Gall wasp biocontrol of invasive *Acacia longifolia*: implications of strong bottom-up effects

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Abstract. The population dynamics of insect herbivore biocontrol agents is central to the successful control of invasive weeds. Although the importance of agent population dynamics is recognized, it is rarely considered in assessments of the biocontrol potential of herbivore agents. Herbivore insect population dynamics are influenced by a combination of top-down effects from natural enemies, bottom-up effects from plant resource availability (resource quality and quantity), and potential interactions between these effects. To better understand the tri-trophic interactions that are likely to determine biocontrol success in a host plant–gall wasp–parasitoid system, the relative importance of top-down and bottom-up effects for the survival of a herbivore biocontrol agent (*Trichilogaster acaciaelongifoliae*), on two *Acacia* host plants in their native range, was estimated using path analysis. On both host plants, there was a strong positive relationship between gall mass per chamber and gall wasp survival and a strong negative relationship between gall mass per chamber and gall parasitism, with parasitoids being less common in large than small galls. There was, however, no significant correlation between parasitism and gall wasp survival and, therefore, no evidence for top-down effects in this system. Strong bottom-up effects of host plant resources on both gall wasp survival and gall parasitism have implications for the spatio-temporal variability of biocontrol success. Such variation should be considered in pre-release assessments and post-release monitoring of gall wasps used as herbivore biocontrol agents.

Key words: biological control; herbivore performance; parasitoid wasps; plant–herbivore interactions.

INTRODUCTION

Knowledge of insect herbivore population dynamics is central to the success of weed management programs using herbivore biocontrol agents (Gassmann 1996, Sheppard 2003, Zalucki and van Klinken 2006). Although the general theory of insect herbivore ecology, especially population dynamics, is well developed, spatio-temporal variation in the strength of insect herbivore–plant interactions is rarely considered when evaluating herbivore agent biocontrol potential (Gassmann 1996, Lakatos et al. 2017). Herbivore insect demography is influenced by a combination of top-down effects from natural enemies (such as parasitoid wasps), and bottom-up effects from plant resource availability (resource quality and quantity; Hunter and Price 1992). Host plant resources (e.g., plant tissue nutrient and water status) and intra-specific competition affect herbivore performance between and within plant individuals (Gassmann 1996, Fritz et al. 2000, Briese 2004, Zalucki and van Klinken 2006, Cornelissen et al. 2008). Introduced herbivore insects may also be exposed to top-down effects because novel generalist natural enemies adopt them as prey (Denoth and Myers 2005, Veldtman et al. 2011). Studies have shown that the relative importance of top-down and bottom-up effects for herbivore performance varies...

In some herbivores, top-down and bottom-up effects may also interact and the influence of host plant resources (such as plant quantity, quality, and morphology) on the relative strength of top-down impacts from natural enemies of the herbivores is well studied (Price et al. 1980, Forkner and Hunter 2000, Denno et al. 2002). In insect gall makers, between- and within-plant variation in gall size positively influences gall maker performance through increased resource quality and quantity, buffering against water loss and protection against parasitoid attack (Price and Clancy 1986, Price 1991, Sumoford et al. 2000, Ito and Hijii 2004, Albarracin and Stiling 2006, László and Tóthmérész 2013). Gall development (growth rate and final size) has been shown to be limited by the resource status of host plant tissue with galls on high-quality host plants, and host plant modules, growing faster and to a larger final size than galls on low-quality host tissue in accordance with the plant vigor hypothesis (Price and Clancy 1986, Price 1991, Albarracin and Stiling 2006). The thick layer of gall tissue is a physical barrier to parasitoids attempting to lay their eggs on developing wasp larvae inside the gall chambers (Price and Clancy 1986). Thus, large galls on high-quality plant tissue are less often parasitized than small galls (Price and Clancy 1986, Albarracin and Stiling 2006). The consequences of plant-mediated top-down effects on herbivore performance remain poorly understood, with only a few examples from biological control of crop pests (Agrawal 2000, Cortesero et al. 2000, Hosseini et al. 2010).

Bottom-up impacts of plant resources on parasitoids in the third trophic level may often have been overlooked in weed biocontrol studies because herbivore agents are traditionally assumed to be introduced to an enemy-free space (Lawton 1985), and thus free from the negative impact of a third trophic level. When generalist enemies are present (e.g., Veldtman et al. 2011, López-Núñez et al. 2017), however, bottom-up impacts of host plant resources on the strength of top-down effects could be essential to herbivore agent survival and the eventual success of biocontrol programs. One such example is the bud-galling wasp *Trichilogaster acaciaelongifoliae* (Froggatt) (Hymenoptera: Pteromalidae; Fig. 1A) that has been used for biocontrol of the invasive Australian *Acacia longifolia* (Andrews) Willd. in

Fig. 1. (A) Reared gall wasp (*Trichilogaster acaciaelongifoliae*) female. (B) Parasitoid (large) and hyper-parasitoid (small) larvae in the gall chamber of a consumed female gall wasp larvae. On the right side, the radial line shows an oviposition trace of a parasitoid extending from the gall surface to the gall chamber.
South Africa (Impson et al. 2011) and Portugal (Marchante et al. 2017) and has been proposed for biocontrol of *A. longifolia* in other parts of the world including New Zealand (Hill 2005) and areas of Australia where *A. longifolia* has spread beyond its native range (Adair 2008). A rich novel enemy assemblage has been shown to be related to the gall wasp in the introduced range in South Africa (Veldtman et al. 2011), potentially exposing the biocontrol agent to strong top-down effects.

By examining the relationship between gall properties, parasitism, and gall wasp survival across multiple sites in the native range, spatial variation in the system can be used to better understand the mechanisms that may be responsible for the success of biocontrol agents in their introduced range. Here, we, therefore, estimated the relative importance of top-down and bottom-up effects within trees for the survival of this herbivore gall wasp biocontrol agent (*T. acaciaelongifoliae*) on two *Acacia* (Fabaceae) host plants (*A. longifolia* subsp. *longifolia* and *A. floribunda* (Vent.) Willd.) across the edge of its native range. We also evaluated the possibility of a direct effect of gall mass per chamber on gall parasitism. We did this to better understand the tri-trophic interactions that are likely to influence the biocontrol success of this gall wasp beyond its native range in Australia (Adair 2008) and elsewhere in the world (Hill 2005, Impson et al. 2011, Marchante et al. 2017).

Based on patterns of top-down and bottom-up effects observed in other gall makers, we predicted that female gall wasp survival at the gall level is positively related to gall mass per chamber (used as a measure of resource availability in galls; Abrahamson and Weis 1997, Stone et al. 2002, Price 2003), negatively related to the presence of parasitoids (Stiling and Moon 2005, Albaracin and Stiling 2006) and negatively density dependent due to intra-specific competition between larvae within the galls (i.e., the individual likelihood of survival decreases with the number of individuals per gall; Fritz et al. 2000). Gall mass per chamber is an informative proxy for resource availability in individual galls in the form of plant tissue quality and quantity, with multiple demonstrated benefits including individuals in large compared to small galls being less often parasitized (Price and Clancy 1986, Albaracin and Stiling 2006), less likely to desiccate (Sumerford et al. 2000), and more likely to have high fecundity (Ito and Hijii 2004). Furthermore, we predicted an interaction between top-down and bottom-up effects with gall mass per chamber being negatively related to the presence of parasitoids in the galls (Price and Clancy 1986, Albaracin and Stiling 2006).

**METHODS**

*The biology and ecology of Trichilogaster acaciaelongifoliae*

*Trichilogaster acaciaelongifoliae* lays its eggs in flower buds and vegetative buds of its host plants and a gall forms, inhibiting flower and phyllode development (Noble 1940, Dennill et al. 1993). When most successful, it limits up to 100% of seed production, inhibits vegetative growth, and occasionally causes tree mortality (Dennill 1985, Hoffmann et al. 2002). Knowledge of the tri-trophic *Acacia*–gall wasp–parasitoid interaction in its native range is, however, limited (Noble 1940, Bashford 2004), and most empirical studies have been conducted in South Africa for biocontrol purposes (e.g., Dennill 1985, Manongi and Hoffmann 1995, McGeoch and Wossler 2000, Veldtman et al. 2011). Both top-down and bottom-up effects apparently influence the population dynamics of this gall wasp (e.g., Dennill et al. 1993, Manongi and Hoffmann 1995, Bashford 2004). Yet no previous study has quantified the relative influences of top-down and bottom-up effects on its performance.

Females of the gall wasp are parthenogenetic and can reproduce without males (Noble 1940), and male-to-female sex ratios vary considerably from about 0.01:1 to 0.94:1 (Noble 1940). Females are therefore critical to gall wasp reproduction and population viability and were the focal individuals of this study. The female larva of the gall wasp induces gall formation (Dorchin et al. 2009), and the gall tissue grows into a thick sphere surrounding the female larva (Fig. 1B; Noble 1940). Each female gall chamber is visible externally as a lobe on the gall surface, and female chambers can, therefore, be counted prior to wasp rearing (Noble 1940). Male larvae develop in chambers in the periphery of the gall tissue (Noble 1940) with no externally visible indication of the number of males in the gall (Noble 1940, Bashford 2004). Male chambers can, therefore, only be observed by
dissecting the gall, killing any developing larvae or pupae. Given that our principle aim was to measure gall wasp survival to adulthood, that is, to record the number of adults emerged, galls could not be dissected immediately to quantify male abundances. By the time all individuals have emerged from the galls, the small peripheral male chambers were no longer visible when galls were dissected. Female chambers are, however, surrounded by substantially larger amounts of both storage and vascular tissue than male chambers (1.5 times more storage tissue and 3.5 times more vascular tissue; Dorchin et al. 2009) and it was, therefore, assumed that males would have a negligible effect on within-gall competition for resources compared to females. Thus, male survival was not estimated and gall wasp survival was quantified as female survival to adulthood. Herein, the terms “chamber” and “gall wasp” are used to refer to female chambers and wasps, respectively, unless specifically mentioned as male.

The natural enemies of the *T. acaciaelongifolii* gall wasp include a rich assemblage of parasitoid species (Hymenoptera). In Australia, the most abundant parasitoids found in association with the gall wasp are members of the Eurytomidae and Torymidae families (Noble 1940, Bashford 2004). Parasitoid-induced gall wasp mortality can be up to 61% (Bashford 2004), but varies substantially, even between neighboring trees (Noble 1940). The reason for such variation, and the impact of high rates of parasitism on gall wasp population dynamics, is unknown. The parasitoids use their ovipositors to penetrate the gall tissue and lay their eggs in the gall chambers (Fig. 1B) and male chambers that lie in the periphery of the gall, close to the surface, are more often parasitized than female chambers (Manongi and Hoffmann 1995). The parasitoid larvae that hatch will consume the gall wasp larva at some point during larval development. Herein, the term “parasitism” refers to parasitoid-induced mortality of gall wasp larvae.

**Study area**

Galls were collected from two *Acacia* host species, *A. floribunda* and *A. longifolia*, across an area of 9370 km² between the Mornington Peninsula (38°20’43” S, 145°00’25” E), Melbourne (37°50’45” S, 145°04’25” E), and Halls Gap (37°08’12” S, 142°31’09” E), Victoria, Australia, which spans the edge of the native range of the *Acacia* host plants (Walsh and Entwistle 1996). The study extent includes three Australian bioregions, the Gippsland Plain (Melbourne and Mornington Peninsula), the Highlands—Southern Fall (Melbourne), and the Greater Grampians (Halls Gap) that vary in climate, geology, and native vegetation (IBRA7; Australian Government Department of the Environment and Energy 2016). We sampled across this large spatial extent with the purpose of capturing natural variation in the system to examine the relationships between gall size and gall wasp survival.

For biocontrol purposes, the primary interest of the study was the *A. longifolia*—gall wasp interaction but given much overlap in the distribution of *A. longifolia* and *A. floribunda* (in the Melbourne region) trees of both host plants were chosen for collection and are dealt with separately in the analysis. Galls were collected from 17 sites (circular and 1 km in diameter) across the three regions (Melbourne: *n* = 15, Halls Gap: *n* = 1 and Mornington Peninsula: *n* = 1). In Halls Gap and Mornington Peninsula, only *A. longifolia* was present while both *A. longifolia* and *A. floribunda* co-occurred across sites in the Melbourne region. *Acacia floribunda* generally occurred in much higher densities across sites (Appendix S1: Table S1) and more *A. floribunda* trees were, therefore, sampled overall (*n* = 44 for *A. floribunda* and *n* = 27 for *A. longifolia*). Up to six trees were sampled within each site except when less than six galled trees were present (Appendix S1: Tables S2 and S3) and these trees were chosen to be as far apart as possible within the extent of the site. The total number of trees (galled and ungalled) per site was counted. For each sampled tree, the circumference at the base was measured and the number of galls per branch was estimated as a mean of ten randomly chosen branches (with a “branch” defined as the terminal 30-cm segment of a shoot following Dennill 1985). The sampled trees were located in urban environments, that is, along road sides and in urban reserves and parks where galls occur in high densities, which enables a relatively high sample size per tree.

**Gall collection and rearing**

Galls were collected for rearing and dissection over a three week period in November 2014 (Table 1) after gall wasp larvae had pupated and
Table 1. Counts of trees, galls, and reared individuals from two host plants, *Acacia floribunda* and *Acacia longifolia*.

<table>
<thead>
<tr>
<th>Gall rearing</th>
<th><em>A. floribunda</em></th>
<th><em>A. longifolia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Trees sampled</td>
<td>44</td>
<td>27</td>
</tr>
<tr>
<td>Galls reared</td>
<td>1416</td>
<td>679</td>
</tr>
<tr>
<td>Gall wasps reared</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>422 (0.65)</td>
<td>250 (0.67)</td>
</tr>
<tr>
<td>Males</td>
<td>72 (0.11)</td>
<td>42 (0.11)</td>
</tr>
<tr>
<td>Male-to-female ratio</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>Parasitoids reared</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Torymidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Megastigmus</em> sp.</td>
<td>90 (0.14)</td>
<td>50 (0.13)</td>
</tr>
<tr>
<td>Monodontomerinae sp.</td>
<td>1 (&lt;0.01)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Eurytomidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eurytoma</em> sp. 1</td>
<td>33 (0.05)</td>
<td>16 (0.04)</td>
</tr>
<tr>
<td><em>Eurytoma</em> sp. 2</td>
<td>27 (0.04)</td>
<td>14 (0.04)</td>
</tr>
<tr>
<td>Pteromalidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Coelocybinae</em> sp.</td>
<td>6 (0.01)</td>
<td>3 (0.01)</td>
</tr>
<tr>
<td>Galls parasitized (%)</td>
<td>11.0</td>
<td>11.7</td>
</tr>
<tr>
<td>Total individuals reared</td>
<td>651</td>
<td>375</td>
</tr>
</tbody>
</table>

*Note:* For each group of individuals reared (gall wasps and parasitoid morphospecies), their proportion out of all individuals reared per host plant (i.e., relative abundance) is given in brackets.

Table 1 shows the counts of trees, galls, and reared individuals from two host plants, *Acacia floribunda* and *Acacia longifolia*. The table lists the number of trees sampled, galls reared, and gall wasps reared for each host plant. For each group of individuals reared, the proportion out of all individuals reared per host plant (i.e., relative abundance) is given in brackets.

Gall rearing was performed in a controlled environment, with galls collected from trees, weighed, and dissected in the laboratory. The number of female and male chambers was counted, and the number of lobes on each gall was estimated. These galls were then stored individually in resealable plastic bags, and gall wasps and parasitoids were reared from the galls at room temperature (~20°C). Emergence of individuals was checked from 25 November, at least once a week for the first three months of rearing and then once a month for the next four months until emergence ended (early July 2015). Emerged individuals were preserved in 96% ethanol and stored for subsequent identification. *Trichilogaster acaciae-longifoliæ* gall wasps were identified using Prinsloo and Neser (2007). Parasitoids were identified to family, or lower taxonomic level when possible, using relevant identification keys (Bouček 1988, CSIRO Division of Entomology 1991, Lawrence and Slipiński 2013) and grouped into morphospecies. A range of inquilines (e.g., Hymenoptera, Lepidoptera, and Coleoptera individuals feeding on the gall tissue; Noble 1940, Bouček 1988, CSIRO Division of Entomology 1991) also emerged from the galls. Whereas parasitoids depend on reaching the gall wasp larvae in the gall chambers to complete their development to adulthood, inquilines complete their development in the tissue of the gall. Inquilines were, therefore, not expected to be restricted by gall size in the same way as parasitoids. Since the focus of this study was to quantify the influence of gall size on gall wasp survival, inquilines were, therefore, not considered further.

To determine if parasitoids preferentially attacked male chambers in the galls (as has been observed in the introduced range; Manongi and Hoffmann 1995), the male-to-female sex ratio of emerged individuals was compared to the ratio of male to female gall chambers in a separate set of dissected galls. If parasitoids attack male and female chambers with the same frequency, the relative number of male compared to female gall wasps should be similar between reared (individuals surviving to adulthood) and dissected (observed number of chambers) galls. Thus, while collecting galls for rearing, 24.6 ± 10.5 (mean ± SD) galls per tree were collected for dissection, from a subset of the sampled trees (n = 11 for *A. floribunda* and n = 12 for *A. longifolia*; see Appendix S1: Tables S2 and S3). These galls were dissected in the laboratory, and the number of female and male chambers was counted.

**Data analysis**

For each reared gall, gall wasp survival to adulthood was quantified as the proportion of chambers from which a gall wasp successfully emerged, that is, the number of emerged adult females divided by the number of female chambers in each gall (estimated as the number of gall lobes). Gall mass per female chamber (i.e., the tissue available to each female gall wasp in the gall) was
quantified as the total mass of each gall divided by the estimated number of female chambers. Gall wasp density was included as a categorical predictor defined as the number of chambers in a gall (one, two, or three chambers). Parasitism was scored as a binary variable, that is, either present or absent in each gall. A binary score was chosen over a density measure because the number of parasitoids in a gall is likely to be highly related to gall wasp sex ratio (Manongi and Hoffmann 1995). Parasitoid density is, therefore, more likely to overestimate the strength of the top-down effect in the system. Parasitoid presence as a measure of parasitism is, therefore, also only an estimate of the likelihood that female gall wasps are exposed to top-down effects. If effects of parasitism on survival are absent, it may be due to either low levels of parasitism overall or low levels of parasitism on female gall wasps specifically.

The hypothesized direct and indirect effects on within-tree variation in gall wasp survival in the reared galls were structured as a causal network (Fig. 2; for detailed description see Appendix S2) and tested as a piecewise structural equation model (piecewise SEM) for each Acacia host (Lefcheck and Duffy 2015). Unlike a traditional path analysis, a piecewise SEM enables the inclusion of random effects and variables with non-normal error distributions (Shipley 2009).

The causal network was divided into component models, which were analyzed separately, before the overall fit of the hypothesized causal network was tested. For each response variable in the causal network (i.e., each variable with at least one ingoing arrow), a separate component model was constructed. Each response variable was then tested as a function of all its hypothesized direct causes (i.e., variables with arrows connecting directly to, and pointing toward, the response variable). The component models of the piecewise SEM were analyzed as mixed models including individual trees as a random effect. A mixed model approach was chosen to correct for spatial aggregation of the data (Zuur et al. 2009) originating from the sampling of multiple galls within each tree and because we were testing the within-tree effect of gall size on gall wasp survival. The component models (Fig. 2) are as follows: (1) a gall wasp survival model (generalized linear model [GLMM] with binomial distribution and logit link function) testing gall wasp survival as a function of gall mass per chamber, gall wasp density, and parasitism; (2) a parasitism model (GLMM with binomial distribution and logit link function) testing parasitism as a function of gall mass per chamber; and (3) a gall mass model (linear mixed model [LMM]) testing gall mass per chamber as a function of gall wasp density. The effect of gall wasp density on gall mass per chamber was included as a path in the causal network because there was a non-linear association between gall mass per chamber and number of chambers in a gall (see Results and Fig. 3).

Pairwise comparisons between all three levels of gall wasp density (i.e., one, two, or three chambers per gall) in the gall mass models were performed with Tukey’s post hoc test. Finally, to test for the importance of the direct link between gall resources and gall parasitism in the tri-trophic interactions of the gall wasp, we also constructed and tested a competing causal network (Appendix S2: Fig. S2). This causal structure did not include the direct link between gall mass per chamber and gall parasitism (see Appendix S2 for further details).

The overall fit of a piecewise SEM is tested with Shipley’s test of d-separation (Shipley 2009). In the d-separation test, a significance for each independence claim in the causal network is found. Any two variables in the causal network that do not share an arrow are included as an independence claim; that is, based on the knowledge used to construct the network, the two variables are considered to be independent.

![Fig. 2. Hypothesized causal network of bottom-up and top-down effects on gall wasp herbivore survival to adulthood. The causal network can be divided into three component models, (i) predictors of gall wasp survival, (ii) predictor of gall parasitism and (iii) predictor of gall mass per chamber.](image-url)
A composite probability of all independence claims is then calculated with a Fisher’s C test statistics using a \( \chi^2 \) distribution. A resulting P-value lower than the chosen significance level (here \( \alpha = 0.05 \)) leads to a rejection of the proposed causal network. If the \( d \)-separation test fails to reject the causal network, the observed data are consistent with the hypothesized causalities. This does not exclude the possibility of other causal structures having created the observed pattern.

Multicollinearity between variables was tested and excluded for the gall wasp survival model (variance inflation factors [vif] \( \leq 1.4 \)). Coefficient estimates (\( B \)) were considered significant at \( P \)-values <0.05. Variance explained by the models is reported as marginal and conditional \( R^2 \) as recommended by Nakagawa and Schielzeth (2013). Marginal \( R^2 \) considers the variance explained by the fixed effects only while conditional \( R^2 \) describes the overall variance explained by the model (including both fixed and random effects).

If marginal \( R^2 \) is low compared to conditional \( R^2 \), and most of the variance explained is due to the random effect, there is large between-tree variation in the magnitude of the tested response.

All statistical tests were performed in R 3.2.2 (R Core Team 2015). Linear mixed models were analyzed in the nlme package (version 3.1-121; Pinheiro et al. 2015), and GLMMs were analyzed with the lme4 package (version 1.1-9; Bates et al. 2015). The overall piecewise SEM fit \( (d\)-separation test), marginal \( R^2 \), and conditional \( R^2 \) were calculated in the piecewiseSEM package (version 1.0.0; Lefcheck 2015) using \( P \)-values based on Satterthwaite’s approximations for the \( d\)-separation test. Tukey’s test was performed in the multcomp package (Hothorn et al. 2008) while vif was calculated using the vif.mer function (available online at https://github.com/aufrank/R-hac ks/blob/master/mer-utils.R).

**RESULTS**

**Rearred and dissected galls**

Between late November 2014 and early July 2015, 651 and 375 individuals emerged from reared *Acacia floribunda* and *A. longifolia* galls, respectively (Table 1). Most gall wasp individuals (female and male) emerged within the first four weeks of rearing from both *A. floribunda* and *A. longifolia* galls (Fig. 4). The reared male-to-female sex ratio was 0.17 on both host species. The male-to-female chamber ratio (mean ± SD) of dissected galls was much higher, at 0.75 ± 0.16 for *A. floribunda* trees and 0.69 ± 0.20 for *A. longifolia* trees. Of the emerged parasitoids, 96% belonged to the families Torymidae (two morphospecies; Table 1) and Eurytomidae (two morphospecies; Table 1) with the remainder being members of the Pteromalidae family (one morphospecies; Table 1). Parasitoids made up 24% and 22% of all individuals reared from *A. floribunda* and *A. longifolia*, respectively (relative abundances of reared individuals from each morphospecies are given in Table 1). Parasitoids emerged throughout the rearing period (up to 33 weeks) from galls of both host plants (Fig. 4).

**Piecewise SEM**

For both host plants, gall wasp survival increased significantly with gall mass per chamber but was unaffected by gall wasp density and the...
test for one- vs. three-chambered galls: Tukey’s test for one- vs. three-chambered galls: \( z = -0.536, P < 0.001 \) for \( A. floribunda \); \( z = -0.679, P < 0.001 \) for \( A. longifolia \); Fig. 3).

The conditional \( R^2 \) was much higher than the marginal \( R^2 \) for both host plants (Table 2iii) indicating that there is large between-tree variation in the size of a one-, two-, or three-chambered gall, respectively (i.e., the variance explained was due to the random rather than the fixed effects). In the other models, conditional \( R^2 \) was not much higher than the marginal \( R^2 \) (Table 2i and ii), indicating that most of the variance was explained by the fixed effects (i.e., gall mass per chamber, gall wasp density, and parasitism) and that the examined relationships were consistent across the sampled trees. For example, for a given gall mass per chamber the likelihood of survival was similar across individual trees within each host plant species. When comparing across hosts plants, the log odds for survival in \( A. floribunda \) galls was more than one and a half times as high as the log odds for survival in \( A. longifolia \) galls (\( B = 1.32 \) vs. \( B = 0.84 \), respectively; Fig. 5). Thus, even though the average gall mass per chamber was higher on \( A. longifolia \) than on \( A. floribunda \) (Fig. 3), survival per mass was higher in \( A. floribunda \) galls (Fig. 6).

When testing the independence claims of the hypothesized causal network (variables not connected by arrows), Shipley’s \( d \)-separation test failed to reject the hypothetical causal network formulated in Fig. 2 (\( A. floribunda \): \( C = 2.76, df = 2, P = 0.252 \); \( A. longifolia \): \( C = 2.66, df = 2, P = 0.265 \); Fig. 5). The proposed causalities therefore provide a valid interpretation of the relative influences of top-down and bottom-up effects on the survival of the gall wasp on both host plants. The competing causal network, excluding the direct impact of gall mass per chamber on gall parasitism, was rejected as a valid explanation of the observed tri-trophic interactions (see Appendix S2).

**Discussion**

Here, we show that there was a strong positive relationship between gall mass per chamber (a proxy for resources provided by the host plant) and the survival of a biocontrol agent to adulthood on two host plant species in its native range, while gall mass per chamber was negatively
Table 2. Direct and indirect top-down and bottom-up effects on larval survival for each host plant species (*Acacia floribunda* and *Acacia longifolia*).  

<table>
<thead>
<tr>
<th>Host plant</th>
<th>Component model</th>
<th>Predictor variable</th>
<th>n</th>
<th>df</th>
<th>B (SE)</th>
<th>P</th>
<th>Marginal $R^2$</th>
<th>Conditional $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. floribunda</em></td>
<td>(i) Call wasp survival</td>
<td>Gall mass</td>
<td>1416</td>
<td>1</td>
<td>1.32 (0.10)</td>
<td>$&lt;0.001$</td>
<td>0.26</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gall wasp density (2 chambers)</td>
<td></td>
<td>1</td>
<td>0.28 (0.14)</td>
<td>0.054</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gall wasp density (3 chambers)</td>
<td></td>
<td>1</td>
<td>0.34 (0.20)</td>
<td>0.082</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Parasitism</td>
<td></td>
<td>1</td>
<td>$-0.41 (0.24)$</td>
<td>0.090</td>
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<td>(ii) Parasitism</td>
<td>Gall mass</td>
<td>1416</td>
<td>1</td>
<td>$-0.77 (0.14)$</td>
<td>$-0.001$</td>
<td>0.12</td>
<td>0.14</td>
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<td>Gall wasp density (2 chambers)</td>
<td></td>
<td>1</td>
<td>$-0.45 (0.04)$</td>
<td>$&lt;0.001$</td>
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<tr>
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<td>Gall wasp density (3 chambers)</td>
<td></td>
<td>1</td>
<td>$-0.53 (0.07)$</td>
<td>$&lt;0.001$</td>
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<tr>
<td><em>A. longifolia</em></td>
<td>(i) Call wasp survival</td>
<td>Gall mass</td>
<td>679</td>
<td>1</td>
<td>0.84 (0.08)</td>
<td>$&lt;0.001$</td>
<td>0.30</td>
<td>0.42</td>
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<tr>
<td></td>
<td></td>
<td>Gall wasp density (2 chambers)</td>
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<td>1</td>
<td>0.25 (0.21)</td>
<td>0.226</td>
<td></td>
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<td></td>
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<td>Gall wasp density (3 chambers)</td>
<td></td>
<td>1</td>
<td>$-0.07 (0.24)$</td>
<td>0.771</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>Parasitism</td>
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<td>$-0.16 (0.35)$</td>
<td>0.639</td>
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<td>(ii) Parasitism</td>
<td>Gall mass</td>
<td>679</td>
<td>1</td>
<td>$-0.74 (0.14)$</td>
<td>$&lt;0.001$</td>
<td>0.25</td>
<td>0.37</td>
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<td></td>
<td>Gall wasp density (2 chambers)</td>
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<td>1</td>
<td>$-0.68 (0.12)$</td>
<td>$&lt;0.001$</td>
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<tr>
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<td></td>
<td>Gall wasp density (3 chambers)</td>
<td></td>
<td>1</td>
<td>$-0.68 (0.16)$</td>
<td>$&lt;0.001$</td>
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</table>

**Notes:** Component models of the path analysis were tested as mixed models (linear mixed models and generalized linear mixed models) to account the sampling of multiple galls within trees. Coefficient estimates ($B \pm SE$) with $P$-values above 0.05 are considered significant (shown in bold). The categorical variable gall wasp density was tested with one-chambered galls as the reference level.

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Fig. 5. Observed patterns of direct and indirect effects on gall wasp survival when tested as a piecewise structural equation model for each of the two host plants (*Acacia floribunda* and *Acacia longifolia*). Dashed arrows indicate non-significant partial regression estimates and full arrows indicate significant partial regression estimates. Unstandardized coefficient estimates are given for each significant partial regression and arrow thickness corresponds to the relative size of the coefficient estimates. Gall mass is defined as gall mass per female chamber. Gall wasp density is tested as a categorical variable (one, two, or three chambers) with one-chambered galls as the reference level; that is, results are shown for both two-chambered (2ch) and three-chambered galls (3ch) tested against one-chambered galls.
related to gall parasitism (here and below, “gall wasp” and “chamber” refer to female gall wasp and female chamber unless specifically mentioned as male). Bottom-up effects were not only directly related to the survival of the potential biocontrol agent, but also related to the strength of the top-down effect of parasitoids in the system. The importance of bottom-up and top-down effects, and their interactions, for herbivore population dynamics are well established (Hunter and Price 1992, Forkner and Hunter 2000, Hunter 2001, Denno et al. 2002, Stiling and Moon 2005), but rarely accounted for in assessments of herbivore agent biocontrol potential (Gassmann 1996, Hoseini et al. 2010). When bottom-up effects are strong, between- and within-tree variation in host plant resources are likely to be a strong predictor of gall wasp survival and establishment in a target range. Furthermore, if gall wasps are exposed to novel enemies when introduced, low resource availability could further suppress gall wasp populations by increasing parasitism in small galls. A strong dependence of biocontrol success on bottom-up effects emphasizes the need for post-release management and monitoring of herbivore agent populations when host plant resource quality is poor or highly variable.

**Top-down and bottom-up impacts on gall wasp survival**

The population dynamics of gall makers are often strongly related to bottom-up effects from host plant resources due to the influence that resources have on gall maker survival, fecundity, and oviposition preferences (e.g., Fritz et al. 2000, Stone et al. 2002, Price 2003). Here, we found a strong relationship between within-tree variation in gall mass per chamber and gall wasp survival. This bottom-up effect was dominant on both host plants, but the likelihood of survival with increasing gall mass per chamber was higher in *A. floribunda* galls, suggesting that *A. floribunda* may provide higher resource quality per unit mass of gall tissue.

Despite a strong effect of gall mass per chamber on survival, there was no evidence that gall wasp survival was density dependent within galls. No previous study has examined density-dependent survival in *A. floribunda*. At the scale of individual gall, gall wasp density had no effect on pupal mass in *A. longifolia* (Dennill et al. 1993). Thus, using a different performance measure (i.e., pupal mass), the results of Dennill et al. (1993) support our finding of absence of intra-specific competition within galls. The gall wasp has, however, been observed to switch to sub-optimal hosts (i.e., the closely related *Acacia melanoxylon* R. Br. and *Parasenianthes lophantha* (Willd.) I. C. Nielsen; Fabaceae) at sites with particularly high gall wasp densities in South Africa (Dennill et al. 1993). Though potentially a rare event, host switching could indicate that intra-specific competition can occur at the tree level in this species (Dennill et al. 1993). Herbivore performance can vary across spatial scales (McGeoch and Price 2005) and life stages (Ohgushi 1995). Thus, negative density dependence could be an important contributor to gall wasp population dynamics without being evident at the scale and with the performance measure used here (i.e., larval survival to adulthood at the scale of individual galls).

No evidence was found for top-down impact of parasitism on female gall wasp survival on either host plant, despite the high abundance of parasitoids among the emerged individuals (24%
and 22% for *A. floribunda* and *A. longifolia*, respectively). Each emerged parasitoid has killed a gall wasp to complete its development (Noble 1940). Thus, the absence of a top-down effect is unexpected, but may be due to high levels of parasitism of male gall wasp larvae. The focus on females in our study may therefore obscure the true strength of the top-down impact from parasitoids. The large discrepancy in male-to-female sex ratio of reared (0.17 for both host plants) compared to dissected (0.75 and 0.69 for *A. floribunda* and *A. longifolia*, respectively) galls suggests that survival of males was low compared to females in the reared galls. Previous findings of a relatively higher exposure of male than female chambers to parasitism due to their peripheral placement in the gall tissue (Manongi and Hoffman 1995) provide further support for the idea. Thus, many of the parasitoids reared in the present study may have emerged from male, rather than female, chambers. Nonetheless, female gall wasps are principally responsible for gall formation (Noble 1940, Dorchin et al. 2009) and their exposure to parasitism is, therefore, critical for determining the gall wasp biocontrol potential.

Gall mass per chamber was negatively related to gall parasitism (Table 2ii, Fig. 5), suggesting that bottom-up effects from gall size can influence the strength of top-down effects on gall wasp survival at the gall level. An effect of gall morphology on parasitoid access to gall chambers has been observed in a range of different gall makers (Weis et al. 1985, Price and Clancy 1986, Albarracin and Stiling 2006). Gall size is also one of the gall morphological traits that is known to be related to the bottom-up effects of host plant quality between and within individual plants (Price and Clancy 1986, Albarracin and Stiling 2006). The rate of development of galls determines the temporal window of opportunity for parasitoids with short ovipositors to reach gall chambers while the final size of the fully developed gall can limit the access of later attacking parasitoids with longer ovipositors (Price and Clancy 1986, Albarracin and Stiling 2006). The weak top-down effects from parasitoids that are often found in gall makers (Stone et al. 2002, Price 2003) may be due to the effectiveness of gall tissue in restricting parasitoid access. Price (2003) suggested that the weak influence of parasitism on the population dynamics of the sawfly *Euura lasiolepis* Smith (Hymenoptera: Tenthredinidae) was due to the combined effects of gall size and toughness in excluding parasitoid species with both short and long ovipositors. The dependence of gall morphology on the resource quality of host plant tissue does, however, suggest that there is potential for modified top-down impact across environmental gradients that affect host plant quality such as soil nitrogen and water status (Albarracin and Stiling 2006, Price and Hunter 2015).

The path analysis provided the first evidence for the importance of gall mass per chamber, parasitism, and the number of chambers per gall for determining gall wasp survival in the system. The tested relationships are, however, likely to represent a subset of a more complex network of causal relationships. Future work may focus on linking plant nutrient quality to both between- and within-tree variation in gall wasp preference and performance to further explore the determinants of biocontrol success in the gall wasp. Although the direct link between gall mass per chamber and the nutrient status of host plant tissue still needs to be demonstrated for this system, the dominant within-tree effect of gall mass per chamber on gall wasp survival that we found suggests that significant variation exists in gall resource availability in this system.

**Implications for biological control**

Indicators of resource availability in this gall wasp–host plant interaction, such as gall size, are likely to be important predictors for its biocontrol potential in an introduced range. Our results suggest that bottom-up effects of gall size will be strongly related to gall wasp survival and through this, the ability of a gall wasp population to establish successfully on invasive host species. Gall makers are often used in biocontrol due to their ability to suppress plant reproduction, especially among species that affect flower development and seed set (Harris and Shorthouse 1996). *Trichilogaster acaciaelongifoliae* is generally considered to be a successful biocontrol agent in South Africa (Impson et al. 2011), where it has become widespread across the invasive range of *A. longifolia* (Veldtman et al. 2010). Impacts on host populations can, however, be inconsistent, with seed production being unaffected in some localities (Dennill and Gordon 1990, Dennill et al. 1999). This low effectiveness has been ascribed to
regional variability in aridity and temperature, suggesting that bottom-up effects do indeed influence biocontrol potential in the introduced range.

In areas where gall mass per chamber is low or highly variable, the composition of native parasitoid assemblages may also be critical to biocontrol success. Even though parasitism was not significantly related to female survival in the native range studied here, plant stress or novel enemy assemblages could result in significant top-down effects in the introduced ranges of potential biocontrol agents (Veldtman et al. 2011). The high exposure of male chambers to parasitism is another potentially important aspect of top-down impacts on gall wasp populations. In the short term, parthenogenetic females sustain population growth and dispersal to new host trees. Long-term exposure of males to high levels of parasitism could, however, make the gall wasp more vulnerable to environmental variability if asexual reproduction affects the adaptive potential of the population (West et al. 1999). This could be especially critical if gall wasps experience a genetic bottleneck when introduced to a target range, which is not uncommon for introduced biocontrol agents (Debach and Rosen 1991). In the introduced range in South Africa, high levels of parasitism (47.6%) have been found in association with the introduced gall wasp (McGeoch and Wossler 2000) and a food web of similar structure and complexity to the one in the native range now exists (Veldtman et al. 2011). Veldtman et al. (2011) also found 33% of the acquired natural enemies of the gall wasp to belong to the same families as its native enemies. High similarity between native and novel food webs suggests that the gall wasps could be experiencing equivalent top-down impacts in native and introduced ranges. However, the strength of the top-down effect will depend on the ability of novel enemies to access gall chambers. If novel enemies in the introduced range on average have longer ovipositors, the impact of top-down effects could be elevated, especially when gall mass per chamber is low. Thus, the choice of optimal release areas should include evaluations of local host plant resource quality and quantity. Biocontrol is more likely to succeed if both herbivore agent population dynamics and the suitability of the release area are assessed prior to agent release (Sheppard 2003, Zalucki and van Klinken 2006, López-Núñez et al. 2017).

A strong impact of bottom-up effects on gall wasp survival suggests that post-release management may need to be implemented in regions where plant condition may result in low gall mass per chamber, or where it is highly variable. This could involve fertilization of low-quality hosts, or continued addition of the biocontrol agent over time. Biocontrol agent establishment under low-resource conditions has been shown to benefit from weed fertilization due to increased herbivore survival and fecundity (Van Hezewijk et al. 2008, Hovick and Carson 2015). However, as Hovick and Carson (2015) showed, if resource addition is too high, the increased resistance of the weed to herbivore damage could reduce the impact of the biocontrol agent while increasing weed abundances (Hovick and Carson 2015). Fertilization of weeds is therefore potentially risky and requires careful management. An alternative strategy may be ongoing targeted release of additional individuals of the biocontrol agent (i.e., agent augmentation). This is likely to be necessary in the early stages of biocontrol establishment, when agent populations are small and vulnerable to stochastic changes in the environment (Grevstad 1996).

The need for post-release management of biocontrol agent populations can only be assessed through continued monitoring of released agents. Long-term post-release monitoring of released agents is often neglected in biocontrol programs despite its importance for the evaluation of biocontrol success (Morin et al. 2009). The strong correlation between gall mass per chamber and gall wasp survival found here provides a potential cost-effective tool for such post-release monitoring of gall wasp agent populations. Gall mass per chamber can be readily measured across multiple sites and used as an indicator of relative gall wasp survival. This measure could be used to predict biocontrol agent population growth or to identify localities and years in which additional management of agent populations is needed.

**Conclusions**

Here, we have shown that the survival of the gall wasp *Trichilogaster acaciaelongifoliae* on two host plant species is strongly affected by withintree variation in gall resource availability and that, by comparison, parasitism plays a minor role. The effect of parasitism on this gall wasp is, however,
mediated by gall mass per chamber and may change with variation in host plant quality and the composition of local parasitoid assemblages. Strong bottom-up effects of factors that determine the survival of herbivorous biocontrol agents have implications for spatio-temporal variability in the success of biocontrol programs and should be considered in pre-release assessments and post-release monitoring of herbivore insect biocontrol agents.

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**Supporting Information**

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2043/full