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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)

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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, parasitism of *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 mas 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* moviles contados en una busqueda de 5 minutos del follaje de las variedades de 'Eureka' y 'Lisbon' y el número 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% mas alta, respectivamente, para los huevos puestos en hojas de 'Eureka' comparado con 'Lisbon', pero estas diferencias no fueron significativas. La morfologia de la superficie de la hoja y el grueso de las tapas de celulas de la hoja de ambas variedades de limón fueron comparados con un microscopio electronico de barrido (SEM). El SEM demostró que el grueso total de la hoja y el grueso de la capa empalizada fue 19.2% y 38.6% mas 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 esponjosa 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 grueso 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 caracteristicas de la hoja o la química del xilema son responsables para la selección del sitio de oviposición de *H. coagulata*. Para los programas

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de cria en masa usando limones como plantas hospederas, se recomienda que se use la variedad 'Eureka' en preferencia a 'Lisbon' por que *H. coagulata* prefiere esta variedad para la oviposición y la actividad forrajera del parasitoide no es afectada adversamente.

The glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), is native to southeastern U.S.A. and northeastern Mexico, and following its establishment in California in the 1980s, it 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

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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 unknowns 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 χ^2 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 (one on the bottom, and one on each of two sides) covered with mesh netting (80 µm Jelliff Corporation, Southport, CT)] 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° ± 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° ± 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% gluteraldahyde 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 undersides 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

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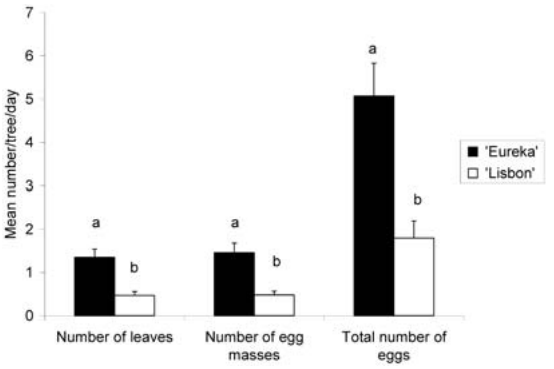


Fig. 1. The mean number of leaves containing *H. coagulata* egg masses, the number of *H. coagulata* egg masses and the total number of *H. coagulata* eggs laid on 'Eureka' and 'Lisbon' lemon trees in the laboratory [different letters indicate significant ($P < 0.05$) differences between cultivars; error bars indicate ± SEM].

H. coagulata egg masses, total *H. coagulata* eggs, percentage parasitism and percentage unknowns were not significantly different between the two cultivars (Table 1). However, the percentage nymphs was significantly higher (5.8% greater) on 'Lisbon' trees compared with 'Eureka' (0%) (Table 1). For egg masses harvested in the field and returned to the laboratory, there were no significant differences in emergence rates between both cultivars for all parameters measured (Table 1).

Gonatocerus ashmeadi and *G. triguttatus* Oviposition Preference

In paired oviposition preference tests in the laboratory, parasitism by *G. ashmeadi* and *G. triguttatus* was respectively 61.7% ± 8.5 and 35.4% ± 12.2 on 'Eureka, and on 'Lisbon' parasitism was 43.1% ± 9.9 and 17.2% ± 7.6, respectively, for *G. ashmeadi* and *G. triguttatus*. These differences were not significant ($t = 1.3$, $n = 15$, $P = 0.22$ and $t = 1.17$, $n = 15$, $P = 0.26$).

Investigation of Leaf Characteristics with SEM

Mean total leaf thickness was higher (19.2%) for 'Eureka' leaves (156.8µm ± 6.2) compared to 'Lisbon' (131.5µm ± 7.3) ($t = 2.63$, $df = 18$, $P < 0.05$) (Fig. 2). The mean thickness of the palisade layer was thicker ($t = 2.32$, $df = 11.1$, $P < 0.05$) in 'Eureka' (38.6%) leaves compared to 'Lisbon' (Fig. 2). There was no difference in cell layer thickness between the cultivars for cuticle ($t = 0.39$, $df = 18$, $P = 0.70$), epidermis ($t = 0.60$, $df = 18$, $P = 0.56$), and spongy parenchyma layers ($t = 1.31$, $df = 18$, $P = 0.21$) (Fig. 2). The underside of 'Lisbon' leaves was smooth, whereas 'Eureka' had many small ridges (Fig. 3). *Homalodisca coagulata* egg placement was between the lower epidermis and spongy parenchyma layer for both cultivars (Fig. 4).

Discussion

Results from this study showed that in the laboratory, the number of *H. coagulata* egg masses laid on 'Eureka' was 204.2% higher than on 'Lisbon'. This indicates that when given a choice under artificial rearing conditions, *H. coagulata* preferred 'Eureka' over 'Lisbon' for oviposition, and suggests that this cultivar should be used for research projects that require large numbers of *H. coagulata* eggs laid on lemon leaves that can be harvested from young potted plants.

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.

Mean variable/tree/sampling event	'Eureka' ± SEM	'Lisbon' ± SEM	Significance
Number of *H. coagulata* nymphs and adults	30.9 ± 1.6	33.0 ± 2.2	$F = 0.96$, $df = 18$, $P = 0.33$
Number of old and new leaves selected	21.0 ± 1.0	24.0 ± 1.1	$F = 3.86$, $df = 1,8$ $P = 0.06$
Emergence from egg masses in the field			
Number of old masses	31.1 ± 2.0	35.1 ± 2.1	$F = 1.87$, $df = 1,2$ $P = 0.18$
Number of old eggs	196.5 ± 13.9	212.8 ± 12.9	$F = 1.04$, $df = 1,12$ $P = 0.31$
Percentage parasitism	42.8 ± 2.4	37.8 ± 2.1	$F = 2.34$, $df = 1,12$ $P = 0.13$
Percentage nymphs	26.5 ± 2.0	32.4 ± 1.7	$F = 5.53$, $df = 1,12$ $P < 0.05$
Percentage unknowns	16.8 ± 1.2	19.5 ± 1.3	$F = 2.39$, $df = 1,12$ $P = 0.13$
Emergence from egg masses in the lab			
Number of new masses	1.3 ± 0.2	1.2 ± 0.2	$\chi^2 = 1.29$, $df = 1$, $P = 0.26$
Number of new eggs	8.0 ± 1.5	7.6 ± 1.5	$\chi^2 = 0.18$, $df = 1$, $P = 0.67$
Percentage parasitism	53.9 ± 7.6	48.9 ± 6.8	$\chi^2 = 1.29$, $df = 1$, $P = 0.26$
Percentage nymphs	5.8 ± 3.8	0.0 ± 0.0	$\chi^2 = 3.00$, $df = 1$, $P = 0.08$
Percentage unknowns	37.2 ± 7.6	43.0 ± 7.1	$\chi^2 = 0.14$, $df = 1$, $P = 0.71$

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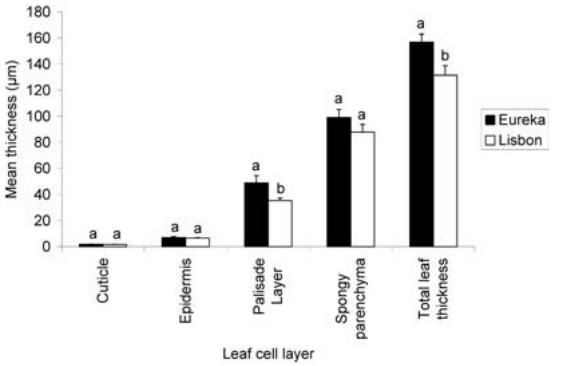


Fig. 2. Mean thickness (µm ± SEM) of the cuticle, epidermis, palisade and spongy parenchyma leaf cell layers within 'Eureka' and 'Lisbon' lemon cultivars [different letters indicate significant ($P < 0.05$) differences between cultivars].

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. coagulata* nymphs and adults, and the number of leaves with *H. coagulata* egg masses between 'Eureka' and 'Lisbon' cultivars. Consequently the oviposi-

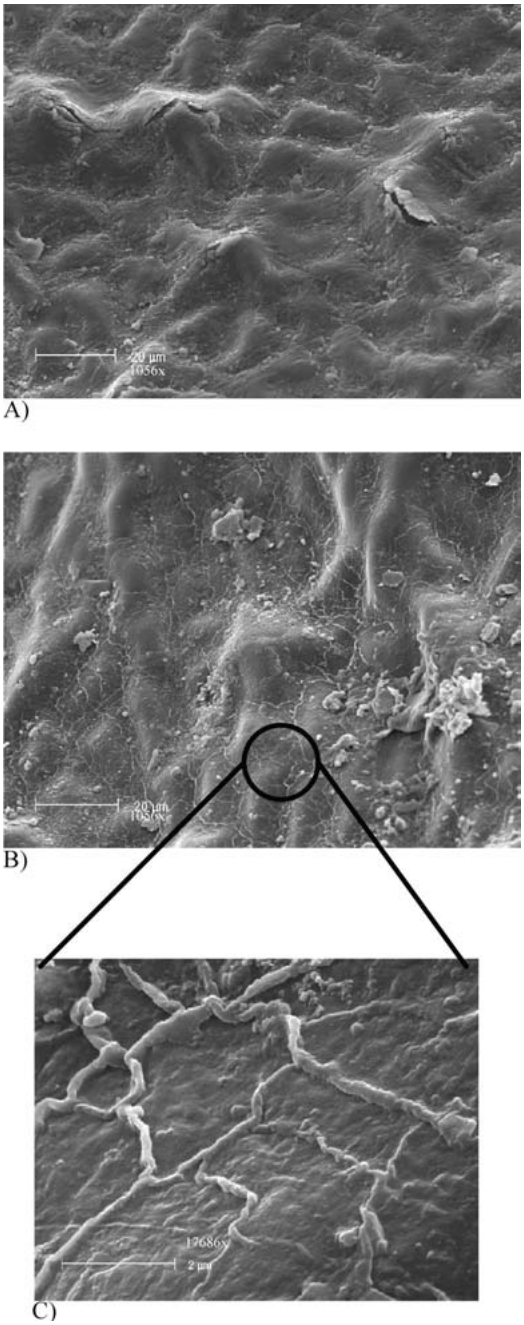


Fig. 3. SEM photographs of the lower leaf surface of 'Lisbon' and 'Eureka' *Citrus limon* cultivars: (A) 'Lisbon' with smooth leaf surface; (B) 'Eureka' leaf surface with many small ridges on the cuticle that may enhance *H. coagulata* tarsal grip during egg laying; (C) Close up of small ridged on 'Eureka' leaves that were not present on 'Lisbon'.

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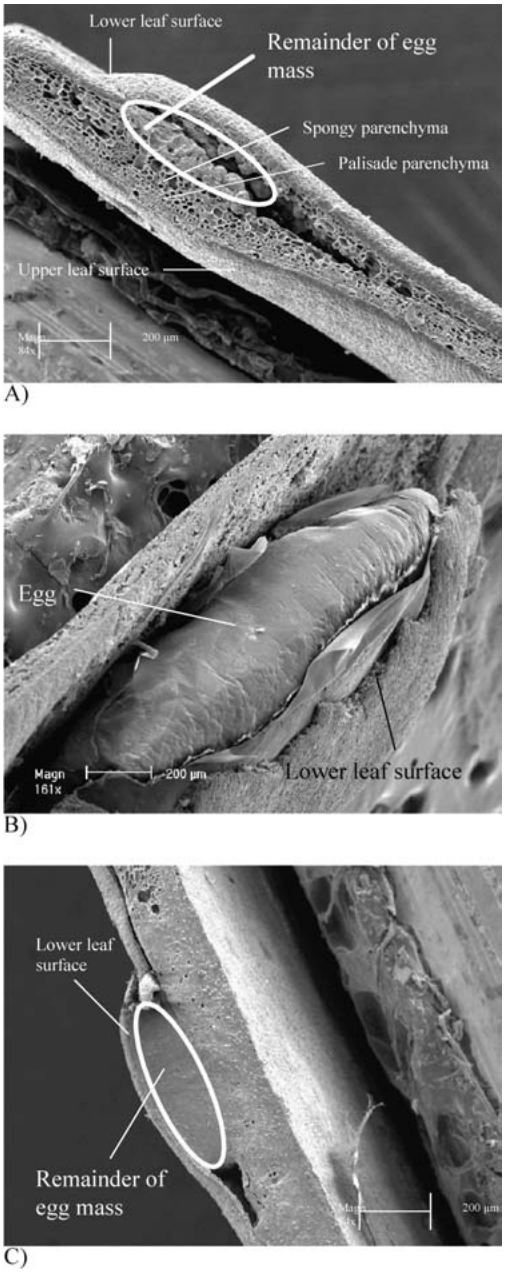


Fig. 4. SEM photographs showing placement of *H. coagulata* egg mass within 'Eureka' and 'Lisbon' lemon leaves: (A) 'Eureka' leaf—egg sliced latitudinally, shows placement of egg between epidermis and spongy parenchyma; (B) 'Eureka' leaf—egg sliced longitudinally, shows placement directly beneath epidermis; (C) 'Lisbon' leaf—egg sliced latitudinally, shows placement of egg between epidermis and spongy parenchyma (note: egg protein filled leaf cells).

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'.

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