Are tritrophic interactions shaped by plant genotype? A field experiment.

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Abstract

In recent years it has become apparent that when studying plant-herbivore interactions, it is important to also consider the multitrophic context, i.e. to what extent generalist and specialist enemies act as bodyguards for the plant. I have performed a field experiment with 25 genets, varying in plant defense levels, of meadowsweet (Filipendula ulmaria) and its herbivore, the chrysomelid beetle Galerucella tenella. Each genet was treated with four different density manipulations of G. tenella (5, 15, 30, and 60 larvae per genet). The experiment was set up on an archipelago island with natural levels of a specialist hymenopteran parasitoid Asecodes parviclava and generalist natural enemies. I set up the following hypotheses: 1) larval survival will be higher on less defended plants than on more defended plants, 2) the proportion of parasitoids will be higher on more defended plants, and 3) larval survival will increase with increasing larval densities. The most important findings of the experiment were an overall very low total survival of larvae (G. tenella pupae and A. parviclava mummies), a generally high parasitism by A. parviclava on more defended plants, and that two groups of generalist predators (hunting and web-forming spiders) were more frequent on less defended plants. My study suggests that herbivore survival may not be higher on less defended plants under situations with abundant occurrence of generalist predators, as they are able to trace high-quality foraging sites.

Keywords

Tritrophic interactions, plant genotype, aggregative behaviour, Galerucella tenella, Asecodes parviclava
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Introduction

Most plants are exposed to a species-rich world of herbivorous arthropods. In response to their damage, plants have evolved various kinds of chemical defenses. These may either be direct defenses in form of harmful substances that deter feeding, or indirect defenses in form of herbivore-induced volatiles. These volatiles can attract natural enemies of the herbivores, such as parasitoids and predators, thus leading to plant-mediated differences of top-down herbivore control (Vet and Dicke 1992, Mumm and Dicke 2010). Volatiles released from damaged plants are frequently specific and the sensory perception of plant volatiles by herbivore enemies is also specific. Thus, enemies exploit the information provided by plant volatiles in searching for their prey or host, in particular specialist parasitic hymenoptera (McCormick et al. 2012). It is generally argued that species-specific chemicals are of large importance for specialist systems. However, to which extent they really affect plant fitness is under debate, since species-specific chemicals in theory are available to all organisms using olfactory cues for prey and host search (Kessler and Heil 2011).

Studies on plant-herbivore interactions have mainly focused upon whether females choose to oviposit on hosts on which their offspring perform best (Gripenberg et al. 2010). However, a number of field studies have revealed a mismatch between female choice and offspring performance. For example, Björkman et al. (1997) found that females of the pine sawfly Neodiprion sertifer preferred to oviposit on trees on which their offspring suffered less from attack by parasitoids over trees on which offspring performance was high. This has resulted in a vivid interest to understand whether parasitoids and predators may function as bodyguards reducing plant damage (Price et al. 1980, Mumm and Dicke 2010). Many insect herbivores show an aggregative behaviour, and in several systems high larval densities are correlated with increased larval growth or pupal weight (Hambäck 2010). These positive feedbacks are assumed to arise because extensive attacks drain plant defenses and indirectly increase plant quality (Hambäck 2010). That means that not only plant quality per se has to be assessed in studies on plant-herbivore interactions. Instead, in order to understand the outcome of plant-herbivore interactions, both resource-based, bottom-up forces and natural enemy-based, top-down forces have to be addressed.

Several studies have demonstrated that plant genotypes shape arthropod community structure and multitrophic interactions (e.g. Johnson 2008, Kos et al. 2011, Abdala-Roberts et al. 2012). However, most studies that have been addressing tritrophic interactions have been conducted under more controlled conditions and with agricultural systems. In contrast the numbers of studies in more natural systems are limited (but see Pöykkö 2011).

In this study I have used the tall herb Filipendula ulmaria, its herbivore, the chrysomelid beetle Galerucella tenella and its hymenopteran parasitoid Asecodes parviclava (Eulophidae). Earlier studies in the Skeppsvik archipelago (Stenberg et al. 2006) have shown that plants in older populations that have been exposed to intense beetle herbivory for a longer time showed higher concentrations of defensive chemicals. Further, a multi-choice experiment showed that these plants were less consumed by adult beetles and less used for oviposition, and finally larvae performed poorly and showed a slower growth rate on plants from well-defended, older host populations while they performed well on plants from less-defended, younger populations. Unpublished work has also shown that more defended
plants show a slower growth rate and a decreased flower production (Lars Ericson, pers. com.). Later studies have shown that *A. parviclava* has a delayed effect as bodyguard on *F. ulmaria*; high parasitism in one year was followed by lower levels of leaf damage and increased seed set for *F. ulmaria* in the following year (Stenberg et al. 2007).

I conducted a field experiment in which I manipulated larval densities of *G. tenella* on experimental plants that showed a broad variation in plant defense. The experiment was set up on a small island with abundant natural occurrence of the host plant and high densities of both *G. tenella* and *A. parviclava*. In that way I wanted to study whether larval survival increased with increasing larval densities and decreasing levels of chemical defense (i.e. increasing plant quality) and whether *A. parviclava* functioned as a bodyguard. It should be added that aggregative behaviour has not been experimentally demonstrated for *G. tenella*, only for the phylogenetically closely related *G. calmariensis* (Hambäck 2010). However, field data on egg laying behaviour show clear aggregated patterns for both species (Lars Ericson pers. comm.)

In accordance with earlier studies on the system (Stenberg et al. 2006) I expected an increase in larval survival on less defended plants. As larval development will take longer time on more defended plants (Stenberg et al. 2006) I expected a higher proportion of parasitized larvae on these plants. The rationale being that a longer development time will not only increase the time of exposure but also that the female wasps avoid to attack larger larvae, as larger larvae show a clear defense behaviour when approached (own observations). For the density manipulations I expected to find that increased densities should result in higher survival rates in accordance with the study by Hambäck (2010) and that this would be more pronounced for plants of more intermediate palatability. I also expected that plants of low resistance already has such a low defense that high larval densities are unlikely to result in any marked change in food quality, while highly defended plants are so toxic that high densities will last for such a short time that they are unlikely to break host defense. However, an alternative hypothesis is also possible, namely that the increased plant damage expected at higher larval densities would attract more female hymenopterans, resulting in an increased parasitism, thus increasing the bodyguard effect.

I set up the following hypotheses: 1) larval survival will be higher on less defended plants than on more defended plants; 2) the proportion of parasitoids will be higher on highly defended plants; 3) Larval survival will increase with increasing larval densities, and this increase will be more marked for plants of intermediate palatability. An alternative hypothesis is that increased larval densities will result in increased parasitation rates.
2 Material and methods

2.1 Study system

Meadowsweet, *Filipendula ulmaria* (L.) Maxim. (Rocaceae), is a tall herb that occurs in abundance on islands in the Skeppsvik archipelago. The archipelago is under isostatic rebound and new islands are continuously formed and colonized by vascular plants which in turn are colonized by various natural enemies (Stenberg et al. 2006). Older populations show a number of traits, such as higher leaf concentration of individual phenolics and condensed tannins, mirroring the effect of past coexistence with its specialist leaf beetle herbivores (Stenberg et al. 2006). This cost of energy results in decreased growth and decreased flower production (Lars Ericson, pers. com.).

*Galerucella tenella* L. (Coleoptera: Chrysomelidae) is a common oligophagous herbivore, with *F. ulmaria* as a major food source (Stenberg et al. 2006). In the innermost part of the archipelago it also feeds on marsh cinquefoil (*Potentilla palustris*) and arctic bramble (*Rubus arcticus*), none of which were present on the experimental island.

Bioassays have shown that *G. tenella* adults prefer to feed and oviposit on less defended *F. ulmaria*, and that larvae developed better and had a higher survival on plants from younger, than from older populations (Stenberg et al. 2006). Adults appear in early June, when they feed on leaves and oviposit on leaves and the floral shoot. Larvae start to emerge in early July and feed on leaves, flower buds and seed embryos. Pupation takes place in the top soil and adults hatch in late July and through most of August.

The hymenopteran parasitoid *Asecodes parviclava* (Thomson) (Eulophidae; earlier *A. mento*; Hansson and Hambäck 2013) is a gregarious koinobiont of *G. tenella*. It also attacks larvae of two other closely related chrysomelids *G. calmariensis* and *G. pusilla*, both monophagous on *Lythrum salicaria*, of which the former also occurs in the archipelago (Hansson and Hambäck 2013, Hambäck et al. 2013). Females oviposit in the larvae and successful parasitoid development leads to the mummification of the host larva. After successful development the parasitoid larvae pupate inside the mummified host larva. The number of pupae within a mummy is highly variable (1-14; Stenberg and Hambäck 2010). The mummies are black and easy to separate from the light yellowish pupae of *G. tenella*. *A. parviclava* is known to be a major cause of mortality of *G. tenella* and the parasitism rates are typically high in the studied archipelago (Hambäck et al. 2006).

Plants used in the experiment were raised from seeds sampled from islands in the Skeppsvik archipelago in fall 2006. Seeds were sown in 3x3x7 cm pots in an unheated greenhouse at the Umeå University campus and germinating seedlings were later in 2007 transferred to plastic pots (7x7x10 cm) and kept for cultivation in the experimental garden. In 2009, 30 genets from mother plants that varied in resistance towards *G. tenella* were cloned by cutting the root stock in pieces and raised in pots (rose pots, diameter 15.5 cm, 1 l). These plants were in 2010 utilized in a bioassay experiment in order to determine the development time of *G. tenella* larvae (see below). Out of this plant material plants from 25 genets were selected in 2011 for the present experiment. Criteria for this selection were that the genets should
represent a wide variation in plant resistance and not vary too much in size, thus excluding small and large plants. A garden soil (Hasselfors P jord) was used throughout the years.

In order to obtain a proxy for plant resistance a bioassay experiment was run in 2010. For each of 30 genets, the survival, development time and pupal weight were determined for 20 *G. tenella* larvae. Newly hatched larvae were individually placed on leaves in Petri dishes and their development were followed daily or every second day. Fresh new leaves were offered every second day. As survival of the larvae showed a broad variation for the different genets the half-life survival of the larvae has been used as a proxy of plant quality (data provided by Lars Ericson).

### 2.2 Experiment

The experiment comprises plants from 25 genets, each replicated 4 times and randomly assigned to one of four larval densities: 5, 15, 30 and 60 larvae per plant. The choice of densities was based on the densities used by Hambäck (2010) and the normal variation of *G. tenella* egg loads in the archipelago. Before the start, the soil in each pot was covered with plant fabric and above that a 3-4 cm sand-soil mixture (7:3), making it easier to later on locate the pupae and mummies. Before pupation the larvae crawl down the floral shoot and hide in the topsoil beneath the host plant. Since the plants remained in their pots throughout the experiment it is very unlikely that they will pupate outside the pot provided that the topsoil has a loose structure (c.f. Hambäck 2010).

Host plants with abundant occurrence of *G. tenella* eggs were sampled from the archipelago, placed in plastic cans with water, and brought to the experimental garden where newly hatched 1st instar larvae of *G. tenella* were placed on the experimental plants. We used four different densities for each genet, namely 5, 15, 30 and 60 larvae per plant. The plants were then transferred to the experimental island on two different dates (14 July, 13 genets) and (16 July, 12 genets).

For the experiment we chose a small open island with large stands of *F. ulmaria* on the higher part. The reason was that high densities of both *G. tenella* and the parasitoid *A. parviclava* had occurred both in 2009 and 2010 which was assumed to increase the possibility that the experimental plants would be exposed to the parasitoid.

In the field, the four plants of each genet were placed together in small open patches, surrounded by stands of *F. ulmaria*. Interplant distance within each patch was 4.5-5 dm which was sufficient to avoid contact between plants. Distance between patches was 4-5 m.

The exposure of the plants in the field was terminated on 26 July when we did not observe that any active larvae remained on the plants. At the same time we also recorded the number of spiders as we observed that they occurred in very high densities. They were classified in two broad groups: web forming and hunters. The plants were brought back to mainland and the sand-soil mixture above the plant fabric was sifted through a net (mesh size 1.0 mm) in order to simplify the search for the light yellowish brown *G. tenella* pupae and the black parasitized mummies. Data was also collected for percentage leaf area damaged and number of intact and consumed flowers (not presented).
In order to obtain data on natural levels of parasitism on the experimental island, 50 *G. tenella* larvae were sampled (25 on 14 July, 25 on 16 July) and reared to pupation.

### 2.3 Statistical analyses

The half-life of larval survival of the genets used in the experiment ranged from 4.0-10.2 days. The genets were divided into three edibility groups, where the half-life of the groups was as follows: group 1 (high edibility) 8-10.2 days, group 2 (intermediate edibility) 6-7.5 days, and group 3 (low edibility) 4.0-5.6 days. Sample sizes were 36, 32 and 32 plants, respectively. I have pooled the data even though the experimental start differed with two days. All statistical analyses were performed using R version 2.15.0 (R Development core team 2012). Data on proportion of surviving pupae were arcsine-transformed, and thereafter analyzed in a two-way ANOVA. Contrasts were performed using Tukey’s Honestly Significant Difference (Tukey’s HSD) test.
3 Results

The overall survival in the experiment was very low and ranged from 5% to 14%, with the only exception of a survival of 33% that was found at the lowest larvae density on plants in the high edibility group (Fig 1).

Figure 1. Proportion surviving *G. tenella* larvae, either forming *G. tenella* or *A. parviclava* pupae, at four different larval densities (5, 15, 30, and 60 larvae per plant) on each of three edibility groups (H: high, I: intermediate, and L: low) of the host plant *F. ulmaria*. (Mean +SE).

Increased larval density reduced the proportion of surviving larvae (p<0.001; Table 1).

*Table 1. Effects of larval density (LD) and host plant edibility (E) on the proportion of added *G. tenella* larvae that survived and either formed *G. tenella* or *A. parviclava* pupae, tested by using two-way ANOVA in R.*

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval Density (LD)</td>
<td>3</td>
<td>6.136</td>
<td>0.0008</td>
</tr>
<tr>
<td>Edibility (E)</td>
<td>2</td>
<td>2.674</td>
<td>0.0746</td>
</tr>
<tr>
<td>LD*E</td>
<td>6</td>
<td>2.379</td>
<td>0.0354</td>
</tr>
</tbody>
</table>
Contrast analysis confirmed that the treatment at lowest larvae density in the high edibility group was the only treatment that significantly differed from the others (Table 2). With exception for the total survival of pupae, no interaction effect between larval density and plant edibility was found in any of the ANOVA tests performed.

Table 2. Tukey’s honest significance test (HSD) of one-way ANOVA on the effect of larval density (LD) on the proportion of total survival (G. tenella + A. parviclava pupae) on F. ulmaria plants of high palatability. A: 5, B: 15, C: 30, and D: 60 larvae per plant.

<table>
<thead>
<tr>
<th>Larval Density</th>
<th>p</th>
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<tbody>
<tr>
<td>A-B</td>
<td>0.043</td>
</tr>
<tr>
<td>A-C</td>
<td>0.0158</td>
</tr>
<tr>
<td>A-D</td>
<td>0.0060</td>
</tr>
<tr>
<td>B-C</td>
<td>0.9745</td>
</tr>
<tr>
<td>B-D</td>
<td>0.855</td>
</tr>
<tr>
<td>C-D</td>
<td>0.9815</td>
</tr>
</tbody>
</table>

The proportion of A. parviclava of the total number of surviving pupae did not differ between the four larval densities, while there was a significant effect of plant edibility (Fig. 2, Table 3). The highest proportion of A. parviclava was found in the low edibility group.

Figure 2. Proportion A. parviclava pupae of the total number surviving pupae (G. tenella or A. parviclava) at four different larval densities (5, 15, 30, and 60 larvae per plant) on each of three edibility groups (H: high, I: intermediate, and L: low) of the host plant F. ulmaria. (Mean + SE).
There was no indication of any differences between the intermediate and high edibility group (Table 3).

Table 3. Effect of larval density (LD) and host plant edibility (E) on the proportion of A. parviclava pupae of the total number of formed pupae (G. tenella + A. parviclava), tested by using two-way ANOVA in R.

<table>
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<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval Density (LD)</td>
<td>3</td>
<td>0.458</td>
<td>0.7124</td>
</tr>
<tr>
<td>Edibility (E)</td>
<td>2</td>
<td>6.612</td>
<td>0.0023</td>
</tr>
</tbody>
</table>

Both larval density and plant edibility had a significant effect on the number of hunting spiders (Fig 3, Table 4 and 5), while only plant edibility had a significant effect on the number of web-forming spiders (Fig. 4, Table 4). For both groups of spiders the highest numbers were found on the most edible plants. This was most marked for the hunting spiders which were more than twice as abundant on plants of the high-edibility compared with the two other edibility groups.

Figure 3. Number of hunting spiders at four different larval densities (5, 15, 30, and 60 larvae per plant) on each of three edibility groups (H: high, I: intermediate, and L: low) of the plant F. ulmaria. (Mean +SE).
The number of hunting spiders increased significantly with larval density. In contrast, the web-forming spiders remained more or less unaffected by larval density within each of the three edibility groups and contrast analysis showed no significance for larval density with one exception (at larval density 30, between the high and intermediate edibility groups; p=0.0189; data not shown).

Table 4. Effects of larval density (LD) and host plant edibility (E) on the number of (A) hunting spiders and (B) web-forming spiders, respectively, tested by using ANOVA in r.

<table>
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<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Larval Density (LD)</td>
<td>3</td>
<td>5.204</td>
</tr>
<tr>
<td></td>
<td>Edibility (E)</td>
<td>2</td>
<td>17.732</td>
</tr>
<tr>
<td>B</td>
<td>Larval Density (LD)</td>
<td>3</td>
<td>0.184</td>
</tr>
<tr>
<td></td>
<td>Edibility (E)</td>
<td>2</td>
<td>10.650</td>
</tr>
</tbody>
</table>

Table 5. Tukey’s honest significance test (HSD) of one-way ANOVA on the effect of edibility (E) on the number of hunting spiders. H: high edibility, I: intermediate edibility and L: low edibility.

<table>
<thead>
<tr>
<th>Larval Density</th>
<th>Edibility</th>
<th>P</th>
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<tbody>
<tr>
<td>5</td>
<td>H-I</td>
<td>0.0661</td>
</tr>
<tr>
<td></td>
<td>L-I</td>
<td>0.8627</td>
</tr>
<tr>
<td></td>
<td>L-H</td>
<td>0.1799</td>
</tr>
<tr>
<td>15</td>
<td>H-I</td>
<td>0.1457</td>
</tr>
<tr>
<td></td>
<td>L-I</td>
<td>1.0000</td>
</tr>
<tr>
<td></td>
<td>L-H</td>
<td>0.1457</td>
</tr>
<tr>
<td>30</td>
<td>H-I</td>
<td>0.0589</td>
</tr>
<tr>
<td></td>
<td>L-I</td>
<td>1.0000</td>
</tr>
<tr>
<td></td>
<td>L-H</td>
<td>0.0589</td>
</tr>
<tr>
<td>60</td>
<td>H-I</td>
<td>0.0106</td>
</tr>
<tr>
<td></td>
<td>L-I</td>
<td>0.7362</td>
</tr>
<tr>
<td></td>
<td>L-H</td>
<td>0.0017</td>
</tr>
</tbody>
</table>
4 Discussion

The overall survival in the experiment was very low, neither the total survival nor the proportion of *G. tenella* pupae increased at higher larval densities, while the number of hunting spiders did. Furthermore, plant quality (edibility) had significant effects on the proportion of *A. parviclava* mummies and the number of hunting and web-forming spiders. Thus both top-down and bottom-up forces interacted and played important roles for the outcome of the experiment.

I found no support for the hypothesis that increasing larval densities implies an increased fitness (survival) of *G. tenella* which contrasts to the study of Hambäck (2010). He reported, for the closely related *G. calmariensis* and *G. pusilla*, an increased fitness at higher larval densities. However, his experiment was run under more controlled conditions in the absence (or near absence) of parasitoid wasps and generalist predators. Instead, the highest total survival in my experiment, 33%, was found at the lowest larval density and on the most edible plants. The observed pattern may depend upon that higher larval densities, as well as the level of plant defense, may attract specialist or generalist natural enemies of the herbivore, thus supporting the hypotheses that they act as bodyguards for the host plant (Price et al. 1980, Vet and Dicke 1992, Mumm and Dicke 2010). That I did not find any positive effect of larval density on the survival of the *G. tenella* larvae also raises the question under which ecological constrains aggregative behavior may be advantageous or not.

The design of the experiment does not allow a critical test of the relative importance of specialist (*A. parviclava*) and generalist (spiders) natural enemies which both occurred at high densities during the experiment. A control sample of 49 *G. tenella* larvae from nearby *F. ulmaria* plants showed that 35 out of 49 (71.4%) sampled larvae were parasitized by *A. parviclava*, and data from both 2010 and 2012 showed that spider densities were about twice as high in 2011. Thus it is tempting to assign the low overall survival in the experiment as a result of strong top-down forces. However the relative importance of generalist predators and parasitoids is not possible valuate due to experimental design.

The bodyguard hypothesis (Price et al. 1980) posits that attacked plants attract specialist, as well as generalist, natural enemies. However, larval density did not have any effect on the proportion of surviving larvae that formed mummies of *A. parviclava*. Instead, the proportion of mummies was significantly higher, around 80%, on plants of the low edibility group. This also means that the proportion of larvae that formed pupae of *G. tenella* was higher on the plants of the mid- and high edibility groups. One explanation for the observed pattern, the higher parasitation rate on defended plants, is that larvae on well-defended plants show a slower growth rate (Stenberg et al. 2006) resulting in an extended exposure time for parasitoid attack. Another and not mutually exclusive explanation may be linked to the egg laying behavior of the female wasps. When they approach a larva, the larva starts to swing the body and female wasps interrupt the attack (own observations). This may explain the lower proportion of *A. parviclava* mummies on less defended plants in the experiment. The high *A. parviclava* densities prevailing during the experiment may also have resulted in so frequent attacks both of larvae at high densities and of small larvae resulting in increased
mortality. Thus a bodyguard effect may have occurred although escaped notice due to the experimental design.

The data on web-forming and hunting spiders revealed very high densities of generalist predators during the experiment. Both groups showed higher densities on less defended plants, and hunting spiders also showed a strong increase with increasing larval densities, while web-forming spiders did not. The observed patterns show that both groups of generalist predators responded upon plant quality, or rather that they responded upon that less defended plant hosts most likely sustain higher densities of prey, thus offering better foraging sites. When spiders occur in high densities, they are known to cannibalize and evict each other from attractive foraging sites (Harwood et al. 2003, Denno et al. 2004). This intraguild competition might play a role in explaining why the two groups of spiders responded differently to the larval density treatment in the experiment.

My results suggest that the positive effect less defended plants have on G. tenella larvae is mitigated in situations when generalist predators are abundant, since these plants are favored as foraging sites. Generalist predators have previously been shown to markedly increase plant fitness (Östman et al. 2003). This increase in fitness may thus be stronger for less defended plants. In cases when generalist predators are more attracted to less defended plants, hymenopteran parasitoids may suffer less from competition when parasitizing larvae on more defended plants. Thus the possibility for the parasitized larvae to avoid attacks from generalists would increase. This would imply a double advantage for A. parviclava to search for hosts on more defended plants.

My study shows that in order to assess plant-herbivore interactions it is important to use a multitrophic perspective invoking both bottom-up and top-down forces and that the outcome of these interactions are likely to be context dependent and vary in space and time (Berger et al. 2012).

5 Acknowledgments

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6 References


### 6.1 Other sources

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