number as well as the size of the windows increases. The exact function of these openings is not known. Perhaps they serve to regulate the atmospheric conditions within the tunnels.

The feeding habit of the larva in the tunnel was also observed. It was usually noted that during the young stages there was no evidence of fibers in the tunnels. Apparently, the larva remained between the fibers of the stalk, feeding only on the pith and not on the fibers. As the larva grew larger, the amount of fibers in the tunnel increased. It is believed that this fiber is the discarded, uneaten portion of the cane tissues, because the larva does not feed on the fibers. Dissection of the crop and midgut showed no fibrous materials. Discarded fibers in the tunnel were the ones subsequently used by the prepupa for the construction of the cocoon.

The larva of *R. obscurus* appeared to be cannibalistic. It is for this reason that usually only one mature larva is found in each tunnel. Because of this cannibalistic behavior, only limited numbers of larva could be reared in a single container.

### STUDIES ON REARING METHODS

During the past few decades, much progress has been made on the rearing of insects on artificial and semi-artificial media. This progress may be attributed to our recent knowledge on insect nutrition. Insect nutrition has been reviewed by Lipke & Fraenkel (1956), and House (1961, 1962); and mass production of insects and artificial diets have been reviewed by Smith (1966), and House (1967). The use of artificial media is of considerable importance in various types of research where insects of uniform ages and genetic background are needed in large numbers.

Although research has been carried out on the rearing of some coleopterous insects on artificial media, no work has been carried out on *R. obscurus*. The current study represents an initial attempt to rear this insect on artificial and semi-artificial media. In the present study, it was found that the development of rearing media for this insect has been extremely difficult because of (i) the long life cycle; (ii) low fecundity; and (iii) lack of basic biological data. More research than that reported herein is needed before large scale mass rearing of *R. obscurus* is feasible.

#### Preparation of non-commercial ingredients

Non-commercial ingredients used in the present investigation were sugarcane powder, shredded sugarcane, and shredded coconut husk. Sugarcane powder was prepared by cutting the cane stalks into small pieces (approximately 1.5 to 2.0 cm long) for oven drying at 72 to 75 C for 24 hours. After drying, they were finely ground by the use of Wiley Cutting Mill No. 1 with 1-mm mesh screen.

Shredded sugarcane and coconut husk were both prepared in the same manner. Mature cane stalks and young coconut fruits were used. The fresh materials were cut into thin slices, about 2.0 cm long, and then chopped in a Waring Blender for a short period to separate the fibrous and non-fibrous fractions. Prolonged blending was avoided because the fibrous materials became too finely chopped. Blended materials were then air-dried in the sun for about 8 to 10 hours before storing in air tight gallon jars for future use.

### **Preparation of media**

All media tested contained agar as binding material. In general, the medium was prepared by dissolving the agar in the necessary quantity of water in a beaker and boiled for about 5 minutes. It was then poured into the Waring Blender. The basic ingredient was then added, and the blender run for about 5 minutes. The antimicrobial agent was then added and the preparation was allowed to cool down to 50 C before the addition of other ingredients such as salt mixture, vitamin mixture, and other preservatives if used. It was again blended for about 3 minutes and then dispensed into sterilized rearing containers. The medium was allowed to cool and harden.

### **Rearing containers**

Various kinds of rearing containers may be used. In the present study, 8-dram shell vials and plastic boxes measuring  $5 \times 5 \times 5$  cm in dimension, with snap covers, were used. For most experiments, the plastic boxes were found to be satisfactory. Another type of rearing container used was a plastic box,  $28 \times 17 \times 4$  cm, with 18 separate compartments and snap cover. This type of container may be suitable for mass rearing because the larvae, being cannibalistic, must be reared individually.

### **Handling of adults**

To obtain eggs, field-collected weevils were maintained in the laboratory in the humidity jars. Twenty pairs of weevils were kept in each jar. This number was found to be optimal, judging from the egg-laying performance and the vitality of the weevils. Too few weevils per jar would be uneconomical; too many would result in overcrowded conditions which would cause the adults to feed on their eggs. Furthermore, overcrowded conditions may not be conducive to mating and oviposition. Equal proportions of males and females were used to ensure fertilization, in spite of the fact that the males were found to be polygamous.

As mentioned earlier, the adults of *R. obscurus* lived for many months without decreasing their fecundity. Although field-collected weevils were found to be productive and may be useful for 4 to 5 months, it is, however, advisable to replace them every 3 months to ensure maximum egg production.

### Handling of eggs and larvae

The usual procedure in most rearing programs, placing the eggs directly on the artificial medium, was not successful with *R. obscurus*. Since low hatching percentage and high larval mortality were encountered, a modified procedure was adopted. Cane pieces, exposed to the adults for oviposition for three days, were removed and then placed vertically in a plastic container matted with moist paper towels. They were kept in the container for one week to allow the eggs to hatch. The larvae were later dissected out and transferred to the rearing medium. By this method, it was found that the larvae could be easily dissected out because of the soft partially decomposed tissues of the cane pieces. Furthermore, the larvae obtained by this procedure appeared to be more vigorous than those obtained from eggs dissected out of the fresh cane pieces.

Although the larvae were known to be cannibalistic, trials were made to rear several larvae in one container. In all cases only one or two of them were able to survive per container.

### Handling of pupae

The prepupa requires fibrous material to construct a cocoon and to pupate. In all of the nonfibrous media, prepupae failed to pupate. After pupation occurred, the cocoons were allowed to remain embedded in the respective media until the adults emerged. The adults were then transferred to the humidity jars.

### Method of evaluation of media

In developing a satisfactory rearing media for *R. obscurus*, it was necessary to test a series of different media. Primary criteria for evaluating the media were its (i) keeping quality; (ii) feeding, tunneling, and pupation suitability; and (iii) contribution to the growth of the insect. Keeping quality is of considerable importance because the larval stage may be as long as 90 days. Therefore, any medium that had poor keeping quality was discarded. In many tests the candidate media were kept under observation for a period of 4 to 7 days without inoculating the larvae. Those that decomposed were discarded immediately. The feeding and tunneling

characteristics were found to be very important because in certain media the larvae failed to bore into the media and died. The size of the larvae and the resulting size of the adults are also important considerations. The ultimate objective is to produce weevils whose size compares favorably with that of the natural population.

### Evaluation of media

With but few exceptions, the media tested for rearing the larvae of *R. obscurus* were modifications of diets developed by other investigators for other species of insects. Therefore, the basic ingredients were the same for most media. A list of media tested in this study is given in Table 5. Each medium was given a performance rating based on criteria already discussed in the preceding section. For convenience a numerical rating was used; 0, poor; 1, satisfactory; and 2, excellent. A medium rated poor failed to produce any adults; one rated satisfactory produced adults but these were smaller than those of the natural population. A medium was rated excellent if it produced adults comparable in size to those of the natural population.

The results of the tests conducted showed that the quality of none of the media came up to the level desired (Table 5). Out of 18 media, 10 were rated poor, 8 satisfactory, and none excellent. These results also indicated that the media developed for other species of insects were generally not satisfactory for rearing the larvae of *R. obscurus*.

The shredded coconut-husk medium (no. 17) and the shredded sugarcane medium (no. 18) proved promising. It appeared that with certain modifications the shredded coconut-husk medium could be rated higher than shown in Table 5. Its major drawback was the unavailability of coconut fruits in quantity. The shredded sugarcane medium showed advantages over the coconut medium because of its excellent keeping quality and suitability for good tunneling by the larvae. Sugarcanes of known varieties are available in large quantities.

TABLE 5. A list of media and their modifications tested for rearing the larvae of *R. obscurus*

Medium no.	Name or description	Modification		Rating*
		Ingredient removed	Ingredient added	
1	Diet for the cotton boll weevil, <i>Anthonomus grandis</i> Boh. (Vanderzant & Davich, 1958)	Soybean protein	Sugarcane powder	0
2	Same as medium no. 1 (Earle <i>et al.</i> , 1959)	Acetone powder of cotton squares	Sugarcane powder	0

TABLE 5. A list of media and their modifications tested for rearing the larvae of *R. obscurus* (Continued)

Medium no.	Name or description	Modification		Rating*
		Ingredient removed	Ingredient added	
3	Diet for the sugarcane borer, <i>Diatraea saccharalis</i> (F.); standard diet (Pan & Long, 1961)	Corn plant powder Dowicide	Sugarcane powder Methyl paraben Methyl paraben	1
4	Same as medium no. 3; improved diet	Dowicide	Corn plant powder	0
5	Puerto Rico Nuclear Center (PRNC) no. 1 for <i>D. saccharalis</i> (Walker <i>et al.</i> , 1966)	Corn fiber	Sugarcane powder	0
6	Same as medium no. 5 PRNC no. 2	Corn fiber	Sugarcane powder	0
7	Same as medium no. 5 PRNC no. 3	Corn fiber	Sugarcane powder	0
8	Louisiana State University diet for <i>D. saccharalis</i> (Hensley & Scuford, 1967)	None	None	0
9	Same as medium no. 8	Wheat germ	Sugarcane powder	0
10	Diet for <i>D. saccharalis</i> (Wong-siri & Randolp, 1962)	Wheat germ	Sugarcane powder	0
11	Vanderzant-Adkisson wheat germ diet for the pink bollworm, <i>Pectinophora gossypiella</i> (Saund.), etc. (Adkisson <i>et al.</i> , 1960)	None	None	1
12	Same as medium no. 11	Wheat germ	70 g wheat germ 70 g sugarcane powder	1
13	Same as medium no. 11	Wheat germ	125 g sugarcane powder	1
14	Same as medium no. 11	Wheat germ	35 g wheat germ 120 g fresh coconut husk	1
15	Same as medium no. 11	Wheat germ	70 g wheat germ 70 g shredded sugarcane	0
16	Same as medium no. 11	Wheat germ	70 g wheat germ 70 g shredded coconut husk	1
17	Same as medium no. 11	Wheat germ	25 g agar 140 g shredded coconut husk 3 g Wesson's salt mixture	1
18	Same as medium no. 11	Wheat germ	25 g agar 140 g shredded sugarcane 3 g Wesson's salt mixture	1

\* Ratings are: 0 = poor  
1 = satisfactory  
2 = excellent



TABLE 6. Composition of media no. 17 and no. 18 for rearing the larvae of *R. obscurus*

Ingredients	Quantity	
	Medium no. 17	Medium no. 18
Shredded coconut husk	140.0 g	—
Shredded sugarcane	—	140.0 g
Agar (non-nutrient)	50.0 g	50.0 g
Wesson's salt mixture	3.0 g	3.0 g
Methyl paraben (in 95% ethyl alcohol)	0.5 ml	0.5 ml
Sorbic acid	0.5 ml	0.5 ml
Vanderzant's vitamin mixture	15.0 ml	15.0 ml
10% KOH solution	10.0 ml	10.0 ml
Water	1,000 ml	1,000 ml

Further tests with media 17 and 18 were being conducted at the time of this writing. The full composition of both of these media is given in Table 6.

In the present investigation it was found that a medium with fiber performed better than one without. The actual role of fiber in the medium is not known, but long strands of fiber in the medium appeared necessary. It was noted that when finely ground sugarcane stalks were used, the larvae failed to develop; tunneling activity was very low. However, when long coarse fibers were incorporated into the medium, larval tunneling, feeding, and development were highly improved.

### Growth and size in various media

Out of 18 media tested, 8 media yielded adults. These were media no. 3, 11, 12, 13, 14, 16, 17, and 18, shown in Table 5. The performance of these media, presented in Table 7, indicated that the highest percentage of yield

TABLE 7. Performance of eight media in which the larvae of *R. obscurus* completed development

Medium no.	No. of larvae tested	Yield of adults (%)	Confidence interval (95%)	Developmental period (days)	
				Mean	Range
3	24	8.3	1.0-26.4	90.5 ± 3.5	87-94
11	18	22.2	7.8-47.6	89.9 ± 4.6	82-94
12	22	13.6	3.6-33.8	90.7 ± 7.4	81-99
13	32	12.5	3.8-27.8	84.5 ± 4.5	78-90
14	16	31.2	13.2-60.8	85.8 ± 5.6	79-94
16	14	42.8	20.4-70.6	88.5 ± 5.6	80-98
17	60	61.7	62.8-77.4	54.3 ± 3.7	45-61
18	16	56.2	30.8-79.0	58.0 ± 4.2	52-66

was obtained from media no. 17 and 18 in which 61.7 and 56.2 percent of the larvae developed into adults. The growth rate of the larvae was also the fastest in these media. The mean larval developmental periods were  $54.3 \pm 3.7$  and  $58.0 \pm 4.2$  days, respectively.

In addition to data on yield and growth rate, those on the size of the adults were also taken. Since it was found that there was a high correlation ( $r = 0.89$ ) between body weight and width of the pronotum, the latter measurements were used as indices of size. The width of the pronotum was measured by Dixon sliding vernier caliper. Although the number of adults obtained were small in most media, it appeared that there was no difference in the size of the adults reared from various media (Table 8). It was also evident that the adults reared from these media were on the average smaller than those obtained from natural populations. However, it might be pertinent to point out, as mentioned in the section on variation in size, that there is considerable size variation of weevils even from field populations.

TABLE 8. Average size of the adults reared from eight media in which the larvae of *R. obscurus* completed development

Medium no.	Number of adults emerged	Width of pronotum (mm)	
		Mean	Range
3	2	$2.4 \pm 0.1$	2.3-2.5
11	4	$2.7 \pm 0.1$	2.6-2.9
12	3	$2.4 \pm 0.1$	2.3-2.6
13	4	$2.8 \pm 0.2$	2.3-3.1
14	5	$2.6 \pm 0.2$	2.3-3.0
16	6	$2.7 \pm 0.3$	2.2-3.1
17	37	$2.7 \pm 0.3$	2.4-3.2
18	9	$2.8 \pm 0.2$	2.3-3.2

## DISCUSSION

The investigation of *R. obscurus* was undertaken to accumulate and record basic information on the biology and rearing of the weevils. This information is extremely necessary and useful for future research along this line, though many aspects of investigation could not be included.

In some studies, a high larval mortality was encountered with sugarcane stalks. The cause of this mortality was not known, but could be attributed to the varieties of cane used. Future studies might indicate that certain susceptible varieties of cane may be more suitable as rearing media than others.

The male to female sex ratio of *R. obscurus* collected in traps was found to be 4:3. This ratio might be influenced by a number of factors. When the adults are sampled from the cavity between the stalk and leaf sheath, the ratio would probably be 1:1 because the adults were usually present in pairs. The time of sampling the adults in fermenting cane traps might also be important. Van Zwaluwenberg (1938) found that there was a predominance of females during the 1st and 2nd weeks, and a predominance of males later. The season at which the sampling is made might also affect the sex ratio for Van Zwaluwenberg (1938) stated that the females tended to be outnumbered by males during the summer months. The true sex ratio should be established by rearing the larvae and observing the sexes of emerging adults.

*R. obscurus* adults in captivity lived as long as 70 weeks. This longevity for adults was probably longer than that of field populations because adults were kept under nearly ideal conditions. Under field conditions, the longevity of the adults might be considerably less.

The present study indicated that *R. obscurus* had a relatively low fecundity. However, field observations showed high adult populations in certain areas. Some of the early investigators attributed this abundance to the high reproductive potential (Koebele, 1900; Muir & Swezey, 1916). The present study failed to show a high reproductive ability of *R. obscurus*. The adult abundance in certain fields may be due to the longevity of the adults and their low mortality rates.

The age structure of *R. obscurus* determined from an adult population in a heavily infested field indicated a small percentage of young individuals, a high percentage of intermediate individuals, and a low percentage of old individuals. This type of age structure suggests a low rate of recruitment of young individuals. The high proportion of adults of intermediate ages, a dangerous situation from an economic standpoint, might be due to the accumulation of adults from each overlapping generation. Whether this type of age structure is typical in the field with mature cane or young cane is not known. The method used to determine the age of the adults, which was unique to a certain extent, was time-consuming because it was based on the number of weeks remaining before a weevil died of old age. A direct method of determining the age of the adults would be useful, but no such method is known at present.

Considerable variation in size of the adults in the field and laboratory was noted in this study. It was not determined whether this variation was due to genetical or nutritional factors. Both may be involved. In the present investigations it was found that no matter how uniform the rearing medium was, there were differences in the sizes and weights of the larvae

and adults. This observation suggests genetic factors. On the other hand, nutritional factors might also be involved; larvae found feeding on the leaf petioles of the pritchardia palm, *Pritchardia martii* (H. Wendl.), and those reared from fresh coconut husk, were usually larger than those obtained from sugarcane.

Color variation of the adults of *R. obscurus* was observed in this study. All color variants were classified into two Groups, I and II. In Group I there was only a single type, and in Group II, five. The dominant color type on Kauai was Group II, Type B; and on Oahu, Group II, Type A. Further investigations should be made to determine the predominance of the various color types in ecologically different areas.

Observations on the circadian activity of the adults of *R. obscurus* indicated that the highest activity occurred between 6:00 to 8:00 P.M. The factors that influence activity during this period were not studied, but it appeared that light might be an inducing factor. The problem of whether other exogenous or endogenous factors were involved was not investigated.

It was observed that the oviposition behavior of *R. obscurus* appeared to be periodic, i.e., after the preoviposition period, egg-laying was intermittent. The term "interoviposition period" was used to denote this non-oviposition period. The time intervals of these "interoviposition periods" were found to be different for females of the same age. The cause of this periodic oviposition behavior is not known. It might be due to hormonal as well as nutritional factors.

The periodicity of activity of the immature stages of *R. obscurus* was not observed. However, it appeared that the larvae were active at night because in the artificial media it was observed that fecal matter and loose uneaten fibers were piled up on the surface of the media at night. The actual spinning of the cocoon was also not observed; however, laboratory observations indicated that it occurred at night.

The artificial media tested in the present study may be classified into two general groups—fibrous and nonfibrous. These investigations showed that the nonfibrous media were generally less satisfactory than the fibrous ones. For unknown reasons, the feeding and boring activity of the larvae were better in the fibrous media. Furthermore, the prepupae rarely pupated in a nonfibrous medium. Future studies should give serious consideration to the inclusion of fibers in the media, even though this practice means more labor and expense.

It was evident from the data that most of the adults reared from artificial media were generally smaller than those in the natural populations. This difference in size is certainly difficult to explain because all the media tested were apparently more nutritious than sugarcane, the natural food of the

weevils. The present investigations suggest that the texture of the media was probably just as important as the nutritional factor for proper larval growth. Perhaps the poor performance of many of the media used in these studies were not due to nutritional factors, but to lack of suitable texture.

### SUMMARY

The present investigations were concerned with the biology and rearing of *R. obscurus*. A humidity jar, which maintained a high humidity, was designed to keep adults in captivity. The larvae were reared on sugarcane, the natural food plant, as well as on artificial and semi-artificial media.

Studies on the immature stages showed that the duration of the egg stage was  $4.6 \pm 0.4$  days. The mean developmental periods of larval, pre-pupal, and pupal stages, for weevils reared on shredded coconut-husk medium, were  $54.3 \pm 3.7$ ,  $7.2 \pm 1.5$ , and  $20.9 \pm 2.8$  days, respectively. Analysis of the data on the growth rates of larvae showed that they agreed closely with those to be expected according to Dyar's Law.

The general structures of the male and female reproductive systems were typical of curculionids. The ovaries were of the synovigenic type, and were asymmetric in regard to the number of ovarioles on each ovary. The sexes could be differentiated by the structure of the rostrum and pygidium. The male to female sex ratio of the trapped adults was 4:3.

The adults were found to be long-lived, however, their fecundity was low. The mean fecundity per female was  $0.9 \pm 0.2$  eggs per day.

Considerable size variation among adults was noted in field populations. The width of the pronotum ranged from 2.0 to 4.0 mm, and the weight from 21.3 to 118.2 mg.

Considerable color variation was also noted among the adults collected in the field. Color variants were classified into two groups and six color types. The occurrence of these color types varied with the locality in which the adults were collected.

The larvae were formerly believed to feed on the pith and as well as the fibrous part of the stalk. The present study showed that the gut contents contained only the pith. Therefore, it was concluded that the larvae fed on the pith and not on the fibers.

The adults of *R. obscurus* showed pronounced periodicity of activity. They were crepuscular, remaining hidden during the day and becoming active in the evening from 6:00 to 8:00 P.M. The females were found to oviposit periodically. The nonoviposition period was referred to as the "interoviposition period."

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The studies on rearing media did not progress as expected, due to the long life cycle of the weevils. However, a total of 18 media were tested. Of these, only 2 were promising. The media tested may be classified into fibrous and nonfibrous media. In general, the former were more satisfactory than the latter. The two media that appeared to be promising were those prepared from shredded coconut husk and sugarcane stalks.

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