Hormaphis hamamelidis and gall size: a test of the plant vigor hypothesis

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We tested the Plant Vigor Hypothesis by determining the distribution of galls formed on leaves of witch hazel, *Hamamelis virginiana*, by the aphid *Hormaphis hamamelidis*, and by determining various factors that affect the fecundity of the gall-forming fundatrices. We also studied the role of the fundatrix in host plant manipulation. While the mean number of galls per leaf was low, galls had an aggregated distribution among leaves. Among trees, the average number of galls per leaf was not related to the mean leaf size, contrary to the preference prediction of the Plant Vigor Hypothesis. While fundatrices preferred the distal leaves of buds, which grew more than the proximal leaves, being on distal leaves conferred no increase in fecundity for fundatrices, contrary to predictions of the Plant Vigor Hypothesis. Gall size was the factor that explained the largest proportion of variation in fundatrix fecundity; fundatrix size explained somewhat less of the variation. Also, gall position on the leaf, number of aphid galls on the leaf, and on which leaf of the bud the gall was located all played small, statistically significant roles in explaining fundatrix fecundity, but their effects were variable between experiments. Removal of fundatrices shortly after galls had enclosed them limited the growth of galls, indicating the role of the fundatrices in gall growth. We compare and contrast this system versus other gall-forming insects, as well as discuss the adaptive significance of the aphid manipulation of the host plant. Much of the data contradict predictions of the Plant Vigor Hypothesis, and we discuss how gall size, as a measure of plant growth caused by insect manipulation, explains the observed patterns.

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Insects that elicit plant galls clearly benefit from manipulating plant development (Meyer 1987, Moran and Whitham 1990a), in terms of protection from harsh environments (Fernandes and Price 1992, Price et al. 1998) and access to superior food (Price et al. 1987, Wool et al. 1999). The success of gall-forming insects depends on their ability to select (Weis and Abrahamson 1986, Weis et al. 1988) and manipulate (Dunn...

Selecting the correct position or tissue is a primary factor influencing galler fitness (Weis et al. 1988), but the particular criteria that maximize fitness are variable among different galler species. Leaf area, gall position and number of galls per leaf are critical to the success of the aphid *Pemphigus betae* (Whitham 1978). Gall position on the leaf, but not leaf area, strongly affects the fecundity of the fundatrices of a *Tetramora* sp. aphid (Akimoto and Yamaguchi 1994). In contrast, the number of galls per leaf, leaf area, and distance from the base of the leaf did not affect the survivorship and fecundity of *Daktulosphaira vitifolii* (Phylloxeridae) females forming galls on the leaves of *Vitis arizonica* (Kimberling and Price 1996).

In several cases, the ability to manipulate host tissue and alter plant physiological processes appears to be the most critical factor for the success of the galling insect (Larson and Whitham 1991, Inbar et al. 1995), but the relative importance and adaptive significance of tissue choice, plant control of gall formation and development, and manipulation by the insect remain unclear (Dodson 1991, Hartley 1998).

We evaluated the relative effects of insect choice and manipulation of plant tissue on the aphid *Hormaphis hamamelidis* (Fitch) (Insecta: Homoptera: Aphididae), whose fundatrices form galls on leaves of *Hamamelis virginiana* L. (Hamamelidaceae). We characterized the distribution of galls among trees and asked if it was related to the average leaf size of trees. Based on the PVH prediction of herbivore preference of larger leaves, we would expect greater gall density on trees with larger leaves. We did a field study to relate the growth of leaves to the distribution of galls, thus testing the PVH prediction of herbivore preference for leaves with greater growth. The PVH predicts that galled leaves should be larger than ungalled leaves, and that galller fecundity should be higher on larger leaves, thus we compared the size of galled and ungalled leaves. Also, two comparative studies tested the hypotheses that gall size, gall position, and leaf area affected fundatrix fecundity, and compared the fecundity and mortality of galls which were alone on a leaf (‘single galls’) and galls from leaves with two or more galls (‘multiple galls’) to test for resource limitation. If resources were limited by total leaf area or the amount of leaf from which resources could be acquired, we would predict strong, consistent leaf area and gall position effects on stem mother fitness, as well as lower fecundity and/or survival for multiple galls. The second study was done over a time course to test for any temporal heterogeneity in fundatrix development or reproduction among galls of different sizes, thus examining the stability of the gall size–fecundity relationship. Lastly, we removed fundatrices from galls to clarify their role in gall formation and growth. We found that gall location and leaf traits have little or no impact on the success of these aphids, whereas insect manipulation of host tissue played a major role in insect success.

**Materials and methods**

**Research system**

Witch hazel *Hamamelis virginiana* L. (Hamamelidaceae) is a common understory tree or shrub in eastern deciduous forests of North America, ranging from Nova Scotia to Florida (Grimm 1983). Winter buds of witch hazel are naked and typically each bud produces one pair of alternate leaves in a growing season, both leaves opening simultaneously in the spring. (The two leaves of each bud will be referred to as either ‘distal’ or ‘proximal’, and this quality will be designated as ‘leaf position’. To avoid confusion, sections of leaf lamina will be referred to as ‘basal’ or ‘apical’.) Leaves are obovate to circular in shape, thus dividing the leaf at one half the maximum length creates two sections of approximately equal area. Thus any effect associated with gall position among leaf halves is not due to an area effect. The witch hazel cone gall aphid, *Hormaphis hamamelidis* (Fitch), forms cone shaped galls on the leaves of witch hazel. *H. hamamelidis* has a monoecious life cycle of three generations, beginning with the fundatrix which hatches from a winter egg and forms a gall in the spring while leaves are still expanding (Lewis and Walton 1958, von Dohlen and Gill 1987). The fundatrix chooses a site on the abaxial side of the leaf, usually on or near the midrib and/or a secondary vein. She then initiates gall formation with repeated brief insertions of her stylet into the leaf in a circular pattern. The gall grows from this area of ‘stinging’ and encloses the fundatrix approximately two weeks after gall formation is initiated (Lewis and Walton 1958; B. Rehill pers. obs.). Eventually, a cone shaped gall 7–14 mm tall is formed on the adaxial side of the leaf, with a small opening, the ostiole, in the bottom of the gall on the abaxial side of the leaf. The fundatrix reproduces parthenogenetically within the gall, bearing the second-generation sexuparae, which mature into alates within the gall; all the aphids feed from phloem in the interior walls of the gall. The second-generation alates leave the gall and bear the third, sexual generation (‘sexuales’) by parthenogenesis. After maturation, the sexuales mate and the female deposits five to ten winter eggs on the bark of the host. The number of second-generation aphids in
the gall provides a measure of the fecundity of each fundatrix.

Study site

Witch hazel trees from a single site in Rothrock State Forest in central Pennsylvania, USA (centered approximately at 40°45.22' N latitude and 77°44.10' W longitude, elevation = 530 m) were used for all the experiments. The site was a mixed deciduous forest dominated by *Quercus rubra* L., *Quercus cocinea* L., *Quercus alba* L., *Acer rubrum* L., and *Acer pennsylvanicum* L. There are large stands of witch hazel in the understory throughout the site, and the entire canopy of most witch hazel trees can be accessed with a 2-m ladder. We sampled all individual witch hazel plants from which any leaves were removed only once, and took no distribution data from any plant from which leaves had been removed.

Methods common to all experiments

Transects: We chose transects in the various experiments to provide a representative sample of trees found in the site.

Leaf sampling: We sampled leaves haphazardly by moving around the tree and removing leaves from the entire canopy.

Leaf measurements: We used a ruler to measure maximum leaf length and width to the nearest mm.

Gall measurements: We measured galls to the nearest 0.05 mm with vernier calipers. Gall diameter was measured as the distance from the bottom of the gall on the abaxial side of the leaf to the tip of the gall on the adaxial side of the leaf. Gall height was measured as the diameter at the base of the gall, approximately perpendicular to the midrib, on the adaxial side of the leaf. Since galls are approximately conical, gall volume was calculated as: 

\[
\text{volume} = \frac{1}{3}\pi \times (\text{gall radius})^2 \times \text{gall height}
\]

We measured gall position on the leaf with a ruler. Gall lateral position was the length of a line perpendicular to the midrib, running from the midrib to the center of the gall, and gall longitudinal position was the distance between the junction of this line and the midrib to the base of the leaf. Relative lateral position was computed as gall lateral position/0.5 × maximum width, and relative longitudinal position was computed as gall longitudinal position/maximum length. The host leaf was also scored for its position in the winter bud, either proximal or distal.

Leaf area: We used a Li-Cor 3100 leaf area meter (Li-Cor, Inc., Lincoln, NE) to measure the leaf area of a sample of ten leaves each from 20 trees within the study site. These data were used to derive the equation:

\[
\text{leaf area} = 0.0068815 \times \text{maximum leaf length} \times \text{maximum leaf width}
\]

Leaf relative size and growth rate

We observed the growth of leaves during the 1999 field season to determine if leaf growth rate influences gall distribution and fundatrix fecundity. On 1 April 1999, before bud break, we removed ten buds from each of three trees. The buds were collected in 70% ethanol, dissected in the laboratory, and the midrib lengths of the two leaves in each bud were measured using an optical micrometer. (Prior experiments indicated that leaf shape was relatively constant within a tree, therefore midrib length constituted a reasonable estimate of leaf size.)

We measured the midrib lengths of ten bud pairs (20 leaves) from each of ten trees, including the three trees from which unexpanded buds were measured, on 7 May (ca 1 week after buds began to open), 18 May (leaves open but still expanding), and 5 June (leaves fully expanded). On the second two dates, once leaf laminae had opened, we also measured leaf widths.
Gall position, gall size, and aphid fecundity 1998

We examined the relative effects of gall size, leaf area, gall position, and gall number per leaf on fundatrix performance. We took eight leaves with no gall, eight leaves with a single gall, and eight to twelve leaves with two or more galls from each of five trees on 7 August 1998. Leaves were flash frozen in liquid nitrogen and kept on dry ice until storage at −80°C. Gall position, gall size, and leaf size were measured, then galls were dissected to census the aphid inhabitants and score for survivorship (no mortality due to fungal infection or predation). We scored leaves as ungalled, singly galled, and multiply galled, and gall type was scored as single (residing alone on a leaf) or multiple (residing with other galls on the same leaf). We measured fundatrix relative fitness as fecundity, the total number of second-generation aphids contained in the gall. We employed an ANCOVA model to analyze the potential effects of gall volume, gall position, and leaf area on fundatrix fecundity. We used the continuous variables gall volume, relative lateral and longitudinal positions, number of galls per leaf, and leaf area as covariates, while the leaf position (distal or proximal leaf of a bud pair) was the categorical variable. We made the a priori decision to include the tree of origin (’tree’) in the analysis as a fixed blocking factor to reduce error variance. We included only galls that had no evidence of predation or abortion (’inhabited galls’: n = 61) in the analysis of fecundity.

Gall position, gall size, and aphid fecundity 1999

We performed an experiment similar to that described above during the 1999 field season to determine the stability of the relationships seen in the 1998 experiment. We also considered fundatrix phenotype (size, and therefore resources) by measuring fundatrix length. We collected galls each week from 10 June through 9 September. Each weekly collection consisted of six leaves with one gall and two leaves with multiple galls from each of six trees. This protocol differed from the previous year’s experiment to better reflect the distribution of galls on leaves seen in both years (ca 80% of galled leaves with a single gall). The trees were randomly assigned to the experiment and none had been used for any prior experiments, and we treated leaves and galls as described for the prior experiment. In addition, we measured fundatrix length with an optical micrometer as an estimate of size. (Fundatrix shape did not vary greatly, thus length was judged a reasonable measure of body size.) For analysis, we placed galls in three size classes with approximately the same number of galls in each class within weeks. Depending on mortality, there were 12–21 galls per size class per week (15.73 ± 0.38 galls/size class/week, mean ± SEM). We included only galls with fundatrices present and no obvious signs of mortality or abortion (n = 76) in the analyses of factors affecting fecundity. Over the entire course of the experiment, we collected a total of 819 galls, with 670 included in various analyses.

Galler removal

We removed fundatrices from developing galls to determine the extent of their influence on gall formation and gall size. In the first fundatrix removal experiment, the fundatrix was removed from one treatment gall on each of five trees along a transect, and there was one untreated, control gall per tree. On 30 April 1998, after galls were initiated but still open, we removed aphids of the treatment galls by suction with a hand-held desoldering plunger (Weller Electronics, Cheraw, SC). Controls were done by touching the tip of the plunger to the gall without applying suction. We scored gall development visually ten days later by noting whether the gall had formed a cavity or was still open.

In the second removal experiment, there were three treatments: fundatrix removal by insecticide injection, sham injection, and unmanipulated galls. We included all three treatments applied on a single branch, and there were 4–15 branches with the treatments on eight trees for a total of 216 galls. Trees were along a ca 200-m transect in the central portion of the site. The treatments were applied on 20 May 1998, after galls had enclosed fundatrices. We injected galls using a 1-ml tuberculin syringe with a blunt tip. The insecticide treatment consisted of filling the gall with a 0.02% pyrethrin/0.20% piperonyl butoxide insecticide solution (Schultz Co., St. Louis, MO). Preliminary experiments indicated that the pyrethrin injection was 100% effective (12 aphids in 12 galls killed two days after injection). We chose pyrethrin for its low phytotoxicity (Wilson et al. 1984), and there were no signs of gall or leaf necrosis due to the injection. We did the sham injections by inserting the tip of an empty syringe into the gall without injection of any material. We measured gall position, gall size, and leaf size on 19 May 1998, and again on 24 June, 25 July, and 25 August 1998. We used gall height as the measure of the continuation of growth because gall diameter did not change among dates (data not shown).

Results

Gall distribution

We found identical patterns in 1998 and 1999. Proportions of galled leaves differed significantly among trees (Fig. 1), ranging in 1998 from 2.3% to 17.5% of leaves with galls, with a mean of 9.8%. Galls were aggregated
among the leaves of the census trees (Table 1); more leaves had multiple galls than expected. More galls were located on distal leaves than proximal leaves (ratios of galls on distal vs proximal leaves; 1998 = 1.94:1; 1999 = 1.73:1; both significantly different from 1:1, P < 0.0001). Trees with larger leaves did not have a higher density of galls; there was no association between a tree’s average leaf size and the mean number of galls per leaf (Pearson product moment; 1998: \(r = -0.32, P = 0.37, n = 10\); 1999: \(r = 0.13, P = 0.72, n = 10\)).

### Table 1. Observed and Poisson expected frequencies of galls per leaf in the 1998 distribution experiment.

<table>
<thead>
<tr>
<th>Number of galls per leaf</th>
<th>Observed frequency</th>
<th>Expected Poisson frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16 669</td>
<td>16 418.84</td>
</tr>
<tr>
<td>1</td>
<td>1392</td>
<td>1806.07</td>
</tr>
<tr>
<td>2</td>
<td>204</td>
<td>99.33</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>3.64</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>0.10</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>0.002</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Leaf relative size and growth rate

Distal leaves were always smaller than proximal leaves in winter buds. In all 30 buds measured, the distal leaf was shorter (mean \(\pm\) SEM = 3.73 \(\pm\) 0.15 mm) than the proximal leaf (mean \(\pm\) SEM = 5.73 \(\pm\) 0.19 mm), and the difference was statistically significant (paired \(t\)-test; \(t = 16.0, df = 29, P < 0.0001\)).

The differences in mean midrib lengths were maintained initially after buds opened, but distal and proximal leaves reached the same size by the time they were fully grown (Table 2). The patterns for leaf areas were the same (data not shown). Thus distal leaves always start smaller than the proximal leaf of the same bud, but on average eventually reach the same size, implying greater growth (and possibly greater growth rate) for distal leaves during leaf expansion.

### Gall position, gall size and aphid fecundity: 1998

Inconsistent with the Plant Vigor Hypothesis, galled leaves were significantly smaller than ungalled leaves (prespecified contrast: \(F = 5.72, df = 1,119, P = 0.018\)), with multiply galled leaves smaller than single-galled leaves (Fig. 2). In a two-way factorial ANOVA, there were significant effects of tree (\(F = 4.03, df = 4,119, P = 0.004\)) and the number of galls on the leaf, i.e. zero, one, or multiple galls/leaf (\(F = 3.58, df = 2,119, P = 0.031\)), but the interaction was not significant (\(F = 1.59, df = 8, P = 0.136\)).

Also inconsistent with the PVH, leaf size was not associated with fecundity (Table 3). Gall volume was the most important factor correlated with fundatrix fecundity, while gall position and the number of galls per leaf played lesser roles. In the ANCOVA model, gall volume explained 14% of the variation in fecundity, but in a linear regression, gall volume alone could explain 25% of the variation in fecundity (Fig. 3). Leaf position (location of the gall on a proximal or distal leaf) was not associated with fecundity, in spite of greater numbers of galls on and the greater growth of distal leaves.

The number of galls per leaf and the gall’s longitudinal position on the leaf each made statistically significant but small contributions to fundatrix fecundity, accounting for 5% and 4% of the variation in fecundity, respectively. Distance from the midrib made no significant contribution. In the ANCOVA model, the slope for number of galls per leaf was positive, indicating fundatrices had higher fecundity on leaves with multiple galls (3.52 more offspring per gall for each additional gall on a leaf). The positive coefficient for relative longitudinal distance indicates a significant trend for fundatrices in galls closer to the apices of leaves to have greater fecundity (19.7 more offspring for a gall located at the apex of the leaf vs the base).
Gall volume was independent of position or leaf area. There were no significant effects of any continuous or categorical variable on gall volume and the ANCOVA model did not explain a significant portion of the variation of gall volume (data not shown). The mean ± SEM gall volume was 21.9 ± 1.4 mm³. Among inhabited galls, there was no significant difference in mean volume between galls in the basal and apical halves of the leaf (Student’s t-test; t = 1.52, df = 59, P = 0.134).

As in the distribution observations, more galls were located on distal leaves; there were twice as many galls on distal leaves (108 total) than on proximal leaves (59 total). Proportions of single and multiple galls found on the two leaves of buds were the same (G = 2.36, df = 1, P = 0.124) and galler survivorship was independent of the leaf of the bud on which the gall was located (G = 2.38, df = 1, P = 0.123). Despite the fecundity advantage for galls located toward the leaf apex, approximately three times as many galls were in the basal half of the leaf as in the apical half (128 basal; 43 apical). The distribution of single and multiple galls did not differ between the two halves of leaves (basal vs apical half, G = 0.34, df = 1, P = 0.560) and there was no difference in survivorship between the galls located in the apical and basal halves of leaves (G = 2.88, df = 1, P = 0.090). Thus differences in distribution among and on leaves were not explained by gall position effects on survival. However, fundatrices on singly galled leaves (50% were inhabited, 21 of 42 total) had higher survivorship than on multiply galled leaves (31% were inhabited, 40 of 129 total; G = 4.85, df = 1, P = 0.028).

An ANCOVA analysis reinforced the importance of gall size and unimportance of leaf size, as well as indicating several minor differences from the previous experiment. This analysis included only the samples collected during the two weekly collections with the greatest mean number of offspring per gall, 22 and 29 July 1999 (Table 4). Leaf area and gall position did not make statistically significant contributions to fecundity. As in the 1998 survey, gall volume was an important correlate of fundatrix fecundity. In the ANCOVA model, gall volume explains ca 20% of the variation in fecundity, but in a simple regression it explains 24% of the variation (Fig. 3). Unlike the 1998 study, the number of galls per leaf had a significant negative effect on fundatrix fecundity, but accounted for only 2.6% of the variation in fecundity. In the greatest contrast with the previous experiment, the leaf of bud (distal or proximal) on which the gall was located explained a significant (8.9%) proportion of fundatrix fecundity. However, fundatrices in galls on the proximal leaves of buds had a higher mean fitness than those on the distal leaves (LS means: 54.1 offspring/gall for proximal leaves, n = 37; 40.3 offspring/gall for distal leaves, n = 39; P = 0.0008), despite the established preference for distal leaves among fundatrices.

The tree on which the galls were located also influenced fundatrix fecundity, indicating possible plant genotypic and/or environmental effects on insect fecun-

### Table 2. Comparisons of the midrib lengths of distal versus proximal leaves of buds in the leaf growth experiment. Results of paired t-tests are for comparisons within dates.

<table>
<thead>
<tr>
<th>Date</th>
<th>Distal leaf Mean ± SEM (mm)</th>
<th>Proximal leaf Mean ± SEM (mm)</th>
<th>df</th>
<th>t_s</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 May</td>
<td>16.2 ± 0.4</td>
<td>20.5 ± 0.6</td>
<td>99</td>
<td>11.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>18 May</td>
<td>57.3 ± 1.5</td>
<td>65.2 ± 1.5</td>
<td>99</td>
<td>10.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>5 June</td>
<td>98.9 ± 2.2</td>
<td>98.8 ± 2.1</td>
<td>99</td>
<td>0.06</td>
<td>0.953</td>
</tr>
</tbody>
</table>

Fig. 2. Mean leaf areas for all leaves in the 1998 aphid fecundity experiment. Consistent with a dose effect, ungalled leaves (n = 39) were larger than leaves with one gall (n = 42) that were larger than leaves with multiple galls (n = 53). Bars with different letters are significantly different (Tukey’s HSD, α = 0.05).
Table 3. ANCOVA analysis of factors affecting stem mother fecundity for the 1998 experiment, including only inhabited galls. The coefficient of determination for the model is 0.434. Relative longitudinal position ranges from 0 (base of the leaf) to 1 (apex of the leaf).

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>P</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>5695.3</td>
<td>11</td>
<td>517.8</td>
<td>3.41</td>
<td>0.014</td>
<td>–</td>
</tr>
<tr>
<td>Tree</td>
<td>583.0</td>
<td>4</td>
<td>147.8</td>
<td>0.86</td>
<td>0.437</td>
<td>–</td>
</tr>
<tr>
<td>Leaf of bud</td>
<td>116.0</td>
<td>2</td>
<td>58.0</td>
<td>0.38</td>
<td>0.664</td>
<td>–</td>
</tr>
<tr>
<td>Gall volume (mm³)</td>
<td>1797.0</td>
<td>1</td>
<td>1797.0</td>
<td>11.85</td>
<td>0.001</td>
<td>0.572</td>
</tr>
<tr>
<td>Relative longitudinal position</td>
<td>678.1</td>
<td>1</td>
<td>678.1</td>
<td>4.47</td>
<td>0.040</td>
<td>19.68</td>
</tr>
<tr>
<td>Relative lateral position</td>
<td>1.2</td>
<td>1</td>
<td>1.2</td>
<td>0.01</td>
<td>0.930</td>
<td>–</td>
</tr>
<tr>
<td>Number of galls on leaf</td>
<td>671.3</td>
<td>1</td>
<td>671.3</td>
<td>4.43</td>
<td>0.041</td>
<td>3.52</td>
</tr>
<tr>
<td>Leaf area (cm²)</td>
<td>489.7</td>
<td>1</td>
<td>489.7</td>
<td>3.23</td>
<td>0.079</td>
<td>–0.29</td>
</tr>
<tr>
<td>Error</td>
<td>7432.8</td>
<td>49</td>
<td>151.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fundatrix length, as an indication of body size and resources, was also an important correlate of fecundity. Body length explained about 13% of the variation in fecundity in the ANCOVA model, and when regressed alone against fecundity, it accounts for 16% of the variation. Body size and gall size appear to influence fecundity independently. While both were significant in the ANCOVA model, there was no significant correlation between them. Gall volume, gall position, leaf area, number of the galls on the leaf, and the tree on which the gall was located did not explain any of the variation in fundatrix length (ANCOVA; P > 0.15).

Similarly, gall volume was not related to gall position on the leaf, leaf area, the number of galls on the leaf, fundatrix length, and the tree on which the gall was located. The mean ± SEM gall volume was 43.3 ± 1.70 mm³, nearly twice the mean of the 1998 survey.

Overall, survivorship (76.1%) was higher than in the 1998 study (35.7%). As in 1998, fundatrices on singly galled leaves (58 of 71, or 81.7% were inhabited) had higher survivorship than those on multiply galled leaves (28 of 42, or 66.7% were inhabited); this difference was marginally significant (G = 3.20, df = 1, P = 0.074). In contrast to the 1998 study, survivorship differed for galls on the two leaves of buds (G = 4.58, df = 1, P = 0.033). Galls on proximal leaves (42 of 49 total, 86%) had higher survivorship than galls on distal leaves (44 of 65 total, 69%).

Galler removal

When fundatrices were removed early (28 April 1998), none of the galls they had initiated formed a cavity, whereas all the control galls enclosed the fundatrix (G = 10.1 using Yates’ correction, df = 1, P = 0.002).

Removing the aphid later by insecticide injection limited gall height. The sham and unmanipulated galls had reached 90% of their final heights on 24 June, when the first offspring appeared in galls as indicated by a weekly sample of galls from the field site (data not shown). By the third sample date, 25 July, the insecticide treated galls were significantly shorter than the sham and unmanipulated galls, a relationship which remained at the last sample date, 25 August (Fig. 5), indicating that the presence of the aphid was necessary for the gall to attain its full size.

These data are consistent with observations from the position and aphid fecundity data from 1998. Inhabited galls were significantly larger than uninhabited galls (one-way ANOVA: F = 19.2, df = 1,70, P < 0.0001).

Discussion

Our tests of the Plant Vigor Hypothesis provided mixed results. Consistent with the preference prediction of the PVH, distal leaves, which undergo greater expansion (and presumably grow more quickly), are consistently...
Fig. 4. Time course of the reproductive output of stem moth- 
ers in three size classes of galls in the 1999 experiment. Galls were divided into the three size classes by week, producing groups with approximately equal numbers of galls within each week. Galls in the largest size class had significantly greater numbers of second-generation aphids than the other two size classes for weeks 7, 8, 9, and 10 of the study, 22 July through 12 August 1999 (two-way ANOVA of tree by size class within each week, using the Tukey-Kramer procedure with \( \alpha = 0.05 \) for pairwise comparisons). Fundatrices in galls of the two smaller size classes never had a significantly greater number of offspring per gall than the fundatrices occupying the largest galls.

preference for gall formation over proximal leaves. However, survival and fecundity of fundatrices on distal and proximal leaves were variable. Contrary to the performance prediction of the Plant Vigor Hypothesis, fundatrices had higher survivorship and fecundity on proximal, not distal leaves. Leaf size did not affect fecundity. Galled leaves were the same size or smaller than ungalled leaves, and leaf area was unrelated to gall density. Altogether, our results provide little support for the PVH, at least in terms of insect performance relative to leaf growth and size.

For Hormaphis fundatrices, stimulating host tissue to produce the largest possible gall is essential. In both years, with two separate sets of host plant individuals, reproductive fitness was related primarily to gall volume, with little or no impact of position on leaf or the choice of leaf within a bud. The removal experiments indicated that the continuous presence of the aphid is required for the gall to complete development and reach full size, a phenomenon observed before in this (Lewis and Walton 1958; R. Gendron, Indiana Univ. of Pennsylvania pers. comm.) and other galling systems (Hovanitz 1959, Dunn 1960, Rohfritsch and Short- house 1982). Gall volume was 90% of maximum by the time offspring appeared, indicating that the second generation mainly benefits from, but does not increase, gall volume. Variation in gall size was not a function of galler phenology; aphid behavior and reproduction were synchronous among galls of various sizes. Along with the observations that larger females tended to produce more offspring but gall size was not related to female size, our evidence suggests that variation in final gall volume, and thus a significant fraction (25% or more) of variation in aphid reproduction, can be attributed to variation in the females’ ability to stimulate gall development. It seems likely that gall size is determined by the interaction of the female’s ability to stimulate gall growth and the responsiveness or inducibility of host tissue, i.e. the amount of host response to a given insect stimulus (Weis et al. 1988).

Table 4. ANCOVA analysis of factors affecting stem mother fecundity, including only inhabited galls, for the 1999 experiment. The coefficient of determination for the model is 0.561.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>P</th>
<th>Slope</th>
<th>Source</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>P</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>15 183.7</td>
<td>11</td>
<td>1380.3</td>
<td>7.43</td>
<td>&lt;0.0001</td>
<td>–</td>
<td>Model</td>
<td>15 183.7</td>
<td>11</td>
<td>1380.3</td>
<td>7.43</td>
<td>&lt;0.0001</td>
<td>–</td>
</tr>
<tr>
<td>Tree</td>
<td>4927.3</td>
<td>5</td>
<td>985.5</td>
<td>5.31</td>
<td>0.0004</td>
<td>–</td>
<td>Tree</td>
<td>4927.3</td>
<td>5</td>
<td>985.5</td>
<td>5.31</td>
<td>0.0004</td>
<td>–</td>
</tr>
<tr>
<td>Leaf of bud</td>
<td>2298.8</td>
<td>1</td>
<td>2298.8</td>
<td>12.38</td>
<td>0.0008</td>
<td>–</td>
<td>Leaf of bud</td>
<td>2298.8</td>
<td>1</td>
<td>2298.8</td>
<td>12.38</td>
<td>0.0008</td>
<td>–</td>
</tr>
<tr>
<td>Gall volume (mm³)</td>
<td>5407.8</td>
<td>1</td>
<td>5407.8</td>
<td>29.12</td>
<td>&lt;0.0001</td>
<td>0.634</td>
<td>Gall volume (mm³)</td>
<td>5407.8</td>
<td>1</td>
<td>5407.8</td>
<td>29.12</td>
<td>&lt;0.0001</td>
<td>0.634</td>
</tr>
<tr>
<td>Stem mother length (mm)</td>
<td>3395.4</td>
<td>1</td>
<td>3395.4</td>
<td>8.29</td>
<td>&lt;0.0001</td>
<td>46.4</td>
<td>Stem mother length (mm)</td>
<td>3395.4</td>
<td>1</td>
<td>3395.4</td>
<td>8.29</td>
<td>&lt;0.0001</td>
<td>46.4</td>
</tr>
<tr>
<td>Relative longitudinal position</td>
<td>386.8</td>
<td>1</td>
<td>386.8</td>
<td>2.08</td>
<td>0.154</td>
<td>15.99</td>
<td>Relative longitudinal position</td>
<td>386.8</td>
<td>1</td>
<td>386.8</td>
<td>2.08</td>
<td>0.154</td>
<td>15.99</td>
</tr>
<tr>
<td>Number of galls on leaf</td>
<td>809.7</td>
<td>1</td>
<td>809.7</td>
<td>4.36</td>
<td>0.041</td>
<td>-4.85</td>
<td>Number of galls on leaf</td>
<td>809.7</td>
<td>1</td>
<td>809.7</td>
<td>4.36</td>
<td>0.041</td>
<td>-4.85</td>
</tr>
<tr>
<td>Leaf area (cm²)</td>
<td>444.6</td>
<td>1</td>
<td>444.6</td>
<td>2.39</td>
<td>0.127</td>
<td>0.09</td>
<td>Leaf area (cm²)</td>
<td>444.6</td>
<td>1</td>
<td>444.6</td>
<td>2.39</td>
<td>0.127</td>
<td>0.09</td>
</tr>
<tr>
<td>Error</td>
<td>11 884.1</td>
<td>64</td>
<td>185.7</td>
<td></td>
<td></td>
<td></td>
<td>Error</td>
<td>11 884.1</td>
<td>64</td>
<td>185.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 5. Mean gall heights for all dates and treatments in the second removal experiment. Bars with different letters are significantly different (Tukey’s HSD, \( \alpha = 0.05 \)). (□ = Insecticide; □ = Sham; □ = Unmanipulated).
Gall size has been shown to play a strong role in galler performance in other systems. Of nine measured variables, gall size was the strongest predictor of fecundity in *Pachypsylla* spp. gallers on hackberry trees (Heard and Buchanan 1998). For *Adelges abietis*, a spruce galling adelgid, the number and average size of emerging adults is positively related to gall volume (McKinnon et al. 1999). The diameter of galls formed by the gall midge *Asphondylia borrichiae* was significantly correlated with midge size, and larger midges had a higher potential fecundity (Rossi and Stiling 1998). Gall size can also affect gall insect survivorship. Weis et al. (1985) found that larger galls of *Eurosta solidaginis* had lower levels of parasitism, an effect also found in tenthredinid (Price and Clancy 1986) and cynipid gall systems (Plantard and Hochberg 1998).

Our results contrast strongly with those of Whitham (1978) who found that fecundity of *Pemphigus betae* fundatrices depended strongly on gall position on the leaf, the area of the galled leaf, and the number of galls on the leaf. The greatest *P. betae* reproductive output was achieved in galls located at the base of the largest possible leaf, with no other galls on that leaf. Whitham (1980) also determined that *P. betae* fundatrices were resource-limited by leaf area and gall position, leading to competition among fundatrices. The pattern for *Hormaphis hamamelidis* is quite different; fecundity is not influenced at all by leaf area, whereas gall position and co-occurrence on the leaf have at most minor effects. Further, for *Hormaphis* fundatrices the effects of gall position and co-occurrence of galls appear minor and quite variable in contrast to the more important and robust relationship between gall volume and fecundity.

In our studies *H. hamamelidis* aphids tended to aggregate despite increased mortality for fundatrices on multiply galled leaves, and in one study experienced slightly increased fecundity when aggregated. Selection of the tissue and/or sites for formation of the largest possible galls would explain such a pattern. Field observations indicate that fundatrices probe unopened buds with their stylets, and may move to other buds or elsewhere on the same bud, sometimes repeating the process several times before settling at the point of eventual gall formation. Fundatrices more frequently chose the distal leaf, which is initially smaller but expands more than the proximal leaf. Females may select leaves (and gall locations) that are more inducible, i.e. leaves that will produce larger galls, based on correlated leaf traits that they can detect before gall initiation (Moran and Whitham 1990b). While the criterion may be leaf expansion or growth rate, not eventual size, our analyses did not detect any differences in gall volume between galls on proximal and distal leaves. Alternatively, preference for different leaves of buds and different halves of leaves, in spite of little or no fecundity or survival advantage in the gall, may be based on survival before gall formation, when fundatrices are exposed to weather and predators.

In general, galler success is likely to be related to the insect’s ability to create a strong sink (Larson and Whitham 1991, Inbar et al. 1995, Larson and Whitham 1997) and sink strength has been linked directly to gall size and fecundity in other aphids (e.g. Burstein et al. 1994). Three observations from this study suggest that fundatrices can establish a sink independent of the host leaf lamina, and may even compete with it. First, being located at the base of the leaf provides no advantage, suggesting that these aphids do not merely intercept resources translocated into and out of the host leaf, but also mobilize resources from locations beyond the leaf. Second, galler fecundity was greater on multiply galled leaves in one experiment. This suggests that two galls can create a stronger sink than one, to the mutual benefit of coexisting aphids, although this cannot be distinguished from the alternative that leaves acting as stronger sinks attract more aphids. Heard and Buchanan (1998) found evidence for intraspecific facilitation in a psyllid–hackberry gall system, and Omer et al. (1995) found that grape phylloxera benefit from aggregation. Third, in the 1998 experiment, galled leaves were generally smaller than ungalled leaves, yet average leaf size was not related to gall density. This pattern suggests that under some circumstances galls can decrease leaf size, and that the galls may thus compete with leaves for incoming materials. Fritz and colleagues (2000) have commented that *Phyllocopta leucitii* galls appear to reduce the size of *Salix discolor* leaves.

The failure of the Plant Vigor Hypothesis to explain our results can be understood by considering the primary beneficial response induced by the gall-forming insect: gall growth. It is this very phenomenon, which defines the guild, that is key to gall insect success. Indeed, there are very few facultative gall formers (Meyer 1987); failure to form a gall means sure death for most gall-forming insects. Plant-galling insects are intimately involved with the processes of plant growth (Price 1991). However for some gall formers, such as *Hormaphis hamamelidis* fundatrices, preference and performance may be more connected to tissue that can be manipulated the most, for example to form the largest galls, than to the growth rate or final size of the galled plant module. Indeed, the consistent finding that fundatrices in the largest and presumably fastest-growing *Hormaphis* galls have the highest fecundities supports such an idea, and is not unique.

Taken together the results of this study suggest several conclusions. The impact of gall location on the leaf is at most relatively minor, accounting for no more than 5% of the variation in fecundity. The presence of (and presumed manipulation of the host by) *Hormaphis* fundatrices is necessary to continue gall formation and obtain maximum gall size, and increased gall size results in increased aphid fecundity. Therefore the aphid appears to manipulate the host to increase its fecundity,
within the limits of the responsiveness or inducibility of the chosen host tissue. It appears that at the scale of the leaf the fundatrix has a major influence on her fecun-
dity through manipulation of host plant tissue. By considering the effects of the primary induced responses of
gall formers, gall size and growth, results not explained by the Plant Vigor Hypothesis can be easily understood. For some gall formers, inducibility, the extent to which host plant tissue can be manipulated beneficially, in concert with the insect’s ability to manipulate the host plant, may be equally or more important than plant module size or growth rate.

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