Mechanisms and traits associated with compensation for defoliation in *Ruellia nudiflora*

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Abstract A full understanding of the ecology and evolution of plant tolerance to damage requires the measurement of a diversity of traits (including multiple fitness-correlates) and underlying mechanisms. Here, we address the compensatory response to defoliation in the perennial herb *Ruellia nudiflora*, measure biomass allocation patterns and relate them to compensation, and address multiple mechanisms and traits that determine compensatory ability. We used maternal full-sib lines of *R. nudiflora* and conducted a defoliation experiment in which half the plants of each line were subjected to removal of 40% of leaf area (the other half remained undamaged). Fitness-correlated traits, physiological traits, and leaf longevity were measured during a 2-month period after defoliation. Using another set of plants, we conducted a second defoliation experiment and measured the concentration of non-structural carbohydrates to test for root-to-shoot carbon mobilization as a compensatory mechanism. *R. nudiflora* showed full compensation in terms of fruit output, and compensatory ability was positively correlated with investment in root biomass in the absence of damage. In addition, defoliated plants produced shorter-lived leaves and had a greater concentration of starch in roots, suggesting that reduced leaf longevity and accumulation of below-ground carbon reserves act as compensatory mechanisms. By measuring multiple fitness-correlates and induced traits, we provide a comprehensive evaluation of *R. nudiflora* compensatory responses to herbivory.

Keywords Defoliation · Leaf longevity · Mechanisms of compensation · Non-structural carbohydrates · Plant physiological traits

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Introduction

Plant compensation for herbivory has been defined as the replacement of plant biomass lost to herbivores (McNaughton 1983), and is mediated by the synergistic action of multiple traits and mechanisms induced upon damage which allow plants to maintain fitness despite tissue loss (Wise et al. 2008). The magnitude of plant compensatory responses depends on multiple factors such as species life form, ontogeny

and timing of damage (Haukioja and Koricheva 2000; Boege 2005; Marquis 1992). Nonetheless, the traits and mechanisms which determine compensatory ability have not always been addressed or well understood (Tiffin 2000; Fornoni 2011) even though this represents a fundamental task to fully understand plant compensatory responses.

Plant investment in below-ground tissue and mobilization of stored (non-structural) carbon reserves from below-ground to above-ground parts upon damage is a common mechanism of compensation, especially in species with large root systems and storage organs (Tiffin 2000). Although, several studies have found a positive relationship between below-ground biomass investment and regrowth capacity following defoliation (e.g., van der Meijden et al. 1988; Hochwender et al. 2000), others have not (e.g., Richards and Caldwell 1985). Changes in leaf physiology and longevity represent another important mechanism of compensation for herbivory (Stowe et al. 2000). For example, studies have frequently found increased leaf photosynthetic rates in defoliated plants (Thomson et al. 2003; Stevens et al. 2008). In addition, defoliation can result in delayed leaf senescence and greater leaf area which in turn increase leaf photosynthetic capacity and mediate above-ground compensatory responses (Meyer 1998; Thomas et al. 2008). Yet, other studies have found that defoliated plants respond to damage by reducing leaf lifespan (e.g., Kouki 2006), and lower leaf longevity has been associated with rapid plant growth (Ackerly and Bazzaz 1995) and greater photosynthetic capacity (Wright et al. 2004) which may influence compensatory ability.

Ruellia nudiflora (Acanthaceae) is a perennial herb found in open disturbed sites in southeast Mexico. In Yucatan, populations of this species exhibit on average 20% of leaf area removed (per plant) by insect herbivores (Ortegón-Campos et al. 2009), and plants usually invest heavily in root biomass (L. Abdala-Roberts, personal observation). In addition, plants typically shed most of their leaves during periods of drought after nutrient resorption has taken place (Cervera and Parra-Tabla 2009), and this may contribute to a reduction in leaf longevity upon leaf damage to save on leaf maintenance costs and stimulate growth. The main goals of this study were to: (1) evaluate the magnitude of compensation for artificial defoliation as well as genotypic variation in

compensation for *R. nudiflora* by measuring a diverse set of vegetative and reproductive traits (i.e., fitness correlates), (2) test for a relationship between root investment and compensatory ability and explore the influence of biomass allocation patterns on compensation, and finally, (3) examine the mechanisms and traits underlying compensation for defoliation in *R. nudiflora* by comparing total non-structural carbohydrates (TNC) in roots, leaves and stems of damaged and undamaged plants, and measuring leaf physiological traits and longevity of damaged and undamaged plants.

Methods

Study species

Ruellia nudiflora (Acanthaceae) is a self-compatible perennial herb that measures between 20 and 30 cm in height and grows in disturbed open or partially shaded areas (Tripp 2007). It occurs from Texas to southeast Mexico and is widely distributed throughout the state of Yucatan (SE Mexico). Each plant produces both flowers that open (chasmogamous, CH) and flowers that do not open and obligately self-pollinate (cleistogamous, CL); overall, this species presents high rates of selfing given that CH flowers exhibit autonomous self-pollination (Abdala-Roberts et al. 2009). Leaves of *R. nudiflora* are attacked by several species of lepidopteran larvae, the most important of which are *Anartia jatrophae* and *Siproeta stelenes* (Lepidoptera: Nymphalidae). Both of this species are generalist herbivores that feed on a number of species of Acanthaceae, Scrophulariaceae, and Verbenaceae (DeVries 1987; Lederhouse et al. 1992).

Seed collection and maternal lines

In January of 2007, between 10 and 15 CL fruits (product of self-pollination) were collected from each of eight randomly selected plants (parental plants) at each of two populations, one located near the town of Yobain (21°14′ N 89°06′ W) and the other near the town of Sinanche (21°13′ N and 89°11′ W) in the state of Yucatan, Mexico. Parental plants were at least 5 m away from each other and distance between populations was of 6.5 km. Mean annual rainfall and temperature at both sites is 450–650 mm and 25.9°C

(Flores and Espejel 1994). Both populations were established on roadsides with herbaceous plant species typical of secondary succession of deciduous forests in northern Yucatan (Arellano-Rodríguez et al. 2003). Fruits from each parental plant were opened and seeds were individually germinated in plastic growing trays. After 3 weeks, in May of 2007, seedlings were transplanted to 1.1-l plastic pots that contained native soil (fertilizer was not used). Pots were then randomly placed in a common garden located at the Campus de Ciencias Biologicas y Agropecuarias of the Universidad Autónoma de Yucatán. Light, humidity, and temperature conditions were homogeneous inside the common garden (photosynthetic active radiation [mean $\pm$ SE] of 784.78 ± 52.1 μmol^{-2} s^{-1}, 12-h photoperiod, and average day temperature, and relative humidity of $30°C \pm 0.67$ and $59\% \pm 2.8$). Once in pots, each plant was given 500 ml of water twice a week which was adequate to maintain soil humidity. All plants from the same parent plant were considered a maternal full-sib line, for a total of eight lines (four per population); each line consisted of 12 plants ($n = 96$). Initial plant position was randomized in the common garden and plants were rotated every 2 weeks.

Defoliation treatment

In July of 2008, once plants were more than 1 year old and had started producing CL flowers (i.e., fully grown; 8–10 expanded leaves), six plants of each line were randomly selected and subjected to removal of 40% of total leaf area. This damage level was chosen to simulate the upper limit of damage intensity observed in some of the sampled populations of *R. nudiflora* (ca. 30–35% of leaf area consumed; Ortegon-Campos, unpublished data). The remaining six plants of each line were not defoliated and acted as controls. All plants had a similar height and number of leaves before defoliation. Leaf area removal was performed with a paperpunch, and the central vein was left untouched during defoliation, mimicking the natural pattern of defoliation. Damage was distributed in 4–5 fully expanded leaves per plant of approximately the same age (determined by a similar position along the main stem). Total leaf area per plant (cm^2) was estimated based on a regression by measuring leaf lengths and areas for a sample of 175 leaves of different sizes randomly chosen from 15 non-experimental plants;

leaf area was measured with a portable leaf area meter (LI-3000A, LI-COR, Lincoln, Nebraska). The resultant regression between leaf length and area allowed us to predict total leaf area based on measurements of leaf lengths for each experimental plant before artificial defoliation (leaf area = 1.191 [length] + 0.944; $R^2 = 0.30$, $P < 0.0001$). Total leaf area was then obtained by summing the area of all individual leaves per plant. Given that the paperpunch removed a known area, we determined the number of holes each plant would receive to achieve a 40% reduction in leaf area. Although, based on the regression the amount of variation in leaf area explained by leaf length was relatively low, thus introducing some error in the calculation of total plant leaf area and in turn the amount of leaf area to be removed per plant, such methodologically induced variation was distributed evenly among lines. This would avoid any bias when detecting defoliation $\times$ genotype interactions, and represents a more realistic defoliation regime given that the level of damage is not constant among plants under natural conditions.

Reproductive, physiological, and vegetative traits

Starting 1 week after defoliation was imposed, weekly censuses were conducted from August 2008 to October 2008, a length of time which approximated an entire growing (and flowering) season. During each census the following were recorded for each plant: number of new leaves produced (taken as a measure of growth) and number of CL fruits produced (most plants did not produce CH flowers during the sampling period). In addition, area-based photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}), mass-based photosynthetic rate (A_{mass}, $nmol$ g^{-1} s^{-1}), conductance (g, $mmol$ H_2O m^{-2} s^{-1}), and water use efficiency (WUE = photosynthetic rate/transpiration) were measured for three fully expanded undamaged leaves per plant every 2 weeks during August 2008 and September 2008. Physiological variables were measured with an infrared gas analyzer (LI-6400, LI-COR, Lincoln, Nebraska) during the morning (09:00 a.m.–11:00 a.m.). Photosynthetic flux density (PPF), air temperature, and relative humidity in the measuring head were 1000 μmol m^{-2} s^{-1}, 30°C and $\sim$55%, respectively. Leaf longevity was assessed by marking three young leaves per plant after defoliation, that were then monitored until senescence occurred, and mean leaf lifetime (weeks) was used

for statistical analysis. In addition, in October of 2008 three fully expanded leaves per plant were collected, dried at 65°C until a constant weight was achieved, and weighed to estimate specific leaf area (SLA = leaf area [cm^2]/leaf weight [g]). Finally, in January of 2009 all plants were harvested and oven-dried at 65°C until a constant weight was achieved. Root and shoot biomass were weighed separately to calculate percent root biomass (root biomass/total vegetative biomass) and total vegetative biomass per plant (excluding fruit biomass).

Non-structural carbohydrates

In July of 2009, 10 fruits were collected from each of 15 *R. nudiflora* plants from the Sinanche population. In November of 2009, fruits were opened, seeds were germinated in plastic growing trays, and a total of 30 seedlings were randomly selected, individually transplanted to 1.1-l plastic pots, and then transported to a common garden (same site used previously) where they were watered 500 ml twice a week for 2 months. In March of 2010, 15 fully grown plants (25–30 cm-height, 10–12 leaves) were subjected to 40% removal of leaf area as described above, and the remaining 15 were not damaged. One month after defoliation, plants of each defoliation treatment were harvested to quantify the concentration of glucose, starch and total amount of non-structural carbohydrates present in leaves, stems, and roots. A period of 1 month after defoliation was considered appropriate to measure changes in carbon reserves due to mobilization because *R. nudiflora* damaged plants initiate production of new leaves 10–15 days after defoliation, and such a response would presumably be associated with carbon mobilization from roots to shoots.

Samples were oven-dried at 60°C for 48 h and ground to a fine powder using liquid nitrogen. Soluble sugars were extracted from 15 mg of dry tissue per sample using 1.5 ml of ethanol (80%) at 27°C for 24 h, and then centrifugating samples at 10,000 rpm for 20 min at 25°C. Starch was extracted from the remaining pellets, after a 1-h incubation with 2.5 ml of sodium acetate buffer (0.2 M, pH 4.5) at 100°C. Extracted starch was then hydrolyzed to glucose by adding 1 ml of sodium acetate and 1 ml of 0.5% amyloglucosidase (A-7420, Sigma, St. Louis, USA), and incubated for 12 h at 55°C. Reactive glucose oxidase/peroxidase (GOD-PAP Ref. 41012, Spin

React, Girona, Spain) was used to quantify simple soluble sugars and starch (Tovar et al. 1990), and sugar concentration was measured at 505 nm using a spectrophotometer (Thermospectronic Genesis 10UV, Madison, WI, USA). Glucose was used as a standard and two replicates were run per sample. Calculation of sample glucose concentration was done with the following equations:

$$\text{Glucose(mg/dl)} = (S_A - B_A/St_A - B_A) \times (\text{SC}),$$

followed by

$$\% \text{Starch} = (\text{mg of glucose} \times 2 \times 0.9/\text{sample weight}) \times (100),$$

where S_A is the sample absorbance, B_A is the blank absorbance, St_A is the standard absorbance, SC is the standard concentration (100 mg/dl), 2 is the dilution factor, and 0.9 is the glucose transformation factor.

Statistical analyses

Leaf and fruit production, physiological traits, leaf longevity, and biomass. We used general linear mixed models with PROC GLM in SAS ver. 9.1 (SAS 2002) which included the following main factors: line (random), defoliation (fixed) and the line × defoliation interaction (random). Although, relevant to test for population-level differences in compensatory ability, the population and population × defoliation effects were not significant in initial analyses for any of the traits measured, and were thus removed from the models.

We used the accumulated number of fruits and new leaves produced per plant for analysis, while for physiological variables values were averaged across leaves and censuses for each plant given that repeated measures analyses previously showed that the defoliation × time interaction was not significant for any of the physiological variables (i.e., the effect of defoliation was constant across censuses). The models used for fruit number and new leaves also considered initial total leaf area before defoliation as a covariate to take into account plant size differences that could influence reproduction and growth capacity. In most cases, the residuals followed a normal distribution ($P > 0.05$; Kolmogorov–Smirnov tests); the only exception was for fruit number which was square root-transformed to achieve normality. If for a given variable the line

effect was significant in the two-way mixed model, we performed separate one-way models (one for each defoliation level) to evaluate the line effect for each defoliation treatment separately to achieve a more conservative estimation of genetic variation among lines (see Fornoni and Núñez-Farfán 2000). In all cases, we used Type III sums of squares and Satterthwaite approximations were employed to determine the number of degrees of freedom when more than one mean square was needed to calculate the F value for a given effect.

Genetic correlations

Compensatory ability was estimated for each line as the difference between the family mean vegetative biomass of damaged plants and the family mean biomass of undamaged plants. Total plant biomass (measured as plant cover) has been shown to be strongly positively associated with fruit production in *R. nudiflora* (Ortegón-Campos et al. 2009). In addition, given reserve storage and possible time lags in fruit production following damage in this species, estimation of compensation based on total vegetative biomass most likely represents a better estimate of long-term reproductive success and life-time fitness than fruit production restricted only to the sampling period. PROC REG in SAS was then used to test if: (1) the average percent root biomass of undamaged plants predicts compensatory ability, (2) average percent root biomass when undamaged predicts total vegetative biomass when damaged, (3) photosynthetic rate (both area- and mass-based) of undamaged plants predicts compensatory ability, and (4) leaf longevity of undamaged plants predicts compensatory ability. Values used for correlations were family means (for damaged or undamaged plants) except for compensatory ability which was calculated as explained previously.

Non-structural carbohydrates

To test for differences in TNC present in roots, stems, and leaves between damaged and undamaged plants, one-way general linear models were used (PROC GLM) which included defoliation as main effect (fixed). Residuals did not show a significant deviation from normality. Separate analyses were conducted for % of glucose, starch and total non-structural sugars present in leaves, stems, and roots.

Results

Effect of defoliation and genotypic variation in compensation

Artificial defoliation significantly reduced leaf lifespan as well as the number of new leaves produced (Table 1; Fig. 1a, b), but did not influence the number of fruits produced (0%: 20.69 ± 2.09; 40%: 21.82 ± 2.33) or total plant biomass (0%: 2.12 ± 0.14 g; 40%: 2.03 ± 0.13) (Table 1), indicating full compensation in terms of reproduction and vegetative biomass. In addition, no effect of defoliation was found on area-based photosynthetic rate (0%: 12.98 ± 0.66 μmol CO_2 m^{-2} s^{-1}; 40%: 12.69 ± 0.59), mass-based photosynthetic rate (0%: 59.21 ± 3.19 nmol g^{-1} s^{-1}; 40%: 60.16 ± 4.22), WUE (0%: 1.82 ± 0.07; 40%: 1.73 ± 0.07), conductance (0%: 0.23 ± 0.02 mmol H_2O m^{-2} s^{-1}; 40%: 0.27 ± 0.02), or SLA (0%: 218.43 ± 4.92 cm^2/g; 40%: 214.01 ± 6.89) (Table 1), suggesting that leaf physiological and morphological traits remained mostly unchanged subsequent to defoliation. In addition, no significant effect of defoliation was observed on percent root biomass (0%: 0.88 ± 0.06; 40%: 0.87 ± 0.07) (Table 1). This last result suggests that although defoliated plants most likely had greater percent root biomass relative to controls after defoliation (due to removal of aboveground leaf tissue), at the time of harvest they had similar values compared to undamaged plants presumably due to an above-ground compensatory response.

With respect to the line effect, significant differences were observed in terms of number of new leaves produced, WUE, and conductance; these differences remained significant after analyzing each defoliation treatment separately ($F_{7,24} \geq 4.14$, $P < 0.01$, $F_{5,6} \geq 3.88$, $P \leq 0.06$ and $F_{5,21} \geq 5.00$, $P < 0.01$ for tests of number of leaves, WUE, and conductance, respectively). For all other traits, however, differences between genotypes were not significant (Table 1). In addition, the genotype × defoliation interaction was also non-significant for most measured variables, the only exceptions being SLA and photosynthetic rate (marginal for area-based) (Fig. 2a, b; Table 1). This suggests that lack of genotypic variation in compensatory ability (biomass and fruit production) as well as in trait changes (e.g., leaf traits) induced by one artificial defoliation event represents a prevalent condition for the genotypes sampled.

Table 1 Two-way mixed model results of response variables measured for maternal full-sib lines of *R. nudiflora* subjected to two levels of artificial defoliation (0 and 40%)

Dependent variable	Source of variation	df	Type III ms	F	P
Leaf longevity	Defoliation	1, 7.12	5.924	9.84	**0.016**
	Line	5, 6.13	1.240	2.17	0.183
	Line × defoliation	5, 11	1.222	0.73	0.410
	Error	11	1.668		
Number of new leaves	Defoliation	1, 6.9	175.77	6.83	**0.03**
	Line	7, 7.7	355.43	13.60	**0.0008**
	Line × defoliation	7, 57	25.76	0.72	0.650
	Initial leaf area	1, 57	293.90	8.24	**0.005**
	Error	57	35.670		
Number of fruits	Defoliation	1, 6.9	0.0014	0.001	0.992
	Line	7, 7.5	2.722	2.23	0.145
	Line × defoliation	7, 57	1.206	0.75	0.634
	Initial leaf area	1, 57	15.34	9.49	**0.003**
	Error	57	1.610		
Total biomass	Defoliation	1, 7.28	0.050	0.08	0.780
	Line	7, 7.31	1.710	2.84	*0.09*
	Line × defoliation	7, 48	0.606	1.33	0.256
	Error	48	0.455		
Percent root biomass	Defoliation	1, 7.41	0.001	0.40	0.545
	Line	7, 7	0.003	0.77	0.627
	Line × defoliation	7, 49	0.004	1.23	0.302
	Error	49	0.004		
Photosynthetic rate (A)	Defoliation	1,5	0.509	0.08	0.792
	Line	5, 5	8.118	1.23	0.412
	Line × defoliation	5, 12	6.595	2.56	*0.084*
	Error	12	2.57		
Photosynthetic rate (A_{mass})	Defoliation	1,5	5.46	0.01	0.910
	Line	5, 5	216.42	0.56	0.732
	Line × defoliation	5, 12	389.02	6.83	**0.003**
	Error	12	56.92		
WUE	Defoliation	1, 5	0.053	6.18	*0.055*
	Line	5, 5	0.215	25.12	**0.001**
	Line × defoliation	5, 12	0.008	0.29	0.909
	Error	12	0.029		
Conductance (g)	Defoliation	1, 5.16	0.007	2.99	0.142
	Line	5, 5	0.042	17.02	0.0037
	Line × defoliation	5, 36	0.0024	0.57	0.719
	Error	36	0.004		
SLA	Defoliation	1, 7	235.81	0.13	0.731
	Line	1, 7	1222.50	0.66	0.701
	Line × defoliation	7, 32	1854.11	3.28	**0.009**
	Error	32	565.85		

P values for significant (*P* < 0.05) and marginally significant (0.05 < *P* < 0.1) effects are shown in bold and italics, respectively
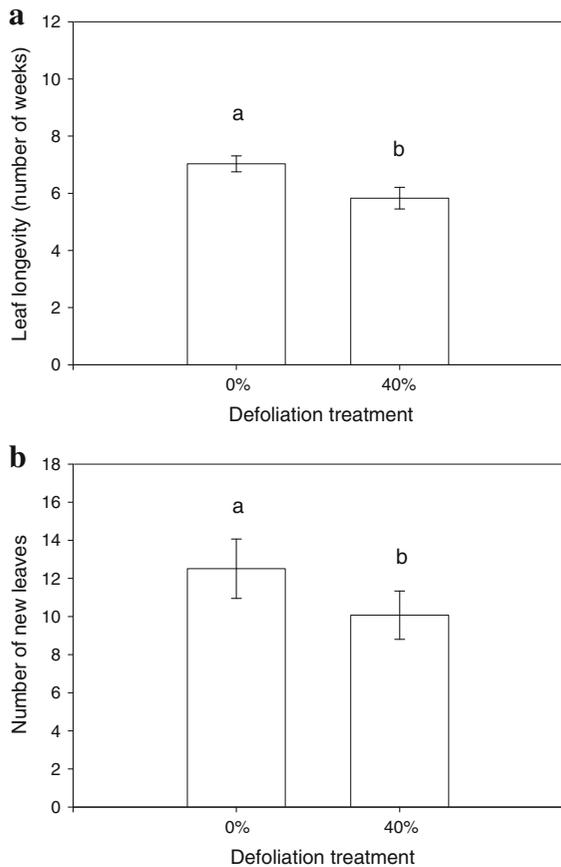
WUE water use efficiency, *SLA* specific leaf area

Fig. 1 Impact of the defoliation treatment on **a** leaf longevity (weeks) and **b** new leaves produced by *R. nudiflora*. *Bars* represent means ± 1 SE and *different letters* indicate significant differences between treatment level means ($P < 0.05$)

Fig. 2 Norms of reaction for maternal full-sib lines of *R. nudiflora* exposed to 0% (controls) and 40% of leaf area removed. **a** Photosynthetic rate per unit of mass (A_{mass}) and **b** SLA. Values shown are means for each line under each defoliation level

Root investment and compensatory ability

A significant positive relationship was found between percent root biomass when undamaged and compensatory ability ($R^2 = 0.58$, $F_{1,7} = 8.45$, $P = 0.02$; Fig. 3a), that is, genotypes which invest proportionally more in root biomass exhibited a greater compensatory response (i.e., same or greater mean value for damaged plants relative to undamaged plants). Similarly, percent of root biomass marginally (and positively) explained biomass when damaged ($R^2 = 0.42$, $F_{1,7} = 4.47$, $P = 0.07$) (Fig. 3b). This result suggests that genotypes which invest more in root biomass will not only compensate more, but will also have more biomass when damaged (i.e., greater fitness), thus linking compensation, via increased investment in roots, to greater fitness in the presence
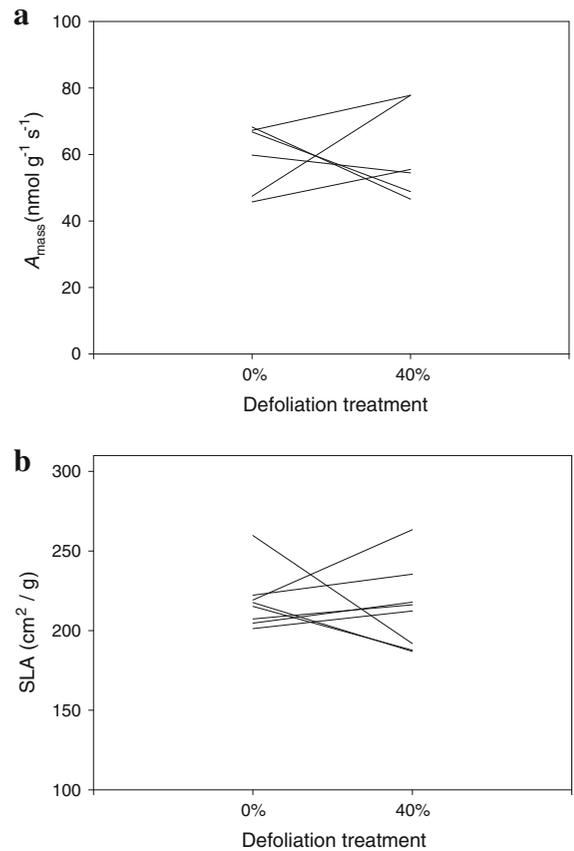
of damage. Finally, photosynthetic rate (area-based: $R^2 = 0.08$, $F_{1,5} = 1.30$, $P = 0.26$; mass-based: $R^2 = 0.20$, $F_{1,5} = 0.92$, $P = 0.37$) and leaf longevity ($R^2 = 0.27$, $F_{1,10} = 1.46$, $P = 0.29$) did not predict compensatory ability.

Changes in allocation of non-structural carbohydrates

Results showed that % of dry mass represented by glucose and starch in leaves and stems did not differ between damaged and undamaged plants ($F_{1,24} \leq 2.50$, $P \geq 0.12$) (Fig. 4). In contrast, the % of glucose and starch in roots differed significantly between damaged and undamaged plants; specifically, and against the expected pattern, defoliated plants had almost double the concentration of starch
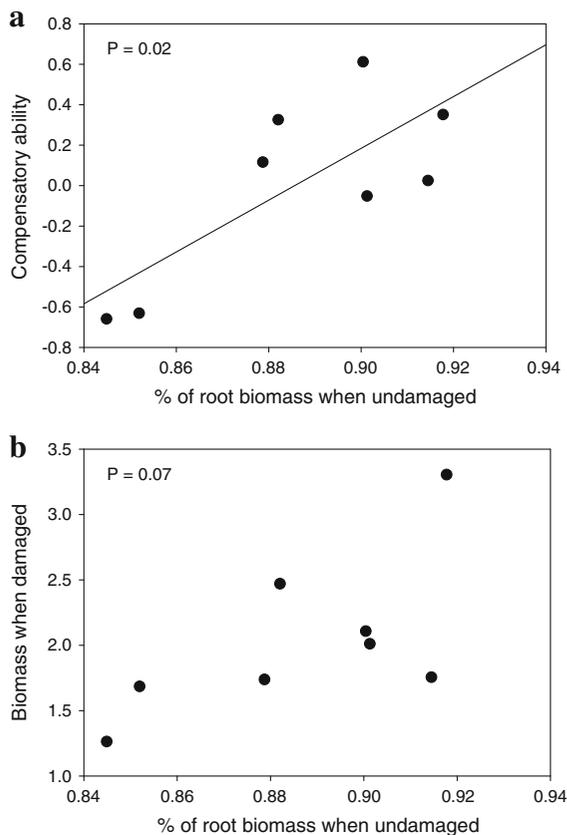
a P = 0.02

b P = 0.07

Fig. 3 Scatter plots showing genetic correlations between **a** percent of root biomass when undamaged and compensatory ability and **b** percent of root biomass when undamaged and biomass when damaged for each genotype

Fig. 4 Concentration of glucose and starch (expressed as % of dry weight) in leaves, main stem, and roots of *R. nudiflora*. Values are means ± 1 SE and *different letters indicate significant differences* between defoliation treatments for each plant part separately ($P < 0.05$)

($F_{1,24} = 13.00$, $P = 0.004$) and close to half the amount of glucose ($F_{1,24} = 19.04$, $P = 0.0002$) in roots than undamaged plants (Fig. 4). The total amount of soluble sugars did not differ between damaged and undamaged plants for leaves, stems, or roots ($F_{1,24} \leq 1.14$, $P \geq 0.29$).

Discussion

Defoliation and genotypic variation for compensation in *R. nudiflora*

Although, the level of artificial defoliation chosen (40% of leaf area removed) is higher than the amount of defoliation *R. nudiflora* plants usually exhibit in natural populations across Yucatan (Ortegón-Campos et a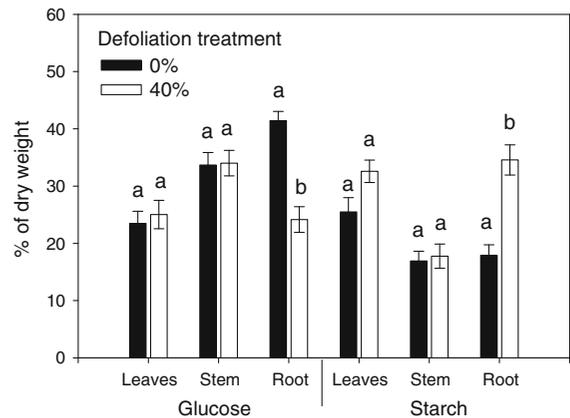l. 2009), damaged plants were able to fully compensate in terms of plant biomass and fruit production. The only evidence of incomplete compensation was given by lower production of new leaves for defoliated plants, but this did not result in reduced biomass or reproduction relative to undamaged plants. Nonetheless, plants were subjected to only one defoliation event and continuous damage throughout a longer period of time may eventually lead to incomplete compensation (Thomson et al. 2003). In addition, environmental conditions such as competition (Mabry and Wayne 1997) or resource availability (Hawkes and Sullivan 2001) may influence *R. nudiflora*'s compensatory ability. Accordingly, recent findings have shown that under natural conditions in the field, a ca. 12% of leaf area lost to folivory on average is enough to cause a significant reduction in fruit production relative to undamaged *R. nudiflora* plants (Ortegon-Campos et al. unpublished data). Based on this, it is likely that low water availability, low but persistent levels of leaf damage throughout the growing season, as well as timing of damage relative to reproduction may have contributed to incomplete reproductive compensation of this species under field conditions. Furthermore, defensive compounds induced by natural defoliation may impose an additional cost to the plant which may constrain compensatory responses (Agrawal et al. 1999), and this may also explain differences in results under field versus common garden conditions. Indeed, previous studies have pointed out that artificial

damage may not elicit a full compensatory response relative to natural leaf damage and this may represent a limitation of this study (Fornoni 2011). Finally, fruit biomass and seed production were not measured and may have provided evidence of incomplete compensation.

With respect to physiological traits, stomatal conductance and photosynthetic rates did not increase for damaged plants despite the fact that there was more root volume available to service the remaining leaf tissue. A possible cause is the water cost of reproduction, i.e., allocation of water to reproductive structures can induce midday water stress of neighboring foliage (Galen et al. 1999). Foliage typically responds to water stress with stomatal closure, which forces a decline in photosynthetic rates. In addition, the size of the reproductive sink is often negatively associated with rates of net foliar photosynthesis (Galen et al. 1999; McDowell et al. 2000; McDowell and Turner 2002). Since, defoliated plants produced the same number of fruits than control plants, the expected increase in conductance and photosynthesis for defoliated plants may have been offset by the fact that a greater proportion of undamaged leaves (chosen for measurement) of defoliated plants was probably neighboring reproductive structures and therefore more water stressed. Such a scenario is reasonable given preferential allocation of water to undamaged leaves, adjacent to which subsequent fruit production would be more likely to occur. This argument, however, remains speculative and further research on the effect of defoliation on within-plant water allocation patterns during reproduction is warranted for *R. nudiflora*.

For both growth and reproduction, the line × defoliation interaction was not significant, which contrasts with results from previous studies (e.g., Mauricio et al. 1997; Agrawal et al. 1999). This finding could reflect low genetic variability within *R. nudiflora* study populations (e.g., influenced by its breeding system) and, as a result, compensatory ability associated with herbivory would be expected to have a limited potential to evolve at present for the studied *R. nudiflora* genotypes (Pigliucci 2001). Such finding, however, should to some extent be taken with caution given the fairly low number of lines used and thus reduced statistical power which may have influenced the detection of significant interactions. We did, however, find a significant line × defoliation interaction for SLA and mass-based photosynthetic rate

which suggests that changes in leaf physiology and morphology may represent induced responses that selection could act on and in turn influence the strength of compensation in this species.

The generally consistent lack of genetic variation in compensation in terms of both growth and reproduction could also suggest that historical factors such as repeated mowing and clearing by humans at the studied *R. nudiflora* populations (L. Abdala-Roberts, personal observation) could have consistently selected for high compensatory ability of all or most of the genotypes studied (Lennartsson et al. 1997). Specifically, investment in below-ground parts and carbon reserves would be favored to increase resprouting capacity subsequent to removal of a large extent of above-ground biomass, and this has been frequently reported for species of grasses which experience predictably high herbivory rates (e.g., Hicks and Reader 1995). This argument, however, remains speculative and cannot be evaluated at this point.

Investment in roots and compensatory ability in *R. nudiflora*

We found a significant positive relationship between percent biomass invested in roots and compensatory ability in *R. nudiflora*, which suggests that stored reserves in roots and investment in below-ground tissue to increase resource acquisition will favor greater tolerance to defoliation as reported in previous studies (e.g., see reviews by Stowe et al. 2000 and Tiffin 2000; see also Schmidt and Baldwin 2009 for example of molecular basis of root allocation for compensation). Because, allocation to root biomass was positively correlated with compensatory ability and biomass when damaged (marginally in the second case), selection for greater compensatory ability can also result in the evolution of increased investment in roots and presumably a greater amount of stored reserves (Hochwender et al. 2000). Nonetheless, for this to take place genetic variation in compensation for growth or reproduction would be necessary, and, except for photosynthetic rate and SLA, this condition was not observed for most traits measured in *R. nudiflora*. Moreover, percent root biomass did not vary significantly among genotypes and this will further constrain the evolution of root biomass allocation in the context of compensatory responses to defoliation. In addition to investment in root biomass,

plant vegetative biomass (i.e., size or vigor) may also be a relevant determinant of plant fitness and compensatory ability. Although, not presented in the results, a positive significant relationship was found between biomass when undamaged and biomass when damaged (sensu Hochwender et al. 2000) as well as biomass when undamaged and fruit number when damaged, suggesting that size and/or intrinsic growth rate is probably a determinant of compensatory ability and fitness in environments with defoliation for this species.

A recent study by Stevens et al. (2008) found a positive relationship between compensatory ability and proportion of biomass allocated to stems (and not to roots) before damage in aspen (*Populus tremuloides*). This result was presumably because the main stem and branches are important sources of stored carbohydrates, and investment in branches in early successional trees such as aspen confers an advantage for light competition. In the case of *R. nudiflora*, however, greater investment in root biomass instead represents a key strategy to overcome herbivore damage, assure reproductive output, and is probably strongly influenced by adaptation to drought-tolerance conditions given the strong seasonality of the subtropical climate present in Yucatan. Based on this, it is reasonable to expect that the total amount and distribution of stored carbon reserves, as well as resource reallocation strategies will differ between herbaceous and woody species (Haukioja and Koricheva 2000).

Changes in leaf longevity and carbon mobilization due to defoliation in *R. nudiflora*

Reduced leaf longevity in response to herbivory has been reported in previous studies (Mabry and Wayne 1997; Kouki 2006; but see studies in Stowe et al. 2000). Accordingly, here we found that damaged plants of *R. nudiflora* produced leaves with a shorter average lifespan compared to undefoliated plants, and such response is presumably designed to reduce leaf maintenance costs incurred by longer-lived leaves as these may act as carbon sinks at some point subsequent to defoliation (Kikuzawa 1995). In addition, the construction of new leaves demands the reallocation of nitrogen from old leaves before leaf senescence, and this will favor a reduction in longevity of pre-existing leaves (Westoby et al. 2002) to achieve an

above-ground compensatory response of defoliated plants. Thus, a reduction in maintenance costs due to lower average leaf longevity, together with nutrient reallocation before leaf senescence (Thomas and Stoddart 1980) may explain the relationship between adjustment of leaf longevity and compensatory ability in *R. nudiflora*.

Alternatively, the observed reduction in leaf longevity upon damage may be simply viewed as the result of physiological constraints on the plant given reduced leaf area and lower allocation to leaf maintenance which would in turn argue in favor of a more indirect and weak connection with compensatory ability. Moreover, the relationship between leaf longevity and compensatory ability was not significant further calling into question the causality between changes in leaf longevity and compensatory responses in *R. nudiflora*. Regardless of this view, however, it is still reasonable to argue that reduced leaf longevity acts as a energy cost-saving strategy which influences levels of carbon storage (which in turn would influence compensatory ability). Finally, on a side note, it is interesting to point out that, given that *R. nudiflora* frequently sheds most its leaves during the dry season (Cervera and Parra-Tabla 2009), this drought-tolerance strategy may also facilitate or serve the dual purpose of reduced leaf longevity upon defoliation which suggests that both water availability and defoliation influence leaf senescence.

Results from the second experiment showed that defoliated plants exhibited almost double the concentration of starch in roots relative to control plants which was against the expected pattern assuming mobilization of carbon reserves from roots to shoots. Moreover, defoliated plants exhibited a significantly lower concentration of glucose in roots and this was presumably due to a conversion of simple sugars to starch in underground tissues. It could be argued that glucose from leaves and shoots was mobilized to underground parts thus contributing to the observed increase in starch concentration in roots of damaged plants. Nonetheless, % glucose was not lower for defoliated plants relative to control plants for either leaves or stems which suggests that most of the glucose that was converted to starch was already present in roots. Thus, instead of carbon mobilization from roots to shoots, we observed an accumulation of carbon reserves in roots based on pre-existing glucose in below-ground tissues of defoliated plants. Such

pattern has been reported in previous studies showing that herbivory (Babst et al. 2005; Schwachtje et al. 2005) and water stress (e.g., drought; Jordan and Habib 1995) can trigger carbon storage to facilitate subsequent regrowth or reproduction after the environmental stress is over. It is also possible, however, that starch reserves were depleted shortly after defoliation (before harvesting of plant tissues) to mediate an above-ground compensatory response and subsequently restored at higher amounts relative to previous conditions. Such a response would have remained undetected given the amount of time chosen for harvesting after defoliation. To assess this possibility, multiple weekly harvests would be necessary to investigate the temporal response in starch allocation patterns.

It is important to also note that carbon storage and mobilization patterns in *R. nudiflora* upon damage may depend on plant ontogenetic stage and the timing of herbivory relative to reproduction. For example, storage may be more likely before or at the beginning of the reproductive season (Marquis et al. 1997; Sosnova and Klimesova 2009) to guarantee enough resources for flower and fruit production. Hence, results from the first experiment may not be directly comparable to the second experiment which was used to measure TNC, given that in the former reproduction had already occurred before defoliation while plants used for TNC quantification were defoliated before reproduction. Based on this, it is possible that a different TNC storage or mobilization pattern may have influenced compensatory responses observed during the first experiment (i.e., mobilization of glucose from roots to shoots). Regardless of this consideration, however, our results show that, as in other herbaceous species, carbon storage can potentially serve as a mechanism to compensate for leaf damage in *R. nudiflora* with mobilization patterns varying as a function of reproductive events.

As a final note, we found that defoliation did not have an effect on photosynthetic rates or SLA in *R. nudiflora* which contrasts with previous findings for other plant species (e.g., Mabry and Wayne 1997; Thomson 2003), and the photosynthetic rate of undamaged plants was not related to compensatory ability. Despite this, as mentioned earlier the line × defoliation interaction was significant for mass-based photosynthetic rate (marginal for area-based photosynthetic rate) and SLA. This suggests that increased photosynthetic rate and SLA can evolve as a

response to defoliation within the populations studied and may represent traits that can help explain compensatory ability in *R. nudiflora*.

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