Fly disturbance suppresses aphid population growth

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Running headline: *Fly suppression of aphid populations*

Abstract. 1. The presence of predators is known to suppress prey populations not only by consumptive but also non-consumptive effects, as it stresses the prey inducing costly changes to behaviour and physiology. However, there is recent evidence that disturbance from non-predacious, non-competing commensals can also negatively affect herbivore performance.

2. We initiated populations of cherry-oat aphids (*Rhopalosiphum padi* L.) with adult aphids in mesocosms containing seedling wheat grass. Following aphid establishment, we added fruit flies (*Drosophila melanogaster* Meigen) at a relatively high density to half of the mesocosms and left the aphids for another five days to reproduce. The experiment was performed over two blocks at 24 ± 4 °C. We hypothesized that a relatively high density of commensals would stress the aphids and reduce their fitness, causing lower population growth and resulting population sizes.

3. Aphid numbers were significantly lower in mesocosms with commensal flies after five days of fly presence across the two experimental blocks, documenting that fly disturbance suppresses aphid fitness and population growth.

4. The negative effect of fruit flies on aphid population growth must have come from the disturbance that flies imposed on the aphids in their search for food, clearly indicating that the flies stressed the aphids. Thus, commensals may in some cases stress herbivores that cannot distinguish between enemies and other active species in their environment, adding to the overall herbivore top-down control through fitness costs.

Key words. commensal species, fitness, fruit flies, non-enemy suppression, *Rhopalosiphum padi*, stress.

Introduction

The mere presence of predators can negatively influence prey fitness and population performance, but it is far less studied how commensals that are neither predators nor competitors affect herbivore populations. Predators stress their prey by inducing fear, which alters prey's behaviour and activates stress physiology (Preisser *et al.*, 2005; Sheriff & Thaler, 2014). This in turn enhances the top-down control of predators on prey (Laundré et al., 2014). Such non-consumptive predator effects are even shown to sometimes be more important for prey population suppression than the direct mortality imposed by predators (Creel & Christianson, 2008), and to have cascading effects on the structure and functioning of ecosystems (Hawlena & Schmitz, 2010). However, interactions in ecosystems are driven by a multitude of species including not only natural enemies and competitors but also commensals that may nevertheless have a suppressing effect via their presence (Eubanks & Finke, 2014; Ingerslew & Finke, 2018). Caterpillar defoliation damage is thus shown to be reduced by the activity of pollinators (Tautz & Rostás, 2008) and grasshoppers (Xi *et al.*, 2013). Although this is recognized, the number of studies reporting examples of suppressive effects of commensals on herbivore reproductive potential and fitness outcomes is limited.

We studied the effects of disturbance from fruit flies on population growth in the bird cherry-oat aphid, *Rhopalosiphum padi* L., a serious global pest in wheat, *Triticum aestivum* L. (Peng *et al.*, 2020). We hypothesized that an abundance of fruit flies would induce costly stress responses in the aphids, resulting in lower aphid population growth. The study suggests an additional effect of commensal insect abundance in suppressing herbivore populations.

Materials and methods

Mesocosms with seedling wheat grass were produced by spreading 4 g of wheat seeds evenly over wet tissue paper on the bottom of transparent plastic boxes (17 cm length × 17 cm width × 11 cm height), yielding approximately 100 seedlings. Four days after sowing, the bottoms including seeds and roots were covered with 2 cm of a 1.5 % agar solution in water to maintain moisture and minimize hiding opportunities, thereby enhancing discovery of aphids of all stages at the final counting. Five days after sowing, 20 adult *R. padi* from our laboratory colony also reared on wheat seedling grass were added to each mesocosm and given 24 h to establish. The following day, 100 ± 5 vinegar flies (*Drosophila melanogaster* Meigen) were added randomly to half of the mesocosms. The flies were reared on Carolina Instant Drosophila Medium Formula 4-24 (Burlington, NC, USA) and yeast (*Saccharomyces cerevisiae* Meyen). In addition, 60 mg of yeast was added to all mesocosms as an initial, protein-rich food source for the flies. Mesocosm tops were covered with paper towel to allow evaporation and aeration but prevent escape of flies and aphids. The paper towels were sealed thoroughly by closing the boxes with the frame of the original box lids from which the interior part had been cut out. Five days after adding flies, the total number of aphids including adults and all instars was counted, and the number of remaining live flies was estimated to the nearest five. The experiment was performed over two blocks in a room with an 18:6 hour light:dark cycle and a temperature of 24 ± 4 °C, using a total of 43 replicates of each disturbance environment. Effects of fly disturbance on aphid population growth across the two blocks were analyzed using analysis of variance (ANOVA) with fly presence or absence as the experimental factor and block as a random factor. The interaction between disturbance and experimental test was insignificant (ANOVA, $F_{1,82} = 0.95$, $P = 0.33$) and therefore excluded from the model. The analyses were performed in JMP 14.0.0 (SAS Institute Inc., Cary, NC, USA).

Results

We found a significant negative effect of fly presence on final aphid population sizes in mesocosms across the two blocks (ANOVA, $F_{1,83} = 5.93$, $P = 0.017$; Fig. 1), documenting that fly presence suppresses aphid population growth. Fly mortality in the mesocosms was high across both blocks (mean ± SE = 83 ± 2 %).

Discussion

Non-predacious suppression of prey by predator presence is well documented (Preisser *et al.*, 2005; Sheriff & Thaler, 2014), but it is less studied how the presence and activity of commensals with no predatory nor competitive impact influence herbivore fitness (Tautz & Rostás, 2008; Eubanks & Finke, 2014; Ingerslew & Finke, 2018). We found that population growth of the aphid *R. padi* was significantly lower in mesocosms containing fruit flies. Since fruit flies do not consume or compete with aphids, our experiment indicates that the activity of the flies stressed the aphids, and we observed many cases where flies shook straws under landing and walked on top of the aphids. This may have resembled predator activity and induced similar costly behavioural and physiological stress responses as shown to predators, including dropping from the straw and shorter time spent feeding (Nelson, 2007), as well as raised metabolic rate and reduced assimilation efficiency (Sheriff & Thaler, 2014). Aphid immune functioning might furthermore have been upregulated in response to raised pathogen risk from exposure to the fly commensals (Schwenke *et al.*, 2016).

As we provided a limited amount of yeast as a food source for the flies, most flies were likely hungry and active in their search for food from early on, and the low fly survival over the experiment clearly shows that the flies had desperately needed energy. Yeast moreover contains a high ratio of protein relative to carbohydrate compared to the ratio required by the flies, and flies have needed carbohydrate both to attain energy and to balance nutrients (Lee *et al.*, 2008). Honeydew is an important energy source for fruit flies in nature (Bateman, 1972), and it is likely that the flies actively foraged for honeydew in our mesocosms and thus induced high disturbance stress on the aphids. In nature, such disturbance could come from many other dipteran species as well as other insects that include honeydew as a dietary supplement (Ossowski & Hunter, 2000; Hung *et al.*, 2015; Bistline-East *et al.*, 2018; van Neerbos *et al.*, 2020). Honeydew from *R. padi* has been found to attract large numbers of dipterans in the field (Monsrud & Toft, 1999). We used only one, relatively high fly density for the proof of concept. However, it would be relevant to perform similar studies at a range of commensal densities to analyze the relationship between commensal density and herbivore suppression, including the minimal density that has a measurable effect. The finding of commensal effects on *R. padi* suggests a general phenomenon, as *R. padi* does not have obvious antipredator responses like e.g. seen in pea aphids (Nelson, 2007). Further studies are required to determine whether the fitness cost in *R. padi* was predominantly caused by lower adult or offspring survival, or by lower individual reproduction under disturbance.

Our study has implications in ecosystem functioning and conservation biological control, as it shows that commensals with no direct consumptive nor competitive effect may have a suppressing effect on herbivore populations via their activity, and thus add to their top-down control. This may in particular be the case for aphids that produce honeydew, which attracts a range of other insects including both commensals and natural enemies (Monsrud & Toft, 1999). In agroecosystems, inclusion of patches with natural vegetation are promoted to increase insect diversity and support the abundance of biological control agents. Our results indicate that the presence of not only predatory but also non-predacious insects benefitting from such patches will aid in the overall control of aphids, further supporting the idea of adding natural patches in agricultural landscapes. Furthermore, aphids including *R. padi* are low-quality prey for generalist predators, which therefore do not prefer this prey (Toft, 2005). Our study suggests maximal suppression of *R. padi* and likely other honeydew producing herbivores when predators and commensals are combined. Further studies on the effect of commensals on herbivore populations are required to fully understand the effect of commensals in ecosystem population dynamics.

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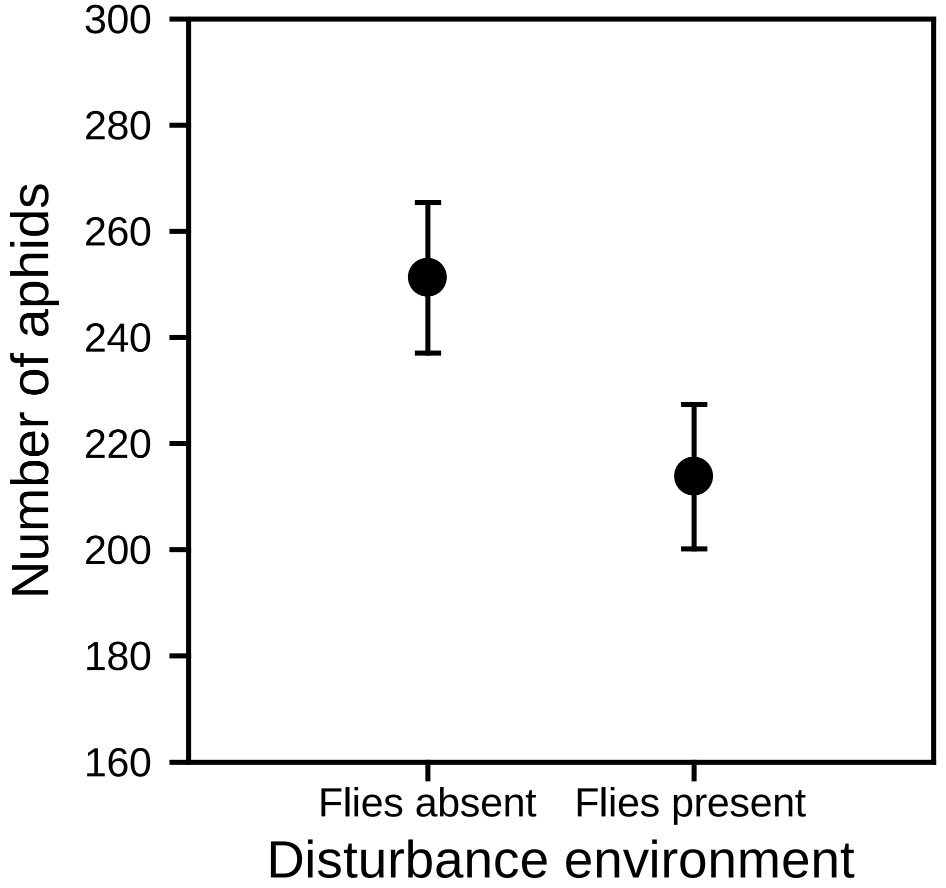

Fig. 1. Number (mean ± SE) of adult and juvenile bird cherry-oat aphids (*Rhopalosiphum padi*) in mesocosms with seedling wheat after six days of establishment and reproduction. Each *R. padi* population was initiated with 20 adult aphids and given one day to establish, after which 100 vinegar flies (*Drosophila melanogaster*) were added to a random half of the mesocosms.