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SHORT COMMUNICATION

Does tropical forest fragmentation affect plant anti-herbivore defensive and nutritional traits?

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Abstract: Leaf traits of tropical tree species are known to operate as intrinsic determinants of insect herbivory. However, we know little about how habitat fragmentation affects these traits and what, if any, are the consequences of this process on herbivory. We tested the effects of forest fragmentation on the leaf traits of sapling of four light-demanding species: *Acalypha diversifolia*, *Hampea nutricia*, *Myriocarpa longipes*, *Siparuna thecaphora*, and two shade-tolerant species: *Pseudolmedia glabrata* and *Garcinia intermedia*, in Los Tuxtlas, Mexico. We also conducted an acceptability assay with a generalist herbivore *Spodoptera frugiperda*. Plant traits did not change with forest fragmentation, but did with plant regeneration mode and species identity. Light-demanding species had significantly higher water content, nitrogen concentration and specific leaf area than shade-tolerant species. The latter had significantly higher leaf strength, carbon concentration and carbon:nitrogen ratio. Acceptability was affected by fragmentation but only in *P. glabrata*; plant tissue from forest fragments was consumed 2.6 times more than that from continuous forest. We conclude that forest fragmentation did not affect leaf traits in this site.

Key Words: defence, forest fragmentation, leaf traits, Los Tuxtlas, shade-tolerance, tropical rain forest

Habitat loss and forest fragmentation across the tropics threaten not only individual components of biodiversity but also species interaction networks (Tylianakis *et al*. 2008). Recent studies have shown that forest fragmentation (distance to the edge, fragment size, etc.) affects plant–herbivore interactions (Wirth *et al*. 2008); however, the underlying mechanisms driving such changes remain poorly understood (Fáveri *et al*. 2008). In a previous study, we reported that herbivory declined in shade-tolerant species in small forest fragments compared with continuous forest, but did not change in the light-demanding species (Ruiz-Guerra *et al*. 2010). Here, we examine intrinsic plant traits (total phenolics leaf strength, specific leaf area, water content, and nitrogen and carbon concentration) in saplings of six species, including the predominant plant regeneration modes in tropical rain forest (light-demanding and shade-tolerant), as factors that may explain variation in herbivory between forest fragments and continuous forest. We hypothesize that light-demanding plant species would not modify their leaf traits between forest fragments and continuous forests, since they are adapted to high-light-availability environments and exhibit low plasticity (Rozendaal *et al*. 2006, Valladares *et al*. 2000). In contrast, shade-tolerant species experience important changes in light availability throughout their ontogenetic stages, and their leaf traits could be expected to show changes in response to the availability of light, and these traits are therefore expected to change between continuous forest and forest fragments (Rozendaal *et al*. 2006). In addition, plants growing in high-light environments produce more carbon-based secondary compounds such as phenolics, which constitute one of the main chemical defences against insect herbivores in the tropical rain forest (Brenes-Arguedas & Coley 2005).

The study was carried out at the Los Tuxtlas Research Station (18°30′N–18°40′N, 95°03′W–95°10′W) and adjacent areas, in Veracruz, Mexico. We compared two

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Table 1. Mean ± SE of leaf traits of of light-demanding species: *Acalypha diversifolia*, *Hampea nutricia*, *Myriocarpa longipes*, *Siparuna thecaphora*, and the shade-tolerant species: *Pseudolmedia glabrata* and *Garcinia intermedia* in continuous forest (CF) and forest fragments (FF) in Los Tuxtlas, Mexico. Values within a row followed by different superscript are significantly different (P < 0.05). Significance was based on mixed models developed for each trait separately.

Plant regeneration mode	Sites	Phenols (mg g^{-1}dry)	Leaf strength (kg cm^{-2})	Specific leaf area (kg m^{-2})	Carbon (%)	Nitrogen (%)	C:N ratio	Water content (g g^{-1})
Light demanding	CF	0.002 ± 0.0003	96.5 ± 5.78	28.7 ± 1.03	43.5 ± 0.77	2.8 ± 0.08	15.8 ± 0.43	4.9 ± 0.716
	FF	0.002 ± 0.0002	100 ± 6.89	32.2 ± 2.26	42.6 ± 0.49	2.8 ± 0.10	15.3 ± 0.40	5.1 ± 0.638
	Mean	0.001 ± 0.0001a	98.5 ± 4.47a	30.4 ± 1.23a	43.1 ± 0.45a	2.9 ± 0.06a	15.6 ± 0.29a	5.0 ± 0.474a
Shade tolerant	CF	0.001 ± 0.0001	264 ± 19.5	16.2 ± 1.00	56.4 ± 2.72	2.1 ± 0.15	27.5 ± 0.85	1.6 ± 0.049
	FF	0.001 ± 0.0001	280 ± 24.3	16.5 ± 1.95	55.9 ± 2.52	2.0 ± 0.13	28.3 ± 0.73	1.6 ± 0.058
	Mean	0.001 ± 0.0001a	271 ± 15.4b	16.3 ± 1.07b	56.1 ± 1.82b	2.0 ± 0.10b	27.9 ± 0.55b	1.6 ± 0.037b

forest types: small fragments (0.3, 3 and 19 ha), with roughly the same age of isolation from the continuous forest (*c.* 20 y), and three sites of continuous forest within the Research station. All sites are located within a restricted altitudinal range, 15–150 m asl, and present the same tropical rain-forest vegetation type (Aguirre & Dirzo 2008). We selected the six species of highest importance values: *Acalypha diversifolia* Jacq. (Euphorbiaceae), *Hampea nutricia* Fryxell (Malvaceae), *Myriocarpa longipes* S. F. Blake (Urticaceae), *Siparuna thecaphora* Poepp. & Endl. (Siparunaceae), *Pseudolmedia glabrata* C. C. Berg (Moraceae) and *Garcinia intermedia* Hammel (Clusiaceae). The former four species are light-demanding, while the latter two are shade-tolerant. We restricted our analyses to saplings (> 50 cm height and diameter < 1 cm).

We collected a set of 10 leaves (position 3 on the phyllotaxis) from each of three to five individual saplings from the six species in each site (N = 167 individuals). In addition we collected another set of two fully expanded leaves in order to estimate leaf strength (N = 216 individuals). Total phenolics were estimated using the modified Prussian blue assay (Waterman & Mole 1994). Leaf strength was measured using a penetrometer (Chantillon, Model 516, New York, USA) (Sanson *et al.* 2001). Area was measured using a portable leaf area meter (CI-202 Bio-Science). To estimate water content, we calculated the difference between fresh and dry weight (plant material was oven-dried at 60°C for 1 wk) relative to the dry weight of each individual leaf. The percentage dry mass of nitrogen and carbon was estimated by combustion using a C/N analyser (TruSpec CN, Leco Corporation 2002). In addition, we conducted an experiment with larvae of the generalist *Spodoptera frugiperda* and tissue of the six study plant species. Two leaf discs of 1 cm in diameter, corresponding to one plant from continuous forest and the other from forest fragments, were simultaneously presented to single third instar larvae of *S. frugiperda* in a Petri dish. To analyse the effect of forest type (fragments *vs.* continuous forest), plant regeneration mode (light-demanding *vs.* shade-tolerant) and species identity on plant traits, we used mixed effects models. All statistical analysis was conducted in R 2.5.2 (R development core team, https://www.r-project.org/).

Contrary to our hypothesis, we found no significant differences in leaf traits between forest types or in the interaction forest type by plant regeneration mode ($F \leq 1.32$, $P \leq 0.331$; $F \leq 0.132$, $P \geq 0.734$; Table 1). Plant responses in highly heterogeneous environments are complex and involve multiple co-occurring biotic and abiotic factors (Valladares *et al.* 2007). In our study site, we have evidence of reduced air temperature, air moisture, soil moisture as well as an increase in light intensity in the forest fragments compared with continuous forest (Ruiz-Guerra unpubl. data). These factors, coupled with the internal limits of the plants (e.g. the increased cost of plasticity in stressful environments), could limit the morphological responses between forest fragments and continuous forest (Valladares *et al.* 2007).

Plant traits varied with plant regeneration mode ($F \geq 9.78$, $P \leq 0.004$). No difference was found in the concentration of phenolics between plant regeneration modes (Table 1). Light-demanding species had higher specific leaf area, nitrogen concentration and water content than shade-tolerant species ($F_{1,27} = 18.2$, $P < 0.001$, $F_{1,28} = 9.79$, $P < 0.01$, $F_{1,28} = 28.5$, $P < 0.0001$, respectively), whereas the shade-tolerant species presented higher carbon concentration and carbon/nitrogen ratio as well as stronger leaves ($F_{1,27} = 14.3$, $P < 0.0001$, $F_{1,28} = 17.5$, $P < 0.001$, $F_{1,28} = 101$, $P < 0.0001$, respectively). These findings are consistent with other studies and are related to the exploitation of forest resources (Poorter *et al.* 2004, Popma *et al.* 1992).

Leaf traits differed significantly among species ($F \geq 29.1$, $P \leq 0.0001$) but not between forest types or in the interaction forest type by species identity ($F \leq 5.55$, $P \geq 0.255$; $F \leq 2.13$, $P \geq 0.07$ respectively). The species follow the same pattern of plant regeneration mode. Differences in leaf traits among species could be related to developmental strategies and phylogenetic constraints

(Martínez-Garza & Howe 2005, Poorter *et al.* 2004). In the food-choice experiment, the herbivore *S. frugiperda* consumed 2.6-times more leaf area from the forest fragments than in continuous forest but only of *P. glabrata* ($F_{1, 44} = 12.0$, P = 0.001). Our results therefore suggest that changes in plant traits are an unlikely explanation for the fragmentation related changes in herbivory we reported previously (Ruiz-Guerra *et al.* 2010). Leaf traits seem to be related to the capture and maintenance of some resources, and are linked to plant fitness in shaded and lit environments rather than to anti-herbivory defences (Houter & Pons 2012, Popma *et al.* 1992, Rozendaal *et al.* 2006).

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LITERATURE CITED

AGUIRRE, A. & DIRZO, R. 2008. Effects of habitat fragmentation on pollinator abundance and fruit set on an abundant understory palm in a Mexican tropical forest. *Biological Conservation* 141:375–384.

BRENES-ARGUEDAS, T. & COLEY, P. D. 2005. Phenotypic variation and spatial structure of secondary chemistry in a natural population of a tropical trees species. *Oikos* 108:410–420.

FÁVERI, S. B., VASCONCELOS, H. L. & DIRZO, R. 2008. Effects of Amazonian forest fragmentation on the interaction between plants, insect herbivores, and their natural enemies. *Journal of Tropical Ecology* 24:57–64.

HOUTER, N. C. & PONS, T. L. 2012. Ontogenetic changes in leaf traits of tropical rainforest trees differing in juvenile light requirement. *Oecologia* 169:33–45.

MARTÍNEZ-GARZA, C. & HOWE, H. 2005. Developmental strategy or immediate responses in leaf traits of tropical tree species. *International Journal of Plant Sciences* 166:41–48.

POORTER, L., VAN DE PLASSCHE, M., WILLEMS, S. & BOOT, R. G. A. 2004. Leaf traits and herbivory rates of tropical tree species differing in successional status. *Plant Biology* 6:746–754.

POPMA, J., BONGERS, F. & WERGER, M. J. A. 1992. Gap-dependence and leaf characteristics of trees in a tropical lowland rain forest in Mexico. *Oikos* 63:207–214.

ROZENDAAL, D. M. A., HURTADO, V. H. & POORTER, L. 2006. Plasticity in leaf traits of 38 tropical tree species in response to light; relationship with light demand and adult stature. *Functional Ecology* 20:207–216.

RUIZ-GUERRA, B., GUEVARA, R., MARIANO, N. & DIRZO, R. 2010. Insect herbivory declines with forest fragmentation and covaries with plant regeneration mode: evidence from a Mexican tropical rain forest. *Oikos* 119:317–325.

SANSON, G., READ, J., ARANWELA, N., CLISSOLD, F. & PEETERS, P. 2001. Measurement of leaf biomechanical properties in studies of herbivory: opportunities, problems and procedures. *Austral Ecology* 26:535–546.

TYLIANAKIS, J. M., DIDHAM, R. K., BASCOMPTE, J. & WARDLE, D. A. 2008. Global change and species interactions in terrestrial ecosystems. *Ecology Letters* 11:1351–1363.

VALLADARES, F., WRIGHT, J. S., LASSO, E., KITAJIMA, K. & PEARCY, R. W. 2000. Plastic phenotypic responses to light of 16 congeneric shrubs from a Panamanian rainforest. *Ecology* 81:1925–1936.

VALLADARES, F., GIANOLI, E. & GÓMEZ, J. M. 2007. Ecological limits to plant phenotypic plasticity. *New Phytologist* 176:749–763.

WATERMAN, P. G. & MOLE, S. 1994. *Analysis of phenolic plant metabolites*. Blackwell Scientific Publications, Oxford. 66–103 pp.

WIRTH, R., MEYER, S. T., LEAL, I. R. & TABARELLI, M. 2008. Plant herbivore interactions at the forest edge. *Progress in Botany* 69:424–448.