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EFFECT OF INSECT DENSITY AND HOST PLANT QUALITY ON WING-FORM IN *MEGAMELUS SCUTELLARIS* (HEMIPTERA: DELPHACIDAE)

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ABSTRACT

Megamelus scutellaris Berg (Hemiptera: Delphacidae) is a South American species that feeds on waterhyacinth, *Eichhornia crassipes* Mart. (Solms). This species exhibits significant wing dimorphism whereby fully winged adults (macropters) are capable of flight while those with reduced wings (brachypters) are not. The wing form is determined by a developmental switch triggered by environmental factors including crowding, host plant quality, temperature, and photoperiod. This study examined the influences of insect density and host plant quality on *M. scutellaris* wing dimorphism, development, and biomass as well as their effects on *E. crassipes*. Two experiments exposed a single generation of *M. scutellaris* to lower and higher densities of conspecifics on low and high quality plants. The first experiment involved transferring second instars to test plants at loads of 50, 5, or 1 g of fresh weight plant biomass per nymph, which resulted in mean densities of 2, 15, and 69 nymphs, respectively, on both low and high quality plants. A second experiment exposed test plants to 2, 4, or 20 *M. scutellaris* adults for 7 days and allowed their progeny to develop into adults which ultimately produced densities of 0, 56, and 352 F₁ adults, respectively, per low and high quality plants. No macropterous adults were produced in any treatment combination in either experiment. Several plant variables were affected by insect densities and plant quality including the mean relative growth rate, the change in leaf number, and the percentage of dead leaves on a plant. *Megamelus scutellaris* appeared to have a relatively high density threshold for macroptery within the range of host quality used in these studies. This may promote more brachyptery which, in turn, may increase the chances of this insect reaching damaging densities in field populations of *E. crassipes*.

Key Words: macroptery, brachyptery, waterhyacinth, *Megamelus scutellaris*, biological control

RESUMEN

Megamelus scutellaris Berg (Hemiptera: Delphacidae) es una especie de América del Sur que se alimenta del jacinto de agua, *Eichhornia crassipes* Mart. (Solms). Esta especie presenta un dimorfismo significativo en las alas mediante el cual los adultos completamente alados (macropteros) son capaces de volar, mientras que los que tienen alas reducidas (brachipteros) no pueden volar. La forma de ala es determinada mediante un interruptor de desarrollo que se desencadena por factores ambientales como el hacinamiento, la calidad de la planta huésped, la temperatura y el fotoperíodo. Este estudio examinó la influencia de la densidad de insectos y la calidad de la planta huésped sobre el dimorfismo de las alas de *M. scutellaris*, el desarrollo y la biomasa, así como sus efectos en *E. crassipes*. Realizamos dos experimentos exponiendo una sola generación de *M. scutellaris* a densidades inferiores y superiores de sus congéneres sobre plantas de alta y de baja calidad. En el primer experimento larvas del segundo estadio fueron transferidas a las plantas de la prueba con cargas de 50, 5, ó 1 g de peso fresco de biomasa de planta por ninfa, que resultó en densidades medias de 2.0, 15 y 69.1 ninfas por planta en las plantas de baja y de alta calidad, respectivamente. En el segundo experimento, las plantas de la prueba fueron expuestas a 2, 4, ó 20 adultos de *M. scutellaris* por 7 días y permitimos que sus progenies se convirtieran en adultos, lo que finalmente produjeron densidades de 0, 56.5 y 352.4 adultos (F₁) por planta en plantas de baja y alta calidad, respectivamente. Adultos macropteros no fueron producidos en cualquier combinación de tratamiento en ninguno de los experimentos. Varias variables de plantas fueron afectadas por la densidad de insectos y la calidad de la planta incluyendo el promedio de la tasa de crecimiento relativa, el cambio en el número de hojas, y el porcentaje de hojas muertas en una planta. *Megamelus scutellaris* parece tener un umbral de densidad relativamente alta para que desarrollen los adultos macropteros dentro del rango de calidad huésped usada en estos estudios. Esto puede promover el desarrollo de los adultos brachipteros que, a su vez, puede aumentar las posibilidades de que este insecto alcanzan densidades dañinas en las poblaciones de campo de *E. crassipes*.

Palabras Clave: macroptery, brachyptery, jacinto de agua, *Megamelus scutellaris*

Many planthopper species in the Delphacidae exhibit significant wing dimorphism to the extent that fully winged adults (macropters) are capable of flight while those with reduced wings (brachypters) are not (Ossiannilsson 1978; Denno et al. 1985). The wing form is determined by a developmental switch that is triggered by environmental factors including crowding, host plant quality, temperature, and photoperiod (Johno 1963; Mochida 1973; Saxena et al. 1981; Denno et al. 1985). Macroptery is usually a density-dependent phenomenon but it often increases when host quality declines (May 1975; Cook & Perfect 1985; Denno et al. 1991). The triggering density threshold for macroptery is variable among and even within species (Denno et al. 1991). Although macropters have the ability to escape suboptimal conditions, this may come at the cost of delayed reproduction, reduced fecundity and longevity, and increased risk of mortality while migrating (Denno et al. 1986; Denno & Roderick 1990; Denno et al. 1989; May 1975; Dingle 1989).

Megamelus scutellaris Berg (Hemiptera: Delphacidae) is a South American species that feeds on waterhyacinth, *Eichhornia crassipes* Mart. (Solms) in Peru, Brazil, Uruguay, and Argentina (Sosa et al. 2005). The insect was recently released in the U.S. as a classical biological control agent for waterhyacinth (Tipping et al. 2011). This species is multivoltine in southern Florida and exhibits the wing dimorphism of both brachypterous and macropterous wing-forms.

The objective of this study was to examine and quantify the influences of insect density and host plant quality on *M. scutellaris* wing dimorphism, development, and biomass as well as their effects on *E. crassipes*.

MATERIALS AND METHODS

All tests were conducted in 37.8 liter aquaria placed in a temperature controlled greenhouse under ambient light and photoperiods. *Megamelus scutellaris* nymphs were collected directly from general quarantine colonies reared in an adjacent greenhouse room. One wk old *M. scutellaris* adults were obtained by exposing plants to adults from quarantine colonies and collecting the appropriately aged F_1 adults that emerged. Plants were grown in a temperature controlled greenhouse maintained within a range of 25-28 °C under ambient light and photoperiod with a standard fertilizer regime of 10.97 g liter⁻¹ of 15-9-12 osmocote and 0.66 g liter⁻¹ of 10% iron chelate.

Experiment 1. Lower Density Feeding by Nymphs

The experimental design was a 3 × 2 factorial arranged in randomized complete block design with 3 insect densities, 2 plant nutrient levels,

and 5 blocks (replications). Insect densities were normalized to account for differences in the biomass of the single test plant by using a ratio of one second instar *M. scutellaris* to 50, 5, or 1 g of fresh weight plant biomass, which resulted in respective mean (±SE) densities of 2.0 ± 0.0, 14.5 ± 0.3, and 64.2 ± 3.2 nymphs per low quality plant, and 2.0 ± 0.0, 15.5 ± 0.6, and 74.2 ± 2.3 nymphs per high quality plant. Second instars were used because first instars suffered high levels of mortality during handling. The high quality plant treatment was created by placing standard fertilized plants in aquaria with 1549.5 mg of 15-9-12 osmocote and 92.5 mg chelated iron in 5 liters of tap water. Low quality plants were derived from plants that were previously held continuously in unfertilized tap water and placed into aquaria with the standard fertilizer regime (309.5 mg of 15-9-12 osmocote and 20.5 mg chelated iron). These fertilizer regimes resulted in leaf tissue nitrogen of 2.6 ± 0.2% and 3.7 ± 0.05% of dry weight biomass for low and high quality plants, respectively.

Eichhornia crassipes test plants were stripped of dead matter and total plant initial fresh weight biomass and leaf counts were taken before placement into aquaria. Insects were added and net tops were placed on the aquaria and secured to prevent insect escape. As nymphs developed into adults they were removed and tallied daily by gender and wing type, placed in a freezer for 7 days, air dried for 24 h, and weighed to determine mean biomass. Plant fresh weight biomass, the number of ramets, and the number of dead and live leaves were measured for each test plant after the last adults had developed and been removed from the aquaria. The third youngest leaf of each plant was removed for carbon and nitrogen analysis. All plant material was dried at 35 °C to a constant weight to determine dry weight biomass. Mean relative growth rate MRGR (mg day⁻¹) was calculated for whole plant biomass using the formula:

$$\text{MRGR} = (\ln W_2 - \ln W_1) / (t_2 - t_1)$$

where W_1 and W_2 are the estimated dry weight biomass at the beginning (t_1) and known dry weight biomass at the end (t_2) of the sampling period, and \ln is the natural logarithm. Dry weight biomass at t_1 was calculated using an estimate of 96% moisture in all test plants.

Experiment 2. Adult and Higher Density Feeding by Nymphs

The experimental design was as above except that 7 d old adults were added to similarly sized plants at densities of 2, 4, and 40 adults (50% female) for 7 days and then removed. The fresh weight biomass of the test plants were not different from one another and insect and plant measurements were taken in the same way as Experiment 1.

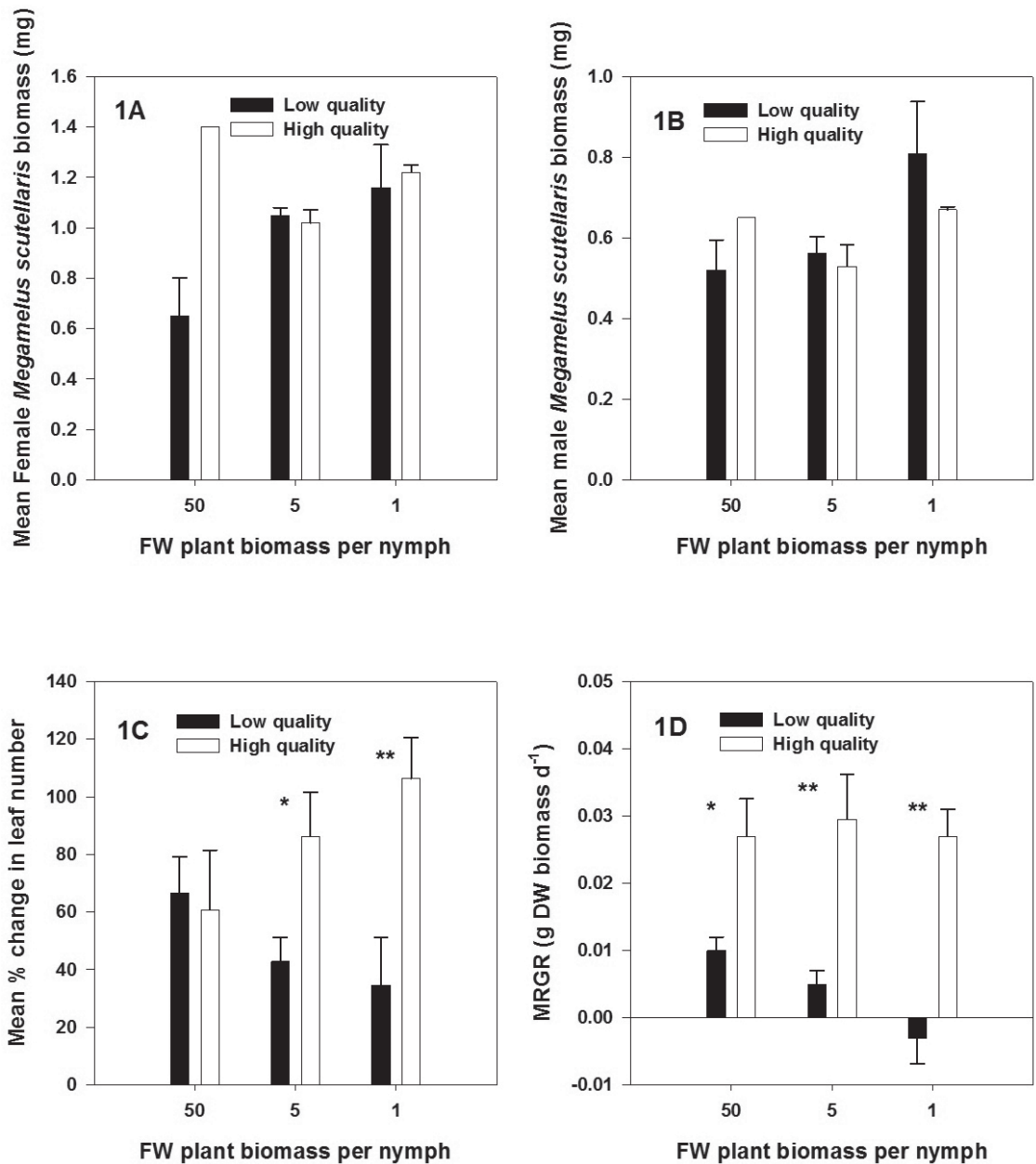


Fig. 1. Effects of increasing nymphal *Megamelus scutellaris* density with two levels of plant quality on: A. adult female biomass of *M. scutellaris*, B. adult male biomass, C. the percent change in leaf number, and D. the MRGR of *Eichhornia crassipes*. *,** Variable means were different within plant quality and insect density at $P = 0.05$ and $P = 0.01$, respectively. ¹FW = Fresh Weight.

Statistical Analysis

Data were tested for normality and homogeneity of variance and transformed as needed prior to analysis. Mean values are presented as arithmetic means with standard errors. The in-

fluence of host quality and insect density and their interactions on insect and plant parameters were examined using two way ANOVA and regression. Variable means were separated post-hoc using t-tests. All statistical analyses were conducted using SAS v9.1 (SAS 2004).

TABLE 1. EFFECTS OF FEEDING BY LOWER DENSITIES OF *MEGAMELUS SCUTELLARIS* NYMPHS ON INSECT AND PLANT VARIABLES WITH PLANT QUALITY AND INSECT DENSITY AS MAIN FACTORS.

Variables	Plant Quality			Insect Density			Quality × Density		
	df	TSS (%)	P	df	TSS (%)	P	df	TSS (%)	P
<i>M. scutellaris</i> mortality (%)	1	<1.0	0.84	2	26.7	0.05	2	14.1	0.18
No. F ₁ adult <i>M. scutellaris</i>	1	<1.0	0.93	2	97.0	<0.0001	2	<1.0	0.89
Adult female biomass (mg)	1	23.8	0.01	2	9.7	0.27	2	29.2	0.03
Adult male biomass (mg)	1	<1.0	0.96	2	27.9	0.05	2	6.7	0.44
Female development time (d)	1	<1.0	0.87	2	12.1	0.35	2	13.9	0.29
Male development time (d)	1	1.2	0.62	2	15.2	0.23	2	13.2	0.28
MRGR (g DW biomass d ⁻¹)	1	60.3	<0.0001	2	1.7	0.64	2	7.4	0.18
Change in leaf number (%)	1	48.8	0.0084	2	1.3	0.88	2	37.5	0.05
No. of ramets per plant	1	71.0	<0.0001	2	1.9	0.51	2	1.9	0.51

Presented are the degrees of freedom (df), the rounded percentage of variance explained by a factor (Total Sums of Squares [TSS] = (100 × factor sums of squares / TSS)), and the level of significance (P).

RESULTS

Experiment 1. Lower Density Feeding by Nymphs

No macropterous adults were produced in any treatment combination. Mortality of nymphs was unaffected by plant quality but negatively affected by insect density with no interaction present (Table 1). Although the number of adults and the sex ratio were unaffected by plant quality, female biomass was greater on higher quality plants and there was a plant quality × insect density interaction (Table 1). This interaction resulted primarily from a difference in magnitude at the low density treatment, a result which should be interpreted cautiously because so few females developed in this treatment combination (Fig. 1A). Despite this, there was a trend of increasing biomass as insect density increased on the low quality plants ($Y = 0.81 + 0.05X$, $r^2 = 0.62$, $P = 0.02$) (Fig. 1A). Across all treatments, females weighed more than males (1.8 ± 0.1 mg vs. 0.6 ± 0.04 mg, respectively) ($t_{38} = 28.7$, $P < 0.0001$). Male biomass was affected only

by insect density but, like females, also increased with increasing density on both low ($Y = 0.47 + 0.007X$, $r^2 = 0.55$, $P = 0.03$) and high ($Y = 0.39 + 0.04X$, $r^2 = 49$, $P = 0.05$) quality plants (Table 1) (Fig. 1B). Mean development time to the adult stage was unaffected by plant quality or insect density regardless of gender (Table 1). In general, male nymphs developed to adulthood about a day sooner than did females ($[13.8 \pm 0.2$ d vs. 14.9 ± 0.5 d] $t_{38} = 3.6$, $P = 0.0009$).

All plant variables were predictably influenced by plant quality but none directly by insect density. There was a plant quality × insect density interaction for the percent change in the number of leaves whereby leaf numbers decreased with increasing insect density for low quality plants but increased for high quality plants (Table 1) (Fig. 1C). Mean RGR was greater with high quality plants than low quality plants although there also was a trend of declining biomass with increasing insect density in the low quality plant treatment ($Y = 0.01 - 0.001X$, $r^2 = 0.43$, $P = 0.07$) (Fig. 1D). No ramets were produced by low qual-

TABLE 2. EFFECTS OF FEEDING BY ADULTS AND HIGHER DENSITIES OF NYMPHS OF *MEGAMELUS SCUTELLARIS* ON INSECT AND PLANT VARIABLES WITH PLANT QUALITY AND INSECT DENSITY AS MAIN FACTORS.

Variables	Plant Quality			Insect Density			Quality × Density		
	df	TSS (%)	P	df	TSS (%)	P	df	TSS (%)	P
No. F ₁ adult <i>M. scutellaris</i>	1	3.0	0.19	2	55.6	<0.0001	2	7.6	0.13
F ₁ female (%)	1	8.9	0.36	2	5.1	0.48	2	<1.0	0.78
Adult female biomass (mg)	1	23.2	0.12	2	<1.0	0.75	2	<1.0	0.79
Adult male biomass (mg)	1	6.5	0.34	2	14.9	0.15	2	34.2	0.04
MRGR (g DW biomass d ⁻¹)	1	13.5	0.04	2	20.4	0.04	2	1.8	0.72
Change in leaf number (%)	1	5.1	0.21	2	24.4	0.03	2	4.9	0.46
Dead leaves (%)	1	6.4	0.14	2	23.7	0.03	2	9.4	0.20
No. of ramets per plant	1	37.5	0.001	2	2.2	0.67	2	<1.0	0.92

Presented are the degrees of freedom (df), the rounded percentage of variance explained by a factor (Total sums of squares [TSS] = (100 × factor sums of squares / TSS)), and the level of significance (P).

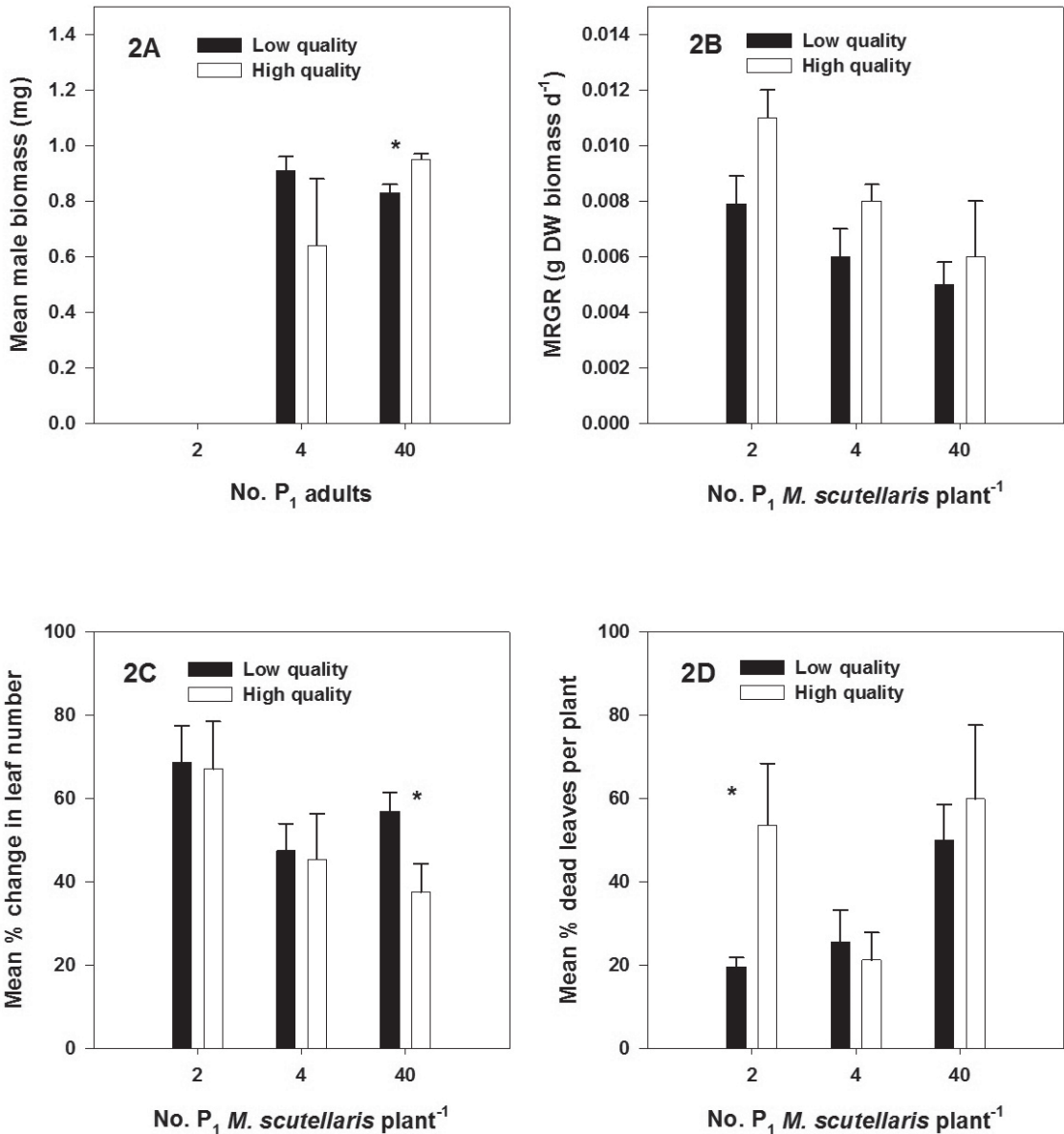


Fig. 2. Effects of increasing initial adult *Megamelus scutellaris* density with two levels of plant quality on: A. adult male biomass of *M. scutellaris*, B. the MRGR of *E. crassipes*, C. the percent change in leaf number, and D. the percentage of dead leaves per plant. *,** Variable means were different within plant quality and insect density at $P = 0.05$ and $P = 0.01$, respectively.

ity plants while those produced by high quality plants (mean \pm SE = 1.4 ± 0.3 per plant) were unaffected by insect density (Table 1).

Experiment 2. Adult and Higher Density Feeding by Nymphs

As in the first experiment, no macropterous adults were produced in any combination of plant

quality or insect density. No F_1 adults were produced in the lowest insect density treatment for unknown reason(s). The total number of F_1 adults produced was unaffected by plant quality but directly affected by insect density (Table 2). Sex ratio, mean female biomass, and mean male biomass were not influenced directly by either plant quality or insect density, although male biomass was influenced by a host quality \times insect density

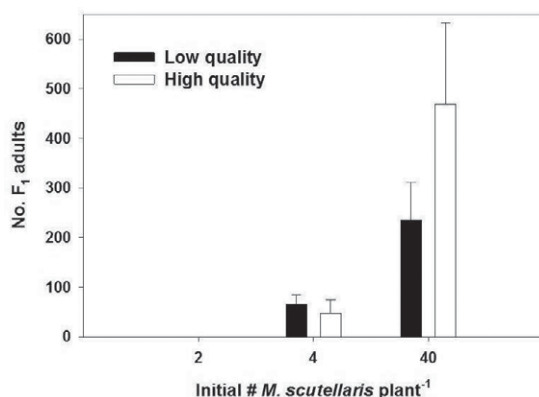


Fig. 3. Mean number of F₁ adults produced from the initial exposure of 2, 4, or 20 adults for 7 days on low and high quality plants of *Eichhornia crassipes*.

interaction (Table 2). This interaction resulted from a change in rank whereby males were larger on low quality plants at low densities while the reverse was true on high quality plants at high densities (Fig. 2A). As in the previous experiment, females were larger than males (1.8 ± 0.1 mg vs. 0.8 ± 0.04 mg, respectively; $t_{24} = 7.7$, $P < 0.0001$).

Several plant variables were affected not only by plant quality, but also insect densities, including MRGR (Fig. 2B), the change in leaf number (Fig. 2C), and the percentage of dead leaves on a plant (Fig. 2D) (Table 2). Although the relationships were not strong, MRGR decreased with increasing insect density with both low ($Y = 0.009 - 0.0002X$, $r^2 = 0.38$, $P = 0.10$) and high ($Y = 0.01 - 0.0004X$, $r^2 = 0.54$, $P = 0.15$) quality plants (Fig. 2B). The percentage of dead leaves per plant increased with increasing insect density with low quality plants ($Y = -20.1 + 4.4X$, $r^2 = 0.78$, $P = 0.003$) (Fig. 2D). Ramets were produced from low quality plants, unlike the first experiment, but more were produced from high quality plants regardless of insect density (Table 2).

DISCUSSION

Although attack by *M. scutellaris* did reduce *E. crassipes* biomass in previous lab studies (Tipping et al. 2011), this insect will probably require high densities in order to negatively suppress the plant in the field. In the lab, plants typically do not exhibit stress until insect densities exceed at least 100 per plant (P.W.T., unpublished data). It is important to understand the influence of density dependent and independent factors on macroptery because of their potential to dilute the impact of this insect on plant populations. Given the numbers of F₁ adults produced in Experiment 2, it appears that *M. scutellaris* has a relatively high threshold for population density within the range

of host quality used in these studies (Fig. 3). Assuming 70 plants per m² in monotypic stands in the field in Florida and, based on the number of F₁ adults produced on plants of lower quality in the high insect density treatment in Experiment 2, the population density of *M. scutellaris* could approach at least 16 K per m² without the formation of macropters, a density that would likely prove damaging to *E. crassipes*.

There was evidence that insect biomass was positively dependent on density (Fig. 1A), indicating that aggregations of *M. scutellaris* may provide an increased level of benefit. Dixon and Wratten (1971) demonstrated that individual *Aphis fabae* Scopoli (Hemiptera: Aphididae) were larger when reared near or within aggregations of conspecifics than when reared alone. This phenomenon has been documented in a variety of insects with different strategies, but only on whole plants indicating that these aggregations somehow modify the plant by creating nutrient sinks or by overwhelming any existent plant defenses (Denno & Benrey 1997; Awmack & Leather 2002).

The lower threshold densities for macroptery seen in this study may promote outbreak levels of the insect which, in turn, may exert a stronger negative influence on waterhyacinth populations since outbreak populations usually consist primarily of brachypters (Zimmerman 1948). Although feeding by *M. scutellaris* after a single generation reduced MRGR of low quality plants in the second experiment by 24% and 37% with low and high insect densities, respectively, MRGR was still positive. However, MRGR was negative in another study when plants were exposed to two consecutive generations of feeding and produced 67% less biomass and 73% fewer leaves compared to the control (Tipping et al. 2011). Future studies will focus on quantifying the relationship between insect population demography, including wing-forms, with landscape level densities in natural field populations of the plant.

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REFERENCES CITED

- AWMACK, C. S., AND LEATHER, S. R. 2002. Host plant quality and fecundity in herbivorous insects. *Annu. Rev. Entomol.* 47: 817-44.
- COOK, A. G., AND PERFECT, T. J. 1985. Seasonal abundance of macropterous *Nilaparvata lugens* and *Sogatella furcifera* based on presumptive macroptery in fifth-instar nymphs. *Ecol. Entomol.* 10: 249-258.

- DENNO, R. F., DOUGLASS, L. W., AND JACOBS, D. 1985. Crowding and host plant nutrition: Environmental determinants of wing-form in *Prokelisia marginata*. Ecology 66: 1588-1596.
- DENNO, R. F., DOUGLASS, L. W., AND JACOBS, D. 1986. Effects of crowding and host plant nutrition on a wing-dimorphic planthopper. Ecology 67: 116-123.
- DENNO, R. F., OLMSTEAD, K. L., AND MCCLOUD, E. S. 1989. Reproductive cost of flight capability: A comparison of life history traits in wing dimorphic planthopper. Ecol. Entomol. 14: 31-44.
- DENNO, R. F., AND RODERICK, G. K. 1990. Population biology of planthoppers. Annu. Rev. Entomol. 35: 489-520.
- DENNO, R. F., RODERICK, G. K., OLMSTEAD, K. L., AND DÖBEL, H. G. 1991. Density-related migration in planthoppers (Homoptera: Delphacidae): the role of habitat persistence. American Natur. 138: 1513-1541.
- DENNO, R. F., AND BENREY, B. 1997. Aggregation facilitates larval growth in the tropical nymphalid butterfly *Chlosyne janais*. Ecol. Entomol. 22: 133-41.
- DINGLE, H. 1989. The evolution and significance of migratory flight, pp. 100-114 In G. J. Goldsworthy and C. H. Wheeler [eds.], Insect Flight. CRC Press, Boca Raton, FL.
- DIXON, A. F. G., AND WRATTEN, S. D. 1971. Laboratory studies on aggregation size and fecundity in the black bean aphid, *Aphis fabae* Scop. Bull. Entomol. Res. 61: 97-111.
- JOHNO, S. 1963. Analysis of the density effect as a determining factor of the wing-form in the brown planthopper *Nilaparvata lugens*. Japanese J. Appl. Entomol. Zool. 7: 45-48.
- MAY, Y. Y. 1975. Study of two forms of the adult *Stenocranus minutus*. Trans. R. Entomol. Soc. Lond. 127: 241-254.
- MOCHIDA, O. 1973. The characters of the two wing-forms of *Javesella pellucida* (F.) (Homoptera; Delphacidae), with special reference to reproduction. Trans. R. Entomol. Soc. Lond. 125: 177-225.
- OSSIANNILSSON, F. 1978. The Auchenorrhyncha (Homoptera) of Fennoscandia and Denmark. Part 1: Introduction, infraorder Fulgoromorpha. Fauna Entomol. Scand. 7: 3-222.
- SAS INSTITUTE. 2004. The SAS/STAT 9.1 User's Guide. Vol. 1-7. SAS Institute, Cary, NC. 5180 pp.
- SAXENA, R. C., OKECH, S. H., AND LIQUIDO, N. J. 1981. Wing morphism in the brown planthopper *Nilaparvata lugens*. Insect Sci. Appl. 1: 343-348.
- SOSA, A. J., MARINO DE REMES LENICOV, A. M., MARIANI, R., AND CORDO, H. A. 2005. Life history of *Megamelus scutellaris* with description of immature states (Hemiptera: Delphacidae). Ann. Entomol. Soc. Am. 98: 66-72.
- TIPPING, P. W., CENTER, T. D., SOSA, A. J., AND DRAY, F. A. 2011. Host specificity assessment and potential impact of *Megamelus scutellaris* (Hemiptera: Delphacidae) and waterhyacinth *Eichhornia crassipes* (Pontederiales: Pontederiaceae). Biocontrol Sci. Tech. 21: 75-87.
- ZIMMERMAN, E. C. 1948. Homoptera: Auchenorrhyncha. Insects of Hawaii 4: 1-268.