



Who holds the reins? Context-dependent resource allocation in the mutualism between fig trees and their fig wasp pollinators

Manasa Kulkarni¹ · Nehal Vijay Naik^{1,2} · Renee M. Borges¹

Received: 22 January 2024 / Accepted: 14 May 2024 / Published online: 27 May 2024
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2024

Abstract

Mutualisms are consumer–resource interactions, in which goods and services are exchanged. Biological market theory states that exchanges should be regulated by both partners. However, most studies on mutualisms are one-sided, focusing on the control exercised by host organisms on their symbionts. In the brood-site pollination mutualism between fig trees and their symbiont wasp pollinators, galled flowers are development sites for pollinator larvae and are exchanged for pollination services. We determined if pollinator galls influenced resource allocation to fig inflorescences called syconia and considered feedbacks from the host tree. We experimentally produced syconia containing only seeds (S), only pollinator galls (G) or seeds and galls (SG) with varying number of introduced female pollinator wasps, i.e., foundress wasps. Biomass allocation to syconia was affected by foundress numbers and treatment groups; SG treatments received highest biomass allocation at low foundress numbers, and both G and SG treatments at high foundress numbers. Seeds are important determinants of allocation at low foundress numbers; galls are likely more influential at high foundress numbers. Most allocation in the G and SG treatment was to the syconium wall, likely as protection from parasitoids and temperature/humidity fluctuations. Dry mass of individual seeds and wasps (except at low foundress numbers) was unchanged between treatment groups, indicating seeds and wasps regulate resource flow into them, with lower flow into galls containing the smaller males compared to females commensurate with sexual dimorphism. We demonstrate the importance of considering the direct role of symbionts in accessing resources and controlling exchanges within mutualisms.

Keywords Biological market · Host sanctions · Gallers · Mutualisms · Resource allocation

Introduction

The interaction between mutualistic partners includes exchange of services and rewards. Often, one partner, the host, receives services from the other, often a symbiont, and rewards the symbiont in exchange (Boucher et al. 1982). Do both partners control exchanges within this trade? Traditionally the control of exchanges has been attributed to one partner, the host (Leigh 2010). The host is usually bigger in size and has access to resources that it can give away as rewards

in exchange for services (Bronstein 2015). Host control of exchanges mainly occurs by partner choice or host sanctions. According to biological market theory, exchanges in mutualisms are comparable to human markets (Noë and Hammerstein 1994). In partner choice, hosts may choose their mutualistic partner based on the quality of service offered (Simms and Taylor 2002; Bshary and Noë 2003). In some mutualisms, after interactions are established, hosts can punish non-cooperative partners by denying allocation and/or selectively allocating resources to cooperative ones via host sanctions (Pellmyr and Huth 1994; Denison 2000; Kiers et al. 2003; Jandér and Herre 2010; Frederickson 2013). Both partner choice and host sanctions work on the same principle—when symbionts provide services to the hosts, feedback on the quality and quantity of services is received by the hosts. Hosts act on this feedback by allocating resources as rewards to cooperative symbionts. Most mutualistic systems have been investigated from the host perspective and consider symbionts as passive participants. They do

Communicated by Robert R. Junker .

✉ Renee M. Borges
renee@iisc.ac.in

¹ Centre for Ecological Sciences, Indian Institute of Science, Bengaluru 560012, India

² Department of Biology (Ecology and Evolutionary Biology), University of Toronto Mississauga, Mississauga, ON, Canada

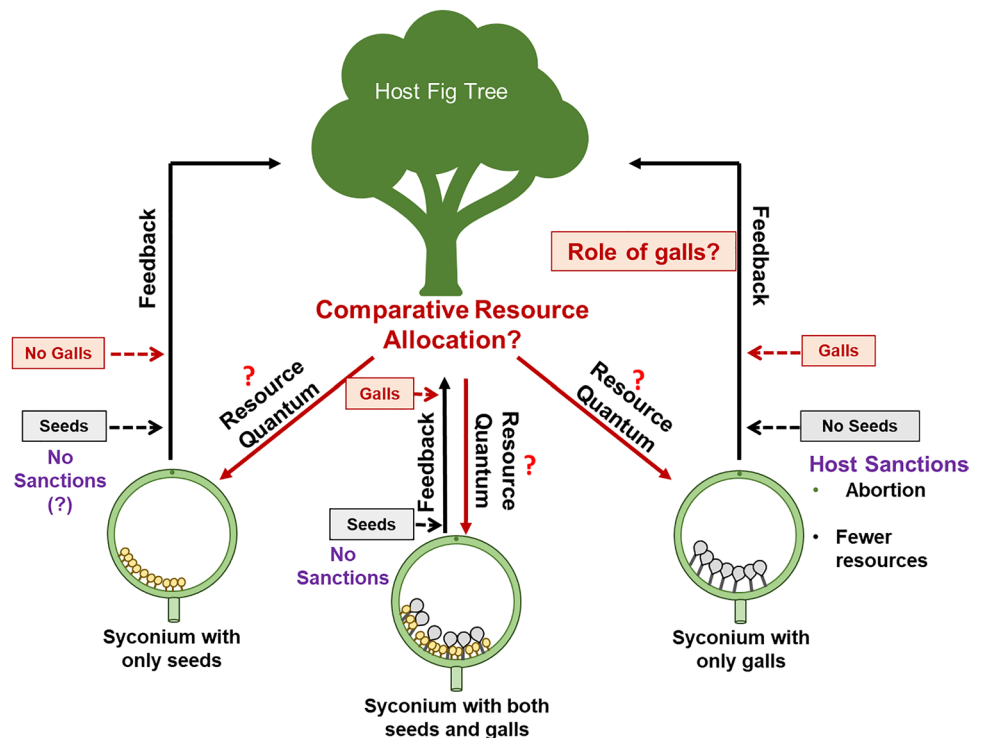
not include any direct control accorded by the symbionts. However, in the mutualism between legumes and rhizobia, negotiations between the partners can set up the terms for exchange and bring in joint control over these exchanges (Akçay and Roughgarden 2007; Akçay and Simms 2011).

In some brood-site pollination mutualisms, as in fig–fig wasp interactions, pollinators form galls within host flowers contained within an enclosed structure called a syconium where pollinator larvae develop. Galls are strong resource sinks and are known to manipulate the host plants to acquire nutrition (Larson and Whitham 1991; Dorchin et al. 2006; Favery et al. 2020). These galler partners can therefore potentially have direct access to resources through host manipulation apart from rewards commensurate with services provided. In the fig system, symbiont cooperation is ensured through sanctions imposed by the tree on non-cooperative pollinator wasps. If pollinators either overexploit the fig by excessive oviposition, and/or subthreshold pollination, sanctions are imposed in one or more of three ways: abortion of whole syconium or reduced number and/or reduced size of the wasp offspring (Herre et al. 2008; Jandér and Herre 2010, 2016; Wang et al. 2014). Host rewards act via enhanced resource allocation to syconia containing more seeds, and thereby to the offspring of presumably more cooperative pollinators (Jandér and Herre 2016). Many studies on host sanctions have thus determined the impact of seed numbers on the fate of pollinator wasps developing in a syconium. Although the number of pollinator galls within a syconium

can also influence resource allocation to the syconium, this has been rarely examined. In dioecious fig species in which wasps develop only within syconia on male trees, syconia that contained no pollinator galls aborted, indicating that wasp oviposition is required to retain syconia in some contexts (Tarachai et al. 2008). Female pollinator wasps that develop within the syconium collect pollen from their natal fig before dispersing and therefore are part of the male function of the tree (Weiblen 2002). Pollen dispersal, therefore, can be considered a pre-paid service offered by the pollinators to the tree. In this case, if the tree imposes sanctions on syconia based solely on seed numbers, it would lose out on potential fitness benefits via pollen dispersal by female wasps developing within the syconia. Therefore, it is important to study the effect of pollinator galls on resource allocation to a syconium and on the occupants of the syconium themselves.

In addition, sanctions are known to be context-dependent, with the number of pollinator wasps entering a syconium being an important context influencing sanctions (Wang et al. 2014). The number of foundresses could determine seed and pollinator gall number that in turn could affect the sanctions imposed by the tree (Fig. 1). The fig system gives us an opportunity to manipulate the type (seeds or galls) and number of occupants within a syconium (Jandér and Herre 2010; Krishnan et al. 2014), which allows us to study context-dependent outcomes of interactions between the fig tree and its pollinator wasps.

Fig. 1 Schema showing possible decisions in resource allocation to fig syconia in the fig–fig wasp interaction



In our study, we addressed the question of mutual control over mutualism outcomes in the fig–fig wasp interaction by considering three possible hypotheses:

Hypothesis 1: Only seeds influence resource allocation to the syconium, i.e., increasing seed number increases allocation to the syconium.

Hypothesis 2: Only pollinator galls influence resource allocation to the syconium; i.e., increasing gall number increases allocation to the syconium.

Hypothesis 3: Both seeds and galls influence allocation.

To test these hypotheses, we conducted two experiments by introducing either manipulated female pollinator wasps that could either only pollinate (produce only seeds) or only oviposit (produce only galls) or unmanipulated wasps (produce both seeds and galls) into syconia. These wasps were introduced to produce syconia with varying numbers of one type of occupant, i.e., seeds or galls (Experiment 1) or in combinations of seeds and galls (Experiment 2) to address the following specific questions:

(1) What is the effect of occupant type (seeds and/or pollinator galls) on syconium abortion? (2) What is the effect of occupant type on resource allocation to the fig syconium? (3) Does the number of foundresses influence the overall effect of occupant types on resource allocation? (4) How is the biomass of an individual occupant affected by the presence of other occupants in a syconium at different foundress numbers?

Predictions of outcomes for the effect of type of occupants on resource allocation to a syconium and its individual

occupants for Experiments 1 and 2 are in Figs. 2, 3, 4, 5, and 6 under the three hypotheses. Since number of foundresses determines seed and gall numbers (Wang et al 2014) which can further impact resource allocation, predictions for low and high foundress densities are presented separately. These predictions are based on the assumptions that, with increasing number of foundresses, the number of both seeds and pollinator galls increase with more seeds being replaced with pollinator galls, assuming that the number of foundresses is not so high that all syconia are aborted. For all experiments, we considered the syconium to be the unit of resource allocation (Jandér et al. 2012). We assume that the host fig tree serves as the source and the syconium as the sink (Fig. 1).

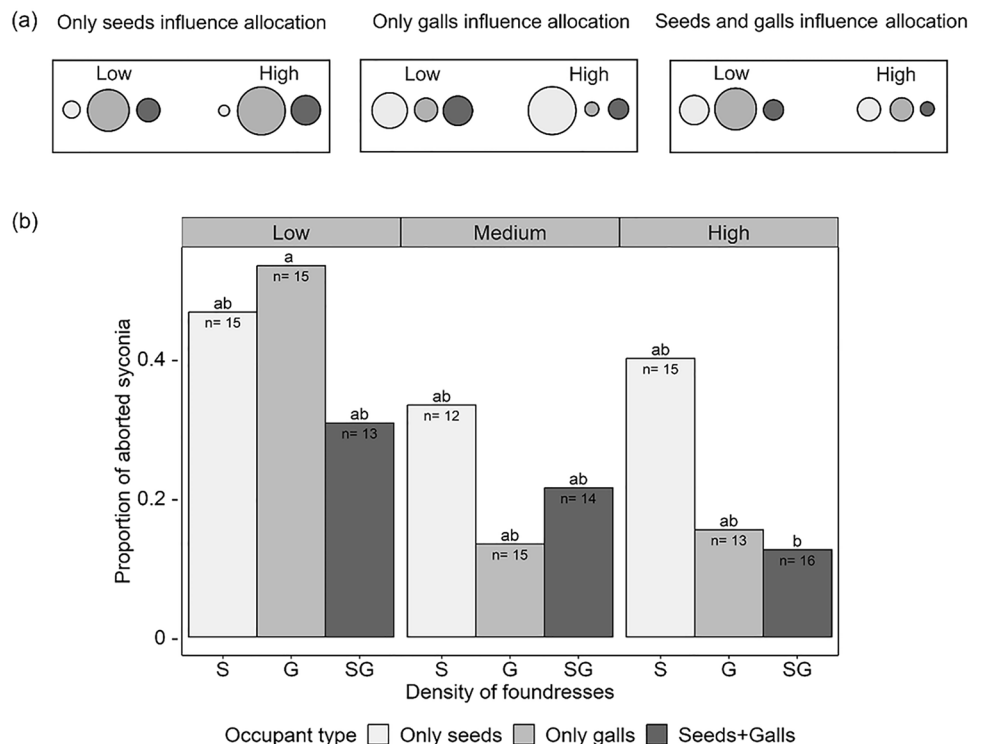
Materials and methods

Study site and study species

The study was conducted on monoecious *Ficus racemosa* Linn. (section: *Sycomorus*) trees located in the campus of the Indian Institute of Science, Bengaluru (12°58'N, 77°35'E), Karnataka, South India, that bear syconia on tree trunks and branches. *Ficus racemosa* is pollinated by the species-specific wasp *Ceratosolen fusciceps* Mayr.

A typical monoecious *Ficus* tree produces many syconia containing 100s–1000s of female flowers lining a cavity and enclosed by a many-layered wall. Male flowers surround the opening of the cavity (i.e., ostiole) which is lined with

Fig. 2 Proportion of aborted syconia of *F. racemosa*. **a** Predicted proportion of aborted syconia under three hypotheses: (i) only seeds influence resource allocation, (ii) only galls influence resource allocation, and (iii) both seeds and galls influence resource allocation. Under each hypothesis, predictions are shown for low and high foundress densities. **b** Results of proportion of aborted syconia under different treatment groups. Vertical bars indicate mean proportion of aborted syconia



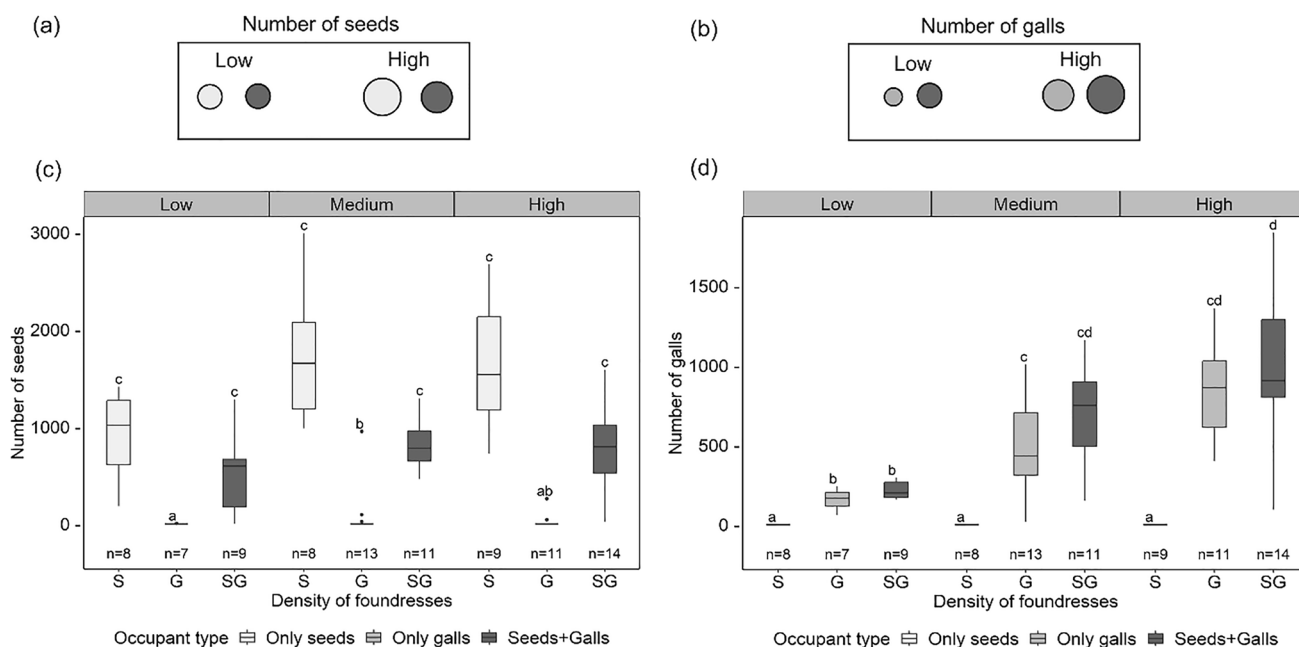


Fig. 3 Predictions for number of seeds (a) and number of galls (b) based on the treatment groups. Results of number of seeds of *F. racemosa* (c) and galls of pollinator *C. fusiceps* (d) in syconia of different treatment groups shown in Box plots. In c and d, horizontal thick lines are median values; lower and upper lines indicate first and third

quartile, respectively; whiskers indicate minimum and maximum values. Letters above error bars indicate significance at $P < 0.05$ (LMM with post-hoc Tukey tests); bars with the same letters are not significantly different. Sample sizes for each treatment group are indicated

bracts. A syconium undergoes development in five phases (A–E) (Galil and Eisikowitch 1968): (i) Phase A: pre-receptive phase, (ii) Phase B: pollen-receptive phase, pollinator wasps enter through the ostiole and pollinate female flowers while also laying eggs in other female flowers, (iii) Phase C: interfloral phase: pollinators and seeds develop, (iv) Phase D: development of wasp progeny is complete, male flowers undergo anthesis, wingless pollinator males eclose, bite openings into female galls, mate and release pollen-carrying females from the syconium by excavating an exit hole through the syconial wall, (v) Phase E: seed dispersal.

Pollination experiments

Manipulation of wasps for pollination

Bunches bearing syconia from four different trees for Experiment 1 and three trees for Experiment 2 (3–6 bunches from each tree) were securely covered with a fine mesh bag during A phase to avoid oviposition by fig wasps outside the experimental conditions. Syconia at the beginning of D phase (when only male pollinator wasps have emerged) were collected in the evening before experimental pollination. Some syconia were cut open and male flowers removed. These are pollen-free or P^- syconia (Jandér and Herre 2010). The rest were left intact (P^+ syconia). All syconia were individually placed in vials covered with gauze to allow female

wasp emergence. In the morning, foundress females were collected in batches from P^- and P^+ syconia into separate vials. From some vials containing wasps from P^+ syconia, ovipositors of the wasps were excised using small scissors (Krishnan et al. 2014). Ovipositor excision was effected by placing 5–10 wasps in a small vial and holding the vial against light. While the wasps faced the closed end of the vial, their ovipositors were quickly excised. Wasps were handled with caution during excision and any damaged or inactive wasps were not used for the experiments. These procedures generated wasps for three treatments (Table 1): Only Seeds (S) (from P^+ syconia with ovipositor-excised wasps, i.e., seed-producing S wasps), only Galls (G) (from P^- syconia with unmanipulated wasps unable to collect pollen and only able to produce galls, i.e., galling G wasps), and Seeds + Galls (SG) (from P^+ syconia, with unmanipulated wasps).

Experimental wasp introductions

To study the effect of syconium occupants on resource allocation, we conducted two experiments.

Experiment 1

To determine the effect of occupant type (seeds vs wasps) and their densities on allocation parameters, different

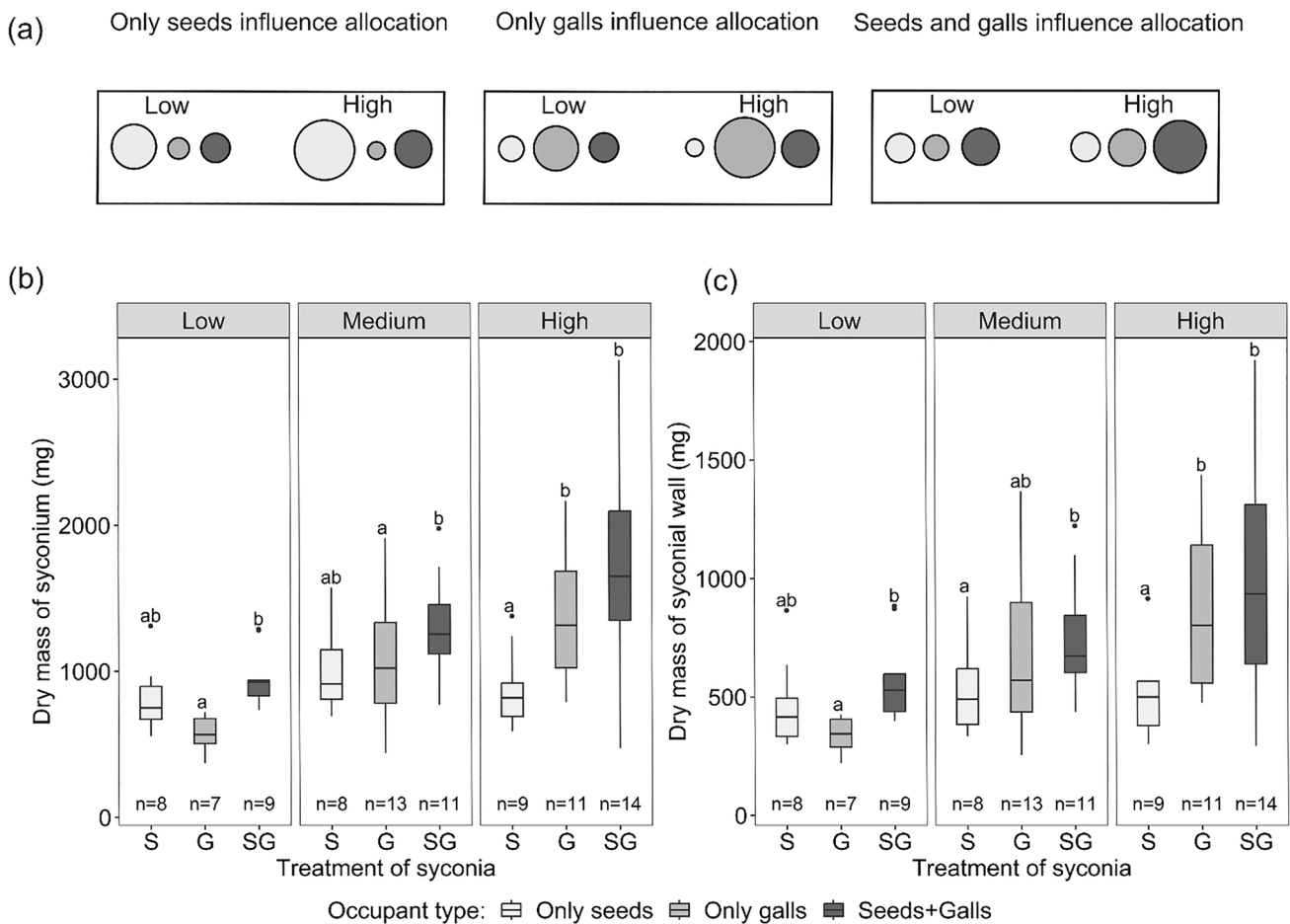


Fig. 4 **a** Predictions for relative dry mass of syconia under the three hypotheses (see legend of Fig. 2). **b** Dry mass of whole syconia, and **c** dry mass of syconial wall. Horizontal thick lines are median values; lower and upper lines indicate first and third quartile, respectively;

whiskers indicate minimum and maximum values. Letters above error bars indicate significance at $P < 0.05$ (LMM with post-hoc Tukey tests); bars with the same letters are not significantly different. Sample sizes for each treatment group are indicated

numbers of foundresses from each of the above-mentioned treatments (S, G, and SG) were introduced into each syconium at B phase. In particular, each syconium treatment had three densities of foundresses (used interchangeably with number of foundresses) with 1 (low), 5 (medium), and 9 (high) foundress wasps introduced into each syconium (Table 1). Wasps were introduced by placing them near the ostiole of syconium with a soft brush and were observed till they entered the syconium. Syconia were allowed to develop till D phase.

We assumed, based on previous studies, that the number of foundresses correlates positively with seed and gall numbers in each syconium (Anstett et al. 1996; Kathuria et al. 1999; Krishnan et al. 2014; Wang et al. 2014). In the SG treatments, increasing foundress numbers increased both seed and gall numbers (see Results), which would make it difficult to determine the effect of one occupant type (e.g., seeds) on the individual mass of the other

occupant type (e.g., wasps). To overcome this, we conducted a second experiment.

Experiment 2

Here, we attempted to keep numbers of one occupant constant while varying the other. A single S wasp was introduced into each syconium with increasing numbers of G wasps (Set 1) and vice versa (Set 2) at B phase (pollen-receptive phase) (Table 1). This experiment was conducted to confirm the effect of increasing density of only one occupant type (seeds or galls) on the individual mass of the other occupant.

Measurement of allocation parameters

Manipulated syconia were observed every morning near the end of C phase and collected before wasp emergence (late

Fig. 5 **a** Predictions for relative percentage water content of syconia (see legend of Fig. 2). **b** Percentage water in syconia of different treatment groups. Horizontal thick lines are median values; lower and upper lines indicate first and third quartile, respectively; whiskers indicate minimum and maximum values. Gray bars on the top indicate treatments of density of foundresses. Letters above error bars indicate significance at $P < 0.05$ (LMM with post-hoc Tukey tests); bars with the same letters are not significantly. Sample sizes for each treatment group are indicated

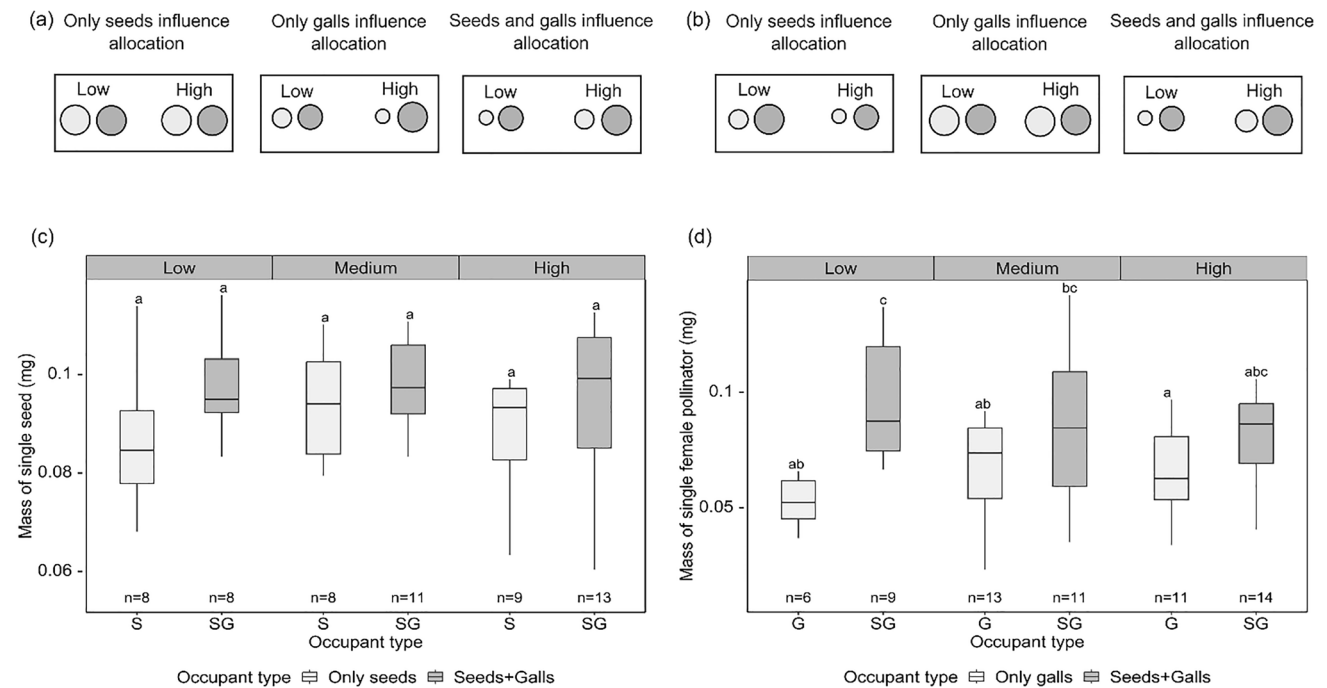
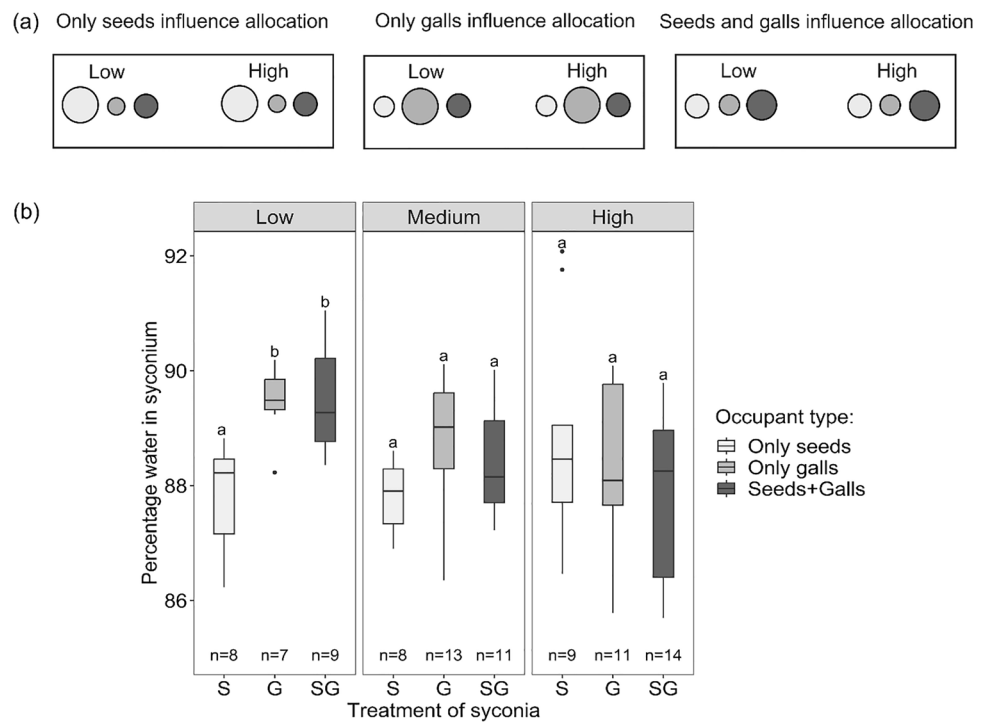


Fig. 6 Predictions (**a** and **b**) and results (**c** and **d**) of dry mass of single seeds (**a**, **c**) and single female pollinator wasps (**b**, **d**) (see legend of Fig. 2). In **c** and **d**, horizontal thick lines are median values; lower and upper lines indicate first and third quartile, respectively; whisk-

ers indicate minimum and maximum values. Letters above error bars indicate significance at $P < 0.05$ (LMM with post-hoc Tukey tests); bars with the same letters are not significantly different. Sample sizes for each treatment group are indicated

Table 1 Treatments and manipulations of female pollinator wasps to compare resource allocation in the presence of different densities of pollinator foundress types

Serial number	Syconia set	Treatment	Focal occupant	Other occupant	Other occupant density	Number of foundresses	Proportion of aborted syconia
1	Set 1	SG0	Seeds	Pollinator	Nil	1 (1S+0G)	0.82
2		SG1	Seeds	Pollinator	Low	2 (1S+1G)	0.69
3		SG5	Seeds	Pollinator	Medium	6 (1S+5G)	0.25
4		SG9	Seeds	Pollinator	High	10 (1S+9G)	0.15
5	Set 2	GS0	Pollinator	Seeds	Nil	1 (1G+0S)	0.69
6		GS1	Pollinator	Seeds	Low	2 (1G+1S)	0.25
7		GS5	Pollinator	Seeds	Medium	6 (1G+5S)	0.08
8		GS9	Pollinator	Seeds	High	10 (1G+9S)	0.14

S foundress is only capable of pollination; G foundress is only capable of laying eggs

C/early D phase). We measured syconium size, dry mass, water content, syconial wall mass and mass of individual occupants as parameters for resource allocation. We chose parameters that are likely to have direct effects on the fitness of mutualists, e.g., fig wasp size, seed size. We assume biomass of each occupant type (seed or wasp) to be indicative of their fitness since wasp size correlates with dispersal ability in pollinator wasps (Venkateswaran et al. 2017) and thereby the ability to breed in another syconium; seed size in general correlates with germination ability (Lamichhane et al. 2018). In addition, we chose parameters such as syconium wall thickness since a thicker wall would protect developing pollinators against parasitoids that oviposit from the exterior and could also act as an environmental buffer for a greater number of wasps developing within. Syconia were weighed, their lengths and widths measured using vernier calipers, and volumes estimated considering an ellipsoid shape. Syconia were dissected into eight equal parts through the ostiole–peduncle axis. Four alternate pieces were chosen, from which galls and seeds were separated. Galls with no exit holes (indicating failed wasp development) were counted as bladders. All components were dried at 75 °C for 48 h and their wet and dry mass were recorded using a Quintix 125D-10IN weighing balance accurate up to 0.01 mg. The number of seeds and wasps were counted. Dry mass and seed/gall numbers were extrapolated for the whole syconium.

Statistical analysis

All analyses were conducted using R-software version 4.1.3 (R Core Team, Vienna, Austria). To estimate the effect of type of occupants on resource allocation parameters and to examine if these effects changed under different foundress densities, generalized linear mixed models (LMMs) were run using the *lme4* package (Bates 2016). Allocation parameters, i.e., volume, dry mass, moisture content of

whole syconium and dry mass of the syconial wall, were the response variables. Different treatment groups of type and density of occupants were categorical predictor variables and tree ID was the random variable. Including bunch ID in the models did not change interpretations and since bunches used for experiments were at similar heights and each bunch included all the treatments, bunch ID was not considered in the models. Since errors were non-normal and skewed except for percent water content, the gamma family of distributions with log link was used. The binomial distribution was used for syconia abortions and arcsine square root-transformed values of proportions with Gaussian distribution were used to compare water contents. The *glmmTMB* package was used with the negative binomial distribution for comparing seed and gall numbers. To compare models with different fixed effects, the maximum likelihood (ML) method and the Akaike Information Criterion (AIC) were used (Online Resource—Table A1). Pairwise comparisons between allocation in treatments of different occupant types under each density category were made with post-hoc Tukey tests conducted using the *emmeans* package. Since log link was used in models for dry mass of syconium and syconial wall, comparisons were back-transformed and expressed as odds ratios.

Results

Abortion of syconia with different types of occupants and density of foundresses

In Experiment 1, only G syconia had significantly higher rates of abortion compared to SG syconia at high foundress density treatment (Fig. 2). However, there was a general trend of a non-significant decrease in rates of syconia abortions at higher densities (Fig. 2). In Experiment 2, abortion proportions were higher compared to Experiment 1 in

general (Table 1). However, the trend remained the same. Proportion of aborted syconia decreased with increasing total number of foundresses comparable to results from Experiment 1 (Table 1). Treatments with only one seed-producing S wasp or gall-producing G wasp had very high rates of abortion (Table 1).

Number of seeds or galls in syconia of different type and density of occupants

Experiment 1

Seed and gall numbers were consistent within treatment groups. There were significantly fewer seeds in the G syconia than in S or SG syconia. There were no galls in syconia of the S treatment (Fig. 3). Both type and density of occupants had a significant impact on seed and gall numbers (Online Resource—Table A1). Seed number was only marginally higher in syconia of the S treatment compared to SG syconia (Fig. 3). However, in both cases, the numbers saturated after the medium number of foundress females and did not increase for the high-density treatment. In the G treatment, gall number increased with foundress number, especially in a comparison of low to high densities (Fig. 3). The proportion of male offspring increased with increasing density of foundresses (Online Resource—Figure A1) which also resulted in an increase in the total number of female offspring (Online Resource—Figure A2). Gall presence reduced seed numbers, but seed presence only slightly increased gall number. Total number of bladders was significantly higher for the G treatment groups compared to the SG treatment (Online Resource—Figure A3), but there was no significant difference in proportion of bladders when analyzed within each density treatment (Online Resource—Figure A4).

Experiment 2

In Set 1, where the focal occupant was seeds, gall number increased with increasing number of G wasps, while there was no significant difference in seed numbers (Online Resource, Figure A5: a). In Set 2, where the focal occupant was pollinators, gall numbers remained almost constant while seed number increased with increasing number of S wasps as expected (Online Resource, Figure A5: b).

Effect of occupant type and density of foundresses on resource allocation

Resource allocation to syconia under different parameters followed a general trend of increased allocation to syconia containing both seeds and galls, followed by syconia containing only galls. GLMM results indicate that tree ID

had a significant effect on all allocation parameters, except percent water content (Online Resource—Table A1). Interaction between occupant type and foundress density had a significant effect on allocation parameters compared to models with only occupant type as predictor variable (Online Resource—Table A1). Under low foundress density, whole syconium dry mass was significantly greater in the SG treatment compared to the G treatment (Fig. 4). For medium foundress density, syconium dry mass was significantly higher for the SG treatment compared to the G treatment (Fig. 4). Under high foundress density, syconia containing galls, either alone (G) or with seeds (SG), had significantly higher dry mass compared to those containing only seeds (S), indicating that gall presence significantly increased biomass allocation to the syconium (Fig. 4). All other allocation parameters, except for percent water content, more or less followed a similar pattern (Fig. 5, Online Resource—Figure A6) with minor differences (Online Resource—Table A2, Table A3). Overall, the results show that the allocation patterns closely match Hypothesis 3 of influence of both seeds and galls on resource allocation. Similar mass of whole syconia in S and SG treatments at low density is likely due to overlap of seed number in these treatments and few galls.

Percent water content was significantly higher in G and SG treatments compared to S treatment only at low density (Fig. 5; Online Resource—Table A2, Table A3). However, at medium and high-density treatments, no significant differences were observed (Fig. 5, Online Resource—Table A2, Table A3). The results suggest the influence of both seeds and galls in acquiring moisture at medium and high-density. However, higher percent moisture in treatments containing galls at low density suggests the greater influence of galls for water acquisition.

Effect of occupant type and foundress density on mass of individual occupants

Experiment 1

Individual seed mass was unaffected by syconial treatment (Fig. 6c). However, LMMs indicated that tree ID had a significant effect on mass of individual seeds and female pollinators (Online Resource—Table A1). There was no significant difference between the dry mass of individual seeds from the S and SG treatments at any foundress density (Online Resource—Table A2) (Fig. 6c). There was no trade-off between numbers and mass of single seeds when compared between different density treatments (Online Resource—Table A1) (Fig. 6c). The dry mass of single female pollinators was significantly different between wasps in G and SG treatments only at low foundress density (Online Resource—Table A2) (Fig. 6d). When individual female pollinator mass was compared

between different density groups, no significant differences were observed (Online Resource—Table A1) (Fig. 6d). Although the mass of single male pollinators was significantly lower than that of female pollinators (Online Resource—Figure A7), their mass across treatment groups was similar (Online Resource—Figure A8). Except for wasp mass under low foundress density, mass of individual occupants was unaffected by type and foundress density.

Experiment 2

Since the dynamics of seed and gall proportions changed with foundress numbers in SG treatments of Experiment 1 (Fig. 3), we examined the results of Experiment 2 to confirm the effect of one occupant on the mass of the other occupant. In Experiment 2, no significant difference was found in mass of single seeds under any density of pollinator wasps or vice versa (Fig. 7) (Mass of single seed: $F = 1.01, P > 0.05$; Mass of single female wasp: $F = 1.08, P > 0.05$; Mass of single male wasp: $F = 0.39, P > 0.05$). Mass of individual seeds or wasps and the ratio of seeds and galls were not correlated (Fig. 8), except for mass of male pollinators when extreme values (Seed:Gall ratio > 20) were removed (Online Resource, Figure A9).

Discussion

Our results show that overall, the allocation of resources to the syconia is flexible and controlled by both seeds and galls depending on the density and type of occupants. Most results agree with Hypothesis 3 according to which both seeds and pollinator galls influence allocation to a syconium. Occupants influence allocation by providing the host tree with feedback on which the tree can act by differential allocation of resources (Jandér and Herre 2016) (Fig. 1). An extreme response of the host tree involves zero allocation resulting in syconia abortion. Although syconia abortions indicate exclusive control of syconia retention by the tree, our results of smaller abortion rates when syconia contain both seeds and galls (SG) or only galls (G) at high foundress density show that the decision to abort or retain syconia is based on number of seeds as well as pollinator galls, indicating effect of seeds and galls. A general trend of high syconia abortions at low foundress densities indicates that not just occupant type but their densities also decide the strength of the feedback and thus the allocation. At low density, no difference between biomass allocation to S syconia and SG syconia indicates that seeds have a higher effect at low foundress density. Previous studies on other *Ficus* species have shown that host trees allocate resources to syconia based on seed number (Jandér and Herre 2010; Wang et al. 2014).

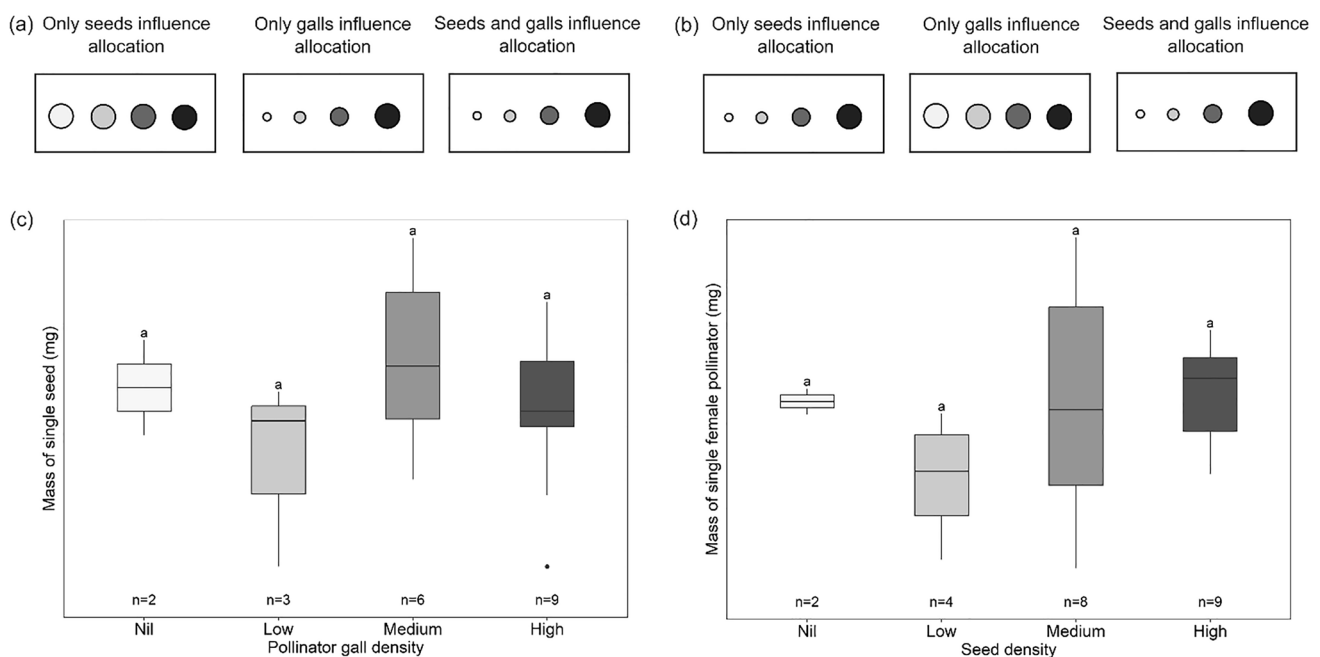
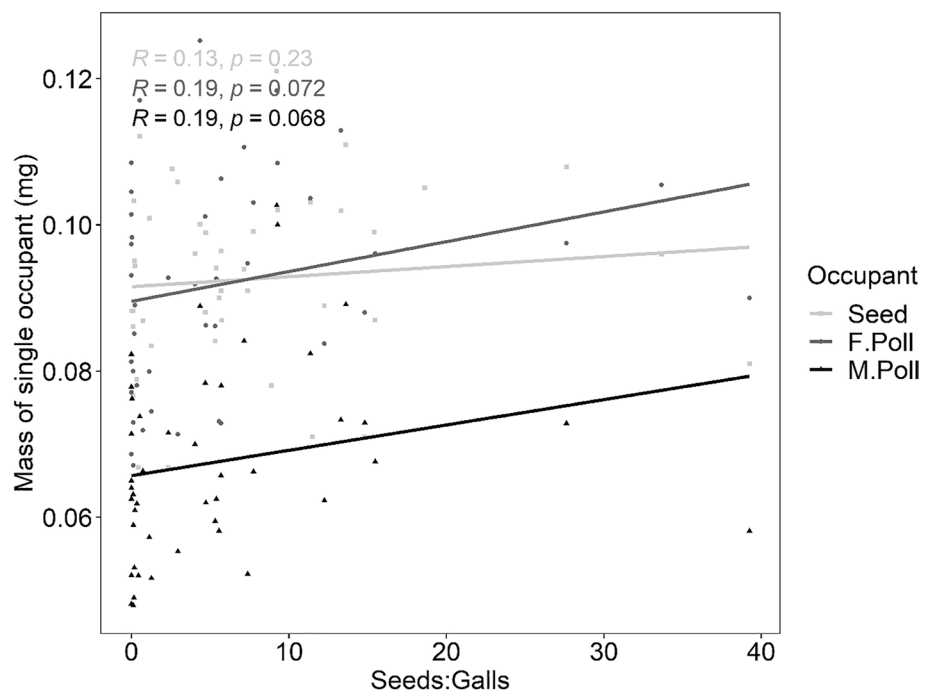


Fig. 7 Predictions (a and b) and results (c and d) of mass of individual seeds (a, c) or pollinator wasps (b, d) (see legend of Fig. 2) in the presence of different densities of the other occupant from Experiment 2. In c and d, horizontal thick lines are median values; lower

and upper lines indicate first and third quartile, respectively; whiskers indicate minimum and maximum values. Letters above error bars indicate significance at $P < 0.05$ (LMM with post-hoc Tukey tests). Sample sizes for each treatment group are indicated

Fig. 8 Dry mass of a single seed and wasp plotted against ratio of number of seeds to pollinator galls. Each point represents pooled and averaged mass of each type of occupant within a single syconium. Regression line for mass of each type of occupant is also represented. $n=40$ syconia



This is true at low foundress density, where galls are very few in number and much of the feedback to the tree from such syconia derives from seeds resulting in SG treatment syconia being perceived the same as G treatment syconia. However, at high foundress density, there was no significant difference between allocation to G syconia and SG syconia, along with an asymptotic number of seeds at medium and high foundress densities. This indicates that galls have more influence at higher foundress densities. Percentage water content of a syconium was higher in the presence of galls at low foundress density indicating galls can also influence intake of water into the syconium. That an S or G syconium containing a single foundress results in syconium abortion suggests direct feedback to the tree from low resource sinks of a few seeds or few galls to influence resources allocated to a syconium.

Two factors can explain the involvement of pollinator galls in allocation decisions to a syconium at higher foundress densities.

1. Pollinator galls are part of the male function of the tree: The host tree gets direct fitness benefits from supporting developing pollinator females. However, the benefit will depend on the number of trees in the pollen-receptive phase in the mating neighborhood, and the number of wasps that can successfully reach receptive syconia on other fig trees. The higher the number of female wasps leaving a fig syconium, the greater the probability that at least a few of them will reach a pollen-receptive syconium and contribute to the male function of the tree. As

foundress number increases, gall number within syconia also increases resulting in a male-biased offspring sex ratio, to reduce inbreeding and local mate competition (Herre 1985; West and Herre 1998; Greeff and Kjellberg 2022). Although there was lower biomass allocation to male wasps compared to females in our study (Online Resource—Figure A7), increased foundress number resulted in a male-biased sex ratio among wasp offspring (Online Resource—Figure A1). The higher allocation to the syconium translates to a thicker wall that can in turn act to protect developing pollinator wasps from environmental fluctuations and parasitoid wasps (Tzeng et al. 2014; Fan et al. 2019).

2. Pollinator galls are strong resource sinks: Insect galls in general are strong resource sinks (Miller and Raman 2019; Borges 2021; Desnitskiy et al. 2023). For example, cecidomyiid galls on the leaves of *Machilus thunbergii* had higher C/N ratios compared to surrounding leaf areas (Huang et al. 2014). A non-gall inducing herbivore *Hellinsia glenni* increased in size when it was introduced into a gall produced by tephritid fly *Eurosta solidaginis* on the plant *Solidago gigantea*, indicating that galls accumulate more resources compared to surrounding tissues (Diamond et al. 2008). The property of galls as resource sinks might be a primary cause for the influence of pollinator galls in resource allocation. However, our results also show that the effect of occupants on allocation was context-dependent; galls were influential only at higher foundress densities, indicating

that galls acting as sinks do not always instigate a significant response.

Given that galls are strong resource sinks and a part of the male function of the host tree, it is important to understand how the host tree ensures cooperation, or in this context, pollination. Host sanctions have mostly been evoked in *Ficus* to explain the cooperation of pollinator wasps in producing seeds in fig syconia. At low foundress density in Experiment 1, the mass of individual wasps was positively affected by the presence of seeds, which acts as an incentive for pollinators to pollinate or else face sanctions. Increased mass of individual pollinator offspring in the presence of seeds at low foundress density indicated that it is beneficial for the pollinator wasps to seek syconia with fewer foundress wasps and this ensures pollination as well, thus reinforcing cooperation between the partners. However, pollinator wasps might not always be successful in finding under-utilized syconia. When we experimentally simulated the scenario of low syconia availability by introducing higher densities of foundresses, host sanctions in terms of allocation to syconia or to individual galls were not observed. Other than the low-density treatment in Experiment 1, the mass of individual seeds and pollinator did not change significantly in the presence of seeds in Experiments 1 and 2. These results suggest (1) that host sanction in terms of lowered wasp mass is not observed at higher foundress densities and (2) that both seeds and pollinator galls (at higher foundress density) have autonomy when it comes to accessing resources as individual occupants. A reason for such autonomy might be that there is a limiting size, below which seeds and pollinator wasps are not viable. A lowered allocation in such a scenario may result in bladders indicating failed wasp development. We recorded higher bladder numbers at high foundress densities, especially in the G treatments, indicating that other forms of sanctions are involved at higher foundress densities. A decreased number of emerging pollinator offspring in the absence of seeds has also been recorded before (Jandér and Herre 2010). In our study, the number of galls was also marginally higher in the presence of seeds indicating a positive relationship between these two occupant types.

Another form of sanction, i.e., syconial abortions in the absence of seeds, was also not pronounced at higher foundress densities in our study. Decreased rates of abortions with increasing number of foundresses have previously been recorded in *F. racemosa* (Wang et al. 2014) in China as we also found in our study. In a gynodioecious species *Ficus hispida*, although male trees showed decreased rates of fig abortions with increasing foundress numbers, in female trees the effect was less pronounced especially under pollen-free foundress treatments (Zhang et al. 2019). This indicates that sanctions differ between

sexes, with male trees showing decreased sanctions against wasps since pollinator wasps develop only in syconia of male trees. Since *F. racemosa* is a secondarily monoecious species, evolved from dioecious ancestors (Weiblen 2000), sanctions in *F. racemosa* seem comparable to male trees of dioecious species. Other than host sanctions, or under conditions where host sanctions are not operating, cooperation between partners can be attributed to other factors. The unbeatable seed hypothesis that suggests that some flowers can only produce seeds (West and Herre 1994), insufficient egg numbers in foundresses (Nefdt and Compton 1996), and interference competition between foundresses for oviposition sites at higher densities (Wang et al. 2009) including even mortal combat (Dunn et al. 2015), are some other factors that might contribute to the coexistence of seeds and galls within the syconium.

Overall, our results show that the fig tree does not have sole control over resource allocation in fig–fig wasp system. Differential control of resource allocation to a syconium depending on its constituents reinforces the idea of context-dependency in mutualisms. It also highlights the importance of considering symbionts and their physiology in understanding the mechanism of resource exchange between the mutualistic partners. Biological market theory implies that individuals of one partner type present varied quality of services and the other partner actively chooses trading partners. Our results highlight not only that each partner plays its role, but also that both partners are actively involved in setting the terms for the interaction that ensures maximum returns to each partner within a given context.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00442-024-05566-3>.

Acknowledgements We thank Yathiraj G. for his help in collecting samples and Mary Sunitha for technical support. We are thankful to Yuvaraj Ranganathan for inputs on the manuscript.

Author contribution statement MK and RMB conceived the study and designed the experiments. MK and NVN conducted the experiments. MK analyzed the data. MK and RMB wrote the manuscript.

Funding This study was supported by a Science and Engineering Board (SERB), Department of Science and Technology, Government of India, grant to RMB. We also acknowledge funding from DST-FIST.

Availability of data and materials All raw data available on request.

Code availability All R code available on request.

Declarations

Conflict of interest We declare that there are no conflicts of interest.

Ethical approval Not applicable.

Consent to participate Not applicable.

References

- Akçay E, Roughgarden J (2007) Negotiation of mutualism: rhizobia and legumes. *Proc R Soc B* 274:25–32. <https://doi.org/10.1098/rspb.2006.3689>
- Akçay E, Simms EL (2011) Negotiation, sanctions, and context dependency in the legume-rhizobium mutualism. *Am Nat* 178:1–14. <https://doi.org/10.1086/659997>
- Anstett M-C, Bronstein JL, Hossaert-McKey M (1996) Resource allocation: a conflict in the fig/fig wasp mutualism? *J Evol Biol* 9:417–428. <https://doi.org/10.1046/j.1420-9101.1996.90404.17.x>
- Bates D (2016) lme4: linear mixed-effects models using Eigen and S4. R package version. 1:1
- Borges RM (2021) Interactions between figs and gall-inducing fig wasps: adaptations, constraints, and unanswered questions. *Front Ecol Evol* 9:685542. <https://doi.org/10.3389/fevo.2021.685542>
- Boucher DH, James S, Keeler KH (1982) The ecology of mutualism. *Annu Rev Ecol Syst* 13:315–347. <https://doi.org/10.1146/annurev.es.13.110182.001531>
- Bronstein JL (2015) *Mutualism*, 1st edn. Oxford University Press, Oxford
- Bshary R, Noë R (2003) Biological markets: the ubiquitous influence of partner choice on the dynamics of cleaner fish-client reef fish interactions. In: Hammerstein P (ed) *Genetic and cultural evolution of cooperation*. MIT Press, Cambridge (**Dahlem Workshop report**)
- Denison RF (2000) Legume sanctions and the evolution of symbiotic cooperation by rhizobia. *Am Nat* 156(6):567–576
- Desnitskiy AG, Chetverikov PE, Ivanova LA, Kuzmin IV, Ozman-Sullivan SK, Sukhareva SI (2023) Molecular aspects of gall formation induced by mites and insects. *Life* 13(6):1347. <https://doi.org/10.3390/life13061347>
- Diamond SE, Blair CP, Abrahamson WG (2008) Testing the nutrition hypothesis for the adaptive nature of insect galls: does a non-adapted herbivore perform better in galls? *Ecol Entomol* 33:385–393. <https://doi.org/10.1111/j.1365-2311.2007.00979.x>
- Dorchin N, Cramer MD, Hoffmann JH (2006) Photosynthesis and sink activity of wasp-induced galls in *Acacia pycnantha*. *Ecology* 87:1781–1791. [https://doi.org/10.1890/0012-9658\(2006\)87\[1781:PASAOW\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87[1781:PASAOW]2.0.CO;2)
- Dunn DW, Jander KC, Lamas AG, Pereira RAS (2015) Mortal combat and competition for oviposition sites in female pollinating fig wasps. *Behav Ecol* 26:262–268. <https://doi.org/10.1093/beheco/aru191>
- Fan K-Y, Bain A, Tzeng H-Y, Chiang Y-P, Chou L-S, Kuo-Huong L-L (2019) Comparative anatomy of the fig wall (*Ficus*, Moraceae). *Botany* 97:417–426. <https://doi.org/10.1139/cjb-2018-0192>
- Favery B, Dubreuil G, Chen M-S, Giron D, Abad P (2020) Gall-inducing parasites: convergent and conserved strategies of plant manipulation by insects and nematodes. *Annu Rev Phytopathol* 58:1–22. <https://doi.org/10.1146/annurev-phyto-010820-012722>
- Frederickson ME (2013) Rethinking mutualism stability: cheaters and the evolution of sanctions. *Q Rev Biol* 88:269–295. <https://doi.org/10.1086/673757>
- Galil J, Eisikowitch D (1968) On the pollination ecology of *Ficus sycamorus* in East Africa. *Ecology* 49:259–269. <https://doi.org/10.2307/1934454>
- Greeff JM, Kjellberg F (2022) Pollinating fig wasps' simple solutions to complex sex ratio problems: a review. *Front Zool* 19:1–23. <https://doi.org/10.1186/s12983-021-00447-4>
- Herre EA (1985) Sex ratio adjustment in fig wasps. *Science* 228:896–898. <https://doi.org/10.1126/science.228.4701.896>
- Herre EA, Jandér KC, Machado CA (2008) Evolutionary ecology of figs and their associates: recent progress and outstanding puzzles. *Annu Rev Ecol Evol Syst* 39:439–458. <https://doi.org/10.1146/annurev.ecolsys.37.091305.110232>
- Huang M-Y, Huang W-D, Chou H-M, Lin K-H, Chen C-C, Chen P-J, Chang Y-T, Yang C-M (2014) Leaf-derived cecidomyiid galls are sinks in *Machilus thunbergii* (Lauraceae) leaves. *Physiol Plant* 152:475–485. <https://doi.org/10.1111/ppl.12186>
- Jandér KC, Herre EA (2010) Host sanctions and pollinator cheating in the fig tree-fig wasp mutualism. *Proc R Soc B* 277:1481–1488. <https://doi.org/10.1098/rspb.2009.2157>
- Jandér KC, Herre EA (2016) Host sanctions in Panamanian *Ficus* are likely based on selective resource allocation. *Am J Bot* 103:1753–1762. <https://doi.org/10.3732/ajb.1600082>
- Jandér KC, Herre EA, Simms EL (2012) Precision of host sanctions in the fig tree-fig wasp mutualism: consequences for uncooperative symbionts. *Ecol Lett* 15:1362–1369. <https://doi.org/10.1111/j.1461-0248.2012.01857.x>
- Kathuria P, Greeff JM, Compton SG, Ganeshiah KN (1999) What fig wasp sex ratios may or may not tell us about sex allocation strategies. *Oikos* 87:520–530. <https://doi.org/10.2307/3546816>
- Kiers ET, Rousseau RA, West SA, Denison RF (2003) Host sanctions and the legume-rhizobium mutualism. *Nature* 425:78–81. <https://doi.org/10.1038/nature01931>
- Krishnan A, Pramanik GK, Revadi SV, Venkateswaran V, Borges RM (2014) High temperatures result in smaller nurseries which lower reproduction of pollinators and parasitites in a brood site pollination mutualism. *PLoS ONE* 9:e115118. <https://doi.org/10.1371/journal.pone.0115118>
- Lamichhane JR, Debaeke P, Steinberg C, You MP, Barbetti MJ, Aubertot JN (2018) Abiotic and biotic factors affecting crop seed germination and seedling emergence: a conceptual framework. *Plant Soil* 432:1–28. <https://doi.org/10.1007/s11104-018-3780-9>
- Larson KC, Whitham TG (1991) Manipulation of food resources by a gall-forming aphid: the physiology of sink-source interactions. *Oecologia* 88:15–21. <https://doi.org/10.1007/BF00328398>
- Leigh EG Jr (2010) The evolution of mutualism. *J Evol Biol* 23:2507–2528. <https://doi.org/10.1111/j.1420-9101.2010.02114.x>
- Miller DG III, Raman A (2019) Host-plant relations of gall-inducing insects. *Ann Entomol Soc Am* 112:1–9. <https://doi.org/10.1093/aesa/say034>
- Nefdt RJC, Compton SG (1996) Regulation of seed and pollinator production in the fig-fig wasp mutualism. *J Anim Ecol* 65:170–182. <https://doi.org/10.2307/5720>
- Noë R, Hammerstein P (1994) Biological markets: supply and demand determine the effect of partner choice in cooperation, mutualism and mating. *Behav Ecol Sociobiol* 35:1–11. <https://doi.org/10.1007/BF00167053>
- Pellmyr O, Huth CJ (1994) Evolutionary stability of mutualism between yuccas and yucca moths. *Nature* 372:257–260. <https://doi.org/10.1038/372257a0>
- Simms EL, Taylor DL (2002) Partner choice in nitrogen-fixation mutualisms of legumes and rhizobia. *Integr Comp Biol* 42:369–380. <https://doi.org/10.1093/icb/42.2.369>
- Tarachai Y, Compton SG, Trisonthi C (2008) The benefits of pollination for a fig wasp. *Symbiosis* 45:29–32
- Tzeng H-Y, Ou C-H, Lu F-Y, Bain A, Chou L-S, Kjellberg F (2014) The effect of fig wall thickness in *Ficus erecta* var. *beehyana* on parasitism. *Acta Oecol* 57:38–43. <https://doi.org/10.1016/j.actao.2013.06.007>
- Venkateswaran V, Shrivastava A, Kumble ALK, Borges RM (2017) Life-history strategy, resource dispersion and phylogenetic associations shape dispersal of a fig wasp community. *Mov Ecol* 5:1–11. <https://doi.org/10.1186/s40462-017-0117-x>
- Wang R-W, Ridley J, Sun B-F, Zheng Q, Dunn DW, Cook J, Shi L, Zhang Y-P, Yu DW (2009) Interference competition and high temperatures reduce the virulence of fig wasps and stabilize a

- fig-wasp mutualism. *PLoS ONE* 4:e7802. <https://doi.org/10.1371/journal.pone.0007802>
- Wang R-W, Dunn DW, Sun BF (2014) Discriminative host sanctions in a fig–wasp mutualism. *Ecology* 95:1384–1393. <https://doi.org/10.1890/13-0749.1>
- Weiblen GD (2000) Phylogenetic relationships of functionally dioecious *Ficus* (Moraceae) based on ribosomal DNA sequences and morphology. *Am J Bot* 87:1342–1357
- Weiblen GD (2002) How to be a fig wasp. *Annu Rev Entomol* 47:299–330. <https://doi.org/10.1146/annurev.ento.47.091201.145213>
- West SA, Herre EA (1994) The ecology of the New World fig-parasitizing wasps *Idarnes* and implications for the evolution of the fig–pollinator mutualism. *Proc R Soc Lond B* 258:67–72. <https://doi.org/10.1098/rspb.1994.0143>
- West SA, Herre EA (1998) Partial local mate competition and the sex ratio: a study on non-pollinating fig wasps. *J Evol Biol* 11:531–548. <https://doi.org/10.1046/j.1420-9101.1998.11050531.x>
- Zhang X, Dunn DW, Wen X, Sun BF, Wang RW (2019) Differential deployment of sanctioning mechanisms by male and female host trees in a gynodioecious fig–wasp mutualism. *Ecology* 100:e02597. <https://doi.org/10.1002/ecy.2597>

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.