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CLASSIFICATION, IDENTIFICATION AND CHARACTERIZATION OF INSECT VIRUSES

E. W. KITAJIMA

Universidade de Brasília, Departamento de Biologia Celular, 70919 Brasília, DF, Brasil

Insect virology in Brazil

Biological control of pests is part of the so-called integrated pest management, together with chemical pesticides, agricultural practices. Among the natural enemies of the pests used for biocontrol, viruses seems to offer some additional advantages due to their high specificity. Certainly the use of viral bioinsecticides poses some difficulties: stability of the viruses; virulence; cost of production and application, etc. besides the need of a carefull preliminary tests to ascertain that the viruses will not constitute risk to other non-target organisms including man. There are several instances of well succeeded cases on the use of virus to control pests (Burgess, 1981), but in restricted circunstance. Probably the best example of wide scale application of a viral bioinsecticide is the use of a baculovirus to control the velvet soybean looper *(Anticarsia gemmatalis)* in Brazil. Up to 500,000 Ha is being treated with viral insecticide with excelente results (Moscardi, 1983). This well succeeded work stimulated other centers in Brazil to consider viruses as another alternative for pest control, and several promising programs have been established: sugar cane borer *(Diatreae saccharalis)* by a granulosis virus-GV (Pavan & Botelho, 1982); cassava caterpillar, *Erynnis ello*, by a another GV (Schmitt, 1984) (Fig. 6, insert); green caterpillar from cashew *(Eacles imperiales magnifica)* with a mixture of densonucleovirus (DNV) (Figs 1-3); and cytoplasmic polyhedrosis virus (CPV) (Figs 4, 5) (Chagas et al., 1982); fall armyworm *(Spodoptera frugiperda)* in corn, with GV (Fig. 6) (Valicente et al., 1988) and NPV (Fig. 7) (Valicente, personal communication); *Sibine* sp., a caterpillar from oil palm, with DNV and CPV (Lucchini et al., 1984); a NPV to control passion fruit caterpillar *Dione* spp. (Kitajima et al., 1986); NPV on gramineae caterpillar *Pseudaletia* sp. (Fig. 8) (Kitajima, personal communication); and more recently, an iridovirus in mole cricket, with a possible pathogenic effect on some termites (Fowler, 1988). For soybeans, besides the baculovirus to control velvet looper, Moscardi & Kastelic (1985) described also granulosis and nuclear polyhedrosis virus in populations of *Spodoptera frugiperda*.

Though in favorable cases, as NPV-, CPV-, entomopox- or iridovirus infections, external symptomatology and simple light microscopy might provide good indications not only for the viral nature, but for the virus type involved in a given epizooty, a precise diagnosis and identification of the virus require a more thoroughful biological, cytological, immunological and molecular works. Because there is not enough trained personnel for this kind of work, it has been suggested to centralize insect virus diagnostic researches in Brasília, using the facilities and researches of both CENARGEN/EMBRAPA and University of Brasília. Brasília's group are working in tight cooperation p. ex. with Moscardi's group from Londrina, to extend the works made with *Anticarsia* to other soybean pests; with Valicente's group at Sorghum and Corn Research Center (Sete Lagoas, MG), to complete the characterization of NPV found in *S. frugiperda*.

Identification and characterization of insect viruses

There are excellent review articles on the subject such as of Smith (1976), Maramorosch (1977), Payne & Kelly (1981), Entwistle (1987), besides the detailed catalog organized by Martignoni & Iwai (1986). We will briefly outline the basic procedures for insect virus identification and update their taxonomy and comment on latent and symbiotic relationship between some viruses and their hosts.

Symptoms – Lack of apetite, changes in behavior, diarrhea, vomit, color alterations, slow movements, reduced rate of growth, large mortality, hanging dead larvae, iridescence, deformations, etc. are symptoms observed in

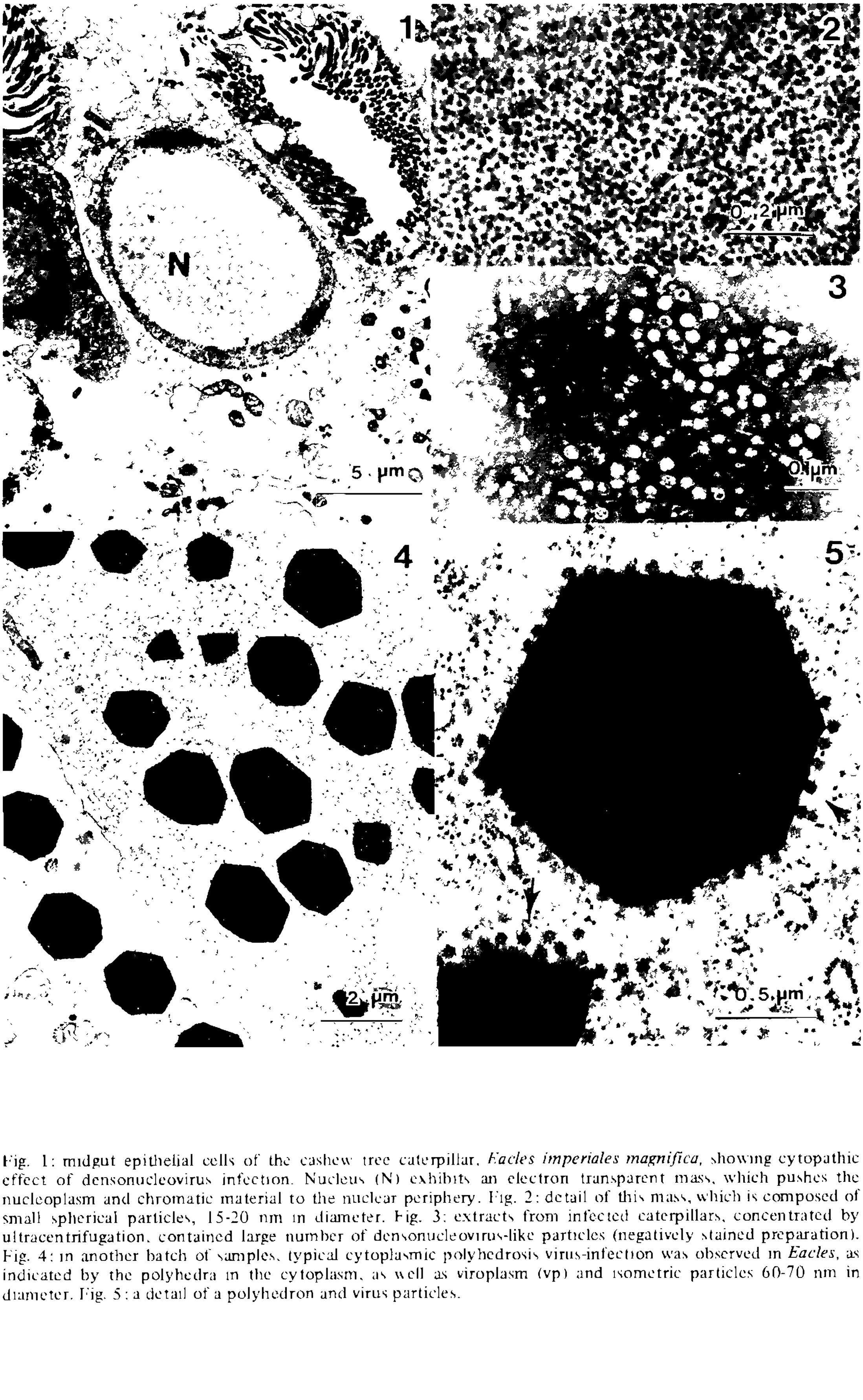
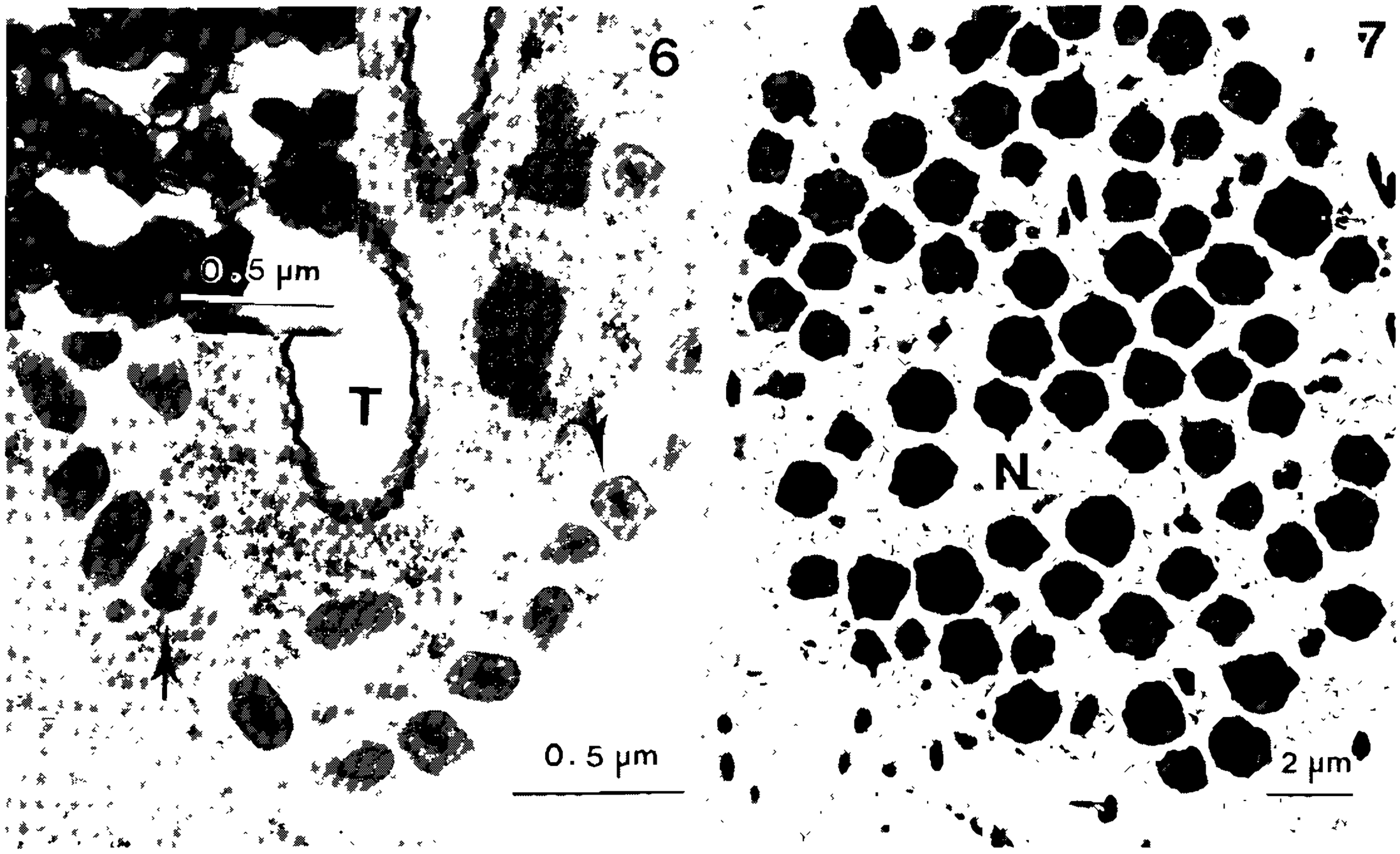


Fig. 1: midgut epithelial cells of the cashew tree caterpillar, *Eacles imperiales magnifica*, showing cytopathic effect of densonucleovirus infection. Nucleus (N) exhibits an electron transparent mass, which pushes the nucleoplasm and chromatic material to the nuclear periphery. Fig. 2: detail of this mass, which is composed of small spherical particles, 15-20 nm in diameter. Fig. 3: extracts from infected caterpillars, concentrated by ultracentrifugation, contained large number of densonucleovirus-like particles (negatively stained preparation). Fig. 4: in another batch of samples, typical cytoplasmic polyhedrosis virus-infection was observed in *Eacles*, as indicated by the polyhedra in the cytoplasm, as well as viroplasm (vp) and isometric particles 60-70 nm in diameter. Fig. 5: a detail of a polyhedron and virus particles.



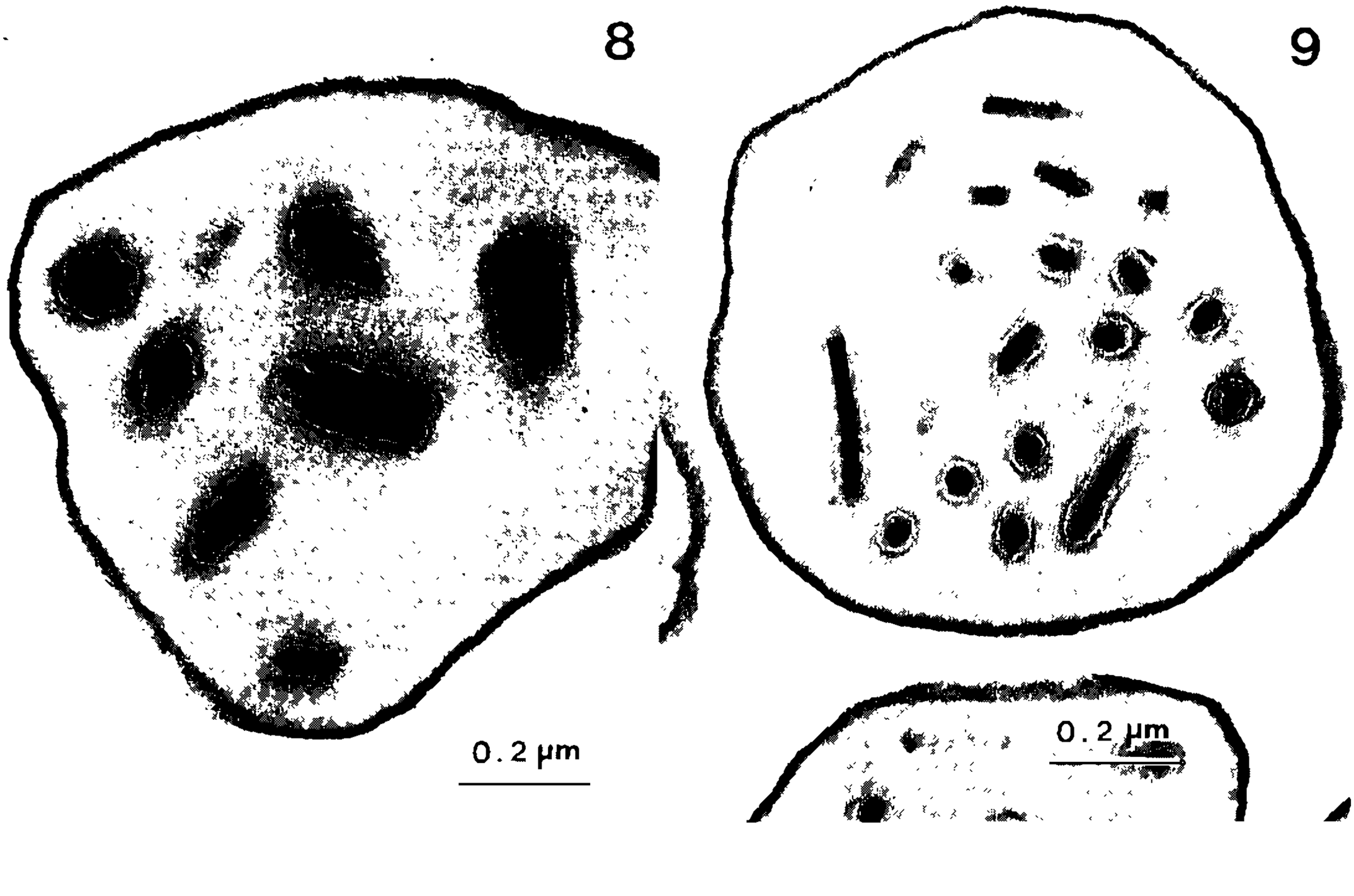


Fig. 6: tracheocyte of fall armyworm, *Spodoptera frugiperda* infected by a granulosis virus, exhibiting a large number of granules scattered in the cytoplasm. Insert shows a negatively stained haemolymph from GV-infected *Erynnis ello*, the cassava caterpillar. Fig. 7: fatbody cell from MENPV-infected *S. frugiperda*. Nucleus is totally taken by polyhedra. Fig. 8: details of a nuclear polyhedron of NPV of multienveloped type (from NPV-infected *Pseudaletia* sp.), in which several nucleocapsids are surrounded by a common membrane. Fig. 9: single enveloped type, where each nucleocapsid is individually surrounded by the enveloping membrane (from NPV-infected *Heliothis armigera*, from Indonesia).

insects, especially in a large number of individuals in a given population, which might suggest an epizooty of viral etiology. However, most of these symptoms might be caused by other pathogens, therefore they alone usually are not enough to confirm viral diseases. Usually more elaborate tests are required for that purpose. It should be mentioned that some anatomical changes visible by naked eye or binoculars, in dissected insects can also provide some clues for virus involvement in the disease.

Light microscopy -- Examination of extracts from dead individuals or tissues from infected insects might offer in favorable cases indications for viral etiology. Nuclear and cytoplasmic polyhedrosis, spheroids, granules and iridovirus crystals are detectable in fresh tissue preparations and sometimes even in unpreserved samples.

However, for many viruses, or in individuals in early phase of infection, light microscopy might be of little help.

Electron microscopy – Whenever available, it is a powerful tool to provide indications for a viral etiology in a given insect pathology. Detection of virus-like particles in extracts, or observation of virions, inclusions and other cytopathic effects consist in strong evidence for virus etiology, and furthermore, depending on the morphology of the observed particles and type of cytopathic effects, it is possible to pinpoint the taxonomic situation of a given virus. Also, the method easily can demonstrate mixed infections. However, since latent infection in insects is quite common, one must be careful to accept quickly the electron microscope evidences. On the other hand, one cannot forget that the presence of viruses is not an indication of disease at all; in cases as that of polydna-viruses in parasitoid wasps, they have a symbiotic relationship and actually, essencial for the life cycle of the wasp.

Biological tests – This is an obvious test to conduct if healthy individuals of the same insect species are easily available. A millipore filter suspension of diseased insect may be either injected or served with food, p. ex.

Serology Immunologic procedures are convenient not only to detect antigens for a given virus in the diseased insects, but also to define the taxonomic position of different, but related viruses. For refined works, however, antisera must be of high specificity and good titer. In the cases of viruses which induce protective protein layers (NPV, CPV, entomopox), it is possible to use antisera a for such a nonconstitutive antigens. Tests can be performed by conventional microprecipitin or double gel diffusion, or by more sensitive methods as ELISA or radioimmunoassay. Use of monoclonal antibodies might enhance small differences between closely related viruses.

Molecular biology approaches – Isolation of protein and nucleic acid components from the viruses, and their characterization (size, molecular weight, S value, density, number of bases or aminoacids, primarily sequences, etc.) are now quite easily made, especially by electrophoretic and/or chromatographic procedures. The quick advance of the molecular techniques, namely genome mapping; isolation and cloning of viral genome or parts of it; DNA analyses by restriction endonucleases (Vlak, 1982); molecular probes, etc. are refining the knowledge of the viral molecules. Some important by-product of such studies was the realization that baculovirus genome might be an efficient expression vector for several valuable messages (Summers & Smith, 1985; Luckow & Summers, 1988).

Latent and "symbiotic" insect viruses – Some pathogenic viruses may occur in insect in latent form, which can be stimulated to disease-causing condition by several means (Smith, 1976). On the other hand, there have been several descriptions of virus-like particles of different types in apparently healthy insects (e. g.: leafhopper – Herold & Munz, 1967; thrips – Paliwal, 1979; beetle – Kim & Kitajima, 1984; aphids – Kitajima, 1976; diptera – Rae & Green, 1968; etc.) (Figs 10-12). Some of them are transmitted vertically (Kitajima et al., 1985). Their characterization is much more difficult, since biological tests are more difficult to perform and most descriptions are limited to their detection in the tissues of the host, where they are accidentally observed; their viral nature has yet to be proved.

One group however has been well characterized, and they are the so-called polydnavirus, somewhat similar to baculovirus, but with segmented genome. These viruses apparently multiply actively only in the calyx epithelium of some parasitoid wasps, being part of the

Fig. 10: viruslike particles in apparently healthy insects, respectively, REO type particles in the ovary of Mexican bean beetle (*Epilachna varivestis*; Coleoptera/Cocheinellidae). Fig. 11: picornavirus type particles between infolding basal membranes of the salivar gland of the green peach aphid, *Myzus persicae* (Homoptera: Aphididae). Fig. 12: non-occluded baculoviruslike particles in the nucleus of midgut epithelial cells of *Diabrotica speciosa* (Colleoptera/Chrysomelid).

calyx fluid after their release from the cell. There are strong evidences that these viruses are required to counteract the immune system of the parasitoid's host (usually lepidoptera larva), although they do not multiply in it, to assure the hatching of the laid egg (Krell et al., 1982). Therefore these polydnaviruses exhibit a simbiotic relationship with these parasitoid wasps.

INSECT VIRUS TAXONOMY

The following groups and families of insect viruses are those approved by the International Committee of Virus Taxonomy (Matthews, 1982).

A. DNA viruses, isometrics, without envelope
1. Family PARVOVIRIDAE (ssDNA, monopartite genome)
Genus *DENSOVIRUS*
2. Family IRIDOVIRIDAE (dsDNA, monopartite)
Genus *IRIDOVIRUS* (smaller)
Genus *CHLORIRIDOVIRUS* (large)

B. DNA viruses, helicoidal, enveloped
3. Family BACULOVIRIDAE (dsDNA, monopartite)
Genus *BACULOVIRUS*
Nuclear polyhedrosis; Granulosis; non-occluded
4. Family POLYDNAVIRIDAE (dsDNA, multipartite)
Genus *POLYDNAVIRUS*
Fusiform nucleocapsid; rodshaed nucleocapsid

C. DNA viruses, complex, enveloped
5. POXVIRIDAE (dsDNA, monopartite)
Group ENTOMOPOXVIRINAE
Genus *ENTOMOPOX*
Subgenus A, B, C
6. Genus *ASCOVIRUS* (dsDNA, monopartite) (Frederici et al., 1983)

D. RNA viruses, isometric, non-enveloped
7. Family PICORNAVIRIDAE (ssRNA, monopartite)
Genus *ENTEROVIRUS*
8. Family CALICIVIRIDAE (ssRNA, monopartite)
Genus *CALICIVIRUS*
9. Family NODAVIRIDAE (ssRNA, bipartite)
Genus *NODAVIRUS*
10. Family REOVIRIDAE (dsRNA, polypartite)
Genus *CYTOPLASMIC POLYHEDROSIS*
11. Family BIRNAVIRIDAE (ssRNA, bipartite)
12. Small isometric RNA viruses, unclassified: Arkansas bee virus, bee acute paralysis, bee chronic paralysis, bee chronic paralysis associated, bee virus X, bee virus Y, black queen cell virus, cloudy wing virus, crystalline array virus, kelp fly virus, sacbrood virus.
13. Small ovoid RNA virus
bee chronic paralysis virus, Drosophila RS

E. RNA virus, helicoidal, enveloped
14. Family RHABDOVIRIDAE (ssRNA, monopartite)
Genus *SIGMAVIRUS*

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