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Authors: Nicola A. Irvin, and Mark S. Hoddle
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OVIPOSITION PREFERENCE OF *HOMALODISCA COAGULATA* FOR TWO *CITRUS LIMON* CULTIVARS AND INFLUENCE OF HOST PLANT ON PARASITISM BY *GONATOCERUS ASHMEADI* AND *G. TRIGUTTATUS* (HYMENOPTERA: MYMARIDAE)

NICOLA A. IRVIN AND MARK S. HODDLE
Department of Entomology, University of California, Riverside, CA 92521, U.S.A.

ABSTRACT

Oviposition preference of *Homalodisca coagulata* (Say) and two of its mymarid egg parasitoids, *Gonatocerus ashmeadi* Girault and *G. triguttatus* Girault, for two *Citrus limon* L. cultivars (‘Eureka’ and ‘Lisbon’) was investigated. In laboratory oviposition choice tests, the number of leaves containing *H. coagulata* egg masses, the number of *H. coagulata* egg masses, and the total number of *H. coagulata* eggs were significantly higher at 187.2%, 204.2%, and 181.7%, respectively, on ‘Eureka’ versus ‘Lisbon’. In the field, there was no significant difference in the number of *H. coagulata* motiles counted in five-minute searches of foliage on ‘Eureka’ and ‘Lisbon’ trees, and numbers of leaves with old (emerged) and new (unemerged) *H. coagulata* egg masses were equivalent between field-planted cultivars. In the laboratory, parasitoid colony oviposition preference for *H. coagulata* egg masses by *G. ashmeadi* and *G. triguttatus* was 18.6% and 23.2% higher, respectively, for eggs laid on ‘Eureka’ leaves compared to ‘Lisbon’, but these differences were not significant. Leaf surface morphology and thickness of leaf cell layers of both lemon cultivars were compared with scanning electron microscopy (SEM). SEM demonstrated that total leaf thickness and the thickness of the palisade layer was 19.2% and 38.6% higher, respectively, in ‘Eureka’ leaves compared to ‘Lisbon’, and that *H. coagulata* egg placement was between the lower epidermis and spongy parenchyma layer for both cultivars. Furthermore, ‘Lisbon’ leaves had a smooth underside, whereas ‘Eureka’ leaves had many small ridges. The thickness and rough surface of ‘Eureka’ leaves may be beneficial for *H. coagulata* oviposition. However, additional research is required to further investigate whether leaf characteristics or xylem chemistry are responsible for *H. coagulata* oviposition choice. For mass rearing programs with lemons as host plants, it is recommended that the ‘Eureka’ cultivar be used in preference to ‘Lisbon’ because *H. coagulata* prefers this cultivar for oviposition and parasitoid foraging is not adversely affected.

Key Words: *Homalodisca coagulata*, Hemiptera, Cicadellidae, Hymenoptera, Mymaridae, oviposition preference, host plant influence, *Citrus limon*.

RESUMEN

La preferencia para la oviposición de *Homalodisca coagulata* (Say) y dos de sus parasitoides de huevos mymaridos, *Gonatocerus ashmeadi* Girault y *G. triguttatus* Girault, en dos variedades de *Citrus limon* L. (‘Eureka’ y ‘Lisbon’) fue investigada. En pruebas del laboratorio donde pueden escoger el lugar de la oviposición, el número de las hojas que tuvieron masas de huevos de *H. coagulata*, el número de masas de huevos de *H. coagulata*, y el número total de huevos de *H. coagulata* fueron significativamente más altos a 187.2%, 204.2%, y 181.7%, respectivamente, en la variedad ‘Eureka’ versus ‘Lisbon’. En el campo, no hubo una diferencia significativa en el número de *H. coagulata* móviles contados en una búsqueda de 5 minutos del follaje de las variedades de ‘Eureka’ y ‘Lisbon’ y el número total de hojas con masas de huevos de *H. coagulata* viejas (emergidas) y nuevas (no emergidas) fueron equivalentes entre las variedades sembradas en el campo. En el laboratorio, el parasitismo de las masas de huevos de *H. coagulata* por *G. ashmeadi* y *G. triguttatus* fue 18.6% y 23.2% más alto, respectivamente, para los huevos puestos en hojas de ‘Eureka’ comparado con ‘Lisbon’, pero estas diferencias no fueron significativas. La morfología de la superficie de la hoja y el grosor de las tapas de células de la hoja de ambas variedades de limón fueron comparados con un microscopio electrónico de barrido (SEM). El SEM demostró que el grosor total de la hoja y el grosor de la capa empalizada fue 19.2% y 38.6% más alto, respectivamente, en hojas de ‘Eureka’ comparado a las hojas de ‘Lisbon’ y que la postura de los huevos de *H. coagulata* fue entre la epidermis menor y la capa del parénquima esponjoso para ambas variedades. Además, las hojas de ‘Lisbon’ tienen el envés liso, mientras que el envés en las hojas de ‘Eureka’ tienen un gran número de pequeñas estrías. El grosor y la superficie áspera de las hojas de ‘Eureka’ puede ser benéfico para la oviposición de *H. coagulata*. Sin embargo, se requiere de investigación adicional para saber si las características de la hoja o la química del xilema son responsables para la selección del sitio de oviposición de *H. coagulata*. Para los programas...
The glassy-winged sharpshooter, Homalodisca coagulata (Say) (Hemiptera: Cicadellidae), is native to southeastern U.S.A. and northeastern Mexico, and has become a significant threat to agricultural and ornamental industries due to its ability to spread the plant pathogenic bacterium, *Xylella fastidiosa* Wells et al. (Hopkins & Adlerz 1988; Purcell & Saunders 1999; Purcell & Feil 2001; Hopkins & Purcell 2002; CDFA 2003; Hoddle et al. 2003). A major classical biological control program has been launched in California against *H. coagulata*, including the mass rearing and release of two mymarid egg parasitoids, *Gonatocerus ashmeadi* Girault and *G. triguttatus* Girault. *Citrus limon* L. (lemon) has been chosen as a host plant for *H. coagulata* rearing and related experiments because it is one of the preferred hosts for *H. coagulata* in southern California and research on this pest is predominately conducted in agro-ecosystems where citrus dominates (Perring et al. 2001). However, several lemon cultivars exist, and two, ‘Eureka’ and ‘Lisbon’, are the most common commercial cultivars. It has been well documented that leafhoppers demonstrate significant oviposition preferences between plant species and between cultivars of the same species (McClure 1980; Stiling 1980; Singh & Agarwal 1988; Catindig et al. 1996; Sharma & Singh 2002). However, when this work was conducted it was not known which cultivar of *C. limon* *H. coagulata* preferred for oviposition under artificial rearing conditions. Consequently, the research undertaken here sought to determine the oviposition preference of *H. coagulata* for two *C. limon* cultivars, ‘Eureka’ and ‘Lisbon’, and whether *G. ashmeadi* and *G. triguttatus* exhibited preferences for *H. coagulata* eggs laid on ‘Eureka’ or ‘Lisbon’ leaves. To assist with interpretation of preference data collected from the laboratory, we conducted field surveys of *H. coagulata* life stages on ‘Eureka’ and ‘Lisbon’ lemons and compared leaf surface characteristics and measured leaf cell layers of both cultivars with scanning electron microscopy (SEM).

**Materials and Methods**

*Homalodisca coagulata* Oviposition Preference in the Laboratory

‘Lisbon’ and ‘Eureka’ trees approximately two years of age and grafted to *Macrophylla* sp. rootstock were obtained from C & M Nurseries, Nipomo, CA. Five trees of each cultivar were pruned to 60 cm in height (mean no. leaves per tree = 43 ± 5.7 for ‘Eureka’ and 37 ± 8.4 for ‘Lisbon’). The trees were potted into 4-litre containers and fertilized every two weeks with Miracle-Gro (20 ml/3.5 liters of water, Scotts Miracle-Gro Products Inc., Marysville, OH). Each of five cages (30 × 60 × 35 cm) received one tree of each cultivar in a randomized design. Cages with trees were held in a greenhouse at 26° ± 2°C and 30-40% RH under natural light. Forty female and ten male *H. coagulata* were collected from the field and placed into cages holding experimental plants. Every two days, approximately 25 field collected female and five male *H. coagulata* were added to each cage. The number of leaves with *H. coagulata* eggs, the number of egg masses, and the number of eggs laid on each cultivar were recorded daily. This study was conducted over the period May 10-24, 2001. Each combination of date and replicates was treated as a block, and a paired comparison *t*-test at the 0.05 level of significance was applied to the data in SAS (SAS 1990).

*Homalodisca coagulata* Oviposition Preference in the Field

A five-minute search for *H. coagulata* eggs on foliage 1-2 m above the ground was conducted on six ‘Eureka’ and six ‘Lisbon’ trees planted as part of a completely randomized block design variety trial at the University of California, Riverside Agricultural Operations Area. Surveyed trees were 17 years of age and cultivar scions were grafted to *Macrophylla* sp. rootstock. Old (emerged) and new (unemerged) *H. coagulata* egg masses found during time searches were harvested from trees and placed in labeled plastic bags. Additionally, a one-minute search for adult *H. coagulata* and nymphs was conducted on foliage within a 1-2 m band above ground around the tree for each replicate.

For collected leaves with emerged eggs, the number of *H. coagulata* egg masses, *H. coagulata* eggs per egg mass, emerged *H. coagulata* nymphs, solitary parasitoid emergence holes, unemerged nymphs and parasitoids, and ‘unemerged unknowns’ (those that could not be identified) were recorded for each lemon cultivar. Leaves with unemerged egg masses were held at 26° ± 2°C and 30-40% RH under a L14:10D photoperiod in 130-ml plastic vials (40-dram Plastic Vial, Thornton Plastics, Salt Lake City, UT) filled with deionized water and 3 ml of antiseptic ([Listerine Antiseptic Mouthwash, Pfizer Inc., New York, NY] (to prevent bacterial rot)) for two weeks to allow *H. coagulata* nymphs and parasitoids to emerge before recording data. This sampling and rearing protocol was repeated every two weeks over the period July 12-October 18, 2001. The total number of
H. coagulata (adults and nymphs) counted on cultivars and the number of leaves with H. coagulata egg masses was log transformed prior to analysis. Data were compared between cultivars by two-way ANOVA in SAS (SAS 1990). For emerged egg masses, percentage parasitism (the number of solitary parasitoid emergence holes + the number of unemerged parasitoids)/(number of H. coagulata eggs) × 100, percentage nymphs (the number of emerged H. coagulata nymphs + unemerged nymphs)/(number of eggs) × 100, and percentage unknowns [(unknowns/number of eggs) × 100] were calculated. The number of H. coagulata eggs was square-root transformed, and egg, H. coagulata nymph, parasitism, and unknown data were compared between cultivars by two-way ANOVA. For unemerged egg masses, percentage parasitism, percentage nymphs and percentage unknowns were calculated as above. The number of H. coagulata eggs was square-root transformed, and all data were compared between cultivars by Friedman’s χ² test. All statistical tests were performed in SAS and means presented here are back-transformed.

Gonatocerus ashmeadi and G. triguttatus Oviposition Preference

Parasitoid colonies were maintained at the University of California, Riverside at 26° ± 2°C and 30-40% RH under a L14:10D photoperiod in cages (50 × 40 × 40 cm) on H. coagulata eggs laid on ‘Eureka’ leaves. Colonies were provisioned with honey-water solution (3:1 Natural uncooked honey, Wild Mountain Brand, Oakland CA). Harvested H. coagulata egg masses on excised leaves were removed from colonies and checked daily for parasitoid emergence to assure uniform age for choice experiments. One ‘Eureka’ and ‘Lisbon’ leaf containing approximately 15 H. coagulata eggs (~24-48 h of age) per leaf were placed through holes in a lid of a 130-ml plastic vial filled with deionized water and 3 ml of antiseptic. A second 130-ml plastic vial with ventilation (three 2-cm holes in a lid of a 130-ml plastic vial filled with deionized water and 3 ml of antiseptic) was inverted and attached to the lid of the vial holding the water and leaves. One newly emerged mated naïve female parasitoid (~24 h old) was placed inside the inverted vial that covered the test material and left for 1 h to forage and oviposit at 26°C ± 2°C and 30-40% RH under 1.2 ± 0.2 log lumens/sqm light before being removed. This set up was replicated 15 times for G. ashmeadi and G. triguttatus. Vials containing leaves with egg masses exposed to parasitoids were held at 26°C ± 2°C and 30-40% RH under a L14:10D photoperiod for three weeks to allow parasitoids to emerge. Percentage parasitism [(the total number of emerged and unemerged parasitoids/total number of H. coagulata eggs) × 100] was calculated for each ‘Eureka’ and ‘Lisbon’ leaf. Females that were not mated (producing male only progeny) were excluded from the analysis. Data were transformed by square root and arcsine transformation and compared between ‘Eureka’ and ‘Lisbon’ cultivars by paired t-tests at the 0.05 level of significance in SAS (SAS 1990). Means presented here are back-transformed.

Investigation of Leaf Characteristics with SEM

One ~2-mm wide leaf section was cut from the middle of each of 10 fully expanded ‘Eureka’ and ‘Lisbon’ leaves (fifth leaf down from the growing tip of branches from containerized trees) with a new razor blade and fixed in 2% gluteraldehyde in 0.1 M phosphate buffer for 2 h. Leaf pieces were washed in buffer and post fixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 2 h. Leaf pieces were then washed in distilled water and dehydrated in an ethanol series and critical point dried. Prepared leaf pieces were mounted on SEM stubs and coated with gold-palladium. Photographs taken at ~800 × magnification for each of the 10 samples for each cultivar of the vertical distribution of leaf cell layer were used to calculate mean thickness of the waxy cuticle, epidermis, palisade layer, and spongy parenchyma layer. Leaf parameters were compared between cultivars by two sampled t-test in SAS (SAS 1990). In addition, SEM was used to examine and photograph the intact leaf surfaces. Finally, ‘Eureka’ and ‘Lisbon’ leaves with H. coagulata egg masses (sourced from the “H. coagulata oviposition preference laboratory trial” above) were prepared for SEM as previously described, and examined to determine where the placement of eggs under the leaf cuticle on the underside of leaves occurred.

RESULTS

Homalodisca coagulata Oviposition Preference in the Laboratory

In the paired choice studies, the number of leaves containing H. coagulata egg masses, the number of H. coagulata egg masses, and the total number of H. coagulata eggs were 187.2%, 204.2% and 181.7% greater, respectively, on ‘Eureka’ trees compared with ‘Lisbon’ (leaves: t = 4.95, df = 76, P < 0.005; egg masses: t = 4.99, df = 76, P < 0.005; total eggs: t = 4.38, df = 76, P < 0.005) (Fig. 1).

Homalodisca coagulata Oviposition Preference in the Field

There was no significant difference in the total number of H. coagulata nymphs and adults and the number of leaves with H. coagulata egg masses between ‘Eureka’ and ‘Lisbon’ cultivars (Table 1). For emerged egg masses in the field, the number of
Table 1. Mean Numbers of Homalodisca coagulata Egg Masses, and Percentage Nymph and Percentage Parasitoid Emergence from H. coagulata Eggs Collected from Field Planted 'Eureka' and 'Lisbon' Lemon Trees.

<table>
<thead>
<tr>
<th>Mean variable/tree/sampling event</th>
<th>'Eureka' ± SEM</th>
<th>'Lisbon' ± SEM</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of H. coagulata nymphs and adults</td>
<td>30.9 ± 1.6</td>
<td>33.0 ± 2.2</td>
<td>$F = 0.96, df = 18, P = 0.33$</td>
</tr>
<tr>
<td>Number of old and new leaves selected</td>
<td>21.0 ± 1.0</td>
<td>24.0 ± 1.1</td>
<td>$F = 3.86, df = 18, P = 0.06$</td>
</tr>
<tr>
<td>Emergence from egg masses in the field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of old masses</td>
<td>31.1 ± 2.0</td>
<td>35.1 ± 2.1</td>
<td>$F = 1.87, df = 12, P = 0.18$</td>
</tr>
<tr>
<td>Number of old eggs</td>
<td>196.5 ± 13.9</td>
<td>212.8 ± 12.9</td>
<td>$F = 1.04, df = 12, P = 0.31$</td>
</tr>
<tr>
<td>Percentage parasitism</td>
<td>42.8 ± 2.4</td>
<td>37.8 ± 2.1</td>
<td>$F = 2.34, df = 12, P = 0.13$</td>
</tr>
<tr>
<td>Percentage nymphs</td>
<td>26.5 ± 2.0</td>
<td>32.4 ± 1.7</td>
<td>$F = 5.53, df = 12, P &lt; 0.05$</td>
</tr>
<tr>
<td>Percentage unknowns</td>
<td>16.8 ± 1.2</td>
<td>19.5 ± 1.3</td>
<td>$F = 2.39, df = 12, P = 0.13$</td>
</tr>
<tr>
<td>Emergence from egg masses in the lab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of new masses</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>$\chi^2 = 1.29, df = 1, P = 0.26$</td>
</tr>
<tr>
<td>Number of new eggs</td>
<td>8.0 ± 1.5</td>
<td>7.6 ± 1.5</td>
<td>$\chi^2 = 0.18, df = 1, P = 0.67$</td>
</tr>
<tr>
<td>Percentage parasitism</td>
<td>53.9 ± 7.6</td>
<td>48.9 ± 6.8</td>
<td>$\chi^2 = 1.29, df = 1, P = 0.26$</td>
</tr>
<tr>
<td>Percentage nymphs</td>
<td>5.8 ± 3.8</td>
<td>0.0 ± 0.0</td>
<td>$\chi^2 = 3.00, df = 1, P = 0.08$</td>
</tr>
<tr>
<td>Percentage unknowns</td>
<td>37.2 ± 7.6</td>
<td>43.0 ± 7.1</td>
<td>$\chi^2 = 0.14, df = 1, P = 0.71$</td>
</tr>
</tbody>
</table>
The reasons for this demonstrated *H. coagulata* oviposition preference are unknown. Scanning electron microscopy (SEM) showed that the underside of ‘Lisbon’ leaves was smooth, whereas ‘Eureka’ had many small ridges on the cuticle that may provide female *H. coagulata* with enhanced tarsal grip during oviposition. However, the depth of these ridges was not quantified. Results also showed that the thickness of the palisade layer was significantly higher (38.6%) for ‘Eureka’ leaves compared to ‘Lisbon’. A thicker leaf structure may be more favorable for *H. coagulata* oviposition.

Female *H. coagulata* preferentially oviposit on lower leaf surfaces, and SEM showed that egg placement occurs between the lower epidermis and spongy parenchyma layer for both lemon cultivars. A thick palisade layer, which is positioned at the top of the leaf, may afford some protection of *H. coagulata* eggs from adverse conditions. For example, solar radiation falls onto the upper leaf surfaces so a thicker palisade layer may insulate eggs from excessive sun exposure. However, the experimental design used in the current study did not standardize ‘Eureka’ and ‘Lisbon’ trees to possess equivalent numbers of leaves, or surface areas, which may have influenced oviposition preference by *H. coagulata* because of varying light intensity. Other factors that influence leafhopper oviposition preference, such as plant chemistry, leaf vein characteristics, number of branches, and trichome densities (Singh & Agarwal 1988; Lit & Bernardo 1990; Denno & Roderick 1991; Andersen et al. 1992; Sharma & Sharma 1997; Sharma et al. 1999; Sharma & Singh 2002) were not investigated in this study and may warrant further research if it becomes necessary to explain in detail factors influencing *H. coagulata* oviposition preferences.

Field results showed that there were no significant differences in the total number of *H. coagu-

lata* nymphs and adults, and the number of leaves with *H. coagulata* egg masses between ‘Eureka’ and ‘Lisbon’ cultivars. Consequently the oviposi-
tion preference for ‘Eureka’ demonstrated in the laboratory was not confirmed in the field. In fact, field results showed that significantly more (5.9%) H. coagulata nymphs successfully emerged from ‘Lisbon’ leaves compared with ‘Eureka’ (leaf thickness of field-planted trees was not investigated in this study). The lack of congruence between laboratory and field studies may be due to differences in environmental factors and tree age between field-planted (~17 years of age) and small containerized trees (~2 years of age). Environmental aspects, such as sunlight, water, soil and nutrients, may affect leaf characteristics and influence host oviposition and utilization (DeReffye et al. 1995), while xylem chemistry varies drastically with plant age and phenology (Andersen et al. 1992, 1995a,b). It has been demonstrated that H. coagulata is very sensitive to changes in nutritional quality (Brodbeck et al. 1990, 1999), and in California Toscano et al. (2003) showed that H. coagulata numbers were over 6-fold higher on young citrus trees compared to older trees. It is also possible that given the very high densities of ovipositing females in citrus at the time this field survey was conducted less preferred lemon cultivars were being used due to shortages of oviposition sites on the most preferred cultivars.

Parasitism by G. ashmeadi and G. triguttatus of H. coagulata eggs masses on ‘Eureka’ and ‘Lisbon’, were not significantly different. The lack of statistical significance suggests that using ‘Eureka’ trees for future experiments with these parasitoids should not detrimentally influence parasitism rate data or fecundity estimates, and demonstrates that parasitoids were not conditioned to oviposit preferentially on ‘Eureka’ over ‘Lisbon’ even though they had been reared on ‘Eureka’.

**SUMMARY**

Based on the results of oviposition preference studies conducted in the laboratory we conclude that the lemon cultivar ‘Eureka’ is preferred over ‘Lisbon’ for oviposition by field-collected H. coagulata females. This cultivar preference by H. coagulata, however, was not observed in the field. Furthermore, the mymarid parasitoids G. ashmeadi and G. triguttatus do not exhibit cultivar preferences indicating that the use of ‘Eureka’ will not adversely affect studies on the reproductive and developmental biology of Gonatocerus spp. It is recommended that for mass rearing programs that require the use of small young containerized lemons to maximize H. coagulata egg mass or Gonatocerus spp. production the ‘Eureka’ cultivar should be used in preference to ‘Lisbon’.

**ACKNOWLEDGMENTS**

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Wang for assistance with statistical analysis. We thank Betsy Boyd, Bryan Carey, and Ruth Vega for assistance in the field and laboratory.

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