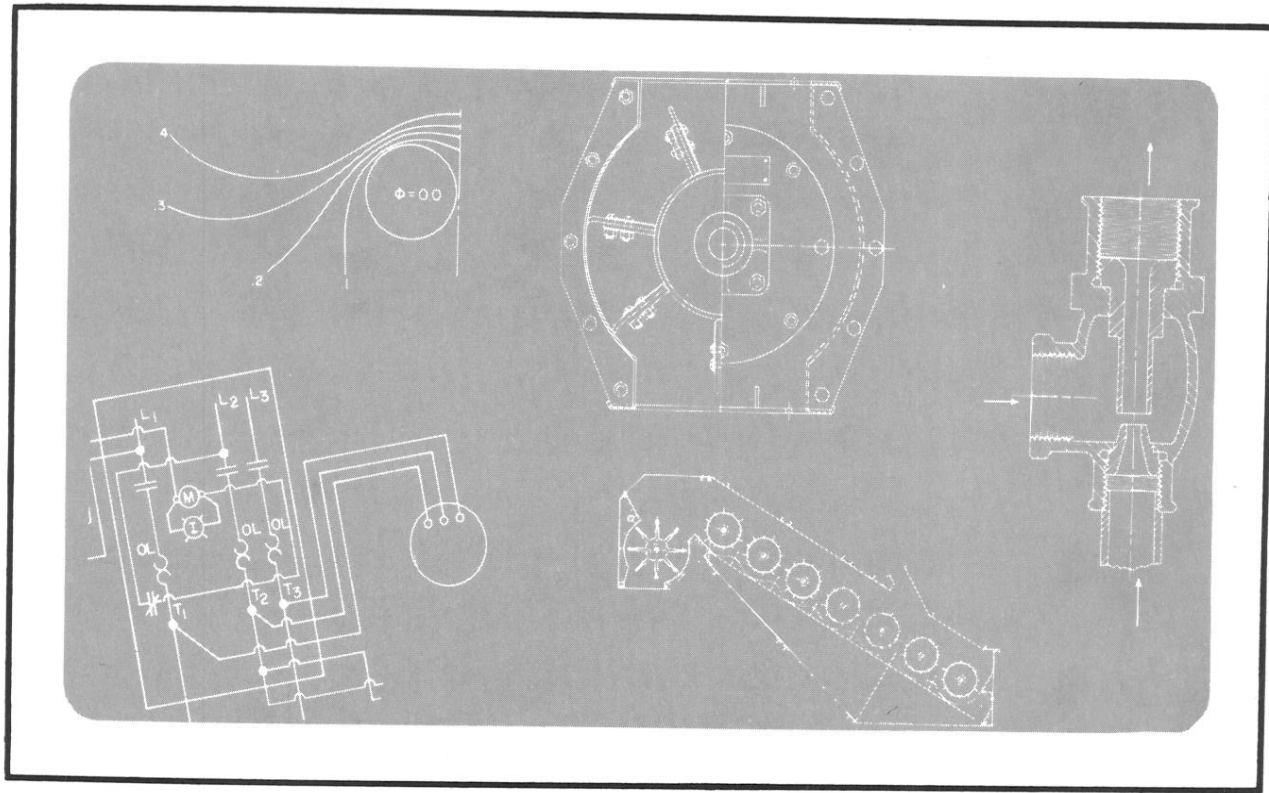


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Techniques for Collecting, Rearing, and Handling Imported Fire Ants

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Techniques for Collecting, Rearing, and Handling Imported Fire Ants

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ABSTRACT

Described are techniques for collecting and handling whole or partial field colonies of red and black imported fire ants, *Solenopsis invicta* Buren and *Solenopsis richteri* Forel; methods and equipment for collecting newly mated queens and for rearing laboratory colonies; and techniques for adding new queens to established colonies, harvesting and separating immatures from colonies, and using vacuum pickup systems with ants. Index terms: ants, imported fire ants, insect collecting, insect handling, insect rearing, *Solenopsis invicta* Buren, *Solenopsis richteri* Forel.

INTRODUCTION

Research and development studies to devise methods for control of red and black imported fire ants, *Solenopsis invicta* Buren and *Solenopsis richteri* Forel, respectively, have been in progress since late 1957, when the U.S. Congress authorized the initiation of a control program. The effective execution of the ensuing investigations on the biology, ecology, behavior, biocontrol, and pheromones of these insects and the evaluation of chemicals and formulations for their control required the development and refinement of techniques for collecting, handling, rearing, and maintaining many colonies of ants in the laboratory.

Methods developed by Khan (1966) and Khan et al. (1967) proved inadequate for producing

colonies of the size and in the numbers required for our studies; thus, new techniques had to be developed. While some of our techniques have been described in scientific papers by personnel of our laboratories, this publication represents the first attempt to collate all these techniques into a single reference and make them available to others conducting research on ants. Numerous changes have been made in the techniques over the past decade, and continued studies will no doubt produce further modifications and refinements of the techniques described.

METHODS OF COLLECTING ANTS IN THE FIELD

Ants for use in laboratory studies can be provided by collecting fragments of colonies from the field as needed or by rearing colonies in the laboratory from newly mated queens collected following nuptial flights in the field. Fragments of colonies containing worker ants, immatures (brood), winged sexuals, and infrequently, the mated queen can be obtained by shoveling a portion of the nest tumulus into a bucket or similar container. The inner walls of the

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FIGURE 1.—Plaster of paris nest for use when separating ants from soil by drying.

container must be carefully coated with Fluon GP-1 (ICI United States, Wilmington, Del.) or dusted with inert talc to prevent escape of the ants. This method of collection is adequate if the queen is not required and if the age of the colony is unimportant. The capture rate of queens from established colonies is increased substantially if collections are made during the early morning hours of bright, sunny days after cool nights from late fall to early spring. Removing a shovelful of the nest tumulus from the side exposed to the sun and spreading the soil on a hard surface as described by Glancey et al. (1975) will often yield a colony queen. Aggregation of the worker ants about the queen makes it easier to locate her. If located in this manner, the queen can be included in the bucket with the other ants from the nest or be placed in a small, separate container, which should be labeled appropriately, for later return to the colony.

After transfer to the laboratory the ants may be fed, maintained, and used directly from the container for various studies, or they may be separated from the soil by the technique described by Markin (1968) in which the soil is spread and dried in large trays, thus forcing the ants to move into moist plaster of paris nests (fig. 1). However, the quickest and easiest method of separating ants from the soil is by slowly dripping

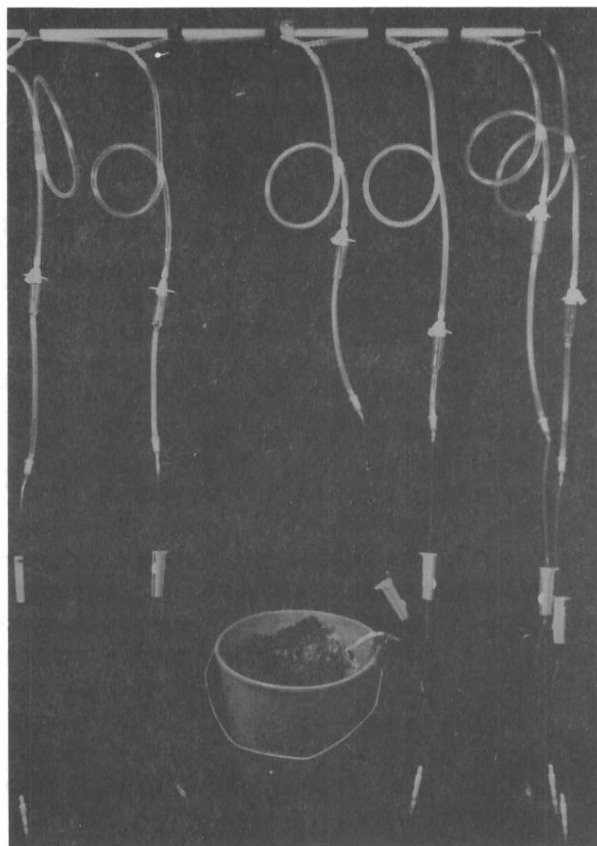


FIGURE 2.—Medical intravenous fluid-drip apparatus as modified for separating ants from soil by flotation.

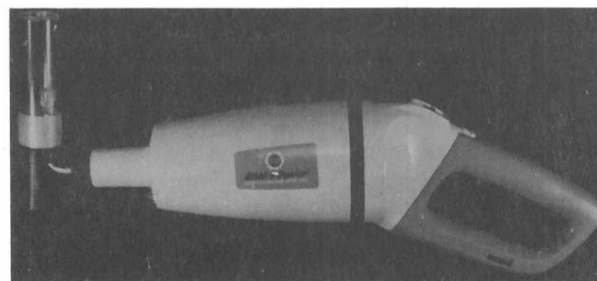


FIGURE 3.—Spot vacuum modified for collecting queen ants.

water (20 to 40 drops per minute) from medical intravenous fluid-drip tubing into the container until the soil is inundated (fig. 2). As the water level rises in the container, the ants will move the brood and queen upward to the soil surface, and the entire mass will eventually float free on the water surface (Jouvenaz et al. 1977). The ants can then be easily transferred to other containers with large spoons or ladles. This technique is

especially useful for collecting inquilines or parasites of the ants, for collecting immatures for pathological studies, and for mass-harvesting ants for pheromone research. Several hundred pounds of ants have been collected by this procedure for extraction of the trail pheromone.

For studies in which only a limited number of worker ants are required, collections may be made by dusting the inner wall of a large vial or small water glass or jar with talc, pushing the container into the nest tumulus until the top is flush with the soil, and leaving it until the desired quantity of ants has fallen into the container. The container can then be removed from the nest with long tongs or with the hands if plastic or rubber gloves are worn for protection.

Colonies of known age and condition can be obtained only by rearing colonies from newly mated queens collected after mating flights, which may occur during any month of the year (Markin et al. 1971, Morrill 1974). However, flights are most numerous and widespread during June through August; thus, queens may be found more easily during this period. Flights may occur as early as 11:30 a.m. and as late as 4:00 p.m., but the majority occur between 1:00 and 3:00 p.m. (Roe 1973). Flights will almost always be preceded by rain on the previous day or in the early morning of the same day. However, winds are often relatively brisk for a day or two after rains, and if such winds exceed 4 to 5 mi/h, flights may be delayed until the winds subside. Also, flights may continue for 2 or 3 consecutive days after fairly heavy rains. As the queens descend from the flight, they can be easily collected from the surface of parking lots, hard-surface roads, etc. After 2 or 3 hours and up to about 48 hours after flight, queens can be found on hard surfaces under pieces of paper or cardboard, tin cans, boards, etc. They may also be found in recently cultivated fields beneath small dirt piles made as the queens excavate the brood chamber from below the soil surface. Collections can be made with light featherweight forceps or an aspirator. Aspirators are commercially available from a number of sources, or they can be made from commercially available materials. We adapted a model 4 cordless spot vacuum (Black and Decker, Towson, Md.) for queen collection by fitting a Plexiglas trap to the intake orifice of the vacuum (fig. 3). Large numbers of queens can be collected quickly without injury with this unit. Immediately after collection the queens are transferred into glass jars or Styrofoam cups containing

moist toweling, which are then capped. In the laboratory the queens may be immediately set up for colony production, or if necessary, they can be held in a refrigerator in a container with moist toweling for several days.

METHODS OF REARING ANTS IN THE LABORATORY

ESTABLISHING AND MAINTAINING COLONY

Newly mated queens must be isolated for egg laying and production of the first workers in the laboratory. A variety of containers, such as shell vials, pill bottles, medicine cups, test tubes, etc., are suitable for this purpose. We use 1-oz clear-plastic cups (Dixie P01-10, American Can, Easton, Pa., or equivalent) and 15- by 125-mm or 16- by 150-mm test tubes (fig. 4). A 5-mm hole is burned through the bottom of each plastic cup with a soldering iron, and a 5- to 7-mm layer of liquefied dental labstone (Ransome and Randolph, Toledo, Ohio) is poured into the bottom. After the labstone has hardened the queens are placed individually into the cups, which are then capped and placed on a bed of wet cotton batting or vinyl sponge. Moisture from the cotton or sponge is wicked-up through the labstone to maintain high humidity in the cups. The test tubes are filled approximately two-thirds full with water, and a saturated cotton plug that snugly fits the bore of the test tube is pushed into the tube so that 3 to 5 cm of the end of the tube remains open and free of water. A single queen is placed in each test tube, and each tube is then isolated in a 5- by 19- by 30-cm enamel or plastic pan. The inner walls of the pan are coated with inert talc or with Fluon to prevent escape of the ants. Fluon is preferable because it adheres to the pan, lasts longer, and does not desiccate the ants as readily as does talc.

The first worker ants will eclose in about 25 to 30 days in a laboratory maintained at 28° C. We have found that about 60% of the queens will eclose workers by the 26th day. Queens that are not laying or are producing only sex brood are usually eliminated to conserve space and reduce labor. Colonies develop most rapidly at 32° C (O'Neal and Markin 1975), but laboratory personnel find this temperature very unpleasant. Colonies can be developed more quickly if larvae and pharate

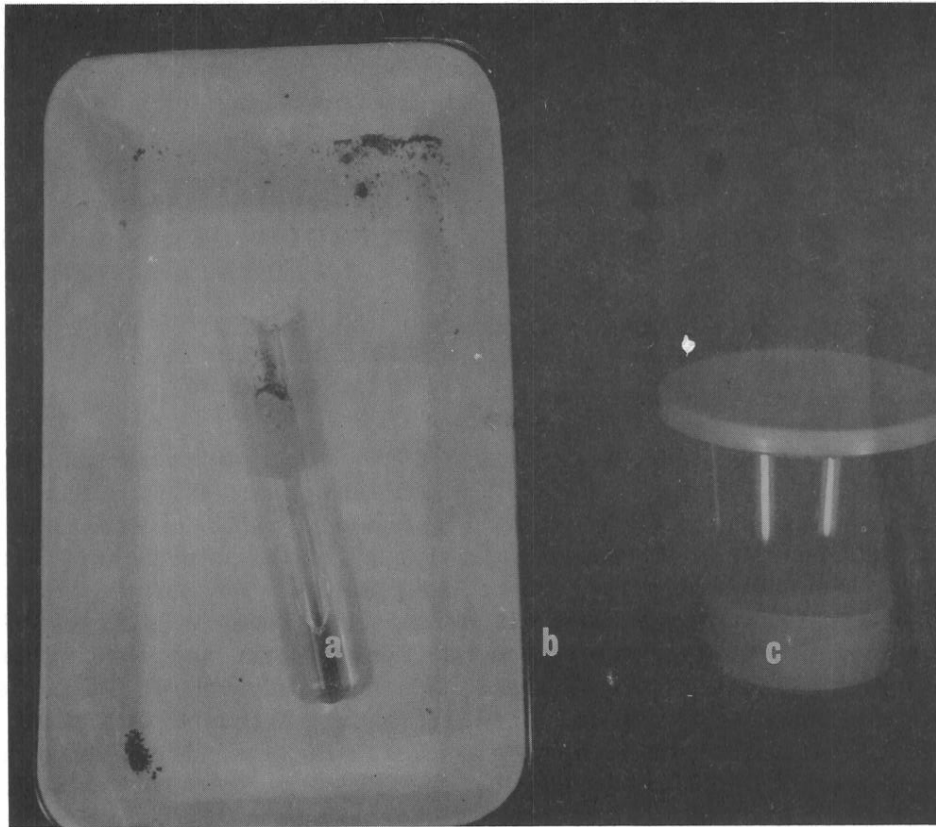


FIGURE 4.—Containers for beginning new ant colonies with newly mated queens. a, Test-tube nest. b, Enamel pan, 5- by 19- by 30-cm. c, One-ounce plastic cup nest.

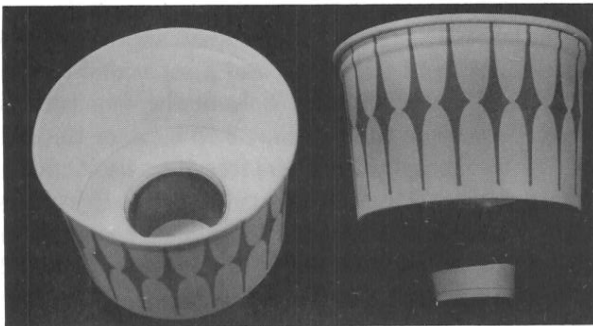


FIGURE 5.—Squat paperboard cup with plastic rearing-cup nest inserted into place.

workers from established colonies are placed with newly mated queens.

The young colonies may be maintained in the initial containers until they number 300 to 500 workers if adequate food and moisture are made available. Food may be placed directly in the bottom of the pans containing the test-tube nests, but placing food into the cup nests increases

mortality of minim workers and queens due to fungal growth on unused food. To alleviate this problem and provide a separate foraging arena, while still conserving space, a 500-ml squat paperboard cup is attached to the top of the plastic rearing cup. A circular hole that exactly corresponds to the outside circumference of the plastic rearing cup just under the top lip is cut in the center of the bottom of the squat cup. The inner wall of the squat cup is coated with Fluon, and the plastic rearing cup is then uncapped and pushed down into the hole in the bottom of the squat cup until it fits snugly (fig. 5). The cups are then returned to the wet cotton or sponge beds. Food can then be placed on the platform formed by the inner bottom of the squat cup.

As the colonies grow larger they are transferred from the plastic cup or test-tube nests to plastic ant nests (fig. 6). The nests are kept in 7- or 12- by 44- by 56-cm plastic nesting trays (Panel Controls, Detroit, Mich.) that provide a foraging arena for the colonies. The inner walls of the trays are coated with Fluon to prevent escape of the ants. Nests of

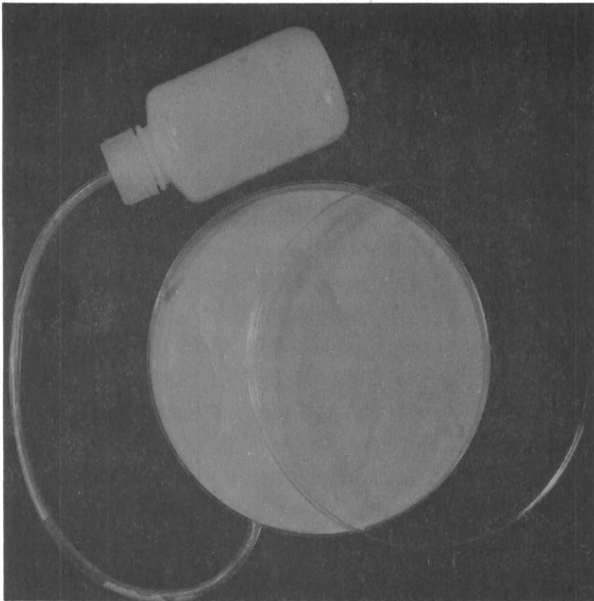


FIGURE 6.—Bishop plastic ant nest for ant-colony maintenance.

the type described by Wilson (1962) can be obtained commercially. These nests, however, are relatively expensive (\$12 to \$20 per unit in 1978) and perform no better than nests assembled in the laboratory from commercially available components for about 50 cents each. We use two designs (Bishop et al. 1980) for fabricating nests in the laboratory. The Bishop nest is made from 15- by 150-mm or 25- by 150-mm disposable plastic petri dishes, and the Williams nest is made from 10- by 100-mm and 25- by 150-mm disposable plastic petri dishes. For the Bishop nest, a 30-cm length of plastic tubing having an inside diameter of 3 mm and an outside diameter of 5 mm is used. It contains a 45-cm piece of crochet yarn that projects 5 and 10 cm beyond the ends of the tubing. The tubing is passed through the sidewall of the bottom half of the petri dish, secured near the center of the bottom with cellophane tape, and then covered with liquefied dental labstone. The other end of the plastic tubing and yarn is passed through a hole in the top of a 125- to 250-ml plastic bottle that is filled with water to serve as a moisture source for the nest. For the Williams nest, a piece of vinyl sponge is cut to fit snugly inside the smaller petri dish, and the sponge is saturated with water. Four 2- to 3-cm holes are made in the top of this dish, the top is replaced, and the dish is then placed in the larger petri dish and covered with liquefied dental labstone. Nests constructed by either design

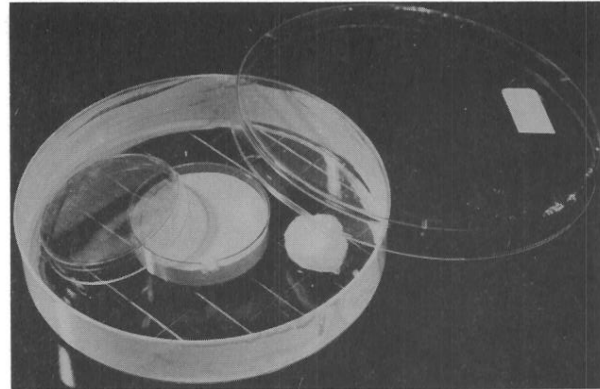


FIGURE 7.—Modified plastic ant nest for small-colony maintenance.

are used for an average of 12 to 15 weeks, with periodic replenishment of the water supply. Water replenishment is accomplished for the Bishop nest by simply refilling the water bottle, but for the Williams nest a hole is drilled through the bottom of the nest into the sponge area, the sponge is saturated with water using a hypodermic syringe with needle, and the hole is then resealed.

Periodically, the nests become dirty or moisture flow becomes impeded, and the nest must be replaced. A new nest is prepared and placed in the tray with the previous nest, and the top of the old nest is removed. The ants will then move by their own volition, particularly if the water bottle for the old nest is removed.

A third type of nest (fig. 7) is also sometimes used for special studies with very small colonies or fragments of larger colonies and for initiation of new colonies by newly mated queens. These nests are made from 15- by 60-mm and 25- by 150-mm disposable plastic petri dishes. A 4- to 6-mm layer of liquefied dental labstone is poured into the smaller dish and allowed to harden. One or more 1- to 2-mm exit holes are burned or drilled through the sidewall of the smaller dish just above the labstone. This dish is then glued in the center of the bottom of the larger dish. The inner wall of the larger dish is coated with Fluon. The labstone is wet to saturation before introduction of the ants, and a wad of wet cotton is placed in the larger dish to help maintain high humidity. Taping the lid securely on the larger dish helps retard drying and provides additional security against escape of the ants.

The rearing trays also become dirty and require changing. A clean tray is prepared as for the

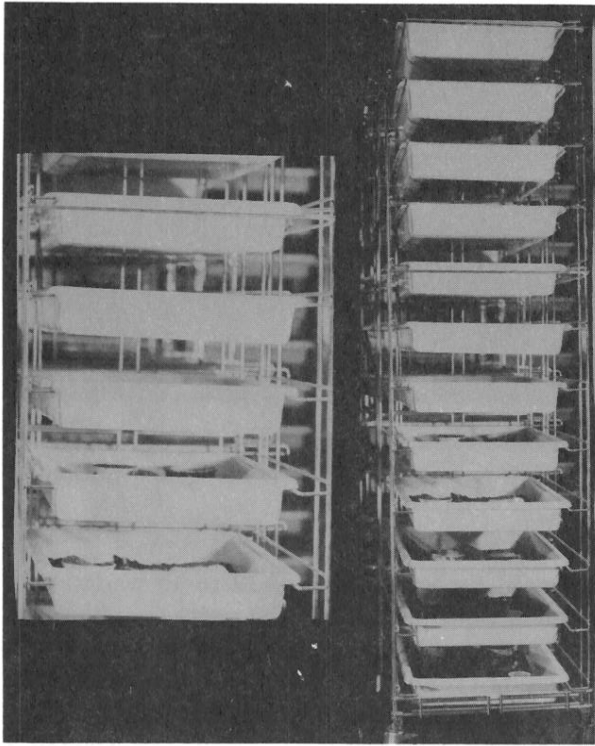


FIGURE 8.—Steel rack for holding trays of ants in laboratory. Inset shows detail of attachment of support rails.

original-colony set up, and the nests containing the colonies are simply transferred to the new tray. Sometimes many worker ants are outside the nests and are left in the old tray as the nests are moved to the new tray. Thus, these ants must be transferred separately to the new tray. Debris in the old tray is loosened from the bottom with a scraper (wide putty knife, paint scraper, etc.), and the debris and remaining ants are shaken to one corner of the tray and poured into a 250- to 500-ml squat cup or beaker that has been coated with Fluon or dusted with talc on the outside. The cup is placed in the new tray, and the ants then begin to work their way up through the debris and exit over the side of the cup into the new tray. After all the ants have left the cup, it is discarded along with the debris.

The trays are placed in chromed-steel racks (fig. 8) for ease of handling in the laboratory and for movement from one laboratory to another. The racks (Old Brazos Forge, Brenham, Tex.) are grocery bread racks that have been slightly modified by rotating the support arms 90° to accommodate the trays. The racks are mobile, will accept up to 12 trays, are collapsible for storage, and can be used for the entire rearing operation from initial

set of newly mated queens to the maintenance of large colonies.

FOOD AND FEEDING

A variety of materials have been utilized for food for laboratory colonies of fire ants. Khan et al. (1967) used meal worms, grasshoppers, peanut butter, and high-meat baby foods. Bhatkar and Whitcomb (1970) reported that a diet of agar, whole egg, honey, vitamins, and minerals was adequate for the imported fire ants, as well as for other species. In addition to using the diet of Bhatkar and Whitcomb, we have used larvae of the greater wax moth, *Galleria mellonella* (L.), the German cockroach, *Blatella germanica* (L.), the American cockroach, *Periplaneta americana* (L.), and crickets, *Gryllus* spp., as well as meat baby food, fried chicken, and peanut butter. We found that, while the ants would survive and reproduce on most of these materials, long-term colony health, growth, and vigor was not as good as desired. In addition, continued feeding of any of these materials resulted in decreased acceptance by the ants. After considerable experimentation with variations of the diet described by Bhatkar and Whitcomb, we found that laboratory colonies did well when consistently fed the following diet:

- 2,000 ml of housefly or stablefly pupae. (Other insects can be used in lieu of fly pupae.)
- 12 whole hen eggs.
- 350 to 450 g of ground beef or all-beef baby food.
- 5 ml of multiple vitamins.
- 60 g of agar.
- 1,500 ml of water.

The quantities given above will make enough diet for a single feeding of 200 mature colonies or a greater number of smaller colonies. Amounts can be adjusted proportionately for more or less diet. The ground meat is cooked and then pureed along with the fly pupae in a heavy-duty food blender. The eggs and vitamins are then added. A portion of the water (100 to 150 ml) is used cold to moisten and soften the agar, and the remainder is heated to boiling and then used to dissolve the softened agar. The pureed ingredients are added to the dissolved agar, mixed thoroughly, and then poured into 20-cm disposable aluminum pie pans to cool. If the diet is not used fairly soon, it is covered with plastic film or aluminum foil to retard drying and then is frozen until needed. Small quantities are kept covered in a refrigerator. The diet is cut into small blocks, the size depending on the

size of the colony to be fed, and placed in the rearing tray where it is accessible to the foraging worker ants. Food for very small developing colonies must be placed closer to the nest itself than is necessary for older, larger colonies. The small blocks of food for very young colonies will remain moist and acceptable to the ants longer if a light coating of paraffin wax is applied by dipping the blocks in melted paraffin.

Williams et al. (1980) found that supplementation of the above diet with 50% honey-water solution increased survival of very young colonies and greatly promoted growth and development in older colonies. Honey can be added directly to the above diet but is more effective when administered separately. The honey-water solution may be administered in small disposable plastic beakers, in culture tubes plugged with cotton, or in micro-capillary tubes from which the ants remove it.

MISCELLANEOUS HANDLING AND STUDY TECHNIQUES

ADDING NEW QUEEN TO ESTABLISHED COLONY

Occasionally the need arises to replace the queen in a colony when the original queen has been lost. This procedure is at best tricky and to date we have not found a fail-safe method. As a general rule, the larger the colony the more difficult it is to replace the queen. Newly mated queens will usually be accepted more readily if adequate time (10 to 14 days) is allowed after flight for histolysis of the wing muscles and initiation of queen pheromone production. Anesthetizing the colony with CO₂ or chilling the colony to immobility in a refrigerator and then introducing the queen while the worker ants are inactive will sometimes enhance acceptance of the new queen. Confinement of the new queen for a brief period with brood from the recipient colony will also sometimes increase acceptability. Continued effort is often the key to introduction of a new queen; a colony may reject several queens and then inexplicably accept one very readily.

HARVESTING AND SEPARATING BROOD FROM COLONY

Various studies require the separation of immatures from the remainder of the colony. This is

easily accomplished by anesthetizing the colony with CO₂, collecting brood from brood piles with a spatula or preferably a suction device, and removing inadvertently collected adults by transfer of brood to successive tissue papers. A suction collector made from a side-arm Erlenmeyer flask and glass tubing works well if suction is gentle and the flask contains loosely wadded tissue paper for padding to prevent injury to the delicate immatures. Some adult worker ants are inevitably included in the initial sample, but these may be easily removed as follows: The anesthetized ants and brood are spread on a large piece of tissue paper (about 40 by 40 cm for up to about a 15-g sample). When the worker ants revive sufficiently to cling to the paper, but before they are active enough to grasp the immatures in their mandibles, the brood is gently rolled or shaken onto a second piece of tissue. This process is repeated several times to produce a sample of brood free or nearly free of adult ants (remaining workers may be removed with forceps). The worker ants clinging to the tissues are simply

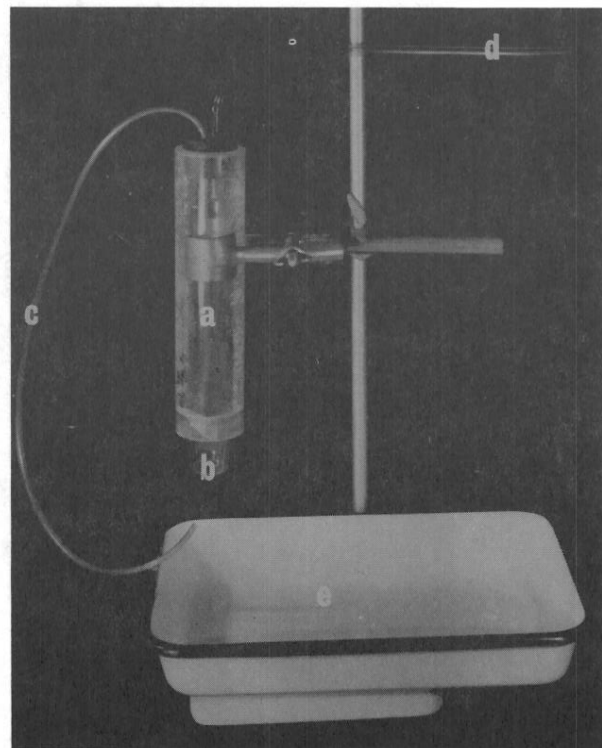


FIGURE 9.—Stationary vacuum pickup system for handling ants. a, Vacuum chamber. b, One-ounce cup for receiving specimens. c, Pickup tube. d, Tube to vacuum source. e, Specimen tray.

reanesthetized in a CO₂ chamber and shaken back into the nest or are disposed of. To prevent the occasional escape of a few workers, we perform separation in a shallow 8.5- by 44- by 56-cm plastic tray with Fluon-coated sides.

VACUUM SYSTEMS FOR HANDLING ANTS

Studies of imported fire ants often require that specimens be transferred individually or in small numbers from one container or substrate to another. Such transfers can be made with forceps, tongue depressors, spatulas, or similar instruments. These methods, however, are tedious and can result in

injury to the specimen. Aspirator systems provide a convenient means of rapidly and safely handling specimens. Such systems are commercially available, but suitable ones can be easily fabricated from readily available components. We have built two systems for our use, a stationary system for the laboratory and a portable system that can be used anywhere that electricity is available for a portable vacuum pump.

The stationary system (fig. 9) is composed of a 20-cm length of acrylic tubing, with an inside diameter of 3.8 cm and an outside diameter of 5.0 cm, fitted at the upper end with a No. 8 rubber stopper having two 5-mm holes. The inner wall of the tube at the bottom end is tapered from 4.5 cm at the end to 4.0 cm at 1.5 cm into the tube

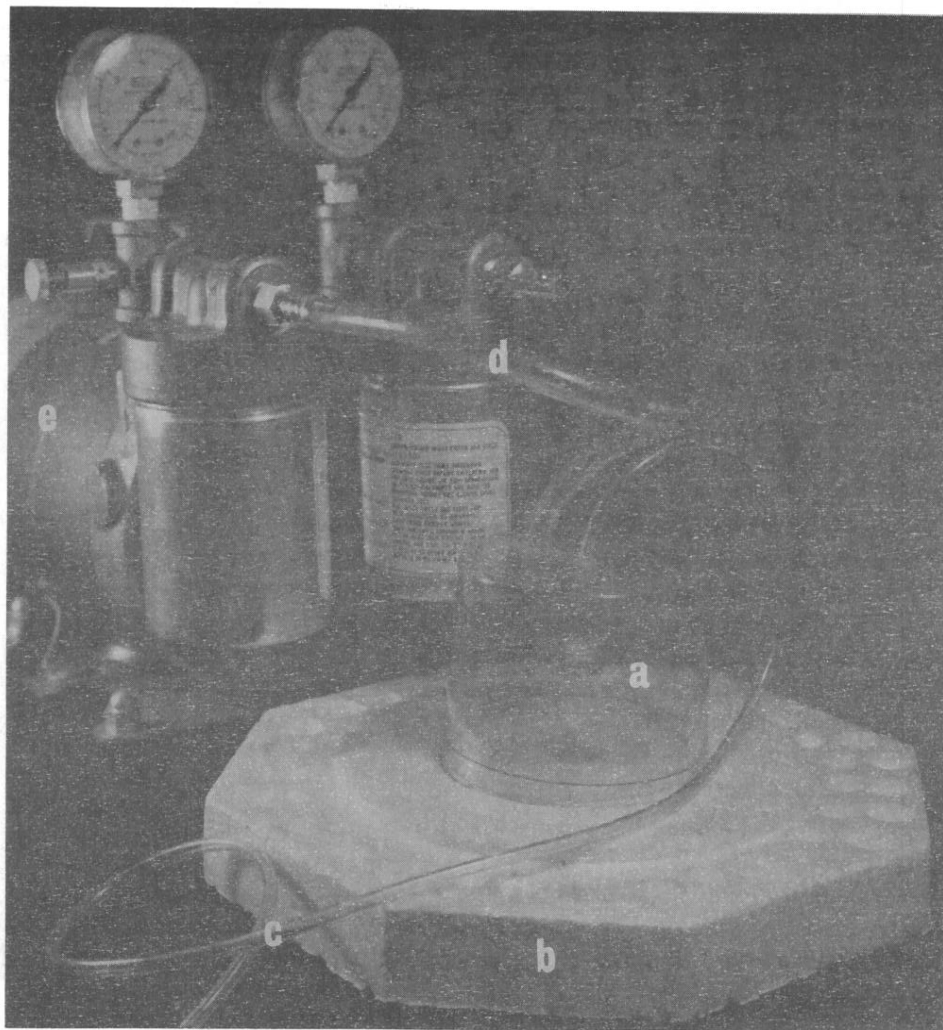


FIGURE 10.—Portable vacuum pickup system for handling ants. a, Plastic freezer-dish vacuum chamber b, Polystyrene base. c, Pickup tube. d, Tube to vacuum source. e, Vacuum pump.

to accommodate a 1-oz plastic cup, resulting in a snug fit between the tube and rimmed top of the cup. The tube assembly or chamber is held vertically by a V-jaw utility clamp secured to a ring stand and is positioned to leave adequate space between the bottom of the tube and the ring-stand base for easy insertion of the plastic cup. A 7.5-cm length of copper tubing having an outside diameter of 5-mm and with one end flared and covered with a small piece of 20-mesh screen soldered over it is pushed through one hole in the rubber stopper and attached via Tygon tubing to a vacuum source. A piece of Tygon tubing about 1 m long, with an outside diameter of 5 mm and an inside diameter of 3 mm, is pushed through the other hole in the stopper until the end inside the chamber hangs just above the plastic cup. This end is weighted to insure that it hangs straight and drops the specimen directly into the cup. The opposite end of this tubing is left free to be moved as necessary to pick up the insects.

The portable system (fig. 10) is made with a 500-ml plastic freezer container, a 5- by 50- by 50-mm block of foamed polystyrene, and two 1- to 2-m pieces of Tygon tubing having inside diameters of 3 mm and 6 mm and outside diameters of 5 mm and 10 mm, respectively. The plastic container is inverted and pressed into the polystyrene near the center of the block to form a groove 4 to 6 mm deep. Two holes are made in the bottom of the inverted container to accommodate snug passage of the tubing. One end of the larger tube is inserted into the container 2 to 3 cm, and the opposite end is connected to the vacuum source. One end of the smaller tube is inserted through the other hole in the container so that the end extends 2 to 3 mm into the specimen receptacle, and the opposite end is left free for necessary movement to pick up specimens. A depression is made in the polystyrene block to hold the specimen container.

Vacuum for the stationary system is usually provided by the piped central system for the laboratory but can be provided by a small portable vacuum pump. Vacuum for the portable system is usually provided by the latter means.

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