LIGHT, WIND, AND TOUCH INFLUENCE LEAF CHEMISTRY AND RATES OF HERBIVORY IN *APOCYNUM CANNABINUM* (APOCYNACEAE)

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Simply visiting and manipulating plants in a way consistent with measurement in typical ecological studies influences the amount of leaf herbivory experienced by some plant species. We examined the mechanistic basis for why *Apocynum cannabinum* is particularly responsive to such visitation and manipulation. In a field experiment, we manipulated both visitation and shading by neighboring plants and measured the resultant changes in plant chemistry, growth, and herbivory. In a greenhouse experiment, we manipulated touch and wind exposure while holding light constant, allowing us to directly test whether the handling causes changes in the plant that might also occur in response to wind exposure. Visitation and neighbor tie back both increased herbivory, shoot biomass, and cardenolide concentration. These changes appear to be mediated by changes in light environment with each treatment. Leaf N and C were also highly responsive to visitation, neighbor tie back, and touch. The strong and similar responses to visitation and neighbor tie back suggest that in this species, visitation acts by reducing aboveground competition through trampling of neighbors; that growth, plant chemistry, and herbivory are extremely sensitive to visitation effects associated with basic ecological measurement; and that competition between plants for light can influence plant-insect interactions. Of even greater importance is the identification that some species are extremely sensitive to even minor changes to their local environment. Such sensitivity may have significant implications for growth in natural communities.

Keywords: Apocynum cannabinum, herbivory, thigmomorphogenesis, visitation effects, competition.

Introduction

Herbivory strongly affects the success of plant species through its influence on plant growth (Louda 1984), reproductive biology (Niesenbaum 1996; Strauss 1997), population demography (Louda and Potvin 1995), and community dynamics (Coley 1983; Fritz and Simms 1992) and has thus been a factor of interest to ecologists for decades. However, recent research suggests that simply visiting and measuring leaf damage by herbivores, a common practice among ecologists, influences the amount of leaf herbivory experienced by some plant species (Cahill et al. 2001). These "visitation effects" have been documented in several community types by many researchers, with mixed results and with different species responding in different ways (Cahill et al. 2002; Schnitzer et al. 2002; Bradley et al. 2003; Hik et al. 2003), but one striking factor from each study is that a small subset of the species tested appear to have a very strong response to visitation, even if most species do not (Hik et al. 2003). This suggests that, regardless of the overall impacts of visitation on herbivory in a community, some species will likely respond strongly. We focus here on why one species, *Apocynum cannabinum*, is particularly responsive to visitation. By understanding the mechanistic basis for such visitation re-

sponses, we anticipate being better able to understand the underlying ecology of plant-herbivore interactions in natural communities.

There have been two main hypotheses for why handling plants might influence herbivory. First, plants exhibit a variety of responses to touch and wind (Jaffe et al. 2002), including changes in secondary chemistry (Cipollini 1998) and herbivory (Cipollini 1997; Cahill et al. 2002). However, in natural systems, plants are routinely brushed by neighboring plants, and it is unclear whether the modest amounts of additional touch associated with ecological research are generally of a magnitude great enough to cause a shift in secondary chemistry. Concerns over impacts of handling plants to take measurements have been expressed before (Niklas 1992; Klaring 1999), and repeated stroking of a plant has been shown to reduce herbivore (aphid) population sizes on individual plants (Van Emden et al. 1990). Second, in order to measure a plant in a natural system, it is first necessary to walk to it. As a result, neighboring plants are inevitably trampled, altering potentially ecologically important factors such as light availability. Changes in light can have direct effects on plant chemistry (Dudt and Shure 1994), growth (Bazzaz 1996), and insect activity (Herrera 1995). Additionally, since herbivory and competition are not independent processes (Agrawal and Van Zandt 2003; Agrawal 2004; Cipollini 2004; Haag et al. 2004), changing the nature of competitive interactions through trampling may indirectly impact herbivory and plant chemistry.

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Recent work provides support that both handling and trampling of neighboring plants influence *A. cannabinum* (Cahill et al. 2002), with trampling increasing leaf herbivory and handling the plants increasing the emergence of a stem borer. Further, both trampling of neighbors and handling of *A. cannabinum* resulted in a variety of effects on plant growth and architecture (Cahill et al. 2002), which, regardless of their impact on herbivory, could have an ecologically relevant impact on plant fitness within the community. Here we present results from two experiments designed to understand the mechanisms that are driving these changes previously found in *A. cannabinum*. In a field experiment, we manipulated both visitation to and handling of focal plants as well as shading by neighboring plants, measuring the resultant changes in plant chemistry, growth, and herbivory. In a greenhouse experiment, we manipulated touch and wind exposure, allowing us to directly test whether the handling of plants that is involved in taking plant measures causes changes in plant growth, chemistry, and palatability similar to those experienced in response to high wind exposure.

Material and Methods

Study System

Apocynum cannabinum L. (Apocynaceae), commonly known as both dogbane and Indian hemp, is an erect perennial herb common in woods, old fields, and other open areas. Stems are reddish, smooth, tough, and branched and grow to 1–2 m. The plant contains a milky white sap containing cardenolides, specifically, the glycosides cymerin and apocynein (Knight and Walter 2002). At our sites, the most abundant herbivore of *A. cannabinum* is *Cycnia tenera* Hübner (Arctiidae), the dogbane tiger moth. Chrysomelid leaf beetles are also known to feed on *A. cannabinum* and sequester their cardenolides for their own defense (Dobler et al. 1998); however, they were not observed at our field sites.

Field Study

Our field studies were conducted on a 5-ha old field that is part of the 55-ha Muhlenberg College Conrad W. Raker Wildlife Preserve in Germansville, Lehigh County, Pennsylvania. The site is fenced and locked with no public access and maintained by mowing every 2–3 yr. The field was not mowed during the growing season of, or immediately before, this research. In addition to *A. cannabinum*, the dominant plants in this field include *Solidago altissima* L. (Asteraceae), *Aster puniceus* L. (Asteraceae), *Achillea millefolium* L. (Asteraceae), *Toxicodendron radicans* L. Kuntz. (Anacardiacese), *Andropogon virginicus* L. (Poaceae), and *Rubus pensilvanicus* Poir. (Rosaceae).

One hundred forty *A. cannabinum* were selected at random as they emerged from the soil in April 2003. Because of the inclusion of a treatment of plant handling in this study, plants were not tagged directly. Instead, each shoot was labeled by spiking an aluminum tag with a 2-in nail in the soil 5 cm from the base of the shoot. Plants were also labeled by plant ID and by treatment with color-coded flags placed within ca. 50 cm of the plant. Thirty-five plants were randomly assigned to each of four treatment combinations:

control, visited, neighbor tie back, and visited and neighbor tie back.

The visitation treatment consisted of treated plants visited and gently stroked once from the base of the plant up toward the top on a weekly basis starting in May and ending in September 2003. This level of handling is similar to that associated with measuring plant height. Control plants did not receive weekly visitations, nor did we walk within 3 m of them throughout the entire experiment. The neighbor tie back treatment consisted of restraining the growth of plants immediately surrounding one-half of the focal plants using a 1-m^2 piece of thin black plastic netting with a mesh size of 1.5 cm^2 (Easy Gardner bird block mesh covering). The tie back treatment was applied in April 2003 and consisted of placing the focal plant through a 3-cm^2 hole in the center of the netting and then anchoring the corners of the netting with long pins, thereby restricting shading from neighboring plants (e.g., Cahill 1999). Control plants did not have their neighbors manipulated.

The experiment was harvested in September 2003. At harvest, PAR was measured with an Accupar Model PAR-80 light ceptometer at the apical meristem of each focal plant alternating sequentially by treatment. Measurements (μmol s^{-1} m^{-1}) were made all on the same day on which there was no cloud cover between 1100 and 1230 hours. All plants were located, plant height and branch number were recorded, and the shoots of each surviving plant were cut at the soil surface and placed into a large sealable plastic bag. In the laboratory, all leaves per plant were counted and assessed individually for herbivory as follows: $0 = 0\%$ leaf area removed (LAR), $1 = 1\% < LAR < 50\%$, $2 = 50\% < LAR < 100\%$, $3 = 100\%$. It was possible to place leaves in category 3 because leaf pedicels were not removed by the herbivore even when the entire leaf area was removed. Herbivory values for each leaf were summed (using the midpoint of each category) to provide an estimate of the total percentage of leaf area that was removed through herbivory for each plant.

All plant material was dried at 60°C for 48 h and then weighed. Leaves were ground on a Wiley mill with a 20-mesh screen and stored in airtight vials at 60°C for no longer that 10 d, and a random subset of samples from 35 plants across all treatments was then prepared for chemical analysis, as described below.

Greenhouse Study

The greenhouse study was performed in the Muhlenberg College greenhouse in Allentown, Pennsylvania, which is screened and temperature controlled and provides supplemental light as needed. In the year before our greenhouse study, *A. cannabinum* root stock was harvested from three different locations within our field site and potted in 100-cm^3 rooting pots with a high porosity peat/bark growing medium. Plants were allowed to grow in the greenhouse for one season and then were overwintered in controlled refrigeration at 4°C. Dead shoots were removed, and pots were placed in the greenhouse for a second season of growth, during which this experiment took place. Pots were placed in trays that were maintained with 5 cm of water to allow plants to continuously access water from below. Before the experiment, initial plant height and leaf number were

recorded. In our fully factorial design, there were two treatment levels, and plants were assigned randomly to each of four treatment combinations, replicating each combination 20 times. The two treatments were touch and wind, crossed in a factorial design. For the touch treatment, plants were gently stroked once from the base of the plant up toward the top three times per week during the 4-wk experiment. Though the intensity of a single-touch event was similar to those in the field studies, the frequency of touch in the greenhouse study was much greater. In the wind treatment, plants were exposed to high-speed oscillating fans for 3 h d^{-1}, with a 30-min on/off cycle that lasted 6 h each morning. Overhead controlled lighting kept light levels constant across treatments. Because plants were potted and on benches, the touch treatment involved only physical manipulation of the plants and did not influence light or soil conditions.

After 4 wk, plants were removed from the greenhouse and placed on a laboratory bench. A subset of plants, up to six to nine per treatment combination, were used in a feeding trial with larvae of *C. tenera*. The larvae were collected from *A. cannabinum* in the field 24 h before the start of the trials, starved, weighed, and then immediately placed on individual plants and enclosed in a bag made of white bridal veil material. After 48 h, caterpillars were removed from plants, starved, and weighed, with leaves counted and categorized by level of herbivory, as described above.

At the end of the experiment, final height, leaf number, and shoot dry biomass were measured for all plants. Plant material was dried at 60°C for 48 h, and leaves were ground on a Wiley mill with a 20-mesh screen and stored in airtight vials at 60°C for no longer that 10 d. Samples from all plants were prepared for C and N analysis, and a random subset of five plants per treatment combination was prepared for cardenolide analysis, as described below.

Leaf Chemical Analysis

Modifying the method of Wiegrebe and Wichtl (1993) for the solid phase extraction of cardenolides, 300 mg of ground, dried plant materials were refluxed for 10–12 min in 20.0 mL 70% methanol using a Thermowell with a Powermite set at 40% power. Five milliliters of 15% lead acetate and 5.0 mL of 4% sodium hydrogen phosphate were added with mixing. The solution was then diluted with 20.0 mL of water and centrifuged at 2500 rpm for 5 min. Ten milliliters of the supernatant were passed through a Baker C_{18} disposable extraction column conditioned with 2×2.0 mL of methanol and then 2×2.0 mL of water using a Baker SPE vacuum manifold. Following this, the SPE column was rinsed with 2.0 mL of water, and the cardenolides were eluted with 2×1.0 mL of methanol; the final 2 mL of extract were collected and stored for analysis. In a glass cuvette, 500 μL of extract were mixed with 500 μL of 2% 3,5-dinitrobenzoic acid, 700 μL of methanol, and 300 μL of potassium hydroxide. A Perkin Elmer Lambda Bio 40 UV-Vis Spectrometer was used to measure the absorbance at 535 nm for 20 min. A blank for each sample, including all of the above except the extract, was also analyzed, and cardenolide concentration (in digitoxin equivalence, mg g^{-1}) was calculated using blank corrected absorbance values and based on a digitoxin standard curve (Dobler and Rowell-Rahier 1994). The concentrations of carbon and nitrogen based on the dry mass of samples were obtained using a Thermo-Finnigen EA1112 Flash CHN analyzer, and C : N ratios were calculated accordingly.

Data Analysis: Significance Values

In the analysis of these two experiments, we conducted many statistical tests on a large number of response variables. A perpetual debate in ecology is over the appropriate use of "corrections" to reduce experiment-wide Type I error rates, and there is no obvious solution that is supported by everyone (Bradley et al. 2003; Cahill et al. 2004; Louda et al. 2004). One approach that can be used would be one MANOVA for the two experiments (field, greenhouse). However, we did not measure every response variable on every plant, and instead we conducted chemical analyses on a small subset of the plants. As a result, each experiment would require several MANOVAs, defeating the goal of a single test. Conceptually, we also doubt the value of a MANOVA in the field experiment because it would be testing the trivially obvious question, "Does anything change in plants if you tie back

Table 1

Field Experiment: Results from ANOVAs to Determine the Effects of Visitation and Neighbor Tie Back on Numerous Response Variables

	Visitation		Tie back		Visit × tie back		
	F	P	F	P	F	P	df
Mean PAR at meristem	**22.85**	**<0.001**	**21.31**	**<0.001**	3.47	0.065	1, 136
Shoot biomass	**5.93**	**0.017**	**4.74**	**0.032**	0.03	0.867	1, 104
Leaf herbivory (%)	**8.14**	**0.005**	**9.15**	**0.003**	1.25	0.267	1, 106
Plant height	2.11	0.149	**6.34**	**0.013**	0.30	0.863	1, 122
Leaf number	3.80	0.054	0.86	0.356	2.37	0.127	1, 111
Cardenolides	**8.17**	**0.008**	**9.15**	**0.005**	0.37	0.547	1, 30
N (%)	0.37	0.547	0.26	0.616	**9.42**	**0.005**	1, 30
C (%)	**67.39**	**<0.001**	0.77	0.387	1.06	0.310	1, 30
C : N	**41.79**	**<0.001**	1.00	0.325	**8.48**	**0.007**	1, 30

Note. All terms in the models were fixed effects, and all F ratios were calculated using the residual MS of the models; df vary among response variables because different numbers of plants were available for the different measures. Terms significant at $P \leq 0.05$ are presented in boldface. N (%) and C (%) refer to per dry mass measures of leaf tissue.

neighbors," and instead the questions of interest are response variable specific. Nonetheless, risks of Type I errors are real; thus, to allow individuals to make their own decisions on the appropriate α value for a given test, we provide actual P values.

Data Analysis: Field Experiment

All analyses were conducted using SPSS ver. 13.0 (SPSS 2004), and all variables were natural log transformed before analysis to normalize the data. To determine the effects of visitation and neighbor tie back on plant growth, leaf chemistry, light availability, and herbivory, several fixed-effects ANOVA were conducted. In each model, visitation $(+/-)$, neighbor tie back $(+/-)$, and their interaction served as fixed effects, with each model containing one of the following response variables (all referring to measures of the target plant): proportion of available light reaching the plant meristem, shoot biomass, height, leaf number, mean leaf herbivory, cardenolide concentrations in leaf tissue, leaf N percent, and leaf C percent.

A separate analysis was conducted to determine which of the response variables measured best explained the observed variation in herbivory found among plants. Herbivory served as the response variable in a forward stepwise regression, with all other measures serving as independent variables. This analysis was conducted without including tie back and visitation treatments in the model, because the goal was to determine whether there were any general patterns related to herbivory rather than to identify those that changed specifically in response to the imposed treatments.

Data Analysis: Greenhouse Experiment

All analyses were conducted as general linear mixed models using SAS version 8.0 (SAS Institute 2001), and all variables (except caterpillar mortality) were natural log transformed before analysis to normalize the data. To determine the effects of touch and wind on a variety of response variables, "Proc Mixed" was used, with rack (=location in greenhouse) as a random effect and touch, wind, and the touch $\times$ wind interaction as fixed effects. Response variables included plant height, leaf number, leaf herbivory, leaf cardenolide concentrations, leaf C percent and N percent (per dry mass), and caterpillar final weight (with initial weight as a covariate). The impact of the treatments on caterpillar mortality was conducted as a generalized linear mixed model using the %Glimmix macro associated with "Proc Mixed" in SAS version 8.0 (SAS Institute 2001), allowing for the binomially distributed response variable, caterpillar status (Littell et al. 1996).

Results

Field Experiment

Both neighbor tie back (TB) and visitation (V) had significant impacts on a variety of plant traits (table 1). Visitation and neighbor tie back increased both the light reaching the focal plant's apical meristem (fig. 1A) and shoot biomass (fig. 1B), with the combined treatment effects being additive for each response variable (table 1).
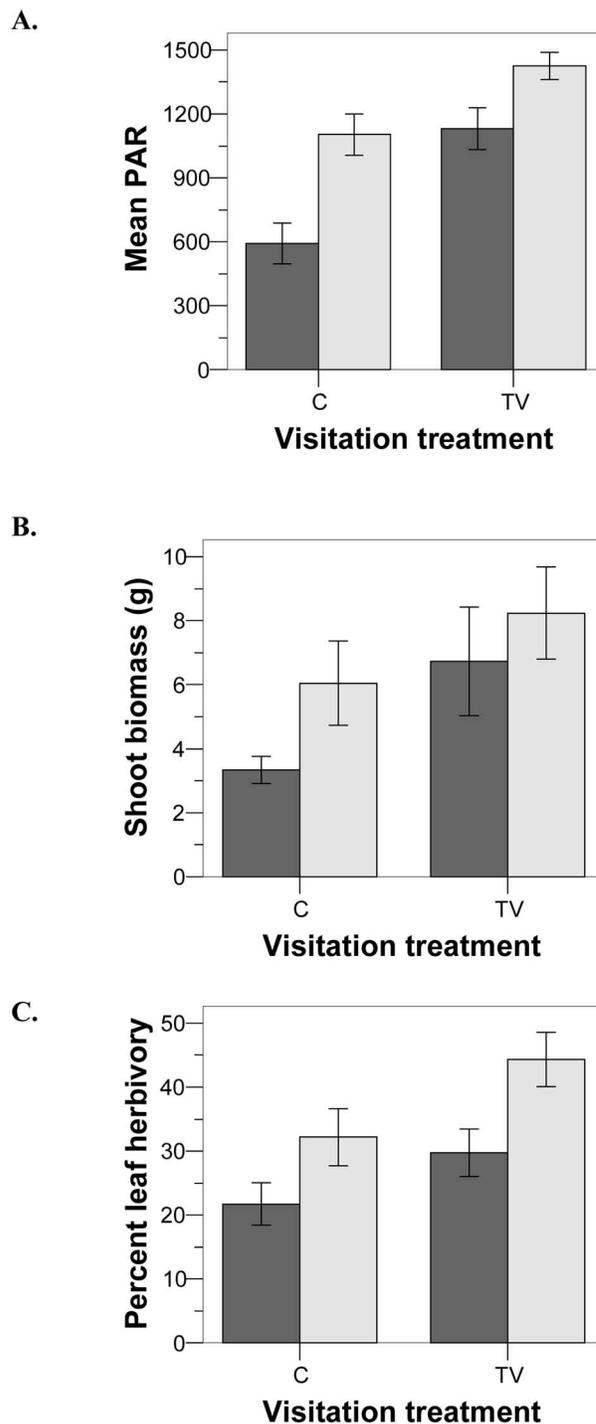
Fig. 1 *A*, Mean light availability (μmol m^{-2} s^{-1} PAR) reaching the apical meristem of focal plants in the field experiment. *B*, Mean shoot biomass at harvest of the focal plants. *C*, Mean percent leaf herbivory (summed over the entire plant) as measured at the end of the field experiment. In all three panels, error bars represent 1 SE. Dark shading represents plants whose neighbors were not tied back, light shading represents the neighbor tie back treatment, *TV* represents plants that were touched and visited, and *C* represents untouched control plants.

A.

B.

C.

D.
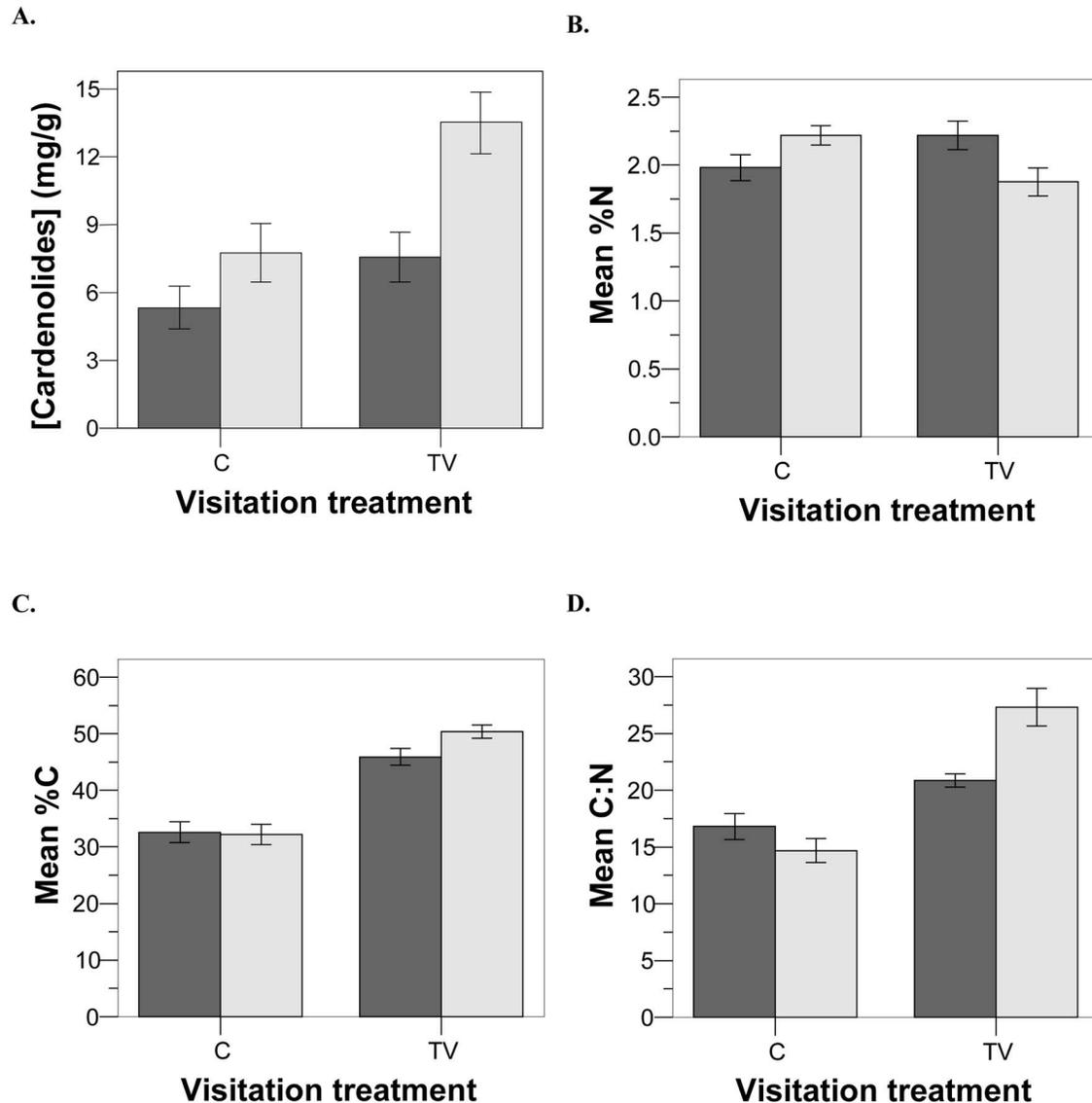
Fig. 2 Mean cardenolide concentrations (*A*), leaf N percent (*B*), leaf C percent (*C*), and leaf C : N ratio (*D*) as measured at harvest from the field experiment. Dark shading represents plants whose neighbors were not tied back, light shading represents the neighbor tie back treatment, *TV* represents plants that were touched and visited, and *C* represents untouched control plants.

Neighbor tie back caused in increase in plant height (mean ± SE cm: control, 63 ± 4; TB, 78 ± 6; V, 72 ± 5; TB and V, 84 ± 5), while neither treatment altered leaf number (table 1). Visitation and neighbor tie back both increased leaf herbivory (fig. 1*C*), with their combined effects being additive (table 1). Visitation and neighbor tie back had additive effects on leaf cardenolide concentration (fig. 2*A*; table 1). There was a significant neighbor tie back × visitation interaction for N percent (fig. 2*B*; table 1). In contrast, visitation, but not tie back, increased C percent (fig. 2*C*; table 1). Not surprisingly, there was a significant tie back × visitation interaction term for C : N ratio, with visitation consistently increasing C : N ratios while neighbor tie back increased C : N ratios only when plants were also visited (fig. 2*D*; table 1).

Across all plants in all treatments, only C : N ratio and leaf number were retained as explanatory variables for the observed variation in herbivory among focal plants (table 2). Increased C : N and smaller leaf numbers were both associated with increased leaf herbivory (table 2). Because these measures were taken at the end of the study, it is unclear whether these factors were causal to the differences in herbivory or whether they were the outcome of herbivory (i.e., herbivores removing leaf blades and leaving plant parts of with higher C : N ratios).

Greenhouse Experiment

Touching and wind treatments had different effects on a variety of plant measures. After controlling for initial differences in height and leaf number (table 3), touching increased plant height (fig. 3*A*) and leaf C percent (fig. 3*B*), while wind

Table 2

Final Model Summary of the Forward Stepwise Regression Conducted to Determine Which Measured Variables Were Most Closely Associated with the Level of Herbivory Experienced by *Apocynum cannabinum* in the Field Experiment

Variable	B (SE)	t	P
Included terms:			
ln(C : N ratio)	0.738 (0.303)	2.435	0.021
ln(final leaf no.)	−0.185 (0.89)	−2.072	0.047
Constant	−3.273 (0.886)	−3.695	0.001
Excluded terms:			
ln(shoot biomass)	. . .	0.744	0.463
ln(light at meristem)	. . .	0.883	0.385
ln(height)	. . .	0.004	0.997
ln(leaf number)	. . .	0.984	0.333
ln([cardenolide])	. . .	1.071	0.293
ln(N) (%)	. . .	1.012	0.320
ln(C) (%)	. . .	1.012	0.320

Note. The overall final model statistics were $R^2 = 0.23$, $F_{2, 30} = 4.47$, $P = 0.020$. B represents the unstandardized regression coefficient for each term included in the model. t represents the t statistic (and associated P) for each independent variable either included or excluded from the model.

increased leaf number (fig. 3C). There were no impacts of either treatment on herbivory, caterpillar growth and survival, cardenolide concentrations, or leaf N percent (table 3).

Discussion

For most response variables, the functional effects of visitation and neighbor tie back on focal plants were the same: increased herbivory, shoot biomass, and cardenolide concentration (figs. 1, 2; table 1). Changes in herbivory and plant size appear to be caused primarily by a reduction in light competition and increased light availability, because touching plants alone had no effect on herbivory, plant size, or cardenolide concentrations (table 3). These findings are consistent with prior work in which leaf herbivory increased when *A. cannabinum* plants were visited weekly, regardless of whether they were touched (Cahill et al. 2002).

Although light is a key determinant of plant growth in productive old field communities through competitive interactions (Cahill 2002) as well as an important factor in plant-insect interactions (Coley 1988; Aide and Zimmerman 1990; Lindroth et al. 1993; Dudt and Shure 1994; Crone and Jones 1999), relatively few studies have explicitly investigated how competition and herbivory interact to affect plant growth in the field (Segarra-Carmona and Barbosa 1990; Reader 1992; Cipollini and Bergelson 2002; Agrawal and Van Zandt 2003; Cipollini 2004; Haag et al. 2004). The majority of these studies have found that either neighbor removal alters herbivory (Cipollini and Bergelson 2002; Agrawal and Van Zandt 2003) or herbivore exclusion alters competitive interactions (Haag et al. 2004), though others have found competition and herbivory to be simply additive in their effects (Reader 1992). Of those studies that found competition to alter herbivory, there is no consistent pattern: Cipollini and Bergelson (2002) found neighbor plants increased herbivory on *Brassica napus*, while both we (fig. 1C) and Agrawal and Van Zandt (2003) found competition reduced herbivory. There are too few studies to generalize as to when competition is likely to increase versus decrease herbivory, though it is interesting that two studies that found that competition increased herbivory, ours and Agrawal and Van Zandt's (2003) study, were conducted on plant species that produce cardenolides. At a minimum, it would be useful to know whether the metabolic pathways in these two families (Aponaceae and Asclepiaseae) are similar. There is evidence that cardenolide accumulation is influenced by light (Eisenbeiss et al. 1999), such that changes in the light environment may have significant impacts on the secondary chemistry of the plant taxa that produce these compounds.

Unique to our study is the fact that we specifically removed only light competition, rather than neighbor presence, and thus are able to conclude that it was shading, rather than belowground competition, that altered herbivory in *A. cannabinum*. The finding that weekly visitations to focal plants have an equivalent impact on plant growth, as does removal of shading by neighbors for the entire growing season, is (fig. 1b), to put it mildly, surprising. Competition is well established as an important determinant of old field community structure (Goldberg 1987). The realization

Table 3

Greenhouse Experiment: Results from ''Proc Mixed'' Conducted to Determine the Impact of Touch and Wind on a Variety of Response Variables in *Apocynum cannabinum*

	Covariate			Wind		Touch		Wind × touch		
	Variable	F	P	F	P	F	P	F	P	df
Final leaf number	Initial number	**144.88**	**<0.001**	**4.09**	**0.048**	0.99	0.323	1.74	0.193	1, 57
Final height	Initial height	**177.23**	**<0.001**	0.08	0.780	**6.99**	**0.010**	3.34	0.072	1, 65
Herbivory	. . .	. . .	. . .	0.01	0.973	0.01	0.970	0.01	0.923	1, 11
Final larval weight	Initial weight	**57.03**	**<0.001**	1.05	0.345	0.19	0.679	0.37	0.567	1, 6
Cardenolides	. . .	. . .	. . .	0.66	0.444	0.01	0.973	0.47	0.516	1, 7
C (%)	. . .	. . .	. . .	0.23	0.633	**8.82**	**0.006**	3.52	0.071	1, 29
N (%)	. . .	. . .	. . .	0.76	0.393	2.28	0.144	0.96	0.336	1, 25

Note. Rack location within the greenhouse served as a random effect in each model. Sample sizes vary among response variable because there were different numbers of plants available for the different plant measures. Terms significant at $P \leq 0.05$ are presented in boldface.

A.

B.

C.
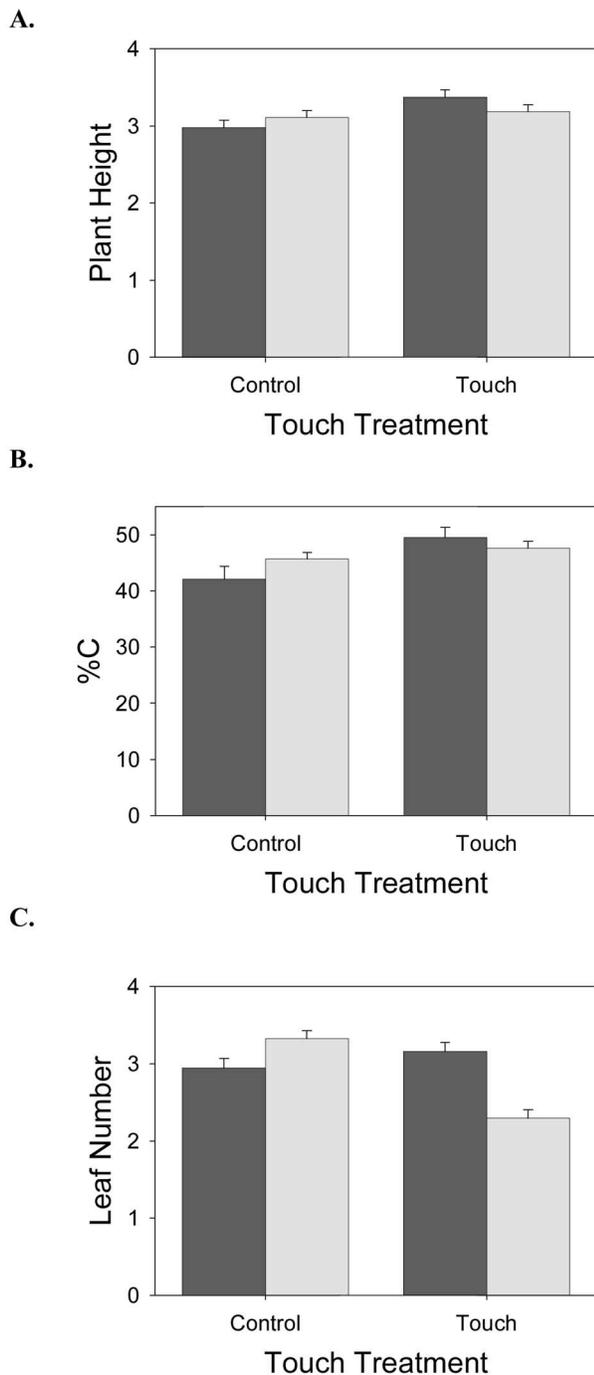
Fig. 3 Mean height (*A*), leaf C percent (*B*), and leaf number (*C*) from the greenhouse experiment. Height and leaf number means presented are adjusted following ANCOVA conducted on ln-transformed data. Dark shading represents the control (no wind), light shading corresponds to the wind treatment, touch refers to plants that were repeatedly stroked, and control refers to plants that were not stroked. Error bars represent 1 SE.

that for at least some species minor disturbances can dramatically alter plant performance suggests our understanding of the contingent nature of competitive outcomes remains limited.

Finding both increased cardenolide concentrations and herbivory in the neighbor tie back and visitation treatments (figs. 1, 2) appears a bit contradictory, because these compounds are toxic to a range of species (Glendinning 1992; Dougherty et al. 1996; Dussourd and Hoyle 2000). However, the dominant herbivore in this study, the dogbane tiger moth *C. tenera*, is a specialist on *Apocynum* sequestering cardenolides as antipredator defenses (Cohen and Brower 1983), and it is possible that defensive chemicals here serve as a feeding stimulant rather than deterrent. Alternatively, other changes in plant chemical composition in response to visitation, neighbor tie back, and touch may have also influenced herbivory. Leaf C percent increased in response to frequent touch in the greenhouse (fig. 3) as well as weekly visitations, but not neighbor tie back, in the field (fig. 2). These results are not due to changes in the light environment because there was no difference in light among treatments in the greenhouse, and neighbor tie back and visitation had similar effects on light (tables 1, 2) but different effects on C percent. Although changes in plant chemistry in response to touch have been previously documented (Jaffe et al. 2002), the idea that a once-weekly handling treatment under field conditions could also cause an increase in leaf C percent is quite surprising.

Percent leaf N also changed with visitation and neighbor tie back treatments (fig. 2); however, potential explanations for the observed patterns are less clear. When applied singly, both treatments resulted in an increase in leaf N percent relative to the control plants; however, when these treatments were applied in combination, leaf N percent did not change relative to the control. Increased leaf N percent may have occurred in both treatments because of increased growth associated with increased light availability. Altered light availability can influence plant phenotypes and nutrient uptake kinetics (Ryser and Eek 2000), which could then alter leaf nutrient composition. However, it is not immediately apparent why these treatments appear antagonistic in terms of leaf N percent while additive for cardenolide concentration, herbivory, and leaf C percent. One possible explanation is that plants subjected to both treatments experienced nearly 45% leaf area removal (fig. 1*C*). This intensity of herbivory is quite high relative to other related studies (Schnitzer et al. 2002; Bradley et al. 2003) and may have put these plants under increased stress. As a result, there may have been earlier senescence and nutrient reallocation to either roots or fruits for these plants, compared with the other treatments. Alternatively, leaf material not consumed on leaves with high levels of herbivory may have lower N because of either herbivore-induced change or selective foraging. This could have resulted in a sampling error; however, we would have expected a similar pattern in the single treatments as well. A more detailed understanding of the flowering and fruiting phenology, coupled with a time series of nutrient budgets, is necessary to more satisfactorily explain these findings. In the multiple regression, it was plant C : N ratios that were most strongly associated with herbivory (more herbivory with higher C : N ratios; table 2), further suggesting that a better understanding of plant chemical changes is needed.

Changes in herbivory can also potentially be explained by shifts in plant morphology. Both neighbor tie back and trampling likely increased the R : FR ratio of light reaching the

focal plants in addition to the overall photon flux. Low R : FR ratios are associated with a general shade avoidance response in many species (Aphalo et al. 1999), which generally consists of increased height and decreased allocation to leaves relative to stems (Schmitt and Wulff 1993). If an herbivore selects hosts on the basis of plant morphology, the neighbor presence causing modified R : FR regimes may result in altered herbivory relative to plants grown without competitors (Agrawal and Van Zandt 2003). However, in contrast to other similar studies (Cipollini and Bergelson 2002; Agrawal and Van Zandt 2003), we found no evidence of a shade avoidance response in *A. cannabinum*, because plants were taller with neighbors tied back than in the control treatment (fig. 1) and leaf number did not vary among treatments in the field (table 1). Further suggesting that *A. cannabinum* does not exhibit the "typical " patterns of growth in response to altered stimuli, plants in the greenhouse were taller when touched and produced more leaves in the wind treatment relative to the control plants (fig. 3). Increased height when neighbors were tied back could have resulted in the plants being more apparent to potential herbivores, though the mechanisms of host detection by this insect are unknown. However, the visitation treatment did not result in increased height (table 1), yet herbivory was still higher relative to the control plots; thus, a shift in morphology is unlikely a causal factor in the change in herbivory found here (fig. 1C).

In addition to the aboveground effects discussed, one might also predict that the visitation treatment could also influence the response variables through belowground processes. Trampling with visitation could result in soil compaction that in turn might affect root performance of the focal plant and thereby influence growth, plant chemistry, and herbivory. However, in this study, we have crossed the visitation treatment with neighbor tie back, which resulted in the same level of herbivory. This means that any soil compaction associated with the visitation treatment did not influence herbivory and further supports that the mechanism here is aboveground, not belowground.

Neighbor tie back and/or focal plant visitation can also alter a variety of factors external to the plant that may influence insect behavior and feeding. These could include a change in volatile profile near focal plants (Hamback et al. 2000), climate (e.g., temperature and humidity) (Stamp and Bowers 1994; Yang and Stamp 1996), disruption of any other associational resistance (White and Whitham 2000), and altered densities of the natural enemies of the herbivore (Stiling et al. 2003).

The goal of the greenhouse experiment was not to replicate what we did in the field but rather to look at the potential effects of touch without changes in light and to determine the influence of touch within the context of regular background physical manipulation induced by wind. We found that both touch and wind resulted in significant changes to a variety of plant characteristics (table 3; fig. 3). Although our wind treatment was similar to that in other studies (Cipollini 1998), we recognize that it may not have exactly simulated field conditions and that the response to wind may be complicated. Our goal here was to examine the effects handling with a more constant background of manipulation that might be caused by wind in the field. We found that the response to the two treatments were not the same, indicating that touch and wind are not equivalent stimuli for a plant. Such contrasting findings for these treatments have been found before (Smith and Ennos 2003), though wind and touch continue to be referred to interchangeably (e.g., Murren and Pigliucci 2005). We suggest that further investigation into the mechanisms behind these observed responses and into why, in this case, touch resulted in an increase in plant size is necessary to more fully understand how plants respond to their local environment, as well as the ecological implications of those responses in natural systems.

Simply visiting and manipulating *A. cannabinum* in a way consistent with measurement in typical ecological studies influenced plant growth, chemistry, and herbivory. It is important to note that the intensity of the handling treatment we imposed is well below that generally used in studies of thigmomorphogenesis (e.g., Patterson 1992; Pruyn et al. 2000; Smith and Ennos 2003). The strong response to experimenter influence suggests caution when studying the ecology of this species. We suggest that, because of the strong impacts of the altered light environment relative to the more subtle effects found in the greenhouse study, visitation effects on herbivory are likely to be greatest in systems in which there is strong competition for light. Similarly, we predict visitation effects will be particularly strong among species that show a high degree of growth or chemical plasticity in response to altered light environments (such as *A. cannabinum*). If this light-mediated hypothesis is true, we would predict highest levels of herbivory in this species to be found in areas of highest light and herbivory to be most variable in areas of most light variation (e.g., old field rather than under an shrub/tree canopy).

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