Aphid–parasitoid community structure on genetically modified wheat

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Since the introduction of genetically modified (GM) plants, one of the main concerns has been their potential effect on non-target insects. Many studies have looked at GM plant effects on single non-target herbivore species or on simple herbivore–natural enemy food chains. Agro-ecosystems, however, are characterized by numerous insect species which are involved in complex interactions, forming food webs. In this study, we looked at transgenic disease-resistant wheat (Triticum aestivum) and its effect on aphid–parasitoid food webs. We hypothesized that alterations on the plant level owing to GM could affect the associated aphid–parasitoid–hyperparasitoid food web. We studied this on two disease-resistant GM wheat lines (Triticum aestivum), their respective non-transformed controls and a conventional line.

2. MATERIAL AND METHODS

(a) Study organisms
Metopolophium dirhodum, Rhopalosiphum padi and Sitobion avenae are common cereal aphids in Europe [5]. They are attacked by primary parasitoids that oviposit inside the living aphid. The parasitoid larva paralyzes inside the dead aphid, forming a ‘mummy’. Primary parasitoids are attacked by two guilds of secondary parasitoids, i.e. hyperparasitoids and mummy parasitoids. Hyperparasitoids attack primary parasitoids before mummification, whereas the more generalist mummy parasitoids attack the already mummified aphid irrespective of whether it contains a primary parasitoid or a hyperparasitoid.

(b) Plant material
We used five wheat lines belonging to three different spring-wheat varieties: Bobwhite, Frisal and Rubli. For Bobwhite and Frisal, each had a transgenic line and its corresponding control line. The transgenic line of Bobwhite (Pm3b#1) carries the transgene Pm3b of hexaploid wheat, which confers race-specific resistance to wheat powdery mildew [6]. The non-segregant line Sb#1 was used as the corresponding control line. The second wheat pair consisted of the non-transformed variety Frisal as a control and the GM Frisal line Chi/Glu(A13) containing the anti-fungal barley seed chitinase and β-1,3-glucanase [7,8]. This line, however, has not been shown to display enhanced resistance to powdery mildew [8]. The variety Rubli is commercially grown in Switzerland.

(c) The convertible glasshouse
The experiments were carried out in a convertible glasshouse that approximates field conditions by exposing the plants to outside environmental temperatures and allowing natural colonization by insects and pathogens. The roof and side walls automatically open under good weather conditions but close during strong winds and rainfall and during the night [9]. Plants were grown in 40 plots (80 × 80 × 80 cm) arranged in two rows, each plot containing the five wheat lines in randomized order. Plants were sown by the end of March and harvested by the end of July. Before sowing, basic fertilizer was added to the soil (per plot: 2008: 5.8 g P, 7.2 g K, 1.7 g Mg, 8.7 g N; 2009: 10.32 g P, 8 g K, 9 g Mg(NO3)2). Plants were watered as required. Flowering ears of all wheat lines were covered with pergamum paper bags to prevent pollen from escaping. No pesticides were applied.

(d) Sampling
Experiments were conducted in 2008 and 2009. Every week, we counted and identified all aphids and collected all mummies on the experimental plants in the central cylinder from mid May until harvest. Parasitoid mummies were stored separately in gelatin capsules at room temperature. After four months, emerged parasitoids were identified to species level using the same keys as Müller et al. [10].

(e) Quantitative food web metrics and data analysis
Traditional binary measures of food web structure are highly sensitive to sample size [11]. Therefore, we used measures based on quantified and have proven to be a useful system for exploring multi-trophic interactions.

Genetic modification (GM) of plants can cause differences between the transformed varieties and their conventional counterpart. For instance, changes in phloem sap composition can affect organisms that feed upon these plants including sap-feeding aphids [3]. In an aphid–parasitoid–hyperparasitoid food web, the effects of host plant traits can cascade up as far as the fourth trophic level [2], even when there are no effects on the herbivores [4].

We hypothesized that alterations on the plant level owing to GM could affect the associated aphid–parasitoid–hyperparasitoid food web. We studied this on two disease-resistant GM wheat lines (Triticum aestivum), their respective non-transformed controls and a conventional line.

1. INTRODUCTION
Insect species that inhabit agricultural ecosystems are involved in complex interactions, forming so-called food webs [1]. The diversity and complexity of such food webs are considered to be important factors that determine ecosystem function and stability. Insect host–parasitoid systems are influenced by plant traits, which can lead to large effects on food web structure [2]. Due to their biology, interactions between aphids, primary parasitoids and secondary parasitoids can easily be
Table 1. Aphid–parasitoid associations across 2 years with respective food web codes in brackets as they appear in figures S1 and S2 of the electronic supplementary material. Numbers in the table correspond to the individuals found for the specific aphid–parasitoid association.

<table>
<thead>
<tr>
<th>Aphid species (year)</th>
<th>Metopolophium dirhodum (21)</th>
<th>Rhopalosiphum padi (22)</th>
<th>Sitobion avenae (23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. avenae (2008)</td>
<td>1</td>
<td>26</td>
<td>6</td>
</tr>
<tr>
<td>R. padi (2008)</td>
<td>290</td>
<td>54</td>
<td>6</td>
</tr>
<tr>
<td>C. clavata (2008)</td>
<td>98</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>D. carpenteri (2008)</td>
<td>18</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>D. laticeps (2008)</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. sp. (2008)</td>
<td>34</td>
<td>5</td>
<td>13</td>
</tr>
</tbody>
</table>

Shannon information theory [12], as described in Bersier et al. [13] and adapted for host–parasitoid webs [2], which use the densities of species and the frequency of interactions: (i) parasitoid diversity, which is equal to species richness when all species are equally abundant but takes on smaller values when abundances are uneven; (ii) link evenness, which equals one when all trophic links have equal frequencies and asymptotically approaches zero for highly uneven frequencies; and (iii) quantitative realized connectance, which is the observed link diversity as a proportion of maximum possible link diversity and is a measure of the complexity of the network. Since secondary parasitoids cannot be unambiguously linked to primary parasitoids [10], we calculated all the food web metrics separately for the aphid–primary parasitoid and the aphid–secondary parasitoid matrices.

We conducted analyses across both years and for each year separately. Parasitoid diversity was square root-transformed; link evenness and realized connectance were arcsine-transformed. Further, we analysed cumulative aphid and mummy abundance, parasitoid hatching success and parasitism rates. Aphid and mummy abundance were used in a generalized linear model with binomial errors. All analyses were done with the statistical software R (R Development Core Team).

3. RESULTS

Three cereal aphid species were recorded in the two study years: M. dirhodum (mean seasonal density per cylinder: 2008: 88.6; 2009: 248.4), R. padi (2008: 66.3; 2009: 76.6) and S. avenae (2008: 2.4; 2009: 17.2). Overall aphid abundance was significantly higher in 2009 ($F_{1,66} = 160.71$, $p < 0.001$) and depended on variety ($F_{2,66} = 5.26$, $p = 0.007$) and on the variety x GM interaction ($F_{1,66} = 4.08$, $p = 0.047$), which was caused by a higher total aphid abundance on Pm3B#1 when compared with its control line Sb#1, whereas abundance was the same on Chi/ Glu(A13) and Frisal. The number of mummies was significantly higher in 2009 when compared with 2008 ($F_{1,66} = 203.23$, $p < 0.001$) and was positively aphid density-dependent ($p < 0.001$, $R = 0.888$). Parasitism rate was low at 2 per cent in both years and negatively correlated with aphid density ($p = 0.023$, $r = -0.253$). Neither wheat variety nor GM or their interaction had an effect on parasitism (variety: $F_{2,66} = 2.10$, $p = 0.130$; GM: $F_{1,66} = 2.70$, $p = 0.105$; variety x GM: $F_{1,66} = 2.04$, $p = 0.158$).

From a total of 3492 mummies collected in both years, 2108 hatched (60.6%). Hatching success was influenced by host aphid ($p < 0.001$; M. dirhodum > R. padi > S. avenae) and by year ($p < 0.001$; 2009 > 2008). We identified 21 parasitoid species, of which eight were primary parasitoid species, seven hyperparasitoid species and six species were mummy parasitoids (table 1). In a parallel field study using the same wheat lines, we found the same aphid and parasitoid species (electronic supplementary material).

The analysis across both years revealed significant annual variation for all the food web metrics except realized connectance of secondary parasitoids (figures 1 and 2). The variety x GMO interaction was significant for link evenness of primary parasitoids ($F_{1,66} = 8.312$, $p = 0.005$). No other differences were found.

Separate analysis of the 2 years revealed several different, inconsistent effects. In 2009, we only found significant effects on the primary parasitoid web. Primary parasitoid diversity significantly differed between the three varieties (figure 1b) and was
highest on Rubli. The variety \times GM interaction was significant for primary parasitoid link evenness (figure 1d). By contrast, in 2008 we only found effects on the secondary parasitoid web: a significant variety effect on link evenness (figure 2c) was found and significantly higher realized connectance on the GM plant lines (figure 2e). The aphid–parasitoid food webs are shown in the electronic supplementary material.

4. DISCUSSION
While other studies have assessed the effects of GM plants on aphids and their parasitoids [14,15], this is the first to look at quantitative food web metrics of an aphid–parasitoid community.

Aphid abundance on the GM line Pm3b#1 was higher when compared with the control line Sb#1. This could directly be due to genetic differences in the plants or to indirect effects caused by the difference in mildew infection. As expected, the Pm3b#1 plants showed enhanced powdery mildew-resistance when compared with the control Sb#1, whereas Chi/Glu(A13) was equally susceptible to powdery mildew as its control line Frisal. In an earlier study we showed that the Pm3b#1 plants did not affect the performance of M. dirhodum in the absence of powdery mildew [16]. We therefore suspect that an indirect effect through mildew causes this difference in aphid abundance.

Parasitism rate was negatively host density-dependent, indicating that parasitoids keep up with the aphid population growth only to a certain point. The early presence of parasitoids in the field therefore seems crucial for keeping aphid populations under control as has been suggested before [17]. Parasitoid diversity remained

Figure 1. Means (±s.e.m.) of the calculated food web metrics of the primary parasitoid web per wheat line and year. Pm3b#1 and Chi/Glu(A13) are the GM lines. Sb#1 and Frisal are their respective non-GM controls. Rubli is the conventional line. (a,b) Parasitoid diversity; (c,d) link evenness; (e,f) realized connectance.
generally unaffected by the wheat lines except for primary parasitoid diversity in 2009, which was higher on Rubli. Higher parasitoid diversity has been linked to higher parasitism rates [18], an effect that was not observed here.

Link evenness was affected in the primary parasitoid and in the secondary parasitoid webs but differently so. Variety affected the secondary web in 2008, whereas GM within the varieties affected link evenness of the primary parasitoid web in different directions in 2009. Food web structure on the transgenic Pm3b#1 line was more dominated by a few stronger links, whereas on the control line Sb#1 they were more evenly distributed. For the GM line Chi/Glu(A13) and its control, this was the other way around. In 2008, connectance of secondary parasitoid food webs was increased on the GM lines when compared with the control lines with potential implications for food web stability [19].

Quantitative food webs allow comparison of the diversity and complexity of species assemblages, which are considered to be the major factors determining ecosystem functioning and stability. Despite the observed changes in community structure among the different wheat lines studied, it is difficult to draw conclusions about the ecological implications of these effects. Observed differences in community structure were inconsistent between the two study years, and there was as much variation between wheat varieties as there was between GM and control lines within varieties. This phenomenon has been observed before in other studies dealing with non-target effects of GM crops [20]. Plant characteristics seem to be more distinct between varieties than between GM plants and the corresponding controls. Therefore, we believe that the observed GM effects are of little ecological significance and fall within the natural variation observed among cultivars.

Figure 2. Means (± s.e.m.) of the calculated food web metrics of the secondary parasitoid web per wheat line and year. Pm3b#1 and Chi/Glu(A13) are the GM lines. Sb#1 and Frisal are their respective non-GM controls. Rubli is the conventional line. (a,b) Parasitoid diversity; (c,d) link evenness; (e,f) realized connectance.
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