Virus and Virus-Like Particles Found in Southern Pine Beetle Adults in Mississippi and Georgia

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Introduction

The Southern Pine Beetle (SPB), *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae), is the most destructive insect pest of pine forests in the southeastern United States and in parts of Mexico and Central America (Payne, 1980). It usually is endemic, but appears periodically in epidemic outbreaks (Blackman, 1949).

During 1995, it was estimated that in Mississippi more than $15 million in timber was lost to the SPB (Nebeker, unpublished), and losses in South Carolina were estimated at more than $100 million (Mike Remion, personal

communication). From 1960-1990, U.S. timber losses to the SPB have been estimated at $901.8 million (Price et al., 1990).

Current methods of minimizing SPB losses, according to Lorio (1980), include these: (1) salvage, (2) cut and leave, (3) fell and spray with insecticides, or (4) pile and burn. Silvicultural treatments, such as thinning, are recommended to prevent or reduce the impact of SPB should an attack occur (Nebeker et al., 1985). Little attention has been given to biological control methods, which may eventually eliminate or complement conventional methods.

The use of biological agents, such as viruses, to regulate SPB populations will require much research before practical applications can be made. Dissemination of self-propagating pathogens of SPB (viruses, bacteria, fungi, protozoa, or nematodes) would provide the first step toward the use of naturally occurring host-specific microorganisms in biological control and would reduce pesticide pollution with subsequent benefits to man and the environment.

There is a general lack of understanding of the interrelationships of insect parasites, predators, and pathogens with the SPB populations. Berisford (1980) presented a general review of the natural enemies of the SPB including pathogens. Sikorowski et al. (1979) found that pathogens of the SPB may play an important role in regulating populations of the SPB. Pabst and Sikorowski (1980) found that under laboratory conditions, three entomophagous fungi were pathogenic to SPB larvae, with *Beuveria bassiana* [(Balsamo); Deuteromycotina] being most virulent.

Thus far, viruses of SPB are unknown and have not been investigated in previous studies. A review of the literature revealed that viruses have been found in coleopterous insects. *Oryctes* baculovirus, nonoccluded virus (subgenus C), was described by Huger (1966, 1972) as a viralpathogen of the larvae of the Indian rhinoceros beetle, *Oryctes rhinoceros* (L.), Dynastidae.

Several entomopox viruses (Entomopoxvirinae) affecting coleopterous insects have been reported from Scarabaeidae by Vago et al. (1968); Bergoin et al. (1968); Goodwin and Filshie (1969); and Goodwin et al. (1991). Entomopox viruses affecting coleopterous insects have been reported from Europe, North and South America, Madagascar, and Australia.

Thomas and Gouranton (1975) studied development of virus-like particles in the crystal-containing nuclei of the midgut cells of *Tenebrio molitor* (L.), Tenebrionidae. Arnold and Barson (1977) reported occurrence of virus-like particles in midgut epithelial cells of the elm bark beetle (*Scolytus scolytus* [F.], Scolytidae). Longworth and Archibald (1975) isolated a virus of black beetle *Heteronychus arator*, F., Scarabaeidae) and Dearing et al. (1980) isolated a small RNA virus from the grass grub (*Costelytra zealandica* [White], Scarabaeidae) (L.).

Lomer (1987) reported that adults of *Strategus aloeus* (L.), Scarabaeidae were capable of supporting *Oryctes* baculovirus similar to that produced in *O. rhinoceros*. Kitajima et al. (1985) found virus-like particles in the cytoplasm of oocytes and eggs of the Mexican bean beetle (*Epilachna varivestis* Mulsant, Coccinellidae), as well as embedded in the nuclei of sperm. They suggested vertical transmission of the virus (vertical transmission; synonym: genetic transmission; the passage of viral genome from one host generation to the next).

Shukla et al. (1984) found a baculovirus of mango nut weevil (*Sternochetus mangiferae* [F.], Curculionidae). This is the first record of a baculovirus in mango nut weevil. Kim and Kitajima (1984) described nonoccluded baculovirus in the spotted cucumber beetle (*Diabrotica undecimpunctata* Mannerheim, Chrysomelidae).

Recently, Wegenstiener and Weiser (1995) reported a new entomopoxvirus in the bark beetle (*Ips typographus* (L.), Scolytidae). *Ips typographus* is a primary insect pest on spruce (*Picea abies* L.) in central and northern Europe.

Insect Viruses

Viral diseases are among the most widely investigated infections in insects. The viral particle (virus, virion) is composed of a protein shell (capsid) that surrounds the nucleic acid. Each virus has only one type of nucleic acid, either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The nucleic acid together with the capsid form the nucleocapsid. Their nucleic acid contains information necessary for their replication in a susceptible host cell. However, the host cells may also be required to supply some of the enzymes necessary for replication. Viruses contain no energy-producing enzyme systems, no functional ribosomes, or other cellular organelles; those are

supplied by the cell in which they replicate (Hull et al., 1989).

Viruses are classified by the International Committee on Taxonomy of Viruses. Today, insect viruses are placed into 12 families, but many remain unclassified (Tanada and Kaya, 1993). Insect viruses may be divided into two groups: occluded and nonoccluded.

Occluded viruses are those in which the virions are occluded in a dense protein crystal (visible in the light microscope). The occlusion body serves to stabilize the virus for prolonged periods outside the host. The occlusion bodies range in size from ~0.5 to 24 µm. Virus particles are released *in vivo* following ingestion of the occlusion body by the insect and solubilization of the matrix protein by combined effects of pH and gut enzymes (Hull et al., 1989). Three families of insect viruses are in this group, Baculoviridae, Entomopoxviridae, and Reoviridae.

Nonoccluded viruses are those in which the virions are not occluded in a dense protein crystal. Examples of these viral families are Iridoviridae, Parvoviridae, and Flaviviridae.

The primary route of viral entrance to the host is via the alimentary tract (mainly midgut) during feeding. Transmission via egg, on the egg surface (transovum) or within the egg (transovarial), is less common. Transovum transmission is more common than transovarial transmission. Transovum transmission may be used to introduce a potential microbial agent such as a virus into the SPB populations.

The objective of the study reported in this bulletin was to determine if there were evidence for a viral presence within SPB populations. This is the first time this type of an investigation on D. *frontalis* has ever been attempted.

Materials and Methods

Adults of SPB were trapped in Georgia and Mississippi over the course of two summers (1993-1994). A total of È100 collections were made. Adults of SPB collected in pheromone traps were used in most of this study. Some adults were also obtained from infested bark that had been placed in emergence chambers at Mississippi State University. The study is based on the gross morphology of virus and virus-like particles, and further studies would be required to characterize viral particles.

Light microscopy was used for detection of virus-caused signs and symptoms and also for detection of occluded viruses.

Two methods of electron microscopy were used for detection of nonoccluded viruses: (1) the phosphotungstic acid negative stain method and (2) the histopathological method (tissue changes associated with disease).

Light Microscopy (LM)

Whole, decapitated SPB were fixed in Perfix® (Fisher Biologicals) and dehydrated through a graded ethanol series. Dehydrated tissues were then cleared in Hemo-De® (Fisher Biologicals) and embedded in fresh Paraplast. Serial 5-µm sections were cut on a rotary microtome (American Optical 820 Spencer Microtome), mounted on glass slides, stained with aematoxylin/eosin, and examined with LM at 100-900x.

Transmission Electron Microscopy (TEM)

Negative stains. To check for virus infection, internal tissue samples of SPB from each collection were macerated in sterile water. A drop of the suspension was placed on a formvar and carbon-coated 200-mesh copper grid and allowed to stand for 5 minutes. The liquid was gently removed with a filter paper wick, stained immediately with 2% phosphotungstic acid for 1 minute, and the grid was then examined with a JOEL TEM at 60 kV.

Histopathology. For histopathological studies, samples of SPB from each collection were decapitated and placed into Karnovsky's fixative in 0.1 M potassium phosphate buffer, pH 7.2, and rinsed with the same buffer. The tissue was then post-fixed in buffered (0.1 M potassium phosphate, pH 7.2) 2% osmium tetraoxide for 2 hours, dehydrated through a graded ethanol series, and embedded in low viscosity Spurr's resin. Thick sections (1 mm) were cut with glass knives on an ultramicrotome (Reichert-Jung Co., Leica, Inc.), mounted on glass slides, stained with borate-buffered-toluidine blue, and examined by light microscopy. Areas of interest, such as decomposed midgut epithelia and fat bodies, were selected and located on the corresponding tissue blocks. Ultrathin sections (60-80 nm

thick) were cut from the selected areas, mounted on formvar-coated 200-mesh copper grids, stained with 0.5% ethanolic uranyl acetate followed by lead citrate, and examined using TEM as for negative stains.

Results

Results of the study imply that viruses are present in the adult populations of the SPB. Although our results are based only on the external morphology of the virus-like particles (VLP), we decided, based on morphological differences of the VLP, to separate them into five families. A brief description of each family accompanies the appropriate electron micrograph. All VLP dimensions are given in nanometers (1 nanometer = one billionth of a meter or 10^{-9} meter).

Family Picornaviridae

The family Picornaviridae is divided into four genera: the Enteroviruses, the Cardioviruses, the Rhinoviruses, and the Aphthoviruses. The virions are small isometric particles 22 to 30 nm in diameter. The type viruses of this family are Cricket paralysis virus, *Drosophila* C virus, and *Gonometa* virus (Evans and Entwistle, 1987). Picorna-like virus was also reported in *Microplitis croceipes* (Creeson) by Hamm et al., 1992. Relatively few picorna-like viruses of insects have been studied in sufficient detail to be classified as picornaviruses by the International Committee on Taxonomy of Viruses (Matthews, 1982). The virions (the infectious unit of the virus) contain a single-stranded RNA. Based on present evidence the replicate schemes of insect picornaviruses mirror those of their mammalian counterparts (Matthews, 1982). The virus replicates in the cytoplasm of cells of the epidermis, alimentary canal, and nerve ganglia. The feces contain a large amount of virus through the rupturing of midgut cells (Tanada and Kaya, 1993).

Family Bunyaviridae

Bunyaviruses are abundantly represented (150 spp.) in the family Bunyaviridae and are classified into 13 subgroups. A number of bunyaviruses cause severe diseases such as encephalitis or hemorrhagic fevers in man and animals (Bouloy, 1991).

All members of the Bunyaviridae family share the same morphological features: the virus particles are spherical (80 to 100 nm in diameter) and possess a membrane envelope from which protrude polypeptide spikes of 5 to 10 nm in length. The Bunyaviridae replicate in the cytoplasm of infected cells. The genome consists of three single-stranded RNA segments. The life cycles of bunyaviruses involve alternate replication in vertebrates and arthropods (Bouloy, 1991). These viruses are omnipresent in the world and have been isolated from various arthropods, mosquitoes, ticks, and sandflies, as well as from vertebrates, including man.

When the virus infects vertebrate cell lines, it usually causes a cytopathic effect leading to cell death. *In vivo*, the infection causes little or no histopathologic changes in arthropods and the virus is passed into the next generation by transovarial (inside the egg) and venereal transmission.

Family Baculoviridae -- Nonoccluded Group

Thus far, only one genus, Baculovirus, has been described. Based on morphological properties, members of this genus are divided into three subgroups: (1) Nuclear polyhedrosis viruses, (2) Granulosis viruses, and (3) Nonoccluded rod-shaped nuclear viruses.

Nonoccluded baculoviruses (NOBV)

Lately, many rod-shaped viruses or virus-like particles of arthropods have been reported in literature that have general properties or structural and other similarities to NOBV (Huger and Krieg, 1991). They also reported that the pertinent hosts belonging to Coleoptera, Lepidoptera, Orthoptera, Diptera, Siphonatera, Hymenoptera, Homoptera, Acarina, Araneina, and Crustacea either suffer from acute infections or are only carriers of the virus without overt signs and symptoms.

But, the majority of the NOBV's described have not been investigated in detail and have been categorized as probable or possible members of this group. At the present time, only three NOBV's of insects have been studied in sufficient detail to warrant their formal inclusion in NOBV's group: the palm rhinoceros beetle virus, Hz-1 (corn earworm) virus, and cricket virus (Huger and Krieg, 1991).

Infectious Flacherie Virus (IFV)

The target of IFV is the goblet cell of the midgut epithelium and the virus multiplies in the cytoplasm, eventually disintegrating the cell. The virus particles are isometric (of equal dimensions, thus appear spherical) and È 27 + 2 nm in diameter. The infectivity of IFV is reduced at pH 2 but unaffected at pH 3 to 12. The virus genome consists of a single molecule of single-stranded RNA. At a late stage of infection, the cytoplasm of the goblet cells is filled with virus particles (Watanabe 1991).

Unknown (recently found) RNA Virus

Many small RNA viruses, with similarities to vertebrate picornaviruses, are ubiquitous in nature and have a wide host range. Many of them cause chronic or inapparent infections, but some are virulent pathogens.

Discussion

We believe that this preliminary report of SPB-VLP is the first record of viruses associated with SPB and *Dendroctonus* (Coleoptera: Scolytidae) in general. Our basic hypothesis is and has been that viruses associated with SPB are important natural control (regulating) agents, which are suitable for control of forest insects. This study also demonstrates the need for additional investigations that will include, in this search for viruses, all life stages of SPB (from egg to adult). Many viruses kill the immature stages of the host, and their association with the adults could be rare or nonexistent. However, demonstration of their association with adults is tremendously encouraging and suggests the need to continue with studies of other life stages.

All VLP found in SPB should be classified and their suitability as biocontrol agents and/or population suppressers established. We found several possible virus infections in various adult tissues, but further study is needed to check tissue specificity and morphology of virus (using the preparations of purified viruses). Also, symptomology associated with infection by different viral infections is unknown.

Even though insect pathogens overcome many of the problems associated with chemical pesticides, they are not extensively used despite their many positive features. The reasons for their lack of use vary, but among the more important ones are that most insect pathogens, including viruses, are unknown and thought to be slow killers; and the public is reluctant to accept biocontrol agents, and viruses in particular, as safe pesticides to control insects. However, one must remember that to date these are considered very host specific. They have excellent potential for future use in pest management as technologies continue to improve for detection.

Again, we emphasize this preliminary report of SPB virus-like particles is the first record of viruses associated with SPB and *Dendroctonus*. We found several possible virus infections in various adult tissues, but further study is needed to determine host specificity and symptomology associated with viral infection. However, we are of the opinion that viruses associated with SPB are important natural regulating agents, which may be suitable for management of this insect.

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