

VIRUS-LIKE PARTICLES IN A FIRE ANT, *SOLENOPSIS* SP.,
(HYMENOPTERA: FORMICIDAE) FROM BRAZIL

S. W. AVERY, D. P. JOUVENAZ, W. A. BANKS, AND D. W. ANTHONY

Insects Affecting Man Research Laboratory, Agr. Res. Serv.,
USDA, Gainesville, Florida 32604

ABSTRACT

Virus-like particles were found by electron microscopy in the adipose tissue of an undescribed species of *Solenopsis* collected in Brazil. The rod-shaped particles were enclosed by double membranes and were present in both the nuclei and cytoplasm of the infected cells.

Interest in the diseases of fire ants, *Solenopsis* spp., was recently renewed by reports that infections of *Thelohania* (Protozoa: Microsporida) had been found in several species of fire ants in South America (Allen and Buren 1974, Allen and Silveira-Guido 1974). Our surveys (unpublished data) also have revealed at least 3 additional microsporidia and several other pathogens associated with fire ants in Brazil and in the United States. However, the only indication of a possible viral infection in Formicidae was reported by Steiger et al. (1969) who found virus-like particles in nerve and fat body tissue of a wood ant, *Formica lugubris* Zett. In this paper, we report observations of virus-like particles in adult fire ants collected in Brazil. These ants were collected from 1 of several colonies that had microsporidian infections, and the virus-like particles were found while examining specimens prepared for ultrastructural studies of the protozoan.

MATERIALS AND METHODS

The host, an undescribed species of *Solenopsis* close to both *Solenopsis invicta* Buren and *Solenopsis saevissima* (F. Smith) (Buren, personal communication), was collected near the Rio Piquiri in Mato Grasso, Brazil. The collection did not include the queen, and there was very little brood. The ants were brought alive to the USDA, Insects Affecting Man Research Laboratory in Gainesville, Florida, and maintained under strict quarantine conditions for several weeks in plastic nests similar to those described by Wilson (1962). They were fed our standard ant diet of macerated laboratory-reared insects, pureed beef, raw eggs, and vitamins in agar.

Gasters from adult workers were examined by phase microscopy for the microsporidian infections. Infected gasters were dissected and fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer for 24 hr at 0°C and an additional 24 hr at 30°C. Then the specimens were postfixated in 1% OsO₄ in 0.1 M cacodylate buffer for 24 hr at 0°C, dehydrated in ethanol series, and embedded in Spurr's low viscosity medium (Spurr 1969). Thick sections were made for phase microscopy; thin sections made for electron microscopy were cut at 60-90 nm with diamond knives and were stained in uranyl acetate and lead citrate (Reynolds 1963). In addition, a 1% phosphotungstic acid at pH 6 was used to make the negative stains of suspensions from approximately 100 ants triturated in a tissue grinder and partially purified

by differential centrifugation. Photomicrographs were made with an electron microscope with an acceleration voltage of 75 kV. Measurements were made directly from the negatives, and the magnification of the electron microscope was calibrated with a carbon replica containing 2160 lines/mm.

RESULTS AND DISCUSSION

Rod-shaped particles were found in the adipose tissue of the ants in association with the microsporidium. Of 6 microsporidian-infected insects examined by electron microscopy, 2 were found to be infected with the particles. A third ant contained particles but was not infected with the microsporidium. A few particles were seen around muscle bundles, but none were found in the epidermis tissue or in the Malpighian tubules adjacent to infected fat body.

The particles were first seen in greatly hypertrophied nuclei of cells in the adipose tissue. The amount of condensed nucleoprotein in these nuclei was considerably less than in normal cells. Some of the cells contained a few particles in the cytoplasm. The second specimen had a higher concentration of the particles, and the nuclei and cytoplasm of these infected cells were completely disrupted (Fig. 1). Approximately 75% of the fat body cells of this specimen contained particles. There was no evidence of occlusion of the particles in a protein matrix in either the nucleus or the cytoplasm. The particles were seen occasionally, however, in tightly packed spheres some of which were bound by a membrane.

The particles were slightly tapered at both ends and were enclosed by double membranes (Fig. 1). The electron dense core was $193 \pm 25 \times 50 \pm 2.5$ nm, and the membrane was 15 nm thick. The whole particle was $252 \pm 12 \times 89 \pm 7$ nm. Particles in the cytoplasm were frequently curved and the membrane appeared to be pulled away from the core. Particles in the suspension from triturated ants were also tapered at each end, but the electron-dense core observed in the thin sections was not always evident in the negative stain preparations.

The virus-like particles described herein do not appear to resemble those described from a wood ant. Steiger et al. (1969) described 2 types of particles from apparently healthy ants: the first were hexagonal in shape and morphologically resembled the iridescent viruses, and the second was spherical in shape. These particles were found in the cytoplasm of nerve and fat body tissue. The particles we have found, however, appear to be morphologically similar to the nonoccluded rod-shaped viruses reported from fat body tissue of the Indian rhinoceros beetle, *Oryctes rhinoceros* (L.) (Huger 1966), and from the gut epithelium of the beetle *Gyrinus natator* L. (Gouranton 1972). Our limited observations also indicate that the development of the virus-like particles from *Solenopsis* may also be similar to those reported from the beetles.

Determination of symptoms in live ants has been inconclusive, and the infection rate could not be estimated. The hypertrophied nuclei and poor cellular integrity of the fat body could be seen in thick sections, and the colony had an increased mortality rate. Because of the dual infection, however, the mortality and cytopathology could not be attributed exclusively to either infection. Very recently similar particles have been found by elec-

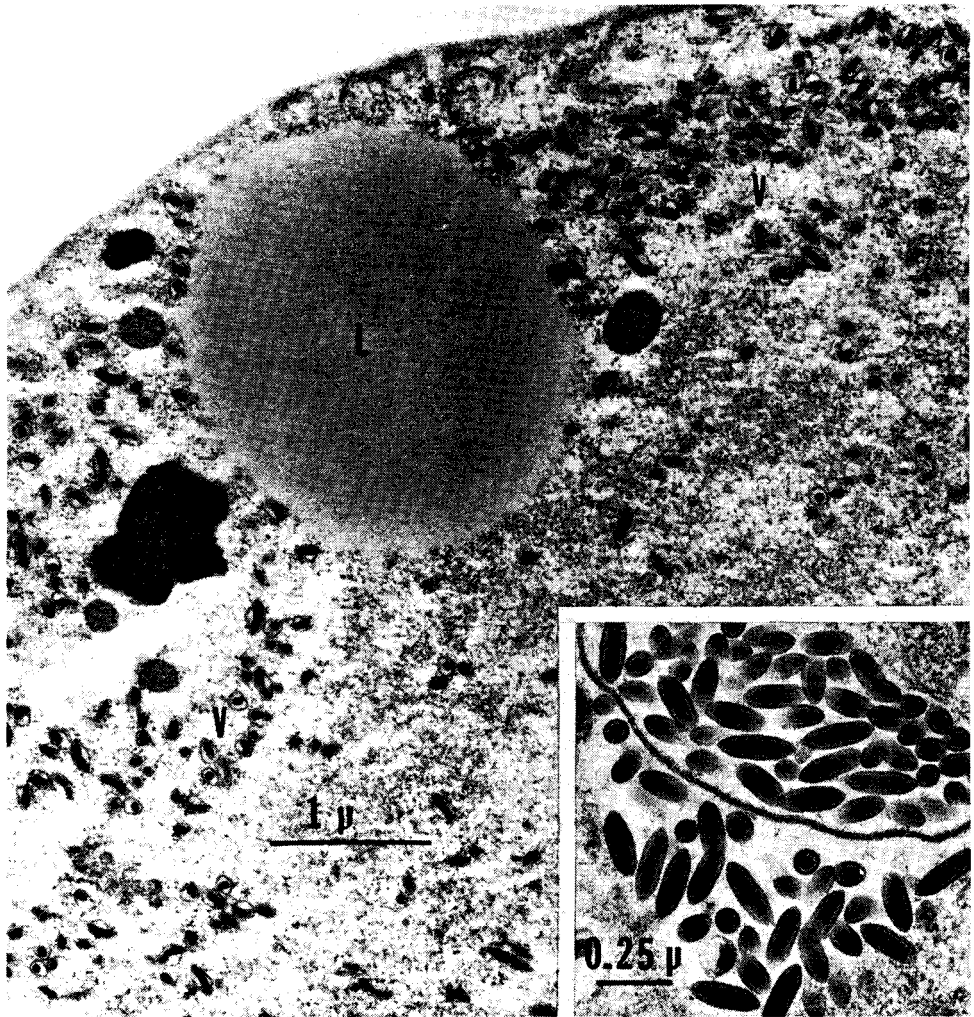


Fig. 1.—Virus-like particles in a fat body cell of *Solenopsis* sp. V, virus-like particles; L, lipid droplet.

tron microscopic examinations of adult workers of *Solenopsis geminata* (F.) collected in Alachua County, Fla. Biochemical and transmission studies will be done as additional material becomes available.

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