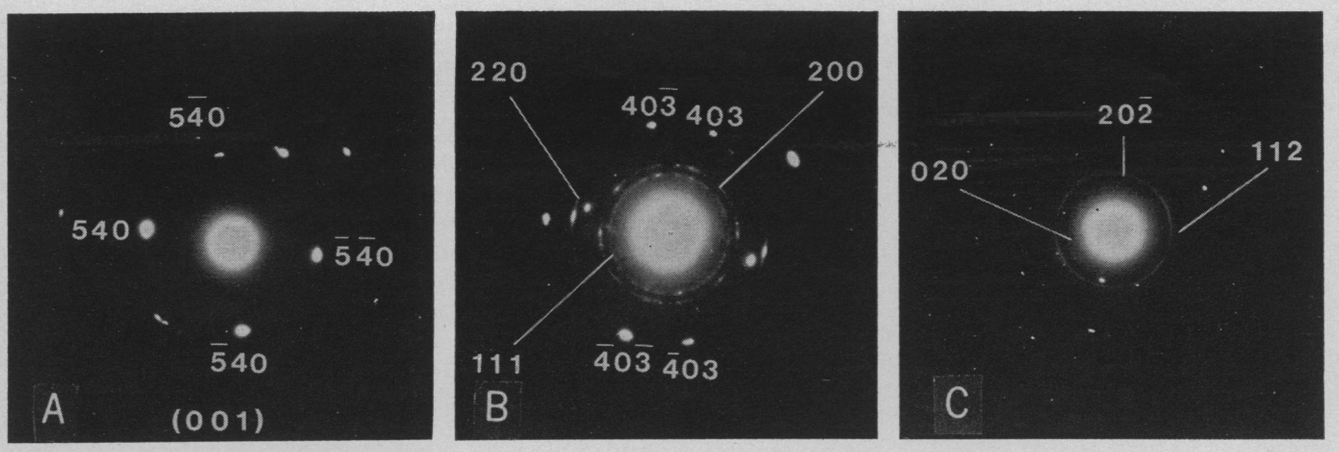

Fig. 2. Electron diffraction patterns of (A) a bipyramid tetragonal crystal of weddellite (calcium oxalate dihydrate) with (001) orientation; (B) the same diagram as (A), after electron bombardment, which still presents spots of weddellite (403) and rings which correspond to lime (111) (200) (220); (C) the crystalline aggregate of (B) which presents the principal rings (020), (20$\bar{2}$), (112) of whewellite (calcium oxalate monohydrate); (D) a dumbbell form (like that in Fig. 1C) which presents a weak ring (020) Ox of whewellite even though very low beam intensity has been used in order to delay the transformation to calcite (104) C and lime (200) L.

To prevent the decomposition of the crystals during observation, the specimens were cooled with liquid nitrogen.

Tetragonal bipyramids are the most abundant forms observed but others with less well-defined crystallization (ellipsoid, biconcave disks, needles, spherulites, and dumbbell forms) were also found, especially in cases of metabolic hyperoxalurias or in healthy individuals on a rich oxalate diet. Tetragonal bipyramids (Fig. 1A) have been recognized as weddellite (*2*). Our analysis by electron diffraction (Fig. 2A) confirms this. The increase in temperature produced by electron bombardment and the high vacuum rapidly convert the weddellite crystals to calcite and then to calcium oxide (Fig. 2B).

Dumbbell forms have been identified as whewellite on the basis of their shapes (*3*). Electron-diffraction ring patterns corresponding to whewellite crystals (Fig. 2, C and D) have been observed in the prismatic crystal aggregates (Fig. 1B), dumbbell-shaped (Fig. 1C), and spherulite forms of calcium oxalate. The patterns of dumbbell forms do not have very sharp rings because low-intensity electron beams are used to delay the transformation to calcite. Dumbbell forms are polycrystalline aggregates with fibrous structures (Fig. 1D).

Dumbbell or sandglass shapes are not exclusive of whewellite. They have also been found in mineral substances of fibrillar structure like hematite (*4*), in some organic polymers (*5*), and in nonpolymeric substances (*6*).

Such aggregates may be produced through a branching or fanning mechanism during the growth of needles in length. The aggregates gradually approach the spherical form with cavities in the center which may remain noticeable in the final spherulite.

Needles, isolated or aggregated in fascicles with irregular morphology, do not have crystalline structures as perfect as those from plant cells (*7*). Dumbbell forms and spherulites with hollowed central areas such as those found in the urinary sediments are stages of the same well-defined development, peculiar to many fibrous crystals.

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Attine Fungus Gardens Contain Yeasts

Abstract. *Yeasts were detected in the fungus gardens of* Atta cephalotes *and* Acromyrmex octospinosus *by scanning electron microscopy and by microbiological techniques.*

Attine ants, by supplying organic substrate, maintain a symbiotic relationship with fungi which in turn serve both as a food source (*1*) and energy reserve for the colony. In this symbiosis the ants and their fungi have developed an efficient means of utilizing plant carbohydrates as an energy source (*1*). The fungus gardens of attine ants consist of organic matter loosely held together by fungus mycelium (*2*). The organisms accepted as true ant fungi are the Basidiomycetes *Agaricus gongylophora, Lepiota* sp., and *Auricularia*

sp. and a member of the Fungi Imperfecti *Tyridiomyces formicarum* (*3*). Our studies with the fungus gardens of the ants *Acromyrmex octospinosus* and *Atta cephalotes* (*4*) indicate that the filamentous fungi of these two species consist of sterile mycelia. No spores, sexual or asexual, have been demonstrated in direct connection with the mycelia. No clamp connections were observed on the mycelium of either garden. However, we have observed the presence of yeast or yeast-like forms on the substrate. We report the organization of the sterile mycelia, yeasts, and substrate material within the gardens of *A. octospinosus* and *A. cephalotes*.

Several developmental stages of the fungus gardens of the two colonies were studied by scanning electron microscopy and microbiological plating. Each of the gardens contains the following recognizable stages: (I) the portion containing newly initiated fungus growth on substrate, (II) the older and more established region of the garden, (III) the freshly discarded garden material, and (IV) refuse material containing later stages of discarded garden material, unacceptable substrate, and dead ants.

Portions of each of these stages were removed aseptically, weighted, and placed in given volumes of sterile water to give an initial concentration of 10^{-2} g of sample per milliliter of water. Subsequent dilutions were made, and 0.1-ml portions were streak-plated with sterile glass rods on petri dishes containing the appropriate mediums. Sabouraud's dextrose agar (*5*), rose bengal agar (*6*), and malt extract agar (*5*) were used to detect fungal growth. Trypticase soy agar (*5*) was used to detect bacterial growth. The colonies of yeasts, filamentous fungi, and bacteria were counted (*7*) and the number of organisms per gram of original sample was calculated in three repetitions of the above procedure.

Results from the platings (Table 1) show that the sterile mycelium is not the only microbial garden inhabitant. In gardens of both *Acromyrmex octospinosus* and *Atta cephalotes* numerous yeasts are present in the newer and older regions. No filamentous fungi other than the structural sterile mycelium are detected in either garden. In the *Atta* garden, bacteria are consistently detected; however, the yeasts are at least ten times more numerous. Examination by light microscopy indicates

Table 1. Average number of colonies observed per gram of material plated.

Sample	Yeasts	Filamentous fungi*	Bacteria
	Acromyrmex octospinosus		
New garden (I)	1.0×10^6	†	†
Old garden (II)	7.2×10^4	†	†
Discarded garden (III)	1.9×10^7	1.3×10^6	6.0×10^8
Refuse material (IV)	1.4×10^6	7.9×10^6	1.4×10^8
	Atta cephalotes		
New garden (I)	4.0×10^4	†	4.0×10^8
Old garden (II)	1.4×10^5	†	5.0×10^8
Discarded garden (III)	5.7×10^7	†	6.0×10^8
Refuse material (IV)	3.2×10^5	1.9×10^6	2.0×10^7

* Exclusive of the sterile mycelium in the garden. † No colonies observed on plates of 10^{-3} dilution.

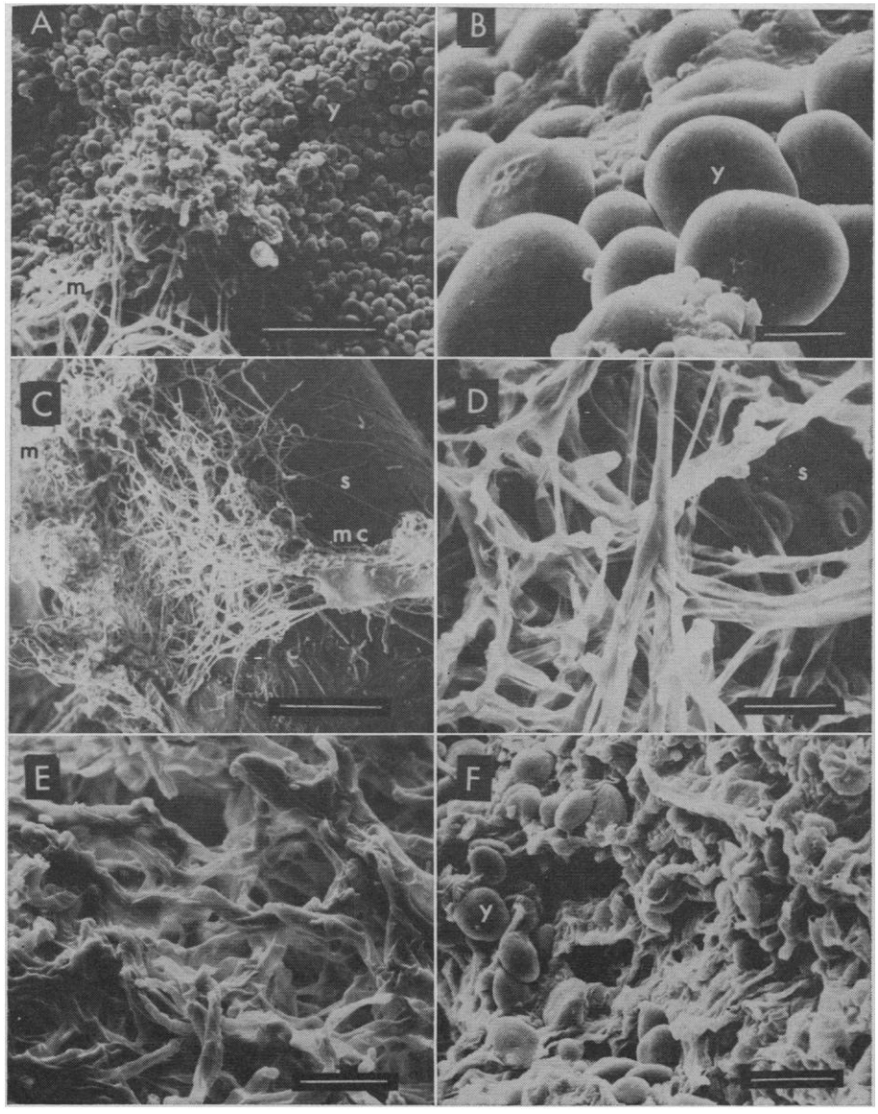

Fig. 1. Scanning electron micrographs of the fungus gardens cultured by the ants *Acromyrmex octospinosus* and *Atta cephalotes*. (A) Yeast-like cells growing on the organic substrate within the older, established region of the *A. octospinosus* garden. Sterile mycelium is present in adjacent areas. Scale is 100 μm. (B) A higher magnification of the yeast-like cells showing their position in the substrate material. Scale is 10 μm. (C) Older mycelial fragment which has been placed on the newly added substrate of *A. cephalotes* garden. Scale is 200 μm. (D) Hyphae of the sterile mycelium within the established garden region of *A. cephalotes*. The substrate material seen below is a leaf fragment. (E) Dense mycelial growth in an older portion of the established region of *A. cephalotes* garden. (F) The depleted garden substrate of *A. cephalotes* which lacks dense, actively growing mycelium. Yeast cells are present on the surface. Scale is 30 μm in D, E, and F. Abbreviations are *y*, yeast-like cells; *m*, mycelium; *mc*, mycelial clump; and *s*, substrate.

each yeast (3 to 25 μm) has a diameter from 2 to 50 times the diameter of any one bacterial cell (0.5 to 1.5 μm). Clearly, in comparison to the yeasts, the bacteria contribute little to the garden biomass. In contrast, both the freshly discarded material (III) and the older refuse (IV) yield a great number of bacteria and filamentous fungi. Yeasts are also present in larger numbers in these fractions than in the garden itself, but preliminary identification (*8*) shows that these species, for the most part, differ from those in the garden proper. Despite repeated contact of the ants with this discarded material, organisms found there do not actively grow in the garden.

Portions of the gardens and refuse at stages equivalent to those studied by plating were removed from the colony, immediately fixed on aluminum specimen holders, and coated with gold-palladium alloy in a vacuum evaporator. Microscopic examination confirmed the presence of numerous yeasts or yeast-like organisms in all parts of the gardens of *Atta cephalotes* and *Acromyrmex octospinosus*. As determined by light microscopy, the maximum range in cell diameter is 2 to 25 μm. The average cell diameter is 4.6 μm in gardens of *Acromyrmex* and 4.9 μm in those of *Atta*. Cells in the range of 2 to 12 μm outnumber the larger cells (15 to 25 μm) by at least 20 : 1 in gardens of *Acromyrmex* and 50 : 1 in those of *Atta*. Although some of the smaller cells could be bacteria, plate counts (Table 1) indicate no significant numbers of bacteria in the gardens.

The yeast cells often are scattered over the surface of the substrate, with mycelium present in adjacent areas (Fig. 1A). These cells are well embedded in the substrate (Fig. 1B). Large cells are present with numerous small cells surrounding them. In some cases the yeast cells are growing on the substrate directly underneath the mycelial mat. In areas of very dense mycelial growth the presence of yeasts on the substrate surface could not be determined. Occasionally the yeasts are present on the substrate with no mycelium closely associated. They were never observed attached to the fungus mycelium.

Stereoscan examination shows that newly added substrate lacks detectable yeast cells, which suggests that yeast cells in the garden are not introduced on the substrate but have originated from parent cells already present in the garden. We believe yeast or yeast-like cells are an integral part of the ant-fungus symbiosis contributing in some way to the relationship.

There is a distinct change in the morphology of the sterile mycelium with age of the garden. Newly added substrate shows mycelial clumps deposited by the ants with mycelial strands radiating outward from the planted piece (Fig. 1C). The substrate is rapidly overgrown by the mycelium. In more established areas of the gardens, individual hyphal forms can be distinguished and the surface of the substrate can be seen below (Fig. 1D). In older parts of the garden the mycelial growth is denser and more compact, individual hyphae are no longer readily distinguished, and the substrate surface is often not visible (Fig. 1E). The depleted substrate discarded by the ants contains only residual hyphal fragments (Fig. 1F).

Thus, the relation of the fungus-growing ants to their gardens is more complex than was suspected. Scanning electron microscopy has allowed the orientation and organization of this complex system to be studied in detail and has demonstrated a highly organized substructure, with a mycelial mat overlying a substrate that has yeast embedded in it. This structure undergoes marked changes in microbial composition with the age of the garden.

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4. The colonies of *Atta cephalotes and Acromyrmex octospinosus* studied are maintained under controlled laboratory conditions and were originally collected from Turrialba, Costa Rica.
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7. Counts were made on dilution plates containing 30 to 300 colonies of microorganisms.
8. Morphological and biochemical characteristics indicate two yeast species in *Acromyrmex octospinosus* garden and five species in the discard and refuse material. Only one of the latter five species is the same as a garden species. Two yeast species occur in the *Atta cephalotes* fungus garden, and the number of species in the discard and refuse material of this ant has not been determined.
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Carbon Dioxide–Fixation in Photosynthetic Green Sulfur Bacteria

Abstract. *The main products of carbon dioxide–fixation in washed suspensions of* Chlorobium thiosulfatophilum *are a polyglucose, α-ketoglutarate, and α-keto-β-methylvalerate. All of these can be formed by a mechanism involving the reductive carboxylic acid cycle. The reductive pentose phosphate cycle appears to play a quantitatively minor role in carbon dioxide–fixation under these conditions.*

It has been commonly believed that the reductive pentose phosphate cycle is the major CO_2-fixing mechanism in all autotrophic bacteria (*1*). Most of the enzymes of this cycle have been demonstrated in cell-free extracts of *Chlorobium thiosulfatophilum*, although some of the activities measured were low (*2*).

In 1966 Evans *et al.* (*3*) described two new ferredoxin-dependent, CO_2-fixing reactions in cell-free extracts of *C. thiosulfatophilum*, catalyzed by pyruvate synthase and α-ketoglutarate synthase, respectively:

$$\text{Acetyl-CoA} + CO_2 + 2\text{Fd} \cdot \text{e} + 2H^+ \rightarrow \text{pyruvate} + \text{CoA} + 2\text{Fd}$$

$$\text{Succinyl-CoA} + CO_2 + 2\text{Fd} \cdot \text{e} + 2H^+ \rightarrow \alpha\text{-ketoglutarate} + \text{CoA} + 2\text{Fd}$$

(CoA is coenzyme A; Fd, ferredoxin; and e, electron.) The authors suggested that CO_2-fixation in *C. thiosulfatophilum* occurs by a reversal of the tricarboxylic acid (TCA) cycle in which α-ketoglutarate synthase replaces α-ketoglutarate oxidase. The primary product of the cycle is acetate. They indicated that the function of the reversed TCA cycle might be synthesis of precursors of amino acids, lipids, and porphyrins, while the reductive pentose phosphate cycle is concerned with carbohydrate synthesis.

We now present more evidence that the reversed TCA cycle operates in intact cells of *C. thiosulfatophilum*. Furthermore, this cycle appears to be responsible for the major part of the