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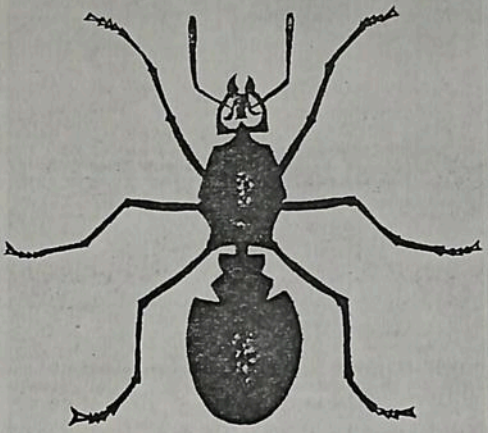
Weber, 1957

FUNGUS-GROWING ANTS AND THEIR FUNGI: *CYPHOMYRMEX COSTATUS*

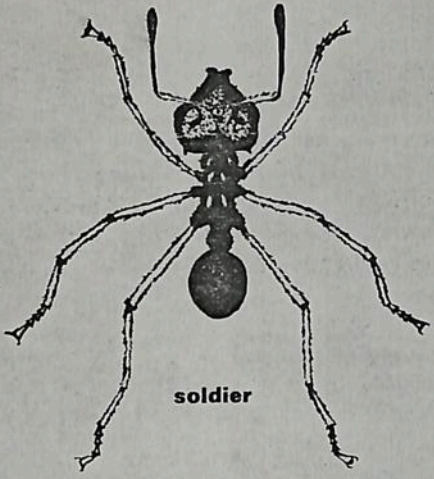
By

NEAL A. WEBER

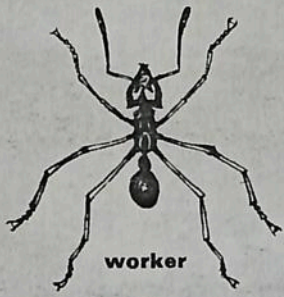
Reprinted from *Ecology*, Vol. 38, No. 3, July, 1957 480-494 3 figs.



Leaf-cutting ant queen



soldier



worker

mushroom grower

THE

NATURALIST IN NICARAGUA:

A NARRATIVE OF

A RESIDENCE AT THE GOLD MINES OF CHONTALES; JOURNEYS IN THE SAVANNAHS AND FORESTS.

WITH

OBSERVATIONS ON ANIMALS AND PLANTS IN REFERENCE TO THE THEORY OF EVOLUTION OF LIVING FORMS.

By THOMAS BELT, F.G.S.,

AUTHOR OF "MINERAL VEINS," "THE GLACIAL PERIOD IN NORTH AMERICA," ETC., ETC.

"It was his faith,—perhaps is mine,—
That life in all its forms is one,
And that its secret conduits run
Unseen, but in unbroken line,
From the great fountain-head divine,
Through man and beast, through grain and grass."

LONGFELLOW.

WITH MAP AND ILLUSTRATIONS.

LONDON:
JOHN MURRAY, ALBEMARLE STREET.
1874.

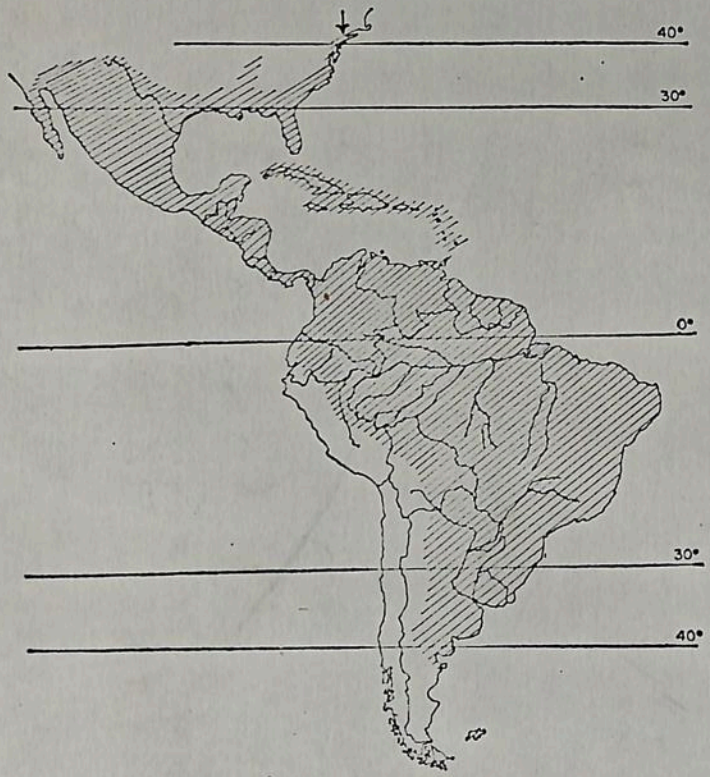


Fig. 5. Distribution of *Attini*.

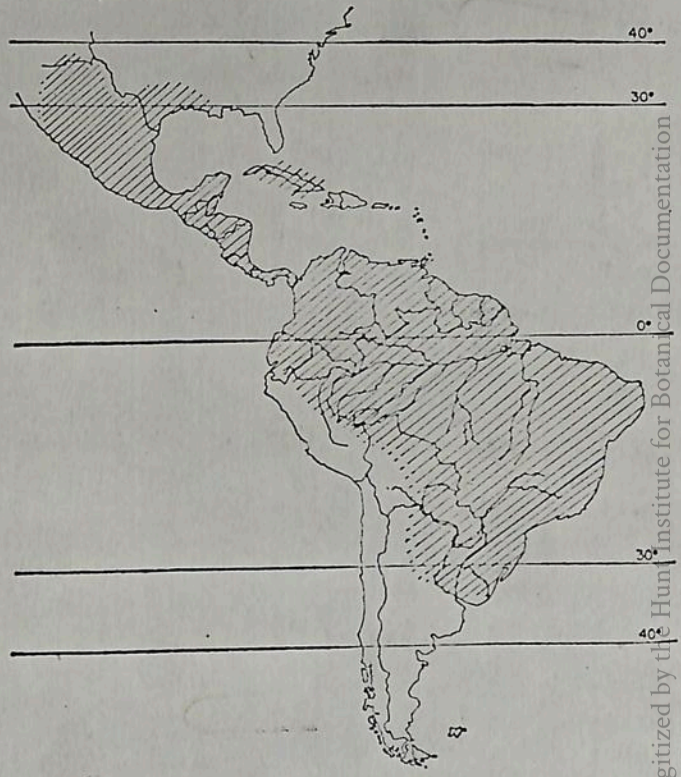
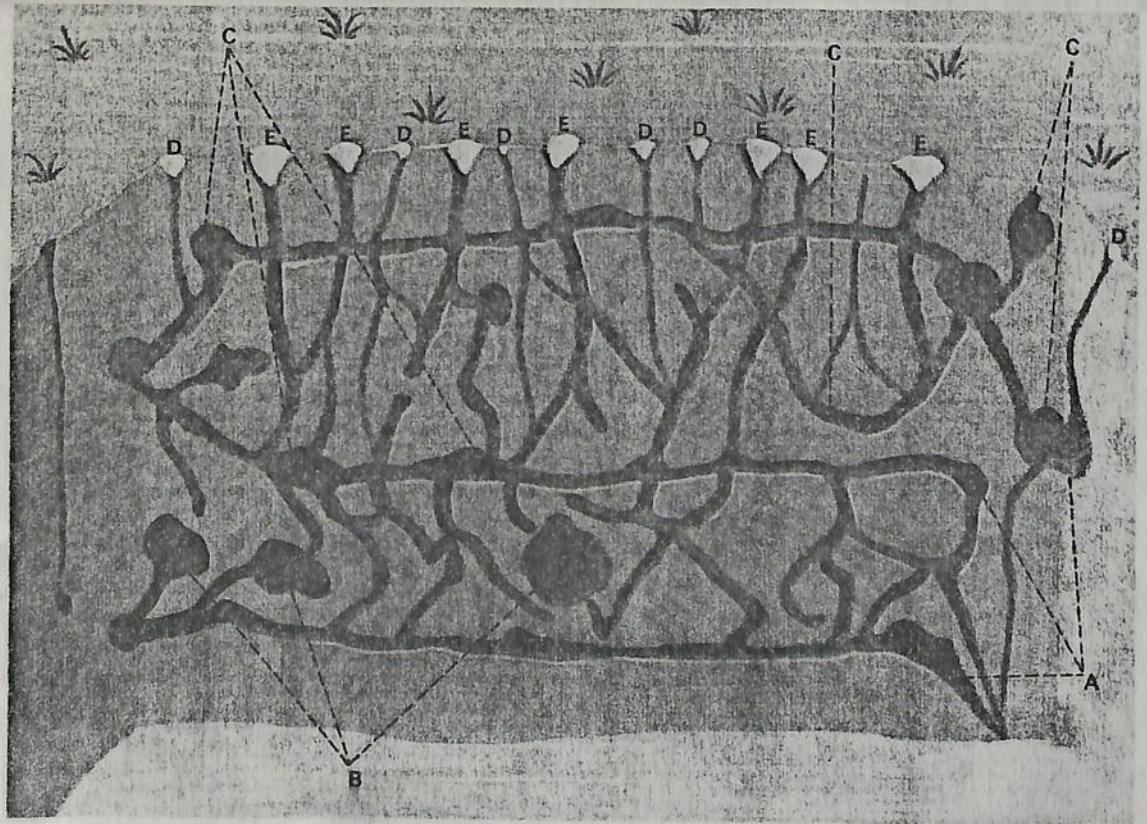


Fig. 6. Distribution of *Atta*.



Schematic diagram of a leaf-cutting and nest. A) main galleries and defence galleries; B) fungus and brood chambers; C) working galleries; D) air holes; E) craters – entrances and exits

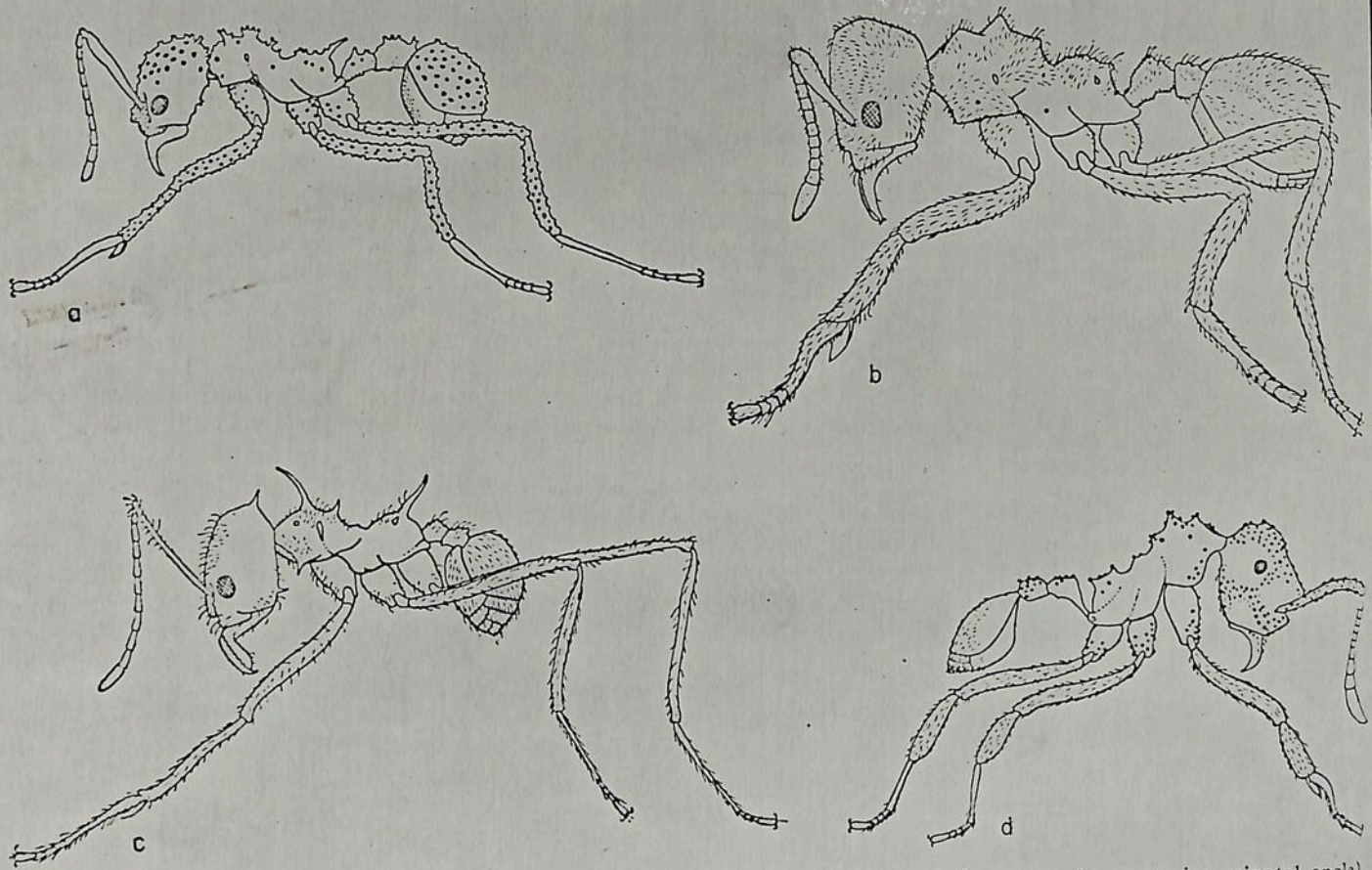


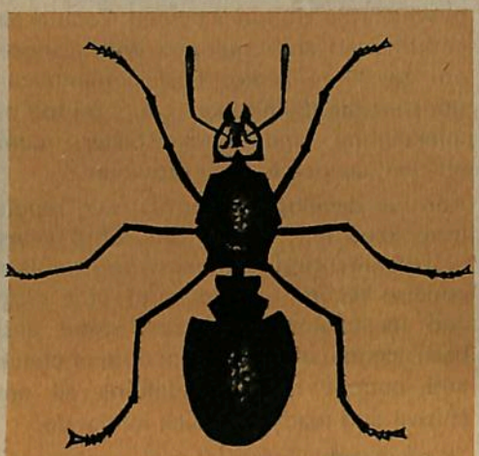
Fig. 4. Outline of representative workers in side view (thorax length is measured from anterior pronotal to posterior epinotal angle). (*a*) *Trachymyrmex arizonensis* Wheeler; length 4.5 mm (thorax 1.6 mm). (*b*) *Sericomyrmex urichi*; length 3.5 mm (thorax 1.5 mm). (*c*) *Atta cephalotes opaca*; length 7 mm (thorax 2.8 mm). (*d*) *Myrmicocrypta ednaella* Mann; length 2.3 mm (thorax 0.85 mm).

Fig. 13 (facing page). Forms of ant fungi. (*a*) Fungus of *Atta colombica tonsipes* Santschi, showing inflated hyphae from an artificial culture in a flask of sterile soil. (*b*) Fungus of *Trachymyrmex jamaicensis* E. André, showing typical staphyla from a fresh fungus garden. The hyphae have been teased apart. The inflations are characteristic of the fungi of the higher attines and are 30 to 50 microns in diameter. (*c*) Fungus of *Cyphomyrmex costatus* Mann which attained the sporophore stage and has been identified as a new species of *Lepiota*. The spores are 5 by 8 microns, and the basidia are 10 microns thick. (*d*) Fungus of *Trachymyrmex septentrionalis* McCook. A fresh staphyla. (*e*) Conidial form of fungus found under certain conditions in the garden of *Trachymyrmex septentrionalis*. The ants immediately leave such gardens. (*f*) A conidiophore of the fungus found in another colony of the same ant under abnormal circumstances. (*g*) Fungus of *Atta cephalotes ithmicola* Weber, showing staphyla forming in an artificial culture on Sabouraud's dextrose agar. The thickest hypha is 39 microns in diameter.

5 AUGUST 1966

"Mushroom growers" plague No. 1 in Brazil

Remarkable life of leaf-cutting ants



Leaf-cutting ant queen

soldier

worker

mushroom grower

The leaf-cutting ants (tribe Attini), which inhabit practically the whole of America, with the exception of Canada, Chile and a few of the islands of the Antilles, are known as mushroom growers. They feed entirely on fungi which they grow in their nests on a compost consisting of fermenting bits of leaves cut from nearby plants. The larger mushroom growers, belonging to the genus Atta, live in enormous colonies of up to 2 million strong. Consequently, huge armies of Atta ants invade fields and plantations, and cut leaves from young plants, causing devastating damage.

In Brazil, leafcutting ants are the No. 1 plague. This article has been written for the Courier by the Bayer Crop Protection agency in Brazil, Messrs. Alianca of São Paulo. Alianca have been investigating the problem of leaf-cutting ants for some considerable time now, and are incessantly searching for effective means of controlling the pests.

The problem is not a new one. As far back as 1560, the Jesuit José de Anchieta drew attention to the devastation caused by leaf-cutting ants. In 1587, Gabriel Soares de Souza wrote in his work "Tratado Descritivo do Brasil" that Bahia could probably be the promised land if it were not for the ants.

The ants! Thousands of words have been written about them, numerous suggestions have been put forward as to how they might be exterminated, and very strict regulations have been issued on their control – but the "sauvas" and the "quenquems", the names by which the leaf-cutting ants and the harvester ants are known in Brazil, have remained despite obvious progress in the field of pest control.

Leaf-cutting ants are now classified in five genera; *Atta* s. str. is divided into several sub-genera. In the southern part of North America including Texas the most injurious species is *Atta texana* which attacks cotton, corn and oranges; in Arizona the chief damage is caused by *Atta versicolor* on corn. Besides *Atta cephalotes* which is distributed from Central Brazil to India, *Atta mexicana* is also a highly dangerous pest of numerous crops. *Atta cotospinosa* attacks rubber trees in British Guiana, cocoa plantations and coconut palms in Trinidad, while in Cuba it invades, in partnership with *Atta insularis*, sugar cane and citrus plantations. In Brazil there are 12 species of leaf-cutting ants.

Harvester ants of the genus *Agromyrmex* are smaller than the leaf-cutting ants, but they are likewise dangerous major pests of many crops. One of the dreaded harvester ant species is *Agromyrmex muticinodes* which is very hard to control. This species usually builds its nests in the thickest of shrubs, under stones or on steep slopes, nearly always some distance away from its "working area". To ferret out colonies of *Agromyrmex muticinodes* is a real task.

On the other hand, it is relatively easy to destroy colonies of leaf-cutting ants provided the nests have only 1, 2, or 3 entry holes. But it is considerably more difficult to control older colonies whose nests are often more than 300 ft. long and 6 feet deep with a very large number of craters and chambers. The main galleries and defence galleries are directly connected with the fungus chambers and the brood chambers. The working galleries branch off from the main galleries, often emerging at the surface well away from the nest, usually close to plants that are cut.

The queen and its state

For perfection, the social organization of ants is probably excelled only by termites in the insect world. Each colony consists of a reproductive caste and a worker caste.

The queen is the most important member in the ant colony. It is the only female

present in the colony with fully developed reproductive organs. The task of the queen is to preserve the species. Its activities are confined entirely to uninterrupted egglaying. The queen stands out against all other ants in the colony by virtue of its length (around 3/4 inch) and its thickness.

The male reproductive forms are somewhat more slender and smaller than the females.

The worker caste includes four groups:

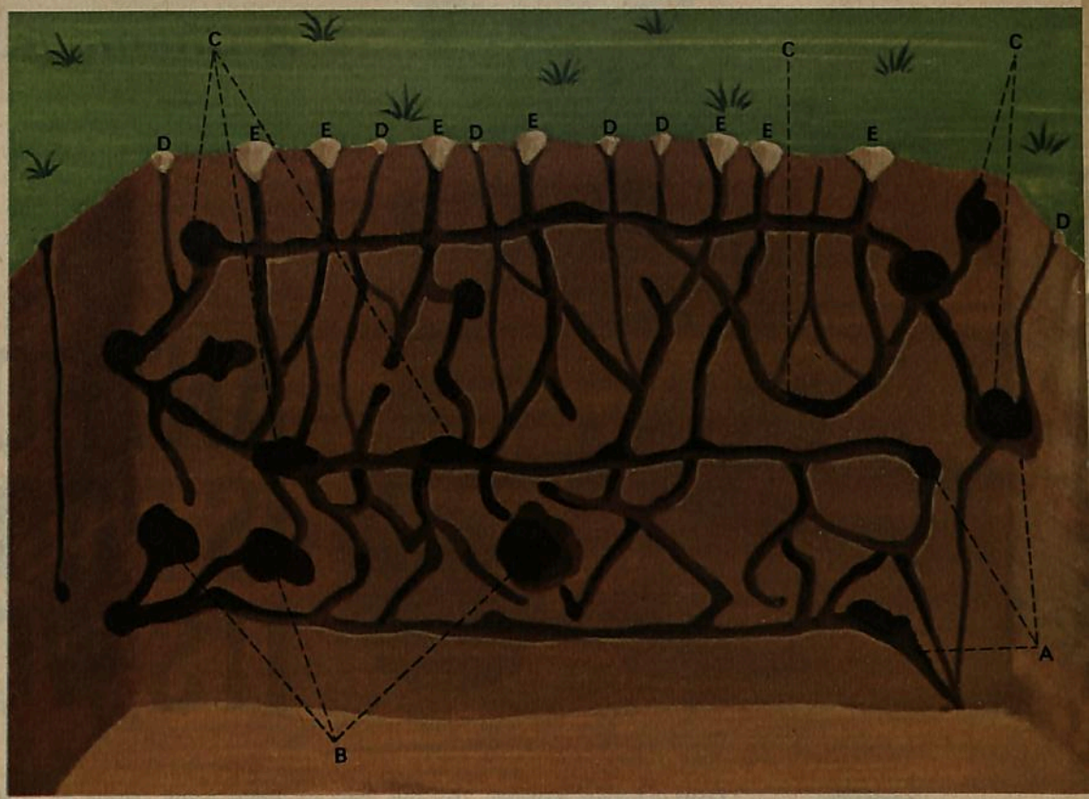
1. the soldiers and the large female workers (10–17 mm long);
2. the medium-sized female workers that act as leaf-cutters and carriers;
3. the small female workers, and
4. the smallest female workers (often only 2 mm long) which act as gardeners and mushroom growers.

The winged males and females swarm out of the nest on hot, sunny days between September and December, usually after a heavy rain fall. Before leaving the nest, each swarming female deposits small pellets of fungus in its throat, which it takes with it to establish a new colony. After mating, the males fall to the ground and die on the same day because they cannot exist alone. If it is not killed and devoured by birds or other enemies in flight, the impregnated female flies to a situation that is attractive to it as a nesting site, alights, tears off its wings, encloses itself in a small cave which it excavates in the soil, and begins to establish a new colony.

After about 4–5 days, the young queen begins laying eggs. After 52 days the larvae pupate and 12–13 days later the first of the smallest workers emerge.

The smallest female workers are later joined by the small, medium-sized and large female workers. Their task is to excavate new tunnels and new chambers. Exactly three months after the queen entered the soil to establish the new colony, the first crater becomes visible. The second crater is made 14 months later, and the other exit and entry holes then follow at short intervals.

An old ant nest may have hundreds of such craters as well as numerous fungus and brood chambers.



Schematic diagram of a leaf-cutting and nest. A) main galleries and defence galleries; B) fungus and brood chambers; C) working galleries; D) air holes; E) craters – entrances and exits

The battle against ants

It is only natural that research workers investigate every possible means of controlling a major pest capable of completely destroying, in a single night, fruit trees, coffee, tea and cocoa shrubs, grape vines, cotton, sugar cane and vegetable fields.

Despite great hopes, biological control did not produce any noteworthy success. Applications of chloroform, corrosive sublimate and sulphur fumes in the entrances to nests did not prove effective either. Quite often, the leaf-cutting ants emerged from their holes only a few days after a control operation, and devastated the fields just as before.

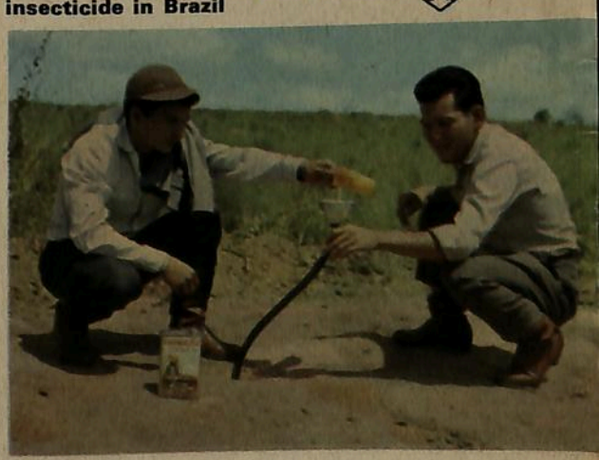
Dusting of nests with certain insecticides such as parathion and DYLOX was found to be a more useful practice. Really good results were later obtained with a compound of ALIANCA. This compound is introduced into the craters through a funnel. The poisonous vapors that form spread to the fungus chambers and all other parts of the nest, so that no ants can escape their deadly action.

The habits of ants differ very greatly, as do the methods of control and their prospects of success.

Will chemical control eventually succeed in eradicating the "greatest plague of Brazil?" We sincerely hope so.

An excavated nest shows no more sign of life

A leaf-cutting ant nest being treated with insecticide in Brazil



Reprinted from SCIENCE, August 8, 1958, Vol. 128, No. 3319, pages 306–307.

Chemical, Insecticidal, and Antibiotic Properties of Fire Ant Venom

The imported fire ant (*Solenopsis saevissima* var. *richteri*) has become an insect of considerable economic importance in the southeastern United States. It has been reported to cause damage to a variety of crops and to attack livestock (*1*). This ant also attacks human beings. The reaction caused by the sting varies with each individual but is generally limited to the area surrounding the wound. An umbilicated pustule develops which is surrounded by a red halo or an edematous painful area (*2*). In some individuals, febrile and allergic systemic reactions have been reported. In at least one case, and possibly in two, reactions to ant stings have been fatal (*2*).

The nature of the venom of the imported fire ant has not been described. The necrotic activity and the characteristic pustule at the site of the sting indicate that the venom is different from any reported insect venom (*2*). We have studied the chemical and physical properties of this venom and have found that it does not resemble the venom of any stinging insect previously studied.

Venom was collected from major workers taken in the field during the fall and winter. The ants were held by the petiole with a forceps while the tip of the abdomen was stroked with a fine capillary until the sting was everted. Droplets of venom issuing from the tip of the sting were collected in the capillary. The procedure was carried out conveniently under low magnification with a dissecting microscope.

The venom is water-insoluble, being less dense than water, in which it disperses as fine milky-colored globules. The absence of ninhydrin-positive reactants indicates it is nonproteolytic. The venom consists of two phases, primarily being composed of an alkaline carrier which suspends fine droplets of a greater density. The alkalinity of the mixture is not due to metal ions. These were determined to be absent by emission spectrographic examination in the Jarrell-Ash 4.8-meter grating spectrograph. The venom is soluble in most organic solvents, but least soluble in ethanol.

Ultraviolet spectrophotometric examination of the venom (in ethanol) in a Beckman DU spectrophotometer showed no peaks, absorption being strongest at the lower wavelengths. Infrared examinations (*3*) were made on a Perkin-Elmer model 21 spectrograph either as a carbon tetrachloride solution or as a film of venom applied directly to the rock salt prism. Only aliphatic C—H stretching was found (3.4 μ), demonstrating the nonaromatic nature of the venom. A carbonyl group (5.70 μ) is present which does not appear to be an open chain, simple ketone (*4*). Both methyl (7.25 μ) and methylene groups are present as well as a possible ether linkage (8.6 μ). The C—H/C=O ratio was found to be much higher when the sample contained small amounts of suspended globules. This indicates that the globular component contributes most or all of the carbonyl-containing compound.

Insecticidal activity was examined by exposing insects to residues, or by topically applying the venom as obtained from the ants. Samples for residual determinations were prepared as acetone or ethanol solutions. The venom was found to be highly toxic to the fruitfly, *Drosophila melanogaster* Meig., the housefly, *Musca domestica* L., a termite, *Kaleotermes* sp., the boll weevil, *Anthonomus grandis* Boh., and the rice weevil, *Sitophilus oryza* (L.). In addition, two species of mites, *Tetranychus telarius* L. and *T. cinnabarinus* Boisd., were highly susceptible. Interestingly, the fire ant is not highly susceptible to its own venom.

The antibiotic activity of the venom was investigated, and it was shown that several types of microorganisms were inhibited by a 1/50 dilution. Tests made by the paper-disk method demonstrated the effectiveness of this venom against *Micrococcus pyogenes, Streptococcus pyogenes, Escherichia coli, Lactobacillus casei,* and a variety of molds. The antibiotic activity of fire ant venom probably explains why the pustules arising at the site of the sting are antiseptic (*2*). A thorough study of the antibiotic properties is now being made.

The toxicities of different samples of venom to *Drosophila* have been found to vary, some samples being at least as toxic as DDT. Highly toxic samples of venom produce an instantaneous paralysis highly suggestive of a nerve poison. The most toxic samples contain a large percentage of the globular component, which suggests that this phase represents the toxic principle.

Recent work on the chemistry of ants has demonstrated the presence of a terpenoid lactone, iridomyrmecin (*5*), in various species of ants in the subfamily Dolichoderinae. Although these ants are in a phylogenetically more advanced subfamily than the fire ant (Myrmecinae) and do not have a functional sting, our infrared data suggest similarities in structure to this lactone. Iridomyrmecin also has been shown to have antibiotic and insecticidal activities (*6*). However, whereas iridomyrmecin produces tremors in insects suggestive of DDT poisoning (*7*), fire ant venom produces a sedative reaction, paralysis being unaccompanied by tremors.

The chemical composition of fire ant venom and the effect of it on malignant cells are being studied.

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14 March 1958

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Trans-2-Hexenal in the Scent Gland of the Hemipteran *Acanthocephala femorata*

THE plant bug, *Acanthocephala femorata* (Fab.), is one of the many hemipterous insects which eject odoriferous materials when disturbed. This large brown coreid, which occurs in the southern United States, is frequently found on citrus plants[1].

The scent gland secretion of *Acanthocephala* adults is stored in a large round reddish-orange sac which lies on the floor of the body cavity covering the metathoracic and first abdominal segments and extending to the second abdominal segment. This sac opens to the outside of the body-cavity through a pair of ostioles each of which is located on a suture between the sternal plate and the pleural plate of the metathorax. The ducts leading from the sac to the ostioles enlarge just inside each ostiole and can be opened to the outside by means of muscle-operated lips. The sac itself contains no obvious musculature for compression which would be required for ejection of the secretion. However, it was observed that ejection always occurred when the metathoracic legs were flexed. If the metathoracic legs were held immobile, no ejection occurred. Indeed, it was observed that movement of metathoracic coxæ from which the femora had been severed was coincident with ejection of the secretion. It is possible that the force required for ejection of the secretion is supplied by coxal pressure during contraction of the metathoracic leg; the secretion thus would be ejected when the lips of the sac ducts are simultaneously opened.

The secretion of *Acanthocephala* adults and nymphs issues as fine droplets. The adult secretion can be sprayed for a distance of at least 8 in. If one side of the body is mechanically stimulated, the spray is unilateral from the stimulated side. However, if the dorsum of *Acanthocephala* is stimulated, the spray is often bilateral.

Samples of clear yellow fluid from the sac were obtained by removing the abdominal dorsum and viscera of *Acanthocephala* and piercing the exposed sac with a fine capillary. Fluid collected in capillaries was identified as *trans*-2-hexenal by infra-red spectrophotometry, mass spectroscopy and the 2,4-dinitrophenylhydrazone derivative.

BLUM M. S., TRAYNHAM J. G. (*)

THE CHEMISTRY OF THE PENTATOMID SCENT GLAND

In many of the Hemiptera, specialized glands have developed from which highly odoriferous compounds are ejected. Because they eject very odoriferous substances when disturbed, the Pentatomidae have been called stink bugs. This paper described the analyses of some of the scent gland components found in the rice stink bug *Oebalus pugnax* (F).

METHODS AND MATERIALS

The contents of the scent gland were collected by piercing exposed glands with fine capillaries.

Infrared analyses were made on a Perkin-Elmer Model 21 spectrophotometer both from a film and from a carbon tetrachloride solution.

Vapor phase chromatographic analyses were performed on a Perkin-Elmer Model 154 B vapor phase chromatograph by injecting 1-50 µl. samples into the inlet of the instrument. Tris-phenoxyphenyl-*n*-dodecyl silane was used as an adsorbant; the instrument was operated at 160° C. Samples were collected by condensing the components in microtubes immersed in liquid nitrogen as they issued from the outlet of the instrument.

Mass spectrographic analyses were made on a modified Consolidated 21-102 analytic mass spectrometer.

2,4-Dinitrophenylhydrazones were prepared by adding the scent gland fluid to 5 ml. of absolute ethanol to which was added a saturated solution of 2, 4-dinitrophenylhydrazine in 2N HCl. The alcohol soluble derivatives were chromatographed employing the method of Gordon *et. al.* (1951).

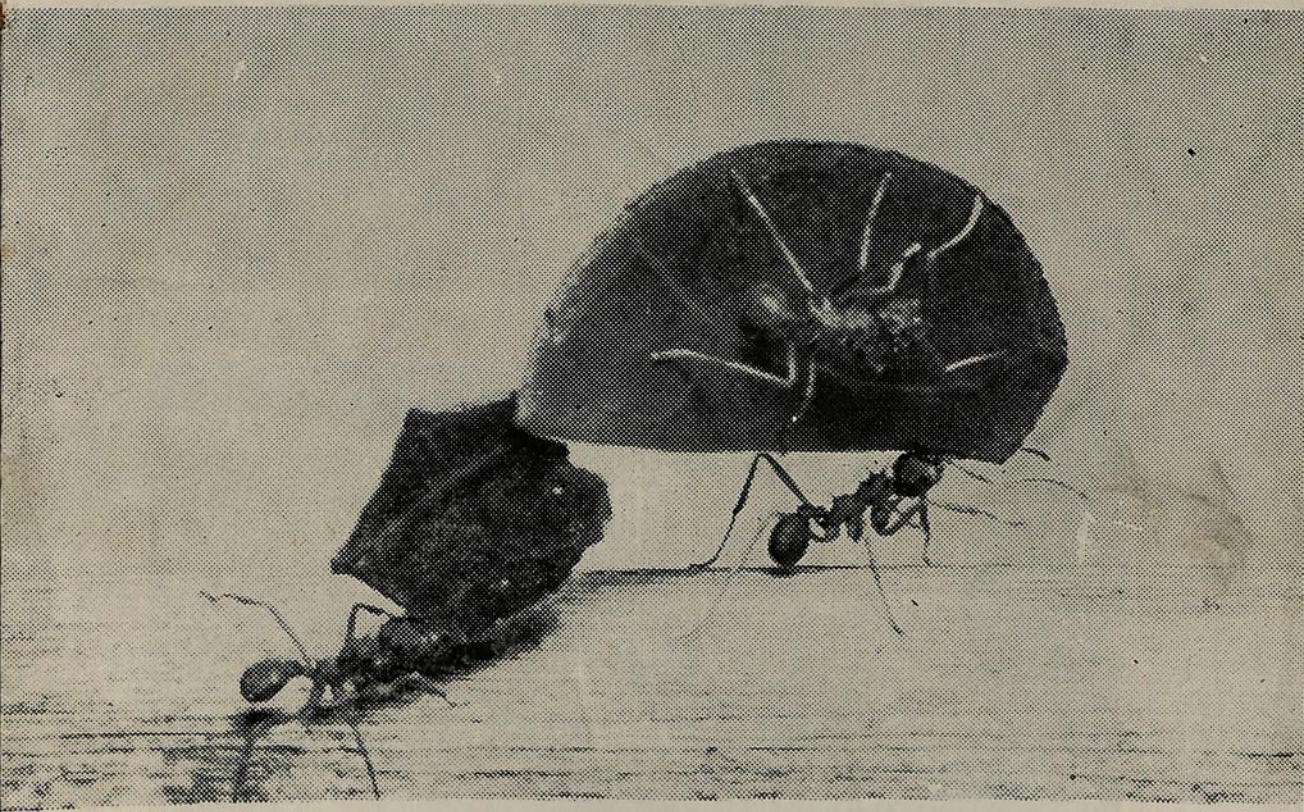
RESULTS

The fluid which had been freshly removed from the scent gland cointained a suspension of orange droplets suspended in a clear fluid. Upon settling, the orange droplets formed a lower layer which consisted of about 40 per cent of the total sample.

(*) *Louisiana State University, Baton Rouge, La., U.S.A.*

LAS HORMIGAS CORTADORAS DE LAS HOJAS (COQUIS)

RECOMENDACIONES PARA SU CONTROL



Circular Nº 48 (Segunda Edición) Enero, 1957

ESTACION EXPERIMENTAL AGRICOLA DE TINGO MARIA

PCEA Programa Cooperativo de Experimentación Agropecuaria
Ministerio de Agricultura.

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17. The control of a single flat reflector on each of the trolleys seems quite feasible. An alternate arrangement would be to use two flat reflectors—the first, on a coelostat mounting, to reflect the incoming starlight onto a path parallel to the earth's rotational axis, the second to reflect the starlight from that path onto the horizontal northbound or southbound path. This arrangement has the advantage that one mirror is fixed and the motion of the other is easier to control. Guiding the single flat reflector is relatively simple, thus this seems an attractive way to construct a telescope of very large aperture.
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Fungus-Growing Ants

A symbiotic relationship exists between an insect and a plant, involving an effective culturing technique.

Neal A. Weber

The fungus-growers are a New World tribe of myrmicine ants, the Attini, that has developed a unique relation with saprophytic plants. The ants eat only the fungus that they culture, and it is not found outside the ant nest. Many animals feed on fungi, and certain beetles and termites grow them in their nests, but the culturing of fungi as described here is believed to be unique. In this process a flourishing growth of one fungus is produced, and of this fungus only, although the medium on which it grows (the substrate) is suitable for the growth of many other kinds of organisms. When the ants are removed, these other organisms multiply and replace the fungus.

The vital part of the attine nest is the fungus garden. It is the abode of the queen and brood as well as of the fungus. Despite the diversity in morphology of the species, the development and care of the garden are fundamentally similar for all varieties.

Fungus-growing is distinguished from leaf-cutting. All members of this tribe subsist solely, in nature, on the fungus that they culture, but some are leaf-cutters and others are not. The latter pick up vegetal particles of suitable size, or insect excrement, and grow the fungus on these. The leaf-cutters go in files, often on well-formed trails, and cut leaves, flowers, or stems. They are most commonly members of the largest species and belong to the genera *Acromyrmex* and *Atta*. Inconspicuous *Trachymyrmex* and *Sericomyrmex* species may also cut leaves and flowers.

It is the purpose of this article to review the chief features of the life of these ants and of the fungus on which they depend. Because of the economic importance of the large species of *Atta*, and particularly of *Atta sexdens* L. in Brazil, a considerable body of literature has grown up, here summarized, and *A. sexdens* may be taken to represent a high expression of this symbiosis. Studies of other species and genera have made significant contributions to the knowledge of the biological role of the attines and are here reviewed.

Species of this tribe were listed by Linnaeus in 1758, and the type genus *Atta* was named by Fabricius in 1804. Latreille called such ants *Oecodoma* in 1818, and this name was used by the early naturalists, such as Bates, Belt, and Smith in Latin America, for the conspicuous leaf-cutters with soldiers now known as *Atta*. Mayr, from 1862 to 1865, originated the generic names *Cyphomyrmex*, *Apterostigma*, *Sericomyrmex*, and *Acromyrmex*, and he has been the chief contributor to the generic classification. Outlines of the wings, heads, and side views of the ants show differences characteristic of the genera (Figs. 1–4).

The tribe has a wide distribution, from approximately 40° north latitude to 44° south latitude (Fig. 5). The economically important *Atta* species have smaller ranges (Figs. 6 and 7). Their general distribution in South

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Nutrients Derived from the Fungus Cultured by the Fungus-Growing Ant *Atta colombica tonsipes*[1,2]

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ABSTRACT

The fungus cultured by the attine ant *Atta colombica tonsipes* Santschi provides the ant with a rich and complete diet. More than 50% of the dry weight of the fungus is available as soluble nutrient. Carbohydrates make up 27% of the dry weight of the fungus; free amino acids 4.7%; protein-bound amino acid 13%; and lipid 0.2%. The carbohydrates consist of trehalose, mannitol, arabinitol, and glucose. No polysaccharides are present. The lipid fraction contains ergosterol as the major sterol.

Attine ants culture a fungus in their nests and utilize this fungus as their primary and possibly sole food source. We are currently engaged in an extensive investigation of the chemical basis for this intriguing symbiotic association. This paper reports the characterization of the nutrients provided by the fungus cultured by *Atta colombica tonsipes* Santschi (det. N. A. Weber).

MATERIALS AND METHODS

Fungus Cultures and Samples.—Pure cultures of the fungus, which has not yet been identified, were obtained by standard isolation techniques from fungus gardens of *A. c. tonsipes*. The authenticity of the fungus as the ants' true food fungus was checked by returning it to the ants and noting that they would readily feed on it. The fungus could be grown under sterile condition either in liquid cultures or on agar surfaces using Sabouraud's or potato dextrose media. Samples were obtained for chemical analysis by scraping the dense mycelial growth off agar cultures, and by filtering liquid cultures. The fungus was freeze-dried to constant weight.

Fungus Extraction.—A sample for carbohydrate analysis was obtained by homogenizing 6.57 g of freeze-dried fungus with 5 portions of methanol, then 5 portions of water. Solvent removal on a rotary concentrator left 3.63 g (55.6% by weight) of solid material, which was analyzed for carbohydrates. A sample for free amino acid analysis was obtained by homogenization of 1.0 g of freeze-dried fungus in water, followed by filtration of the extract. A sample for total amino acid analysis was prepared by refluxing 1.0 g of freeze-dried fungus with 6 N HCl for 24 hr, filtering, evaporating to dryness repeatedly, and finally dissolving the residue in 5.0 ml of 1:1 water:ethanol. An extract of lipid was obtained by homogenizing 10.31 g of freeze-dried fungus 3 times with 3:1 ether:methanol, filtering the mixture through a sintered-glass funnel, drying the filtrate with magnesium sulfate, and removing solvent on a rotary concentrator, leaving 20.6 mg of solid lipid residue.

Carbohydrate Analyses.—Silyl ethers of the extracted carbohydrates were prepared with Tri-Sil® (Pierce Chemical Co., Box 117, Rockford, Ill.). Acetates were prepared by a routine treatment with acetic anhydride and pyridine. Components of the

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carbohydrate mixture were identified by comparison of retention times of the components of the silylated mixture with authentic standards on a 6-ft×⅛-in. 10% SE-30 on AW-DMCS Chromosorb® W GLC column (180°C for monosaccharides, 250°C for disaccharides), and of the components of the acetylated mixture with authentic standards on a 6-ft×⅛-in. 6% Carbowax® 20 M/Terephthalic Acid on AW-DMCS Chromosorb® W GLC column (240°C) and on a 6-ft ×⅛-in. 6% LAC-728 on AW-DMCS Chromosorb W GLC column (200°C). Identifications were further confirmed by actual isolation of mannitol from the extract, found in every respect to be identical to authentic mannitol [mp 165°C, lit. mp 166°C (Heilbronn 1965); $[\alpha]_D^{25°}$ −0.7±1.0° (0.06 g/100 ml, water), lit. $[\alpha]_D^{25°}$ −0.49° (water (Heilbronn 1965); $[\alpha]_D^{25°}$ + 30±4° (0.05 g/100 ml, sat. borax), lit. $[\alpha]_D^{25°}$ + 28° (sat. borax) (Stanek et al. 1963)]. Also, treatment of the carbohydrate mixture with phenylhydrazine, followed by silylation, removed peaks from the GLC trace assigned to glucose, but did not remove those assigned to xylitol, arabinitol, mannitol, and trehalose.

Quantitative analyses were obtained by adding a known amount of sucrose to the carbohydrate extract prior to silylation. Quantitative analyses were then obtained by measuring the areas under the peaks caused by each component of a programmed GLC run (175–300°C at 4°/min) of the silylated mixture on the 10% SE-30 column, using the peak resulting from the sucrose derivative as the internal standard. A polysaccharide analysis was obtained by noting the difference in the amount of glucose present before and after hydrolysis with 10% HCl (Adams 1965). Since there was no difference it was concluded that no polysaccharides were present in the extract.

Amino Acid Analyses.—Dr. Thomas Hernandez, Department of Pharmacology and Dr. Roland Coulson, Department of Biochemistry, Louisiana State University, School of Medicine, New Orleans, made the analyses using standard ion exchange chromatographic techniques.

Sterol Isolation.—The lipid extract (20.6 mg) was chromatographed over 30 g of Florisil® (7% water). A sterol-containing fraction (16 mg) was eluted with 25% ether in petroleum ether. Final purification by a digitonide precipitation (Schoenheimer and Dam 1933) gave 9.0 mg of sterols. The major component of this sterol mixture (87%) was identified as ergosterol by comparison of the retention times of the trisilyl ethers (prepared using Tri-Sil) of the components of the sterol mixture with authentic samples on a 6-ft×⅛-in. 3% QF-1 on GasChrom Q GLC column (200°C). Two unidentified sterols and a trace of cholesterol made up the remaining 13% of the mixture.

Table 1.—Nutrients derived from fungus cultured by *Atta colombica tonsipes.*

Nutrient	mg/g dry fungus
Trehalose	160
Glucose	47
Mannitol	51
Arabinitol	14
Protein-bound amino acid	130
Free amino acid	47
Lipid (major sterol-ergosterol)	2
Total wt of nutrients identified	451

Table 2.—Amino acids derived from fungus cultured by *Atta colombica tonsipes.*

Amino acid	mg amino acid/g dry fungus: Protein-bound	Free
Aspartic acid	14.12	1.48
Threonine	5.75	1.40
Serine	5.99	2.45
Glutamic acid	15.12	3.67
Glutamine		1.74
Proline	7.20	1.64
Glycine	13.77	2.94
Alanine	5.41	3.76
Cystine	5.04	0.00
Valine	10.64	3.51
Methionine	2.45	.86
Isoleucine	4.67	2.52
Leucine	9.84	4.57
Tyrosine	4.41	2.81
Phenylalanine	7.33	3.86
Ornithine	1.27	.46
Lysine	5.22	2.69
Histidine	5.81	1.87
Arginine	7.13	3.92
Tryptophan		.00
Total amino acids	130.14	47.29

RESULTS AND DISCUSSION

Table 1 lists the nutrients which have been isolated from the mycelia of a 35-day-old potato-dextrose agar culture of the fungus which is ordinarily grown in the nests of *A. c. tonsipes.* Table 2 shows the amino acid distribution of the free and protein-bound amino acids derived from the fungus mycelia. The distribution of sugars varies somewhat with age and carbon source in the growth medium. As the fungus ages, trehalose increases at the expense of mannitol. Glucose is present in only small amounts in the extract when the fungus is grown in media containing glycerol, mannitol, glucitol, mannose, and fructose rather than glucose as the carbon source, but is present, though to a lesser extent, when the fungus is grown on sucrose. Trehalose, arabinitol, and mannitol are always present. We were able to detect no polysaccharides in the extract of soluble carbohydrates.

The amino acid distribution recorded in Table 2 would doubtless show some variation depending upon the nature of the growth medium. The distribution is completely different from the amino acid distribution of the potato dextrose medium on which the fungus was grown and hence does represent the total amino acid pool of the fungus.

The lipid component of the fungus, though minor, is of critical importance to the ant, since insects require a dietary source of sterols. The major sterol of the food fungus is ergosterol, a commonly encountered fungal sterol.

To be sure, the fungus cultured in the laboratory on potato dextrose agar is not biochemically identical to the same fungus grown on leaves in the nest by *A. c. tonsipes*. Consequently, the nutrients listed in Table 1 and 2 may not represent precisely the diet of the ants in their natural state. However, these results certainly suggest the type of diet being provided the ants by their food fungus, and it is indeed an excellent one, high in carbohydrates, in a form readily available as an energy source, and high in protein which is rich in the essential amino acids.

Subsequent papers in this series will discuss the integration of the carbon and nitrogen metabolisms of the ants and their fungus, and the role of antibiotics and growth-promoting substances in the ants' fungus-culturing activities.

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Seasonal Occurrence and Physiology of *Culex tarsalis*[1] in Foothills of Fresno County, California[2]

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ABSTRACT

Field data from a 5-year study indicate that there is a movement of *Culex tarsalis* Coquillett into foothill areas adjacent to the San Joaquin Valley in Fresno County in fall, and that overwintering of females takes place there. During this overwintering period, females are gonotrophically inactive and usually contain well-developed fat bodies. Females fed and became gonotrophically active in January, and there was a general movement out of the foothill area later. Almost all females captured during the inactive overwintering period were inseminated and only a small proportion of this population was parous.

Culex tarsalis Coquillett is one of the more intensively studied mosquitoes in western United States because it is a primary vector of endemic viral encephalitides. There are numerous publications that record observations on *C. tarsalis* in California.

An extensive study was made of flight and dispersal of this species in the Sacramento Valley by Bailey et al. (1965). They concluded that there is a definite autumn movement to the hills west of the Valley. Stuntz (1952[6]) had hypothesized earlier that *C. tarsalis* females overwinter chiefly in hilly country, and that they fly back into the Sacramento Valley early in the year.

In the San Joaquin Valley, most work has been done near Bakersfield. Bellamy and Reeves (1963) reported that blood engorgement and egg development commenced in January in that area after being greatly diminished in autumn and early winter. Experimental data showed winter survival of parous females, but field observations indicated that this circumstance was uncommon. Reduction in numbers of parous females in overwintering populations in the Bakersfield area was substantiated by Burdick and Kardos (1963) and Nelson (1964). Brookman (1950[7]) noted the inactivity of winter populations and described seasonal occurrence in the Bakersfield area; population peaks occurred in summer and early autumn.

There are fragmentary field observations on the biology of *C. tarsalis* in Fresno and other counties. Abell (1959) conducted a study of mosquito populations in a Fresno County foothill area similar to the one in which our study was made. His 1-year study was concerned primarily with immature stages. He found *C. tarsalis* larvae to be most abundant (though

[1] Diptera: Culicidae.

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The Chemical Basis for the Attine Ant-Fungus Symbiosis. Absence of Antibiotics[1]

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ABSTRACT

The theory that attine ants maintain pure cultures of their food fungi by application of antibiotics to their fungus gardens has been rendered untenable. Extracts prepared from the ant *Atta colombica tonsipes* Santschi, from its wild fungus gardens, from expended fungus gardens, and from laboratory cultures of its fungus have been assayed for antibiotic activity against a wide spectrum of microorganisms, and no significant growth-inhibiting activity has been detected. The conclusion is unavoidable that the mechanism by which the attines prevent contamination of their fungus cultures by alien microorganisms does not involve the elaboration of antibiotic substances by the ants, the fungus, or any unspecified 3rd symbiont.

Attine ants culture a fungus in their nests and utilize this fungus as their food source (Belt 1874; Muller 1874; Wheeler 1907; Weber 1958, 1966). The more primitive genera such as *Cyphomyrmex* and *Apterostigma* use insect feces, insect carcasses, and vegetal debris as substrate for their fungus cultures. The intermediate genera *Sericomyrmex* and *Trachymyrmex* supplement the debris they collect with leaves and flowers which they cut from live plants, while the most highly evolved genera, *Acromyrmex* and *Atta*, provide almost all the substrate for their fungus gardens by extensive leaf-cutting activities (Weber 1958, 1966). The fungus gardens of the ants are flourishing cultures of a fungus which appears to be peculiar to attine nests (Moeller 1893; Weber 1966). The ants succeed in maintaining their fungus cultures in spite of possible contamination by alien microorganisms present in the surrounding soil or carried into the nest on substrate or on the ants themselves. If the ants are removed from their gardens, rapid degeneration follows as alien bacterial or fungal contaminants overwhelm the food fungus (Moeller 1893; Weber 1956, 1957, 1966). It is clear that the ants are actively culturing their fungus and are essential for its viability. In the maintenance of their fungus gardens, the application of fecal, and probably salivary material, is a general behavioral characteristic of the attines (Huber 1905; Weber 1958, 1966). Weber (1947) pointed out that the chemical milieu provided by the ants must be a key factor in the maintenance of their gardens. He has subsequently reiterated and expanded this view, and has suggested the possibility that antibiotic and/or growth-promoting agents may be present in the salivary and fecal material (Weber 1954, 1955, 1956, 1958, 1966).

At Michigan we have undertaken an extensive study of the chemical basis for the intriguing symbiosis between attine ants and their food fungi (Martin et al. 1969). In this paper we describe experiments designed to establish the role of antibiotics in this symbiosis. Not only did it appear to be an a priori possibility that the ants were applying antibiotics in their fecal and salivary material, as Weber has suggested, but it seemed possible also that antibiotics might be produced by the fungus when grown in the chemical medium provided by the ants, or even that some unknown 3rd constituent, such as a minor bacterial or protozoan symbiont present in the fungus garden, might be the actual source. It was our initial hope that by studying the attine ant-fungus system we would be led to a rich source of new and possibly useful antibiotics.

It is worth noting in anticipation of our results that there have been only a few reports of antibiotic activity in insect products. Iridomyrmecin, produced by several ants, has been found to exhibit weak antibiotic activity (Pavan 1955). Also 10-hydroxy-Δ^2 decenoic acid, present in the royal jelly of the honey bee, is weakly antibiotic (Blum et al. 1959). In addition, antibiotic activity has been reported in the hemolymph of the large milkweed bug, *Oncopeltus fasciatus* Dallas (Frings et al. 1948, Barfknecht 1964[5]), the exudate of the physogastric queen of the fungus-growing termite *Termes redemanni* Wasmann (Sannassi and Rajulu 1967), and in extracts of *Sarcophaga* larvae (Nicholls 1912), the greater wax moth, *Galleria mellonella* (L.) (Oliver 1947), and the red flour beetle, *Tribolium castaneum* (Herbst) (DeCoursey et al. 1953).

MATERIALS AND METHODS

Ants of the species *Atta colombica tonsipes* Santschi (determined by N. A. Weber) were collected in the summer of 1966 in the vicinity of Gamboa, Canal Zone (Steiner and Martin 1967). The ants were kept deep frozen from a few hours after their capture until extracts were prepared from them. Fungus gardens were collected from nests of *A. c. tonsipes* in the vicinity of Gamboa in the summer of 1967. These fungus gardens were either frozen or dried shortly after being collected and were stored either frozen or dried until extracts were prepared from them. Refuse, or expended fungus garden cast out of the nest by the ants, was collected from the same nests in the summer of 1967, dried, and stored dry until extracts were prepared. The fungus was also cultured artificially using liquid and agar potato dextrose media.[6]

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[5] C. Barfknecht, 1964. Investigation of the blood antibiotic in the giant milkweed bug. Ph.D. dissertation (Part I, 23 p.). University of Kansas, Lawrence.

[6] We thank Professor Neal A. Weber of Swarthmore College for the initial inoculum from which our fungus culture was started.

Table 1.—Results of Antibiotic bioassay on materials derived from *A. c. tonsipes*, its fungus and its refuse.

Extract no.	Material extracted	Solvent	Bioassay results
1	*A. c. tonsipes*[a]	Ether	No growth-inhibiting activity
2	"	Methanol	"
3	"	Water	"
4	Refuse[b]	Ether	"
5	"	Methanol	"
6	Cultured fungus[c]	"	"
7	Residue from culture medium[d]	Water	"
8	Fungus garden (frozen)[b]	Ether	Weak inhibition of 3 strains of *Staphylococcus* and 2 species of *Mycobacterium*
9	" (dried)[b]	"	"
10	" (frozen)[b]	Methanol	Weak inhibition of *M. butyricum*
11	" (dried)[b]	"	"
12	" (frozen)[b]	Water	"
13	" (dried)[b]	"	Weak inhibition of *M. butyricum*, 2 strains of *Proteus* and 3 strains of *Pseudomonas*

[a] Tested as 1% solutions and at full strength.
[b] Tested as 10% solutions.
[c] Tested as 5% solutions.
[d] Tested as 1% solutions.

Extracts were prepared by homogenizing the material being extracted with 2 portions of solvent in a Waring Blendor®, filtering, and removing solvent at reduced pressure and at temperatures not exceeding 60°C. The crude residues so prepared were assayed for antibiotic activity. In addition, the residue remaining after removal of water from a liquid potato dextrose culture medium in which the *tonsipes* fungus had been growing was tested for activity.

Antibiotic bioassays were conducted on the extracts described by the standard impregnated filter-disc method using as test organisms various bacteria and fungi grown on appropriate nutrient agars. For extracts 1–7 (Table 1), the following test organisms were used: *Aerobacter* sp. (3 strains), *Bacillus cereus*, *Escherichia coli* (3 strains), *Proteus* sp. (3 strains), *Pseudomonas* sp. (4 strains), *Serratia* sp., *Staphylococcus aureus* (3 strains), *Mycobacterium butyricum*, *M. marinum*, *M. phlei*, *M. smegmatis*, *Aspergillus fumigatus*, *Candida albicans*, *Mucor corymbifera*, *M. pusillus*, *M. racemosus*, *Paecillomyces varioti*, *Penicillium* sp. (2 strains), and *Saccharomyces cerevisiae*. For extracts 8–13, 2 additional strains of *E. coli*, *Proteus* sp., and *Staphylococcus aureus*, and 3 additional strains of *Pseudomonas* sp. were tested.

RESULTS

Table 1 presents antibiotic bioassay results for all the extracts prepared. Extraction of whole ants with ether, methanol, and water gave materials (entries 1–3) which were found to be completely devoid of any antibiotic activity, even when tested at full strength. On the assumption that the exhausted fungus gardens cast out of the nest as refuse by the ants might contain a residual quantity of any substance applied to the growing garden by the ants, ether and methanol extracts of this material were prepared and tested (entries 4 and 5), and were found to be completely devoid of activity. As a matter of course, an ether extract of laboratory cultured fungus (entry 6) and the water-soluble residue from a liquid culture medium in which the fungus had been growing (entry 7) were tested for antibiotic activity. Both were completely inactive. Finally, an actively growing fungus garden, collected from a thriving colony of *A. c. tonsipes*, was extracted with the 3 solvents ether, methanol, and water (entries 8–13). In such a fungus garden everything in the ant-fungus system should be present; adult ants, brood, fungal mycelium, substrate, and any unknown additional symbionts. Only very weak inhibition of a few specific test organisms was observed in the antibiotic assay. The extracts had no effect on most of the test organisms, and in a few cases even enhanced their growth. The antibiotic activity observed in extracts 8–13 is much too weak and of far too restricted a nature to be regarded as of any real significance in the ant-fungus symbiosis. Therefore, our experiments have failed to demonstrate significant antibiotic production by any organism involved in the *A. c. tonsipes*-fungus garden system. Of course, there remains the remote possibility that an extremely unstable antibiotic is produced by some constituent in the symbiosis, and that we failed to detect any activity because of decomposition prior to testing.

DISCUSSION

The theory that attine ants maintain the purity of their fungus gardens by the application of antibiotics is is an extremely appealing one. It has been so frequently cited, both in popular and technical publications, that it has come to be accorded more the status of an established fact than a tentative hypothesis, which is all that Weber intended it to be. The results reported in this paper render that theory untenable. Indeed, this negative result may be generalized further by stating that any theory explaining the maintenance of uncontaminated fungus cultures by attine ants, which depends upon antibiotic production by any of the various organisms involved in the sym-

biosis, is ruled out. We must then reconsider some of the questions which the antibiotic theory seemed to answer. How do attine ants prevent contamination of their fungus gardens? What is the significance of their application of fecal material to the gardens? What is the nature of the chemical agents present in the fecal material, and what role do they play in the fungus culturing process? Subsequent papers will describe studies on the chemical basis of the symbiosis and will provide answers to these puzzling questions.

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Drosophila lowei, a New American Member of the *Obscura* Species Group[1,2]

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ABSTRACT

A new species, *Drosophila lowei* (Diptera: Drosophilidae), from the mountains of Arizona and Colorado, is described. The species is of particular interest because it is the 5th American species to be described in the *obscura* subgroup of the *obscura* Fallén species-group, subgenus *Sophophora*, and is clearly related to *D. pseudoobscura* Frolowa and *D. persimilis* Dobzhansky & Epling. *D. lowei* may usually be distinguished from *D. pseudoobscura*, with which it is sympatric, by its smaller size, proportionately longer wings, and its darker eye color. The chromosomes of *D. lowei* differ from those of *D. pseudoobscura* by a pericentric inversion in chromosome II. Hybrids between the 2 species are sterile. Ecologically *D. lowei* prefers the more temperate and cooler habitats above 7000 feet elevation and has its population peak in late summer and fall. There is evidence that the species undergoes a reproductive diapause at that time.

At present there are 4 American species of *Drosophila* in the *obscura* subgroup of the *obscura* Fallén species-group, in the subgenus *Sophophora* (Diptera: Drosophilidae). They are *D. pseudoobscura* Frolowa, *D. persimilis* Dobzhansky & Epling, *D. miranda* Dobzhansky, and *D. frolovae* Wheeler (Patterson and Stone 1952, Buzzati-Traverso and Scossiroli 1955). The present report describes the 5th American species in the *obscura* subgroup, *D. lowei*, n. sp., first encountered by one of us (W.B.H.) in the Santa Catalina Mountains near Tucson, Ariz., in 1960 (Heed et al. 1962). The species is smaller, with proportionately longer wings and darker eyes than *D. pseudoobscura*. Detailed examination showed that it was distinctly different from *D. pseudoobscura* in several respects, but closely related to it. Several cultures of *D. lowei* were established after the species was recognized, but none could be maintained for longer than 1 year. During 1960 and 1961, data on the chromosomes and hybrids with *D. pseudoobscura* were collected. On July 15, 1961, the species was

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[2] Endorsed and communicated by Floyd G. Werner. Accepted for publication July 8, 1968.

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FUNGUS-GROWING ANTS AND THEIR FUNGI: *TRACHYMYRMEX SEPTENTRIONALIS*

By

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