

and Smith (1957) also found that susceptible mites lay more eggs than resistant mites, but they were unable to reach any conclusions as to differences in egg viability or rates of development.

These differences in reproductive rates support the theory that each of the populations comprises a distinct strain of *T. urticae*, and that each strain is biologically as well as toxicologically different from the S mites and from one another.

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Rearing Stored-Product Insects for Laboratory Studies: Lesser Grain Borer, Granary Weevil, Rice Weevil, *Sitophilus zeamais*, and Angoumois Grain Moth^{1, 2, 3, 4}

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ABSTRACT

Cultures of *Rhyzopertha dominica* (F.), lesser grain borer; *Sitophilus granarius* (L.), granary weevil; *S. oryzae* (L.), rice weevil; *S. zeamais* Motschulsky; and *Sitotroga cerealella* (Olivier), Angoumois grain moth, were reared in a room maintained at 80±2°F and 55±5% relative humidity on Ramona wheat contained in gallon jars. The moisture of wheat used as media was adjusted to contain 13±0.3% moisture content. Systematic rotation of the lesser grain borer was based on food consumption; rotation of cultures of the other species was based on the age of insects in each.

A 30-ml measure of lesser grain borer adults (approximately 9000 live insects) emptied onto 2 quarts of wheat resulted in an average of 29,000 insects per jar in 8 weeks.

Parent stock of *Sitophilus* spp. was left on 2½ quarts of media 1 week, and the same stock was used for 3 consecutive weeks to establish new cultures. Starting with a 40-ml measure of granary weevil adults (approximately 7000), cultures of this species yielded an average of 10,000 insects in 7 weeks; cultures started with a 27-ml measure of the rice weevil (approximately 6200) and a 30-ml measure of *S. zeamais* (approximately 5200) yielded an average of 12,000 insects in 6 weeks. Adult progeny of the Angoumois grain moth began emerging during the third week after 300 moths were placed on 2½ pints of wheat. Emergence reached its peak 5 weeks after initial infestation, and an average of 2850 moths was produced in each culture.

Procedures vary throughout the world in laboratories rearing large numbers of stored-product insects, as each laboratory uses methods most suitable to its special needs, facilities, and personnel. Although the need for standardization of techniques is well known, especially those used to rear insects for tests with insecticides, it is seldom that thorough descriptions of insect-rearing procedures are given in the literature. However, such information is essential, since the physiological condition of test insects reared under different conditions may be the basic reason for differences in results from various laboratories.

To carry out work on the relative susceptibilities of stored-product insects to insecticides, we have to rear many species under known conditions. Procedures

described by various authors in the symposium edited by Campbell and Moulton (1943) were used initially, but the many species required made it essential to evaluate management of our cultures critically and develop standardized methods for the most efficient use of time and space. Methods developed for rearing various groups of stored-product insects for our laboratory studies will be described as they become available.

It is the purpose of this paper to present data obtained on production, comments on management, and a description of methods we have adopted to rear *Rhyzopertha dominica* (F.), lesser grain borer; *Sitophilus granarius* (L.), granary weevil; *S. oryzae* (L.), rice weevil; *S. zeamais* Motschulsky, and *Sitotroga cerealella* (Olivier), Angoumois grain moth. All 5 species develop inside grain and are reared systematically on the same kind of media.

GENERAL METHODS.—*Food*.—Like most laboratories, ours use the cheapest kind of food suitable for insect cultures that is readily available at all times. Ramona wheat of good quality which has not been treated

¹ Coleoptera: Bostrichidae.

² Coleoptera: Curculionidae.

³ Lepidoptera: Gelechiidae.

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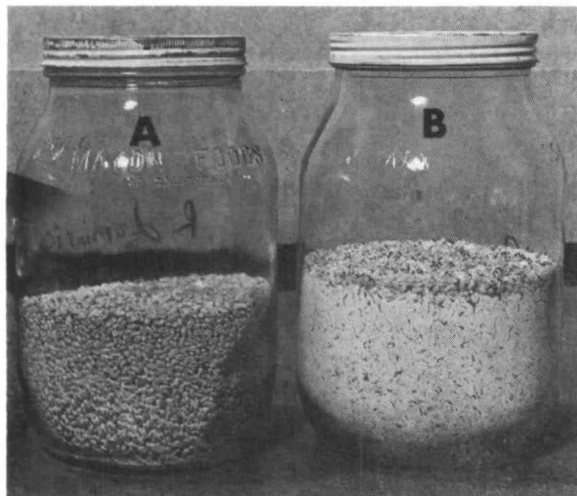


FIG. 1.—Appearance of lesser grain borer cultures 1 week (A) and 8 weeks (B) after initial infestation.

with insecticides is obtained locally and provides a satisfactory diet for all species considered in this report. When purchased, the moisture content, determined with a Steinlite 500 RC, Model 90, Electronic Moisture Tester, usually ranges from 10 to 11%, but may be lower.

Before use as media, wheat is stored at 5°F for 2 weeks to eliminate possible contamination by undesired insects. Then water is added, as needed, to increase the moisture content to $13 \pm 0.3\%$. Batches of 100 lb are blended carefully in a concrete mixer as water is added to the wheat; 3 batches are prepared each time to provide food for several weeks. Moistened wheat is stored in large, airtight metal drums and allowed to temper at least 3 days before use.

Temperature and Humidity.—The culture room is maintained at $80 \pm 2^\circ\text{F}$ and $55 \pm 5\%$ RH. Electric heaters and refrigeration are thermostatically controlled to maintain the desired temperature. Humidity control consists of solenoid actuated steam (connected to steam line), hair-element-type humidistat, and a fan to blow steam into the air. This system is similar to one used by Dr. D. L. Lindgren, Department of Entomology, University of California, Riverside, and it provides approximate ($\pm 5\%$) control of relative humidity in the range desired.

Light.—Lights in the rearing room remain on constantly during each work day. They are off at night.

Culture Containers.—A gallon jar covered with a metal lid is used to hold each culture. Ventilation is provided with a 2-in-diam screened opening in the lid; 30-mesh brass screen is used to prevent escape of the insects. Filter paper is placed inside lids used for the Angoumois grain moth.

Prevention and Control of Mites.—An infestation of predaceous mites caused the most serious problem experienced with *Sitophilus* sp., but this difficulty was eliminated with dicofol described by Strong et al. (1959). Our original stock of Angoumois grain moths was infested with an unidentified mite. Mite-free cultures were started by treating wheat containing developmental stages with dicofol (Kelthane®) in the same manner as it was used for *Sitophilus* spp. One treatment controlled mites and subsequent direct exposure to dicofol was unnecessary.

Cultures of the lesser grain borer have not been infested by mites. However, as a standard precaution to prevent mite infestations, lids of jars are covered with fresh dicofol-treated cloths held in place by rubber bands at the time all new cultures are established. Cloths are soaked in a dicofol-acetone solution made from 1 part by volume of 42% dicofol EC and 3 parts of acetone. Treated cloths are dried before use.

Prevention of Contamination by Other Insects.—During the process of screening to obtain parent stock or collecting for experiments, insects are removed from the rearing room and handled in another area as a precaution to prevent contamination. Mixed cultures of species easy to separate are a nuisance and should be avoided, but closely related species such as the rice weevil and *S. zeamais* require special precautions. In addition to the usual careful cleaning of equipment after use with each species, cultures of the rice weevil and *S. zeamais* are set up on separate days. At regular intervals samples are taken from cultures and inspected for purity.

SPECIFIC CULTURE PROCEDURES.—The basic rearing procedures we follow are described here. Comments on production and management for specific uses are made later.

The Lesser Grain Borer.—Systematic rotation of the lesser grain borer is based primarily on food consumption. Eight weeks after initial infestation, cultures have developed to the stage that most of the media has been devoured (Fig. 1 B). The 5 oldest cultures are removed from shelves when they have reached this stage; 3 are held for use in experiments during the following week, and 2 are used to establish 5 new cultures. Contents of the latter are screened with a U.S. Standard Sieve no. 10 to separate insects from wheat as shown in Fig. 2. Dust is removed from insects by screening with a no. 20 sieve. A 30-ml measure of lesser grain borer adults (approximately 9000 live insects) is emptied onto 2 quarts of wheat to establish each of the new cultures set up each week.

Ordinarily, screening of a few spoonfuls of material supplies more insects than needed in tests at 1 time, but an entire culture may be screened at any desired time from the 8th to the 9th week following initial infestation. To insure collection of active, apparently healthy insects, small quantities are placed



FIG. 2.—An 8-week-old culture of lesser grain borer is being screened by hand with a U.S. Standard Sieve no. 10; a no. 20 sieve is used next to separate dust from the insects.

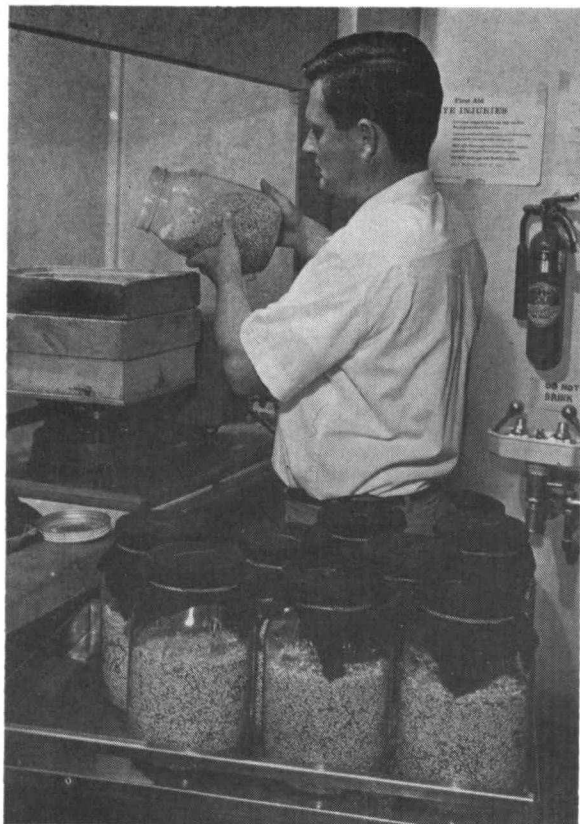


FIG. 3.—Cultures of *Sitophilus* spp. are being screened to separate adults from media with a machine adapted for this use.

in the center of large petri dishes, and the cover is placed over the dish to prevent the insects' flight. Only adults moving to the edge of petri dishes are collected for experimental use.

Granary Weevil.—For convenience, this species is reared in multiples of 5 cultures. Six weeks after initial infestation adults begin emerging from wheat. Cultures are removed from shelves at the end of 7 weeks; 3 are held for test use during the following week, and 2 are used to provide insects for new cultures. When these are removed each week, jars containing developing stages are thoroughly shaken to prevent an adverse environment created if left undisturbed (Morrison 1964). Then they are moved forward on the shelves so cultures are always arranged according to dates of infestation. Parent stock is left on media only 1 week for egg laying.

Five new cultures are set up each week; 2 contain insects used for the 1st time as parent stock, 2 have insects used for the 2nd time, and 1 has insects used for the 3rd and final time. A simple coding system is followed to permit reuse yet to avoid the use of very old insects as parent stock. This is best explained with a description of the procedure we follow in using the 2 cultures removed from shelves each week to provide insects for new cultures.

The 2 cultures are screened (Fig. 3) to separate insects from media. Then a 40-ml measure of granary weevil adults (approximately 7000) is emptied onto 2½ quarts of wheat in each of 2 jars to establish 2 new cultures; the figure 1 is written on the

treated cloth covering the containers to show that parent stock has been used only once. One week later these are screened and the parent stock is used to establish 2 new cultures; the figure 2 is written on the covers. When these are screened 1 week later, a 60-ml measure of the insects obtained from the 2 jars is used to establish 1 new culture and the figure 3 is written on the cover. After 1 week this culture is screened and the insects are discarded. Thus, each week, as new insects are introduced into the rearing cycle from the 2 cultures removed from shelves for this purpose, older insects are discarded.

Although *Sitophilus* spp. may be screened from media in many ways, a device used in the laboratory of Professor Donald A. Wilbur, Department of Entomology, Kansas State University, Manhattan, appeared especially suitable for this purpose. A Roto-matic Experimental Sifter with an electric timer was obtained from General Mills Equipment Company, Kansas City, Missouri, and adapted for use in screening *Sitophilus* spp. (Fig. 3). An entire culture is placed in the top tray over a 10-mesh stainless-steel screen. This tray holds the wheat. Insects fall into the second tray onto a 20-mesh screen, and dust falls into the pan at the bottom. The gentle circular motion of the machine rapidly separates insects from media without injury.

Rice Weevil.—This species is reared in multiples of 3 cultures. Each is started by placing adults (approximately 6200) contained in a 27-ml measure onto 2½ quarts of wheat. The parent stock is held on media 1 week for egg laying. It is then removed by screening (Fig. 3).

Five weeks after initial infestation of media, adults begin emerging from wheat. The oldest cultures are removed from shelves at the end of 6 weeks; 2 are held for test use during the following week, and 1 is used to provide insects for a new culture. Cultures remaining on shelves are thoroughly shaken and moved forward to keep them arranged according to age.

Three new cultures are established each week; 1

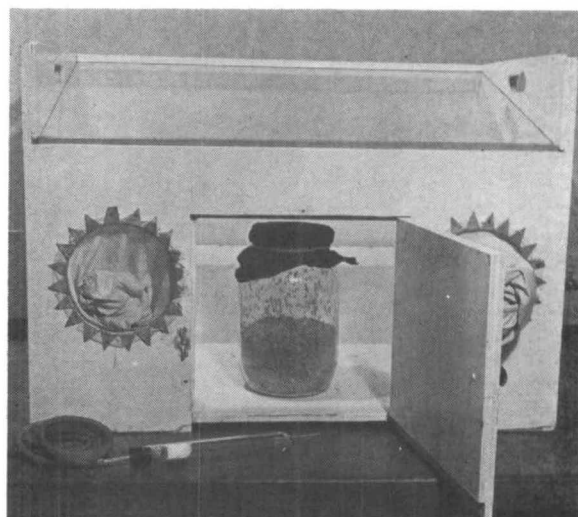


FIG. 4.—A culture of Angoumois grain moths ready for release inside a sleeve cage. The glass tube in front of the cage is used to collect adults. The culture jar has been tilted to disturb moths and appears to contain more media than is used in cultures.

Table 1.—Production of cultures of 4 species of grain beetles on wheat in gallon jars.

Species	Weeks from initial infestation to use	No. live adult insects/culture (thousands)	
		Mean	Range
Lesser grain borer	8	29	28–31
Granary weevil	7	10	9–11
Rice weevil	6	12	11–13
<i>Sitophilus zeamais</i>	6	12	10–14

with insects used for the 1st time as parent stock, 1 with insects used for the 2nd time, and 1 with insects used for the 3rd and final time. Obviously this procedure is accomplished simply by using parent stock for 3 consecutive weeks. A coding system similar to that described for the granary weevil is followed. As the older parent stock is screened from media and discarded, fresh insects are introduced into the cycle each week from the culture removed from shelves to provide stock for this purpose.

Sitophilus zeamais.—This species also is reared in multiples of 3 cultures. Each is started by emptying a 30-ml measure of adults (approximately 5200) onto 2½ quarts of wheat. Otherwise the procedure is exactly the same as that described for the rice weevil.

Angoumois Grain Moth.—New cultures are started by placing 300 moths on 2½ pints of media. The moths are collected in a sleeve cage (Fig. 4) with glass tubes made for this purpose, anesthetized with CO₂, and released on wheat. Containers are placed on their sides at the time parent moths are introduced. They are kept in this position during the 1st week to allow the maximum surface area for egg deposition. Then they are set upright. We usually rear this species in multiples of 6 cultures, but as many as desired may be started each week.

New adults begin emerging from wheat during the 3rd week, and emergence reaches its peak 5 weeks after initial infestation. Cultures are discarded at the end of 6 weeks. Any moths capable of flying from the container into the sleeve cage may be collected and used to start new cultures, but only moths of a known age are used in laboratory tests with insecticides. All moths are removed from each jar at a designated time prior to such use so that moths collected for exposure to insecticide treatments will be within the age limits desired.

PRODUCTION.—Since a knowledge of production is essential for efficient management of cultures, measurements were made to determine the number of insects grown. Small volumes of insects were taken from cultures of each species of grain beetles. These were counted to obtain basic figures for estimating total populations. Then, during each of 3 consecutive weeks, all insects were collected from 1 culture of the lesser grain borer, 3 cultures of the granary weevil, 3 cultures of the rice weevil and 2 cultures of *S. zeamais*. Insects from each were measured separately, and an estimate of the total population per culture was calculated (Table 1).

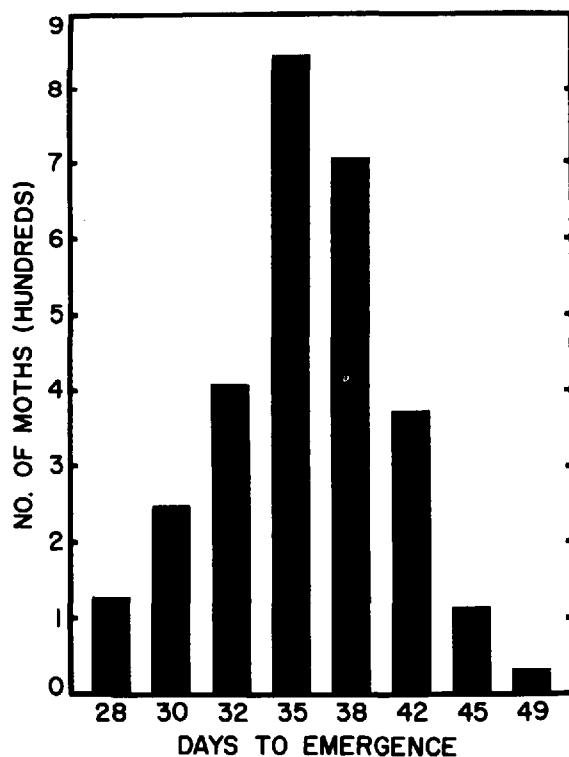
Moths were collected periodically, counted, and removed from 3 cultures of Angoumois grain moths from the time they began emerging up to 7 weeks after initial infestation. Total production (Fig. 5)

ranged from 2622 to 3208 moths, with an average of 2850 moths/culture.

COMMENTS.—Data given in Table 1 and Fig. 5 serve as a guide in scheduling routine experiments according to insects available or, if special work is planned, we can estimate the number of extra cultures needed and proceed accordingly. Other innovations can be made easily to provide insects for specific purposes without changing basic rearing procedures.

Adult Angoumois grain moths should be handled as described for use in experiments, since moths of this species have a relatively short life span, but cultures could be set up more often to provide peak populations over a period of several days each week. Cultures of *Sitophilus* spp. may contain adult insects ranging from 1 day to 2 weeks old when used in tests. If desired, we might screen adults from media periodically, and insects collected could be held for use when they reached the exact age required.

We have not experienced difficulty with the procedure described for rearing the lesser grain borer, but cultures of this species are less flexible to manage. Since adults promptly burrow into grain (Fig. 1 A) and are not readily removed by screening, the age of adult insects taken from cultures for use in experiments is not known. However, for critical work requiring insects of a known age, parent stock could be placed on a medium containing approximately 10% ground wheat mixed with whole wheat kernels as described by Strong and Sbur (1964). After the desired exposure for deposition of eggs into this medium, it could be separated from adults and whole kernels of wheat by screening. Then if the ground

**FIG. 5.**—Mean number of adults collected from cultures of Angoumois grain moths at various intervals after initial infestation.

wheat containing eggs of the lesser grain borer should be dispersed in a fresh batch of wheat, insects of a known age would be available for use in experiments when adults emerged.

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A Bioassay for Detecting Compounds Which Stimulate or Deter Feeding by the Sweetclover Weevil^{1, 2}

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ABSTRACT

A bioassay employing sweetclover root disks impregnated with various plant extracts has been developed. The bioassay has been used to demonstrate the distribution of substances influencing feeding in fractionated water-methanol-chloroform extracts of *Melilotus infesta*

Guss. and *M. officinalis* L. Lam. leaves. Indications are that substances responsible for the resistance of *M. infesta* or the susceptibility of *M. officinalis* to feeding by adult sweetclover weevils, *Sitona cylindricollis* Fähræus, reside in the water-methanol fraction.

Among 19 *Melilotus* species screened by Manglitz and Gorz (1964), *M. infesta* Guss. was the only species not fed upon to an appreciable extent by the adult sweetclover weevil, *Sitona cylindricollis* (Fähræus). The weevil resistance of *M. infesta* was confirmed by Gross and Stevenson (1964) and Radcliffe and Holdaway (1964).

Since the resistance of *M. infesta* is manifested by a refusal of the adult weevils to feed extensively on this species, it appears that resistance may result from either a lack of attractiveness or the presence of deterrent substances. Thus, studies to determine the chemical nature of this resistance should focus first on detecting substances which would stimulate or deter weevil feeding.

One prerequisite for a study of the chemical nature of resistance is a suitable bioassay for selecting biologically active fractions in various plant extracts. This paper describes such a bioassay and gives results obtained from preliminary experiments using the bioassay.

MATERIALS AND METHODS.—One of the first requirements in the development of an effective bioassay is a suitable means of presenting the extracts or fractions to the weevils for feeding. For a bioassay involving the sweetclover weevil, which feeds exclusively from the leaf margins, the requirements for the feeding medium are: (a) the medium must be thin enough to allow feeding on the edges but rigid enough to support the weight of the weevils; (b) the consistency of the medium must permit chewing by

the weevil; (c) the medium must be relatively inert so as to prevent appreciable feeding on the untreated material and to avoid the presence of substances which alter the feeding response of the weevil to the active chemical components; and (d) except for changes caused by feeding, the material should remain constant in size and shape so that the extent of feeding can be readily determined. Many materials, including pith from the Japanese elder as used by Thorsteinson (1955), agar blocks, and disks of potato tuber, carrot, sweet potato roots, cabbage, and celery hearts were tested as media for the bioassay, but all were unsuitable with respect to one or more of the foregoing requirements. Disks cut from sweetclover roots and subjected to certain pretreatments are the most satisfactory of the bioassay media thus far tested.

First-year plants of field-grown Evergreen sweetclover (*M. alba* Desr.) provided the roots used in preparing the bioassay disks. The plants were dug in November 1965, and fleshy branch roots from 12 to 15 mm in diam were selected. Sections approximately 20 mm long, cut from the central portion of these roots with a 9-mm cork borer, were sliced into disks 0.1 mm thick with a hand microtome. The disks were washed several times in distilled water, suspended in water (50 ml/300 disks) in a 250-ml flask, frozen, and lyophilized. To inactivate enzymes and remove alcohol and chloroform-soluble constituents, the lyophilized disks were refluxed for 4 hr with boiling 100% ethanol followed by 4 hr of extraction with chloroform and 4 hr with methanol in a Soxhlet thimble. After treatment, the disks were stored under methanol at -20°C until used.

Prior to use in the bioassay, disks were attached to no. 1 stainless-steel insect pins cut to a length of 13 mm. The 5 disks used for each treatment were placed in a 50-mm watch glass containing 0.15 to 1 ml of plant extract which had been evaporated nearly to dryness under vacuum. Sufficient water was added to nonaqueous extracts or fractions so that the disks were still damp after the organic solvents had evaporated. Petri plates 15 mm deep and 95 or 145

¹ Coleoptera: Curculionidae.

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