

Current Opinion in Insect Science

Competitive interactions in insect parasitoids: effects of microbial symbionts across tri-trophic levels --Manuscript Draft--

Short Title:	Microbe-mediated competition in insect parasitoids
Keywords:	parasitoid competition; microbe-mediated effects; parasitoid-associated symbiont; herbivore-associated symbiont; extrinsic competition; intrinsic competition; fighting arena
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Abstract:	Competition for hosts is a common ecological interaction in insect parasitoids. In the recent years, it has become increasingly evident that microorganisms can act as "hidden players" in parasitoid ecology. In this review, we propose that parasitoid competition should take in consideration the microbial influence. In particular, we take a tri-trophic perspective and discuss how parasitoid competition can be modulated by microorganisms associated with the parasitoids, their herbivore hosts or the plants attacked by the herbivores. Although research is still in its infancy, recent studies have shown that microbial symbionts can modulate the contest outcome. The emerging pattern is that microorganisms not only affect the competitive traits of parasitoids but also the fighting arena (i.e. the herbivore host and its food plant), in which competition takes place. We have also identified important gaps in the literature which should be addressed in future studies to advance our understanding about parasitoid competition.
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14 **Abstract**

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316 become increasingly evident that microorganisms can act as “hidden players” in parasitoid ecology. In this
417 review, we propose that parasitoid competition should take in consideration the microbial influence. In
518 particular, we take a tri-trophic perspective and discuss how parasitoid competition can be modulated by
619 microorganisms associated with the parasitoids, their herbivore hosts or the plants attacked by the
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1022 competitive traits of parasitoids but also the fighting arena (i.e. the herbivore host and its food plant), in
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1726 **Keywords:** parasitoid competition, microbe-mediated effects, parasitoid-associated symbiont, herbivore-
1827 associated symbiont, extrinsic competition, intrinsic competition, fighting arena
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30 **1.Introduction**

31 Competition for limited resources is a ubiquitous interaction within the animal kingdom. In the case of
32 insect parasitoids, competition has been instrumental for unravelling fundamental ecological aspects such
33 as species coexistence and how communities are structured in food webs [1]. In addition, competition
34 among parasitoids is crucial from an applied perspective given that it may affect pest suppression and
35 eventually the outcome of biological control programs [2].

36 Parasitoid competition can occur: i) among adults when foraging or exploiting hosts, the so-called
37 “extrinsic” competition; ii) among larvae developing within the same host, the so-called “intrinsic”
38 competition [3–7] . Intrinsic competition among parasitoids of the same species is known as
39 superparasitism and among different species multiparasitism [1]. In solitary parasitoids, the first larval
40 instar generally uses its mandibles to kill the competing parasitoid whereas, in gregarious parasitoids,
41 supernumerary larvae are usually eliminated via scramble competition [4,8] (but see [9]). Finally, contest
42 resolution may also occur via physiological suppression when the parasitoid larva (or the ovipositing
43 female) releases inside the host factors that are either directly toxic to the competitor or impair indirectly
44 its development by altering the host nutritional milieu [3,10,11].

45 Recent studies made evident that parasitoid competition does not only depend on the identity of the third-
46 trophic level organisms, but also on the herbivore host and plant species [12–15]. In other words, while
47 parasitoids are the focal players, both the herbivore and its food plant represent the fighting arena in which
48 parasitoid competition takes place [3]. However, this tri-trophic perspective might not be enough to truly
49 unravel the complexity of parasitoid competition. Microorganisms that virtually colonize all animals and
50 plants have been shown to act as “hidden players” in several ecological interactions [16–19]. Parasitoids
51 are not an exception, as they are associated with bacteria, fungi and especially with a wide array of viruses
52 which are involved in parasitoid reproduction, suppression of host immunity, and behavioral manipulations
53 of their hosts [20]. Therefore, parasitoid-associated microorganisms could also directly or indirectly affect
54 parasitoid competitive interactions. Similarly, herbivore-associated microbial symbionts, such as
55 *Hamiltonella defensa*, can modify the fighting arena and should also be taken into account when studying
56 parasitoid interactions [21,22]. Finally, we argue that plant-associated microorganisms could have
57 cascading effects on parasitoid competition via bottom-up, tri-trophic effects.

58 Thus, in this opinion paper, we propose that parasitoid competition should be viewed in the light of
59 microbial influence. In particular, we take a tri-trophic perspective and discuss how parasitoid competition
60 can be modified by microbes associated with 1) the parasitoids, 2) the herbivore hosts and 3) the plants. In
61 this review we use the term “host” to indicate the organism in which parasitoid larvae develop. Such term
62 has also been used in the literature when referring to the insect that harbors a microbial symbiont, but we
63 avoid using this terminology here to prevent confusion.

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2.1 Microbe-mediated competition in parasitoids

2.1 Effects of parasitoid-associated microbes on parasitoid competition

66 Microbial symbionts associated with parasitoids include viruses, bacteria, and to a lesser extent fungi [20].
67 The majority of the parasitoid-associated viruses are Polydnaviruses (PDVs) which have established obligate
68 mutualistic associations with their parasitoid partners [23]. Briefly, PDVs suppress the immune response
69 system of the herbivore host, e.g., by overcoming egg encapsulation [24]. Parasitoid-associated bacteria
70 include reproductive manipulators such as *Wolbachia*, *Cardinium*, *Rickettsia*, and *Arsenophonus* [25–27].
71 Only a few fungi have been described in parasitoids: the most detailed case study refers to a

73 Saccharomycotina yeast-like organism found in the egg parasitoid *Comperia merceti* although its functional
174 role is not fully understood [28].

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375 These parasitoid microbial symbionts can affect several aspects of parasitoid–host ecology, such as
476 parasitoid intrinsic rate of increase, superparasitism, incidence of host-feeding, host range, host-
577 exploitation [20], and, eventually, might impact the intra- and interspecific competitive abilities of
678 parasitoids. For instance, *Wolbachia* bacteria may affect intraspecific larval competition in the egg
879 parasitoid *Trichogramma kaykai*, possibly due to the longer developmental time and higher mortality of
980 infected parasitoid larvae [29]. In contrast, infection of a Saccharomycotina yeast does not affect
1081 intraspecific competition in the egg parasitoid *Comperia merceti*, although the yeast also induces a cost in
1282 terms of longer developmental time in infected parasitoids [28].

13
1483 Microbial symbionts can induce superparasitism behavior with consequences for parasitoid competition.
1584 For example, *Leptopilina boulardi* and *L. heterotoma* are parasitoids of *Drosophila* flies that naturally
1685 coexist in the field [30]. Only *L. boulardi* (but not *L. heterotoma*) can be infected with a viral symbiont
1786 called *Leptopilina boulardi* filamentous virus (LbFV) [31]. Under controlled laboratory conditions, *L. boulardi*
1987 outcompeted *L. heterotoma* in the absence of LbFV, whereas the parasitoid species coexisted when *L.*
2088 *boulardi* was infected by LbFV. As the viral symbiont induces superparasitism and egg wastage in *L.*
2189 *boulardi*, the resulting reduced host exploitation abilities allow the coexistence of the inferior competitor *L.*
2390 *heterotoma* [31].

24
2591 Another possible way in which symbionts can affect parasitoid competition includes differential host-killing
2692 rate, e.g., via host feeding. Using the parasitoid *Neochrysocharis formosa* as model organisms, Ye et al.
2793 [32] report increased host feeding by thelytokous, *Rickettsia*-infected, wasps that may eventually preempt
2894 hosts for the *Rickettsia*-free strain of the same parasitoid species.

30
3195 Finally, symbiont-mediated effects on parasitoid host exploitation abilities may impact competition
3296 between *Aphytis melinus* and *A. chrysomphali*, important natural enemies of the major citrus pest
3397 *Aonidiella auranti* [13,33]. *Aphytis melinus* is arrhenotokous (i.e. males are produced from unfertilized eggs)
3498 while *A. chrysomphali* is thelytokous (i.e. females are produced from unfertilized eggs) due to infection with
3599 *Wolbachia* [33]. *Aphytis melinus* is the stronger competitor and has partially displaced *A. chrysomphali* in
3700 several parts of the world where both parasitoid species coexisted [12,34]. Nevertheless, infection with
38101 *Wolbachia* allows *A. chrysomphali* to produce females even on low quality (i.e. small size) hosts, whereas *A.*
39402 *melinus* requires hosts above a certain size threshold to produce female progeny [13,34,35]. Interestingly,
4103 in absence of its competitor, *A. chrysomphali* showed preference for high quality (i.e. large size) hosts.
4204 Thus, the presence of the symbiont is probably mediating the competitive interactions between *Aphytis*
43105 species, allowing the weaker competitor to produce females regardless of host size and eventually to
44106 coexist in sympatry with the stronger competitor.

46 47 2.2 Effect of herbivore-associated microbes on parasitoid competition

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498 Herbivore insects harbor a range of microbes which are acquired from the environment or are maternally
509 transmitted. Among the maternally-transmitted microbes, several endosymbiotic bacteria provide
5110 protection against parasitoids. The best-known examples of such protective bacteria are *Hamiltonella*
5211 *defensa* [36] and *Regiella insecticola* [37] in aphids and *Spiroplasma* sp. in *Drosophila* flies [38].
5312 Endosymbiotic bacteria can protect their insect partners by enhancing their immune system and by
5413 producing toxins against the immature parasitoids. The protective effects of these endosymbionts are,
5514 however, highly specialized. For example, the protection of aphids by *H. defensa* depends on the aphid and
5615 parasitoid genotypes, as well as on the bacterial strain [22]. This highly specific defense provided by
5716 endosymbiotic bacteria of herbivores against parasitoids can mediate the competition between parasitoid
6017 species that attack the same herbivore host.

118 One of the best-known cases of competition between parasitoid species mediated by endosymbiotic
119 bacteria occurs among parasitoids of the pea aphid *Acyrtosiphon pisum*. McLean and Godfray [39]
120 demonstrated that *H. defensa* mediated the intrinsic competition in pea aphids multiparasitized by
121 *Aphelinus abdominalis* and *Aphidius ervi* because the bacteria is known to impact the former parasitoid
122 species more strongly than the latter. The same endosymbiont also mediated competitive interactions
123 between *A. ervi* and *Praon pequodorum* but in a more subtle way. In North America, the pea aphid was
124 parasitized by a complex of native parasitoid species until the introduction of *A. ervi* [40]. This species
125 successfully parasitized the pea aphids and displaced all the native species except *P. pequodorum*, a
126 parasitoid that is superior at larval competition but less efficient in searching for aphids. Kraft and
127 colleagues [41] demonstrated that *H. defensa* does not influence the larval competition between these two
128 parasitoids because the superior *P. pequodorum* is not constrained by a strain of *H. defensa* known to
129 affect *A. ervi*. The authors argued that symbiont-conferred resistance specific towards *A. ervi* may give an
130 advantage to *P. pequodorum* in terms of adult competition which, in turn, facilitated persistence of the
131 latter species in the field. Another example of competition mediated by *H. defensa* comes from a field
132 experiment. Rothacher and colleagues [42] demonstrated that infected aphids harbored a higher diversity
133 of parasitoid species than uninfected aphids, most likely because the dominant parasitoid *Lysiphlebus*
134 *fabarum* was affected by *H. defensa*.

135 The presence of endosymbiotic bacteria in aphids can also increase the incidence of superparasitism.
136 *Aphidius ervi* tends to superparasitize the pea aphid *A. pisum* when it is defended by *H. defensa*, likely
137 because superparasitism reduces the parasitoid mortality caused by the bacteria [43]. This last result also
138 demonstrates that: i) superparasitism can be beneficial for parasitoids [44] and ii) parasitoids can detect
139 the presence of symbiotic bacteria in the herbivore host. The effects of the protective bacteria are not only
140 confined to the infected herbivore, but they can also cascade to the plant which, in turn, might also modify
141 the competition between parasitoid species. In this sense, Frago and colleagues [45] demonstrated that
142 aphid endosymbionts attenuate the volatiles released by *Vicia fabae* plants attacked by infected *A. pisum*
143 leading to a reduced recruitment of its parasitoid *A. ervi*. Whether other parasitoid species are differently
144 affected by these microbe-mediated changes and, therefore, modulate competition remains unknown.

145 Endosymbiotic bacteria can also affect the apparent competition between parasitoid species [46]. The
146 presence of *H. defensa* in *A. pisum* did not reduce the absolute abundance of other two species of aphids,
147 *Megoura viciae* and *Aphis fabae*, in microcosm cages. However, their parasitoids became extinct likely
148 because the high abundance of *A. pisum* infected with *H. defensa* reduced the searching efficacy of the
149 other parasitoids.

150 2.3 Effect of plant-associated microbes on herbivore quality and consequences for parasitoid competition

151 Plant quality can affect the fitness of immature parasitoids and their competitive abilities via direct effects
152 of toxic plant compounds or via indirect effects through herbivore immunity and nutritional quality [47–49].
153 There is evidence showing that the identity of the first trophic level can affect parasitoid interspecific
154 competition, possibly due to indirect effects in terms of host food quality. For example, the intrinsic
155 competition between the larvae of the parasitoids *Hyposoter ebeninus* and *Cotesia glomerata* is affected by
156 the plant identity [15]. In fact, *C. glomerata* larvae compete better when their pierid hosts feed on *Brassica*
157 *nigra* than on *Brassica oleracea* [15].

158 While the effect of plant species in parasitoid interactions has been documented, we are not aware of any
159 study that has investigated how plant-associated microbes affect parasitoid competition. However, it is
160 increasingly reported in the literature that plant-growth promoting bacteria and fungi do not simply
161 stimulate plant growth but also confer resistance against a wide range of herbivores by enhancing plant
162 defenses via the Jasmonic Acid and/or Salicylic Acid signaling pathways [16,50]. Therefore, it is plausible to
163 argue that plant-associated microbial symbionts can modify host quality and further modulate parasitoid

164 competition. We expect that plant-associated microbes are likely to play a minor role compared to
165 herbivore-associated and parasitoid-associated microbes because plant-mediated effects might be diluted
166 across the tri-trophic food chain. Nonetheless, this research field deserves to be explored.

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168 3. Conclusions

169 Although research on microbe-mediated competition in insect parasitoids is still in its infancy, recent
170 studies have shown that microbes can modulate the contest outcome. The emerging pattern is that
171 microorganisms do not only affect the competitive traits of parasitoids but also the fighting arena (i.e. the
172 herbivore host and its food plant) in which competition takes place.

173 The role of parasitoid-associated microbes seems to be context-specific and species-dependent. For
174 example, evidence from reproductive manipulators hints that microbial symbionts associated with a given
175 parasitoid species can either be beneficial (i.e. *Wolbachia* for *A. chrysomphali*) or detrimental (i.e. LbFV for
176 *L. boulandi*) in interspecific competition. Nonetheless, a large group of parasitoid-associated symbionts
177 which are expected to be beneficial for their parasitoid partners - in the context of competition - are
178 Polydnaviruses (PDVs): in fact, these viral symbionts suppress herbivore immunity and prepare the host
179 nutritional milieu for the offspring development of the associated parasitoid species [23,51]. Whether and
180 how PDVs modulate competition in parasitoids has not yet received the deserved attention.

181 Studies that have investigated the role played by herbivore-associated microbes on parasitoid competition
182 are so far restricted to protective symbionts such as *H. defensa*. These studies have pointed out that the
183 outcome of larval competition among parasitoid species with different degree of susceptibility to protective
184 symbionts depends on the infection status of their herbivore hosts [39]. Although investigating the role of
185 facultative protective symbionts in the context of parasitoid competition is certainly interesting, it is also
186 important to point out that these bacteria are only a small fraction of the total microorganisms that are
187 known to colonize insect herbivores. Thus, in order to advance our understanding of parasitoid
188 competition, further efforts should be made focusing on other herbivore-associated microorganisms. For
189 example, parasitoid competition has largely been studied in caterpillar hosts (see reviews [3,4]) but how
190 caterpillar-associated microbes modulate the strength of intra- and inter-specific competition has lagged
191 behind.

192 To date, the role of plant-associated microbes has not been explored in parasitoid competition. Yet in this
193 review we speculated that competition between parasitoid larvae developing inside the same herbivore
194 host can be modulated by plant microbes across the tri-trophic food web via effects on herbivore quality.
195 However, competition between adult parasitoids searching for herbivore hosts can also be affected if plant-
196 associated microorganisms differently affect parasitoid attraction towards hosts and food sources. One
197 possible way this could be achieved is by exploiting bacteria and yeasts (or the volatiles they produce) in
198 floral nectar making flowers more attractive to parasitoids [52–54].

199 We are only recently starting to unravel the role of microorganisms in insect competition. Yet, it is
200 becoming increasingly evident that, in order to better understand the role of competition on parasitoid
201 ecology, we need to place parasitoid interactions in a tri-trophic perspective and consider also the role
202 played by microbial symbionts across different trophic levels. By doing so, we will be able to advance our
203 current knowledge of parasitoid competition and its implications in biological control.

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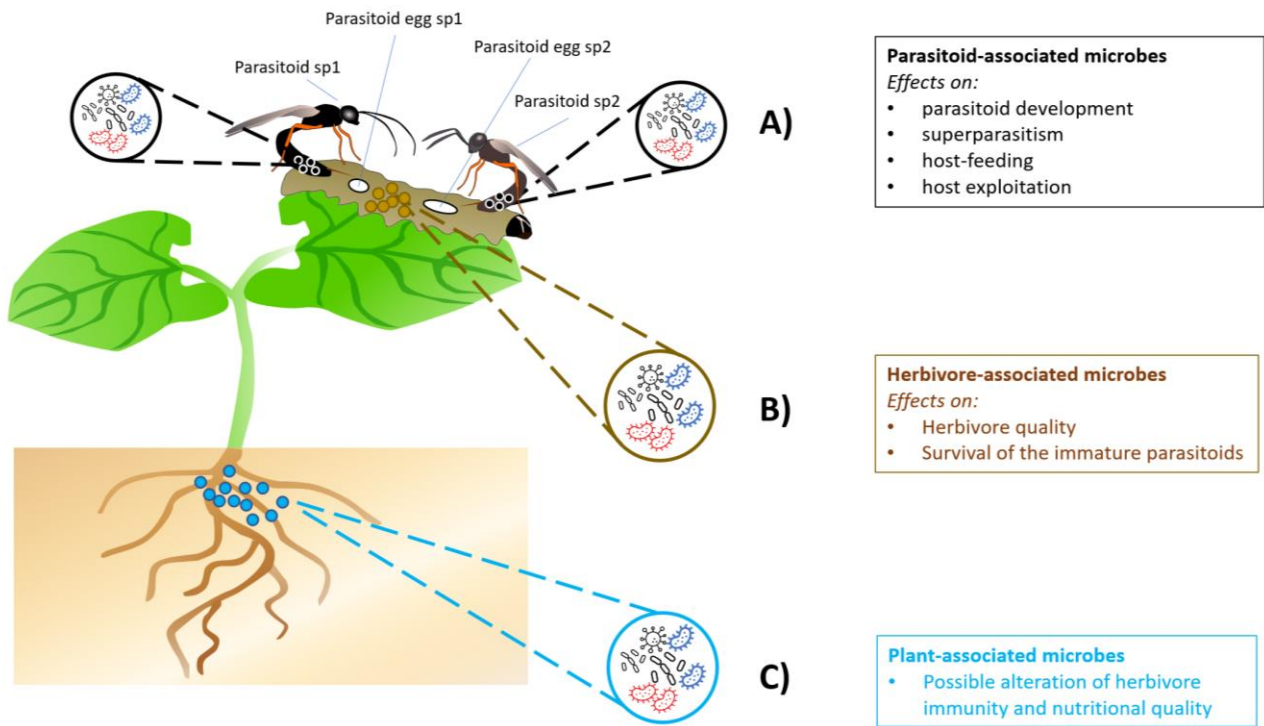
Box 1: Microbe-mediated facilitation in parasitoids

We broadly refer to interspecific facilitation for those situations in which a parasitoid species may benefit from interspecific competition. Given the nature of the antagonistic interactions between hosts and their parasitoids, a common case of facilitation may occur when a parasitoid species that is a superior competitor but poorer at suppressing host defenses interacts with a weaker competitor that is better at host exploitation [4]. Examples of interspecific facilitations are rare in the parasitoid literature probably because such phenomenon is overlooked [15,55]. Nonetheless, we argue that microbial symbionts associated with parasitoids may promote interspecific facilitation. Indirect evidence from such hypothesis emerged from manipulative experiments carried out by Vinson and Stoltz [56]. They showed that eggs of the parasitoid *Campoletis sonorensis* develop better inside *Trichoplusia ni* hosts when injected together with *Hyposoter exiguae* PDVs than with *C. sonorensis* PDVs. Another case of potential facilitation in parasitoids was found in multiparasitized pierid hosts where the presence of *C. glomerata* larvae benefited the superior competitor *Hyposoter ebeninus* [15]. Differences in the ability to suppress host immunity might be the underlying mechanisms promoting facilitation, as the superior larval competitor *H. ebeninus* suffers more from egg encapsulation compared to *C. glomerata*. Taking into account that both *H. ebeninus* and *C. glomerata* evolved obligate symbiotic mutualisms with PDVs, it is tempting to suggest that parasitoid-associated viral symbionts are the hidden players promoting interspecific facilitation, although this hypothesis still remains to be tested. Finally, whether herbivore-associated microorganisms can promote facilitation is largely unknown.

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233 **Figure 1**



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 235 **Overview of microbe-mediated competition in insect parasitoids.** A) Parasitoid-associated microbes can
 236 modulate intra- and inter-specific competition by affecting parasitoid's developmental rate, incidence of
 237 superparasitism, host-feeding and host-exploitation. B) Herbivore-associated microbes (e.g. protective
 238 endosymbionts) can affect competition among parasitoid species developing within the same herbivore
 239 host by: i) producing compounds that are selectively toxic for parasitoid larvae; ii) enhancing herbivore
 240 immune system and thus reducing host quality. C) Plant-associated microbes are also likely to have
 241 cascading effects on parasitoid competition via alteration of the herbivore immunity and/or nutritional
 242 quality. Herbivore-associated and plant-associated microorganisms can potentially shape the strength of
 243 adult competition among parasitoid species if microorganisms differently affect attraction of foraging
 244 parasitoids towards herbivore hosts and food plants

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Dicke M, Cusumano A, Poelman EH: Microbial symbionts of parasitoids. *Annu Rev Entomol* 2020, 65:171–190 ****Comprehensive overview of the microbial symbionts associated with parasitoids and the role they play in parasitoid ecology**

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Patot S, Allemand R, Fleury F, Varaldi J: An inherited virus influences the coexistence of parasitoid species through behaviour manipulation. *Ecol Lett* 2012, 15:603–610 **** This study showed that interspecific competition between two parasitoid species is affected by a parasitoid-associated viral symbiont acting as reproductive manipulator**

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Conflict of interest

The authors declare that the content of this manuscript was not affected by any financial, commercial, legal, or professional interest.

Highlights

- Microbial symbionts should be considered in parasitoid competition
- Microorganisms affect competitive traits of parasitoids
- Microorganisms alter the fighting arena where parasitoids compete