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A Novel Method to Induce Oviposition of the Glassy-Winged Sharpshooter (Hemiptera: Auchenorrhyncha: Cicadellidae)[1]

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J. Entomol. Sci. 40(2): 246–249 (April 2005)

Key Words vector, induced oviposition, insect culture

The glassy-winged sharpshooter, *Homalodisca coagulata* (Say), has a native distribution that includes the southeastern US and northern Mexico (Turner and Pollard 1959, USDA Tech. Bull. 1188. 28 pp.). The accidental introduction of *H. coagulata* into regions of California, Tahiti and Hawaii has created great concern because it is a vector of the xylem-limited bacterium *Xylella fastidiosa* Wells et al. This bacterium is the causal agent for many economically-important plant diseases including phony peach, Pierce's disease of grape, plum leaf scald, citrus variegated chlorosis and several other diseases termed leaf scorches (Hopkins and Purcell 2002, Plant Dis. 86: 1056-1066). A classical biological control program using inundative releases of large numbers of egg parasitoids in the genus *Gonatocerus* (Hymenoptera: Mymaridae) in an effort to manage introduced populations of *H. coagulata* has been initiated in California (Jones 2001, Proc. Pierce's Disease Res. Symp. December 5-7, California Department of Food and Agriculture, pp. 50-51.). Maintenance of large cultures of *H. coagulata* for egg production essential for the mass rearing of *Gonatocerus* parasitoids is difficult and time consuming because few host plant species adequately and efficiently support all life stages of *H. coagulata* (Brodbeck et al. 2004, Environ. Entomol. 33: 165-173).

Female *H. coagulata* oviposit by inserting their eggs in masses into leaf tissue, although we have also observed egg masses on stems and fruits of several species of plants including okra (*Hybiscus esculentus* L.) and cotton (*Gossypium hirsutum* (L.). In the summer of 2000 during collecting trips required to augment our cultures of *H. coagulata*, we observed the majority of the newly collected females ovipositing on the leaves of holly plants in the holding cage after transportation from the field by automobile. Oviposition behavior of caged females has been observed by us and others (Hix 2001, Calif. Ag. July/August: 19-23) to occur during daylight hours; however, this behavior has yet to be observed in field populations (R. Mizell, pers. obs.). The occurrence of this oviposition behavior by the high proportion of newly collected

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gravid females led to investigations into the effects of stress on the oviposition behavior of *H. coagulata* as a means to manipulate their reproductive output.

Twenty gravid female *H. coagulata* were field-collected from crape myrtle, *Lagerstroemia indica* L., using a sweep net on six different days between the dates of 1 April and 3 June 2004. Females were judged gravid if their abdomens were swollen and distended. Ten females were randomly chosen from those collected and placed immediately into a wooden-framed cage covered with nylon screen (15 × 15 × 58 cm) that was provisioned with one 3-w old cotton plant, (*Gossypium hirsutum* (L.) 'Deltapine 88'), or glabrous soybean, (*Glycine max* (L.) 'D90-9216'), plant. The remaining ten females were placed into a clear plastic tube (30.5 × 3.8 cm) capped with nylon screen. A commercial hair dryer (Conair Corp. East Windsor, NJ 08529, mod. #0941) was used to direct a flow of warm air (40°C, 5.0 mps) through the tube for 15 min (Fig 1). Airflow through the tube was measured with a wind speed indicator (Turbo Meter, Davis Instruments, Hayward, CA). Preliminary tests indicated that airflow temperatures above 40°C were lethal to leafhoppers after only a few minutes of exposure (data not shown). After airflow treatment, females were placed into a wooden cage with either of the two plant species as described previously. Cages of treated and untreated females were placed in a laboratory (25°C, 35% RH) with ambient photoperiods ranging from 12.5:11:5 LD to 14:10 LD corresponding to 1 April and 3 June 2004, respectively, at the North Florida Research and Education Center (N 30.5, W 84.6). Plants were examined for egg masses the next morning at 1000 h (EST). Females were collected from both cages and dissected to quantify numbers of mature, chorionated oocytes in the lateral and or median oviducts. Each paired test with treated and untreated female *H. coagulata* using either cotton or soy plants for an oviposition substrate, was conducted three times. Host plant effects on oviposition

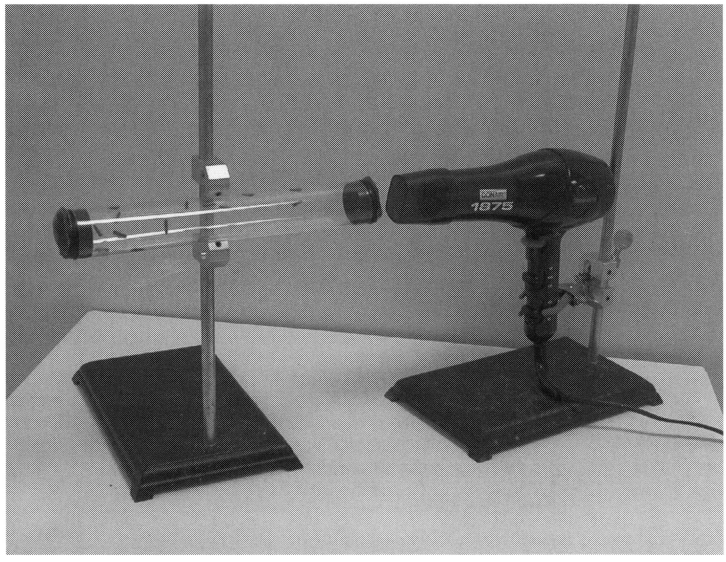

Fig. 1. Airflow apparatus used to induce oviposition in gravid female *H. coagulata*.

were analyzed by ANOVA (SAS Institute 1999, version 8.0, SAS Institute, Cary, NC) with total eggs per plant as the experimental unit, as we could not accurately quantify eggs deposited per individual female because of the unknown quantity of eggs within each gravid female prior to each test as well as which females were responsible for oviposited eggs. Paired t-tests were used to compare the differences between the number of eggs oviposited, number of mature chorionated oocytes not oviposited and total eggs between cohorts of treated and untreated females.

Host plant had no effect on the number of eggs oviposited by treated ($F = 0.84$; df = 1, 4 $P < 0.42$) or untreated females ($F = 0.03$; df = 1, 4 $P < 0.88$). Paired t-test analysis showed that field-collected, gravid *H. coagulata* oviposited a significantly higher proportion of their compliment of eggs following warm air treatment when compared to untreated females (Table 1). Targeted dissections of the ovaries indicated that females treated with the warm airflow had fewer chorionated oocytes within their reproductive structures than females that were not treated (Table 1). Examination of the ovarioles of all females revealed a range of 0 to 24 mature chorionated oocytes, indicating that some gravid females oviposited their entire compliment of chorionated eggs.

Although the explanation for this inducible oviposition by *H. coagulata* has not been fully investigated and was not the goal of this study, we hypothesize that this phenomenon occurs due to stress caused by rapid and excessive desiccation from exposure to the flow of warm dry air. High temperatures alone are not responsible for induction of oviposition in gravid *H. coagulata*. These insects survive and reproduce by feeding solely on xylem fluid, which is 99% water (Andersen et al. 1989, Entomol Exp. Appl. 50: 148-159). Culture cages of *H. coagulata* within non-air-conditioned green houses often reach temperatures of 45°C for 5 h per day during the summer months in north Florida without noticeable increases in mortality of adults or nymphs from cages normally maintained in greenhouses with indoor temperatures of 30°C. When removed from access to xylem tissue or other sources of water, adult *H. coagulata* do not survive more than 24h at 28°C, 45% RH (Tipping, pers. obs.).

DeCoursey and Webster (1952, J. Econ. Entomol. 45: 1030-1034) first reported the phenomenon of stress-induced oviposition in gravid female mosquitoes, *Ochlerotatus sollicitans* (Walker). A variety of chemical agents, including pesticides, caused female mosquitoes to oviposit more eggs than untreated controls. Other examples of insects that increase their oviposition following exposure to stress include the the Angoumois grain moth, *Sitotroga cerealella* (Oliver), and western corn rootworm, *Diabrotica virgifera virgifera* LeConte (DeCoursey and Webster 1952, Mabry and Spenser 2003, Entomol. Exp. Appl. 109: 113-121).

Table 1. Means (±SE) of number of eggs oviposited and retained by gravid *H. coagulata* after treatment with warm air flow. Values within rows followed by different letters are significantly different; $P < 0.05$. n = six replications

	Treated	Untreated	Pr > |t|
Proportion of eggs oviposited	54.4 ± 4.4a	28.2 ± 5.3b	0.002
Eggs oviposited per female	13.7 ± 2.3a	5.9 ± 1.2b	0.015
Total oviposited + mature oocytes	194 ± 18.9a	187.2 ± 17.1a	0.696

Hypothetically, insects that oviposit their remaining eggs as they are dying could potentially maximize their reproductive output. Stress in insects can be induced by a variety of conditions including temperature extremes, malnutrition, and desiccation. We propose that the behavior of *H. coagulata* to oviposit when stressed by desiccation is a strategy to maximize reproductive potential because females are unable to reabsorb mature chorionated oocytes (Tipping et al. 2004, Proc. Pierce's Disease Res. Symp. December 7-10, California Department of Food and Agriculture, pp. 150-152). In normal field conditions, *H. coagulata* oviposits on an extremely diverse variety of host plants including many that are not suitable for successful development of the immatures (Brodbeck et al. 1996, Arch. Ins. Physiol. Biochem. 32: 65-83). A broad ovipostional host range may not necessarily be disadvantageous to neonates, as we have documented adaptations that allow immature *H. coagulata* to efficiently relocate to suitable hosts (Tipping et. al. 2004, Florida Entomol. 87: 372-379). Stress-induced oviposition thus appears consistent with both the reproductive physiology and the nutritional ecology of *H. coagulata* due to the inability of females to reabsorb oocytes and the high vagility of immatures.

The capability to induce oviposition in gravid *H. coagulata* by stress with an ordinary inexpensive commercial hair dryer can be a valuable tool for researchers interested in obtaining large numbers of uniform-aged eggs. Use of this method to induce oviposition provides researchers with a tool to acquire a greater amount of eggs from each female collected, regardless of the initial number of mature ova within the reproductive system. This technique also can be useful for sentinel field sampling for the presence of *Gonatocerus* parasitoids as well as for initiation and maintenance of cultures of *Gonatocerus* parasitoids. Finally, collection of many egg masses in a short period of time may also be instrumental in the creation or augmentation of existing cultures of *H. coagulata*.

In summary, gravid *H. coagulata* females were induced into ovipositing a significantly greater proportion of their eggs 24 h after treatment with a directed flow of warm air (40°C, 5.0 mps) for 15 min than untreated females. Treated and untreated females oviposited 54.5% and 28.2% of their eggs, respectively, regardless of host plant.

The authors thank Morgan Kalberlow, Terry Riddle and Tim Jones for assistance with this project. The California Department of Food and Agriculture as well as the University of California, Davis provided funding for this research. Contribution of the Florida Agricultural Experiment Station Journal Series number R-10335.