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The objectives of this research include (1) establishing laboratory methods for rearing trombiculid mites, (2) identifying the ecological requirements of the freeliving adult stages, and (3) ascertaining the biotic associations of freeliving stages. The first year's work concentrated on locating foci of chiggers and quantifying densities of unengorged, infective larvae. Over 600 vertebrate hosts were examined, from which fourteen species of chigger were identified. The washing method of chigger recovery from hosts

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was improved and quantified, by measuring recovery of known larval densities. Laboratory cultures of Eutrombicula spp. were started from engorged larvae. In field work, vinyl pastic squares were used in a removal trapping technique to measure chigger population sizes. Major foci of Eutrombicula were found in decomposing pine logs. Field experimentation is using pine logs in 4 x 4 M pens containing lizard hosts, for continued study of chigger bionomics. Sampling of the biota associated with trombiculids was initiated using tullgren extraction and flotation of soil samples with a Ladell apparatus.

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Ecological Requirements of Chigger Mites,

by

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INTRODUCTION

Chiggers, the larval stage of trombiculid mites, are vectors of disease in some parts of the world and are pests of major importance. Bites of some chigger species produce a mild to severe dermatitis in man and some animals. Chiggers are vectors of scrub typhus, a rickettsial disease, in parts of the asiatic-pacific area (Traub and Wisseman 1974). In Vietnam, scrub typhus was responsible for more "fevers of unknown origin" in American troops than any other tropical disease except malaria (Nadchatram and Dohany 1974). In the United States chiggers (genus Neoschoengastia) are responsible for economic losses in turkey production (Kunz 1969). Chiggers causing dermatitis in humans (pest chiggers, genus Eutrombicula) affect use patterns in recreational areas. In sensitive individuals, dermatitis caused by chigger infestations may result in hospitalization. As a result of their importance to man, chiggers have been the subject of an extensive (for acarines) taxonomic literature, devoted to the parasitic larval stage. Little is known of the free-living nymphal and adult stages, except that they are non-parasitic predators living in soil or similar habitats. The last major taxonomic work on the postlarval stages of trombiculids was published 20 years ago (Crossley 1960).

The general life history of trombiculids is now well known (Loomis 1956). Eggs develop through a deutoval stage and hatch into the hexapod larval stage, the "chigger." Hosts of the parasitic chigger stage are

vertebrates: amphibians, reptiles, birds and mammals. Host specificity varies among the hundreds of known chigger species, from highly specific to extremely broad. The taxonomy of the group is based upon the larval stage. Engorged larvae drop from the host and pass through protonymph (quiescent), and adult (active, bisexual) stages. The active forms (deutonymph and adult) are predators on small arthropods and their eggs. These active postlarval stages are little known. Most have not been collected, reared or described. Most knowledge of postlarval stages has been gained from laboratory rearings, starting with engorged larvae obtained from vertebrate hosts. To rear trombiculids to the adult stage, a suitable food for the predaceous deutonymphs must be found. A variety of arthropod prey species have been successful in laboratory rearings (Lipovsky 1954). Some success has been achieved with foods which trombiculids might not encounter under natural conditions. For example, Nadchatram (1965) used culicine mosquito eggs as a standard laboratory food for deutonymphs and adult trombiculids. American workers have used, among other things, eggs or immatures of the collembola Sinella curviseta, a species easily bred in the laboratory (Lipovsky 1951). Using these foods, trombiculids have been reared in the laboratory and a few species induced to reproduce (Nadchatram 1968). The establishment of laboratory culture of chiggers has been hampered by (1) lack of a suitable food for postlarvae and (2) lack of knowledge of abiotic requirements of postlarvae.

Few trombiculid species have been collected as postlarval stages (Crossley 1960), but conclusions about the postlarval habitat may be drawn

from host associations of the chiggers themselves. These interpretations are based upon the range of vertebrate hosts on which the chigger species occurs, and knowledge of the ecology of each host (Loomis 1956, Crossley 1960). Some highly host-specific species appear to be nest inhabitants as postlarvae. For those chiggers which occur on a variety of vertebrate hosts, frequency analysis may suggest which hosts are most likely to encounter chiggers. Postlarval habitats may then be inferred from knowledge of the habitats of the hosts. The most extensive analysis of this type was performed by Nadchatram (1970) for Malayan chiggers. He recognized seven ecological groups of trombiculids, based on occurrence in local habitats, host associations and color of unfed larvae. Adult trombiculids were then located in the field, in two of the suggested field habitats for the seven groups.

Research reported here was undertaken to address questions about the ecology of freeliving stages of chigger mites, by identifying their habitats, the biotic and abiotic environmental factors which predispose certain areas to the presence of certain chigger species, and the general ecological requirements of the adult stages. Necessary correlates are knowledge of the host associations, and suitable laboratory rearing methods so that biotic and abiotic factors can be evaluated experimentally.

In this first year we have initiated laboratory and field research. These are reported briefly in the following section. All research is in early stages, and interpretations are subject to revision as the research projects progress. Our initial objectives this past year have been (1)

identification of chigger-vertebrate host associations in this region as a basis of determining postlarval habitats, (2) quantification of methods of chigger detection, recovery and postlarval recovery, (3) initiation of rearing procedures for trombiculids.

PROGRESS IN RESEARCH PROJECTS

Trombiculid - Vertebrate Host Associations

During the past year, we examined over 600 species of vertebrate hosts for chiggers, and identified fourteen different trombiculid species from our region (Table 1). The most intensive effort was made during the summer, and concentrated within the Georgia Piedmont. We emphasize that these are first year results, and do not consider the list of species exhaustive. We are now redesigning our trapping effort to concentrate on particular habitats which have proved to be productive of chiggers. The purpose of this effort is identification of habitats and environmental factors predisposing them to the presence of trombiculids. Examining hosts for larvae is the most productive method for detecting the presence of most trombiculid species.

Chiggers were removed from vertebrate hosts with a modification of the washing method of Lipovsky (1951) and Henry and McKeever (1971). This method involves refrigerating hosts and then washing them in containers of detergent. Henry and McKeever (1971) filtered the washing solution and obtained good recovery of parasitic mites. We preferred decanting the wash water into a separatory funnel since we desire live undamaged larval

trombiculids. We tested the efficiency of the separatory funnels and various concentrations of detergents by adding known numbers of chiggers (Eutrombicula alfreddugesi). Different concentrations ranging from 0.07% to 2.8% of Palmolive dishwashing liquid and Tween-80 were tested to identify the precipitation time of chigger mites, optimum detergent concentration and efficiency of recovery. Three drops, 3 ml, 6 ml, 12 ml and 24 ml of the detergents were mixed with 900 ml water and placed in separatory funnels. Twenty live, free-living chiggers which had been captured in the field were then placed in the separatory funnels. Five ml of 95% ethanol were added to the top of each separatory funnel to break up any foam and free mites possibly trapped there. Ten ml of solution was then drained from the bottom of the funnel at 15 second, 30 second, and 1, 2, 3, 4, 5, 10, 15, 20 and 30 minute, and one hour intervals to measure precipitation and recovery rates. Also, the addition of 200 ml of 95% ethanol was tested as a possible scavenging agent to speed precipitation. Results showed that Palmolive dishwashing detergent was superior to Tween-80 at each concentration tested (Chi-square test, Alpha = 0.05). Addition of more than 5 ml of 95% ethanol reduced recovery of chiggers drastically: addition of 25 ml dropped recovery to less than 50%. Palmolive dishwashing detergent, in concentrations of 1.3% in the 900 ml wash water, yielded 100% recovery of added chiggers in 15 minutes or less. In practice, we are using a 30 minute settling time to allow for interference by hair or other debris in the settling process. The double-wash procedure of Lipovsky (1951) and the mechanical agitation recommended by Henry and McKeever (1971) are utilized to maximize chigger recovery from vertebrate hosts.

Quantification of "Chigger" Samplers

Foci of infective larvae of the genus Eutrombicula (pest chiggers) are readily located using "chigger" samplers. These various black objects have been used to collect unengorged chiggers. Wharton and Fuller (1952) used bakelites caps from reagent bottles. Loomis (1956) used plexiglas-acrylic plastic squares to locate chiggers. Crossley and Proctor (1970) reported the use of small squares of prepainted black masonite baseboard material. These devices were placed in grass or soil surface. Within a few minutes chiggers could be seen running on the surface of the sampler. Generally chigger samplers have been used as a survey technique, but Loomis (1956) used them to quantify activities of Eutrombicula alfreddugei. Loomis collected eight species of trombiculids with chigger samplers. Thus, we have adopted the chigger sampler as a major method for detecting the habitat of the effective larvae. These habitats identifications form the starting point for seeking the presence of postlarval stages in the habitats found to be effective. We constructed chigger samples from black vinyl baseboard material, cutting 100 cm^2 (7 x 14 cm) rectangles. After 1 - 2 minutes exposure, chiggers (if present) are readily seen running on the shiny black surface. The flexible samplers can be inserted into a bottle of soapy water to remove and collect the chiggers.

We attempted to quantify the efficiency of our chigger samplers by using the removal trapping method of Menhinick (1963). With this method, sequential samples are taken with a short interval between them. Assuming that probability of trapping does not change and migration is not signifi-

cant, the number trapped should decrease through time according to a negative exponential relationship (Menhinick 1963). Once a location containing active larvae had been established, chigger samplers were used repeatedly at 5 minute intervals. Results were inconsistent. All larvae could be removed by the repeated trapping within 10 minutes or so; however, more larvae appeared within an hour (or at least by the next day). Laboratory experiments suggest to us that the interval used was too short. We may have been exhausting the response of the population to the chigger sampler.

In the laboratory, we investigated response of Eutrombicula alfreddugesi to chigger samplers as a function of time and light intensity. Chiggers were collected from the surfaces of chigger samplers in the field using a micro-aspirator collecting bottle. A 20 ml scintillation vial was coated with plaster of Paris-charcoal mixture on the bottom and lower one-third of the sides. The top two-thirds of the vial was coated with teflon to restrict the chiggers to the lower part of the vial. Finely tapered glass tubing was used for collecting and suction tubes, inserted through a stopper. The suction tube was protected by extremely fine-meshed plastic cloth. Only light suction (by mouth) was necessary to remove live chiggers from the samplers. No mortality due to the collecting method was detected. Two hundred field-collected chiggers were placed in a 14 x 28 cm plastic box with teflon-coated sides. The bottom of the box contained a 2-3 cm layer of chopped fresh bermuda grass. A plastic chigger sampler was then placed in the box and the number of chiggers appearing on the sampler was

recorded each minute for a twenty minute period. The experiment was repeated in light intensities of 32, 350, 500 and 720 foot-candles of incident light. The sampler was not removed from the box. In all experiments the peak number of chiggers occurred between 2 and 5 minutes after initiation and declined to 0-1 per sampler after 15 minutes. Maximum number on the sampler was 5-6 chiggers.

Light intensities were not statistically significant, but we do not consider this aspect of the experiments to be conclusive. Field observation showed that active Eutrombicula larvae were found in shaded areas of logs rather than direct sunlight ($P = 0.05$, t test). We interpret the results of the time trials to show that the response of larval chiggers to the samplers became exhausted within a few minutes. Such a reduction in response with repeated sampling has been noted for Daphnia (Stearns 1975) and for zooplankton in general (Hutchinson 1967, McNaught and Hasler 1964). Thus the reduction of chigger catches on the samplers appears due to reduction in trapping efficiency rather than to reduction in population size. To evaluate this conclusion we returned to the field and took repeated samples (with removal) with half-hour intervals rather than five minute intervals between samples. The results showed a negative exponential decrease in number of chiggers caught over a 4 hour period. The sites which were sampled did not produce any further chigger activity, at least for the next 48 hours. These results are consistent with the suggestion, based on laboratory experimentation, that the immediate reduction in trappability was due to exhaustion of the response to the chigger samplers. However, the practicality of removal trapping as a means for surveying population

size seems questionable due to the long (four-hour) period required to obtain the information. On the other hand, the efficiency of the samplers seems higher under field conditions, ranging between 40% and 60% in these experiments. The largest numbers of chiggers were inevitably seen on the first sampling. These results suggest that a single, carefully-timed sample with the chigger sampler may yield a population estimate that is satisfactory for our purposes of locating foci of active chiggers and assigning relative intensities to foci. We intend to publish the results of these trapping experiments following another summer's evaluation of the technique.

Identification of Postlarval Habitats

During the past year, chigger samplers proved useful in locating postlarval habitats. The technique was most useful, however, in locating huge populations of Eutrombicula alfreddugesi. This species was found on a variety of animal hosts and in grass habitats, but largest numbers and most consistent collections were made from old, dry pine logs. Samplers placed on the soil surface beside pine logs rarely collected E. alfreddugesi. This finding was unexpected, since previous work (Loomis 1956, Crossley 1960, D. Johnson, personal communication, 1980) has shown species of Eutrombicula to be abundant in oak logs. Eutrombicula alfreddugesi has occasionally found in fescue meadows, but more often within a few centimeters of fence posts (Robina pseuddacacia). Pine logs (primarily Pinus taeda and P. echinata) of varying ages were found to be inhabited by Eutrombicula.

Logs with bark still tightly adhering to the bole, and logs with bark entirely missing, were equally infested. The common host for these chiggers would appear to be one of the lizard species (principally Sceloporus undulatus or Eumeces laticeps) which uses pine logs in our region. In any case, we have located at least one of the principal habitats for Eutrombicula alfreddugesi in our area.

Current research is investigating the pine log habitat in several ways. We have initiated a routine sampling program in which pine logs are destructively sampled, their ages recorded by decay stage, and arthropod fauna enumerated. When chigger samplers reveal infective trombiculid larvae during the summer, log samples will be further stratified by presence or absence of chiggers and of lizards as hosts. Some log-lizard associations found to harbor chiggers will be nondestructively sampled. In addition, we have established some experimental sites in a set of 4 x 4 m pens (Figure 1). The pens have sheet metal walls capable of retaining lizards such as Sceloporus undulatus. Logs found to have infective chiggers were placed in pens last fall, together with chigger-free lizards (Sceloporus undulatus) (Figure 1). The lizards successfully overwintered and we are now monitoring this experiment with chigger samplers and by examination of the lizards for chiggers.

Two techniques are under investigation for extracting postlarval trombiculids from soils or similar habitats. A rack of 24 large temperature-controlled tullgren funnels was constructed and is in use. Postlarval trombiculids are occasionally recovered in routine tullgren extraction, but the efficiency is unknown and may be low. An alternative method is flotation

(Cockings 1948), in which postlarvae are recovered by dissolving soil in water agitated with current or in a salt solution. Postlarval trombiculids are covered with dense, highly branched setae which permit them to float readily. A Ladell apparatus was constructed after the design of Lawson and Merritt (1979) (Figure 2) and is now being tested as a collection method for adult trombiculids.

Rearing Methods for Trombiculids

Several cultures of Eutrombicula spp. have been initiated, both as individual experiments and for mass rearings. Single experiments are using 5-dram vials lined with charcoal-plaster of Paris mixture (Crossley 1960) and other small containers. Eggs of the collembolan Sinella curviseta are used as food for nymphs and adults. Mass cultures are maintained in terraria (Figure 3) lined with charcoal-plaster of paris mixture. Large populations of Sinella curviseta are maintained in the terraria to provide food for the postlarval stages. Lizards (Anolis, Sceloporus or Eumeces) are kept in the terraria as hosts for the larval chiggers. Activated yeast is added for the collembolans and Tenebrio larvae are introduced for the lizards; otherwise, the terraria need only water to maintain the culture. When the mass cultures begin producing large numbers of postlarvae they can be used to evaluate the efficiency of the tullgren extractors for postlarval trombiculids.

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Manuscript Prepared During This Contract Period

Wicht, M. C. and D. A. Crossley, Jr. Some observations and improvements in chigger collecting methods.

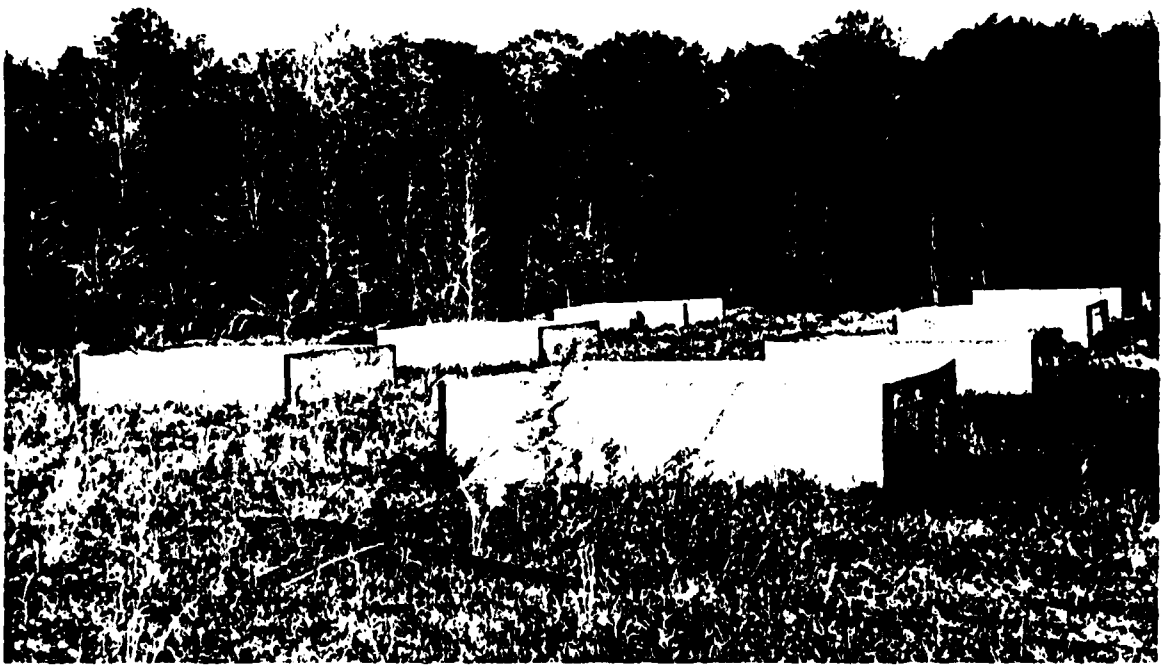
Presentations During This Contract Period

"Some observations and improvements in chigger collecting methods." M. C. Wicht and D. A. Crossley, Jr. Annual Meeting, Entomological Society of America, Atlanta, Georgia, November 30, 1980.

Table 1. Associations between vertebrate hosts and trombiculid species collected in Georgia (USA) Summer 1980 - Winter 1981 (Identification tentative)

Trombiculid Species	Vertebrate Hosts	Season
Euschoengastia peromysci	*Peromyscus leucopus*	Autumn-Winter
Euschoengastia lanceolata	*Peromyscus leucopus* *Sigmodon hispidus*	Summer
Eutrombicula splendens	Reptiles, Mammals	Summer
Eutrombicula lipovskyana	*Anolis carolinensis*	Summer
Eutrombicula alfreddugesi	Reptiles, Mammals	Summer
Eutrombicula batatas	*Signodon hispidus*	Summer
Comatacarus americanus	*Blarina carolinensis*	Summer
Parasecia gurneyi	*Signodon hispidus*	Autumn
Trombicula lipovskyi	*Ochrotomys nutalli* *Sigmodon hispidus* *Peromyscus leucopus*	Autumn-Winter
Neoschoengastia americana	*Sylvilagus floridanus*	Summer
Fonsecia palmella	*Elaphe obsoleta*	Autumn
New Species #1	*Pitymys pinetorum*	Summer
New Species #2	*Peromyscus leucopus*	Summer
New Species #3	*Eumeces laticeps*	Summer

FIGURE 1. SMALL SHEET METAL PENS AVAILABLE FOR EXPERIMENTAL STUDIES OF CHIGGER MITES. TOP, ARRAY OF PENS ADJACENT TO FOREST. BOTTOM, PINE LOGS CONTAINING EUTROMBICULA ALFREDDUGESI INTRODUCED INTO A PEN.



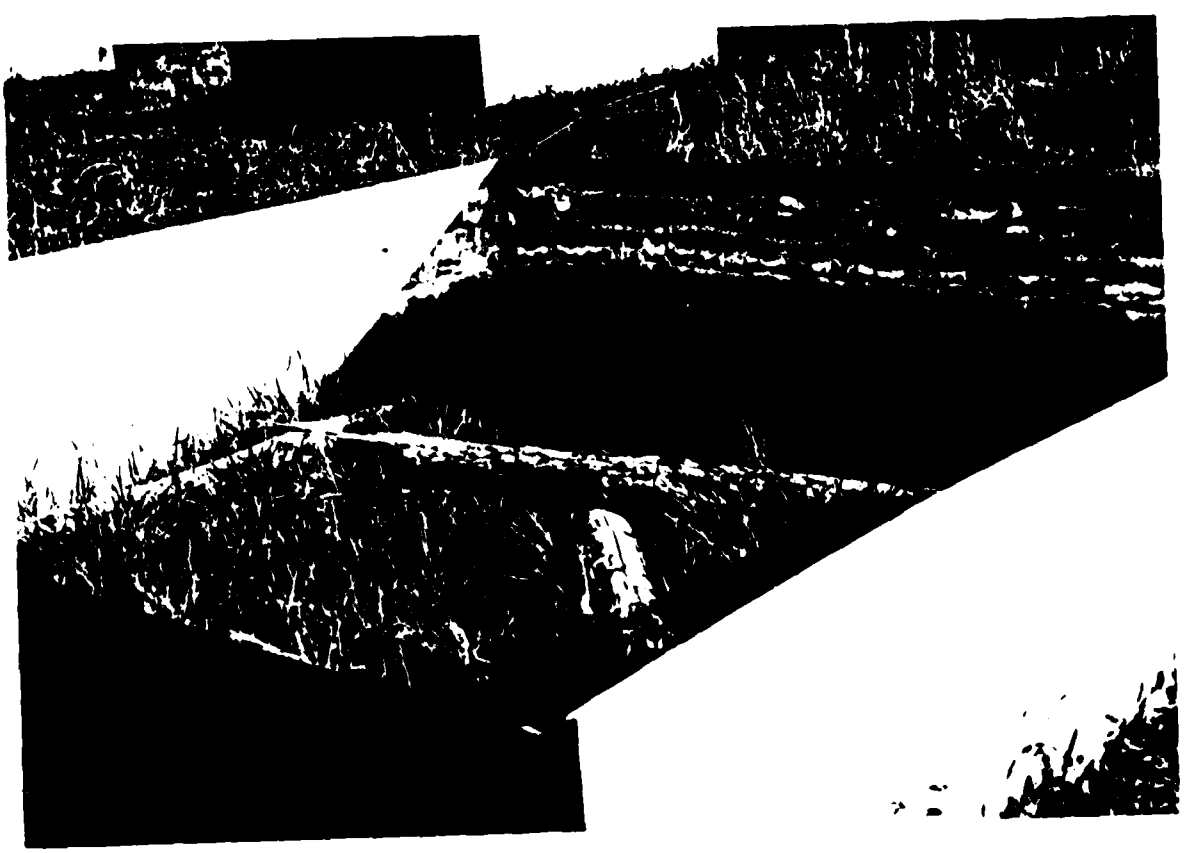


FIGURE 2. MODIFIED LADELL APPARATUS USED FOR FLOTATION OF SOIL SAMPLES CONTAINING POSTLARVAL TROMBICULIDS

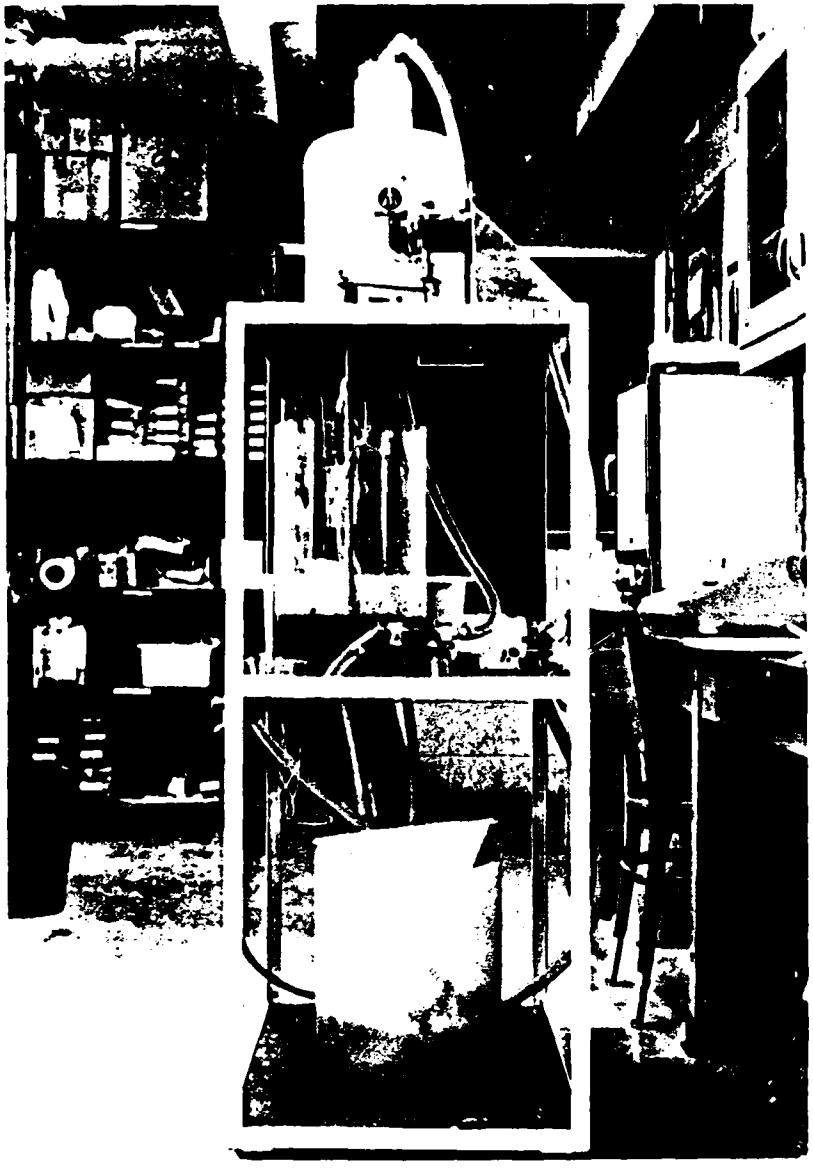
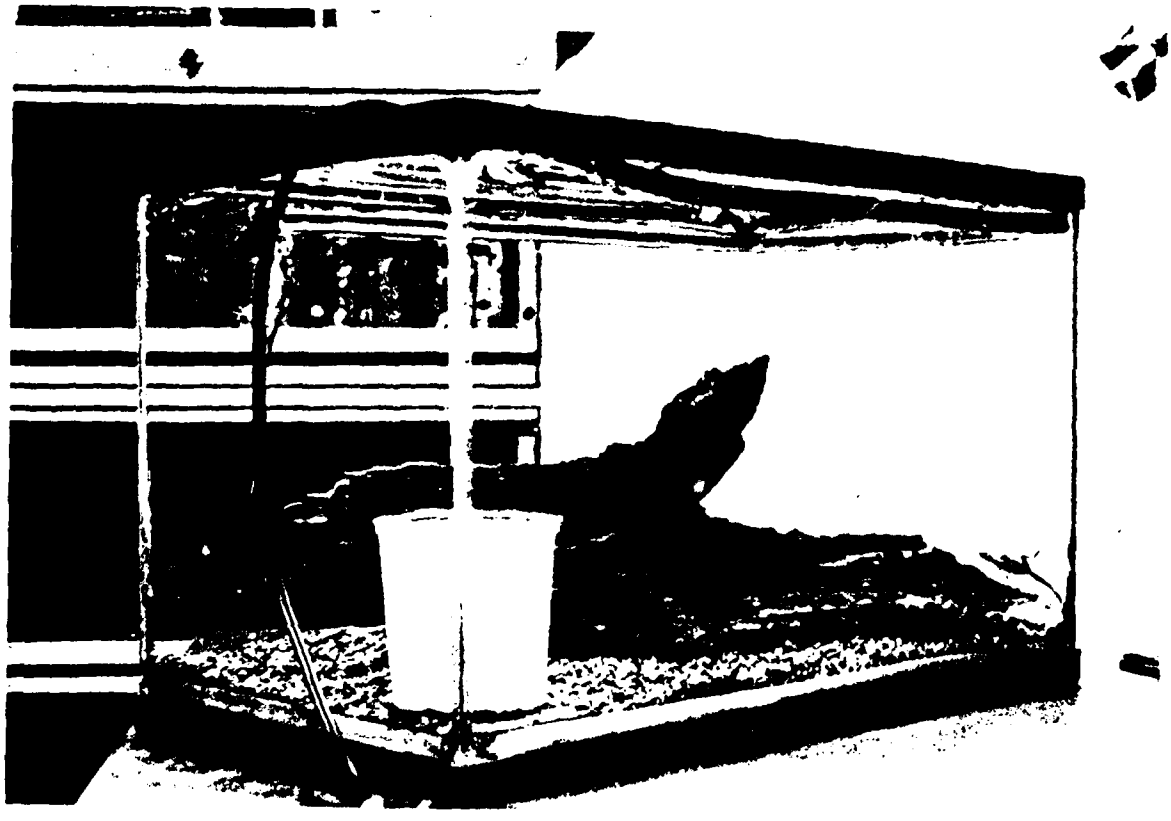
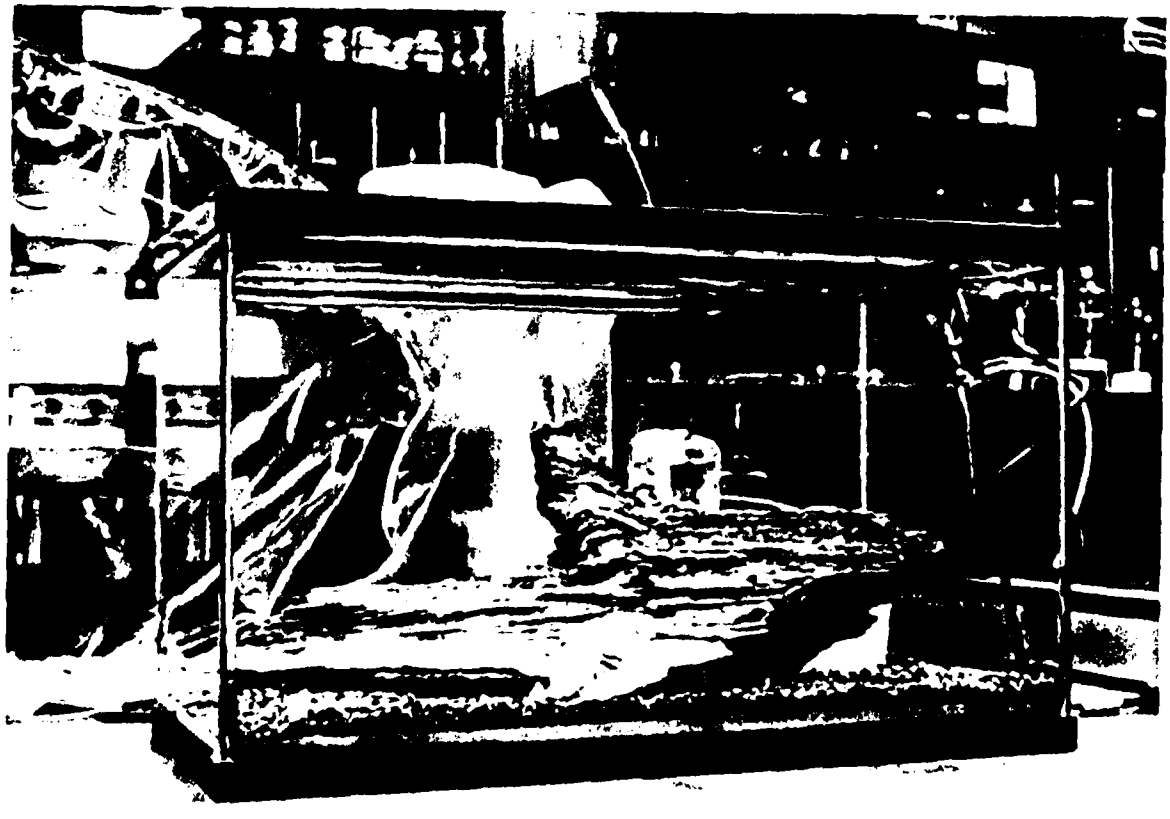


FIGURE 3. TERRARIUM USED FOR MASS CULTURE OF EUTROMBICULA ALFREDDUGESI.



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