ISOLATION AND CHARACTERIZATION OF GWSS PATHOGENIC VIRUSES

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INTRODUCTION

Insect pathogens such as insect-selective viruses often cause natural epizootics in the field and can be used as a biological control agent or vector for carrying plant or crop protection agent. In order to identify an epizootic, scientists have classically waited for an outbreak of disease and then tried to isolate the causative agent from diseased insects. Alternatively, one can take advantage of the fact that in any population there are multiple infective agents and look for these agents using biochemical and molecular methods. At present there are no known viral epizootics of GWSS. In this project, we are attempting to isolate and characterize viral pathogens of the GWSS using a new approach we call 'virus mining". In this approach we assume that GWSS pathogenic viruses will be found and can be isolated from any given population as long as the population examined is sufficiently large. Additionally, we are attempting to establish continuous GWSS cell lines that can efficiently support the replication of the GWSS viruses that we isolate. A susceptible cell line is a key to the further characterization and manipulation of a virus and will be beneficial in other areas of GWSS study.

OBJECTIVES

1. Isolate and characterize viruses infective against GWSS.
2. Establish continuous cell lines from embryonic GWSS tissues.

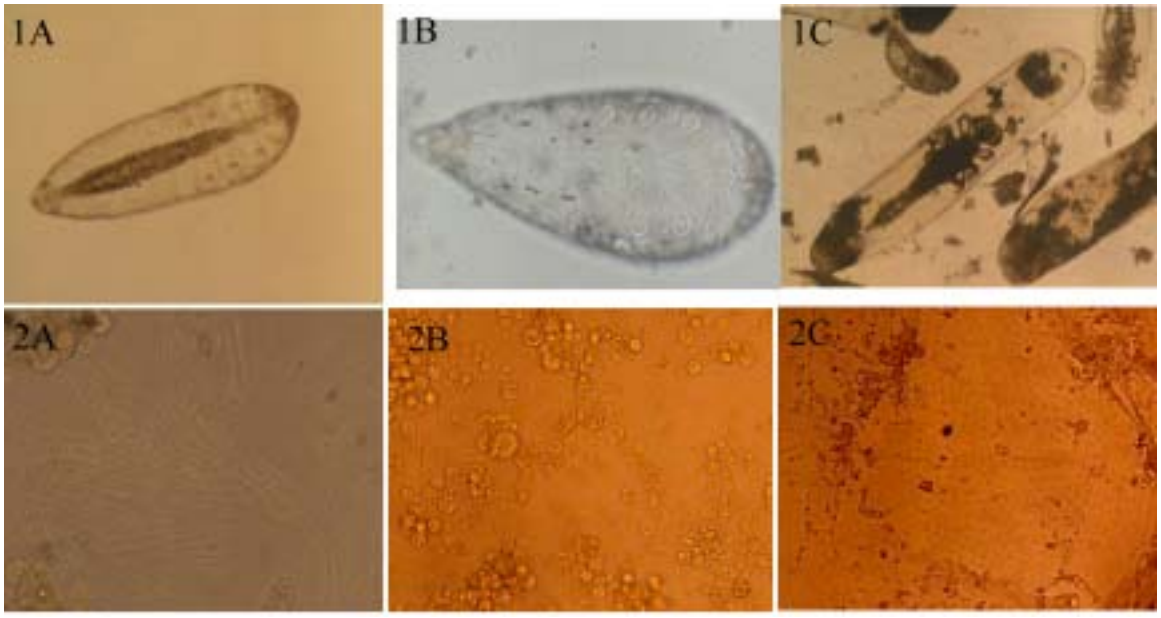
RESULTS AND CONCLUSIONS (April-September of 2001)

GWSS adults (Objective 1) and egg masses (Objective 2) were field-collected during two outings, one on April 25-26 (Filmore, CA) and the other on August 15-17 (Riverside, CA) of 2001. Adults from the April collection consisted of insects that had overwintered whereas those collected during August were the progeny of the overwintering insects. At present the effect of overwintering on virus ecology is unknown. GWSS showing unusual behavior or morphology indicative of virus infection were not observed. Egg masses from the April collection generally contained from 10-16 eggs whereas the egg masses from August contained significantly less, generally only 4-8. Interestingly, although an attempt was made in the field to select egg masses that were not parasitized by wasps (i.e., masses with any dark-colored eggs, eggs with holes, etc. were not collected), roughly 40% of the primary cultures showed wasp larvae (Figure 1A), pupae (Figure 1B) or adults (Figure 1C). This suggested that parasitization of one or more egg per mass occurs at significantly higher levels in the field. Additionally, during the August outing, female GWSS bearing white spots on their forewings were collected for oviposition in rearing cages. White wing spots are an indicator of oviposition within 3 minutes to 36 hours (Hix, 2001). Egg masses were stored at 5ºC under humidity for less than 48 h prior to processing for the generation of primary cultures.

In preliminary experiments to isolate a GWSS pathogenic virus (Objective 1), approximately 1000 GWSS adults were homogenized and resuspended in PBS buffer. The average adult weighed 31.3±6.8 mg. Following ultracentrifugation through a 20-50% sucrose gradient at least 7 distinct major and minor bands were observed. Three very minor bands were collected, resuspended in PBS, and pelleted. Following phenol and chloroform extractions, the bands were found to contain nucleic acid. Endonuclease and ribonuclease treatments and agarose gel electrophoresis indicated that the bands contained RNA of approximately 800 nucleotides. Extractions were performed with three additional batches of approximately 1000 adults each and similar results were obtained. Since all of the experiments produced similar results, we don't believe that the isolated RNAs are of virus origin. It appeared that the bands were composed of ribosomes and polyribosomes on the basis

of abundance, density, and size of the RNAs. We are currently processing larger numbers of adults (approx. 3,000-5,000) per batch and will use the currently identified bands as markers. We will also subject potentially virus-containing bands to scanning electron microscopy.

Egg masses from both collection trips were surface sterilized and dissociated in culture medium in order to generate primary cultures (Objective 2). Three out of 17 primary cultures established from the April egg masses and 1 of 11 primary cultures from the August egg masses have survived and are proliferating. The lower survival rate of the primary cultures from the egg masses collected in August may be partly due to the reduced amount of embryonic tissue (resulting from fewer eggs per egg mass). The following parameters appear to be the best for the establishment of GWSS primary cultures: the egg mass should contain at least 10 eggs (per 1.5 ml of medium) at an early stage of development (see Fig. 1A of Hirumi and Maramorosch, 1971); 30 second sterilization with 70% ethanol; aseptic dissociation with forceps in ExCell 401 medium (JRH Biosciences) supplemented with 20% fetal bovine serum (FBS) followed by gentle pipeting, and culture at 28ºC in darkness. Sterilization with formaldehyde (2%) and mild trypsinization (0.01%) appeared to be detrimental. LH, TC-100, IPL-41 and TNM-FH media each supplemented with 20% FBS were less optimal. At present, the GWSS primary cultures are mainly composed of fibroblast-like (Figure 2A), round (Figure 2B), and epithelial (Figure 2C) cells with a doubling time of 2-3 week.



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