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Myrmecological Techniques

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ABSTRACT — An ant colony collection-maintenance unit is described and various adaptations of it are described and illustrated. These adaptations may be used to study food preference, interspecific fighting, or predation. Methods of feeding and watering colonies and examination of individual ants are also included as well as maze constructions and colony-recognition devices that may be attached. Only techniques for observation of ant behavior are presented; experimental results are omitted.

Wheeler (1910) stated, "I am convinced that there is no form of entomological work more fascinating than collecting ants, for these insects are everywhere abundant and no two of their colonies ever present the same picture to the observer."

This fascination for ant study has been shared by a host of myrmecologists. Goetsch (1957) contended that he was drawn to the study of ants partly by a desire to find out how much truth there was in the old ant legends. Wheeler and Wheeler (1963) stated, "Certainly the pursuit of any branch of natural history may be recommended as an avocation to our youth, to convalescents, to our tired business men or in fact to anyone who craves a hobby or surcease from the nerve-wracking routine of city life, or a valid excuse for remaining as many hours as possible in the open air of the woods and fields." This report is intended as an aid to those who, like the quoted scholars, would ponder the mysteries of ant societies in the laboratory.

Although some investigators take a considerable amount of equipment into the field, most methods of collecting and preserving ants (alive or dead) are relatively simple. The nature and the amount of equipment varies from collector to collector and depends upon the nature of the project. Although no list could be considered to be standard, one of considerable detail can be found in Wheeler and Wheeler (1963). The study of ants in the laboratory involves the use of artificial nests and some auxiliary equipment. Wheeler (1910) described many artificial nests such as Swammerdam's used around 1737. This unit consisted of a dish containing some earth covered with a pane of glass and set in a larger dish containing some water. Other units described were those of Huber, Lubbock, Janet, Viehmeyer, Fielde, Buckingham and Santschi. The last unit has been a very popular one for over sixty years. It is constructed of wet plaster of Paris and glass plates. Wheeler and Wheeler (1963) describe Gregg's nest as being the best all-purpose type. Forrest (1962) describes a unit which consists of plastic sandwich boxes and flexible plastic tubing.

All of these artificial nests have one very serious defect in that ants cannot be collected directly into the unit. In most cases, soil and ants, plus leaves and other debris are scooped into a plastic or cloth bag to be worked over later. Anyone who has tried to find ants in a substantial amount of soil and then transfer them to any of these artificial nests will quickly realize that this procedure is extremely time consuming and frustrating.

In 1964, Glass and Hamrum reported on a unit that was used both for the collection and maintenance of ants. Complete instructions for building this unit are found in their paper. In general, the unit is actually an over-sized aspirator, the body of which is a pint or quart Ball (or Kerr) jar partially filled with dirt; and the suction part of which is the bulb from an infant enema syringe of 250 ml. capacity. The unit (Fig. 1) has such a powerful aspirating action that workers, eggs, larvae, pupae, winged forms, food stored by the ants and myrmecophiles are quickly drawn into the jar. A colony can be sampled in a matter of minutes or seconds. The jar bottom is tapped against the palm of the hand to force the ants to the bottom of the jar. The aspirator head is removed and the jar quickly closed by screwing the Ball ring, with a special clear plastic insert (in place of the normal Ball lid) to the neck of the jar. The plastic disc insert (Fig. 2) has three one-quarter inch holes punched in it. Two of the holes are plugged with Tygon tubing stoppers. The third hole is plugged with a Tygon tubing air vent, the bore of which is packed with steel wool. When returning to the laboratory, the two stoppers are replaced with two Tygon tubing feeders. The artificial nest is then complete (Fig. 3). We have collected over 850 colonies of ants by this method and at the present time are maintaining 260 colonies in the laboratory. Many of them have larvae present.

Wheeler (1910) states, "Anyone who wishes to gain a thorough knowledge of ants will do well not to pin his faith in any one of them (artificial nests), but will select the form best adapted to the special problem at hand." This statement was made at a time when nests were clumsy and of limited use. Forrest (1962) showed that plastic sandwich containers could be utilized for holding soil, as feeding units or as general observation

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chambers. All of these units could be connected together with plastic tubing.

This paper will attempt to show that one can use the Glass and Hamrum collection and maintenance unit to investigate most phases of ant activity, since a large amount of auxiliary equipment can be built from the same materials as those making up the nest. The use of the unit and its modifications regarding taxonomic problems has been reported by Glass and Hamrum (1966). This paper will be concerned with the construction and design of the auxiliary equipment and with certain techniques needed to study various phases of ant activity. It will not be concerned with actual experiments conducted in the laboratory. It is hoped that this paper will be of interest to teachers of biology, entomology and ecology; to amateur and professional myrmecologists and to those who offer courses in biological techniques, or need some projects for independent study.

Removal of Ants From the Units

Ants may be removed from the units for study under a microscope. The air vent is removed from the celluloid disc cover and a drinking straw aspirator is used to remove the ants from the nest. The clear, acetate straw has a 35° bend at one end (Fig. 4). If a small plug of steel wool is forced up the bore of the straw it becomes lodged at the bend. This plug prevents one from aspirating ants into his mouth. This piece of equipment may also be made from one-quarter inch O.D. glass tubing.

Immobilizing Ants

If one desires to immobilize ants in the nest, CO_2 gas can be introduced into the unit. One-fourth inch O.D. Tygon tubing is used to conduct the gas into the unit through the hole occupied by the air vent. The nest may be cleared of gas by unscrewing the cap with the plastic disc, and blowing one's breath into the jar.

Sometimes it is difficult to see the immobilized ants as they rest upon the dark soil surface. In this case it is preferable to aspirate active ants from the nest with a mouth-aspirator and then to tap them on to the cold surface of a thermal anesthetizer (Fig. 5). This unit may be placed under a binocular microscope and the ants studied at will.

Care of Living Ants

Any number of liquid foods can be pipetted or drawn into the Tygon tubing feeders. One can use egg-yolk, honey and sugar mixture; plant juices such as diluted Karo syrup, blueberry juice, apple juice and maple syrup; or animal products such as honey, homogenized earthworm or crayfish (or the filtrate) and fruit flies. Solid foods such as chopped walnuts, peanut butter, bits of meat or insect pupae can be dropped onto the surface of the soil in the nest. Care should be taken not to introduce an excess of food into the nest since a mold problem might develop.

The first feeding units used with the nest were Tygon tubing "circles" (Fig. 6). They can be most aptly described as being self-leveling feeders. The feeding unit used at the present time is made by slipping a six-inch length of Tygon tubing over a length of wire bent into the shape of a cane (Fig. 7). The straight end of the wire is placed in a holder which is placed in a hot-air oven at 290°F for five minutes. We use holders that have room for 30-40 wires. After removing from the oven, the form is inverted and the tubing-wire complexes are dumped into cold water. After a few minutes the tubing is removed. Each bit of tubing will remain in the cane shape. Most ants can enter a cane feeder of one-fourth inch O.D. Tygon tubing. Feeders for carpenter ants should be one-half inch O.D. Obviously, the holes in the plastic lid of the nest will have to be enlarged to receive them. The cane feeders are filled by pulling a liquid food into them through a hypodermic syringe attached to the end of the cane. A small, non-perforated glass bead of sufficient diameter is forced into the handle end of the cane before the syringe is removed. This bead will keep the liquid food from running out of the feeder when it is in place. Since the food surface is above the ant while it is feeding, it will fall away from the food if it slips or is pushed by other ants. We have found that these cane feeders will provide the ants with an abundance of food and minimize the chances of becoming entrapped in the food.

Drinking Water and Soil Moisture

Ants will live for a long time without food but not without water. Ants often drink and require a humid atmosphere. A cane-type feeder will supply the drinking water and at periodic intervals the soil in the nest may be moistened by pipetting water into the unit. Recently we have devised a unit which serves both as a source of drinking water and of water for moistening the soil. A 12 dram vial serves as a reservoir. The vials have a flexible plastic top. A regulator adds water, drop by drop, to the nest. This device consists of a one and one-half inch piece of one-fourth O.D. Tygon tubing through which the loop end of a doubled cotton string has been threaded. Approximately one-fourth inch of the loop is forced over the lip of the tubing and is held in place by a one-half inch O.D. tubing collar approximately three-fourths of an inch long. Two or more discs of blotting paper, one-fourth inch in diameter and a small disc of brass screen are forced into the bore of the collar. A small length of one-fourth inch O.D. tubing holds all discs in place. A small air inlet is punched into the side of the plastic top. One may obtain more drops per unit of time by using more blotting paper discs in the regulator or by obtaining more blotter-water surface in some other manner. We have used regulators (Fig. 8) that have delivered from one ml. to 8 mls. over a 72-hour period. Through the use of the cane-type feeders and the watering device, an artificial nest can be supplied with an abundance of food and water. It is possible to leave artificial nests unattended for weeks by using these devices. This time-saving aspect is a factor if one wishes to maintain between 300 and 400 colonies in the laboratory at one time.

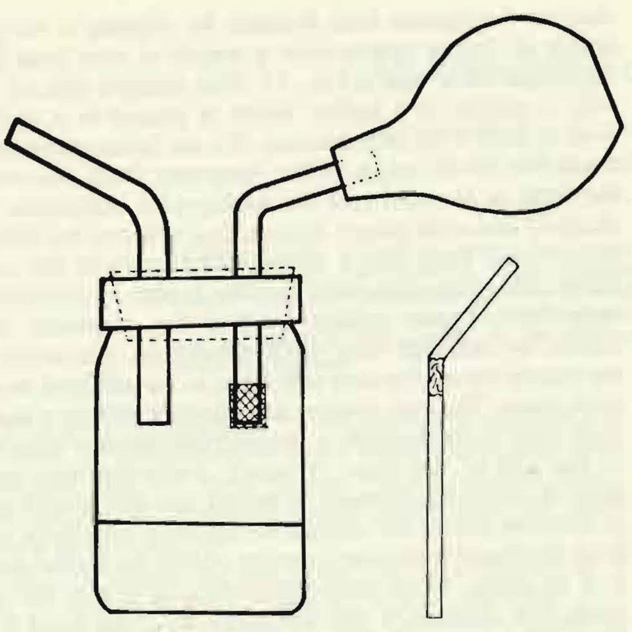


FIG. 1. Collection Unit FIG. 4. Mouth Aspirator

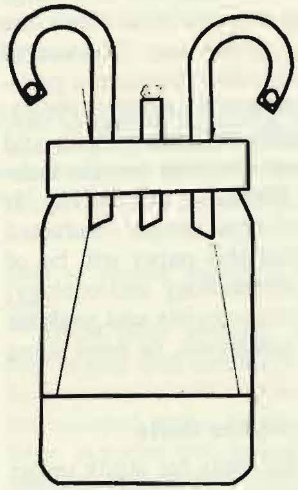


FIG. 3. Maintenance Unit

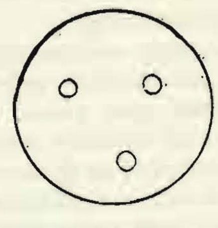


FIG. 2. Plastic Disc Insert

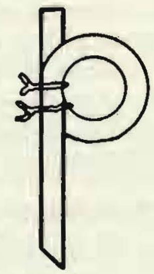


FIG. 6. Circle Feeder

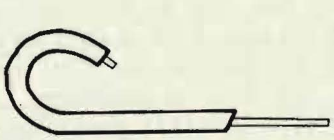


FIG. 7. Cane Feeder

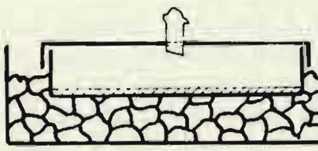


FIG. 5. Thermal Anesthetizer

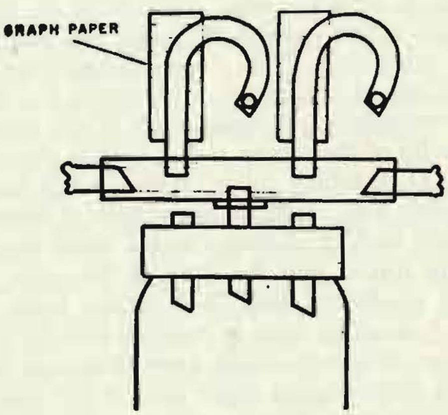
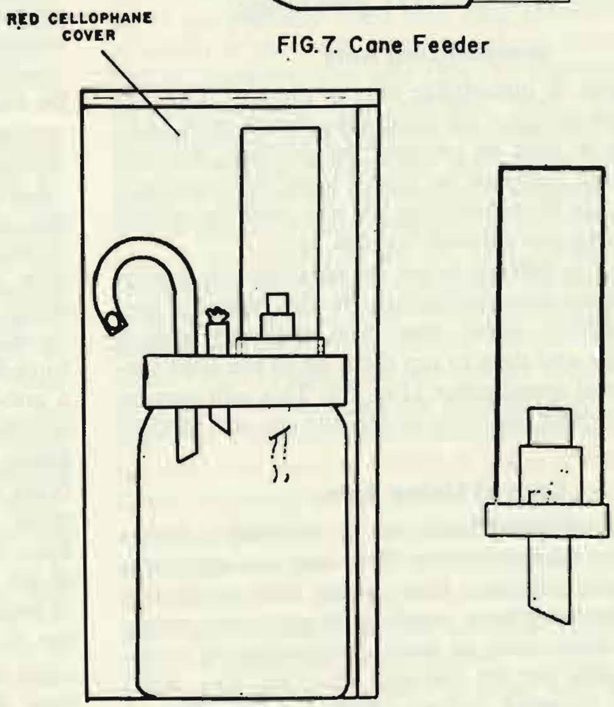


FIG. 9. Food Choice Unit



(a) In Place (b) Detail

FIG. 8. Watering Device

Food Choice Units

If several one-quarter inch holes are bored through one side of a length of one-half inch O.D. Tygon tubing, cane-shaped feeders can be placed into these holes. The ends of the tubing are closed with stoppers. An entrance to the food-choice unit is provided for by a small length of one-quarter inch O.D. Tygon tubing, the free end of which is placed into a hole in the plastic lid of the artificial nest. Small strips of graph paper can be cemented to the cane feeders (Fig. 9) to measure the volume of a food consumed. It different foods are placed in the different cane feeders, food-choice experiments can be run.

Auxiliary Food Units

Units containing cultures of live insects, other arthropods, annelids, etc. can be connected to the artificial nest. These units consist of Ball jars with modified lids. *Drosophila* cultures can be grown in pint jars filled with fly food to a depth of one and one-half inches. These culture jars are capped with the regular Ball ring and a 40 mesh brass screen disc the size of the Ball lid. A piece of celluloid, one-half inch wide and two inches long is folded over the edge of the brass mesh disc and stapled in two places. A quarter-inch hole, punched through the celluloid and brass screen, accepts some Tygon tubing that will connect the unit with the nest. (Fig. 10) Many species of ants can catch adult *Drosophila* of the mutant stocks vestigial, miniature and curved, and will carry larvae and pupae back to their nest. Any small animal that can be grown in a pint "culture" jar, i.e. flour beetles, meal-worms, etc. can be used in predator-prey experiments.

Interspecific Fighting and Predation

Two or more ant nests can be connected by means of Tygon tubing. If the ants used in the experiment are of unequal size, a device can be constructed which will allow the smaller ant to have free passageway from unit to unit (Fig. 11), but which will confine the larger species to its nest. Observations on raiding and interspecific fighting can be made with this equipment.

Colony Odor

Colony odor experiments can be conducted by using two artificial nests connected together in such a manner that the passage of ants can be controlled or limited in a manner different from that just described. Such a controlling device is shown in Fig. 12. A small magnet holds a steel BB shot against the inner wall of one-half inch O.D. Tygon tubing. The magnet is held in place by a small rubber band. With the BB in such a position, ants can pass freely through the tubing. If the magnet is pulled away from the tubing or moved upwards to a new position, the steel BB will fall and cover the opening of the smaller O.D. tubing. Access to and from the various units can be controlled by means of this device. One might wish to use electromagnets as a modification.

An artificial nest containing a colony of ants can be connected to an empty nest. If a number of ants are allowed to pass up into the observation chamber (Fig. 12) and then are prevented from returning to their home, they will be forced to live in the new nest for a period of time. Food and water can be supplied to the new nest. If the "isolated" ants are fed a diet different from that of the mother colony they might take on a different colony odor. If, at some later time, ants from both units are trapped in the observation area, any fighting can be noted.

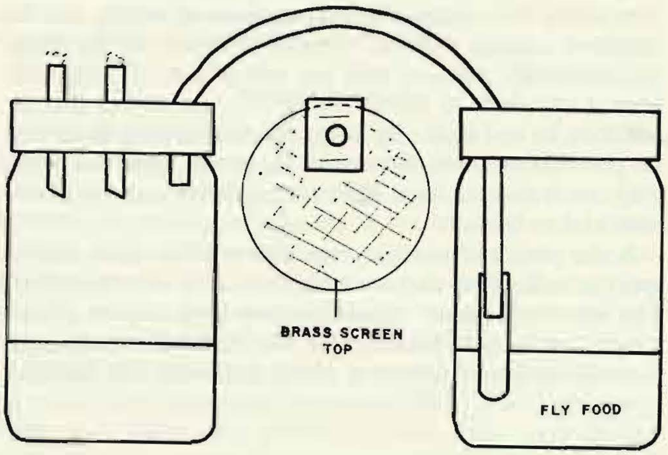


FIG. 10. Nest & Drosophila Unit

FIG. 11. One-way Device

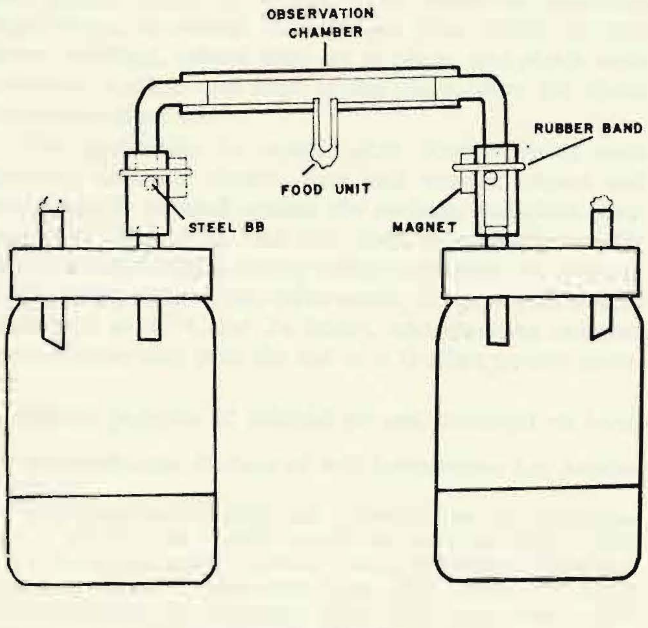


FIG. 12 Colony Odor, Fighting

Ant Mazes

It is possible to construct an ant maze of almost any complexity by using sections of one-fourth and one-half inch O.D. Tygon tubing, steel BB's, small magnets and rubber bands. Plastic tubing T's can be constructed from the two different sizes of tubing. Introduction of new pieces of tubing into the maze complex will remove the odor trails left by any of the workers which has touched its gaster to the wall of the tubing. If one desires to have ants travel long distances, long sections of tubing can be wrapped around a jar or a paper cylinder. If the nests and tubing are covered with red cellophane, all areas will seem to be dark to the ants (Fig. 8), since they are insensitive to red light. By using this equipment it should be possible to study the means by which ants find their way: such as by a fixed light source, odor trails or landmarks of some sort.

Some pieces of auxiliary equipment to be used with a specific collection and maintenance unit are described. The list of equipment represents only a small part of that which can be constructed from the materials mentioned. A study of a specific phase of ant behavior will demand a special piece of equipment. At the present time we are working with ant-aphid relationships and have designed some equipment for this study. We hope that the availability of this inexpensive equipment will stimulate many individuals to become interested in ant behavior.

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