CELL CULTURE BASED PROPAGATION OF THE GLASSY-WINGED SHARPSHOOTER AS A METHOD TO REPLICATE *HOMALODISCA COAGULATA VIRUS-01* FOR VECTOR MANAGEMENT

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ABSTRACT
Invasive species management is crucial in agricultural production. The glassy-winged sharpshooter (GWSS, *Homalodisca vitripennis*) is the primary vector for *Xylella fastidiosa*, a xylem-limited bacterium. A dicistroviridae virus known as *Homalodisca coagulata virus-01* (HoCV-01*)* has been identified that infects and increases mortality rates in GWSS when used alongside chemical insecticides. The virus requires an insect host for propagation, thus making a cell culture approach a logistical and economical method towards producing a biological control agent. In this study, we have developed a system for large-scale propagation of GWSS cell cultures from primary cell cultures. Mass production of GWSS cells via culturing techniques will allow us to produce HoCV-01 virus in large enough quantities to be utilized as a method of insect control.

LAYPERSON SUMMARY
The glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*) is an invasive leafhopper species that has been the source of great economic loss in agriculture throughout California and the southwest. GWSS are the primary mode of transmission of *Xylella fastidiosa*, the causal agent of Pierce's disease. Developing a biological control agent for this disease has become increasingly vital towards controlling insect populations and overcoming the problems of harmful environmental effects and insect resistance to chemical insecticides. Utilizing a virus that has been shown to increase mortality in GWSS colonies, this work focuses on a cell culture based approach to propagate GWSS cell lines that act as vectors to grow live virus, which can then be extracted. Since the target virus cannot grow outside of the insect, successful cultivation of GWSS cells is critical to the development of a viral based biological control agent

INTRODUCTION
The xylem-limited bacterium *Xylella fastidiosa (Xf)* is the causal agent in Pierce's disease (PD) and is principally transmitted by the glassy-winged sharp shooter (GWSS, *Homalodisca vitripennis),* an invasive leafhopper species. GWSS are indigenous to the southern United States and northeastern Mexico but have successfully invaded new territory including California and the Hawaiian island of Oahu (Hunnicutt, 2008). PD has been a source of great economic loss in many agricultural arenas throughout North America, particularly in grape vineyards. Introduction of GWSS into new vineyards has been linked directly with an increase in PD (Perring et al., 2001). GWSS are voracious xylem feeders, enabling them to rapidly spread the plant pathogen and making control of this invasive pest crucial to disease management. The ability of GWSS to cover large ranges presents a great risk to agriculture within the United States as well as internationally if this invasive species is not managed.

Use of other vector management techniques such as insecticides that are non-specific has lead to additional problems including insecticide resistance and residue contamination, whereas utilizing a virus that is already present within GWSS populations to develop a viral biological control presents a target specific option for pest management (Hunnicutt et al., 2006). A dicistroviridae virus known as *Homalodisca coagulata virus-01* (HoCV-01*)* has been identified that infects and increases mortality rates in GWSS. The focus of this study was to expand on previously unsuccessful attempts to rear infected glassy-winged sharpshooters to adulthood by utilizing cell culture techniques versus live insect colonies.

OBJECTIVES
1. Successfully propagate primary cell lines of glassy-winged sharp shooters via tissue cell culture techniques.
2. Establish production of the target virus HoCV-01 in vitro.
3. Amplify extracted HoCV-01 to develop biological control agent.

RESULTS AND DISCUSSION
The GWSS cell cultures of established cell lines were obtained from the USDA Agricultural Research Service and centrifuged at 350rpm for six minutes in a 15 amp 5804 R centrifuge (Eppendorf™, Hamburg, Germany). A loose pellet was observed in each tube **(Figure 1)**. The supernatant was drawn off without disturbing the pellet and then 8mL of fresh medium, classified as modified Wayne Hunter-2, WH2, Honey bee cell culture medium (Hunter, 2010), was added to disturb each pellet. The suspended cells were transferred to 25cm^2 tissue culture flasks (4mL per flask) that have been treated to promote cell attachment (Corning®, Lowell, MA), and kept in an incubator at an approximate temperature of 24 degrees

Celsius and 53% humidity. All handing of cell cultures took place inside a Purifier Class II Biosafety Cabinet (LABCONCO™, Kansas City, MO) culture hood.

Culture flasks were examined using an inverted microscope (Olympus DP30BW, IX2-SP, IX71) at 10X magnification. Cells were observed to attach to the substrate within *48 hours* of initial transfer. Over a 10-day period the flasks were examined every-other day, and at the end of the period fibroblasts had developed and were observed across the flask surface **(Figure 2)**.

With successful cultivation in flasks, the cells were transferred to 48-well sterile tissue culture plates, with lids, that have been treated to promote cell attachment (GREINER CELLSTAR®, Monroe, NC). Transfer of cells was completed using 0.25% Trypsin EDTA (Invitrogen™, Carlsbad, CA) to detach cells from the culture surface, centrifugation in a 15 amp 5804 R centrifuge (Eppendorf™, Hamburg, Germany) at 350rpm for six minutes, and introduction of 0.5mL of fresh medium per well.

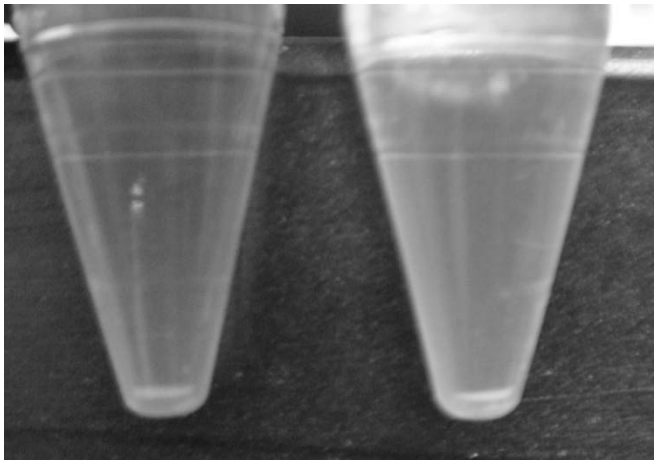

Figure 1. Initial GWSS cells received formed loose pellets upon centrifugation in preparation for transfer to cultures.

CONCLUSIONS

Successful cultivation of GWSS cells is critical to the development of a viral based biological control agent. We now have a system for mass production of cells. In this future, we plan to inoculate cell culture plates with HoCV-01 and extract viral titers until optimal viral extraction time frames are determined and use this method of cell culturing to mass produce the novel HoCV-01 virus as an alternative method for disease control. The results of this work are essential in furthering methods of management of the insect vector for PD, reducing the prevalence of the disease and lessening its economic impacts.

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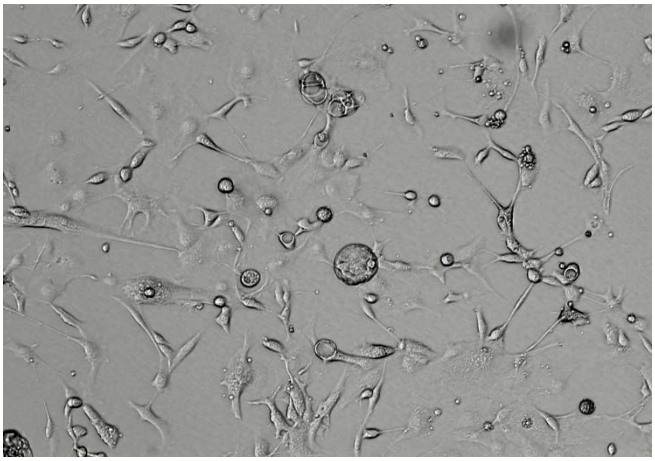

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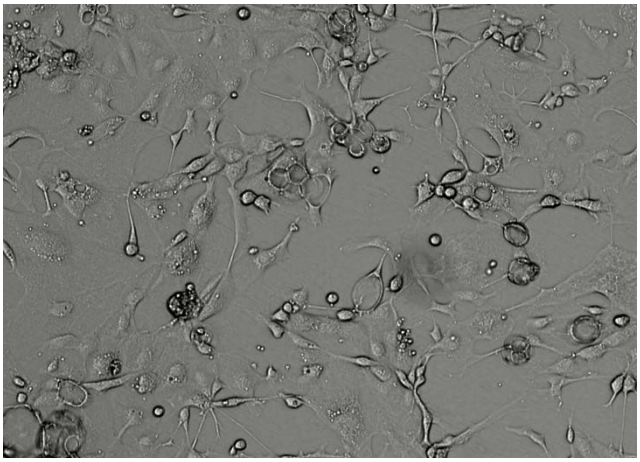

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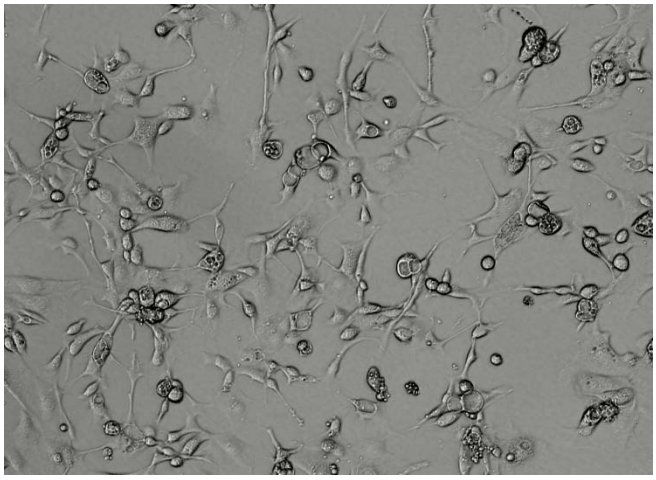

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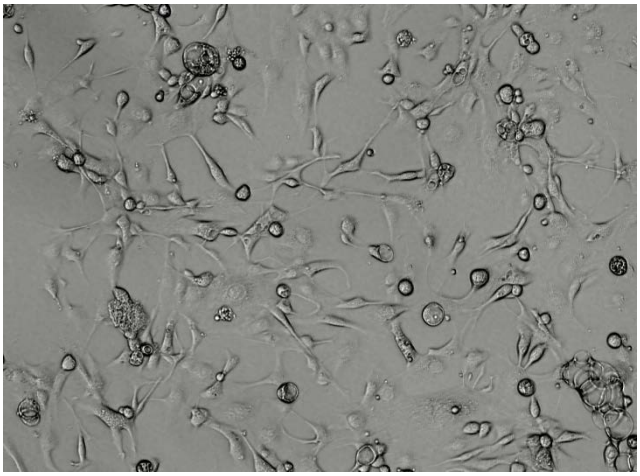

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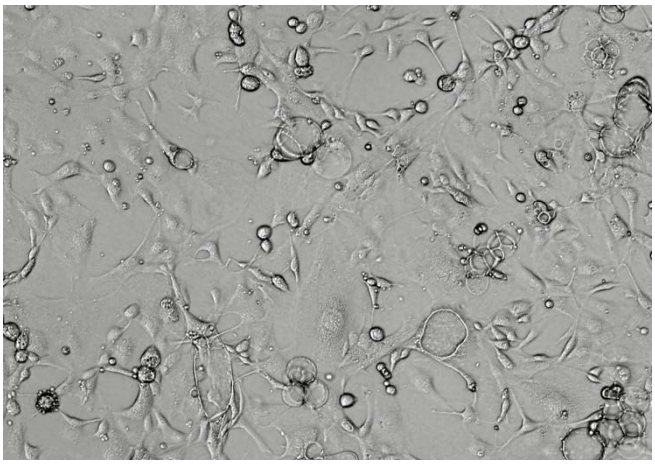

DAY 10

Figure 2. Timeline of growth of GWSS cell cultures over the 10-day incubation period. Substrate attachment and formation of fibroblasts was observed on day 2, 48-hours after initial culture introduction. Continued growth was observed over the 10-days until both fibroblast and monolayer development was observed across the growth surface.