VIRUSES IN LABORATORY-REARED CACTUS MOTH, *CACTOBLASTIS CACTORUM* (LEPIDOPTERA: PYRALIDAE)

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The cactus moth, *Cactoblastis cactorum* (Lepidoptera: Pyralidae: Phycitinae), is a non-native species threatening a variety of native cacti, particularly endangered species of *Opuntia* (Zimmerman et al. 2001), on the coast of the Gulf of Mexico. *Cactoblastis cactorum* populations have expanded from Florida northward along the Atlantic coast as far as Charleston, SC, and westward along the Gulf of Mexico to Dauphin Island, south of Mobile, AL. It is feared that further movement to the west will allow *C. cactorum* to enter the US desert Southwest and Mexico, particularly the latter. Numerous cactus species, especially those of the genera *Opuntia* and *Nopalea*, are native to the U.S. and Mexico. Local economies based on agricultural and horticultural uses of cacti could be devastated by *C. cactorum* (Vigueras & Portillo 2001).

A bi-national control program between the US and Mexico is being developed, utilizing the sterile insect technique (SIT). In the SIT program, newly emerged moths are irradiated with a ^{60}Co source and released to mate with wild individuals. The radiation dose completely sterilizes the females and partially sterilizes the males. When irradiated males mate with wild females, the F1 progeny of these matings are sterile. In order for the SIT program to succeed, large numbers of moths must be reared from egg to adult on artificial diet in a quarantined rearing facility (Carpenter et al. 2001). Irradiated insects must then be released in large numbers at the leading edge of the invasive population and at times which coincide with the presence of wild individuals available for mating. Mortality from disease in the rearing colony disrupts the SIT program by reducing the numbers of insects available for release.

The *C. cactorum* colonies at the insect rearing facility at the Crop Protection and Management Research Laboratory, USDA-ARS, Tifton, GA, were established in Mar and Dec, 2001, from larvae and eggs sticks collected from the wild in the U.S. and South Africa, respectively. The US colony was reared on *Opuntia ficus-indica* cladodes, whereas the South African colony was reared on artificial diet based on white kidney beans. Occasional infection and mortality due to microsporidia were detected by light microscopy in 2004. However, by late 2004, we noted mortality of large, late instars in which no microsporidia or other disease agents could be detected by light microscopy. In later generations, progressively younger and smaller larvae also were affected. By 2004, mortality levels greater than 50% occurred in most rearing containers of insects on bean diet, in spite of sanitation measures (i.e., cleaning all rearing containers with 1% sodium hypochlorite solution) that were in place. Affected larvae crawled out of the diet, climbed up the sides of the rearing container, died, and turned black. Dead larvae were frequently attached to the container wall by a small portion of the midbody, with the anterior and posterior ends hanging free. Disease problems were limited almost exclusively to the South African colony reared on kidney bean diet.

Larvae were submitted to the University of Georgia's Veterinary Diagnostic and Investigational Laboratory, Tifton, GA, for detection and identification of possible viruses by negative stain transmission electron microscopy (NSTEM) (Styer et al. 1987). Of 123 normal and diseased larvae examined by NSTEM, 40.6% (50/123) were positive for an icosahedral virus (IV) approximately 24 nm in diameter (range, 19-34 nm) (Fig. 1), 0.8% (1/123) were positive for cytoplasmic polyhedrosis virus (CPV), and 58.6% (72/123) were negative for viruses. The 24 nm IV was much more prevalent in the South African colony than in the US colony, although there was no correlation between morbidity or mortality and the presence of the 24 nm IV. The single larva positive for CPV was from the US colony on bean diet. In NSTEM preparations, the 24 nm IV appeared as scattered individual particles, small to medium aggregates and small aggregates that were complexed with granular material and/or short membrane strands or vesicles (Fig. 1) and was frequently present in massive numbers. Aggregates and complexes were more common in preparations from stunted larvae than from large, moribund, or dead larvae. The size range of the 24 nm IV (19-34 nm) suggests that it may belong to the Picornaviridae (van Regenmortel et al. 2000).

Because the 24 nm IV was initially observed in larvae of the South African colony only, a brief study was performed to determine whether diet had any effect on larval mortality or the presence of the virus. Thirty larvae from the South African colony raised on bean diet (10 dead and 20 alive) were examined by NSTEM, as were 32 larvae from the US colony raised on bean diet (11 dead and 21 alive) and 22 larvae (all alive) from the US colony on cladodes. All of the South African larvae raised on bean diet were positive for the 24 nm IV,

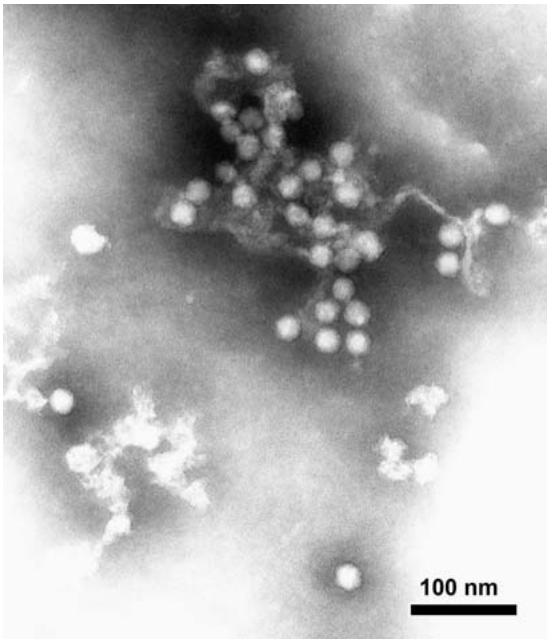

Fig. 1. Electron micrograph of negatively stained, 24nm diameter, icosahedral viruses from a larva of *Cactoblastis cactorum*. The virions occur singly and as small aggregates complexed with membranous material. Bar, 100 nm.

as were 14/32 (44%) of the US larvae on bean diet. No viruses were detected in the 22 larvae from the US colony reared on cladodes. Incidence of the 24 nm IV differed significantly in larvae reared on bean diet vs those reared on cladodes (χ^2 =13.92, $df = 1, P = 0.0002$). Although diet and perhaps the different microenvironments created by the diet did relate to the expression of an apparently latent virus infection among US larvae, there was no correlation between mortality and the presence of the 24 nm IV among larvae raised on the kidney bean diet.

In an effort to correlate infection by the small IV with pathological changes and to check for the presence of viruses not detected by NSTEM, tissues from 23 of the 123 larvae examined by NSTEM were prepared for ultrastructural examination (UTEM). Tissues from one half of each of these larvae were dissected in McDowell and Trump's modified Karnovsky's fixative and processed for ultrastructural examination by a microwave-assisted method similar to that detailed by Giberson (2001). Tissues examined included midgut, fat body, hypodermis, Malpighian tubules, nerves, heart, pericardium, blood cells, and silk glands. Larvae selected for UTEM included apparently normal, healthy larvae and moribund, stunted and darkly pigmented larvae, as well as larvae that were positive for CPV, or the 24 nm IV, or were virus negative by NSTEM.

Light microscopic examination of 0.5 µm thick sections stained with toluidine blue-O failed to identify any pathological changes that were correlated with the presence of the 24 nm IV observed by NSTEM. For example, midgut epithelial cells of both virus positive and virus negative larvae were commonly vacuolated and blebbed into the midgut lumen and their cytoplasm frequently contained large numbers of irregular cytoplasmic inclusion bodies which proved to be small to large, often complex lysosomes (Fig. 2A and 2C). Cytoplasmic polyhedra and CPV virions (Fig. 2A and 2B) were observed in midgut epithelial cells of 3 larvae from the US colony, including the larva that was CPV-positive by NSTEM. By both light microscopy and UTEM, CPV polyhedra were intensely stained to almost unstained structures approximately 0.5 µm in diameter (range, 0.2-1.2 µm) that were scattered within a lightly stained virogenic stromata. On UTEM, numerous CPV virions were present within and along the margins of the developing polyhedra. Tamashiro & Huang (1963) described a cytoplasmic polyhedrosis virus with polyhedra 262-880 nm in diameter from *C. cactorum* from Hawaii. Identification of the CPV in the Tifton *C. cactorum* colonies was beyond the scope of this study.

An unenveloped icosahedral virus with a maximum diameter of 50 nm was encountered in a large number of otherwise unremarkable nuclei of midgut epithelial cells of four larvae from the US colony on diet (4/23 or 17%) (Fig. 2D). These larvae had been collected from a single rearing cage on the same date and were quite dissimilar in appearance (e.g., a small, stunted, normally pigmented larva; a large, presumably healthy larva; a small, moribund, black-colored larva; and a large, recently dead, black-colored larva). The 24 nm IV was not detected by UTEM, even in tissues of larvae in which large numbers of virions were present in NSTEM preparations.

The low incidence of viruses and the absence of significant mortality in the cladode-reared *Cactoblastis* colony suggests either that rearing the larvae on artificial diet introduces stress that allows expression of disease or that the cladodes, the natural food, confer protection against expression of viral disease. In either case, it is clear that the food plays a major role in expression of at least the 24 nm icosahedral virus in *Cactoblastis* and that the development of a satisfactory artificial diet that suppresses it is critical to mass rearing of this insect.

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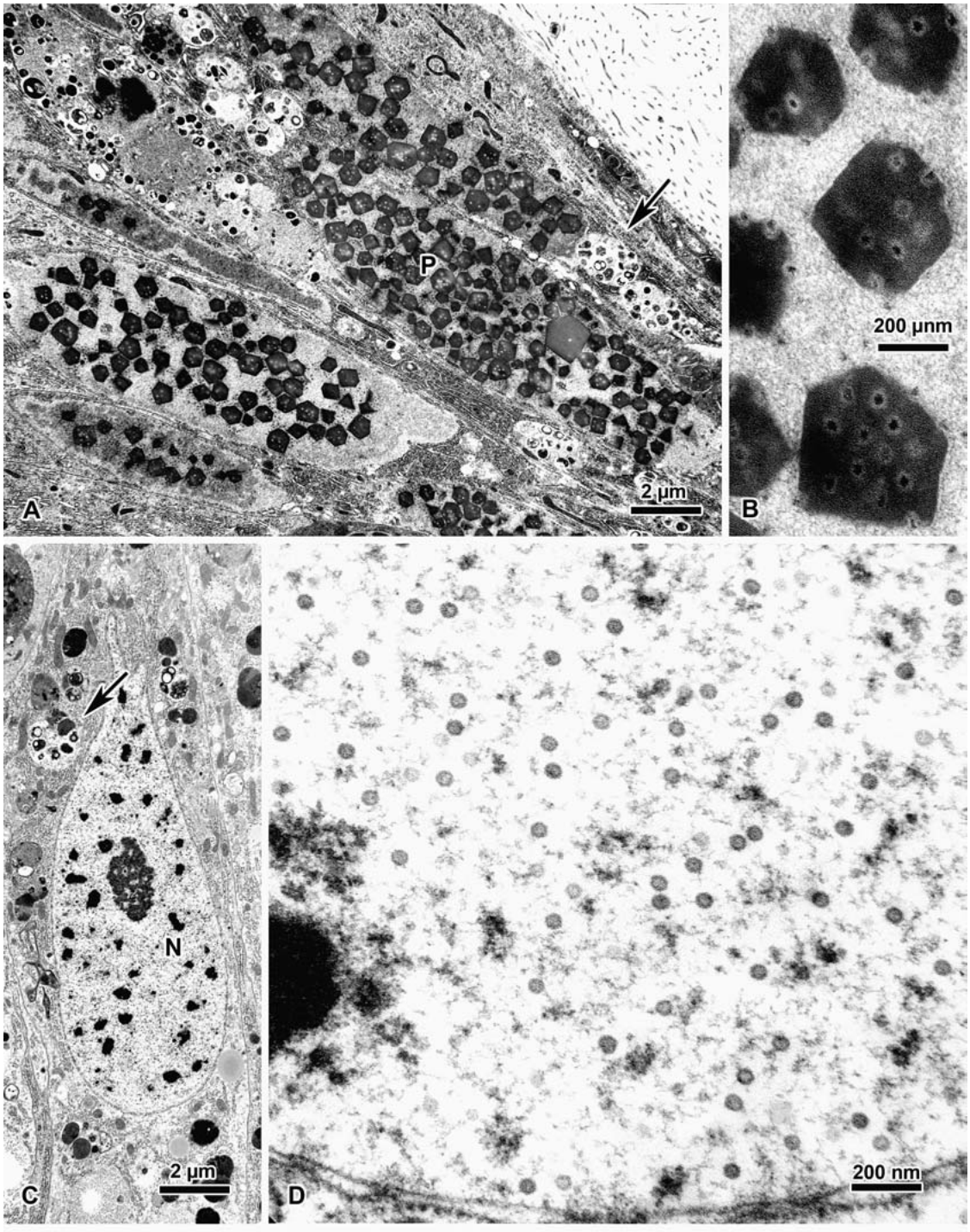

Fig. 2. Electron micrographs of the cytoplasmic polyhedrosis virus (CPV) and the approximately 50 nm diameter intranuclear virus that were observed in midgut epithelial cells of larvae of *Cactoblastis cactorum*. A. CPV-infected cells. P, CPV polyhedra embedded in the fibrogranular virogenic stromata; L, midgut lumen; arrow, lysosomes. Bar, 2 µm. B. CPV polyhedra with occluded virions. Bar, 200 nm. C. A cell infected with the 50 nm icosahedral virus. N, apparently normal nucleus; arrow, lysosomes. Bar, 2 µm. D. A portion of the nucleus in C showing scattered 50 nm diameter icosahedral viruses. Bar, 200 nm.

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