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MODIFIED REARING AND MAINTENANCE TECHNIQUES FOR *MANTISPA VIRIDIS* (NEUROPTERA: MANTISPIDAE)

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Abstract

Improved techniques were developed for the rearing and maintenance of *Mantispa viridis* Walker. These have proven highly successful and have eliminated problems encountered by other researchers working on mantispine developmental studies, and should allow subsequent studies to be conducted under standardized conditions. These techniques also have been used in maintaining colonies of beaded lacewings (Neuroptera: Berothidae), various spider egg parasitoids and predators, and are adaptable for other arthropods that require individual confinement.

Resumen

Se desarrollaron tecnicas mejoradas para la crianza y mantenimiento de *Mantispa viridis* Walker. Estas tecnicas tuvieron mucho exito y se eliminaron problemas encontrados por otros investigadores; de la misma forma estas tecnicas permitieron que se realizaran estudios subsiguientes en condiciones normales. Estas tecnicas se utilizaron tambien para mantener colonias de los insectos de encaje con borlas (Neuroptera: Berothidae), y para varios parasitos de huevos de arañas. Estas tecnicas pueden ser adaptadas para la crianza de otros artropodos que requieran confinamiento individual.

Most previous developmental data reported for members of the Mantispinae (Neuroptera: Mantispidae) are difficult to compare intra- and interspecifically due to studies having been conducted under uncontrolled conditions of temperature, photoperiod, relative humidity (RH) and larval rearing techniques. The exception is a standardized rearing technique developed by Redborg & MacLeod (1983, 1985). They constructed rearing cells by drilling wells into a hardened mixture composed of powdered, activated carbon and plaster of Paris. Spider eggs were added to the wells, a first instar mantispid was placed in each, and the top was sealed with a glass cover. Egg incubation

and larval development were monitored under controlled conditions of 25°C, 80% RH, and 16L:8D photoperiod. Their standardized rearing procedure allowed the calculation of mean developmental data for each stage of three species, *Climaciella brunnea* (Say) (Redborg & MacLeod 1983), *Mantispa sayi* Banks (as *Mantispa uhleri* Banks), and *Mantispa viridis* Walker (Redborg & MacLeod 1985).

This report presents the modification of laboratory techniques used to rear and maintain colonies of *M. viridis*. These rearing procedures, while based on the work of the preceding authors, eliminate several problems they encountered and provide a savings of both time and space.

Materials and Methods

Rearing Materials

Larval Rearing Containers. Five dram plastic pill-bottles with "child-proof" caps (Inventive Packaging Corp., Denver, CO) were used to rear individual *M. viridis* larvae. The top 6 mm of these have a flange 1 mm wider than the bottom portion. Pill-bottle size was reduced by shaving 3.4 cm off the bottom using a flat wood boring bit (2.54 cm diameter) set in a drill press. This produced a flanged rearing container 2.5 cm high by 2.5 cm top outer diameter (Fig. 1). Reducing the size of the containers served two purposes. First, it decreased the interior volume from approximately 19.2 ml to 9.3 ml, thereby reducing the amount of substrate needed. Second, it made the container considerably less prone to being knocked over.

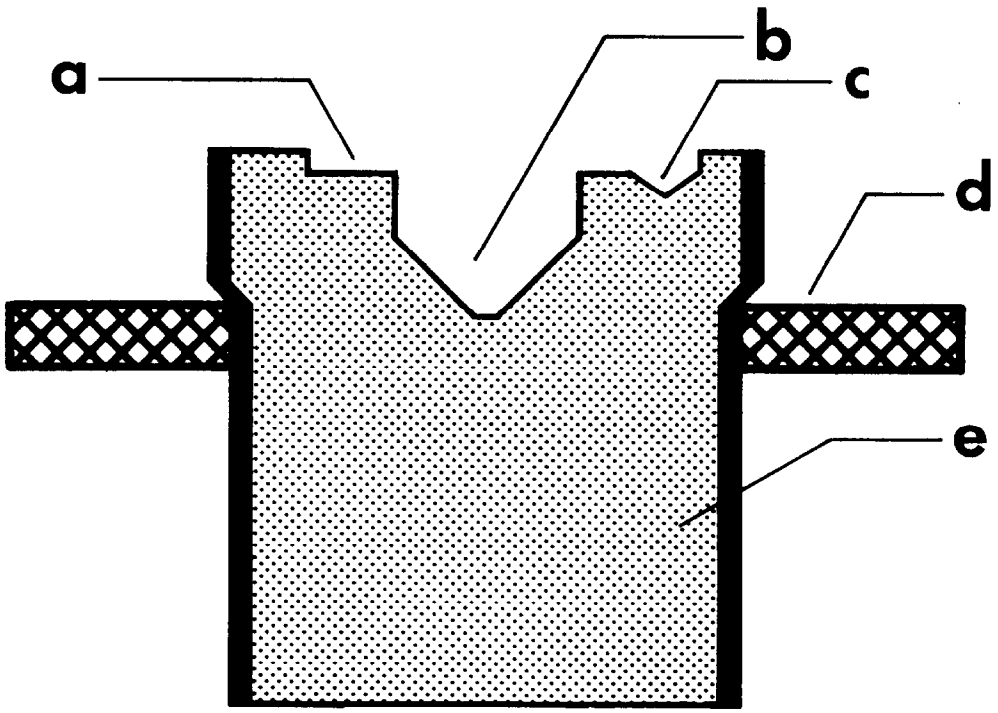

Fig. 1. Rearing container for *M. viridis* larvae, cross section. a, top-molded, cover slip seating well (15.8 mm diameter by 0.8 mm deep); b, rearing cell (8.3 mm diameter by 7.1 mm deep); c, cover slip access well (3.2 mm diameter by 1.6 mm deep); d, acrylic support; e, activated carbon/plaster of Paris substrate.

To construct a rearing cell that would effectively confine first instar *M. viridis* larvae, a built-in seal for the top of the rearing cell was incorporated. Using a number 9 cork borer, 15.8 mm diameter circles of 0.8 mm thick polypropylene were cut out and appressed to the centers of 3.8 cm squares of masking tape. The top of a cut-down container was then attached to the masking tape so that the polypropylene circle was centered within the opening. Each container was then filled with a wet mixture of 1 part powdered, activated carbon to 9 parts plaster of Paris (Redborg & MacLeod 1985). A 5:4 (wt:v) ratio of substrate to distilled water produced an optimum consistency for mixing and pouring. To eliminate air pockets trapped in the wet substrate, each container was tapped several times on a flat, hard surface. An hour later, substrate-filled containers were transferred to an environmental chamber (35°C) and held for an additional 48 h to complete drying. When the substrate was completely dry, the masking tape with the polypropylene circle still attached, was peeled away leaving a smooth outer surface bordering a 15.8 mm diameter by 0.8 mm deep molded well. This well prevented a cover slip from sliding off the rearing cell as it would be seated below the top surface. Using an 8.3 mm diameter drill bit set into a drill press, a 7.1 mm deep hole that would serve as the rearing cell was drilled in the center of the molded well. A second hole, 3.2 mm diameter by 1.6 mm deep, was drilled at the junction of the top surface and molded well. This provided access to facilitate removal of the 12 mm diameter circular glass cover slip used to seal the rearing cell. Compressed air was used to remove pulverized substrate material produced by the drilling.

Finished rearing containers were supported inside a closed, rectangular plastic box (18.7 x 13.5 x 8.4 cm) (Stock No. T69C, Tri-State Molded Plastics, Inc., (TSMP), Dixon, KY) by means of a 3.2 mm thick acrylic sheet in which 20 evenly-spaced 23.8 mm holes had been drilled (Fig. 2). Each rearing container fit snugly into the plastic support reducing the possibility of jarring the cover slip from the rearing cell. The 6 mm lip of the rearing container that projected above the surface of the acrylic support facilitated manipulation of individual containers. Relative humidity (RH) was maintained at 80% within the plastic box with a saturated water solution of KBr (Solomon 1951).

To test the effectiveness of this design, twenty newly eclosed (<12 h old) first instar *M. viridis* from a single clutch of eggs were placed individually in twenty cells. Cells were examined after 24 h and the presence or absence of larvae recorded. The test was replicated six times using larvae produced by six different wild-caught females.

Spider Eggs. Eggs of numerous spider species belonging to the families Theridiidae, Lycosidae, Salticidae, and Araneidae were used to rear *M. viridis*. However, nonaggglutinate eggs of theridiids such as the house spider, *Achaearanea tepidariorum* (C. L. Koch), and the black widow, *Latrodectus mactans* (F.), were easiest to employ. Within 72 h of deposition, eggs of these species separate from each other and could be poured from an opened egg sac into a rearing cell or storage container. Eggs which remained attached to the silken lining of egg sacs could be freed by carefully dislodging them with forceps or a moistened camel's-hair brush.

Field-collected egg sacs and those obtained from laboratory colonies of mated female spiders were opened and the eggs collected in 50 mm x 9 mm plastic petri dishes having tight-fitting lids (Falcon Co., Oxnard, CA). Bottoms and sides of petri dishes were lined with 55 mm diameter filter paper disks to prevent eggs from "jumping out" of, or adhering to, the sides of the dish due to static electricity present on the plastic surface. Colonies of *A. tepidariorum* and *L. mactans* were maintained in an environmental chamber at 27.8 ± 2°C, 80 ± 5% RH with a 14L:10D photoperiod. Spiders were fed either *Drosophila melanogaster* Meigen or *Musca domestica* L. adults depending on the size of the spider.

Eggs which were not immediately used to rear *M. viridis* were stored for later use at –20°C (Redborg and MacLeod 1985). Prolonged storage (> 3 months) of small eggs,

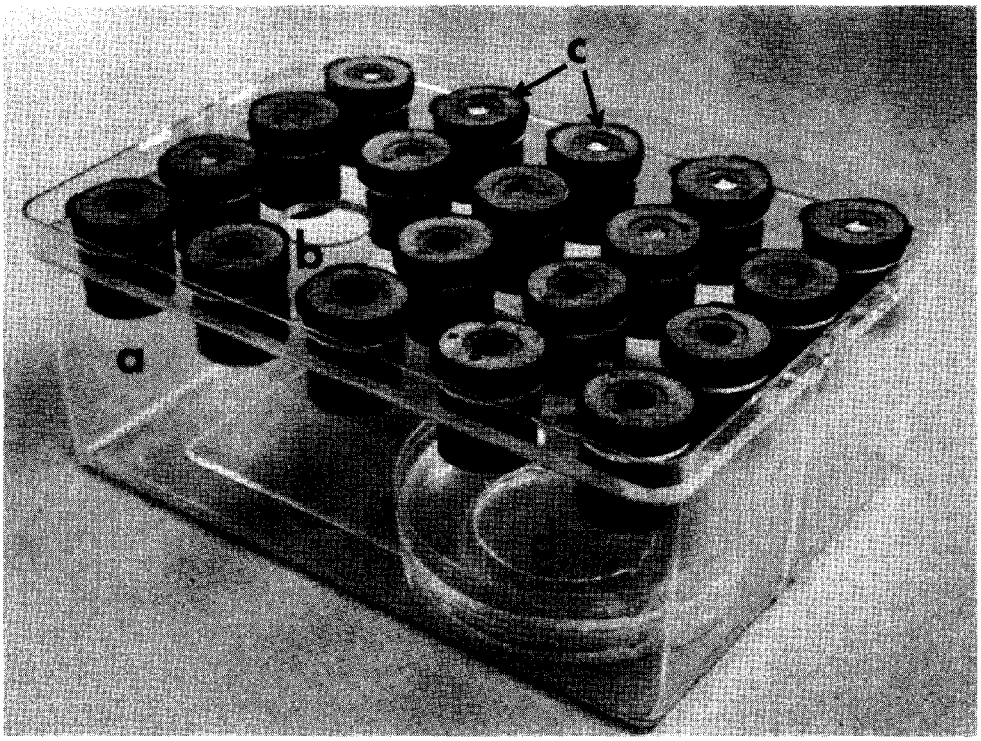

Fig. 2. Maintenance container for *M. viridis* larvae, top removed. a, plastic box; b, acrylic support; c, rearing containers; d, plastic Petri dish containing a saturated water solution of KBr.

such as those of *A. tepidariorum*, resulted in complete desiccation of the eggs. Larger eggs, such as those of *L. mactans*, could be stored for longer than 6 months with no appreciable desiccation.

Freezer-stored eggs were held at room temperature for at least 2 h before being used to rear *M. viridis*. This allowed the eggs to thaw completely and any water to evaporate that initially condensed on the chorions. Damaged and desiccated eggs were discarded to reduce the possibility of fungus developing on the eggs within a sealed rearing cell.

Adult Maintenance Containers. Seven dram, plastic vials fitted with snap-cap lids (Cat. No. 133157-1, American Scientific Products, McGraw Park, IL) were used to house individual adults. Bottoms of these vials had been cut away and replaced with 25 mesh/cm Saran® (Style 5038400, Chicopee, Gainesville, GA) screening to ensure adequate ventilation. Two-thirds of the interior wall was lined with filter paper to provide a surface where pharate adults could undergo the final ecdysis. Vials were supported inside a plastic box (Stock No. T69C, TSMP, Inc.) as described previously (Fig. 3).

Adults were allowed to mate in 506 ml capacity, round (10.5 cm diameter by 7 cm deep), plastic containers with screw-top lids (Stock No. T40C, TSMP, Inc.). Two 5 cm diameter holes were cut in opposite sides of the container and a 7.6 cm diameter hole was cut in the lid. These openings were covered with Saran® screening (25 mesh/cm) to provide ventilation.

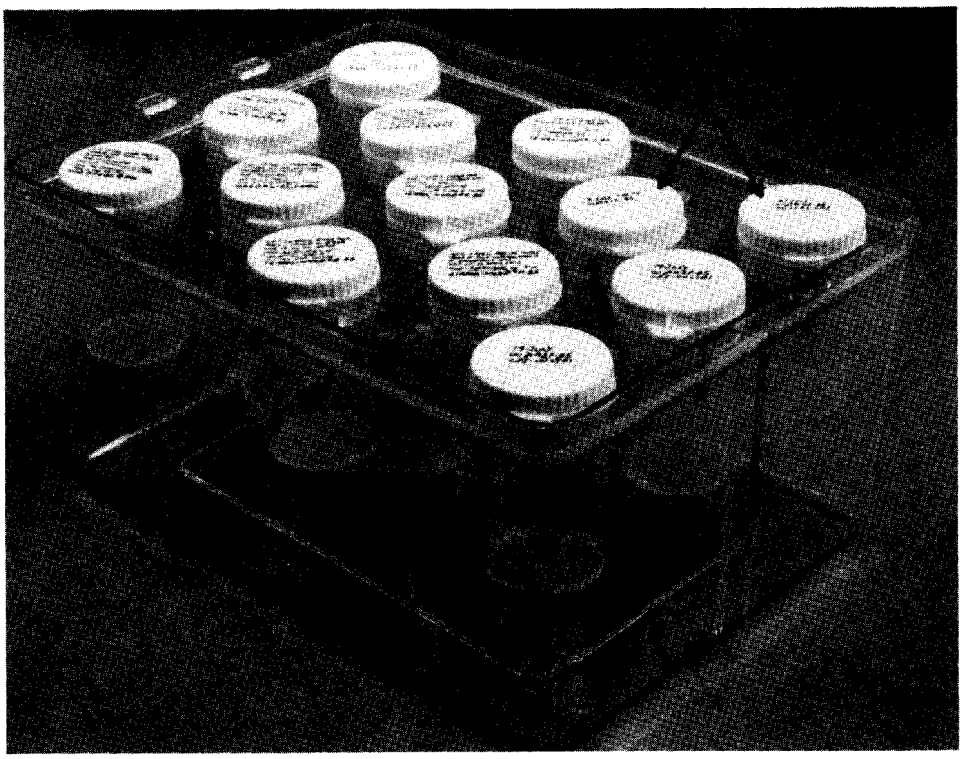

Fig. 3. Maintenance container for *M. viridis* adults, top removed. a, plastic box; b, acrylic support; c, bottom screened, 7 dram vials; d, plastic Petri dish containing a saturated water solution of KBr.

Rearing and Maintenance

Larval Rearings. Larval rearings were initiated by introducing either fresh or freezer-stored eggs into a rearing cell. In general, enough eggs were added to fill the cell one-half to two-thirds full. Using a camel's-hair brush moistened with distilled water, a first instar *M. viridis* was transferred into a cell and the top was sealed with a glass cover slip. Sealed rearing containers were placed in the plastic box and transferred to an environmental chamber maintained at 25°C with a 16L:8D photoperiod. Five days after the third instar had spun a cocoon, the cover slip was carefully removed from the rearing cell and the cocoon was transferred to an adult maintenance container.

Adult Maintenance and Reproduction. Adult male and female *M. viridis* were maintained individually in 7 dram, ventilated vials as described previously (Fig. 3) and were fed one housefly per day. Interior sides of vials containing females were lined with 8.9 mm x 3.7 mm sheets of clear acetate (Ful-Vu Report Covers, Cooks' Inc., Darby, PA). The interior surface of the cap was lined with a 2.5 cm diameter acetate circle. The clear liner allowed direct observation of female oviposition and feeding behavior, and provided an easily removed and replaced oviposition substrate. This facilitated making egg counts either directly with the aid of a stereomicroscope or indirectly by photographing the clutch of eggs and counting the number of eggs from a black and white print (Redborg & MacLeod 1985). Females rarely oviposited on the screened bottoms of the vials and usually this occurred with females >90 d old. In such cases, screens were easily removed and replaced.

For mating, a single pair of 4 to 10 day old, recently fed adults was placed into a mating container. All pairings were initiated during the photophase cycle under conditions of 27.8 ± 2°C, 60 ± 10% RH, and 15L:9D photoperiod.

Egg Incubation. Acetate vial liners with attached eggs were transferred to 7 dram, snap-cap vials which had the bottom surfaces replaced with fine-mesh polyester cloth. Before closing the vial, the top was sealed with Parafilm® to prevent larvae from crawling over the lip of the vial and becoming lodged between the lip and cap. Alternatively, a 1 cm band of Fluon® applied to the top interior sides of the vial effectively prevented the highly mobile first instars from escaping. Egg clutches were incubated in a plastic box containing a saturated water solution of KBr and maintained under the same environmental conditions as adults.

Results and Discussion

These techniques for rearing and maintaining laboratory colonies of *M. viridis* offer several advantages over those developed and employed by Redborg & MacLeod (1985). Of primary importance in rearing mantispids is the initial establishment of first instar larvae. The small size of unengorged *M. viridis* first instars (approximately 1.3 mm long by 0.1 mm thick) and their mobility presents the problem of containing larvae within a defined area while allowing direct observation of larval development.

Redborg & MacLeod (1985) addressed this problem by covering the top of the larval rearing cell with a 1 cm square glass cover which had been cut from a standard microscope slide. To completely seal the rearing cell, the substrate surrounding the open cell was moistened with distilled water. They noted that sealing the rearing cell with water could cause condensation within the cell, promoting the growth of a mold lethal to mantispid larvae.

The design presented here eliminates this potential problem by employing a seal for the rearing cell that does not require the addition of water. Because the cover slip rests on the molded flat surface surrounding the rearing cell, contact between the glass and edge of the substrate is virtually complete (Figs. 1 and 2), providing an effective barrier against larval escape. None of the 120 larvae used to test the efficiency of this design escaped. Further evidence for the effectiveness of this seal comes from the number of successful mantispid rearings recorded over a 4 year period. Less than 1% (n = 237) failed because the first instar escaped. The cover slip also functions as a surface on which engorged third instar larvae could attach an irregular scaffolding of silk lines which serve as a framework for the silken cocoon in which pupation occurs.

As an additional benefit, size of the rearing cell can be varied without decreasing the effectiveness of the seal. Cells have been used which range in size from 4.8 mm deep by 3.2 mm top diameter to 11.1 mm deep by 10.3 mm top diameter to rear *M. viridis*. In no instance did varying the size of the rearing cell affect the integrity of the seal.

Maintaining adult *M. viridis* in 7 dram, ventilated vials supported within a plastic box offers considerable space savings within an environmental chamber. Because each box is a self-contained unit, they can be stacked, transported to another location, or easily manipulated. These features would be of considerable value to any researcher who has limited facilities (i.e., a single environmental chamber) or who must share space with other individuals.

To determine the fecundity and fertility of *M. sayi*, Redborg & MacLeod (1985) used filter paper to line the interior top and sides of glass jars in which individual females were kept. They counted the number of eggs per clutch from a black and white photograph taken after eclosion and after the filter paper had been stained with India ink. In contrast, we found that clear acetate provides a suitable oviposition surface and offers several

advantages over filter paper linings. The behavior of *M. viridis* females can be observed both during oviposition and feeding. As mentioned previously, fecundity and fertility can be calculated either by directly counting the number of eggs per clutch using a stereomicroscope or from a photograph of the clutch. In both instances the acetate does not have to be stained or colored prior to counting or photographing eggs, but merely placed against a dark background.

While developed specifically to rear and maintain colonies of *M. viridis*, these techniques are not limited to this species. They have proven equally suitable for incubating eggs, rearing larvae, and maintaining adults of two other mantispid species, *Mantispa interrupta* Say and *Mantispa pulchella* (Banks). With few or no modifications this rearing technique should prove equally adaptable to other mantispid species.

With minor changes, adult rearing containers have been used to rear and maintain colonies of three species of beaded lacewings (Neuroptera: Berothidae) (Brushwein 1987, unpublished data), numerous predators and parasitoids of spider eggs (Diptera and Hymenoptera) (Brushwin unpublished data), and six species of external spider parasitoids (Hymenoptera: Ichneumonidae: Polysphinctini) (Brushwein unpublished data). This demonstrates the utility and adaptability of these rearing techniques. They should be suitable for many other insect species.

Acknowledgments

We thank Kevin Hoffman, Gloria McCutcheon, Tom Skelton and three anonymous reviewers for their comments and suggestions on earlier drafts of this manuscript. We thank Frances Scarborough for making editorial comments on the Spanish summary. J. R. Brushwein's current address is 517 Lake Ave., Lehigh Acres, FL 33936. This is Technical Contribution No. 3098 of the South Carolina Agricultural Experiment Station, Clemson University. Address reprint requests to J. D. Culin.

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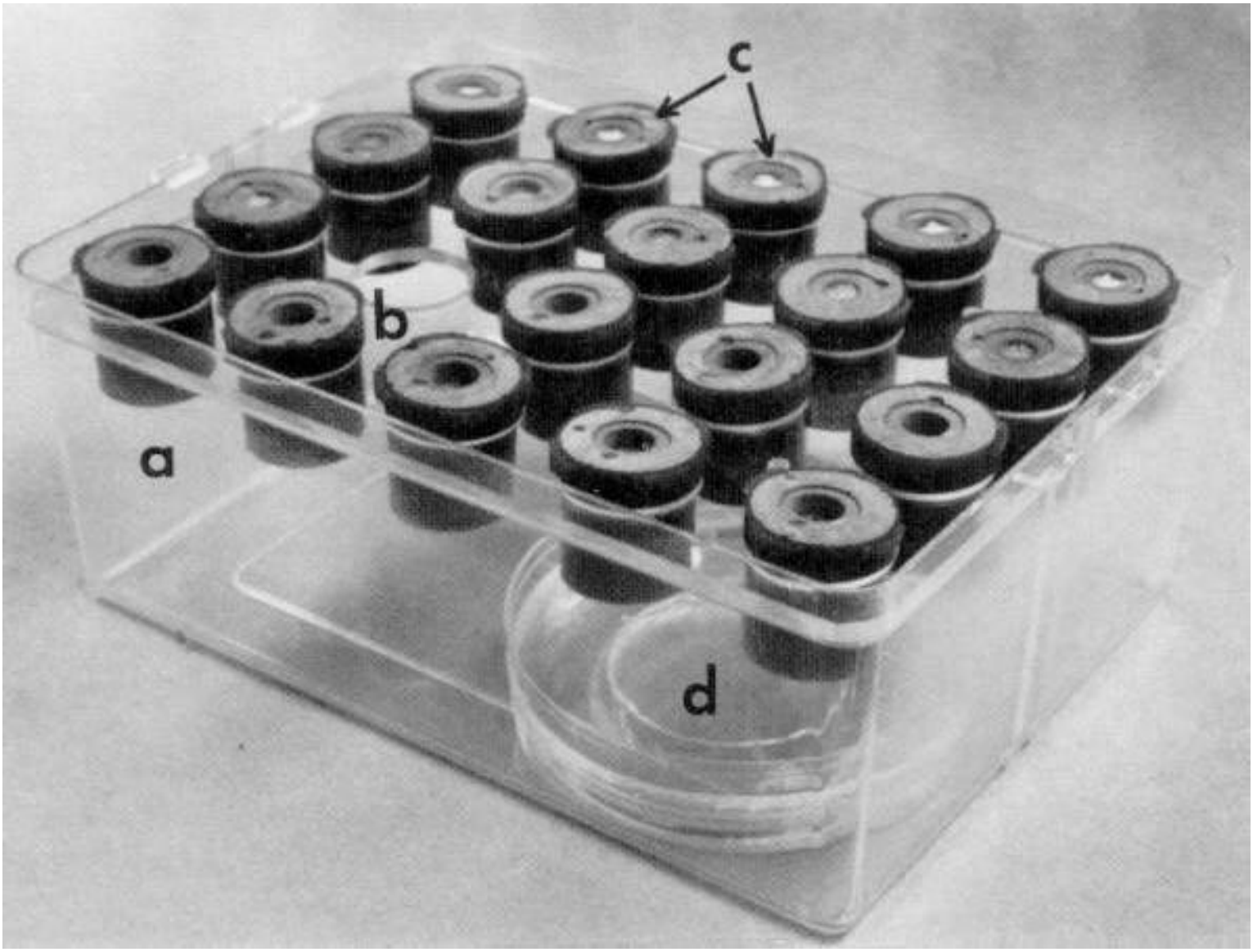
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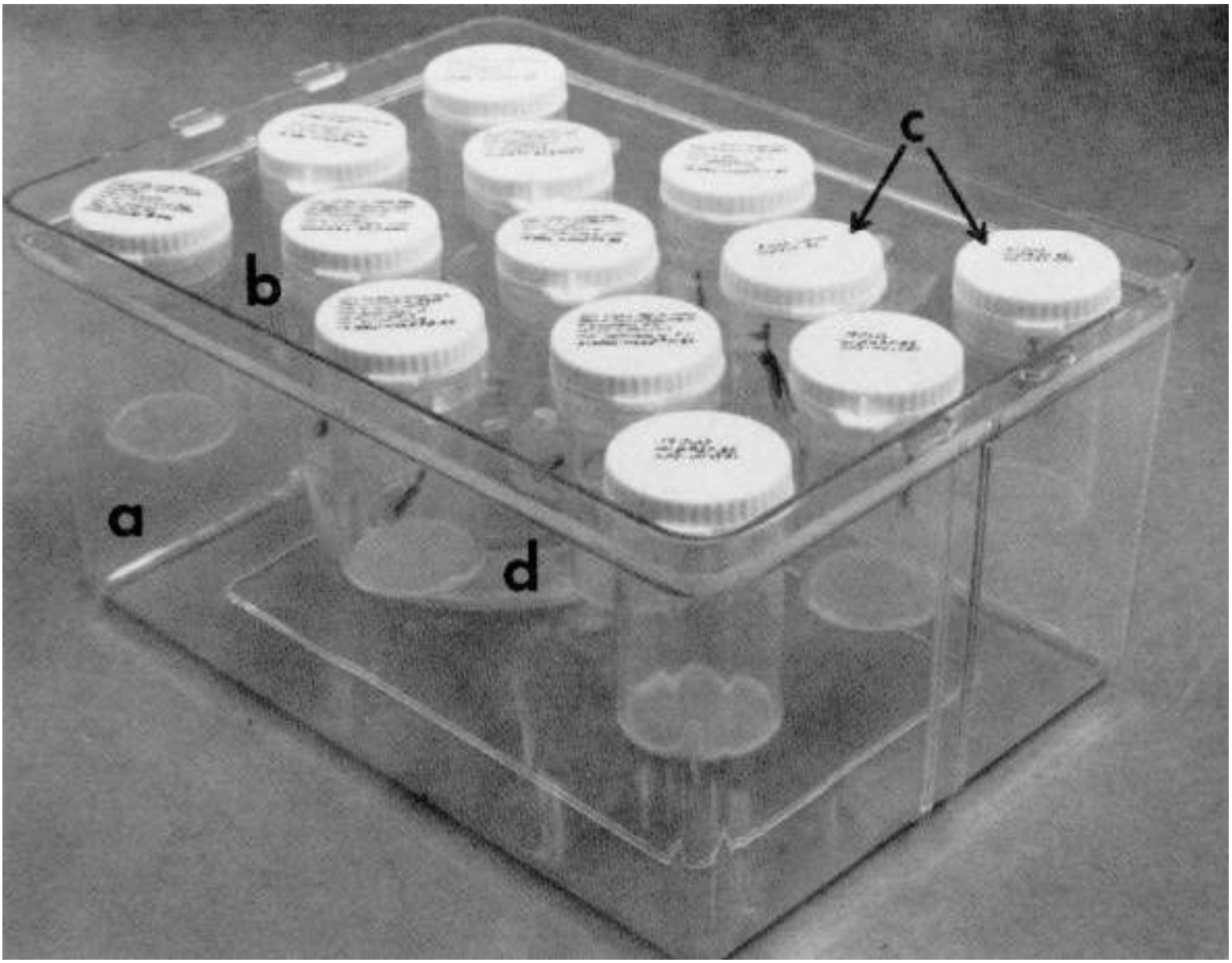
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