Differential effects of nematode infection on pollinating and non-pollinating fig wasps: Can shared antagonism provide net benefits to a mutualism?

Justin Van Goor1,2 | Finn Piatscheck1,3 | Derek D. Houston1,4 | John D. Nason1

Abstract

1. Species pairs that form mutualistic associations are also components of broader organismal community networks. These interaction networks have shaped the evolution of individual mutualisms through interspecific interactions ranging from secondarily mutualistic to intensely antagonistic. Our understanding of this complex context remains limited because characterizing the impacts of species interacting with focal mutualists is often difficult. How is the fitness of mutualists impacted by the co-occurring interactive network of community associates?

2. We investigated this context using a model interaction network comprised of a fig and fig wasp mutualist, eight non-pollinating fig wasp (NPFW) antagonists/commensals and a nematode previously believed to be associated only with the pollinator wasp mutualist.

3. Through repeated sampling and field observations, we characterized the ecological roles of these mutualist-associated organisms to identify key antagonists. We then investigated how potential nematode infection of NPFWs could impact wasp survival across key life stages and, in turn, inferred how this influences the fitness of the fig–pollinator mutualists.

4. Unexpectedly, we found all Ficus petiolaris-associated NPFWs to be the targets for nematode infection, with infection levels sometimes exceeding that of pollinators. Experimental data collected for the most abundant NPFW species suggest that nematode infection significantly reduces their longevity. Further, comparisons of nematode loads for emerging and successfully arriving NPFWs suggest that infection severely limits their dispersal ability.

5. Through these observations, we conclude that this infection could impact NPFWs more severely than either mutualistic partner, suggesting a novel role of density-dependent facultative mutualism between figs, pollinator wasps and the nematode. This antagonist-mediated suppression of other network antagonists may present an ecologically common mechanism through which antagonists can present net benefits for mutualists’ fitness.
1 | INTRODUCTION

Mutualisms, or reciprocally beneficial interspecific interactions, are ubiquitous in nature and strongly influence ecological processes that, in turn, have shaped the trajectories of organismal evolution (Kiers et al. 2010). Therefore, understanding the ecology and evolution of mutualistic associations is a crucial component to understanding ecosystem function (Bronstein, 2015). To date, the majority of theoretical (Archetti, 2019; Ferriere et al. 2002) and empirical (Heil et al. 2009; Nelson et al. 2018; Paterson et al. 2010) studies have focused on the pairwise interaction between obligate mutualistic partners. Virtually all mutualistic species pairs, however, are members of more complex networks of organismal interactions that may range from secondarily mutualistic to neutral, or strongly antagonistic in nature (Fath, 2007; Melián et al. 2009). This context is often lacking (though expanding; see Nuismer et al. 2018 and Arroyo-Correa et al. 2019), but necessary for a clearer understanding of the ecological and evolutionary dynamics of mutualistic systems (Hall et al. 2020; Levine et al. 2017).

Generally, ecological theory predicts that interaction with community-level associates stabilizes or enhances mutualism fitness (Banerjee et al. 2020; Chagnon et al. 2020; Jones et al. 2009; Morris et al. 2003). However, works estimating negative (Bachelot & Lee, 2020; Ferriere et al. 2002; Mougi & Kondoh, 2014) and neutral (Arizmendi et al. 1996; Bronstein, 2001) effects also exist, showcasing a presumed role for context dependence in the diversity of species assemblages. The body of empirical research evaluating the role of interaction networks on mutualism fitness is limited, but growing (Song et al. 2020; Thompson & Fernandez, 2006). One impediment to investigating the effects of community-level antagonism on mutualism fitness is that lifetime fitness in many systems is difficult to quantify (Bronstein, 2015; West et al. 1996). This can be alleviated by focusing on model systems in which all intimately interacting species are known, ecological roles as mutualists and exploiters are well-understood and key components of lifetime reproductive success are easily estimated.

One such model system is the fig–fig wasp obligate nursery–pollination mutualism. *Ficus* species (more than 750 world-wide, Berg, 1989) produce a nearly closed, urn-shaped inflorescence (a fig) that attracts wasps through volatile floral compounds (Wang et al. 2016). Figs are entirely reliant on typically host species-specific fig wasps (Hymenoptera: Agaonidae, many genera) for pollination services, and pollinator wasp larvae develop within a subset of the fig’s ovules (Janzen, 1979). Pollinators have short adult life spans (<60 hr; Kjellberg et al. 1988), but excellent dispersal capabilities, exploiting wind currents to reach receptive trees that are often located many kilometres from their natal trees (Harrison & Rasplus, 2006; Nason et al. 1998).

In addition to obligate mutualistic relationships with pollinating wasps, individual species of *Ficus* are subject to exploitation by a diversity of non-pollinating fig wasp (NPFW) genera (multiple Families; Bouček, 1993). Each fig species typically supports at least one, and often several, NPFW species (Compton & Hawkins, 1992) and, like pollinating wasps, many are host fig specific (though exceptions exist, see Farache et al. 2018) and appear to be attracted to receptive figs by the same volatile blends produced to attract pollinators (Proffit et al. 2018). In contrast to the pollinator, which enters and oviposits inside the fig, all Neotropical NPFWs oviposit from the fig’s outer surface by inserting their ovipositors through the fig wall (Elías et al. 2008). Depending on the species, most NPFWs parasitize pollinators and/or other non-pollinators, while others consume developing seeds or induce galls within the fig wall in close proximity to developing pollinators (Segar et al. 2018). Thus, many NPFW species have negative fitness impacts on the fig–pollinator mutualism (Borges, 2015; Zhang et al. 2020; Zhang & Li, 2020).

To date, the majority of research investigating antagonist effects on the fig–fig pollinator mutualism has focused on NPFWs. Equally pervasive, but much less studied, are nematodes associated with fig pollinators (genera including *Schistonchus*, *Pristionchus*, *Ficophagus*, *Caenorhabditis* and others; Davies et al. 2017; Martin et al. 1973; Susoy et al. 2016; Vovlas & Larizza, 1996; Woodruff & Phillips, 2018). Entomopathogenic nematodes of the genus *Parasitodiplogaster* (Diplogastridae) are pantropical associates of pollinating fig wasps (Poinar & Herre, 1991). The life history of *Parasitodiplogaster* is tightly coupled with that of their pollinating wasp hosts, which they rely upon for energy, transport to a new fig and subsequent reproductive success. For a description of the life cycle of figs, pollinator wasps, NPFWs and *Parasitodiplogaster*, see Figure 1. *Parasitodiplogaster* nematodes require transport to a new fig at each generation, and it is thus necessary that their impacts on female pollinator wasp survival are not so great as to prohibit successful dispersal to trees bearing receptive stage figs (Gupta & Borges, 2019; Herre, 1995; Van Goor et al. 2018). Despite this constraint, the virulence of nematode infection varies across species as a function of host-wasp species population density (Herre, 1993) and can range from avirulent or commensal (Shi et al. 2019; Van Goor et al. 2018), to virulent (Herre, 1993, 1995), reducing host offspring production by up to 15%.

While infection by *Parasitodiplogaster* nematodes can negatively influence the fitness of pollinating fig wasps (definitive hosts), the incidence and fitness effects of nematode infection on co-occurring NPFWs have not been described. Although pollinators and NPFWs share the same developmental space within the fig and are both exposed to infective juvenile nematodes while emerging from a mature fig, infection of NPFWs should be maladaptive for the nematodes. Pollinator wasp hosts enter figs to lay their eggs, granting infective nematodes access to the next generation of emerging hosts.

**KEYWORDS**

antagonism, dispersal, fig nematode, fig wasp, fitness limitation, mutualism, non-pollinating fig wasp, *Parasitodiplogaster*
Conversely, all Neotropical NPFWs oviposit from the exterior of the fig, precluding associated nematodes access to the interior of the fig and new hosts. Therefore, nematode infection of NPFWs is a behaviour that should be strongly selected against (Giblin-Davis et al. 1995; Krishnan et al. 2010; Vovlas & Larizza, 1996). Surprisingly, however, *Parasitodiplogaster* has been reported to infect multiple Mexican (Van Goor et al. 2018) and Panamanian (personal observation) NPFW species. If nematodes negatively impact the fitness of NPFWs that interact antagonistically with figs and their pollinators, they could have previously unappreciated benefits for the fitness and persistence of the fig–pollinator mutualism (as has been described for ants associated with fig communities, Bain et al. 2014). Further, the tightly co-occurring nature of this model system allows unprecedented ability to evaluate the complex role of interacting antagonists of varying ecology on focal mutualist-partner reproductive success over space and time.

Here, we investigate how *Parasitodiplogaster* nematode infection may limit NPFW fitness and, in turn, potentially benefit the mutualistic partnership between figs and wasps (Figure 2). If nematode infection of NPFWs is widespread and significantly reduces NPFW reproductive ability, we can hypothesize a previously undescribed secondarily mutualistic association between nematodes and their fig–fig wasp hosts. First, we quantify the level of antagonism between NPFW species and the fig–pollinator mutualists in the Sonoran Desert rock fig *Ficus petiolaris* to identify which NPFWs may limit mutualism fitness. We then determine the incidence and number of nematodes infecting those NPFWs to identify the ecological relevance of this infection. Finally, we estimate the fitness effects of infection through its potential impacts on key components of NPFW life history such as dispersal ability and longevity.

FIGURE 1  Life cycle: Pre-receptive phase monoecious figs (a) grow on *Ficus* branches before the development of internal female flowers. The fig is then receptive to pollinating wasps (b), which enter the fig through a terminal pore (ostiole) and then pollinate a subset of flowers and oviposit eggs into another subset before dying. If the pollinator was infected by nematodes they will molt from infective juveniles to consumptive adults that feed on wasp tissue before molting again into reproductive adults (1). They will then aggregate outside the pollinator and form mating clusters before female nematodes disperse throughout the fig to lay eggs and die. During (b) and early (c) phases, NPFW species oviposit eggs from the exterior of the fig into developing wasp galls, unpollinated florets or developing seeds. During the inter-floral phase (c), pollinator and NPFW offspring larvae develop in galls, nematode eggs develop on these galls and seed development takes place. In male-phase (d), male flowers develop within the fig, adult male pollinators and NPFWs emerge to inseminate females and release them from their galls, and infective stage juvenile nematodes position themselves on wasp galls. In later male-phase, female pollinators collect pollen from flowers and juvenile nematodes perform nictation behaviour to contact and infect hosts (2). Using a hole bored by pollinator males, female pollinators, NPFWs and nematodes all exit the fig to start the cycle anew. As fig seeds reach maturity, the fig becomes pumped with sugar to promote herbivory and seed dispersal (e). Total developmental time for the *Ficus petiolaris* community (a–e) is between 6 and 10 weeks (personal observation).

2 | MATERIALS AND METHODS

2.1 | The *F. petiolaris* system of Northwestern Mexico

*Ficus petiolaris* is a monoecious (male and female flowers and function in the same individual, albeit strongly protogynous) rock-strangling fig that is widespread throughout Baja California and mainland Mexico. The nine census sites investigated in this study are located in the states of Baja California and Baja California Sur (Table S1 and Figure S1), where *F. petiolaris* is the only native fig species. *Ficus petiolaris* is obligately
FIGURE 2 Hypothesized interaction schematic between figs, pollinators, NPFWs and Parasitodiplogaster nematodes. The solid green arrow indicates the mutualism between figs and their pollinating fig wasps. NPFWs have demonstrable antagonistic and fitness-reducing effects (solid red arrows) against both figs and pollinating fig wasps. Fig wasp nematodes can range from virulent to relatively benign against pollinator hosts (dotted red arrow). The interaction between fig nematodes and NPFWs is not understood but is hypothesized to be antagonistic (solid red arrow) due to the average length of time infective nematodes would likely be associated with these wasps. It is hypothesized that nematode-induced reduction of NPFWs may directly benefit pollinating wasps and their host fig trees

pollinated by an undescribed Pegoscapus wasp (Agaonidae), which has been shown to be a single species based on phylogenetic analyses (Satler et al. 2019). Pegoscapus wasps die inside figs after pollination and oviposition, and the number of foundress wasps contributing offspring to each fig can be counted. This Pegoscapus species is subject to parasitism by a single species of Parasitodiplogaster nematode, whose 28S rDNA sequences form a single, well-supported clade that clusters with other publicly available Neotropical Parasitodiplogaster sequences (Van Goor et al. 2018). No other fig-associated nematode genera have been observed in F. petiolaris figs.

In addition to a pollinator wasp and associated Parasitodiplogaster, F. petiolaris in Baja California is the host to eight chalcidoid NPFW species. This community is comprised of three Idarnes species (Sycophaginidae, Satler et al. 2020); one from species group flavicollis (ovule galler) and two from species group carme (kleptoparasites or parasitoids, Farache et al. 2018). These three species are referred to herein as Idarnes flavicollis and Idarnes carme species 1 and 2 respectively. Additionally, there are two species of Heterandrium (Pteromalidae), both of which gall F. petiolaris ovules (Duthie & Nason, 2016), and one species of Ficicola (Pteromalidae) that generates large galls protruding from the receptacle into the interior of the fig and which may spatially impact developing seeds or larvae (Conchou et al. 2014). Finally, one species of Physothorax (Torymidae) and one species of Sycophila (Eurytomidae) are parasitoids that develop within other fig wasp larvae (Farache et al. 2018).

2.2 Which NPFWs antagonize the F. petiolaris mutualism?

Mature F. petiolaris trees were geo-referenced at nine sites along a latitudinal gradient spanning 741 km of the Baja California peninsula. Mature, wasp-releasing figs were sampled from each site and measured, pollinating foundress wasps counted, pollinating and NPFW offspring produced per fig collected and the presence/absence of juvenile nematodes assessed. These study sites were visited at four time points (2012–2014) to ensure adequate sample sizes of wasp-producing figs in both wet (October–December) and dry (May–July) seasons. The wasp offspring were preserved in 95% ethanol and figs were air-dried. Pollinators and NPFWs per fig were tallied by species and sex. A subset of the dried figs were cut into quarters and suspended in 90% ethanol to determine seed production.

We analysed the effects of NPFWs and nematodes on two primary fitness components of the mutualism: pollinator offspring and seed production per fig. We used a GLMM with Poisson errors and a log-link function to study the pollinator offspring production per fig as a response variable against the predictor variables site, tree nested within site, season (wet or dry), pollinator foundress count, fig volume (mm$^3$), nematode infestation (presence or absence) and the number of NPFW offspring produced by each of the eight NPFW species. These GLMM analyses were conducted using the glmer function in the lme4 package (Bates et al. 2015) for R (R Core Team, 2020).

We used a second GLMM to model seed production per fig as a function of the same predictor variables as in the preceding model. The directionality and significance of the association observed between species offspring production in these models can allow for the inference of ecology and potential antagonism against figs and pollinators, but should be viewed in the appropriate ecological context (Raja et al. 2015). In general, larger, more productive figs may produce more pollinators, seeds and NPFW offspring, making positive correlations between interacting associates a meaningful null hypothesis. However, significant positive associations could also predict kleptoparasitism or parasitoid infection, while significant negative associations could suggest competition for resources. In all models, the predictor variables site, tree nested within site and season were treated as random effects because of the over-dispersion of pollinator offspring and seed counts observed between trees within sites over time. The glmer function does not report $p$-values for random effect variables or associated nested terms.

2.3 Frequency of interaction between nematodes and NPFWs

We sampled F. petiolaris-associated wasps of each NPFW species emerging from mature figs across each study site and through time. For wasps from nematode-infested figs, the thoracic and abdominal cavities were dissected using 0.25-mm diameter tungsten
needles to determine the presence and number of infective juvenile nematodes.

2.4 | Does nematode infection influence NPFW life history?

Previous investigations have shown that Parasitodiplogaster infection only marginally influences Pegascopus pollinator fitness in F. petiolaris, notably when the pollinator is overexploited (>10 nematodes per host, Van Goor et al. 2018). Because NPFWs are not ideal hosts for nematodes, their role in potential NPFW life-history limitation has not been investigated. A key fig wasp life-history stage potentially impacted by Parasitodiplogaster infection is the process of dispersal from the natal fig to receptive new figs. To examine this effect, the number of nematodes infecting NPFWs emerging from mature, nematode-infested figs was compared to the number of nematodes infecting successfully dispersed NPFWs arriving at receptive figs at two sampling sites. If nematode infection decreases NPFW dispersal, we predict that successfully dispersed wasps will contain fewer nematodes than wasps emerging from nematode-infested figs. The comparison of infection rates between emerging and successfully dispersed wasps of each NPFW species was conducted by exact test using the poisson.test function in R.

To investigate possible NPFW longevity reductions due to nematode infection, we conducted longevity experiments (see Van Goor et al. 2018) and used dissections to determine infection incidence and the number of nematodes per host compared to the hour in which the individual wasp died. Survivorship curves comparing the hour of mortality for nematode-infected and uninfected NPFWs were analysed using a log-rank test. We conducted a GLM with Poisson errors and a log-link function for the number of nematodes extracted per wasp host against the predictor variables longevity study, hour and individual fig to determine the effect of nematode infection on reduced longevity for infected NPFWs with adequate sample sizes. These analyses were conducted in JMP® Pro 14 (SAS Institute Inc., Cary, NC, 1989–2021).

3 | RESULTS

3.1 | Which NPFWs antagonize the F. petiolaris mutualism?

The four field collections conducted from 2012 to 2014 yielded a total of 2,187 mature, wasp-producing figs. We obtained an average of 260.1 figs (range 166–373) per site, with each fig producing an average of 84.7 (range 2–503) fig wasps. Of these wasps, 40.1% (36.5 per fig) were pollinators and 59.9% (48.2 per fig) were NPFWs. All eight NPFW species were observed at all sites except for Physothorax and Sycophila, which were absent at one and two sites respectively. The three Idarnes species were the most abundant NPFWs, collectively accounting for an average of 49.6% of all wasps observed across the F. petiolaris sites, with Idarnes flavicollis as the most common (24.2% of all wasps, Table S2). Illustrative of the high abundance of NPFWs in the F. petiolaris interaction network, we observed only 15 (0.69%) figs in which NPFWs were absent. Interestingly, 160 (7%) of the mature figs surveyed contained zero pollinating foundresses and no pollinating wasp offspring, yet still produced NPFW of all genera. Nematode infestation was not observed in any of these zero-foundress figs.

In the pollinator GLMM (n = 2,187, df = 11, Log Likelihood = −29,544.2, full model details available in the Supplement), we found pollinator offspring per fig to be significantly associated with fig volume (positive), pollinator foundress count (positive) and nematode infestation (negative) (all p-values < 0.001). Figs infested with nematodes produced significantly fewer pollinator offspring (p < 0.001), though this effect size was small (mean of 36.80 and 37.17 pollinator offspring in infested vs. uninfested figs).

Interestingly, pollinator offspring production had a range of significant (both positive and negative) and non-significant associations with NPFWs (Table 1).

Of the mature figs collected, a total of 120 (60 from two sites) were examined to investigate potential antagonistic effects of NPFWs with fig seed production. The fig seed model (GLMM, n = 120, df = 12, Log Likelihood = −827.20) revealed a highly significant positive relationship between seed production per fig and the predictor variable fig volume (p = <0.001). Although seed production was not significantly associated with pollinator foundress count (p = 0.746), it had a significant positive association with pollinator offspring production (p = 0.048). Interestingly, seed production was found to be significantly higher (by 10.3%) in figs with nematode infestation (p < 0.001). As in the pollinator GLMM, significant associations between fig seed production and NPFWs ranged from positive to negative, while others were not significant (Table 1).

3.2 | Frequency of interaction between nematodes and NPFWs

Parasitodiplogaster infestation was observed in 36% (780 of 2,187) of all mature figs sampled, varying between 12% and 80% depending on individual study site and collection trip. From the infested figs, a total of 2,791 emerging pollinators and NPFWs (range of 4 to 1,182 individuals depending on species) were dissected to determine the presence and number of infective juvenile nematodes. With the exception of Sycophila (presumably due to very low sample sizes), all NPFW species were found to be infected by Parasitodiplogaster, with the incidence of infection in individual wasps varying substantially among species, ranging from 6.7% to 39.6% (Table 2). Interestingly, the number of nematodes per infective event also varied substantially among NPFW species. Idarnes flavicollis and Heterandrium 1, in particular, experienced the highest incidences of nematode infection (39.6% and 27.8% respectively) and also highest average nematode loads (2.52 and 2.32 nematodes per host). These nematode loads...
TABLE 1  GLMM results for the Ficus petiolaris community showing associations in offspring production between wasp species pairs and with fig seed production. The analysis of F. petiolaris seed production represents a subset of the total dataset. + indicates a significant positive association (p = <0.05), − indicates a significant negative association (p = <0.05) and ns indicates non-significance (p = >0.05)

| Fig seeds | Pegoscapus | Idarnes flavicollis | Idarnes carme 1 | Idarnes carme 2 | Heterandrium 1 | Heterandrium 2 | Ficicola | Physothorax | Sycophila |
|-----------|------------|---------------------|-----------------|-----------------|----------------|----------------|----------|------------|----------|
| Fig seeds | /          | /                   | /               | /               | /              | /              | /        | /          | /        |
| Pegoscapus| +          | /                   | /               | /               | /              | /              | /        | /          | /        |
| Idarnes flavicollis | −          | −                   | /               | /               | /              | /              | /        | /          | /        |
| Idarnes carme 1 | ns         | +                   | −               | /               | /              | /              | /        | /          | /        |
| Idarnes carme 2 | +          | +                   | +               | +               | /              | /              | /        | /          | /        |
| Heterandrium 1 | ns         | −                   | +               | −               | +              | /              | /        | /          | /        |
| Heterandrium 2 | +          | +                   | +               | +               | +              | /              | /        | /          | /        |
| Ficicola   | +          | ns                  | +               | ns              | ns             | ns             | /        | /          | /        |
| Physothorax| −          | −                   | −               | ns              | ns             | ns             | /        | /          | /        |
| Sycophila  | ns         | −                   | −               | ns              | ns             | ns             | +        | /          | /        |

TABLE 2  Infection of Ficus petiolaris-associated pollinator (Pegoscapus) and NPFW (all other genera) wasp species by Parasitodiplogaster nematodes. Results are from wasps emerging from mature, nematode-infested figs and are pooled across all site and year collections

| Wasp species | Wasps dissected | Percent infected | Average nematodes per host | Median nematodes per host | Maximum nematodes per host |
|--------------|----------------|------------------|----------------------------|---------------------------|---------------------------|
| Pegoscapus   | 1,182          | 61.5%            | 4.03                       | 3                         | 50                        |
| Idarnes flavicollis | 573          | 39.6%            | 2.52                       | 2                         | 21                        |
| Idarnes carme sp. 1 | 460          | 8.5%             | 1.21                       | 1                         | 4                         |
| Idarnes carme sp. 2 | 135         | 13.3%            | 1.56                       | 1                         | 5                         |
| Heterandrium sp. 1 | 110          | 27.8%            | 2.32                       | 1                         | 15                        |
| Heterandrium sp. 2 | 122          | 18.8%            | 1.65                       | 1                         | 4                         |
| Ficicola     | 104           | 6.7%             | 1.57                       | 1                         | 4                         |
| Physothorax  | 59            | 8.5%             | 1.60                       | N/A                       | N/A                       |
| Sycophila    | 4             | 0%               | N/A                        | N/A                       | N/A                       |

were significantly greater than in other NPFW species, though not as high as in pollinators (Table S3).

### 3.3  Does nematode infection influence NPFW life history?

Nematode infection of NPFWs appears to be widespread and may impact their fitness through reduced longevity and dispersal ability, but this effect has not been previously examined. A total of 281 NPFWs of various species were collected as they were arriving at receptive fig trees. Although we were able to sample six of the eight NPFW species associated with F. petiolaris (Table S4), only Idarnes flavicollis and Idarnes carme 1 and 2 (the most common NPFWs, Table S2) were collected frequently, with sample sizes of 111, 86 and 71 individuals respectively. In each of these Idarnes species, the incidence of nematode infection in individuals arriving at receptive fig trees (3.6%, 1.2% and 2.8% respectively) was lower than in the population as a whole (Table 3). Further, arriving wasps had fewer infective nematodes per individual than did wasps departing nematode-infested figs (poisson test, p-values = <0.001, 0.005 and <0.001 respectively; Table S3).

Indeed, virtually all arriving infected wasps were infected by only a single juvenile nematode (Table S4), compared to a mean of 2.5 nematodes per infected emerging Idarnes flavicollis wasps (Table 2). Thus, in these three NPFW species we found that both the rate of infection and the number of nematode individuals observed per infective event were lower in wasps that successfully dispersed to receptive figs as compared to wasps emerging from infested figs (Table 3).

Three separate controlled longevity trials were conducted between 2014 and 2016, which produced NPFWs of all associated genera from a total of 50 figs (29 infested and 21 uninfested). Of these longevity figs, dissection efforts were conducted for between four and 410 NPFW individuals (based on species availability) to confirm nematode infection status and nematode infection load. Meaningful samples sizes of infected wasps (>10 individuals) were only possible for three common NPFW species: Idarnes flavicollis (174 longevity participants dissected and 33 infected), Idarnes carme species 1 (410 dissected and 30 infected) and Heterandrium species 1 (64 dissected and 14 infected). Of these three species, nematode infection was found to significantly reduce the longevity of Idarnes flavicollis (Figure 3, log-rank test, n = 33, df = 1, chi-square = 4.824, p = 0.028) but not Idarnes carme species 1 or Heterandrium species 1.
TABLE 3  Infection and dispersal data for the most abundant NPFWs associated with Ficus petiolaris. To estimate the percentage of the total wasp population infected with nematodes, we multiplied percentage of infected wasps emerging from infested figs by the percentage of all figs found to be infested with nematodes (36%). To estimate the rate of failure of nematode-infected wasps to disperse, we divided the percentage of individuals arriving with nematodes by the percentage emerging with nematodes. Finally, to estimate the percentage of NPFW individuals removed from the population due to nematode infection, we multiplied the total percentage of individuals infected by the failure to disperse rate.

| Non-pollinator wasp species | Percent infected wasps emerging from infested figs | Percent of total wasp population infected | Percent infected wasps dispersed to receptive figs | Failure rate of infected wasps to disperse to receptive figs | Percent of wasps excluded due to nematode infection per generation |
|-----------------------------|---------------------------------------------------|------------------------------------------|-----------------------------------------------|----------------------------------------------------------|---------------------------------------------------------|
| Idarnes flavicollis         | 39.6%                                             | 14.3%                                    | 3.6%                                          | 0.91                                                      | 13%                                                     |
| Idarnes carme species 1     | 8.5%                                              | 3.1%                                     | 1.2%                                          | 0.86                                                      | 2.7%                                                    |
| Idarnes carme species 2     | 13.3%                                             | 4.8%                                     | 2.8%                                          | 0.79                                                      | 3.8%                                                    |

4 | DISCUSSION

Nematode infection of F. petiolaris pollinators, while common and significantly negatively associated with offspring production, is of relatively benign effect, limiting pollinator production by less than 1% each generation (consistent with Gupta & Borges, 2019, Shi et al. 2019 and Van Goor et al. 2018). In F. petiolaris, it has been previously observed that nematode infection does not appear to limit pollinator longevity, dispersal ability or offspring production except infrequently when many (10 or more) nematode individuals infect the same host (Van Goor et al. 2018). Here, we found a significant positive association between nematode infestation and seed production (an increase of 10%). This surprising, apparently beneficial effect has not been previously reported and the mechanism responsible for increased seed production is not currently understood. Further, we found that nearly all NPFWs associated with F. petiolaris are targets for nematode infection which may severely limit their dispersal ability and longevity, and therefore suppress their exploitation of the fig–fig wasp mutualism. If real, these previously overlooked mutualistic associations may act as drivers for community-network dynamics and may have bolstered the fig–fig wasp mutualism over evolutionary time-scales, particularly in species burdened with many NPFWs. Further, similar ‘hidden’ interactions may underlie interaction network dynamics in other Arthropod-rich communities, especially those in which spatially intimate taxa utilize similar energetic and reproductive resources in ephemeral environments.

4.1 | Which NPFWs antagonize the F. petiolaris mutualism?

Biological communities are structured and modulated through organismal network interactions ranging from mutualistic to antagonistic (De Andreadzi et al. 2019). Individual species may enact profoundly higher influence on focal species than others, which has been predicted to either stabilize or destabilize network structure (Bachelot & Lee, 2020; Heil et al. 2009; Montesinos-Navarro et al. 2017). A more robust understanding of network-level interactions can provide essential nuance to the evolutionary history and trajectory of whole communities (Paterson et al. 2010). Like the vast majority of monoeocious fig systems, the NPFW community associated with F. petiolaris is speciose (Bouček, 1993). Interestingly, in F. petiolaris, the total production of NPFWs per fig typically outnumbers the production of pollinating wasp mutualists (Table S2). We observed that 7% (160) of all figs surveyed produced NPFW offspring in the absence of a pollinating foundress, and that all NPFW genera were produced in these figs. The fact that Parasitodiplogaster nematodes were not observed in any of the zero-foundress figs reinforces our contention that NPFW species are not vectors for nematode transmission to the interior of receptive figs, as has been previously suggested (Giblin-Davis et al. 1995; Jauharlina et al. 2012; Vovlas & Larizza, 1996).

Within the NPFW community of F. petiolaris, three wasps of the genus Idarnes were particularly common, consisting of nearly...
Pollinating fig wasps are the ‘appropriate’ hosts for Parasitodiplogaster nematodes because these wasps enter receptive figs and secure reproductive space for nematodes. Indeed, we observed here that pollinators are infected more frequently (Table 2) and with significantly higher nematode loads (Table S3) than NPFW hosts. However, certain NPFW species are infected more frequently and tend to have higher nematode loads than other NPFWs, despite the fact that all NPFWs emerge within the fig cavity. Interestingly, this appears to correspond with the NPFW species that are most abundant and have the most fitness-limiting effects on the mutualism (Idarnes flavicollis, Physothorax and Heterandrium 1) and those that emerge from reproductive galls at the same time as pollinators (personal observation). To ensure their own reproductive opportunities, nematodes that infect pollinators should delay their potentially fitness-limiting behaviour until their host has successfully arrived at a receptive fig. Pollinating fig wasps are relatively short lived (typically <60 hr, Kjellberg et al. 1988; Van Goor et al. 2018), meaning that nematode-moulting cues (Figure 1) have likely evolved in response. However, F. petiolaris NPFWs have much longer life histories (mean 150–350 hr depending on species, unpublished data) in which they search for receptive figs or oviposit into multiple figs (Ghara et al. 2014). Compared to the pollinator, NPFWs infected with nematodes spend significantly more time infected, which may lead to more detrimental effects on longevity, dispersal ability and overall fitness. This prolonged interaction between nematodes and their ‘incorrect’ host provides important context that has been described to benefit mutualism fitness in theoretical models (Banerjee et al. 2020; Guimarães et al. 2017; Song et al. 2020).

### 4.3 Does nematode infection influence NPFW life history?

NPFWs that fail to arrive at receptive figs are incapable of reproducing. While the pollinators frequently arrive at receptive figs infected with nematodes, we only infrequently observed infected NPFWs successfully arriving at receptive F. petiolaris figs. When NPFW infection was observed (in only seven of 275 wasps) it was typically with a single nematode, and in one instance two (Table S4). This strongly suggests that NPFW antagonists of F. petiolaris do not have the same tolerance to nematode infection as pollinating mutualists, and that nematode infection may severely limit NPFW dispersal ability, longevity and, consequently, reproductive capabilities in natural environments.

Efforts were also made to investigate the Parasitodiplogaster infection effects on NPFW longevity. We conducted longevity observations on three of the more abundant NPFWs but were only able to identify potential longevity limitations for the most common and most antagonistic NPFW (Idarnes flavicollis). The non-significance of longevity observations for other NPFWs may represent false negatives due to low sample sizes and to the artificial conditions in which the trials took place (small plastic vials). Notably, all participant wasps,
infected and uninfected, remained relatively stationary within these vials until they died. These conditions do not appropriately represent the natural stresses NPFW wasps experience as they disperse, oviposit and avoid predation throughout their life spans, and so should be treated with interpretive caution. However, the clear longevity reduction present for *Idarnes flavicollis* despite these experimental conditions could suggest a broader, more profound role that nematode infection may represent for NPFW fitness in nature and may suggest a mechanism capable of explaining our arrival observations.

Alternatively, one could assume that the non-significant NPFW longevity curves of *Idarnes carne* species 1 and *Heterandrium* species 1 suggest a truly benign effect due to nematodes. NPFWs in *F. petiolaris* and other fig communities live much longer than pollinators and visit and likely oviposit into multiple figs. Perhaps the infection pattern we have observed here can be explained simply by nematodes disembarking their NPFW hosts once they arrive at a receptive fig. Like many nematodes, *Parasitodiplogaster* is extremely desiccation sensitive and will perish within minutes of outside exposure (personal observation), making their ability to exit a wasp and enter through the thick fig wall seemingly unlikely. Further, if this hypothesis was supported, it could be expected that one would observe significantly fewer nematodes within NPFWs that survive longer in longevity experiments (more opportunities to disembark). However, this notion is not supported for any of the examined NPFW species (Tables S4–S9; Figure S3); it appears as if the nematodes that enter a NPFW remain there at least until the time of wasp mortality.

It was previously estimated that pollinators average four nematodes per infection event and lose 2.8% of the general population each generation due to *Parasitodiplogaster* exploitation (Van Goor et al. 2018). Here, we find that the NPFW species associated with *F. petiolaris* are not only subject to nematode infection, but they are likely sensitive to infection by even a single nematode. With data from the more abundant arriving *Idarnes* wasps, we similarly estimated net losses due to infection, finding that they are similar to substantially higher than those suffered by pollinators (Table 3). Of the three *Idarnes* species, *Idarnes flavicollis*, which has been identified as the most abundant and one of the most antagonistic NPFWs associated with *F. petiolaris*, loses an estimated 13% of the general population due to nematode infection every generation. The two *Idarnes carne* species also show loses, though less dramatic, and it is likely (with increased sampling) that all of the *F. petiolaris*-associated NPFWs suffer fitness limitations due to nematode infection. Similar patterns of infection have been observed in NPFWs associated with five other host fig species in Panama (personal observation), suggesting that nematode impacts on NPFW communities are much more widespread than previously recognized (including in two separate *Ficus* subgenera). In particular, NPFWs associated with *F. popenoei* may be infected more frequently than pollinators (Table S7) and may experience more detrimental fitness limitations (Tables S8 and S9).

In aggregate, this suggests that nematode infection may remove a sizeable proportion of the total wasp-antagonist community in each generation. This may be explained as an indirect effect (Gillespie & Adler, 2013; Guimarães et al. 2017) or may represent a novel density-dependent facultative mutualism between *Parasitodiplogaster* nematodes, *Pegoscapus* pollinating wasps and *F. petiolaris*. Ultimately, this may present a mechanism through which antagonist communities are modulated over shared network resources in other non-fig systems as well, especially those characterized by multiple arthropod species co-occurring in ephemeral environments or within the same host. Such ‘the enemy of my enemy is my friend’ scenarios may be much more common in nature than currently appreciated, but requires careful future evaluation.

5 | CONCLUSIONS

*Parasitodiplogaster* infection of NPFWs associated with *F. petiolaris* is ubiquitous and may have substantial ecological and evolutionary consequences for fig wasp community dynamics. This community context is essential to understanding the evolution of interspecific interactions (Palmer et al. 2010) and may represent just one puzzle underlying ecological modulation within this and other arthropod communities. In addition to NPFW suppression, *Parasitodiplogaster* nematodes may be able to clear figs of harmful and widespread *Fusarium*-like fungal infections that are capable of eliminating entire crops from fig trees (Michalides et al. 1996). The mechanism underlying this secondarily mutualistic behaviour of *Parasitodiplogaster* for fig systems is currently unknown, but will be the target of future research. Together, these interactions suggest more ecologically profound roles that *Parasitodiplogaster* (and perhaps other nematodes) may provide for their hosts. Similar facultative mutualisms may potentially act as hidden drivers for community-network dynamics elsewhere, especially in lesser-studied, invertebrate-rich assemblages.

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AUTHORS’ CONTRIBUTIONS

All the authors collected the data, performed the analyses, assisted with the development of the manuscript and have approved the current draft of this manuscript. In addition, J.V.G. developed the conceptual design of the study, provided funding for data collection and wrote the manuscript; F.P. performed GLMM analyses and prepared the figures; D.D.H. prepared the figures; J.D.N. developed the theoretical and conceptual design of the study, provided funding for data collection and provided analytical assistance.
DATA AVAILABILITY STATEMENT
Data and code used within this manuscript will be publically available on the Dryad Digital Repository https://doi.org/10.5061/dryad.djh9w0w08 (Van Goor et al. 2021).

ORCID
Justin Van Goor https://orcid.org/0000-0001-7498-9315
Finn Piatsock https://orcid.org/0000-0002-1796-6288
Derek D. Houston https://orcid.org/0000-0002-2730-1159

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