

Effect of nutrient quality and leaf age of water hyacinth, *Eichhornia crassipes*, on the development of its co-evolved herbivore, *Eccritotarsus catarinensis* (Hemiptera: Miridae)

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Specialist herbivores have evolved adaptations to overcome plant defensive chemicals and thus prefer younger leaves with their higher nutritional value, whereas generalist herbivores are unable to overcome these chemical defences, and opt for older leaves which have fewer nutrients, but are less defended (Center & Wright 1990). Plants such as the invasive aquatic weed *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae) (water hyacinth) continuously produce young, unfurling leaves that require high concentrations of digestibility-reducing chemicals in their tissues to ensure protection against herbivory (Center & Wright 1990). These defensive chemicals are products of secondary metabolism (Berenbaum 1995) and ensure that young leaves are typically unpalatable to herbivores and resistant to disease (Center & Wright 1990). The younger leaves of *E. crassipes* (aged relative to the tip of the stem, with youngest leaf closest to the apex) contain higher concentrations of nitrogen (N), phosphorus (P), potassium (K), and magnesium (Mg), all of which are important nutrients for insect herbivores, and low concentrations of calcium (Ca) and manganese (Mn), which form part of the plant's structural defences (*i.e.* toughened leaves) against herbivory (Center & Wright 1990).

Eccritotarsus catarinensis (Carvalho) (Hemiptera: Miridae), a long-lived sap-sucking bug that was released in 1996 to control water hyacinth in South Africa, is a specialist herbivore that is able to take advantage of the high nutrient content of young water hyacinth leaves (Hill *et al.* 1999). Damage is caused by the four nymphal instars and adults that feed gregariously on the undersides of leaves by piercing and draining them of chlorophyll, leading to chlorosis and eventual death of the leaves (Hill *et al.* 1999). Total developmental time from egg to adult requires 23 days, with nymphal development typically taking 15 days, and adults living as long as 50 days (Hill *et al.* 1999). Since the performance of *E. catarinensis* on leaves of different age and

nutrient quality has not yet been quantified, the influence of these factors on insect development was thus investigated.

Methods for testing the mirid's performance on water hyacinth at different nutrient levels were adapted from Coetzee *et al.* (2007). Cultures of water hyacinth were grown under ambient outside conditions at Rhodes University, Grahamstown in two different plastic 3000 l pools, where one pool served as the high nutrient treatment and the other as the low nutrient treatment. The high-nutrient pool was treated with 1 kg of Multicote[®] fertilizer (15 % N, 3 % P, 12 % K and 7 % S; 5:1:4) (20 mg N/l), and 11.2 mg/l commercial iron chelate (13 % Fe), while no fertilizer was added to the low-nutrient pool. Approximately 280 plants were grown at these nutrient levels for four weeks. Leaves at position two and five from the apex of the plant represented 'young' and 'old' leaves, respectively (with about 12 days in age difference), and were used for nutrient comparisons. The carbon and nitrogen content of leaf two and five from each of five plants from each nutrient treatment (20 in total) was quantified after the four week growth period using a C-N analyser (Europa Scientific, ANCA-SL) at IsoEnvironmental, Rhodes University. One hundred adult *E. catarinensis* were placed onto one test plant in a 5 l tub, covered with mesh netting and left for 24 hours to permit oviposition. Adults were then removed and the date of oviposition was recorded. Once hatched, ~25 individual nymphs were harvested from the tub and reared separately on water hyacinth leaf discs in Petri dishes at 27 °C; with the leaf discs representing each of the treatments (*i.e.* young and old leaves, from the high and low nutrient treatments). Mortality and developmental time for each nymph replicate was recorded from oviposition to adulthood through daily monitoring.

General linear models in STATISTICA ver. 11 were used to compare the mean carbon and nitrogen concentrations between the different leaf treatments and the mean developmental times of

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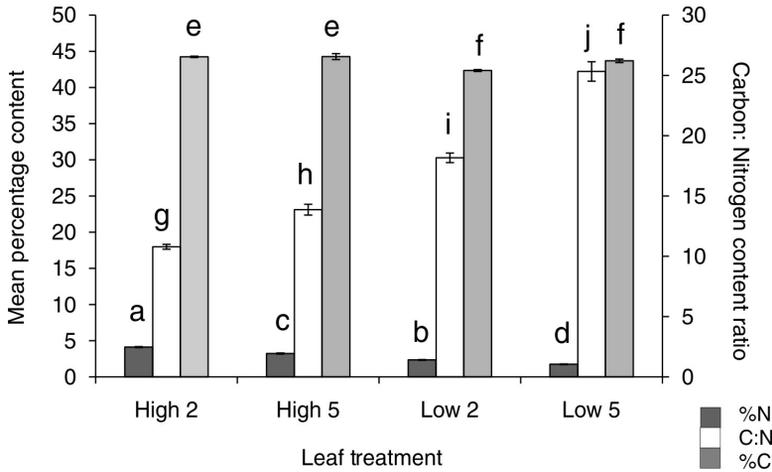


Fig. 1. Mean (\pm S.E.) percentage carbon and nitrogen content, and C:N ratio, of water hyacinth leaf samples from different nutrient treatments. 'Low' and 'High' represent the nutrient level of the water in which the sampled plants were grown, while '2' and '5' represent the leaf positions on the plant, indicating their age. Means compared by GLM factorial ANOVA, with different letters indicating significant differences.

E. catarinensis nymphs reared on the different leaf treatments by a factorial ANOVA with a Fisher's (LSD) *post hoc* test. A chi-square test was used to compare differences in *E. catarinensis* mortality rates between the four leaf treatments.

Carbon:nitrogen content analysis confirmed the significant difference in nutritional value between the different leaf treatments (Fig. 1). Water nutrient level, and thus leaf nutrient content, had the highest influence on nitrogen and carbon content. The % N was significantly higher in the high nutrient treatment than in the low nutrient treatment ($F_{(1,20)} = 178.306$, $P < 0.00001$), and significantly higher in younger leaves than in older leaves

($F_{(1,20)} = 37.346$, $P = 0.000015$). In contrast, the % C was only significantly different between leaves from the different nutrient levels ($F_{(1,20)} = 5.99$, $P = 0.0263$) and not between younger and older leaves within the same nutrient treatment. The C:N ratio was significantly higher in the low-nutrient treatments ($F_{(1,20)} = 84.577$, $P < 0.00001$), and in the older leaves ($F_{(1,20)} = 24.926$, $P = 0.000133$).

Both leaf age and plant nutrient content had a significant impact on *E. catarinensis* development and survival. Nymphal developmental time (Fig. 2) was significantly shorter on young leaves compared to old leaves ($F_{(1,56)} = 10.79$, $P = 0.008$).

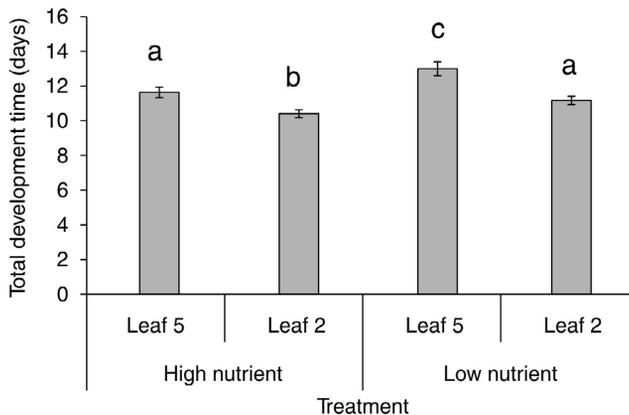


Fig. 2. Mean (\pm S.E.) developmental time to adulthood of *Ecclitotarsus catarinensis* nymphs fed on young and old water hyacinth leaves treated with high and low levels of nutrients. 'Low' and 'High' represents the nutrient level of the water in which the host plants were grown, while '2' and '5' represent the leaf positions on the plant, indicating their age. Means compared by GLM factorial ANOVA, those followed by the same letter were not significantly different.

Nymphs reared on high nutrient leaves developed significantly faster than those reared on low nutrient leaves ($F_{(1,56)} = 22.84, P < 0.00001$) (Fig. 2). Total developmental time of nymphs fed on low nutrient, young leaves was not significantly different from that on high nutrient, old leaves ($F_{(1,56)} = 1.09, P = 0.3018$). Therefore, plant nutrient quality played less of a role than leaf age in the development of *E. catarinensis*. The shorter developmental time of nymphs reared on the younger leaves may be caused by higher concentrations of nutrients (N, P, K, Mg) in these leaves, which provide higher concentrations of amino acids and minerals for increased growth rates (Center & Wright 1990).

Survival of nymphs fed on low quality older leaves, where only 23.8 % survived to adulthood, was significantly lower than that on any other leaf treatment ($\chi^2 = 81.118, \text{d.f.} = 3, P < 0.00001$). This was due to the nutrient-poor status of these leaves, which was insufficient to sustain most of the nymphs until adulthood. The survival of nymphs reared on young and old leaves at high nutrient levels was similar (65 % and 70 %, respectively), whereas those reared on younger leaves of low quality expressed significantly higher survival (85 %) than the other leaf treatments ($\chi^2 = 81.118, \text{d.f.} = 3, P < 0.00001$). Increased nymphal survival on young, low quality leaves implies that these leaves yield higher performance due to the higher nutrient levels (nitrogen content), and lower structural defences (carbon content) as denoted in Fig. 1. High quality young and old leaves ensured very similar nymphal survival, yet this was much lower than survival on low nutrient young leaves. The high nutrient levels of the leaves may have facilitated the production of increased levels of plant defensive chemicals to hinder the performance of the insects.

Younger leaves yielded significantly shorter nymphal development times, in comparison to those of nymphs reared on older leaves. This complies with previous studies conducted on the specialist herbivore *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae), which showed a particular preference for younger leaves of water hyacinth with higher nutrient content (Center & Wright 1990). The shorter nymphal development time allows faster emergence of adults, thus more rapid maturation, mating and reproduction in a shorter time period. This increases the growth of the insect population, which is greatly advantageous to biological control efforts.

Similar nymphal development times on low quality young leaves, and high quality old leaves suggest a similar nutrient composition of these leaves. The high nutrient content of the water in which the plants were grown allowed for surplus nutrient allocation to the older leaves, thus older leaves of higher nutrient quality produced faster nymphal development than that on leaves of plants grown in low nutrient quality water. This nymphal development time matched that of the low quality younger leaves, where nutrients are typically allocated to younger leaves to ensure maximum survival of the plant, which in turn provide sufficient nutrients to the specialist herbivore for increased development and growth.

The major role played by plant chemistry in plant-insect interactions has been studied extensively within an ecological and evolutionary context. The higher concentration of nutrients in the younger leaves facilitates increased allocation of plant metabolites to those leaves, which deter generalist insect herbivores, but stimulate feeding by specialist herbivores, such as *E. catarinensis* (Edwards & Wratten 1983). In particular, specialist herbivores have evolved unique features to overcome these defensive metabolites during the co-evolution of plants and insects (Futuyma & Slatkin 1983). Plant chemicals (including nutritional, toxic and antidigestion chemicals) perform simultaneous sequential reactions in the process of defence, with some chemicals and reactions having greater importance than others (Duffey & Stout 1996). Because of this, specialist herbivores are continuously contending with the plant enzymes that their hosts produce; thus the value of the nutritional plant chemicals must outweigh the functions of the potentially harmful toxins and antidigestion compounds produced. In light of this, the increased developmental rate of *E. catarinensis* on young, highly nutritious water hyacinth leaves, along with confirmation that younger leaves contain higher levels of nutritional nitrogen concentrations, explains the success observed with this well-adapted insect (Coetzee *et al.* 2008). The insect herbivore has shown remarkable evolutionary adaptations to the arms race with the world's worst water weed.

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