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SEPARATE EFFECTS OF HUMAN VISITATION AND TOUCH ON PLANT GROWTH AND HERBIVORY IN AN OLD-FIELD COMMUNITY[1]

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Although animal scientists have long been aware that methods used to measure an experimental system can affect the subject of measurement, similar confounding effects of commonly used field methods have only recently been acknowledged by plant ecologists. Here we demonstrate significant effects of weekly visitation (walking up to a focal plant) and handling (taking morphological measures) on plant growth and herbivory in an old-field community. Of the three species examined, *Apocynum cannabinum* was the most severely affected by our treatments. For *Apocynum*, weekly visitations resulted in a positive relationship between initial and final size, which did not occur in the unvisited plants. Visitation also increased leaf herbivory, resulting in a reduced leaf:stem biomass ratio. Handling the plants nearly doubled the proportion of individuals with a stem borer emergence hole. Growth of the other species in this study, *Potentilla recta* and *Erigeron philadelphicus*, was altered by either visitation or visitation plus handling. Visiting plants in order to observe them and touching them as one would when making morphological measurements can have important biological consequences. We suggest that plant ecologists treat repeated entry into a natural system as a research method, subject to the same scrutiny and justification as all other experimental methods.

Key words: experimental methods; herbivory uncertainty principle; old-field; thigmomorphogenesis; visitation effect.

That the act of scientific measurement can influence one's measures is well known (Heisenberg, 1927), and ecologists have long recognized that their research activities can alter the biological systems they study. For example, capturing and handling animals can influence survival rates or behavioral activity in a variety of taxa, including butterflies (Singer and Wedlake, 1981; Mallet et al., 1987), fish (Wallin and Van Den Avyle, 1995), mammals (Pietsch et al., 1999; Place and Kenagy, 2000), and birds (Sedinger et al., 1997; Cox and Afton, 1998). Regular visits to bird nests may influence rates of nest predation (MacIvor, Melvin, and Griffin, 1990; Major, 1990), and taking measures of plant height in a greenhouse can alter stem growth (Klaring, 1999).

Cahill, Castelli, and Casper (2001) demonstrated that in an abandoned hayfield weekly visits to plants and the minimal handling necessary to measure their height can alter the intensity of leaf herbivory some plants experience. In six species tested, one showed enhanced damage following touch, a second showed decreased damage, and four showed no difference. The variability and unpredictability of the results across species led to the naming of this effect as ''the herbivory uncertainty principle'' (Cahill, Castelli, and Casper, 2001). Although that study is apparently the first to show changes in herbivory associated with modest research activity in the field, Niklas (1992) has warned about potentially misleading experimental results if plant handling is not standardized among experimental treatments.

Lacking from Cahill, Castelli, and Casper (2001) was any attempt to determine whether herbivory was altered due to direct effects of handling the plants or whether plant–herbivore interactions were indirectly affected when researchers trampled neighboring vegetation. Differentiating between the two is important to understanding the full implications of ''visitation effects'' for the design and interpretation of field studies.

Touching plants can elicit a variety of morphological and chemical responses (Jaffe and Forbes, 1993; Jaffee, Leopold, and Staples, 2002). The production of secondary plant compounds can change following mechanical stimulation (Jaffe and Forbes, 1993; Cipollini, 1997, 1998), potentially altering herbivore behavior and/or feeding (Cipollini, 1997), particularly if this touch results in physical damage to the plant (Bolter et al., 1997; Karban and Baldwin, 1997). Mechanical stimulation through touch by researchers or browsers or through stem flexures associated with wind can cause decreased plant height, increased stem thickness (Jaffe and Forbes, 1993; Cipollini, 1999; Pruyn, Ewers, and Telewski, 2000), and changes in root placement and structural properties (Goodman and Ennos, 1998; Niklas, 1998). A common explanation is that a more stocky body plan decreases the chances of future damage in a windy environment (Jaffe and Forbes, 1993; Niklas, 1998), but because several species show morphological responses inconsistent with this explanation, mechanically induced changes likely function in other capacities as well. Changes in stem elongation (Bown and Zhang, 2000; Coutand et al., 2000) and gene expression (Mauch et al., 1997) can occur rapidly following stem flexure (1 h or less), and some evidence suggests that mechanical disturbance early in a plant's life may influence growth patterns later in life—even after cessation of the disturbance (Thellier et al., 2000). The strength of thigmomorphogenetic effects can vary among plant parts within a single plant, with the strongest effects occurring on the most sensitive parts (e.g., unfolding leaves) and not

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necessarily on those parts handled most (Turgeon and Webb, 1971).

Methodological descriptions of field studies in plant biology rarely acknowledge that plants were often measured while researchers were standing on their neighbors, with potentially significant biological consequences. Repeated visits to the same individuals over the growing season can create paths through the vegetation, particularly obvious in productive habitats. Such disturbances may affect the growth of the focal plant and/or its risk of herbivore attack. For example, trampling adjacent vegetation should reduce aboveground competition by increasing the light levels, while also altering a suite of other microclimatic variables. Changes to the abiotic environment may influence the focal plants directly or the associated insect community. Standing near an individual can result in breakage of roots and soil compaction, impeding root growth (Goss, 1977). Physical damage to aboveground plant parts in either the focal plants or their neighbors potentially results in the release of volatile chemicals that could attract herbivorous insects (Bolter et al., 1997), increase predation of herbivore eggs (Kessler and Baldwin, 2001), alter parasitoid behavior (Roese et al., 1996; Pare and Tumlinson, 1997), or induce plant defenses in neighboring plants (Preston, Laue, and Baldwin, 2001). In short, there are numerous mechanisms by which research activity could directly or indirectly alter plant growth and herbivory.

We conducted a field experiment using three common plant species in order to separate the direct effects of handling plants from the indirect effects of visitation on both plant growth and degree of herbivory. We subjected marked plants to three treatments: (1) unvisited controls, (2) visiting once weekly without touching the plants, and (3) visiting and touching the plants once weekly. We were unable to touch the plants without also visiting them, and thus a factorial design was not used. At harvest we scored leaf damage caused by insect herbivores and measured a number of morphological plant traits that might have been affected by our treatments, allowing us to assess whether these two fundamental experimental methods influence plant growth and/or herbivory.

MATERIALS AND METHODS

Field site description and study species—Fieldwork was conducted in a 2.5-ha section of the Laurels Conservation Preserve in Chester County, Pennsylvania, USA. The field site was last cut for hay in 1994, 5 yr prior to this study and is bounded on three sides by wood lots. At the time of this study, the vegetation was dominated by grasses (e.g., *Festuca* spp., *Dactylis glomerata*), Canada thistle (*Cirsium arvense*), and poison ivy (*Rhus radicans*), with invasion by *Rosa multiflora*. Plant growth is nutrient limited, with increased growth occurring with either fertilization or elimination of root competition (Cahill, 1999; Cahill and Casper, 2000). At peak growth, the vegetation forms a dense canopy approximately 1–1.5 m in height, with <20% of the available light reaching the soil surface (Cahill, 1999). This is the same field site used in the prior study of visitation effects (Cahill, Castelli, and Casper, 2001).

Three focal species, *Apocynum cannabinum*, *Potentilla recta*, and *Erigeron philadelphicus*, were used in this experiment. Two species exhibited a significant response to being visited and touched in prior work: in *Apocynum cannabinum*, handling increased leaf damage, and in *Potentilla recta*, handling decreased leaf damage (Cahill, Castelli, and Casper, 2001), and we wanted to determine whether visitation or touch elicited those responses. As they are not a random subset of all species in the system, caution is warranted in extrapolating results to the entire plant community. *Erigeron philadelphicus* was common in the field but had not been used in the previous visitation experiment. Both *A. cannabinum* and *P. recta* are herbaceous perennials, while *E. philadelphicus* is biennial. Both *A. cannabinum* and *E. philadelphicus* have the ability to spread vegetatively through stolons. All three species can reach 70 cm in height at this site, though *P. recta* is generally shorter (J. F. Cahill, personal observation).

Experimental design—Between 31 May and 4 June 1999, 30 experimental blocks were established throughout the field. All blocks were approximately 15 m wide and ranged in length from 7 to 22 m (mean ± 1 SD = 14.9 ± 4.03 m). Block boundaries were chosen arbitrarily, using the criterion that no block be located closer than 15 m to a wooded area nor <5 m from the nearest adjacent block and were not based upon obvious differences in species composition, stem density, or physiognomy. Within each block, up to ten shoots of each focal species were chosen by marking individuals as they were encountered. Each shoot was marked by tying flagging tape around the base of the plant and placing a pin flag within 20 cm of the plant stem. Leaf number was counted for each plant, giving a measure of initial size. Marked shoots were separated by at least 50 cm from all other marked shoots of any species. Future mention to marked ‘‘plants’’ in this study refers to individual ramets, rather than genets, as multiple ramets from the same genet may have been used.

Due to variation in abundance within the field site, not all species occurred within every block, and the number of plants marked per species and the total number of marked plants per block varied. On average, each block contained 18.6 marked plants (SD = 5.9) for a total of 558 focal plants. There were an average of 8.6 marked *A. cannabinum* plants (SD = 2.5) per block in 27 blocks, 5.6 (SD = 3.6) marked *P. recta* plants per block in 26 blocks, and 6.5 (SD = 3.1) marked *E. philadelphicus* plants per block in 28 blocks.

Blocks were randomly assigned to each of three visitation treatments: (1) NV, not visited, in which blocks were only entered during the experimental setup and harvest. (2) V, visited, in which blocks were entered once weekly and all marked plants visited. Visitation entailed walking close enough to each plant to view the flagging tape at the base of the stem. As the field is densely vegetated, this often involved temporarily pushing the neighboring vegetation aside. The marked plants were not touched directly. This degree of disturbance is the absolute minimum necessary to conduct a demographic survey of marked individuals in this system. (3) VT, visited and touched, in which all marked plants were visited once weekly and touched. The touch treatment was designed to simulate the handling involved in measuring leaf number and plant height and not ‘‘natural’’ disturbances such as wind flow or brushing by neighboring vegetation or browsers. The actual touch treatment consisted of gently stroking each plant from base to top of stem one time each 7 d. This degree of handling is extremely minor when compared to the handling generally conducted in greenhouse studies investigating plant responses to touch (e.g., Cipollini, 1999; Pruyn, Ewers, and Telewski, 2000).

Because it was necessary to maintain the visited and unvisited treatments in spatially segregated areas, we applied the same treatment to all marked plants within a block. Due to an error in the field, 11 blocks were originally assigned to the NV treatment, V = 9, and VT = 10. During the course of the experiment, the land managers mowed a path through one of the NV blocks, and we eliminated it from the study.

After 9 wk of treatment, measures of light availability were taken, and the shoots of the marked plants in all 30 blocks were harvested (25–29 July 1999). Within each block, two living marked shoots of each species were randomly selected for light measures and for identification of associated invertebrates. Prior to harvesting these plants, photon flux density (LI-COR Quantum Sensor, Lincoln, Nebraska, USA) was measured (1) above the canopy, (2) immediately above the apical meristem of the marked plant, and (3) at the soil surface. After scoring these plants for leaf damage (see details below), a plastic bag was secured over each plant and the stem clipped at the soil surface. We originally planned to identify insects found on each of these plants, but the samples rapidly deteriorated, and this determination was not possible. Due to this decomposition, biomass could not be measured on these plants.

Harvest of the remaining plants consisted of (1) recording whether each marked plant was dead or alive, (2) scoring leaf damage on all living plants, and (3) clipping the plant at the soil surface. Leaf damage due to herbivory by invertebrates was estimated using visual estimation on a 0–4 scale, with

TABLE 1. Proportion of light reaching the soil surface (photosynthetically active radiation [PAR] at soil surface/PAR above the canopy) and focal plant meristem (PAR just above the apical meristem of marked plants/PAR above the canopy). Included in each generalized linear mixed model are two random effects, Block(Treatment) and species × Block(Treatment). Excluding these effects significantly reduced the fit of the model for both the Meristem/Canopy measure (significance of reduction in −2 Res Log-Likelihood [Little et al., 1996] = 0.036) and the soil/canopy measure ($P < 0.001$), indicating significant variation in light penetration among blocks.

Effect	Soil/Canopy			Meristem/Canopy		
	df	*F*	*P*	df	*F*	*P*
Treatment	2, 26	0.41	0.668	2, 26	6.89	0.004
Species	2, 37	0.48	0.622	2, 37	6.69	0.003
Treatment × Species	4, 37	0.69	0.601	4, 37	2.71	0.045

"0" representing <1% of the leaf area missing, "1" = 1–25% missing, "2" = 26–50%, "3" = 51–75%, and "4" = >75% of leaf area missing. At the beginning of the study, most plants would have scored a 0, and no plant would have scored greater than 1. Eggs of the dogbane tiger moth (*Cycnia tenera*) were found on the underside of the leaves of *A. cannabinum* at the start of the experiment. Plants were not excluded from the study based upon the occurrence of eggs, nor did we record whether each plant did or did not have eggs present when initially marked.

Plant shoots were separated into leaf and stem biomass, dried at 70°C for 48 h, and weighed. During the summer, we noticed many stems of *A. cannabinum* inside and outside of the study were dying. Upon examination, we found a hole (<1 cm diameter) near the base of the stem on these plants. By dissecting individuals not used in this study, we found a living stem borer inside one plant, which was later identified as a larval form of a stem boring moth (genus *Papaipema*, most likely *P. baptisiae* (P. Goldstein, personal communication, [Field Museum, Chicago, Illinois]). During harvest and again prior to weighing, the stems of *A. cannabinum* were examined for the presence or absence of an emergence hole.

At harvest there were some instances when flagging tape and a pin flag were found, but no marked plant was within the tape. We counted these plants as dead. Because their true fate was unknown, this may have overestimated the actual mortality rate. There was no evidence that the flagging tape routinely came undone or was chewed by animals during the course of the study, with >95% of marked plants recovered. There was also no evidence of widespread damage by mammalian or mollusk herbivores, and thus discussion of herbivory here refers specifically to insect damage.

Statistical analyses—All analyses included the main (fixed) effects of species identity and visitation treatment (NV, V, VT). Because treatments were applied to whole blocks, blocks were the units of replication and were treated as a random factor nested within the visitation treatment. All analyses were conducted using PROC MIXED in SAS, which uses a log likelihood function to account for error associated with random effects (Littel et al., 1996; SAS, 1999). Because not all species were tagged within each block, the ANOVA is unbalanced, and type III sums of squares were used.

The main effects of visitation and species identity on mortality and the amount of leaf herbivory were examined in separate analyses. For mortality, we calculated the proportion of each species that died in each block. For the amount of leaf herbivory, we used the mean herbivory score per block for each species (0–4). These response variables were calculated similarly to those used by Cahill, Castelli, and Casper (2001). Mean damage was log transformed to satisfy the assumption of normality. Due to a large number of "zeros" in the survival data set, no transformation resulted in normalizing the data, and thus this model was run with that assumption violated. To compare the results of this study directly to the findings of prior work (Cahill, Castelli, and Casper, 2001), the analyses of leaf damage and survival were repeated using only the NV and VT visitation treatments.

The effect of the visitation treatment on total plant biomass (ln transformed) was determined using a similar statistical model as in the prior analyses. However, initial leaf number differed as a function of species identity and visitation treatment (PROC MIXED: Species × treatment, $F_{4,37} = 3.29$, $P = 0.02$). Therefore, differences in final biomass could have resulted either from differences in starting size or from effects of visitation. To separate these confounding factors, initial leaf number (ln transformed) was used as a covariate. The total aboveground biomass of each marked plant was treated as a separate data point. Because we detected a significant covariate × treatment × species interaction, we compared the slopes of the lines describing the relationship between initial leaf number and final biomass among treatments for each species separately using the ESTIMATE function in PROC MIXED.

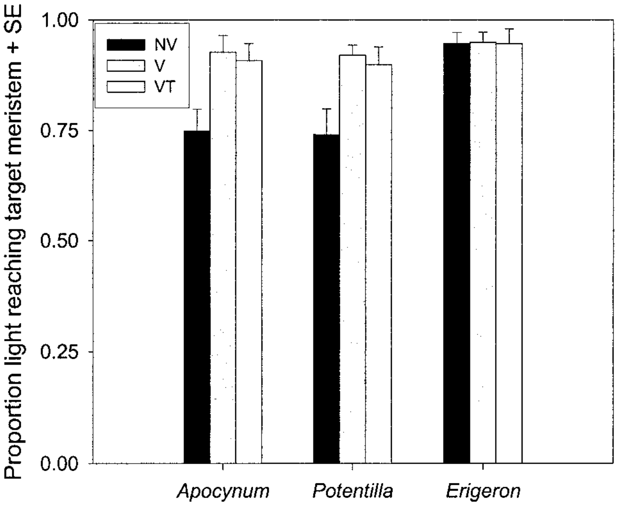

Fig. 1. Effects of species identity and visitation treatment on the proportion of available light reaching the apical meristem of the marked plants at the end of the experiment. The proportion of light reaching the apical meristem increased with visitation (V, VT) for *A. cannabinum* and *P. recta*. The three treatments consisted of: NV = plants were not visited during the study, V = plants received weekly visitation, and VT = plants received weekly visitation and were touched to simulate the taking of morphological measures.

To determine whether visitation influenced relative allocation of biomass between leaves and stems, an analysis was performed using leaf biomass (ln transformed) as the response variable and stem biomass (ln transformed) as the covariate. In this analysis there were significant two-way covariate × treatment and covariate × species interactions, and differences in slopes were again determined using the ESTIMATE function. Initial leaf number was not a significant covariate of final leaf biomass and was not included in the statistical model.

An additional analysis was conducted to determine whether the probability that an individual of *A. cannabinum* possessed an emergence hole of the stem borer varied as a function of visitation treatment. Because the response variable was the presence or absence of a hole, a generalized linear mixed model using the %GLIMMIX macro in PROC MIXED was used to allow for binomially distributed data (Littel et al., 1996).

Separate analyses examined the two light measures as a function of species identity and treatment: the proportion of light reaching both a focal plant's meristem (light at meristem/light above canopy) and the proportion reaching the soil surface (light at soil surface/light above canopy). Both models included block(treatment) as a random effect.

RESULTS

Treatment effects on light availability—The proportion of available light reaching the marked plant's apical meristem varied as a function of species identity and visitation treatment (Table 1; Fig. 1). More light reached the meristem of both *A. cannabinum* and *P. recta* when visited (V) or visited and touched (VT) than when unvisited (NV; Fig. 1; protected least

TABLE 2. Generalized linear mixed model (GLM) results for analysis of (1) initial leaf number × final shoot biomass as a function of species identity and visitation treatment and (2) leaf : stem biomass allocation as a function of species identity and visitation treatment. Included in each GLM are two random effects, Block(Trt) and Spp × Block(Trt). Excluding these effects significantly reduced the fit of both GLMs ($P < 0.001$).

	Total biomass (covariate = initial leaf number)			Leaf biomass (covariate = stem biomass)		
Effect	df	*F*	*P*	df	*F*	*P*
Covariate (Cov)	1, 180	69.10	<0.0001	1, 178	185.50	<0.0001
Treatment (Trt)	2, 26	0.66	0.5234	2, 27	2.72	0.840
Species (Spp)	2, 33	5.37	0.0096	2, 32	20.62	<0.0001
Trt × Spp	4, 33	3.27	0.0232	4, 32	4.98	0.0031
Covar × Trt	2, 180	0.74	0.4771	2, 178	4.42	0.0133
Covar × Spp	2, 180	0.77	0.4639	2, 178	3.94	0.0212
Covar × Trt × Spp	4, 180	3.33	0.0167	4, 178	2.01	0.0944

squares differences comparisons, $P < 0.01$). This indicates that visitation alone resulted in changes in the local size hierarchy, allowing for focal plants to become closer to the top of the local canopy. Visitation had no effect on light reaching the meristem of *E. philadelphicus*, probably because these plants were as tall or taller than the surrounding vegetation in all treatments (Fig. 1). The proportion of light reaching the soil surface was not affected by species identity or visitation treatment (Table 1), indicating that any gap made by visitation was filled by either the focal plant or surrounding vegetation.

Effects on plant growth and insect herbivory—The relationship between initial leaf number and final shoot biomass varied as a function of species identity and treatment (Table 2). For *A. cannabinum*, final plant size was related to initial size only when visited (V and VT, not NV; Fig. 2) *Potentilla recta* showed the opposite response with a stronger relationship between initial and final size in the NV treatment than in the VT treatment (Fig. 2), with the V treatment not significantly different from the other treatments. Neither visitation nor handling affected the relationship between initial size and final biomass for *E. philadelphicus*.

Mean leaf damage per block did not vary as a function of the visitation treatment (Table 3; Fig. 3). However, it was clear from both visual examination of graphed data and posthoc tests that there was greater leaf damage in *A. cannabinum* when it was visited (V or VT) than when left alone (NV). When the same analysis was repeated, using only the NV and VT treatments (as in Cahill, Castelli, and Casper, 2001), the species × treatment interaction was significant (Table 3). These results are consistent with those of our prior study in which *A. cannabinum* experienced more damage in a VT treatment than when unvisited. No significant effect of treatment was found for *P. recta* (Fig. 3), contradicting our earlier findings (Cahill, Castelli, and Casper, 2001) that *P. recta* exhibited decreased leaf damage when visited and touched.

Mean mortality per block varied as a function of the visitation treatment and by species (Table 3; Fig. 3). In general, mortality was greatest in the V treatment and lowest in the NV and VT treatments (Fig. 3). When species were examined in posthoc tests, individuals of *A. cannabinum* were less likely to die when they were handled (VT) than when they were not touched (NV or V). Mortality was highest for *E. philadelphicus* when they were visited (V) compared to unvisited controls, and plants that were visited and touched (VT) had a mortality rate intermediate between the other two treatments.

The relative allocation of biomass between stems and leaves varied as a function of species identity and visitation treatment (Table 2; Fig. 4). The nonsignificant covariate × treatment × species interaction suggests that treatments had similar effects on this relationship across species (Table 1). However, upon visual inspection and contrasts of slopes, it is clear that the visitation treatment did not affect stem : leaf relationships for *P. recta*, but it affected the relationship in *A. cannabinum* and *E. philadelphicus* in similar ways. In both cases, smaller plants tended to have less leaf biomass per unit stem biomass in the VT treatment than in the NV treatment, with the V treatment intermediate. Overlaying the herbivory damage category associated with each plant onto a scatter plot provides an indication that more than one mechanism appears to be generating these patterns. For *A. cannabinum*, plants in the VT treatment group with low leaf : stem ratios (Fig. 3) tended to have damage scores of 4, particularly plants with small stems. This pattern suggests that the difference in slopes was driven by leaf tissue loss to herbivores. In contrast, for *E. philadelphicus* (Fig. 3), there appears to be no pattern between stem biomass and damage class. Thus, the differences in slopes probably resulted from differences in plant biomass allocation rather than enhanced leaf herbivory.

The proportion of *A. cannabinum* individuals with an emergence hole from the stem borer *P. baptisiae* varied significantly as a function of the visitation treatments (%GLIMMIX in PROC MIXED: treatment effect, $F_{2,23} = 3.86$, $P = 0.036$; Fig. 5). Nearly 50% of plants had an emergence hole in the VT treatment, while <30% had such a hole when not touched (Fig. 5).

DISCUSSION

We draw three main conclusions from this study. First, visitation alone and the combination of visiting and handling of plants affected plant performance differently, demonstrating both direct and indirect biological consequences of research activity. Second, effects on plant performance included changes in mortality, herbivory, and plant growth. Third, the three species responded to the treatments differently, with the strongest evidence for indirect visitation effects occurring for *A. cannabinum*.

Species-specific responses—*Apocynum cannabinum*—When marked shoots of *A. cannabinum* were visited (touched or not), they experienced increased leaf damage by invertebrate herbivores and a stronger relationship between initial and final plant size than did the unvisited controls (Fig. 2). Significant effects of visitation on growth and herbivory in the absence of touch are likely due to changes in local conditions

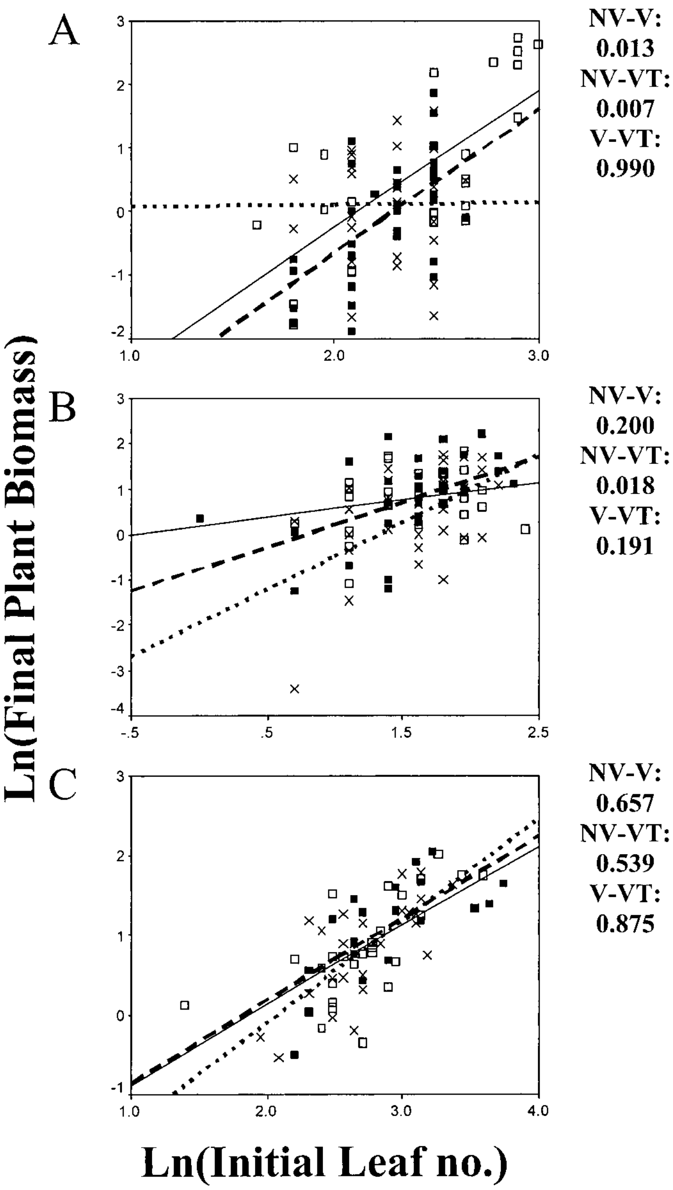

Fig. 2. Final plant biomass as a function of initial plant size and visitation treatment for (A) *A. cannabinum* (NV, $R^2 < 0.01$; V, $R^2 = 0.35$; VT, $R^2 = 0.50$), (B) *P. recta* (NV, $R^2 = 0.29$; V, $R^2 = 0.26$; VT, $R^2 = 0.05$), and (C) *E. philadelphicus* (NV, $R^2 = 0.52$; V, $R^2 = 0.49$; VT, $R^2 = 0.45$). Symbols correspond to: NV = "*x*" + dotted line, V = filled squares + dashed line, VT = open squares + solid line. In the generalized linear mixed model, there was a significant covariate × species × treatment interaction. Listed are *P* values for each pairwise comparison of slopes, as determined through the ESTIMATE function in PROC Mixed in SAS. For *P. recta*, there is an apparent outlier that represents a single very small plant at the end of the study from the NV treatment. When this data point is removed, the three way interaction is still significant ($P < 0.05$); however, the slopes of the lines in that panel are homogeneous. See Fig. 1 for an explanation of the NV, V, and VT treatments.

associated with trampling the neighboring vegetation. The V and VT treatments increased light levels at the apical meristem for *A. cannabinum* (Fig. 1), which could influence the growth and visibility of the focal plants and/or the abundance and behavior of herbivorous insects. We did not measure any other microclimatic variables (e.g., temperate and humidity), any of

TABLE 3. Generalized linear mixed model (GLM) results for mean leaf damage and survival per block using (1) all three visitation treatments (NV, V, VT) and (2) using only two treatments (NV, VT). Excluding the V treatment allows for direct comparison with the results of a prior study (Cahill, Castelli, and Casper, 2001). Included in each GLM are two random effects, Block(Trt) and Spp × Block(Trt). Excluding these effects significantly reduced the fit of both models ($P < 0.001$).

	Three treatments (NV, V, VT)			Two treatments (NV, VT)		
Effect	df	*F*	*P*	df	*F*	*P*
Survival						
Treatment (Trt)	2, 28	3.75	0.036	1, 18	0.01	0.916
Species (Spp)	2, 36	15.68	<0.001	2, 21	8.31	0.002
Trt × Spp	4, 28	0.93	0.456	2, 21	0.04	0.675
Leaf damage						
Treatment	2, 28	1.85	0.176	1, 18	3.62	0.073
Species	2, 33	94.80	<0.001	2, 19	94.25	<0.001
Trt × Spp	4, 33	1.34	0.276	2, 19	3.57	0.049

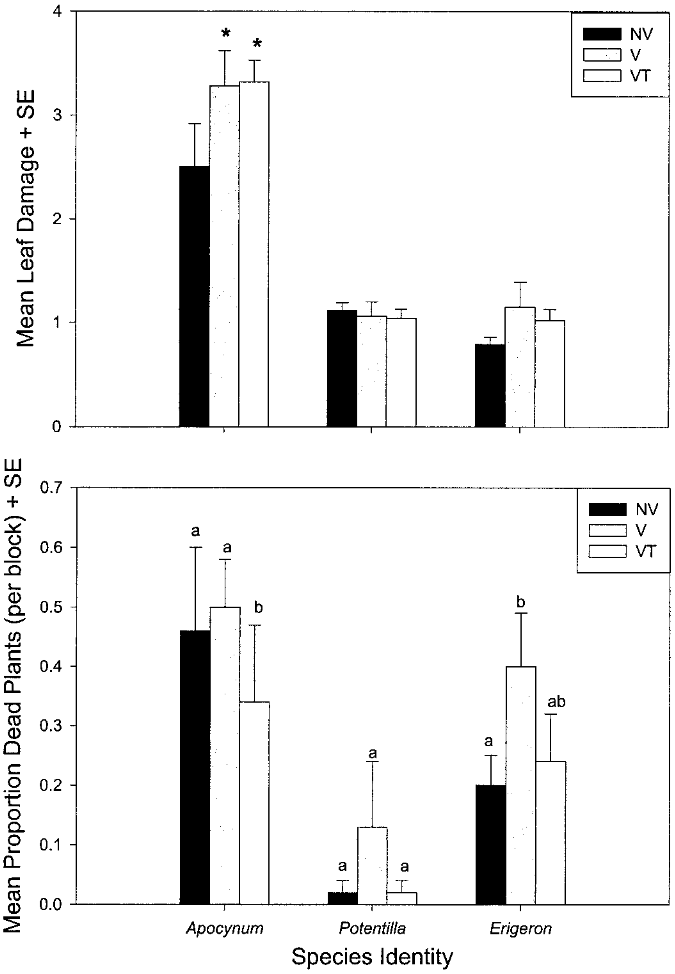

Fig. 3. Mean leaf damage and mortality as a function of species identity and visitation treatment. For each experimental block, mean leaf damage and mortality were calculated for each species separately and then the average values of those block means were calculated. For *A. cannabinum*, mean leaf damage increased with visitation (V and VT) and survival decreased when plants were visited and touched. Mortality increased in *E. philadelphicus* with visitation. See Fig. 1 for an explanation of the NV, V, and VT treatments.

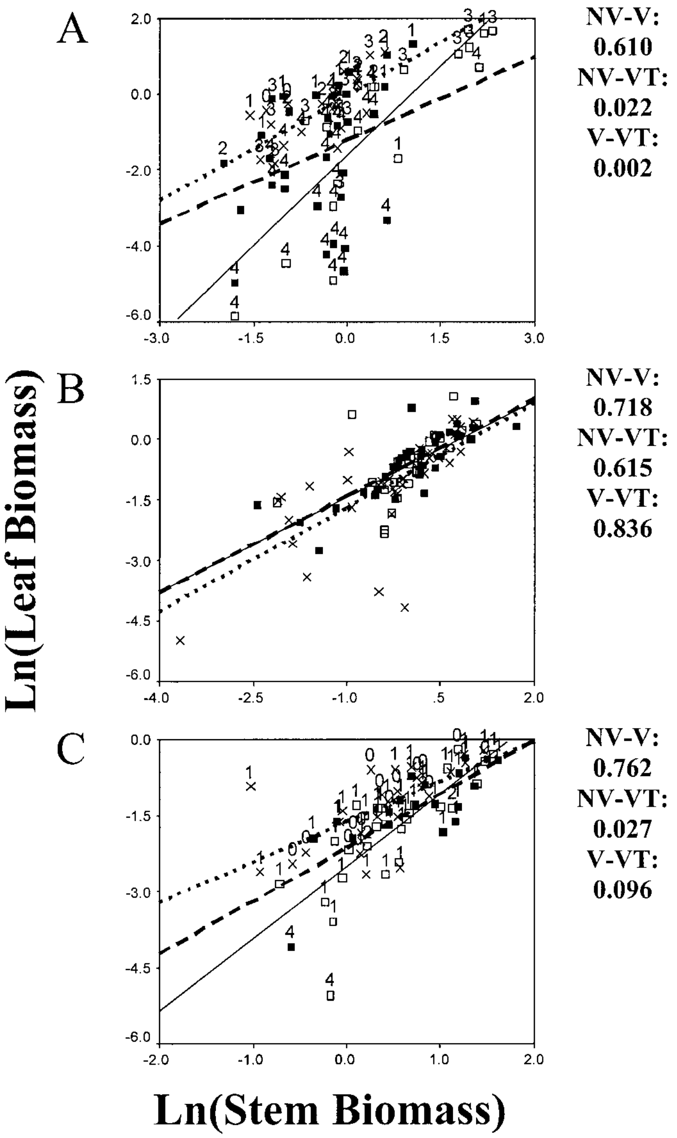

Fig. 4. Leaf biomass allocation as a function of stem biomass and visitation treatment for (A) *A. cannabinum* (NV, $R^2 = 0.51$; V, $R^2 = 0.18$; VT, $R^2 = 0.65$), (B) *P. recta* (NV, $R^2 = 0.50$; V, $R^2 = 0.68$; VT, $R^2 = 0.35$), and (C) *E. philadelphicus* (NV, $R^2 = 0.42$; V, $R^2 = 0.62$; VT, $R^2 = 0.60$). Symbols correspond to: NV = "*x*" + dotted line, V = filled squares + dashed line, VT = open squares + solid line. Listed are *P* values for each pairwise comparison of slopes, as determined through the ESTIMATE function in PROC Mixed in SAS. For both *A. cannabinum* and *E. philadelphicus*, smaller plants in the VT treatment had reduced leaf : stem ratios relative to small plants in the NV treatments, while larger plants had similar leaf : stem ratios regardless of visitation treatment. See Fig. 1 for an explanation of the NV, V, and VT treatments.

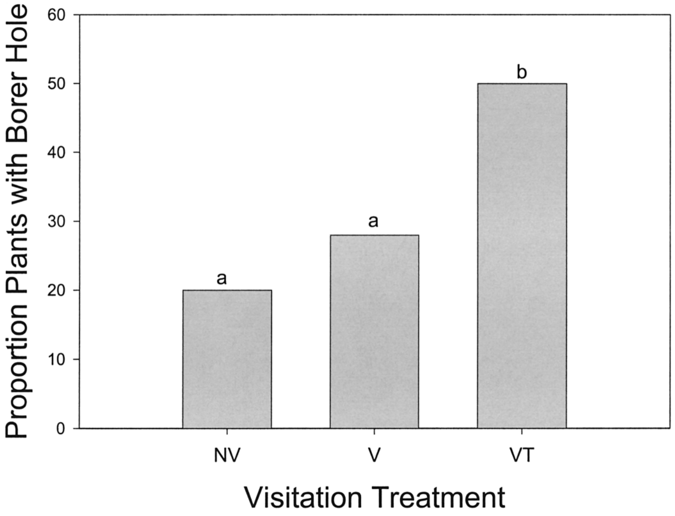

Fig. 5. Proportion of all marked *A. cannabinum* plants that had a stem borer emergence hole at the end of the study as a function of visitation treatment. A generalized linear mixed model with binomially distributed data was conducted to determine the effects of visitation of the occurrence of borer holes. Columns with similar letters are not significantly different in post hoc tests ($P > 0.05$). See Fig. 1 for an explanation of the NV, V, and VT treatments.

which could be more directly involved in causing the observed patterns. Additionally, the plant wounding associated with trampling likely altered plant volatile production. Whether or not those changes influenced herbivore, parasitoid, or predator abundances and feeding rates is unknown.

Initially large plants in the V and VT treatments reached a larger final size than did initially large plants in the NV treatment while initially small plants in the V and VT treatments remained smaller than those in the NV treatment. This pattern is not due to changes in final biomass among treatments (TRT), as that did not vary (GLM without initial size as a covariate; $\mathrm{TRT}_{2,20}$ $P > 0.35$). Instead, this pattern is likely due to the combined and opposing effects of elevated leaf tissue damage and increased light availability. Larger plants may have been better able to increase growth in response to increased light levels (e.g., occupy the small gap that was formed) despite increased damage from herbivores (Fig. 3). The growth of smaller plants appears either to be more hampered by an equivalent intensity of leaf damage or these plants were simply too short to overtop their neighbors. Because shoot competition is generally size asymmetric (Weiner and Thomas, 1986; Schwinning and Weiner, 1998), it is surprising that initial size did not influence final growth in the unvisited plots. This result contradicts the widespread assumption that initial starting size is a strong determinant of final plant performance.

The strong effects of visitation on herbivory in *A. cannabinum* are likely due to changes in feeding by the specialist herbivores of this species. The dogbane beetle, *Chrysochus auratus*, and larvae of the dogbane tiger moth, *Cycnia tenera*, both fed on *A. cannabinum* during the study, with the vast majority of the damage caused by *C. tenera* (J. F. Cahill, personal observation). Both species specialize on plants in the genera *Apocynum* and *Asclepias*, sequestering cardenolides produced by the host plant to deter predators (Cohen and Brower, 1983; Dobler, Daloze, and Pasteels, 1998). How the visitation treatments altered the interactions between *A. cannibinum* and these specialist herbivores is unclear, but there are four main possibilities: (1) The search behavior of these herbivores may be tightly coupled to plant visibility, which likely increased with visitation. (2) The release of volatile chemicals from trampled neighbors could have recruited more herbivores to the general area. (3) Changes in the light environment due to trampling may directly alter insect feeding due to species-specific preferences for certain micro-climatic conditions. (4) Changes in the light environment might indirectly alter insect feeding, mediated through a change in plant defense chemistry. As the majority of the leaf herbivory on *A. cannabinum* was by *C. tenera* larvae (which do not fly), any changes in foliar or volatile chemistry likely resulted in stimulation of feeding, rather than increased herbivore population size. The phytochemistry and feeding behavior of *A. cannabinum* and its herbivores are poorly understood, and future study is warranted.

Handling also had important consequences for *A. cannabinum*. Touched plants were the most likely to have a stem borer emergence hole (Fig. 5) and differed from the NV and V treatment groups in the relative proportion of leaf vs. stem biomass (Fig. 4). Eggs of the stem borer (*P. baptisiae*) are laid on the underside of leaves in the fall, overwinter in the litter layer, and find hosts in early spring (Forbes, 1954) before the initiation of the treatments in this study. Therefore, touching shoots of *A. cannabinum* must have increased the probability of emergence of borers already within the plant stem, rather than the likelihood that the focal plants attracted these insects. One possible explanation could be that borer emergence is related to plant size, and a survey outside of the treatment blocks did indicate that the probability of a plant having an emergence hole increased with plant height (J. F. Cahill, J. P. Castelli, and B. B. Casper, unpublished data). However, we cannot directly determine the relationship between plant size and emergence in our study, as many plants with a borer hole were dead at harvest, and thus plant size could not be accurately measured. There are, however, two indirect lines of evidence that suggest the observed patterns were not a simple function of plant size: (1) borer emergence differed between the V and VT treatments, even though plant growth responses between these two were identical (Fig. 2), and (2) initial leaf number was not a significant term in a separate GLMM ($P = 0.26$), indicating that initial size of the focal plants did not alter borer emergence. These results suggest that the observed increase in borer emergence was likely due to touch, independent of plant size. Handling the plants may have changed host plant quality either through changes in defense chemistry or nutrition. Stem borers feed on tissue with low nutritional value, and small changes to the plant can have significant consequences for borer success (Hedin et al., 1984, 1993). It is important to note that an emergence hole could indicate either successful larval development or an attempt of the larvae to find a new host plant, and thus it is unclear whether handling benefited or harmed the stem borers.

More than 90% of the dead stems of *A. cannabinum* in all three treatments had a stem borer emergence hole, yet the VT group, which had the highest incidence of borer emergence, had the lowest overall mortality. This pattern of shoot mortality is inconsistent with the idea that handling increased borer growth and development success, which should have resulted in greater plant damage and mortality. There are two possible explanations: (1) The mortality results may have been an artifact of when the plants were harvested, and if the harvest occurred a few weeks later, mortality may have increased among stems with an emergence hole, eliminating or reversing the observed differences among groups. Alternatively, (2) handling may have been detrimental to borer growth causing them to search for new host plants, thereby decreasing handled plant mortality. Without further study of this specific plant–insect interaction we cannot differentiate between these alternative hypotheses.

Erigeron philadelphicus—For *E. philadelphicus*, VT changed the relative allocation of biomass between stems and leaves, with a reduced leaf : stem ratio in the VT treatment, particularly among the smaller individuals. Because there was little herbivory, this result must be due to changes in allocation. The V treatment demonstrated an intermediate response, which suggests this result was driven in part by indirect effects of visitation, which may have been enhanced in the VT treatment by the increased disturbance of handling the marked shoots. However, the proportion of available light reaching the apical meristem did not vary among treatments for *E. philadelphicus*, which was as tall as the surrounding vegetation. Nonetheless, changes in light levels might still play a role, as the smaller plants primarily showed the greatest change in biomass ratio. However, based on established stem elongation responses to red : far red ratios (e.g., Casper, Cahill, and Hyatt, 1998), we would expect the stem : leaf ratio to decrease with more light, not increase. Alternatively, *E. philadelphicus* may have responded directly to being touched, resulting in increased stem : leaf ratios (a thigmomorphogenetic response). We note that touching plants nine times over a period of 2 mo is an extremely mild touching regime compared to treatments generating touch-induced responses in greenhouse studies (e.g., Cipollini, 1999; Pruyn, Ewers, and Telewski, 2000).

Potentilla recta—In contrast to the findings for *A. cannabinum*, the unvisited *P. recta* plants had a stronger relationship between initial and final plant size than did the plants that were visited and touched, with the V treatment intermediate to the NV and VT treatments. However, this finding is strongly influenced by a single data point that represents a particularly small NV individual. When this data point is removed, we find no differences among the slopes of the different treatments. As there is no a priori reason to reject that data point, it is unclear whether this finding is biologically meaningful or a statistical artifact.

Relationship to prior findings—Some of our results are consistent with those of our prior study involving two of these same species (Cahill, Castelli, and Casper, 2001), and some are not. In that study, which had only a no visitation (NV) and a visit and touch (VT) treatment, *A. cannabinum* showed elevated leaf herbivory with visiting and touching as it did in this study. For *P. recta*, visiting and touching decreased the amount of leaf herbivory in the earlier study, which contrasts with the lack of a herbivory response to visitation treatments in this study. This finding suggests that some visitation effects are dependent on the external biological environment, rather than purely species-specific plant responses. This element of temporal unpredictability complicates a researcher's ability to account for visitation effects in field studies.

Implications and future directions—The implications of our work are clear. Field biologists need to determine the impact of their experimental methods on their study organisms. We have shown that even the simple act of walking up to a plant in order to make observations can have biological consequences, with touching plants either further enhancing the effects of visitation or causing additional effects. Many longitudinal studies of plants require frequent handling in order to obtain such performance measures as height, leaf area, flower number, or damage caused by herbivores. In some cases, taking measurements less often and sacrificing temporal information may be preferable to making more frequent measurements with less accurate results. At the very least, it is important to know whether longitudinal and horizontal approaches give the same answer; if they do not, one cannot assume a longitudinal study is ‘‘better’’ (Jackson et al., 1999), as the measurements may have caused, rather than captured, the observed differences.

By separating effects of visitation from those of handling, this work has made a first step towards understanding the mechanisms responsible for the patterns originally reported by Cahill, Castelli, and Casper (2001). Now that we know researchers can have biologically significant direct and indirect effects, further work must be conducted to understand the specific mechanisms that were operating. For instance, many studies investigating induced responses of plant defense systems require a high level of touch or damage to see induction, but our results suggest that modest levels of touch, in the field, may alter herbivory (*A. cannabinum*) and leaf : stem allocation patterns (*E. philadelphicus*). That such minor handling may have ecological relevance is quite surprising as one could expect the background levels of touch (wind, browsing, neighbors) to swamp out our mild treatment. Our results suggest that plants, or their insect herbivores, may possess the ability to respond to minor stimuli even under field conditions. A better understanding of touch-induced changes in plant secondary chemistry and growth and the feeding behavior of associated herbivores could lead to a better understanding of why plants respond to visitation and handling. In particular, more information regarding thigmo responses under field conditions is urgently needed.

The effects of visitation on herbivory, even without handling, suggest the possibility of indirect interactions mediated through changes in plant volatile profiles as a function of trampling neighbor vegetation (Preston, Laue, and Baldwin, 2001). Further differentiation of the effects of trampling on light availability from its effects on plant chemistry would provide valuable information about the mechanisms that underlie plant–insect interactions in natural systems. It is important to note that researchers are not the only agents of trail creation in natural systems. A wide variety of ungulates create trails as conduits from bedding and feeding sites. Whether herbivory and plant growth vary predictably as a function from trail edge is unclear, and whether researchers are simply mimicking these natural disturbances is unknown. Regardless of the exact mechanisms involved in this study, basic techniques such as observing and measuring plants must be viewed as research methods, whose confounding effects should be addressed in the development of appropriate experimental designs.

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