

PROGRESS ON THE DEVELOPMENT OF A MONOCLONAL ANTIBODY SPECIFIC TO GLASSY-WINGED SHARPSHOOTER EGG PROTEIN: A TOOL FOR PREDATOR GUT ANALYSIS AND EARLY DETECTION OF PEST INFESTATION

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INTRODUCTION

Effective control of glassy-winged sharpshooter (GWSS) will require an integrated pest management approach. A major component of true integrated pest management is the exploitation of the pest's natural enemies, which, when utilized to their greatest potential, can also increase the effectiveness of chemical, mechanical, and cultural control. Unfortunately, very little information exists on predaceous enemies of GWSS. Evidence of predation on GWSS has been observed in the field (JRH, pers. obs.); however, the GWSS predator complex and its impact on GWSS mortality are unknown. A useful technique for identifying a pest's natural enemy complex is through the use of predator gut content immunoassays employing pest-specific antibodies (Greenstone 1996).

Over the past decade we have developed a library of MAbs specific to the egg stage of *Lygus hesperus*, *Pectinophora gossypiella*, and *Bemisia argentifolii* (Hagler et al. 1991, 1993, 1994) for use in studying egg and adult female predation in the field (Hagler et al. 1992; Hagler and Naranjo 1994a,b). Our MAB library provided an avenue to qualitatively identify and assess the impact of over a dozen predator species on populations of key insect pests; provided a quick, efficient, and cost effective technique for screening numerous predators in a conservation biological control program (Hagler & Naranjo, 1994a,b; Hagler, 2002); and provided a method to compare the efficacy of *in vitro*-reared predators with that of their wild counterparts in an augmentative biological control program (Hagler and Naranjo 1996).

Attempts to monitor GWSS populations and their natural enemies in Southern California are complicated by the presence of a native species of sharpshooter, the smoke tree sharpshooter (STSS), *Homalodisca lacerta*. The eggs of this species are virtually indistinguishable by the naked eye from GWSS eggs. Thus it is difficult to separate the relative rates of predation and parasitism of GWSS and STSS in areas where these two species overlap. The similarity also prohibits positive identification of GWSS eggs intercepted during quarantine inspections of plant shipments. A pest-specific MAb can be used to accurately identify pests that are difficult to differentiate visually. For example, Greenstone (1995) developed an egg-specific MAb diagnostic test that differentiates *Heliothis virescens* from *H. zea*. Pest control advisors have used this MAb in a squashblot immunoassay to rapidly and positively screen field collected eggs. Early detection of *H. virescens* infestations is critical for effective and environmentally sound pest management. A MAb specific to GWSS egg would be an invaluable tool for early monitoring of pest infestation and decision-making in pesticide application. To date, we have developed a series of antibodies specific to GWSS. In this report we describe the antibodies that are currently available for mass screening the GWSS predator complex.

OBJECTIVES

1. Develop a GWSS monoclonal antibody based enzyme-linked immunosorbent assay (ELISA) to:
 - a) Identify key predators of GWSS by analyzing their gut contents for GWSS remains.
 - b) Differentiate GWSS eggs from taxonomically and visually similar species.

RESULTS AND CONCLUSIONS

Parental Hybridoma Cell Lines:

Over a dozen parental GWSS hybridoma cell lines were screened by ELISA for reactivity against GWSS and STSS eggs, nymphs and adults as well as the adult or larval (lepidopterans) stage of 15 other insect species. The majority of cell lines were reactive to the GWSS and STSS egg stage. Additionally, 3 of the cell lines showed reactivity to the GWSS and STSS adult female lifestage. None of the hybridoma cell lines reacted to the other 15 insect species tested (Figure 1).

From the original GWSS hybridoma cell lines examined, 3 hybridomas were selected for additional cloning. The cell lines selected were 1D4, 6C4, and 6D5. These 3 cell lines were selected because: 1D4 only responded to the GWSS and STSS egg stage; 6C4 only responded to the GWSS and STSS egg and adult female stages; and 6D5 had a stronger reaction to the STSS egg stage than the GWSS egg stage (Figure 1). Additionally, each cell line yielded a weak response to the other insects tested. Sub-cloned cell lines 1D4-1D8 and 6D5-2H1 have been mass-produced and are now ready for use for screening potential predators of GWSS and STSS. We collected predators every other week (June through October) from three different locations in California. This winter we will assay them by sandwich ELISA for the presence of GWSS egg antigen.

The fact that these antibodies react to the egg stage of both species should not affect our predator evaluations because the sites we collected from did not contain STSS (see Objective 1). However, these antibodies will help us fulfill our second goal, that is, a MAb capable of differentiating GWSS from STSS. Next year we will select other potential parental cell lines (Figure 1) and clone them to try to obtain an antibody specific only to GWSS.

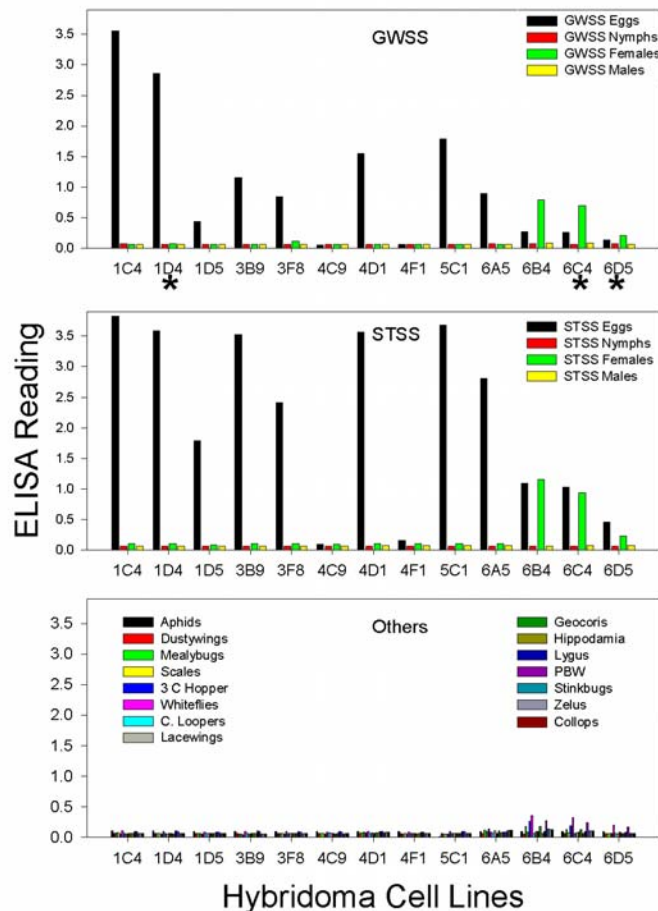


Figure 1. Parental hybridoma cell lines screened for reactivity against GWSS (top), STSS (middle), and other insect species (bottom). Those cell lines marked with an asterisk below them have been sub-cloned, screened for reactivity, and mass-produced.

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