

PREVALENCE AND PREDICTABILITY OF HANDLING EFFECTS IN FIELD STUDIES: RESULTS FROM FIELD EXPERIMENTS AND A META-ANALYSIS¹

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Various effects on plant growth associated with handling or touching plants are well documented from greenhouse and laboratory studies, but are generally unknown or ignored under field conditions. We examined the prevalence of the effects of handling, at levels typical of many ecological experiments, on aboveground biomass and damage by invertebrate herbivores for a total of 16 common species from three plant communities in western Canada. Significant effects of handling were observed in the alpine meadow and grassland, but not in the boreal forest. Handling reduced aboveground biomass and increased the mean intensity of invertebrate leaf damage for most species. A meta-analysis of the relationship between plant traits and response to handling indicated that woody plants and species without strong chemical or conspicuous morphological defenses were most strongly affected. Overall, our results indicate that potentially confounding effects of routinely sampling plants in the field are widespread and merit further investigation.

Key words: alpine meadow; boreal forest; grassland; meta-analysis; observer effect; old field; thigmomorphogenesis; visitation.

The physiological, biochemical, and morphological responses of plants to chronic mechanical stimulation or disturbances such as touching, wind, and rain are termed thigmomorphogenesis (Jaffe, 1973; Jaffe and Forbes, 1993). Many aspects of thigmomorphogenesis have been well documented in laboratory (Niklas, 1992; Kraus, Kolloffel, and Lambers, 1994; Cipollini, 1998), greenhouse (Goodman and Ennos, 1998), horticultural (Autio, Voipio, and Koivunen, 1994), plantation or nursery (Jacobs, 1954; Prunyn, Ewers, and Telewski, 2000), and some field (Larson, 1965; Grace, 1977; Cordero, 1999) studies. Until recently however, the broader implications of potential responses by plants to handling during routine sampling typical of many ecological field studies have largely been ignored.

Cahill, Castelli, and Casper (2001) demonstrated that the simple act of visiting and touching marked plants during the course of an experiment in an old field in eastern Pennsylvania, USA, could influence the growth and the intensity of herbivory experienced by these plants. These results suggested that studies involving repeated visitation of individuals to measure patterns of growth, herbivory, and competition are difficult to conduct in an unbiased manner that does not mask the true response to the factor under investigation. While the implications of this finding could have profound consequences for how ecologists interpret and conduct field studies, Cahill, Castelli, and Casper (2001) also indicated that the generality of their results had yet to be demonstrated in different plant communities. Explicit testing of the generality of this phenomenon is essential to determine whether handling effects are

widespread or only of localized concern (e.g., Schnitzer et al., 2002).

We tested the hypothesis that visiting and touching plants influences aboveground growth and/or leaf damage caused by invertebrate herbivores. First, to determine the prevalence of handling effects, we conducted parallel field experiments over the course of 8–10 wk in three different communities in western Canada, including an alpine meadow, a boreal forest understory, and a fescue grassland. Second, in order to assess the predictability of these responses, we conducted a meta-analysis that included 22 species from four sites—three from this study and the old field site described by Cahill, Castelli, and Casper (2001). We determined whether particular plant traits associated with growth form or morphological and chemical defense were characteristic of observed responses to handling under field conditions.

MATERIALS AND METHODS

Study areas—Boreal forest and alpine meadow communities were located in the southwest Yukon, Canada (61° N, 138° W; see Hik, McColl, and Boonstra [2001] for more detail). This region is characterized by long, cold winters and short (about 3 mo), dry summers, typical of subarctic continental climate regimes. The alpine meadow was located at an elevation of 2000 m (800 m above the timberline) in the Ruby Ranges. Dominant vascular species include *Salix polaris*, *S. reticulata*, *Carex consimilis*, and *Dryas octopetala*. The boreal forest (about 850 m) was located approximately 30 km south of the alpine site in an open forest dominated by white spruce (*Picea glauca*), scattered shrubs including dwarf birch (*Betula glandulosa*) and grey willow (*Salix glauca*), and an understory dominated by fescue (*Festuca altaica*), bluebells (*Mertensia paniculata*), Arctic lupine (*Lupinus arcticus*), and bearberry (*Arctostaphylos uva-ursi*). The grassland (about 700 m) was located at Kinsella (53° N, 111° W) in central Alberta, Canada, on the southern edge of the aspen parkland, approximately 150 km southeast of Edmonton and 1850 km southeast of the Yukon sites. Aspen parkland is a savanna-type habitat, with vegetation consisting of small stands of aspen (*Populus tremuloides*) and large tracts of grassland vegetation. Within the grassland areas (which were used in this study), the dominant grasses include plains rough fescue (*Festuca campestris*) and blue grama grass (*Bouteloua gracilis*). Common forbs include yarrow (*Achillea millefolium*), prairie and pasture sage (*Artemisia ludoviciana* and *A. frigida*), and northern bedstraw (*Galium boreale*). This grassland was

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TABLE 1. Experimental species in alpine meadow, boreal forest, grassland, and old field communities. Effect of touching on old field species were reported by Cahill, Castelli, and Casper (2001).

Community	Species ^a	Abbreviation	Growth form	Chemical defense ^b	Morphological defense ^b
Alpine	<i>Carex consimilis</i> Holm	CACO	Graminoid	0	0
Alpine	<i>Polygonum viviparum</i> L.	POVI	Herb	0	0
Alpine	<i>Artemisia norvegica</i> Fries subsp. <i>saxatilis</i> (Bess. ex. Hook.) Hall & Clem.	ARNO	Herb	0	0
Alpine	<i>Salix reticulata</i> L.	SARE	Woody	1	0
Alpine	<i>Dryas octopetala</i> L.	DROC	Woody	0	0
Boreal	<i>Festuca altaica</i> Trin.	FEAL	Graminoid	0	0
Boreal	<i>Mertensia paniculata</i> (Ait.) G. Don var. <i>paniculata</i>	MEPA	Herb	0	0
Boreal	<i>Epilobium angustifolium</i> L.	EPAN	Herb	1	0
Boreal	<i>Achillea millefolium</i> L. subsp. <i>borealis</i> (Bong.)	ACBO	Herb	1	0
Boreal	<i>Arctostaphylos uva-ursi</i> (L.) Spreng. s.l.	ARUV	Woody	1	0
Grassland	<i>Festuca campestris</i> Rydb.	FECA	Graminoid	0	0
Grassland	<i>Artemisia ludoviciana</i> Nutt.	ARLU	Herb	1	0
Grassland	<i>Galium boreale</i> L.	GABO	Herb	0	0
Grassland	<i>Achillea millefolium</i> L.	ACMI	Herb	1	0
Grassland	<i>Thermopsis rhombifolia</i> (Nutt.) Richards	THRH	Herb (legume)	1	0
Grassland	<i>Rosa arkansana</i> Porter	ROAR	Woody	0	1
Old field	<i>Apocynum cannabinum</i> L.	APCA	Herb	1	0
Old field	<i>Linaria vulgaris</i> P. Mill.	LIVU	Herb	1	0
Old field	<i>Solanum carolinense</i> L.	SOCA	Herb	1	1
Old field	<i>Potentilla recta</i> L.	PORE	Herb	0	0
Old field	<i>Cirsium arvense</i> (L.) Scop.	CIAR	Herb	0	1
Old field	<i>Poa pratensis</i> L.	POPR	Graminoid	0	0

^a Names and authorities for alpine meadow and boreal forest species follow Cody (2000), grassland species follow Moss and Packer (1994), and old field species follow Rhoads and Block (2000). All species were common native perennials.

^b Assessment of gross chemical and morphological defenses (1 = present or strong, 0 = absent or weak) was based on field observations and other sources including Jung, Batzli, and Seigler (1979), Elias and Dykeman (1982), Looman (1983), Willard (1992), Kershaw (2001), and J. Andruchow (unpublished manuscript).

moderately grazed by cattle in the autumn for over 20 yr; however, cattle were not present during the course of this experiment.

Experimental methods—We selected species in each community on the basis of their relative abundance and diversity of growth forms, including three graminoids, eight herbs, one legume, three woody subshrubs, and one shrub (Table 1). In each community we established 20 blocks, each approximately 30 m long × 10 m wide, and spaced approximately 10–15 m apart. In the alpine meadow and boreal forest, five individual plants of each study species (25 marked plants per block) were selected at random and marked by placing a small loop of drab-colored (brown or gray) wire around the base of each plant. A numbered flag marker was placed at approximately 10 cm away from the marked plant to facilitate relocation. For clonal species, individual ramets were tagged rather than whole genets. All species had five plants marked in each block, except for *Polygonum viviparum* (four blocks had four individuals) and *Achillea borealis* (two blocks had four individuals), such that the total number of plants marked was 496 in the alpine meadow and 498 in the boreal forest.

In the grassland, ten individuals of each species were tagged within each block (60 marked plants per block) with a small length of flagging tape tied at the base of each plant stem. A numbered flag marker was placed approximately 15 cm away from the marked plant to facilitate relocation. All six species had ten plants in each block except for *Galium boreale* (one block had five individuals and one block had no individuals) and *Thermopsis rhombifolia* (one block had six individuals), so that a total of 1181 plants were tagged.

Each block was randomly assigned to one of two treatments, not handled (Control) or visited and touched every week (Touch). The touch treatment entailed walking up to the marked plant and gently stroking its entire length once, simulating our technique for measuring plant height or leaf number. All plants were initially tagged, and the number of leaves (alpine and boreal only) and plant height (or shoot length in the case of woody subshrubs) were measured. Height for most species was measured from the base of the stem to the tip of the longest leaf. For graminoids, the longest leaf was measured from

the base to the tip, and for subshrubs, length of the stem was measured from the point where the loop of wire or flagging was attached. No additional measurements were made until the end of the experiment when final height and final aboveground biomass (dry mass) were recorded.

Aboveground biomass was measured by clipping at ground level, drying plants at 60°C to constant mass, and weighing each plant. We also recorded whether plants showed any evidence of invertebrate herbivory, which could be readily distinguished from vertebrate herbivory. In the alpine meadow and boreal forest invertebrate herbivore damage was infrequent and recorded in three categories (0 = <10%, 1 = 10–50%, and 2 = >50% leaf area lost). In the grassland, we observed comparatively elevated levels of total leaf area lost to invertebrate herbivores and consequently estimated damage using five categories (0 = <1% leaf area lost, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = >75%). All plants, including the ones we marked, were free of herbivore damage at the beginning of the experiment.

Plants in the alpine meadow were initially marked between 23 and 25 June 2000. The handling treatment commenced on 30 June and weekly thereafter, for a total of eight visits over the course of the growing season. Final plant height was measured on 7 August, and aboveground biomass was harvested on 19–21 August. Plants in the boreal forest were initially marked 9–11 June and handled every week thereafter. They were remeasured and harvested on 15–16 August. Plants in the grassland were initially marked 2–4 June, handled every week thereafter, and remeasured and harvested on 14–18 August.

Statistical analysis—Each of two response variables, aboveground biomass and leaf damage, were analysed using Type III tests of fixed effects in a generalized linear mixed model (GLM: PROC Mixed: SAS Institute, 2000; Littell et al., 1996). The main model consisted of the Treatment (Touch and Control plants), Species (five species in alpine and boreal, six species in grassland), Treatment × Species interaction, Block (nested within Treatment), and the Species × Block(Treatment) interaction. Both the Block(Treatment) and Species × Block(Treatment) terms were treated as random effects. Results for each community were analyzed separately because there were no common

TABLE 2. Effect of touching on aboveground biomass in alpine meadow, boreal forest, and grassland communities.

Effect ^a	Alpine			Boreal			Grassland		
	df	F	P	df	F	P	df	F	P
Ln(initial height)	1, 387	17.49	<0.001	1, 381	56.22	<0.001	—	—	—
Ln(initial leaf no.)	1, 387	60.79	<0.001	1, 381	18.08	<0.001	—	—	—
Species	4, 72	30.18	<0.001	4, 72	25.14	<0.001	5, 90	79.68	<0.001
Treatment	1, 18	5.12	0.04	1, 18	0.29	0.60	1, 18	4.67	0.04
Species × Treatment	4, 72	1.49	0.21	4, 72	1.10	0.37	5, 90	0.23	0.95

^a Results of Type III tests of the fixed effects in a generalized linear mixed models (PROC Mixed; SAS Institute, 2000). The response variable in each analysis is Ln(aboveground biomass). Included in each model are two random effects, Block(Treatment) and Species × Block(Treatment). Excluding these effects did not significantly alter the fit of the model in either the alpine (significance of reduction in -2 Res Log-Likelihood [Littel et al., 1996]: Block[Treatment] = 0.50; Species × Block[Treatment] < 0.16), or the boreal site (Block[Treatment] = 0.08; Species × Block[Treatment] = 0.16). Ln(initial height) and Ln(initial leaf number) were used as covariates in both the alpine meadow and boreal forest, but not in the grassland (see MATERIALS AND METHODS).

species. Post-hoc means comparisons were adjusted to maintain an overall value of $\alpha = 0.05$.

In alpine meadow and boreal forest communities, the analysis of plant biomass included two covariates, log(initial plant height) and log(initial leaf number), to control for initial differences in plant size. There were no significant interactions between these covariates and any of the terms in the GLM. In the grassland there were significant Species × Covariate interactions, violating a major assumption of analysis of covariance (Underwood, 1997). As a result, the GLM used for the biomass data from the grassland was not adjusted for initial size. Importantly, however, initial plant height (initial leaf number was not recorded for all species) did not differ between treatments (PROC MIXED: Treatment $F_{1,18} = 0.04$, $P = 0.837$; Treatment × Species $F_{3,89} = 1.62$, $P = 0.162$).

All species were included in the analysis of leaf damage even if they experienced little or no leaf damage. Leaf damage was analyzed as the mean score given to each of the marked plants for each species within each block. Data from alpine meadow and boreal forest were not normally distributed and no appropriate transformation could be applied; however, a log transformation was used to normalize grassland data.

Meta-analysis of plant traits—We conducted a meta-analysis to determine the effects of gross plant traits on responses to handling using Metawin v.2.0 software (Rosenberg, Adam, and Gurevitch, 2001). We included all 22 species from our grassland, boreal forest, and alpine meadow (see above) and old field (Cahill, Castelli, and Casper, 2001) experiments (Table 1). The aboveground biomass data from the old field were not included in Cahill, Castelli, and Casper (2001), but these plants were harvested in a manner identical to that described above for the other species.

The three characters we chose for this meta-analysis included growth form (graminoid, herbaceous, woody), the presence or absence of conspicuous gross morphological defenses (i.e., thorns or spines, but not silica), and a qualitative assessment of chemical defenses, based on a survey of documented antiherbivore compounds or medicinal uses (see Table 1). In the case of chemical defenses, there is little specific quantitative information available for most species; consequently, we viewed this only as a preliminary attempt to look for broad patterns related to chemistry in the data set.

Because we used all of the results from our own experiments (including unpublished data) there was no publication bias in this analysis. We assessed the overall effect size using log response ratios (Rosenberg, Adam, and Gurevitch, 2001) and used the relevant comparisons in our database to evaluate the effects of each class variable. Due to the nested design of our experiments, the sample sizes indicated the number of replicate blocks not the number of plants. The 95% confidence intervals were calculated using the bias-corrected bootstrap method (Rosenberg, Adam, and Gurevitch, 2001). All 22 species were included in the biomass comparisons, but only the 18 species showing any herbivore damage were included in the herbivory analysis because it is not possible to estimate log response ratios with zeros in the denominator.

RESULTS

Effects of visitation on plant biomass—In alpine meadow, boreal forest, and grassland communities, final plant biomass differed significantly between species (Table 2; Fig. 1), a result that we anticipated given the range in growth forms. More importantly, for almost all species, the general effect of handling was to reduce plant biomass (Fig. 1). Significant main effects of handling were observed in the alpine meadow and grassland, but not in the boreal forest (Table 2). There were no significant interaction effects between Species and Treatment in any community. The only significant post-hoc result was for *Polygonum viviparum* in the alpine meadow ($P = 0.002$), and the consistent effect of handling in reducing plant biomass accounted for the significant main effects of Treatment in the GLM (Fig. 1).

Effects of visitation on damage by invertebrate herbivores—In all three communities, mean leaf damage varied significantly between species (Table 3; Fig. 1). In the alpine meadow, leaf damage varied as a function of Treatment ($P = 0.009$) and the Species × Treatment interaction was also significant ($P < 0.001$). However, a number of assumptions of ANOVA models were violated because so few species in the alpine showed signs of herbivory (Fig. 1), so we conducted the analysis using only species showing leaf damage (damage score > 0). In this case, only the Treatment effect was significant ($P = 0.003$) and not the interaction ($P = 0.071$). In the grassland, only the interaction was significant ($P = 0.043$; Table 3). Treatment effects on leaf damage in the boreal forest ($P = 0.7442$) were not significant, nor were any post-hoc Species × Treatment contrasts at this site. A post-hoc analysis of leaf damage indicated that handling significantly increased insect herbivory for *Salix reticulata* in the alpine ($P = 0.002$) and *Festuca campestris* in the grassland ($P = 0.001$).

Meta-analysis of handling responses based on conspicuous plant traits—The meta-analysis provided somewhat contradictory results between the among-groups test of effect sizes and whether or not the 95% confidence intervals overlapped zero. Handling had an overall negative effect on plant biomass (Fig. 2). The effect sizes did not vary as a function of growth form or defense; however, the mean effect size was different from zero for woody plants (not herbs or grasses) and chemically or morphologically “undefended” species. In contrast, the overall effect of handling on damage by herbivores (Fig.

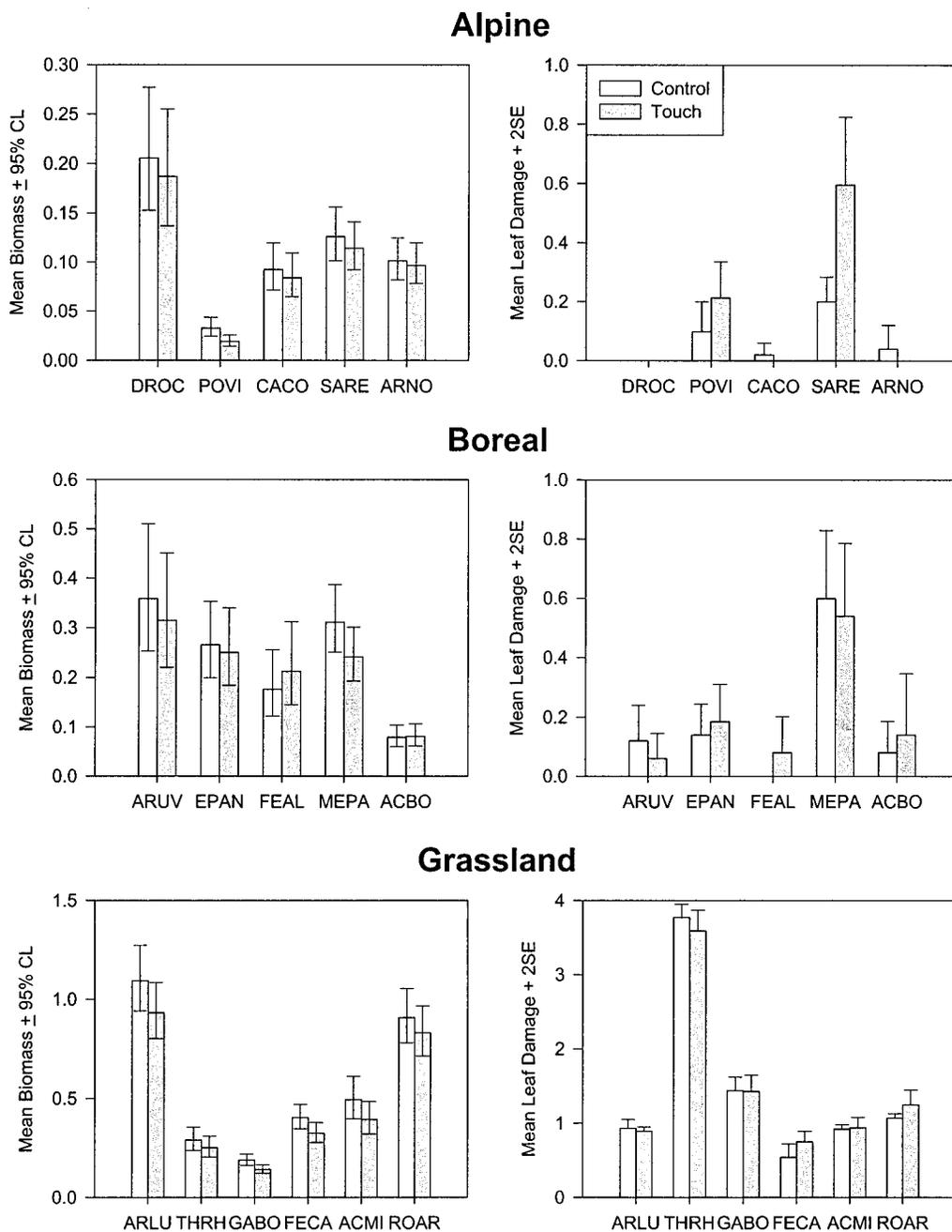


Fig. 1. Aboveground biomass and leaf damage by invertebrate herbivores of plants that were either not visited (Control, open bars) or visited and touched weekly (Touch, shaded bars) during summer 2000 in alpine meadow, boreal forest, and grassland communities. Biomass values are backtransformed following statistical analysis. Species abbreviations are indicated in Table 1.

TABLE 3. Effect of touching on invertebrate leaf damage in alpine meadow, boreal forest, and grassland communities.

Effect ^a	Alpine			Boreal			Grassland		
	df	F	P	df	F	P	df	F	P
Species	4, 71	24.29	<0.001	4, 71	15.60	<0.001	5, 90	124.52	<0.001
Treatment	1, 18	8.52	0.009	1, 18	0.11	0.744	1, 18	2.32	0.145
Species × Treatment	4, 71	6.90	<0.001	4, 71	0.42	0.791	5, 90	2.40	0.043

^a Type III tests of the fixed effects using generalized linear mixed models (PROC Mixed; SAS Institute, 2000). The response variable is mean leaf damage of each species in each block. Included in these models are two random effects, Block(Treatment) and Species × Block(Treatment). Excluding these effects did not significantly alter the fit of the model in either the alpine or boreal forest (significance of reduction in -2 Res Log-Likelihood: all *P* > 0.45).

2) showed significantly more variation in effect sizes among species than expected by chance ($Q_1 = 39.77$, $df = 1$, $P < 0.001$). Handling did not vary significantly among growth forms ($P = 0.166$), chemical defense ($P = 0.48$), or morphological defense ($P = 0.238$); however, woody plants experienced higher levels of herbivore damage when handled. There was significantly greater variation in effect sizes among species with conspicuous chemical defense ($P = 0.003$) or without morphological defense ($P = 0.002$), than would be expected by chance.

Even though our assessment of chemical defense was based on qualitative observations, the overall pattern was robust to alternative categorizations. For example, alpine *Artemisia norvegica* has few terpenoids, is relatively nonaromatic, and is a highly preferred forage species of mammalian herbivores in the Yukon (J. Andruchow, unpublished manuscript) and was therefore categorized as not having strong chemical defense (in contrast to *A. ludoviciana* in the grassland). However, switching this categorization did not change the results for effect size in the meta-analysis.

DISCUSSION

Prevalence of handling effects—We found significant effects of handling on plant growth and herbivory in two of three communities when we selected five or six common species and measured their response during a single season (Table 2). While no significant effects or trends were observed in the boreal forest, handling decreased the size of alpine plants and increased leaf damage. In the grassland site, handling decreased plant size and interacted with species identity to influence leaf damage (Tables 2 and 3; Fig. 1). At the species level, these effects were generally more subtle. Although a significant post-hoc effect on aboveground biomass was found for only one species (*Polygonum viviparum*), 14 of the 16 species tested displayed a trend towards decreased plant biomass with handling.

Across all species in all experiments, weekly handling reduced growth by 10% (Fig. 2). Such a reduction in aboveground biomass following handling, at an intensity common to many studies in plant ecology, may have potentially significant implications for the study of a variety of ecological questions. Many ecological processes, ranging from competition for light (Schwinning and Weiner, 1998) to pollinator attraction (Lortie and Aarssen, 1999), are dependent upon plant size. If the simple act of monitoring marked plants during a field study influences their size, then it may also influence the strength of these connected processes.

The overall trends describing the effects of handling on leaf damage by invertebrates showed that seven of the 12 damaged species have a trend towards increased leaf damage with touching, while five of 12 species have a trend in the opposite direction. The meta-analysis indicated more variability in the effects of handling on herbivory than expected. This variability in response to handling suggests that complex sets of plant-insect interactions determine leaf damage in a species-specific manner, supporting the initial claim of uncertainty in response to handling plants (Cahill, Castelli, and Casper, 2001). Plant responses to handling are likely to be subtle and infrequent or ecologists would have paid greater attention to them before, but at present the expression and magnitude of any effects remain difficult to predict (e.g., Schnitzer et al., 2002; Wenny, 2002).

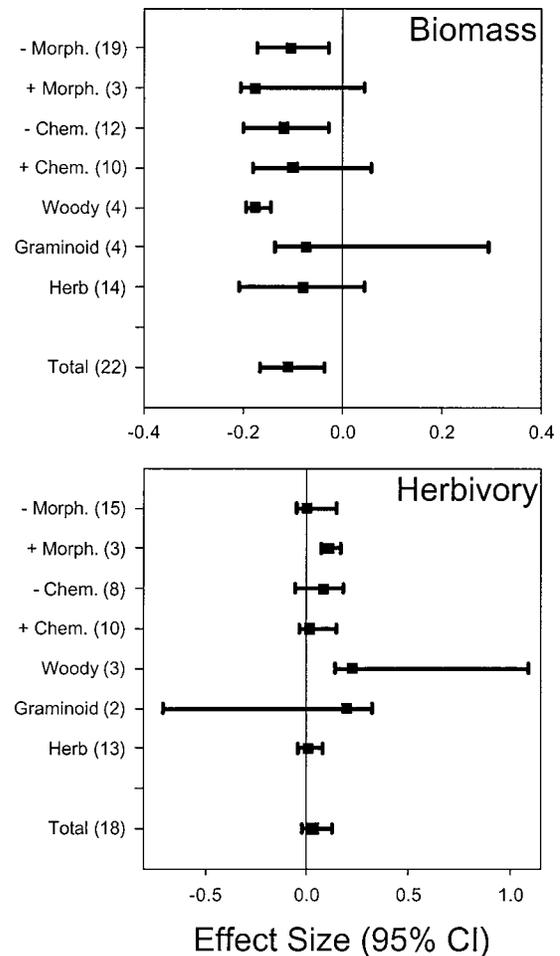


Fig. 2. Differences in effects size of handling for biomass and herbivore damage based on growth form, gross chemical defense traits, and gross morphological defense traits (see Table 1). Data are means of the overall effect size using log response ratios ($\pm 95\%$ bias-corrected bootstrap confidence intervals).

Possible mechanisms—There are several ecological mechanisms that could account for our results, including both direct effects of handling the plants and indirect effects associated with trampling the neighboring vegetation (Cahill, Castelli, and Casper, 2002). Visual inspection of our marked plants clearly indicated that the pattern of leaf damage resulted from invertebrate herbivory, rather than from handling the plants. However, handling could cause a variety of less obvious effects, including subdermal cell damage and breaking of trichomes or other epidermal structures, both of which could potentially decrease aboveground biomass. Indeed, the growing literature on thigmomorphogenesis suggests that what ecologists generally consider passive effects may actually have significant consequences as plants can respond to extremely minor touch stimuli (Jaffe, Leopold, and Staples, 2002). For example, stem elongation can be altered within minutes following even modest amounts of stem flexure (Coutand et al., 2000), suggesting that even minor handling could potentially affect stem growth rates. Short bursts of touching increase peroxidase activity in bean leaves (Cipollini, 1998) and can alter patterns of carbon and biomass allocation in *Brassica napus* (Cipollini, 1999). Similarly, differences in metabolic allocation

to different functions (e.g., structural vs. labile defenses) could contribute to the patterns we observed, but further studies will need to address this issue.

Most studies documenting thigmomorphogenic effects in plants use much more intensive stroking regimes than we did. For instance, Cippolini (1998) applied up to 40 strokes/d for 35 d, while our plants were handled a maximum of 8–9 times over 10 wk. Because our modest handling was sufficient to cause changes in allocation, the implication is that plants in the field are much more sensitive than previously imagined based on greenhouse experiments.

Visiting and touching a marked plant also entail the unavoidable act of stepping on some of its neighbors or disturbing the canopy. In prior work, simply visiting marked individuals of *Apocynum cannabinum* was sufficient to elevate leaf herbivory to the level found when other individuals were both visited and handled (Cahill, Castelli, and Casper, 2002). Visiting marked plants can result in a variety of potentially important effects, including (1) increased soil compaction and reduced root growth, (2) increased light reaching the marked plant, (3) alteration of microclimatic conditions. While increased availability of light has been observed to increase growth of some boreal species (e.g., Lieffers and Stadt, 1994) and competition for light generally limits growth of grassland species (e.g., Liira and Zobel, 2000), we observed a consistent decrease in aboveground biomass suggesting that our results are not due to benefits associated with changes in the light environment in these communities.

In both woody and herbaceous plants, there is increasing evidence that thigmomorphogenesis is not a whole-plant phenomenon, but is usually characterized by a highly localized response (Goodman and Ennos, 1998; Pruyn, Ewers, and Telewski, 2000). Because we only measured aboveground biomass of our study species, we cannot determine whether our treatment resulted in no effect on whole-plant biomass or rather simply a reallocation of biomass from shoot to root. Many plants alter patterns of allocation, over different time scales, following disturbance (Aerts and Chapin, 2000). While patterns of plasticity in some species appear to be ontogenetically constrained (McConnaughay and Coleman, 1999), the partitioning of available photosynthate between roots and shoots is dependent upon dynamic source–sink interactions (e.g., Minchin, Thorpe, and Farrar, 1994). For example, allocation responses to physical touching are known for *Cecropia schreberiana* saplings in two wind regimes in an elfin cloud forest (Cordero, 1999), where the wind-exposure treatment produced several thigmomorphogenic responses, including reductions in plant stature and crown area, changes in allocation patterns, and increased root : shoot ratio, leaf abrasion, and leaf epinasty. Aboveground handling may also influence the biomass and mechanical properties of roots of herbaceous plants (Goodman and Ennos, 1998).

There is some evidence that touching plants or trampling vegetation may result in the production and release of a variety of chemicals that influence leaf palatability and/or insect abundance. For example, in response to touch, many species initiate gene expression producing chemicals associated with herbivory (e.g., Braam et al., 1997; Baldwin et al., 2001). The costs of producing these secondary defensive compounds may reduce allocation to structural biomass. In response to wounding, which could be caused by either trampling or herbivory, some species have been observed to release a variety of compounds, which are either cues to herbivores or parasitoids of the her-

bivores (Pare and Tumlinson, 1997; Walling, 2000; Kessler and Baldwin, 2001). Consequently, changes in secondary chemistry could alter both the herbivores and parasitoid abundance that explain both the increases and decreases in leaf herbivory that were found in this study.

Some species may also be adapted to high levels of physical disturbance or herbivory, which could mask the effects of touching. In contrast to our results in the alpine and grassland, the aboveground biomass of plants in boreal forest did not decrease following touching (Fig. 1). Many boreal species are reported to be fairly tolerant of herbivory and show varying degrees of compensatory growth within a season, including *Arctostaphylos uva-ursi* (del Barrio, Luis-Calabuig, and Tarrega, 1999), *Epilobium angustifolium* (Michaud, 1991), and *Festuca altaica*, *Mertensia paniculata*, and *Achillea millefolium* (Hicks and Turkington, 2000). These species appear to be able to activate dormant stems or leaves immediately after damage, thereby perhaps minimizing the impacts of handling within a season.

Predictability of handling effects—Particular plant traits or characteristics may help to predict the differential performance of species to handling effects and alert researchers to the potential for problems. Similar comparative approaches have been used to examine the relationship between plant traits and various environmental factors such as fertility and disturbance (Craine et al., 2001), invasiveness (Rejmánek and Richardson, 1996), and grazing (Diaz, Noy-Meir, and Cabido, 2001).

Although we observed significant effects of handling under field conditions, these results are not easily understood in terms of simple plant traits (Fig. 2). While woody plants appeared to have a stronger response to handling than graminoid or herbaceous species, it was unclear why this was observed. Even our results for closely related taxa in different communities appear to indicate that very different responses (positive, negative, neutral) are possible. For example, the four graminoid species in our studies responded differently to handling. Two species showed no significant effects of handling. *Carex bigelowii* (syn. *consimilis*), a widespread arctic species, tolerates relatively intensive grazing (Jonsdottir, 1991; M. Tait and D. S. Hik, unpublished data), which may allow this species to minimize the within-season effects of touching. Similarly, in the old field, *Poa pratensis*, a perennial grass introduced from Europe that is able to withstand continuous heavy grazing (Looman, 1983), did not show any effect of handling. Boreal *Festuca altaica* also appears to be tolerant of herbivory (John and Turkington, 1997), and this was the only species to show any trend of increasing biomass in response to touching (Fig. 1). However, in the grassland, *Festuca campestris* is also adapted to long-term grazing by vertebrates (Looman, 1983), yet was the only species in that community to show significant post-hoc effects of touching on leaf damage by invertebrates.

Further work is necessary to understand why species responses might vary in different communities or under different environmental conditions. A systematic selection of species in future handling trials may help to resolve this question. Specifically, we suggest that species showing variable strong and weak responses to handling be investigated more closely to determine the relationships among plant traits, environmental conditions, and plant responses. Our meta-analysis results (Fig. 2) also suggest that there is more variation among species in the herbivory response compared to the biomass response,

indicating that more detailed examination of specific plant–herbivore interactions would be helpful.

Implications for field ecologists—There are still many outstanding questions about the potential effects of handling and visitation in ecological studies. As outlined above, the mechanisms by which plants respond to touching are unclear, and this information will be essential to understanding how best to minimize these confounding effects in experimental studies in the field and greenhouse. Schnitzer et al. (2002) have recently shown that handling may also increase rates of foliar pathogen damage in old field communities. Modification of a particular sampling regime may reduce or eliminate these problems, but there is still little information about dose responses of handling plants in the field (Telewski and Pruyn, 1998; D. S. Hik et al., unpublished data).

We imposed what most ecologists would consider to be a minor handling treatment and found ecologically relevant responses, both in terms of biomass and insect herbivory. The generally consistent pattern of decreasing biomass for all species in the alpine and grasslands suggests that the interpretation of experimental results of many studies may not be compromised. Also, studies involving a single measurement or harvest should not be affected. However, if one is interested in the study of a process that is size dependent, our results suggest that it may be very difficult to get a “true” measure of the process if plants need to be regularly visited. Additionally, although only a few species showed increased levels of herbivore damage following touching, the uncertainty of these responses could easily have a significant influence on some experimental studies. While handling effects for a particular species may be relatively consistent, comparisons among species may be less reliable. Even if the effects of handling plants are infrequent, the restrained caution urged by Cahill, Castelli, and Casper (2001) and Schnitzer et al. (2002) must be emphasized in light of our present results.

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