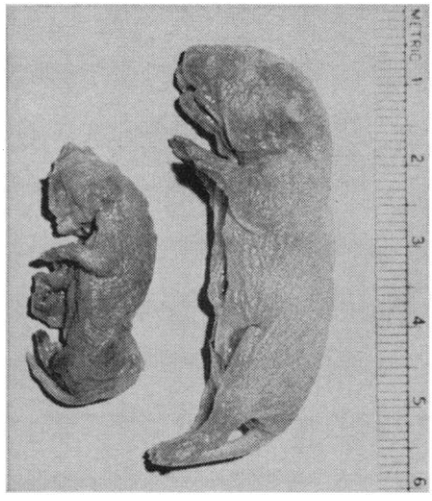

Fig. 1. Two stillborn fetuses of a rat (No. 2-5-2) injected with LSD early in pregnancy: one is fully developed; the other, immature.

Gross examination of the young from all experiments revealed some overall stunting of development in the case of three stillborn and one that survived for 6 weeks (Fig. 1), but no other abnormalities. Offspring from rats treated with LSD weighed at birth as much on the average as the controls; later some of them grew as well, but others failed to develop at the same rate. For example, average control offspring weighed 64 g at 10 days, while an average offspring of treated rat No. 2-4-1 weighed only 44 g; of treated rat No. 2-4-2, 44 g; and of treated rat No. 2-6-1, 46 g. The stunted offspring of treated rat No. 2-6-2 weighed only 54 g at 36 days, while his apparently healthy littermates weighed 80 to 106 g, averaging 96.5 g.

Our results possibly may be explained by the recent finding of chromosomal abnormalities in cultured cells grown in the presence of LSD (*4*).

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Diets for Rearing the Ambrosia Beetle Xyleborus ferrugineus (Fabricius) in vitro

Abstract. *Female ambrosia beetles placed on media containing sucrose, yeast extract, casein, starch, wheat germ, cottonseed oil, salt mixture, agar, water and cacao sawdust or powdered cellulose excavated galleries, oviposited, and produced progeny that developed to maturity. Several generations have been raised in the laboratory on media inoculated with ambrosia fungi by the beetles.*

The Scolytidae is one of the most destructive families of insects in the world. Although much research on important species of this family has been conducted (*1*, *2*), the means by which they nutritionally utilize their natural woody substrate are essentially unknown. The associations of microorganisms with Scolytidae in wood were reported in early studies of this family (*1*, *3*), but the nature of any nutritional symbiosis between the microorganisms and the beetle remains obscure (*4*).

Study of the nutrition of this family has lagged behind similar studies of many other important families of insects largely because it is difficult to investigate Scolytidae within intact woody tissue. Efforts to rear scolytids through their entire life cycle have failed, although various media have permitted larval development for several bark beetles (*5*).

We report here successful techniques for rearing *Xyleborus ferrugineus* (Fabricius) and *X. posticus* Eichhoff on several artificial media under laboratory conditions. These are two ambrosia beetles that attack cacao, *Theobroma cacao* Linnaeus, in tropical areas of the Western Hemisphere.

Most of the experiments on rearing scolytids on artificial media involved *X. ferrugineus*, but *X. posticus* was studied on diets containing cacao sawdust. Five media were used (Table 1); these will be referred to as diets A, B, C, D, and E.

The ingredients of diet A were mixed in a Waring Blendor, placed in tubes (approximately 15 ml per 20- by 150-mm culture tube), plugged with cotton, and autoclaved. For the other diets, all ingredients, except water and the aqueous extract of cacao bark, were mixed dry in a beaker, and then the liquid ingredients were added to make a uniform slurry. The extract of cacao bark (diet D) was prepared by macerating 100 g of air-dried cacao bark in 1 liter of distilled water in a Waring Blendor and filtering the resulting liquid through six layers of cheesecloth. Diets B through E were placed in tubes (approximately 12 ml per 20- by 150-mm culture tube) and the tubes were plugged with cotton and autoclaved. Most tubes of media were then used immediately after cooling.

In studies with diet A, female beetles, newly emerged from the host tree, were introduced (one per tube) and observed daily for activities and development of progeny. The cultures were held in a laboratory at 24.5°C until eggs were observed; then the tubes were divided into two groups (72 and 77 tubes, Table 2). One group (77 tubes) was continued under the uncontrolled light conditions of the laboratory, and the other (72 tubes) was held in continuous dark, except during observation. After 60 days the medium was carefully removed from each tube and the progeny were counted.

In studies with diets B through E, two female beetles were released in each tube. The beetles used on diet B had emerged from cacao trees, and were held in fresh cacao sawdust for 4 days during transport from Costa Rica to Wisconsin. Beetles used on diets C through E were progeny from rearings on diet B. All cultures were maintained in the dark at 22°C except during observations.

As progeny matured in diet B, the tubes were unplugged and inverted as a group over a beaker containing four sheets of moistened filter paper in the bottom. The group of culture tubes (17 tubes, Table 2) was wrapped tightly in aluminum foil to stimulate emergence of progeny from the darkened tubes into the lighted beaker. The beaker was held in the laboratory at 22°C under uncontrolled light.

The culture tubes containing maturing adults on diets C through E were unplugged, wrapped with foil, and each attached mouth to mouth to a similar tube containing a moistened plastic-foam disc. The progeny were removed and counted twice each week.

Beetles mined extensively through each diet. In most tubes, major portions of the brood galleries were excavated adjacent to the tube wall, thus facilitating observations of insect activity and development. The total numbers

Table 1. Composition of various diets used for rearing *Xyleborus ferrugines* in vitro. Values are grams of solid ingredients or milliliters of liquid ingredients.

Ingredient	Diet				
	A	B	C	D	E
Sucrose	15.0	30.0	13.4	18.8	18.8
Yeast extract	10.0	20.0	8.9	12.5	12.5
Casein	10.0	20.0	8.9	12.5	12.5
Starch	10.0	20.0	8.9	12.5	12.5
Wheat germ	5.0	10.0	4.5	6.3	6.3
Cottonseed oil	5.0	10.0	4.5	6.3	6.3
Salt mixture (Wesson)	1.3	2.5	1.1	1.6	1.6
Agar	40.0	80.0	36.0	50.0	50.0
Cacao sawdust (fresh)	200.0	550.0			
Cacao sawdust (dry)			179.0		
Cellulose powder				250.0	250.0
Cacao bark extract				845.0	
Water (distilled)	1000.0	1000.0	1000.0	155.0	1000.0

Table 2. Production of *Xyleborus ferrugineus* on various semiartificial diets. Rearings with diet A were held at 24.5°C at Turrialba, Costa Rica; rearings with diets B through E were held in darkness at 22°C at Madison, Wisconsin.

Diet	In vitro generation	Females per tube	Tubes (No.)	Tubes with brood	Rearing terminated (days)	Total progeny			
						Mature		Per tube*	
						Female	Male	Range	Mean
A†	1	1	77	47	60	484	15	1–68	10.9
A‡	1	1	72	35	60	532	14	2–65	17.1
B	1	2	18	17	84	564	10		33.8
C	2	2	45	24	95	296	9	1–33	12.7
D	2	2	20	14	119	572	11	7–93	41.7
E	2	2	20	14	119	480	10	6–74	35.0

* Immature stages included in data for diet A; only emerged adults included for diets B through E. † Uncontrolled light conditions. ‡ Continuous darkness.

of mature progeny found in the tubes at 60 days on diet A and those that emerged from the tubes containing diets B through E are presented in Table 2. Fifty-five percent of the maternal females produced brood on diet A. On this diet, eggs were first observed 7 days after introduction of the females; larvae, pupae, and adults were observed at 14, 17, and 22 days, respectively. After 60 days, the progeny were: adults, 96.7 percent; pupae, 1.5 percent; and larvae, 1.8 percent. The sex ratio of progeny adults on diet A was 1 male to 35 females, about normal for this species. The mean numbers of progeny per female in darkness as compared with uncontrolled light were 17.1 and 10.9, respectively.

Diets B through E were each satisfactory for the completion of brood development. The approximate 12 ml of diet per culture tube appeared adequate for the broods of two females. The highest percentage (94 percent) of successful cultures (produced progeny) was on diet B; however, the greatest mean number of adult progeny per tube was on diet D. The insects did not require cacao tissue or extract in the diet (diet E). However, the addition of extract of cacao bark to the cellulose-based diet D did stimulate a quicker initiation of gallery construction than occurred in diet E.

Eggs were first observed 4 days after placing females on the diets. Oviposition occurred most frequently at the terminals of galleries. In observations made 14 days after females were released on diets D and E, the number of eggs per ovipositional site averaged 5.6, but ranged to 23. After ovipositing at one location, the female frequently excavated one or more branch galleries and laid again. With maturity, the larvae became more mobile, and they dispersed throughout the gallery system. By the 4th week after initiation of insect cultures, all stages of progeny were observed in galleries.

Initial emergences of progeny adults from the inverted tubes generally occurred during the 4th and 5th weeks after the cultures were established. The peaks in emergence of progeny occurred on all diets during the 7th or 8th week. During this period, production on diets B, D, and E averaged greater than one mature beetle per tube per day. Emergence continued through 17 weeks from diets D and E, indicating that the maternal female continued to oviposit for several weeks or that mature female progeny extended the original galleries and oviposited. The ratio of males to females in the progeny at Madison was approximately 1 to 48. Infrequently, dead progeny (usually males) or living callow males and females would fall from the inverted culture tubes, but more than 95 percent of the individuals were mature and appeared normal in size and activity.

No provisions were made to inhibit the growth of microorganisms on the media after the beetles were introduced. In essentially every culture tube mycelial growth of a species of *Fusarium* was obvious by the 4th day after introduction of the beetles. Mycelial growth occurred first next to the galleries but mycelial mats eventually covered the entire surface of the media. The ambrosial organisms for *X. ferrugineus* have not yet been identified.

Rearing trials with *X. posticus* were also successful on diets containing cacao sawdust, but survival and progeny production were less than with *X. ferrugineus*.

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