PLANT-INSECT INTERACTIONS IN CHANGING ENVIRONMENTS

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DIPL.-BIOL. DAVID JOACHIM GLADBACH
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1. Referent: Prof. Dr. Teja Tschardtke

2. Korreferent: Dr. Carsten F. Dormann

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“..., what good is it to argue about whether HOLISM or REDUCTIONISM is right, when the proper way to understand the matter is to transcend the question, by answering MU“.

Douglas R. Hofstadter - *Gödel, Escher, Bach: An Eternal Golden Braid*

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CHAPTER 1

General introduction

INTRODUCTION

TROPHIC INTERACTIONS: CENTRAL FOR ECOSYSTEM FUNCTIONING AND SERVICES

One quarter of all extant organisms are herbivorous insects (Strong *et al.* 1984; Mayhew 2001), which shape ecosystems in interaction with plants (Weisser and Siemann 2004). Herbivory as ecosystem function can decrease plant biomass and productivity (Matson *et al.* 1997) and thereby change environmental conditions and diminish resources for species (Jones and Lawton 1995). Fluctuations within herbivore populations are passed to other trophic levels by cascading up and down the food chain. Increasing populations of herbivorous insects may reduce plant biomass on one hand, and at the same time provide sufficient resource for predators (i.e. biocontrol agents). Because herbivorous insects occupy a major trophic position in food chains, (human induced) environmental changes causing fundamental shifts of this trophic level can affect directly linked trophic levels antagonistically and thereby potentially cause instability of ecosystem functioning (Chapin *et al.* 1997).

Effects of human induced changes to landscape structure, agriculture (intensification), and climate have been identified to affect ecosystem functions with increasing frequency. Publications and citations of these terms in combination with ecosystem function have at least doubled since 2004. For climate change the publication record increased from 30 (2004) to 87 (2009), citations increased from 1100 to 3100 between 2005 and 2009 (Web of Science® Citation Report 2010).

The expansion of agricultural land affects landscape scale patterns (*sensu* Turner and Gardner 1991) and decreases structural complexity and habitat diversity (Turner 1989; Wrבka *et al.* 2004). Consequently distribution patterns of insects may alter (Rand *et al.* 2006) and thereby change ecosystem functions (Kareiva and Wennergren 1995; Hooper *et al.* 2005).

Locally agricultural intensification includes the application of fertilizers and pesticides that reduce biodiversity within habitats and alters local conditions for insects. Interactions may be more stable in complex agricultural systems (Van Emden and Williams 1974; Balvanera 2001; Gurr *et al.* 2003; Casula *et al.* 2006; Schmitz 2009), whereas in intensively managed systems, trophic interactions are often dominated by few species and thereby, susceptible to environmental change (Straub and Snyder 2006; Straub *et al.* 2008).

Climate change is expected to increase the global mean surface air temperature by 1.8-5.8°C (2090 to 2099 relative to 1980 to 1999), with additional changes in other climate change drivers such as increasing CO₂ levels or extreme weather events (IPCC 2007). Recent studies, have shown that increases in CO₂ may alter plant-insect interactions in various ways (Lincoln *et al.* 1986; Lincoln and Couvet 1989; Stiling *et al.* 1999; Stiling and Cornelissen 2007). Elevated temperature is generally assumed to increase herbivory (Bale *et al.* 2002), while altered water conditions are discussed controversially (Mattson and Haack 1987; Morecroft *et al.* 2002).

Effects of landscape structure, habitat type and local management, or climate change have been well studied with respect to trophic interactions. However, since the simultaneous

investigation of different levels of environmental change is difficult, studies are scarce and the interplay of these factors remains hard to predict. While few studies have shown the interaction of landscape and local processes and their effect on patterns of trophic interactions (Cushman and McGarigal 2004; Dauber *et al.* 2005; Schweiger *et al.* 2005), I am not aware of studies that investigated interaction effects of different climate change drivers or climate change with landscape or field management on ecosystem functions. Predictions about the quality of future ecosystem functions require mechanistic studies of how trophic interactions respond to interactions of the different types of environmental changes.

BIOTIC ECOSYSTEM SERVICES – THE BESS PROJECT

The work that is contained in this thesis was integrated in the umbrella project BESS, launched as *Helmholtz Young Investigators Group*, a cooperation between the Center for Environmental Research –UFZ, Leipzig and the Department of Agroecology, University Göttingen. Ecosystem services in general comprise naturally provided processes that support human life and can be divided into provisioning services (e.g. crops), regulating services (eg. pollination, biocontrol) and cultural services (e.g. recreation, Carpenter *et al.* 2009). Type, quality and extend of services are a trade off, driven by the characteristics of the service providing ecosystem (Foley *et al.* 2005). As increasing human requirements push ecosystem services beyond the limits of their

Figure 1.1. Human requirements (top) and current condition (bottom) of ecosystem services. Ecosystem services are split into provisioning, regulating and cultural services (plotted left to right) based on the Millenium Ecosystem Assessment. Black radial lines indicate the tendency of human requirement and condition of the service respectively. Figure adapted from Carpenter *et al.* 2009.

capacity, scientists aimed to pinpoint gaps between human requirements and the actual state of ecosystem services in the course of the Millenium Ecosystem Assessment (www.MAweb.org, Fig. 1).

Within this framework BESS focuses on the regulating services - biocontrol and pollination in specific. Both services are related to food production and thus are of major importance for human wellbeing. However, owing to agricultural intensification the condition of pollination and biocontrol services are underperforming with respect to human requirements. In this context the aim of BESS is (1) to acquire the understanding of mechanisms that steer these ecosystem services and subsequently (2) to provide tools and methods to manage and improve these important ecosystem services at a landscape scale. Three sub-projects have been initiated:

- i) Pollination of wild bees is investigated with respect to landscape context and source habitats within agricultural landscapes
- ii) The work of this thesis constitutes the plant-insect interaction and biocontrol part of the BESS Project. Details are given in the section chapter outline.
- iii) A modelling group uses the field data from projects i) and ii) and available information from the literature to compute spatial explicit models. These models serve as a test of how different steering tools may change the services at a landscape scale.

CHAPTER OUTLINE AND MAIN RESULTS

This thesis comprises three studies approaching the effects of co-acting environmental change factors on trophic interactions. Hereby the focus was on plants and insects with optional inclusion of a predator level. Main questions considered the interaction effects of

- i) landscape context and local habitat type on the spillover of pest organisms and biocontrol agents from crop into non-crop habitats.
- ii) Climate warming and high vs. low field management on tri-trophic interactions in wheat.

Figure 1.2. *Meligethes aeneus*

- iii) Different climate change drivers on the performance of insect herbivores in heathland.

The second chapter deals with the investigation of how spillover of rape pollen beetles (Fig. 1.2) and their parasitoids from oilseed rape fields (Appendix 1.1) into cropland habitats (wheat fields and field margin) and noncrop habitats (fallow, grassland, wood margin) is affected by landscape composition. The study was conducted in the vicinity of Göttingen on a gradient of eight landscape sectors (Fig. 1.3). Within these sectors

Figure 1.3. Landscape gradient: (left) Homogeneous landscape consisting of large agricultural fields. (right) Heterogeneous landscape characterized by patchy distribution of crop and non-crop habitats with different landscape elements (hedgerows, woodlots).

we recorded different landscape parameters and the colonization of potted wild mustard plants by rape pollen beetles and parasitoids. Both landscape and local factors affected the abundance of rape pollen beetles and parasitoids. Rape pollen beetle infestation and parasitism rates were lower in noncrop habitats and higher in wheat fields and field margins. In contrast, beetles and parasitoids responded differently to landscape scale parameters. Parasitism rates were not related to landscape simplification, but benefited from increasing proportions of oilseed rape. In contrast, we found more rape pollen beetles on plants in simple landscapes, which we attribute to multi-annual population built-ups resulting from long-term oilseed rape planting (as part of the crop rotation). Parasitoid spillover from cropland affects trophic cascades on wild plants outside cropland, which can be expected to be a widespread effect shaping non-crop food webs.

The third chapter reports of the experimental elevation of temperature (using shields for passive warming) and the resulting effects on tri-trophic interactions in fertilized vs. unfertilized wheat and wheat monocultures vs. mixed cultures (with undersowings). The experiment was designed as a full factorial split-plot, mixed and monocultures were manipulated on the highest level and divided into fertilized and unfertilized strips. Within each of the fertilizer treatments we established the warming treatment, yielding a total of 8 treatments (8 treatments, 6 replicates, $n = 48$ plots, Appendix 1.2). We measured the number of cereal aphids and their natural enemies (Fig. 1.4) in relation to yield of

Figure 1.4. Aphids and biocontrol agents in wheat: (top) *Araneus spec.* (bottom) *Metopolophium dirhodum* (Walker)

Figure 1.5. *Lochmaea suturalis*

wheat plants weekly from wheat flowering to dough ripening. Fertilization increased yield, but also aphid density, which negatively affected yield. Predator-prey ratios were highest in diversified wheat and related to reduced aphid population growth. Elevated temperature enhanced aphid growth strongest in fertilized monocultures, leading to reduced yield. Our results show that diversified crops and reduced fertilization may become an important tool to prevent pest outbreaks in future warmer climates.

The fourth chapter contains a report of multiple independently manipulated climate change drivers (CO_2 , temperature, drought) and their effects on herbivorous insects. The study was conducted at the CLIMAITE research facility near Brandbjerg, DK, in heathland ecosystem consisting of a heather (*Calluna vulgaris*) grassland mosaic. The experiment was designed as a full factorial split-plot with 6 blocks $\times$ 2 CO_2 treatments $\times$ 4 warming/drought treatments = 48 plots (Appendix 1.3). We exposed a total of 432 ($n = 9$ per plot) larvae of heather beetle (*Lochmaea suturalis* THOMSON, APPENDIX 1.4) to ambient vs. elevated drought, temperature and CO_2 (plus all combinations) for five weeks. Weight and survival were recorded weekly. Larval growth and survival were significantly reduced by elevated CO_2 and drought, which most distinctly affected insect herbivores. Warming had negative effects on growth but not on survival. Combined CO_2 and drought significantly reduced growth, while interactions of warming with other global change drivers were inconsistent. Co-acting climate change factors modified each others effects on herbivorous insects, introducing complexity that has to be treated carefully when making predictions. In conclusion insect herbivore populations may respond negatively to future climate.

CONCLUSIONS

In this experimental series shifts within trophic interactions as a result of environmental changes were shown. However, predictions or generalisations about the fate of ecosystems services under environmental change have to be treated with care, because all of the investigated factors of environmental change varied in their effects depending on the responding organism. Insects with different grades of specialisation responded to different aspects on a landscape scale – generalists benefited from landscape complexity, while specialist responded to the presence of their preferred food resource. The same was true for habitat diversity on a local scale, specialists did not benefit from diversity but rather from uniform and abundant resource of the crop they are specialized on. Climate change (i.e. temperature) had adverse effects on different insects. Aphids benefited from warming, while others were affected negatively.

Environmental change factors that act in concert caused complex responses of ecosystems.

We showed that even temperature elevation at the lower scale of predicted change can cause fundamental shifts in established interactions. Extrapolating from local field management effects we may conclude that large scale patterns such as landscape structure may interact with climate change drivers and thus can be used as a tool for insect population management in future. However, species specific responses to experimentally controlled changes highlight the complexity with which future environmental change affects ecosystems.

APPENDIX

CONTENT

Appendix 1.1. Oilseed rape fields in the vicinity of Göttingen

Appendix 1.2. Experimental field site in Göttingen

Appendix 1.3. CLIMAITE field site at Brandbjerg, DK

Appendix 1.4. Larva of *Lochmaea suturalis*

Appendix 1.1. Scattered oilseed rape fields in an agricultural landscape in the vicinity of Göttingen. Spillover effects were investigated from oilseed rape fields into cropland habitats (wheat fields and field margin) and noncrop habitats (fallow, grassland, wood margin).

Appendix 1.2. Experimental setup for manipulation of trophic interactions in wheat. Treatments for 48 plots comprise (i) herbicide treatment: monoculture - front left, mixed culture - front right; (ii) fertilizer treatment applied as alternating strips: unfertilized wheat - light green shading; fertilized wheat - dark green shading; (iii) shields for passive warming (greenhouse foil) and control (gauze). Further details are given in chapter 3.

Appendix 1.3. CLIMAITE field site at Brandbjerg, DK. The facility consists of 12 octagons in 6 blocks. The octagon in front is with ambient CO₂ treatment; elevated CO₂ is applied with plastic tubes tied to the surrounding metal frame and supplied from a CO₂ gas tank. Each octagon is divided into 4 plots with single and combined warming and drought treatments. At sunset curtains for passive night time warming are pulled along the guard rails over the two plots at the far end. The drought curtain is drawn along a suspended beam (see distant octagons) over the two plots at the left hand side. Note the white gauze bags for caging the heather beetle larvae during the experiment. White pins indicate subplots of other experiments and locations of sensors for recording of abiotic conditions. Further details and pictures are given in chapter 4.

Appendix 1.4. Larval stage II of *Lochmaea suturalis* feeding on *Calluna vulgaris*.

CHAPTER 2

*Spillover cascades: crops affect
trophic interactions on wild plants
in surrounding habitats*

ABSTRACT

Ecosystem processes in agricultural landscapes are often triggered by resource availability in crop and noncrop habitats. We investigated how oilseed rape (OSR, *Brassica napus*, Brassicaceae) affects noncrop plants in managed systems and semi-natural habitat, using trophic interactions among wild mustard (*Sinapis arvensis*, Brassicaceae), rape pollen beetles (*Meligethes aeneus*, Nitidulidae) and their parasitoids (*Tersilochus heterocerus*, Ichneumonidae). We exposed wild mustard as phytometer plants in two cropland habitat types (wheat field, field margin) and three noncrop habitat types (fallow, grassland, wood margin) across eight landscapes along a gradient from simple to complex (quantified as % arable land). Both landscape and local factors affected the abundance of rape pollen beetles and parasitoids. Rape pollen beetle infestation and parasitism rates were lower in noncrop habitats and higher in wheat fields and field margins, whereas beetles and parasitoids responded differently to landscape scale parameters. We found the hypothesized spillover from OSR crop onto wild plants in surrounding habitats only for parasitoids, but not for pollen beetles. Parasitism rates were not related to landscape simplification, but benefited from increasing proportions of OSR. In contrast, rape pollen beetles benefited from simple landscape structures, presumably due to multi-annual population built-ups resulting from long-term OSR planting (as part of the crop rotation). In conclusion, we showed that parasitoid spillover from cropland affects trophic cascades on related wild plants outside cropland, which has been not shown so far, but can be expected to be a widespread effect shaping noncrop food webs.

Key-words: habitat identity, herbivory, large-scale effects, local effects

Authors:

David J. Gladbach

Andrea Holzschuh

Christoph Scherber

Carsten Thies

Carsten F. Dormann

Teja Tscharntke

INTRODUCTION

Landscape-scale patterns (*sensu* Turner and Gardner 1991) often affect ecosystem processes locally (Kareiva and Wennergren 1995; Hooper *et al.* 2005). Spatial configuration and composition of ecosystems and habitat diversity (Turner 1989) have been shown to be interwoven with land-use intensity (e.g. Wrabka *et al.* 2004), and may influence, as landscape effects, important trophic interactions such as biocontrol and herbivory (Gardiner *et al.* 2009) or pollination (Ricketts *et al.* 2008). Hence, both ecosystem services and dis-services (Zhang *et al.* 2007) in different agro-environments are promoted or constrained to various extents depending on landscape effects. Further, local effects such as habitat type and quality (habitat identity) influence diversity and population size of organisms (Matter & Roland 2002; Haynes *et al.* 2007; Zaller *et al.* 2008). Thus, local interactions are triggered by factors such as resource availability at both the landscape and the local habitat scale. However, most studies focus either on just local effects or the distribution of only one habitat type within a landscape (Meyer *et al.* 2009). Only few studies have shown the interaction of landscape and local processes and their effect on patterns of insect diversity or trophic interactions (Cushman and McGarigal 2004; Dauber *et al.* 2005; Schweiger *et al.* 2005). As a simultaneous investigation of local and landscape patterns is difficult, an experimental approach introducing the same study system in a wide range of habitats and landscapes is a suitable, but little explored technique.

Here, we study the rape pollen beetle *Meligethes aeneus* (Fabricius 1775), which is one of the most important pest organisms in oilseed rape (*Brassica napus*, L.) (Büchi 2002; Alford *et al.* 2005). Published studies focus on pollen beetles and their parasitoids on OSR and emphasise that landscape context influences trophic interactions in cropland (e.g. Thies and Tscharnkte 1999; Ricketts *et al.* 2008; Büchi 2002; Bianchi *et al.* 2006; Thies *et al.* 2008), whereas wild Brassicaceae have been considered only in their potential role as alternative host plants and not as a substitute resource when OSR fields are not longer available. Thus examples of crop-noncrop spillover are almost absent (Rand *et al.* 2006). However, spillover may shape trophic interactions and thus we focus on the effect of cropland such as OSR on wild plants in noncrop habitats as well as in crop systems. Although existing theories predict the spillover of insects from crop to non-crop areas (Tscharnkte *et al.* 2005; Rand *et al.* 2006; Rand and Louda 2006), we are not aware of studies that actually test how the huge and functionally important crop-related populations distribute across different habitats in the landscape shaping food webs,

In the present study we investigated how landscape composition affects crop-noncrop spillover and associated trophic interactions. We quantify flower herbivory by rape pollen beetles and its parasitism by an ichneumonid wasp (Nilsson 2003) in different habitat types across a gradient of landscape complexity. Spillover may vary due to the source capacity as well as the attractiveness of the destination. We hypothesize:

- (i) Both habitat type and landscape characteristics influence the spillover of pollen beetles (*Meligethes aeneus*) and their parasitoids (*Tersilochus heterocerus*, Thomson 1889) across the crop-noncrop interface.

- (ii) Increasing proportion of oilseed rape as source habitat increases populations of specialised parasitoids more than their generalist hosts (following Thies *et al.* 2008), thereby affecting parasitism rates.

METHODS

EXPERIMENTAL SETUP

The study was conducted after the flowering period of OSR from 1st June to 15th July 2006 in the vicinity of Göttingen, Lower Saxony, Germany (51° 32' N, 9° 56' E). The regional landscape pattern varies from intensively managed, simply structured landscapes that undergo a large inter-annual change (arable land up to 90%) to complex, extensively managed landscapes with a high proportion of near-natural, perennial habitats (i.e. fallow, wood margin; arable land <20%). Eight landscapes (i.e. landscape sectors) were chosen along this gradient of land-use intensity (Appendix 2.1). There was no spatial correlation in the land-use gradient of the landscapes. Within each of the landscapes, we established study plots in five major habitat types (cereal field, field margin adjacent to cereal field, fallow, grassland, wood margin adjacent to crop field), yielding a total of 40 plots.

The locations of the habitats within landscapes were chosen as near to each other as possible, in order to achieve maximum similarity with respect to landscape-scale parameters. Distance of habitats to the nearest OSR crops (mean $197 \pm \text{SE } 22\text{m}$) was tested in lme models (described below) and did not vary significantly ($F_{4,21} = 1.81$, $P = 0.147$). In each habitat we established (1st June) a plot of three wild mustard plants (*Sinapis arvensis*), which is a native rural plant found ubiquitously in agricultural and semi-natural habitats flowering from April to October. Phytometers were grown in pots under standardised conditions in the same soil (standard garden soil, watered every second day), before the start of the experiment. Thus we achieved a maximum of phytometer similarity among habitats and landscapes. Excluding differences in plant quality was particularly important for this study, because it can change the oviposition rate of rape pollen beetles (Hopkins and Ekbom 1996). From the time when rape pollen beetles dispersed from the fading OSR fields the phytometers were freely accessible for rape pollen beetles and their parasitoids, so that differences due to local and landscape effects would be recognizable through population density and parasitism rates.

STUDY SPECIES

All developmental stages of rape pollen beetle *Meligethes aeneus* (Coleoptera: Nitidulidae) feed on pollen. *M. aeneus* is one of the economically most important pest species on OSR, preventing seed development and hence causing loss of yield but the species also feeds on a range of other plant species, such as wild mustard, our phytometer (Ekbom and Borg 1996; Cook *et al.* 2006). In late April adults start moving into (not yet flowering) OSR crops for maturity feeding and subsequent oviposition. In field conditions the reproductive period usually lasts 2 months, but rape pollen beetles have been shown to exhibit reproductive plasticity in dependence of

environmental or host plant conditions (Ekbom and Borg 1996; Billqvist and Ekbom 2001). In ideal conditions oviposition may take place until October and also observations of beetles laying eggs in the year of their hatching have been observed (Bromand 1983). Larvae of rape pollen beetles develop in flowers, drop to the ground on maturity, pupate and emerge after one to five weeks. Beetles live on a variety of plants (Hokkanen 2000; Gurr *et al.* 2003; Lehrman *et al.* 2008) when OSR crops have faded. Adults of the first generation die after egg laying, second generation beetles move into hibernating sites under herbaceous vegetation or moist woodland debris (Müller 1941; Nilsson 1988; Williams 2004). The larvae of the rape pollen beetle are attacked by the univoltine parasitoids, *Tersilochus heterocerus*, *Phradis interstitialis*, and *Phradis morionellus* (Hymenoptera: Ichneumonidae), of which the last is rare. The parasitoids are specialised on rape pollen beetles and attack host larvae in the first (*Phradis* spp.) and second (*T. heterocerus*) instar. After parasitizing the larvae in the flower, both endo-parasitoid species kill their host larvae after they drop to the ground before pupation in the soil (Jourdheuil 1960). Parasitoids peak and start egg deposition in May during OSR flowering (Williams 2006). They overwinter in their hosts in the ground and subsequently emerge from the soil of the last year's OSR crop area.

DATA COLLECTION

Samples of rape pollen beetles were collected from the phytometers at flowering of wild mustard (27. June 2006) by clipping all flowering stalks and storing them in plastic cups at -22°C . The number of parasitized larvae was determined by dissecting the rape pollen beetle larvae under a binocular (Zeiss, Stemi SV 11). Parasitoid eggs were assigned to the parasitoid species by their typical pigmentation (black - *T. heterocerus*; white - *Phradis* spp., Foerster 1868). Because we found only 1 - 2 individuals of *Phradis* spp. in two different landscapes, only *T. heterocerus* was included to further analyses. After peak ripening, we collected all remaining *Sinapis* plants and stored them at 1°C (15. July 2006). Plant damage to seed set was quantified by counting the number of pods and the podless stalks that remained after rape pollen beetle herbivory.

Landscape parameters were estimated on the basis of the official digital thematic maps (ATKIS – Digitales Landschaftsmodell 25/1; Landesvermessung und Geobasisinformation, Hannover, Germany 1991–1996) containing areal measures of arable land, grassland, forests, hedgerows, garden land and settlement. In addition, we mapped habitat types in the field during the season, allowing a specific classification of land use in the areas with arable land (Appendix 2.4). Data were digitized and analysed in ArcView 3.2 (ESRI Redlands, CA, USA) in a radius of 750 m around each experimental patch, since this scale has been shown to be appropriate for the studied host–parasitoid interactions (Thies *et al.* 2003). Habitats were also characterised by vegetation surveys using the Braun-Blanquet scale (Braun-Blanquet, 1964). The surveys were conducted within two weeks at the beginning of the study period on five randomly chosen plots per habitat of 2x2 meters each. Turboveg 2.79 (Hennekens and Schaminée 2001) was used to transform Braun-Blanquet data into plant percentage cover data and to analyse number and abundance of plant species (Appendix 2.2).

DATA ANALYSES AND STATISTICS

Variation of adult rape pollen beetle numbers on the sampled phytometer may, in part, be due to diurnal activity patterns. Therefore we included only the rape pollen beetle larvae in our analyses. Missing phytometer plants due to mammalian herbivory, were treated as NA in the statistical analyses. Although being standardized, the phytometers had different numbers of flowering stalks by the time we collected the samples of rape pollen beetles. This variation, however, was not correlated to habitat type (Appendix 2.3) or any landscape parameters (Appendix 2.1). To account for the varying sample size we analyzed our data with the larval abundance divided by the number of flowers in the respective samples. Larvae per flower and parasitism rates in experimental patches were arcsine square-root transformed and landscape and habitat type effects were tested in linear mixed effects models (lme, Pinheiro *et al.* 2009) in R 2.9.1 (R Development Core Team 2009). Obtaining normally distributed residuals after the transformation, we could use the more established and widely used lme models instead of generalized linear mixed models for analysing non-normal data, which are more difficult to fit appropriately (Bolker *et al.* 2009).

Maximal models contained the following landscape-scale predictors: (i) % arable land, selected a priori based on our experimental design; (ii) % oil seed rape (OSR), as important predictor due to the focus of spillover from OSR into other habitats; (iii) a small set of additional landscape-scale predictors shown to be important based on inspection of zero-order correlation matrices (Murray and Conner 2009), namely the mean perimeter to area ratio (MPAR) and the number of arable land patches in the landscape (Appendix 2.4). MPAR gives information about the complexity of a landscape (see Bianchi *et al.* 2006). Low values of MPAR indicate large patches in a landscape whilst landscapes with high MPAR values are characterised by many small patches of fields and habitats, thus the number of different resource types tends to increase with MPAR. Local effects comprised habitat type and the number of larvae per flower. The maximum models were fitted as

$$y = \mathbf{X}\beta + \mathbf{Z}b + \varepsilon \quad \text{eqn. 1}$$

where y is the response variable (larvae per flower, parasitism rate), β represents the coefficients for local and landscape effects, b is the random effect term for landscapes $\mathbf{Z}$ and ε the normally distributed error (Pinheiro and Bates 2000). Two-way interactions were included. The random effect *landscape* (with eight levels) was included to represent nesting of habitats in landscapes. Landscape measures and habitat type were ordered according to the spatial scale (i.e. large-scale effects prior to habitat type).

For each response variable, we used the stepwise AICc function, an information-theoretical approach for small sample sizes (Burnham and Anderson 2002), to find the minimal adequate model. Two maximum models were calculated for the response variable parasitism rate: first we included conservatively only the design variables (i.e. landscape effects and habitat type); the second included also the number of larvae per flower which is a possible predictor for parasitism rates but was not initially included in the experimental design.

Seed number, seed weight, fruit set and destroyed buds were estimated per plant. These plant performance measurements were transformed, if necessary, before we fitted lme-models with landscape effects (see above), habitat type, larval infestation, podless stalks and parasitism as explanatory variables. To examine habitats for differences in species richness and evenness we applied an ANOVA with Tukey's HSD post-hoc test (Hothorn *et al.* 2008). Means were calculated as arithmetic means, unless stated otherwise.

RESULTS

RAPE POLLEN BEETLE LARVAE

The minimal adequate model (Appendix 2.5) for the prediction of rape pollen beetle larvae per flower included mean perimeter-area ratio (MPAR) and habitat type as explanatory variables. Rape pollen beetle larvae responded negatively to landscape complexity (MPAR) on the landscape scale ($F_{1,25} = 16.36$, $P = 0.001$, Fig. 2.1). High larval numbers were observed in simple landscapes (MPAR = 0.05), whilst observations were low in complex landscapes (MPAR = 0.20; Appendix 2.1). Other landscape parameters had no significant effect on larval density. Habitat type significantly affected larvae per flower ($F_{4,25} = 3.13$, $P = 0.032$). Cereal fields (0.34 ± 0.05) and field margins (0.27 ± 0.05) had higher numbers of larvae per flower than fallow (0.21 ± 0.05), grassland (0.16 ± 0.03) and wood margin (0.14 ± 0.04 ; Appendix 2.3). Significant differences of larvae per flower occurred only between cereal fields and field margins on the one hand and fallow, grassland and wood margin on the other hand (Tukey test, Fig. 2.1).

Figure 2.1. The back-transformed data of larvae per flower as a function of the significant mean perimeter-area ratio (MPAR: low values indicate simple, high values complex landscape structure) for each of the five tested habitats. The lines represent model predictions of larval infestation for each of the habitats. Wheat and field margin (dashed lines) had significantly higher larval infestation than grassland, fallow and wood margin (solid lines). Lines of the same type do not differ significantly from each other (Tukey's HSD).

Figure 2.2. Back transformed data of parasitism rates as a function of percentage of oilseed rape crop area for each of the tested habitats. The lines represent model predictions of parasitism rates in each of the habitats. Parasitism rates were significantly higher in wheat fields and field margins (dashed lines) than in grassland, fallow and wood margins (solid lines). Lines of the same type do not differ significantly from each other (Tukey HSD). Also significant was the increase with OSR crop area.

PARASITISM RATES

At the landscape scale, parasitism rates responded only to % OSR crop area ($F_{1,23} = 13.06$, $P = 0.002$; Fig. 2.2, Fig. 2.3, Appendix 2.5). Locally, host density (i.e. larvae per flower; $F_{1,2} = .84$, $P = 0.015$) and habitat type ($F_{4,21} = 2.88$, $P = 0.047$) were significant predictors. Since these two factors correlated with each other, we calculated two different models, the design model with % OSR and habitat type (Fig. 2.2), and the model that included % OSR crop area and larvae per flower (Fig. 2.3). Parasitism rates increased stronger with larval density if OSR crop area was high as shown by the interaction between both factors ($F_{1,23} = 6.09$, $P = 0.022$; Fig. 2.3). In landscapes with a low OSR crop area of 2%, parasitism was not observed, or only at rates below 10%. Parasitism rates by *T. heteroceris* increased with larvae per flower and increasing proportion of OSR crops (up to a maximum of 50%, Fig. 2.3). Plots without parasitism were found in landscapes with an average OSR crop area of 7%, whereas plots with parasitized rape pollen beetles occurred in landscapes with an average OSR crop area of 9% (Fig. 2.3).

PLANT PERFORMANCE

The seed set of *S. arvensis* plants was not significantly influenced by any of the landscape variables nor by habitat type, the minimal adequate model was the null-model (Appendix 2.5). A lme-model including the number of branches as a measure for plant size and podless stalks as a measure for herbivory explained the weight of all seeds of a plant (i.e. yield) best. Total seed weight increased significantly with the number of branches ($F_{1,19} = 8.88$, $P = 0.008$) and decreased with the number of podless stalks ($F_{1,19} = 4.77$, $P = 0.042$). Surprisingly, the amount of rape pollen beetle larvae per flower however could not be linked to the number of podless stalks ($P = 0.633$).

DISCUSSION

We showed that both landscape and local factors affect the abundance of rape pollen beetles (*M. aeneus*) and its parasitoid (*T. heterocerus*). Whilst the reaction to habitat type was similar, both species responded to different landscape parameters. We found the hypothesized spillover from OSR crop onto wild plants in surrounding habitats only for parasitoids, but not for pollen beetles, providing one of the first experimental evidence for plant-herbivore cascades triggered by organisms emigrating from cropland.

HABITAT TYPE EFFECTS ON RAPE POLLEN BEETLE LARVAE

Twice as many rape pollen beetle larvae per flower were found on phytometer plants in cropland habitats such as wheat fields and adjacent field margins in comparison to numbers in the semi-natural habitats (grassland, fallow, wood margin). The higher colonisation of phytometers in cropland habitats may be the result of a population built-up of this crop inhabiting species (Rand and Tscharrntke 2007). Crop rotation with periodic availability of mass-flowering resources has been shown to change landscape-wide densities of associated organisms (Thies *et al.* 2008). In contrast near-natural habitats provide resources constantly, but on a lower level, thus preventing the establishment of high pollen beetle abundances.

Figure 2.3. Back-transformed parasitism rates as a function of the interaction between larval infestation and three categories of oilseed rape crop area (indicated by the shaded areas and the percentage values at the top). Graphs are overlapping for OSR crop area to avoid too distinct separations of this continuous variable. Parasitism rates responded positively to larval densities and OSR crop area. The synergistic interaction between larvae per flower and rape crop area is apparent in the increasing slope from left to right.

HABITAT TYPE EFFECTS ON PARASITISM

Similar to the results for the rape pollen beetle larvae density, parasitism rates were high in wheat fields and field margins and low in the other three habitat types, i.e. parasitoids profited more than their hosts in crop systems. Parasitism rates increased with larval density (as for example in Elzinga *et al.* 2007), indicating that higher numbers of larvae are easier detectable. Since *T. heterocerus* is highly specific to the olfactory signals of (infected) Brassicaceae (Lewis and Tumlinson 1988; Vet *et al.* 1995; Renwick 2002; Wackers 2004; Jönsson *et al.* 2005; Jönsson and Anderson 2007), parasitoids should be less attracted to alternative (i.e. not Brassicaceae) host plants infested with rape pollen beetles. As infestation of *S. arvensis* with rape pollen beetle larvae depends on habitat type, concentration of the herbivores on the phytometer are cascading up to parasitoids. Hence, parasitism rates responded to larval density mediating habitat type.

LANDSCAPE-SCALE EFFECTS ON RAPE POLLEN BEETLE LARVAE

The numbers of rape pollen beetle larvae on the phytometer plants decreased with an increase of landscape complexity, which is in line with some previous studies (Thies and Tschardt 1999; Thies *et al.* 2003; but see Zaller *et al.* 2008b). Simple landscapes may support a population build-up of rape pollen beetles over many years, because they consist of large arable crop patches (including OSR) with uniform resources (Grilli and Bruno 2007). Complex landscapes, in contrast, have scattered small patches of different resource types and thus are likely not to provide enough supply for local mass-populations.

In contrast to our expectation that OSR fields are the main source for spillover of rape pollen beetles, we found no correlation between OSR area and infestation rates of the rape pollen beetle larvae. This is in line with Thies *et al.* (2008), but in contrast to other studies suggesting pest pressure to increase with cropping area (Jonsen and Fahrig 1997; den Belder *et al.* 2002; Klug *et al.* 2003). The lack of response to OSR crop area may be a result of large-scale dispersal patterns (Thies *et al.* 2008), intraspecific larval competition on the phytometers (Nilsson 1988; Ekbom 1998; Hokkanen 2000), or due to abscission of heavily infected buds (Williams 2004).

LANDSCAPE-SCALE EFFECTS ON PARASITISM

Oilseed rape appeared to be a great source of parasitoids in June. The positive correlation between parasitism rates and OSR crop area indicates that the parasitoids shift from OSR to other (more limited) resources in the landscape after depletion of hosts in OSR fields. This supports similar results of Thies *et al.* (2008), who showed that reductions of OSR between years enhanced parasitism. Apart from the positive effect on parasitism rates, OSR crop area interacted significantly with the number of rape pollen beetle larvae. The increase of OSR crop area intensified the positive response of parasitism rates on host density, i.e. larvae per flower. This is possibly the consequence of an easier detection at higher host densities combined with a higher availability of parasitoids from larger OSR areas. Despite these spillover and concentration effects on the landscape scale, mean parasitism rates remained at a low level (< 20%, Fig. 2.2

and 2.3). Two mechanisms may explain this pattern. First, parasitoid populations peak in May (Williams 2006). With a limited lifespan of 1-2 weeks during the summer (Nilsson 2003), the observed parasitism rates may be the result of a declining population. Second, Elzinga *et al.* (2007) reported that parasitoids occur with lower frequency in small patches compared to larger ones. The three phytometer plants established in the habitats constitute a small patch in contrast to the OSR and this is why the observed parasitism rates ranged below the ones that are usually observed in OSR crops (*T. heteroceris* parasitism rate 0.2 - 0.3, Thies *et al.* 2003). Thies *et al.* (2008) considered the regional population pool (i.e. the pool present within OSR areas) more important for biological control than local management (in their case establishing field margins), and stated that parasitoids in agricultural landscapes can be strongly influenced by inter-annually changing crops. Thus, the lack of response of parasitism to the area of semi-natural habitats in a landscape can be best explained with the specialisation of *T. heteroceris* which binds the parasitoid strongly to the area of OSR. Further, the response of parasitoids, but not herbivores, to changes in OSR area with corresponding changes in parasitism rates supports the general idea that parasitoids are more sensitive to disturbances and environmental change than their hosts (Kruess and Tscharntke 1994; Holt *et al.* 1999; Elzinga *et al.* 2007).

SEED SET OF *SINAPIS ARVENSIS*

The decrease of total seed weight per phytometer plant with an increase of podless stalks indicated that herbivory negatively affected the yield of *S. arvensis*. Podless stalks are a typical sign of herbivory by rape pollen beetles (Thies and Tscharntke 1999). However, the number of rape pollen beetles or their larvae was not related to the number of podless stalks. This may have been caused by the fact that the observed herbivory is not only due to the rape pollen beetle numbers at the time of collection, but also a result of continuous herbivory throughout the experiment. Second, loss of ripe pods due to maturity of the phytometer plants (Bruce *et al.* 2002) may account for variation that remains unexplained.

CONCLUSIONS

More than habitat type, landscape-scale factors explained the observed plant-herbivore-parasitoid spillover cascade. Mortality of rape pollen beetles by parasitism was higher in simple landscapes with a high proportion of OSR crops. The response of parasitism to host density profited particularly well from a larger proportion of OSR in the landscape. Positive density dependence in parasitoids is widespread and a basis of successful biological control stabilizing prey populations at low levels (Hassell and May 1974). According to our results, parasitoid spillover from cropland may significantly influence trophic cascades in wild habitats, which is rarely shown, but should be widespread shaping natural food webs.

APPENDIX

CONTENT

Appendix 2.1. Dependent and independent variable measures averaged over all habitat types

Appendix 2.2. Average vegetation characteristics for habitat types

Appendix 2.3. Dependent and independent variable measures averaged over all landscapes

Appendix 2.4. Correlation matrix of model variables and landscape-scale parameters.

Appendix 2.5. Maximal and minimal models from the stepwise AICc analysis

Appendix 2.1. Measures are averaged over all habitat types and given as mean values $\pm$ 1SE for each landscape.

	Barterode	Bodensee	Grossenrode	Meensen	Reinshof	Roringen	Settmarshausen	Varlosen
larvae	97.20 $\pm$ 71.99	117.60 $\pm$ 93.13	65.50 $\pm$ 67.1	92.00 $\pm$ 99.62	32.25 $\pm$ 58.52	64.00 $\pm$ 56.47	53.80 $\pm$ 73.92	120.80 $\pm$ 188.58
flowers	768.60 $\pm$ 374.39	405.80 $\pm$ 86.64	667.75 $\pm$ 675.44	475.60 $\pm$ 207.95	306.50 $\pm$ 222.35	360.40 $\pm$ 190.04	361.00 $\pm$ 264.88	382.00 $\pm$ 298.83
parasitism rate	0.14 $\pm$ 0.23	0.00 $\pm$ 0.01	0.00 $\pm$ 0.01	0.05 $\pm$ 0.04	0.00 $\pm$ 0	0.08 $\pm$ 0.08	0.06 $\pm$ 0.07	0.08 $\pm$ 0.11
number of patches	138.60 $\pm$ 9.63	90.20 $\pm$ 3.83	199.80 $\pm$ 26.34	80.20 $\pm$ 27.49	95.40 $\pm$ 15.76	112.60 $\pm$ 8.26	239.60 $\pm$ 60.58	171.20 $\pm$ 7.56
mean patch size (ha)	1.28 $\pm$ 0.09	1.95 $\pm$ 0.08	0.87 $\pm$ 0.15	2.36 $\pm$ 0.61	1.89 $\pm$ 0.37	1.57 $\pm$ 0.12	0.77 $\pm$ 0.21	1.03 $\pm$ 0.05
habitat diversity (Simpson Index)	0.71 $\pm$ 0.22	1.06 $\pm$ 0.06	1.46 $\pm$ 0.06	1.18 $\pm$ 0.28	0.69 $\pm$ 0.2	1.47 $\pm$ 0.19	1.72 $\pm$ 0.11	1.48 $\pm$ 0.11
number of patches arable land	88.80 $\pm$ 14.36	48.20 $\pm$ 3.7	45.00 $\pm$ 5.48	24.60 $\pm$ 2.07	40.80 $\pm$ 8.9	34.00 $\pm$ 2.92	22.00 $\pm$ 6.78	52.40 $\pm$ 7.47
MPAR	0.11 $\pm$ 0.02	0.05 $\pm$ 0	0.15 $\pm$ 0.01	0.10 $\pm$ 0.03	0.21 $\pm$ 0.04	0.05 $\pm$ 0.01	0.16 $\pm$ 0.01	0.08 $\pm$ 0.01
% arable land	83.50 $\pm$ 6.03	66.70 $\pm$ 4.04	43.71 $\pm$ 5.18	55.82 $\pm$ 20.31	84.69 $\pm$ 4.85	36.81 $\pm$ 4.86	18.34 $\pm$ 1.26	41.21 $\pm$ 6.17
% fallow	1.42 $\pm$ 1.16	3.85 $\pm$ 1.44	2.35 $\pm$ 0.55	2.82 $\pm$ 0.72	1.14 $\pm$ 0.67	6.92 $\pm$ 1.17	8.69 $\pm$ 2.57	14.88 $\pm$ 2.97
% natural near habitats	7.58 $\pm$ 4.27	24.02 $\pm$ 4.82	43.65 $\pm$ 8.68	36.06 $\pm$ 19.73	5.02 $\pm$ 1.86	45.78 $\pm$ 5.99	60.82 $\pm$ 6.34	35.23 $\pm$ 4.23
% cereal crops	64.88 $\pm$ 2.72	46.56 $\pm$ 4.51	26.79 $\pm$ 11.29	36.93 $\pm$ 14.12	63.21 $\pm$ 4.85	25.96 $\pm$ 2.24	14.83 $\pm$ 1.55	28.54 $\pm$ 1.9
% OSR	12.74 $\pm$ 3.48	3.59 $\pm$ 1.29	9.99 $\pm$ 4.08	8.66 $\pm$ 3.46	6.80 $\pm$ 4.45	10.73 $\pm$ 2.68	3.47 $\pm$ 1.05	10.28 $\pm$ 4.1
% grassland	2.99 $\pm$ 2.82	11.64 $\pm$ 1.24	11.97 $\pm$ 6.01	10.62 $\pm$ 5.9	1.75 $\pm$ 0.97	9.26 $\pm$ 13.06	34.09 $\pm$ 3.98	29.81 $\pm$ 1.36
% wood	2.99 $\pm$ 2.38	12.07 $\pm$ 4.56	28.66 $\pm$ 13.52	22.63 $\pm$ 9.35	0.02 $\pm$ 0.01	31.96 $\pm$ 17.65	22.68 $\pm$ 10.37	2.18 $\pm$ 4.3

Presence in landscape			Vegetation				
Habitat	Mean	Standard error	Shannon index	Species richness	Plants	Relative presence in plots	Mean cover in % (in plots where present)
cereal field (ha)					<i>Triticum aestivum</i>	100.00	86.00
					<i>Viola arvensis</i>	28.33	2.29
					<i>Alopecurus myosuroides</i>	16.67	22.60
					<i>Papaver rhoeas</i>	13.33	2.88
					<i>Fallopia convolvulus</i>	8.33	2.40
				2.91	<i>Fumaria officinalis</i>	8.33	1.80
		2.91			<i>Aphanes arvensis</i>	6.67	14.00
			0.23		<i>Cirsium arvense</i>	6.67	3.50
					<i>Poa pratensis</i>	6.67	2.50
					<i>Brassica napus</i>	6.67	2.00
field margin (m)					<i>Dactylis glomerata</i>	91.67	12.00
					<i>Poa trivialis</i>	81.67	24.04
					<i>Poa pratensis</i>	73.33	17.30
					<i>Elymus repens</i>	55.00	10.58
					<i>Alopecurus pratensis</i>	51.67	18.90
		1733.85	1.67	12.40	<i>Taraxacum Sec. Ruderalia</i>	51.67	4.39
					<i>Arrhenatherum elatius</i>	45.00	14.41
					<i>Bromus hordeaceus</i>	45.00	9.85
					<i>Festuca pratensis</i>	40.00	6.21
					<i>Galium aparine</i>	33.33	4.80

Appendix 2.2. continued

Presence in landscape		Vegetation				
Habitat	Mean	Standard error	Shannon index	Species richness	Plants	Relative presence in plots (in %)
fallow (ha)	5.26	0.74	2.01	13.55	<i>Poa trivialis</i>	65.00
					<i>Arrhenatherum elatius</i>	63.33
					<i>Poa pratensis</i>	50.00
					<i>Dactylis glomerata</i>	46.67
					<i>Taraxacum Sec. Ruderalia</i>	45.00
					<i>Cirsium arvense</i>	40.00
					<i>Festuca rubra</i>	38.33
					<i>Elymus repens</i>	38.33
					<i>Holcus lanatus</i>	36.67
					<i>Bromus hordeaceus</i>	33.33
grassland (ha)	14.02	1.95	1.86	14.32	<i>Poa pratensis</i>	81.67
					<i>Poa trivialis</i>	75.00
					<i>Alopecurus pratensis</i>	73.33
					<i>Taraxacum Sec. Ruderalia</i>	61.67
					<i>Dactylis glomerata</i>	60.00
					<i>Cerastium holosteoides</i>	58.33
					<i>Lolium perenne</i>	38.33
					<i>Holcus lanatus</i>	36.67
					<i>Veronica arvensis</i>	36.67
					<i>Arrhenatherum elatius</i>	35.00
wood margin (m)	9311.50	1393.62	1.67	12.08	<i>Dactylis glomerata</i>	80.00
					<i>Galium aparine</i>	73.33
					<i>Poa pratensis</i>	53.33
					<i>Arrhenatherum elatius</i>	46.67
					<i>Geum urbanum</i>	46.67
					<i>Poa trivialis</i>	43.33
					<i>Elymus repens</i>	43.33
					<i>Fraxinus excelsior</i>	43.33
					<i>Bromus sterilis</i>	41.67
					<i>Veronica hederifolia</i>	36.67
						Mean cover in % (in plots where present)
						6.33
						37.53
						8.10
						11.36
						4.04
						4.13
						20.74
						12.26
						30.77
						2.70
						10.35
						28.02
						26.25
						7.30
						7.08
						2.29
						39.04
						5.77
						2.23
						25.95
						10.85
						3.84
						6.50
						22.18
						2.64
						15.92
						11.96
						3.08
						22.52
						2.86

Appendix 2.3. Measures are averaged over all landscapes and given as mean values $\pm$ 1SE for each habitat type.

	Cereal field		Fallow		Field margin		Grassland		Wood margin	
larvae	163.00	± 136.32	38.57	± 46.56	100.00	± 81.22	68.63	± 78.86	27.86	± 28.39
flowers	603.00	± 343.44	287.00	± 137.62	452.88	± 234.38	549.25	± 532.29	402.00	± 174.65
parasitism rate	0.09	± 0.08	0.01	± 0.03	0.13	± 0.18	0.01	± 0.03	0.00	± 0.01
number of patches	140.63	± 56.37	137.63	± 65.04	147.25	± 83	152.38	± 61.84	126.88	± 35.49
mean patch size (ha)	1.46	± 0.63	1.58	± 0.8	1.49	± 0.68	1.31	± 0.49	1.49	± 0.43
habitat diversity (Simpson Index)	1.21	± 0.38	1.23	± 0.39	1.17	± 0.42	1.32	± 0.46	1.18	± 0.38
number of patches arable land	48.00	± 26.27	39.38	± 16.45	47.88	± 22.48	44.75	± 22.26	42.38	± 20.47
MPAR	0.10	± 0.05	0.12	± 0.07	0.11	± 0.05	0.13	± 0.07	0.12	± 0.05
% arable land	55.52	± 23.34	56.10	± 22.18	56.08	± 25.19	49.83	± 26.53	51.70	± 25.32
% fallow	4.82	± 4.28	5.47	± 4.47	4.68	± 4.04	5.91	± 5.37	5.40	± 6.22
% natural near habitats	31.02	± 20.28	29.87	± 17.17	30.02	± 19.87	34.33	± 22.68	36.12	± 23.03
% cereal crops	38.01	± 18.81	39.87	± 15.01	38.63	± 19.89	38.53	± 20.91	37.28	± 21.66
% OSR	9.92	± 4.72	8.79	± 4.35	9.74	± 4.19	5.35	± 2.77	7.61	± 4.96
% grassland	12.30	± 12.63	14.07	± 12.59	12.30	± 13.1	19.29	± 13.95	12.13	± 10.92
% wood	15.95	± 15.33	13.47	± 11.53	15.12	± 17.26	10.68	± 12.76	21.76	± 17.98

Appendix 2.4. The correlation matrix shows r^2 -values of dependent variables for the most important landscape-scale parameters. Bold values have been used as predictor variables in the maximal models. Landscape-scale parameters were measured in all landscapes on a radius of 750 m.

	Larvae/flower	Parasitism rate
Landscape structure metrics		
mean edge/patch	−0.044	−0.041
mean edge/patch arable land	−0.033	−0.213
mean patch size	0.037	−0.152
mean patch size arable land	−0.033	−0.208
MPAR	−0.367	−0.131
MPAR arable land	−0.006	0.158
number of patches	−0.039	0.115
number of patches arable land	0.040	0.361
total edge	−0.091	0.247
total edge arable land	−0.002	0.273
wood edge	−0.075	−0.071
Landscape composition metrics		
% arable land	−0.05	0.092
% beans	−0.3	−0.169
% cereal field	−0.105	0.080
% fallow	0.250	−0.025
% grassland	0.139	−0.071
% maize	0.192	−0.14
% OSR	0.155	0.482
% orchard	0.012	0.250
% perennial near–natural habitats	0.033	−0.117
% potatoes	−0.089	−0.038
% sugar beet	−0.198	−0.113
% urban area	−0.192	0.018
% water	−0.22	−0.146
% wood	−0.041	−0.094
% shrubs	−0.194	0.079
crop diversity (Simpson Index)	0.109	−0.028
crop evenness	0.113	0.086
habitat diversity (Simpson Index)	0.064	−0.148
habitat evenness	0.077	−0.114
number of crop types	0.080	0.269
number of habitat types	0.088	0.096

Appendix 2.5. The maximal and minimal adequate models from the stepwise AICc analysis were calculated with landscape as random factor and are given for the respective response variable. The maximal models were fitted with the following landscape-scale predictors: % arable land + MPAR + % OSR + number of arable land patches.

Dependent	Model	Model fixed factors	Factors	numDF	denDF	F-value	P-value	AICc
larvae/flower	maximal model	landscape factors + habitat identity + landscape predictors * habitat identity						69.14
	minimal model	MPAR + habitat identity	MPAR	1	25	16.36	< 0.001	-41.86
			habitat identity	4	25	3.13	0.032	
parasitism	maximal model	landscape factors + habitat identity + landscape predictors * habitat identity						167.75
	minimal model	%OSR + habitat identity	%OSR	1	21	12.03	0.002	-12.75
			habitat identity	4	21	2.88	0.047	
parasitism	maximal model	landscape factors + larvae/flower + landscape factors * larvae/flower						93.48
	minimal model	(%OSR + larvae/flower)^2	% OSR	1	23	13.06	0.002	-18.48
			larvae/flower	1	23	6.84	0.015	
			%OSR:larvae/flower	1	23	6.09	0.022	

CHAPTER 3

*Heating up trophic interactions in
crops with low vs. high intensity
management*

ABSTRACT

Global warming and agricultural intensification can affect trophic interactions including biological pest control. We tested effects of elevated temperature (using shields for passive warming) in fertilized vs. unfertilized wheat and wheat monocultures vs. wheat diversified with non-crop plants in a full factorial design (8 treatments, 6 replicates, n= 48 plots). We quantified density of cereal aphids and their natural enemies in relation to wheat yield. Fertilization enhanced yield strongly, but also aphid density, which negatively affected yield. Predator-prey ratios were highest in diversified wheat and related to reduced aphid population growth. Warming enhanced aphid growth strongest in fertilized monocultures leading to reduced yields. Our results show that diversified crops and reduced (adapted) fertilization may gain importance as a tool to prevent pest outbreaks in future warmer climates.

Key Words: climate change, intercropping, pest management, *Sitobion avenae*

Authors:

David J. Gladbach

Carsten Thies

Teja Tscharntke

Carsten F. Dormann

INTRODUCTION

Tritrophic interactions can affect functioning of agricultural and natural ecosystems (Holland and Thomas 1997; Matson *et al.* 1997; Migui and Lamb 2007). Agricultural management such as intercropping compared to monocultural planting and fertilization has been shown to affect yield in agroecosystems (Theunissen 1994; Praslicka and Mistina 2004; Zehnder and Hunter 2008). Changing climate, in interaction with farming practices, will affect tritrophic interactions in the future (e.g. Vandermeer *et al.* 1998; Bale *et al.* 2002; Flynn *et al.* 2006; Adler *et al.* 2007). Warming as the principal driver of climate change has been reported to shorten insect development times (Bale *et al.* 2002), reduce larval mortality (Netherer and Schopf 2010) and thus benefit insect abundance and diversity (Wilf and Labandeira 1999). Warming is assumed to increase herbivory (Currano *et al.* 2008; Delucia *et al.* 2008), but consequences for agroecosystems are difficult to predict as responses of insects of different trophic positions to warming may interact (Adler *et al.* 2007). Interactions may be more stable in complex agricultural systems (e.g. Van Emden and Williams 1974; Balvanera 2001; Gurr *et al.* 2003; Casula *et al.* 2006; Schmitz 2009), whereas in intensively managed systems, trophic interactions are often dominated by few species and thereby, susceptible to environmental change (Straub and Snyder 2006; Straub *et al.* 2008).

In the present study we tested how trophic interactions change in differently managed patches of a wheat field (*Triticum aestivum*) in response to warming. Experimental management included herbicide, fertilizer and temperature manipulation in a full factorial split plot. In the resulting eight treatment combinations we measured numbers of aphids, different biocontrol agents and yield of wheat plants as ecosystem functions. We hypothesise that:

- (i) Monocultures in herbicide treated plots support higher aphid densities (Root 1973; Perrin 1976; Risch *et al.* 1983; Sunderland and Samu 2000), while mixed cultures support higher density of biocontrol agents (Smith 1976). Yield of wheat plants can thus be higher in mixed cultures (Sarker *et al.* 2009).
- (ii) Fertilization effects on to yield will outweigh negative effects of increased aphid growth and population densities (White 1993; Dixon 1997; Zehnder and Hunter 2008).
- (iii) Increase of insect population growth rates due to warming (Holopainen J.K. and Kainulainen P. 2004; Netherer and Schopf 2010) leads to particularly large changes of trophic interactions in intensively managed plots.

METHODS

EXPERIMENTAL DESIGN AND TREATMENTS

The experiment was conducted at a research site of the University, city of Göttingen, Lower Saxony, Germany (51°32'N, 9°56'E), between 1st and 28th of July 2008. The average daily temperature in July was 17.9°C with a daily maximum of 32.9°C and nightly minimum of 8°C. Prior to the experiment the site was used repeatedly for agricultural experiments, but remained

unfarmed in 2007. The site was milled and treated with Roundup®UltraMax twice in March to reduce weed pressure. At 10th of April summer wheat (*Triticum aestivum* forma Passat) was sown with 450 seeds per m² on an area of 12x40 m. The sown area was irrigated for 2 h in the evenings at least three times per week, depending on the weather conditions to secure ideal conditions for germination and growth of early wheat seedlings. From jointing stage onwards we watered twice per week in periods of dry weather conditions.

The experiment was designed as a full factorial split plot manipulating herbicide (monocultures vs. mixed cultures), fertilizer (fertilized vs. unfertilized), and temperature (warming vs. no warming). The study contained six blocks (replicates) with 8 plots of one square meter (with one meter spacing), giving a total of 48 plots. Plots were at least one meter from the edge of the wheat field. Each block was half monoculture and half mixed culture. Both, mono- and mixed cultures were split into fertilized and unfertilized strips, which contained a warmed and a non-warmed plot.

The fertilizer treatment was applied in two steps; the first (09.04.2008) was Blaukorn® fertilizer (NPK, 14 + 7 + 17) with 500 kg/ha, the second (22.04.2008) with mineral fertilizer (27% N, 330 kg/ha). The herbicide treatment was also applied in two steps with Accord®SuperPack at BBCH 13 - 29 (Biologische Bundesanstalt, Bundessortenamt and Chemical industry, Zadoks *et al.* 1974; Lancashire *et al.* 1991) within one week after each of the fertilizer applications. Herbicide treated fields developed into monocultures with few wild herbs, whereas wild herbs grew up in untreated fields (Appendix 3.1). Passive warming with shields that were hung 10 - 15 cm above the wheat tillers increased the temperature near the top of the shoots by 1 - 1.5 degrees when compared to ambient plots (Appendix 3.2). Shields were made of greenhouse-foil with regular pinholes (to be permeable for rainwater) clamped on a wooden frame of one square meter. Ambient plots were equipped with a similar construction that had gauze instead of greenhouse-foil to control for treatment artefacts (e.g., heavy rain events might wash insects off the plants). All shields were established on the 29th June. All plots received 500 aphids of the species *Sitobion avenae* (Fabricius) grown under standardized conditions by the Kaatz Biotech AG at wheat flowering on the 1st of July.

MEASUREMENTS

Numbers of aphids and biocontrol agents were recorded weekly (four times) for each plot until dough ripening at the end of July, by counting all individuals on 50 tillers. Immigration of additional aphids was negligible, since only single individuals of other cereal aphid species (i.e. *Rhopalosiphum padi* (L.), *Methopolophium dirhodum* (Walker)) were encountered. Biocontrol agents included species from the Chrysopidae, Coccinellidae, Dermaptera, parasitic Hymenoptera, Syrphidae, and web-building spiders. All organism groups apart from the parasitoids were used for calculating the predator-prey ratio as a measure of overall biocontrol. Yield of wheat plants was estimated by drying and weighing the seeds of 10 spikes per plot.

For each plot we established a series of additional measurements to evaluate the treatment effects. Temperature was recorded with ibuttons (Thermochron® DS1921) approximately

10cm below the wheat spikes at a resolution of 8 measures per 24h from 01.07.-30.07.2008. Temperature differences between elevated and control plots were calculated for each week. We thus averaged the difference between treatment temperatures and a reference temperature from the experimental field for each measure. The effect of the fertilizer treatment on plants was assessed after two weeks with a Hydro N-Tester, a handheld device that measures the nutritional state (i.e. percent nitrogen) of a plant by light transmittance of chlorophyll (Olivier *et al.* 2006; Naud *et al.* 2009) in the leaves (unfertilized plots, $n = 24$: $336.33 \pm \text{SD } 19.59$; fertilized plots, $n = 24$: $497.91 \pm \text{SD } 19.59$). All nitrogen values in this study are given as the corresponding dimensionless transmission values of the Hydro N-Tester, as no correction values for the used wheat variety were available (“Sortenkorrekturwerte”: <http://www.ages.at>). Tiller density, percent wheat and wild herb cover were recorded after the experiment to avoid disturbance of aphid colonies.

ANALYSES AND STATISTICS

Numbers of aphids are per 100 tillers unless stated otherwise. Aphid population growth was calculated as growth relative to the number of aphids at the first aphid count. Aphid populations showed the typical collapse at dough ripening in time step 4. As this collapse was not related to any of our treatments and thus would have distorted the patterns arising from the experimental treatments, we excluded time step 4 from all statistical analyses. Predator-prey ratios were calculated as the ratio of all predators to aphids. Parasitism rate was calculated as the ratio of parasitized aphids to the sum of parasitized and non-parasitized aphids.

Growth rate, predator-prey ratio and parasitism rate were taken as response variables in linear mixed effects models (lme: Pinheiro *et al.* 2009) in R 2.10.1 (R Development Core Team 2009). Growth rates were log-transformed, predator-prey ratios and parasitism rates were square root transformed. Obtaining normally distributed residuals after the transformation, we used the more established lme models instead of generalized linear mixed models for analysing non-normal data, which are more difficult to fit appropriately (Bolker *et al.* 2009). The order of fixed effects was time, herbicide, fertilizer, and temperature, plus three-way interactions between all terms. Because plots were sampled repeatedly over time, we included random intercepts for plots nested in block, herbicide and fertilization treatment and random slopes for time. Variance functions (Pinheiro and Bates 2000; Zuur *et al.* 2009) were used to account for heteroscedasticity. A modified version of the stepAIC function (Venables & Ripley 2002), corrected for small sample sizes (Burnham and Anderson 2002), was used to find the minimal adequate model for each response variable. Additional lme models were fitted with the described random effect structure to estimate the effect of biocontrol agents on aphids and of nutritional state and aphids on yield. Structural equation models (Grace and Bollen 2005) were calculated with AmosTM (Arbuckle 2007).

RESULTS

YIELD

Yield was almost three times higher in fertilized plots (326 ± 42 g/m², $n = 24$) than in unfertilized plots (110 ± 21 g/m², $n = 24$, Tab. 3.1a, Fig. 3.1a). While increasing exponentially with the nutritional state of the wheat plants (Tab. 3.1a¹), yield was reduced by aphids (Tab. 3.1a¹, Fig. 3.2). Warming increased yield in fertilized mixed cultures, but decreased yield in fertilized monocultures, whereas the inverse pattern was observed in unfertilized plots (Tab. 3.1a: herbicide $\times$ fertilizer $\times$ temperature, Fig. 3.1a).

Figure 3.1. Response of the three trophic levels to management type and temperature treatment. Yield (a) was estimated at the end of the experiment and is given in g/m². Back-transformed measures of growth rate relative to week 1 (b), predator-prey ratio (c) and parasitism rate (as percent parasitized aphids, d) are plotted over time for the different treatment combinations. Symbols for warming and no warming are the same in all plots.

APHIDS

Overall aphid numbers increased from wheat flowering in the first week and collapsed after milk ripening in the third week. Highest numbers were reached in the third week in fertilized and warmed monocultures (mean 547 ± 57 , $n = 6$, Appendix 3.3), lowest numbers were recorded in unfertilized and non-warmed mixed cultures (mean 54 ± 7 , $n = 6$, Appendix 3.3).

Growth rates of aphid populations increased significantly over time (Tab. 3.1b, Fig. 3.1b). Growth was stronger in fertilized and warmed (Tab. 3.1b, Fig. 3.1b) plots. Monocultures had a strong trend of supporting higher aphid growth than mixed cultures, which became significant in the interaction with time (Tab. 3.1b: time $\times$ herbicide, Fig. 3.1b).

BIOCONTROL

Predator-prey-ratios varied over time (Tab 3.1c, Fig. 3.1c) and increased particularly in mixed cultures (Tab. 3.1c: time $\times$ herbicide, Fig. 3.1c). Unfertilized plots and warmed plots had higher predator-prey ratios (Tab. 3.1c, Fig. 3.1c). The fertilizer effect was slightly stronger in warmed plots (Tab. 3.1c: fertilizer $\times$ temperature, Fig. 3.1c). Predator-prey ratios negatively affected growth rates of aphids in the third time step ($F_{1,23} = 4.51$, $P = 0.04$).

Parasitism rates varied over time and decreased in the third time step (Tab. 3.1d, Fig. 3.1d). In contrast to predator-prey ratios parasitism rates were higher in monocultures (Tab. 3.1d, Fig. 3.1d) and did not respond to warming (and were hence dropped from the minimal model). Unfertilized plots had higher parasitism rates (Tab. 3.1d, Fig. 3.1d). Overall biocontrol (combined predator-prey and parasitism) was best in unfertilized (fertilizer $F_{1,11} = 16.54$, $P < 0.001$) and mixed cultures (herbicide $F_{1,5} = 9.49$, $P = 0.05$).

DISCUSSION

In this study we investigated the effect of warming on a tritrophic system in different field management systems. Warming and field management practices affected aphid density and growth as well as their natural enemies in wheat crops. Monocultures benefited aphids, while mixed cultures supported biocontrol agents (hypothesis i). Positive effect in fertilized plots increased both aphids and yield (hypothesis ii). Warming increased aphid population growth rates, but aphids could only take advantage of warming when nutritive requirements were met in fertilized plots (hypothesis iii).

CROPPING SYSTEMS AT AMBIENT TEMPERATURES

Wheat plants in fertilized plots were larger, had more tillers, higher yields and possibly higher carrying capacities for aphids (Rotem and Agrawal 2003; Zehnder and Hunter 2008). The enhanced aphid growth in fertilized plots is in line with literature (Weibull 1987; Awmack and Leather 2002) predicting elevated fertility and per capita growth of aphids when plant quality increases in terms of nitrogen availability. Although fertilization increased aphid densities and growth, this negative impact on the wheat plants was outweighed by the direct positive effect of fertilization on yield, only at a given nutritional state the negative effect of aphids on yield

Figure 3.2. Higher nutritional state increased both, aphids (not shown in this graph) and yield. At a given nutritional state an increase of aphids negatively affects yield. Black points represent high aphid numbers, blank points low aphid numbers. For divisions in high and low aphid numbers nutritional value was divided into four discrete sections (0 - 300, 300 - 400, 400 - 500, 500 - 600) and aphid numbers within each section were distributed by value into two equal-sized groups. The regression lines are for low (broken) and high (solid) aphid numbers. Values of nutritional value are the transmission measures of the Hydro N tester.

was visible (Fig. 3.2).

Predator-prey ratios and parasitism rates were lower in fertilized plots than in unfertilized ones. As absolute numbers of mummies and predators tended to increase in fertilized plots, this reduction in potential biocontrol is due to an over proportional increase of aphid numbers with nutrititional value of the wheat plants. Yield in monocultures and mixed cultures differed only slightly, which seems to be unusual but may be the result of a combination of effects. In fertilized plots competition may not have severely affected wheat plants, as intercropped wheat was reported to gain competitive advance by fertilizing (Ghaley *et al.* 2005). Remaining handicaps due to competition may have been outweighed by improved reduction of aphids in mixed cultures. Furthermore yield in monocultures may be negatively affected by the application of herbicides, so that advantages and disadvantages were counterbalanced between the two systems in this study.

The significant interaction between time and the herbicide treatment indicates limited aphid growth in mixed cultures at higher densities. Non crop plants in mixed cultures impeded aphid distribution between wheat tillers by masking visual and olfactory cues, which has follow-up consequences: (1) Dispersing aphids are likely to die on non crop plants (Theunissen 1994). (2) Increased aphid aggregations on single tillers may have aggravated (local) competition (despite comparatively low numbers in the plot) and reduce aphid fertility. (3) Increased aggregations of aphids on single wheat tillers surrounded by non crop plants also may easier attract the more abundant predators (Harwood *et al.* 2003), which was shown by increased predator-prey ratios in mixed cultures. Spiders contributed particularly to predator-prey ratios (not shown) in mixed crops, which is in line with literature (Sunderland and Samu 2000) confirming the effective (spider) predation of aphids in mixed cultures.

Table 3.1. Results of minimal adequate models for the given response variables. Two models were calculated for yield, the design model and another for the effect of aphids.

	numDF	denDF	F-value	P-value
a) Yield/m ²				
herbicide	1	5	1.07	0.348
fertilizer	1	10	19.66	0.001
temperature	1	20	2.78	0.111
herbicide x fertilizer	1	10	7.45	0.021
herbicide x temperature	1	20	1.61	0.219
fertilizer x temperature	1	20	2.36	0.140
herbicide x fertilizer x temperature	1	20	6.12	0.023
a ¹) Yield/m ²				
nutritional value	1	21	37.36	<0.0001
aphids/m ²	1	21	29.78	<0.0001
nutritional value x aphids/m ²	1	21	16.40	0.001
b) Aphid growth				
time	1	68	17.68	0.000
herbicide	1	5	5.63	0.064
fertilizer	1	11	294.62	<0.0001
temperature	1	68	5.86	0.018
time x herbicide	1	68	21.08	<0.0001
herbicide x temperature	1	68	3.00	0.088
c) Predator-prey				
time	2	108	28.03	<0.0001
herbicide	1	5	5.38	0.068
fertilizer	1	10	9.40	0.012
temperature	1	108	3.87	0.052
time x herbicide	2	108	6.80	0.002
time x fertilizer	2	108	1.46	0.236
time x temperature	2	108	1.12	0.329
herbicide x fertilizer	1	10	0.13	0.723
herbicide x temperature	1	108	1.37	0.244
fertilizer x temperature	1	108	3.48	0.065
herbicide x fertilizer x temperature	1	108	2.42	0.123
d) Parasitism rate				
time	2	118	7.11	0.001
herbicide	1	5	8.57	0.033
fertilizer	1	10	5.17	0.046
herbicide x fertilizer	1	10	3.43	0.094

Parasitism rates contributed little to biocontrol in mixed cultures, which is in contrast to other studies (e.g. Praslicka and Mistina 2004). Parasitoids (Hymenoptera: mainly Aphidiidae) are highly specialized on aphids in wheat fields (Sigsgaard 2002; Brewer and Elliott 2004; Thies *et al.* 2005) thus their host finding ability may have been diminished in mixed cultures. Furthermore monocultures had higher aphid densities, so resource concentration (Sheehan and Shelton 1989) may have positively affected parasitism rates.

CROPPING SYSTEMS AT ELEVATED TEMPERATURES

Warming caused higher predator-prey ratios and thereby, appeared to enhance top-down control (Fig. 3.3). The largest functional change between systems in ambient and elevated temperatures was the negative effect of aphids on yield (Fig. 3.3). In warmed conditions monocultures had a positive effect on aphids on one hand, but a less negative effect (than in non warmed plots) on biocontrol on the other, which led to increased impact of biocontrol on aphids (Fig. 3.3). However, these main tendencies counterbalance each other, which will be discussed subsequently.

Like in non-warmed plots fertilization was a major driver in the warmed systems. As stated in hypothesis (iii) responses of aphids and yield to other factors were stronger in fertilized plots. Capped responses in unfertilized plots (Fig. 3.1a,b) were possibly due to a lack of nutrients. However, our results from fertilized plots indicate increased risks of pest outbreaks with warming and a potential key role of mixed cultures in insect population management.

While yield was similar in mixed and monocultures in non-warmed plots, warming led to a reduction of yields in monocultures relative to mixed cultures. In warmed and fertilized plots aphids benefited from reduced development times (Bale *et al.* 2002; Holopainen and Kainulainen 2004; Adler *et al.* 2007; Currano *et al.* 2008) and increased growth rates (Fig. 3.1b) leading to particularly high aphid densities in monocultures (Appendix 3.1) and thereby reducing yield significantly (Fig 3.2, Fig 3.3). Limitation of aphids in mixed cultures may have counteracted potential negative yield effects of competition between wheat and non-crop plants. Higher abundance of biocontrol agents in warmed mixed cultures led to higher predator-prey ratios thus increasing potential biocontrol of aphids (Fig. 3.3). Higher activity of predators (Soares *et al.* 2003) may have contributed to improved and effective biocontrol.

However, this improved biocontrol potential is mainly due to ground living predators, as parasitism showed no response to warming, and contributed little to increased aphid reduction in warmed plots. As parasitoids are highly mobile (Powell and Poppy 1999) they could shift easily between warmed and non-warmed plots (which is also indicated by the somewhat erratic crossing of warming and no warming curves in Figure 3.1). Development times of mummies may decrease with temperature, and activity of adults is likely to increase when warming is not locally confined (Hance *et al.* 2006). As the warming treatment in our experiment was at the lower limit of expected future temperature elevation, the observed patterns may become even more apparent in the future than we showed.

CONCLUSIONS

At actual temperatures an intensive field management maximizes yield although aphids profit likewise from monocultures and fertilized plants. It seems however, that in monocultures population growth of aphids will exceed suppression potential of predators at future warmer climates. In contrast the more complex systems proved to be a warrant for biocontrol, and intercropping may gain importance as an effective practice to prevent pest outbreaks in future warmer climate.

In general warming seemed to increase the strength of interactions between trophic levels (Fig. 3.3). This may lead to a higher sensitivity of whole ecosystems, because tighter links may cause even smaller shifts in population densities due to environmental changes, to cascade further up or down the food chains.

Figure 3.3. Intensity (thickness) and quality (“+” and “-” Symbols) of interactions between trophic levels and treatments are plotted for warmed versus non-warmed systems. The quality of effects of herbicide and fertilizer are given with respect to the application of both factors.

APPENDIX

CONTENT

Appendix 3.1. Plant species cover and occurrence in mixed- vs. monocultures

Appendix 3.2. Temperature data for warming, no warming and field reference

Appendix 3.3. Aphid densities per 100 tiller in different treatment combinations

Appendix 3.1. Cover of the 10 most common plant species in plots. Percent values for presence in plots are given with respect to a total n = 24. Minimum cover for all non crop species was zero.

Plant species	Present in mixed culture plots (%)	Mean cover	Maximum cover
<i>Triticum aestivum</i>	100.00	39.10	90.00
<i>Calystega sepium</i>	91.67	13.50	50.00
<i>Plantago lanceolata</i>	41.67	32.00	60.00
<i>Lotus corniculatus</i>	25.00	19.90	70.00
<i>Vicia hirsuta</i>	37.50	14.60	45.00
<i>Polygonum aviculare</i>	16.67	1.50	2.00
<i>Latyrus pratensis</i>	33.33	17.20	70.00
<i>Epilobium tetragonum</i>	29.17	7.00	30.00
<i>Poa spec.</i>	33.33	31.00	70.00
<i>Elymus repens</i>	33.33	19.20	45.00
Plant species	Present in monoculture plots (%)	Mean cover	Maximum cover
<i>Triticum aestivum</i>	100.00	41.50	90.00
<i>Calystega sepium</i>	75.00	8.70	30.00
<i>Plantago lanceolata</i>	25.00	30.00	40.00
<i>Lotus corniculatus</i>	16.67	2.00	5.00
<i>Vicia hirsuta</i>	20.83	4.25	10.00
<i>Polygonum aviculare</i>	16.67	1.00	1.00
<i>Latyrus pratensis</i>	25.00	1.80	5.00
<i>Epilobium tetragonum</i>	33.33	3.00	8.00
<i>Poa spec.</i>	25.00	24.00	40.00
<i>Elymus repens</i>	8.33	12.00	23.00

Appendix 3.2. Temperature data are given for a reference without shield, as average over all plots with warming shields and gauze shields.

	Daily mean (°C)	Daily maximum (°C)	Night minimum (°C)
Reference (n = 6)			
week 1	19,20	25,64	12,73
week 2	16,04	21,37	11,53
week 3	14,80	19,19	10,73
week 4	21,01	27,06	14,01
Warming (n = 24)			
week 1	20,37	28,92	12,68
week 2	16,95	22,70	11,63
week 3	15,68	20,10	10,87
week 4	22,28	31,35	13,98
No warming (n = 24)			
week 1	18,95	25,14	12,54
week 2	15,94	21,17	11,45
week 3	14,75	19,08	10,69
week 4	20,78	26,59	13,84

Supporting information 3.3. Aphid density is plotted as aphids per 100 tillers over time for the different management types. The different curves in each plot represent the temperature treatment, with $n = 6$.

CHAPTER 4

*Effects of warming, drought and
elevated CO₂ on performance of
an insect herbivore in heathland*

ABSTRACT

The impact of climate change on herbivorous insects can have far-reaching consequences for ecosystem functioning. However, experiments investigating the combined effects of multiple climate change drivers on herbivorous insects are scarce. Here we independently manipulated three climate change drivers (CO₂, temperature, drought) in a Danish heathland ecosystem dominated by heather (*Calluna vulgaris*) and grasses. The experiment was a full factorial split-plot with 6 blocks × 2 CO₂ treatments × 4 warming/drought treatments = 48 plots. We exposed a total of 432 larvae (n = 9 per plot) of the heather beetle (*Lochmaea suturalis* THOMSON), an important herbivore on heather, to ambient vs. elevated drought, temperature and CO₂ (plus all combinations) for five weeks. Larval growth and survival were strongly and significantly negatively affected by elevated CO₂ and drought. Warming had negative effects on growth but not on survival. Combined CO₂ and drought significantly reduced growth, while interactions between warming, CO₂ and drought had varying effects. Overall, our study shows that i) drought was the most important factor for this insect herbivore, ii) the effect of climate change drivers on the herbivore was dependent on other co-acting factors, iii) insect herbivore populations can respond negatively to future climate,

Key-words: multiple climate change drivers, FACE experiment, *Lochmaea suturalis*

Authors:

David J. Gladbach

Karen Stevnbak Andersen

Søren Christensen

Anders Michelsen

Kristian R. Albert

Teis Nørregård Mikkelsen

Claus Beier

Christoph Scherber

INTRODUCTION

Herbivorous insects account for about one quarter of all extant organisms (Strong *et al.* 1984; Mayhew 2001) and are essential to ecosystem structure and functioning (Weisser and Siemann 2004). Ecosystem process rates such as herbivory may be altered significantly under climate change (Currano *et al.* 2008). The global mean surface air temperature is expected to increase by 1.8-5.8°C (2090 to 2099 relative to 1980 to 1999), with additional changes in other climate change drivers such as increasing CO₂ levels or extreme weather events (IPCC 2007). In recent studies, effects of global change drivers on herbivorous insects have been studied mostly in single-factor manipulative experiments rather than multi-factorially. For example, studies have shown that increases in CO₂ may alter plant-insect interactions in various ways (Lincoln *et al.* 1986; Lincoln and Couvet 1989; Stiling *et al.* 1999; Stiling and Cornelissen 2007). Elevated temperature (Bale *et al.* 2002) and altered water conditions (Mattson and Haack 1987; Morecroft *et al.* 2002) have also been considered independently. However, although several studies have started to investigate the combined effects of global change drivers on ecosystem processes (Shaw *et al.* 2002; Pritchard *et al.* 2007), studies on the joint consequences of different climate change drivers on herbivorous insects are scarce (but see Dury *et al.* 1998; DeLucia *et al.* 2008). Multifactor studies, however, will be key to a better mechanistic understanding of plant-herbivore interactions and may function as basis for predicting trophic interactions with respect to climate change.

In the present study we independently manipulated atmospheric CO₂ concentration, near-surface air temperature, and summer drought in a replicated field experiment (Mikkelsen *et al.* 2007). The experiments were conducted in nutrient-poor heather vegetation dominated by *Calluna vulgaris* (L.) and *Deschampsia flexuosa* (L.) Trin. We recorded growth and survival of larvae of an important specialist herbivore, the heather beetle *Lochmaea suturalis* (Chrysomelidae), which shows outbreaks and is a major threat to heather worldwide. We directly measured the response of insect individuals on heather to multiple climate change effects under field conditions. Elevated CO₂ may alter the chemical composition of plants (Peñuelas and Estiarte 1998; Awmack and Leather 2002) and thus reduce the nutritive value for herbivores (Lincoln *et al.* 1986). Consequently, we tested the following hypotheses:

- (i) Elevated atmospheric CO₂-concentrations negatively affect growth and survival of *L. suturalis* larvae because of nitrogen dilution (Roth and Lindroth 1994; Goverde and Erhardt 2003).
- (ii) Prolonged drought negatively affects plant quality (Brenes-Arguedas *et al.* 2009) and hence negatively affects larval growth and survival (Scriber and Slansky 1981).
- (iii) Warming positively affects larval growth and survival due to higher metabolic rates (Netherer and Schopf 2010).
- (iv) Interactive effects of global change drivers on herbivores may reinforce each other (e.g. CO₂ and drought) or act antagonistically (e.g. temperature and CO₂, temperature and drought).

METHODS

SITE DESCRIPTION

The experiment was conducted at the CLIMAITE research site at Brandbjerg (55°53'N, 11°58'E), Denmark between 1st June to 10th July, 2008. The site is located on nutrient poor sandy soils with unmanaged dry heath/grassland mosaic consisting of heather shrubs (*Calluna vulgaris*, 30% cover) and grasses (*Deschampsia flexuosa*, 70% cover). The annual mean temperature was 8°C and the precipitation averaged 600 mm (www.DMI.dk).

EXPERIMENTAL DESIGN AND TREATMENTS

The Brandbjerg field site was designed as a full factorial experiment combining the effects warming (T), drought (D) and CO₂ (CO₂) to mimic a possible climate scenario in Denmark in the year 2075. Climate manipulations (T, D, CO₂), an ambient control (A) and all combinations of them (TD, TCO₂, DCO₂ and TDCO₂) were established in October 2005. The study design contained six blocks (replicates) consisting of two octagons (7 m diameter), each divided into four plots (split-plot design), yielding a total of 48 plots (Fig. 4.1a). CO₂ was manipulated at the octagon level, while drought, elevated temperature, a combination of both treatments and a plot without drought or warming were applied within octagons (Fig. 4.1b).

CO₂ was elevated to 510 ppm using a free air carbon enrichment (FACE) system (Miglietta *et al.* 2001). Passive night time warming (Beier *et al.* 2004) with curtains (height 50 cm) covering the vegetation from sunset to sunrise increased the average temperature (at 20 cm above ground surface) by 1-2°C. The drought treatment was implemented using curtains (controlled by rain sensors) that were activated during May 2008 to simulate a prolonged summer drought. For further detailed information on the facility, treatments and the experimental design see Mikkelsen *et al.* (2007).

STUDY ORGANISM AND MEASUREMENTS

The heather beetle *Lochmaea suturalis* is a strictly monophagous insect herbivore which feeds on *Calluna vulgaris* both as larvae (Fig 4.1c) and as adults (Mohr 1966). Outbreaks have been reported from northern Europe (Gimingham 1972) in which larvae of *L. suturalis* can reach densities of up to 2000 individuals/m² and cause complete defoliation and death of heather (Brunsting 1982).

300 adults of *L. suturalis* were caught near Großalmerode (Germany, 55°15'N, 9°47'E) in a *Calluna* heathland after they mated in April. The specimens were kept in 6 l plastic boxes ("Faunabox", 27 × 18 × 18 cm, Savic, Belgium) using standard protocols (Melber 1989) on the 5th of May 2008. Females successively lay up to 300 eggs between end of April and August. Egg batches were transferred to petri dishes where the larvae hatched after 6-11 days and were fed on small pieces of *Calluna* branches. This was done until we transferred approximately 900 larvae to the field site in Denmark on the 28th of May 2008. Before the start of the experiment on the 1st of June heather beetle larvae were divided into classes according to their weight. Each of the 48 plots received the same amount of larvae from each weight class (9 in total).

Figure 4.1. Aerial photograph (a, Copyright by Kim Pilegaard) of the experimental FACE site at Brandbjerg, DK. Curtains were drawn over the plots for illustrative purposes only; (b) split-plot design of the three treatments CO₂ (CO₂), drought (D) and warming (T) and all combinations, including an ambient control (A), adapted from Mikkelsen *et al.* (2007); (c) 2nd instar larva of *Lochmaea suturalis* feeding on a *Calluna* plant (Copyright by C. Scherber).

Gauze mesh bags (length 30 cm, diameter 13 cm) were tied around individual heather twigs to prevent the *L. suturalis* larvae from leaving the plant during the experiment. For comparability between plots we used heather twigs of the same size (estimated with a scale paper). Larvae were collected from four plots within one octagon, weighed and returned to the plants before proceeding to the next octagon. This practice minimized the time during which the larvae were separated from the plants. Individuals lost were counted as alive for the survival analysis. For weighing we used a Mettler AJ100 fine scale accurate to 0.1 mg, placed on a granite block inside the field station. Measures of fresh weight and survival were recorded weekly during larval development. The experiment was terminated when larvae were close to pupation in the litter layer (after 5 weeks). All larvae being alive by the end of the experiment were recollected and kept in a freezer for further analyses.

For additional information of the treatment effects structural equation models (not shown) were calculated with the following measures of June 2007: carbon and nitrogen content of green leaves from plant individuals in the same plots were analyzed using an Eurovector CN analyzer coupled to an Isoprime isotope ratio mass spectrometer (ambient CO₂: 26.46 ± 0.74, elevated CO₂: 30.45 ± 0.90, $F_{1,5} = 13.897$, $P = 0.01$, Appendix 4.1). Condensed tannin of green leaves was extracted with methanol and analyzed with a spectrophotometer (ambient CO₂: 39.50 ± 2.27 mg/g, elevated CO₂: 45.74 ± 2.60 mg/g, $F_{1,5} = 10.23$, $P = 0.02$, Appendix 4.2). Soil moisture was continuously measured in each plot using TDR-probes (see Mikkelsen *et al.* 2007).

ANALYSES AND STATISTICS

Larvae relative growth rates were calculated from larval weights as indicator of larval development (Hoffmann and Poorter 2002; Awmack *et al.* 2004) using the formula

$$\text{RGR} = (\ln W_{t(x)+1} - \ln W_{t(x)}) / (D_{t(x)+1-t(x)}), \quad \text{eqn 1}$$

where RGR is relative growth rate (Stamp and Yang 1996), $W_{t(x)}$ and $W_{t(x)+1}$ the weights (in mg) for time step x and D the time between two time steps. Because of high larval mortality, the 5th time step was excluded from the growth rate analyses to keep the design balanced. Larval weights were pooled for each plot and time step. Kaplan-Meier survivorship for each plot and time steps 1-5 was calculated using the survfit function from the survival package (version 2.35-7, 2008) in R 2.10.1 (R Development Core Team 2009). Survival rate and relative growth rate were taken as response variables in linear mixed effects models (lme models, Pinheiro *et al.* 2009) to account for the split-plot design of the experiment. The order of fixed effects terms was time, CO₂, drought, temperature, plus two- and three-way interactions between all terms. Because plots were visited repeatedly over time, we included random intercepts for plots nested

Table 4.1. Performance of *Lochmaea suturalis* larvae. Start and end weights as well as number of remaining larvae are given as means of the six treatment replicates (A = ambient control, T = elevated temperature, D = elevated drought, TD = elevated temperature and drought, CO₂ = elevated CO₂, CO₂T = elevated CO₂ and temperature, CO₂D = elevated CO₂ and drought, CO₂TD = elevated CO₂, temperature and drought). Overall values are grand means of all plots (N = 48).

Treatment	Mean weight [mg] ± SE at experimental start	Mean weight [mg] ± SE after 4 weeks	Mean number of larvae ± SE after 5 weeks
A	1.96 ± 0.05	10.45 ± 1.60	6.33 ± 0.33
T	2.01 ± 0.09	7.57 ± 2.10	6.33 ± 0.84
D	1.99 ± 0.05	4.20 ± 1.62	4.33 ± 0.92
TD	1.97 ± 0.08	9.40 ± 0.9	4.33 ± 0.61
CO ₂	1.92 ± 0.09	5.83 ± 1.17	2.83 ± 0.70
CO ₂ T	1.98 ± 0.06	6.27 ± 1.09	5.83 ± 0.71
CO ₂ D	1.94 ± 0.06	8.40 ± 0.7	2.50 ± 0.72
CO ₂ TD	1.99 ± 0.06	4.80 ± 1.28	2.33 ± 0.84
Overall	1.99 ± 0.03	7.16 ± 0.77	4.70 ± 0.67

in block and CO₂ treatment, and random slopes for weeks 1 - 4(5). For 48 plots this yielded 192 (response variable: RGR) and 240 (response variable: survival rate) degrees of freedom for errors in the full model. Variance functions (Pinheiro and Bates 2000; Zuur *et al.* 2009) were used to account for heteroscedasticity. A modified version of the stepAIC function (Venables & Ripley 2002), corrected for small sample sizes (Burnham and Anderson 2002), was used to find the minimal adequate model for each response variable.

RESULTS

GROWTH

At the beginning of the experiment, larvae weighed approximately 2.0 mg and reached an average final weight of 7.2 mg (Tab. 4.1). Elevated CO₂, prolonged drought, and elevated temperature all significantly decreased larval weight (Tab. 4.1).

Relative growth rates increased significantly over time and peaked after three weeks (Tab. 4.2, Fig. 4.2). Relative growth in elevated CO₂ ($F_{1,5} = 15.20$, $P < 0.05$), drought and warming plots were significantly lower compared to the ambient plots (Tab. 4.2, Fig. 4.2). In addition, there were significant two-way interactions between time and CO₂, time and drought and time and warming (Tab.4.2, Fig. 4.2). In combined CO₂ and drought conditions larvae grew slowly (Average RGR in week 1 and 2: D 0.027 ± 0.01 ; DCO₂ 0.045 ± 0.01) particularly in weeks three and four (D 0.01 ± 0.01 ; DCO₂ 0.00 ± 0.02 , Appendix 4.3). Larvae in plots with combined elevation of drought and warming gained on average 0.03 ± 0.06 mg per day. This is less than in plots with warming, but more than in drought-only plots in the second half of the experiment (drought $\times$ temperature $F_{1,107} = 5.18$, $P < 0.05$; time $\times$ drought $\times$ temperature $F_{3,107} = 5.87$, $P < 0.001$, Appendix 4.3).

Figure 4.2. Relative growth rate of *Lochmaea suturalis* larvae over time for all plots with elevated treatments (n=24, broken lines) and controls (n=24, solid lines).

Table 4.2. ANOVA tables of the minimal adequate models for relative growth rate (RGR) and survival rate.

Response variable: RGR	numDF	denDF	F-Value	P-value
time (weeks)	3	107	6.953	<0.0001
CO ₂	1	5	15.201	0.011
drought	1	107	11.464	0.001
warming	1	107	26.597	<0.0001
time × CO ₂	3	107	4.978	0.002
time × drought	3	107	5.995	<0.0001
time × warming	3	107	3.001	0.033
CO ₂ × drought	1	107	7.996	0.005
CO ₂ × warming	1	107	2.085	0.151
drought × warming	1	107	5.182	0.024
time × CO ₂ × drought	3	107	2.030	0.114
time × drought × warming	3	107	5.887	<0.0001
CO ₂ × drought × warming	1	107	3.811	0.053
Response variable: Survival rate				
time (weeks)	4	210	38.101	<0.0001
CO ₂	1	5	8.476	0.033
drought	1	210	65.911	<0.0001
warming	1	210	3.161	0.076
time × CO ₂	4	210	1.325	0.261
time × drought	4	210	5.032	<0.0001
CO ₂ × drought	1	210	0.450	0.498
CO ₂ × warming	1	210	16.186	<0.0001
drought × warming	1	210	0.430	0.508
CO ₂ × drought × warming	1	210	16.555	<0.0001

SURVIVAL

Five weeks after the start of the experiment an average of 4.7 ± 0.67 *Lochmaea suturalis* larvae still remained in the plots (Tab. 4.1). While both, elevated CO₂ and prolonged drought had negative effects on larval abundance after 5 weeks, abundance was slightly higher under elevated than ambient temperature (but only when warming was combined with elevated CO₂, Tab. 4.1).

Resembling a type II response (Pearl 1928), Kaplan-Meier survivorship declined significantly with time (Tab 4.2, Fig 4.3). Type III responses of survival were observed in elevated CO₂, and drought (Tab. 4.2, Fig. 4.3). Survival rates in drought plots dropped significantly in the second week (Tab. 4.2, Fig. 4.3). Warming increased survival only slightly (Fig. 4.3). However, warming increased survival rates to levels of ambient plots in combination with CO₂ (Tab. 4.2, Appendix 4.4). Finally the three-way interaction of CO₂, drought and temperature yielded the lowest survival rates in the experiment (0.25 ± 0.059 after 5 weeks, Tab. 4.1, 4.2, Appendix 4.4).

Figure 4.3. Survival rate of *Lochmaea suturalis* larvae over time for all plots with elevated treatments (n=24, solid lines) and ambient plots (n=24, broken lines). Thin lines show the confidence intervals at 5% and 95%.

DISCUSSION

We investigated main effects and interactions of the climate change drivers CO₂, warming and drought on growth and survival of larvae of a chrysomelid beetle. Insect larval stages are generally considered most sensitive to environmental changes. Given the strong treatment effects on larval stages, it can further be assumed that fecundity and fitness correlates in herbivorous insects may shift considerably under global change.

All global change drivers adversely affected growth and survival of *Lochmaea suturalis* larvae, with the exception of temperature, which only affected growth. Two- and three-way interactions of global change drivers in most cases amplified the negative impacts of main effects on growth or survival.

CO₂ AND DROUGHT EFFECTS

In general, growth was particularly strong at intermediate larval stages and reduced when larvae were closer to pupation. This result is consistent with studies on different insect herbivores (e.g. Berger *et al.* 2006). Confirming our hypotheses (i) and (ii), elevated CO₂ and drought reduced growth rates. Analyses of leaf carbon and nitrogen content indicated that CO₂ increased the C/N ratios of *C. vulgaris* leaves and thus reduced food plant quality for *L. suturalis* (comp. Scriber and Slansky 1981). It is likely that nitrogen dilution due to enhanced plant growth in elevated CO₂ (Lincoln *et al.* 1986) may have lowered food conversion efficiency of *L. suturalis*, resulting in slower development (Roth and Lindroth 1994; Lawler *et al.* 1996; Goverde and Erhardt 2003).

There was no effect of drought on leaf C/N, but plants under drought stress (reduced soil water content) often build tougher and therefore harder digestible leaf structures (Lincoln *et al.* 1993; Awmack and Leather 2002; Rouault *et al.* 2006), which may be an explanation for reduced larval growth in drought plots. However, consumption of plant material by the larvae was too small to record differences in the field.

Nitrogen dilution effects on growth rates may also be responsible for reduced larval survival (Brunsting and Heil 1985) because of elongated development times (Coviella and Trumble 1999). Analyses of tannins showed that the heather plants growing under elevated CO₂ contained higher concentrations of leaf tannins than those in ambient CO₂. In combination with prolonged small larval stages, which are most sensitive to foliar quality (Rouault *et al.* 2006), higher tannin concentrations may additionally have increased mortality, particularly among smaller larvae. Reduced soil water content under drought conditions may also have affected leaf water content negatively (Mattson and Haack 1987). In consequence, survival rates in drought plots dropped in week one and two, as particularly young developmental stages of *L. suturalis* depend on elevated levels of microclimatic humidity (Melber and Heimbach 1984; Melber 1989).

Confirming the sensitivity of small larval stages to food plant quality survival rates in the first two weeks were similar in combined CO₂ and drought and in plots with CO₂ or drought applied separately. Obviously the negative effect of these main effects was at a maximum for small larvae. Only as larvae outgrew the first stage during the progress of the experiment, the negative effects of the combined CO₂ and drought treatment further reduced survival. However, in combined CO₂ and drought larvae ceased to grow in week three. Hence, low nutritive values of *C. vulgaris* may lead to lowered food conversion efficiency, which in combination with low consumption rates may be insufficient to yield energy in excess to what is required for foraging.

WARMING EFFECTS

Warming had no effect on leaf nitrogen or tannin concentration in heather. In insects, increased temperature causes higher metabolic rates and can thereby increase growth, fecundity and survival. However, warming effects on *L. suturalis* larvae in our study were less pronounced

than the distinct effects of CO₂ and drought, and depended on presence and absence of the other climate change drivers. For instance, and in contrast to hypothesis (iii), warming adversely affected larval growth, but reductions remained moderate in comparison to CO₂ or drought effects. These results are in line with findings of Melber (1989), who reported that at high temperatures larvae move closer to the (cooler) ground in order to evade unfavourable conditions. However, while warming may increase consumption and metabolic rates, it also leads to faster tannin accumulation in the larvae. In the face of unchanged survival rates, increased detoxification of tannins may take place at the cost of growth (Barbehenn *et al.* 2009). This assumption is supported by increased survival in combined CO₂ and warming. We suggest that the detoxification efficiency of defensive metabolites increases with temperature (Mattson and Haack 1987), but becomes significant only when concentrations of carbon-based toxic agents increase (Lawler *et al.* 1996).

However, the negative effects of warming increased in combination with CO₂ and drought, and survival of *L. suturalis* larvae dropped to the lowest levels of the study. Possibly larvae could compensate only certain levels of tannin or nitrogen dilution that were exceeded in the three-way interaction of all climate change drivers. Warming may on the one hand increase metabolism rates and thus benefit insect herbivores, but on the other for example greatly reduce relative air humidity when combined with drought. Although we could not exactly determine the mechanism, warming may have introduced unexplored side effects of the climate change factors that lead to qualitatively different impacts on herbivore insects.

This is supported by the fact that the combination of drought and warming increased growth relative to the drought-only treatment, while all other combinations or main effects of climate change drivers lead to reduced growth.

CONCLUSIONS

In the present experiment, drought effects were slightly stronger than CO₂ and clearly dominated over those of warming. CO₂ and drought always negatively affected survival and growth. Warming was less consistent – main effects were not obvious or slightly positive, while particularly interactions with drought had negative impacts on insect herbivores.

We showed clearly that complexity of insect responses increases with the number of combined climate change drivers that may enhance or weaken each others effects. Thus our results are in contrast to earlier hypotheses that e.g. assumed warming to result in increasing insect populations (Coley 1998; Himanen *et al.* 2008; Klapwijk *et al.* 2010). According to our results climate change may not promote but reduce insect populations. Increasing plant C/N ratios may increase the duration of insect developmental stages that are particularly sensitive to drought conditions and may particularly suffer from so called “extreme weather” events with predicted prolonged drought periods, which may be aggravated by warming (Rouault *et al.* 2006). However, generalisations of the effects of single climate change drivers should be handled with care, as the effect of a climate change driver demonstrably depends on the concert of co-acting global change drivers.

APPENDIX

CONTENT

Appendix 4.1. Carbon to nitrogen ratio in leaves of heather (*Calluna vulgaris*) in dependence CO₂, drought, and warming and all treatment combinations

Appendix 4.2. Leaf tannin concentration of heather (*Calluna vulgaris*) in dependence CO₂, drought, and warming and all treatment combinations

Appendix 4.3. Relative growth rate of *Lochmaea suturalis* larvae in dependence CO₂, drought, and warming and all treatment combinations

Appendix 4.4. Survival rate of *Lochmaea suturalis* larvae in dependence CO₂, drought, and warming and all treatment combinations

Appendix 4.1. Carbon to nitrogen ratio in leaves of heather (*Calluna vulgaris*). The graph panels are ordered according to the split-plot design of the experiment. C/N ratios are given for each treatment in elevated (open triangles) and ambient (solid circles) CO₂ octagons. Thus each graph includes only the subset of plots ($n = 6$) with the respective treatment. The single CO₂ effect is obtained when comparing both symbols in the control panel. Other single effects are obtained when comparing the solid circles of different panels. Two and three way interactions with CO₂ can be taken from the open triangles in all but the upper left panel.

Appendix 4.2. Leaf tannin concentration of heather (*Calluna vulgaris*). The graph panels are ordered according to the split-plot design of the experiment. Tannin concentrations are given as mg per g for each treatment in elevated (open triangles) and ambient (solid circles) CO₂ octagons. Thus each graph includes only the subset of plots ($n = 6$) with the respective treatment. The single CO₂ effect is obtained when comparing both symbols in the control panel. Other single effects are obtained when comparing the solid circles of different panels. Two and three way interactions with CO₂ can be taken from the open triangles in all but the upper left panel.

Appendix 4.3. Relative growth rate of *Lochmaea suturalis* larvae. The graph panels are ordered according to the split-plot design of the experiment. Relative growth rates are given for each treatment in elevated (broken lines) and ambient (solid lines) CO₂ octagons. Thus each graph includes only the subset of plots ($n = 6$) with the respective treatment. The single CO₂ effect is obtained when comparing both curves in the control panel. Other single effects are obtained when comparing the solid lines of different panels. Interactions with time can be seen along each curve. Two and three way interactions with CO₂ can be taken from the broken curves in all but the upper left panel.

Appendix 4.4. Survival rate of *Lochmaea suturalis* larvae. The graph panels are ordered according to the split-plot design of the experiment. Survival rates are given for each treatment in elevated (broken lines) and ambient (solid lines) CO₂ octagons. Thus each graph includes only the subset of plots ($n = 6$) with the respective treatment. The single CO₂ effect is obtained when comparing both curves in the control panel. Other single effects are obtained when comparing the solid lines of different panels. Interactions with time can be seen along each curve. Two and three way interactions with CO₂ can be taken from the broken curves in all but the upper left panel. Thin lines show the confidence intervals at 5% and 95%.

SUMMARY

Plant-insect interactions are essential to structure and functioning of terrestrial ecosystems. Shifts within interactions, or their removal from ecosystems due to human caused environmental changes, can have far-reaching consequences for agroecosystems and near-natural systems. Environmental change in this context has three aspects: (1) The expansion of agricultural land decreases structural complexity and habitat diversity on a landscape level. (2) Agricultural intensification due to the application of fertilizers and pesticides often reduces biodiversity within habitats and alters local conditions for insects. (3) Climate change modifies abiotic (e.g. temperature, CO₂ concentration, relative air humidity) and biotic (e.g. concentration of defensive metabolites in plants, nutritional value of plants) conditions for insects on different levels. Predictions about the quality of future ecosystem functions require mechanistic studies of how trophic interactions respond to environmental changes. This thesis comprises three studies approaching the mentioned aspects of change:

(1) In eight landscapes sectors in the vicinity of Göttingen we investigated how landscape composition affects spillover of rape pollen beetles and their parasitoids from oilseed rape fields on wild mustard phytometer plants in cropland habitats (wheat fields and field margin) and noncrop habitats (fallow, grassland, wood margin). Parasitism rates were not related to landscape simplification, but benefited from increasing proportions of oilseed rape. In contrast, rape pollen beetles benefited from simple landscape structure, presumably due to multi-annual population buildup resulting from long-term oilseed rape planting (as part of the crop rotation). We showed that parasitoid spillover from cropland affects trophic cascades on related wild plants outside cropland, which has been not shown so far, but can be expected to be a widespread effect shaping noncrop food webs.

(2) We tested effects of elevated temperature (using shields for passive warming) in fertilized vs. unfertilized wheat and wheat monocultures vs. wheat diversified with undersowings in a full factorial split-plot (8 treatments, 6 replicates, $n = 48$ plots). We measured number of cereal aphids and their natural enemies in relation to yield of wheat plants. Fertilization increased yield, but also aphid density, which negatively affected yield. Predator-prey ratios were highest in diversified wheat and related to reduced aphid population growth. Elevated temperature enhanced aphid growth strongest in fertilized monocultures, leading to reduced yield. Our results show that diversified crops and reduced fertilization may become an important tool to prevent pest outbreaks in future warmer climates.

(3) We independently manipulated three climate change drivers (CO₂, temperature, drought) in a Danish heathland ecosystem dominated by heather (*Calluna vulgaris*) and grasses. The experiment was a full factorial split-plot with 6 blocks $\times$ 2 CO₂ treatments $\times$ 4 warming/drought treatments = 48 plots. We exposed a total of 432 ($N = 9$ per plot) larvae of heather beetle (*Lochmaea suturalis* THOMSON) to ambient vs. elevated drought, temperature and CO₂

(plus all combinations) for five weeks. Overall, this study shows i) that drought was the most important factor for insect herbivores, ii) how the effect of climate change drivers depend on other co-acting factors, iii) that insect herbivore populations may respond negatively to future climate.

In this experimental series we showed clear shifts within trophic interactions as a result of environmental changes. While effects of landscape and local management varied with specialisation of organisms, climate change (i.e. temperature) benefited insects with shorter generation cycles. Extrapolating from local field management effects we may conclude that large scale patterns such as landscape structure may interact with climate change drivers and can be used as a tool for insect population management in future. However, species specific responses to experimentally controlled changes highlight the complexity with which future environmental change affects ecosystems.

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PUBLICATIONS

MANUSCRIPTS

Krewenka K, Gladbach DJ, Holzschuh A, Evaars J, Dormann CF, Tschartnke T (in prep.)
Spatiotemporal patterns of hoverflies in crop vs. non-crop habitat types - implications
of functional traits.

Krewenka K, Holzschuh A, Gladbach DJ, Hellmich S, Dormann CF, Tschartnke T (in prep.)
Spatiotemporal patterns of bees and predatory wasps in different habitat types.

Stevnbak K, Gladbach DJ, Scherber C, Christensen S, Mikkelsen TN, Beier C (in prep. for
Nature) Grasshoppers eat less at future CO₂ but stimulate belowground activity more,
due to relieved N-limitation.

Gladbach DJ, Stevnbak K, Christensen S, Michelsen A., Mikkelsen TN, Beier C, Tschartnke
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Gladbach DJ, Thies, C, Tschartnke T, Dormann CF (submitted to *Global Change Biology*,
May 2010) Heating up trophic interactions in crops with low vs. high intensity
management.

Gladbach DJ, Holzschuh A., Scherber C, Thies C, Dormann CF, Tschartnke T (submitted to
Oecologia, May 2010) Spillover cascades: crops affect trophic interactions on wild
plants in surrounding habitats.

Gladbach A, Gladbach DJ, Quillfeldt P (submitted to *Journal of Avian Biology*, February 2010)
Male achromatic wing colouration is related to body condition and female reproductive
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CONTRIBUTIONS TO CONFERENCES

Gladbach DJ, Thies C, Scherber C, Holzschuh A, Dormann CF, Tschardt T (2009) Effects of local and landscape features on multitrophic plant-insect interactions. Poster, Conference of the German Society of General and Applied Entomology, Göttingen.

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CURRICULUM VITAE

DAVID GLADBACH

BORN IN COLOGNE, 14TH MAY 1977

2006 – 2010 **PhD study** at the Department of Crop Sciences, Agroecology, Georg-August-University in Göttingen, funded by the Helmholtz Association (VH-NG-247) under supervision of Prof. Dr. Teja Tscharnkte

„Plant-insect interactions in changing environments”

2000 – 2005 **Diploma studies** in Biology at the Friedrich-Schiller-University in Jena, Main subject: Ecology, Minor subjects: Zoology, Environmental Law

Diploma thesis (2004 – 2005) at the Institute of Ecology, Friedrich-Schiller University in Jena, under supervision of Prof. Dr. Stefan Halle

*„Experimental study of dispersal and immigration behaviour in common voles, *Microtus arvalis*”*

Erasmus Exchange Study (2001 – 2002) at the University of Wales in Bangor, UK

1998 – 2000 **Undergraduate studies** at the Friedrich-Schiller-University in Jena

1997 – 1998 **Civil service** at „Schullandheim Ratsgymnasium Bielefeld“ on Langeoog

1988 – 1997 **Grammar school**, „Marienschule Opladen“, Leverkusen-Oplanden

1984 – 1988 **Primary school**, „Ernst Moritz Arndt Schule“, Burscheid-Hilgen

DECLARATION OF ORIGINAL AUTHORSHIP

I, David Gladbach am the overall author of this thesis including the manuscripts presented in chapters 2 to 4. Development of main ideas, data collection, statistical analysis, and writing of the manuscripts is my original work. The respective co-authors contributed to various parts of the studies and provided comments on the manuscripts. Any material from other resources has been properly and fully acknowledged.

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