13 The Use and Usefulness of Artificial Herbivory in Plant–Herbivore Studies

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13.1 Summary

Artificial damage is a popular method in plant–herbivore studies, because the use of real herbivores is often laborious and because it may be virtually impossible to use herbivores in many experimental setups. We made a literature search of studies that tested whether natural and artificial damage have similar effects on plants. Of 46 studies found, 33 (72%) reported a significant difference between responses to artificial and natural herbivore damage in at least one of the statistical tests included. The studies contained 280 statistical tests, of which 99 (35%) showed a significant difference between artificial and natural damage. Phytochemical responses to artificial and natural damage were different in 41% of the statistical tests and 75% of the studies found at least one significant difference. Plant resistance, measured as secondary damage, herbivore performance, fungal growth in damaged tissue or plant attractiveness to parasitoids of herbivores, differed in 60% of the statistical tests and 85% of the studies had significant differences. Growth, reproduction and physiological responses to artificial and natural damage differed in 20–30% of statistical tests and 50–83% of studies had significant differences. Thus, studies on plant tolerance (growth and reproduction after damage) more often showed similar effects for artificial and natural damage than studies on plant resistance to herbivory, but even in tolerance studies artificial and natural damage often have different effects. Some studies indicated that application of herbivore saliva and careful imitation of timing and spatial pattern of damage helped in reaching the same effect with simulations and natural damage.
13.2 Introduction

In many experimental settings, artificial damage has several practical benefits over the use of real herbivores (Hjältén, Chap. 12, this Vol.). The extent of damage and the location of damaged parts can easily be controlled, and collateral damage to other than target tissues can be minimized. The removed biomass can be collected and measured. Furthermore, there is no need to collect and rear herbivores. The use of artificial damage enables efficient experimental designs, with balanced sample sizes of experimental groups and a low variation of treatment intensity within each experimental group. For these reasons, artificial damage is used more often in herbivory research than real herbivores.

Artificial damage does not, however, always adequately mimic natural damage (Baldwin 1990; Hjältén, Chap. 12, this Vol.). Many types of herbivory are not applicable for simulations. Damage by stem borers, miners, galling insects, root feeders or sucking insects is seldom tried to simulate. However, even when the apparent damage pattern is easy to reproduce, several characteristics of natural herbivory may be difficult to simulate, such as the timing of damage, herbivore host choice, location of damage within a plant, and subtle details of damage by herbivore mouth parts. Herbivore saliva can also play a role in plant responses (Walling 2000). The effects of trampling, defecating and urinating, dispersal of plant pathogens by herbivores, herbivore enemies and competitive release due to damage of neighbouring plants are seldom taken into account in herbivore simulations. The study of these and other effects of herbivory on plant community and ecosystem processes requires specific experimental designs. Indirect effects are often outside the scope of herbivore studies that use artificial damage, which usually concentrate on the hierarchical level of plant individuals.

In spite of many potential pitfalls, artificial damage continues to be a popular method in much of herbivory research. To control whether the effects of natural and artificial damage differ, several studies have included both types of treatments in experiments. Our aim is to review the tests that compare the plant response to natural and artificial herbivory. We examine how frequently natural and artificial damage give different responses. We also discuss what traits show differential responses to artificial and natural damage, and how the difference in response is affected by the type of herbivore and plant.

13.3 Material and Methods

We carried out a search from the Institute for Scientific Information (ISI) Web of Science database (www.isiknowledge.com, 1975–2002) with search words
'artificial damage', 'artificial herbivory', 'simulated herbivory', 'simulated damage', 'natural herbivory' and 'natural damage'. We extended the search further by checking the reference lists of relevant articles that we found from the ISI database.

From articles, we recorded the method of artificial damage and the herbivore species of natural damage, type of response variables used, target tissue and sample size. We classified response variables as physiological and phytochemical responses, growth and reproduction responses, and resistance to secondary herbivory (other than chemical defence). From each statistical test, we checked whether there were pairwise significant differences among control, natural damage and artificial damage groups. In cases where there were several types of artificial damage, we chose the one that was closest to natural damage in pattern and timing. If there were no a posteriori tests, the results could usually not be used, but in some cases, a multiway ANOVA design enabled us to deduce the pairwise comparison among experimental groups. When applicable, we also used information from the studies that did not have any undamaged control group. We used treatment means and standard deviations to calculate the effect size as a standardized mean difference statistic $d = J(\bar{X}_N - \bar{X}_A)/s$, where $\bar{X}_N$ and $\bar{X}_A$ are the means for natural and artificial damage, respectively, $s$ is the pooled standard deviation and $J$ is a correction term that removes the bias due to small sample size (Gurevitch and Hedges 1993). The equation $d = 2J\sqrt{(\chi^2/\left(N - \chi^2\right))}$, where $N$ is the total sample size, was used for frequency data. The sign of $d$ was assigned as negative if artificial damage had a stronger effect than natural damage to the expected direction from the control group, and positive otherwise.

We recorded whether the statistical tests of the same study belonged to the same experiment or to several independent experiments. Independent experiments were defined as distinct experiments with their own plant individuals, herbivores and separate statistical tests. Multiple years of data were considered as separate experiments if each year was tested separately, and one experiment if there was a common test of the whole data set. One experiment usually consisted of tests of many traits that were measured from the same plants. We made a general evaluation for each study and for each experiment of whether a significant difference in response to artificial and natural damage was found in any of the statistical tests. Thus, if one of the tests was significant, the whole study was assigned as showing a significant difference. When original studies discussed results of artificial and natural damage, differences were sometimes considered as irrelevant or minor, although they were statistically significant. We do not necessarily disagree with the interpretation of original tests, but to be consistent we followed the results of the statistical analyses.

The results are reported with studies, experiments and statistical tests as sampling units. It should be noted that only at the level of study, and possibly
at the level of experiment, do the sample units represent observations that are independent in a statistical sense. Statistical tests belonging to the same experiment are associated, which has to be kept in mind when interpreting the testwise results. Testwise reports, however, give important insights into many of the questions discussed in the text. Furthermore, different experiments in the article are not independent in a strict sense, and the same caveat applies even to different studies with the same plant species. Similar to many ecological reviews, there is only a limited number of species in the data set and some popular model species are used in many studies. The only real solution to this problem of representativeness is to carry out more studies to cover a broad scale of taxa.

In some cases, the authors have known before or expected that the effects of artificial and natural damage differ. In the case of *Nicotiana-Manduca sexta*, Baldwin (1988) showed that artificial damage induces a stronger nicotine production than natural damage. Two later studies that continued to elucidate the system are included in this review (McCloud and Baldwin 1997; Kahl et al. 2000). Agrawal (1998), Agrawal et al. (1999) and Agrawal and Sheriffs (2001) compared real herbivory and rapid leaf clipping because earlier data suggested that clipping does not induce resistance. Turlings et al. (1990) used natural herbivory and artificial damage, with or without caterpillar regurgitants, to study volatile release and parasitoid attraction. These studies present a further test of natural and artificial damage, but there is a tendency for them to show a significant difference between the two damage types. A less clear case is the series of articles on birch herbivory (Haukioja and Neuvonen 1985; Hartley and Lawton 1987, 1991; Neuvonen et al. 1987; Hartley 1988; Hartley and Firn 1989; Hanhimäki and Senn 1992). Early studies showed a difference in response to artificial and natural damage. This was often confirmed in later studies, although it is not clear whether a specific result was a priori predicted. For some of the comparisons of the effect of natural and artificial herbivory, we present results with all studies included and with a data set without the studies where the authors apparently had a clear prior expectation of the result. In general, the results were approximately similar with both data sets.

### 13.4 Commonness of Differences Between Natural and Artificial Herbivory

We found 46 papers that tested the difference between responses to artificial and natural herbivore damage (Table 13.1). Thirty-three of the studies (72%) reported a significant difference between responses to artificial and natural herbivore damage in at least one of the statistical tests included. Thirteen of the studies found no difference between responses to different damage meth-
ods. Many of the papers presented results from several independent experi-
ments. When each experiment is evaluated separately, 50 of the total 73 ex-
periments (68%) showed a difference between artificial and natural herbivory
and there was no difference in 23 experiments (32%) in any of the tests of the
experiment. Finally, we checked how many statistical tests showed a sig-
nificant difference in responses. Of 280 statistical tests, 99 (35%) showed a
difference and 181 (65%) showed no difference between responses to natural and
artificial herbivory. The pattern was similar even if studies where a significant
difference between natural and artificial damage was a priori expected (see
Sect. 13.3) were not taken into account. Of the tests with no specific expecta-
tion of the result, 23 of 34 studies (68%) and 77 of 238 statistical tests (32%)
showed a difference between natural and artificial damage. It is thus common,
although not ubiquitous, that responses to natural and artificial herbivory dif-
fer.

Obviously, with a higher number of statistical tests within a study or an
experiment, the probability that at least one of the tests is significant is
increased. The studies including tests with a significant difference in response
to artificial and natural herbivory contained more statistical tests than stud-
ies without any difference [Wilcoxon rank sum test, $W=304.5$, $P=0.027$;
median and range of number of tests: studies showing a difference 4 (1...37)
tests, studies with no difference 2 (1...5) tests].

Another factor potentially affecting the likelihood of finding a significant
difference is the sample size. Ultimately, all ecological experiments would
show a significant (although possibly biologically irrelevant) difference
among experimental groups if the sample size is high enough, since there are
always differences among ecological samples. Some of the studies had aston-
ishing sample sizes, e.g. 2200 plants in Juenger and Bergelson (2000), showing
a significant difference. There was no significant difference in sample sizes of
experiments with negative and positive results [Wilcoxon rank sum test,
$W=587.5$, $P=0.886$; median and range of sample size: significant difference 44
(12...2000), no difference 44 (12...105)].

The analysis of effect sizes (Table 13.1), however, suggested that there is a
publication bias for too many studies with a low sample size showing a differ-
ence between artificial and natural damage. The relationship of sample size
vs. effect size should reveal in a scatterplot a funnel-type pattern, with small
sample sizes displaying more variation around the mean effect than large
studies, but without any decreasing trends or without any large holes on the
left side of the plot near the x-axis (Palmer 1999). There was a decreasing
trend between sample size and effect size (Spearman correlation, $R_s=-0.268$,
$P=0.001, N=149$). Further examination revealed that the publication bias was
due to chemical response variables ($R_s=-0.541$, $P=0.001, N=35$). The other
types of response variables did not show any association between sample size
and effect size (resistance $R_s=-0.136$, $P=0.465$, $N=31$; growth $R_s=0.005$,
$P=0.977, N=45$; reproduction $R_s=0.046$, $P=0.791, N=35$; physiological traits:
Table 13.1. References included in the review. General evaluation of difference in response to artificial and natural damage, response variables, plant and animal taxa and effect sizes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Difference in response to artificial and natural damage</th>
<th>Type of response variables</th>
<th>Response traits measured</th>
<th>Plant species</th>
<th>Plant family</th>
<th>Plant functional group</th>
<th>Animal species</th>
<th>Animal taxon</th>
<th>Effect size (mean and range) (%)</th>
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<tbody>
<tr>
<td><strong>Insects; grasses</strong></td>
<td>Yes</td>
<td>Chemical and physiological</td>
<td>Water potential; carbohydrate, protein, proline and chlorophyll content</td>
<td><em>Hordeum vulgare</em></td>
<td>Poaceae</td>
<td>Grass</td>
<td>Schizaphis graminum</td>
<td>Homoptera</td>
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<td>Poaceae</td>
<td>Grass</td>
<td>Chilo partellus</td>
<td>Lepidoptera</td>
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<td>Woodhead (1981)</td>
<td>Yes</td>
<td>Growth</td>
<td>Seedling size</td>
<td><em>Triticum aestivum</em></td>
<td>Poaceae</td>
<td>Grass</td>
<td>Melanoplus sanguinipes</td>
<td>Orthoptera</td>
<td>16.2 (~84.1...102.4)</td>
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<td>Capinera and Roltsch (1980)</td>
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<td>Resistance</td>
<td>Parasitoid activity</td>
<td><em>Zea mays</em></td>
<td>Poaceae</td>
<td>Grass</td>
<td>Spodoptera exigua</td>
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<tr>
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<td>Chemical</td>
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<td><em>Brassica napus</em></td>
<td>Brassicaceae</td>
<td>Herb</td>
<td>Phyllotreta crucifera</td>
<td>Coleoptera</td>
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<td>Bodnaryk (1992)</td>
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<td></td>
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<td>Delia floralis</td>
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<td>Brassicaceae</td>
<td>Herb</td>
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<td>Diptera</td>
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<td>Yes</td>
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<td>Herb</td>
<td>Marmora albapalpella</td>
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<td>Growth</td>
<td>Shoot growth</td>
<td><em>Epilobium latifolium</em></td>
<td>Onagraceae</td>
<td>Herb</td>
<td>Plathypena scabra</td>
<td>Lepidoptera</td>
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<tr>
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<td>Physiological</td>
<td>Leaf water loss</td>
<td><em>Glycine max</em></td>
<td>Fabaceae</td>
<td>Herb</td>
<td>Plathypena scabra,</td>
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<td>Leaf water loss</td>
<td><em>Glycine max</em></td>
<td>Fabaceae</td>
<td>Herb</td>
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<td>Treatment</td>
<td>Type</td>
<td>Response</td>
<td>Species</td>
<td>Genus</td>
<td>Family</td>
<td>Herbivore</td>
<td>Herbivore</td>
<td>Value (Range)</td>
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<td>Poston et al. (1976)</td>
<td>No</td>
<td>Physiological</td>
<td>Net carbon exchange</td>
<td>Glycine max</td>
<td>Fabaceae</td>
<td>Herb</td>
<td>Plathypena scabra, Cynthia cardi</td>
<td>Lepidoptera</td>
<td>-17.1...15.8</td>
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<td>Welter (1991)</td>
<td>No</td>
<td>Growth and physiological</td>
<td>Photosynthesis, plant growth</td>
<td>Lycopersicon esculentum</td>
<td>Solanaceae</td>
<td>Herb</td>
<td>Manduca sexta</td>
<td>Lepidoptera</td>
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<td>Quinn and Hall (1996)</td>
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<td>Growth and physiological</td>
<td>Leaf and nodule production, Volatile release</td>
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<td>Nicotiana sylvestris</td>
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<td>Manduca sexta</td>
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<td>Yes</td>
<td>Resistance</td>
<td>Leaf damage, oviposition</td>
<td>Raphanus raphanistrum</td>
<td>Brassicaceae</td>
<td>Herb</td>
<td>Pieris rapae, Phyltetra spp., Oryctolagus cuniculus</td>
<td>Coleoptera, Lepidoptera, Lagomorpha</td>
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<td>Agrawal et al. (1999)</td>
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<td>Chemical and reproduction</td>
<td>Glucosinolates, flowering, seed set, pollen production</td>
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<td>Leaf length, seed production</td>
<td>Sinapis alba, Brassica napus</td>
<td>Brassicaceae</td>
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<td>Davis and Boyd (2000)</td>
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<td>Chemical</td>
<td>Content of C, N, Ni and glucosinolates</td>
<td>Streptanthus polygaloides, S. insignis</td>
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<td>Plant family</td>
<td>Plant functional group</td>
<td>Animal species</td>
<td>Animal taxon</td>
<td>Effect size (mean and range) (%)</td>
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<td>Insects; shrubs and trees</td>
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<td><em>Betula pendula</em></td>
<td>Betula</td>
<td>Tree</td>
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<td><em>Apocheima pilosaria</em></td>
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<td>Resistance</td>
<td>Herbivore feeding preference</td>
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<td>Betulaceae</td>
<td>Tree</td>
<td><em>Eriocnoria spp.</em></td>
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<td>Phenolic content, protein precipitation ability</td>
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<td>Tree</td>
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<td>Lepidoptera</td>
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<td>Betulaceae, Rosaceae</td>
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<td><em>Gossypium hirsutum</em></td>
<td>Malvaceae</td>
<td>Shrub</td>
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<td>23.5 (−6.9...54.5)</td>
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<td>Heil et al. (2001)</td>
<td>Yes</td>
<td>Chemical</td>
<td>Extrafloral nectar production</td>
<td><em>Macaranga tanarius</em></td>
<td>Euphorbiaceae Tree</td>
<td></td>
<td><em>Scarabaeidae spp., Xenocatantops humilis</em></td>
<td>Coleoptera, Orthoptera</td>
<td>−170.8</td>
</tr>
<tr>
<td>Hodge et al. (2000)</td>
<td>No</td>
<td>Growth</td>
<td>Leaf retention</td>
<td><em>Macroptera exelsum</em></td>
<td>Piperaceae</td>
<td>Tree</td>
<td><em>Cleora scriptaria</em></td>
<td>Lepidoptera</td>
<td>5.4</td>
</tr>
<tr>
<td>Britton (1988)</td>
<td>No</td>
<td>Growth</td>
<td>Leaf area</td>
<td><em>Pinus contorta</em></td>
<td>Pinaceae</td>
<td>Tree</td>
<td><em>Neodiprion sertifer</em></td>
<td>Hymenoptera</td>
<td>2.0</td>
</tr>
<tr>
<td>Study</td>
<td>Treatments</td>
<td>Response Variables</td>
<td>Plant Species</td>
<td>Plant Family</td>
<td>Life Stage</td>
<td>Herbivore Class</td>
<td>Effect Size</td>
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<tr>
<td>Litvak and Monson (1988)</td>
<td>Yes</td>
<td>Chemical, Monoterpene production, Protein and tannin content</td>
<td>Pinus ponderosa</td>
<td>Pinaceae</td>
<td>Tree</td>
<td>Lepidoptera</td>
<td>95.6 (−33.7...505.6)</td>
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<td></td>
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<tr>
<td>Faeth (1986)</td>
<td>Yes</td>
<td>Chemical</td>
<td>Quercus emoryi</td>
<td>Fagaceae</td>
<td>Tree</td>
<td>Lepidoptera, Coleoptera, Orthoptera, Hymenoptera</td>
<td></td>
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</tr>
</tbody>
</table>

**Algae and their herbivores**

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatments</th>
<th>Response Variables</th>
<th>Plant Species</th>
<th>Plant Family</th>
<th>Life Stage</th>
<th>Herbivore Class</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pavia and Toth (2000)</td>
<td>Yes</td>
<td>Chemical, Phlorotannin content</td>
<td>Ascophyllum nodosum</td>
<td>Fucaceae</td>
<td>Brown alga</td>
<td>Gastropoda</td>
<td>144.3 (−17.6...318)</td>
</tr>
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<td>Toth and Pavia (2002)</td>
<td>No</td>
<td>Chemical and growth, Growth, phlorotannin content</td>
<td>Laminaria hyperborea</td>
<td>Laminariaceae</td>
<td>Brown alga</td>
<td>Gastropoda</td>
<td>−3.6 (−48.3...40.8)</td>
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</table>

**Mammals**

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatments</th>
<th>Response Variables</th>
<th>Plant Species</th>
<th>Plant Family</th>
<th>Life Stage</th>
<th>Herbivore Class</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teague (1988)</td>
<td>No</td>
<td>Growth</td>
<td>Acacia karroo Castor sp.</td>
<td>Fabaceae</td>
<td>Herb</td>
<td>Capra hircus</td>
<td>−21.6</td>
</tr>
<tr>
<td>Reichman and Smith (1991)</td>
<td>No</td>
<td>Growth</td>
<td>Lolium perenne</td>
<td>Poaceae</td>
<td>Grass</td>
<td>Geomys bursarius</td>
<td>−230.5</td>
</tr>
<tr>
<td>Howe et al. (1982)</td>
<td>Yes</td>
<td>Growth</td>
<td>Ipomopsis aggregata</td>
<td>Polemoniaceae</td>
<td>Herb</td>
<td>Sigmodon hispidus</td>
<td>−6.9 (−45.2...43.2)</td>
</tr>
<tr>
<td>Juenger and Bergelson (2000)</td>
<td>Yes</td>
<td>Growth and reproduction</td>
<td>Ipomopsis aggregata</td>
<td>Polemoniaceae</td>
<td>Herb</td>
<td>Thamomys sp.</td>
<td>−6.9 (−45.2...43.2)</td>
</tr>
<tr>
<td>Paige (1992)</td>
<td>Yes</td>
<td>Growth and reproduction</td>
<td>Ipomopsis aggregata</td>
<td>Polemoniaceae</td>
<td>Herb</td>
<td>Odocoileus hemionus, Cervus elaphus</td>
<td>28.9 (−59.7...149.8)</td>
</tr>
<tr>
<td>Paige (1999)</td>
<td>Yes</td>
<td>Growth and reproduction</td>
<td>Ipomopsis aggregata</td>
<td>Polemoniaceae</td>
<td>Herb</td>
<td>Odocoileus hemionus, Cervus elaphus</td>
<td>75.9 (−43.1...193.2)</td>
</tr>
<tr>
<td>Maschinski and Whitham (1989)</td>
<td>Yes</td>
<td>Reproduction</td>
<td>Ipomopsis arizonica</td>
<td>Polemoniaceae</td>
<td>Herb</td>
<td>Rodentia, Artiodactyla</td>
<td>−36.6 (−85.8...15.5)</td>
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</tbody>
</table>

* Sign of effect size was assigned as negative if artificial damage had a stronger effect than natural damage to expected direction from control group, and positive otherwise.
too low sample size for the test). It was not possible to further isolate a specific type of chemical studies behind the publication bias. Chemical studies with a high effect size and low sample size included several types of plants such as birch (Hartley and Firn 1989), pine (Litvak and Monson 1998), barley *Hordeum vulgare* (Cabrera et al. 1995) and the brown alga *Ascothylum nodosum* (Pavia and Toth 2000). The response traits ranged from volatile compounds and phenolics to primary metabolites such as protein and carbohydrate content. The publication bias of chemical studies remained significant even if we removed the studies with prior expectation of a difference between natural and artificial damage (see Sect. 13.3). There may thus be missing chemical studies with a low sample size and negative result that have not been published. A negative result in this case means that artificial damage has a stronger effect on the expected direction than natural damage. Due to publication bias of the chemical studies, other results, where the whole data set is included, could also be biased. The results remained, however, very similar even when the chemical studies were removed from the analysis (difference of at maximum three percentage units; results not shown).

### 13.5 Strength of the Effect of Natural and Artificial Damage

Of 280 statistical tests, 155 showed a difference between the control and either natural damage, artificial damage, or both. In 56 tests, both damage types resulted in a similar and a significant difference to the control group. Assuming that statistical power of the experiments was sufficient to reveal biologically important differences, these 56 cases with a difference to the control group but no difference between damage methods are ideal to support the use of artificial damage. There were 106 tests with no differences between artificial damage, natural damage and the control group, and an additional 19 tests with no difference between two damage types but where the control group was missing. These results can be used to support the use of artificial damage.

Other types of test results are more problematic. In several tests, both damage methods resulted in a significant difference from the control group, but one of the treatments was significantly stronger than the other one. Whether this is acceptable depends on the goals of the study and on the actual difference between the treatments. In cases where both treatments differed from the control, natural damage had a significantly stronger effect in 30 tests and artificial damage in 10 tests.

Also, in other respects, natural damage often had a stronger effect than artificial damage. Of the total of 99 statistical tests showing a difference between natural and artificial damage, natural damage had a significantly stronger effect in 77 tests. In 43 of these 77 tests, there was no significant difference between the control group and the artificial damage treatment. There
were only six tests where artificial damage produced a significant effect but natural damage did not. Thus, there was a considerable proportion of tests where the effect of herbivory would not have been detected if only artificial damage had been used.

Perhaps the most problematic cases are those where natural and artificial damage have an effect in opposite directions from the control group. Six tests (2%) produced this result. Two of the tests used a chemical response variable, one test dealt with growth and three tests with reproduction.

13.6 Responses of Different Types of Response Traits to Artificial and Natural Damage

The effect of herbivory is mediated to other plant characteristics through physiological and chemical processes (Voelckel and Baldwin, Chap. 17, this Vol.). It is thus possible that physiological and phytochemical traits are more sensitive in showing the difference between responses to artificial and natural damage than other plant traits such as growth and reproduction. The review results give some support to the hypothesis that phytochemical response often differs between artificial and natural damage. Phytochemical response differed between artificial and natural damage in 75% of the studies, 75% of the experiments and 41% of the tests. It must be noted, however, that chemical studies are difficult to interpret due to publication bias (see Sect. 13.4). It is possible that when sample size has been low, significant results have been published more often than non-significant results.

Most chemical traits used in the tests were secondary compounds with putative defensive function or enzymes involved in their biosynthesis. We found only three studies that tested the response of other types of chemical traits. Davis and Boyd (2000) did not find any difference between the effects of artificial and natural damage when studying nickel, carbon and nitrogen content and the carbon:nitrogen ratio after damage. Ni, C and N were studied for their possible role in resistance to herbivory. Faeth (1986) tested how leaf protein content is affected by damage. He found no difference in responses to artificial and natural damage. Cabrera et al. (1995), on the other hand, found a difference between the effects of different damage types on soluble protein content in leaves, and also on the levels of soluble sugar, free proline and chlorophyll. However, the method of artificial damage of Cabrera et al. (1995) was not an especially close imitation of natural herbivory. They used aphids as natural herbivores and artificial damage consisted of wounding leaves with microcapillary tips twice a day.

Physiological responses were more often similar for artificial and natural damage than phytochemical responses. Response variables included extrafloral nectar production (Heil et al. 2001), photosynthetic rate (Poston et al. 1976;
Welter 1991) and water status (Ostlie and Pedigo 1984; Cabrera et al. 1995). Production of extrafloral nectar was higher after artificial damage, or after jasmonic acid spraying, than after natural damage of similar extent (Heil et al. 2001). Otherwise physiological responses were closely similar after natural and artificial damage. Ostlie and Pedigo (1984) found a difference in water loss in the comparison of one of two herbivore species, but the difference disappeared 16 h after damage. Cabrera et al. (1995) also found differences in water status, but, again, wounding with microcapillary tips can hardly be expected to have the same effect on water potential as aphid damage.

Resistance to secondary herbivory was often different after artificial and natural damage. In 85 % of the studies, 84 % of the experiments and 60 % of the statistical tests, induced resistance of the damage methods was significantly different. Resistance studies include several cases where the test was replicated in several papers using the same study system, with a high probability of observing a difference between natural and artificial damage (see Sect. 13.3). However, significant differences between artificial and natural damage were even more common in the studies without a prior expectation of the result (results not shown). Resistance studies included herbivore bioassays of plant quality after damage (Haukioja and Neuvonen 1985; Hartley and Lawton 1987; Neuvonen et al. 1987; Hanhimäki and Senn 1992; Anderson and Alborn 1999; Anderson et al. 2001), fungal growth in damaged tissue (Hatcher et al. 1994, 1995), secondary damage on plants exposed to herbivores (Agrawal 1998; Agrawal and Sherriffs 2001) and plant attractiveness to parasitoids of herbivores (Turlings et al. 1990; Mattiacci et al. 1994).

There may be an adaptive basis in the ability of plants, herbivores and herbivore enemies to discriminate between artificial and natural damage. From a plant's point of view, herbivore repellents are important after herbivore damage but not after other types of damage. Herbivores may benefit from either avoiding or favouring previously damaged leaves. For enemies of herbivores, it is even important to discriminate between damage types of different herbivore species (Takabayashi and Dicke 1996).

Plants often have a good tolerance to herbivory in terms of growth and reproduction (Strauss and Agrawal 1999; Stowe et al. 2000). It is expected that tolerance buffers growth and reproduction responses so that artificial and natural damage have more similar effect than other types of response variables. As predicted, growth and reproduction were less sensitive to different damage methods, but even for these response variables together, 59 % of studies and 26 % of tests showed a difference. Growth (23 % of the tests significantly different) seemed to be somewhat less sensitive to the choice of the damage method than reproduction (30 % of the tests significantly different).
13.7 Simulations of Mammalian and Invertebrate Herbivory

Because human tools are more of the size of mammalian jaws than insect mouthparts, one would expect that mammalian herbivory is easier to simulate than insect herbivory. The data set gives a possibility to test this hypothesis for tolerance traits, i.e. growth and reproduction after damage. For the other types of response traits, only invertebrates were used as natural damage agents in the papers of the data set. There were 7 studies, containing 57 statistical tests, with mammalian herbivory. In 5 studies and 22 statistical tests (39%), there was a significant difference between the damage methods. Invertebrate herbivores were used in 10 tolerance studies; half of the studies showed a significant difference in a response to artificial and natural damage. Of 72 statistical tests of tolerance traits in invertebrate studies, 11 (15%) showed a significant difference in a response.

According to these results, it is not easier to simulate mammalian damage than invertebrate damage. It must be noted, however, that only a few plant–vertebrate systems have been used in the studies of the data set. Four mammalian studies dealt with compensation and overcompensation of two Ipomopsis species, I. aggregata and I. arizonica. Natural and artificial damage often had a differential effect on growth and reproduction of Ipomopsis species, but it is not clear whether there were problems in sampling or in the experimental treatments of mechanically damaged plants (Bergelson et al. 1996; Paige 1999). The effect of natural damage may also be biased if the possibility that herbivores choose large plants could not sufficiently be taken into account in the statistical analysis (Bergelson et al. 1996). Mammalian saliva may, similar to insect saliva, affect plant growth and metabolism (Bergman 2002), but the effect of mammalian saliva was not tested in the current papers.

Invertebrate studies of tolerance consisted of a broader scope of plant–herbivore systems. Half of the studies did not observe any difference between artificial and natural damage. Simulated damage and natural herbivory of tobacco hornworm Manduca sexta had similar effects on growth and photosynthesis of tomato (Welter 1991). The effects of European pine sawfly Neodiprion sertifer on the growth of lodgepole pine (Britton 1988) and the effects of gastropod damage on the growth of the kelp Laminaria hyperborea (Toth and Pavia 2002) were similar to the effect of artificial damage. Quinn and Hall (1996) could artificially simulate the defoliation and denudation of the legume Medicago sativa by alfalfa weevil Hypera postica and Hodge et al. (2000) showed that artificial damage and herbivory of Cleora scriptaria, a geometrid moth, had the same effect on leaf loss of Macropiper excelsum. In some of the studies, the lack of significant differences may be due to low sample size and few and insensitive response variables compared to mammalian studies. In two of the studies, a significant difference between two damage types was anticipated (Agrawal 1998;
Agrawal et al. 1999). Agrawal and Sherriffs (2001) studied the combination of vertebrate and insect herbivory. The damaging agents were lepidopteran larvae, whereas the response trait was secondary damage by *Pieris rapae*, flea beetles and rabbits. Although induction had a somewhat different effect on rabbit and insect herbivory, the general result was that artificial damage was a poor proxy of natural herbivory.

### 13.8 Attempts of Exact Simulation

Some studies have tested several methods of artificial damage to find the most adequate simulation. Gavloski and Lamb (2000) studied how damage by three herbivores of cruciferous plants with different damage patterns can be simulated. They used concentrated or dispersed defoliation of cotyledons of *Sinapis alba* and *Brassica napus*, with or without meristem removal, to simulate damage by the flea beetle *Phyllotreta cruciferae*, diamondback moth *Plutella xylostella* and bertha armyworm *Mamestra configurata*. There were some significant differences in leaf growth between artificial and natural damage when the appropriate artificial damage pattern was compared with natural damage, but only for the low damage level of *Mamestra configurata* and only for the first of the four observation dates, 8–9 days after damage. For the other herbivores and the later observation dates, responses to artificial and natural damage were similar. Both damage methods resulted in similar compensation in leaf growth, less compensation for the flea beetle damage than the other two herbivores, and better compensation by *S. alba* than by *B. napus*. Gavloski and Lamb (2000) thus showed that, in their system, when the amount of biomass removal and the pattern of removal (dispersed or concentrated, with or without meristem removal) are reproduced by punch or scissors, artificial damage produces acceptable results.

Gavloski and Lamb (2000) did not try to mimic the temporal pattern of herbivory. In contrast, Baldwin (1988) simulated the temporal, in addition to spatial, pattern of herbivory when he studied the effect of *Manduca sexta* damage on *Nicotiana sylvestris*. The response variable was the alkaloid content, which appeared to be more sensitive to the damage method than the growth and reproduction traits used by Gavloski and Lamb (2000). Damage increased the alkaloid content, and the increase was larger after simulated damage than after real herbivory. The response of artificial damage was closest to natural herbivory with a careful imitation of temporal and spatial pattern of *M. sexta* damage, where leaf removal was performed with microscissors at the same pace as damage by larvae. Later work has shown that both artificial and natural damage increase jasmonic acid concentrations in the leaves, but chemical compounds in herbivore saliva block the induction of alkaloids (McCloud and Baldwin 1997; Kahl et al. 2000; Musser et al. 2002).
After applying saliva to wounded tissue, artificial and natural damage resulted in similar induction of alkaloids.

In addition to alkaloid induction, oral secretions are important for the release of volatile compounds that attract enemies of herbivores. Turlings et al. (1990), Dyer et al. (1995), Alborn et al. (1997), and Kahl et al. (2000) have studied whether plant odours from damaged plants attract Cotesia marginiventris parasitoids. They observed that plants damaged by Spodoptera exigua caterpillars released volatiles that were better attractants than volatiles from artificially damaged plants. When larval regurgitant was added to artificially damaged leaves, they were as attractive to parasitoids as Spodoptera-damaged leaves. The use of herbivore saliva in combination with artificial damage is thus a potential method to improve the quality of herbivore simulations. It is very difficult to collect enough insect saliva for large experiments, but treatments may become easier if the bioactive chemicals of saliva are identified (Moon et al. 1994; Alborn et al. 1997; Musser et al. 2002).

13.9 Conclusions

The literature review shows undeniably that artificial damage often has a different effect on plants than natural damage. Should ecologists for this reason avoid using artificial damage? Our review indicates that it is worthwhile to make an effort to use real herbivores whenever possible (see also Hjältén, Chap. 12, this Vol.). Our opinion, however, is that artificial damage still has its place in the toolbox of the ecologists. There were only a few cases, about 3%, of the worst possible outcome where artificial and natural damage had opposite effects on plants. Furthermore, the difference in the effects of artificial and natural damage is not always undesirable but may give interesting insight into plant responses to herbivory. For instance, Agrawal (1998) and Agrawal et al. (1999) noted that clipping with scissors did not induce the production of secondary compounds, but natural damage did. Artificial damage can then represent the effect of biomass removal and natural damage the effect of both biomass removal and induction. Jasmonic acid spraying can be used to induce resistance without any biomass removal (Agrawal et al. 1999). Studies on the effects of oral secretions of herbivores (Dyer et al. 1995; Alborn et al. 1997; McCloud and Baldwin 1997; Musser et al. 2002; Voelckel and Baldwin, Chap. 17, this Vol.) are other examples of the importance of artificial damage as an experimental method.

At the community and ecosystem level, the most important effects that artificial damage should succeed in simulating correctly are often the ones affecting biomass and population dynamics of interacting species. Variation among response variables is important in this respect. The differences
between artificial and natural damage were somewhat smaller for growth and reproduction than for the other types of response variables. There is thus hope that artificial damage has fairly similar effects to natural damage on the relative biomasses of different plant species in ecosystem studies. On the other hand, bioassays showed that herbivores often make a distinction between artificially and naturally damaged plants. The difference between natural and mechanical damage in induced responses may be important if secondary damage is extensive. A test of the adequacy of artificial damage for dominant plant species and their main herbivores would increase the reliability of the simulation method, but is very laborious in species-rich ecosystems. If artificial damage is used in ecosystem studies, it is of course important to take into account such problems as the difficulty of simulating many types of herbivore damage or other effects of herbivores than biomass removal, for instance impact on nutrient cycling (Hjältén, Chap. 12, this Vol.).

Ecosystem studies are typically interested in such system variables as measures of nutrient cycling and energy flow, productivity and biomasses of different trophic levels and functional types. We did not find controlled experiments that measure such characteristics and compare artificial and natural damage at the ecosystem level. Plant response traits nearest to the ecosystem variables are growth and reproduction responses, which are related to primary production and producer biomass. Because there was a tendency for the difference between responses to artificial and natural damage to decrease when the scope extended from cell and tissue level (chemical responses) to whole-plant level (growth and reproduction), it could be speculated that the difference between responses perhaps could be even smaller when the focus is at the level of ecosystems, while benefits of artificial damage – better control of damage level and experimental design – remain the same. Many types of feedback mechanisms, however, make it difficult to extrapolate from responses of individual plants to community and ecosystem level. On the other hand, if there are differences between responses at the ecosystem level, it opens up interesting possibilities. For instance, if natural damage induces herbivore defence and artificial damage does not, it could be possible to build up experimental designs to study whether induced secondary chemicals really play a role in the ecosystem context by decreasing herbivore pressure, affecting decomposition or by acting in some other way.

More generally, there are plenty of methods in biology that interfere with the natural function of biological systems. Interference often has an effect that can only partially be predicted and explained. For instance, plants are grown in growth chambers, greenhouses and common gardens, although it is known that biological processes work in a different way in artificial environments than in natural ones. Studies commonly discuss experimental artefacts as potential sources of error. Because plants can be exposed to both natural and artificial damage, it is possible to test whether the error is large or not.
We classified plant responses to growth, reproduction, resistance, chemical responses and physiological responses. These are emergent properties, so there is no general answer to the question of whether it is legitimate or not to use artificial damage. The review shows that it is good practice to carry out an experiment with both damage methods irrespective of the type of response variable, because when ecologists have tested the adequacy of herbivory simulations, in less than a third of the studies artificial and natural damage produced the same response.

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