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Infection of the Diaprepes Root Weevil, *Diaprepes abbreviatus* (Coleoptera: Curculionidae) by an Iridovirus

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Introduction

The Diaprepes root weevil (DRW), *Diaprepes abbreviatus* (L.), is now regarded as a principal threat to sustainability of the citrus industry in Florida. Since its introduction into Florida in 1964, the weevil has spread throughout the citrus producing areas of peninsular Florida. Adult females oviposit on leaves. Neonate larvae fall to the ground and burrow into the soil where they feed on progressively larger roots as the larvae grow. Tree decline occurs over time as primary roots are damaged and infected by root rot pathogens. Tree death results when the structural root or root crown is girdled. Feeding damage by adults on leaves is considered secondary. Few effective and environmentally appropriate control options are available to growers for controlling such subterranean pests. We undertook a search for pathogens of DRW for use in generating new management strategies for control of DRW. We report the first known viral pathogen of DRW and discuss its potential for controlling this pest.

Methods

Insects were obtained from a colony at the U.S. Horticultural Laboratory, Ft. Pierce, FL. IIV-6 was obtained from Dr. J. Kalmakoff, University of Otago, Dunedin, New Zealand. IIV-6 was amplified in third instar *Tricoplusia ni*, purified, sterilized, and stored at - 40°C.

DRW were exposed by stabbing, microinjection, and *per os*. Controls were inoculated with sterile water. Stabbing was with insect pins dipped in sterile water or in purified IIV-6. Microinjections used a glass 25 µl syringe and a 30G1/2 gauge needle. Weevils inoculated *per os* were fed a 20 % sucrose solution or purified virus in a 20% sucrose solution over 24 h. To verify infectivity, inoculum was made from homogenates of IIV-6 infected larvae. The homogenate was centrifuged and the pellet was processed for DNA extraction. The supernatant was centrifuged and sterilized through a 0.45 µm membrane syringe filter. Larvae were inoculated by injection with 2 µl of the sterile filtrate.

Inoculated DRW were dehydrated, fixed, sectioned and viewed by transmission electron microscopy using standard techniques.

Results and Discussion

- Adult and larval DRW were infected with IIV-6 by 3 inoculation methods: puncture, micro-injection, and *per os* (Fig. 1);
- IIV-6-infected female DRW produced IIV-6-infected eggs (vertical transmission) (Fig.1);
- Horizontal transmission between adults has also been demonstrated (Fig. 2);
- Infected DRW showed virus in cells lining the tracheae and in muscle and nerve tissues (Figs. 2, 4, 5);
- The virus was 120 - 130 nm and icosahedral, as reported for IIV-6;
- PCR analysis detected viral DNA in virus-exposed individuals. We developed a primer set to a highly conserved region of the capsid protein gene from several insect Iridoviruses posted in GenBank. Primer products generated were sequenced. Sequence analysis showed amplimers were 99% identical to Iridescent virus type 6 (IIV-6) major capsid protein gene. The high homology confirmed presence of IIV-6 infection in exposed weevils. Filter-sterilized supernatant from homogenates made from IIV-6-infected larvae resulted in IIV-6 infections when injected into healthy larvae (Fig. 3).
- **This is the first report of a viral infection in DRW. Transovarial passage and *per os* infections indicate potential for development of IIV-6 as an agent for**

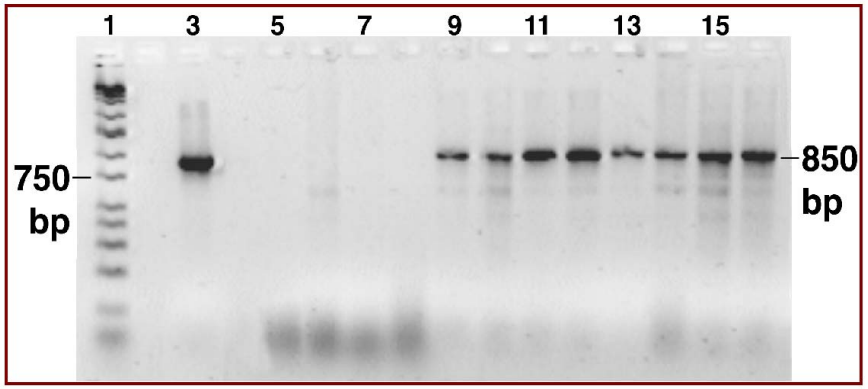


Figure 1. Gel of amplified DNA from *D. abbreviatus*.

Lane	Sample
1	Ladder wide-range DNA marker (50 - 10,000 bp).
2, 4, 5	Blanks (2 & 4) and water control (5).
3	Positive control (purified IIV-6).
6, 7	Adult (6) and larva (7) injected with 2 µl water.
8	Healthy egg control.
9, 10	Adult (9) and larva (10) inoculated by insect pin.
11, 12	Adult (11) and larva (12) injected with 2 µl homogenate from larva with patent IIV-6 infection.
13, 14	Adult (13) and larva (14) inoculated *per os* with purified IIV-6 in 20% sucrose.
15, 16	Eggs from adult females inoculated by injection (15) or *per os* (16).

biological control of DRW.

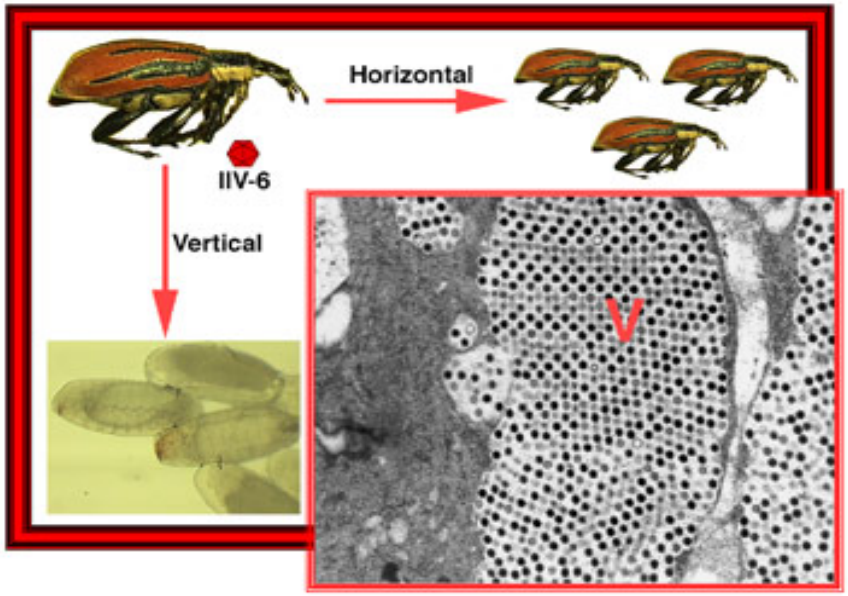


Figure 2. Vertical and horizontal transmission of IIV-6 have been demonstrated. **Inset:** Transmission electron micrograph of an array of IIV-6 particles (V) in a muscle cell of *D. abbreviatus*.

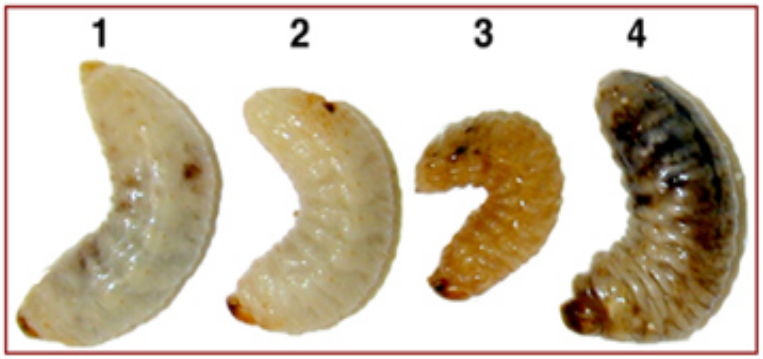
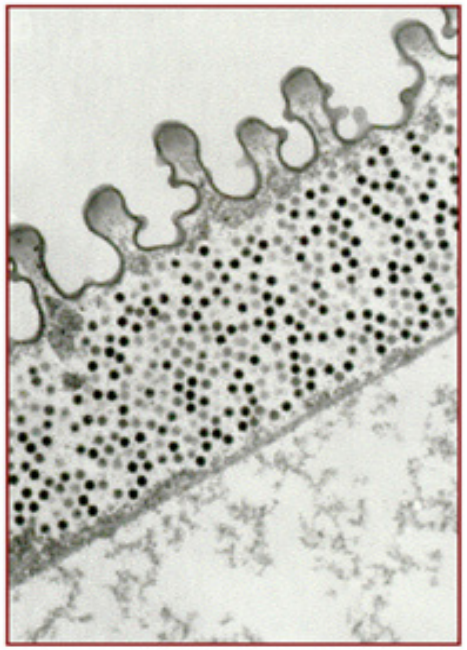


Figure 3. 1) Control (injected with water); 2) Covert IIV-6 infection; 3) Early stage of patent IIV-6 infection; 4) Late stage of patent IIV-6 infection.



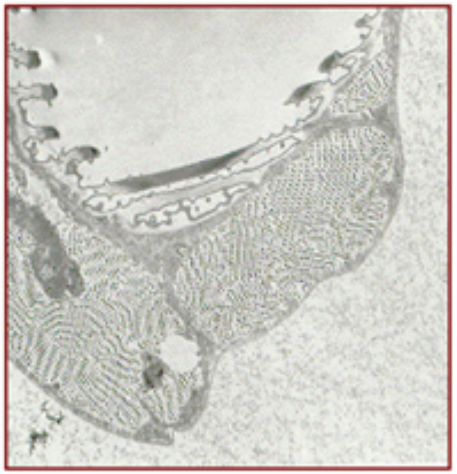


Figure 5. TEM of tracheal epithelial cells of *D. abbreviatus* infected with IIV-6.

Figure 4. TEM of tracheal epithelial cells of *D. abbreviatus* infected with IIV-6.

References

Devauchelle, G., Attias, J., Monnier, C., Barray, S., Cerutti, M., Guerillon, J., and N. Orange-Blange 1985. *Chilo* iridescent virus. Current Topics in Microbiology and Immunology 116:38-48.

Hunter-Fujita, F. R., Entwistle, P. F., Evans, H. F., and N. E. Crook. 1998. Characteristics of insect pathogenic viruses. *In* "Insect Viruses and Pest Management". pp. 1-26. John Wiley and Sons. New York.

Kelley, D. C. and J. S. Robertson. 1993. Icosahedral cytoplasmic deoxyriboviruses. J. Gen. Virol. 20: 17-41.

Lapointe, S. L. and J. P. Shapiro. 1999. Effect of soil moisture on development of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Fla. Entomol. 82: 291-299.

Marina, C. F., Arrendondo-Jiménez, J. I., Castillo, A., and T. Williams. 1999. Sublethal effects of iridovirus disease in a mosquito. Oecologia 119, 383-388.

Williams, T. 1996. The Iridoviruses. Adv. Virus Res. 46: 345-413.

Woodruff, R. E. 1964. A Puerto Rican weevil new to the United States (Coleoptera: Curculionidae). Fla. Dept. Agric., Div. Plant Industry Entomol. Circ. No. 30, 2 pp.