Lobesia botrana as a preferred host of Campoplex capitator, the most occurring larval parasitoid in European vineyards

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With 2 figures

Abstract: Parasitoids are major biological agents in crop protection, and understanding their preference towards specific host species is a key aspect of successful pest control. In the present study, we have examined the host preferences of the larval parasitoid Campoplex capitator (Hymenoptera: Ichneumonidae), one of the major natural enemies for tortricid grapevine pest populations in European vineyards. Using a test choice between its two main and sympatric hosts, we wanted to determine whether C. capitator females that emerged from Lobesia botrana (Lepidoptera: Tortricidae) also prefer its natal host L. botrana, or Eupoecilia ambiguella (Lepidoptera: Tortricidae) as host; and consequently, if it could be a good candidate as a bio-agent for these species. Our results distinctly showed that naïve C. capitator females preferred L. botrana over E. ambiguella as host, as they directly sought out new L. botrana hosts. Overall, it reveals that C. capitator could be a very efficient parasitoid to control L. botrana populations. Our results might also suggest the importance of natal host in parasitoid host preference, which should be considered in the future when mass-rearing bio-agents.

Keywords: grapevine moth, host species preference, larval parasitoid, choice test

1 Introduction

With more than 900,000 known species, insect herbivores represent nearly a quarter of all terrestrial macroscopic biodiversity, acting as a dominant component of all terrestrial food webs on Earth (Resh & Cardé 2009, Sauvion et al. 2017). While they are well regulated by both host plant and natural enemies within their ecosystems (Kaplan et al. 2016), agroecosystems often break this balance – through agriculture intensification, monoculture development and pesticide use – and favor the emergence of pest species (Wilby & Thomas 2002). Even if only 2% of insect herbivores species are considered as pests (Dhaliwal et al. 2010), they are responsible for an estimated crop loss of up to 50% (Thacker 2002, Aggarwalim et al. 2006) and are consequently a major human concern, with implications for public health and agriculture. At a crucial time when farmers struggle to increase or at least maintain yields while reducing pesticide use for environmental and health issues, biological control and integrated pest management strategies appear to be the best current alternatives (Brewer & Goodell 2012).

Biological control is based on population regulation and specially on interactions between a host – here, insect herbivore – and its natural enemies (e.g. parasitoids, predators, pathogens) (Van Driesche et al. 2008). It includes, for example, the release of one or more natural enemies into a targeted agroecosystem to control a growing pest population. Parasitoids – specially hymenopterous parasitoids – are key biological agents, and have been the most common type of natural enemy used in crop protection (Van Driesche et al. 2008, Giunti et al. 2015). Parasitoids evolve in a complex environment and therefore rely on a variety of stimuli (e.g. visual, vibrational and olfactory cues) to locate a suitable habitat with host, food and/or mates (see Giunti et al. 2015 for reviews). Multiple factors can influence parasitoids’ host foraging, including host size and density, but also importantly their learning capacity (Lin & Ives 2003, Giunti et al. 2015, Morgan et al. 2016). While there is a heritable component for habitat preference, it remains unclear as to exactly when this preference development occurs (i.e. during early life stages, after emergence, or both), despite numerous theories informing optimal parasitoid foraging (Barron 2001,
Davis and Stamps 2004, Giunti et al. 2015). The notion of Natal Habitat Preference Induction (NHPI) has been proposed by Davis & Stamps (2004) to better understand such processes, and states that animals prefer to develop in a similar habitat to the one they experience in early life stage. The learning process associated with this notion is mostly related to chemical cues coming from the natal habitat which are memorized during ontogeny (Corbet 1985, Ruther et al. 2002). For instance, females of the parasitoid *Psytalia concolor* (Hymenoptera: Braconidae) exhibit different oviposition preferences depending on their natal host – i.e. females did not always prefer to oviposit in their natal host (Giunti et al. 2016). Altogether, this raises one of the most important challenges in biological control: the plastic specificity between the bio-agent and targeted host.

In order to conduct an efficient and sustainable pest population control, the parasitoid should be able to successfully target its pest species (Van Driesche et al. 2008), even in an environment susceptible to host several pest species. Agroecosystems, such as vineyards, can host a large number of pests living in sympatry (Vogelweith & Thiéry 2018), and as such, investigating host preference in parasitoids is crucial for biological control. The grape berry moth *Eupoecilia ambiguella* (Hubn.) (Lepidoptera: Tortricidae) and the European grapevine moth *Lobesia botrana* (Den. & Schiff.) (Lepidoptera: Tortricidae) are two polyphagous grapevine pests (reviewed: Thiéry et al. 2018). These two species are responsible for significant damages in vineyards either directly by attacking grape bunches – a larva is able to damage between 2 and 10 berries depending on the grape cultivar – or indirectly by favoring the development of several fungi, such as *Botrytis cinerea*, *Aspergillus carbonarius*, and *Aspergillus niger* (Delbac & Thiéry 2016, Thiéry et al. 2018). *Eupoecilia ambiguella* mostly occurs in central Europe while *L. botrana* is widely distributed but mostly occurs at high density in Mediterranean climates (Thiéry 2008). However, in several European vineyards, these two species can share common distributional range where they often live in sympatry, as in Beaujolais vineyards for example (North of Lyon, France) (Vogelweith et al. 2014). The density of each species within vineyards can vary from 15:75 to an equal distribution 50:50 (unpublished data). Despite such differences, biological control of *E. ambiguella* and *L. botrana* has always been the same, mostly performed through the release of the egg parasitoid *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) (Vogelweith et al. 2014, Thiéry et al. 2018). For the past decade, the larval parasitoid *Campoplex capitator* (Hymenoptera: Ichneumonidae) has drawn more and more attention (Marchesiniand & Dalla Monta 1994, Xuéreb & Thiéry 2006). Cosmopolitan and very plastic in its ecological requirements (Moreau et al. 2010), this parasitoid is very efficient in regulating grapevine pest populations, with sometimes a level of parasitism rate up to 90% in *L. botrana* (Vogelweith et al. 2013). But, attempts to mass-rear *C. capitator* for the control of grapevine pests has always failed, chiefly because of the limited information about its biology/ecology, including the limited understanding of its ability to switch from the rearing-host (e.g. *L. botrana*) to hosts encountered in the field (e.g. *L. botrana* and *E. ambiguella*) (unpublished data). For instance, the larval-pupal parasitoid *Psytalia concolor* (Hymenoptera: Braconidae) reared on the fruit fly *Ceratitis capitata* (Diptera: Tephritidae) (Canale & Benelli 2012) did not show any difference in oviposition behaviors and host acceptance when provided either with its rearing host *C. capitata* or the alternative host pest *Bactrocera oleae* (Diptera: Tephritidae) (Canale & Benelli 2012).

Here we seek to determine, for the first time, whether *C. capitator* females emerged from wild populations of *L. botrana* prefer *E. ambiguella* or *L. botrana* as host in a choice test, and consequently whether it could be a good candidate as bio-agent to regulate grapevine pest populations.

## 2 Material and methods

### 2.1 Model insects

Larvae of *E. ambiguella* and *L. botrana* used in this experiment came from two independent rearing facilities, maintained diapause-free at the French National Institute for Agricultural Research (INRA) (Villenave d’Ornon, France) for more than 15 years (see Vogelweith et al. 2017). These two strains were based on multiple adults reared in flying cages to which wild individuals were periodically added. Larvae of each species were provided with *ad libitum* semi-artificial diet (Vogelweith et al. 2015) and maintained in similar boxes (18 × 11.5 × 7 cm) under the same standard laboratory conditions (22 ± 1 °C, 70 ± 10% RH, and a L16:D8 photoperiod) at a density of approximately 100 individuals per 300 ml of diet. Corrugated papers were placed within each box, allowing the larvae to hide, as they did in grape berries.

Female parasitoids of *C. capitator* were obtained from wild parasitized *L. botrana* larvae collected on Grenache cultivar in a French vineyard (N 42° 44' 7.063", E 2° 52' 56.441"; Perpignan, France) in May 2013 (see Vogelweith et al. 2013). Sampled larvae were reared individually in glass tubes (70 × 9 mm diameter) closed with cotton plugs under the conditions stated above and checked daily until emergence from either an adult moth or a parasitoid. Different parasitoid species emerged from larvae and after identification (Thiéry 2008), only *C. capitator* females (easily visually identifiable with their ovipositor) were kept for this experiment. Parasitoid females were trans-
ferred to clean glass tubes provided with a droplet of honey and cotton plugs soaked with water, but otherwise kept at the same conditions. All C. capitator females used in this experiment were tested two days after their emergence, naïve (no prior oviposition) and were kept virgin because females of this species can also reproduce parthenogenetically, producing in such case only males (Morris & Fellowes 2002, Thiéry & Xuereb 2004).

2.2 Experimental design
A total of 22 C. capitator females were tested to determine their preference between E. ambiguella and L. botrana. Tests were carried out in an experimental arena constituted of a Petri dish (95 mm diameter, 62 mm high) divided into two equal zones (Fig. 1): one zone dedicated to E. ambiguella, and the second dedicated to L. botrana. At the center of each zone was taped a piece of corrugated paper containing a 4th instar larva of either E. ambiguella or L. botrana (Fig. 1). Prior to the test, all larvae were checked to ensure they were at the same instar (4th instar) based on the larval head capsule size (1.2 ± 0.1 mm) (Delbac et al. 2010), and without physical damages. Then, larvae were moved to the small piece of corrugated paper using a paintbrush washed with 90% ethanol after each larva. The female parasitoid was then released in the middle of the experimental arena. Its behavior was recorded until the first attack (defined as the first oviposition attempt) or for a maximum of 30 min. During this time-period, the first species visited, the time spent by the parasitoid on each side of the arena (E. ambiguella versus L. botrana sides), the number of visits to each larva and the chosen larva (i.e. larva which suffered from an oviposition attempt) were recorded. As female C. capitator can perform multiple oviposition attempts in few seconds, inflicting serious injuries to the larva, a test was ended after the first attack. Each test was performed with a different female parasitoid and host larvae. The position of each species in the experimental arena was exchanged every time and a new arena was used for each repetition to avoid potential influence of cues/odor from both parasitoid and larvae. The experiment was performed for two hours (four individuals during 30 min per day), every day during six days, at the sunset – time-period where parasitoids are the most active – at 24 ± 1 °C, 60 ± 10% RH and under the ambient photoperiod conditions.

2.3 Statistical analyses
All data analyses were conducted using the software R v3.5.1 loaded with car and lme4 packages. The proportion of time spend by the female parasitoid on each side was tested using a Chi-squared test for given probabilities where the proportion of time spent on the L. botrana side was compared to a probability of 0.5. The number of visits per host species was tested using a generalized linear model (GLM) with poisson distribution, and the species (L. botrana or E. ambiguella) entered as explanatory categorical factors. Finally, the first species visited as well as the choice made by the female parasitoid were tested using a generalized linear model (GLM) with binomial error distribution, and the species (L. botrana or E. ambiguella) entered as explanatory categorical factors.

3 Results
The first choice (i.e. the first larva visited) by C. capitator females was almost always L. botrana (95.45% of the cases; F1 = 44.72; p < 0.0001). Moreover, females spent significantly more time on L. botrana side of the experimental arena (χ²1 = 7.68; p = 0.006; IC 95% = [0.59; 0.94]; Fig. 2a) and visited more often L. botrana larvae compared to E. ambiguella larvae (F1 = 10.11; p = 0.001; Fig. 2b). Finally, C. capitator females almost exclusively attacked L. botrana larvae (F1 = 31.18; p < 0.0001; Fig. 2c).

4 Discussion
Although host preference in parasitoids has been well studied, whether a parasitoid prefers its natal host or an alternative host in an agroecosystem hosting sympatric pest species remains unexplored. Based on a choice test experiment, our results clearly showed that C. capitator females emerged from wild population of L. botrana preferred the latter as host over E. ambiguella. Indeed, they went mostly directly to the L. botrana side, spent more time on this side and visited
it more often compared to *E. ambiguella* larvae. Finally, *L. botrana* larvae were almost exclusively choose/attacked by *C. capitator* females.

At least three non-mutually exclusive hypotheses could explain such preference. The first hypothesis relies on the notion of NHPI (Davis & Stamps 2004), meaning that *C. capitator* females emerged from *L. botrana* prefer to lay their eggs in *L. botrana* larvae due to special stimuli received in early-life in their native habitat. Mounting evidence suggests the importance of the cues learned during ontogeny on parasitoid choice (Morris & Fellowes 2002, Bodino et al. 2016). Such learning can take place after emergence or during pre-adult stages (when the parasitoid in still on/inside its host) and/or during adult emergence (Giunti et al. 2015), as illustrated by the endoparasitoid *P. concolor*. Females from the latter excised from their host just before emergence – and consequently did not have contact with a natal host in early adult life – were unable to show a preference for the natal host (Giunti et al. 2016). Our results are consistent with the NHPI hypothesis even though we cannot determine whether *C. capitator* females are using pre- or post-emergence learning. The same experiment should be performed with *E. ambiguella* as natal host to confirm that statement. Parasitoid learning could also occur through an associative learning where they learn to associate cues from their environment to increase their foraging efficiency (Hoedjes et al. 2011). In our study, parasitoid cocoons were removed from their environmental complex and placed under control conditions, meaning that associative learning was not tested here. As such, we do not know the consequences of host species choice upon *C. capitator* fitness. This point should be investigated in a further field survey where *C. capitator* live in an environment where *L. botrana* and *E. ambiguella* are in sympathy.

Secondly, parasitoids can also inherit natal environmental preferences related to a local adaptation that may result in a parasitoid population specializing on one host (Gutiérrez-Ibáñez et al. 2007, Zepeda-Paulo et al. 2013, Bodino et al. 2016). A previous study suggested a local adaptation of *L. botrana* larvae to existing parasitoid pressure (Vogelweith et al. 2013). Since *L. botrana* and *C. capitator* co-evolved in vineyards for many years, it is also likely that *C. capitator* have adapted to local host species. Indeed, *C. capitator* used in this experiment came from an area where *L. botrana* are predominant compared to *E. ambiguella* (Ricaud 2013).

Finally, the third hypothesis involves the defense strategies used by *E. ambiguella* and *L. botrana* larvae (Vogelweith et al. 2014). Despite many similarities in their ecological requirements and their natural enemies, these two species have been shown to invest differently in the defense mechanisms which might influence parasitoid choice (Brodeur et al. 1996, Vogelweith et al. 2014). Indeed, even though *L. botrana* larvae have been shown to express strong behavioral defenses, their cuticle and immune system are less resistant to parasitoids compared to *E. ambiguella’s* (Vogelweith et al. 2014). Therefore, the choice performed by *C. capitator* is very consistent, and could be the result of a local adaptation of *C. capitator* to *L. botrana* larvae in the field, as mentioned in the second hypothesis.

In conclusion, these results show that biological control by augmentation – relying on the release the of natural enemies (i.e. *C. capitator*) that occur in the agroecosystem – could be very efficient against *L. botrana* populations due to the high specificity of its parasitoid. However, it might be less efficient against *E. ambiguella* populations, at least if *C. capitator* has *L. botrana* as natal host. More investigations about the relationship between *C. capitator* and *E. ambiguella* should be performed in order to consider this parasitoid
as a potential bio-agent. But, it is important to recall that studies and field reports indicated that C. capitator is parasitizing E. ambiguella in European vineyards (Colombera et al. 2001, Théry et al. 2011, 2018, Rusch et al. 2015), even if parasitism success is higher in L. botrana than in E. ambiguella when the ratio of both species in the population is close to 50:50 (Vogelweith et al. 2014). Further investigations should be done in order to determine whether it would also be a good candidate to control E. ambiguella populations. Altogether, this study is one of the first to reveal C. capitator’s potential as a valuable and efficient bio-agent for the control of grapevine moths in European vineyards.

Conflict of interest

The authors declare no competing financial interests.

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