1 **Leafhopper Viral Pathogens: Ultrastructure of salivary gland infection of**
2 **Taastrup-like virus in *Psammotettix alienus* Dahlbom; and a novel**
3 **Rhabdovirus in *Homalodisca vitripennis* (Germar) Hemiptera: Cicadellidae**

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15 **Abstract**

16 Viruses that are pathogenic to insect pests can be exploited as biological control agents. Viruses that are
17 pathogenic to beneficial insects and other arthropods, as in honey bees, silk worms, and shrimp, cause
18 millions of dollars of losses to those industries. Current advances in next generation sequencing
19 technologies along with molecular and cellular biology have produced a wealth of information about
20 insect viruses and their potential applications. Leafhoppers cause economic losses as vectors of plant
21 pathogens which significantly reduce the worlds' food crops. Each year more viruses are discovered
22 primarily through the use of next generation sequencing of the leafhopper hosts. The diversity of viruses
23 from leafhoppers demonstrates a wide range of taxonomic members that includes genomes of DNA or
24 RNA from families like: Reoviridae, Iridoviridae, Dicistroviridae, Iflaviridae, and others yet to be
25 classified. Discussed is a recent viral pathogen isolated from the leafhopper *Psammotettix alienus*, name
26 Taastrup Virus. Taastrup virus (TV) is a novel virus with a RNA genome, a Filovirus-like morphology,
27 being tentatively placed within the *Mononegavirales.* Adult *Psammotettix alienus* infected with TV,
28 showed the highest concentration of virions in salivary glands, consisting of a principal gland (type I-VI-
29 cells) and an accessory gland. Examination of thin sections revealed enveloped particles, about 1300 nm
30 long and 62 nm in diameter, located singly or in paracrystalline arrays in canaliculi of type III- and IV-
31 cells. In gland cells with TV particles in canaliculi, granular masses up to 15 µm in diameter were present

32 in the cytoplasm. These masses are believed to be viroplasms, the sites for viral replication. TV particles

33 were observed at the connection between a canaliculus and the salivary duct system. A TV-like virus

34 with strongly similar morphology was discovered in the ornamental plant, *Liriope*, near Fort Pierce,

35 Florida, USA. When the virus was inoculated to a leafhopper cell culture, HvWH, made from the glassy-

36 winged sharpshooter, *Homalodisca vitripennis* (Germar), the cells rapidly degraded with 100% mortality

37 in 48 hours. These two instances are the only reported cases of this newly discovered viral pathogen of

38 leafhoppers.

39 **Keywords:** Biological control, Cell culture, *Cicadellidae;* Glassy-winged sharpshooter;

40 *Mononegavirales;* Pathogen*;* Rhabdovirus, Salivary glands; Taastrup virus

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1. Introduction

43 Insects are commonly infected with multiple viruses and traditional methods of detection and

44 isolation, such as observation of sick or dead insects in the field can miss many pathogens as

45 diseased insects fall to the ground and are quickly scavenged by ants or other predators. The use

46 of insect cell cultures to 'capture' virus by inoculation of crude preparations made from insects

47 collected from the field, can propagate virus if the cell lines are permissive to that specific virus

48 (Hunter et al, 2001) however, these methods only capture a small fraction of the potential pool of

49 viruses that are infecting insects and other arthropods. Mining of the genetic sequences now

50 available, for new insect viruses, has proven to be a rich resource full of undiscovered, insect

51 pathogens (Katsar et al, 2007; Valles et al, 2004, 2008; Liu, et al, 2011). Even so, serendipity

52 still provides many new discoveries for the prepared mind. Recently a Taastrup-like leafhopper-

53 infecting virus was discovered in the ornamental flowering plant, Genus: *Liriope,* Fort Pierce,

54 Florida, USA (Fig. 1). The plant had a mixed infection with a Tospovirus that produced the

55 traditional ring-like symptoms on the leaves (Fig. 2). During these examinations, a Taastrup-like

56 virus was identified. Current research efforts are focused on identifying potentially new

57 leafhopper pathogens which may have a function as biological control agents.

58 Rhabdoviruses (Family: Rhabdoviridae) in the Order *Mononegavirales*, are enveloped viruses

59 with a nonsegmented negative-strand RNA genome. Currently this Order includes four

60 families— Rhabdoviridae, Bornaviridae**,** Filoviridae, and Paramyxoviridae. Rhabdoviruses have

61 a diverse host range including humans, vertebrate animals, and invertebrates in the aquaculture

62   and agricultural industries. The International Committee on Taxonomy of Viruses, ICTV,

63   recognizes nine genera in the family Rhabdoviridae (Dietzgen et al. 2011). These include

64   viruses considered to be plant viruses transmitted by arthropod vectors of the genera

65   *Nucleorhabdovirus* (10 species) and *Cytorhabdovirus* (9 species); with members in the genera

66   *Ephemerovirus (5 species)*, *Lyssavirus (12 species)*, *Novirhabdovirus (4 species)*, *Perhabdovirus*

67   *(3 species), Sigmavirus (7 species)*, Tibrovirus *(2 species), Vesiculovirus (10 species), and one*

68   *unassigned genus (6 species),* which have been isolated from various vertebrate and invertebrate

69   hosts (www.ictvonline.org/virusTaxonomy.asp). Although more than 150 rhabdoviruses have

70   been identified from a wide range of hosts worldwide, most still remain to be assigned to a genus

71   within a family due to the high amount of genetic diversity among them (Kuwata et al, 2011).

72   Filovirus-like particles of Taastrup virus (TV) have been described from a population originating

73   from France of apparently healthy leafhoppers belonging to the species *Psammotettix alienus*

74   Dahlbom (Hemiptera: Cicadellidae)(Lundsgaard, 1997). *Psammotettix* leafhopper species are

75   extensively listed in the literature as feeding on grasses and occur worldwide. The type specie of

76   the Genus, *Psammotettix maritimus* (Penis), feeds on *Convolvulus* (Haupt, 1929; DeLong, 1973).

77   Examination of plant and leafhopper tissues showed flexuous particles, 55-70 nm in diameter

78   and 600 or 1100 nm long, consist of an inner coiled nucleocapsid about 30 nm in diameter and

79   surrounded by a membrane with pronounced surface projections inserted (Lundsgaard, 1997),

80   thus resembling members of Filoviridae morphologically. The RNA genome has partly been

81   sequenced, which demonstrated both, that TV belongs to the virus Order *Mononegavirales,* but

82   which has not been officially classified into any of the established families of this virus order

83   (Bock *et al*., 2004). However, TV isolated from the leafhopper *P. alienus,* in Denmark,

84   AY423355, was inferred to belong to the family Rhabdoviridae, in the genus *Cytorhabdovirus*

85   based on analyses of the L polymerase gene (Bourhy et al., 2005). The hosts for *P. alienus* are

86   grasses and cereals (Raatikainen and Vasarainen, 1976). The insects salivate during probing,

87   penetration, and plant fluid ingestion (Backus, 1985), thus it is likely that the salivary glands play

88   a key role in the epidemiology of TV. Thus, a more in-depth study was conducted on the

89   ultrastructure of organs from infected leafhoppers with results presented here.

90

2. Experimental Section

The following experiments were performed in order to determine the distribution of virus in leafhoppers taken from a TV-infected population. Two fractions were made from ten randomly chosen insects. One fraction consisted of heads (including salivary glands) and the other fraction of thorax, abdomen, wings and legs. Each of the fractions was extracted in 1 ml distilled water. After one cycle of differential centrifugation (5 min at 1800 x g and 20 min at 13,000 x g), the sediments were dissolved in 20 µl (head fraction) or 100 µl (body fraction) of negative staining solution (0.5% ammonium molybdate, pH 7). Preparations were made on Formvar® mounted nickel grids (400-mesh) and the number of TV particles in ten grid squares was counted in a transmission electron microscope (JEOL JEM-100SX). The mean number of particles for the head fraction was 9.1 [standard deviation (SD) = 4] and zero particles for the body fraction. In the next experiment, the heads from ten insects were severed and two fractions prepared, namely: a salivary gland fraction (SG) and a fraction representing the remaining part of the head (H). After differential centrifugation, both sediments were dissolved in 20 µL staining solution. The mean number of particles per grid square was 34.3 (s = 5) for SG and 0.3 (s = 0.7) for H, showing the salivary glands to be the most important site for accumulation of TV particles.

Ten adults taken in random from the TV infected population were dissected in Ringer's solution (0.7% NaCl, 0.035% KCl, 0.0026% CaCl$_2$). Each pair of salivary glands was divided in a left and a right part with the purpose of checking one part for presence of particles by negative staining electron microscopy (extraction in 10 µl 0.5% ammonium molybdate) and the other corresponding part by ultrastructural analysis. The salivary glands were fixed, dehydrated, and embedded essentially according to Berryman and Rodewald (1990). In brief, the specimens were transferred to fixative (3% formaldehyde, 0.3% glutaraldehyde, 100 mM phosphate buffer, pH 7.0) and maintained in fixative for at least 2 h. After washing (3.5% sucrose, 0.5 mM CaC1$_2$, 100 mM phosphate buffer, pH 7.4), the aldehyde groups were quenched with 50 mM NH$_4$Cl dissolved in washing solution) for 1 h. The specimens were then washed (3.5% sucrose, 100 mM maleate buffer, pH 6.5) and post-fixed with 2% uranyl acetate in the same maleate buffer for 2 h. After dehydration in acetone (50%, 70%, 90%), the specimens were infiltrated in LR Gold (The London Resin Co., England) containing 0.5% benzoin methyl ether (3 changes) and polymerized under near ultraviolet light (Philips TW6). Fixation and dehydration (including 50% acetone) was done

121 at 5-10 °C and dehydration (from 70% acetone), embedding, and polymerization performed at -

122 20°C. Thin sections (60-70 nm) were collected on 200-mesh nickel grids, stained with saturated

123 uranyl acetate for 5 min and lead citrate (1 mM, pH 12) for 1 min, and examined with a JEOL

124 100SX transmission electron microscope operated at 60 kV. As controls, specimens were prepared

125 in the same way from a healthy population of *P. alienus.*

126 Field collected *Liriope* which had symptoms of a tospovirus-like infection (Figs. 1, 2) were

127 prepared for examination by TEM as mentioned above. Upon discovery of a Taastrup-like virus

128 infection a crude sap inoculum was prepared. Solutions were prepared from *Liriope* plants with

129 and without symptoms and the resulting inoculum dispensed onto leafhopper cell cultures

130 produced from the glassy-winged sharpshooter, *Homalodisca vitripennis*, HvWH, Hunter, USDA.

131 The crude plant sap preparation used 2 g of leaf tissues homogenized in Histidine, monohydrate

132 buffer, pH 6.5, which was the same buffer used for insect cell cultures (Hunter and Polston 2001;

133 Hunter et al, 2001; Marutani et al, 2009). The solution was then centrifuged in 15 mL tubes at 600

134 xg for 4 min to pellet the plant debris. The supernatant was collected and filtered through a 0.45

135 μm filtration unit, and then filtered again through a 0.22 μm filtration unit. The filtered sap

136 inoculum was then added to adherent leafhopper cell cultures at 1 mL per 25 cm^2 flask (Corning®,

137 Inc), and let stand for 10 minutes. After this time 4 mL of fresh insect medium was added (Table

138 1). Leafhopper cell cultures were observed daily for cell pathogenicity.

139 Leafhopper cell line maintenance: HvWH, were cultivated in H2G Leafhopper medium, modified

140 from WH2 honey bee media (Hunter 2010) (Table 1). Culture methods in (Kamita et al, 2005;

141 Biesbock et al, 2014). Prior to adding the fetal bovine serum, the medium was passed through

142 sterilizing filtration units (500 mL, 0.22 μm filter, Corning), then fetal bovine serum was added to

143 make a 10% concentration. Aliquots of 5 mL of medium were placed on the counter for three days

144 at room temperature to test for bacterial contamination. Fungin™ a new soluble formulation of

145 Pimaricin (InvioGen, Cat. No. ant-fn-2, 200mg) was added to the culture medium to inhibit mold

146 growth. Cultures were maintained in Corning 25 cm^2 tissue culture flasks treated with

147 CellBIND™ to promote cell attachment (Corning®, Lowell, MA) (Hunter 2010). Culture flasks

148 were kept in an incubator at 24°C and examined using an inverted microscope (Olympus IX70) at

149 10x, 20x, 60X magnifications. Complete medium change was done every 10 days without

150 disturbing the culture surface and cultures were passed when approximately 80% confluent. Cell

151   passage used 0.25% Trypsin EDTA solution (Invitrogen^{TM}, Carlsbad, CA) to dissociate cells by

152   exposure for 2 to 5 min to achieve complete dissociation. Adherent cells had medium gently

153   pipetted across the culture surface to detach cells. Once cells were dissociated, they were collected

154   into 15 mL centrifuge tubes and centrifuged for three min at a force of 400xg, in a clinical

155   centrifuge, (IEC, Centra CL2, Thermo Electron Corp., Milford, MA 01757). The supernatant was

156   drawn off and the cell pellet gently resuspended in 4 mL of fresh medium. Cells were seeded at 2

157   mL per 25 cm^2 flask, being split at a 1:2 ratio. Fresh medium was added to make a final volume of

158   5 mL per flask. Newly passed cultures were left untouched for 48 hours to let cells settle and attach

159   to the flask substrate.

160

3. Results and Discussion

161

162   Sogowa (1965) has described the morphology of leafhopper salivary glands in detail, but *P. alienus*

163   was not included and has not been described elsewhere. The morphology of these glands was

164   therefore studied and described here. The naming of the glandular cells made by Sogowa (1965)

165   is followed. The salivary glands of P. *alienus* are lying in the head and prothorax and consist of

166   two pairs of glands, each made up of a complex principal gland (Fig. 3, I-VI) and an U-formed

167   accessory gland (Fig. 3, AG). The lettering III, IV, and V all represents single cells, but the areas

168   designated I, II, and VI in Fig. 3, represents one to more cells each (the exact number not

169   determined). The accessory salivary gland (AG) consists of many cells. According to the

170   ultrastructure of the canaliculi in type III-cells (see later), these cells are subdivided in four type

171   IIIa-cells and two type IIIb-cells. The orientation of the principal salivary gland within the insect

172   showed one IIIb-cell pointing downward and the other IIIb-cell against the front. The I- and II-

173   cells (anterior lobe) form the proximal part and the IV- through VI-cells (posterior lobe) the distal

174   part of the gland. The overall length of a typical principal salivary gland (from the tip of the lower

175   IIIb-cell to the tip of the upper IIIa-cell) is about 550 µm.

176   Negative staining electron microscopy revealed TV particles in glands from two of 10 examined

177   insects. By ultrastructural analysis, unusual structures, as described below, were detected in glands

178   from the same two individuals. Rod-shaped particles were observed either singly or in

179   paracrystalline arrays in canaliculi of type III- and IV-cells (Fig. 4 -5). The type III-cells are easily

180 distinguished from other salivary gland cells by not having secretory vesicles in the cytoplasm.
181 However, two types of III-cells could clearly be distinguished in *P. alienus* according to the
182 structure of canaliculi. In the one type, here designated IIIa, the content of the canaliculi is granular
183 (Fig. 3 and 4A), whereas the other, designated IIIb, has canaliculi filled with many small
184 membrane-bound spherules containing electron-opaque granules (Fig. 3 and 4B). The IV-cells
185 could easily be identified from all other salivary gland cells by a visible line pattern in their
186 secretory vesicles (Fig. 4C).

187 The particles in the canaliculi were often seen with a membrane-like bleb at the one end (Fig. 4D).
188 These blebs are probably remnants of viral envelopes remaining after a presumed budding process.
189 For particles for which both ends could be seen, the mean length - not including the bleb - was
190 calculated to be 1294 nm (s = 22). In cross section, an electron-opaque inner hollow core (about
191 31 nm in diameter) is seen surrounded by an outer faint electron opaque layer, separated by a
192 translucent layer (Fig. 4D, 5A). The diameter of particles was calculated to be 62 nm from particles
193 laying in paracrystalline arrays. The morphology and dimensions for the particles observed here
194 in canaliculi are in agreement with the negative stained particles presented previously
195 (Lundsgaard, 1997). This taken together with the correlation found between presence of particles
196 in extracts and presence of particles in canaliculi among the 10 examined leafhoppers. The
197 particles observed in canaliculi are identical to those described in the former paper. So, the inner,
198 hollow core is probably the helical nucleocapsid of TV and the outermost faint layer the G protein
199 spikes. In cross sections through particles of Rhabdovirus and Filovirus (Murphy and Harrison,
200 1979; Geisbert and Jahrling, 1995), an electron opaque layer, thought to be the virus membrane,
201 is present between the layer of spikes and the inner nucleocapsid core. The analogous position of
202 this electron opaque layer is translucent in the present TV particles. This discrepancy can be
203 explained by use of osmium tetroxide for post-fixation (known to preserve and stain membranes)
204 in the studies on rhabdo- and filoviruses, whereas osmium tetroxide was omitted in the present
205 investigation. Osmium tetroxide was omitted in order to preserve the antigenic activity for future
206 research on localization of viral antigens in these specimens. Aggregates of particles, 75-80 nm in
207 diameter, were described from head cells of *P. alienus* (Lundsgaard, 1997). These particles were
208 seen located in the cytoplasm (not in canaliculi as presented here) and their central, electron opaque
209 core was not seen hollow (as are the present TV particles), suggesting that the particles described
210 previously were not TV particles.

211   In the cytoplasm of type III- and IV-cells, possessing canaliculi with TV particles, fine granular

212   masses up to 15 μm in diameter were observed (Fig. 4A). These masses do not contain ribosomes

213   or other cellular organelles and their presence was positive correlated with presence of TV particles

214   in nearby canaliculi. Granular masses (viroplasms), believed to be viral replication sites, have been

215   observed in cells infected with Rhabdovirus (Murphy and Harrison, 1979; Conti and Plumb, 1977)

216   and Filovirus (Geisbert and Jahrling, 1995). The granular masses present in the cytoplasm of

217   salivary glands with TV particles are thus suggested to be viroplasms, the sites for TV replication.

218   Because a nuclear localization signal has been identified in the G-protein gene of TV (Bock *et al*.,

219   2004), nuclei close to viroplasms were carefully examined for abnormalities as compared with

220   uninfected controls. Neither budding through nuclear membranes nor abnormality of nucleoplasm

221   were observed during the present investigation.

222   In several cases, two glandular cells in contact with each other could contained both, viroplasms

223   in the cytoplasm and TV particles in canaliculi, while the other neighboring cell was uninfected

224   (Fig. 4B). These uninfected cells were of a type, which were infected elsewhere. TV particles were

225   never seen between such cells or in the space between the plasma membrane and the basal lamina,

226   so, the delivery of mature TV particles appeared only to take place by budding of nucleocapsids -

227   synthesized in viroplasms - into canaliculi destined for saliva excretion. TV-particles or viroplasms

228   were neither observed in type I-, II-, V-, and VI-cells of principal salivary glands, nor in accessory

229   salivary glands from infected leafhoppers. TV-particles or viroplasms were not seen in salivary

230   glands from leafhoppers originating from a TV-free population of *P. alienus*.

231   This investigation shows that salivary glands are the primary site of TV synthesis in adult *P.*

232   *alienus*. One possible mode of TV acquisition relies on virions excreted with saliva into the plant

233   tissue, which are passively delivered into the plant during feeding. Then TV is being ingested by

234   uninfected nymphs and/or adults which become infected. Former investigations (Lundsgaard,

235   1997) have shown that host plants used for rearing TV infected leafhoppers, do not become

236   infected, and so, the plants probably function as passive host acquisition for leafhopper vectors

237   which then are able to transmit TV post replication latent period.

238   A Rhabdovirus, with strong similarity to the Taastrup virus was discovered in an ornamental plant,

239   *Lirope spp.*, grown in Florida, USA (Hunter and Adkins 2009; Hunter et al, 2009). A crude sap

240   extract was prepared and screened on leafhopper cell cultures, *Homalodisca vitripennis* (Germar)

241   glassy-winged sharpshooter (Hemiptera: Cicadellidae), for pathogenicity (Fig. 5C,D). The plant,

242   *Liriope*, belongs to the lily family and is one of the most popular ground covers in Florida (Fig.

243   1).   *Liriope* is a perennial, herbaceous, mat-forming plant that grows to about 30 cm tall.

244   Leafhopper cell cultures inoculated with the virus-laden sap from infected *Liriope* plants resulted

245   in massive cell death, 100% mortality in 48 hrs. Control cells inoculated with non-symptomatic

246   plant sap inoculum showed no significant changes in cell morphology, adherence, or survival.

247   Examination using TEM showed viroplasms-like structures in inoculated leafhopper cells treated

248   with virus-infected *Liriope* sap, at 30 hrs post treatment (Fig. 5D). Attempts to sap inoculate non-

249   infected *Liriope*, from the same field plots failed to produce symptoms. However, in the original

250   *Liriope* plant with symptoms, the TV-like virions were observed at significant numbers in plant

251   tissues to suggest replication (Fig. 2).

252

253   **4. Conclusions**

254   As genomic techniques continue to improve more viruses are rapidly being discovered (Babayan

255   et al, 2018; Hunter et al, 2006; Hunnicutt et al, 2008; Liu, et al, 2011; Nouri et al, 2018; Rosario

256   et al, 2018). The taxonomic group which includes Taastrup viruses and other related virus

257   (Taastrup virus---Viruses; ssRNA viruses; ssRNA negative-strand viruses; Mononegavirales;

258   Rhabdoviridae; Cytorhabdovirus; unclassified Cytorhabdovirus) continues to have new members

259   discovered across a diverse range of insect Orders, from Hemiptera to Lepidoptera (Ma, et al,

260   2014). Insect infecting viruses are still examined for use as biological control agents (Lacey et

261   al, 2015; Szewczyk et al, 2006), but applications of viruses have moved beyond just being

262   pathogens. The advances in molecular biotechnology, low cost sequencing, bioinformatics

263   software, and virus discovery pipelines to analyze large genetic datasets (Babayan et al, 2018;

264   Bigot et al, 2018) have made virus discovery easier (Greninger 2018). Along with the rapid

265   increase of virus discovery, has been the expansion of novel molecular biology applications for

266   viruses, used in whole, or in part (promoters/peptides) across agriculture, human medicine, and

267   material sciences (Hunter et al, 2019; Kolliopoulou et al, 2017; Wen and Steinmetz 2016). Virus

268   can be used to delivery 'genetic' payloads, to produce RNA suppression of specific genes such

269   as toxins in plants, or critical genes in an insect pest (Andrade and Hunter 2016, 2017;

270   Kolliopoulou et al, 2017; Zotti and Smagghe 2015; Zotti et al, 2018). Since sap inoculation into

271   uninfected plants was not successful, it may be that the virus requires inoculation by an infected

272   leafhopper. If this is the mode of transmission, the rate of transmission would be low, and may

273   explain the severity of infection due to random exposure in nature. The analyses of the infection

274   pathway in leafhopper salivary glands (*Psammotettix alienus* Dahlbom), along with the capacity

275   to induce 100% mortality in leafhopper cell cultures [*Homalodisca vitripennis* (Germar)]

276   suggests Taastrup-like virus may have an early evolutionary association with leafhoppers. The

277   rapid mortality observed in leafhopper cells when inoculated may also be why infected

278   leafhoppers are rare, and difficult to collect in field sampling efforts.

279

Conflict of Interest

281   "The authors declare no conflict of interest".

282

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289

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431   **Tables and Figures**

432   **Table 1.** Hunter's Leafhopper Cell Culture Medium, H2G, components. **[1A, B]** *Homalodisca*

433   *vitripennis* (Germar) (Hemiptera: Cicadellidae) cells in culture started from embryonic tissue

434   from eggs. Adult glassy-winged sharpshooter leafhopper.

435   **Figure 1.** Healthy non-infected *Liriope* is a plant belonging to the lily family and is one of the

436   most popular ground covers in Florida. It is also called 'Lilyturf' or 'monkey grass' and is a

437   native plant of Eastern Asia.

438   **Figure 2.** Flowering plant, genus, *Liriope,* with ring-like symptoms typical of a tospovirus

439   infection-(A). Electron microscopy examination of plant tissues however, showed a mix

440   infection with one virus having distinct morphology similar to Taastrup virus (B- Taastrup-like

441   virus Florida strain); Plant tissues showed typical virus infection associated with the plant-

442   infecting Rhabdoviruses (C).

443   **Figure 3.** Drawing of salivary glands from *Psammotettix alienus* seen from the insect axis (left)

444   or from outside (right). The salivary glands consist of a principal gland (cell type I to VI) and an

445   accessory gland (AG), both connected to the principal duct (PD). According to ultrastructure of

446   canaliculi, the type III-cells are differentiated in four type IIIa-cells (pointing upwards and

447   backwards) and two type IIIb-cells (pointing forwards and downwards).

448   **Figure 4.** Section through a type IIIa-cell from *P. alienus* infected with Taastrup virus. Aggregates

449   of virus particles (white arrowheads) are located in canaliculi **(c)** close to granular masses **(gm)** in

450   the cytoplasm. Bar = 2 µm. **[4A];** Section through a type IIIb- (right) and a type IV-cell (left) from

451   *P. alienus* infected with Taastrup virus. Aggregates of virus particles (black arrowhead) are located

452   in a canaliculus of the type IIIb-cell (inset). Note the vesicles with electron opaque granules in the

453   canaliculus, characteristic of type IIIb-cells. The neighboring cell (type IV) has cytoplasm **(cp)**

454   with rough endoplasmic reticulum together with electron opaque secretory vesicles (sv). No virus

455   particles were observed in any of the canaliculi (c) of this cell. Bar = 2 µm. Inset bar = 500 nm.

456   **[4B];** Section through a type IV-cell from another infected leafhopper other than the one examined

457   in Fig. 4. Virus particles (white arrowhead) are located in canaliculi **(C)** of this cell. Note the dark

458   lines in the secretory vesicles **(sv)**, which are characteristic for the type IV-cells. **Cp** =cytoplasm.

459   Bar = 1 µm. **[4C];** Longitudinal section through aggregates of virus particles located in a

460   canaliculus of a type IIIa-cell from *P. alienus* infected with Taastrup virus. Small blebs (white

461   arrowheads) are seen at one end of several particles. Bar = 1 μm. **[4D].**

462

463   **Figure 5.** Transverse section through virus particles located in a canaliculus of a type IIIb-cell

464   from *P. alienus* infected with Taastrup virus. An electron opaque tube with a hollow centre is

465   seen surrounded by a translucent layer and outermost a faint electron opaque layer. TEM

466   micrograph, Bar = 100 nm. **[5A];** Close-up where a canaliculus opens into the salivary duct

467   system. Oblique sections through some virus particles are seen (white arrowhead). Bar = 500

468   nm. **[5B];** Light microscope image of Taastrup-like virus infected leafhopper cells (*H.*

469   *vitripennis*) (**5C**) and transmission electron microscopy image (**5D**) of cell culture from glassy-

470   winged sharpshooter, *Homalodisca vitripennis* (Germar), HvWH infected with a Taastrup-like

471   virus (**5C**, 30x magnification) (Hunter and Adkins 2009). TEM of leafhopper cell with

472   viroplasms-like structures in infected cells (**5D**, image provided by Diann Achor, University of

473   Florida, Electron Microscopy Core, Lake Alfred, FL).

Table 1. Hunter's Leafhopper Medium, H2G, components.

Grace's Insect medium (supplemented, 1X)	210 mL
0.06M L-histidine monohydrate buffer solution pH=6.5	290 mL
Medium 199 (10X)	10 mL
Medium 1066 (1X)	17 mL
Hank's Balanced Salts (1X)	33 mL
L-Glutamine (100X)	1.5 mL
MEM, amino acid mix (50X)	1.5 mL
Pen-Strep (w/ Glutamine)	2.5 mL/500 mL
Nystatin	1.0 mL/500 mL
Gentamycin	1.5 mL/500 mL
Dextrose	1.8 g
Fetal Bovine Serum	10% of final volume

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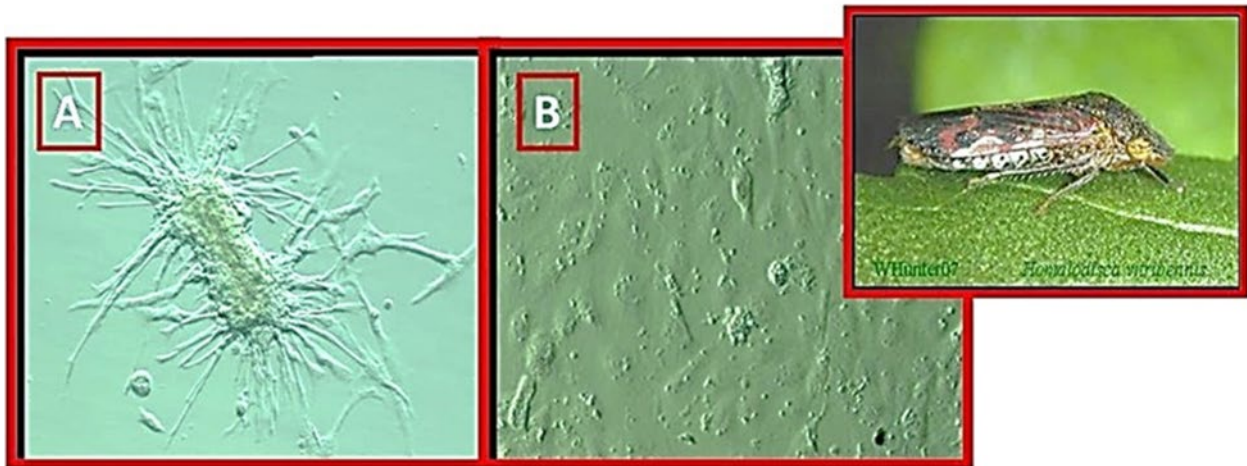
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476   most popular ground covers in Florida. It is also called 'Lilyturf' or 'Monkey grass' and is a

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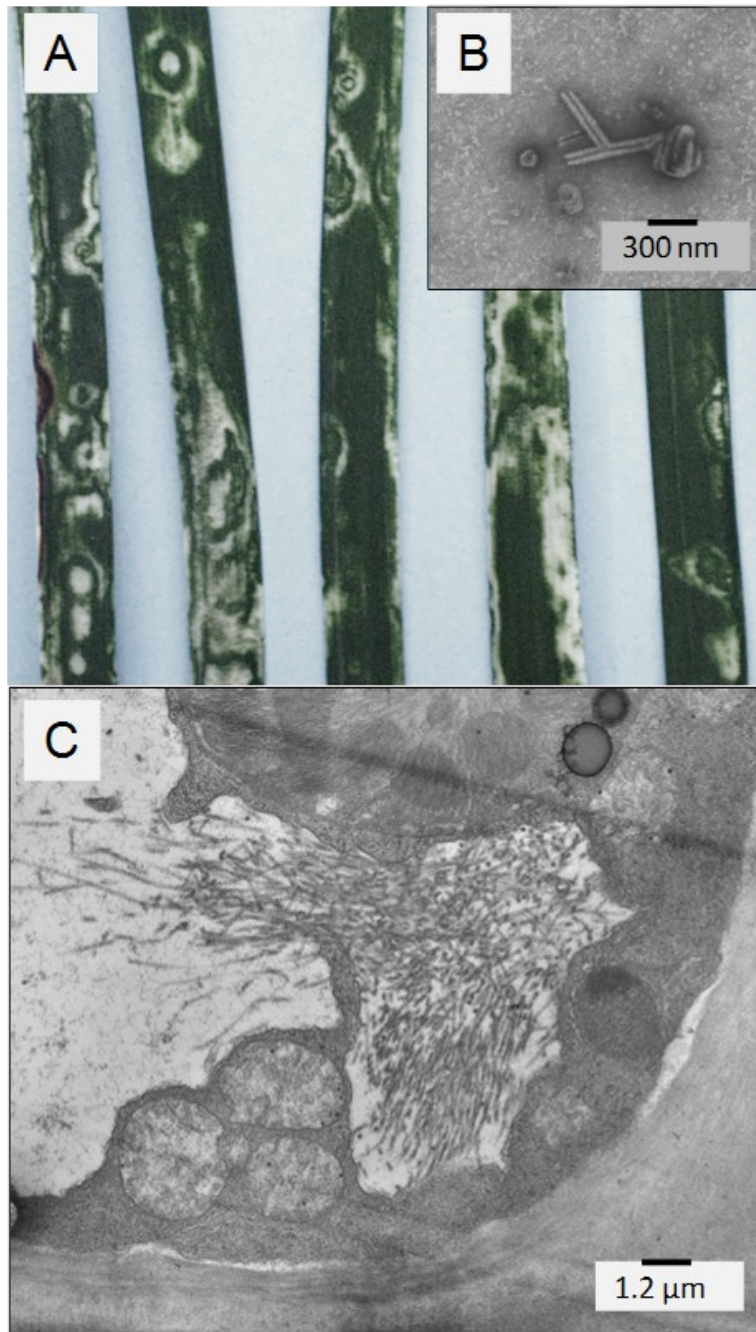

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490   or from outside (right). The salivary glands consist of a principal gland (cell type I to VI) and an

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493   backwards) and two type IIIb-cells (pointing forwards and downwards).
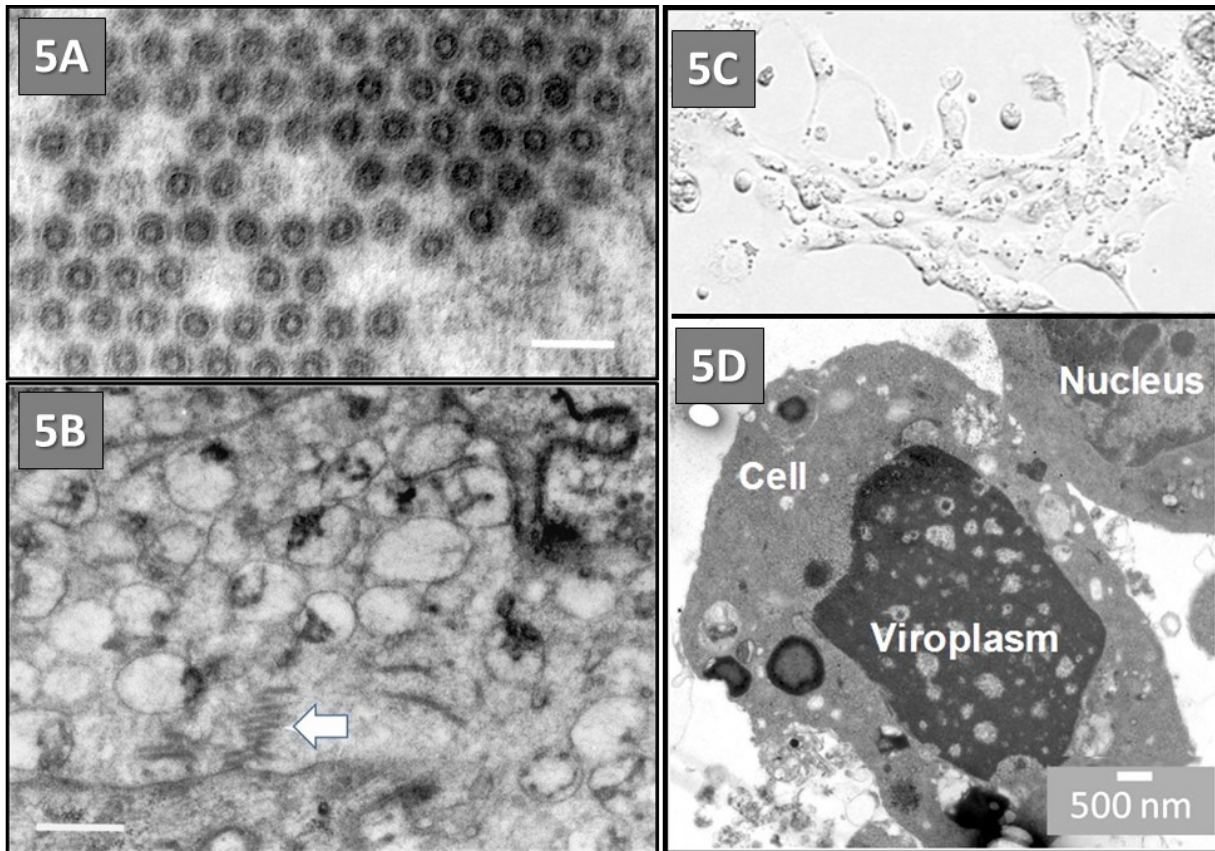
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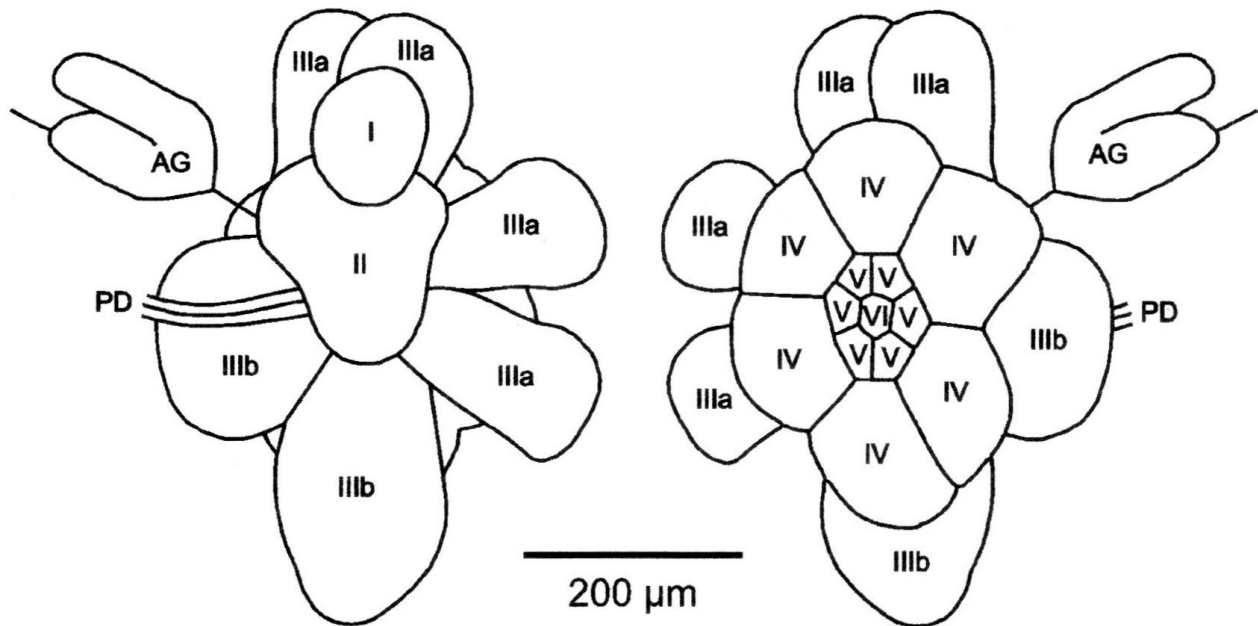
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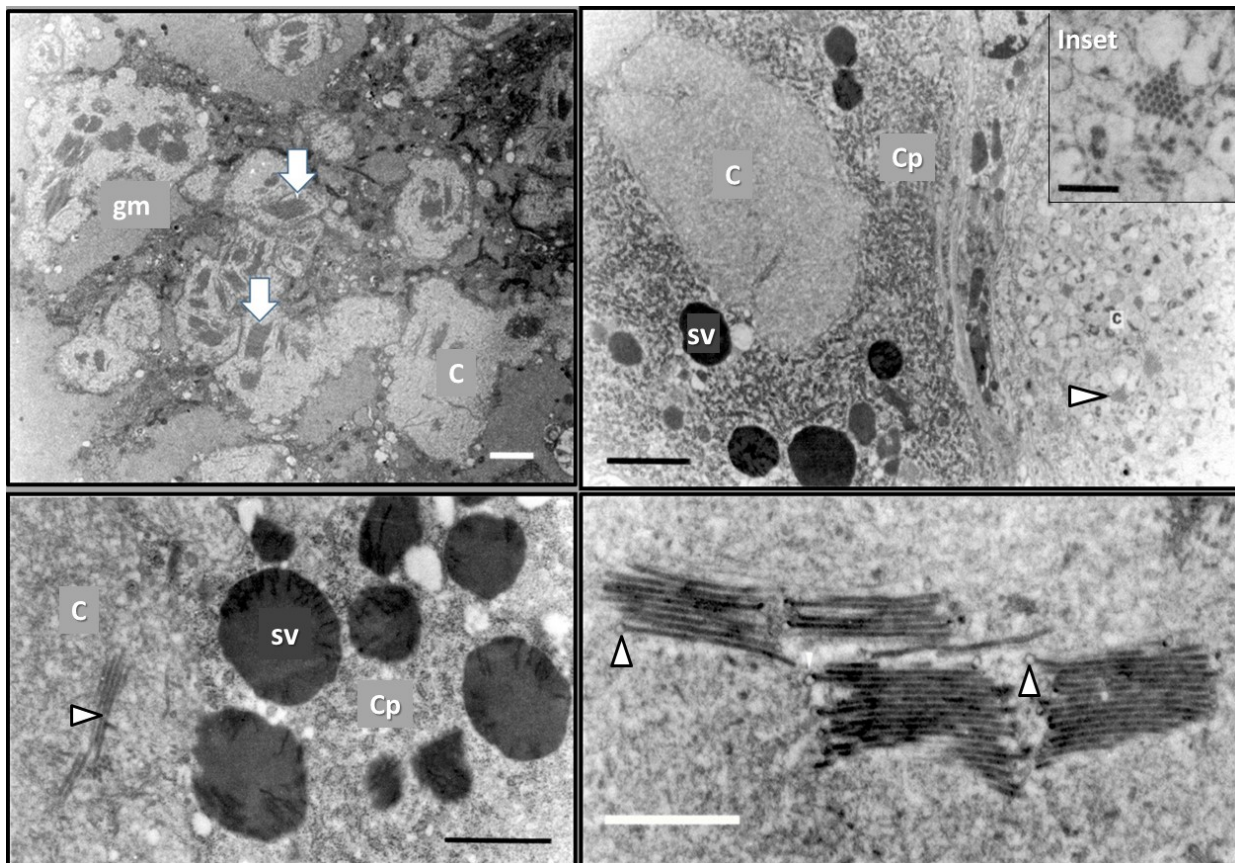
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516   Bar = 100 nm. **[5A]**; Close-up where a canaliculus opens into the salivary duct system. Oblique

517   sections through some virus particles are seen (white arrowhead). Bar = 500 nm. **[5B]**; Light

518   microscope image of Taastrup-like virus infected leafhopper cells (*H. vitripennis*) (**5C**) and

519   transmission electron microscopy image (**5D**) of cell culture from glassy-winged sharpshooter,

520   *Homalodisca vitripennis* (Germar), HvWH infected with a Taastrup-like virus (**5C**, 30x

521   magnification) (Hunter and Adkins 2009). TEM of leafhopper cell with viroplasms-like structures

522   in infected cells (**5D**, image provided by Diann Achor, University of Florida, Electron Microscopy

523   Core, Lake Alfred, FL).

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