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Artificial Defoliation Induces Trichome Production in the Tropical Shrub *Cnidoscolus aconitifolius* (Euphorbiaceae)[1]

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ABSTRACT

Induction of plant defenses and their spatial variability are key subjects in the field of ecology and evolution of defensive traits in plants. Nevertheless, induction has been more commonly studied under controlled environments, ignoring other factors that might influence this process in natural settings. The main goal of this study was to determine if artificial defoliation induces trichome production in three natural populations of the tropical shrub *Cnidoscolus aconitifolius*. First, we performed trichome counts for each population before imposing artificial defoliation to assess differences in trichome loads between populations. Trichome densities (trichomes/cm^2) were quantified for leaf blades, petioles, and flower stalks. To determine if defoliation induced trichome production, three defoliation treatments (0% leaves defoliated or controls, 50% of total leaves defoliated, and 100% defoliation) were applied once at the beginning of the reproductive season. Trichome counts were performed on each structure every ~20 d during a 3-mo period after the application of treatments. Trichome counts showed significant differences in trichome densities between populations for all three structures. In turn, artificial defoliation increased trichome density. Significant differences among treatments were found for trichome densities on leaf blades and petioles. In both these structures, the 100 percent defoliation treatment differed significantly from control plants, presenting higher trichome densities. In addition, the treatment × population interaction was not significant for leaf blades and petioles, indicating that induction is a generalized response in this species, at least at the study sites. These results indicate that trichomes in *C. aconitifolius* are inducible due to defoliation.

RESUMEN

La inducibilidad de defensas en plantas y su variabilidad espacial, son temas clave en el campo de la ecología y evolución de las respuestas defensivas en plantas. No obstante, el mecanismo inductivo ha sido estudiado principalmente en ambientes controlados, ignorando algunos factores que pudieran influenciar este proceso en sistemas naturales. El principal objetivo del presente estudio fue determinar si los tricomas son un rasgo inducible por defoliación artificial en tres poblaciones naturales de *Cnidoscolus aconitifolius*. Se calcularon las densidades de tricomas (tricomas/cm^2) en láminas foliares, pecíolos y pedúnculos para determinar posibles diferencias en abundancia de tricomas entre poblaciones. Los tratamientos de defoliación (0% o controles, 50% y 100% de hojas defoliadas) se aplicaron una sola vez al inicio de la temporada reproductiva. Se realizó un seguimiento de las densidades de tricomas cada ~20 días durante tres meses. Hubieron diferencias significativas entre poblaciones en las densidades de tricomas de las tres estructuras. Se observaron diferencias significativas entre los tratamientos para láminas foliares y pecíolos. Para ambas estructuras, el tratamiento de 100% difirió significativamente del control, presentando las densidades mas altas de tricomas. La interacción población x tratamiento no resultó significativa para láminas foliares y peciolos, indicando que todas las poblaciones bajo estudio respondieron de la misma manera a la defoliación. Los resultados indican que los tricomas en *C. aconitifolius* son inducibles por defoliación.

Key words: Cnidoscolus aconitifolius; *defoliation; herbivory; induction; México; spatial variation; trichomes.*

PLANT SPECIES HAVE EVOLVED A SERIES OF DEFENSIVE STRATEGIES in response to herbivore damage (Pullin & Gilbert 1989, Zangerl & Bazzaz 1992, Strauss & Agrawal 1999, Rautio *et al.* 2002). One defensive strategy is the use of trichomes, which represent a physical and/or chemical defense for plants (see Levin 1973 for review of initial studies), and several studies over the past decade have reported their role as resistance traits against herbivores (Pollard 1986; Pullin & Gilbert 1989; Ågren & Schemske 1993, 1994; Mauricio & Rausher 1997; Agrawal 1999, 2000; Traw & Dawson 2002; Valverde *et al.* 2002). Their ecophysiological role in leaves has also been shown (Duke 1994, Raven *et al.* 1999, Pérez-Estrada *et al.* 2000)

Because biotic selective forces (*i.e.*, herbivory) acting on plant defenses may vary spatially (Marquis 1992; Thompson, 1994, 1997), plant defenses, specifically trichome presence and abundance, may vary accordingly (van Dam *et al.* 1999, Hare & Elle 2001). In addition to biotic and/or abiotic forces determining spatial variation in plant defense *per se*, one mechanism (commonly elicited by the former forces) contributing to this variation (usually at a finer time scale) is induction, defined in this context as an increase in defense levels in response to damage (Adler & Karban 1994). Inducible defenses have been postulated as a mechanism by which plants reduce defense costs, because defenses are relaxed in the absence of herbivore damage (Karban & Myers 1989, Karban & Baldwin 1997). Several studies have shown that trichomes are inducible structures and that herbivore damage can act as an elicitor of trichome production (Pullin & Gilbert 1989; Agrawal 1999, 2000; Traw & Dawson 2002). Most of these studies, however, have been carried out under controlled environments. Further research needs to focus on experiments in natural populations to compare results (greenhouse vs field experiments) and generate more realistic conclusions. In addition, very few studies addressing these topics have been conducted in tropical systems, and it is only by studying plant defense induction under different ecological settings that we will obtain a full understanding of this process in plant communities.

Induction of flower and leaf production in response to defoliation has been shown for the study species, *Cnidoscolus aconitifolius* (Euphorbiaceae; Parra-Tabla *et al.* 2004), which makes it reasonable to

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predict that this species' defensive traits, specifically trichomes, might also be inducible due to defoliation (not to mention other abiotic factors such as annual rainfall). In addition, herbivory on *C. aconitifolius* has shown to differ substantially across sites in Yucatán, México (Ortegón 2004), which might be a factor causing trichome loads to vary spatially.

The present study addresses the following questions: (1) Are trichomes in *C. aconitifolius* inducible by artificial defoliation? (2) Does this induction response vary among different parts of the plant (*i.e.*, leaf blades, petioles, flower stalks)? (3) Does trichome density vary among natural populations of the tropical shrub *C. aconitifolius*?

METHODS

Cnidoscolus aconitifolius is a tropical shrub, 3–5 m tall, distributed throughout most of the Yucatán Península. Both leaf blades and petioles are each 10–20 cm long; blades are shallowly or deeply lobed with three to seven lobes per leaf (Standley & Steyemark 1949). This species is monoecious with flowers arranged in inflorescences with dichotomic ramification, and stalks 15–40 cm in length (as in *C. spinosus*; see Bullock 1982). *C. aconitifolius* presents glandular trichomes on most of its aerial structures, which produce stinging compounds (L. Abdala-Roberts, pers. obs.). These compounds (*i.e.*, serotonin) have shown to confer resistance against herbivores (Pollard & Briggs 1984) and are found in other species of the same genus (*e.g.*, *C. texaunus*; Lookadoo & Pollard 1991).

Three natural populations of *C. aconitifolius* were selected, each one located in a different site in the state of Yucatán, México (Fig. 1). Each population was composed of ~50 individuals, of which 24 were tagged ($N = 72$ experimental plants in total). The populations at these sites were chosen because they have shown to differ in characteristics that were relevant to the current study, such as herbivore damage (see Table 1), which could lead to differential levels of trichome induction/abundance among sites. Field work was carried out from July 2002 to September 2002.

SITE DESCRIPTION AND HERBIVORES.—One of the populations, situated in the locality of Xmatkuil, was present in a pasture with artificial irrigation (the study area had been used for livestock feeding during previous years). Plants in Dzemul were located in a deciduous cactus forest (Flores & Espejel 1994), while in Hobonil, they were located in a semideciduous medium height forest (Flores & Espejel 1994). All sites have similar herbivore species composition (mostly Lepidoptera, Orthoptera, and Homopotera); however, Lepidopteran larvae, which constitute the most important herbivores of *C. aconitifolius* (in terms of amount of damage inflicted), have shown greater variation in species composition and abundance across study sites (Rodríguez 2004) compared to other herbivores. For example, *Chioides catillus albofascicatus* and *Anteos maerula* (both Lepidoptera, and the most important herbivores of this plant) have shown to vary in abundance between sites during the reproductive season of *C. aconitifolius*. Thus, despite overall similarities in species composition, natural herbivory rates have shown to differ between populations at each site (ranging from an average of 5–35% of total leaf area lost per plant (Ortegón 2004), with total defoliation being relatively common in some cases; V. Parra-Tabla, pers. obs.). Population differences could be a result of variation in lepidopteran abundance and composition across sites.

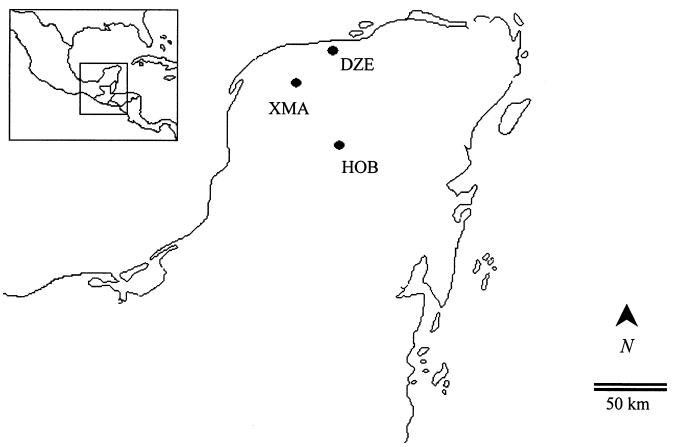

FIGURE 1. *Cnidoscolus aconitifolius* study sites.

RESPONSE VARIABLES.—Trichome density in leaf blades, petioles and flower stalks were the response variables measured. Leaf blades were sampled because it was expected that plants would respond to damage by producing trichomes on leaf blades to defend their photosynthetic tissue. Petioles were sampled because they represent a structure used by lepidopteran larvae while feeding (*i.e.*, *Anteos maerula* larvae); first the larvae wraps around the petiole compressing it until it blocks the flow of latex to the leaf blade; subsequently it starts feeding from the tip of the blade (while hanging from the petiole), and continues until it has finished eating the entire leaf. This feeding behavior results in potentially high amounts of leaf biomass lost to these herbivores (L. Abdala-Roberts, pers. obs.), and thus, petiole defense seems a reasonable mechanism to decrease herbivory by these insects. Finally, we expected that plants would also induce trichome production on flower stalks to defend reproductive structures and increase fitness in the presence of herbivores.

TRICHOME DENSITY CALCULATIONS.—Both absolute trichome numbers and area were quantified for each plant part (trichomes can be counted without the use of a microscope; they range from 0.25 to 1 cm in length). Before calculating trichome densities in leaf blades, we first measured the area of ~70 leaf blades from each site (leaf area meter model CI-202, CID, Inc.®), as well as their length and width (flexometer; Truper®, USA). Regression equations were obtained for each site (expected area = $\lambda + \beta$ [length × width]) to evaluate if length × width products were good estimates of leaf blade area. Regressions for each population showed a good estimate of area using length × width (R^2 ranged from 0.78 to 0.87, $P < 0.01$ in all cases; $N = 70$–73). The lengths × widths recorded for every individual were transformed to expected areas, which were then used to obtain trichome densities (see Núñez-Farfan & Dirzo 1994).

Trichome counts on petioles and flower stems were conducted at three different segments (each of 1 cm) along the length of the structure—(1) distal measure: 1 cm before the petiole joined the leaf blade or the flower stalk started to ramify; (2) medial: exactly at the midpoint of the structure; and (3) proximal: 1 cm before the petiole or flower stalk joined the immediate branch. This sampling method was necessary because

TABLE 1. *Characterization of study sites. DZE = Dzemul, HOB = Hobonil, XMA = Xmatkuil; Coords. = coordinates (INEGI 1994); AR = annual rainfall (CNA 2002); MAT = mean annual temperature (CNA 2002); RAL = range of total leaf area lost to herbivory (Ortegón 2004); MTD = mean trichome density ± SE (see results, Spatial variation; LB = leaf blades; P = petioles; FS = flower stalks); different letters indicate significant differences between populations for each structure (P < 0.05; Tukey).*

					MTD (trichomes cm^2)		
Site	Coordinates	AR (mm)	MAT (°C)	RAL	LB	*P*	FS
DZE	89°20'N, 21°14'W	600–700	24–26	10–20%	10.52 ± 0.02a	5.13 ± 0.02a	15.9 ± 0.05a
HOB	88°58'N, 20°5'W	1000–1200	26–28	15–35%	5.02 ± 0.52b	6.81 ± 0.47a	23.4 ± 1.34b
XMA	89°37'N, 20°52'W	1000–1200	24–26	5–10%	14.5 ± 0.62c	19.1 ± 2.80b	22.9 ± 3.00b

trichome distribution on both these structures was not homogeneous, being biased to higher densities at the distal portion (L. Abdala-Roberts, pers. obs.). Trichomes were counted along the surface of each segment, resulting in three counts, which were then averaged to obtain a single value (mean number) for every sampled petiole or flower stalk. Segment diameter was calculated once for any given petiole or flower stalk with an electronic caliper (Vernier®, ±0.01 mm) and area was obtained using height = 1 (1 cm segments). Finally, mean number of trichomes was divided by the area to obtain trichome density.

TRICHOME INDUCTION: DEFOLIATION TREATMENTS.—To evaluate trichome inducibility, three defoliation treatments were applied once on experimental plants at all three populations at the beginning of the reproductive season (start of July 2002). Leaves were clipped with scissors at the base of the petioles. The treatments were: natural herbivory or controls (0% defoliation), 50 percent defoliation (half of total leaf number randomly clipped per plant), and 100 percent defoliation (all leaves clipped per plant). Eight plants were randomly chosen for each treatment at every site ($N = 24$ per treatment across sites).

Previous defoliation treatments with this species have only reported significant results (for female flower production and leaf production) using high levels of defoliation (*i.e.*, 75% defoliation; Parra-Tabla *et al.* 2004). In addition, total defoliation has been reported repeatedly at least for one of the study sites (Hobonil), which indicates that the treatment of 100% defoliation can be representative of natural herbivory in some cases. Both these reasons (the plant's response to high defoliation in previous studies and natural total defoliations) made it reasonable and necessary to apply high intensities of defoliation (50% and 100%) on the study plants. Trichome counts were performed every 3 weeks on three new leaf blades and petioles for each plant after initial defoliation. Flower stalk counts were conducted only twice during the entire study, once in July, and again in September.

A one-way ANOVA (treatment, three levels; covariate: leaf number) was performed to determine if initial differences between treatments existed. Two-way repeated measures ANOVAs (RMANOVA) were carried out (population and treatment, three levels each; covariate: leaf number; $N = 72$) for each structure because we were following trichome numbers for the same plants during the entire sampling season (Scheiner & Gurevitch 1993, Zar 1996). Tukey tests were performed to detect which treatments differed significantly. Finally, one-way ANOVAs (treatment, three levels; covariate: leaf number) were performed for each sampling date (five in total) to detect when significant differences took place throughout the sampling season. Tukey tests were carried out for the sampling dates that did present significant differences.

SPATIAL VARIATION.—Trichome counts were performed on experimental plants at the beginning of the reproductive season (first week of July 2002), prior to treatment application, and trichome densities were compared between populations for all three structures. A one-way multivariate analysis of variance (MANOVA) was conducted (site, three levels; covariate: leaf number; $N = 72$) using trichome densities in all three structures as response variables. MANOVA was chosen because trichome densities in all three structures are correlated (Scheiner & Gurevitch 1993). A multiple comparison test (Tukey) was performed to determine which populations differed for each structure.

All statistical analyses were performed with SYSTAT for Windows (1997), v. 10 (SPSS Inc®). Data previously showed normal distribution and homoscedasticity for all analyses.

RESULTS

TRICHOME INDUCTION.—No initial differences in trichome densities were detected between treatments for any structure ($P > 0.05$ in all cases). In addition, a RMANOVA with leaf blade areas as response variable (treatment and population as main effects) was performed to assess a possible effect of defoliation on leaf size. This was done because a decrease in leaf size due to herbivory has shown to act as a confounding effect when trying to detect trichome induction (decreased areas lead to greater trichome densities while in fact absolute trichome numbers are not increasing; see Agrawal 1999, Traw & Dawson 2002). Although this analysis showed no difference between treatments (df = 2, 60, $F = 2.88$, $P = 0.068$), or a temporal pattern (df = 2, 120, $F = 2.45$, $P = 0.09$) in leaf size, differences can be considered marginal. Because of this, an additional RMANOVA (treatment, population) was performed with trichome absolute numbers, and showed significant differences between treatments (df = 2, 60, $F = 4.88$, $P = 0.01$).

The RMANOVA to test for an induction response showed significant differences in trichome densities between treatments for leaf blades (df = 2, 59, $F = 4.88$, $P = 0.011$; Fig. 2a). No differences were found for the treatment × population interaction ($P = 0.94$). The 100 percent defoliaton treatment had the highest density (1.47 ± 0.16 trichomes/cm^2), and differed significantly ($P < 0.001$; Tukey) from the control (1.14 ± 0.10 trichomes/cm^2); Figure 2a. Significant

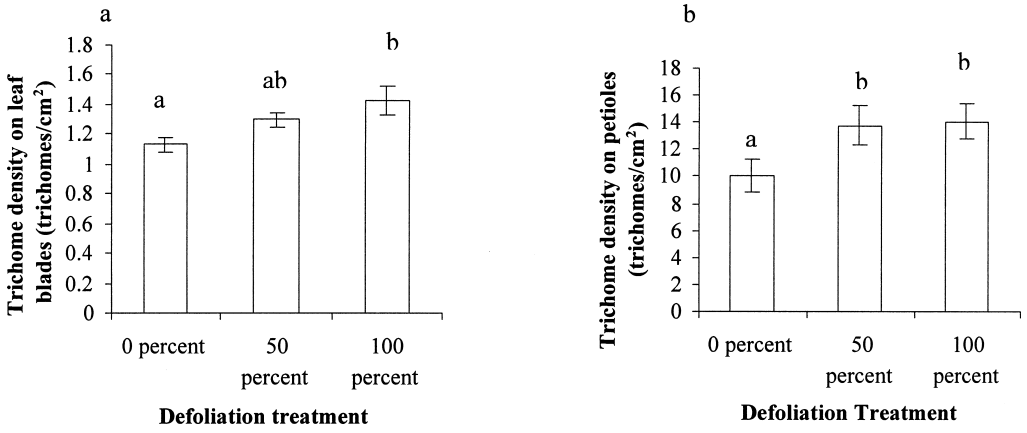

FIGURE 2. Differences in leaf blade (a) and petiole (b) trichome densities for each defoliation treatment. Bars represent the means ± SE for each treatment. Different letters indicate significant differences ($P < 0.001$; Tukey).

differences were also observed at the temporal scale (df = 8, 236, $F = 4.76$, $P < 0.001$), indicating an increase in time for trichome densities on leaf blades (Fig. 3a). Univariate ANOVAs for each sampling date showed significant differences between treatments only during two sampling dates ($F \geq 3$, $P \leq 0.05$ for both periods). The 100 percent treatment plants significantly differed from controls for both dates ($P < 0.05$ in both cases; Tukey; Fig. 3a).

The RMANOVA for petioles indicated significant differences in trichome density between treatments (df = 2, 59, $F = 3.57$, $P = 0.03$). No differences were found for the treatment × population interaction ($P = 0.142$). The 100 percent treatment plants had the highest densities (13.5 ± 1.34 trichomes/cm^2) and differed significantly from controls (10.28 ± 1.03 trichomes/cm^2); the 50 percent treatment also differed from controls ($P = 0.002$ and $P < 0.001$ respectively; Tukey; Fig. 2b). However, the temporal pattern was not significant ($P = 0.08$), indicating that induction in petioles was less pronounced compared to leaf blades (even though this result is marginal; Fig. 3b). Two sampling dates exhibited significant differences between treatments ($F \geq 3$, $P \leq 0.05$ for both dates): the first sampling date had significant differences between the 100% defoliation treatment and controls ($P < 0.05$; Tukey), while the fourth sampling date showed differences between the 100% treatment and controls, as well as between the 50% treatment and controls ($P < 0.05$ in both cases; Fig. 3b).

No differences were found between treatments for trichome densities in flower stalks (df = 2, 34, $F = 2.36$, $P = 0.10$). The treatment × site interaction, however, was significant (df = 4, $F = 2.19$, $P = 0.04$), with 50% defoliated Xmatkuil plants having the highest trichome densities across all sites and treatments (Fig. 4), although significant differences were not detected.

SPATIAL VARIATION.—Multivariate analysis of variance indicated significant differences in trichome densities between populations for all three structures (Wilk's $\lambda = 0.247$, df = 6, 118, $F = 19.8$, $P < 0.001$). For each structure the following was found: significant differences for

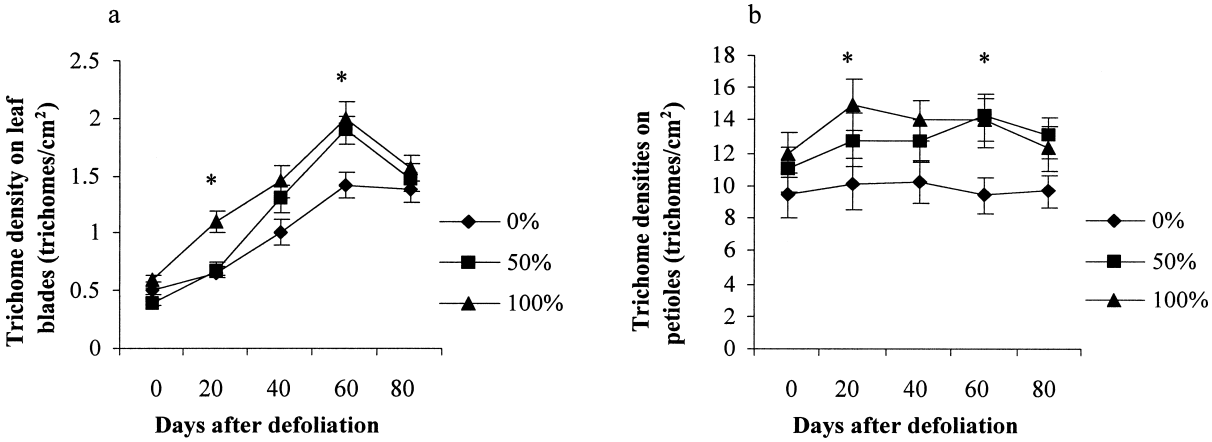

FIGURE 3. Temporal pattern of trichome densities for leaf blades (a) and petioles (b). Values correspond to means ± SE for each treatment at each sampling date. *Sampling dates with significant differences between treatments ($P < 0.05$; Tukey).

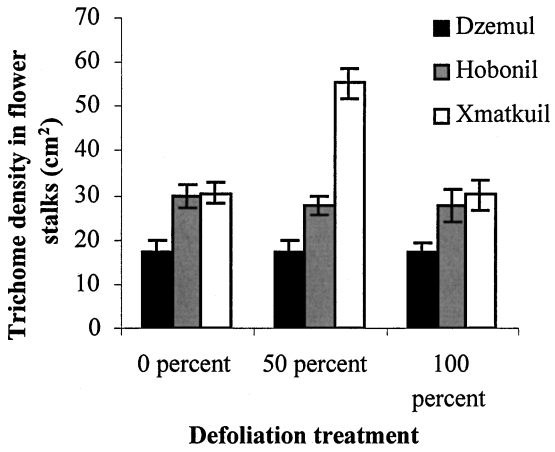

FIGURE 4. Flower stalk mean trichome densities ± SE for defoliation treatment at each site.

leaf blades (df = 2, 61, $F = 37.14$, $P < 0.001$), petioles (df = 2, 61, $F = 31.67$, $P < 0.001$), and flower stalks (df = 2, 61, $F = 6.53$, $P < 0.001$). See Table 1 for results of Tukey tests between populations for each structure. The number of leaves was not significant in any case ($P > 0.05$).

DISCUSSION

Our study demonstrated that artificial defoliation induces trichome production in leaf blades and petioles in a short period of time (~30 d to detect significant differences between treatments), and a successive relaxation after 3 mo (Fig. 3). In addition, for both these structures, the population × treatment term was not significant, indicating that the induction response was the same across populations (same pattern of increase), which is evidence of a generalized response mechanism for trichome induction in this species.

Populations of *C. aconitifolius* differed in trichome density for all structures under study, indicating spatial variation for this trait. Plants in Xmatkuil exhibited the highest overall trichome densities (Table 1). Average leaf herbivory rates reported for this site were the lowest of the three sites (Parra-Tabla *et al.* 2004, Ortegón 2004), suggesting that trichomes may act as a resistance mechanism lessening herbivore attack (Pullin & Gilbert 1989; Ågren & Schemske 1993, 1994; Mauricio 1998; Agrawal 1999, 2000; Fordyce & Agrawal 2001; Valverde *et al.* 2002), although other unmeasured abiotic factors such as rainfall or soil nutrients could be strongly influencing trichome abundance. In addition to spatial variation of biotic (and nonmeasured abiotic) forces acting on trichomes as an important cause for spatial variation of this trait, any conclusion about trichome variation between sites must also consider their inducibility as an additional source of plant defense variability. Trichome induction (shown here to be driven in part by leaf damage) can determine how abundant trichomes are at a given site in a relatively short period of time, rapidly leading to differential defense levels across sites if herbivore rates vary between them (in contrast to constitutive defenses, for example, in which case within-population changes in phenotype/genotype composition would take longer to establish even if herbivore rates differed considerably between sites).

In this study, we considered natural herbivory rates corresponding to a 3-mo period immediately before the study's sampling season (Table 1). We expected that if trichome induction and relaxation were detected for our experimental plants, sites with previously low herbivory (*i.e.*, Xmatkuil) would have plants that had relaxed their defenses and exhibited relatively low trichome numbers. The opposite would be expected for sites with previously high herbivory (*i.e.*, Hobonil). However, this pattern was not observed, since plants from Xmatkuil for example, had the highest trichome counts. Because these herbivory measurements were made on non-experimental plants, the specific history of attack of the sampled plants was not known. Results could, therefore, be misleading when correlating trichome induction and abundance with herbivory intensity, specially if they are not representative of damage for the entire population (for Xmatkuil at least). Moreover, these herbivory rates do not include several previous seasons, which would be necessary to reconstruct more adequately the herbivore damage history of the populations (although this species has shown immediate response to damage not only here, but also for flower production and leaf growth (Parra-Tabla *et al.* 2004), indicating a strong "current response component" regardless of conditions during previous seasons). Independently of the limitations of the herbivory rates employed, maybe in addition to herbivory, other unmeasured variables influence trichome abundance (considering their role in plant ecophysiology and metabolic cost), such as water and nutrient availability (Woodman & Fernandes 1991). Nonetheless, the degree to which abiotic factors could affect trichome densities remains unknown and their influence is merely speculative. Only field measurements of percentage humidity, evapotranspiration, and soil nutrients, as well as greenhouse experiments controlling these factors might answer this question.

Trichome densities of Dzemul plants occupied an intermediate place compared to the other two sites, which is possibly due the fact that this site exhibits intermediate levels of herbivory (compared to the other sites). Hobonil was the site with the highest herbivory rates; however, it exhibited the lowest trichome densities of all three populations for leaf blades and petioles. Nevertheless, plants in Hobonil had the highest trichome densities for flower stalks (Table 1), which could be reflecting a response to the elevated natural herbivory in this site. Low trichome numbers on leaf blades and petioles could be indicating other factors affecting trichome densities in these structures.

Trichome induction in *C. aconitifolius* was present in leaf blades and petioles; however, the temporal pattern of trichome increase was less marked for the latter. For both structures, induction was most clear under the 100 percent defoliation treatment, meaning that herbivory must be high to induce trichome production. Nevertheless, this study focuses only on one season, and natural defoliation could, on average, be higher if several seasons are taken into account, not to mention accumulated amounts of constant herbivory over several years (*i.e.*, chronic herbivory; see Cobb *et al.* 2001), which could eventually lead to higher trichome loads, even though damage is not very high each season.

Contrary to leaf blades and petioles, overall trichome induction in flower stems was not detected. One reason for this was probably that trichome densities in flower stalks were already very high before

plant defoliation. Consequently, an induction response would have been difficult to detect because these structures were previously highly defended. Nevertheless, the treatment × population interaction was significant, and this result was due to plants in Xmatkuil that responded to the 50 percent defoliation treatment (Fig. 4). This is evidence of trichome induction, although the response was considerably more limited to that of leaf blades where the induction response was present in all populations and exhibited a marked temporal pattern.

To conclude, herbivory can induce trichome production in *C. aconitifolius*, and apparently represents a relevant selective force determining trichome abundance in natural populations of this plant species. However, its importance as a primary factor determining trichome induction and abundance will depend on: its own intensity and duration over time, and the plant part under study (the clearest induction response for leaf blades, and the most limited for flower stalks). These factors should be taken into account when studying glandular trichomes, as their combined consideration will help to better understand the prevalence and inducibility of this defensive trait.

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LITERATURE CITED

ADLER, F. R., and R. KARBAN. 1994. Defended fortresses or moving targets? Another model of inducible defenses inspired by military metaphors. Am. Nat. 144(5): 813–832.

AGRAWAL, A. A. 1999. Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. Ecology 80(5): 1713–1723.

———. 2000. Benefits and costs of induced plant defense for *Lepidum virginicum* (Brassicaceae). Ecology 81(7): 1804–1813.

ÅGREN, J., and D. SCHEMSKE. 1993. The cost of defense against herbivores: An experimental study of trichome production in *Brassica rapa*. Am. Nat. 141(2): 338–350.

———, ———. 1994. Evolution of trichome number in a naturalized population of *Brassica rapa*. Am. Nat. 143(1): 1–13.

BULLOCK, S. H. 1982. Componentes fenológicos del sistema de entrecruzamiento monóico de *Cnidoscolus spinosus* (Euphorbiaceae) en Jalisco. Bol. Soc. Bot. Mex. 42: 1–9.

COBB, N. S., R. T. TROTTER, and T. G. WHITHAM. 2001. Long-term sexual allocation in herbivore resistant and susceptible Pinyon Pine (*Pinus edulis*). Oecologia. 20 pp. Available at: http://www2.nau.edu/~bah/BIO326/Cobb.pdf.

DUKE, S. O. 1994. Glandular trichomes: A focal point of chemical and structural interactions. Int. J. Plant Sci. 155(6): 617–620.

FLORES, S., and I. ESPEJEL. 1994. Tipos de vegetación de la Península de Yucatán. Etnoflora Yucatanense. Fascículo 3. UADY. México. 135 pp.

FORDYCE, J. A., and A. A. AGRAWAL. 2001. The role of plant trichomes and caterpillar group size on growth and defense of the Pipevine swallowtail *Battus philenor*. J. Anim. Ecol. 70: 997–1005.

HARE, J. D., and E. ELLE. 2001. Geographic variation in the frequencies of trichome phenotypes of *Datura wrightii* and correlations with annual water deficit. Madroño 48(1): 33–37.

INEGI. 1994. Carta topográfica. 1: 1 000 000. México.

KARBAN, R., and J. MYERS. 1989. Induced plant responses to herbivory. Ann. Rev. Ecol. Syst. 20: 331–348.

———, and I. T. BALDWIN. 1997. Induced responses to herbivory. University of Chicago Press, Chicago, Illinois, 319 pp.

LEVIN, D. A. 1973. The role of trichomes in plant defense. Q. Rev. Biol. 48(1): 3–15.

LOOKADOO, S. E., and A. J. POLLARD. 1991. Chemical contents of stinging trichomes of *Cnidoscolus texanus*. J. Chem. Ecol. 17(9): 1906–1916.

MARQUIS, R. J. 1992. The selective impact of herbivores. *In* R. S. Fritz and E. L. Simms (Eds.). Plant resistance to herbivores and pathogens, ecology, evolution and genetics, pp. 301-325. University of Chicago Press, Chicago, Illinois, 590 pp.

MAURICIO, R. 1998. Costs of resistance to natural enemies in field populations of the annual plant, *Arabidopsis thaliana*. Am. Nat. 151(1): 20–28.

NÚÑEZ-FARFÁN, J., and R. DIRZO. 1994. Evolutionary ecology of *Datura stramonium* L. in central Mexico: Natural selection for resistance to herbivorous insects. Evolution 48(2): 423-436.

ORTEGÓN, I. 2004. Variación espacial en la composición química de ceras epicuticulares de *Cnidoscolus aconitifolius*. Tesis de Maestría. Centro de Investigación Científica de Yucatán.

PARRA-TABLA, V., V. RICO-GRAY, and M. CARBAJAL. 2004. Effect of defoliation on leaf growth, sexual expression and reproductive success of *Cnidoscolus aconitifolius* (Euphorbiaceae). Plant Ecol. 173: 153–160.

PÉREZ-ESTRADA, L. B., Z. CANO-SANTANA, and K. OYAMA. 2000. Variation in leaf trichomes of *Wigandia urens*: Environmental factors and physiological consequences. Trends Ecol. Evol. Physiol. 20: 639–632.

POLLARD, A. J. 1986. Variation in *Cnidoscolus texanus* in relation to herbivory. Oecologia (Berlin). 70: 411–413.

——, and D. BRIGGS. 1984. Genecological studies of *Urtica dioica* L.III. Stinging hairs and plant-herbivore interactions. New Phytol. 97: 507–522.

PULLIN, A. S., and J. E. GILBERT. 1989. The Stinging Nettle, *Urtica dioica*, increases trichome density after herbivore and mechanical damage. Oikos 54: 275–280.

RAUTIO, P., A. MARKKOLA, J. MARTEL, J. TUOMI, and E. HÄRMÄ. 2002. Developmental plasticity in birch leaves: Defoliation causes a shift from glandular to non-glandular trichomes. Oikos. 98: 437–446.

RAVEN, P. H., R. F. EVERT, and S. E. EICCHORN. 1999. Biology of plants. 6th edn. Freeman, New York, 944 pp.

RODRÍGUEZ, F. P. 2004. Estudio comparativo de poblaciones de *Cnidoscolus aconitifolius* Mill. (Euphorbiaceae) de Yucatán, México: fenología, gremio de polinizadores, éxito reproductivo, y estructura genética. Tesis de Licenciatura. Universidad Autónoma de Yucatán.

SCHEINER, S. M., and J. GUREVITCH. 1993. Design and analysis of ecological experiments. Chapman Hall. New York, New York, 445 pp.

STANDLEY, P. C., and J. A. STEYEMARK. 1949. Flora of Guatemala. Vol. 24, part VI. University of Chicago Press, Chicago, Illinois 440 pp.

STRAUSS, S. Y., and A. A. AGRAWAL. 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecol. Evol. 14(5): 179–185.

SYSTAT. 1997. SYSTAT for Windows, Version 10. SPSS Inc., 699 pp.

THOMPSON, J. N. 1994. The coevolutionary process. University of Chicago Press, Chicago, Illinois, 376 pp.

———. 1997. Evaluating the dynamics of coevolution among geographically structured populations. Ecology 78(6): 1619–1623.

TRAW, M. B., and T. E. DAWSON. 2002. Differential induction of trichomes by three herbivores of Black Mustard. Oecologia 131: 526–532.

VAN DAM, N. M., J. D. HARE, and E. ELLE. 1999. Inheritance and distribution of trichome phenotypes in *Datura wrightii*. J. Hered. 90(1): 220–227.

VALVERDE, P. L., J. FORNONI, and J. NÚÑEZ-FARFÁN. 2002. Defensive role of leaf trichomes in resistance to herbivorous insects in *Datura stramonium*. J. Evol. Biol. 14: 424–432.

WOODMAN, R. L., and G. W. FERNANDES. 1991. Differential mechanical defense-herbivory, evapotranspiration, and leaf hairs. Oikos 60: 11–19.

ZANGERL, A. R., and F. A. BAZZAZ. 1992. Theory and pattern in plant defense allocation. *In* R. S. Fritz and E. L. Simms (Eds.). Plant resistance to herbivores and pathogens, ecology, evolution and genetics, pp. 363–391. University of Chicago Press. Chicago, Illinois, 590 pp.

ZAR, J. H. 1996. Biostatystical analisis, 3rd edn. Prentice-Hall. New York, 662 pp.