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Biological Control 37 (2006) 108–118
Biological Control
www.elsevier.com/locate/ybcon

Development and application of a glassy-winged and smoke-tree sharpshooter egg-specific predator gut content ELISA ☆

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Received 6 July 2005; accepted 22 December 2005

Abstract

The recent invasion of southern California by the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), has triggered a statewide control effort. Management of GWSS will include biological control using resident and imported natural enemies. Currently, very little information is available on the role of generalist predators in suppression of GWSS eggs, nymphs or adults. We have developed a sharpshooter egg-specific monoclonal antibody (MAb) for use as a diagnostic tool for predator gut content analysis. The MAb was tested by an indirect enzyme-linked immunosorbent assay (ELISA) for specificity to the different life stages of GWSS, smoke-tree sharpshooter (STSS), *Homalodisca liturata* Ball (Hemiptera: Cicadellidae), and various life stages of 27 other arthropod species. We found that the MAb only reacted to the egg stage of both sharpshooters and, to a lesser extent, to the adult stage of gravid GWSS and STSS females. Moreover, the ELISA was more responsive to younger GWSS eggs than older ones. Laboratory trials were conducted to determine how long GWSS egg antigen remained detectable in the guts of the green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and the ladybird beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) using both an indirect and sandwich ELISA format. We found that GWSS egg antigen was detectable for up to 30 and 12 h in the guts of *C. carnea* and *H. axyridis*; respectively, and that the sandwich ELISA was much more sensitive than the indirect ELISA. Finally, 98 field-collected lacewings were examined for sharpshooter remains using our sharpshooter-specific sandwich ELISA. The assay detected sharpshooter egg antigen in 8.2% of the lacewings examined. This work represents a first step towards identifying the GWSS predator complex.

Keywords: ELISA; *Homalodisca coagulata*; *Homalodisca liturata*; *Harmonia axyridis*; *Chrysoperla carnea*; Leafhopper; Pierce's disease; Biological control; Predator–prey interactions; Gut content analysis; Monoclonal antibody

1. Introduction

The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), is a polyphagous pest native to the southeastern region of the United States. It was first reported in California in 1989 (Sorenson and Gill, 1996) and has since spread throughout southern California (Blua et al., 2001). GWSS feeds on the plant's xylem fluid and can acquire and transmit *Xylella fastidiosa* Wells (Xanthomonadales: Xanthomonadaceae)

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doi:10.1016/j.biocontrol.2005.12.015

(Redak et al., 2004). This xylem-limited bacterial pathogen is responsible for several devastating plant diseases in California such as Pierce's disease in grape (Almeida and Purcell, 2003a), almond leaf scorch (Almeida and Purcell, 2003b), and oleander leaf scorch (Costa et al., 2000). Another vector of *X. fastidiosa* is the smoke-tree sharpshooter (STSS), *Homalodisca liturata* Ball (Hemiptera: Cicadellidae). The STSS is native to California and a close relative to GWSS (Burks and Redak, 2003). Both species partially overlap in the host plants they share and in geographic ranges (Blua et al., 2001). However, while GWSS has been associated with epidemic outbreaks of Pierce's disease (Redak et al., 2004), STSS and other native leafhopper species that are known vectors of *X. fastidiosa* have only been associated with sporadic disease outbreaks (Purcell and Frazier, 1985). Moreover, Pierce's disease occurrence associated with the native leafhopper vectors have rarely been of economic significance because they are usually limited to localized portions, especially the edges of fields (Purcell and Frazier, 1985).

A multi-disciplinary research program is currently underway to develop new management techniques for *X. fastidiosa* epidemics in California (Tariq et al., 2004). A key component to this area-wide program will be to identify key natural enemies of the GWSS [National Research Council (NRC, 2004)]. To date, egg parasitoids, particularly *Gonatocerus* spp. (Hymenoptera: Mymaridae), are considered the most effective GWSS natural enemies and have been the subject of numerous studies (de León et al., 2004; de León and Jones, 2005; Irvin and Hoddle, 2005; Triapitsyn et al., 1998, 2003; Vickerman et al., 2004). However, very little effort has been expended on identifying key predators of GWSS (NRC, 2004).

Identifying the impact of insect predators can be challenging as they are usually small, elusive, nocturnal or cryptic. Direct visual field observations of predation are rare and often difficult to obtain. While predation studies using enclosures can provide some indication of predator impact, it fails to recreate natural conditions and can result in an overestimation of predation. This may be especially true for GWSS generalist predators because sharpshooter adults and nymphs are highly mobile and may easily escape predation in the field. Furthermore, GWSS may be unpalatable to some spider species, which would still catch GWSS in their webs or kill them under enclosed conditions (K. Daane, pers. obs). A more valid method to qualitatively identify predators of key pests in nature is by the molecular analysis of predator gut contents for pest remains (reviewed in Sheppard and Harwood, 2005; Symondson, 2002). The state-of-the-art predator stomach content analyses include both monoclonal antibody (MAb)-based enzyme-linked immunosorbant assays (ELISAs), which detect prey-specific proteins (Agustí et al., 1999; Greenstone and Morgan, 1989; Greenstone, 1996; Hagler et al., 1991, 1993, 1994; Schenk and Bacher, 2004; Symondson and Liddell, 1993), and polymerase chain reaction (PCR)-based assays, which detect prey-specific DNA (Agustí et al., 2003a,b; Chen et al., 2000; Hoogendoorn and Heimpel, 2001; Kasper et al., 2004; Zaidi et al., 1999). While pest-specific ELISAs have been used for over a decade to identify predators of agricultural pests (Hagler and Naranjo, 1994a,b, 2005; Hagler et al., 1992; Symondson et al., 1999), PCR-based techniques have only recently been implemented for gut content analysis of predators (reviewed in Sheppard and Harwood, 2005; Symondson, 2002). ELISA-based gut content assays possess important advantages over PCR-based assays. First, MAb-based ELISAs can be species and life stage-specific, which provides a higher level of precision to document predation (Hagler and Naranjo, 1996). Second, ELISA-based gut content assays are more suitable for screening large numbers of predators because they are less tedious, time consuming, and expensive than PCR-based assays once the pest-specific MAb has been developed (Chen et al., 2000).

Efficient molecular gut content analyses have important applications to the field of biological control. Because these methods are highly sensitive and rapid, they are powerful tools for acquiring crucial information needed to develop biological control programs targeting arthropod pests (e.g. Morris et al., 1999) or weeds (Bacher et al., 1999; Schenk and Bacher, 2004).

The main objectives of this study were to: (1) develop a MAb-based ELISA specific to GWSS egg protein, (2) determine how long GWSS egg antigen can be detected in the gut of green lacewings, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and ladybird beetles, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), and (3) examine a small number ($n = 98$) of field-collected lacewings by ELISA to determine the proportion of individuals feeding on GWSS eggs in nature.

2. Methods and materials

2.1. Antibody production

Hybridoma development was contracted out with Maine Biotechnology Services (Portland, ME). Five female mice (BALB/c, 10–12 weeks old) were immunized by intraperitoneal injections with crude GWSS egg protein (2.0 μg/ml). Each mouse received three booster injections every three weeks. The titer of each mouse sera was assayed by indirect ELISA (Hagler et al., 1991) to determine their response to GWSS egg antigen (ca. 2.0 μg/ml egg protein). The mouse yielding the highest immuno-response (1:2000-fold dilution) was selected for hybridoma fusion. Techniques leading to the production of hybridoma cell lines secreting antigen-specific MAbs were identical to those described by Hagler et al. (1991, 1994).

The screening of clones was conducted at the USDA-ARS, Western Cotton Research Laboratory (WCRL), Phoenix, Arizona, USA. A total of 50 supernatants of parental fused hybrid cells were examined by indirect ELISA. Single GWSS eggs were homogenized in 500 μl TBS buffer (Tris-buffered saline; pH 7.4). Fifty microliters of GWSS egg homogenate were placed in each well of a