ASSOCIATIVE LEARNING OF HOST-PLANT CHEMICAL STIMULI IN IMMATURE GLASSY-WINGED SHARPSHOOTERS

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ABSTRACT
Olfactory conditioning may provide the immature glassy-winged sharpshooter (GWSS) with a mechanism by which they can effectively track their host-plants in space and time. In the presence of vanilla scent, nymphs that ingested vanilla-flavored xylem fluid were significantly more attracted to a neutral visual target than were nymphs that had ingested unflavored xylem fluid. These results are consistent with our previous finding that GWSS response to visual stimuli is enhanced by exposure to host-plant odor.

INTRODUCTION
The nutritional requirements of immature glassy-winged sharpshooters (GWSS) constrain their diet to plants with low amide- and high amino acid concentrations in the xylem fluid (Brodbeck, et al. 1999). To obtain a balanced level of nutrients, nymphs may frequently need to switch host-plants (Brodbeck, et al. 1999, Redak et al. 2004). Locating host-plants that are physiologically-suitable with respect to providing adequate levels of xylem nutrients may require that the nymphs integrate information from several types of host-plant stimuli (Harris and Foster 1995). For example, nymph response to foliar colors (*sensu* Prokopy and Owens 1983, Tipping et al. 2004) is enhanced by the presence of host-plant volatiles (Patt & Sétamou 2006). The ability to learn to recognize stimuli associated with suitable host-plants would facilitate detection and location of host-plants whose distribution varies temporally and spatially within the nymphs' environment (Behmer et al. 2005, Pompilio et al. 2006).

The goals of our ongoing study are to determine whether nymphs can associatively learn to recognize olfactory stimuli produced by host plants, and, if so, to evaluate the relative importance of olfactory conditioning in host-plant recognition.

To provide nymphs for testing, second- to fourth instars were placed on cowpea (*Vicia unguiculata*) sprigs for 1.5 days. The cut-ends of the sprigs were immersed either in hydroponic solution containing a low concentration of vanilla extract, or, as a control, in hydroponic solution alone. After removal from the sprigs, the nymphs' responsiveness to a pale green disk in the presence of vanilla extract odor was tested in an olfactometer (Patt & Sétamou 2006) using no-choice tests. In preliminary tests with blank air, 44% of nymphs from the control group jumped to the pale green target, demonstrating that innate attraction to this color is low. An increased response to the pale green target in the presence of vanilla odor would indicate that the nymphs had developed an attraction to vanilla scent via their previous feeding on vanilla-flavored xylem fluid.

OBJECTIVES
1. Determine whether nymphs can associatively-learn to recognize olfactory stimuli produced by host plants.
2. Evaluate the relative importance of olfactory conditioning in host-plant recognition.

RESULTS
Vanilla extract constituents were detected by gas chromatography-mass spectrometry analysis of ethanolic extractions made from vanilla-treated cowpea sprigs.

Nymphs that fed on plant sprigs with vanilla-flavored xylem fluid were significantly more attracted to the pale green target than nymphs that fed on control sprigs with non-flavored xylem fluid (Figure 1). However, there was no difference between individuals in the experimental and control groups with respect to the amount of time they required to orient- and jump to the visual target (Figure 2).

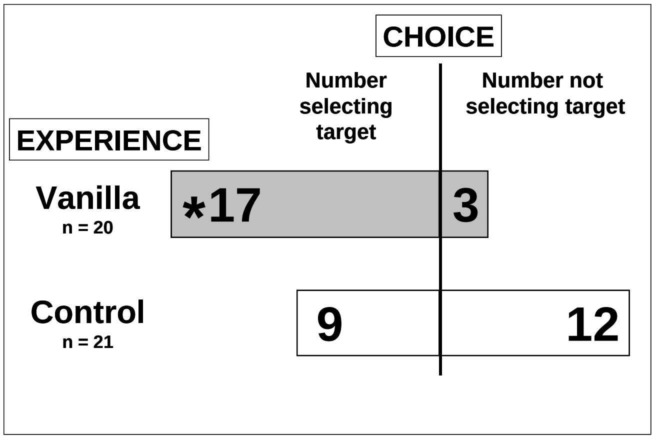

Figure 1. Results of no-choice assays showing the numbers of nymphs that either jumped to a pale green disk ('selecting target') or failed to jump to the disk ('not selecting target'). Nymphs had experience feeding on cowpea sprigs with their cut ends immersed either in hydroponic solution with vanilla extract ('Vanilla') or hydroponic solution alone ('Control'). Significant levels of choice within the same bar are indicated by * = $P \leq 0.01$ (G-test).

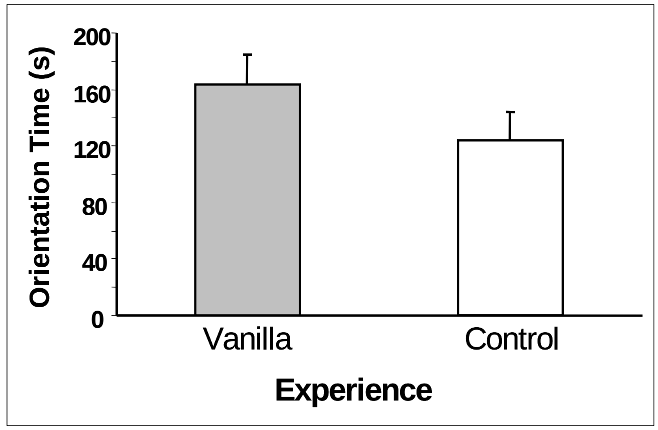

Figure 2. Mean time ($\pm$ SE) required by nymphs in each test group to detect and orient to a pale green target in the presence of vanilla scent. N = 17 nymphs in the vanilla group and 9 nymphs in the control group. Means are similar (T-test, P = 0.1933).

CONCLUSIONS

Nymph response to a non-attractive color was enhanced following ingestion of a novel flavor, indicating that immature GWSS are capable of olfactory conditioning. Rapid population growth of GWSS may depend on the close proximity of host plants suitable for successful juvenile development (Redak et al., 2004). Therefore, understanding the mechanisms by which nymphs locate their host-plants is fundamental to developing vegetation management programs aimed at suppressing their population growth and dispersal in complex landscapes. A manuscript describing the design of the behavioral assay in more detail and the results of ongoing experiments will be submitted for publication in the near future.

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EVALUATION OF BLUE-GREEN SHARPSHOOTER FLIGHT HEIGHT

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ABSTRACT

Flight heights of blue-green sharpshooters (BGSS) were monitored in Napa Valley vineyards from March through September for three seasons (2004-2006) using pole towers to position yellow sticky panel traps at heights up to 24 feet. Towers were located adjacent to vineyards at the edge of a riparian zone. Eleven towers were monitored in 2004 and 2006; twelve were monitored in 2005. Trap catches in 2004 were considerably greater than in 2005 and 2006. For the March-May period, 76-99% of the catches were made at 15 feet or lower. These data support the possible use of screen or natural barriers to reduce the number of BGSS entering vineyards in the spring.

INTRODUCTION

Where the blue-green sharpshooter, *Graphocephala atropunctata,* (BGSS) is the primary vector of Pierce's disease (PD), control measures should be aimed at reducing the number of BGSS entering vineyards (Goodwin and Purcell 1992), especially early in the growing season. Early-season infections (March-May) are responsible for most chronic cases of PD vectored by BGSS (Purcell 1975, 1981). Infections resulting from BGSS feeding later in the growing season are not likely to result in PD because most will be eliminated with normal pruning. This is unlike the situation with PD caused by glassy-winged sharpshooter (GWSS) feeding, where chronic infections may occur nearly year-round (Almeida and Purcell 2003).

Vector control measures in the North Coast include the use of insecticides (Goodwin and Purcell 1992) as well as management of riparian plant communities to reduce the number of favorable BGSS breeding host plants (Insley, E., et al. 2000).

Another method of reducing vector numbers is to block their flight into vineyards through the use of physical barriers. This could include the use of tall fences made with insect screening materials, as well as natural barriers created by planting dense stands of conifers or other non-host tree species. Both of these approaches are already being employed in a few vineyards in the North Coast, although there are currently no data to show their impacts. The use of barriers has also been suggested as a management tactic to keep GWSS out of vineyards (Blua and Morgan 2003).

For barriers to be effective, they would need to block the majority of BGSS from entering vineyards, since small numbers of insects can still lead to significant disease development (Purcell 1979). Unfortunately, little is known about the overwintering behavior of BGSS and its preferred winter plant hosts (Purcell 1976). Therefore, it is not clear how tall a barrier would need to be in order to be effective. Most trapping by both researchers and growers has been done from the ground at the 5-6 foot level.

This project addresses the question of BGSS flight height by installing and monitoring pole towers that can accommodate yellow sticky panel trapping up to a height of approximately 24 feet.

OBJECTIVES

1. Evaluate the predominant flight height of BGSS entering vineyards from adjacent riparian habitats through the use of yellow sticky panel traps positioned at heights from 5 to 24 feet.

RESULTS

Eleven pole towers were installed and monitored in the Napa Valley in 2004 and 2006; twelve towers were monitored in 2005. Two of the towers monitored in 2004 were not used in 2005 due to the low number of BGSS trapped at those locations. Three additional towers were installed in 2005. One tower used in 2004 and 2005 was not used in 2006 due to low trap counts. Eight towers were monitored in the same locations in all three seasons. Tower locations covered a distance of approximately 25 miles from the Carneros region in southern Napa County to the outskirts of Calistoga at the north end of Napa Valley. Towers were positioned along riparian zones adjacent to vineyards that had a history of PD.

A diagram of a pole tower is shown in Figure 1. Towers were 25 feet in height, constructed from Schedule 40 PVC pipe with a pulley at the top and a rope running through it. Yellow sticky panel traps were attached to clips on the rope at the following heights: 24 feet, 20 feet, 15 feet and 10 feet. An additional trap at 5 feet was clipped to a metal stake mounted in the ground.