Cross-kingdom interactions matter: fungal-mediated interactions structure an insect community on oak

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Abstract

Although phytophagous insects and plant pathogens frequently share the same host plant, interactions among such phylogenetically distant taxa have received limited attention. Here, we place pathogens and insects in the context of a multитrophic-level community. Focusing on the invasive powdery mildew Erysiphe alphitoides and the insect community on oak (Quercus robur), we demonstrate that mildew–insect interactions may be mediated by both the host plant and by natural enemies, and that the trait-specific outcome of individual interactions can range from negative to positive. Moreover, mildew affects resource selection by insects, thereby modifying the distribution of a specialist herbivore at two spatial scales (within and among trees). Finally, a long-term survey suggests that species-specific responses to mildew scale up to generate landscape-level variation in the insect community structure. Overall, our results show that frequently overlooked cross-kingdom interactions may play a major role in structuring terrestrial plant-based communities.

Keywords

Erysiphe alphitoides, indirect defence, indirect interactions, invasive species, Microsphaera alphitoides, multi-trophic interactions, plant–fungus–insect interactions, trait-mediated interactions, tripartite interactions

INTRODUCTION

Phytophagous insects and plant pathogens are among the most speciose groups worldwide (Strong et al. 1984; Agrios 2005), and several studies suggest that they frequently interact within local communities (Hatcher 1995; Johnson et al. 2003; Rostás et al. 2003; Simon & Hilker 2003; Stout et al. 2006). Indeed, in one of the earliest articles demonstrating pathogen–arthropod interactions, Karban et al. (1987) asked ecologists to switch focus from interactions among closely related species – which were traditionally presumed to interact the strongest (Darwin 1859; Gause 1934) – to interactions between phylogenetically distant species. Despite this wake-up call, Stout et al. (2006) concluded in a recent review that pathogen–plant–insect interactions still receive limited attention, and identified several gaps in our current knowledge. Most notably, few studies have examined interactions between plant-feeding insects and plant pathogens in the field or placed such interactions in a wider community context (Stout et al. 2006). A key question is how cross-kingdom interactions scale up to affect the realised community structure at various spatial scales (Hatcher 1995; Stout et al. 2006).

Pathogens have the potential to modify the structure of phytophagous insect communities by differentially affecting the performance of local community members, and by modifying the interactions between them. However, while laboratory experiments have convincingly demonstrated that the impact of host plant pathogens on individual insect species can range from negative to positive (Rostás et al. 2003; Stout et al. 2006), few studies have experimentally assessed whether or not different species within the same community respond in a similar way to the very same host pathogen (Stout et al. 2006).

Such differential effects of pathogens on herbivore performance may be either direct, plant-mediated, or mediated by natural enemies (Cardoz et al. 2003; Turlings & Wäckers 2003; Stout et al. 2006). In addition to the effects of pathogens on herbivore performance, the presence of plant pathogens may also modify the strength with which insect herbivores are attracted to individual plant units. For example, herbivores may prefer to oviposit on either infected or non-infected leaves (Simon & Hilker 2005) or host-plants (Hatcher et al. 1994; Biere et al. 2002; Röder et al. 2007). Likewise, the rate of emigration may differ between infected and non-infected plants (Röder et al. 2007). Importantly, if insect taxa respond differently to the presence of a plant pathogen, fungal-mediated interactions may generate variation in local community structure (Moran & Schultz 1998; Kluth et al. 2001).

In this article, we use a series of detailed experiments to pinpoint the diversity of direct and indirect interactions between three plant-feeding guilds: leaf miners, free-feeding insects and the oak powdery mildew Erysiphe alphitoides. We then use two large field experiments to understand whether fungal-induced changes in resource selection affect the distribution of a specialist herbivore at two spatial scales: among leaves within a single tree, and among trees within a landscape. Finally, we use long-term spatially explicit observations to assess whether or not herbivore-specific responses to mildew infection translate into predictable patterns in insect community structure across the landscape. For comparison, we conducted a set of similar
experiments and observations to assess the impact of early-season herbivory by free-feeding insects on our focal insect taxa.

**MATERIALS**

**Study site and taxa**

The pedunculate oak *Quercus robur* is the only oak species in Finland and sustains a large community of specialist insect herbivores. In our study area, four plant-feeding guilds are prominent: a biotrophic plant pathogen, free-feeding insects, leaf miners and gallers (for details of the study system, see Appendix S1).

The plant pathogen *E. alphitoides* (Griffon & Maublanc) U. Braun & S. Takamatsu 2000 (formerly *Microsphaera alphitoides*) attacks the young oak leaves in early spring (Edwards & Ayres 1982). High densities of the species in Europe were first observed in 1907 (Hariot 1907), as followed by a rapid epidemic spread across Europe (Foëx 1941; Mougou et al. 2008). This study system then has the potential to reveal the impact of an invasive fungal pathogen on the insect community (Desprez-Loustau et al. 2007).

Tens of species feeding on oak are multivoltine. Their first generations typically attack the expanding oak leaves at around the same time as the infection is initiated by powdery mildew (Feeny 1970). Subsequent generations can be found in the summer and autumn. Hence, while the first generation feeds on the leaves when mildew infection is absent or just established, later generations may face heavily infected leaves.

In addition to free-feeders, more than twenty specialist late-season leaf miner and galler species feed on the oak trees (see Tack et al. 2010 for more details). While some of the species are found in the early spring, their peak abundance is later in the season than that of free-feeding insects.

**Experiments and observational data**

To explore the effects of powdery mildew on the insect herbivore community, we combined a series of experiments with long-term observational data. The methods are subdivided to those addressing direct and indirect local interactions (Fig 1A), those addressing resource selection by adult insects (Fig 1B), and those examining realised effects on local community structure (Fig 1C). All experiments, the interactions addressed, and the responses examined are summarised in Table 1.

**Interactions within the local community**

To identify the types of interactions occurring locally, not only within the same community but among members of different guilds (Fig 1A), we performed a series of experiments aimed at establishing direct and plant-mediated interactions among fungus and insects (Experiments 1 and 2; arrows 1a,1b and 2 in Fig 1A), at quantifying the impact of a free-feeding herbivore on a late-season leaf miner (Experiment 3; arrow 3 in Fig 1A), and at assessing parasitoid-mediated interactions among the fungus and the leaf miner (Experiment 4; arrow 4 in Fig 1A).

**Experiment 1:** The effect of mildew infection on food consumption and utilisation by Tischeria ekebladella: To investigate how mildew infection on host leaves affects the performance of *T. ekebladella* (arrow 2 in Fig 1A), we enclosed a single moth pair in a muslin bag tied around a branch tip. Moths were hatched from leaf mines collected in the early spring from various locations on the island of Warrikast (where the experiment was conducted). As the bags were introduced before mildew could be detected, we distributed the bags across multiple trees (n = 12) in the hope of including both infected and non-infected leaves. Once mildew infection developed to a scorable level, we selected a set of focal mines on healthy (n = 52 on four trees) and infected (n = 52 on eight trees) leaves. As the presence of conspecifics on the same leaf may affect larval performance (Tack et al. 2009), we focused on mines occurring singly on the leaf.

To measure mine growth, we recorded the outline of the focal mines approximately every 11 days (27 July, 7, 18 and 30 August, 10 and 20/21 September 2007) using a fine marker and a transparent acetate sheet. The mine outlines were subsequently scanned and the areas measured in cm² using ImageJ (Rasband 1997–2011). To detect differences in larval weight among leaf miner individuals on healthy and mildew-infected leaves, we dissected the larvae from the leaves on the last measuring date (coinciding with the end of the growing season). Larvae were then oven-dried at 80 °C for 24 h and weighed to the nearest microgram.

As specific leaf area, leaf consumption per unit area and growth efficiency [measured as the ratio (larval weight/leaf weight consumed per unit area × final mine area)] may differ among mildew-infected and healthy leaves, we also assessed leaf mass. For this purpose, we punched a single leaf disc (Ø 10 mm) from within the leaf mine and two leaf discs from outside the mined area. These leaf discs were subsequently dried and weighed to the nearest microgram. The weight of the two leaf discs outside the leaf mine were averaged before analysis.

**Experiment 2:** The effect of mildew infection on the larval growth rate of *Acrornicta psi*: To assess the effect of *E. alphitoides* on late-season free-feeding insect herbivores (arrows 1a and 1b in Fig 1A), we used fifth-instar larvae of *A. psi* for a feeding trial. Larvae were reared until the fifth instar in enclosures and fed oak leaves randomly collected across multiple host individuals. Offspring of three wild-caught female moths were randomly assigned to two treatments implemented in individual 10-cm diameter Petri dishes. In a no-choice setting, larvae were fed with either two mildew-infected (n = 20 larvae) or two non-infected (n = 23 larvae) leaves. Leaves were replaced every 24 h to prevent them from drying. During a period of 96 h, we measured larval weight gain (‘weight at the end of the experiment’ – ‘initial weight’) as well as leaf consumption (‘leaf weight before’ – ‘leaf weight after’). Feeding treatments were continued until all larvae had pupated, after which we weighed and sexed the pupae.

**Experiment 3:** The effect of early-season herbivory on survival and weight of *T. ekebladella*: To investigate how leaf damage inflicted by free-feeding herbivores in the early spring directly and indirectly affect the performance of larvae of the leaf miner *T. ekebladella* feeding later in the season (arrow 3 in Fig 1A), we used field-collected lepidopteran larvae to establish damage treatments. We then allowed moths of *T. ekebladella* to oviposit on a single branch tip on each of 25 trees representing three treatments: (1) control treatment, i.e. a tree without any experimental damage, (2) direct treatment, i.e. a tree where a free-feeder had been introduced to the *T. ekebladella*-branch earlier in the season and (3) direct treatment, i.e. a tree where a free-feeder had been introduced on a branch tip next to the *T. ekebladella*-branch earlier in the season. In the direct and indirect treatment, realised damage levels were more than twice as severe on experimentally damaged branches as on control branches (50% vs. 21% of leaves damaged respectively). A single pair of *T. ekebladella* was introduced in a muslin bag tied around each branch tip (see Tack et al. 2009 for
Figure 1 Ecological interactions and patterns addressed in this study. Panels A and B show potential interactions among members of three dominant plant-feeding guilds: free-feeding insects (represented by a larva of *Acronicta psi*, left in A), the oak powdery mildew (*Erysiphe alphitoides*, centre in A and shown as powdery structure in B and C), and leaf miners (represented by a mine and larva of *Tischeria ekshladella* right in A and moths in B). Panel A represents local interactions, where arrows illustrate specific interactions explored in this study. The performance of the free-feeder may be directly affected by the consumption of mildew mycelium, spores, or infected epidermal cells (arrow 1a). Alternatively, the free-feeder is affected by changes in the host plant induced by the mildew (arrow 1b). Since the leaf miner *T. ekshladella* and the oak powdery mildew do not feed upon the same leaf tissues, their interaction is mediated by changes in the host plant (arrow 2). Early-season herbivory by free-feeding insects may induce changes in the host plant that affect the late-season leaf miner (arrow 3), whereas mildew-induced changes in the plant may affect the natural enemies of the leaf miner (arrow 4). Panel B illustrates effects of mildew on resource selection by insects, including oviposition preference within (arrow 5) and among trees (left side of panel B). Panel C shows how interactions depicted in A and B scale up to affect the structure of local insect communities on oak trees. Galls and leaf mines on the leaves represent some of the target taxa identified in Appendix S1. Drawing by Ika Österblad.
more details). At the end of the growing season, we scored the survival of all leaf miner larvae ($n = 1194$) and dried and weighed all live individuals (as described above). In addition, we recorded the number of conspecific larvae, free-feeding damage ($0/1$), mildew infection ($0/1$), and leaf abscission ($0/1$) for each leaf occupied by $T. ekebladella$.

**Experiment 4: Rate of parasitism on the leaf miner** $T. ekebladella$ on healthy, mildew-infected and insect-damaged leaves

To test whether parasitoids differentially affect leaf miner larvae on healthy, insect-damaged, or mildew-infected leaves (arrow 4 in Fig. 1A), we introduced a moth pair ($\alpha^\beta$) of $T. ekebladella$ in a closed muslin bag (10 trees; three bags per tree). The bags and adult moths were removed before the eggs hatched. Hence, parasitoids had access to the leaf mines during the entire developmental stage of the host larvae. (No egg parasitoids are known for $T. ekebladella$.) In autumn, we recorded the number of larvae, mildew infection ($0/1$) and damage by free-feeding herbivores ($0/1$) for each leaf miner occupied leaf. To assess parasitism rate, we reared adult insects from leaf mines by placing mined leaves individually in plastic cups and storing them in a cellar for hibernation ($n = 895$ leaf mines). A tissue paper was added to each cup to decrease humidity. In early spring, the material was placed at room temperature to stimulate the emergence of hosts and parasitoids.

**Resource selection**

To investigate the impact of the fungus on the resource selection of insects across spatial scales, we examined whether or not moths of $T. ekebladella$ select healthy vs. infected leaves within trees (Experiment 5; arrow 5 in Fig. 1B), and how the infection status of the full tree affects the number of moths attracted to it (Experiment 6; left-hand part of Fig. 1B). In a similar vein, we also examined whether or not previous herbivory altered resource selection in $T. ekebladella$ at both spatial scales.

**Experiment 5: Resource selection at the leaf level**

To explore how the mildew affects the spatial distribution of leaf mines within trees, we assessed whether moths of $T. ekebladella$ actively avoid or prefer mildew-infected leaves (arrow 5 in Fig 1B) in a constrained-choice setting. To this end, we re-analysed a subset of the data from an experiment conducted in 2004 (see Gripenberg et al. 2007). Here, we enclosed part of the foliage on each of five oak trees in muslin bags (two shoots per bag, ten bags per tree; 729 leaves in total; this subset is referred to as the ‘within tree’ treatment of the ‘Tree pairs’ material in Gripenberg et al. 2007). A pair of moths ($\alpha^\beta$) was later introduced in each bag for approximately 4 days. After removing the moths, we counted the number of eggs laid on each leaf. Three weeks later, we visually assessed the presence or absence of mildew – and of herbivore damage – on each leaf.
Experiment 6: Resource selection at the tree level. At a scale larger than leaves within trees, mildew may affect the preference of moth females with respect to tree individuals. To examine the resulting effect of tree-level mildew infection intensity and herbivore damage on the number of moths attracted to previously unoccupied trees, we artificially removed all T. ekebladella from a set of 63 small trees (1–3 m) in the autumns of 2005, 2006 and 2007. These trees were located on the island Wattkast, Finland (see Gripenberg et al. 2008 for more detailed information and a map). We revisited each tree in each subsequent autumn to establish the colonisation pressure of T. ekebladella, as reflected by the abundance of leaf mines. At the same time, we assessed the intensity of mildew infection and of insect herbivory of the tree. For mildew, we used four categories: 0 = no mildew found on the tree; 1 = small amounts of mildew present on the tree; 2 = parts of the tree with substantial amounts of mildew, other parts uninfected; 3 = the whole tree infected. For insect damage, we used four comparable categories: 0 = no herbivory; 1 = light herbivory, only few leaves have been chewed upon; 2 = moderate herbivory, obvious that some leaves have been chewed upon, but most leaves intact; 3 = more than 30% of the leaves have been chewed upon.

Realised effects on insect community structure
To assess whether or not the structure of the natural insect community varies with the mildew infection level of the oak tree (Fig. 1C), we conducted yearly surveys on an additional set of trees between 2003 and 2006. More specifically, we recorded the insect community on 88 small trees (1–3 m) on the island of Wattkast (Fig. 1 in Appendix S1). For each tree, we recorded the level of mildew infection (using the categories outlined in the previous section), and the abundance of each of nineteen leaf mining and galling insect species on each of 20 shoots per tree.

As the relative isolation of each tree will likely affect the number of potential immigrants, the number of leaf mines observed on a tree was related both to its mildew infection status and to its connectivity to other trees (for a definition of connectivity, see Appendix S1).

Statistics
To analyse the data, we used the framework of generalised linear mixed-effects models (Littell et al. 2006). All models were fitted with procedure GLIMMIX in SAS 9.2. For continuous data, we assumed a normal distribution with an identity link. If necessary, data were log-transformed prior to analysis to achieve homoscedasticity and normality of residuals. For count data, we assumed a Poisson distribution with a log link, and for binominal data, we assumed a binomial distribution with a logit link. To derive degrees of freedom in mixed models we used the Kenward–Roger adjustment (Littell et al. 2006). For models with multiple interactions, we used the principle of backwards stepwise model simplification to arrive at a minimum adequate model, where variables were retained when \( P < 0.1 \) (Crawley 2007). All model structures, their responses and link functions are summarised in Table 1. A more detailed description of statistical analyses applied can be found in Appendix S2. In reporting the results, we offer empirical means for factors in simple models and least-squares means for factors in models including strong random effects, interactions and covariates. All means and their standard errors are reported in the original scale of the response.

## RESULTS

Our experiments revealed a perplexing diversity of cross-kingdom interactions. The qualitative effects are summarised in Table 2, with quantitative details given below.

### The effect of mildew infection on the food consumption and utilisation by T. ekebladella

In Experiment 1, the difference in size among leaf mines on leaves with and without mildew varied through time (interaction Treatment \( \times \) Time; \( F_{5,96.74} = 7.21; P < 0.001 \)). More specifically, the area of the leaf mine was significantly larger on mildewed leaves during periods 3, 4 and 5 (Fig. 2). However, we detected no significant difference in the mine area in the early development (periods 1 and 2; Fig. 2), in the final leaf mine area (period 6; Fig. 2), or in the final larval weight among leaves with and without mildew (mean ± SE: 1.25 ± 0.08 g vs. 1.09 ± 0.06 g respectively; \( F_{1,19.38} = 2.56; P = 0.13 \)).

The specific leaf area and the leaf mass consumed per unit area did not detectably differ among healthy and mildew-infected leaves (\( F_{1,7,96} = 0.03, P = 0.87 \); and \( F_{1,3,17} = 0.01, P = 0.94 \) respectively); neither did growth efficiency differ among healthy and mildew-infected leaves (\( F_{1,8,61} = 0.00, P = 0.96 \)).

### Table 2  A summary of effects uncovered with respect to individual interactions represented in Figure 1

<table>
<thead>
<tr>
<th>Effect of:</th>
<th>Interaction (Fig 1)</th>
<th>Observed effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mildew on free-feeding herbivore</td>
<td>1a &amp; b</td>
<td>Growth rate of herbivore decreases***, pupal mass decreases*, growth efficiency decreases***, leaf consumption unaffectedNS</td>
</tr>
<tr>
<td>Mildew on leaf miner</td>
<td>2</td>
<td>Mine size at dates 3*, 4** and 5** increases, mine size at date 1NS, 2NS and 6NS,</td>
</tr>
<tr>
<td>Free-feeder on leaf miner</td>
<td>3</td>
<td>Larval weight lower on herbivore-damaged leaves*, but not on systemic leavesNS; larval survival unaffectedNS</td>
</tr>
<tr>
<td>Mildew on parasitism rate of leaf miner</td>
<td>4</td>
<td>Parasitism rate increases**</td>
</tr>
<tr>
<td>Mildew on resource selection of leaf miner</td>
<td>5</td>
<td>Abundance of leaf mines at both leaf level** and tree level** decreases</td>
</tr>
<tr>
<td>Mildew on insect community structure</td>
<td>Panel 1C</td>
<td>Insect abundances lower on mildew-infected trees in 2008***, species-specific responses to mildew in 2004** and 2006*</td>
</tr>
</tbody>
</table>

Asterisks denote significance levels, with interaction- and test-specific details given in the text: NS \( P > 0.05 \); * \( P < 0.05 \); ** \( P < 0.01 \); *** \( P < 0.001 \).
The effect of mildew infection on the larval growth rate of *A. psi*

In Experiment 2, the free-feeding larvae gained mass faster when fed control leaves than when fed mildew-infected leaves (means ± SE: 0.19 ± 0.01 g vs. 0.13 ± 0.01 g respectively; *F*1,39 = 15.15; *P* = 0.0004; Figure S1). As leaf consumption did not detectably differ among treatments (*F*1,39 = 0.45; *P* = 0.51), the increased growth rate can be fully attributed to a higher growth efficiency (*F*1,39 = 20.42; *P* < 0.0001). Pupal weight was higher on the healthy leaves than on mildew-infected leaves (means ± SE: 0.188 ± 0.006 g vs. 0.165 ± 0.007 g respectively; *F*1,38 = 6.5; *P* = 0.02).

The effect of early-season herbivory on survival and weight of *T. ekebladella*

In Experiment 3, the majority of leaf miner larvae survived (ca. 80%), and no differences in survival were detected among larvae on branch tips exposed to direct earlier herbivory (least-squares mean ± SE: 0.79 ± 0.04), leaves on branch tips next to tips exposed to earlier herbivory (0.80 ± 0.04) and leaves on control branch tips (0.79 ± 0.05; *F*2,19.37 = 0.05; *P* = 0.95). Survival did not detectably differ among leaves previously damaged by herbivores and undamaged leaves (least-squares means ± SE: 0.79 ± 0.03 and 0.81 ± 0.04 respectively; *F*1,500 = 0.00; *P* = 0.95). Whether a leaf was infected or not by mildew had no significant effect on survival (least-squares mean ± SE: healthy leaves: 0.81 ± 0.04; mildew-infected leaves: 0.79 ± 0.03; *F*1,237.4 = 0.39; *P* = 0.53), but survival decreased with conspecific density and with leaf abscission (*F*1,430.9 = 16.72, *P* < 0.0001 and *F*1,501 = 7.16, *P* = 0.008 respectively).

The weight of larvae was not affected by the treatment applied (*F*2,19.06 = 0.70; *P* = 0.51). However, larval weight increased with mildew infection (least-squares means ± SE; healthy leaves: 0.81 ± 0.04; mildew-infected leaves: 1.14 ± 0.04; *F*2,548.5 = 5.02; *P* = 0.03), whereas larval weight decreased with conspecific density (*F*1,797.5 = 59.80, *P* < 0.0001) and with leaf abscission (least-squares means ± SE; attached leaves: 1.13 ± 0.03; abscised leaves: 0.98 ± 0.06; *F*1,797.7 = 9.36, *P* = 0.002 respectively). Larval weight was lower on leaves previously damaged by other herbivores than on undamaged leaves (least-squares means ± SE: 1.08 ± 0.04 g vs. 1.14 ± 0.04 respectively; *F*1,835 = 4.67; *P* = 0.03).

Parasitism rate of the leaf miner *T. ekebladella* on healthy, mildew-infected and insect-damaged leaves

In Experiment 4, parasitism rate was higher on mildew-infected than on healthy leaves (means ± SE: 0.34 ± 0.03 vs. 0.19 ± 0.02 respectively; *F*1,891 = 7.63; *P* = 0.006; Figure S1), whereas damage by free-feeding insects had no detectable effect (means ± SE: undamaged leaves: 0.22 ± 0.02; herbivore-damaged leaves: 0.24 ± 0.03; *F*1,891 = 0.34; *P* = 0.56). Parasitism rate per capita increased with an increase in the density of conspecific moth larvae (*F*1,891 = 4.01; *P* = 0.05).

Resource selection at the leaf level

In Experiment 5, females of the leaf miner *T. ekebladella* oviposited more eggs on leaves without mildew infection than on those with mildew infection (a difference of 34%; least-squares means ± SE: 1.46 ± 0.44 and 1.09 ± 0.34; *F*1,726 = 9.57; *P* = 0.002). No significant difference in oviposition preference was detected between undamaged leaves vs. leaves damaged by insects (least-squares means ± SE: 1.41 ± 0.41 and 1.13 ± 0.37; *F*1,726 = 1.80; *P* = 0.18).

Resource selection at the tree level

In Experiment 6, the number of *T. ekebladella* leaf mines encountered on individual trees varied strongly among years (*F*2,184 = 8.10; *P* = 0.0004; Fig. 3). Although we found a consistent and negative

![Figure 2](image_url)  
Figure 2 The performance of the leaf miner *Tischeria ekebladella* feeding on healthy and mildew-infected leaves. Shown are mine areas (log-transformed) as a function of time. Data points are jittered horizontally to reduce overlap, and horizontal bars indicate treatment means for each time period. For each sampling date, a star indicates a significant difference (*P* < 0.05) in mine area among healthy and mildew-infected leaves.

![Figure 3](image_url)  
Figure 3 Resource selection by *Tischeria ekebladella* at the level of trees with different intensity of mildew infection. Each symbol represents the number of leaf mines encountered on a single tree experimentally cleared of previous leaf mines, and the curves refer to year-specific estimates from a generalised linear model. Dots for different years are offset horizontally for visual clarity.
relationship between the number of mines and the strength of mildew infection on the tree (F_1,184 = 9.19; P = 0.003; Fig. 3), we did not detect any effect of herbivore damage (F_1,184 = 0.33; P = 0.57). Year-specific regressions of leaf mine numbers on mildew infection level were significant for 2 of 3 years (P = 0.02 in both 2006 and 2007).

**Realised effects on insect community structure**

In 2 of 6 years, individual herbivore taxa responded differently to the presence of the powdery mildew (as suggested by a significant interaction between ‘Species’ and ‘Mildew’; P = 0.001 and P = 0.02 in 2004 and 2006 respectively; Fig. 4). This finding indicates that the herbivore community structure differed significantly among trees with different mildew infection levels. In 2008, we detected no interaction, but a significant main effect of the factor ‘Mildew’ (P < 0.001), with the lowest abundances on the most heavily infected trees. Herbivore taxa were differentially affected by ‘Spatial connectivity’ in 3 of 6 years (as revealed by a significant interaction between ‘Species’ and ‘Spatial connectivity’ (2003: P < 0.001, 2004: P < 0.0001, 2008: P = 0.003), whereas abundances were consistently lower (across species) on more isolated trees in 2 of 6 years (as suggested by the significant main effect ‘Spatial connectivity’: P = 0.01 and P < 0.0001 in 2005 and 2007 respectively). Overall, these results show that both mildew and spatial context are important factors in structuring local insect communities across the landscape.

**DISCUSSION**

This study demonstrates that the ecological interactions among members of different kingdoms may be an important force in structuring local communities in nature. Here, we found that individual herbivore species were differently affected by the same fungus, that interactions between the fungus and the insects may be mediated by both the plant and natural enemies, and that the trait-specific outcome of individual interactions ranged from negative to positive (see Table 2 for a summary of the effects uncovered). Moreover, resource selection by insect herbivores was shown to be modified by fungus infection of the host plant. Through these trait- and species-specific effects, infection by mildew has the potential to shape the composition of local insect communities across the landscape – as was empirically demonstrated by our survey of insect communities across 88 trees of variable infection levels.

**Interactions among fungus and plant-feeding insects structure the local insect community**

Our experiments revealed a broad range of ecological interactions among individual community members on oak. Of these, the interactions between the fungal pathogen and the insect herbivores were particularly versatile in character. Although early-season herbivory left little or no trace on different performance measures for late-season insect herbivores, fungal infection had a detectable impact. Interestingly, our findings suggest that different insect taxa respond differently to the presence of the powdery mildew. More specifically, fungal infection reduced the growth rate, growth efficiency and pupal mass of the free-feeding caterpillar A. psi (arrows 1a and 1b in Fig. 1). This will likely decrease its fitness, as body size has been shown to predict female fecundity in multiple lepidopteran species (Honěk 1993; Tammaru et al. 1996). In contrast, fungal infection increased the growth rate, decreased the developmental time and increased the parasitism rate of the leaf miner T. ekebladella (arrows 2 and 4 in Fig. 1). Taken together, these results support not only a significant impact of a pathogenic fungus on the fitness of insect herbivores, but also emphasise the wide range of ecological outcomes: mildew may improve individual performance measures for given insect taxa while impairing other performance measures.

The finer mechanisms behind the patterns detected remain to be elucidated. In principle, the negative impact of the oak powdery mildew on the free-feeder A. psi may be direct (as caused by the consumption of spores, mycelium and infected epidermal cells) or mediated by changes in plant chemistry (Hatcher 1995). However, a direct effect seems unlikely, as no toxic effect of spores or mycelium has been shown in powdery mildews – on the contrary, some arthropods have specialised on, or increase growth when, consuming this resource (e.g. Yarwood 1943; English-Loeb et al. 1999; Mondy & Corio-Costet 2004). A more clear-cut picture then emerges for the interaction between the fungus and the leaf miner. Since T. ekebladella only feeds upon the palisade parenchyma, it has no direct contact with the ectoparasitic powdery mildew, which only penetrates the epidermal cells with its feeding organs (Braun 1987). Hence, the plant-mediated interaction may be caused by interactions between the response pathways triggered by pathogens and herbivores (Bostock 1999; Kessler & Baldwin 2002; Nimchuk et al. 2003; Taylor et al. 2004). Moreover, leaf miner performance may be affected by independent mildew-induced changes in oak leaf physiology, nutritional quality and chemistry (Hatcher 1995; Hají et al. 2009).

Overall, plant-mediated indirect interactions between insect herbivores and pathogenic micro-organisms have recently received increased attention due to the realisation that in natural settings, any plant will have to deal with multiple enemies at the same time (Hatcher 1995; Stout et al. 2006; Röder et al. 2011). Yet few studies have focused on enemy-mediated interactions among pathogenic fungi and insect herbivores (Omacini et al. 2001; Biere et al. 2002; Turlings & Wäckers 2003; Stout et al. 2006). Here, our findings
demonstrate that the leaf miner *T. ekebladella* suffered increased parasitism on mildew-infected leaves in a natural setting (arrow 4 in Fig. 1). Possible explanations in our study system include that altered volatile emissions from mildew-infected plants may affect herbivore natural enemy responses (Cardoza et al. 2003; Turlings & Wäckers 2003; Hare 2011), that differences in the larval phenology of the host on healthy and mildew-infected leaves may change the ‘window of opportunity’ for parasitism (note differing patterns of growth in Fig. 2A), or that the nutritional quality of larvae on healthy and mildew-infected leaves may result in selective parasitism.

**Resource selection by insects**

Our studies of insect oviposition revealed an effect of mildew presence on the behaviour of ovipositing female moths. This effect was evident both in the number of leaf mines encountered at the level of individual leaves within trees, and in the level of leaf miner recolonisation of full trees experimentally cleared of leaf mines in the previous year.

Within trees, the spatial distribution of mildew affects the fine-scale distribution of the herbivore, as *T. ekebladella* preferred to lay eggs on leaves without mildew. Notably, these differences in oviposition choice at a small scale reflected spatial variation in the risk of becoming parasitised, and may hence reflect an evolutionary adaptation to parasitism-mediated natural selection. As the difference in parasitism rate among mildew-infected and healthy leaves was relatively large (corresponding to a 1.8-fold increase in the risk of becoming parasitised), it implies that mildew may have a marked impact on local insect performance.

At a between-tree level, fewer leaf mines were found on trees characterised by heavier mildew infection. This pattern may be caused by a shift in migration rates, with lower immigration and/or higher emigration from mildew-infected trees. Interestingly, our findings suggest that mildew-induced changes in movement and oviposition decisions may contribute to shape the distribution of a specialist insect both within the local community (i.e. among leaves within a single tree) and across the landscape. An effect of powdery mildew fungus on spatial dynamics has also been demonstrated in the Glanville fritillary butterfly (*Melitaea cinxia*) system, where mildew infection was shown to affect the local extinction risk of the herbivore (Laine 2004) and the colonisation rate of its parasitoid (van Nouhuys & Laine 2008).

**Implications for herbivore community structure**

The most intriguing finding of our study is that the complex interactions among a pathogenic fungus and insect herbivores examined above actually scale up to affect the structure of the larger herbivore community. Our detailed experiments showed that individual species responded differently to the presence of the same plant pathogen. They also revealed how mildew affected multitrophic interactions among taxa. Together, they hence suggest that mildew may drive the relative abundances of different species in different directions, thereby moulding the structure of the full community. Indeed, the results from our long-term observational study support this prediction: different insect species are differentially affected when species are confronted with the very same plant pathogen, and overall community structure across the landscape is affected by mildew infection level.

**CONCLUSIONS**

Overall, our multitrophic-level perspective on interactions within local oak communities demonstrates that effects among phylogenetically distant taxa are abundant and diverse, and that they may be mediated by both the host plant and by natural enemies. Where previous research has demonstrated that insect species may respond to the presence of a pathogenic fungus in a laboratory setting, our study places these findings in the context of more diverse natural communities (see also Johnson et al. 2003; Rostás et al. 2003; Stout et al. 2006). In such communities, individual insect species react very differently to the presence of the same plant pathogen, and the ecological outcome can range from negative to positive depending on the specific trait and species examined (see Table 2). As a likely result, it seems that different species respond differently to plant pathogens (e.g. Moran & Schultz 1998; Kluth et al. 2001; Röder et al. 2007; Mouttet et al. 2011). As the literature reveals that insects vary widely in their response to host plant quality (Strong et al. 1984), induced responses (Karban & Baldwin 1997), and indirect defences (Turlings & Wäckers 2003; Hare 2011), we expect that similar complexities are likely to emerge in future studies. These results emphasise that the traditional focus on herbivore–herbivore interactions or pathogen–pathogen interactions should be supplemented by work bridging both fields in a natural setting. As herbivore–herbivore interactions appear to play a rather minor role in structuring our focal communities (see also Tack et al. 2009), the current study illustrates how interactions across kingdoms may be crucial for our understanding of terrestrial plant-based communities. Strikingly, the epidemic invasion of a fungal pathogen in northern Europe – now approximately 100 years ago – may have had a profound impact on the structure, spatial ecology and evolutionary trajectory of the native insect community.

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**AUTHORSHIP**

AJMT conceived the idea of combining materials from studies designed and implemented by AJMT, SG and TR; AJMT analysed the data, and all authors contributed to the manuscript.

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