Vegetation density as non-consumable resource: an important factor mediating oviposition site selection of herbivorous insects

Abstract. The herbaceous vegetation of habitats comprises physical, non-consumable features, which may affect the susceptibility of herbivores towards their natural enemies. We studied the effect of vegetation density on herbivore oviposition by analysing field data with null models and evaluating the results in a laboratory bioassay. The generalist leaf beetle, *Galeruca tanaceti*, preferentially deposits its egg clutches on non-host plants such as grasses. Earlier studies revealed that oviposition within structurally complex vegetation reduces the risk of egg parasitism. Consequently, leaf beetle females should prefer patches with dense vegetation for oviposition to increase their reproductive success. Particularly, in this study the following two hypotheses were tested: (A) within habitats the number of egg clutches in patches with different grass stem densities is directly proportional to the number of stems in these areas, because beetle females encounter grass stems by chance. (B) The number of egg clutches in areas with high stem densities is disproportionately higher than in low-density areas, because beetle females actively select to oviposit in high-density patches, but avoid low-density patches. The statistical analysis of the field data revealed that stem density and vegetation height are significantly positively correlated with egg clutch presence. In the field a disproportionately high presence of egg clutches was determined in patches with high stem densities. However, in a laboratory two-choice bioassay females did not deposit a disproportionately high number of egg clutches in high-density areas, but the results indicated that stem encounter rate has an influence on oviposition site selection. However, according to the field results both stem density and vegetation height trigger oviposition site selection of the herbivore. Therefore, this study made evident that non-consumable resources can impose a strong influence on oviposition strategies of herbivorous insects.

Introduction

The properties of host plants, especially their structural and chemical features, are well-investigated biotic factors, which affect the relationship between insects and plants (Bernays and Chapman 1994, Schoonhoven *et al.* 2005). However, natural habitats of herbivorous insects do not only comprise host plants, but also non-host plants. A diverse vegetation composition of habitats can profoundly affect the behaviour of herbivorous insects and thereby their abundance, reproduction and survival (e.g. reviewed in Andow 1991, Agrawal *et al.* 2006). Non-host plants within diverse vegetation might exert several adverse influences on the orientation and performance of phytophagous insects by their chemical and physical characteristics. For example, non-host plants may chemically disrupt the olfactory orientation to host plants by releasing attractant, repellent or masking odours (Thiery and Visser 1986, Held *et al*. 2003, Amarawardana *et al*. 2007). Physically non-host plants may camouflage visual host plants cues (Finch and Collier 2000, Hambäck 2003) or they may hinder the herbivore to access the host plant (Litsinger *et al*. 1991, Coll and Bottrell 1994). Furthermore, non-host plants may indirectly affect herbivores *via* natural enemies, which they may provide with shelter or alternative food sources (e.g. reviewed in Landis 2000, Langellotto and Denno 2004).

On the other hand, non-host plants may also constitute important so-called non-consumable resources for herbivores (Dennis *et al*. 2006). For instance, structural features of the vegetation can be crucial for survival, when certain non-consumable substrates are needed, e.g. for roosting, mate location, overwintering or as pupation sites (Wiklund 1984, Bernays and Chapman 1994, Dennis 2004, Veldtman *et al*. 2007). Several insect species are known to oviposit on non-host plants, which might not only allow successful overwintering, but may also be a strategy to provide eggs with enemy-free space (Obermaier *et al*. 2006, Veldtman *et al*. 2007). Moreover, non-host plant species may emit odorants that disturb the host and prey location process of predators and parasitoids (e.g. Monteith 1960, Wäckers 2004, Bukovinszky *et al*. 2007). The vegetation structure in the vicinity of plants chosen for oviposition can reduce the foraging success of natural enemies, because access to prey or hosts is hampered through visual or physical interference (Tschanz *et al*. 2005, Obermaier *et al*. in press). Thus, beyond the provision of nutritional resources habitats with

enhanced structural complexity may considerably support the survival and performance of herbivorous insects.

When studying the influence of physical vegetation structure on herbivore behaviour, bioassays in the laboratory allow for the specific testing of possible underlying mechanisms, which might explain the observed patterns in the field. In combination with null models or a set of multiple alternative hypotheses (null hypotheses) it is feasible to assess whether the observed pattern of the species distribution differs from a random arrangement. In ecological studies null models, represented by an assembly of statistical methods, have proven to be an important and flexible tool for revealing patterns and uncovering key processes (Gotelli and Graves 1996, Gotelli 2001, Peres-Neto *et al.* 2001).

Here, we investigate the effect of vegetation density on the oviposition site selection of the polyphagous leaf beetle, *Galeruca tanaceti* L. (Coleoptera: Chrysomelidae). The leaf beetle was chosen as model organism to elucidate the effects of non-consumable vegetation structures on herbivore performance. The tansy leaf beetle lays eggs in clutches on vertical plant structures (mainly non-consumable dried grass stems), well above the ground, in grasslands in autumn (Obermaier *et al.* 2006). The eggs are subject to parasitism by the specialised egg parasitoid, *Oomyzus galerucivorus* Hedqvist (Hymenoptera: Eulophidae). Previous studies have shown that high and complex structured vegetation reduces the risk of parasitism and might therefore provide 'enemy-free space' for the leaf beetle (Meiners and Obermaier 2004, Obermaier *et al.* 2006, Obermaier *et al.*, unpublished data). In the present study we examine whether single vegetation structure parameters can determine the oviposition site selection of the leaf beetle in heterogeneous vegetation. In a field survey combined with a laboratory experiment the following hypotheses are tested: (1) Leaf beetle females encounter grass stems used for oviposition by chance. Thus, the number of egg clutches rises directly proportional with an increasing stem density (the number of egg depositions reflects the ratio of stems present in areas of low compared to high stem density); (2) females prefer sites with high stem densities for egg deposition (the number of egg depositions is disproportionately high in areas with high stem density compared to low stem density areas).

Material and methods

Life-history of *G. tanaceti*

The polyphagous tansy leaf beetle *Galeruca tanaceti* L. (Coleoptera: Chrysomelidae) feeds on species of the families Asteraceae, Brassicaceae, Caryophyllaceae, Dipsacaceae, Liliaceae, Lamiaceae, Polygonaceae and Solanaceae (Lühmann 1939, Prevett 1953, Obermaier and Zwölfer 1999). The female leaf beetles oviposit well above the ground on dry vertical structures, mostly grass stems, within the herbaceous vegetation layer in autumn (Scherf 1966, Obermaier *et al.* 2006). Food plants are rarely used for oviposition. The females oviposit about once a week after being fed *ad libitum* in the laboratory at 22°C (B. Randlkofer, personal observation). The gravid females are unable to fly and have to walk up the plant structures for oviposition. The eggs are deposited in clutches and are subject to parasitism by the specialised egg parasitoid *Oomyzus galerucivorus* (Hymenoptera: Eulophidae). Hibernation takes place in the egg stage. In the following spring the beetle larvae hatch and three weeks later pupation takes place. The emerging adults enter a reproductive diapause until September (Siew 1966, Meiners *et al.* 2006).

Field survey

Analysis of the vegetation structure

The field study was conducted in autumn 2004 in Northern Bavaria, Germany (latitude 50°03'N, longitude 10°35'E) at three semi-arid grassland sites ranging from 1300 to 12000 m^2 that were unmown (site Lichtlein and Sechsthal) or grazed by sheep occasionally (site Schafhof). The vegetation structure of microhabitats (r = 0.1 m) around egg clutches (oviposition plots) was compared to the vegetation structure of microhabitats without egg clutch occurrence (control plots). In order to choose the oviposition and control plots randomly, random points were generated using a geographic information system (GIS, ESRI™ ArcView 3.2, Redlands, USA). A handheld GPS device (Garmin$^®$ GPS 12 Personal Navigator$^®$, Garmin International Inc., Olathe, USA) was used to locate these random points within the study site. A

sample of 60 oviposition plots and 50 control plots was analysed on the sites Lichtlein and Schafhof, whereas on the site Sechsthal a sample of 32 control and 33 oviposition plots was analysed. On two study sites (Lichtlein, Sechsthal) the sample of investigated oviposition plots represented all occurring egg clutches within the sites. Several structural parameters were recorded (Sundermeier 1999): percentage horizontal plant cover (at 30 and 50 cm height), percentage vertical plant cover, mean and maximum vegetation height. The number of plant stems suitable (in terms of stability and straightness) for egg deposition was also recorded.

Distribution of egg clutches with regard to stem density

Next we investigated whether egg clutches were found at a different proportion in areas with high plant stem density than can be explained by chance. For this purpose we first determined the proportion *P(O|D)* of egg clutches found in high-density areas:

$$P(O \mid D) = \frac{number\ of\ oviposition\ plots\ in\ high\ stem\ density}{total\ number\ of\ oviposition\ plots} \tag{1}$$

However, this quantity alone is not sufficient to solve the initial question. Additionally, the proportion *P(D)* of high-density areas itself has to be considered, otherwise a high proportion of egg clutches in high densities could just result from a disproportionate presence of high-density areas:

$$P(D) = \frac{number\ of\ control\ plots\ in\ high\ stem\ density}{total\ number\ of\ control\ plots} \tag{2}$$

The null hypothesis predicts that a single stem in an area with a high stem density has the same probability to have an egg clutch deposited on than a stem in a low-density area. In this case the number of oviposition events in areas with high stem density would be proportionally higher compared to areas with few stems (the alternative hypothesis predicts a disproportionately higher number of egg clutches in areas of high stem density). In order to be able to discriminate between the two hypotheses the probability ratio ρ of expressions (1) and (2) can be used:

$$\rho = \frac{P(O|D)}{P(D)} \tag{3}$$

This ratio expresses the relative number of oviposition plots in high-density areas. According to Bayes' theorem this ratio has to be equal one, if egg clutches are distributed randomly within high- and low-density areas. A result larger than one indicates that more egg clutches are found in areas with high stem density than expected according to the natural occurring distribution of high-density areas (disproportionately more oviposition sites in high-density areas). This would suggest an oviposition preference for areas with a high stem density.

In order to categorise the microhabitats correctly a threshold had to be set, which defines the minimum number of stems necessary to allocate the investigated plots as high-density plots. The randomly chosen control plots without egg clutches were considered as a representative sample reflecting the real proportion of areas with high plant stem density within each study site. Thus, for each study site the mean number of plant stems occurring in control plots was calculated and defined as a general threshold between high- and low-density plots. Plots containing the same or a higher number of stems than the mean were classified as located in high-density areas. Based on this threshold, the number of oviposition and control plots located in areas with a high plant stem density was determined.

By setting the threshold for high-density patches at the mean number of plant stems per control plot for each site the resulting margin might not represent the appropriate threshold for the leaf beetle. Therefore several arbitrary thresholds were set and the resulting ratios ρ were calculated according to equation (1) to (3).

Two-choice bioassay

The test arenas (27 × 26 cm) consisted of floral foam covered with filter paper. Each of the six arenas in use was placed in a plastic box (height = 16 cm). The edges were painted with liquid Teflon (Dyneon™, Dyneon GmbH & Co. KG, Burgkirchen, Germany) to prevent the beetles from escaping. Dried grass stems (*Dactylis glomerata*), cut uniformly to a height of 30 cm, were arranged in two fields in natural occurring densities (Fig. 1): Field A (26 × 9 cm) contained 10 stems (stem density =

0.04 stems per cm²), field B (13 × 9 cm) contained 20 stems (stem density = 0.17 stems per cm²).

Eggs of *G. tanaceti* were collected in the environs of Berlin and Würzburg (Germany) in March 2005. The egg masses were transferred to a climate chamber (25°C, 60% RH, 18L:6D, 1000 lux) and kept there from emergence to pupation of the larvae. Larvae and adult beetles were fed with Chinese cabbage (*Brassica pekinensis*). Adult beetles were kept at 15°C from 7 am to 7 pm and 8°C from 7 pm to 7 am, 70% RH, 12L:12D and 800 lux to break diapause and to induce egg production.
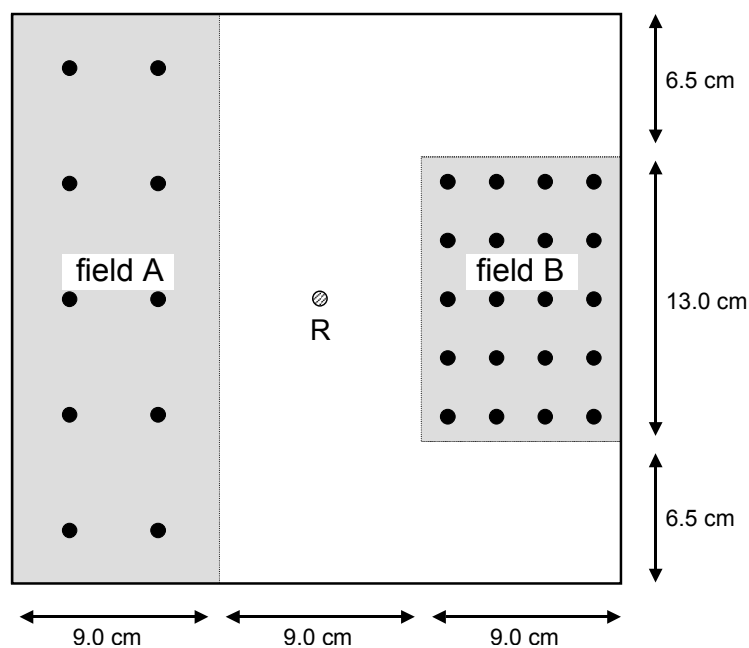

Fig 1. Schematic drawing of the two choice bioassay arena. Grey areas are supplied with different numbers of grass stems according to the drawing. R = release point of the beetle individuals.

Five gravid female leaf beetles were released in the centre of the arena for each trial and allowed to oviposit within the arena for three consecutive days. Pieces (up to 300 cm²) of Chinese cabbage were distributed equally over the floor of the arena to provide the beetles with food. After a female had oviposited during the course of a trial, a naive individual replaced it. The trial was replicated 36 times in a green house in 2005. Each egg deposition, which occurred during the test, was counted individually.

Four different outcomes can be expected for this experiment. 1. If the female beetles respond to plant stem density (stems per unit area) the number of egg clutches should raise directly proportional to stem density (null hypothesis A in accordance with the field survey – stem density field A : stem density field B = 1:4), as field B contains four times more stems per unit area than field A (Fig. 1). 2. A disproportionately high number of egg clutches deposited in areas with high stem density (indicated by a 1:4+x ratio of egg clutches) would imply a preference due to an active choice of high-density areas (alternative hypothesis B in accordance with the field survey). 3. The absolute number of stems, not related to any reference value like area, could control the egg deposition behaviour. If the number of stems alone is responsible for a given distribution of egg clutches, the expected ratio for the two-choice test is 1:2 (absolute number of stems field A : absolute number of stems field B). 4. The beetles' behaviour could be independent of any vegetational parameter and area size alone might be responsible for a given distribution of egg clutches. In this case the expected oviposition ratio would be 2:1 (size field A : size field B).

Statistical analysis

Single parameter logistic regression models were used to predict the probability of egg deposition by *G. tanaceti* depending on vegetation structure parameters (Hosmer and Lemeshow 1989). Correlating parameters with significant influence on egg deposition were summarised with principal component analysis. The goodness-of-fit of the habitat models was determined by considering the coefficient of determination R^2 after Nagelkerke (1991). In order to measure the classification accuracy of the models irrespective of a particular threshold, the Receiver Operating Characteristic curve (ROC-curve) (Hanley and McNeil 1982, 1983) and the resulting Area Under Curve (AUC-value) was used. The AUC-value provides a measure of the diagnostic accuracy of the model (Zweig and Campbell 1993) and varies from 0.5 (determination by chance: null-model) to 1.0 (perfect classification) (Zweig and Campbell 1993, Bonn and Schröder 2001; see also Meiners and Obermaier 2004). All analyses concerning the logistic regression were performed in SPSS 13.0 for Windows. The comparison whether the calculated probability ratios ρ differ significantly from a random distribution ($\rho = 1$) was carried out with the Fisher exact test. For the bioassay data the

chi-squared goodness-of-fit test (Statistica ´99 Edition) was used to compare expected ratios with observed values. *P*-values below 0.05 indicate that the expected ratios differ significantly from the observed ones. This allows for the rejection of the underlying hypothesis, i.e. the assumed mechanism leading to the given egg clutch distribution in the bioassay.

Results

Analysis of the vegetation structure

All vegetation structure parameters investigated showed a significant influence on egg clutch occurrence, but the strength of their impact was not consistent for all study sites (Table 1).

Table 1. The recorded variables of the vegetation structure for the investigated study sites. Positive (+) or negative (–) effects are given, when models of univariate logistic regression showed a significant influence ($P < 0.05$) of the variable on leaf beetle oviposition. n.s.= no significant model was calculated for this variable.

Tested variable	Lichtlein	Schafhof	Sechsthal
Vertical cover herbaceous layer (%)	n.s.	+	n.s.
Vertical cover grass (%)	n.s.	n.s.	-
Horizontal cover at 30 cm (%)	n.s.	+	n.s.
Horizontal cover at 50 cm (%)	+	+	n.s.
Mean vegetation height (cm)	+	+	n.s.
Maximum vegetation height (cm)	+	+	+
Stem density (no. of stems per plot)	+	+	+

Two out of seven recorded parameters best explained the choice of oviposition site by *G. tanaceti* for all three investigated sites. These were the variables stem density and maximum vegetation height (Table 2), which both influenced the egg clutch occurrence significantly positively. The maximum vegetation height was on average 20 cm higher for microhabitats containing egg clutches compared to microhabitats

Table 2. Model characteristics for significant univariate logistic regression models for the parameters (a) maximum vegetation height and (b) stem density for the investigated study sites.

Site	n oviposition	n control	Mean ± SE oviposition	Mean ± SE control	$R^2_{Nagelkerke}$	P_{model}	Coefficient ± SE	AUC ± SE	P_{ROC}
(a) Maximum vegetation height (cm)									
Lichtlein	61	51	82.3 ± 2.2	61.8 ± 3.2	0.279	< 0.001	0.052 ± 0.012	0.748 ± 0.047	< 0.001
Schafhof	60	50	44.9 ± 1.8	29.0 ± 1.7	0.379	< 0.001	0.102 ± 0.021	0.812 ± 0.041	< 0.001
Sechsthal	32	33	86.9 ± 3.7	62.2 ± 5.4	0.241	< 0.001	0.035 ± 0.011	0.732 ± 0.063	0.001
(b) Stem density (number of stems per plot)									
Lichtlein	61	51	15.6 ± 1.5	9.6 ± 1.5	0.093	0.005	0.051 ± 0.020	0.690 ± 0.051	0.001
Schafhof	60	50	13.7 ± 1.1	6.8 ± 0.8	0.294	< 0.001	0.186 ± 0.044	0.796 ± 0.043	< 0.001
Sechsthal	32	33	11.8 ± 1.5	6.9 ± 0.8	0.170	0.003	0.128 ± 0.052	0.684 ± 0.066	0.011

without egg clutches. Similarly, the mean stem density in oviposition plots exceeded the mean stem density of control plots on average by 5 to 7 stems per plot. When both parameters were combined to a single variable through principal component analysis the resulting models for the investigated sites showed even higher statistical validity for two study sites (Table 3). For the site Lichtlein a logistic regression model for the variable maximum vegetation height alone had the highest statistical power.

Table 3. Model characteristics for significant univariate logistic regression models with the combined factors stem density and maximum vegetation height (summarised with principal component analysis) for the investigated study sites.

Site	$R^2_{Nagelkerke}$	P_{model}	Coefficient ± SE	AUC ± SE	P_{ROC}
Lichtlein	0.197	< 0.001	0.800 ± 0.214	0.726 ± 0.049	< 0.001
Sechsthal	0.305	< 0.001	1.311 ± 0.389	0.773 ± 0.058	< 0.001
Schafhof	0.427	< 0.001	2.455 ± 0.497	0.837 ± 0.038	< 0.001

Distribution of the egg clutches with regard to stem density

In the field the probability of oviposition increased with increasing stem density (Table 2). However, we wanted to test whether this increase was directly proportional to the increase in the number of stems and can therefore be simply explained by chance, as suggested by the null-hypothesis, or whether there were disproportionately more egg clutches in areas with high stem density. Our analysis showed that egg clutches were deposited in areas with high stem density in higher proportions than compared to the overall occurrence of high-density areas on the study sites (Table 4). This result strongly supports the alternative hypothesis and indicates that the leaf beetle females seem to deliberately choose microhabitats with a high stem density for oviposition. The mean number of stems per control plot in the field determined the categorisation of plots with high and with low stem density. For control plots of the sites Schafhof and Sechsthal a threshold of 7 stems per microhabitat and for the site Lichtlein a threshold of 10 stems was calculated. All plots above the threshold were defined as plots located within high stem density areas, all below as plots located within low stem

Table 4. The distribution of the egg clutches in areas of high stem density at the study sites. Shown are calculated values for the proportion of oviposition plots relative to the proportion of control plots located in areas of high stem density (ratio ρ) for site-specific thresholds of high stem density. Threshold = mean number of stems in control plots. P-values are given for the Fisher exact test analysing whether the calculated probability ratios ρ differ significantly from a random distribution ($\rho = 1$).

Site	Threshold	n control plots	n oviposition plots	n high-density control plots	n high-density oviposition plots	Proportion high density control plots	Proportion high density oviposition plots	Ratio ρ	P
Lichtlein	≥ 10	51	61	18	35	0.35	0.57	1.63	0.023
Sechsthal	≥ 7	33	32	16	23	0.48	0.72	1.48	0.077
Schafhof	≥ 7	50	60	21	52	0.42	0.87	2.06	< 0.001

density areas at the study sites.

The resulting probability ratio ρ exceeded significantly a value of 1.0 at the site Schafhof with $\rho = 2.06$ (95% CI range: 3.27-26.06, odds ratio = 8.77, $P < 0.001$) and the site Lichtlein with $\rho = 1.63$ (95% CI range: 1.07-5.72, odds ratio = 2.45, $P = 0.023$), at the site Sechsthal a strong tendency was detected with $\rho = 1.48$ (95% CI range: 0.87-8.71, odds ratio = 2.67, $P = 0.077$). As it is not known, which is the perceptual threshold for high stem density of the beetle, arbitrary thresholds were assumed and the respective ratios ρ were calculated for the recorded values of stem densities (Fig. 2). It is evident that any randomly defined threshold leads to a ratio ρ above 1.0 and therefore to a disproportionately higher egg density in high-density areas on all sites investigated.
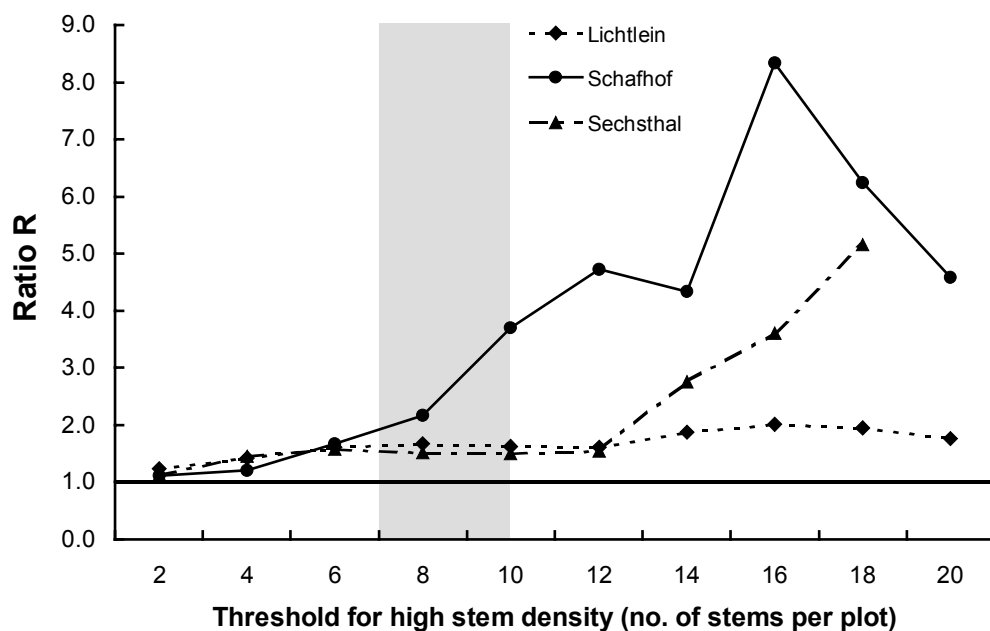

Fig 2. The distribution of the egg clutches in areas of high stem density at the study sites. Shown are calculated values for the proportion of oviposition plots relative to the proportion of control plots located in areas of high stem density (ratio ρ) for different arbitrary presumed thresholds of high stem density. The value of 1.0 would indicate that the increase in egg clutches per area is directly proportional to the increase in stem number (null hypothesis). The grey bar indicates the actual thresholds of stem density derived from the mean number of stems recorded within control plots.

Two-choice bioassay

In the bioassay arena leaf beetle females laid more egg clutches in the field with the higher number of stems (field B) (Table 5, Fig. 1). The observed ratio of egg deposition in fields A and B was 13:18. Only the expected ratio of 1:2 in case of an effect of the number of stems alone on oviposition did not differ significantly from the observed ratio ($\chi^2 = 0.62$, df $= 1$, $P = 0.430$). This implies that the absolute number of stems is affecting the oviposition of *G. tanaceti*. The observed ratio differed significantly from an expected ratio of 1:4 ($\chi^2 = 3.72$, df $= 1$, $P = 0.054$), i.e. egg clutches within the arena were not deposited directly proportional to stem density (null-hypothesis). Likewise, it differed significantly from a ratio of 2:1 ($\chi^2 = 4.17$, df $= 1$, $P = 0.041$), thus the size of the area can be excluded as a key factor for egg deposition.

Table 5. Test of the variables stem density, absolute number of stems and size of the area with stems for oviposition site choice of *G. tanaceti* in a two-choice bioassay arena. Given are the ratios of the available stem densities (number of stems per unit area), the number of stems and the areas of the two fields (A and B) offered to the beetles. Expected values were calculated according to these ratios on the basis of the total number of eggs laid during the test. In order to compare expected values with the observed number of egg depositions the chi-squared test of goodness-of-fit was used.

| | | Explaining factor | | |
	Field	Stem density	Absolute number of stems	Area
Expected ratio	A : B	1 : 4	1 : 2	2 : 1
Expected values with $n = 31$	A : B	6 : 25	10 : 21	21 : 10
Observed egg depositions	A : B	13 : 18	13 : 18	13 : 18
χ^2		3.72	0.62	4.17
df		1	1	1
P		0.054	0.430	0.041

Discussion

For many insects, host plants are the most essential resources for growth and reproduction. Thus, in the past many studies on herbivore-plant interactions focused exclusively on the analysis of the abundance and distribution pattern of host plants (e.g. Bernays and Chapman 1994). However, lately it has been pointed out that this approach leads to a definition of habitats for herbivorous insects, which is too restricted. Specifically, it was criticised that non-consumable resources (structural elements) have been disregarded (Dennis 2004, Dennis *et al*. 2006). The results of the present study demonstrate that vegetation structure in general can profoundly affect egg deposition behaviour of herbivores. The distribution of *G. tanaceti* egg clutches was strongly influenced by stem density and maximum vegetation height. These two parameters could be extracted as key factors for oviposition site selection independently for all three investigated grassland sites. Oviposition becomes more likely as stem density and vegetation height are increasing. These findings are in accordance with previous studies that revealed a positive influence of vegetation complexity, i.e. vegetation height and horizontal vegetation cover, on leaf beetle egg clutch occurrence in the field (Meiners and Obermaier 2004, Obermaier *et al*. 2006).

Other studies, which investigated the effect of vegetation density on oviposition preference of herbivores mostly focused on host plant density, i.e. the quantity of the food resource (Thompson and Quisenberry 1995, Shea *et al*. 2000, Nomakuchi *et al*. 2001, Stiling and Moon 2005). Up to now, the reported findings are highly variable; they either reveal a positive effect, no effect or a negative effect of the host plant density on the choice of the oviposition site and herbivore abundance (cf. Rhainds and English-Loeb 2003). The majority of these studies left aside the effect of the vegetation structure *per se* within natural habitats, which are composed of host plants and non-host plants. Studies that investigate the density of the entire vegetation of a natural habitat, including non-consumable structures and its effect on phytophagous insects, are rare (Langellotto and Denno 2004, Tschanz *et al*. 2005). In contrast to Tschanz *et al*. (2005), the results presented here revealed a positive relationship between leaf beetle egg deposition and the density of the vegetation.

In order to gain a more detailed insight in the mechanism that underlies the egg laying behaviour of *G. tanaceti* in heterogeneous vegetation, we asked whether the

observed increase in oviposition probability in areas with high stem density is only by chance, i.e. directly proportional to the increase in stem density as suggested by the null hypothesis, or whether the leaf beetle deliberately chooses to oviposit in high stem density areas (disproportional higher number of egg clutches in areas with high stem density). The field survey revealed that the leaf beetle females showed indeed a clear preference for areas with high plant stem density, as more eggs were deposited in high-density areas than expected by chance. The ratio of the proportion of oviposition plots located in high-density areas relative to the proportion of control plots in high-density areas (ratio ρ) was always above one (Table 4). This is even true independently of any hypothesised threshold of the mean stem number separating high-density areas from low-density areas (Fig. 2). Moreover, the disproportionately high egg deposition in high stem density areas does hint to a preference, which is probably due to an active choice by the leaf beetle females possibly implicating that the insects are able to perceive stem density as a measure.

To test the significance of stem density as single factor for the choice of egg deposition sites of *G. tanaceti* a laboratory experiment was conducted. In a two-choice bioassay the response of egg laying leaf beetles towards two different grass stem densities was examined. In contrast to the field results, the observed number of egg depositions in the arena experiment was not disproportionately higher in the high-density area of the arena, i.e. leaf beetle females seem not deliberately choose to deposit their egg clutches in high-density areas. Still, the absolute number of stems seemed to be a regulating factor, as the stem encounter rate influenced oviposition site selection of the leaf beetle females within the arenas. However, the setup also might have been too simplistic regarding the number of parameters tested. According to the field observations, it is possible that the female beetles react to both, the effect of the parameters maximum vegetation height and stem density. Our results and previous findings (Meiners and Obermaier 2004) suggest a synergistic effect of the parameters maximum vegetation height and stem density.

Concluding, this study shows the importance to scrutinise a hypothesis both in the field and the laboratory. Null-models, as represented by our null-hypotheses, with no additional assumptions necessary to explain the observed patterns (e.g. Gotelli and Graves 1996), can be useful tools in clarifying such mechanistic questions. The results underline that non-consumable vegetation features can profoundly influence herbivore reproductive strategies.

Acknowledgements

References

Agrawal AA, Lau JA, Hambäck PA (2006) Community heterogeneity and the evolution of interactions between plants and insect herbivores. *Quarterly Review of Biology* **81**: 349-376.

Amarawardana L, Bandara P, Kumar V, Pettersson J, Ninkovic V, Glinwood R (2007) Olfactory response of *Myzus persicae* (Homoptera: Aphididae) to volatiles from leek and chive: Potential for intercropping with sweet pepper. *Acta Agriculturae Scandinavica Section B - Soil and Plant Science* **57**: 87-91.

Andow DA (1991) Vegetational diversity and arthropod population response. *Annual Review of Entomology* **36**: 561-586.

Bernays EA, Chapman RF (1994) Host-plant selection by phytophagous insects. 1st ed. New York: Chapman & Hall.

Bonn A, Schroeder B (2001) Habitat models and their transfer for single and multi species groups: a case study of carabids in an alluvial forest. *Ecography* **24**: 483-496.

Bukovinszky T, Gols R, Hemerik L, van Lenteren JC, Vet LEM (2007) Time allocation of a parasitoid foraging in heterogeneous vegetation: implications for host-parasitoid interactions. *Journal of Animal Ecology* doi: 10.1111/j.1365-2656.2007.01259.x.

Coll M, Bottrell DG (1994) Effects of nonhost plants on an insect herbivore in diverse habitats. *Ecology* **75**: 723-731.

Dennis RLH (2004) Just how important are structural elements as habitat components? Indication from a declining lycaenid butterfly with priority conservation status. *Journal of Insect Conservation* **8**: 37-45.

Dennis RLH, Shreeve TG, Van Dyck H (2006) Habitats and resources: The need for a resource-based definition to conserve butterflies. *Biodiversity and Conservation* **15**: 1943-1966.

Finch S, Collier RH (2000) Host-plant selection by insects – a theory based on "appropriate/inappropriate landings" by pest insects of cruciferous plants. *Entomologia Experimentalis et Applicata* **96**: 91-102.

Gotelli NJ (2001) Research frontiers in null model analysis. *Global Ecology and Biogeography* **10**: 337-343.

Gotelli NJ, Graves GR (1996) Null models in ecology. Washington: Smithsonian Institution Press.

Hambäck PA, Pettersson J, Ericson L (2003) Are associational refuges species-specific? *Functional Ecology* **17**: 87-93.

Hanley JA, McNeil BJ (1982) The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* **143**: 29-36.

Hanley JA, McNeil BJ (1983) A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology* **148**: 839-843.

Held DW, Gonsiska P, Potter DA (2003) Evaluating companion planting and non-host masking odors for protecting roses from the Japanese beetle (Coleoptera: Scarabaeidae). *Journal of Economic Entomology* **96**: 81-87.

Hosmer DW, Lemeshow SL (1989) Applied logistic regression. 1st ed. New York: Wiley.

Landis DA, Wratten SD, Gurr GM (2000) Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* **45**: 175-201.

Langellotto GA, Denno RF (2004) Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. *Oecologia* **139**: 1-10.

Litsinger JA, Hasse V, Barrion AT, Schmutterer H (1991) Response of *Ostrinia furnacalis* (Guenée) (Lepidoptera: Pyralidae) to intercropping. *Environmental Entomology* **20**: 988-1004.

Lühmann M (1939) Beiträge zur Biologie der Chrysomeliden. 4. Beobachtungen an *Galeruca tanaceti* Lin. *Entomologische Blätter* **35**: 91-95.

Meiners T, Obermaier E (2004) Hide and seek on two spatial scales – vegetation structure effects herbivore oviposition and egg parasitism. *Basic and Applied Ecology* **5**: 87-94.

Meiners T, Randlkofer B, Obermaier E (2006) Oviposition at low temperatures – late season negatively affects the leaf beetle *Galeruca tanaceti* (Coleoptera: Galerucinae) but not its specialised egg parasitoid *Oomyzus galerucivorus* (Hymenoptera: Eulophidae). *European Journal of Entomology* **103**: 765-770.

Monteith LG (1960) Influence of plants other than food plants of their host on host-finding by tachinid parasites. *Canadian Entomologist* **92**: 641-652.

Nagelkerke NJD (1991) A note on a general definition of the coefficient of determination. *Biometrika* **78**: 691-692.

Nomakuchi S, Masumoto T, Sawada K, Sunahara T, Itakura N, Suzuki N (2001) Possible age-dependent variation in egg-loaded host selectivity of the Pierid butterfly, *Anthocharis scolymnus* (Lepidoptera: Pieridae): a field observation. *Journal of Insect Behavior* **14**: 451-458.

Obermaier E, Heisswolf A, Randlkofer B, Meiners T (2006) Enemies in low places – insects avoid winter mortality and egg parasitism by modulating oviposition height. *Bulletin of Entomological Research* **96**: 337-343.

Obermaier E, Heisswolf A, Randlkofer B (in press) Comparison of habitat preference in a generalist and a specialist herbivorous beetle on multiple spatial scales. Pp. 39-50 in Schröder B, Reuter H, Reineking B (eds) Multiple Skalen und Skalierung in der Ökologie. Serie: Theorie in der Ökologie. Frankfurt am Main: Peter Lang Verlag.

Obermaier E, Zwölfer H (1999) Plant quality or quantity? Host exploitation strategies in three Chrysomelidae species associated with Asteraceae host plants. *Entomologia Experimentalis et Applicata* **92**: 165-177.

Peres-Neto PR, Olden JD, Jackson DA (2001) Environmentally constrained null models: site suitability as occupancy criterion. *Oikos* **93**: 110-120.

Prevett PF (1953) Notes on the feeding habits and life-history of *Galeruca tanaceti* L. (Col., Chrysomelidae). *Entomologist's Monthly Magazine* **89**: 292-293.

Rhainds M, English-Loeb G (2003) Testing the resource concentration hypothesis with tarnished plant bug on strawberry: density of hosts and patch size influence the interaction between abundance of nymphs and incidence of damage. *Ecological Entomology* **28**: 348-358.

Scherf H (1966) Beobachtungen an Ei und Gelege von *Galeruca tanaceti* L. (Coleoptera, Chrysomelidae). *Biologisches Zentralblatt* **1**: 7-17.

Schoonhoven LM, van Loon JJA, Dicke M (2005) Insect-plant biology. 2nd ed. Oxford: Oxford University Press.

Shea K, Smyth M, Sheppard A, Morton R, Chalimbaud J (2000) Effect of patch size and plant density of Paterson's curse (*Echium plantagineum*) on the oviposition of a specialist weevil, *Mogulones larvatus*. *Oecologia* **124**: 615-621.

Siew YC (1966) Some physiological aspects of adult reproductive diapause in *Galeruca tanaceti* (L.) (Coleoptera: Chrysomelidae). *Transactions of the Royal Entomological Society London* **118**: 359-374.

Stiling P, Moon DC (2005) Quality or quantity: the direct and indirect effects of host plants on herbivores and their natural enemies. *Oecologia* **142**: 413-420.

Sundermeier A (1999) Zur Vegetationsdichte der Xerothermrasen nordwestlich von Halle/Saale. Berlin: J. Cramer.

Thiery D, Visser JH (1986) Masking of host plant odour in the olfactory orientation of the Colorado potato beetle. *Entomologia Experimentalis et Applicata* **41**: 165-172.

Thompson RA, Quisenberry SS (1995) Rice plant-density effect on rice water weevil (Coleoptera: Curculionidae) infestation. *Environmental Entomology* **24**: 19-23.

Tschanz B, Schmid E, Bacher S (2005) Host plant exposure determines larval vulnerability – do prey females know? *Functional Ecology* **19**: 391-395.

Veldtman R, McGeoch MA, Scholtz CH (2007) Fine-scale abundance and distribution of wild silk moth pupae. *Bulletin of Entomological Research* **97**: 15-27.

Wäckers F (2004) Assessing the suitability of flowering herbs as parasitoid food sources: flower attractiveness and nectar accessibility. *Biological Control* **29**: 307-314.

Wiklund C (1984) Egg-laying patterns in butterflies in relation to their phenology and the visual apparency and abundance of their host plants. *Oecologia* **63**: 23-29.

Zweig MH, Campbell G (1993) Receiver-operating characteristic (ROC) plots – a fundamental evaluation tool in clinical medicine. *Clinical Chemistry* **39**: 561-577.