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SOME FACTORS INVOLVED IN A
METHOD FOR REARING THE BOXELDER BUG,
LEPTOCORIS TRIVITTATUS SAY.

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE
Robert C. Wilkinson, Jr.
1950



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Some Factors Involved in a
Method for Rearing the Boxelder Bug,
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of the requirements for

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Ray Hutson
Major professor

Date July 20, 1950

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SOME FACTORS INVOLVED IN A METHOD FOR REARING
THE BOXELDER BUG, LEPTOCORIS TRIVITTATUS SAY.

By

ROBERT C. WILKINSON, JR.

A THESIS

Submitted to the school of Graduate Studies of Michigan
State College of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

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Department of Entomology

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Acknowledgement

I would like to offer personal appreciation to those members of the college entomological and botanical staffs who gave information and suggestions essential to the solution of the problem.

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Introduction

At the beginning of the fall term in 1949, it was suggested that the writer investigate possibilities for rearing the boxelder bug, Leptocoris trivittatus Say.

The reason for considering the project as material adequate for use in a thesis, after first attempts failed, was the importance attached to the feasibility of utilizing these insects for chemical spraying tests. The suitability of the boxelder bug for this purpose was based on its perennial abundance, coupled with the fact that the various stages offer considerable resistance to control by common, chemical means.

The problem, simply, was one of determining how to propagate the boxelder bug in an inside laboratory, with commonly available materials and equipment, in such a manner that the various stages could be drawn from surplus stock to be used for testing throughout the year.

The following is a record of such an attempt.

CLASSIFICATION, DESCRIPTION, AND SYNONYMY

CLASSIFICATION:

Phylum Arthropoda

Class Hexapoda

Order Hemiptera (Heteroptera)

Family Coreidae (Corizidae)

Genus Leptocoris Hahn 1831

Species trivittatus Say 1825

DESCRIPTION (3): (Plate I, immediately following)

"Elongate-oval, depressed above, subconvex beneath. Above fuscous-black, very finely pubescent, subopaque; ocelli, narrow, median line and broader, marginal stripe of pronotum behind transverse impression, also its hind margin very narrowly, clear red; costal and apical margins, and usually the nervures of elytra, dorsum and inner wings, red, or in part orange-yellow; membrane fuscous; under surface fuscous-black, the margins and middle of abdomen, the sides of sternal pleurae and the coxae, red; eyes brown. Head and pronotum minutely granulate-punctate, the latter with an elevated, narrow, median line behind the transverse impression; beak reaching hind coxae, joint (1) as long as head, (3) and (4) subequal, (2) longest. Scutellum and elytra similarly punctate, length 11-13.5 mm., width 3-4 mm."

Plate 1. Drawings of boxelder bug (after E.O. Essig (9), p. 333)

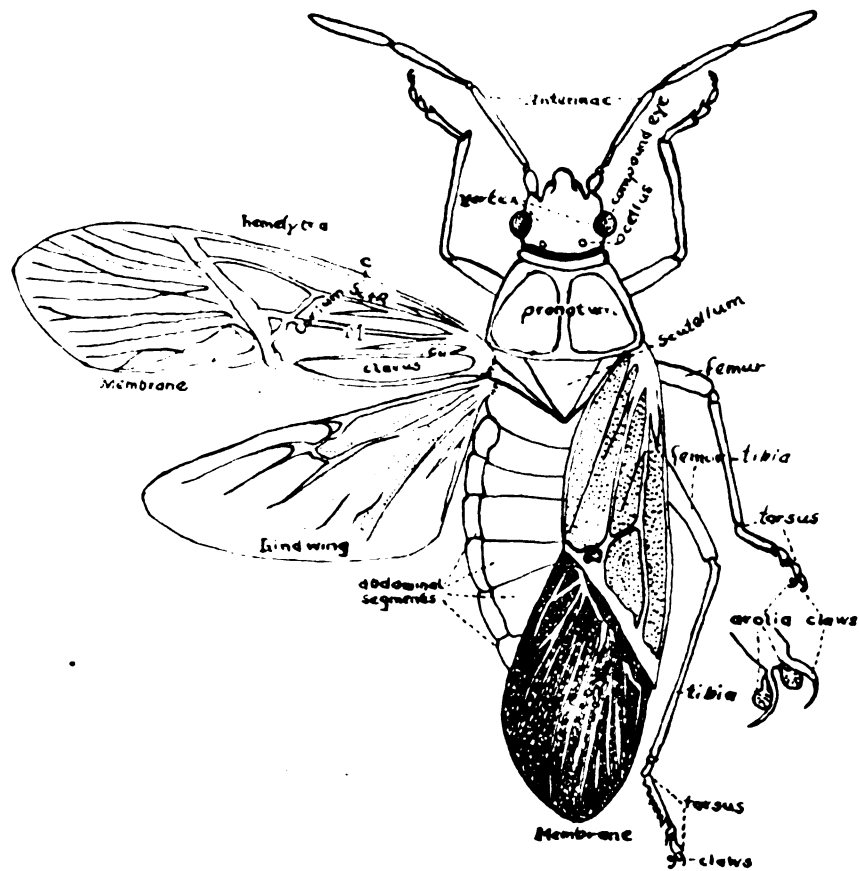


Figure 1. Dorsal view of external anatomy. X7 .

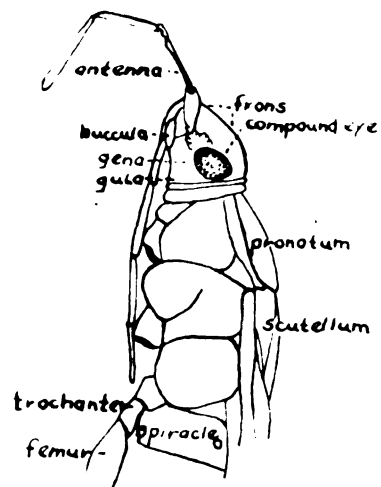


Figure 2. Lateral view of head and thorax. X7 .

SYNONYMY (9):

Leptocoris trivittatus (Say) 1825

Serinetha trivittata (Say)

Lygaeus trivittatus Say

Review of Literature

Thomas Say (28) gave the historical description of Leptocoris trivittatus in 1825, while Blatchley (3) pictured the insect in detail a century later, together with definitions of the pertinent family, tribe, and genus. Van Duzee (40) catalogued the complete classification. Essig (9) has listed a brief synonymy, but excluded dates and sources.

Although many brief, general accounts and notes have been published to date, Smith and Shepard (32) have apparently written the only complete discussion of the life history and food habits.

The internal anatomy of this insect was made the subject of a study by Wooley (41), and Payne (25), (26) described the genitalia and associated phenomena. Kay (11), (12) investigated some of the microscopic life found in the body tracts.

Peterson (27) has included valuable information on insect-rearing, much of it based on a pioneer paper concerning temperature and humidity effects by Smith (31). A method of humidity control, with tables, was authored by Stevens (35).

Bare (1), Lomax (17), McDaniel (21), Metcalf and Flint (22), and Munro and Post (24) have written about the principle control measures which have proven more or less effective against the boxelder bug.

REVIEW OF HISTORY, HABITS, AND CONTROL

HISTORICAL:

Originally described as inhabiting the Missouri territory, yearly insect-population reports have indicated progressive north and eastward extension of the insects' range.

This spread has been attributed to homestead plantings of the boxelder, Acer negundo L., which, with its five varieties, covers most of the United States.

HABITS:

After overwintering under rocks, debris, or in houses, the adults leave hibernation during early spring to feed, mate, and lay eggs in the opening buds, on flowers, seeds, or in bark crevices of pistillate boxelder trees.

The nymphs emerge in two weeks time, feed, and molt five times before reaching maturity in about two months time. Total life cycles require from fifty to seventy-eight days time. In warmer climates two generations occur each year.

Other than a housewifely regard of the bug as a nuisance, little importance is attached to the pest, economically speaking. The insect does not ordinarily attack humans, however, Usinger (39) reported such an attack after fasting, and observed feeding on beefsteak. He also raised nymphs through several instars on blood serum.

Knowlton observed feeding on moribund bees, while cannibalism on devitalized bugs is common in the laboratory.

Ordinarily, nymphs and adults feed on the foliage, tender shoots, flowers, and fruit of boxelder, with occasional attacks on ash and maple. The nymphs descend or fall to the ground, where they have been observed to feed on twenty-three known species of weeds and grasses.

Feeding on immature and ripe fruit of apple, cherry, peach, plum, and grape by nymphs and adults have caused much damage at times in the West. Hutson (10) found all stages attacking everbearing strawberry foliage and fruit with resultant death of many of the plants.

CONTROL:

A brief survey of the methods used for control is included here, since the reason for undertaking such a study was based on ultimate use of the insect for insecticidal tests.

Nymphs have been killed by drenching them with cold water, kerosene, and oil, or by spraying with soap solutions of nicotine. Dusting with 4 percent nicotine has been effective.

All stages have been controlled by drenching masses with kerosene emulsions, hot water, and solutions of Dreft.

Various contact sprays such as pyrethrum, derris, thiocyanate, and nicotine, in concentration twice as strong as those used for aphids, have been tried with some success. Use of two-percent solutions of chlordane, lindane, toxaphene, and a nitroparaffin compound has shown excellent results (24).

Methods of Study

Semi-dormant, adult bugs were collected from outdoors during the fall and stored in a two-quart mason jar, with small perforations in the lid, at temperatures just above freezing. Additional stock was obtained from outside sources during the winter.

Boxelder seed, referred to as seed in the following discussion, was collected from trees before frost occurred, and during the winter. A series of tests was carried out to determine what temperature was best suited for storage, but viability of seed stored at room temperature was not found to be appreciably different from that of seed subjected to single temperatures, or combinations of temperature changes above and below freezing.

The bugs were bred in different kinds of containers, and offered various, available foods.

Those cages containing plants were either placed directly under, and two feet distant from, two banks of continuously-operating, fluorescent lights, or were exposed to sunlight from a south-facing window. Other cages were exposed either to the fluorescent rays at a five to ten-foot distance, or were exposed to sunlight.

Data was taken throughout the work regarding preference for egg-laying sites, rates of egg-laying and hatching, dates of ecdysis, and time required for total life cycle. Results were checked by repetition whenever successful rearing was obtained.

Eggs were removed daily from the cages and placed in covered petri dishes lined with a bottom covering of filter paper, moistened with wet cotton.

Indoor room conditions were checked regularly during the processes. A Tycoos Hygrodeik was set up, and relative humidity readings taken with a twelve-inch fan creating the required air current. A revolving-drum-type thermograph recorded temperatures, and was found to vary not more than plus or minus one degree Fahrenheit from readings on two standard, liquid thermometers, placed at different points in the room.

LIST OF MATERIAL USED

EQUIPMENT:

1. Tycos hygrodeik.
2. Electric fan.
3. Thermograph.
4. Two standard, liquid thermometers.
5. Two banks of dual fluorescent lights.
6. Refrigerator.
7. Dissecting microscope with grid lens.

REARING CONTAINERS:

1. Glass battery jars.
2. Two-quart mason jars.
3. Cardboard ice-cream containers.
4. Circular, wire screen cages.
5. Wire screen cage, $1\frac{1}{2}'$ x $2'$ x $2'$.
6. Glass lamp chimney cages.
7. Glass terrarium.
8. Petri dishes.
9. Test tubes.
10. One-half pint milk bottles.
11. Miscellaneous glass jars.
12. Cotton.
13. Filter paper.
14. Cheesecloth.
15. Vaseline.

ITEMS FOR FEEDING AND WATERING:

1. Cotton.
2. Glass vials.
3. Syracuse watch glasses.
4. Boxelder seed and assorted foods.
5. Flower pots in water containers.
6. Scotch tape, string, sticks, and pins for fastening food in containers.

PROCEDURE, RESULTS, AND DISCUSSION

TESTS CARRIED ON IN A "FIXED" TEMPERATURE ROOM.

Temperature:

The temperature maintained was 80° F., plus or minus 2°. Optimum range for a large number of temperate-zone species is reported to be from 75° to 80° F. (5).

To assume that this temperature prevailed both in and outside of the cages would be erroneous. The following table allows for more accurate estimate of conditions (27).

Table 1.--Estimating temperature changes within rearing containers.

Condition created	:	Internal temperature changes.
Exposure of uncovered screen or glass cages to sun or other light.	:	Increase of 2° over surrounding air.
Covering cages with 38 x 44 weave cotton gauze under light.	:	Increase up to 10° over surrounding air.
Covering cages with gauze in shade.	:	Increase is negligible.
Inclusion of plants in cages.	:	Maximum decrease of 7° F.

Relative humidity:

The humidities varied widely, from a low of 33 percent to a maximum of 77 percent, with a mean of 57 percent. An increase of 5 percent over room humidity might be expected in gauze-covered cages. Inclusion of plant material would also increase humidities in the containers.

Light:

Cotton gauze had been measured to cut down light intensities from 75 to 45 candle power. Those gauze-covered cages placed directly under light sources probably did not receive appreciably more light than glass cages placed farther away.

Food and feeding habits:

Boxelder seed was offered in glass containers, with water made available. Results are tabulated below.

Table 2.--Feeding on boxelder seed.

Seed preparation	:	Feeding
Water soaked.	:	All stages refused.
Boiled nearly to dryness.	:	All stages refused.
Whole, dried.	:	Adults and older nymphs fed readily.
Hulled.	:	Adults and older nymphs fed.
Sprouted.	:	All stages fed on unmolded food.
Chopped, dried.	:	All stages fed on unmolded food.
Green.	:	All stages fed on unmolded food.

Boxelder seedlings were grown either in flower pots covered with cylindrical screen, or glass, lamp-chimney cages, or in a cotton gauze-covered terrarium.

All stages fed intermittently on seedlings, especially the midribs of the more succulent leaves. Stems were infrequently attacked. Green foliage, detached from pistillate trees, was attacked, but molded readily. Dried seed in cages with plants was preferred to foliage.

Everbearing strawberry plants were grown in wire cages, in lamp-chimney cages, and in a terrarium.

Table 3.--Results of feeding on strawberry plants.

Conditions	:	Feeding
Plants and seed, plus water.	:	Slight.
Plants plus seed.	:	Periodic.
Plants plus water.	:	Some.
Plants.	:	Periodic.

Table 4.--Miscellaneous foods.

Food offered	Resultant feeding	
	Adults	Nymphs
Germinated red snap beans in test tube.	Slight.	Slight.
Red snap bean plants in terrarium (with seed and lettuce).	Slight.	Slight.
Milkweed seeds and plants in wire cage.	None.	None.
Forcing lettuce plants with seed.	Slight.	None observed.
Sliced cucumber, and dried, rolled oats.	Not observed.	None.
Karo corn syrup, in water solutions of 10, 20, 30, 40, and 50 percent concentration through wick in small vial.	None.	None.
Fruit fly agar infected with mold.	Not observed.	None.
Shepherd's Purse, <u>Capsella bursa-pastoris</u> (L.) Medic. and other unidentified weeds.	Slight.	Slight.
Whole apples, with water made available.	Some.	Old nymphs, some.
Cut apples, and water.	Some.	All stages, some.
<u>Euphorbia humistrata</u> Engelm.	Slight.	Heavy.

Preference for egg-laying sites:

Eight copulating pairs of bugs were included in a cheesecloth-covered battery jar containing water. Seed and various common materials were placed in the container in several positions.

Table 5.--Egg-laying site preference.

Place eggs were laid	:	Number laid
Wings of boxelder seeds fastened to sides of jar.	:	271
Cheesecloth top.	:	127
String supporting seeds.	:	92
Jar walls and bottom.	:	48
Toweling in bottom of jar.	:	35
Water fountain.	:	8
Cheesecloth ball on side of jar.	:	1
Cotton in bottom and on sides of jar.	:	0
Total	:	589

Egg-laying and hatching rates:

Table 6.--Egg-laying and hatching.

Conditions	: Average number eggs laid : per female.	: Percent of eggs : hatched.
Eight couples in cheesecloth- covered battery jar, with seed and water. (Fig. A, p. 18)	74/15 days laying time.	87 percent (sample of 47)
One couple in jar as above. (Fig. B, p. 18)	195/20 days laying time.	48
One couple in jar as above. (Fig. D, p. 19)	312/30 days laying time.	45
Seeds and seed- lings in lamp- chimney cage.	48	46
Terrarium contain- ing seed, water, strawberry, and boxelder seedlings.	355 total several couples.	61
Lamp-chimney cage with strawberry and seed.	77	62
Cheesecloth-covered battery jar contain- ing water supply, chopped seed, and bottom-covering of filter paper moistened with cotton.	142 (placed in con- tainer)	50

FIG. A: RATE OF EGG-LAYING: 8 FEMALES: 589 EGGS

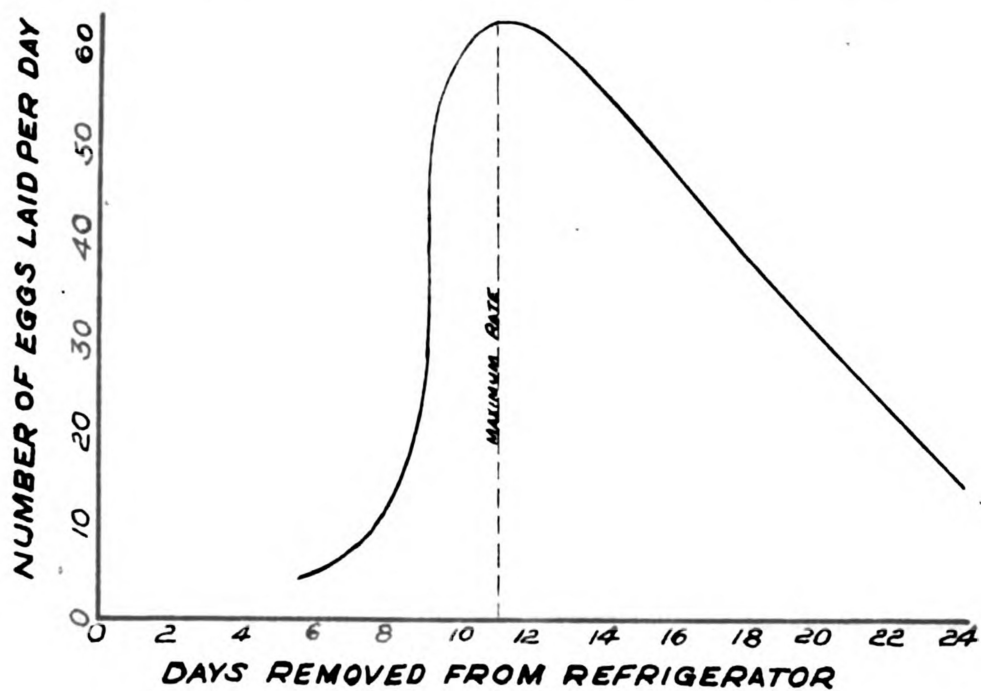


FIG. B: RATE OF EGG-LAYING: ONE PAIR BUGS: 195 EGGS

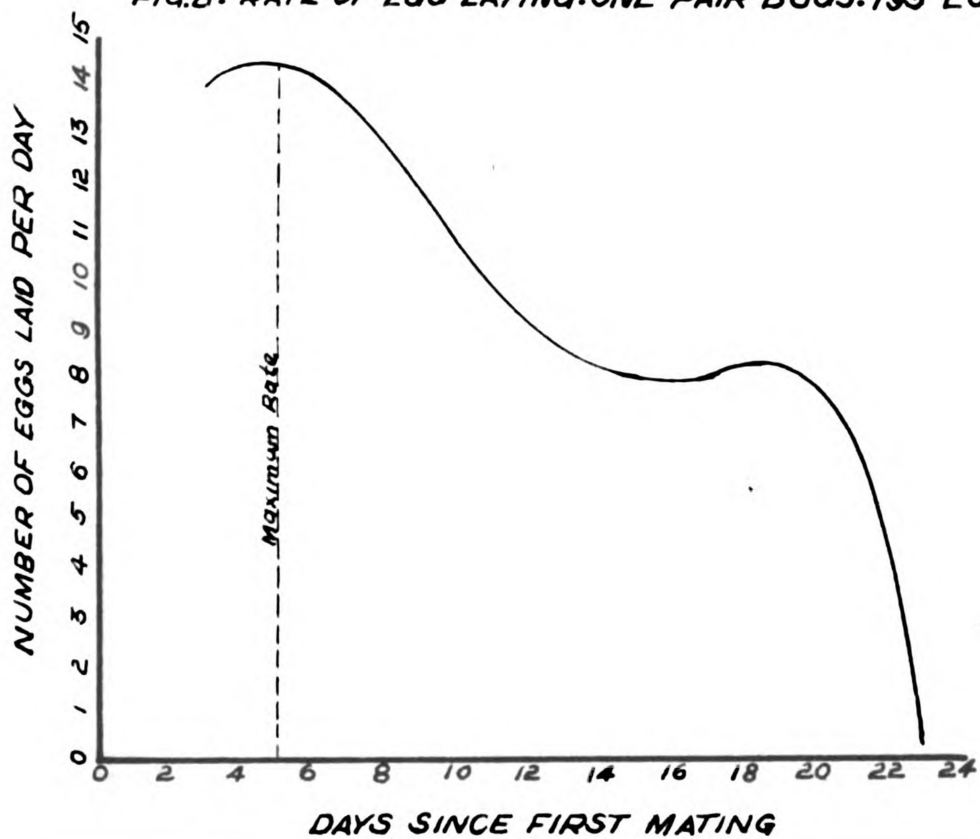


FIG.C:EGG-LAYING RATE:ONE FEMALE: 183 EGGS

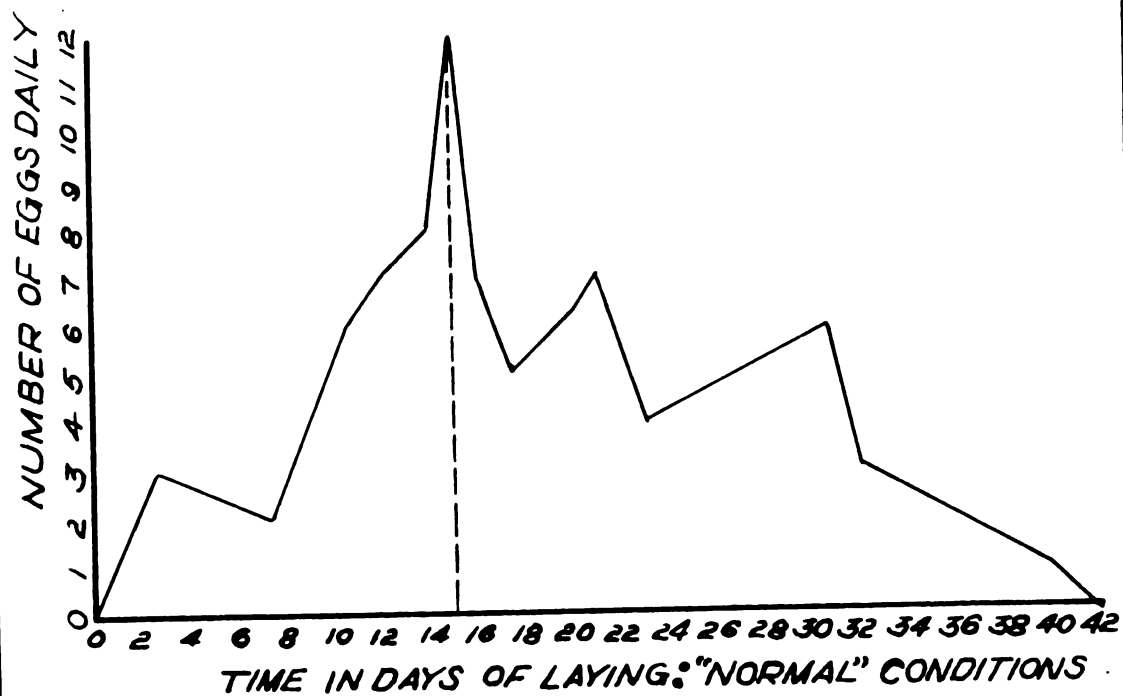
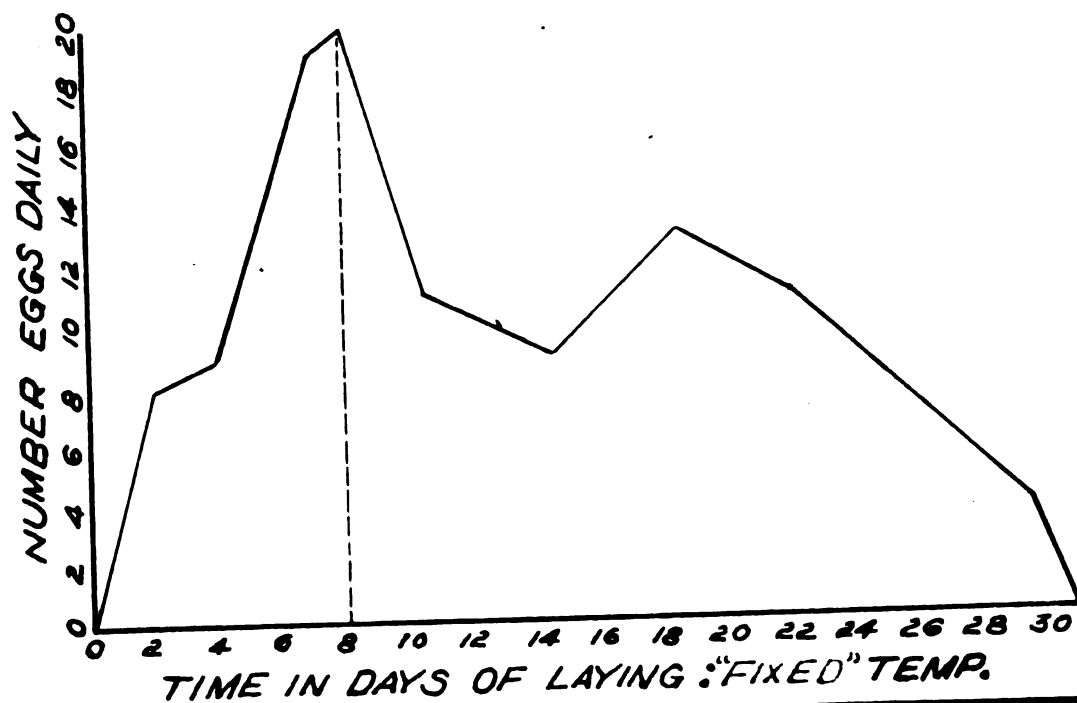


FIG.D:EGG-LAYING RATE:ONE FEMALE:312 EGGS



Days required to hatch:

An average of five to six days was required for the hatching of eggs. (Graph, Fig. E., p. 21.)

Nymph development:

Eggs from one parent were hatched in small, glass dishes, with sprouted seed for food.

Daily counts taken of the living offspring showed that the death-rate was highest during the first four days after hatching. (Graph, Fig. F., p. 21.)

A number of nymphs were raised individually in small containers, with chopped seed for food, in order to observe molting.

Table 7.--Summary of molting rates.

Instar number	:	Average number of days before ecdysis.
I	:	4
II	:	4
III	:	6
IV	:	3
V	:	5
Total		22

FIG.E:EGG-HATCHING RATE: BASIS 94 EGGS

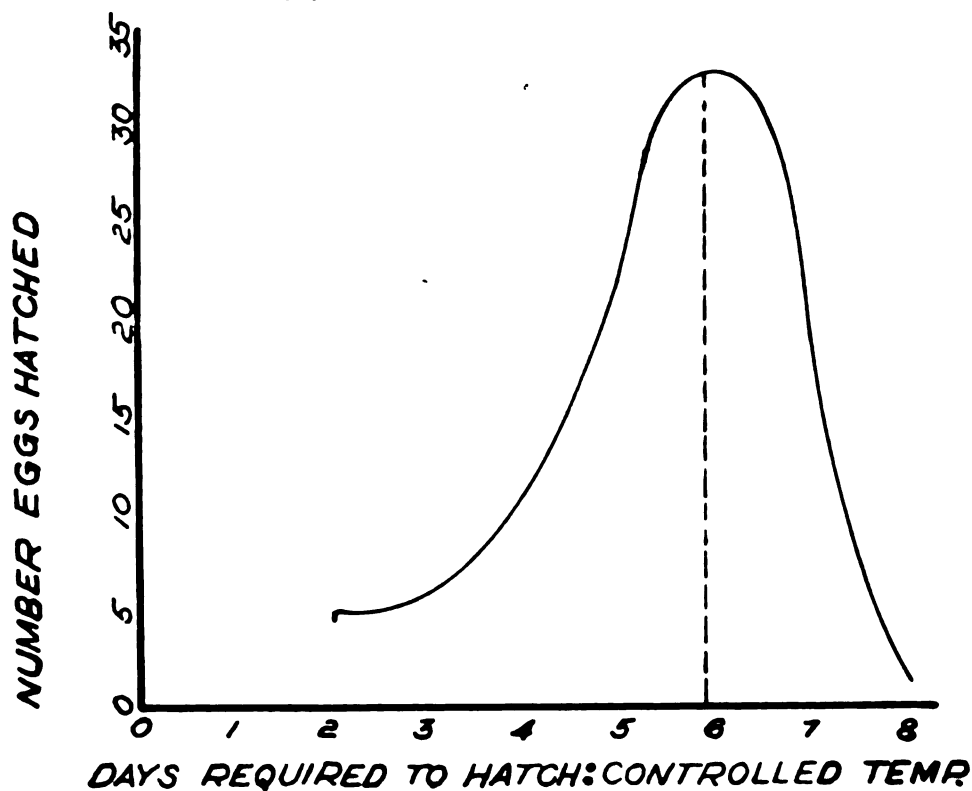
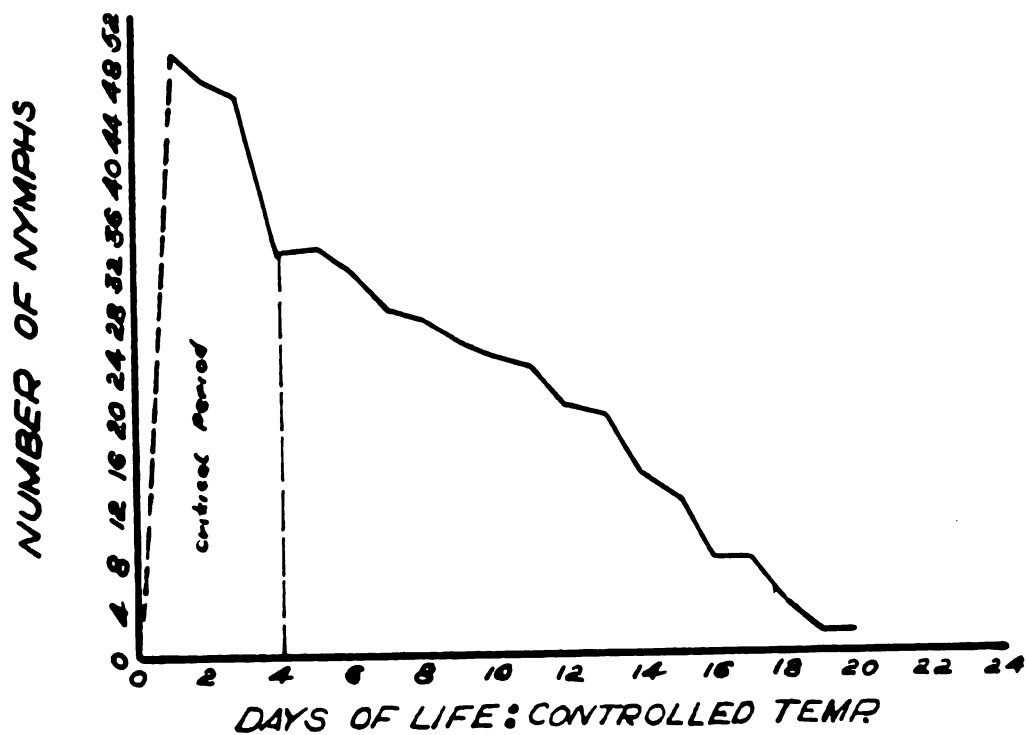


FIG.F: MORTALITY OF NYMPHS: BASIS 94 NYMPHS



The only nymphs which survived to maturity were those fed on chopped seed. Two-thirds of the nymphs thus fed developed to the final stages.

To measure the nymphs, 50 specimens were put in test tubes with green seed for food. After each molting, measurements were made under an adjustable beam of light, using a lens grid calibrated to tenths of a millimeter mounted in a dissecting microscope.

Table 8.--Measurement of nymph instars.

Stage	Length in mm.			
	Min.	Max.	Mean	
Eggs	1.5	1.5	1.5	
I	1.5	3.0	2.4	
II	3.0	5.2	4.0	
III	4.4	6.2	5.6	
*IV	5.5	7.9	7.0	
V	7.7	10.7	9.2	
Adults	10.0	13.5	12.0	

*Black wing-pads become prominent.

TESTS CARRIED ON UNDER "NORMAL" CLASSROOM CONDITION:

Temperature:

Temperatures, as recorded from April to June, were never below 68° F., or above 81° F.

Moisture:

Relative humidities varied from 27 to 80 percent with a mean of 56 percent during the daytime.

Light:

Containers were exposed to direct sunlight during the greater part of the day.

Table 9.--Egg-laying and hatching.

Conditions	Number of eggs laid per female.	Percent of hatching.
Cheesecloth- covered battery jar containing seed and water. (Fig. C, p. 19)	183/42 days laying time.	20
Cheesecloth- covered lamp- chimney cage over seeds and seedlings.	35	29
Cheesecloth- covered battery jar with chopped seed, water, and moist filter-paper- bottom lining.	125 (placed in jar)	72
Cardboard, ice- cream container with moist cotton.	50 (placed in con- tainer)	50
Two-quart mason jar with green seeds and foliage.	3	0

Nymph survival:

The only condition under which nymphs survived to maturity was that in which they were fed on chopped seed in a battery jar, with water made available. Two-thirds of the nymphs completed their life cycles.

TESTS CARRIED OUT UNDER OUTDOOR CONDITIONS:

Conditions:

Two breeding couples were placed outdoors in a 1½' x 2' x 2' wire screen cage containing seeds and seedlings. The cage was exposed to weather on the south side of a building during May and June.

Results:

Twenty-six eggs were laid per female, with 13.4 percent hatching, and one nymph developing to maturity.

FUNGUS GROWTH:

The sudden, heavy mortality of nymphs led to belief that parasitic fungi might be present. Mycelial growths appeared on dead bugs, foliage, and other objects during periods of high humidity.

Inspection of material and dead bugs revealed species of Alternaria and Rhizopus to be present. A particularly persistent fungus, with yellowish conidiophores, was identified by Doctor E. S. Beneke as Aspergillus sulphureus (Class Ascomycetae).

Since Thom and Church (36) made mention of entomoparasitism by the Aspergilli, it was decided to try to determine if this last species had any appreciable effect on the growth of nymphs.

For this purpose, six large petri dishes were outfitted with a bottom-covering of filter paper, a supply of chopped and whole seed, and a sterilized source of water. Three of the fountain wicks were then contaminated with samples from a culture of A. sulphureus. Ten nymphs of various instars, from an outside source, were put into each of the dishes, which were then placed under a continuously-operating light.

Table 10.--Results of infection of water supply with A. sulphureus.

Infected water supply		Non-infected water supply	
Dish number	Number dead after 8 days.	Dish number	Number dead after 8 days.
I (10 nymphs)	7	I	3
II	6	II	9
III	6	III	8
Total	19	Total	20

No correlation between presence of fungus and mortality was observed.

RELATIVE HUMIDITY:

Humidity is probably the most important, single factor that can be varied to retard fungus growth. The majority of the Aspergilli, for instance, grow well and sporulate most abundantly within the optimum, insect-rearing range (75° - 80° F.), while temperature is known to be a critical factor in growth for only a few species of this group. ((37), pp. 45-7).

Below relative humidities of 70 percent, little danger exists from mold development in most materials, according to Curtis and Clark (7). To allow for an increase of 5 percent in cheesecloth-covered cages, the maximum allowable relative humidity in the room should be near 65 percent.

Steps to prevent high humidities from occurring should include circulation of air, inclusion of absorptive material in containers, such as filter paper, use of the most permeable cage-covering practicable, and selection of wide-mouthed vessels, such as battery jars, for propagation.

TEMPERATURE:

Maintenance of a constant rearing temperature is necessary not only for obtaining stable humidities, but also to avoid water condensation on surfaces within the cages. To allow for an approximate increase of 5° F. in a cheesecloth-covered battery jar over outside temperatures, the room temperature should probably not vary more than two degrees from a mean of 72° F. for optimum conditions.

LIGHT:

During rearing the use of continuously-operating, artificial light appears to be an adequate, if not superior, substitute for sunlight, since a greater number of eggs were laid in shorter periods of time (in most cases).

SUMMARY

METHODS:

Boxelder bugs were introduced into cages containing various foods. Observations were made on food habits, reproduction, and mortality factors under different conditions of light, moisture, and temperature.

RESULTS:

Feeding habits:

All stages will feed on finely-chopped, dry seed, sprouted seed, seedlings, strawberry foliage, bean plants, weeds, and cut apple.

Chopped, dry seed is the most suitable food for nymphs.

Adults and large nymphs will feed on whole seed, hulled seed, and whole apples.

All stages do not feed on water-soaked seed, boiled seed, milkweed seeds and plants, cucumber, corn syrup, fruit fly agar, and rolled oats.

Dry, whole seed is the most suitable food for adults and older nymphs.

Egg-laying sites:

Seed fastened to the side of a battery jar is the most preferred place for laying.

Egg-laying and hatching rates:

Rates of egg-laying varied from zero to 312 per female under all conditions.

Under the same favorable conditions females in separate containers laid from 74 to 312 eggs apiece.

Eight females in a single container laid noticeably less eggs per female than did single females under the same favorable conditions.

The percent of hatching varied from zero to 87 percent under all conditions.

In most cases, from 40 to 60 percent of eggs will hatch under favorable conditions.

Rearing in exposed, outdoor cages is unsatisfactory for egg-laying and hatching.

Nymph survival:

Two-thirds of nymphs raised on chopped, boxelder seed survived; other methods failed.

Nymph mortality:

The most critical period in raising nymphs is during the first instar.

Nymph molting:

Five moltings took place during a period of 22 days.

AN AVERAGE LIFE SCHEDULE FOR ARTIFICIALLY-REARED BOXELDER BUGS.

Conditions:

Temperature: 80° plus or minus 2° F.

Humidity: high.

Food: Adults: whole, dried seeds.

Nymphs: chopped seeds.

Water: available in cotton-wick vial.

Table 1.--Average life schedule.

<u>Number of days from</u> <u>dormancy.</u>	<u>:</u> <u>:</u> <u>:</u>	<u>Activity</u>
0-3		Adults feed.
3		Copulation begins.
6		First eggs laid.
10-11		First eggs hatch, and greatest rate of egg- laying.
16		First ecdysis.
21		Second ecdysis.
24		End of egg-laying.
26		Third ecdysis.
31		Fourth ecdysis.
35		Fifth ecdysis.
39		Final ecdysis, and emer- gence of adults.

OPTIMUM CONDITIONS TO BE MAINTAINED:

Temperatures should vary little from a mean of 72° F.

Relative humidities should be kept below 65 percent.

Air circulation should be maintained.

Continuous light should be maintained for most rapid reproduction.

CONTAINERS:

Battery jars covered with cheesecloth are the best known rearing containers for boxelder bugs.

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