NATURAL HISTORY NOTES AND CAPTIVE MANAGEMENT OF LEAF-CUTTING ANTS IN THE GENUS *ATTA*

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Synopsis: Zoos and other educational institutions are increasingly recognizing that live colonies of leaf-cutting ants, *Atta* species (Figs 1-2), are valuable and highly effective public educational displays (Morgan 1991a, b, 2001, Powell 1991). Relevant aspects of *Atta*'s biology and behavior are summarized to provide a framework for husbandry discussions. Captive colony acquisition is considered including field collecting techniques and regulatory requirements for holding or importing *Atta*. The basic principles of captive management are outlined, and a simple system used at the Insectarium for keeping colonies is described. These concepts and techniques can be extended to designing and managing public display colonies.

INTRODUCTION

The display potential of *Atta* was first recognized in 1938 when the Bronx Zoo temporarily displayed a live colony (Ditmars, 1938). In 1978, the Cincinnati Zoo began permanently exhibiting *Atta* in our newly opened Insectarium, and was then the only or one of very few institutions displaying these ants. By 1991, at least 21 organizations in North America and Europe showcased *Atta* (Morgan 1991b), and by 2001 this increased to more than 50 institutions including zoos, museums, science centers and universities (Morgan 2001). The rapidly growing popularity of leaf-cutting ant displays has been fueled largely by the ants' remarkable biology, especially their astonishing behavior of actively cutting and carrying countless leaf fragments along organized trails back to their nests. Six species of *Atta* have been exhibited: *A. cephalotes*, *colombica*, *laevigata*, *mexicana*, *sexdens* and *texana* (Morgan 1991b, 2001).

NATURAL HISTORY OF *ATTA*

The biology and behavior of *Atta* has been the subject of numerous publications and detailed reviews including Wheeler (1907), Wilson (1971), Weber (1972, 1982), Lofgren and Vander Meer (1986), Hölldobler and Wilson (1990), and Wirth et al. (2003a).

A central feature of *Atta* biology is fungus farming; the ants grow a mutualistic fungus for food within their nest chambers (Figs 3-4, 8-9). The precise identity of the fungus has long been elusive since it rarely produces fruiting bodies needed for taxonomic classification (Hölldobler and Wilson 1990), though it appears to belong to the basidiomycete family Lepiotaceae (Chapela et al. 1994; Mueller et al. 1998; Johnson 1999). To culture their fungus, *Atta* workers cut large quantities of fresh plant material and are commonly called leaf-cutting ants (Fig 1). Leaf fragments are grasped in the ants' mouthparts and carried high overhead somewhat like tiny green umbrellas, thus the foragers are also sometimes called parasol ants (Fig 2).

Taxonomic Relationships: The genus *Atta* (Hymenoptera: Formicidae) is part of the large and diverse ant subfamily Myrmicinae. It further belongs to the highly specialized tribe Attini, whose members are commonly called attines. The tribe embraces 12 more-or-less closely related genera together containing over 200 species (Wirth et al. 2003a). All attines grow a mutualistic fungus for food within their nests.

Fungus-Growing Ants: The attines reflect an intriguing evolutionary progression. At one extreme are socially primitive species residing in tiny colonies with monomorphic or same-sized workers. At the other extreme are highly evolved forms living in populous colonies with polymorphic or size-specialized workers. All collect and use various organic materials to culture their fungus. However, the primitive species do not cut leaves, but instead gather rotten wood bits, dead insects, caterpillar droppings, and the like.

Leaf-Cutting Ants: The two most advanced attine genera are A*cromyrmex* and *Atta*. Both harvest mostly fresh foliage to culture fungus. *Atta* represents the pinnacle of attine evolution (Hölldobler and Wilson 1990). There are 15 recognized species of *Atta*, though the genus is in desperate need of taxonomic revision (Fisher and Cover 2007).

Biogeography: *Atta* is native only to the New World and found primarily in humid Neotropical habitats, including both forests and savannahs. However, a few species have adapted to harsher climates, such as *A. mexicana* which occurs in the hot dry deserts of Mexico and extreme southern Arizona, and *A. texana* found exclusively in semi-temperate regions of Texas and Louisiana.

Some species have extremely limited geographic ranges while others occur broadly. The two widest-ranging species are *A. sexdens* and *cephalotes*. Both are found throughout much of tropical Central and South America (Weber 1972) and in a variety of habitats. *Atta cephalotes* in Trinidad, for example, nests in coastal or low elevation habitats in loose sandy soil, on densely forested mountainsides in thick heavy clay earth, or in highly disturbed regions (pers. obs.).

Social Organization: *Atta* demonstrates one of the most complex social organizations known for any kind of ant (Hölldobler and Wilson 1990). Its colonies typically consist of a single fertile queen and numerous sterile female workers. Large, reproductively mature colonies also often contain alates or winged males and virgin queens.

Queens: The massive queens, over 20 mm long in most species, dwarf even the largest workers (Figs 3, 9). Queens are specialized to mate in the air and then initiate new colonies (Fig 6). Once the first few workers are present, queens continue to lay eggs at a steadily increasing rate, eventually supporting worker populations consisting of a million or more individuals.

Worker Polymorphism: The workers are strongly polymorphic, ranging from tiny *minors* (Fig 4) through intermediate *medias* to relatively massive *majors* (Fig 5). The workers of *A. sexdens*, for example, vary in body length from about 2-15 mm, have a corresponding 8-fold increase in head width, and a 200-fold increase in dry weight, permitting a high degree of task specialization based on size. Workers also specialize on various tasks depending on their age, with younger workers tending to remain within the nest and older workers venturing outside.

Worker Task Specialization: The workers perform at least 20 distinct tasks needed for fungus gardening and colony maintenance. The minors characteristically remain in the nest and serve as either fungus gardeners and nursemaids, or within-nest generalists. The medias work both inside and outside the nest as excavators and foragers. And the powerful majors tend to stay inside the nest until needed as defenders, and quickly respond to disturbances with painful bites.

Nest Structure and Function: Established nests are large, extend 2-6 meters or more into the ground, and may contain hundreds of fungus gardens. Nest architecture is complex and adapted to control the fungus gardens' environment. Subterranean garden chambers provide the high humidity required for fungal growth. Central passageways above the gardens passively vent heat and stale air produced by fungal decomposition, while the nest is ventilated in turn with fresh air drawn in through peripheral tunnels. A lower system of passages drains the gardens to prevent flooding. In dry periods the ants may reduce or close nest openings, abandon gardens in the upper tiers, and move deeper into moist soil.

Colony Reproduction: Mature colonies annually produce numerous alates (Fig 6). Mass mating flights often occur at the start of a rainy season. The young queens carry a bit of fungus from their parental nest within their mouthparts. Each queen typically mates with several males and stores enough viable sperm in a spermatheca to last her lifetime of 20 plus years (Weber 1972). The males die shortly after mating, while the queens shed their wings and quickly excavate small shallow cavities. Each queen rears her first brood (eggs, larvae, and pupae) and fungus garden in isolation using nutrients from her own body: fecal secretions to nourish the embryonic fungus garden, and trophic or nutrient-rich eggs for the larvae. Developing workers assume domestic duties and soon begin to forage for plant material. Colony growth is slow the first year, and then proceeds rapidly, with reproductive maturity reached in about five years.

Foraging and Fungal Substrate Selection: The plant material used by leaf-cutting ants to culture their gardens is called *fungal substrate*. Successful foragers emit a powerful pheromone from their poison gland via the otherwise non-functional sting to attract nest mates to desirable plants. Other pheromones are used in long-term orientation along odor trails. Regularly used trails leading from the nest are soon cleared of debris becoming well-defined ant highways that increase foraging efficiency. Ants from large colonies often forage along trails stretching 100 m, and sometimes 200 m or more from their nests.

Atta naturally uses a diversity of plant species but clearly demonstrates a preference for certain types (Cherrett 1968, Rockwood 1976, Bowers and Porter 1981, Waller 1982, Lofgren and Vander Meer 1986, and Wirth et al. 2003a). Most *Atta* species, including *A. cephalotes*, *colombica*, *mexicana*, *sexdens*, and *texana*, harvest mainly dicots, though some have specialized on grasses (monocots), and a few, such as *A. laevigata*, will take both dicots and grasses.

Dicot specialists seem to prefer introduced or agricultural cultivars over native plants, woody over herbaceous plants, new leaves and flowers over mature leaves, foliage with a relatively high sugar content, and sun-drenched versus shaded leaves, presumably because of their higher nutritional value. Plants with leaves which are very tough, densely pubescent (hairy) or containing sticky saps are often avoided.

Fungal substrate is collected and manipulated assembly-line style. Workers straddle leaves, measure portions with their leg span, then rotate and shear with sharp mandibles. Large nests are characterized by long populous columns of foragers returning with fresh plant material.

Gardening Behavior: Plant fragments brought into the nest are processed by a series of progressively smaller workers. The substrate is broken apart, macerated finely, treated with enzymes, and added to the upper and outer regions of the gardens. The ants fertilize their gardens with fecal droplets, meticulously weed out undesirable foreign micro-organisms (Currie and Stuart 2001), and harvest portions used for food. Old and exhausted pieces of the gardens are removed from basal regions and discarded as debris.

Fungus Garden Structure and Function: The gardens look like globular masses of grey, sponge-like material, and are typically 10-25 cm in diameter (Figs 3-4, 8-9). The ants' gardening behavior determines the appearance of a garden, which varies along its height. The upper and outer regions are formed into relatively large gray cells with thin granular walls. The base of the garden is compacted from the weight above and consists of smaller, yellow-brown cells. As a garden matures, it becomes peppered with light colored clusters of fungal mycelium (Fig 4). These nutrient-rich growths are the principal diet of the colony, particularly the developing larvae, though the workers also ingest sap from cut leaves. Both the queen and brood are held within the maze of fungal chambers.

Symbiotic Relationships: The ants and their fungus evolved in close association and now form an obligate mutualism where both are interdependent and benefit. The fungus provides the ants with nutritious food while the ants disperse and shelter the fungus, bring it substrate, and protect it from micro-organisms. Neither partner can survive for long without the other. They are also part of an even more complex symbiosis involving a third obligate mutualist and a host-specialized parasite (Currie et al. 1999). The cultured fungus gardens are host to the virulent parasitic fungus *Escovopsis* (Ascomycotina) and quickly succumb if the parasite is left unchecked. It is countered by a filamentous bacterium, *Streptomyces*, which lives only on the ants' body surfaces and produces an antibiotic that suppresses the growth of *Escovopsis*.

Ecological Roles: Harvesting leaves on behalf of their fungus, leaf-cutting ants are dominant herbivores in the Neotropics. In natural systems their impact is largely beneficial. Native plants tend to be pruned rather than defoliated. For example, *A. colombica* in Panama harvested a maximum of 40% of the leaves from certain plants (Wirth et al. 2003a). This increased environment heterogeneity by creating small light gaps in the forest canopy, allowing a more diverse plant community to thrive. Nest excavations also improve poor tropical soils by turning over vast quantities of earth, introducing nutrients and increasing aeration and drainage.

Pest Status: Since some leaf-cutting ant species thrive in disturbed sites, such as those cleared for farms, pastures and settlements, colony density there often becomes much higher than in natural systems (Cherrett and Pergrine 1976, Wirth et al. 2003b). Correspondingly, these ants are *the* single most destructive agricultural pest in the Neotropics, and are despised by both large scale and subsistence farmers. Nests also frequently undermine roadways and buildings causing structural collapse. Altogether they are responsible for several billion dollars of economic damage annually (Lofgren and Vander Meer 1986).

CAPTIVE COLONY ACQUISITION

Regulatory Considerations: Many countries have policies regulating the importation and internal movement of plant pests and wildlife. The focus here is on the United States.

Agricultural Regulations: The United States Department of Agriculture, Animal & Plant Health Inspection Service, Plant Protection & Quarantine (USDA-APHIS-PPQ) mandates that a Non-indigenous Species' Permit must submitted and approved before non-native plant pests can be legally imported or moved across state lines. For example, a permit would be required for keeping any of the tropical *Atta* species, or for *A texana* if moved or held outside the areas of Texas or Louisiana where it occurs naturally.

USDA-APHIS-PPQ Non-indigenous Species Permits typically specify a number of conditions. For example, institutions must draft Standard Operating Procedures (SOPs), file these with the USDA, and are subject to periodic inspections to ensure compliance. All employees working with the permitted species must be properly trained and understand the importance of effective containment.

Specific conditions for leaf cutting ants state that public displays or other colony containment must be constructed from durable materials and prevent the escape of even the smallest workers. Queens must be maintained in designated nest chambers with physical excluders that prevent their movement but not that of the workers.

The permitting process has changed in recent years and is now predominantly an electronic format. A single person, typically an institutional representative, must be designated as the permit holder and pass a low-level security clearance. Additional details on USDA permit applications are on their website (http://www.aphis.usda.gov/plant_health/permits/index.shtml).

Wildlife Regulations: All wildlife imported into the United States is subject to inspection by the U.S. Fish & Wildlife Service to ensure compliance with international treaties and protection of endangered species. Depending on the country of origin, an Export Permit may also be required by the Fish & Wildlife Service. For more information see their website (http://www.fws.gov/).

Necessity and Justification for Field Collection: New *Atta* colonies can not be started from captive colonies since the mating flights of most ants follow environmental conditions that are nearly impossible to duplicate in the laboratory (Hölldobler and Wilson, 1990). Thus, captive colony availability ultimately depends on some method of field collection.

Attempting to collect large colonies, deeply entrenched, populous and well-defended, is almost always a no-win situation. Numerous painfully-biting soldiers quickly respond to nest disturbances and drive intruders away. Much more practically, captive colonies are typically started by excavating and collecting nests that are about one year old. Less commonly, newly mated queens can be collected and induced to initiate colonies. Neither activity negatively impacts wild populations since both life stages are naturally subject to extremely high mortality rates (Wirth et al. 2003a), while colony populations are already excessively elevated in many regions due to human disturbance.

Finding Year-old Colonies: In many parts of the Neotropics, young *Atta* colonies can be found in clearings around buildings and farms, or along road sides, especially in the vicinity of large established nests (pers. obs.). Year-old colonies can frequently be identified by their surface profile. They have only a single nest opening often surrounded by a characteristic columnar turret (Weber 1972) (Fig 7). The nest entrance is about 1 cm in diameter and may be open with active workers, open but apparently inactive, or temporarily sealed with soil bits and debris (pers. obs.). Oftentimes a few fresh leaf bits littering the vicinity help confirm the identity of an inactive nest entrance. In loose sandy soils, year-old nest entrances are sometimes surrounded by small conical mounds (pers. obs.). The characteristic turrets disappear with age and the formation of multiple nest openings.

Year-old colonies consist of a single fungus garden (about 4-6 cm diam) often located 10-20 cm below ground surface in a small spherical cavity (Fig 8). The queen usually clings to the side or top of the fungus garden (Fig 9), and the worker population might be anywhere from a few dozen to perhaps a 100 or more individuals. Generally only minors and medias, but no major workers, are present.

Excavating Year-old Colonies: A shovel is used to dig a roughly 25 cm deep trench just to one side of the nest entrance. Then a hand trowel is employed to carefully scrape away the soil laterally, eventually exposing the fungus garden chamber (Fig 8). Paper towels are formed into an appropriately-sized padded nest cavity within a small plastic field container, and slightly moistened to provide humidity for the garden. A tablespoon serves to gently lift out the fragile garden and resident ants, and to transfer all to the field container (Fig 9). A small hand-held aspirator (Table 1) is often used to collect additional workers that may have left the nest because of disturbance, particularly for smaller colony populations. If a garden accidentally breaks apart during collection, it is roughly pieced together in the field container and will be repaired by the workers. Container lids have a few tiny puncture holes for air exchange. Colonies may be held in field containers for up to a week or more, and are offered a few fresh leaf pieces daily. Once reaching the Insectarium they are transferred into permanent laboratory housing.

Collecting and Initiating Colonies with Newly Mated Queens: Newly mated queens are rarely though sometimes easy to collect from the wild as they wander over the ground surface or begin digging their nest burrows. The principle difficulty is the need to be in the field immediately following the mating flights, which occur infrequently and are not easy to predict.

Fertilized queens can be identified by their lack of wings, which they shed shortly after mating. Such queens can be induced to initiate colonies in captivity if placed in small moist chambers as described by Weber (1972), Mintzer and Vinson (1985) and Mintzer (1987).

CAPTIVE MANAGEMENT OF *ATTA* COLONIES

The laboratory maintenance of *Atta* colonies was detailed by Weber (1972, 1976, 1979, 1982) and summarized by Hölldobler and Wilson (1990). The design and care of public display colonies was briefly described by Ditmars (1938), Morgan (1991b, 2007), and Powell (1991).

The basic husbandry requirements of all *Atta* species appear to be virtually identical (Morgan 1991b, 2001). Captive *Atta* colonies are extremely adaptable. They are typically maintained on fresh and processed plant materials that are not available to them in nature. They also can thrive in an endless variety of containment and nesting situations as long as their basic environmental requisites, especially humidity and temperature, are met.

It can not be overemphasized that the needs of the fungus gardens are much stricter than those of the ants, thus the primary husbandry goal is to focus on maintaining an optimal environment for fungal growth. This is best accomplished with a garden chamber design that both enhances and helps the ants regulate fungal microenvironment. The shape and size of garden chambers are relatively unimportant. Given an optimal environment, the ants will readily grow their fungus in any small protective cavity. Indeed, rapidly growing colonies will even sometimes construct fungus gardens in relatively exposed sites (pers. obs.).

Conventional Housing and Containment: Weber (1972, 1976) employed small, clear acrylic plastic containers, called garden chambers, to house an *Atta* colony and its fungus gardens. The clear containers allowed monitoring of fungal growth and observation of colony activities.

To accommodate an expanding colony, garden chambers were linked together with tunnels made from clear tubing. Interconnecting tunnels can be almost any length, but an optimal width is about 2.5 cm (1 in) in diameter. Wider passageways may be used by the ants as gardening sites, while much narrower tunnels restrict the free flow of workers and substrate. Plant material was placed in a separate container, called the foraging arena, also connected to the nest with tubing.

In such a system, where garden chambers and foraging arena are not otherwise contained, it is crucial that all components be extremely well constructed and fit precisely. The ants are adept and relentless chewers, will eventually open and escape from any small space, and begin to forage outside of their enclosure. Plastic sealant can be used to temporarily repair small cracks or holes used by escaping ants. A useful technique employed at the Insectarium involves first firmly-fitting the escape hole with small pieces of ultra fine (40-100 mesh/inch) stainless steel cloth (Table 1 lists source), which the ants can not chew through, then applying the sealant.

Insectarium Laboratory Housing: At the Insectarium, surplus or reserve colonies are maintained as small populations. Colonies are each housed within single glass-sided aquaria, which serve as foraging arenas, and in turn contain one or sometimes several garden chambers. We do not interconnect multiple garden chambers with tubing as all are contained within the overall foraging arena, and the workers readily move back and forth between nest chambers.

Foraging Arenas: Standard 19 or 38 liter (5 or 10 gal) glass aquaria are used to house and contain colonies residing in single garden chambers (Fig 11). Colonies can be kept relatively small and maintained this way for many years. If desired, larger tanks can be used for more populous colonies residing in several garden chambers. For example, our main back-up colony lives in a 151 liter (40 gal) tank housing six garden chambers.

To deter escape, all silicon sealant in the inner corners of the aquaria, which can give ants a foothold, is meticulously removed with a straight-edged razor blade. Then, to create a slippery barrier, about a 15 cm (6 in) wide band of 3-in-One multi-purpose oil is applied to the glass around the upper inner tank perimeter at weekly intervals, or more frequently if needed (Table 1 lists oil manufacturer and usage notes).

Garden Chambers: Small clear acrylic boxes are modified to serve as garden chambers (Figs 10-12; Table 1 lists source and specifications). For moisture control, each garden chamber is fitted with a 2 cm thick base layer of Hydrostone (Fig 10; Table 1 lists manufacturer and usage notes). Cured Hydrostone is porous and absorbs water, acting both as a moisture reservoir and to wick away any excessive condensation. The plastic bottom of the garden chamber is drilled with four 12 mm (1/2 inch) holes, one near each corner, that extend part way up into the Hydrostone base layer to allow for drainage.

While not essential, we usually cut and fit a rectangular piece of 8-mesh/inch hardware cloth (Table 1) to lay flush atop the Hydrostone base. This creates a thin spacer that slightly elevates the bottom of the fungus garden above a possible wet surface. We also add a small amount of tan mortar color (Table 1) to the Hydrostone during preparation to give it a natural earth tone.

Entrance Holes and Queen Exclusion: Each fungus garden chamber has a single entrance hole drilled in the box lid. This entrance hole is normally about 1 cm in diameter unless the box houses the queen. Then we create a slot-shaped entrance hole (about 4 x 15 mm) by drilling several overlapping holes in a line with a standard 11/64 inch drill bit and smoothing the edges. This opening (Fig 12) readily allows the passage of workers with plant material but not the queen, as per our USDA-APHIS-PPQ permit conditions.

Moisture Requirements: The fungus gardens need a constant, highly humid atmosphere, preferably at or near the saturation point (100% RH). Weber placed clean moist sand in containers housing small colonies, which have less ability to regulate moisture levels, but noted that sand was unnecessary for most large colonies with established gardens. A related problem is the accumulation of water within garden chambers from condensation, which is detrimental to the fungus if left unchecked.

The ants, in contrast, tolerate a wide range of humidity levels. They will survive in a dry atmosphere as long as they have access to drinking water. Weber recommended that humidity levels in the foraging arena be kept relatively dry (20-50% RH) to prevent the formation of molds. At the Insectarium, foraging arenas are kept a little more humid (50-60% RH) and regular debris removal eliminates mold problems. In a relatively humid atmosphere, plant material remains fresher longer and the ants require less or no drinking water.

At the Insectarium we do not always provide drinking water, especially for larger well-established colonies, but the provision of water can be advantageous for small colonies or during the drier winter months. When offered, drinking water is provided in shallow dishes or trays half-filled with course gravel, with the water level kept near that of the gravel surface, preventing the workers from slipping, falling in and drowning in deeper water.

Temperature Requirements: Ambient temperatures of 25-27ºC (77-80ºF) are ideal for fungal growth. Temperatures that are somewhat lower (20ºC; 60ºF) or higher (30ºC; 86ºF) are suboptimal for fungus grown in laboratory cultures (Weber, 1972). The ants, however, function well within a significantly broader range of temperatures. Their metabolism slows at cooler temperatures, and if briefly chilled in a standard household refrigerator (about 3ºC; 37ºF) they become torpid, but will recover when warmed. However, temperatures that are below freezing (0ºC; 32ºF) or above 35ºC (95ºF) are lethal to the ants.

Illumination of Observation Colonies: *Atta* colonies readily adapt to lighting at normal room intensities, and can have additional illumination to highlight the fungus gardens for public viewing provided that the gardens are not overheated. The type of lighting fixtures and their placement can be important considerations since florescent bulbs produce significantly less heat than incandescent bulbs. Observation nests should never be exposed to direct sunlight since this will quickly cause overheating and injure or kill the colony.

At the Insectarium, we illuminate our public display fungus garden chambers with two 75 watt incandescent floodlights shining down on the viewing windows from about 2 m (6 ft) away. This causes a several degree heating effect within the garden chambers, but since our building is maintained at about 24ºC (75ºF), the net result is an optimal temperature for fungal growth.

Seasonal Fungus Garden Management: Effective fungus garden management is the key to successfully keeping *Atta*. Fungus gardens are routinely monitored to distinguish healthy, actively growing gardens from ones that are shrinking or otherwise failing. Management practices are directed towards helping the ants maintain an optimal environment for fungal growth.

Seasonal variables may have a profound affect on management strategy. For example, in Cincinnati, spring and summer bring relatively high atmospheric humidity and moisture-laden plant material. Consequently, moisture potentially builds up within the garden chambers, and if this results in condensation and pooling, this excess moisture must be eliminated. Conversely, winter brings lower atmospheric humidity and reduced plant moisture, and garden desiccation must be prevented.

Garden chamber moisture levels are controlled by regulating moisture input and output. Excess moisture can often be regulated simply by a garden chamber design that provides adequate drainage. However, increasing ventilation (nest chamber openings) and offering relatively dry substrate can also be a part of the management strategy. Conversely, fungal garden moisture levels are often adequately maintained by vigorous colonies simply by providing them with moist substrate. However, hydrating the Hydrostone with water and reducing ventilation can also be useful in some situations.

Substrate Source and Use: It is vital that all plant material provided to the ants be free of insecticides, fungicides or other contaminants. While captive colonies often flourish even when fungal substrate choices are limited, we prefer to routinely vary the substrate to help ensure that a diversity of plant-based nutrients is potentially available to the fungus gardens. The source of the substrate we give to the ants is strongly dependant upon the season.

Growing Season: During the warmer months we offer foliage from various landscape trees, shrubs, vines, ground covers and weeds. Since these plants are managed by the Zoo's Horticultural Department we know that all are pesticide-free. Substrate acceptability was determined by experimentation and useful choices available to us include plant species in about 40 genera and 25 families (Morgan 1991b). Some of our most heavily utilized plants, based on both ant preference and ready availability on Zoo grounds, are listed in Table 2. Plant material is typically provided in the form of small branch-like cuttings, as opposed to stripping off the leaves, since this helps keep the foliage fresher longer.

Winter Season: During the cold months we depend predominantly on winter-hardy landscape plants, especially *Euyonomus* and *Viburnum* species (Table 2), and to a lesser degree on interior plantscape and greenhouse plants. We also utilize grocery produce, particularly apple wedges, which the ants readily visit and harvest because of the high moisture and sugar content. Grocery produce always has a risk of pesticide contamination. Thus before use we thoroughly cleanse all produce with a fruit and vegetable wash, such as Mom's Veggiewash or Fit (Table 1). The ants will also readily accept certain prepared foods, for example steamed rice or raisons, either dried or cooked in water to increase water content. All can be refrigerated to conveniently extend shelf life.

Utilization and Disposal: The amount of substrate needed depends on colony size, and can be determined by experimentation. We like to provide amounts that keep our colonies, especially our public display colony, foraging all day long. Captive colonies of all sizes respond favorably to a daily provisioning of fresh substrate, and we routinely offer fresh substrate daily. However, if necessary, colonies that are well established can easily go several days without substrate.

Prior to adding fresh substrate, the older partially or completely denuded branches are briefly shaken to dislodge many of the clinging ants and removed. For this job, some keepers prefer to wear beekeeping gloves (Table 1) that cover the forearms to deter bites. The branches are cut into shorter lengths with hand-held pruners, placed into a durable plastic storage bin, and moved into an ultra-cold deep freezer to kill any remaining ants. They are frozen at -29ºC (-20ºF) for 72 hours before disposal, as per our USDA-APHIS-PPQ permit conditions.

Waste Removal and Population Control: These topics are considered together since they often go hand-in-hand. For example, a shop vac (high-suction vacuum hose and canister) can be used to simultaneously remove colony waste and surplus workers. All debris, old plant matter and ants removed from containment are deep frozen as above before disposal.

Colonies form debris piles consisting of dry degraded yellowish-brown fungus bits, dead workers, and unused leaf fragments. Debris piles are usually placed away from the active garden chambers, typically at the far end of a foraging arena. It is possible to encourage a colony to place nest debris in a specific location by initially seeding that location with a small pile of debris. Our display colony, for example, places virtually all of its nest debris in a 151 liter (40 gal) tank connected to the nest with clear tubing. The colony is, in effect, toilet trained.

Waste consisting of unused cut leaves, fallen plant fragments and dead workers also tends to accumulate in separate foraging arenas. Both types of debris can be quickly and effectively removed with a shop vac, along with any ants that happen to be in the way.

Healthy captive colonies exhibit exponential population growth that eventually leads to excessive workers. Periodically removing excess workers is a much better and less stressful husbandry strategy than, for example, limiting the amount of substrate offered to a colony. We predetermine how large (i.e., how many garden chambers) we want to maintain for specific colonies, keep the colonies growing exponentially by providing abundant substrate, and remove excess workers and new fungus gardens with a shop vac as necessary.

Emergency Fungus and Brood Augmentation: Despite best management intent, situations can arise where fungus gardens and/or worker populations decline sub-optimally, especially in very young colonies which are not well established. After ensuring that rearing environments are optimized, two additional options are available to keepers that can help reverse declines.

Fungus Augmentation: Captive *Atta cephalotes* colonies will readily accept fungus pieces from other colonies (pers. obs.). It is important that all workers from the donor colony be first removed to prevent fighting with ants from a recipient colony or attacking its queen. Even the tiniest workers can be effectively removed with a finely-pointed Rubis forceps (Table 1). Also, fungus should only be offered in amounts that can be properly tended by the recipient ants. Conceivably, excessive amounts might be prone to attack by the parasitic fungus *Escovopis*.

Brood Augmentation: Most ants will readily accept and care for brood of their own species taken from other colonies, and offering such brood can bolster small or struggling populations (Morgan 1991c, 2004). Providing pupae, rather than larvae, is best since these require no further feeding and soon produce young workers. *Atta cephalotes* also accepts brood from other colonies (pers. obs.). However, the workers in disturbed colonies frequently protectively clump around their brood. Since pupae lack cocoons and are delicate, the adhering workers must be removed with painstaking care before the pupae can be transferred to a recipient colony.

Staffing and Keepers: Leaf-cutting ants are complicated social insects and can not be managed like many of the hardy solitary or gregarious insect species commonly kept in zoos or other live insect exhibits. Asking keepers to only be responsible for cleaning and providing substrate to the ants each day will eventually lead to colony failure. It is vital that keepers be:

- Interested in leaf-cutting ants and willing to learn as much as possible about their sophisticated social organization and complex symbiotic relationships.
- Vigilant, trained in basic husbandry techniques and colony manipulations, and responsive to changing colony needs.
- Able to regularly check and assess fungus garden growth and health, forager response to plant offerings, seasonal changes in nest micro-environment, etc., and make appropriate management adjustments as necessary.
- Available most days with minimal turnover in trained personnel.
- Tolerant of occasional painful bites by large workers and soldiers.

Any less stringent standards for staffing set the stage for minor husbandry issues to be missed or ignored, then eventually becoming serious colony-threatening problems.

PUBLIC DISPLAY COLONIES

The techniques and concepts described above can be extended to designing and managing public educational display colonies. Indeed, a highly effective though somewhat schematic exhibit could be created simply by employing the Insectarium reserve colony housing system on a larger scale. For example, two large glass aquaria could be linked with clear tubing, with one tank serving to house 8-10 standard garden chambers, or a similar number of naturalistic nest chambers abutting the glass, and the second tank functioning as a foraging arena. Managing populous captive colonies using a relatively simple set-up gives both keepers and institutions valuable practice and experience working with *Atta* before investing time and resources into a complex naturalistic display.

TABLES

Table 1: Products mentioned in the text and usage notes.

Aspirator: small, portable suction device with 9 dram styrene receptacle available from Bioquip, 2321 Gladwick, Rancho Dominguez, CA 90220; USA Phone: (310) 667-8800; Fax: (310) 667-8808; www.bioquip.com.

Beekeeping gloves: long-sleeved gloves available from the Walter T. Kelly Co., 807 West Main Street, Clarkson, KY 42726-0240; Ph: (270) 242-2012; www.kellybees.com.

Clear acrylic boxes: about 80 ounce or 19x14x10 cm (7.5x5.25x3.75 in) containers with friction-fitted lids manufactured by Pioneer Plastics, P.O. Box 6, Dixon, KY 42409; www.pioneerplastics.com; catalog number 079-C. This plastic is relatively thin and brittle, and must be firmly supported when drilling or fabricating to avoid fractures.

Fit: fruit and vegetable wash manufactured by HealthPro Brands Inc., Cincinnati, Ohio 45242. Washing solution for removing surface contaminants from fruits and vegetables; removed by rinsing with water.

Hydrostone: gypsum cement manufactured by the U.S. Gypsum Co., 125 S. Franklin St., Chicago, IL 60606; Ph: 800-487-4431 for technical information. Hydrostone is sold as a bagged white powder and used like plaster-of-Paris, but is much more durable when cured. We mix Hydrostone and water in about a 2:1 ratio to make a slurry that is then poured into new garden chambers or other moulds. The mix heats as it cures and hardens, usually within 30 minutes, and overnight is ready to use.

Hardware cloth: 1/8 inch mesh galvanized metal screen; F.P. Smith Wire Cloth Company, 10112 Pacific Avenue, Franklin Park, IL 60131; Ph: (800) 323-6842.

Mom's Veggiewash: fruit and vegetable wash manufactured by Mom's Veggiewash, P.O. Box 214, Troy, NY 12180; www.veggiewash.com. Washing solution for removing surface contaminants from fruits and vegetables; removed by rinsing with water.

Mortar color: concentrated powdered dye, manufactured by Solomon Grind-Chem Service, Old Water Works Plant, P.O, Box 1766, Springfield, IL 62705; Ph: 217-522-3112. Small amounts (i.e., 1 tsp/2 gallons) are added to a Hydrostone slurry until the desired color is achieved.

Rubis forceps: finely-pointed Swiss-made forceps available from Bioquip, 2321 Gladwick, Rancho Dominguez, CA 90220; USA Phone: (310) 667-8800; Fax: (310) 667-8808; www.bioquip.com.

Stainless steel mesh cloth: F.P Smith Wire Cloth Company, 10112 Pacific Avenue, Franklin Park, IL 60131; Ph: (800) 323-6842.

3-in-One multi-purpose oil: light-weight, house-hold lubricant manufactured for the WD-40 Co., San Diego, CA 92110; www.wd40.com. The most effective slippery barrier is obtained when oil is applied to clean glass; before re-application, old debris-laden oil is best wiped away with paper towels. Thin smears are often sufficient; if oil rivulets later run down tank sides then too much was applied.

Table 2: Fungal substrate plants frequently used for rearing *Atta* at the Insectarium. Usage is based on high acceptability to the ants and ready availability on Zoo grounds; many other plant species are also used less frequently.

Border Privet, *Ligustrum obtusifolium* (Oleaceae)

Euonymous, *Euonymous* species (Celanstraceae)

Forsythia flowers, *Forsythia* species/cultivars (Oleaceae)

Foxgrape, *Vitis labrusca* (Vitaceae)

Northern Catalpa, *Catalpa speciosa* (Bignoniaceae)

Red Mulberry, *Morus rubra* (Moraceae)

Tree-of-Heaven, *Ailanthus altissima* (Simaroubaceae)

Viburnum, *Viburnum* species/cultivars (Caprifoliaceae)

Willow, *Salix* species (Salicaceae)

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Fig 1: Foragers of *Atta cephalotes* cutting leaf fragments for fungal substrate.

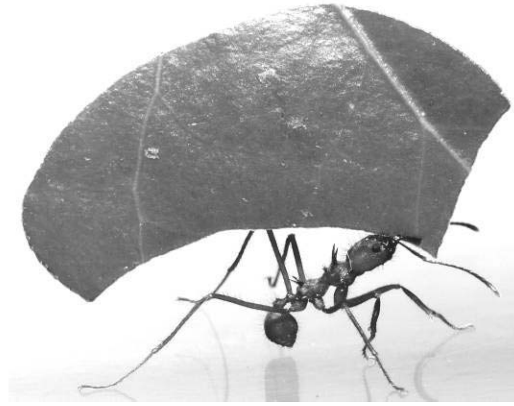

Fig 2: Nest-bound forager with overhead leaf fragment, or parasol ant.

Fig 3: Queen clinging to a fungus garden surrounded by worker ants.

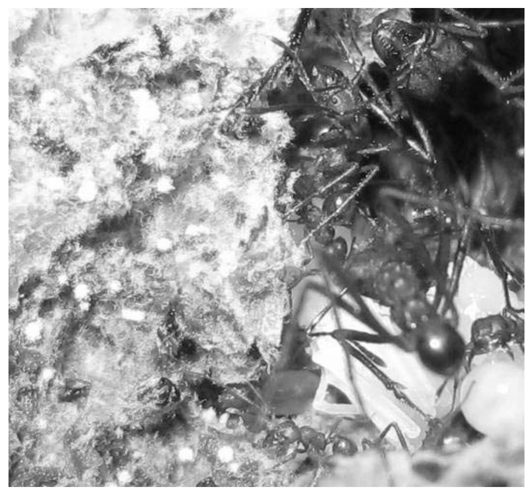

Fig 4: Fungus garden with hyphal tufts; note minor worker at bottom center.

Fig 5: Major worker or soldier; adductor muscles in the head power the sharp mandibles.

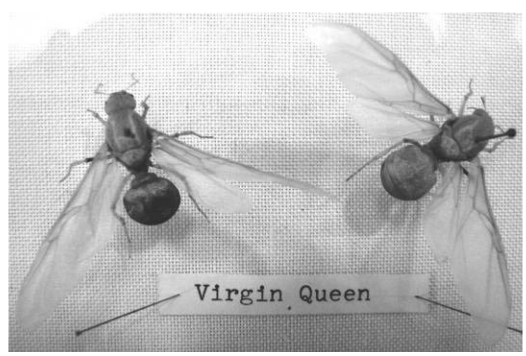

Fig 6: Winged, unmated queens; wings are shed shortly after the mating flight.

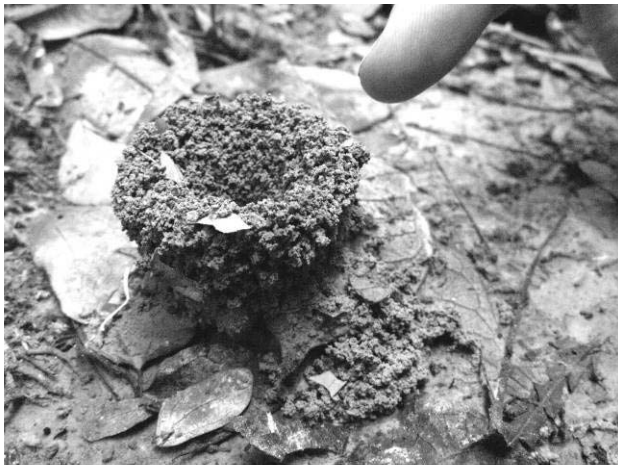

Fig 7: Characteristic turret-like nest entrance of a one-year old *Atta* colony.

Fig 8: Ground cut away of a one-year old nest showing single fungus garden.

Fig 9: Entire one-year old colony (queen, workers and fungus garden) on a tablespoon.

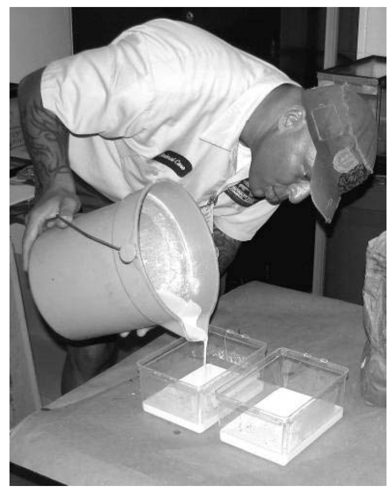

Fig 10: Pouring liquid Hydrostone to form nest garden chamber bases.

Fig 11: Overhead view of a garden chamber within a five-gallon aquarium, the foraging arena.

Fig 12: Queen-excluding nest entrance hole confining the queen.