

Connectivity counts: disentangling effects of vegetation structure elements on the searching movement of a parasitoid

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Abstract. 1. A heterogeneous habitat structure can have a profound impact on foraging carnivorous arthropods. In the present study, we examined which elements of complex vegetation structure influence the searching movement of a parasitoid model organism.

2. Previous field work showed that tall and dense vegetation reduces the parasitism success of the eulophid egg parasitoid *Oomyzus galerucivorus* while the probability of host egg deposition increased close to plant tips.

3. In laboratory bioassays, dried grass stems were arranged according to the natural situation in different setups. The wasps' walking time on stems increased with increasing stem height and density. High stem density decreased the walking time of the parasitoids on the ground and an increased stem height reduced the propensity to fly to the ground. Connectivity had a minor positive effect on the number of stem contacts, but considerably reduced the number of wasps reaching the upper part of grass stems by two-thirds.

4. Thus, although enhanced vegetation complexity enhances walking activity of the parasitoids in the vegetation, laying eggs at the tip of long grass stems in dense vegetation can be an adaptive strategy for the host, as it maximises the number of connections between plant parts to cross by parasitoids before reaching the host. The connection points disorient the wasps, which lose time, reverse their direction or fly away.

Key words. egg parasitoid, habitat complexity, host searching, movement ability, *Oomyzus galerucivorus*, plant canopy, spatial plant structures, walking behaviour.

Introduction

A variety of physical and chemical properties of plants, on which hosts or prey are present, directly influence the mobility of natural enemies (Price *et al.*, 1980; Coll *et al.*, 1997; Romeis *et al.*, 1998). In particular physical vegetation traits, for example plant architecture, are known to affect the movement behaviour and searching activity of parasitoids and predators in either positive or negative ways (Grevstad & Klepetka,

1992; Frazer & McGregor, 1994; Weisser, 1995; Clark & Messina, 1998; Legrand & Barbosa, 2003). The architecture of single plants influences notably enemies that are small in size such as parasitoids (Price *et al.*, 1980; Gardner & Dixon, 1985; Stadler & Völkl, 1991). Several studies specifically investigated the host finding behaviour of parasitoids using artificial plant models varying in their degree of structural complexity (Andow & Prokrym, 1990; Lukianchuk & Smith, 1997; Gingras & Boivin, 2002; Gingras *et al.*, 2002). Other studies used natural plants to analyse the effect of plant architecture on foraging success of parasitoids (Cloyd & Sadof, 2000; Gingras *et al.*, 2003). Most investigations describe that

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a high degree of plant structural complexity has a negative impact on foraging efficiency of parasitoids.

However up to now, the majority of studies dealing with the influence of plant structure on the movement patterns of parasitoids concentrated on the architecture of single plants only (but see Gols *et al.*, 2005; Romeis *et al.*, 2005). The entire structure of the environment, e.g. a habitat patch, and its influence on natural enemy movement is generally neglected in investigations of parasitoid-host and other arthropod interactions (Casas & Djemai, 2002). Within a habitat the vegetation structure is often assumed to be entirely homogenous, but the structural complexity of sub-patches may vary strongly in their degree of complexity (Langellotto & Denno, 2004; Meiners & Obermaier, 2004; Tschanz *et al.*, 2005). Vegetation structure may significantly alter predator-prey interactions simply by modifying the movement tracks of predators (Kareiva & Perry, 1989; Vohland, 1996). Consequently, the movement ability of an insect depends on behavioural traits and vegetation structure, because both interact and lead to distinct movement patterns (Neuvonen, 1999; Goodwin & Fahrig, 2002). This may affect the time predators or parasitoids spend on a plant, and subsequently the co-occurrence of natural enemies and their victims (Casas & Djemai, 2002 and references therein).

To elucidate the role of vegetation structural parameters in parasitoid foraging, we investigated the movement patterns of *Oomyzus galerucivorus* Hedqvist (Hymenoptera: Eulophidae), a specialised egg parasitoid of the polyphagous leaf beetle *Galeruca tanacetii* L. (Coleoptera: Chrysomelidae). Like other egg parasitoids, the small wasps (c. 1.5 mm in size) mostly walk up and down plants during their search for host eggs. In general, the probability of leaf beetle oviposition increases in patches with high stem density in the field (Meiners & Obermaier, 2004; Randlkofer *et al.*, 2009). Earlier field studies also revealed that a dense and complex vegetation structure reduces the parasitism success of the egg parasitoid on a small spatial scale ($r = 0.1$ m) (Obermaier *et al.*, 2008). Furthermore, leaf beetle egg clutches, which are deposited high up on the plants used for oviposition, are less likely to be parasitised (Obermaier *et al.*, 2006). In the present laboratory study, we classified vegetation structure using the three structural parameters 'density' (number of plant stems per unit area), 'height' (plant size), and 'connectivity' (number of direct contacts between different plant individuals). The classification of these three vegetational parameters was chosen with respect to a previous characterisation of the architecture of single plants (Andow & Prokrym, 1990; Gingras *et al.*, 2002). The impact of each individual parameter on the movement patterns of *O. galerucivorus* females was experimentally investigated in bioassays. Thereby, we aimed at gaining insight into mechanisms potentially acting in the field.

Specifically, we asked in our study (i) whether a high degree of structural complexity constricts the movement of the egg parasitoid, and thus, decreases the searching activity within structures of high complexity or on plant parts relevant for host encounter and (ii) whether it is possible to single out one structural parameter that has the strongest influence on the movement patterns.

Materials and methods

Life history of Oomyzus galerucivorus

Oomyzus galerucivorus is known to parasitise several species of *Galeruca* throughout Europe (Sinacori & Mineo, 1993). The main host of the specialised egg parasitoid in Germany is *Galeruca tanacetii*, a polyphagous leaf beetle. In autumn, the leaf beetle females oviposit well above the ground on dried vertical plant structures, mostly on non-consumable grass stems, within the herbaceous vegetation layer, to reduce overwintering mortality (Scherf, 1966; Obermaier *et al.*, 2006). Shortly after their deposition, *O. galerucivorus* parasitises the leaf beetle eggs. As a result of their minute size (c. 1.5 mm) the egg parasitoids are considered poor flyers and have to search for host egg clutches, mainly by walking up and down vertical plant structures within the vegetation. Host faeces at the bottom of plants left behind by female beetles after oviposition act as an arrestant cue, and intensifies the host search, but the odour of the faeces alone does not elicit any response in the wasps (Meiners *et al.*, 1997 and unpublished). The parasitoid larvae hibernate in the host eggs and adults emerge next spring. As the leaf beetle is an univoltine species, the adult parasitoids have to outlast 5–6 months until host eggs are available (Meiners *et al.*, 2006). The parasitism rate of leaf beetle eggs by *O. galerucivorus* can reach up to 89% (Meiners *et al.*, 2006). However, overall parasitism rates fluctuate between sites and years with some sites being heavily parasitised in one year but not experiencing any parasitism pressure in another year (B. Randlkofer, unpublished).

Study insects

Parasitoids were reared from *G. tanacetii* egg masses collected in the nature reserve 'Hohe Wann' in Northern Bavaria, Germany. Egg clutches were kept separately in glass vials (10 ml, 32 × 24 mm) in a climate chamber at 20 °C, 60% RH and LD 18:6 h photoperiod to initiate the eclosion of the parasitoids. Afterwards the parasitoids were transferred to a climate chamber with 10 °C, 70% RH and LD 18:6 h until testing. The animals were provided with honey water as food and moist filter paper within the rearing Petri dishes. Females and males were not separated until shortly before the bioassay, to ensure that mating could take place.

Experimental design

The base of the test arenas (24 × 24 cm) consisted of floral foam covered with filter paper. Dried grass stems (*Dactylis glomerata* L., Poaceae), with the top part cut off and blades removed, were used to build six different types of vegetation structures. Dried, non-consumable grasses are the preferred oviposition substrate of the leaf beetle host of *O. galerucivorus* and the main component of the vegetation in the habitat at the time of host search. To vary the complexity of the vegetation structure, arena designs with a low or high degree of vegetation density, with a short or tall height of grass stems or with low

or high connectivity were created. In the centre of each arena one grass stem was placed, and around it four concentric circles with a spacing of 2 cm were drawn. Thus, the inner circle had a diameter of 2 cm, whereas the outermost circle had a diameter of 16 cm. Within these circles the grass stems were uniformly distributed in distance (Fig. 1).

For the low-density design 13 grass stems were used in total (circle 2: four stems, circle 4: eight stems), whereas for the high-density design 51 grass stems were used in total (circle 1: 5, circle 2: 10, circle 3: 15, circle 4: 20). Thus the high-density design had about four times more stems than the low-density design. In order to test the influence of height on the movement patterns of the egg parasitoid, short and tall grass stems were used (short height: 15 cm, tall height: 45 cm). The effect of connectivity was tested in arenas with high stem density and an equal stem height of 45 cm. Connections (length *c.* 1.5 cm) between the grass stems were inserted using bast fibres at three different heights (8, 23, and 38 cm). In the low-connectivity design, 10 connections in total per level between two single grass stems were randomly tied (circle 1: one connection, circle 2: two connections, circle 3: three connections, circle 4: four connections). In the high-connectivity design, 50 connections in total per level were tied between grass stems, i.e. each grass stem within a circle was connected *via* bast fibres with its neighbour. Overall, in the high-connectivity design a total of 150 connections were created, whereas in the low-connectivity design a total of 30 connections were created.

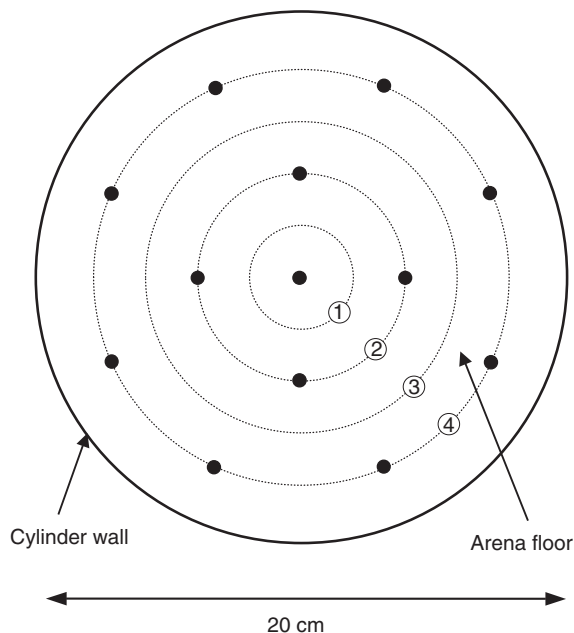


Fig. 1. Schematic representation of the bioassay arena (top view) for observations of the searching behaviour of *Oomyzus galerucivorus* in different vegetation structure types. Numbers and dotted lines indicate circles where the grass stems were arranged according to the chosen design. Black dots = position of dried grass stems (here for the low-density design).

All arenas were covered with a plexiglass cylinder with a diameter of 20 cm and with a height of 50 cm. A lid with very fine plastic gauze (mesh size 0.5 mm) was used to close the cylinder at the top. Experiments were conducted under uniform illumination provided by a full spectrum plant lamp (60 W light bulbs; General Electric Company, Niskayuna, New York) simulating daylight conditions. The light bulbs were covered with translucent parchment paper to dampen the brightness of the lamps.

Behavioural observations

In all experiments female parasitoids were mated, fed and naïve with respect to experience with vegetation structures and oviposition. The test individuals were 7–34 days old. At this age, female parasitoids used for rearing were observed to successfully parasitise leaf beetle eggs in a long-day light cycle (B. Randlkofer, unpublished). The maximum life span for the parasitoids observed in the laboratory was up to 4 months. The female parasitoids were released singly on the floor in the centre of the arena and each female was only used once. Their behaviour (walking, flying, resting, and grooming) and their position (floor, stems, and cylinder wall) inside the arena were recorded for 5 min. Every stem contact was registered, which implied that a parasitoid had either walked up a grass stem or had landed on it after a short flight. In order to specify the position of the parasitoids on stems, the 45-cm-long grass stems were subdivided into three 15-cm sections (lower, middle, and upper section). Before the first parasitoid was released into the arena, the floor was sprayed with water, which was repeated in regular intervals. After each completed observation the arena was rotated for 90° to avoid the influence of potential side effects. Two corresponding arena designs (e.g. low and high density with short stems, etc.) were usually used for simultaneous observations and after five consecutive observations, the arena types were exchanged between the two observers. The observations were recorded using the event encoder software 'The observer 3.0' (Noldus Information Technology, Wageningen, The Netherlands). Experiments were undertaken in a constant temperature room during daytime from 09:30 to 16:00 hours. The average abiotic test conditions were 22 ± 2 °C, $71 \pm 6\%$ RH, 1013 ± 7 hPa.

Mode of movement (flying versus walking behaviour)

Two distinct categories were defined in order to distinguish what kind of influence the vegetation structures have on different modes of movement and thus, on the wasps' movement patterns. All observed individuals showing walking and/or flying behaviour during the observations were allocated either to the 'non-flying' or 'flying' category for each of the tested arena designs (see Table S1). Flying wasps were defined either as individuals which showed flying behaviour in addition to walking behaviour while foraging, or as individuals which flew to the wall of the cylinder and reentered the arena. Non-flying wasps were defined as individuals that did not fly at all during the 5 min of the experiments or which foraged

for an undefined time by walking, then flew straight to the cylinder and stayed there. Only the walking time of 'non-flying' individuals was considered for further analyses of the time which the parasitoids actively spent searching within the arena. Generally, the majority of parasitoids (*c.* 70%) showed walking behaviour only, and walking time data of 'flying' and 'non-flying' individuals were rather similar. Analysing only the walking time of 'non-flying' individuals facilitated data interpretation. Movement from position A to position B can only be reliably predicted for walking individuals, because reaching position B is always dependent on the previous position A, whereas for flying individuals changing from one position in space to another is arbitrary and difficult to predict.

Statistical analyses

The influence of stem density and height (i.e. the four setups: low/high density, short/tall stems) as well as the three degrees of connectivity (i.e. the three setups: no/low/high connectivity with tall stems and high density; the high density-tall stems setup without bridges between grass stems is considered here as 'no connectivity') on the walking time on the floor and on the stems, were analysed with univariate analysis of variance (general linear model = GLM – univariate procedure in SPSS). Differences in walking time between the lower, middle, and upper stem part were tested using repeated measures ANOVA (general linear model = GLM – repeated measures procedure in SPSS), with stem part as a within-subject variable and density, height, and connectivity as between-subject factors. Prior to repeated measures ANOVA procedures the assumption of sphericity was tested with Mauchly's test. The Greenhouse–Geisser epsilon was used to correct for violations of the sphericity assumption and to adjust the degrees of freedom accordingly. To investigate the effect of connectivity on the number of parasitoids reaching higher stem parts, χ^2 tests were performed. The number of wasps staying on or leaving the lower stem part and the number of wasps reaching or not reaching the upper stem part in the different connectivity setups were analysed. Statistical calculations were conducted with SPSS for Windows 17.0 (SPSS Inc., Chicago, Illinois). We used GLM models with Poisson error distribution (link = log) to analyse the effect of density and height or connectivity on the number of wasps flying from the stem to other stems, the floor or the cylinder (STATISTICA for Windows version 5.5; StatSoft, Inc., Tulsa, Oklahoma). Variations in the number of stem contacts were analysed using the Kruskal–Wallis test and Dunn's multiple comparison post hoc test and were conducted in GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, California).

Results

General observed behaviour and movement within the arenas

After the gentle release in the centre of the arena the majority of the parasitoids started to walk around on the floor immediately. When the wasps came in direct contact with a

stem they usually started to climb up, with resting/grooming stops in between. In the setups without any connections between the stems they usually did not move downwards before reaching the tip. At the tip of the stem the individuals spent a considerable amount of time inspecting it, and commonly switched over to resting and grooming activities after some time (B. Randlkofer, pers. obs.). In the 'non-flying' group a small proportion of the wasps (10–37%) left the tip of the stems by flying away to the cylinder wall and stayed there. The wasps in the 'flying' group behaved similarly but used short flights or hops to move between stems, or to fly away from the grass stems after reaching the tip (29–90%). In the connectivity setups the connection points served sometimes as resting places and wasps did not move further upwards, but occasionally moved backwards.

Effect of density and height on parasitoid searching movement

In the laboratory, high stem density significantly decreased the time *O. gallerucivorus* females spent walking on the floor compared with low stem density (Table 1A, Fig. 2a). Further, the height \times density interaction shows an increase in walking time on the floor in low density arenas from short to tall stems, which is not present in the high density arenas. However, height alone showed no significant effect on walking time on the floor. In contrast, the searching activity on the grass stems was positively affected by stem height and density, which also showed a significant interaction effect (Table 1A, Fig. 2b). Also, walking time at low stem densities increases more from short to tall stems in the low than in the high-density design. Overall the parasitoids enhance walking activity when the complexity (density or height) increases. Stem density or height did not considerably influence the number of stem contacts when comparing all four setups (Kruskal–Wallis test, $H = 5.800$, $P = 0.122$, Fig. 2c).

As the highest probability of egg clutch encounter is close to the tips of grasses in the field, we analysed the searching activity on different parts of the tall grass stems within low or high density. The walking time of the parasitoids depended on their respective position on the stem. The average walking time

Table 1. The effect of (A) stem density and height and (B) the degree of connectivity on walking time on the floor (Fig. 2a) and on the stems (Fig. 2b). Univariate analysis of variance performed with a general linear model.

Source	Walking on floor			Walking on stems		
	d.f.	<i>F</i>	<i>P</i>	d.f.	<i>F</i>	<i>P</i>
(A)						
Model	3	4.366	0.006	3	25.791	<0.001
Density	1	6.352	0.013	1	18.115	<0.001
Height	1	1.105	0.295	1	48.935	<0.001
Density \times height	1	4.631	0.033	1	6.528	0.012
Error	160			156		
(B)						
Connectivity degree	2	2.241	0.112	2	2.152	0.122
Error	94			94		

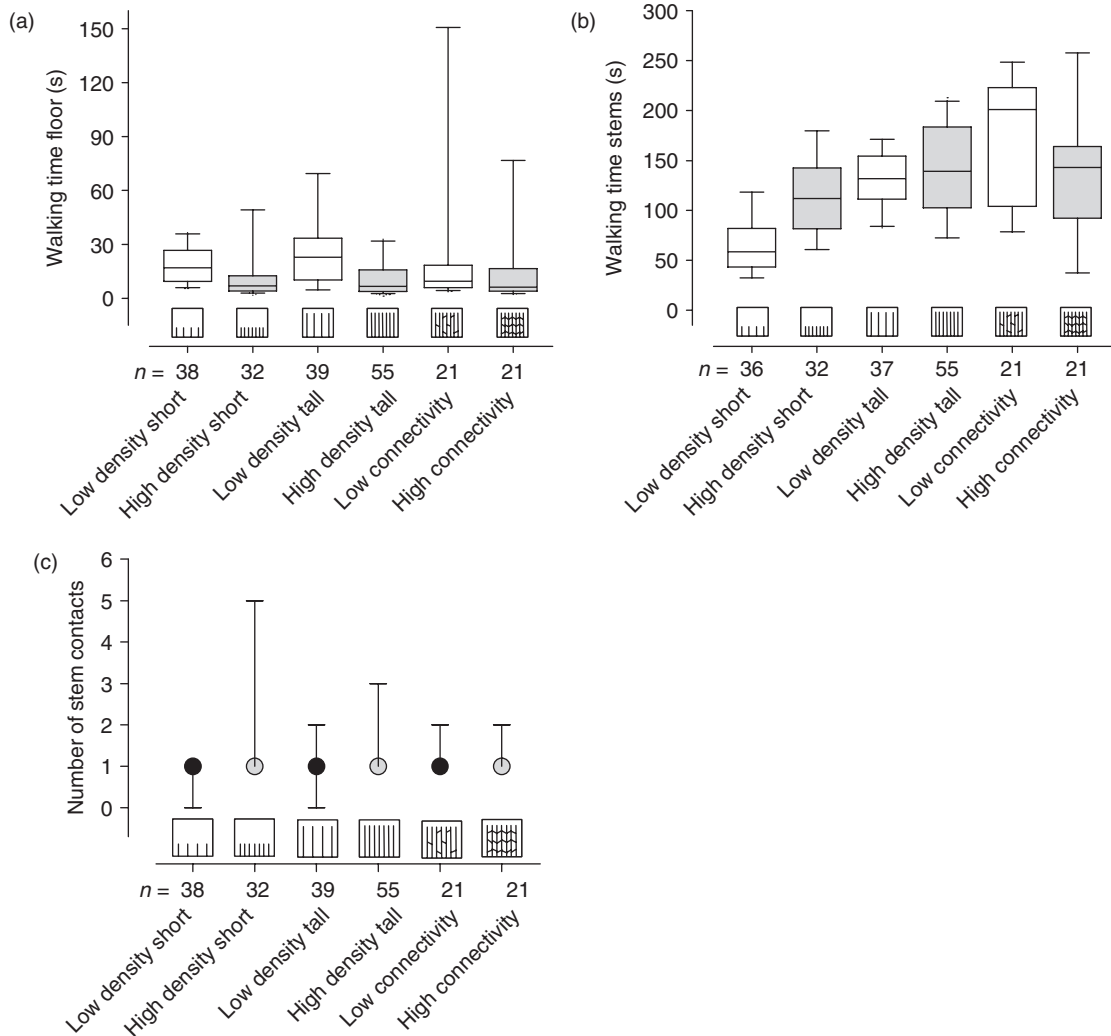


Fig. 2. Searching behaviour of *Oomyzus galerucivorus* females in vegetation structures composed of short (15 cm) or tall (45 cm) grass stems at a low/high density or possessing a low/high degree of connectivity at an overall high density during the observation period of 300 s. Only data of individuals which foraged within the arena by walking were considered (= 'non-flying' individuals). (a) The total time that the parasitoids spent walking on the floor or (b) on the stems. (c) Total number of stem contacts (median \pm range). The horizontal line within the box represents the median, the upper and lower border of the box are the 25th and 75th percentiles and the error bars show the 10th and 90th percentiles.

on the lower, middle, and upper 15 cm of grass stems ranged from 30 (middle) to 50 s (lower/upper) per stem part and was not significantly influenced by stem density (Table 2A, Fig. 3).

In general, the parasitoids that showed flying behaviour flew more often between the stems in high stem density by trend (Wald $\chi^2 = 3.202$, d.f. = 1, $P = 0.074$; Table 3), whereas height had no effect on stem-to-stem flights (Wald $\chi^2 = 0.110$, d.f. = 1, $P = 0.741$). Stem-to-floor flights were more frequent in the arenas with short stems (Wald $\chi^2 = 6.567$ d.f. = 1, $P = 0.010$) and were not influenced by density (Wald $\chi^2 = 0.027$, d.f. = 1, $P = 0.869$). Flying behaviour from the stems to the cylinder wall was neither affected by height (Wald $\chi^2 = 0.513$, d.f. = 1, $P = 0.47$) nor by density (Wald $\chi^2 = 0.145$, d.f. = 1, $P = 0.703$).

Effect of connectivity on parasitoid searching movement

When comparing the three degrees of connectivity (i.e. no connectivity = high density tall setup without bridges between stems, low connectivity, and high connectivity setup) no influence on walking time on the floor or on the stems was detected (Table 1B, Fig. 2a + b).

However, in an analysis of walking time for the three stem parts (lower, middle, and upper) connectivity significantly affected the time the parasitoids spent walking (Table 2B, Fig. 3). In general, walking time also depended on the parasitoid's position on the stem in the different connectivity setups. Moreover, wasps in the low connectivity design needed significantly more time to move from one stem part to another

Table 2. The effect of (A) stem density and (B) the degree of connectivity (no, low, and high) on walking time on different parts of the stems (Fig. 3). Repeated-measures analysis of variance performed with a general linear model.

Source	d.f.	MS	F	P
(A)				
<i>Within-subject effects</i>				
Stem part	1.649	5637.763	6.731	0.003
Stem part × density	1.649	528.526	0.631	0.504
Error (stem part)	138.545			
<i>Between-subject effects</i>				
Density	1.166	675.062	1.166	0.283
Error	84.000			
(B)				
<i>Within-subject effects</i>				
Stem part	1.557	3986.473	3.571	0.042
Stem part × connectivity	3.114	478.025	0.428	0.740
Error (stem part)	107.443			
<i>Between-subject effects</i>				
Connectivity	2.000	3082.387	3.540	0.034
Error	69.000			

than the wasps in the no connectivity design (Bonferroni's post-hoc test, $P = 0.029$). Regarding the number of stem contacts, connectivity had a moderate positive effect (Fig. 2c). The wasps in the high connectivity setup visited on average slightly more stems (mean = 1.29) than wasps in the no connectivity setup (mean = 1.07) (Kruskal–Wallis test, $H = 7.308$, $P = 0.026$, Dunn's multiple comparison post-hoc test: $P < 0.05$). The number of stem contacts in the low connectivity setup (mean = 1.19) lay in between the no and high connectivity setup and did not differ significantly from each other.

Nevertheless, fewer wasps reached the upper stem part of tall grass stems in the two connectivity designs compared with the no connectivity design with tall grass stems (Fig. 4; $\chi^2 = 32.024$, d.f. = 4, $P < 0.0001$). While 98% of the wasps

in the no connectivity setup left the lower stem part by walking upwards, 81% in the low connectivity setup and only 67% in the high connectivity setup did so. Similarly almost all (95%) of the wasps reached the upper stem part in the no connectivity setup, whereas only 62% (low) and 33% (high) of the wasps reached the upper part in the connectivity setups.

Regarding the wasps showing flying behaviour, the number of flying events from stem to stem increased with the degree of connectivity by trend (Wald $\chi^2 = 4.621$, d.f. = 2, $P = 0.099$; Table 3). Flight events from the stems to the floor were not different between the three connectivity setups (Wald $\chi^2 = 2.958$, d.f. = 2, $P = 0.228$). Contrary to the high connectivity setup, in the low and no connectivity setups the parasitoids showed a tendency to leave the vegetation structures by flying away from the stems to the wall of the cylinder (Wald $\chi^2 = 5.147$, d.f. = 2, $P = 0.076$).

Discussion

Earlier field investigations in this parasitoid–host system showed that host egg clutches are deposited more frequently in patches with high stem density than compared with low density patches (Meiners & Obermaier, 2004; Randlkofer *et al.*, 2009). Moreover, the success of *O. galerucivorus* to parasitise eggs is reduced within patches of tall and dense vegetation on a small spatial scale in the field ($r = 0.1$ m) (Obermaier *et al.*, 2008). Host egg clutches are usually located close to the tips of plants. The higher the egg clutches are attached to plants, the lower the probability of parasitism (Obermaier *et al.*, 2006). In the present study, we aimed at gaining insight into the mechanisms responsible for this effect, focussing exclusively on the influence of vegetation structure.

In the laboratory we reproduced situations in which we could assess the influence of three structural parameters: stem density, height, and connectivity. In the following, we discuss these three aspects using an approach which considers a foraging wasp as a particle moving in a geometrically complex

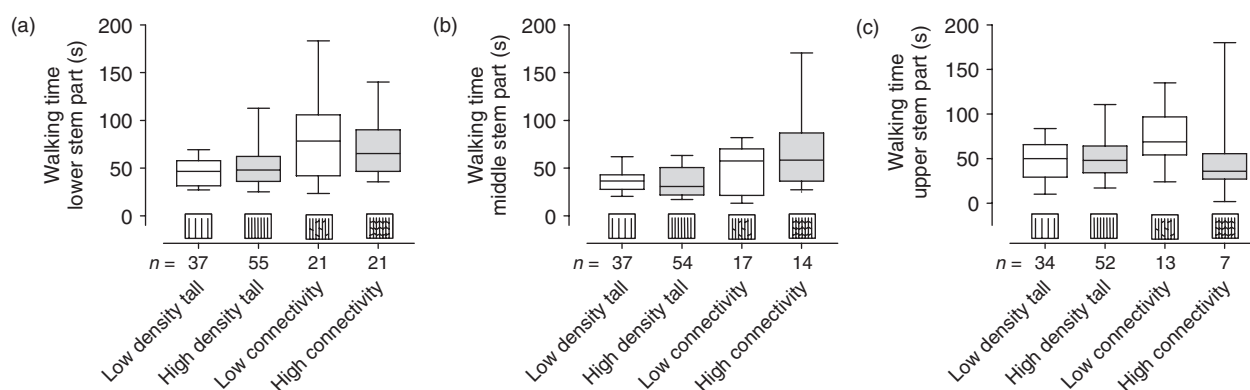


Fig. 3. Searching behaviour of *Oomyzus galerucivorus* females in vegetation structures composed of 45-cm tall grass stems at a low/high density or low/high connectivity at an overall high density. Only data of individuals which foraged within the arena by walking were considered (= 'non-flying' individuals). The time that the parasitoids spent walking on (a) the lower 15 cm, (b) the middle 15, and (c) the upper 15 cm of grass stems. The horizontal line within the box represents the median, the upper and lower border of the box are the 25th and 75th percentiles, and the error bars show the 10th and the 90th percentiles.

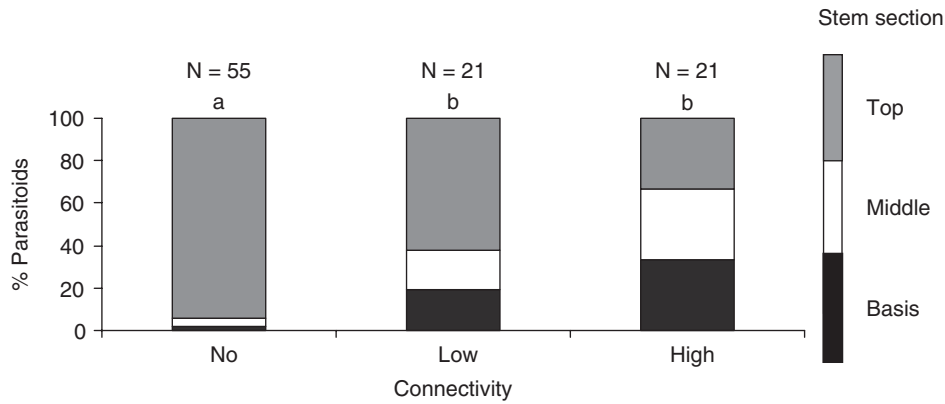


Fig. 4. Proportion of wasps not leaving the basal stem section (black bars), reaching the middle section and staying there, or entering the upper stem part and staying there (grey bars) in the three connectivity designs. N: number of wasps tested. Different letters indicate significant differences in a χ^2 test ($P < 0.001$ after Bonferroni's correction).

Table 3. Weighted marginal mean numbers (\pm SE) of flights per wasp between different locations within the arenas of different designs: (A) density and height, and (B) connectivity. Data shown refer to translocation events displayed by wasps assigned to the category 'flying individuals' that showed both repeated flying and walking behaviour during the experiments.

Design	N	Stem to stem	Stem to floor	Stem to cylinder
(A)				
Low density	39	0.26 \pm 0.07*	0.21 \pm 0.07	0.75 \pm 0.08
High density	46	0.50 \pm 0.07*	0.15 \pm 0.05	0.91 \pm 0.04
Short stems	32	0.38 \pm 0.09	0.34 \pm 0.09**	0.75 \pm 0.08
Tall stems	53	0.40 \pm 0.07	0.08 \pm 0.04**	0.92 \pm 0.04
(B)				
No connectivity	14	0.44 \pm 0.09	0.03 \pm 0.03	0.88 \pm 0.06
Low connectivity	14	0.14 \pm 0.10	0	1
High connectivity	34	0.71 \pm 0.13	0.21 \pm 0.11	0.29 \pm 0.13

*Trend ($P < 0.074$) between the two marked setups;

**significant difference ($P < 0.010$).

environment. This approach allowed us to take movement decisions at regular distance intervals and has a long history in statistical physics (Casas & Djemai, 2002). From the parasitoid's point of view, the probability of reaching host eggs is equivalent to the probability to walk up to the top of tall plant stems in order to find host eggs within a given time period. Like many other small egg parasitoids, *O. galerucivorus* searches for host egg clutches mostly by walking up and down plant stems and leaves. Thus, successful parasitism mainly depends on the allocation of searching time to specific places within the habitat. Consequently, the likelihood of encountering a host egg clutch on a plant might decrease with a shortened duration of the search (cf. Casas & Djemai, 2002).

High stem density significantly reduced the time the parasitoids spent walking on the floor, but did not significantly increase the number of stems visited. Time limitations determined by the experimental conditions seemed not to be the reason for this finding. Furthermore, density interacted

with height. With increasing stem number and stem height the walking time of the parasitoids on the stems in total augmented as well. All females did reach the tip of the stems in the tall stems design without any connections between the stems. Therefore, the extra time to cover the extra distance is not sufficient to explain the reduced parasitism success observed in the field on tall grasses. The reason is obviously not due to the grass height.

A similar retaining effect of dense and tall vegetation on the wasps could be seen for the individuals that showed flying behaviour. They tended to fly more often from stem to stem in higher grass stem densities and flew significantly less to the ground in tall stem than in short stem environments.

Connectivity between plant parts had a strong impact on the probability to reach the tip of the grass stems; significantly fewer wasps reached the upper stem parts in high connectivity. The connection points served sometimes as resting places which led to no further upwards movement within the observational period. Sometimes, the wasps moved backwards. Generally, a greater proportion of the wasps in the low and high connectivity setups showed backwards movement than the ones in the no connectivity setup. A higher number of connections between grass stems apparently constrained the wasps which prevented them from reaching the relevant upper parts of plant stems. Even the somewhat enhancing influence of high connectivity on the number of stem contacts does not affect that result. Furthermore, walking time on the upper stem part within the high-connectivity design in comparison to the low-connectivity design tended to be considerably reduced, but the effect was not significant. However, the few wasps that reached the tips of grass stems in high connectivity did so within the first half of the total observation time. Therefore, a limited observation period seems not to explain the reduced searching time in high connectivity compared with low connectivity.

The behavioural observations of the parasitoids in periods of 5 min should help to evaluate the effect of the vegetation parameters on their host search success in the field. Despite their long life-time as adults, the parasitoid females have

only a limited amount of time for host search in the field. Host egg masses are available for about 2 months (September/October) (Meiners *et al.*, 2006). Furthermore, the parasitoids are restricted by temperature (solar radiation) to a few hours, sometimes minutes, each day. Moreover, even at places with high host egg mass density, females have to search several hundreds or thousands of stems to encounter a host egg mass. Thus, the results of our 5-min assay should at least indicate which vegetation parameters might 'protect' host eggs in the field. Studies with a longer time frame and a bigger natural-like setup could confirm the effect of connectivity on this host–parasitoid interaction.

So far, only a few studies dealt with entire plant patches and the effect of habitat vegetation structure on host searching behaviour. These studies focused on the movement behaviour of predatory walking arthropods, such as coccinellid beetles and predaceous mites. The experiments were conducted with artificially created plant canopies, wherein individual plants were arranged in a way that leaves between plants could touch and form 'bridges' for the walking predators (Kareiva & Perry, 1989; Skirvin & Fenlon, 2003). In contrast to our manipulative experiments, both investigations report that bridges of touching leaves facilitated the movement of the carnivores within the plant canopy, allowing them to move more easily through the canopy and disperse faster. However, a study investigating the impact of plant density supports our results. Risch *et al.* (1982) detected that the foraging success of a predaceous lady beetle was reduced with increasing plant density in a green house study, because the beetles spent a greater amount of time on plants without prey. The authors attribute the results to the fact that increasing plant density elevates plant surface area and subsequently leads to an increase in structural complexity. Furthermore, the results of a computer simulation on predator movement revealed that the proportion of plant canopy searched is mediated by the complexity of the vegetation structure, with a lower proportion of the canopy visited on more complex structured parts (Skirvin, 2004). Coll and Bottrell (1996) found that the density of the parasitoid *Pediobius foveolatus* is reduced by the presence of tall corn plants in a bean-corn intercrop reducing the immigration rate and enhancing the emigration rate of parasitoids. Short maize plants did not interfere with the immigration of the wasps, demonstrating that plant species diversity *per se* had no influence on the population dynamics, but probably that enhanced plant structure (non-host plant height) affected the insects.

Both the chemical diversity and the structural complexity of the vegetation affect foraging arthropods (Randlkofer *et al.*, 2010). Under natural conditions also, *O. gallerucivorus* is of course not only confronted by complex vegetation structures but also by different chemical and even visual stimuli, which the parasitoid could employ during host search. Until recently, it is not known that naïve parasitoids from populations used in the here presented experiments, do respond to any host or host related odours (Meiners *et al.*, 1997). Only experienced parasitoids are attracted by the odours of yarrow, *Achillea millefolium*; a major tansy leaf beetle host plant. Field experiments revealed that the presence of *A. millefolium*

can enhance the likelihood to find host patches for the parasitoid (Meiners & Obermaier, 2004). However, non-host plant volatiles can mask host plant odours and interfere with a host search, as leaf beetles prefer to oviposit in patches with high plant species diversity (Randlkofer *et al.*, 2007). Furthermore, the parasitoids do not recognise the host eggs from a distance, not even in close range of about 2 cm (Meiners *et al.*, 1997; Hilker & Meiners, 1999). But previous laboratory studies with *O. gallerucivorus* revealed that the direct contact to faeces from adult leaf beetles on filter paper arrests female parasitoids (Meiners *et al.*, 1997). Female wasps climbed up grass stems that were treated with host faeces at their foot more frequently than untreated grass stems. This result indicates that the contact with host faeces might guide the wasps during host search in the field once they encountered a host patch, but still leaves the parasitoids with the challenge to cope with complex vegetation structures.

Recent bioassays elucidated that the parasitoids are attracted visually to vertical lines in a distance of a few centimetres (but avoid dark areas and dense line patterns) (Meiners, unpublished). This may result in an unequal distribution of parasitoids between patches of low and high vegetation density.

From field data and laboratory assays it is known that the beetles choose oviposition sites on the basis of stem height (Randlkofer *et al.*, 2009). It is advantageous for the leaf beetle host to place its eggs at the top of long stems, not because of the length *per se*, but because long plant stems ensure the presence of many connections between plant parts. This finding is underlined by a highly significant correlation between connectivity and mean/maximum vegetation height (Obermaier *et al.*, 2006; E. Obermaier, unpublished). The connections confuse the wasps, which lose time, reverse their movement direction or fly away. Therefore, this study emphasises the effect of single vegetation parameters on natural enemies of herbivorous insects.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference:

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Table S1. Total number of wasps tested. Wasps were separated into the groups "non-flying" and "flying" individuals

for each of the tested arena designs. “Flying” wasps are defined as individuals which showed flying behaviour while foraging or flew to the wall of the cylinder and re-entered the arena. “Non-flying” wasps are defined as individuals which did not fly at all during the 5 mins observation time of the experiments or which foraged for an undefined time by walking and then flew straight to the cylinder and stayed there, i.e. decided to leave the patch.

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