EFFECTS OF HOST QUALITY ON FLIGHT MUSCLE DEVELOPMENT IN *NEOCHETINA EICHHORNIAE* AND *N. BRUCHI* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Neochetina eichhorniae Warner and *N. bruchi* Hustache, biological control agents of *Eichhornia crassipes* (Martius) Solms-Laubach, are usually incapable of flight but occasionally develop indirect flight muscles enabling dispersal. This reportedly alternates with oögenesis and is reversible. We examined host quality as a possible explanation for the transitions between these 2 states by allowing populations of the 2 species to develop on plants differing in nutritive quality and then examining the status of their ovaries and flight muscle development. The leaf nitrogen content of the plants increased directly with fertilizer treatment levels but herbivory by the weevils changed the pattern of variation. *Neochetina eichhorniae* suppressed overall nutritive quality while still enabling tissue nitrogen levels to increase with fertilizer treatments. *Neochetina bruchi*, however, negated these effects and tissue nitrogen levels failed to correlate with fertilizer treatments. As a result, herbivore intensity (the number of weevils per plant) and the proportion of the populations that responded in one way or the other (either oögenesis or flight muscle development) differed between the 2 species. Very few *N. eichhorniae* responded in the lowest fertilizer treatment and none produced flight muscles. This increased in the intermediate treatments to about an 80% response with most individuals reproductive. At higher levels, the overall response declined somewhat with an increasing proportion becoming dispersive. Very few *N. bruchi* developed flight muscles except in the highest fertilizer treatment. The frequency of reproductive *N. bruchi* varied little across fertilizer treatments, tracking host quality instead. We conclude that transitions from reproduction to dispersal in these 2 species are not in response to low nutritive quality of the plant tissue and require adequate nutrition to occur. Host quality, however, is affected by a multitude of factors, including the intensity of herbivory, which complicates interpretation of nuanced responses.

Key Words: biological control, invasive plants, herbivory, dispersal, fecundity, reproductive biology

RESUMEN

El agente de control biológico de *Eichhornia crassipes* (Martius) Solms-Laubach, *Neochetina eichhorniae* Warner y *N. bruchi* Hustache, usualmente no son capaces de volar pero ocasionalmente desarrollan musculos indirectos de vuelo para permitir su dispersión. Ha sido reportado que este se alterna a oögenesis y es reversible. Examinamos la calidad del hospedero como una explicación posible de las transiciones entre estos 2 estados al permitir que poblaciones de las 2 especies se desarrollen sobre plantas que varian en su calidad nutritiva para luego examinar el estatus de sus ovarios y el desarrollo de los musculos de vuelo. El contenido de nitrógeno en las hojas de las plantas aumentó directamente con los niveles de tratamiento de fertilizante pero la alimentación de los picudos (gorgojos) cambió el patrón de la variación. *Neochetina eichhorniae* suprimio la calidad nutritiva total mientras que permitia que los niveles de nitrógeno en los tejidos aumentaran con el tratamiento de fertilizante. Sin embargo, *Neochetina bruchi*, anulo estos afectos y los niveles de nitrógeno de los tejidos no correlacionaron con los tratamientos de fertilizante. Por conseguiente, la intensidad herbívora (el numero de picudos por planta) y la proporción de la población que respondió en una manera u otra (por oögenesis o desarollo de musculos de vuelo) varian entre las dos especies. Muy pocos de los *N. eichhorniae* respondieron en el tratamiento de menor nivel de fertilizante y ninguno de ellos producieron musculos de vuelo. Este aumentó en los tratamientos intermediarios hasta una respuesta cerca de 80% con la mayoria de los individuos reproductivos. En los niveles más altos, la respuesta total se redujo con una proporción mayor de ellos dispersandose. Muy pocos de los *N. bruchi* desarrollaron musculos de vuelo, menos en el tratamiento de alto nivel de fertilizante. La frecuencia de individuos reproductivos de *N. bruchi* varia poco en los tratamientos de fertilizantes, apuntando en su lugar a la calidad de hosperdero. Concluimos que la transición de reproducción a dispersión en estas 2 especies no son en respuesta de la calidad baja nutritiva de los tejidos de la planta y requieren nutrición adecuada para suceder. Sin embargo, la calidad de hospedero, es afectada por una multitud de factores, incluyendo la intensidad de herbivoros, que complica la interpretación de las erráticas respuestas.

The South American weevils *Neochetina eichhorniae* Warner and *N. bruchi* Hustache (Coleoptera: Curculionidae) were released in Florida during 1972 and 1974, respectively, to aid in the control waterhyacinth (*Eichhornia crassipes* (Martius) Solms-Laubach: Pontederiaceae), a floating aquatic weed (Center 1994). Populations established readily and dispersed widely with *N. eichhorniae* most often the predominate species (Center & Dray 1992; Center et al. 1999). Their early presence at sites distant from release areas was puzzling in that the adult weevils were generally believed incapable of flight. The subsequent discovery of large numbers of *N. eichhorniae* at street lights (Center 1982; Stark & Goyer 1983) refuted this notion and explained the nearly ubiquitous occurrence of this species throughout the Southeast. Stark & Goyer (1983), however, were not able to induce the weevils to fly, so flight behavior remained inexplicable. Buckingham & Passoa (1985) then discovered that these species underwent a periodic degeneration and regeneration of indirect flight muscles, which seemed to alternate with oögenesis, similar to the rice water weevil (*Lissorhoptrus oryzophilus* Kuschel) (Palrang & Gregarick 1993). They related flight muscle regeneration to temperature, with a threshold for development at about 21°C.

We have observed an apparent connection between flight muscle development in *Neochetina* spp. populations and the intensity of herbivory on the plants on which they resided (Center & Dray 1992). We also have noticed that weevils from high quality plants (as exemplified by the nitrogen content of the leaf tissue) exhibited a higher frequency of flight muscle development than those from poor quality plants (unpublished data). We therefore hypothesized that the nutritive quality of the plant tissue influenced indirect flight muscle development and factored into this oögenesis-flight syndrome. To test this, we grew plants in 5 different fertilizer regimens to vary plant quality and released both species into these cultures during spring 2008. The progeny of these weevils were recovered at the end of the summer and dissected to assess indirect flight muscle development and reproductive status. The nitrogen content of the leaf tissue was assessed near the beginning and towards the end of the study to quantify the nutritional quality of the plants.

Materials and Methods

Stock cultures of waterhyacinth (*Eichhornia crassipes* (Martius) Solms-Laubach: Pontederiaceae) were cultivated outdoors in 20 concrete mesocosm tanks (0.8 m wide × 2.2 m long × 0.65 m deep, water depth 0.5 m, vol. 0.88 m^3). Individual rosettes from these cultures were then equally distributed among 60 identical tanks during 3-4 Mar 2008 until each contained 21 plants. All tanks were fertilized with 22 g of Peter's 20:20:20 N:P:K fertilizer and 18 g of Miller 10% Fe Chelate. The plants were allowed to grow to full coverage. The water was then drained and replaced with clean, unfertilized well water. The numbers of rosettes in each tank were counted and then reduced to 120 plants of approximately equal size during 8-10 Apr 2008.

The plants were retained in unfertilized water for about 3 weeks to induce deficiency symptoms. Slow-release fertilizer (Scott's Osmocote Plus 15-9-12 N:P:K, Southern 8-9 month formulation) was then added at 5 different treatment rates: 30, 150, 270, 390, and 510 g/tank. We knew from previous studies that this procedure produced varying concentrations of nitrogen in the plant tissue and that these levels affected fecundity of the weevils (Center & Dray, in press). Each rate was applied to 4 tanks for each herbivory by fertilizer treatment during 23-24 Apr 2008 which provided 4 replicates for each treatment combination. The fertilizer was dispensed in screen packets containing ballast and floatation and tethered to an anchor so that they floated upright within the root zone of the plants. Iron chelate (Miller® Iron Chelate DP 10% Fe) was added at rates of 1.06, 5.29, 9.53, 13.76, and 18.00 g/tank in the low to high fertilizer treatments, respectively. The fertilizer packets were replaced after 3 months (22-24 Jul 2008) when iron chelate was again added. Air temperature and relative humidity were monitored at 30-min intervals in 5 tanks with iButton® Data Loggers Model DS1923-F5 suspended at the level of the plant canopy in an inverted cup. Water temperature was monitored with a separate Data Logger in a sealed capsule suspended within the root zone of the plants.

The leaf blade was excised from the youngest mature leaf of each of 3 plants in each tank on 2 Jun, by which time a complete leaf turnover would have occurred, and again on 4 Aug 2008. They were weighed fresh, dried at 53.4 ± 0.1°C and 5.8 ± 0.1% RH to constant weight, and weighed again. The dried leaves were composited into a single sample per tank and ground in a Willey mill to pass a 40-mesh screen. Samples of the ground tissue were analyzed with a C-H-N analyzer (Perkin-Elmer® Series II CHNS/O Analyzer Model 2400) and compared against tomato leaf standards to determine % N.

Both species of weevils (*Neochetina eichhorniae* and *N. bruchi*) were obtained at a local field site in western Broward County, FL (26.55115°N, 80.70675°W). They were sorted by species and gender and placed on the plants during 21 Apr to 13 May 2008, until an infestation level of 24 pairs/tank was achieved. All tanks were infested with an equal number of weevils on each release date. Each species was released into 20 tanks. An additional set of 20 tanks was used as a no-weevil control to determine if herbivory affected plant quality.

The intensity of the weevil infestations (adults/plant) was estimated on 26 Aug 2008 when 10 randomly selected plants in each tank were non-destructively examined to count adults. Five observers each counted adult weevils on 2 plants in each tank.

All plants in each tank were submersed on 9-12 Sep 2008 under a mesh-covered frame that conformed to the inside dimensions of the tanks. Weevils, which represented progeny of the original stock, were collected as they surfaced, sorted to species and gender, counted, and 10 females of each species from each tank were dissected to ascertain reproductive status (Grodowitz et al. 1997). Those remaining were preserved in 70% ethanol in vials labeled to identify the source tank. A sample of 100 individuals was used to determine sex ratios in cases where populations were large; otherwise sex ratios were based on the total population.

Approximately 10 weevils of each sex and species from each tank were later dissected to assess development of the dorsal indirect flight muscles. The weevils were pinned onto beeswax in well plates and covered with alcohol. The elytra were removed to expose the dorsal meso- and metanotal sclerites. The sclerites were removed to reveal the underlying longitudinal flight muscles. If they were present, they were rated as partially or fully developed depending on the extent of the width of the thorax occupied, as per Buckingham & Passoa (1985). They were rated as absent when there was no sign of development. For purposes of analysis, the partial and fully developed categories were combined.

Frequency data were analyzed by the CATMOD procedure in SAS (SAS Institute 2004) following a hierarchical approach for multi-way contingency tables of categorical data in which the presence of interactions is the primary interest (Sokal & Rohlf 1995; Gotelli & Ellison 2004). Higher-order interactions were analyzed first and those that failed tests of significance (Maximum Likelihood Estimates and Wald χ^2) were deleted. This iterative process continued so as to maximize the value of the joint multinomial likelihood function. Yates continuity correction (Sokal & Rohlf 1995; Gotelli & Ellison 2004) was applied to data sets in which more than 5% of the cells contained zeros. Herbivore intensities were square root transformed before being analyzed by a paired *t*-test. Sex ratios were compared against a theoretical 1:1 ratio across species and fertilizer levels with heterogeneity *G*-tests to produce ANOVA-like tables (Sokal & Rohlf 1995) that partition main effects from interactions.

Leaf nitrogen data were analyzed by analysis of covariance (ANCOVA, Proc GLM; SAS Institute 2004) to compare the least squares means and slopes of the regressions of leaf nitrogen content on fertilizer treatments for each herbivore treatment (control, *N. bruchi*, or *N. eichhorniae*) towards the end of the study and the initial pooled data, prior to a herbivore effect, at the beginning of the study. The initial data were compared among herbivory treatments and found not to differ which justified pooling the data into a single regression. The regression model was $\hat{Y} = a + b\mathrm{H} + c\mathrm{F} + d\mathrm{H} \cdot \mathrm{F} + e$ where $\hat{Y}$ represents the leaf nitrogen estimate, H the herbivory treatment (*N. bruchi*, *N. eichhorniae*, control, or initial), F represents the fertilizer treatment, $H \cdot F$ is the fertilizer by treatment interaction testing for equal slopes, and e is error. The Y-intercept is designated "a" while "b", "c", and "d" are regression coefficients.

Results

Air temperature averaged 27.0°C (range 17.5-38.0°C) during the study. Relative humidity at the top of the plant canopies within the tanks averaged 95% (range 38-100%). Water temperature averaged 28.3°C (range 22-33°C). Water temperature probably best approximated thermal exposure of the weevils and was continuously above the threshold for flight muscle development as determined by Buckingham & Passoa (1985). Day length began at 12.93 h on 21 Apr, peaked at 13.78 h on 21 Jun, then decreased to 12.37 h on 12 Sep.

Fertilizer treatments directly affected the nutritive quality of the waterhyacinth leaf tissue ($F = 74.95$, $P = <0.0001$, 7, 112 *df*) so that mean values at the beginning of the study ranged from 2.2% to 4.2% N. However, high levels of herbivory induced stress on the host plants resulting in deterioration of plant quality. As a result, the nitrogen concentrations in the leaf tissue changed but the effect of the fertilizer varied depending on the herbivory treatments (Fig. 1; fertilizer by herbivore interaction $F = 11.4$, $P = <0.0001$, 3 *df*). Tis-

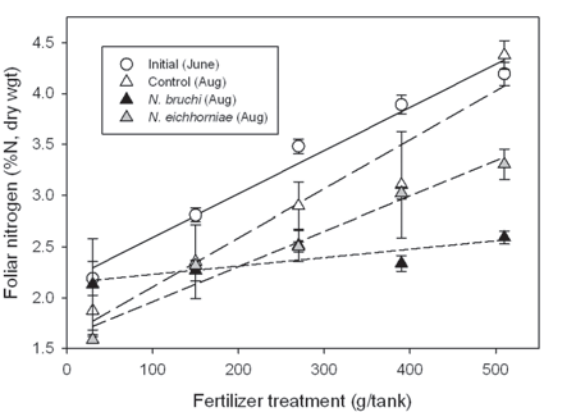

Fig. 1. Leaf tissue nitrogen levels (%N) compared among herbivory and fertilizer treatments near the beginning (Jun) and end (Aug) of the study.

sue N levels in the control (no herbivory) treatments late in the study responded to fertilizer treatments in a similar manner as they had earlier (i.e., the slopes of the regressions did not differ; $F = 0.77$, $P = 0.3836$, 1 *df*). However, overall leaf tissue nitrogen levels were depressed somewhat (least squares means 3.31% in Jun vs 2.92% in Aug, $P = 0.005$) probably due to growth, increased root competition, and increased structural biomass during the interim period. Foliar N concentrations also increased in response to increasing fertilizer treatments in the *N. eichhorniae* treatments. The slope of the regression did not change relative to initial readings ($F = 1.55$, $P = 0.2165$, 1*df*) although overall levels were suppressed (least squares means 2.55 vs 3.31% N, $P = <0.0001$). Fertilizer treatment had no effect on foliar N concentrations in tanks subjected to herbivory by *N. bruchi* (i.e., the slope of the regression did not differ from 0) by the end of the study in marked contrast to the earlier data, the control, and the *N. eichhorniae* treatment. The least squares means differed from the control (2.37% N vs. 2.92% N, $P < 0.0001$) and from the initial values (2.37% vs. 3.31%) but not from *N. eichhorniae* (2.55% N, $P = 0.1822$). However, a comparison of slopes for *N. bruchi* and *N. eichhorniae* regressions indicated that fertilizer affected the 2 species quite differently ($F = 11.31$, $P = 0.0011$). The most pertinent findings from this analysis are that (1) fertilizer level directly affected the nutritive quality of the plant tissue but the effect was modified by herbivory, (2) herbivory by both weevil species suppressed leaf tissue nitrogen concentrations, and (3) *N. bruchi* negated the effects of fertilizer on leaf nutritive quality whereas *N. eichhorniae* did not.

The 2 *Neochetina* species differed on most parameters except sex ratios, which were virtually identical among fertilizer levels ($G = 7.698$, $P = 0.6468$, 10 *df*) and showed a slight deficit of females (52% males: $G = 5.089$, $P = 0.0244$, 1 *df*). The lone exception among the 10 treatment combinations was for *N. bruchi* at the lowest fertilizer level wherein females comprised only 32% of the population ($G = 3.340$, $P = 0.072$, 1 *df*). Herbivore intensity was consistently lower in *N. bruchi* than in *N. eichhorniae* treatments ($t = 3.48$, $P = 0.0251$, 4 *df*), with the differences between species most apparent in the highest and lowest fertilizer treatments (Fig. 2). For *N. bruchi*, intensity was low (about 1 adult/plant) in the lowest fertilizer treatment but very similar (about 3 adults/plant) among treatments at the remaining levels. In contrast, intensity of *N. eichhorniae* herbivory increased directly with fertilizer level, from about 3 adults/plant in the lowest to nearly 7 adults/plant in the highest treatment.

Host quality may affect both oögenesis and flight muscle development possibly as alternate responses. Thus, it may be revealing to first view

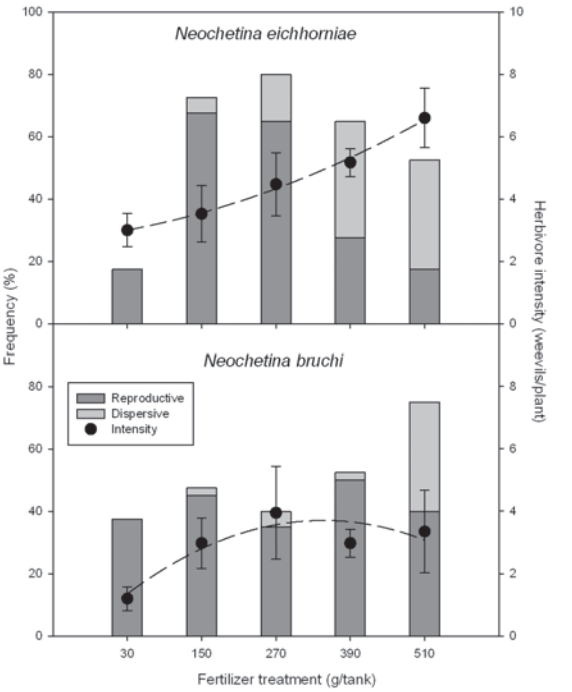

Fig. 2. The relative frequency of *N. eichhorniae* (top) or *N. bruchi* (bottom) females with either functioning ovaries (reproductive) or indirect flight muscles (dispersive) compared across fertilizer treatments contrasted with herbivore intensity (means ± S.E.) in respective treatments. The bars represent the sums of the 2 responses. The dashed lines represent second order polynomial regressions fitted to mean herbivore intensity values.

the data in terms of the percentage of the females exhibiting either a flight muscle or an oögenesis response as opposed to no response (Fig. 2). The 2 species responded similarly, overall, when viewed in this manner (*N. bruchi*: 50.5%, *N. eichhorniae*: 57.5%; Wald $\chi^2 = 1.49$, $P = 0.2224$, 1 *df*), but their responses to the various fertilizer treatments differed (species × fertilizer Wald $\chi^2 = 25.03$, $P < 0.0001$, 4 *df*). The response by *N. eichhorniae* was meager in the lowest fertilizer treatment, with only 17.5% responding, but then increased with increasing fertilizer peaking at the middle treatment at about 80% before declining at higher levels. A proportional shift from reproduction toward dispersal occurred as fertilizer increased. In contrast, *N. bruchi* varied little among all but the highest fertilizer treatment, which was marked by a large proportional increase in dispersive individuals.

When considered independently, flight muscle production was unaffected by gender alone (Wald $\chi^2 = 2.32$, $P = 0.1276$, 1 *df*) or in combination with other factors (gender × fertilizer Wald $\chi^2 = 5.07$, $P = 0.2803$, 4 *df*; gender × species Wald $\chi^2 = 0.18$, P

= 0.6746, 1 *df*), so these were dropped from further analysis. Overall, *N. bruchi* and *N. eichhorniae* differed in the proportions of adults with flight muscles (9% vs 19%, respectively, Wald χ^2 = 6.29, P = 0.0121, 1 *df*), and this difference varied according to fertilizer level (species × fertilizer Wald χ^2 = 15.96, P = 0.0031, 4 *df*).

About 40% of females of both species were reproductive, but fertilizer treatment affected the 2 species differently (species × fertilizer Wald χ^2 = 27.24, P < 0.0001, 4 *df*). Very few female *N. eichhorniae* were reproductive in the lowest fertilizer treatment, whereas a maximal number were reproductive in the second lowest and middle treatments (Fig. 1). The frequency of reproductive *N. eichhorniae* progressively declined at higher fertilizer levels. In contrast, reproductive female *N. bruchi* were relatively evenly distributed across fertilizer levels (Fig. 2).

Discussion

Flight muscles are energetically and materially expensive to produce and their production constrains fecundity (Marden 2000). Hence, the development of full flight musculature probably requires adequate nutrition and would therefore be expected to occur more frequently in weevil populations fed high quality plant tissue. Alternatively, dispersal allows species to meet nutrient demands by moving to higher quality resources (Huberty & Denno 2006), so a greater frequency of flight muscle development might instead occur in response to nutritionally inadequate plant tissue. Our data supports the first hypothesis, that flight ability in *Neochetina* spp. is linked to high host plant quality as reflected in the nitrogen content of the tissue. However, plant quality is influenced by a multitude of factors including nutrient supply, inter- and intraspecific plant competition, the growth stage of the plant, and herbivore intensity. These factors interact to greater or lesser degrees causing nuanced patterns in field populations. For example, increased plant quality leads to increased fecundity and ultimately greater herbivore intensity which feeds back to reduce plant quality. Flight muscle development in this circumstance may be seen as a response to increased herbivore density when, in fact, it may be due to deterioration of the host resource. It may not be possible to segregate these subtle effects, but it is clear that flight muscles in both *Neochetina* species are not developed in response to low host plant quality as evidenced by the lack of flight muscle incidence in our lowest fertilizer treatments.

In previous studies, we found *N. bruchi* to be more sensitive to host quality than *N. eichhorniae* (Center & Dray 2010) so we were surprised by the lack of response to fertilizer treatments on the part of *N. bruchi*. However, the fact that herbivory by this species suppressed the effects of fertilizer suggests that they responded to the resultant nutritive quality rather than the actual experimental treatment. The developmental period of *N. bruchi* is shorter than that of *N. eichhorniae* (DeLoach & Cordo, 1976) so it is possible that the population development was more advanced with their response to fertilizer treatments having occurred earlier. This comports well with Buckingham & Passoa (1985) who reported that flight muscles appeared earlier in *N. bruchi* (6 d post-emergence for males, 7 for females) than in *N. eichhorniae* (11 d post-emergence for males, 12 for females). Similarly, female *N. bruchi* were fecund within 5 d of emergence whereas female *N. eichhorniae* did not produce eggs until d 7.

We cannot rule out the possibility that increased herbivore intensity affected flight muscle development in the case of *N. eichhorniae*. In this instance, herbivore intensity increased with increasing fertilizer treatments along with tissue nitrogen and the proportion of dispersive individuals among those that responded. In the case of *N. bruchi* herbivore intensity varied little among fertilizer treatments as did the frequency of reproductive individuals, which mirrored tissue nitrogen levels at the end of the study. Flight muscle frequency was low in all but the highest fertilizer treatment, which also tracked tissue nitrogen levels. However, the exceptional result in the highest fertilizer treatment, in terms of the combined response and the increased proportion of dispersive individuals, was not readily explainable in terms of host plant quality. Perhaps this relates to the earlier history in that treatment wherein tissue N levels were initially quite high but decreased late in the study. Thus, it may be change in tissue N that triggers the dispersive response rather than the actual nutrient status at any given point in time. This merits further investigation.

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