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Bureau of Entomology and Plant Quarantine

PROCEDURE FOR MASS REARING OF ADULTS OF TIPHIA VERNALIS ROH., A JAPANESE BEETLE PARASITE

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INTRODUCTION

With the extension of the range of the Japanese beetle as a serious pest, the official agencies of a number of States have given attention to various phases of the problem, including the liberation of parasites to aid in the natural control of the beetle. A summary of available information on methods of rearing Tiphia vernalis Roh., one of the more effective of the parasites of the Japanese beetle, is therefore given herein, for the benefit of any who may be interested.1/

Although Tiphia vernalis is the most effective of the four species of Tiphia which have been reared in quantity at Moorestown, N. J., it is unquestionably the most difficult to rear with a sufficient degree of efficiency to justify the expense and effort involved. For this reason most of the parasites of this species liberated in the field have been secured from shipments of field-collected material from Japan, which proved very successful, or have consisted of females collected in this country from established colonies that resulted from earlier liberations. However, considerable attention has been given by the Moorestown laboratory to the development of rearing technique for various species of Tiphia. The procedure outlined herein for T. vernalis would be, with some modification, suitable for T. popilliavora Roh.

It is suggested that those interested might well consult the discussion on "Methods for Rearing Tiphiids and Scoliids," by J. L. King, in "Culture Methods for Invertebrate Animals," Comstock Publishing Company, Ithaca, N. Y., pages 502-505, 1937.

1/ This outline contains material that was furnished to workers of the University of Maryland in the spring of 1940, in connection with a program of mass rearing which they undertook that season.

STOCK OF INSECT MATERIAL

Host Supply

As has been said, Tiphia vernalis is at best difficult to rear, and assuring an adequate supply of healthy third instars of the Japanese beetle is one of the first prerequisites to success. The grubs may be obtained in several ways, but, provided the soil is not too hard or the grubs too deep, a coarse two-pronged hand weeder has been found to be an admirable means of scratching the grubs loose from the grass roots and soil with a minimum of effort, time, and injury to the grubs. If the digging is done at a time of year when the grubs are found at some depth, a grubbing hoe is a more practical implement.

As the grubs are dug, every effort should be made to prevent them from coming into contact with one another, as they quickly bite on contact, and the resulting wound is injurious and often fatal. The use of a roomy box in which they may be placed as they are dug is recommended. A generous layer of soil must be provided between each two layers of grubs, and a clump or two of sod, into which the grubs may crawl, placed in each box is an excellent means of reducing grub movement and the consequent injury through contact. Experience has shown that overcrowding a grub box results in a waste of material. At Moorestown boxes measuring 18 by 12 by 7 inches are used, and when full they contain approximately 500 grubs. These boxes of grubs are stored at 45° F. until needed.

The number of factors influencing the cost of obtaining host material makes an exact estimate impossible. The quantity of grubs available in the soil is but one of these factors. At the Moorestown laboratory experience has shown that for efficient digging the grub population should be well above 10 grubs per square foot. In some cases it may be profitable to transport the diggers considerable distances in order to find continuous digging where the population is sufficiently high.

The depth at which the grubs are found is a strong limiting factor. The deeper they are, the more soil must be moved before actual production begins. From May 1 to the first week in June, and again from the first week in September to early in October, grubs may be scratched from the top 3 inches of soil and grass roots with a hand weeder, as mentioned above. Before June and after October 10 one may expect to find them from 3 to 6 inches deep, when it is necessary to use a grubbing hoe (mattock), a much slower and more laborious procedure.

The type of soil, the degree of moisture, and the temperature are all factors which the experienced grub digger knows have a direct bearing, not only on the quantity of grubs obtainable per hour, but also on the number that are unavoidably injured in the digging.

Given optimum soil conditions during May or September, with a soil population of 10 to 50 grubs per square foot, an experienced workman can average from 200 to 250 grubs per hour. If a wage rate of 50 cents per hour is assumed, we strike an arbitrary cost of .0025 to .002 cent per grub, under the above conditions.

Parasite Material

The female parasites used in the rearing work may be either field collected or laboratory reared. In the case of field-collected females, the fact of previous mating must be assumed, and there is always the possibility that the individual's reproductive potential has been reduced through previous oviposition. This is more likely to be true in the case of females collected some time after field emergence has begun. Laboratory-reared females may be positively mated, and, in theory at least, should be capable of 100 percent egg production. However, oviposition records of both field-collected and laboratory-reared females indicate that the former, if collected early in the flight period, are about 40 percent more efficient in egg production than laboratory-reared material. (This estimate is based on an average oviposition rate of 50 eggs per female.)

PARASITIZATION OF HOST GRUBS

Preparation

When the female Tiphia are ready to be placed with host material the rearing cans are prepared as follows: A 6-ounce, round tin can (see Equipment and Materials) is divided into 6 equal-sized, vertical compartments by inserting screen partitions described in the list of equipment. After a small quantity of soil has been sprinkled on the bottom of the can the host grubs are introduced, one in each compartment, or fewer if desired. The can is then filled level with field soil which has been sifted through a 6-mesh screen, and the soil is compacted by firm pressure with some instrument of convenient size to fit into the can, such as the bottom of a bottle (fig. 1, B). The soil used in the cans should be sufficiently moist so that when squeezed in the hand it will form a ball and yet be easily friable. The purpose in compacting the soil in the rearing cans is to provide a medium of sufficient stability to permit the grub to form its natural soil cell, without which the parasite is usually unable to complete parasitization successfully.

For ease in handling, the cans are packed in trays of any convenient size. At the Moorestown laboratory the flats used for this purpose hold 35 cans. After the cans containing the grubs in the compacted soil have been placed in the flat, the surface of the soil in the cans is thoroughly moistened, though not soaked, by spraying with plain water from a pressure spray can. On the

surface of the soil is now placed a piece of heavy waxed paper 1/2 inch square, upon which is placed food for the parasite. This food may take the form of a drop of honey-and-water solution (1-10) or a crumb of fondant made by kneading together confectioner's sugar and honey until a dry, friable candy is obtained. The rearing can is now ready to receive the parasite. The females, being sufficiently hardy to withstand moderate handling, can best be introduced with the bare fingers of the right hand while the can cover is poised in the left hand, ready to be quickly applied. (See instructions under Manipulation.) After being covered, the can is placed in the tray, which is held at approximately 75° F. for from 24 to 48 hours, depending upon the number of host grubs in each can and upon the fecundity of the females being used. This latter point can be determined only by actual performance.

In handling the trays of rearing cans, as in moving them to and from storage, care must be taken to keep them level and not subject them to any rough handling that will cause the liquid or semiliquid food to be jarred from the piece of waxed paper onto the soil, where it would be quickly lost.

Disposal of Parasitized Host Grubs

Following the oviposition period, the cans are opened for examination and for transfer of the parasites to fresh rearing-can units. The routine procedure usually adopted is described under Manipulation. When examination of a host grub shows it to be parasitized, as manifested by an egg attached ventrally, usually behind the third pair of legs, the grub is placed in one of the individual compartments of the cross-section flat used for storage of parasitized host material during cocoon formation, described under the heading Equipment and Materials.

As will be explained, this flat contains 196 compartments. As a partial insulation against desiccation of the inner rows of compartments, the outside row is filled with soil but does not contain any host material. Thus the capacity of each flat becomes 144 parasitized grubs. At the time the outer row of compartments is filled with soil the others are filled about one-third full and the flat is ready to receive the parasitized grubs. The soil used here should be the same as that described for use in the rearing cans.

After one parasitized grub has been placed in each of the partly filled compartments, 4 or 5 kernels of wheat are added to each compartment, and the entire flat is filled with soil. The wheat germinates in the moistened soil, and the small seedlings furnish food for the host grubs. This soil is now firmly compacted by strong pressure on each individual compartment. This operation is facilitated by the use of a "comb" (fig. 1, G), a piece of hard wood 18 inches long and 1 inch thick, so notched that each "tooth"

is 1 inch square. When the "comb" is properly made to fit the cross-section flats, a whole row of compartments can be compacted at once, as the cross-section partitions fit into the intervals between the "teeth" of the "comb" and the "teeth" themselves just fit into the compartments. When the compacting has been completed, the additional space thus created is filled with soil packed in by hand. The flat is now thoroughly wetted by spraying with water and stored at approximately 75° F. Each flat is marked with the date upon which it was filled, and the flats are stacked one directly on top of another. This prevents the escape of host material and reduces evaporation.

Manipulation

In mass rearing, as in most other cases of large-scale production, a systematic performance and division of duties among workers tends to conserve time, effort, and expense.

Assuming a contemplated program whereby the number of females in rearing cans is maintained at 1,000 during the greater part of the rearing period (6 weeks), the division of labor in the laboratory and the number of workers involved would be somewhat as follows:

Rearing cans are assembled in the trays, supplied with grubs, filled with soil, and the soil compacted by one team of 5 men. The tray is then passed on to a second team, comprised of 3 men, who spray the cans with water and supply the waxed-paper squares and the food; members of this group also supply these trays of fresh cans to the transfer men as they are needed. In connection with the work of the team first mentioned, it should be pointed out that in handling the grubs preparatory to placing them in the rearing cans caution must be exercised continually to prevent the grubs from injuring one another. This point can not be too strongly emphasized, as one careless worker can easily reduce the efficiency of the entire process by holding too many grubs in his hand at one time. This is a perfectly natural tendency of all workers in their efforts to work rapidly at this particular operation. Eight grubs in the hand, together with a little soil, is not too many if the worker is fast and quickly empties his hand.

The transfer men, of whom there are 4, operate individually and are waited upon by the other teams. Each transfer man has a large empty tray directly in front of him which serves as his dumping tray, and just beyond this is a supply of freshly loaded cans (fig. 2). On his left is a tray to receive emptied cans, while on his right is a tray of the old cans from which the Tiphia are to be transferred as the contents are dumped.

With a little experience the worker soon becomes adept at removing the lid from the old can, dumping its contents in front

of him, placing the emptied can on his left while with his right hand he is picking up the Tiphia which has just been dumped, and quickly placing her in a fresh can, adding the lid, and replacing it in its tray. As fast as the dumping tray is filled it is passed along to the 3-man team of examiners, and the transfer man is supplied with another tray.

"Practice makes perfect," and until some degree of dexterity is attained in transferring the females from one can to another, care must be taken to refrain from cutting the newly placed female in two when the lid is placed on the can. The lid must be added without very much fitting or adjusting, as the Tiphia which has just been placed in the can quickly seeks to escape through the small opening through which she can see light as the lid is being fitted, and the result is nearly always a mutilated female.

The examiners separate the parasitized grubs from the unparasitized ones and place the former in a previously prepared cross-section flat. As soon as a flat has received its full complement of parasitized grubs and food seeds it is filled with soil, compacted, labeled, and stored. Whenever possible, the team that is preparing the rearing cans should also prepare and pack the cross-section flats so that only one source of soil will be necessary. However, the task of loading the rearing cans is not a simple one, and it is sometimes necessary to have a separate team of 2 men to care for the flats.

If the cocoon-formation flats are dated and stacked as they are filled, it will, of course, be necessary to restack them in reverse order before they are broken down for cocoon removal; otherwise the first flats filled, and therefore the first to form cocoons, will be at the bottom of the stack.

In case there are fresh females to be placed in rearing cans, this operation can usually be attended to by the team that supplies the water, waxed paper, and food.

The following statement shows the estimated cost of the above-mentioned unskilled labor (17 men) plus 1 technical supervisor for a 6-week period:

17 men (unskilled labor) for 561 man-days at $4 per day	$ 2,244
1 technical supervisor for 6 weeks	375
Total estimated cost of laboratory help	$ 2,619

HANDLING THE COCOONS

Cocoon formation of this species should be complete in 25 to 28 days. After such a period the flats are withdrawn from storage for cocoon removal. This somewhat laborious task is performed as follows:

Onto a large ash can (galvanized iron) is fitted a specially constructed wooden hopper, upon which rests a square-frame, 6-mesh sieve. This sieve bears on wooden ledges of the hopper so that it may be easily agitated by hand, the soil thus sifted through passing down the hopper into the ash can (fig. 3).

When the cocoon-formation flat is brought from storage it is inverted on the square frame of the sieve, and the tray portion of the flat is lifted free of its contents. Cocoons which have been formed against the bottom of the tray will now be visible, embedded in the exposed surface of soil in the cross section (fig. 4). These cocoons should be very carefully removed with a pair of forceps and placed in individual 2-dram vials. Before removal of cocoons from the flat is begun, it is well to have an ample supply of vial trays already filled with clean cocoon vials. All handling of cocoons is done with great care, since denting or crushing of a cocoon nearly always kills the parasite larva within. Insofar as possible the cocoons are handled by the looser filaments surrounding it. This operation is one in which even long experience does not develop speed. When a fungus-infected or obviously diseased cocoon is handled, the forceps should be flamed before being used again for handling a healthy cocoon.

The cocoon-bearing soil is removed by knocking the cross-section partitions against the rim of the sieve frame. Pushing the soil from the compartments is likely to result in damage to the cocoons. As small quantities of soil and cocoons are jarred into the sieve, the cocoons are removed as they are discovered, and the soil is gradually sifted through into the ash can. The wheat sprouts are removed by hand and carefully searched for cocoons. When repetition of this process finally empties the cross section another one takes its place.

The individual vials into which the healthy cocoons are placed for storage are contained in light, screen-bottom trays (fig. 3, E), 216 per tray. Across the bottom of the inside of the tray are wooden strips at intervals of about 1-1/2 inches and thick enough so that when the open ends of the cocoon vials rest on a wooden strip and the closed end rests on the screen bottom, the vials recline at an angle of about 20°. Each worker, as he picks a cocoon from the loosened contents of the cross-section flat, places it directly in a vial. For this purpose a tray of empty vials should be close by his soil-sifting equipment. As the trays of vials are filled with cocoons they are placed immediately in racks in storage cellars of constant temperature so adjusted at biweekly intervals that the average temperature of the field soil at a 3-inch depth is maintained.

Some time before adult emergence from the cocoons is expected the vials are capped with specially made circular screen caps (fig. 5). These caps are of fine brass-wire gauze made with a

special die which produces a tiny basin-shaped cap, the bottom of which is "dimpled" in order to give elasticity to the cap when it is forced into the open neck of the vial.

HANDLING THE ADULTS

When it is time for the emergence of the adult Tiphia from the cocoons, the vials are examined daily. The trays are partially withdrawn from the racks, and the reclining position of the vials permits the inspection of a tray at a glance (fig. 6). If any adults have emerged they can be readily seen and the vials quickly removed from the tray. When emergence records are desired, individual record cards for its contents are kept in each tray and summarized at the close of the work. Where a large quantity of material is being reared, the daily volume of vials removed from the trays is large as emergence reaches its peak, and should be handled in any convenient container so that the caps are not accidentally knocked from the vials. The day's emergence having been taken, the sexes can be readily separated if desired before the Tiphia are removed from the vials.

Mating

Removal of the adults from the emergence vials and their subsequent handling when in large numbers is effected in the following manner: The vials are uncapped, and adults of both sexes are placed in wood-frame, muslin hoods, in front of windows, for mating (fig. 7). Each hood must be constantly attended by a worker who removes the mating couples as soon as union is complete. This is done by putting the pair into a wide-mouth shell vial, stoppering the vial, and setting it out of direct sunlight until mating is complete and the pair have separated. The individual worker must decide for himself the maximum amount of material that can be handled in the hood at one time under prevailing conditions of light and temperature. The optimum temperature for mating appears to be approximately 82° F. and is best when not in direct sunlight. The Tiphia will mate in sunlight, but if the sunlight falls through the window glass onto the muslin hood the temperature quickly reaches a point where the adults become excessively active and mating is reduced. Invariably a small percentage of females refuse repeated efforts of males to mate. These females are removed from the hood and stored until the following day, as it has been found that the great majority of females will mate within 20 to 30 minutes, and that the time spent in trying to mate the remainder on that day is wasted.

When mating pairs in the vials are seen to have separated, they are placed in the hood, one sex at a time, and collected en masse by a simple suction device made from an ordinary hair drier by removing the heating element and attaching an adapter to the air-intake port. As the glass receiving tube of the collector

becomes about one-fourth full it is removed, and the adults are emptied into a storage or liberation can, as the case may be. If the mated females are to be used for rearing purposes instead of for liberations, they should be allowed to remain in the vials where mating took place and only the males removed. From these vials the females can be readily shaken into the rearing cans, to which the cover is quickly added. Here again caution is necessary to prevent mutilation of the female when the cover is placed on the can. Glass vials containing living specimens must never be left where they will be in direct sunlight, as the heat within the vial quickly kills them.

ESTIMATE OF APPROXIMATE RESULTS TO BE OBTAINED WHEN 200,000 PARASITIZED HOST GRUBS ARE USED

As has been said, Tiphia vernalis is a most difficult species to rear through to the adult stage with a satisfactory degree of efficiency. From past experience and recorded data, an estimation of the approximate results that might be expected from 200,000 parasitized grubs is 12,000 adult females, and is based on the following calculations:

Total number of grubs needed from the field ---	500,000	(100%)
Number of grubs to be parasitized ----------	200,000	(40%)
Number cocoons formed ----------	100,000	(50%)
Adult emergence ----------	30,000	(30%)
Adult FEMALE emergence ----------	12,000	(40%)

It is estimated that the number of females which would be needed in order to obtain the desired number of 200,000 parasitized grubs would be 4,000.

Since the above figure of 12,000 females represents 120 colonies, it may be seen that if 4,000 females (40 colonies) were to be withheld each year for propagation purposes the program would permit the liberation of 80 colonies annually.

The foregoing estimates are made on a basis of one generation annually. Recent experiments with a multiple-generation program have proved that such a plan is impracticable. Several lots of laboratory-reared cocoons were stored under differing temperature schedules for the purpose of determining which schedule would permit the earliest forced emergence of physically normal adults.

It was found that the difference between the development within cocoons stored under schedules providing a comparatively brief period of minimum temperatures followed by periods of increasingly high temperatures and in cocoons stored at average soil temperatures was hardly perceptible. Furthermore, when emergence was finally forced from this experimental material it was unsatisfactory in every respect. Emergence continued in small numbers

over a period of several weeks, a fact which in itself would greatly complicate any attempt at mass rearing of multiple generations. In the second place, the order of emergence of the sexes was reversed, the bulk of the early emerging adults being females. Very few of these females lived until males could be obtained for mating, and on the few occasions when both sexes were available at the same time neither seemed interested in the other.

Briefly, the general conclusions reached were that, owing to the length of the developmental period which appears necessary at hibernation temperatures, a normal second generation of adults could not be successfully obtained in the laboratory more than a month in advance of field emergence. This fact could be used to advantage if and when liberations were desired in the warmer regions of the South, but it renders impracticable the mass rearing of multiple generations in the laboratory.

When host material is abundant it is the practice to expose the grubs to parasitization only once, as this assures material of maximum acceptability. In the foregoing tabulation it is estimated that of 500,000 grubs originally dug, 300,000 are either lost, injured, or refused as host material by the parasite. Of the 200,000 parasitized grubs, there is assumed to be a 50-percent mortality of parasite material before time for formation of cocoons. Between the time the cocoons are formed and the time for emergence there is a 70-percent mortality, some of which occurs in the immature stage and much of it in the fully formed adult stage. It is estimated that 4 out of every 10 laboratory-emerged adults are females.

When, as frequently happens, a limited supply of host material necessitates the repeated use of grubs not parasitized on the first exposure, these grubs should be subjected to selective sorting at the same time they are examined for parasitization. If all grubs in the prepupal stage as well as those showing mechanical injury or any lack of normal vitality are culled out, the percentage of successful parasitization of the remaining material should be fully as high as that on the material at the first exposure.

In handling the acceptable unparasitized grubs, the examiner must again guard against physical contact of the grubs with one another in the container which receives them following examination.

EQUIPMENT AND MATERIALS

NEEDS ESTIMATED ON THE BASIS OF A PROGRAM FOR 1,000 FEMALES

Weeders for digging grubs ---------- One per man.

Grub hoes (mattocks) ---------- One per man.

Mechanical hand counters for counting the grubs as they are dug and placed in the boxes ---------- One per man.

Grub boxes (capacity 500 grubs) ---------- 250

Rearing cans. Six-ounce, circular, pressed metal can with even fitting lid (can to measure 2-3/4 inches in diameter by 2 inches deep); capacity 1 female parasite and 4 to 6 host grubs ---------- 2,000

Screen partitions. These are made by cutting strips of 8-mesh screen 2-5/8 inches long by 1-1/4 inches wide, and riveting 3 of them together by a single rivet through the center. The strips are then separated so that a 6-spoke wheel is formed. When this is fitted into the can the spokes form the partitions separating the can into 6 compartments. The object of the screen partitions is to provide a barrier through which the grubs cannot penetrate from one compartment to another but which will not hinder the movement of the parasite through the soil. The partitions, being 1/4 inch less in height than the depth of the can, permit the soil in the can to be readily compacted ---------- 2,000

Implement (flat-bottom round bottle) that will fit into the can for use in compacting the soil ------- 3

Heavy waxed paper cut into 3/4-inch squares ------ Several pounds.

Honey-and-water solution or fondant of honey and confectioner's sugar for parasite food ---------- 3 pounds.

Trays for handling rearing cans. Capacity 35 cans. Galvanized trays 20 inches long by 14 inches wide by 2 inches deep. Trays that contain the cross-section flats may also be used ---------- 570

Spray can. Ordinary pressure sprayer to be used for moistening rearing cans and cocoon-storage flats -- 1

Ash cans, galvanized iron. Approximately 20 to 25 gallon capacity ---------- 6 to 8

Wooden hoppers for use in sifting contents of cocoon-formation flats. A four-sided wooden hopper 28 inches by 20 inches at the top and tapering to 9 by 9 inches at the bottom, which fits into the ash can. The side rims of the top of the hopper should be fitted with ledges or sliding surfaces in which the sieve frame rests while being agitated. All measurements given for this item are outside measurements	4
Sieves, 6-mesh screen on a frame of about 3/4-inch stock. (Outside measurements 20-1/2 inches by 18-3/4 inches by 3-3/4 inches)	4
Cocoon-formation flats (18 by 18 by 2-1/4 inches), capacity 144 grubs. The partitions consist of wood strips 2 inches wide by 18 inches long by 3/16 inch thick, so notched and fitted together that there are 14 compartments on a side. In all there are 196 compartments 1 inch square and 2 inches deep. Before being used these cross sections are treated by soaking them in hot paraffin to retard rot. The trays in which these cross sections fit are of medium-weight galvanized stock and 18 inches square by 2-1/4 inches deep (inside measurements)	350
Cocoon-storage vials. 2-dram homeopathic vials to contain 1 cocoon each	25,000
Trays for vial storage (18-1/2 by 17-1/2 by 2-1/4 inches), capacity 216 vials. These trays are of light-wood frame with 16-mesh copper-screen bottoms. Across the inside of the trays and against the screen bottoms are strips of wood 3/4 inch wide by 1/2 inch thick. These strips are spaced 1-1/2 inches apart. The vials are laid in the trays in a sloping position with the neck resting on these wood strips	115
Vial caps (described in text)	30,000
Wood frame and muslin mating hood	4
Electric suction collector (adapted from ordinary hair drier)	3
Large-mouth shell vial. These are made by putting plaster-of-paris bottoms in glass cylinders 3 inches long by 7/8 inch in diameter.	1,000
Corks to fit plaster-bottom vials	1,500

Forceps	5 pairs.
Alcohol lamps or Bunsen burners	4
Implement for compacting soil in cross-section flats (described in text)	1
Storage cans for storing males and extra females. Capacity 100 females. The same cans that are used in liberation work are fitted with the same food and water device resting on a screen disc atop a 2-3 inch medium of damp sphagnum moss	200
Sphagnum moss	1 bale.
Food blocks and water bottles	one each per can.

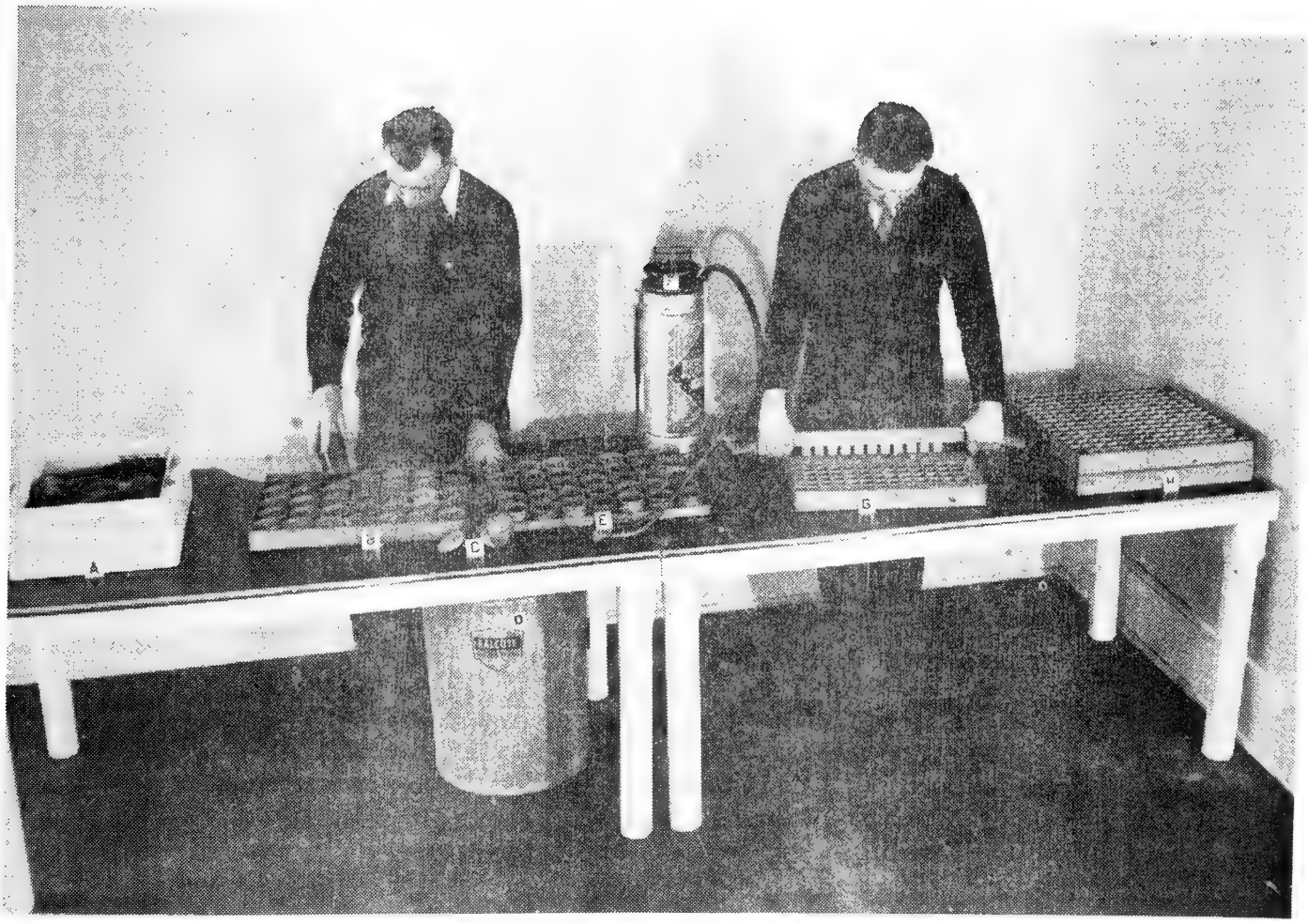

Figure 1.--Compacting soil in oviposition cans and cocoon -formation flats. The letters refer to the various equipment as follows: A, Box containing fresh grubs from the field; B, worker using bottle to compact soil in freshly loaded oviposition cans; C, empty oviposition can; D, ash can containing fresh soil for use by both workers; E, oviposition cans ready to be loaded; F, pressure sprayer; G, worker using "comb" to compact soil in cocoon-formation flats; H, cross-section flats containing parasitized grubs ready to be filled with soil and compacted.

Figure 2.--Transfer man at work. The letters refer to the various equipment as follows: A, Tray of oviposition cans containing soil, host grubs, and female Tiphia; B, cans freshly loaded with soil and host grubs and showing waxed paper squares on surface of soil; C, worker has dumped a can from A preparatory to placing a parasite in a fresh can from B; D, tray to receive emptied cans.

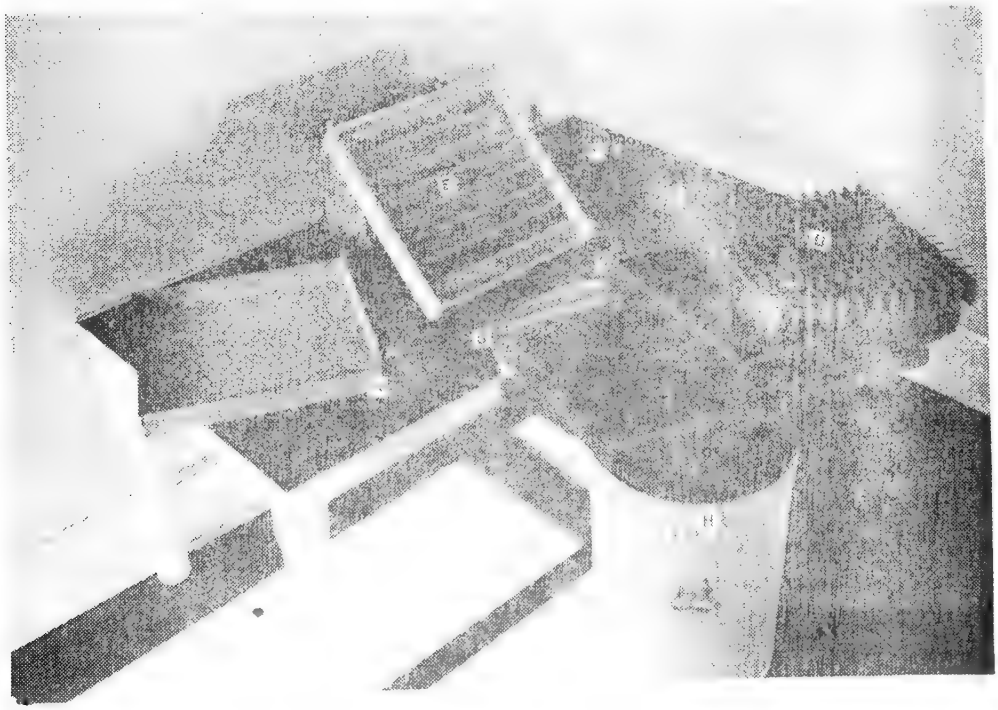

Figure 3.--Equipment set up for extracting cocoons from cocoon-formation flats. A, Empty flat from which cross section has been removed; B, wooden hopper; C, sieve in position on hopper; D, cross section in position for extracting cocoon-laden soil; E, tray for cocoon-storage vials in position to receive cocoons; F, alcohol lamp; G, forceps; H, ash can into which fits hopper B.

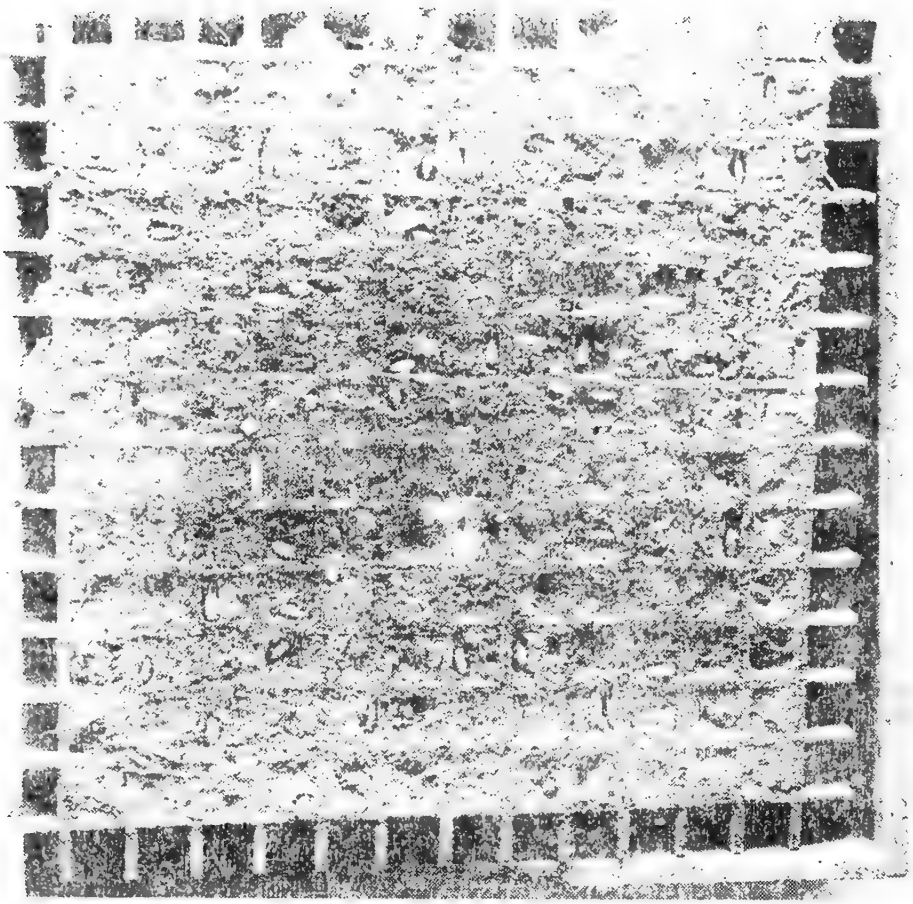

Figure 4.--Cocoon-laden cross section in position on sieve frame after flat was inverted and tray removed. Note how many of the cocoons were formed at the bottoms of the compartments and in contact with the bottom of the tray.

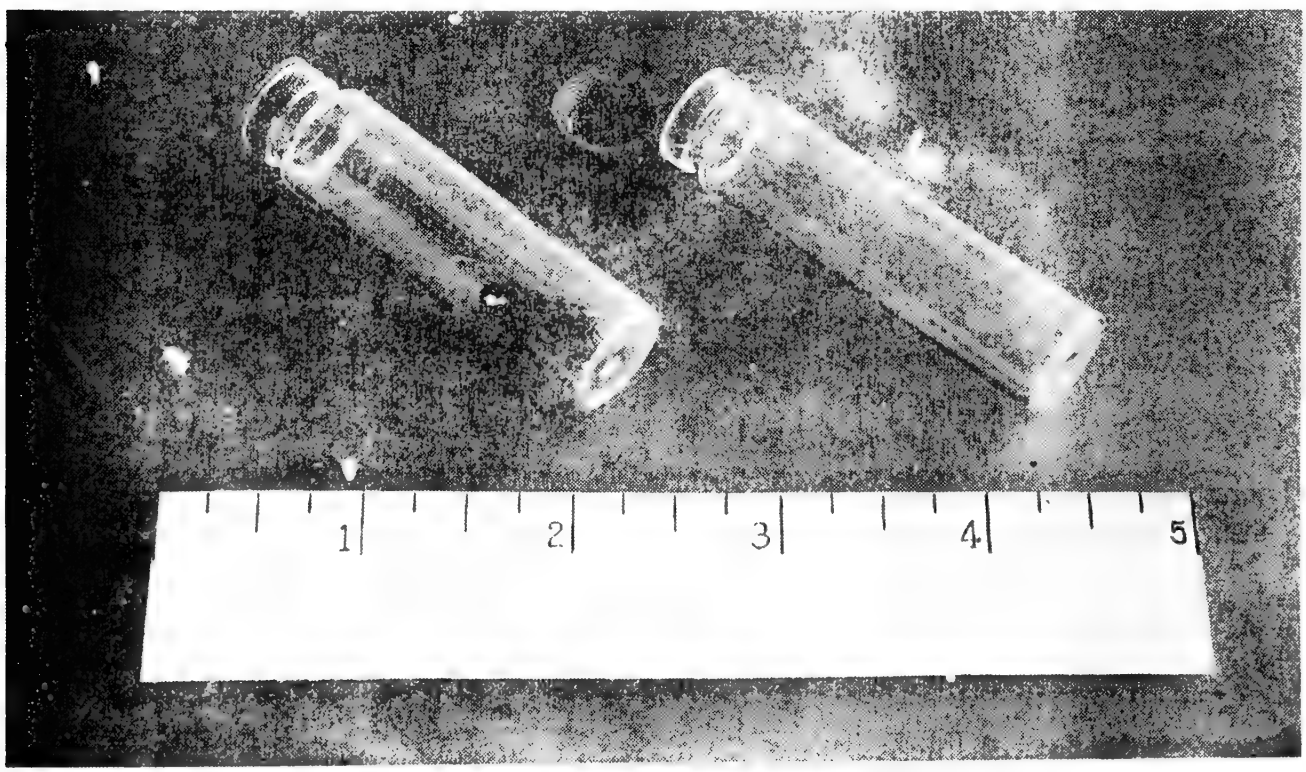

Figure 5.--Cocoon-storage vials with screen caps.

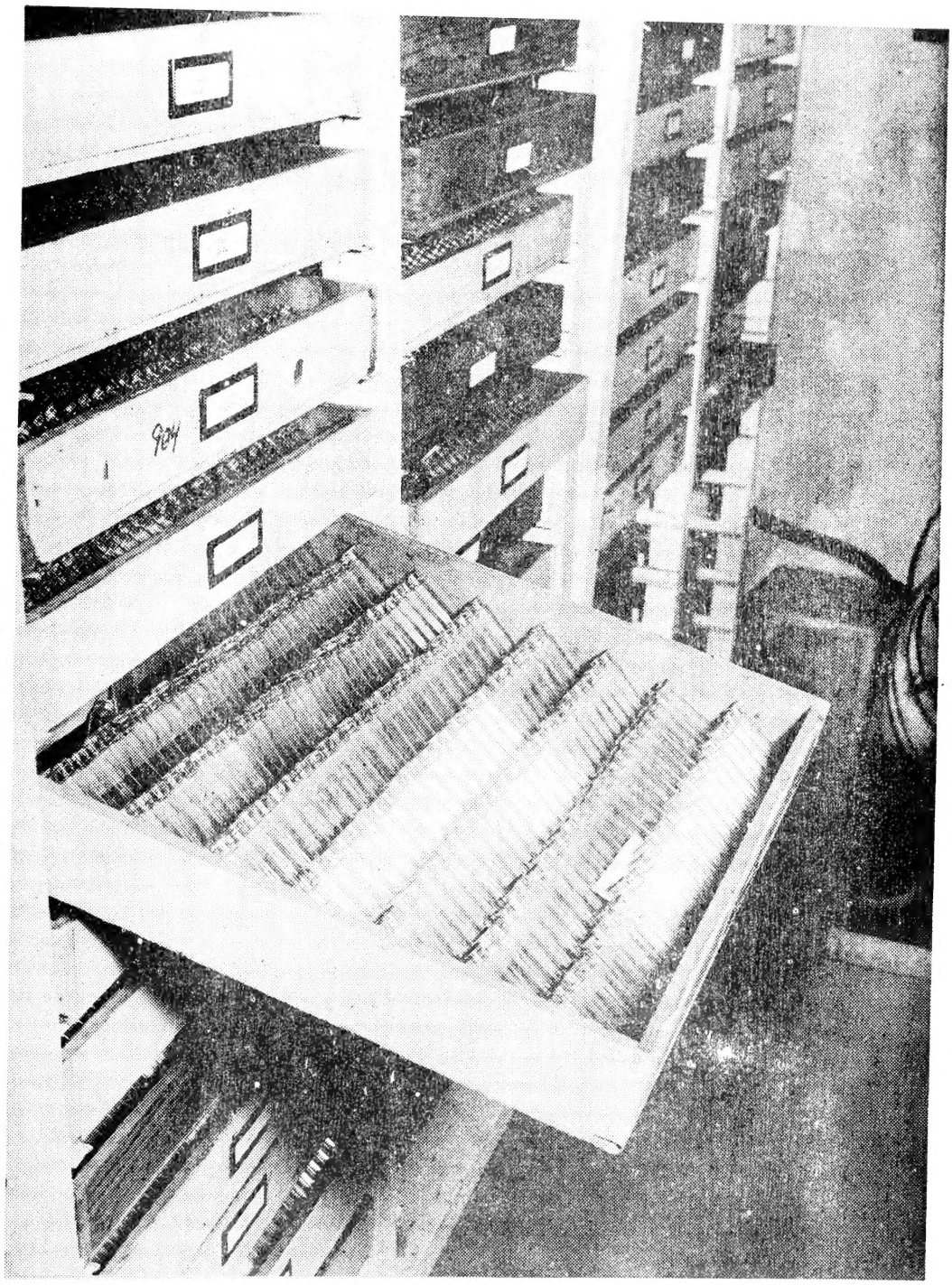

Figure 6.—Tray of cocoon storage vials partly withdrawn from rack in storage cellar.

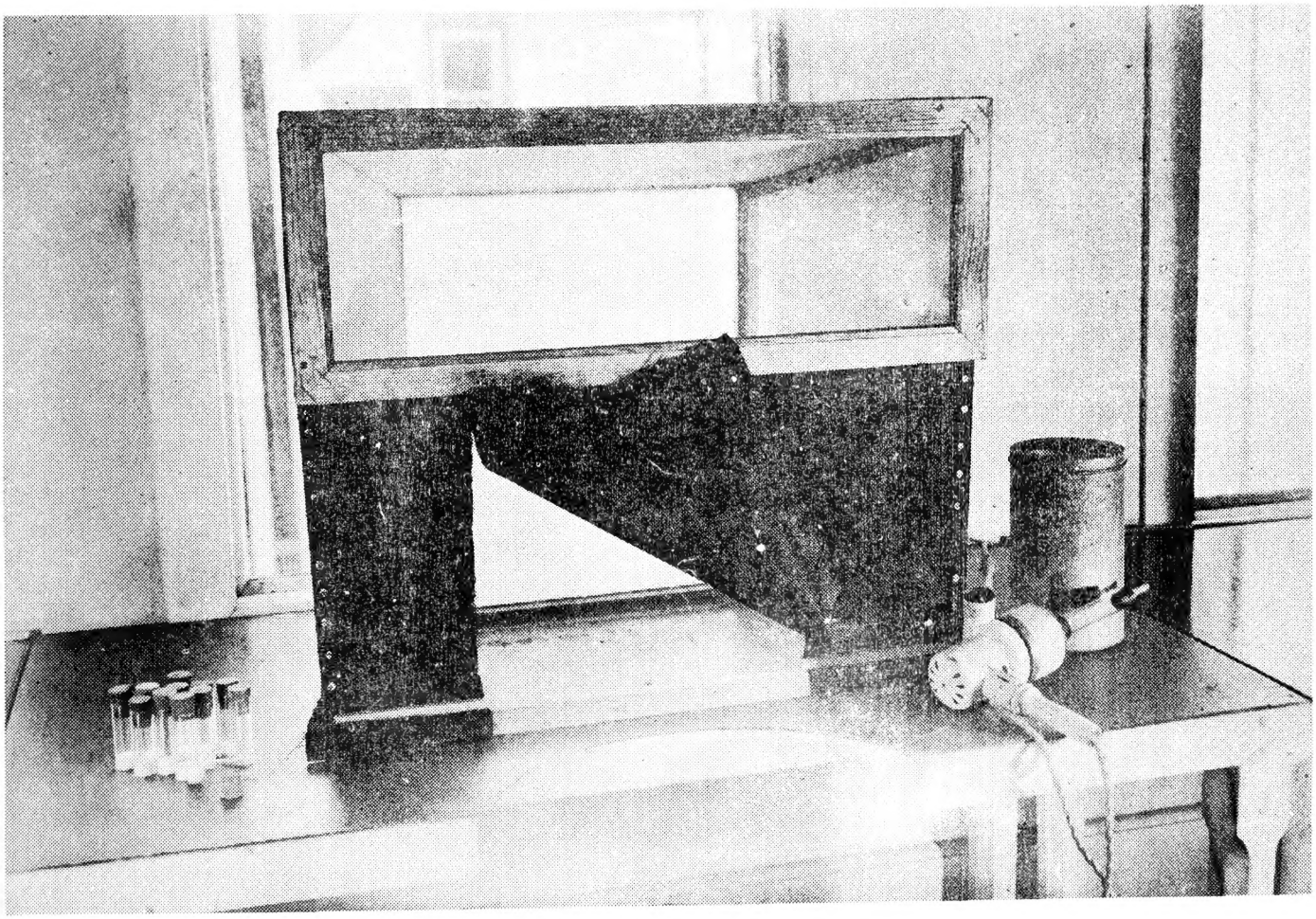

Figure 7.--Equipment used for mating Tiphia adults in the laboratory, showing plaster-bottom vials, mating hood, suction collector, and can for storing adults.

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