TAILORING COMPLEXITY

Multitrophic interactions in simple and diversified habitats

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Dit onderzoek is uitgevoerd binnen de onderzoekschool Production Ecology and Resource Conservation.

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Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus van
Wageningen Universiteit,
Prof. Dr. Ir. L. Speelman,
in het openbaar te verdedigen
op maandag 28 juni 2004
des namiddags te 13.30 uur in de Aula

Bukovinszky, Tibor (2004)

Tailoring complexity
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Thesis Wageningen University - with references with summary in Dutch

ISBN: 90-8504-049-3

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Abstract

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Reduction of herbivore populations is more often observed in vegetationally diverse than in simple ecosystems. Consequently, plant-species mixtures (i.e. intercropping) may be used to manage pest problems in agro-ecosystems, thereby contributing to the development of ecologically sustainable crop production. Compared with monotypic stands, increasing vegetation diversity may suppress herbivore populations through the reduction in the apparency and quality of crop plants and by the increased success of natural enemies. Unfortunately, the highly variable responses of both herbivores and natural enemies to intercropping provide insufficient information to make generalisations for the selection of pest-suppressive plant-mixtures. An explanation for this lack of information is that predictions of the hypotheses explaining the pest-suppression are relevant at the population level, while the underlying mechanisms at the behavioural (i.e. functional) level remain largely untested. In this thesis I attempted to further our understanding of how vegetation diversity leads to pest suppression in agro-ecosystems. The aims were (1) to understand the cause(s) of lower herbivore numbers in vegetationally more diverse cropping systems compared to monocultures and (2) to study the behavioural bases of the responses of natural enemies to vegetation diversity. The studied system included Brussels sprout (*Brassica oleracea* gemmifera), its herbivores, and *Diadegma semiclausum*, a parasitoid of the diamondback moth. Vegetational diversity was characterised by mixing Brussels sprout with either barley (*Hordeum vulgare*) or mustard (*Sinapis alba*). Numbers of several herbivore species were reduced when Brussels sprout was mixed with barley. A study showed that the plant competition in the species mixture influenced herbivore responses by altering plant quality compared with the monocrop. Field and simulation studies showed that responses of herbivores in diversified habitats were influenced by species-related differences in foraging behaviour. Behavioural and analytical studies showed the importance of inter- and intraspecific variation in volatiles of both damaged and undamaged plants in the attraction of the parasitoid *D. semiclausum*. Plant mixtures interacted with the searching behaviour and time-allocation of wasps. Compared with pure Brussels sprout patches, mustard attracted and retained individuals longer, whereas barley reduced their tendency to enter the plant patch. Although both mustard and barley reduced the tendency of wasps to locate hosts on Brussels sprout, parasitoids improved their foraging efficiency through oviposition experiences and became equally efficient in finding further hosts. In conclusion, this thesis demonstrates the importance of foraging behaviour in explaining variable responses of herbivores and parasitoids to plant-species mixtures.

Keywords: vegetational diversity, intercropping, plant quality, herbivores, *D. semiclausum*, foraging behaviour, time-allocation;

“Ki gépen száll fölébe, annak térkép e táj,
s nem tudja, hol lakott itt Vörösmarty Mihály;
annak mit rejt e térkép? Gyárat s vad laktanyát,
de nékem szöcskét, ökröt, tornyot, szelíd tanyát;
az gyárat lát a látcsőn és szántóföldeket,
míg én a dolgozót is, ki dolgáért remeg,”

“For him who flies above it, a map is all he sees,
this living scape of being but symbols and degrees;
the reader of the maplines has neither known nor felt
the place where the great Mihály Vörösmarty dwelt;
what’s hidden in the map? Yes, barracks, mills, and arms,
but for me crickets, oxen, steeples, quiet farms;
with field-glasses he marks the crops and industries,
but I, the trembling laborer, the forest trees,”

Miklós Radnóti – *Nem tudhatom…* (*I know not what…*)
January 17, 1944.

translated by Zsuzsanna Ozsváth and Frederick Turner

Chapter

1

General introduction - Cross-scale management of functional diversity for sustainable pest control in agro-ecosystems

T. Bukovinszky

General introduction - Cross-scale management of functional diversity for sustainable pest control in agro-ecosystems

1. Introduction

Nature's services constitute the basis of our existence on Earth. Some of these services like food, pharmaceuticals, clear water and air and fossil energy are obvious, whereas some indirect services like prevention from pest and disease outbreaks remain obscure to many of us. This is because indirect ecosystem services are emergent properties of complex interactions of species with their biotic and abiotic environment (e.g. Levin, 1999). Many of Nature's services are renewable, but only as much as system processes allow them to be. Unfortunately, the rate of depletion of ecosystem services by humans exceeds their rate of renewal and worse, we disassemble the driving processes responsible for their delivery (Levin, 1999, Holling et al., 2002).

Natural pest control is an ecosystem service, success of which is related to land-use and species richness in the system (Wilby and Thomas, 2002). Despite of the increasing development and intensification of pest control measures, pre-harvest crop losses in agro-ecosystems increased from 35% to 42% in the period of 1965 to 1990 (Pimentel, 1991, Lewis et al., 1997). Estimates of crop losses in developing countries including post-harvest losses may reach 60-70% (Thomas, 1999). If we were able to understand the link between biodiversity, land use and pest suppression, we could be able to develop pest management strategies that are not only productive, but ecologically sustainable as well.

The sustainability of a system is its "capacity to create, test and maintain adaptive capability", whereas its adaptive capacity (i.e. resilience) is a "measure of its vulnerability to unexpected or unpredictable shocks" (Holling, 2001). Therefore sustainable control of pests should be a strategic combination of practices that draw on the pest-suppressive properties of natural ecosystems without impairing their adaptive capacity. A locally adaptive management practice may be maladaptive at a regional level, because it does not consider processes taking place at different scales (Lewis et al, 1997, Levin, 1999, Holling et al., 2002). For example, local environmental friendly production systems may not be sustainable at a large scale, simply because they reduce the number of resource types (i.e. plant species) and associated food-webs from where they receive natural pest control. This is especially true for cropping systems where annual disruption of habitats represents a sink for species occurring in the region. Pest control services in these systems depend on the organismal diversity in surrounding landscape elements.

Conservation ecology (CE) and conservation biological control (CBC) both focus on understanding the relationship between species diversity and function, but each developed independently into different directions. While CE focuses on the preservation of species in food-webs, CBC is often more concerned with the enhancement of local populations of natural enemies for pest control. However, to manipulate local dynamics of pests and natural enemies, sustainable management of agro-ecosystems must rely on an understanding of relationships between species diversity and functioning of species. Hence, CBC should become a combination of applied food-web dynamics and habitat management for the suppression of local pest populations (Polis and Winemiller, 1996, Kean et al., 2003).

In this chapter I will review ecological theory relevant to the prevention of pest outbreaks and describe a conceptual framework for the development of sustainable pest management strategies. First, the importance of species diversity, and spatial and temporal processes for the functioning of natural systems is summarised and the impact of agricultural intensification on these processes is discussed. Next, a concept for integrating principles from CE and CBC is described, which embraces aspects of how to draw on inherent pest management properties of ecosystems without destabilising them, making the management practice sustainable. Finally, the objectives of this thesis with a short overview of theory are presented, followed by a description of the research questions.

2. Species diversity and community stability

It is known that species-diverse ecosystems often exhibit positive properties, compared to simple ecosystems, like more efficient use of resources, compensatory growth and low fluctuation in population density (Ewel 1986, Tilman and Downing, 1994, Tilman et al., 1996, but see Pfisterer and Schmid, 2002). An additional property often observed is protection against pests. In early studies it was found that pest outbreaks were more frequent in boreal forests than in tropical ecosystems, and greatly simplified ecosystems often displayed violent fluctuations in population densities, whereas diverse natural communities showed less dramatic oscillations (Odum, 1953, Elton, 1958).

Oscillations in resource-consumer dynamics are essential, because more species may exist “on the oscillations of other species”, whereby competitive chaos contributes to higher species diversity on limited resources (Huisman and Weissing, 1999). However, food webs vary largely with respect to the strength of interaction between species (Polis and Strong, 1996), so that dynamics of communities are often influenced by a few species that, via strong feedback loops, have a disproportionately large impact on the dynamics of the system (Paine, 1966, Estes and Palmisano, 1974). Until recently, weak to intermediate interactions between species that are more abundant and tend to fluctuate widely, received little attention (Berlow, 1999, McCann, 2000). However, because they dampen oscillations of strong resource-consumer interactions, they play an important role in determining community stability by decreasing the chance of extinction, i.e. keeping densities away from zero (McCann et al., 1998, but see Berlow, 1999). Therefore in diverse ecosystems “individual species take up the slack for each other, owing in part to independent statistical fluctuation, and no doubt in part to reduced competitive pressure" (Levin, 1999). Consequently, a system may respond to external changes in a gradual way until resilience (i.e. stability) is diminished to the point where strong links are being lost, resulting in catastrophic shifts to alternative states (Scheffer et al., 2001, Scheffer, 2002). This, in principle, leads to the conclusion that all species in an ecosystem are "sacred" and important in the maintenance of stability (McCann, 2000).

When a species is freed from the "control" of top-down and bottom-up forces, an upsurge in its abundance may occur (i.e. pest outbreaks). The lower incidence of pest outbreaks in diverse ecosystems was initially explained by the presence of more natural enemies, which controlled densities of individual herbivore species (MacArthur, 1955, but see McCann, 2000). In general there is little or no correlation between species diversity of a community and population density of individual species (McCann, 2000). However, functional properties of diverse systems may be better correlated with population density. Rodriguez and Hawkins (2000) found no relationship between species diversity of a parasitoid food-web and levels of parasitism. However, when the dataset was re-analysed so that functional characteristics of trophic relationships were considered (i.e. number of links, net interaction effects), parasitism rates were influenced by food web structure, and hyperparasitoids with weak interactions had a strong influence on parasitism (Montoya et al., 2003). In their analysis, a higher food-web complexity resulted in lower parasitism rates (Montoya et al., 2003). In another study carried out in an agricultural food-web, increasing the richness of natural enemies led to a greater reduction of pest populations through facilitative interactions between parasitoids and predators. When predators were absent in the food web, parasitism rates by a specialist parasitoid were low, because of the higher density of a non-host herbivore relative to the host. However, when predators were included in the food web, they reduced the densities of the non-host, so the host became more "accessible" and the mortality of herbivores increased in a non-additive way leading to higher crop yield (Cardinale et al., 2003).

These examples illustrate that species-function in food-webs is influential in determining the abundance of individual species, where not only strong, but weak, indirect interactions are also important. Although with higher species diversity a reduction in the density of single species is little correlated, one may expect a reduction in unpredictable fluctuations, that is, in the "uncertainty" of pest outbreaks.

Because there is often overlap between the function of species comprising a community, stability is rather determined by function than by identity of species (Hooper and Vitousek, 1997, Peterson et al. 1998). This conclusion was deduced from the generally non-linear relationship frequently observed between species diversity and ecosystem stability. Subsequent theories assumed an increasing overlap in the ecological functioning with increasing species diversity (Ehrlich and Ehrlich, 1981), demonstrating that species-diverse systems may exhibit functional redundancy. Idiosynchrasy may exist between diversity and function especially in systems where species diversity is low and species show little overlap in function. Idiosynchrasy shows that the strength of interactions may vary between species, and that strong interactions are often “drivers” of community dynamics (Lawton, 1994, Walker, 1995). These concepts often concentrated on diversity-stability relationships at a local scale. However, ecosystem functioning is not restricted to local communities and distribution of species across scales is influenced by how they “observe” and function in their environment (Levin, 1992, Ritchie and Olff, 1999). The degree of functional overlap between species across spatial and temporal scales may reinforce ecosystem stability, termed as cross-scale resilience (Peterson et al., 1998).

3. Spatial processes and trophic interactions

Agro-ecosystems are less species-diverse than semi-natural and natural ecosystems (Duelli et al., 1999), which is related to the limited number of resource types (i.e. plant species) available and to the management of resources that alters competitive situations and selectively favours a few herbivores that reach pest status. Consequently, much of organismal diversity in agro-ecosystems is associated with the surrounding landscape elements, so the question rises how fragmentation of semi-natural and natural habitats and habitat complexity influence pest control services in adjacent agro-ecosystems.

3.1. Large-scale processes and population interactions

It is a combination of landscape characteristics, like the quality and geometry of landscape elements, and characteristics of species, like their dispersal ability, that largely determine the persistence of species in a landscape. Limited dispersal itself can lead to spatial aggregation of organisms (Tilman et al., 1997), but changes in geometry of landscapes have drastic effects on the spatial distribution of organisms (Plotnick and Gardner, 2002). Landscape fragments influence the distribution of species by limiting their dispersal via changing the rate of dispersal of individuals and by representing reflecting boundaries (e.g. river basins). Because of their size and greater carrying capacity, large habitat patches have more immigrants and fewer emigrants than small ones (MacArthur and Wilson, 1967, Tscharntke and Brändl, 2003). In natural terrestrial landscapes, fragment size, shape and isolation are confounded characteristics determining connectivity of landscape elements and thereby species load. When fragments are large, shape and isolation distance have less importance, because connectivity is high (Krawchuk and Taylor, 2003). When patch size is reduced, configuration becomes important in determining connectivity between patches.

A species may compensate for habitat-loss until a threshold in isolation and fragment size (i.e. critical habitat size) is reached. This threshold is related to the dispersal ability of the species to gather enough resources and the carrying capacity of the habitat to provide them with resources (With and Crist, 1995; Tscharntke et al., 2002). Exceeding this threshold results in the local extinction of populations and even species (Plotnick and Gardner, 2002, With et al., 2002, Tscharntke and Brändl, 2003). By determining species diversity, spatial structuring of resources also mediates interspecific interactions (Maron and Harrison, 1997); prey-predator population dynamics is influenced by the geometry of the environment wherein species interact (Cuddington and Yodzis, 2002), so that dynamics may shift from near-equilibrium states to extinction of both prey and predator populations. Food-chain length and trophic structure are vulnerable to fragmentation, with parasitoids often being more vulnerable than their host (Hunter, 2002a). Loss of higher trophic levels may lead to pest outbreaks. For example, fragmentation of forest ecosystems led to large-scale defoliation by the forest tent caterpillars, because parasitoids with lower dispersal ability were de-coupled from their host (Roland and Taylor 1997).

By influencing interspecific interactions, and by preventing the rapid spread of single traits to all populations across a region, spatial structuring determines co-evolutionary selection forces across landscapes (Thompson, 2001). Due to uneven distribution of interacting species across landscapes, a genetic trait may be adaptive at locations where two species coexist and interact (i.e. co-evolutionary hotspots), and maladaptive at locations where the interaction does not take place (Thompson, 2001). Therefore, spatial heterogeneity of landscapes have implications for the development and spread of resistance to pesticides in pest species in a landscape. Large-scale monocultures are expected to aggravate problems with the developments of pest and disease resistance, whereas heterogeneous landscapes may act as reservoirs for susceptible pest/disease genotypes and slow down the evolution of resistance (Wissinger, 1997).

Annual agro-ecosystems are considered ephemeral, where the yearly destruction of habitat has implications for biological control. It is expected that life-history strategies of insects will be differently influenced by an increase of crop habitat. In annual agro-ecosystems r-selected herbivores with short life-span, fast development and high reproductive capacity (i.e. strongly fluctuating populations) are favoured over later successional K-selected species with longer life-span (Wissinger, 1997, Landis and Marino, 1999). Moreover, pests with shorter developmental time have less natural enemy species that phenologically overlap with the development of the host than herbivores with longer life-span, so biological control of short-lived herbivores may decrease when species are randomly lost (Wilby and Thomas, 2002).

3.2. Small-scale processes and victim-exploiter dynamics

Responses of organisms at small spatial scales are stronger influenced by individual cases of variation than those at large scales, so predictions on the responses of organisms are also more difficult to make at small scales (Levin, 1992). At large scales limited dispersal is a determining factor, and life history traits correlating well with dispersal of animals (e.g. body size), may provide sufficient information to quantify effects of spatially heterogeneous environments on species coexistence (Ritchie and Olff, 1999). At small scales, individuals are within their dispersal domain, and behavioural differences at the individual level may become increasingly influential (Bergelson and Kareiva 1987, Bommarco and Banks 2003).

3.2.1. Structural complexity

Small-scale structural complexity of the environment is influential to foraging success of parasitoids (Casas and Djemai, 2002), e.g. an increasing complexity of plant surface may lead to a decrease of parasitism (Andow and Prokrym, 1990, Gingras et al., 2002). Huffaker's classic study showed that the increasing complexity of the environment increased the persistence of predator-prey systems (Huffaker, 1958, Huffaker et al., 1963), leading to the prediction that complexity "stabilises" predator-prey dynamics (but see Bierzychudek, 1988). A study on an aphid-ladybird system found a destabilising effect of spatial heterogeneity on prey-predator dynamics, leading to prey outbreaks (Kareiva, 1987), where the outcome of the dynamics depended on the behavioural details of ladybirds (i.e. searching behaviour) and on the distribution of the prey (Kareiva and Odell, 1987). A recent study established that the greater persistence of a predator-prey system in spatially heterogeneous environments, resulted from the average probability that the prey found unoccupied patches, the average probability that predators found the prey, and the stochasticity of individual colonisation events (Ellner et al., 2001). The essential process allowing persistence of the population was isolation by distance, where subdivision of habitats provided refuges for the prey relative to the predator (Ellner et al., 2001).

3.2.2. Chemical ecology, direct and indirect plant defences

Living elements of the habitat, like the plants, are not just neutral media wherein organisms travel and interact, but they convey information that can influence interactions between species. In natural ecosystems, low plant apparency, direct (i.e. bottom up) and indirect (i.e. top-down) defences, all protect plants from excessive herbivore damage, so a complementarity between bottom-up and top-down forces acting on herbivores may exist (Power, 1992, Ode et al., 2004). Apart from using direct (i.e. structural and allelochemical) defences against herbivores, plant-derived infochemicals are perceived and used in multitrophic contexts (Price et al., 1980) and thereby play an important role in indirect defences of plants against herbivores (Vet and Dicke, 1992, Dicke and van Loon, 2000).

Plant-derived infochemicals are used by parasitoids in the process of host location (Vinson, 1976, Vet and Dicke, 1992, Tumlinson et al., 1993, Völkl and Sullivan, 2000), but as they do not guarantee the presence of a suitable host, they provide incomplete information. Therefore, parasitoids in complex habitats may often be faced with the reliability-detectability problem (Vet et al., 1991): volatiles from the host's plant may be easy to detect, but they are less reliable signals of the host's presence. Host-related cues are reliable signals of the host's presence, but as they are small in quantity, their detectability is far below to that of plant cues. However, plant volatiles that are induced by the feeding of herbivores (so called herbivore-induced volatiles) reliably predict the host's presence and are detectable from greater distances than the host itself (Vet et al., 1991, Vet and Dicke, 1992). This way induced plant volatiles may be important mediators of host/prey-parasitoid/predator interactions (e.g. DeMoraes et al., 1998, Kessler and Baldwin, 2001).

The efficient use of plant cues in a habitat with host and non-host infested plants of different species may however, be hampered by a high level of "background noise" relative to the "signal" (e.g. Dicke et al., 2003). Therefore, behavioural differences of parasitoids in the processing of plant-derived infochemicals may influence population-level interactions and could potentially influence persistence of host-parasitoid communities (Vet, 2001, Vos et al., 2001, Shiojiri et al., 2001). Differences between parasitoid species in their responses to plant-derived cues, might lead to differences in parasitism levels even in closely related cruciferous habitats (Geervliet et al., 2000). Interestingly, information constraints of searching parasitoids to discriminate between volatiles from host- infested and non- host infested plants may result in a more prudent exploitation of hosts contributing to the persistence of communities with more parasitoid species (Vos et al., 2001).

These examples illustrate that the functioning of species (i.e. searching behaviour), may exert strong effects at the population and community levels. Besides, these observations also highlight that any generalisations on how small-scale complexity of habitat affects stability of predator-prey interactions is not possible without information on the behavioural and biological details of the system, which will determine if habitat heterogeneity will synchronise or asynchronise host/prey-parasitoid/predator interactions leading to persistence, pest outbreaks or extinction of populations.

4. A concept for Conservation Ecology and Conservation Biological Control

The above overview indicates that stability of ecosystems and the occurrence of pest outbreaks is an emergent property of complex interactions within and between trophic levels, and that processes at different scales may mediate these interactions. This also explains why pest control measures that traditionally concentrated on applying unilateral "therapeutic" treatments to eliminate pests, often failed because they were neutralised by countermoves within the system, e.g. removal of a pest species is followed by the development of another herbivore to pest status (for discussions see Lewis et al., 1997, Thomas, 1999).

In order to be successful, management policies should take multilateral approaches not only at a local but also at a regional scale. Based on the concept of cross-scale resilience and a better understanding of the relationship between species diversity and function, we may identify potential feedback loops that help us to re-enforce ecosystem functions in disturbed agricultural landscapes.

In this concept, principles of CE and CBC are integrated (fig. 1). Agricultural production inherently generates disturbance, which can disassemble the regional species pool. Therefore, management practices must reduce source and sink effects of local populations to preserve species and maintain food-web interactions at a regional scale. Conservation ecology has a central role in the preservation of species interactions, by determining the maximum scale and level of disturbance that an agro-ecosystem shall exert on the regional species pool. Thus, methods to estimate ecosystem functions (i.e. resilience) across landscapes need to be developed (e.g. Peterson, 2002).

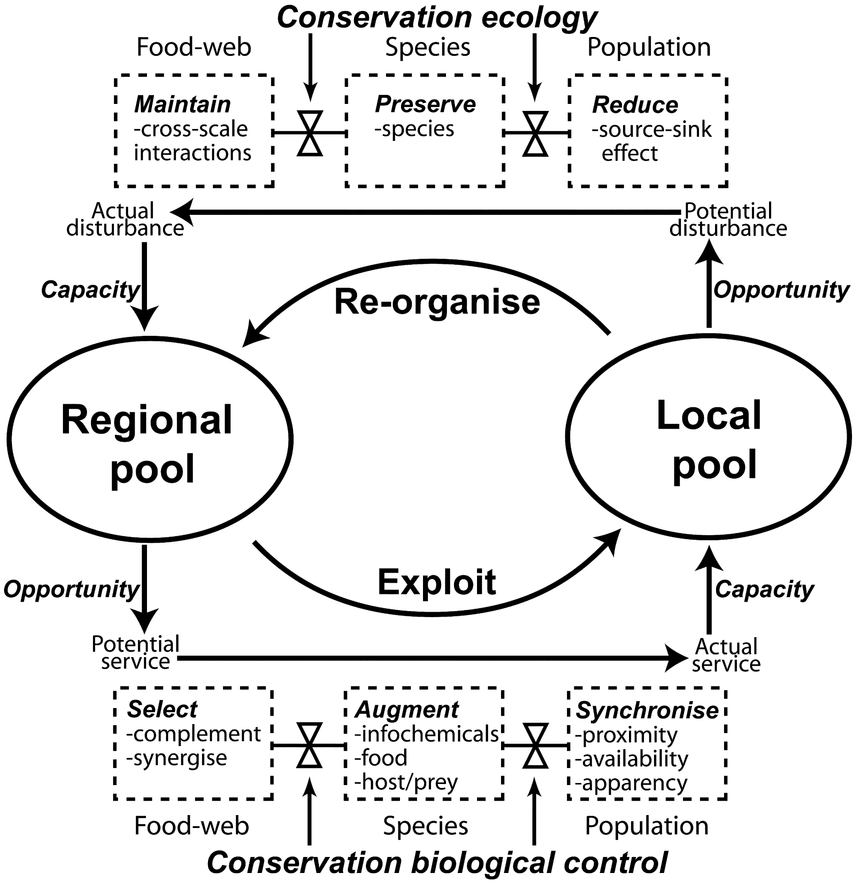
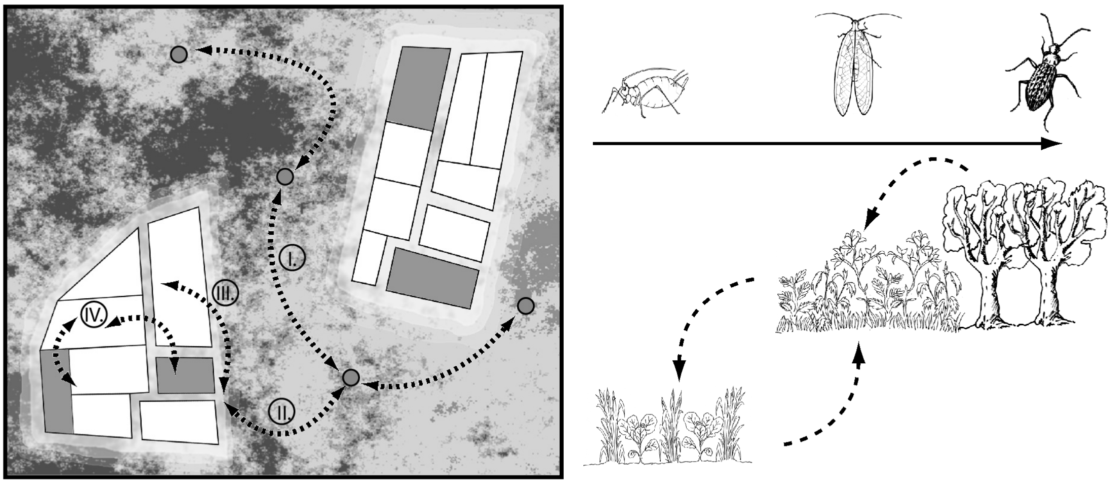


Figure 1. Integration of principles of conservation biological control (CBC) and conservation ecology (CE) to utilise pest reduction properties in local (i.e. agricultural) production systems while maintaining resilience in regional (i.e. natural) ecosystems. The regional (i.e. natural) pool provides opportunities for CBC, which "tailors" them to local management practices via managing resources across scales (from food-web to population levels). This creates disturbance that could destabilise system processes and reduce their capacity to generate further opportunity. However, CE can reduce disturbance and maintain resilience. Note that CBC provides active tools for utilisation, whereas CE rather preserves the systems innate capacity to re-generate.

By preserving food-web interactions, natural ecosystem processes decrease the chance for unpredictable pest outbreaks, and generate opportunity for CBC in local agro-ecosystems. For example, non-crop landscape elements surrounding crops may provide valuable biocontrol services, so the spatial management of late-successional habitats is of high importance (fig. 2a). Permanent semi-natural habitats (i.e. ecotones) that typically surround crop fields (i.e. hedgerows, woodlots, field boundaries) and fallows are important biotopes for preserving natural enemies and recruiting them for biological control in adjacent crop fields (Ewel 1986, Wissinger 1997, Letourneau and Goldstein, 2001). Many generalist natural enemies (e.g. chrysopids, carabids) associated with annual cropping systems are "cyclic colonisers", because they colonise crops and return to the proximal non-crop habitats for overwintering or in times of disturbance (e.g. Leather et al. 1993, Wissinger 1997), so the temporal connectivity of crop and non-crop elements is an important factor influencing species diversity in crop fields (fig. 2b).



(a) (b)

Figure 2. The concept of cross-scale management of functional diversity for pest control. **(a)** Processes at spatial scales are presented as nested sets of interactions. I. Connectivity of habitats maintains food-web dynamics at a meta-population level, where dots represent local populations. Semi-natural i.e. ecotone areas are borders important in II. preservation of species and III. recruitment of later successional species into agro-ecosystems. Pest control in annual cropping systems benefits from IV. facilitating and synchronising natural enemies between and within crop fields, where fallow cultivation (grey plots), flowering margins and intercropping provide the tools. **(b)** The temporal (i.e. successional) connectivity of natural and semi-natural (i.e. ecotone) habitats, for the annual cyclic colonisation of agro-ecosystems by later successional species. The solid arrow represents direction of succession of vegetational and insect communities, from annual cropping systems to semi-natural and natural climax communities. Dashed arrows represent interchange of species between habitats.

Indeed, fraction, configuration and connectivity of non-crop landscape elements can have threshold values for providing sufficient biological control to adjacent crop fields (With et al. 2002, Den Belder at al. 2002, Bianchi and van der Werf, 2003).

However, as greater species diversity does not necessarily lead to higher level of biological control in adjacent crop fields (Rosenheim, 1998), management tools should be provided that “tailor” regional services to maximise pest control in local habitats and create minimum disturbance. In the following sections an overview is given of how pest-suppressive properties of local agro-ecosystems may be increased by selective augmentation of natural enemies and reduced apparency of crop plants for herbivores.

4.1. Selection of controlling measures

The terms synergy and complementarity may be used to describe how interactions between species in a food-web increase mortality of a target pest. Synergism is when the interaction between two species enhances function (i.e. pest control) in a non-additive way, whereas complementarity increases function additively (Thomas, 1999, Wilby and Thomas, 2002). Several examples of synergism and complementarity are known that may reduce pest populations (see Lewis et al., 1997, Thomas, 1999, Cortesero et al., 2000, Wilby and Thomas, 2002). Synergy and complementarity of interactions between trophic levels, like direct and indirect lines of defences, may be used to combat pests on crop plants. For example not only those traits that directly influence herbivores could be used to protect crops, but traits that are responsible for the attraction of plants for natural enemies (i.e. infochemicals, extra-floral nectars) should also be preserved (e.g. Loughrin et al., 1995). Herbivores that are developing on sub-optimal diets often suffer higher mortality by natural enemies (Benrey and Denno, 1997). Thus synergy between direct and indirect defence can be reached by using a combined approach of plant defences that slow down herbivore development thereby creating opportunity for parasitoids to reduce pest populations. Partial or tissue-specific resistance in crops against herbivores, or botanical pesticides that slow down herbivore development, but do not kill them, allow population build-up of natural enemies (see Lewis et al., 1997, Charleston, 2004). Cultivars can be selected that not only hinder herbivore development but even boost natural enemy activity (van Lenteren and de Ponti, 1990, Bottrell et al., 1998).

Synergy and complementarity of interactions can be considered within trophic levels, when natural enemies complement or enhance each others performance. Natural enemies attacking a herbivore species need to be selected in such a way that all developmental stages of the herbivore exploited by different parasitoid species are controlled (Wilby and Thomas, 2002). Natural enemies may be selected so that they increase each others performance in a non-additive way, known as predator-predator facilitation (Cardinale et al., 2003).

4.2. Augment and synchronise

Knowledge from plant-insect interactions can be applied to engineer spatial heterogeneity and composition of vegetation for the *augmentation* of natural enemies to increase the efficiency of biological control (Kean et al., 2003). Such tools involve the manipulation of food sources, or the provision of alternative host or prey (Landis et al., 2000, Wilkinson and Landis, in press). However, the selective enhancement of natural enemies requires in-depth knowledge of the biology of the species in the ecosystem. Inappropriate subsidy of resources may increase pest pressure, either by disrupting the activity of natural enemies, benefiting the antagonists of natural enemies (i.e. hyperparasitoids) or the pest itself (Kean et al., 2003, Wilkinson and Landis, in press). Practices attracting natural enemies into crop fields include the use of sugar sprays to aggregate e.g. ladybird beetles in specific areas (Hagen and Tassan, 1970, van der Werf et al., 2000), or selectively providing floral nectar that is favoured by parasitoids but not by the pest (Wäckers, 2001, Landis et al., 2000, Winkler et al., 2003, Wäckers, 2004).

In annual cropping systems, biological control may be too late because natural enemies arrive in the habitat when pest populations have already built up (van Lenteren, 1980). *Synchronising* natural enemy and herbivore populations may circumvent this problem, if augmentative techniques are applied in time and in the adequate proximity to the target crop (Landis et al., 2000, Wäckers and Steppuhn, 2003). An aspect often overlooked in biological control is the management of natural enemies in times when hosts are not present in the field. Alternative food sources and host or prey should be available when the target pest is scarce, allowing natural enemy populations to persist (Landis et al., 2000, Wilkinson and Landis, in press). During cultivation time, management practices may “circulate” natural enemy populations between plots to increase biocontrol over larger areas. Mechanical practices may be efficiently used to facilitate dispersal of beneficials to distant plots or back to overwintering sites (Burgio et al., 2000).

Practices to synchronise herbivore and natural enemy populations include the application of trap crops and polycultures, and generally lead to a decrease in pest numbers (Hokkanen, 1991, Andow, 1991, Smith and McSorley 2000). Herbivores may use infochemicals in the process of host-plant location. Traditional management strategies like trap cropping make use of the greater attractivity of herbivores towards “decoy” plants than towards the crop, which enables farmers to reduce pest pressure and pesticide applications (Hokkanen, 1991, Potting et al., 2002). As foraging parasitoids also use plant-derived infochemicals, management of infochemicals may provide perspectives to target pests by natural enemies via the application of plant-species mixtures. The study of chemo-ecological relationships in a tritrophic context led to the development of a stimulo-deterrent diversionary strategy in Africa, where the intercropped plant molasses grass attracted parasitoids to the vicinity of cereal crops and at the same time repelled the stemborer pests (Khan et al., 1997, Gohole, 2003). Plant-species mixtures may reduce herbivore numbers simply via structural diversification (Finch and Collier, 2000). Undersowing or intercropping crops with companion plants can hamper movement and foraging behaviour of herbivores leading to pest reduction (Andow, 1991, Altieri, 1994, Finch and Kienegger, 1997, Hooks and Johnson, 2003).

Undersowing with clover may create a refuge for natural enemies by providing alternative host/prey, thereby leading to increased biological control efficiency at low pest population levels in annual cropping systems (Lewis et al., 1997). However, vegetation diversity may also interfere with the efficiency of natural enemies, particularly specialists, in finding their hosts (e.g. Gingras et al., 2002, Sheehan, 1986). Species mixtures may be used, that simultaneously reduce host-plant apparency for the pest and increase parasitism rates. It will be obvious that to reach such complementarity, in-depth knowledge on the behavioural details of the system is required.

5. Research objectives

The aim of my research was to specify the ecological conditions necessary for the suppression of herbivores and augmentation of natural enemies, using vegetation diversification in agro-ecosystems. The experimental work focussed on elucidating the interaction between vegetationally diverse habitats (i.e. plant-species mixtures) and herbivore and natural enemy populations. The experimental system, the specific aims of the research and the hypotheses are described below, including the outline of the experimental approach used in the different chapters.

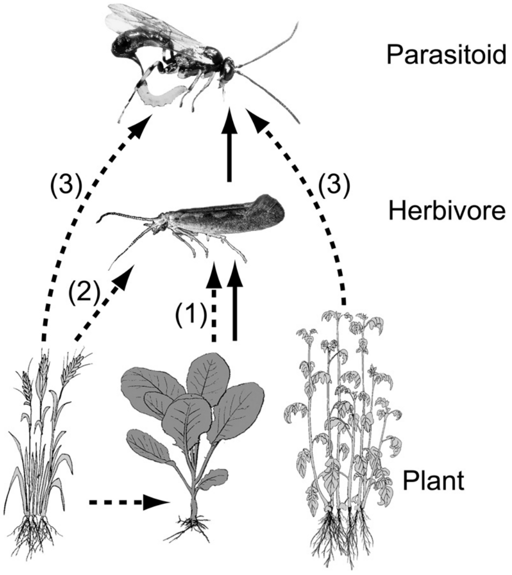


Figure 3. The tritrophic context studied in this thesis. Solid arrows represent trophic relationships. The plant species were Brussels sprout (*Brassica oleracea* gemmifera), with the companion plants malting barley (*Hordeum vulgare*) and white mustard (*Sinapis alba*). The herbivore species were *Plutella xylostella*, *Pieris rapae* and *Brevicoryne brassicae*. The parasitoid studied was *Diadegma semiclausum*, a specialist parasitoid of *P. xylostella*. Dashed arrows represent the relationships studied. The results of (1) indirect and (2) direct effects of malting barley on herbivore populations on Brussels sprout are presented in chapters 3 and 4, respectively. The effects of (3) barley and mustard as a companion plant on the searching behaviour of *D. semiclausum* are presented in chapters 5,6 and 7 (see text for further explanation).

5.1. The experimental system

The experimental system focussed on the insects associated with Brussels sprout plants (*Brassica oleracea* L. var gemmifera) (fig. 3). The entomofauna of *Brassicaceae* is distinct and well-studied in a multitrophic context and it is one of the model systems for research on agro-biodiversity (Root, 1973, Price et al., 1980, Kareiva, 1985, Geervliet, 1997, Perfecto and Vet, 2003, Hooks and Johnson, 2003). There were various specialist herbivores associated with Brassicas that were studied in different phases of the research. The prominent ones were the diamondback moth (*Plutella xylostella* L.), the cabbage aphid (*Brevicoryne brassicae* L.) and the cabbage butterflies (*Pieris brassicae* L. and *P. rapae* L.). The natural enemy species studied was *Diadegma semiclausum* (Hellén) (Ichneumonidae, *Campopleginae*), a specialist parasitoid of *P. xylostella*.

The responses of herbivores and the parasitoid to intercropping were studied when Brussels sprout was associated with the companion plant species malting barley (*Hordeum vulgare* L. cv Video) or white mustard (*Sinapis alba* L. cv Carnaval). We chose the malting barley as a companion plant, because it has an entomofauna distinctly different from that of Brussels sprout, so barley presumably did not provide direct subsidy (i.e. alternative food, host) for the higher trophic levels under study. *Sinapis alba* was chosen because it was a crucifer with a similar entomofauna and comparable secondary chemistry to that of the Brussels sprout.

This thesis tried to give answers to two major questions: (1) What are the cause(s) of lower herbivore numbers in vegetationally more diverse cropping systems compared to monocrops? (2) How do natural enemies respond to vegetation diversity?

5.2. Responses of herbivores to vegetation diversity

Many, often partly overlapping hypotheses have been formulated to explain the lower pest abundance in intercropping systems compared to monocultures. According to the “host-plant-quality” hypothesis, mixing plant species adversely affects nutritional quality and chemical suitability of the host plants, compared with those grown in monocultures (Bach, 1981, van Lenteren, 1998). However, many studies examining principles of pest reduction in intercropped systems, solely attribute “pest-suppressive” effects to the higher degree of plant diversity and disregard the influence of plant density and concomitant differences in plant growth and quality (Smith and McSorley, 2000, Hooks and Johnson, 2003). As a result, the effects of interspecific plant competition with the direct effects of plant diversity on herbivore responses remain confounded (Chapter 3).

Other hypotheses describe the direct, negative effects of vegetation diversity on the ability of herbivores to find, stay and accumulate on host plants when compared to pure stands. The most frequently cited one is the “resource-concentration" hypothesis, which predicts that specialist herbivores are more likely to immigrate and stay in pure than in diverse stands (Root, 1973). This "trapping " effect may be the result of several factors like patch size, purity, plant density, patch shape, distance between patches and plant size (Pimentel, 1961, Root, 1973, Cromartie, 1975; 1981; Stanton, 1983; Grez and Prado, 2000). The resource concentration hypothesis explains differences in herbivore numbers by the differences in the abundance and arrangement of host plants, where non-host plants may have a neutral effect on the herbivore. For example, Kareiva (1985) showed that the greater accumulation of flea beetles in large host plant patches may be explained by lower emigration rates from large than from small patches, where movement behaviour explained the effect of patch size.

In several studies an active interference by the companion plant with searching herbivores is supposed. These mechanisms included physical obstruction, visual camouflage, masking of host plant odours, producing repellent volatiles or even altering the volatile profile of host plants (for overviews see Vandermeer, 1992, Finch and Collier, 2000,). Frustratingly, many of these hypotheses were made without an explicit testing of the underlying mechanisms (but see Garcia and Altieri, 1992, Finch and Kienegger, 1997). Recently, Finch and Collier (2000) put forward the "appropriate-inappropriate" landing theory, which stated that associated plants in close proximity to host plants reduced herbivore numbers by interfering visually and structurally with the host-plant finding and acceptance behaviour of herbivores. Because it was based on detailed comparative observations of herbivores, this theory incorporated behavioural details and eliminated much of the misconceptions and untested assumptions from previous hypotheses. Although numbers of all herbivore species were reduced in their experimental undersown system, this reduction differed with the insect species studied. It was not explicitly pointed out, but the variable response might have indicated possible differences in the sensory modalities between the species studied. Despite of the ample information present on the foraging behaviour of many herbivore species, there has been limited effort to explore the responses of local populations of herbivores to patch size and vegetation composition as functions of foraging behaviour (Chapter 4).

5.3. Responses of natural enemies to vegetation diversity

The "enemies hypothesis" is one of the earliest hypothesis formulated concerning the explanation of lower pest numbers in mixed cropping, and it states that lower abundance of herbivores in vegetationally diverse than in pure stands is explained by an increased success of top-down regulation by natural enemies (Root, 1973). This enhanced control by natural enemies is explained by a compounded effect of several factors. (1) Diverse habitats offer important requisites for parasitoids and predators, such as nectar and pollen sources, that are not or insufficiently available in monocultures. (2) Populations of generalist predators and parasitoids are less likely to fluctuate because of the greater diversity of host or prey species within complex environments. (3) Specialist predators and parasitoids are less likely to fluctuate widely, because the refuge provided by a complex environment enables their prey/host species to escape widespread annihilation

(Huffaker, 1958, Huffaker et al., 1963, Root, 1973). The predictions of the enemies hypothesis were rarely supported by experimental data and great variation from negative, neutral to positive effects of habitat diversification on parasitism levels were observed (Sheehan, 1986, Russell, 1989). Sheehan (1986) pointed out that agro-ecosystem diversification could have negative effects on the searching and foraging behaviour of specialist enemies like parasitoids.

The basis of this contradiction is that the predictions of the "enemies hypothesis" are based on population (i.e. numerical) responses of natural enemies, rather than on behavioural (i.e. functional) responses. Besides, most of the studies testing the hypothesis, collected data on parasitism rates, while host densities were not standardised (Sheehan, 1986). Clearly, the behavioural responses of natural enemies to vegetation diversity should be understood before responses at the population-level are interpreted.

However, predictions on parasitoid foraging in nature may be misleading when studies are restricted to single plant attributes (e.g. volatiles) in laboratory bioassays, while disregarding effects of other factors (e.g. plant structure) (Hunter, 2002b). Another factor overlooked in this respect is that parasitoids may increase their foraging success in complex environments by learning habitat cues (Papaj and Vet, 1990, Vet et al., 1990, Vet et al., 1995). The only study so far by Perfecto and Vet (2003) found that experience of foraging parasitoids with cues from mono- and dicultures indeed led to differences in initial host-encounter rates. Hence, the effect of habitat diversity on natural enemies should be based on more realistic experimental set-ups, where the effects of experience should also be considered (Chapters 5,6,7).

5.4. Outline of chapters

In **chapter 2**, field observations are described that investigated whether intercropping Brussels sprout with barley influenced populations of herbivores and natural enemies. In field and semi-field experiments in **chapter 3**, I describe whether differences in host-plant quality, as a result of competition between plants, confound the direct effects of plant diversity on herbivore responses. In a combination of field and simulation studies in **chapter 4**, I applied basic knowledge on the foraging strategies of three specialist herbivores, the cabbage aphid (*Brevicoryne brassicae*), the cabbage butterfly (*Pieris rapae*) and the diamondback moth (*Plutella xylostella*), to explain differences in their responses to small-scale fragmentation of their habitat. In **chapter 5**, responses of *D. semiclausum* to inter- and intraspecific variation in volatile profiles of crucifers and a non-crucifer species were described by using a combination of Y-tube olfactometer bioassays and a chemical analysis of headspace volatiles from plants. The effects of density, species composition and configuration of plants on the searching efficiency of *D. semiclausum* to locate host patches was studied in different semi-field set-ups in **chapter 6**. In another semi-field set-up in **chapter 7**, I investigated how experiences with encountering host patches affect subsequent foraging success and time-allocation of *D. semiclausum* in different plant species compositions. In **chapter 8**, the findings from the thesis are discussed, with an emphasis on how results may be used to specify the ecological conditions for conservation and augmentation of natural enemies by diversification of the agro-ecosystems.

Acknowledgements

The helpful suggestions of Joop C. van Lenteren and Lousie E.M. Vet greatly improved earlier versions of this chapter. Hajnalka Tréfás helped drawing figures 2b and 3.

Chapter

2

Influence of intercropping Brussels sprout with malting barley on abundance of insect herbivores and natural enemies

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Influence of intercropping Brussels sprout with malting barley on abundance of insect herbivores and natural enemies

Abstract

Abundances of herbivore and natural enemy populations were measured and compared in a Brussels sprout malting barley row-intercrop and a Brussels sprout monoculture. Insect counts were recorded by yellow water traps and individual plant observations. The herbivores monitored were the cabbage aphid (*Brevicoryne brassicae*), the green peach aphid (*Myzus persicae*), the eggs and larvae of the cabbage moth (*Mamestra brassicae*), larvae of the diamondback moth (*Plutella xylostella*) and larvae of the small cabbage butterfly (*Pieris brassicae*). The natural enemy species monitored were larval and pupal counts of hoverflies, adult counts of the aphid parasitoid *Aphidius* spp. and *Diaeretiella rapae*, and rates of parasitism of *P. xylostella* by *Diadegma* spp. and that of *B. brassicae* by *D. rapae*. Intercropping reduced abundances of both winged and apterous forms of *B. brassicae* and *M. persicae*. Yellow traps caught more adults of *P. xylostella* in the mono- than in the intercrop. Abundance of *P. xylostella* larvae and pupae were higher in the monocrop early in the season, but their abundance in the intercrop was equal to or higher than those in the monocrop in the second half of the observation period. Intercropping reduced egg and larval numbers of *M. brassicae*, but larval counts of *P. rapae* were not different between inter- and monocropped plots. Overall densities of syrphid larvae were higher in mono- than in intercropped plots, but this difference diminished and average abundance of pupating larvae was higher in the inter- than in the monocrop in the second half of the season. Numbers of *Aphidius* spp. and *Aphelinus* spp. were higher in the inter- than in the monocropped plots. The rates of parasitism by *Diadegma* spp. and *D. rapae* were not influenced by the cropping system. Results are discussed with focus on possible mechanisms to be investigated in forthcoming chapters.

Keywords: intercropping, herbivores, natural enemies, pest suppression

1. Introduction

The observation that herbivore damage and outbreaks are frequently lower in vegetationally diverse than in monotypic cropping systems has been fascinating ecologists for several decades (Elton, 1958, Root, 1973, Risch et al., 1983, Andow, 1991, Smith and McSorley, 2000, Hooks and Johnson, 2003). Originally, two hypotheses have been formulated to explain the lower herbivore numbers: the "enemies hypothesis" and the "resource concentration hypothesis" (Root, 1973). The enemies hypothesis predicts a higher mortality of herbivores in diverse than in simple systems because of the increased success of natural enemies. This increased success is due to the larger amounts of alternative resources (i.e. host, prey, food) and the more suitable microhabitats that vegetationally diverse habitats provide, leading to greater survival and success of natural enemies compared to monotypic stands. Moreover, herbivore-natural enemy dynamics are expected to fluctuate less in diverse than in simple habitats, because the refuge provided by a complex environment enables their prey/host species to escape widespread annihilation (Huffaker, 1958, Huffaker et al., 1963, Root, 1973, but see Kareiva, 1987, Ellner et al., 2001).

The resource concentration hypothesis predicts that specialist herbivores are more likely to locate and stay in pure than in diverse stands. Several mechanisms responsible for this effect have been reported like (1) physical obstruction, (2) visual camouflage, (3) masking of host plant odours, (4) olfactory repellence and (5) reduced host plant quality for herbivore development (Vandermeer, 1992, Finch and Collier, 2000, Hooks and Johnson, 2003). In addition, the recently formulated appropriate-inappropriate landings theory states that non-host plants in close proximity to host plants reduce herbivore numbers by interfering visually and structurally with the host-plant finding and acceptance behaviour of herbivores (Finch and Collier, 2000). Although experimental evidence supporting the two hypotheses have accumulated in the past decades, considerable variation both in herbivore and natural enemy responses was found, which prevented any generalisations to be made (Risch et al., 1983, Sheehan, 1986, Russell, 1989, Andow, 1991, Hooks and Johnson, 2003).

The entomofauna of *Brassicaceae* is distinct and well-studied and it became the model system for research on agro-biodiversity (Root, 1973, Price et al., 1980, Kareiva, 1985, Geervliet, 1997, Perfecto and Vet, 2003, Hooks and Johnson, 2003). Agricultural diversification tactics of cruciferous crops involved intercropping, undersowing with living and dying mulches, trap cropping and flowering field edges (for reviews see Smith and McSorley, 2000, Hooks and Johnson, 2003). However, the ecological mechanisms responsible for the responses of arthropods to diversified *Brassica* cropping systems were not always tested and remained poorly understood.

In this study, the effect of vegetational diversification on insect herbivores and their natural enemies in a Brussels sprout – barley intercropping, a combination that has not been tested before, were compared. The main objective was to provide a starting point for a series of manipulative studies that would provide in-depth knowledge on the mechanisms determining herbivore and natural enemy responses to agro-ecosystem diversification.

2. Materials and Methods

2.1. *Experimental fields*

An intercropping of Brussels sprout (*Brassica oleracea gemmifera* L. cv. Maximus) and malting barley (*Hordeum vulgare* L. cv. Video) was established, with a monoculture of Brussels sprout as control. Barley was sown the same day when the 5-6 week old Brussels sprout plants were transplanted to the field on 21 May 2001. Plants were arranged according to an additive row-intercrop design, where 3 rows of barley were placed between every two rows of Brussels sprout. Row and plant spacing of Brussels sprout was 75 cm and 50 cm, respectively, both in the mono- and in the intercrop. Plots (30m x 30m) of each treatment were established with three replications at two locations. The isolation distance between plots was a path of at least 7 m wide sown with a grass mixture of *Lolium* spp. and *Poa* spp. The locations were in the vicinity of Wageningen (The Netherlands), about 15 km distance from each other. One of them was located in a woodland composed of *Quercus* spp., *Fagus silvaticus* and pine trees in the direct vicinity and with community gardens at a few hundred meters distance. The other location was surrounded over several square kilometres by a mosaic of intensively cultivated crops i.e. maize, potatoes, wheat and large pastures. Plots at both locations received the same amount of organic fertiliser and soil cultivation. No chemical treatment was applied throughout the season.

2.2. *Sampling by yellow water traps*

Yellow traps (15cm diameter and 1.5cm deep) filled with water containing surfactant were used to monitor flying insects. Three traps were placed into each plot. The traps in the intercropped plots were placed in the Brussels sprout rows to make sure that they operated in similar direct surroundings in the different treatments (Fig.1.). As the height of vegetation increased, traps were adjusted to the level of the top of the canopy. Traps were emptied twice or three times a week. Aphids were identified to species, parasitoids to genus level. The sampling period was 5 June till 18 July [from 15 to 58 days after planting (DAP)]. Because of logistic reasons, sampling was carried out at one location only.

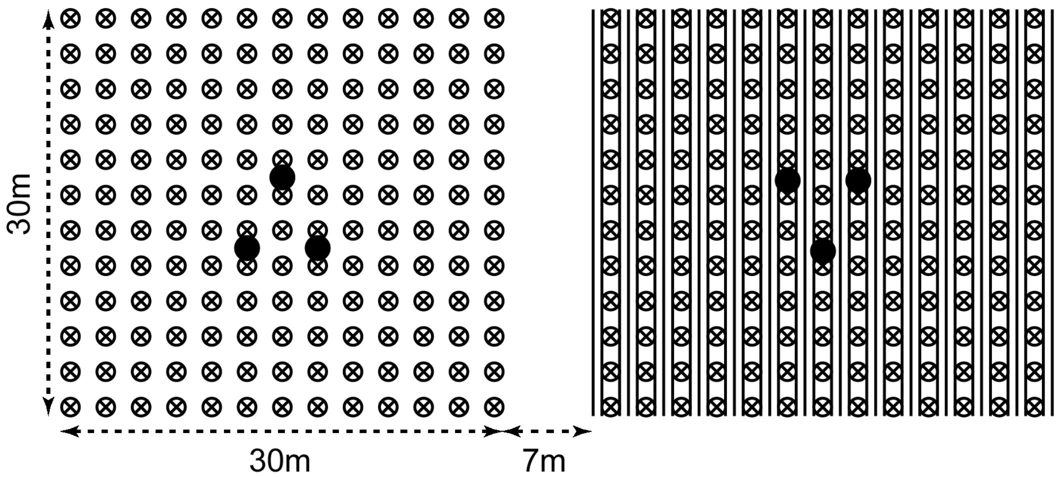

Figure 1. Schematic representation of the placement of yellow traps in the plots. Dots with crosses represent Brussels sprout and lines represent barley. Black dots are yellow water traps placed 6m distance from each other and at least 9m from the field edge. Note that plant and row spacing and the distances between plots are not on scale.

2.3. Insect counts

Fifteen randomly selected Brussels sprout plants per plot at both sites were sampled once a week. Winged and apterous forms of *M. persicae* and *B. brassicae*, larval and pupal numbers of *P. xylostella* and larval numbers of *M. brassicae* per plant were recorded. Syrphids, as important predators of aphids, were monitored and their larval and pupal stages were distinguished. The apparent rates of parasitism of *P. xylostella* by *Diadegma* spp., were recorded as the fraction of unhatched parasitoid cocoons of the total number of larvae and pupae of the hosts present on the plants. The apparent parasitism of *B. brassicae* by *Diaeretiella rapae* was also recorded as the fraction of unhatched parasitoid mummies of the total number of aphids present on the plants. Sampled plants were marked and avoided in later sampling. Observations took place from 30 DAP to 104 DAP. The leaf area of Brussels sprout plants was monitored each week throughout the season. Three randomly selected plants per plot were removed, and the leaf area per plant was determined with a leaf area meter (LI-COR®).

2.4. Data analysis

Data from yellow traps, individual plant observations, leaf area data, and counts from suction traps were normalised by $\log_{10}$ transformation [$\log_{10}(x+1)$]. Parasitism data were arcsine– square root transformed [$\arcsin(\sqrt{(x)})$] . A Mixed Model for Analyses of Variances with compound symmetry or autoregressive structure on the covariance matrices was used (Littell et al., 1996) (PROC MIXED, with REPEATED statement, SAS 8.0). In the model, location was treated as a random factor whereas plots were considered as experimental units with repeated observations. The selection between the fitted models for final inferences was based on the fit statistics of Akaike`s Information Criterion and Schwarz` Bayesian Criterion (Littell et al., 1996). The yellow trap data did not contain a random factor, and therefore they were analysed by an ANOVA. Due to damage in the trap material inflicted by heavy rainfall, data from 24, 25, 28, 30 and 58 DAP were not considered in the analysis.

3. Results

3.1. Yellow trap catches

Results of yellow trap catches showed that all examined species responded to cropping system. The three herbivores were caught in higher numbers in mono- than in intercropped fields. Catches of *M. persicae* (P<0.004, df=1, F=9.2), *B. brassicae* (P<0.001, df=1, F=106.4) and *P. xylostella* (P<0.001, df=1, F=47.9) were higher in Brussels sprout mono- than in intercropped plots (fig. 2.)

Interestingly, catches of the two observed aphid parasitoid groups showed the opposite tendencies than catches of the herbivores. Significantly more *Aphidius* spp. were caught in inter- than in monocropped sprout (P<0.001, df=1, F=196.6). *D. rapae* was caught in significantly higher numbers in intercropped sprouts when compared to the monocultures (P=0.001, df=1, F=12.4).

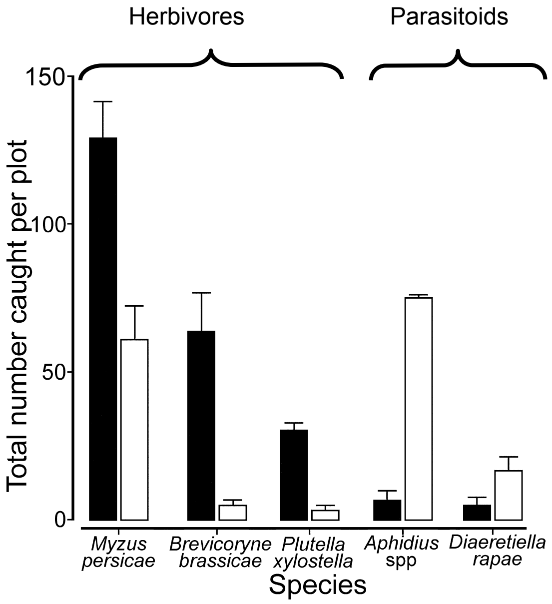

Figure 2. Average (±SE) of total catches per plot of *M. persicae, B. brassicae, P. xylostella, Aphidius spp.* and *D. rapae and Aphelinus spp.* collected from yellow water traps in inter- (white bars) and monocrops (black bars).

3.2. Herbivores on Brussels sprout

Intercropping influenced abundance of *M. persicae* and *B. brassicae*. Significantly lower abundance of the winged forms of *M. persicae* was recorded on inter- than on monocropped plants (fig. 3a, P<0.001, df=1, F=53.8), indicating a lower colonisation of Brussels sprout. This finding was in line with the tendencies observed from yellow trap catches. After 72 DAP hardly any winged forms were recorded. When densities of apterous forms were included in the comparison (fig. 3b.), the difference could be observed until the end of the observation period (P<0.001, df=1, F=93.5).

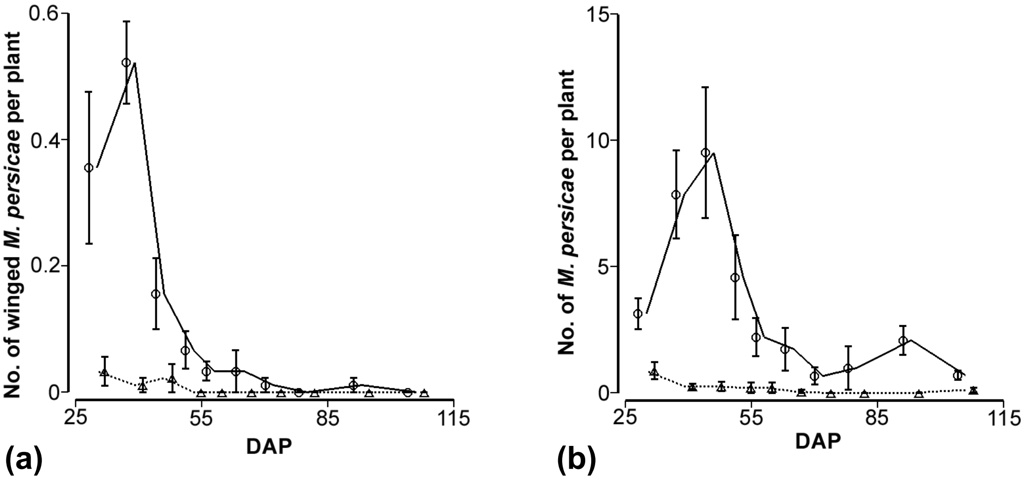

Figure 3. Average number (±SE) of **(a)** winged forms and **(b)** all forms of *Myzus persicae* L. per plant in inter- (dotted line, triangles) and monocropped (solid line, circles) plots at different days after planting (DAP).

Winged morphs of *B. brassicae* showed significantly lower abundance on inter- than on monocropped Brussels sprout plants (fig. 4a, P<0.001, df=1, F=30.8). Their maximum abundance was reached at a later time (53 DAP) than winged forms of *M. persicae* (39 DAP). When densities of apterous forms were included in the comparison (fig. 4b.), the difference further increased (P<0.001, df=1, F=88.1). However, differences in abundance between the mono- and intercrop gradually diminished after 87 DAP and counts did not differ during the last two sampling dates (93 and 106 DAP, fig. 4b).

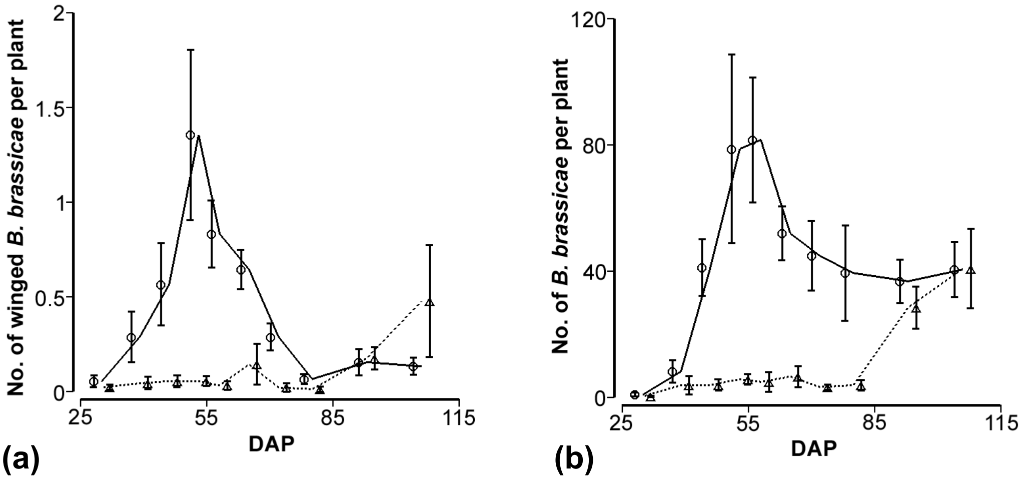

Figure 4. Average number (±SE) of **(a)** winged forms and **(b)** all forms of *Brevicoryne brassicae* L. per plant in inter- (dotted line, triangles) and monocropped (solid line, circles) plots at different days after planting (DAP).

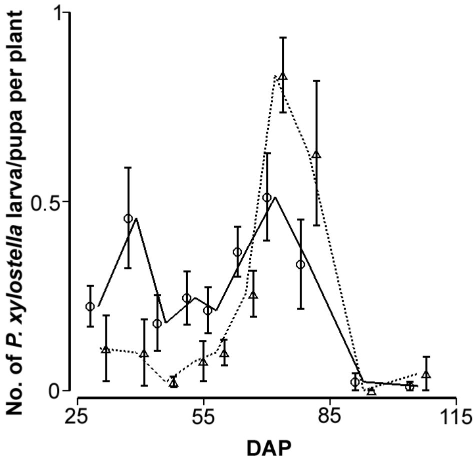

Figure 5. Average number of (±SE) larvae and pupae of *Plutella xylostella* L. per plant in inter- (dotted line, triangles) and monocropped (solid line, circles) plots at different days after planting (DAP).

The observed lepidopteran herbivores responded to intercropping in different ways. Overall densities of larvae and pupae of *P. xylostella* did not differ significantly between the treatments (fig. 5., P=0.115, df=1, F=2.52). However, the significant interaction between cropping system and observation date showed that differences in population levels between cropping systems changed across the observation period (Cropping system*DAP: P=0.003, df=9, F=3.0). Early in the season larval and pupal densities were higher in the mono- than in the intercrop, but these differences diminished from 65 DAP onwards.

Egg (fig. 6a, P<0.001, df=1, F=16.87) and larval (fig. 6b, P<0.001, df=1, F=174.0) counts of the cabbage moth *M. brassicae* were lower in inter- than in monocropped plots. Although larval counts showed considerable variation over time, differences between treatments existed until the end of the season.

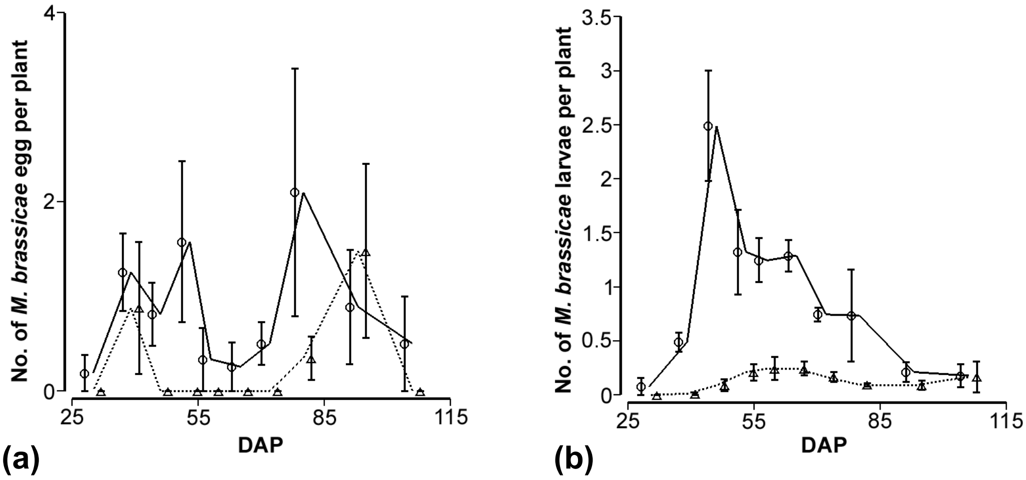

Figure 6. Average number (±SE) of **(a)** eggs and **(b)** larvae of *Mamestra brassicae* L. per plant in inter- (dotted line, triangles) and monocropped (solid line, circles) plots at different days after planting (DAP).

Larval densities of the small cabbage butterfly *P. rapae* were not influenced by cropping system (P=0.151, df=1, F=2.1) and there was no interaction with DAP (Cropping system*DAP: P=0.16, df=9, F=0.17) showing similar tendencies throughout the observation period.

3.3. Natural enemies

Overall counts of hoverfly (Syrphidae) larvae were higher in mono- than in intercropped plots (fig 7a, P=0.015, df=1, F=6.2). However, the difference between treatments diminished and abundance was equal to or higher in the inter- than in the monocrop from 72 DAP onwards (Cropping system*DAP: P<0.001, df=9, F=8.7). Syrphid pupae occurred at 58 DAP and reached higher numbers in the inter- than in the monocrop from 72 DAP until the end of the experiment (fig. 7b, P<0.001, df=1, F=59.0).

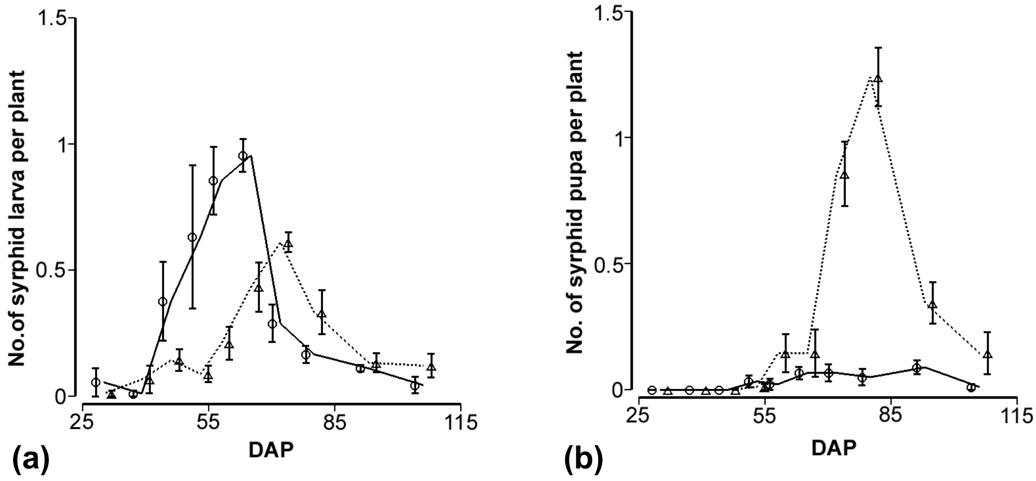

Figure 7. Average number (±SE) of **(a)** larvae and **(b)** pupae of *Syrphidae* per plant in inter- (dotted line, triangles) and monocropped (solid line, circles) plots at different days after planting (DAP).

Apparent rates of parasitism by hymenopteran parasitoids of *B. brassicae* and *P. xylostella* were measured. Parasitism of *B. brassicae* by *D. rapae* increased from the start of the observation period and reached its average maximum of ± 40 percent at 72 DAP, after which it decreased till the end of the experiment (fig. 8a). Parasitism levels by this species did not differ between inter- and monocropped plots (P=0.926, df=1, F=0.01). *P. xylostella* larvae were mostly parasitised by *D. semiclausum* and rarely by *D. fenestrale*. Parasitism levels were low in the first half of the observation period and gradually increased towards the end of the experiment reaching levels close to 100 percent at the end of the observation period (fig. 8b). Parasitism levels by *Diadegma* spp. did not differ between inter- and monocropped plots (P=0.381, df=1, F=0.9).

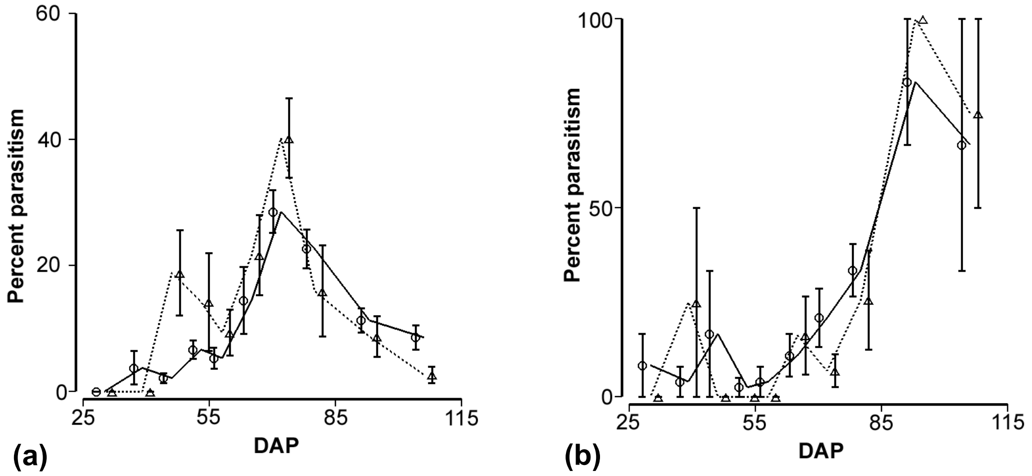

Figure 8. Percent parasitism (±SE) of **(a)** *Brevicoryne brassicae* L. by *Diaeretiella rapae* M`Intosh and **(b)** of *Plutella xylostella* L. by *Diadegma* spp. per plant in inter- (dotted line, triangles) and monocropped (solid line, circles) plots at different days after planting (DAP).

3.4. *Plant size*

Brussels sprout plants were smaller in intercropped plots than in monocultures (Fig. 9) (P<0.001, df=1, F=133.4). The difference in plant size increased until 72 DAP, when the leaf surface of monocropped plants was 2 fold higher than that of intercropped plants.

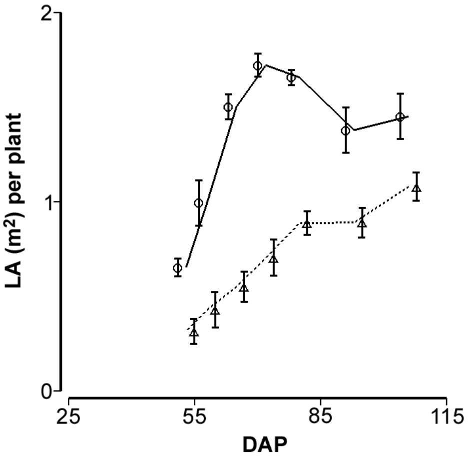

Figure 9. Plant growth expressed as leaf area (LA; ±SE) of inter- (dotted line, triangles) and monocropped (solid line, circles) Brussels sprout plants at different days after planting (DAP).

Later in the season (±90 DAP) the leaves of the monocropped plants started to senesce and their leaf area decreased. Leaf area of intercropped plants continued to increase until the end of the observation period and the difference between treatments was decreasing (Cropping system*DAP: P<0.001, df=6, F=10.1).

4. Discussion

4.1. *Herbivores*

Yellow trap catches and individual plant observations revealed a reduction in the numbers of winged forms of *M. persicae* and *B. brassicae* in the intercrops when compared to monocultures, indicating differences in colonisation rates. Intercropping also reduced numbers of apterous forms of both species. Studies carried out in other intercropping systems also reported reduction in numbers of these aphid species. Broccoli intercropped with living mulches (Costello and Altieri, 1995), and undersowing white cabbage or collard with clover reduced abundance of *M. persicae* and *B. brassicae* when compared to monocultures (Lehmus et al., 1996, Finch and Kienegger, 1997). Undersowing Brussels sprout with spurrey (Theunissen and Den Ouden, 1980), with clover

(Vidal, 1997) or with black mustard (Bukovinszky et al., 2003), reduced population densities of *B. brassicae*.

Yellow traps caught less *P. xylostella* moths, indicating a lower colonisation of Brussels sprout plants in the inter- than in the monocrop. However, these tendencies were reflected in larval and pupal counts at the beginning of the observation period only, and differences diminished later on. The lower egg and larval counts of *M. brassicae* suggested a reduced colonisation by this species in the inter- than in the monocropped plots. Other studies reported lower abundance of *P. xylostella* in intercropping systems with labiatae herbs (Dover, 1986), subterranean clover (Finch and Kienegger, 1997), strawberry clover (Theunissen and Schelling, 1996), red clover (Åsman et al., 2001), or with indian mustard (Åsman, 2002; Charleston and Kfir, 2000).

Finch and Kienegger (1997) demonstrated that population sizes of *B. brassicae, P. xylostella* and *M. brassicae* on collard plants were reduced by undersowing it with clover. This effect was explained by the structural properties of the non-crop plant possibly interfering with host searching and acceptance behaviour of herbivores. They concluded that at least 50% of the vertical profile of the target plant should be covered by the non-crop plant for sufficient reduction in colonisation. In another study, intercropping cabbage with high clover reduced oviposition by *P. xylostella* when compared to intercropping with short clover or monocultures of cabbage (Åsman et al., 2001). Since the height of barley reached the height of sprout plants early in the season (25-30 DAP), it might have reduced numbers of colonising *M. persicae*, *B. brassicae* and *P. xylostella* via structural interference. Interestingly, intercropping did not influence larval densities of *Pieris rapae*. In other studies *P. rapae* responded to intercropping practices in different ways. Intercropping collards with clover reduced the number of eggs laid by *P. rapae* (Finch and Kienegger, 1997). However, in the study of Maguire (1984) collards surrounded by tomato received more eggs than collards surrounded by other collards. It seems that *Pieris rapae* responds rather to the size of resource patches than to the species composition of surrounding vegetation (Maguire, 1983; Clough et al., 2002). Its foraging behaviour compared to other crucifer specialists may be responsible for the observed patterns in abundances (Root and Kareiva, 1984; Hern et al., 1996). To unravel why changing structure of surrounding vegetation represents a greater barrier for some species and less to others, studies are needed in which the different effects of spatial patterns of the habitat on foraging behaviour of herbivores can be separated (Chapter 4).

Later in the season differences in population levels of *B. brassicae* and *P. xylostella* diminished. Although the barley already died off, it was difficult to explain this by the lack of structural effects of intercropped vegetation alone, because winged forms of colonising aphids were already rare. If aphids did colonise plots late in the season, it was expected to increase their abundances equally in the different treatments. An explanation could be a possible difference in quality between mono- and intercropped plants. Monocropped Brussels sprout grew faster and attained greater leaf area than intercropped sprout, but this tendency seemed to change at the end of the season. Several studies suggested that differences in herbivore populations between mono- and intercrop might be related to changes in plant quality, but few studies actually have tested this assumption (Gold et al., 1990, Smith and McSorley, 2000, Hooks and Johnson, 2003). Thus, studies are needed to investigate the possible role of host plant quality in the responses of herbivores to intercropping (Bukovinszky et al., 2004).

4.2. Natural enemies

Syrphids on Brussels sprout responded to intercropping differently early and late in the season. Larvae were more abundant in the monoculture early in the season, but they reached equal to or higher abundance with more individuals pupating on the inter- than on the monocropped plants. Although syrphids are generalist predators that exploit both Brussels sprout and cereal habitats, they may prefer cereals (Tenhumberg and Poehling, 1995), where there is not only a greater diversity of hosts, but where temperature and humidity may also favour their egg laying behaviour (Wahbi, 1967, Ankersmit et al., 1986). Syrphid larvae late in the season could have eventually aggregated on intercropped Brussels sprouts that still supported increasing *B. brassicae* populations. As a result, the number of syrphid larvae declined later and more syrphids pupated on inter- than on monocropped sprout. Dying mulches have been reported to increase abundance of natural enemies on adjacent crop plants, often increasing efficiency of biological control (Hooks and Johnson, 2003). Our finding suggests that in intercropping systems, the phenology of the non-crop relative to the crop plants should be considered to increase natural enemy abundance and success in the crop.

Results from yellow trap catches suggested greater abundance of *Aphidius spp*. and *Diaeretiella rapae* in the inter- than in the monocropped plots. These species exploit aphids on both plant species. Barley harboured cereal aphids throughout the season (data not shown), so parasitoids could have exploited more host species in the inter- than in the monocrop. However, a greater abundance of *D. rapae* did result in higher parasitism rates in the inter- than in the monocrop. Moreover, rates of parasitism of the diamondback moth by *Diadegma* spp., did not differ between mono- and intercropped plots. It is possible that barley did not have a positive effect on *Diadegma* spp., like providing alternative food (nectar) and hosts that could have enhanced the activity of these specialist parasitoids. *Diadegma* spp. reached higher levels of parasitism late in the season, when barley started withering already. It could be that characteristics of the intercrop (e.g. microclimate, presence of aphid honeydew) were not different anymore from those in the monotypic stands that would have differentially influenced parasitism levels. Other studies also showed that the greater abundance of natural enemies in intercropping might not always lead to better biological control (Vidal, 1997, Gohole, 2003). Structural and infochemical effects of diverse vegetation may interact with searching behaviour of parasitoids and can influence parasitism levels (Price, 1988, Andow and Prokrym, 1990, Khan et al., 1997). Clearly, more mechanistic behavioural studies are needed to explain how foraging success of natural enemies is influenced by vegetation diversity (Chapters 5,6,7).

In conclusion we may say that intercropping with barley reduced the abundance of most of the studied herbivore species on Brussels sprout. This reduction in abundance was concomitant with a greater abundance of two aphid parasitoid species. However, no differences in herbivore mortality due to parasitism were detected. In the forthcoming chapters, I investigate several possible mechanisms influencing success of herbivores and their natural enemies in vegetationally diverse and in monotypic systems.

Acknowledgements

This study is part of the project “Enhanced biodiversity” funded by the Earth and Life Sciences of the Dutch Scientific Organisation (NWO-ALW, projectnumber: 014-22.031). Preparation of the field layout by the experimental farm of the Wageningen University (Unifarm) is acknowledged.

Chapter

3

Plant competition in pest-suppressive intercropping systems complicates evaluation of herbivore responses

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Plant competition in pest-suppressive intercropping systems complicates evaluation of herbivore responses

Abstract

In the light of current theories on the effects of intercropping on pest reduction, population responses of the diamondback moth (*Plutella xylostella*), the cabbage aphid (*Brevicoryne brassicae*) and the life history traits of the large white butterfly (*Pieris brassicae*) were studied in a Brussels sprout (*Brassica oleracea gemmifera*)/malting barley (*Hordeum vulgare*) additive row intercrop and a Brussels sprout monoculture. More *P. xylostella* adults were caught in the monoculture than in the intercrop. Numbers of *P. xylostella* larvae and pupae per sprout plant were lower in intercropped plots than in monocultures. However, more larvae and pupae were found per m^2 leaf area in the inter- than in the monocrop. Both the densities per plant and per m^2 leaf area of *B. brassicae* populations were lower in the inter- than in the monocrop. After the barley withered and competition with Brussels sprout abated, aphid densities became higher in the inter- than in the monocrop. These findings may be explained by interspecific plant competition resulting in stressed sprout plants with a smaller size and delayed phenology relative to monocropped plants. Effects of differences in plant nutritional quality on herbivore performance were studied by offering leaves of inter- and monocropped sprout plants to larval *P. brassicae*. Performance and food utilisation were significantly better on leaves from the intercrop (lower dry weight consumption, higher growth rates) than from the monocrop. Defoliation rate was also higher on leaves of intercropped plants than on monocropped ones. The studies indicate that plant stress and consequent changes in developmental rate and nutritional quality of plants are playing a role in herbivore population responses to intercropping. It is argued that such confounding effects of plant competition in intercropping designs can hamper the evaluation of herbivore responses in pest-suppressive agro-ecosystems.

Keywords: intercropping, interspecific competition, “host-plant-quality hypothesis”, *Brevicoryne brassicae, Plutella xylostella, Pieris brassicae;*

1. Introduction

Increasing within-field vegetation diversity (e.g. intercropping) often reduces pest insect populations compared with monocultures (Andow, 1991; Altieri, 1994; Smith and McSorley, 2000). Many often partly overlapping hypotheses have been formulated to explain differences in pest abundance in intercropping systems compared to monocultures. Most frequently cited is the “resource-concentration" hypothesis, which predicts that the spatial configuration of diverse vegetation makes host plants in intercrop less “susceptible” to herbivore attack, by directly interfering with visual and olfactory components of host-plant location and movement of herbivores (Root, 1973; Stanton, 1983). The recently formulated “appropriate-inappropriate” landing theory states that associated plants in close proximity to host plants may directly disrupt the host-plant finding and acceptance behaviour of herbivores (Finch and Collier, 2000). According to the “host-plant-quality” hypothesis intercropping adversely affects nutritional quality and chemical suitability of the host plants of herbivores, compared with monocultures (Bach, 1981; Van Lenteren, 1998), often causing reduced herbivore performance.

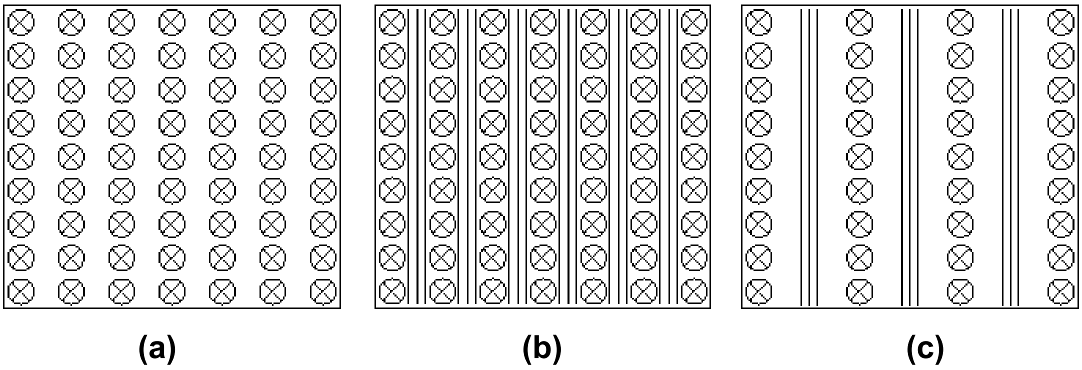

Figure 1. A schematic drawing of a (**a.**) monoculture, (**b.**) an additive and (**c.**) a substitutive (replacement) row intercropping. Dots represent main crop, lines are rows of the associated crop.

Many case studies purporting to test the underlying principles of pest reduction in intercropped systems disregard the influence of plant density and attribute pest “suppressive” effects to the higher degree of plant diversity alone (Smith and McSorley, 2000). Furthermore, many different intercropping designs are being used for comparisons with monocultures (Fig. 1). “Substitutive” (replacement) designs (Fig. 1c) are inapplicable because they confound the effects of resource concentration with the effect of treatment (Andow, 1991). Additive designs (Fig. 1b) are widely used for comparisons, because here the density of the target plant is the same in the mono- and intercrop. However, addition of an associated crop can lead to plant competition and thus to changes in host-plant quality of the target crop. Although the importance of plant biomass in the comparisons of herbivore densities has been pointed out (Root, 1973; Andow, 1991), many studies neglect the differences in plant growth and quality. Although error variance, caused by differences in plant size, can be eliminated by analysis of covariances (Andow, 1991), differences in arthropod responses to altered host plant quality are not accounted for. As a result, the confounding effects of interspecific competition with the direct effects of plant diversity on herbivore responses remain a scarcely studied problem (Hooks and Johnson, 2003). The issue in this study was whether differences in host-plant quality, as a result of competition between plants are confounding the direct effects of plant diversity on herbivore responses.

Brussels sprout/malting barley intercrop was used to study the effects of vegetation diversity on population sizes of the diamondback moth (*Plutella xylostella* L.) and the cabbage aphid *(Brevicoryne brassicae* L.). In a second experiment the effects of intercropping were evaluated on the nutritional quality of host plants for one of the specialist herbivores on crucifers, the large white butterfly (*Pieris brassicae* L.).

2. Materials and Methods

2.1. *Experimental fields*

An intercropping of Brussels sprout (*Brassica oleracea gemmifera* L. cv. Maximus) and malting barley (*Hordeum vulgare* L. cv. Video) was established, with monoculture of Brussels sprout as control. Barley was sown on May 12 and 5-6 week old Brussels sprout plants were transplanted to the field on May 19th 2000. Plants were arranged according to an additive row-intercrop design, where 3 rows of barley were placed between every two rows of Brussels sprout (Fig. 1b). Row and plant spacing of Brussels sprout was 75 cm and 30 cm, respectively, both in the monocrop and the intercrop. Plots (80m x 50m) of each treatment were established and replicated twice at two locations in the vicinity of Wageningen (The Netherlands).

2.2. *Plant growth*

The leaf area of Brussels sprout plants was monitored each week throughout the season. Five randomly selected plants per plot were removed and the leaf area per plant was determined with a leaf area meter (LI-COR®).

2.3. *Insects populations*

Adults of *P. xylostella* were monitored by using transparent cylindrical sticky traps (glue: Tanglefoot®). Nine traps were placed 15 cm above the ground in the canopy layer of Brussels sprout, in each of the inter- and monocropped plots at one of the two locations. After five days, traps were removed and any moths or parasitoids caught were identified. Numbers of *Diadegma semiclausum* (Hellén) and *Diaeretiella rapae* M`Intosh caught in sticky traps were recorded as the main parasitoids of *P. sylostella* and *B. brassicae*, respectively. New traps were set out a week later. Trapping was done for three weeks from July 5 to August 9 [47 and 82 days after planting (DAP)]. Fifteen randomly selected Brussels sprout plants per plot were sampled each week starting 35 DAP. Larval and pupal densities of *P. xylostella* and numbers of *B. brassicae* were recorded per plant and per leaf area (m^2). The apparent levels of parasitism of *P. xylostella* by *Diadegma* spp. were also recorded as the fraction of unhatched parasitoid cocoons of the total larvae and pupae of the host present on the plants, for four weeks starting 73 DAP.

2.4. Herbivore development

Plants from both treatments were selected randomly and taken to the laboratory, where they were offered to larvae of *P. brassicae.* The experiment was carried out from August 10 (83 DAP) until September 2, using the culture of *P. brassicae* maintained at the Laboratory of Entomology at Wageningen University. As size and leaf numbers of sprout plants were different in mono- and intercropped plots, leaves from three levels (upper, middle and lower levels) were taken for further treatments within the monocrop vs. intercrop comparison. Neonate caterpillars (n=20) were transferred individually into Petri dishes and were provided *ad libitum* with leaf discs throughout their development (16:8 L:D, 70% RH, 21 °C). Fresh leaves were provided every day. A balance of 0.1-mg accuracy (Sartorius®) was used to measure fresh weight of caterpillars on a daily base. Pupal and adult weights were measured once at the end of the experiment. Larval development was compared by determining the larval instar once every three hours in each moulting period. Consumption by caterpillars was recorded (both as cm^2 leaf area and mg dry weight) by scanning the remaining leaf discs every day and measuring them using the imaging software Scion Image Beta 4.02 (Scion Corporation®). Fifteen fresh leaf discs of each treatment were weighed every day, dried at 50 °C for 4 days and re-weighed. The Bradford-technique (Jones, 1989) was used to determine the water-soluble protein content of the leaves once at the start and once at the end of the experiment.

2.5. Data analysis

Leaf area data, *P. xylostella* and *B. brassicae* counts were normalized by $\log_{10}$ transformation [$\log_{10}(x+1)$]. Parasitism data were arcsine–square root transformed $[\arcsin(\sqrt{(x)})]$. A General Linear Mixed Model for Analyses of Variances with compound symmetry or autoregressive structure on the covariance matrices was used (Littell et al., 1996) (PROC MIXED, with REPEATED statement, SAS 8.0). In the model, location was treated as a random variable whereas plots were considered as experimental units with repeated observations. The selection between the fitted models for final inferences was based on the fit statistics of Akaike`s Information Criterion and Schwarz` Bayesian Criterion (Littell et al., 1996). Because eight weeks had passed between the last and the preceding observation date on aphid counts and leaf area measurements, these data were not included in the model. Instead, comparisons were carried out using a Mann-Whitney U-test. Sticky trap counts of adult *P. xylostella* and counts of parasitoids were analysed by General Linear Model procedures.

Growth rates of *P. brassicae* caterpillars were compared by estimating slopes of the larval growth curves using the GLIM (Generalised Linear Model): lnWeight=$\beta_0+\beta_1$*time and comparing the predicted slopes (β_1) (SAS PROC GENMOD). Developmental times were compared across three larval instars (2nd, 3rd, and 4th) by using a proportional hazard model for time-structured data (Kalbfleisch and Prentice, 1980). Larval consumption, pupal and adult weights and leaf quality data were analysed by GLIM procedures. Analyses were performed by using SAS 8.0 (SAS Institute Inc.) and SPSS 10.0.5. (SPSS Inc. 1989-1999).

3. Results

3.1. Plant growth

Brussels sprout plants were smaller in intercropped plots than in monocultures (Fig. 2) (P<0.0001, df=1, F=347.33). These differences increased until mid-August (75 DAP), when the leaf surface area of monocropped plants was 10 fold higher than that of the intercropped ones.

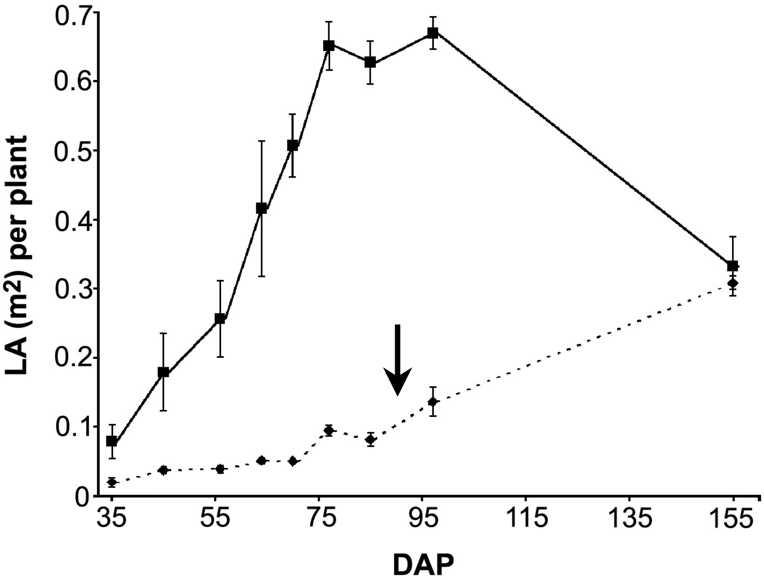

Figure 2. Plant growth expressed as leaf area (LA; ±SE) of inter- (dotted line) and monocropped (solid line) Brussels sprout plants at different days after planting (DAP). Arrow indicates when barley withered in the intercrop. Some error bars were smaller than the size of the data symbol.

Both growth and developmental rates of the intercropped plants were suppressed by interspecific competition. Barley began to wither 90 DAP, competition for water abated and leaf area of Brussels sprout in the intercrop continued to increase. About the same time, the leaves in the monoculture started to senesce and the leaf area decreased. Late in the season (155 DAP), the leaf area of inter- and monocropped Brussels sprout plants did no longer differ (Mann-Whitney U-test, P=0.773).

3.2. Insect populations

Significantly more *P. xylostella* adults were caught in monocropped than in intercropped plots, e.g. there was a more than 10 fold difference 65 DAP (Fig. 3a) (P<0.0001, df=1, F=123.21). Larval and pupal counts of *P. xylostella* per plant were significantly lower in the intercrop (P<0.0001, df=1, F=36.06).

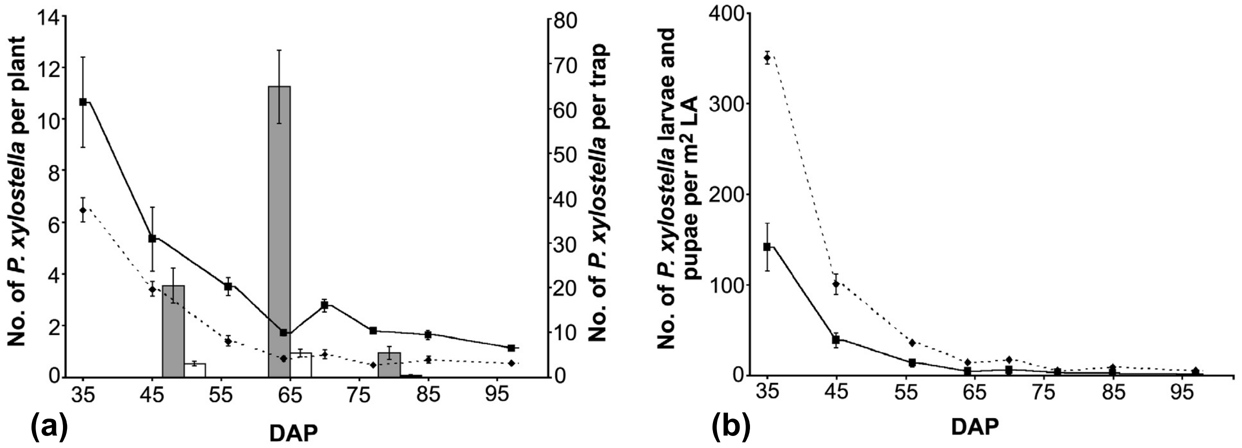

Figure 3. **(a)** Average number (±SE) of larvae and pupae of *Plutella xylostella* L. per plant (lines) and number of adults per trap (bars), in inter- (dotted line, white bars) and in monocropped (solid line, grey bars) plots. **(b)** Average number (±SE) of larvae and pupae per m^2 leaf area in inter- (dotted line) and monocropped (solid line) plots at different days after planting (DAP).

However, when the densities were calculated per m^2 of leaf area, larger numbers were found in the intercrop than in the monoculture (P<0.0001, df=1, F=72.15). This difference was very pronounced in the first half of the season, but disappeared later on (Fig. 3b).

B. brassicae numbers in the monocultures showed an exponential increase early in the season (Fig. 4a), lower numbers of aphids being observed in inter- compared with monocropped plots (P<0.0001, df=1, F=291.56). This tendency seemed consistent and did not change when differences in plant size were considered (Fig. 4b) (P<0.0001, df=1, F=62.74). However, at the last sampling date (155 DAP), *B. brassicae* reached significantly higher numbers in the inter- than in the monocrop, both as number per plant (Mann-Whitney U-test P=0.021, Fig. 4a) and number per m^2

leaf area (Mann-Whitney U-test P=0.043, Fig. 4b). When this tendency was observed, the leaf area of Brussels sprout in the monocrop was decreasing due to senescing leaves, while the leaf area of Brussels sprout in the intercrop was still increasing.

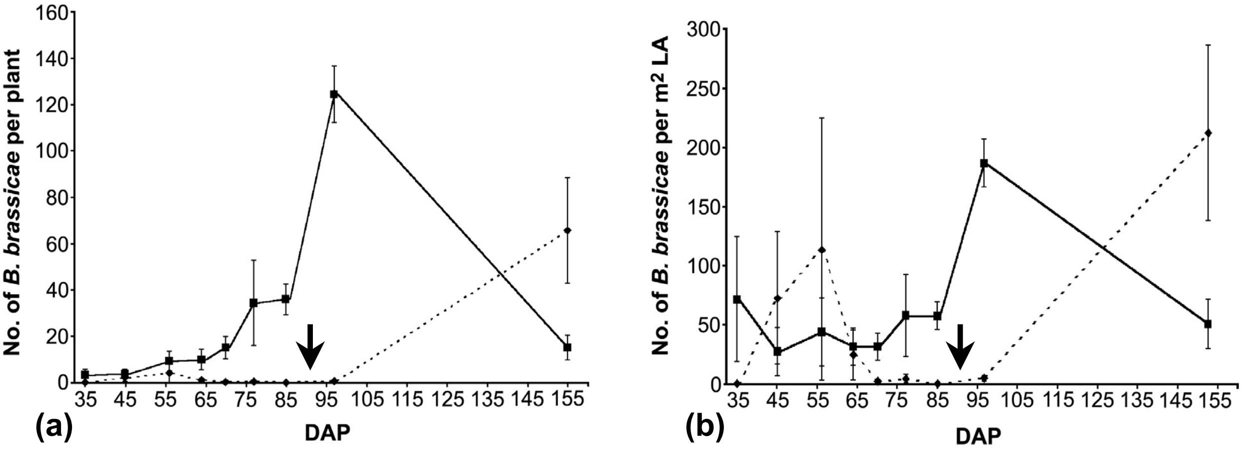

Figure 4. Average number (±S.E.) of *Brevicoryne brassicae* L. **(a)** per plant and **(b)** per m^2 leaf area in inter- (dotted line) and monocropped (solid line) plots at different days after planting (DAP). Arrow indicates when barley withered in the intercrop.

Significantly more *Diaeretiella rapae* individuals were caught by sticky traps in the mono- than in the intercrop, resulting in a 20 and 10 fold difference 50 DAP (F= 23.32, df=1, P<0.0001) and 82 DAP (F=23.81, df=1, P<0.0001), respectively. Significantly more *D. semiclausum* were caught in the mono- than in the intercrop, resulting in a 8 and 6 fold difference 50 DAP (F= 8.13, df=1, P<0.008) and 82 DAP (F=8.89, df=1, P<0.0005), respectively. The average parasitism of *P. xylostella* by *Diadegma* spp. ranged from 25% to 32% and did not differ between treatments (F=0.59, df=1, P=0.449).

3.3. Herbivore development

Cropping system influenced food consumption and utilisation of caterpillars (Table 1). Larvae consumed more foliage on intercropped than on monocropped plants (Fig. 5a). The extent of this defoliation was smaller on the upper leaves than on the lower ones, as leaf level had a significant effect. The interaction between cropping system and leaf level was significant, suggesting that leaf consumption across the three leaf levels was influenced by cropping system (Table 1). Caterpillars consumed more dry mass to develop on monocropped than on intercropped plants (Fig. 5b). Larvae on the lower leaves consumed significantly more dry mass than on upper ones. Differences between mono- and intercropped plants were consistent over leaf levels, as the interaction was not significant.

Table 1. Likelihood Ratio statistics (type 3 analysis) on the effects of inter- or monocrop, sex and leaf level (lower, middle, upper) on larval consumption, growth rates, pupal and adult weights of *Pieris brassicae* L. The nominal variables X1 and X2 denote the three leaf levels.

	Source	df	Chi^2	P
Consumption (cm^2 Leaf Area)[a]	Sex	1	1.38	0.2405
	Cropping system	1	126.42	<0.0001
	X1	1	135.74	<0.0001
	X2	1	140.88	<0.0001
	Cropping system*X1	1	34.20	<0.0001
	Cropping system*X2	1	13.22	<0.0003
Consumption (mg dry weight)[a]	Sex	1	0.52	0.4711
	Cropping system	1	26.33	<0.0001
	X1	1	82.45	<0.0001
	X2	1	68.39	<0.0001
Larval growth[a]	Sex	1	3.77	0.0523
	Cropping system	1	9.99	0.0016
	X1	1	110.88	<0.0001
	X2	1	83.58	<0.0001
Pupal weight (mg)[a]	Sex	1	9.72	0.0018
	Cropping system	1	0.65	0.4212
	X1	1	76.46	<0.0001
	X2	1	73.30	<0.0001
	Cropping system*X1	1	12.51	0.0004
	Cropping system*X2	1	12.22	0.0005
Adult weight (mg)[a]	Sex	1	0.08	0.7712
	Cropping system	1	0.04	0.8367
	X1	1	29.37	<0.0001
	X2	1	41.99	<0.0001
	Cropping system*X1	1	6.50	0.0108
	Cropping system*X2	1	6.07	0.0137

[a]- Non-significant interactions are omitted from the model.

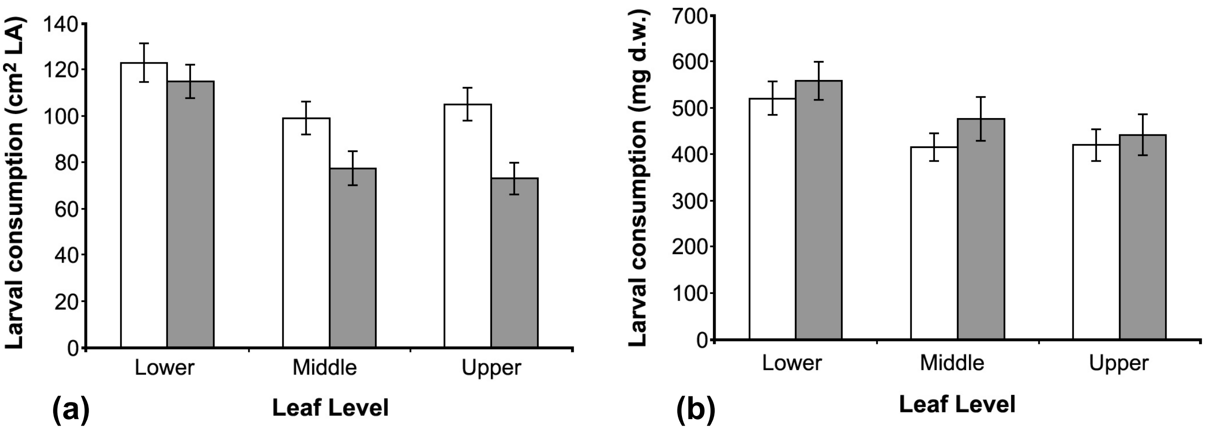

Figure 5. (a) Leaf area and **(b)** dry matter consumption of *Pieris brassicae* L. at different leaf levels (lower, middle, upper) on inter- (white bars) and monocropped (grey bars) Brussels sprout (average±S.D.).

There was a significant effect of cropping system on *P. brassicae* growth rates (Table 1), which were slightly higher on inter- than on monocropped plants (Fig. 6a). Caterpillars feeding on the upper leaves attained higher growth rates than on lower ones. The interaction between leaf level and cropping system was not significant, indicating that the differences between the two cropping systems were consistent over the three leaf levels. Cox regression analysis showed that the overall developmental time of caterpillars was not influenced by the cropping system (Wald-test=0.680, df=5, P=0.410). Developmental times differed for different leaf levels, the lower leaves supporting slower growth than middle and upper ones (Wald-test=96.255, df=5, P<0.0001). Interactions between leaf level and cropping system were not significant (X1*Cropping system, Wald-test= 0.736, df= 7, P= 0.981, X2* Cropping system Wald-test= 0.17, df= 7, P= 0.889), indicating that changes across leaf levels were similar in intercropped and monocropped plots.

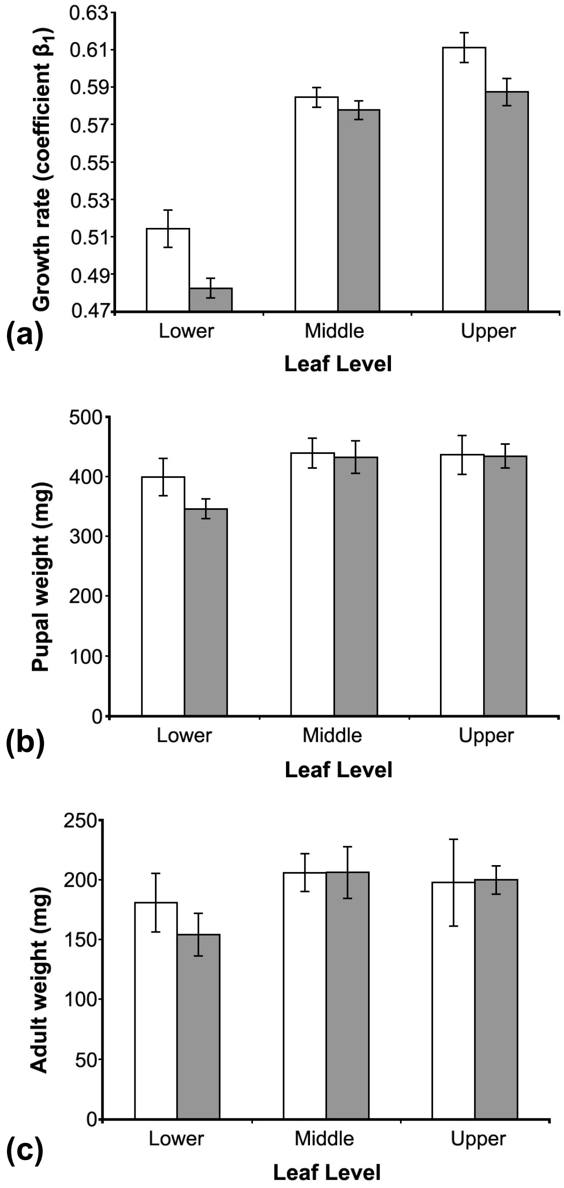

Figure 6. (a) Larval growth rates, **(b)** pupal and **(c)** adult weights of *Pieris brassicae* L. on lower, middle, upper leaves of inter- (white bars) and monocropped (grey bars) Brussels sprout (average±S.D.).

Pupal and adult weights did not differ between inter- and monocrop (Table 1; Fig. 6b and c). Pupae and adults developing on the lower leaves were smaller than on the middle and upper leaves, whereas the mean weights of pupae and adults on middle and upper leaves were almost identical (Fig. 6b and c). Pupae and adults on the lower leaf level were heavier on intercropped than on monocropped plants, as there was a significant interaction between cropping system and leaf level. Among all the dependent variables examined, sex had a significant effect on pupal weight only (Table 1). Males and females responded similarly to cropping system and leaf level, as there was no significant "Cropping system*Sex" interaction. Males were heavier than females, which was in contrast with earlier findings (Karowe and Schoonhoven, 1992).

The fresh weight and water content (mg/cm^2) of intercropped leaves were lower than those of monocropped plants (Table 2). As dry weight of leaves in the intercrop was smaller, the percentage water content does not reflect this tendency. Both the fresh and dry weight of the leaves were significantly influenced by leaf level, the lower leaves being generally heavier than the upper ones (Tables 2, 3). Moreover, the differences over leaf levels were influenced strongly by cropping system. The water-soluble protein content of intercropped and monocropped plants did not differ (Table 3).

Table 2. Average (± SD) fresh and dry weight, water and protein content of Brussels sprout leaves collected from inter- an monocropped plants at three leaf levels.

Leaf Level	**Treatment**	**Fresh Weight** n=165 (mg/cm^2)	**Dry Weight** n=165 (mg/cm^2)	**Water Content** n=165 %	**Protein Content** n=10 g/g d.w.
Upper	Monocrop	30.2±2.4	5.8±0.4	80.8±0.6	0.27±0.026
	Intercrop	25.1±1.4	4.0±0.3	83.9±1.2	0.27±0.016
Middle	Monocrop	33.1±1.9	5.9±0.5	82±0.9	0.21±0.02
	Intercrop	25.7±1.8	4.3±0.3	83.3±0.7	0.22±0.02
Lower	Monocrop	34.4±2.4	4.9±0.6	85.7±1.2	0.16±0.01
	Intercrop	26±1.2	4.3±0.2	83.2±0.8	0.17±0.02

Table 3. Likelihood Ratio statistics (type 3 analysis) on the effects of inter- or monocrop and leaf level (lower, middle, upper) on fresh and dry weight, water and nitrogen content of Brussels sprout leaves. The nominal variables X1 and X2 denote the three leaf levels.

	Source	df	Chi^2	P
Fresh weight (mg/cm^2)[a]	Cropping system	1	445.92	<0.0001
	X1	1	90.89	<0.0001
	X2	1	8.03	0.0046
	Cropping system*X1	1	39.59	<0.0001
	Cropping system*X2	1	3.94	0.0473
Dry weight (mg/cm^2)[a]	Cropping system	1	658.12	<0.0001
	X1	1	27.33	<0.0001
	X2	1	79.38	<0.0001
	Cropping system*X1	1	107.08	<0.0001
	Cropping system*X2	1	92.49	<0.0001
Water content (%)[a]	Cropping system	1	99.83	<0.0001
	X1	1	103.13	<0.0001
	X2	1	75.72	<0.0001
	Cropping system*X1	1	167.41	<0.0001
	Cropping system*X2	1	82.24	<0.0001
N-content (g/g dry weight)[a]	Cropping system	1	2.49	0.1143
	X1	1	117.88	<0.0001
	X2	1	51.70	<0.0001

[a]- Non-significant interactions are omitted from the model.

Upper leaves contained most of the protein whereas the bottom leaves had the least. There was no interaction between cropping system and leaf level, so the protein content of intercropped leaves was similar to those of monocropped leaves.

4. Discussion

Populations of both *P. xylostella* (Dover, 1986; Theunissen and Schelling, 1996) and *B. brassicae* (Theunissen and Den Ouden, 1980; Vidal, 1997) were negatively influenced in several intercropping trials. The present observations showed that populations of *P. xylostella* and *B. brassicae* were lower in the Brussels sprout/malting barley intercropping system then in the Brussels sprout monocrop. The natural enemies observed did not contribute to differences in herbivore densities between the treatments. In the intercrop, plant size, shape and leaf area changed due to interspecific plant competition. Differences in plant size can complicate even the simplest comparison of herbivore population size and pressure, since the reference unit (plant) is not standardised in the treatments. Schellhorn and Sork (1997) found that differences in the densities of specialist herbivores between weedy and monocropped collards were not reflected in damage levels and concluded that plant competition interfered with their attempts to reduce herbivore damage. In our study the significantly reduced populations of *P. xylostella* larvae potentially inflicted higher pressure (numbers per cm^2) on Brussels sprout in the inter- than in the monocrop.

Once the host plant is located, the oviposition behaviour of herbivores may be influenced by host-plant quality (Wolfson, 1980). Herbivores respond to changes in size and architecture of their host plant. The size of the substrate influenced oviposition behaviour of the cabbage moth (*Mamestra brassicae* L.) (Rojas and Wyatt, 1999), whereas for the cabbage root fly (*Delia brassicae* W.) (Kostal, 1993) and the cabbage butterfly (*Pieris rapae* L.) (Ives, 1978; Jones and Ives, 1979) the shape of the substrate was decisive in making oviposition choices. The size and phenological stage of Brussels sprout in the intercrop was different from those plants in the monocrop and it might have influenced host plant selection and acceptance by *P. xylostella* moths and *B. brassicae* alatae. Changes in the shape and size of individual plants may influence behavioural responses of herbivores in diverse habitats in other ways. Finch et al. (2003) found that the height and leaf surface areas of plants in the neighbourhood of the host plant are important factors determining oviposition on cauliflower plants by the cabbage root fly. Consequently, plant spacing and neighbourhood may alter the effects of the cropping system on foraging behaviour of insect herbivores not only through changing the shape and size of the crop plant alone. The size of associated plants relative to the size of the crop plant may also influence herbivore behaviour.

Sub-optimal host plant quality may influence herbivore abundance, as was demonstrated in the cassava/cowpea intercropping system, where cassava plants maintained lower whitefly densities as a result of plant competition (Gold et al., 1990). Differences in fresh weight and water content of leaves suggest that Brussels sprout plants intercropped with malting barley may have endured drought stress. As barley was taller than Brussels sprout within 20 DAP, the lower dry weight of Brussels sprout leaves possibly reflected a lower photosynthetic activity in the intercrop than in the monocrop. Although larvae of *P. brassicae* consumed more area of intercropped leaves, dry weight consumption was still below of those larvae feeding on monocropped leaves because of the lighter leaves in the intercrop. The findings that larvae attained higher growth rates and the same overall pupal and adult weights by consuming less dry weight indicate a better food utilisation in the inter- than in the monocrop. These responses of *P. brassicae* to intercropping were stronger at the lower leaf level, where even pupal and adult weights were lower in the monoculture than in the intercrop. This shows that leaf age and cultivation practice were interacting factors influencing herbivore performance. The finding that herbivore response to drought stress is more drastic when the plant tissue they feed on is older has been previously demonstrated (Wearing, 1967; Björkman and Larsson, 1999).

Although within-plant differences in protein content might explain the observed differences in consumption and growth rates of larvae at the three leaf levels, differences of protein levels alone could not explain differences in nutritional suitability between inter- and monocropped plants.

However, food utilisation of herbivores is also influenced by the levels of other nutrients, like carbohydrates, that may also change in stressed plants (Holtzer et al., 1988). Besides the absolute amounts of single nutrients, ratios of proteins and carbohydrates may also be important determining food utilisation of herbivores and may even influence their responses to plant allelochemicals (Simpson and Raubenheimer, 2001). Therefore, plant competition in intercropping systems may influence food utilisation of herbivores in several ways.

In contrast with *P. brassicae*, plant competition in the intercropped plots appeared to affect *B. brassicae* populations in a negative way. Although moderate drought stress can facilitate development of *B. brassicae* by increasing amino-acid concentration of the host plant (Miles et al., 1982), Brussels sprout plants exposed to a strong deficit of turgor make the cabbage aphid restless eventually rejecting the plant (Wearing and Van Emden, 1967). Clough et al. (2002) used the same crop combination at the location of our study site and eliminated interspecific competition by surrounding Brussels sprout patches of different sizes with malting barley. Although less *B. brassicae* colonised Brussels sprout patches associated with barley, population densities throughout the season were not different compared with the monocrop. These findings give further support to the hypothesis that in the present study, plant stress in the intercropped plots had a negative effect on aphid populations. Reversed tendencies in aphid numbers at the end of the season could be explained by differences in the nutritional condition of foliage due to delayed crop phenology in the intercrop. While monocropped plants eventually lost their leaves towards the end of the season as sprout formation commenced, intercropped plants still produced vigorous leaves by then free from drought stress, supporting increasing B. *brassicae* populations.

5. Conclusions

Resource concentration is a collective term that includes factors like patch size, plant density, plant diversity, patch shape, distance between patches and plant size (Cromartie, 1975; 1981; Stanton, 1983; Grez and Prado, 2000). This study demonstrates that additive intercropping designs can introduce interspecific plant competition, which changes the size, architecture and nutritional suitability of the host plant. Consequently, plant competition is a factor that may strongly influence herbivore responses to resource concentration and may affect subsequent herbivore success in diverse systems. This complicates comparisons at the higher trophic levels.

Herbivores often perform better on physiologically stressed plants (White, 1974) or on plant parts with greater vigour (Price, 1991). While consumption and growth rates of *P. brassicae* were enhanced by plant stress and delayed plant phenology, populations of the cabbage aphid were adversely influenced, supporting the theory that different guilds of herbivores may respond to host-plant stress very differently (Björkman and Larsson, 1999). Hence, other representatives of different feeding guilds (e.g. sucking, chewing, browsing etc.) should be tested to develop a general idea of herbivore response to host–plant quality in a specific cropping system. As altered growth rates and phenology of plants can affect population dynamics of herbivores long after competition has stopped, long - term effects of competition - induced changes in plant quality on herbivore responses may be an important factor to be considered when additive intercropping designs are evaluated. Gold et al. (1990) argued that indirect effects of plant competition might be an important factor responsible for the highly variable responses of herbivores to intercropping. This may be further supported by the fact that pathways of primary and secondary compounds in plants are interrelated, so plant stress can unpredictably affect constitutive and induced defences against herbivores (Dale, 1988).

With strong plant competition intercropping is clearly inapplicable, but lower levels of plant competition may still have significant effects on the physiological and behavioural responses and population dynamics of herbivores. Because competitive relationships are different in every plant combination, the mechanistic evaluation of different intercropping systems in conventional field trials is virtually impossible. There is a need for combining fieldwork with a laboratory approach comprised of behavioural and physiological studies to separate the direct effects of increasing vegetation diversity from the confounding effects of plant stress.

Acknowledgements

This study is part of the project “Enhanced biodiversity” funded by the Earth and Life Sciences of Dutch Scientific Organisation (NWO-ALW, projectnumber: 014-22.031). The help of Gabriella Kiss, Michael Brewer, Yde Jongema and Karin Winkler in collecting field and laboratory data is appreciated. Bertha Koopmanschap is thanked for showing how to measure protein content of leaves. The help of Gerrit Gort with mixed models, Joep Burger with generalised linear models and Lia Hemerik with the Cox regression is gratefully appreciated. We are grateful to Rosemary Collier, the PhD discussion group of Entomology and the two anonymous reviewers for constructive criticisms on the manuscript. Preparation of the field layout by the experimental farm of the Wageningen University (Unifarm) is acknowledged.

Chapter

4

The role of foraging behaviour in the spatial dynamics of herbivores in heterogeneous habitats

T. Bukovinszky, R.P.J. Potting, Y. Clough, J.C. van Lenteren and L.E.M. Vet

The role of foraging behaviour in the spatial dynamics of herbivores in heterogeneous habitats

Abstract

Experimental data on the relationship between plant-patch size and population density of herbivores within fields often deviate from predictions of the theory of island biogeography and the resource concentration hypothesis. We argue that features of foraging behaviour can explain differential responses of specialist herbivores to habitat heterogeneity. In a combination of field and simulation studies, we applied basic knowledge on the foraging strategies of three specialist herbivores, the cabbage aphid (*Brevicoryne brassicae*), the cabbage butterfly (*Pieris rapae* L.) and the diamondback moth (*Plutella xylostella* L.), to explain differences in their responses to small-scale fragmentation of their habitat. In our field study, populations of specialist herbivores with different foraging strategies responded to different sizes of host plant patches (9 plants and 100 plants) in different ways. The densities of winged cabbage aphids were independent of patch size. Egg-densities of the cabbage butterfly were higher in small than in large patches. Densities of diamondback moth adults were higher in large than in small patches. When patches in a background of barley were compared to those in grass, densities of the cabbage aphid and the diamondback moth were reduced, but densities of the cabbage butterfly were not influenced. Foraging strategies of the three species were implemented in an individual-based simulation framework. Individuals of a contact searcher species (cabbage aphid) recognised a host plant only after landing. The visual searcher (cabbage butterfly) and the olfactory searcher (diamondback moth) had different sensory capabilities for in-flight detection of host plants. These strategies were studied by varying the parameters that differed among species; i.e. displacement speed (distance travelled per unit time) and arrestment responses to host plants. Simulated patch size-density relationships were similar to those observed in the field. The importance of foraging behaviour in the responses of herbivores to spatial heterogeneity of the habitat is discussed.

Keywords: Brevicoryne brassicae, Pieris rapae, Plutella xylostella, patch size, population density, foraging behaviour, individual-based spatial modelling;

1. Introduction

Understanding the relationships between species abundance and spatial heterogeneity of the habitat is a primary concern of conservation ecologists, and of increasing interest for agro-ecologists. Large-scale agricultural land-use leads to species loss due to habitat fragmentation (Kruess and Tscharntke, 1994; Tscharntke et al., 2002; Hunter, 2002, Tscharntke and Brändl, 2004). At the same time large-scale agricultural areas are known to suffer larger pest outbreaks than finely grained habitats (i.e. polycultures) that are embedded in diverse landscape mosaics (Ewel, 1986, Andow, 1991). Consequently, much ecological research is devoted to explore the relationships between area and isolation of habitat patches, species-richness and population densities of individual species. While species richness-habitat area relationships are often positively correlated, studies show deviances on how densities of individual species are influenced. Empirical studies and analyses of literature data demonstrated that responses of animals to changing patch sizes are not uniform; studies found strongly positive, negative, or no correlation between patch size and densities of individual species (Bender et al., 1998, Connor et al., 2000, Bommarco and Banks, 2003). Because they are relevant to different spatial scales, the theories formulated to explain how patch size affects densities of individual species often contradict experimental data (Connor et al., 2000, Bowman et al., 2002), which further complicates any generalisations to be made.

The resource concentration hypothesis explains responses of herbivores at smaller spatial scales (i.e. local population) with differences in dietary breadth and movement behaviour of individuals as the determining factors (Root, 1973). It applies to specialist insect herbivores only; densities of generalist species should be independent of patch size. It implies that insect movement at small scales is responsible for lower immigration into and higher emigration out of smaller patches compared to larger patches. Differences in emigration rates as a result of movement behaviour within patches appeared to strongly affect densities of herbivores in host plant patches of different sizes (Kareiva, 1985). However, differences in dietary breadth and movement alone may provide insufficient prediction of patch responses of herbivores. Different specialist herbivores were found to respond to changing scales of habitat fragmentation in different ways (Banks, 1998). Moreover, different specialist herbivores on the same plant species were found to respond to patch size differently and non-linear patch size-herbivore density relationships were observed (Bach, 1986, 1988ab). Variation in herbivore responses to small-scale habitat fragmentation has been found, even when the same plant-herbivore system was examined (Cromartie, 1975, Maguire, 1983, Grez and Gonzalez, 1995).

The experimental designs often differ with regard to spatial scale, which is known to alter responses of herbivores to resource patchiness (Bommarco and Banks, 2003). For example local population sizes of the specialist small cabbage white butterfly (*Pieris rapae* L.) are not only influenced by the size of patches but also by the isolation distance among them (Fahrig and

Paloheimo, 1988). All these examples underline the need to acquire knowledge on how responses of herbivores are influenced by resource patchiness at different spatial scales.

Because of the short time that host plants in agricultural landscapes are available for insect population development, categorising differences in immigration behaviour among species, e.g. orientation type, aerial vs. ground level movement, may help to explain local population densities in response to patchiness in annual agricultural cropping systems (Bowman et al., 2002). Despite of the ample information on the foraging behaviour of many herbivore species, there has been limited effort to explore the responses of local populations of herbivores to patch size and vegetation composition as functions of foraging behaviour. In a combination of field and simulation studies, we applied basic knowledge on the foraging strategies of three specialist herbivores, the cabbage aphid (*Brevicoryne brassicae* L.), the cabbage butterfly (*Pieris rapae* L.) and the diamondback moth (*Plutella xylostella* L.), to explain differences in their responses to small-scale fragmentation of their habitat. Densities of the three specialist herbivores were followed in a field experiment, so we could compare species responses of colonising populations and population densities across the season. A spatially explicit individual-based model was used to simulate the effects of behavioural differences on herbivore response to resource patchiness. It seems that the three species use a combination of sensory modalities, but each to a different extent.

Cabbage aphid. Because of their low flight-speed, aphids are more passive travellers when compared to insects with larger body size. As a result, drifting with the wind is an important characteristic of their dispersal over greater distances (Compton, 2002). When leaving the air column, aphids use visual cues and land on yellow-green objects looming up against their flight path (Kennedy et al., 1961). Visual cues are useful to distinguish high- (trees, shrubs) and low-lying (grassy) green elements but are unlikely to be specific for plant species (Kennedy et al., 1959, Compton, 2002, but see Moericke, 1969). After landing in the vegetation, aphids engage in short-distance (attack) flights close to the vegetation, until a host plant is found (Moericke, 1955, Kennedy et al., 1961, Compton, 2002). Field observations showed that many aphid species, including the cabbage aphid, recognise the host plant only after landing on it (Kennedy, 1959). Although there is evidence that volatiles from the host may elicit a sensory response of aphids that some species may use to increase their chances of host location (Pickett et al., 1992, Powell and Hardie, 2001), the success of host plant location in many species may be extremely low (Ward et al., 1998). Although the cabbage aphid was found to respond to volatiles of its host plant in olfactometer bioassays (Petterson, 1973, Nottingham et al., 1991) the presence of host-plant odours did not affect its targeted (host-response) flight in flight chamber bioassays (Nottingham and Hardie, 1993). Therefore the role of olfactory cues in the long-range orientation of *B. brassicae* is likely to be very limited.

Cabbage butterfly. The searching pattern of the small cabbage butterfly is described as random and its pre-oviposition behaviour involves the use of visual and volatile cues for directed search at small scales (Hern et al., 1996). The importance of visual stimuli in the host plant finding of *Pieris* spp. is supported by their ability to learn optical traits of suitable host plants upon a contact-chemosensory reward (Traynier, 1984, 1986, van Loon et al., 1992). Jones (1977), Root and Kareiva (1984), and Yamamura (1999) described the movement pattern of *P. rapae* in spatially heterogeneous crucifer fields. During an egg-laying bout a female lays one or two eggs per plant. Following oviposition, females often fly above the vegetation and travel long distances before the next landing. Even when host plants are abundant, there is a minimum distance that females travel between successive egg-laying bouts, estimated to be between 1.33m and 3m (Root and Kareiva, 1984, Yamamura, 1999).

Diamondback moth. It is suggested that diamondback moths use volatile cues to locate their host plants (Palaniswamy et al., 1986, Aude Couty, personal communication). Ovipositing females lay more than one egg on a host plant and the presence of conspecific eggs and larvae do not influence oviposition rates (Groeters et al., 1992, Gonring et al., 2000). When host plants are abundant, diamondback moth females do not travel between ovipositions (Justus and Mitchell, 1996). Once inside the habitat, females are likely to be arrested, engaging in short distance flights to neighbouring plants.

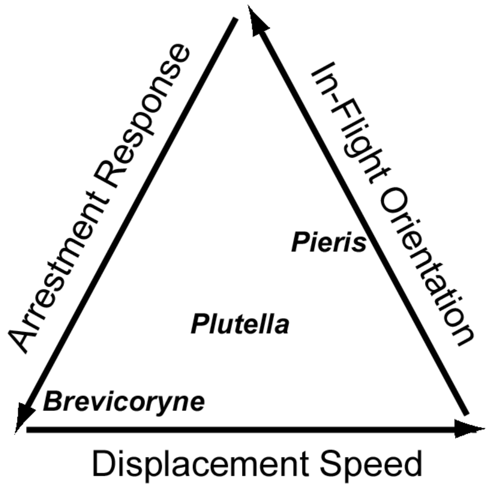

Figure 1. A representation of the differences in the three main characteristics describing foraging behaviour of the three specialist herbivores.

Clearly, there are characteristic differences between the three species with respect to the distance an individual travels per unit of time (i.e. displacement speed), the in-flight orientation (i.e. sensory capability) towards host plants and arrestment response to plants once inside the habitat. These differences are illustrated on figure 1. The distance an individual travels per unit of time (i.e.

displacement speed) is the smallest in the cabbage aphid, while that of the cabbage butterfly is the largest among the three species. Once their host plant is found, the cabbage aphid and the diamondback moth show a strong arrestment response by staying or hopping to neighbouring plants, whereas the cabbage butterfly engages in longer-distance flights between ovipositions. In the simulations, we assumed that the cabbage aphid has no in-flight orientation capability to host plants and is able to identify its host only after landing. We assumed that that the cabbage butterfly is capable of in-flight orientation and that its ability to detect a host is not influenced by patch size. We assumed that the diamondback moth is capable of in-flight orientation, but to a lesser extent than the cabbage butterfly, and its ability to detect a host plant depends on the air-borne concentration of volatiles, which is positively related to the size of host plant patches. In order to illustrate these differences in sensory modalities, rather than indicating presence-absence of the type of sensory input used, from here on, we use the terms ‘contact’, ‘visual’ and ‘olfactory’ searchers for the cabbage aphid, the cabbage butterfly and the diamondback moth, respectively.

2. Materials and Methods

2.1. *Field study*

The monitoring of field populations was carried out in Wageningen, The Netherlands, in the summer of 2001. The experimental field was subdivided into three 100m x 50m blocks laid out in a row (fig. 2). Half (50m x 50m) of each block was sown on 11 May with either barley (*Hordeum vulgare* cv Video) or grass (mixture of *Lolium* spp.). The grass was kept short by mowing throughout the season. Brussels sprout (*Brassica oleracea* gemmifera cv Maximus) patches were planted three weeks after the barley was sown (reached ±20cm height). Two patches consisting of 100 plants and two patches consisting of 9 plants were placed in every plot. Plant spacing was 50 cm. An edge of 75 cm around sprout patches was kept free of vegetation to minimise the effect of interspecific competition on Brussels sprout plants.

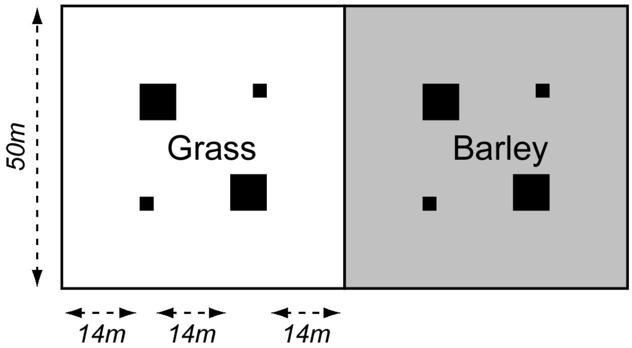

Figure 2. The layout of the field experiment. Four patches of Brussels sprout (3x3 and 10x10 plants two of each size) were embedded in fields (50mx50m) of barley or mown grass each replicated three times (not shown).

2.2. *Plant observations*

We monitored host plant patches for the occurrence of herbivores six times between July 9 and September 24. The monitoring was non-destructive, plants were checked for insects but nothing was removed (3 plants in 9-plants patches, 15 in 100-plants patches). The species recorded were the cabbage aphid (*B. brassicae*), eggs of the small cabbage white butterfly (*P. rapae*) and larvae and pupae of the diamondback moth (*P. xylostella*). Winged (alatae) morphs of the cabbage aphid were distinguished from the wingless (apterae) ones, enabling us to follow the densities of colonisers. Monitoring of colonising *P. xylostella* adults was carried out between July 1 and August 4. Transparent cylindrical sticky traps (h=21cm, d=9cm, glue: Tanglefoot®) were placed within the canopy of Brussels sprouts plants (two in 9-plant patches, four in 100-plant patches). They were collected every week and replaced by new ones. Trap material was taken to the laboratory for identification.

Hover-fly densities were recorded by counting syrphid eggs and larvae per plant. Rates of parasitism of the cabbage aphid by *D. rapae* and parasitism of the diamondback moth by *Diadegma* spp. were followed by recording the intact (not hatched) parasitised specimens as the proportion of the observed number of the host on a plant.

2.3. *Data analysis*

Densities per plant averaged for each plot were considered as experimental units (n=6). Herbivore counts per plant and per trap were [ln(value+1)] transformed. Data on rates of parasitism of the cabbage aphid and the diamondback moth were $[\arcsin(\sqrt{(x)})]$ transformed. Due to the low incidence of the diamondback moth early and late in the season, data of four weeks (census 2-5) were analysed for the comparisons of parasitism by *Diadegma* spp. To test the effect of patch size and vegetation background over time, a General Linear Mixed Model was used (proc mixed, repeated measurements, using SAS 8.0), where the three blocks were considered as random factors (Littell et al., 1996). Compound symmetry and autoregressive covariance structures were fitted to the correlation matrix of observed data. The selection between the fitted models for final inferences was based on the fit statistics of Akaike`s Information Criterion and Schwarz` Bayesian Criterion (Littell et al. 1996).

2.4. *Simulation study*

We used the spatially explicit simulation framework of Potting et al. (2002), which included behavioural-based stochasticity allowing us to study the interactions between vegetation structure and insect behaviour.

2.4.1. Spatial environment

We studied the effects of vegetation structure by establishing patches of different sizes in a spatially explicit environment (grid-size 500x500), where a grid-cell represented either a host or a non-host plant. In every simulation run 250 insects were randomly seeded in the environment, after which the model kept track of the position of each individual. The total number of cells covered by each patch-size was kept constant by varying the number of patches in the environment: 192 patches of 3x3, 48 patches of 6x6, 12 patches of 12x12, and 3 patches of 24x24.

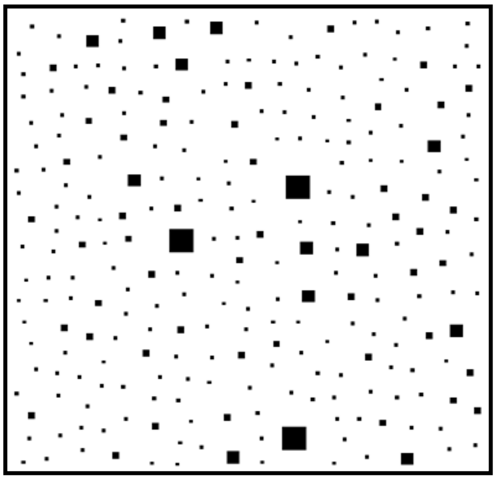

Figure 3. An example of the simulation environment, where habitat patches of four sizes (3x3, 6x6, 12x12, 24x24) occupying the same total area were randomly distributed in the arena. A more uniform distribution of patches was achieved by standardising minimum isolation distance among neighbouring patches.

In this case calculating patch size-density relationships would yield the same tendencies as patch size-number of visits relationships. Results are presented as the total number of times that herbivores visited host plant patches of a given size. Patches were randomly re-distributed in the environment after every 5 simulation runs. For a uniform distribution of patches, a minimum isolation distance between neighbouring patches was defined (16 cells, fig. 3). To keep population densities constant across the treatments and to avoid undesired “edge effects”, individuals were not allowed to emigrate from the environment, but “mirrored” to the opposite side of the environment. Each computer run simulated the displacement of 250 insects for 50 time steps. We carried out 25 runs for each patch scenario.

2.4.2. Foraging behaviour

Insects were randomly searching for host plants in the environment. Three foraging strategies have been simulated. The "contact searcher" (cabbage aphid) discriminated between host and non-host plants only after landing. (fig. 4a). The other two foraging strategies simulated herbivores with different sensory capabilities for in-flight detection of host plants. Here, the apparency of a host plant was simulated by a probability that an individual will detect it upon encounter. The "visual searcher" (cabbage butterfly) was able to detect host plants while flying (fig. 4b). This was implemented as a gliding box, referred to as a "search window", which was moving along the flight-path of the individual. Any host plant inside the search window was detected with the same probability (64%), independent of patch size. Visual searchers could detect hosts from a single row of cells below their flight path, up to search windows of 3, 5 and 7 cells wide. There is a minimum distance that a cabbage butterfly travels between successive egg-laying bouts (Root and Kareiva, 1984, Yamamura, 1999). We simulated this behaviour by activating the search-window at a later point along the flight-path. The "olfactory searcher" (diamondback moth) was implemented by setting the probability of detecting a host plant proportional to the area of the patch where the plant was situated (fig. 4c). We assumed that large patches emitted more volatiles and were detected with a higher probability (64% in 24x24 patches) than small ones (1% in 3x3 patches). Unlike visual searchers, olfactory searchers were only able to detect host plants on a row of cells on their flight path.

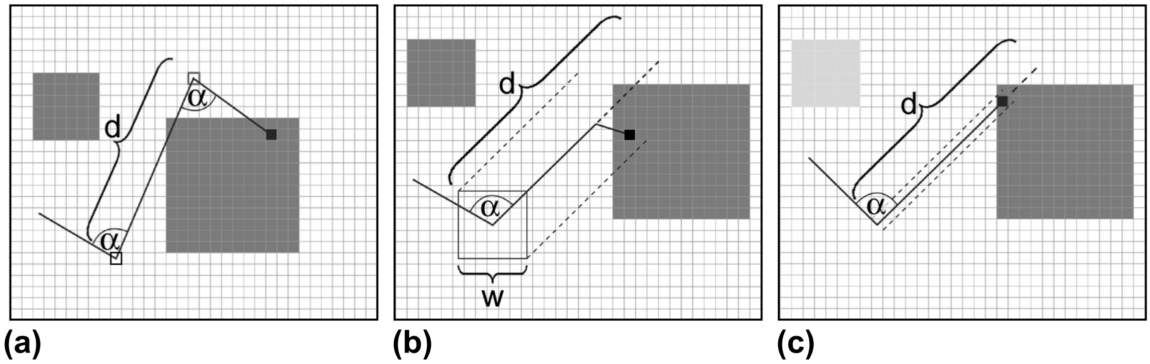

Figure 4. Examples of the three foraging strategies. Grey patches indicate host plants. *α*-is the direction of the flight path between two successive random take-offs. *d*-is the maximum distance covered in one bout and was chosen random between *0* and *d*. **(a)** A "contact searcher" identifies its host plant upon landing. **(b)** A "visual searcher" is able to detect host plants in its flight path, where *w* is the width of search window. Cells in patches of different sizes were detected with the same probability. **(c)** An "olfactory searcher" that detects large patches with greater probability (dark grey) than small (light grey) ones proportional to their area.

Within each foraging strategy we studied the effect of varying displacement speed and arrestment response. The displacement speed was defined as the maximum distance covered between two landings and was set at 1, 10 or 20 cells. The arrestment response was defined by a given probability for "hopping" behaviour. Hopping meant that individuals either stayed in the cell or moved to one of the eight neighbouring cells. When individuals did not hop, a flight was initiated. The effect of arrestment response was studied at probability values of 50%, 75% and 95%. Where the three foraging strategies were compared, simulated individuals had a displacement speed of 10 cells, an arrestment response of 75%, and in-flight detection included one cell only. Throughout these scenarios, vegetation background was composed of non-host plants, where the likelihood to get arrested in a cell was very low. Therefore the arrestment response in the background vegetation was set to 1%.

The effects of changing quality of vegetation background on patch responses of herbivores were studied in a separate scenario. Here an individual could discriminate between host and non-host plants upon contact, and could either stay in the current cell or move to one of the eight neighbouring cells only. The probability to move inside the patches was set to 75%. We studied the relationships between patch size and number of visits, when individuals in the background vegetation had either 1% or 50% probability for movement into neighbouring cells.

3. Results

3.1. Field study

Patch size and vegetation background were important factors influencing population densities of the herbivore species. However, responses of the three herbivore species were quite different.

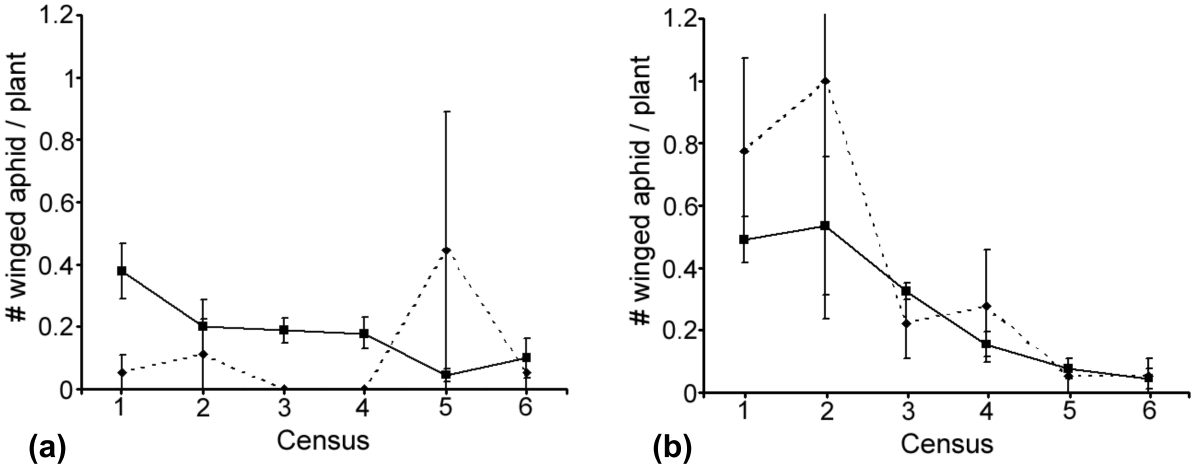

Figure 5. Average (±SE) number of winged cabbage aphid (*B. brassicae*) per plant observed in Brussels sprout patches of different sizes (- - ◆ - -small patch, —■—-large patch) embedded in **(a)** barley or **(b)** grass as vegetation backgrounds.

Immigrating cabbage aphids attained higher density in patches embedded in grass than in barley (P=0.007, df=1, F=7.66, fig. 5). Patch size did not have a significant effect on densities of winged morphs (P=0.773, df=1, F=0.08) and there was no interaction between patch size and vegetation background. When densities of all morphs were compared, neither patch-size (P=0.29, df=1, F=1.13) nor vegetation background (P=0.453, df=1, F=0.57) had an influence. Neither patch size (P=0.911, df=1, F=0.01) nor vegetation background (P=0.574, df=1, F=0.32) influenced densities of hover-fly eggs and larvae. Apparent rates of parasitism of the cabbage aphid by *D. rapae* were lower in small patches than in large ones (P=0.004, df=1, F=8.8), but vegetation background had no influence (P=0.643, df=1, F=0.22).

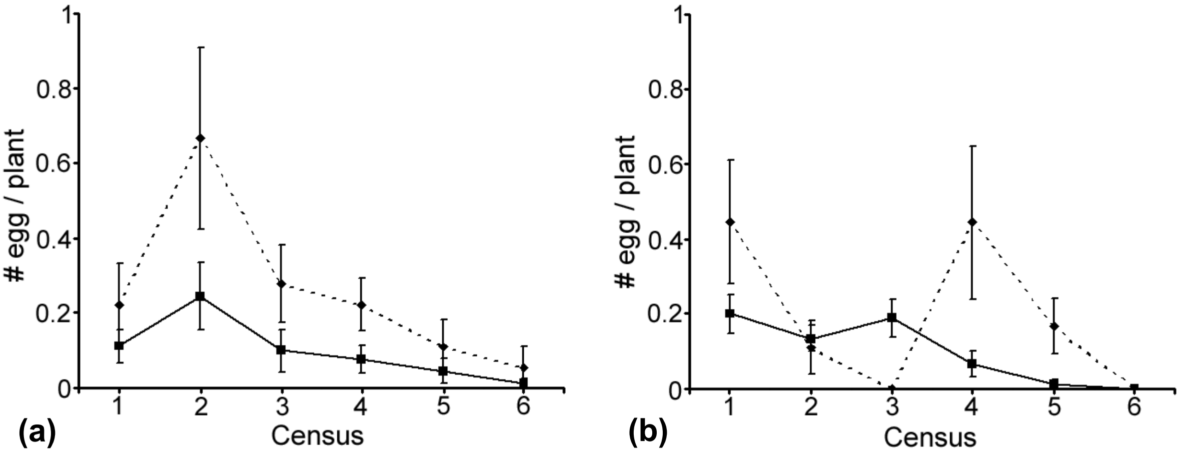

Figure 6. Average (±SE) number of cabbage butterfly eggs (*P. rapae*) per plant observed in Brussels sprout patches of different sizes (· · ◆ · ·-small patch, —■—-large patch) embedded in **(a)** barley or **(b)** grass as vegetation backgrounds.

Egg densities of the small cabbage white butterfly showed greater colonisation of small host-plant patches than large ones (P=0.001, df=1, F=10.77, fig. 6). The presence of barley in the vegetation background did not influence the average number of eggs per plant (P=0.362, df=1, F=0.84, fig. 6ab).

Densities of adult diamondback moths were higher in large patches than in small ones (P<0.001, df=1, F=18.23. fig. 7). Vegetation background had a clear effect, as traps in barley (fig. 7a) caught fewer specimens than traps in grass (P<0.001, df=1, F=27.71, fig. 7b). The interaction between patch size and vegetation background was significant (P=0.003, df=1, F=10.16), indicating that barley did not only result in reduced overall numbers of moths, but also increased the difference between small and large patches. Responses of immigrating adults to patch size had no effect on larval and pupal densities of subsequent generations (P=0.617, df=1, F=0.25). Patches in barley supported lower larval population densities compared with patches in grass throughout the season (P<0.001, df=1, F=12.27, data not shown).

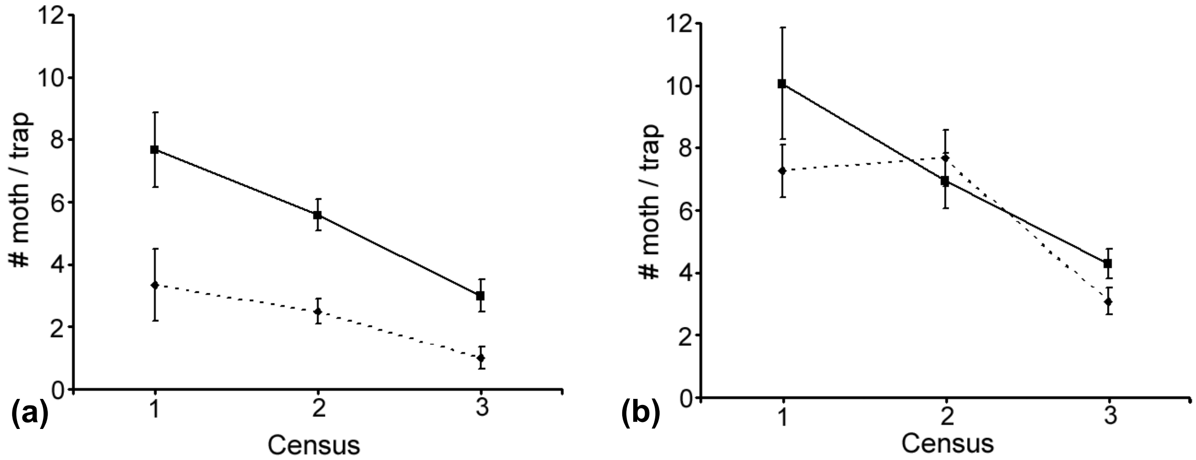

Figure 7. Average (±SE) number of diamondback moth (P. *xylostella*) caught per trap in Brussels sprout patches of different sizes (· · ◆ · ·-small patch, ━■━-large patch) embedded in **(a)** barley or **(b)** grass as vegetation backgrounds.

Neither patch size (P=0.254, df=1, F=1.32), nor vegetation background (P=0.143, df=1, F=2.2) influenced apparent rates of parasitism of the diamondback moth by *Diadegma* spp.

3.2. Simulation study

3.2.1. Foraging behaviour and patch response

Simulating different searching strategies with the default parameters resulted in different number of visits in patches of different sizes (fig. 8).

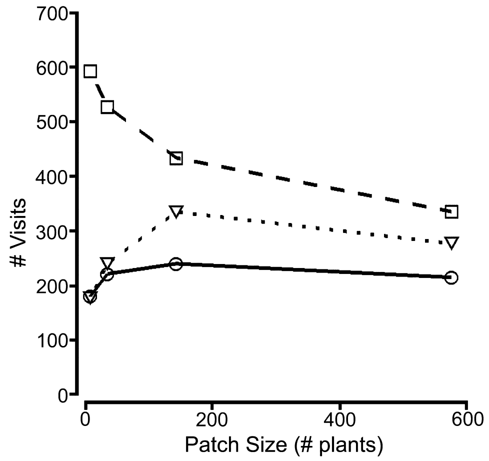

Figure 8. Simulated relationships between patch size and number of visits for herbivores with different foraging strategies. ─⊖─-contact searcher; -⊟--visual searcher; ··▽··-olfactory searcher; Displacement speed of individuals was set to a maximum of 10 cells, arrestment response in host plant patches was set to 75% and window size (for the visual and olfactory searcher) was set to 1 cell.

For the contact searcher, the relationship between patch size and number of visits appeared to be non-linear, with lowest average values in patches of 9 plants and with a highest mean density in patches of 144 plants. A further increase of patch size to 576 plants resulted in a decrease in the number of visits per plant. For a visual searcher the number of visits per plant was negatively related to patch area with the highest frequency of visits in the smallest (9 plants) patches and with lowest frequency in patches of 576 plants. For an olfactory searcher with a higher probability of patch finding for large patches than for small ones, the pattern was similar to that observed in case of the contact searcher. There was an increase of visits per plant until an asymptote was reached in patches of 144 plants followed by a decrease towards a patch size of 576 plants.

Changing behavioural parameters within a foraging strategy had different effects on the relationships between patch size and number of visits. For a contact searcher, increasing displacement speed led to an increase of visits in patches of intermediate to large sizes, while there was less increase of visits in patches of 9 plants (fig. 9a). In case of a visual searcher increasing displacement speed led to an overall increase of visits in patches of all sizes (fig. 9b). However, this increase was stronger in small patches than in large ones, so individuals with a greater displacement speed showed a stronger negative relationship between patch size and number of visits than less mobile individuals. For an olfactory searcher a third pattern emerged (fig. 9c), where an increasing mobility gave rise to an increasingly positive patch size-visits relationship.

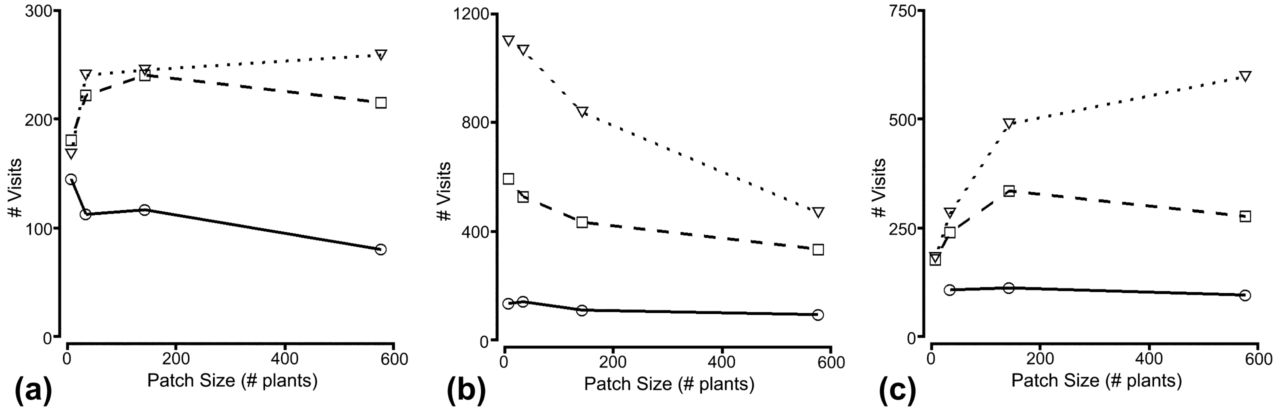

Figure 9. Simulated relationships between patch size and number of visits for herbivores with different displacement speed in the three searching strategies. The maximum distance covered per flight bout was set to —○— -1 cell, -□- -10 cells and ··▽·· -20 cells. **(a)** -Contact searcher; **(b)** -Visual searcher; **(c)** -Olfactory searcher; Arrestment response in host plant patches was set to 75% and window size (for the visual and olfactory searcher) was set to 1 cell.

An increase in the arrestment response of a contact searcher from 50% to 95% increased number of visits in patches (fig. 10a).

This increase was stronger in patches of 36 and 144 plants than in patches of 9 and 576 plants, resulting in an asymptotic relationship between patch size and number of visits.

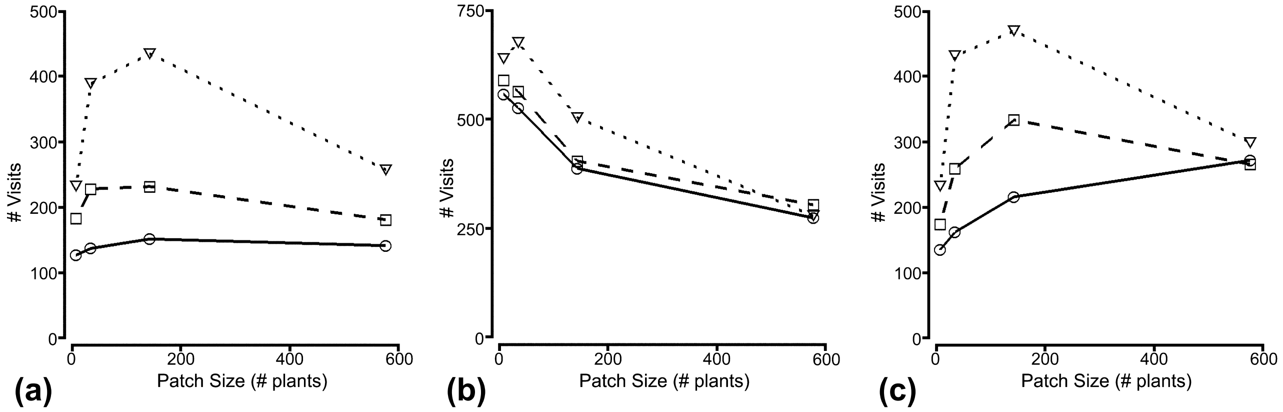

Figure 10. Simulated patch size – number of visits relationships for herbivores with changing arrestment responses in the patches of host plants when the three searching strategies were implemented. The probability of being arrested in a given cell was set to —⊖— -50%, -⊟- - 75% and ··▽··- 95%. **(a)** -Contact searcher; **(b)** -Visual searcher; **(c)** -Olfactory searcher; Displacement speed of individuals was set to a maximum 10 cells and window size was 1 cell (detection on the flight-path only).

For a visual searcher increasing arrestment response did not influence the negative patch size-number of visits relationship (fig. 10b). For the olfactory searcher an arrestment response of 50% resulted in a positive patch size-number of visits relationship, with most visits observed in patches of 576 plants (fig. 10c). Increasing arrestment response to 75% and 95% increased number of visits in all patches, but it revealed an asymptote in patches of 144 plants.

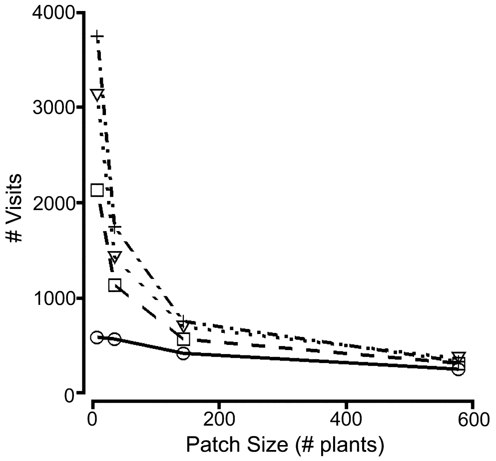

Figure 11. Simulated patch size-number of visits relationships for a visual searcher with changing size of search window. The width of the search window was set to —⊖— -1 cell (detection on the flight-path only), -⊟- -3 cells, ··▽··-5 cells, and -·+·- -7 cells. Displacement speed of individuals was set to a maximum of 10 cells and arrestment response in host plant patches was set to 75%.

Increasing in-flight orientation of the visual searcher increased number of visits in small patches relative to large ones (fig. 11). Activating the search window at increasing distances from the starting point a cell of the flight path did not change the overall negative patch size-number of visits relationship, but decreased the number of visits more in small patches than in large ones (data not shown).

3.2.2. Vegetation background and patch response

When the probability to move within the background vegetation was 1%, the number of landings per patch was lower than when it was set to 50%. At 1% probability for movement in non-host cells, individuals were unlikely to cross the background and travel from one patch to the other. Due to the greater perimeter of smaller patches, emigration rates from smaller patches were higher than from larger ones, leading to a positive patch size-number of visits relationship (fig. 12).

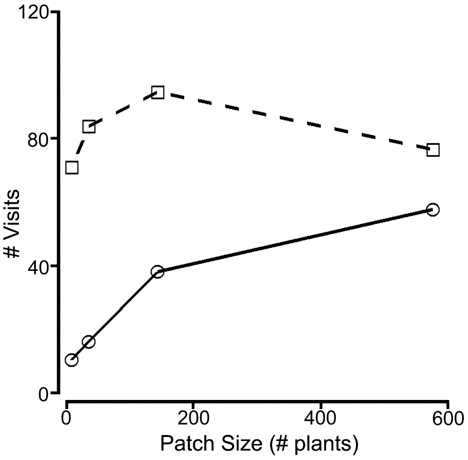

Figure 12. Simulated patch size-number of visit relationships when the probability to move among patches was changing. Individuals moved to neighbouring cells only, where the probability to move within non-host cells was set to —⊖— -1% or -⊟- -50%. Probability to move within the host plant patches was set to 75%.

Increasing the probability to move within the background-vegetation led to an overall increase of visits in patches of all sizes. Patch response also changed, as individuals could now bridge distances among patches. This resulted in differences in immigration rates, more individuals arriving into the more numerous smaller patches than into fewer larger ones.

4. Discussion

4.1. *Patch size*

We observed population responses of three herbivore species, specialists on crucifers, to host plant patches of different sizes embedded in mown grass or barley as background vegetation. Densities of winged cabbage aphids were independent of patch size, which was also observed by Grez and Gonzalez (1995). Egg densities of the cabbage butterfly were lower in large patches than in small ones, agreeing with findings of studies on densities of *Pieris* species in relation to patch size or perimeter of patches (Cromartie, 1975, Maguire, 1983, Muriel and Grez, 2002). Diamondback moth densities were higher in large patches. These results support earlier findings, where higher densities of diamondback moth were found in large patches (Maguire, 1983, but see Grez and Gonzalez, 1995). Subsequent larval densities showed no differences between different patch sizes.

The simulation experiment showed that the different patch size-density relationships as observed in the field experiment could be attributed to the different foraging strategies of the insects. In the simulation environment, a contact searcher with no in-flight detection abilities, responded to patches independent of their size. When the arrestment response increased, large patches retained more individuals than small ones, simply because they lost fewer individuals via emigration through the borders of the patch. This may lead to a more positive patch size-density relationship, but not always. As large patches were spatially less available displacement speed was a controlling factor in patch size-density relationships. When displacement speed was low, few large patches were found, resulting in low visitation of large patches. So patch response of a contact searcher was both determined by its ability to travel between patches of different sizes (i.e. displacement speed) and by its emigration behaviour (determined by its arrestment response). Given their low maximum flight-speed aphids are unlikely to actively travel to plants that are several meters away, and once the host is found they would not visit neighbouring plants either (equivalent to a strong arrestment response) (Compton, 2002). Therefore such a foraging behaviour would lead to aphid densities likely to be independent of patch size, as we also observed in our field experiment.

The visual searcher showed a negative patch size-density relationship, which was the result of its response to linear dimensions of patches: smaller patches with greater perimeter-area ratios were more likely to be encountered. This patch response was more pronounced when the displacement speed of individuals was greater, leading to an increased accumulation on smaller patches. This effect was so dominant that increasing the arrestment response was not likely to change this relationship. Therefore a great displacement speed coupled with a good ability to detect host plants from large distances would increase encounter rates of herbivores with small habitat patches. Hence the searching tactics of *P. rapae* explains the aggregation to small patches in our field experiment.

The olfactory searcher revealed complex interactions between behaviour and spatial parameters of the environment. The responses of an olfactory searcher are positively related to the size of the patch, but a positive patch size-density relationship exists only when all patch sizes are spatially available, so at a high displacement speed. However, increasing arrestment response is likely to increase densities in patches of intermediate size. These results suggest that in a given spatial environment, between- (i.e. displacement speed) and within-patch (arrestment response) behaviour together determine patch responses of individuals. So, an olfactory searcher with a high displacement speed and a strong arrestment response may respond to patch sizes as was observed for the diamondback moth in our field experiments.

4.2. Background vegetation

In the field experiment, the three species gave different responses to the different vegetation backgrounds. Densities of immigrants of the cabbage aphid and the diamondback moth were reduced by barley. Egg densities of the cabbage butterfly were not influenced by background vegetation. Background vegetation also influenced patch responses of diamondback moth, as differences between densities in small and large patches were increased by barley in the background.

The simulation study demonstrated that background vegetation can alter patch responses simply by interfering with the mobility of insects when travelling between patches. Besides interfering with mobility, barley, being taller than the Brussels sprout plants, might have altered the olfactory and visual perception of patches by herbivores when compared to mown grass. It could have interfered with the foraging behaviour of herbivores in two ways. First, barley decreased the linear dimensions of the patches, as plants were no longer “looming up” from the background. Hence the perception of physical (dimensional) visual and olfactory cues from host plant patches could all have been altered by changing composition of vegetation background. The overall negative effects of barley on immigrating cabbage aphids and diamondback moths could be the result of such a mechanism. The lack of response of the cabbage butterfly to vegetation background could be due to its ability to fly out of the habitat and search above the vegetation during daytime (Root and Kareiva, 1984), so visual dimensional properties of the patches were not altered by vegetation background from the perspective of this species. Second, the presence of barley might have interfered with foraging behaviour of herbivores at the level of host-plant acceptance. Structural complexity of surrounding non-host plants could influence acceptance of host plants by herbivores (Finch and Collier, 2000, Finch et al., 2003). Because of its greater complexity, the presence of the barley could make herbivores loose host plants easier than in grass. As a greater proportion of host plants were surrounded by barley in small than in large patches, the lowest densities of diamondback moth in small barley patches, might also be explained by the greater interference of barley with host-acceptance behaviour of a herbivore.

4.3. Foraging behaviour and patch response

Results of this study show that patch responses of local herbivore populations are influenced by the behavioural characteristics of the species studied. This supports earlier studies arguing that predictions of the resource concentration hypothesis alone cannot explain the variation in herbivore responses to resource patchiness. Bowman et al. (2002) explained patch responses of organisms based on differences in immigration behaviour into patches (i.e. orientation type, aerial- vs. ground-level dispersers). Our results showed that putative differences in the relative importance of stimulus modality (i.e. contact, visual, olfactory) could affect between-patch behaviour of species resulting in different patch responses. Within-patch behaviour, simulated here by arrestment response, also influenced patch responses via affecting "diffusion" of individuals in the environment. Therefore characteristics of resource exploitation (e.g. egg distribution, solitary vs. gregarious traits) that influence within-patch behaviour, could be important traits determining patch responses of herbivores. Using basic knowledge on searching strategies of different herbivore species that deal with similar adaptive constraints under similar conditions, may enable us to define "behavioural groups" that show similar patch responses. For example *Colias* butterflies that forage in a different ecosystem (Stanton, 1983), show egg-laying behaviour similar to *Pieris* butterflies that might result in similar spatial dynamics. The minimal number of behavioural and life-history characteristics that are required to make valid predictions for patch responses remains to be determined.

4.4. Spatial scale and foraging behaviour

Responses of herbivore populations to fragmented habitats is comprised of scale-dependent processes. At large scales limited dispersal is the determining factor, and life history parameters (e.g. body size), correlating well with limited dispersal of animals, may provide sufficient information to formulate scaling laws to quantify effects of habitat fragmentation (Ritchie and Olff, 1999). Responses of organisms at small spatial scales are stronger influenced by individual cases of variation than those at large scales, so predictions on the responses of organisms are more difficult to make (Levin, 1992). At small scales individuals are within their dispersal domain and have more freedom to choose and accumulate on more preferred parts of a habitat, so behavioural characteristics become more influential (Bergelson and Kareiva, 1987, Garcia and Altieri, 1992, Bommarco and Banks, 2003). Hence, the common denominator that mediates the impact of different behavioural characteristics on patch size-density relationships is spatial scale. This explains why at small scales other attributes (e.g. patch size) of the habitat may be more important than at large scales (e.g. isolation between patches) for the same species (Krawchuk and Taylor, 2003). Therefore, an understanding of interactions between foraging behaviour and scale may not only help us explain the high variance found in herbivore responses to small-scale habitat manipulation techniques (Grez and Gonzalez, 1995, Maguire, 1983, Cromartie, 1975, Banks, 1998). It could also help us to specify the spatial scale at which the impact of habitat characteristics (e.g. patch size, connectivity, patch shape) on local population densities of a given species is changing.

Acknowledgements

This study is part of the program “Enhanced biodiversity” funded by the Earth and Life Sciences of Dutch Scientific Organisation (NWO-ALW, projectnumber: 014-22.031). We are grateful to Hajnalka Tréfás for her help in the collection of the field data. The authors thank Jeffrey A. Harvey and Joop. J. A. van Loon for critically reviewing the manuscript. Preparation of the field layout by the experimental farm of the Wageningen University (Unifarm) is acknowledged.

Chapter

5

Variation in plant volatiles and the attraction of the parasitoid *Diadegma semiclausum* (Hellén)

T. Bukovinszky, R. Gols, M.A. Posthumus, L.E.M. Vet and J.C. van Lenteren

Variation in plant volatiles and the attraction of the parasitoid *Diadegma semiclausum* (Hellén)

Abstract

Differences in allelochemistry of plants may influence their ability to attract parasitoids that protect them against herbivores. We studied responses of *Diadegma semiclausum*, a parasitoid of the diamondback moth (*Plutella xylostella*) to inter- and intraspecific variation in odour blends of crucifers and a non-crucifer species. Uninfested Brussels sprout (*Brassica oleracea* gemmifera), white mustard (*Sinapis alba*), a feral *Brassica oleracea* and malting barley (*Hordeum vulgare*) were compared for their attractivity to *D. semiclausum* in a Y-tube bioassay. Volatiles from all four plants were more attractive to the parasitoid than clean air. However, when plants were tested against each other, parasitoids preferred volatile blends of the three cruciferous plants over those of malting barley. Wasps also discriminated between uninfested crucifers: mustard was as attractive as feral *Brassica oleracea* and both were more attractive than Brussels sprout. Attractivity of uninfested plants was compared to that of plants infested by the host *P. xylostella*. Host-infested mustard and Brussels sprout were more attractive than uninfested conspecifics. Interestingly, the volatile blends of uninfested mustard and infested Brussels sprout were equally attractive. We also compared the volatile composition of different plant sources by collecting headspace samples and analysing them with GS-MS. Similarity between volatile profiles were determined by hierarchic clustering and non-metric scaling based on the Horn-index. Due to the absence of several compounds in its blend the volatile profile of barley showed great dissimilarity from those of crucifers. The volatile profile of mustard was distinctly different from those of the two Brassicas. The volatile profile of feral *Brassica oleracea* was different from infested Brussels sprout, but showed overlap with uninfested Brussels sprout. The volatiles of infested and uninfested Brussels sprout were highly similar and mainly quantitative differences were found. *Diadegma semiclausum* appeared to discriminate based on subtle quantitative differences in volatile composition of odour blends of infested and uninfested plants.

Keywords: Diadegma semiclausum, Plutella xulostella, Hordeum vulgare, *Sinapis alba, Brassica oleracea*, parasitoid attraction, headspace volatiles;

1. Introduction

Plant-derived infochemicals are important for parasitoids of herbivores in the process of host location (Vinson, 1976, Vet and Dicke, 1992, Tumlinson et al., 1993, Völkl and Sullivan, 2000). The dietary breadths of herbivores and their parasitoids may both influence to what degree plant volatiles are used in this process (Vet and Dicke, 1992). In general, volatiles from uninfested and damaged plants are easier to detect by a parasitoid than cues from the host, but they are weaker predictors of the host's presence (Vet et al., 1991). On the other hand, information from the host itself (i.e. faeces, frass, silk) is a more reliable tell-tale of the host's presence, but is usually much more difficult to detect than plant-derived volatiles. The solution to this reliability-detectability problem may rely in herbivore-induced volatiles, which are emitted upon damage by the host, being both reliable and detectable. In natural ecosystems, host-parasitoid interactions take place in habitats composed of several to many plant species, where both the expectancy of the host's presence and the specificity of volatile infochemicals may show great between- and within-plant variance (Vet, 1999). Variation in plant volatiles between and within plant species can be greater than between damaged and undamaged plants (Geervliet et al., 1997), and such differences can be reflected in attractance of parasitoids to plants (Elzen et al. 1983, 1986, Fox and Eisenbach, 1992, Geervliet, 1997). Differential ability of plants to attract natural enemies may be even responsible for further dietary specialisation of herbivores exploiting specific plant taxa like the family Cruciferae (Yano, 1994). For example opposing choices at the plant-level could benefit the herbivore and lead to enemy-free space shaping host-parasitoid interactions (Fox and Morrow, 1981, Fox and Eisenbach, 1992, Bigger and Fox, 1997, Gratton and Welter, 1999, Oppenheim and Gould, 2002). If host-parasitoid interactions in ecosystems are to be understood, foraging behaviour of parasitoids must be studied in relation to inter- and intraspecific variation in plant-derived infochemicals.

Crucifers are characterised by the presence of glucosinolates and their volatile breakdown products, which play a role in host searching behaviour of parasitoid species foraging on hosts associated with plants of this family (Read et al., 1970, Reed et al., 1985, Smid et al., 2002). Other studies show effects of inter- and intra specific variation in volatiles of cruciferous plants on their attractivity to parasitoids. Fox and Eisenbach (1992) found that wild crucifers were preferred by *Diadegma insulare*, a parasitoid of *Plutella xylostella* L., when compared with collards. Higher levels of parasitism of *P. xylostella* by *Cotesia plutellae* were observed on common cabbage than on Chinese cabbage, which was attributed to differences in attractivity of plant volatiles (Liu and Jiang, 2003).

Breeding programmes involving glucosinolate chemistry largely focus on mammalian toxicity, and the demands of consumers in the utilisation of plant organs (Mithen, 2001). The few efforts to select for resistance in breeding programmes have focussed on direct defences, ignoring the effects on the third trophic level (Dicke, 1999a, Bradburne and Mithen, 2000). It is possible that breeding programmes of crucifers have changed the apparency of plants not only for herbivores but also for those parasitoids, which use plant-derived infochemicals in habitat and host location. Therefore domesticated plants might have altered capacity to attract natural enemies, but experimental evidence is needed to test this assumption (Loughrin et al., 1995, Benrey et al., 1998, Cortesero et al., 2000).

The family Brassicaceae has undergone extensive breeding programmes, resulting in huge variation both in the composition and the allocation of plant chemicals in different organs (Benrey et al. 1998, Mithen, 2001). We chose members of the family *Cruciferae* to study how inter- and intraspecific variation in volatile blends of plants influences their ability to recruit parasitoids for protection against herbivores.

We examined the responses of *Diadegma semiclausum* (Hellén), a specialist parasitoid of the diamondback moth (*P. xylostella*) in olfactometer bioassays. The observed behavioural differences were related to the variation in volatile blends of plants by collecting, analysing and comparing headspace volatiles. The uninfested plants compared were Brussels sprout, a naturalised population of previously cultivated *Brassica oleracea*, white mustard and malting barley. The latter species was included in the comparisons as a species not related to crucifers and a non-host for *P. xylostella*. Comparisons of host-infested and uninfested plants involved Brussels sprout and mustard.

2. Materials and Methods

2.1. Plants

Plants used were malting barley (*Hordeum vulgare* L. cv Video), white mustard (*Sinapis alba* L. cv Carnaval), Brussels sprout (*Brassica oleracea* L. gemmifera cv Cyrus) and a naturalised population of *Brassica oleracea* L. This feral population was found in a roadside hollow in 2001 and it probably "escaped" from a local farm, but it is unknown how long it has previously been growing in the wild (Harvey et al., 2003). Plants were reared in a greenhouse compartment at L16-D8 photoperiod, 20-28 ºC and 40-80% R.H. Plants were sown in ca. 1.2 litre pots filled with standard compost (Lentse Potgrond®) with no extra fertiliser added. To standardise the leaf area and biomass of the plants, a different number of plants per pot were grown for each species; eighteen for barley, nine for mustard, and one for Brussels sprout and the feral *B. oleracea*. Age, average leaf area and fresh weight of test plants are listed in table 1. The first flower buds of mustard just started to develop when plants were tested. Before testing a plant, the pot with excess soil was removed and below-ground plant parts were wrapped in aluminium foil. The plant was then used for the behavioural assay or the collection of headspace samples.

Table 1. Average (±SD) leaf area (LA) and fresh weight of plants per pot used in the bioassays. Plant age is given in weeks after sowing, n is the number of pots measured.

	Brussels sprout **6-7 weeks** n=17	**Barley** **3-4 weeks** n=10	**Wild crucifer** **6-7 weeks** n=16	**Mustard** **3-4 weeks** n=5
LA (cm^2)	539.4(43.5)	703.1(14.1)	499.1(19.5)	690.3(44.8)
FreshWeight (g)	24.2(2.04)	22.6(0.2)	23.2(1.5)	28.2(1.7)

2.2. Insects

Diadegma semiclausum (Hellén) was collected from Brussels sprout fields in a woodland area in the vicinity of Wageningen (The Netherlands) and was maintained on *P. xylostella* reared on Brussels sprout (8D: 16L photoperiod, 20 ± 2º C and 70% R.H). In the rearing cages, parasitised host larvae were allowed to pupate on paper stripes, which were transferred into a plastic cage with no host or plant material present. Wasps emerging from cocoons were provided *ad libitum* with water and honey. Mated females of 5-10 days of age with no oviposition experience were used in olfactometer bioassays. To obtain infested plants (mustard or Brussels sprout), 20 second and early third instar larvae of *P. xylostella* were evenly distributed over a test plant, 14-16 hours before the experiment.

2.3. Y-tube bioassay

To test behavioural responses of individual *D. semiclausum* females to plant volatiles, a glass Y-tube (ø 3.5cm) olfactometer was used (for details see Takabayashi and Dicke, 1992). The Y-tube had a stem section of 22cm long. The two arms of the Y-tube were connected to glass vessels containing the odour source. The volume of the containers was five-litre in all comparisons except those involving the feral *Brassica*. As this plant had long petioles, we used two containers of 30 litres to accommodate plants. When an odour source was compared to clean air, a piece of cotton-wool humified with water was placed in an empty container. While the five-litre containers were directly attached to the olfactometer, the two 30-litre containers were attached by a silicon hose. Air was filtered over charcoal led into each container at 4 l/min. The air was extracted at the base of the olfactometer at 8 l/min. The olfactometer was illuminated from above with high frequency fluorescent lights at an intensity of 30-35 μmol photons/m^2/sec. Wasps were individually tested in the olfactometer, and each wasp was used only once. In order to increase their motivation to search for hosts (Geervliet, 1997, Potting et al., 1999), females were transferred from the cage into the Y-tube on a piece of Brussels sprout leaf, damaged by the host but not containing the host itself or its products. The observation started by releasing the wasp at the base of the Y-tube, at four centimetres distance from the opening. Wasps were either walking or flying towards the odour source. A finish line was drawn one centimetre from the sieve at the end of each arm. Once a wasp crossed the finish line and did not return to the junction for at least 15 seconds, this was considered a choice. Wasps that did not make a choice within ten minutes after release and wasps that did not reach even the junction of the olfactometer in five minutes were considered as non-responding individuals. Odour sources were replaced after every ±6 females tested, and at least eight plants have been tested for each combination of plants. The odour sources were exchanged after testing ±3 females, and the olfactometer was turned 180° around its longitudinal axis after every ±10 females tested.

The attractiveness of the odour blends of the following uninfested plants were tested against clean air and each other: Brussels sprout, feral *B. oleracea*, mustard and barley. Odour blends of host-infested Brussels sprout and mustard were compared to uninfested conspecifics, and uninfested mustard was compared to host-infested Brussels sprout. The tests were carried from March till September 2003. A binomial test was used to determine whether preferences of parasitoids were significantly different from a non-preference situation ($p=q=0.5$, two-tailed, $\alpha=0.05$).

2.4. Collection and analysis of headspace volatiles

Headspace volatiles were collected from all plant-sources tested in Y-tube bioassays (except for the *Plutella*-infested mustard) and were analysed by GS-MS. Four to five samples of each plant-source were taken in the period of June-August 2003. Plants prepared for sampling (as described in section "*Plants"*) were transferred into 30-litre collection flasks. Pressurised air was filtered through silica gel, molecular sieves 4A and 13X (Linde), and activated charcoal before entering the flask. The air inlet, air outlet, filters and sampling jar were connected with ø 0.8cm teflon tubing. The system was purged for 1 hr at an airflow rate of 500 ml/min to remove volatile contaminants. Subsequently, volatiles were collected in a glass tube containing 90mg Tenax-TA (20/35 mesh) for 4-5 hrs at a flow rate of 150-250 ml/min. Blanks were taken in duplo from empty collection containers. The collected volatiles were released from the Tenax by heating the trap in a Thermodesorption Cold Trap Unit (Chrompack) at 250°C for 10min and flushing with helium flowing at 12ml/min. The released compounds were cryo-focussed in a coldtrap (0.52mm ID deactivated fused silica) at a temperature of –85°C. By ballistic heating of the cold trap to 220°C the volatiles were transferred to the analytical column (DB5, 60m X 0.25mm ID, 0.25-μm film thickness), which was connected to a Finnigan MAT 95 mass spectrometer. The temperature of the column oven was programmed from 40°C (4 min hold) to 250°C (4 min hold) at a rate of 4 °C/min and the initial helium velocity was 25cm/sec. The mass spectrometer was operated in the 70 eV EI ionisation mode and was scanning from mass 24 to 300 at 0.7 sec/decade. Compounds were identified by comparison of the mass spectra with those in the Wiley 7th/NIST98 library and in the Wageningen Mass Spectral Database of Natural Products and by checking the retention index. Emission rates were measured by quantifying peak areas. Compounds are presented as peak area per litre of trapped air per gram above-ground fresh weight.

Those compounds that were not present in the blank and were detected in at least two replicate samples, were included in the analysis. The detected quantities of individual compounds within a sample were considered as variables, and dissimilarities among the 24 plant samples were calculated based on the Horn-index (average link method) (see Krebs, 1989, eqn.1.). We chose this index because the calculated similarity among the volatile blends is supposedly little affected by the number of compounds included in the analysis (Krebs, 1989).

$$R_0 = \frac{\Sigma[(x_{ij}+x_{ik}) \log(x_{ij}+x_{ik})] - \Sigma(x_{ij} \log x_{ij}) - \Sigma(x_{ik} \log x_{ik})}{[(N_j+N_k) \log(N_j + N_k)] - (N_j \log N_j) - (N_k \log N_k)} \quad \text{eqn1.}$$

Where R_0 is the Horn's index of similarity for samples j and k, x_{ij} and x_{ik} are the amounts detected of compound i in sample j and sample k, where N_j is Σx_{ij} the total amount of volatiles in sample j and N_k is Σx_{ik} the total number of compounds in sample k. To illustrate the dissimilarities in volatile profiles, hierarchical clustering and non-metric scaling were used. Analysis was performed by the Syntax 5.1 program package (Podani, 1997).

3. Results

3.1. Y-tube bioassay

When volatiles of different plants were tested against clean humidified air, 60% to 95% of females made a choice. Females preferred volatiles from all the plant sources to clean air ($P<0.05$, fig. 1.).

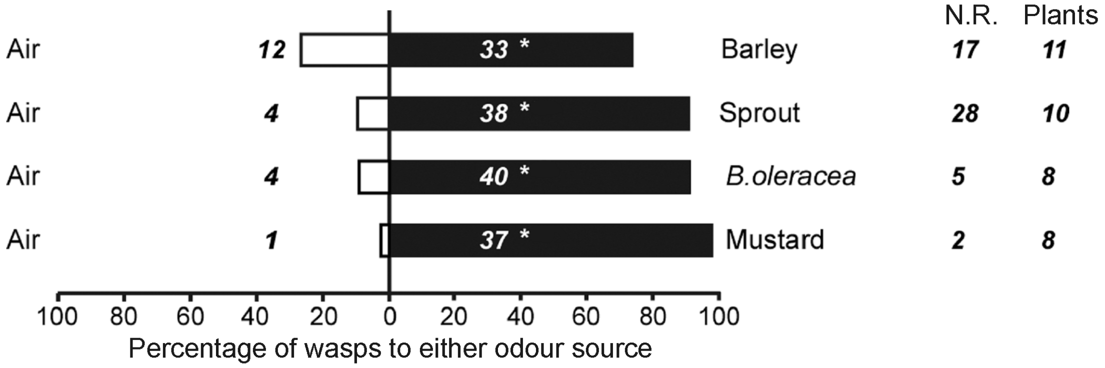

Figure 1. The percentage of female *D. semiclausum* choosing either odour source when volatiles from uninfested plants are compared to clean air. Asterisks indicate statistically significant preferences within tests (*-P<0.05). Numbers next to the graph are the number of non-responding (N.R.) individuals and the number of plants tested.

When offered the choice between blends of uninfested plants, 61% to 88% of the tested females made a choice (fig. 2). Females clearly discriminated between volatiles from different species of uninfested plants. All cruciferous plants were preferred over malting barley (P<<0.05). Wasps discriminated between the different cruciferous plants as well. Females preferred both mustard (82% of choices) and the feral *B. oleracea* (74% of choices) to Brussels sprout (P<0.05, fig. 2). Females were equally attracted to mustard and the feral *B. oleracea*.

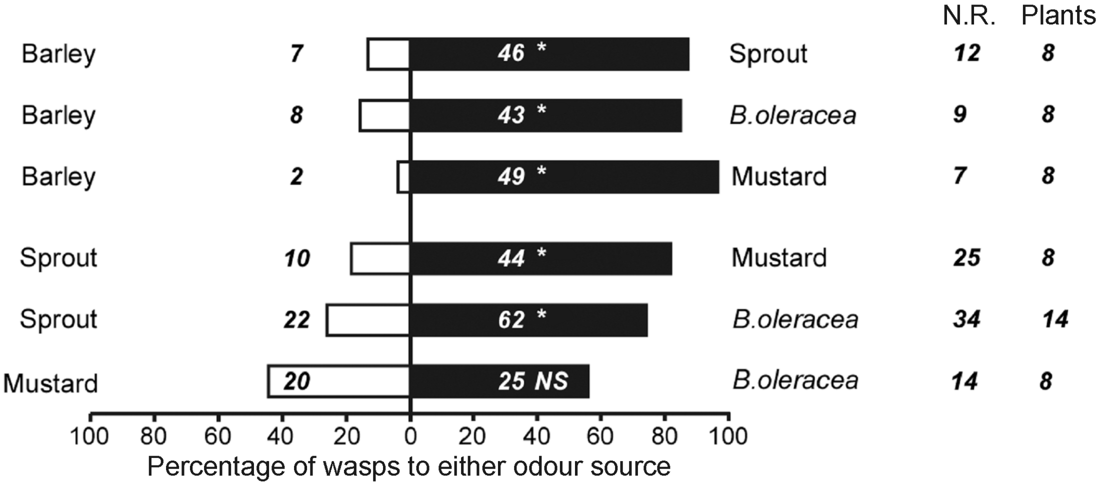

Figure 2. The percentage of female *D. semiclausum* choosing either odour source when volatiles from uninfested plants are compared. Asterisks indicate statistically significant preferences within tests (*-P<0.05). Numbers next to the graph are the number of non-responding (N.R.) individuals and the number of plants tested.

When females were exposed to volatiles from uninfested and host-infested cruciferous plants, 88% to 99% of individuals responded (fig. 3). Wasps discriminated between infested and uninfested conspecifics. Females preferred both mustard (91% of choices) and Brussels sprout (83% of choices) to uninfested conspecifics. Interestingly, when females were offered the choice between host-infested Brussels sprout and uninfested mustard plants, no preference was found.

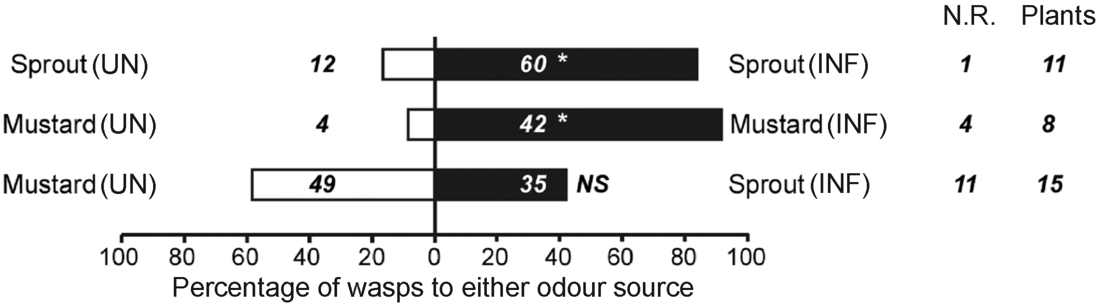

Figure 3. The percentage of female *D. semiclausum* choosing either odour source when volatiles from uninfested (UN) plants (Brussels sprout and mustard) and from *P. xylostella*-infested plants (INF) are compared. Asterisks indicate statistically significant preferences within tests (*-P<0.05). Numbers next to the graph are the number of non-responding (N.R.) individuals and the number of plants tested.

3.2. *Headspace volatiles*

In the 24 samples analysed across the treatments, 70 compounds were detected (table 2). The compounds were identified as ketones, alcohols, aldehydes, esters, terpenoids, sulphides, nitriles and others. Fifty-nine compounds were detected in *Plutella*-infested Brussels sprout, 48 in uninfested Brussels sprout, and 46 both in the feral *B. oleracea* and in the mustard. In the headspace of barley, only 15 compounds were detected. Both quantitative and qualitative differences in headspace volatiles of tested plants were found.

Table 2. Volatile compounds detected in the headspace of Brussels sprout infested by larvae of *P. xylostella* (n=5), uninfested Brussels sprout (n=5), feral *B. oleracea* (n=5), mustard (*S. alba*) (n=5) and in uninfested barley (n=4). Amounts of individual compounds are given as average peak area (±SE) per litre of trapped air per gram above ground biomass.

	Compound[a]	B. sprout+ *Plutella*	Brussels sprout	*B. oleracea*	*S. alba*	Barley
Ketones						
1	3-pentanone$^{\Delta, \#, \circ}$	2.3±1.6	-	-	-	-
2	2-hexanone	3.1±1.7	1.5±0.8	0.5±0.2	0.1±0.1	0.2±0.2
3	1-cyclopropyl-2-propen-1-one$^{\circ}$	10.7±4.9	1.9±1	0.9±0.6	1.3±0.5	-
4	3-heptanone	4.5±1	3.3±1.1	2.5±0.4	1.3±0.6	-
5	2-heptanone	1.9±0.4	1.7±0.4	1.1±0.3	0.7±0.2	-
6	2-methyl-2-cyclopenten-1-one (t)	6.4±4.8	0.8±0.8	0.5±0.5	-	1.7±1
7	3-octanone$^{\circ}$	2.4±1.6	0.2±0.2	2.6±2.3	-	-
8	1,7-octadiene-3-one, 2-methyl-6-methylene-	8.6±2.8	5.4±2.7	2±0.9	0.7±0.5	-
Alcohols						
9	1-penten-3-ol$^{\Delta, \square, \circ, \#}$	117.5±60.9	18.6±11.4	1.2±0.7	4.6±0.9	-
10	3-pentanol$^{\Delta, \square, \circ}$	21.9±11.5	2.6±0.9	0.7±0.2	0.3±0.2	-
11	3-methyl-3-buten-1-ol	0.7±0.4	-	-	-	-
12	3-methyl-1-butanol$^{\circ}$	2.6±0.8	-	-	6.5±1.6	28.3±6.2
13	2-methyl-1-butanol	-	-	-	1.1±0.5	-
14	1-pentanol$^{\Delta, \square, \circ}$	8.2±4	1.5±0.7	1.3±0.9	0.8±0.1	0.4±0.4
15	(Z)-2-penten-1-ol$^{\Delta, \square}$	12.5±8.7	1.1±0.7	0.2±0.1	-	-
16	3-methyl-2-pentanol$^{\Delta, \square}$	1±0.8	-	1.3±0.7	-	-
17	(Z)-3-hexen-1-ol $^{\Delta, \square, \circ, \times, \#}$	358.8±252.9	46±22.5	9.5±2.6	11.3±2.8	17.5±8.2
18	1-hexen-3-ol	5±1.9	2.5±1.1	-	0.2±0.2	-
19	1-hexanol$^{\Delta, \square, \circ}$	28.3±18.2	5.3±2.5	3.9±2.1	2.3±0.4	1.6±0.6
Esters						
20	3-methyl-1-butyl acetate$^{\circ}$	0.8±0.4	-	-	6.5±2.5	1.3±0.6
21	2-methyl-1-butyl acetate	-	-	-	0.4±0.2	-
22	(Z)-2-penten-1-yl acetate	182.2±112.3	24.4±16.9	0.1±0.1	1.7±1	-
23	pentyl acetate$^{\circ}$	17.6±10.8	2.7±1.7	-	1.3±0.5	-
24	(Z)-3-hexen-1-yl acetate$^{\Delta, \circ, \times, \#, \blacktriangle, \bullet}$	2175.8±1325.7	381.7±256.2	46.1±17.4	231±73.4	-
25	hexyl acetate$^{\Delta, \circ, \#, \blacktriangle}$	111.5±76.8	18.2±11.6	4.8±1.9	11.5±3.6	-
26	(Z)-3-hexen-1 yl-propanoate $^{\circ}$	2.2±1.2	-	-	-	-
27	heptyl acetate$^{\circ}$	4.7±2.3	1.5±1	0.3±0.3	0.9±0.5	-
28	2-ethylhexyl acetate	33.9±10.4	37.5±10.5	11.3±4.7	6.4±1.5	5.5±2.7
29	(Z)-3-hexen-1-yl butanoate	3.6±2.8	-	-	0.2±0.2	-
30	methyl salicylate$^{\Delta}$	1.5±0.6	0.8±0.6	0.9±0.6	3.2±1.8	-

Table 2. *(continued).*

31	(Z)-3-hexen-1-yl 2-methylbutanoate	6.9±3.7	0.4±0.4	-	0.3±0.2	-
32	(Z)-3-hexen-1-yl 3-methylbutanoate	3.2±2.3	-	-	3±0.6	-
<u>*Terpenoids*</u>						
33	*α*-thujene[×, Δ, ○, ●]	83.8±29	49.7±17.8	27.9±6.1	1.5±0.8	-
34	*α*-pinene[Δ, ×, #, ▲, ●]	19±4	11.5±2.3	6.1±1.8	0.7±0.3	-
35	thuja-2,4,(10)-diene	4.4±1.3	2.7±0.4	1.4±0.4	-	-
36	sabinene[Δ, ○, ×, #, ▲, ●]	595.9±212.2	345.7±112.2	233.5±45.1	31.2±10.6	-
37	*β* -pinene[Δ, ○, ×, #, ▲]	24.4±7.2	15.1±4.5	7.5±1.5	2.4±1	-
38	myrcene[Δ, ○, ×, #, ▲]	150.4±69.9	79.2±36.7	59.9±19.4	12.1±5.4	8.8±1.7
39	linalooloxide A, dehydroxy-	-	-	2.7±1.8	-	-
40	linalooloxide B, dehydroxy-	-	-	3.5±2.5	-	-
41	*α*-phellandrene	0.4±0.3	0.7±0.4	-	0.1±0.1	-
42	*α*-terpinene[Δ, ●]	8.9±4.5	6.4±2.7	0.6±0.5	0.6±0.6	-
43	limonene[Δ, ×, #, ●]	510.8±209.5	257.2±107.2	188.4±49	18.9±7.7	6.6±1.3
44	*β*-phellandrene[Δ, ×, #]	9.6±3.6	4.9±1.8	3.4±1	1.2±0.6	3±1.2
45	1,8-cineole[Δ, ○, ×, #, ▲]	255±79.1	127.9±40.6	41.2±8.9	39.6±14.6	-
46	2-oxo-1,8-cineole	-	-	-	2.3±1.6	-
47	(E)-*β*-ocimene [×, ●]	1.8±0.8	1.4±1.1	0.8±0.5	-	-
48	*γ*-terpinene[Δ, ○, ●]	10.1±4.2	7.9±3.8	2.5±0.6	0.6±0.6	-
49	trans-4-thujanol	36±15.5	14±7.5	10.1±3.2	0.2±0.1	-
50	*α*-terpinolene[●]	2.5±1.1	1.7±0.8	1.4±0.9	0.2±0.1	-
51	linalool oxide B	-	-	0.9±0.6	-	0.7±0.7
52	p-cymene[Δ]	2.1±1	0.9±0.5	0.5±0.2	0.1±0.1	-
53	linalool[Δ, □, ×, #]	3.9±3.9	-	-	-	53±9.1
54	cis-4-thujanol	10.8±4.8	3.4±3.3	3.3±2	-	-
55	(E)-3,4,8-dimethyl-1,3,7-nonatriene[Δ, ○, ●]	34.7±17.8	1.4±0.5	47.8±27.1	1.9±1.2	-
56	terpinen-4-ol	1.6±0.8	0.6±0.4	-	-	-
57	*α*-terpineol	0.6±0.4	0.4±0.4	0.2±0.2	3.2±1.7	1.3±0.7
58	cis-*β*-elemene, [Δ, ×, #]	-	-	73.8±57.5	-	-
59	germacrene D tent[Δ, ●]	-	-	-	3.2±1.2	-
60	(E,E)-*α*-farnesene [○, ×]	-	-	41.6±29.9	0.4±0.4	-
61	*β*-bisabolene	-	-	5.3±4.6	-	-
62	carvone	1.2±1.2	1.1±0.7	-	-	-
<u>*Aldehydes*</u>						
63	(E)-2-hexanal[Δ, ○, #, ▲, ●]	2.9±2.1	-	-	-	-
<u>*Sulphides*</u>						
64	dimethyl trisulphide[Δ, □, ○, ×, #, ▲]	1.7±0.5	1.2±0.3	0.8±0.3	0.5±0.1	-
<u>*Nitrogen containing*</u>						
65	benzyl cyanide[Δ]	11.5±5.1	0.8±0.8	-	-	-
66	indole[Δ, ×]	7.8±4.7	-	-	1.7±1.1	-
<u>*Unknown*</u>[b]						
67	unknown ^{81b,135,150}	-	-	-	-	7.9±0.8
68	unindent ^{mixt}	1.6±1.3	1.9±0.8	-	-	-
69	unk ^{107,108b,150}	2.9±1.3	1.3±0.8	0.1±0.1	-	-
70	unk ^{79,107,150b}	2.3±1.1	2.9±1.3	-	-	-
<u>Total amount</u>		4939.7±2586	1503.2±647	857.8±255	420.1±130	139.1±23

[a]- References related to volatile analysis on crucifers. [Δ]- compound detected by Geervliet et al., 1997; [□]- compound detected by Agelopoulos et al., 1995; [○]- compound detected by Mattiacci et al., 1994; [×]- compound detected by Tollsten and Bergström, 1988; [#]- compound detected by Blaakmeer et al., 1994; [▲]- compound detected by Agelopoulos and Keller, 1994; ●- compound detected by Shiojiri et al., 2001;
[b]-characteristic mass peaks, b denotes the base peak

Dissimilarities among the volatile profiles of different plants are presented in figure 4. The smallest total amount of volatiles was emitted by barley, followed by mustard, feral *B. oleracea,* uninfested Brussels sprout and host-infested Brussels sprout. The volatile profile of barley showed the greatest dissimilarity among the treatments, with only 15 of compounds detected out of the 70. Fourteen of these compounds were also present in the volatile blend of at least one of the other plant sources. The dominant compounds produced by barley were linalool, which was also present in smaller amounts in infested Brussels sprout, and 3-methyl-1-butanol, which was detected in smaller amounts both in the volatile blends of mustard and infested Brussels sprout.

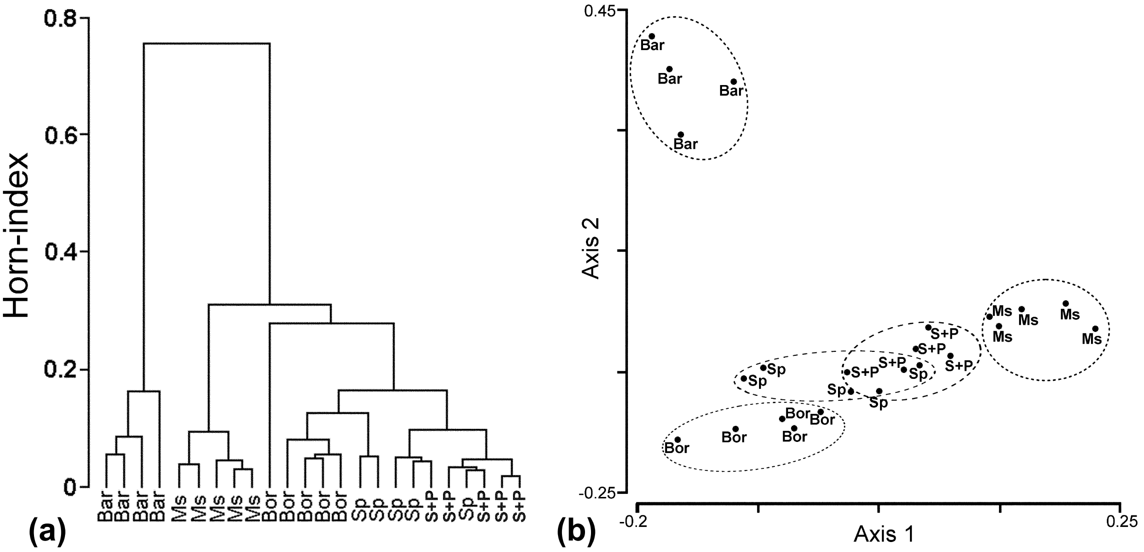

Figure 4. Dissimilarities in volatile profiles of plants. **(a)** Hierarchic clustering and **(b)** non-metric scaling of dissimilarities in volatile profiles based on the Horn-index. "Bar"-barley, "Ms"-mustard, "Bor"-feral *B. oleracea*, "Sp"-uninfested Brussels sprout, "S+P"-Brussels sprout infested by *P. xylostella* larvae.

The volatile blend of mustard was more similar to the other crucifers than to barley but it still grouped out from the Brussels sprout and the feral *B. oleracea* samples (fig. 4). The 46 compounds detected in mustard were greatly overlapping with those detected in Brussels sprout and the feral *B. oleracea*. Only a few compounds like 2-methyl-1-butyl acetate, 2-oxo-1,8-cineole and germacrene D were detected in mustard alone. The compounds dominantly produced by mustard were the so-called green-leaf volatiles (GLVs) like (Z)-3-hexen-1-yl acetate, (Z)-3-hexen-1-ol and hexyl acetate. Other compounds that were detected in mustard were the terpenoids 1,8-cineole and sabinene. These GLV's and terpenoids also dominated the odour blend of uninfested Brussels sprout, but were produced by infested Brussels sprout in greater quantities (table 2). Volatiles like (Z)-3-hexen-1-yl 3-methylbutanoate, and other compounds like 3-methyl-1-butanol, 3-methyl-1-butyl acetate, and indole were detected in mustard and infested Brussels sprout only.

Volatile profiles of infested and uninfested Brussels sprout plants showed the highest similarity. 59 compounds were detected in the headspace of Brussels sprout infested by *P. xylostella*, 11 of these compounds were not detected in the headspace of uninfested Brussels sprouts (table 2).

Compounds produced in infested Brussels sprout in higher amounts than in uninfested plants were 1-penten-3-ol, (Z)-3-hexen-1-ol, (Z)-2-penten-1-yl acetate, (Z)-3-hexen-1-yl acetate, hexyl acetate and benzyl cyanide. The volatile profile of feral *B. oleracea* was different from that of infested Brussels sprout, but was very similar to that of uninfested Brussels sprout. Some compounds like linalool oxide B, dehydroxylinalool oxide A dehydroxylinalool oxide B were present in the feral *B. oleracea* only. *β*-bisabolene and cis-*β*-elemene were detected in the feral *Brassica oleracea* only (table 2). (E,E)-*α*-Farnesene was found in high amounts in the feral *B. oleracea* and mustard only, whereas 3-methyl-2-pentanol was present in the volatile blend of the feral *B. oleracea* and that of infested Brussels sprout only.

4. Discussion

4.1. Responses of D. semiclausum to inter-and intra- specific variation in volatiles

Diadegma semiclausum is attracted to volatiles emitted by undamaged and host-infested cabbage (Ohara et al., 2003). In this study, *D. semiclausum* preferred all tested plant species, including barley, over clean air. Yet, when uninfested plants of different species were tested against each other, females clearly preferred volatile blends of crucifers to barley. In a comparable study, *Cotesia kariyai* a parasitoid of the noctuid *Pseudaletia separata*, was attracted to several unrelated non-host food plants, which was explained by the presence of GLVs in the headspace of the different plant species (Takabayashi et al., 1991). Except one compound, all the compounds produced by barley, were also present in the headspace of crucifers, including the GLV (Z)-3-hexen-1-ol and terpenoid compounds, which could explain the preference of wasps towards barley odours compared with clean air.

Parasitoids further discriminated between odours of uninfested crucifers. Despite of the lower amounts of total volatiles and the fewer compounds produced by both mustard and the feral *Brassica*, these plants were still more attractive to *D. semiclausum* than Brussels sprout was. Some alcohols and terpenoids emitted by mustard were present in this species only, and some alcohols, esters and indole were detected both in mustard and infested Brussels sprout. The GLV (Z)-3-hexen-1-yl acetate, known to be produced by herbivore-infested Brussels sprout in greater quantities than by uninfested plants (Blaakmeer et al., 1994, Mattiacci et al., 1994, Geervliet et al., 1997), was the dominant compound that mustard produced. White mustard also contains specific aromatic hydroxy-benzyl and benzyl glucosinolates (McCloskey and Isman, 1993, Hopkins et al., 1998, Mewis et al., 2002), and aromatic derivatives of these glucosinolates were detected in headspace of uninfested mustard (Tollsten and Bergström, 1988). We did not detect these derivatives in our samples, possibly due to differences in methodology for collecting and analysing samples. Tollsten and Bergström (1988) used cut plants for example, whereas we used potted plants.

Females did not discriminate between odour blends of plants based on quantitative differences in volatile production only. This is suggested by the finding that the greater amounts of plant volatiles produced did not mean stronger attraction of *D. semiclausum* towards plants. While qualitative differences in volatile blends were probably important for females to discriminate between odours of barley and crucifers, differences in quantity and ratios of compounds could be important in the discrimination between mustard and Brussels sprout. The volatile profile of the feral *B. oleracea* was very similar to that of uninfested Brussels sprout, with a few compounds detected in the feral *B. oleracea* exclusively. Yet, wasps were able to discriminate between these lines of *B. oleracea*, indicating that subtle differences in volatile profiles of uninfested plants could be important in the attractivity of these plants to *D. semiclausum*.

4.2. Responses of D. semiclausum *to host-infested Brussels sprout*

Brussels sprout plants damaged by the hosts were more attractive for the parasitoids *Cotesia glomerata, C.rubecula*, *C.plutellae* and *D. semiclausum* than artificially damaged or intact Brussels sprout plants (Steinberg et al., 1992, 1993, Blaakmeer et al., 1994, Geervliet 1997, Shiojiri et al, 2000, Ohara et al., 2003). Our results also suggest that *D. semiclausum* assessed changes in volatile blends as a result of herbivore damage. The result that infested Brussels sprout plants emitted GLVs in a generally greater amount, but similar to those of uninfested plants, was in line with findings of other studies (Blaakmeer et al., 1994, Geervliet et al., 1997, Reddy and Guerrero, 2000, Smid et al., 2002). Compounds like 3-pentanol, linalool, (E)-2-hexenal and indole were mostly detected in *Plutella*-infested Brussels sprout. Some of these GLVs elicited olfactory responses and were held responsible for the attraction of the parasitoids *Cotesia rubecula*, *C. glomerata* and *C. plutellae,* and also for the predatory lacewing *Chrysoperla carnea* (Geervliet et al. 1997, Reddy et al., 2002, Smid et al., 2002). The volatile profile of *Plutella*-infested Brussels sprout shared great similarities with volatile profiles of white cabbage and Brussels sprout, when infested by different *Pieris* spp (Geervliet et al., 1997, Blaakmeer et al., 1994).

Based on dissimilarities in the volatile profiles, wasps were able to discriminate between infested and uninfested Brussels sprout plants. Interestingly, such discrimination was not observed when infested Brussels sprout was compared to mustard, although dissimilarities in volatile profiles were greater here. Hence, we may not hypothesise that females could not discriminate between mustard and infested Brussels sprout because the differences were too small. Instead, they might not discriminate, because the “values” of volatile information (that is the expectancy of the host’s presence) from these plant-sources were similar. Based on studies on *Leptopilina heterotoma*, parasitoid of *Drosophila* spp., Vet et al. (1998) hypothesised that parasitoids may actively choose not to discriminate among subtle, quantitative differences and rely on qualitative differences until they learn to discriminate via experiences. Our data suggest that such a mechanism may play a role in the searching behaviour of *D. semiclausum*: While host location by inexperienced females on Brussels sprout was hindered by the greater preference of wasps to search uninfested mustard, oviposition experiences enabled females to locate subsequent host-infested plants with equal efficiency irrespective of neighbouring plant species (Chapters 6,7). In complex habitats, a host species may exploit different plant species, depending on its food-plant preference. Mustard is a preferred food plant of *P. xylostella* and is used as a trap crop (Palaniswamy et al., 1986, Talekar and Shelton, 1993). *P. xylostella* develops faster and reaches greater body weight on white mustard than on Brussels sprout (R. Gols, unpublished). Hence, if the presence of *P. xylostella* on these plants is different, it could be a viable strategy for *D. semiclausum* to have a preference towards volatile blends that reflects the food-plant preference of the host. In the field however, the same plant is often infested by different herbivore species. Parasitoids, like *C. glomerata* could not discriminate between volatiles of plants infested either by hosts or non-hosts (Vos et al., 2001). If this is true for *D. semiclausum,* it could be another explanation of the high initial preference for mustard odours: once inside the habitat, less time is wasted on searching among plant-herbivore communities till a suitable host-patch is found.

As yet, we have no information on how the volatile composition of mustard is influenced by herbivore damage, nor on the discriminatory ability of the parasitoid *D. semiclausum* with respect to hosts and non-hosts on the same plant species. However, plants in natural ecosystems are rarely uninfested and finding a suitable host on the more preferred mustard could result in a preference for uninfested mustard over infested Brussels sprout. This could magnify any effects of heterospecific plants on host-parasitoid dynamics. It would also mean that the importance of herbivore-induced synomones, as solution to the reliability-detectability problem for this parasitoid, would be ultimately influenced by vegetation diversity of the habitat. An interesting topic future research could be to study how inter- and intraspecific variation in plant volatiles influences the ability of this parasitoid to discriminate between plants infested by hosts and non-hosts.

4.3. Volatile attraction and indirect defences

Results from this and other studies may indicate that leaf vegetables that are selected for low concentrations of allelochemicals (i.e. Brussels sprout) could have a lowered ability to attract the specialist parasitoids than their wild ancestors (Fox and Eisenbach, 1992). As plant breeding and biological control were developing independently, we have limited information on what mechanisms are responsible for triggering different responses of parasitoids between plant species and genotypes within plant species (van Lenteren et al., 1995, Bottrell et al., 1998, Cortesero et al., 2000). The finding that the feral *Brassica oleracea* was more attractive than cultivated conspecifics might indicate that artificial selection in cultivated plants have changed their ability to attract natural enemies compared with wild relatives. However, further studies are needed to explicitly test this hypothesis. Our results indicate that volatile traits responsible for the attraction of natural enemies to plants could be valuable to consider in breeding programmes aiming to enhance biological control. Although some traits that increase attractivity for the parasitoid may also attract herbivores, such traits could still be valuable tools in developing pest-suppressive diversification strategies (i.e. companion planting, intercropping), if the role of increasing plant species diversity in host-parasitoid interactions are better understood.

Acknowledgements.

This study is part of the project “Enhanced biodiversity” funded by the section Earth and Life Sciences of the Dutch Scientific Organisation (NWO-ALW, project number: 014-22.031). We thank Wouter Tigges for rearing the parasitoids, Marcel Dicke and Jeffrey A. Harvey for constructive comments on the manuscript. J.A. Harvey provided the feral *B. oleracea* plants.

Chapter

6

Plant composition of the habitat influences searching behaviour of the parasitoid *Diadegma semiclausum* (Hellén)

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Plant composition of habitat influences searching behaviour of the parasitoid *Diadegma semiclausum* (Hellén)

Abstract

The searching behaviour of *Diadegma semiclausum* was studied in semi-field set-ups of different plant composition and configuration. The set-up consisted of a group of plants that surrounded the target Brussels sprout plant infested by the host *Plutella xylostella*. We studied (1) the effect of plant density *per se* on foraging behaviour in monotypic set-ups where the target plant was surrounded by uninfested conspecifics in low (8 plants) and high (20 plants) densities. (2) The effect of plant species composition and spatial arrangement were studied by interspersing uninfested sprout plants by malting barley or white mustard in different horizontal (row, mix) arrangements. We tested (3) the effect of vertical arrangement on foraging behaviour by varying the height of mustard plants relative to the Brussels sprout plants. No effect of density of conspecific plants or horizontal arrangement of plants were found on the efficiency of wasps to locate the target plant. When the effect of plant composition was tested, wasps in the monotypic stand located the target plant the fastest, followed by barley and the mustard set-ups. When compared to the monotypic stand, an increase in percent of visits on the barley explained the lower efficiency of females to find the target plant. However, percent of visits alone did not explain the lower efficiency to find the target plant in the mustard. A higher searching activity on mustard also contributed to the delay in locating the target plant. The organisation of behaviours was similar in the mustard and the monotypic set-up, but different in barley. Plant height seemed to influence foraging behaviour of females. Irrespective of its relative height to Brussels sprout plants, tall mustard decreased the tendency of wasps to locate the target plant when compared to short mustard plants. The importance of structural and volatile complexity of the habitat on foraging behaviour of parasitoids is discussed.

Key words: *Diadegma semiclausum, Plutella xylostella*, vegetation diversity, foraging behaviour, host location, proportional hazards model;

1. Introduction

Insect parasitoids are evolved to find their host in a heterogeneous environment, where their target is nested across several spatial levels. These levels cover the range from the landscape-level vegetation patches to between-plant (neighbourhood of different plant species), down to within-plant (e.g. individual leaf or leaflet) patches. Consequently, when emerging outside the habitat of their host, the host selection process of parasitoids involves a chain of hierarchic steps until successful parasitism is achieved (Vinson 1998, Vinson 1976). In this hierarchic process, parasitoids use different cues from plants (i.e. infochemical, visual) for the long-range orientation towards their host (Vet et al. 1991, Vet and Dicke 1992, Vet et. al., 2002). When searching within the habitat of their host or landing on the host's plant, visual, structural and geometric properties of the host's plant and its neighbourhood are also important (Vinson, 1976, Need and Burbutis, 1979, Andow and Prokrym 1990, Wäckers, 1994). The structure, heterogeneity and connectivity of leaf surface may influence parasitoid searching efficiency and subsequent parasitism (Andow and Prokrym, 1990, Barbosa and Benrey, 1998, Gingras et al. 2002, Gingras and Boivin, 2002, Casas and Djemai, 2002). Therefore, in natural habitats with a diverse plant composition, neighbouring plants enveloping host-infested target plants may influence the way cues from host plants are being perceived by the searching wasp. This local environmental effect on parasitoid foraging efficiency may play an important role in shaping local host-parasitoid interactions.

The greater success of natural enemies in the control of local herbivore populations were often held responsible for less frequent pest outbreaks in vegetationally diverse natural than in simple agro-ecosystems (Elton, 1958, Root, 1973, Risch et al., 1983, Sheehan, 1986, Russell, 1989). However, great variation from negative, neutral to positive effects of habitat diversification on parasitism levels were observed, even when the same tritrophic system was studied (Russell, 1989, Perfecto and Vet, 2003). Differences in parasitism are emergent properties of complex individual (e.g. behavioural) level interactions between trophic levels (Vet, 2001, Dicke et al., 2003). Although Price et al. (1980) drew attention to the study of natural enemies in a multitrophic context, little effort was made to study foraging behaviour of carnivores in relation to plant composition of habitats (Dicke et al., 2003). Most experimental studies are restricted to tri-trophic interactions that are examined within a single species plant-host complex under laboratory conditions (Hunter, 2002b, but see Dicke et al., 2003). Although some studies do investigate the influence of neighbouring plant species, they concentrate on single attributes (e.g. volatiles) in simplistic laboratory bioassays and disregard effects of other factors (e.g. structure) (Hunter, 2002b). Hence, predictions on parasitoid foraging in nature may be misleading, when observations are based on traits of single plant species. The presence of the few existing studies on parasitoid foraging behaviour in diverse habitats (Khan et al., 1997, Gohole, 2003, Perfecto and Vet, 2003) underlines the need to study foraging behaviour of parasitoids in more realistic experimental set-ups. This was the reason why we investigated the effects of different components of diverse vegetation on the behaviour of *D. semiclausum*, foraging for *P. xylostella* on Brussels sprout plants.

The diamondback moth (*Plutella xylostella* L.) is a herbivore specialised on cruciferous plants, with a worldwide distribution and economic importance (Saucke et al., 2000, Talekar and Shelton, 1993). *Diadegma semiclausum* (Hellén) is its specialist parasitoid and one of its most important biological control agents (Verkerk and Wright, 1996). *D. semiclausum* relies on infochemicals both from the plant and the host during host selection (Davis 1987, Ohara et al., 2003).

We hypothesised that the species composition of plants and their configuration influence the efficiency of *D. semiclausum* to locate host patches. The experimental set-up consisted of a group of plants surrounding a host-infested (target) Brussels sprout plant. The target plant was considered a host-patch, because the whole plant contained hosts in a uniform distribution and females immediately encountered the first host when landing on the target plant. To test our hypotheses we followed the foraging behaviour of *D. semiclausum* in a semi-field environment, where host-infested target plants were surrounded by different species of neighbouring (i.e. companion) undamaged plants. We investigated if efficiency of females to locate the target plant was influenced when (1) surrounding uninfested Brussels sprout plants (i.e. non-target plants) were present in low and high densities. The tendency of females to start searching the set-up and to find the target plants were compared in experiments (2) non-target plants were interspersed with the companion plants malting barley or white mustard. Here, the effect of habitat configuration was also tested by arranging the companion plants in the set-up in two different horizontal (row, mix) arrangements. (3) In a third experiment, we varied the height of mustard relative to the target and non-target plants to test if vertical configuration of the neighbourhood relative to the target plant influenced the foraging behaviour of *D. semiclausum*

2. Materials and Methods

2.1. Parasitoids

Diadegma semiclausum was collected from Brussels sprout fields in the vicinity of Wageningen (The Netherlands) and was reared on Brussels sprout infested with larvae of the diamondback moth. The rearing was maintained in a climate room (20 ± 2º C, 70% RH and L16-D8 photoperiod). Sprout plants infested with the host were obtained from a mass rearing maintained at our laboratory. Parasitoid cocoons were collected weekly and were transferred into a clean insect cage (37cm x 40cm x 30cm) with no plant material or host present. To obtain mated females we transferred newly emerged wasps, seven females and seven males into glass tubes (20 cm long, ø 5 cm) that were covered by gauze at both ends. Water and honey was administered *ad libitum* on the gauze. The day before an observation, females were separated from males and were transferred to another cage (42cm x 22cm x 22cm). For the observations five to ten day old females without oviposition experience were used.

2.2. Plants

We used the crucifer Brussels sprout (*Brassica oleracea* L. gemmifera cv. Cyrus) as a host plant for *P. xylostella.* White mustard (*Sinapis alba* L. cv. Carneval) was used as a cruciferous companion plant, and malting barley (*Hordeum vulgare* L. cv. Video) as a non-cruciferous companion plant. All plants were grown in a greenhouse (20-28ºC, 40-80% RH. and L16-D8 photoperiod). Brussels sprouts (one plant per pot) reached a biomass of about 30 to 35 g in 4-5 weeks. To obtain similar plant biomass per pot for barley and mustard plants in the different set-ups (20-25 g in 4-5 weeks), we varied the number of plants per pot with companion plant species. We used nine plants per pot for mustard and 18 plants per pot for barley.

2.3. Experimental set-up

We conducted the three experiments in a ‘flight chamber’ (for details see Wiskerke & Vet 1994) installed in a greenhouse compartment under the same conditions as mentioned in the “plants” section but without additional illumination. No fans were used to provide directed airflow. The greenhouse compartment was continuously ventilated so there was a turbulent air-current inside the tent. The basic set-up consisted of nine Brussels sprout plants in three rows of three plants with only the centre plant (target plant) containing the hosts (fig. 1a). Fifteen third instar *P. xylostella* were evenly distributed over three (top, middle, and bottom) leaves of the target plant in the late afternoon a day before the experiment. These densities were comparable with moderately high densities found in local cabbage fields (Bukovinszky, personal observation). In all three experiments, only the target plants contained hosts, all other plants were undamaged. Experiments I and II were carried out from May to September 2002, and experiment III was carried out from September to November 2002. Within these two observation periods different set-ups were randomly used and at least two treatments were tested on the same day.

2.3.1. Experiment I- Effect of plant density

This experiment was performed to see if changing plant density *per se* influenced searching efficiency of *D. semiclausum*. We compared the behaviour of females in low (9 sprout plants) and high-density (21 sprout plants) monotypic stands (fig 1ab).

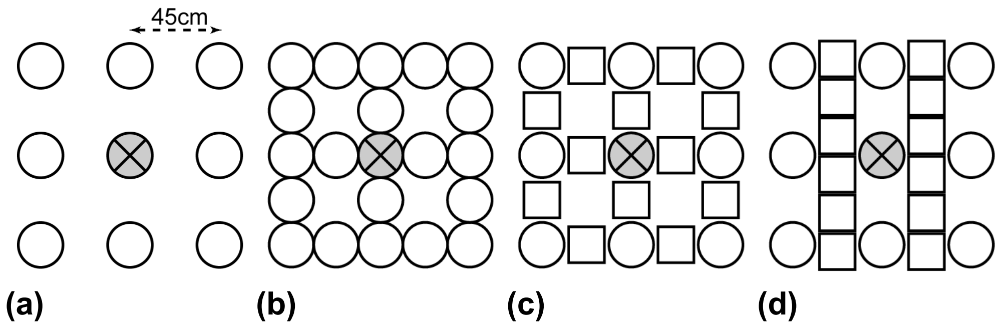

Figure 1. The experimental set-up. ⊗ –target (host-infested) Brussels sprout plant; ○ -non-target (uninfested) B. sprout plants; ☐ -Companion (uninfested) plants that were either mustard, barley or in the monotypic set-up B. sprout; Monotypic set-ups were in a **(a)** low or a **(b)** high density arrangement. Companion plants were either in the **(c)** mixed or in the **(d)** row arrangement. The target plant was at 90cm distance from the release station.

2.3.2. *Experiment II- Effect of plant species composition and horizontal arrangement*

In the second experiment we increased species diversity by replacing sprout plants with either barley or mustard plants as illustrated in figure 1bc. We also varied the horizontal spatial arrangement of plants. Brussels sprout plants were interspersed with twelve pots of mustard or barley (mixed configuration, fig 1c), or were arranged as two rows of six plants between the sprout plants (row configuration, fig1d). The two resulting data sets were compared to the high-density sprout data set from experiment I (fig 1b).

2.3.3. *Experiment III- Effect of vegetation height*

In the third set-up, we examined if the vertical configuration of the companion plant relative to the Brussels sprout plants, influenced the success of females in finding the target plant.

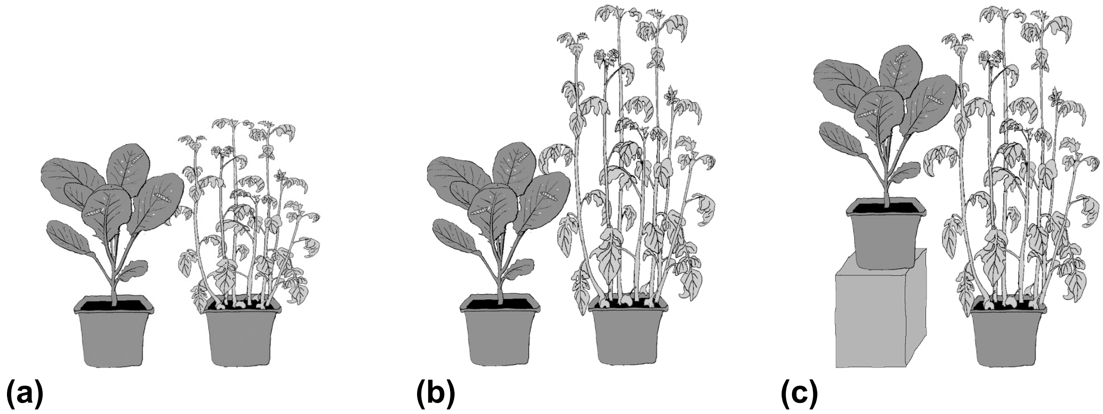

Figure 2. Illustration of the vertical arrangement of target plants and companion mustard plants (experiment III.). The **(a)** short mustard plants were 3 weeks old, so their height was the same as the Brussels sprout plants. The **(b)** tall mustard plants were 4 weeks old and were either 20 cm taller than sprout plants or sprout plants were **(c)** elevated to a relative height comparable to the short mustard.

In this experiment 3 and 4 week old mustard plants were used as short and tall plants, respectively. Plants were placed on the table in a row arrangement (fig. 1d) and short mustard as companion plants served as the control set-up (fig. 2a). In two treatments the tall mustard plants were either placed on the table (fig. 2b), or on a 20 cm high platform elevating them to the height of the mustard comparable to the control set-up (fig 2c).

The difference between 3 and 4 weeks old plants was mainly the elongation of internodes and to a lesser extent an increase in leaf area. Plant height was 21.3±2.7 (mean±SD) cm for short and 40.2±2.7 cm for tall mustard and leaf area per pot (9 plants) was 677.8±139.6 cm^2 for short and 812.7±98.6 cm^2 for tall mustard (n=54).

2.4. Behavioural observations

We put a female parasitoid in a vial on the release station made of a glass cylinder (30 cm long, ø 15 cm) placed at a height of 10 cm and situated 90 cm from the target plant. A leaf disc damaged by the host but without the host itself, was put in the vial with the female to increase her motivation to search for hosts (Geervliet 1997, Potting et al. 1999). Each observation started when the wasp left the release platform and ended when it arrived at the target plant. To compare the tendency of wasps to start searching and their efficiency in host-finding in the different set-ups, times were measured from taking off until the first landing in the vegetation, and until the arrival at the target plant (fig. 3).

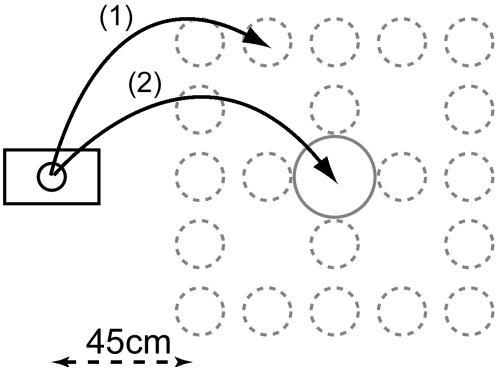

Figure 3. The two variables recorded for comparison by the proportional hazards model. The (1) tendency to start searching was compared by recording the time from leaving the release platform until landing in the set-up. The (2) foraging efficiency was recorded as the time from leaving the release platform until the first landing on the target plant.

To record the foraging behaviour and position of wasps in the set-up, a handheld microcomputer (Workabout Psion PLC 1995$^©$), with The Observer software 3.0 (Noldus Information Technology 1993$^©$) was used. The recorded behavioural elements are described in table 1. Four behaviours were recorded: flying, walking, standing still and grooming.

Table 1. The description of the behavioural elements recorded in the experiments.

Element	Description
Fly	The parasitoid is airborne. This behaviour included both the close-range inspection of plants by hovering and flight behaviour between plants.
Walk	The wasp moves on the plant surface with a more or less constant speed.
Groom	The wasp stands still and cleans the antennae with its forelegs, or the wings and the abdomen with its hind legs.
Stand still	The wasp is motionless.

The position of the parasitoid was recorded as being either on the non-target or on the companion plants. The distinguished levels of resolution of the recorded behavioural elements is demonstrated in figure 4. The time spent on the table and on the fabric of the tent was not considered in the analysis. Each wasp was used only once. Plants infested by *P. xylostella* were used 1-5 times. Females that flew to the ceiling before finding the target plant and did not return in the set-up within five minutes were discarded from the analysis. At least 25 observations were carried out in each set-up.

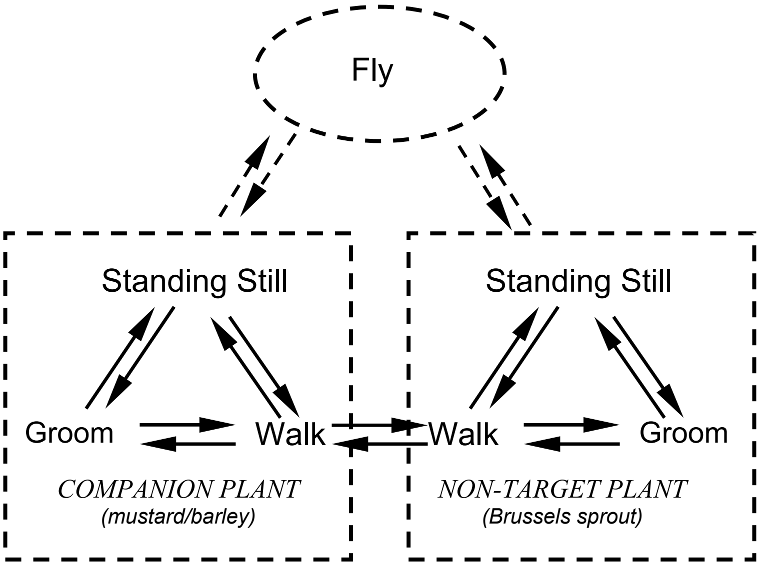

Figure 4. Distinguished levels of resolution of behavioural observations until arrival to the target plant. The spatial compartments (rectangles) were companion plants (mustard/barley) or non-target plants (non-infested B. sprout). Arrows represent possible transitions from one behaviour or spatial compartment to the other. Flight could follow any of the observed behavioural elements (dashed arrows). Note that only one spatial compartment (non-target plant) was considered in the high and low density monotypic stands (see fig 1ab).

2.5. *Data analysis*

2.5.1. *Plant location*

To analyse the time-structured data (i.e. start searching the set-up, arrival to the target plant), the Cox`s proportional hazards model was used (Cox, 1972, Kalbfleisch and Prentice, 1980, Ormel et al., 1995). The model is described by the hazard model formula that models the effect of p fixed (time-independent) covariates z_i ($i=1\ldots,p$) on the hazard rate for the event performed by an individual at time t. The general form of the proportional hazards model is given in eqn 1.

$$h(t;z) = h_0(t)\exp\left\{\sum_{i=1}^{p}\beta_i z_i\right\} \qquad \text{eqn 1}$$

It is assumed that for different values of z_i, the hazard rates $h(t;z)$ are proportional, where $h(t;z)$ is the probability per second for the event to occur, and h_0 is the observed baseline hazard. The β values are coefficients representing the relative strength of the covariates z_i, thus exp (β_i) is the effect on the hazard rate of increasing z_i with 1. The hazard at time t is the product of the baseline hazard $h_0(t)$ and the exponential linear sum of $\beta_i z_i$. When for different values of z_i the hazard rate is not proportional, the extended model for time dependent covariates may be used (Kleinbaum, 1996). The general form of the model with fixed and time-dependent covariates is described in equation 2, where it is assumed that the hazard at time t depends on the value of $z_j(t)$ at the same time, where δ_j is the relative strength of the covariate $z_j(t)$.

$$h(t;z(t)) = h_0(t)\exp\left\{\sum_{i=1}^{p1}\beta_i z_i + \sum_{j=1}^{p2}\delta_j z_j(t)\right\} \qquad \text{eqn 2}$$

2.5.2. *Selection of covariates*

The summary of the covariates used in the three experiments and their coding are presented in table 2. In the three experiments the fixed covariates used were plant density (low, high), plant composition (monotypic, mustard, barley), configuration (mixture, row) and height of plants (short, tall, elevated). These covariates were handled as categorical variables and were coded 0,1, where the number of covariates depended on the factor levels. The control in the three experiments were the low and high density monotypic set-ups and the short mustard set-up, respectively. The control was considered to have baseline hazards for the occurrence of the event and were coded 0,0. While exploring the different set-ups, experiences with companion plants may influence the efficiency of wasps to locate the target plant. Therefore visits to companion plants may be considered as repeated choices that a wasp made during searching. We studied this effect in experiments II and III by including the visits to companion plants as a time-dependent covariate. The time-dependent covariate was expressed as the percent of the cumulative visits to the companion plants of total visits to both compartments (table 2). To estimate the value of δ_i for the time-dependent covariate z_j, the value of this covariate was defined for each of the failure times of each individuals at risk. Analysis was carried out by SAS 8.02 (PROC PHREG, ©SAS Inc.).

Table 2. Coding of the different covariates in the three experiments where effects on arrival tendencies to target plants have been estimated.

Experiment I.	**Covariate (z_{ij})**	**Code**
Z_1	Plant density	0-Low (9 plants), 1-High (21 plants)
Experiment II.		
Z_1	Plant species	*0*-B. sprouts, mustard, *1*-barley;
Z_2	Plant species	*0*-B. sprouts, barley, *1*-mustard;
Z_3	Configuration	*0*-Mixed, *1*-row;
Z_4	% Visits to companion plant	$c(t)/c(t)+n(t)*100$[§]
Z_5	Interaction Z_1*Z_4	*0* or $c(t)/c(t)+n(t)*100$
Z_6	Interaction Z_2*Z_4	*0* or $c(t)/c(t)+n(t)*100$
Experiment III.		
Z_1	Height	*0*-short, tall, *1*-elevated;
Z_2	Height	*0*-short, elevated, *1*-tall;
Z_3	% Visits to companion plant	$(c(t)/c(t)+n(t))*100$[§]

[§]- where c(t) is the cumulative number of visits on companion plants and n(t) the cumulative number of visits on non-target Brussels sprout plants between time 0 and t.

2.5.3. Searching behaviour

The behaviour of wasps in experiments I and II were compared. Four behavioural elements (fly, walk, groom, stand-still) were extracted from the dataset. As a preliminary analysis did not detect any differences between the low and the high density monotypic stands and the row and mixed configurations, individuals within the monotypic, barley and mustard set-ups were pooled for further analysis. Analysis by Kruskal Wallis ANOVA followed by Mann-Whitney U-tests were used to detect differences between groups. To correct for type I error (Sokal and Rohlf, 1995), the sequential Bonferroni procedure was used (Rice, 1989).

To describe the organisation of behavioural elements in the different set-ups the data was subjected to socio-metric analysis. All individuals within a set-up were pooled, and first order markovian transition matrices were constructed, where row and column entries corresponded to preceding and succeeding behavioural elements, respectively. Pooling of data was necessary so that the expected values in the matrix were larger than 0 and less than 20% of the entries were smaller than 5. The diagonals of these matrices were logical zeros, as behavioural elements could not follow themselves. The expected values of the cells were found by the iterative proportional fitting method of Goodman (1968) and were compared to the observed values by log-likelihood statistics (G-test). When significant deviations from expected values were found within a table, it was truncated into 2x2 contingency tables and individual G-tests were carried out, corrected by the sequential Bonferroni procedure for table-wide α levels (Rice, 1989). Yates correction for continuity was applied throughout (Sokal and Rohlf, 1995). Behavioural kinetograms were constructed to illustrate differences in the behavioural flows within and between the three set-ups.

3. Results

Increasing the number of conspecific plants in the set-up from nine to twenty one did not significantly influence arrival times on infested plants (expβ=0.974, P=0.92, df=1, Chi^2=0.01, fig. 5).

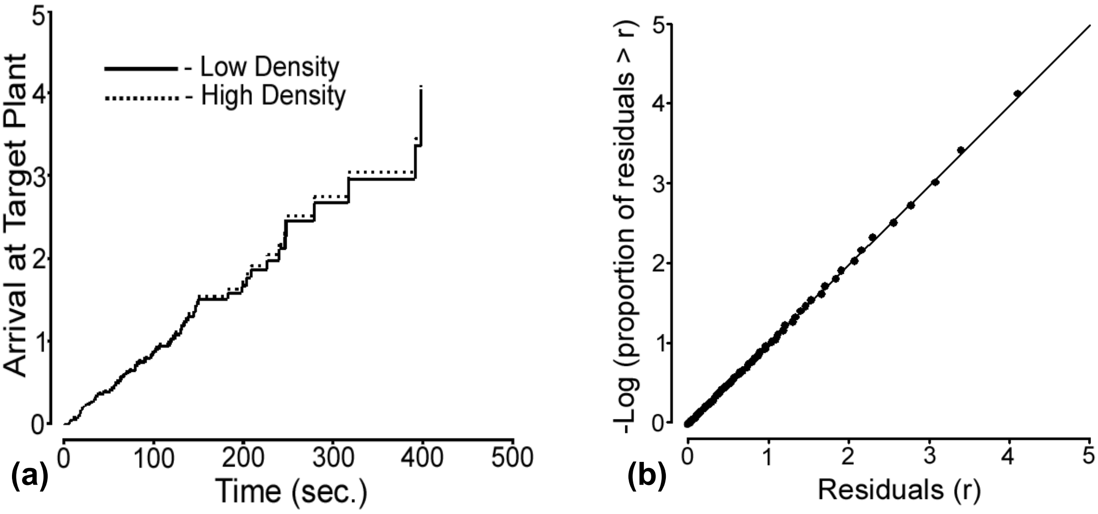

Figure 5. (a) Tendency of wasps to find the target plant containing hosts, when Brussels sprout plants are surrounded by low (solid line, 8 plants) or high densities (dotted line, 20 plants) of conspecifics. **(b)** Graphical test for goodness of fit. When the model fits, the dots should be on a straight line through the origo with a slope of 45°.

However, replacing Brussels sprout plants with heterospecifics (mustard or barley) influenced searching parasitoids in different ways. Parasitoids showed different tendencies to start searching the set-up. After taking off, wasps in the mustard started to search the fastest, although this difference was not significant when compared to the monotypic set-up (fig. 6, table 3). Wasps showed a lower tendency to start searching in the vegetation in the barley set-up when compared to the high-density monoculture, but this difference was also not significant. However, when compared to the mustard, wasps in the barley started to search significantly later (expβ=0.521, P= 0.03, df=3, Chi^2=9.29, fig. 6). The arrangement of plants in the arena (mix, row) had no effect on landing tendencies.

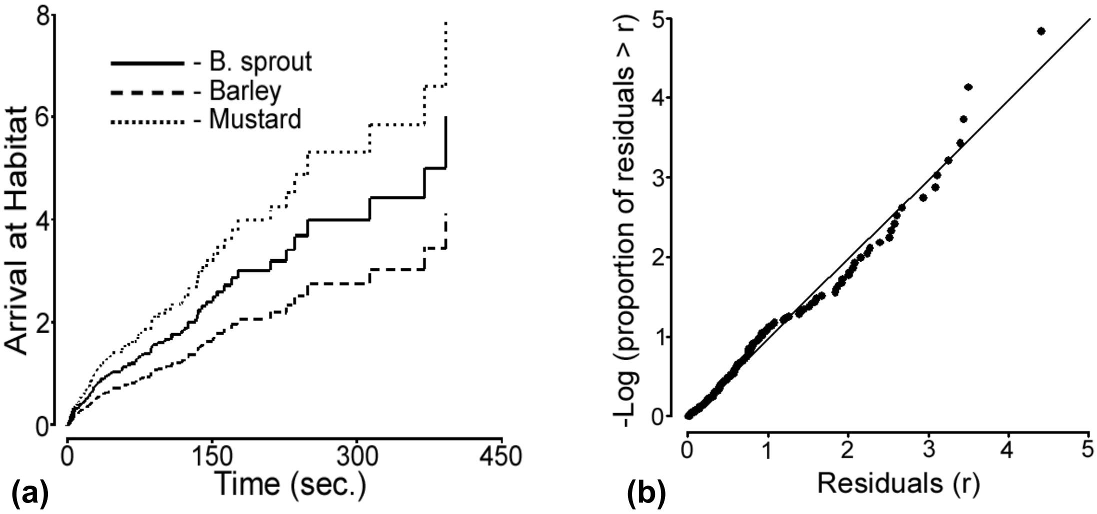

Figure 6. (a) Tendency of wasps to start searching in the set-up, when Brussels sprout plants are surrounded by conspecifics (solid line), or mixed with barley (dashed line) or mustard (dotted line). **(b)** Graphical test for goodness of fit. When the model fits, the dots should be on a straight line through the origo with a slope of 45°.

Table 3. Effects of different plant species (barley, mustard) and arrangement of plants (mix, row) on tendencies to land on the vegetation for the first time.

Covariates	**Chi^2 (df)**	**Estimated effect (exp β)**
Barley	1.1(3)ns	0.754
Mustard	1.86(3)ns	1.447
Configuration (Mix/Row)	0.7(3)ns	0.843

ns –not significant, * -P<0.05, ** -P<<0.05;

While the configuration did not influence the time until arrival at the target plant, plant-species composition did (table 4). Wasps arrived at the target plant earlier in the monotypic stand compared to the barley or the mustard set-ups (fig. 7). The lowest arrival tendencies were observed in the mustard set-up. When we included the percent of visits to the companion plants, it had a significantly positive effect on the tendency to arrive at the target plant (table 4): an increasing probability of arrival was found with an increasing number of visits to companion plants. However, including the percent of visits to companion plants, the effects of both plant species on arrival tendencies stayed significant, suggesting that visits to companion plants did not fully explain the effect of plant species on arrival at the target plant.

Table 4. Effects of covariates (companion plant species, configuration, visits on companion plants) on arrival tendencies of wasps to target (infested) plants. For formulation of the last covariate, see materials and methods.

Covariates	Chi^2 (df)	Estimated effect (exp β)	Effect on arrival
Barley	12.99(4)**	0.332	↓
Mustard	28.11(4)**	0.120	↓
Configuration (Mix/Row)	1.67(4)ns	0.763	
% Visits to companion plant	14.28(4)**	1.012	↑

ns –not significant, * -P<0.05, ** -P<<0.05;

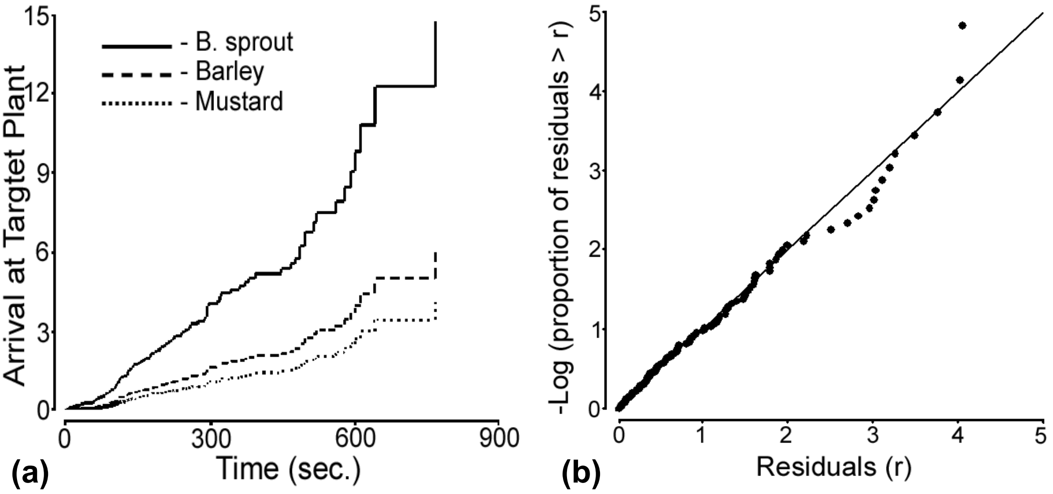

Figure 7. (a) Tendency of wasps to find the target plant when Brussels sprout plants are surrounded by conspecifics (solid line), or mixed with barley (dashed line) or mustard (dotted line). **(b)** Graphical test for goodness of fit. When the model fits, the dots should be on a straight line through the origo with a slope of 45^{o}.

We investigated whether visits to companion plants depended on the plant species, by testing the interactions between the fixed and the time-dependent covariates. This meant that the data was stratified (divided) into subgroups according to different values of the fixed covariate (for details see Hemerik et al., 1993), assuming that for each level of the fixed covariate, there was a different baseline hazard on which the effect of the time-dependent covariate was tested. Adjusting for visits to either companion plant species by stratification explained the effect of landing frequencies in case of barley (percent of visits on companion plants was not significant anymore Chi^2=2.79, df=5, P=0.73), but did not in case of mustard (percent of visits to companion plants stayed significant Chi^2=12.46, df=5, P=0.03). Interestingly, the estimated effect of visits to barley increased the hazard rate of arrival to target plants (expβ=1.44), whereas visits to mustard had a negative effect (expβ=0.69). These results mean that the differences in arrival tendencies to the target plant in the mustard set-up cannot be fully explained by the differences in the frequency of visits on companion plants. Other behavioural elements involved in searching that were not considered as covariates influenced arrival tendencies differently in the barley and in the mustard combinations.

Analysing the respective behavioural elements may help us explain what differences, other than frequency of visits on the companion plants, influenced arrival tendencies of females at the target plants. The behavioural elements performed by individuals most frequently and for the longest time were flying and walking and differences in these behaviours were detected between the set-ups (table 5). Females in the monotypic set-up showed the lowest number of flying and walking behaviours. Females in the barley flew and walked more often and longer than females in the monotypic stand, and these behaviours were even more frequent and longer in the mustard set-up (table 5).

Table 5. Average frequency and duration (both with ±SE) of behavioural elements in the monotypic, the barley and the mustard set-ups.

	Monotypic n=62	**Barley** n=50	**Mustard** n=50
Frequency			
Fly*	3.4(0.4)$^{\mathbf{a}}$	6.5(0.8)$^{\mathbf{b}}$	16.0(2.1)$^{\mathbf{c}}$
Walk*	2.0(0.4)$^{\mathbf{a}}$	4.2(0.9)$^{\mathbf{b}}$	13.8(2.1)$^{\mathbf{c}}$
Groom	1.2(0.3)	1.6(0.3)	1.2(0.2)
Stand still	0.8(0.3)	1.1(0.3)	1.2(0.3)
Duration			
Fly*	71.3(6.8)$^{\mathbf{a}}$	154.1(16.4)$^{\mathbf{b}}$	191.7(18.7)$^{\mathbf{c}}$
Walk*	15.1(4.0)$^{\mathbf{a}}$	23.8(5.3)$^{\mathbf{b}}$	80.4(11.0)$^{\mathbf{c}}$
Groom	8.2(2.2)	8.3(1.7)	3.9(0.8)
Stand still	4.2(1.8)	7.5(2.3)	2.9(0.8)

*- significant difference detected by Kruskal Wallis ANOVA. Numbers in a row followed by the same letter are not significantly different (Mann-Whitney U-test).

The structure of behavioural flows are presented in the behavioural kinetograms of figure 8. The observed behavioural transitions within the three set-ups were significantly different from expected transitions (G-test, $P<0.05$). This indicated that females did not deploy different behavioural elements randomly while searching. When the transitions were tested around single behaviours, certain behavioural transitions were significantly present or absent. The organisation of the behaviour of females searching in the monotypic and the mustard set-ups were similar, with the same transitions being significantly present or absent (fig. 8). Flight behaviour was often followed by a short stop, which most probably continued in walking or in a few occasions in grooming (fig. 8a). Walking was often followed by grooming or flying. The behaviour of females was characterised by the absence of transitions from flying to grooming and from walking to standing still.

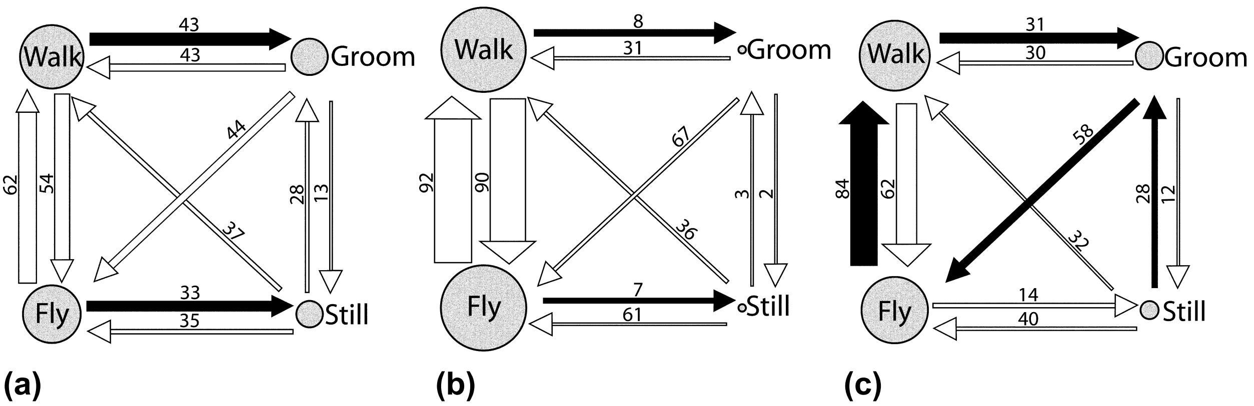

Figure 8. Behavioural kinetograms of *D. semiclausum* females searching in the **(a)** monotypic, **(b)** in the mustard and **(c)** in the barley set-ups until the target plant is found. The size of the circles is proportional to the overall frequency of the behavioural elements. The thickness of an arrow is proportional to the likelihood of occurrence of each behavioural transition. The black arrows are significantly positive deviations from expected behavioural transitions. Transitions with significantly negative deviations from expected values are not shown. Numbers are percentage transitions to succeeding behaviours.

Mustard increased the frequency of transition between walking and flying (fig. 8b), but probably because it was proportional to the frequency of occurence of the behavioural elements, this transition stayed non-significant. The organisation of the behavioural flows of females searching in the barley was unlike those searching in the monotypic and in the mustard set-ups (fig. 8c). After landing on a plant, females rarely stood still, but rather started short duration walking. Grooming was often preceded by walking and stand still, and was often followed by flight behaviour.

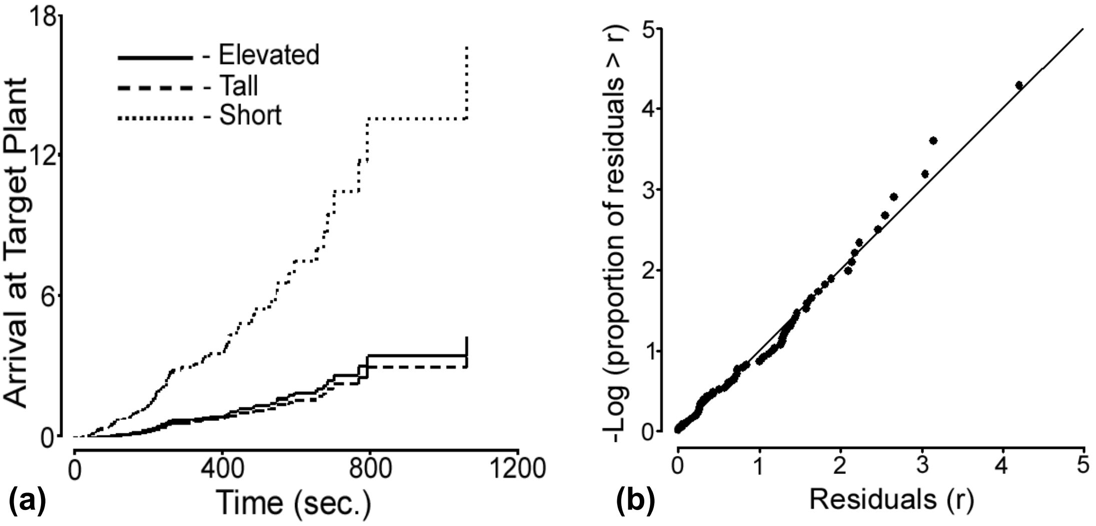

Figure 9. Tendency of wasps to find the target plant, when the nine Brussels sprout plants were interspersed by twelve mustard plants of different vertical arrangements. **(b)** Graphical test for goodness of fit. When the model fits, the dots should be on a straight line through the origo with a slope of 45^{o}.

Table 6. Effects of covariates (vertical arrangement, visits on companion plants) on arrival tendencies of wasps to target (infested) plants.

Covariates	**Chi^2 (df)**	**Estimated effect (exp β)**	**Effect on arrival**
Tall mustard	23.39(3)**	0.182	↓
Elevated mustard	24.65(3)**	0.176	↓
% Visits to companion plant	10.34(3)*	1.013	↑

ns –not significant, * -P<0.05, ** -P<<0.05;

When compared to the set-up where Brussels sprout plants were surrounded by short mustard, wasps in the set-up with tall mustard arrived at target plants later (table 6, fig. 9). Elevating Brussels sprout plants in the tall mustard to the same relative height as in the short mustard set-up, did not influence arrival tendencies. The percent of visits to the mustard plants significantly increased arrival times to target plants (table 6.).

4. Discussion

4.1. Foraging behaviour and habitat composition

Here we demonstrated a differential effect of plant species composition of the host's habitat on the foraging behaviour of the parasitoid *Diadegma semiclausum*. As females were not influenced by the different number of conspecifics plants in the set-up, the observed differences in foraging behaviour in the barley and the mustard set-ups were due to species-related effects and not simply to changes in the biomass or leaf area of plants. Females entered the mustard set-up faster than the monotypic and the barley set-ups. The longer flights before landing for the first time might indicate an increased need to orientate, when barley surrounded Brussels sprout plants than when mustard did. The lower arrival tendencies to the target plant in the diverse set-ups were concomitant with a greater visitation of barley or mustard plants and an increase in the frequency and the duration of flying and walking behaviours. We think that mustard plants interacted with foraging behaviour of females via its attractive volatile blends, whereas barley rather influenced them through its structural properties. This assumption is also supported by the differences in which barley and mustard differentially influenced foraging females: percent of visits to the companion plants did not equally influence the arrival tendencies in the two set-ups, wasps in mustard spent more time flying and walking and differences were observed in the organisation of behavioural flows.

4.2. Volatile and structural properties of habitat

The attraction of parasitoids to undamaged and non-host plant species has been demonstrated earlier (Elzen et al., 1983, Geervliet, 1997, Takabayashi et al., 1991, Perfecto and Vet, 2003). Similar to herbivores, the olfactory responses of carnivores may be altered, if odours from host-infested plants are mixed with those from non-host infested plants or even from plants that are not food for the host (Monteith, 1960, Vos et al., 2001, Dicke et al., 2003). Volatile interference may be expected, when the volatile compounds of plants that are responsible for the attraction of parasitoids towards their prey or host, show overlap with those compounds released by the other plant (Dicke et al., 2003). Due to subtle differences in odour blend composition the parasitoid *Cotesia glomerata* is unable to discriminate between volatiles of plants infested by hosts from those infested by non-hosts (Geervliet et al., 1996, Vos et al., 2001). *D. semiclausum* prefers volatiles from undamaged white mustard to intact Brussels sprout and it does not discriminate between volatiles of host-infested Brussels sprout and undamaged white mustard (Chapter, 5). Due to their higher preference and responsiveness for mustard volatiles, females restricted their initial searching on these plants, so arrival to the target Brussels sprout plant was the end of a searching process, where most of the mustard plants were inspected first.

When volatile blends from different plant species were offered in separate odour fields to wasps (Chapter 5), they easily discriminated between volatiles from undamaged barley and crucifers. Based on our knowledge on the volatile information, we would not expect differences in foraging efficiency observed here between the monotypic and the barley set-ups. The ability of parasitoids to discriminate between odours of host-infested and undamaged plants may be differentially influenced when it is presented in the volatile background of single plant species as compared to volatile mixtures. (Dicke et al., 2003). Most of the compounds that were detected in barley were also present in either undamaged or host-infested Brussels sprout (Chapter 5). In this study the volatiles from barley and Brussels sprout were most probably mixed due to diffusion and turbulent airflow, which presented females with a different situation than in our earlier olfactometer bioassay. Although the volatile fields in the two experiments were not comparable, we can rule out the possibility that barley odours repelled females (Chapter 5). On the other hand, if the effect of barley was due to volatile attractivity, a similar effect is to be expected as observed in case of mustard, which was not the case here. Changing geometric properties (i.e. diversity and connectivity of surface structures) influences searching behaviour of *Trichogramma* parasitoids (Romeis et al., 1998, Gingras et al., 2002). Barley plants were taller (with 15±5cm) than sprout plants and their morphology was also quite different. Females often attempted to start searching in the set-up by descending in the vegetation from above, which often resulted in landings on the tip of the leaves of barley plants. Based on the results of present and previous studies, we think that structural properties of the barley set-up, and not volatile masking, were responsible for hindering the wasps to find the target plant.

Placing the plants in different horizontal arrangements did not influence foraging success of *D. semiclausum*. In small-scale field studies, the shape of plant patches and background vegetation influenced the predation of aphids by coccinellid beetles (Grez and Prado, 2000). The lack of response of *D. semiclausum* to horizontal arrangement of plants indicates that the searching behaviour of this parasitoid is not influenced by small-scale changes in habitat configuration. However, row or mixed arrangement did not change the overall structural complexity of the set-up and changes in the direct neighbourhood of the target plants were minor. When short or tall mustard plants surrounded the target plant, the change in structural complexity was greater, which was concomitant with the change in foraging behaviour. As internodal distance within mustard plants drastically increased in a week, tall mustard plants could have provided females a greater surface to search than short ones. Because of the small distance of only 1-3 cm between leaf levels, females in the short mustard plants did not search the entire leaf surface when foraging on a plant. Emission of volatile compounds may differ with the development of plant organs and with plant age (Tollsten and Bergström, 1988). Therefore, a possible effect of differences in volatile profiles of plants of different age-classes might have contributed to the observed differences between short and tall mustard, which aspect needs further study.

4.3. Habitat composition and local host-parasitoid interactions

The host selection of parasitoids is hypothesized to be influenced both by their host-range and the food-plant range of their host (Vet and Dicke, 1992). The finding therefore that *D. semiclausum* is attracted to volatiles of cruciferous plants may not be surprising (Legaspi, 1984, Ohara et al., 2003, Chapter 5), because its host is also specialised on cruciferous plants (Yano, 1994, Åsman, 2002). Although *P. xylostella* has a wide dietary range within the family Cruciferae (Bigger and Fox, 1997, Åsman et al., 2001), it does not prefer all crucifer species equally (Talekar and Shelton, 1993, Charleston and Kfir, 2000). *P. xylostella* prefers volatiles, develops faster and reaches greater body weight on white mustard than on Brussels sprout (K. Winkler, unpublished, R. Gols, unpublished). The higher preference of *D. semiclausum* for mustard compared to Brussels sprout makes us assume that this parasitoid may prefer mustard because of the higher encounter probability and possibly better quality of the host on this plant species in the field (Chapter 5). Local population interactions of hosts and parasitoids are often determined by the ability of herbivores to escape into enemy-free space (Gratton and Welter, 1999, Oppenheim and Gould, 2002). High-density populations of *P. xylostella* often have broader dietary range than low-density populations, and top-down regulation by natural enemies may play a role in this (Fox and Eisenbach, 1992, Bigger and Fox,1997). Studying host parasitoid interactions in set-ups with different plant composition may therefore provide information on the importance of host-selection behaviour on local host-parasitoid interactions.

However, parasitoids may undergo rapid adaptation to local host populations (Price, 1981, Vos and Hemerik 2003), and phenotypic plasticity in host-selection behaviour enables them to exploit hosts on different host plant species (Brodeur et al., 1998, Geervliet et al., 1998, Daza-Bustamante et al., 2002). Therefore learning from experiences with encounters with hosts and non-hosts is a factor that strongly influences foraging success of parasitoids in complex habitats (reviewed in Vet et al 1995, Perfecto and Vet, 2003). These experiences may involve the learning of complex volatile and visual stimuli (Vet et al., 1995).

Here we only looked at arrival tendencies of parasitoids with no oviposition experience. However, learning may have influenced the foraging efficiency and time-allocation of parasitoids in the different set-ups. At present, we have no information on how parasitoids learn and increase their foraging efficiency in different habitats. The only study so far that dealt with the effect of experience with hosts in set-ups with two plant species, focused on arrival tendencies into host patches exclusively (Perfecto and Vet, 2003). In our forthcoming paper, we attempt to give a more complete picture on the effect of habitat diversity on the time-allocation of *D. semiclausum* within the habitat (Chapter 7).

Hunter (2002b) called for observing insect responses to plant attributes in more realistic situations that can be extrapolated to responses under natural conditions. Our results highlight the complexity of how volatile and structural properties of plants may envelope target plants and influence the “apparency” of hosts for foraging parasitoids. Compared to our earlier studies that were based on volatile attractivity only, a lower foraging efficiency of females in the barley set-up is somewhat unexpected. Such findings underline the need to observe the behaviour of insects in more realistic set-ups (see also Wiskerke and Vet, 1994, Vos et al. 1998), but also acknowledges the fact that a mechanistic separation of causal relationships may only be achieved by means of manipulative experiments.

Acknowledgements

This study is part of the project “Enhanced biodiversity” funded by the section Earth and Life Sciences of the Dutch Scientific Organisation (NWO-ALW, project number: 014-22.031). We thank Wouter Tigges for maintenance of the parasitoid rearing, Matthijs Vos for his constructive comments on the manuscript and Mike Keller for providing us the software for the analysis of the transition matrices.

Chapter

7

Time-allocation of the specialist parasitoid *Diadegma semiclausum* (Hellén) in vegetationally diversified habitats

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Time-allocation of the specialist parasitoid *Diadegma semiclausum* (Hellén) in vegetationally diversified habitats

Abstract

We analysed the time-allocation of the parasitoid *Diadegma semiclausum* with respect to arrival and leaving tendencies in set-ups of different plant species composition. The set-up consisted of a group of plants surrounding two host-infested (target) Brussels sprout plants. The surrounding plants were either malting barley or white mustard interspersed with sprout plants, whereas the control monotypic set-up consisted of sprout plants alone. Barley and mustard negatively influenced the arrival tendencies of parasitoids to the first target plant, which was in line with our earlier findings. Females located the second target plant with the same efficiency in the different set-ups. Subsequent analysis of the time spent in different parts of the set-ups revealed that parasitoids in the mustard flew longer than parasitoids in the other two set-ups. Females in the mustard tended to spend more time on target plants over successive visits than females in the monotypic and the barley set-ups. Although the type of set-up did not influence the total number of hosts encountered, in the mustard set-up it took parasitoids longer to encounter the hosts than in the monotypic set-up. Barley had no influence on encounter tendencies with the hosts. Compared to the monotypic set-up, barley had no influence on leaving tendencies from the set-up. Parasitoids had lower leaving tendencies from the mustard than from the monotypic and the barley set-ups. The number of visits to target plants had a positive effect on leaving tendencies in general, but it was not responsible for the lower leaving tendencies from the mustard set-up. We conclude that plant species composition of habitats can influence time- allocation and foraging efficiency of parasitoids. Implications of these possible effects on host-parasitoid interactions and efficiency of biological control are discussed.

Key words: *Diadegma semiclausum*, habitat diversity, foraging behaviour, time-allocation;

1. Introduction

In natural habitats the apparency of a host to its parasitoid is a compounded effect of the apparency of the host-infested plant and that of the host itself (Godfray, 1994). Hence, habitats comprised of many plant species may influence foraging behaviour of parasitoids and subsequent host-parasitoid interactions differently than monotypic habitats. The “enemies hypothesis” predicts that natural enemies are more successful in vegetationally diverse than in pure habitats, such as monoculture agro-ecosystems, resulting in lowered herbivore populations in diverse stands (Root, 1973). Some of the predictions of this hypothesis may be plausible for generalist natural enemies, i.e. diverse habitats harbour a greater diversity or prey/host and alternative food sources. This hypothesis also assumes that because of the greater availability of refuges, the host/prey is more likely to escape widespread annihilation in diverse than in simple systems, so populations of specialist natural enemies, like many parasitoids are expected to fluctuate less widely. This way herbivore populations are checked earlier and outbreaks are more often prevented in diverse than simple systems. The general prediction that structural complexity “stabilises” is already questionable because it depends on the biological and behavioural details of the species studied (Kareiva, 1987, Kareiva and Odell, 1987, Bierzychudek, 1988, Ellner et al., 2001). Besides, it was argued that searching behaviour of specialists, like many parasitoids, are negatively influenced by vegetation diversity (Sheehan, 1986). Furthermore, the hypothesis did not consider that habitat elements (i.e. plants) are not just neutral media in which organisms interact. After all, the habitat conveys information (i.e. infochemicals) that influences foraging of herbivores and their natural enemies and thus multitrophic interactions (Price et al., 1980, Sheehan, 1986, Vet and Dicke, 1992, Perfecto and Vet, 2003).

In the process of host location parasitoids use infochemical and visual cues that are derived from the host itself or its direct environment, such as the plant on which the host feeds (Vinson, 1976, 1998, Nordlund et al., 1988, Vet and Dicke, 1992, Wäckers and Lewis, 1994, Wäckers, 1994). Depending on the dietary breadth of its host, the parasitoid may discriminate between the volatile-blends of a few to several different plant species, which can be useful in the location of the hosts’ habitat (Bradburne and Mithen, 2000, Elzen et al. 1983, 1986, Takabayashi et al., 1998). Especially useful for host location are plant-volatiles emitted in response to herbivore feeding-damage (Vet et al., 1995, Turlings et al., 1990, Potting et al., 1995, Mattiacci et al., 1994, Steinberg et al., 1993). However, as the presence of hosts is not guaranteed, volatiles provide only incomplete information (Vet et al., 1991). Host-related cues are more reliable signals of the host’s presence, but as they are small in quantity, their detectability is far below to the level of plant cues (Vet et al. 1991). Herbivore-induced plant volatiles, being highly reliable and well detectable, may solve this reliability-detectability problem and can provide an efficient way for parasitoids to locate hosts.

In natural diverse ecosystems, composed of several to many plant species, both the expectancy of the host's presence and the specificity of volatile infochemicals in host location may show great between- and within-plant variance (Dicke, 1999b, Vet, 1999). In fact, variation in plant volatiles between different plant species and varieties can be greater than between herbivore-damaged and undamaged plants (Geervliet et al., 1997). Therefore, the efficient use of specific plant cues in a habitat with many non-target plant species and herbivores present may be hampered, because of the expectedly high "background noise". Olfactory disruption by odour masking has been suggested for natural enemies (Monteith, 1960), but it has rarely been tested (but see Dicke et al., 2003). Besides the complexity of volatile information, other aspects like a greater geometric complexity of the vegetation surface surrounding the host-patch may negatively affect host location by parasitoids in diverse habitats (Andow and Prokrym, 1990, Romeis et al., 1998, Gingras and Boivin, 2002). Due to the fewer constraints in the process of volatile information until a host is found and the lower structural complexity, natural enemies may colonise simple agro-ecosystems more readily than diverse systems.

Depending on the specificity of volatile plant information used by a parasitoid, its behaviour may be differentially influenced by the species of plants neighbouring the target host-infested plants (Chapters 5,6). Another factor that will influence a parasitoid's efficiency in complex environments is their ability to phenotypically adapt through the process of learning. Parasitoids can learn habitat cues and thus have the potential to learn to discriminate between signal and noise (Papaj and Vet, 1990, Vet et al., 1990, Vet et al., 1995). Perfecto and Vet (2003) found that experience of foraging parasitoids with cues from mono- and dicultures, led to differences in initial host-encounter rates. This finding suggests that different plant species neighbouring target plants may determine time-allocation of parasitoids between host patches and influence their subsequent foraging success in different ways.

The objective of the present study was to investigate how plant species composition in a habitat influences time- allocation and foraging efficiency of the parasitoid *Diadegma semiclausum* (Hellén). The foraging behaviour of *D. semiclausum* was recorded in a set-up that consisted of two host-infested plants that were spatially separated and were surrounded by either uninfested host plants or a combination of uninfested host plants and a second plant species. To analyse time-allocation, the observation period was divided into time until arrival at one of the host-infested plants and from arrival at a host plant until departure from the set-up.

2. Materials and Methods

2.1. Parasitoids

Diadegma semiclausum was collected from Brussels sprout fields in the vicinity of Wageningen (The Netherlands) and was reared on Brussels sprout infested with larvae of the diamondback moth. The rearing was maintained in a climate room (20 ± 2º C, 70% RH and L16-D8 photoperiod). Sprout plants infested with the host were obtained from a mass rearing maintained at our laboratory. Parasitoid cocoons were collected weekly and were transferred to a clean insect cage (37cm x 40cm x 30cm) with no plant material or host present. To obtain mated females we transferred newly emerged wasps, seven females and seven males into glass tubes (20 cm long, ø 5 cm) that were covered by gauze at both ends. Water and honey was administered *ad libitum* on the gauze. The day before an observation, females were separated from males and were transferred to another cage (42cm x 22cm x 22cm). For the observations five to ten day old females without oviposition experience were used.

2.2. Plants

We used the crucifer Brussels sprout (*Brassica oleracea* L. gemmifera cv. Cyrus) as a host plant for *P. xylostella.* White mustard (*Sinapis alba* L. cv. Carneval) was used as a cruciferous companion plant, and malting barley (*Hordeum vulgare* L. cv. Video) as a non-cruciferous companion plant. All plants were grown in a greenhouse compartment (20-28ºC, 40-80% RH and L16-D8 photoperiod). To obtain similar plant biomass per pot for barley and mustard plants in the different set-ups (20-25 g in 4-5 weeks), we varied the number of plants per pot with companion plant species. We used nine plants per pot for mustard and 18 plants per pot for barley.

2.3. Experimental set-up

Experiments were conducted in a ‘flight chamber’ installed in a greenhouse compartment (for details see Wiskerke and Vet, 1994), where the conditions were as described in section 2.2. No fans were used to provide directed airflow in the set-up. As the greenhouse compartment was continuously ventilated, there was a turbulent air-current inside the tent. Brussels sprout plants were arranged on a table in three rows of five plants with the central plants host-infested (i.e. target plants) (fig. 1). Four leaves of two target Brussels sprout plants were infested by one third instar larva of the host *Plutella xylostella*, 16 hours prior to the experiments. In the three set-ups the 15 Brussels sprout plants were interspersed by 22 pots of uninfested plants, that were either the companion plants barley or white mustard, or in the monotypic set-up Brussels sprout. Within the observation period different set-ups were randomly used, and when possible two treatments were tested on the same day. The experiment was carried out from late March till mid August 2003.

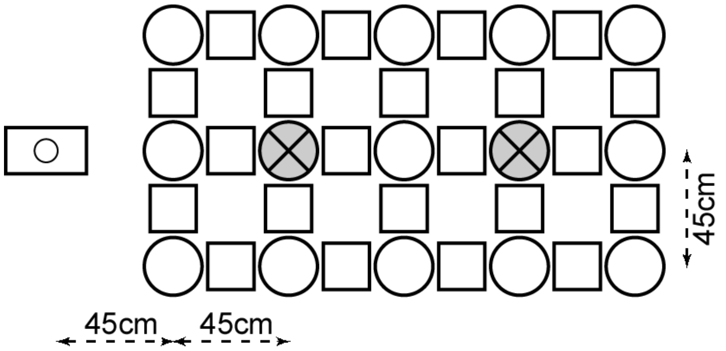

Figure 1. The spatial arrangements of the experimental set-up. ⊗ –target (host-infested) Brussels sprout plant; ○ -uninfested Brussels sprout plants; ☐ -uninfested plants: mustard, barley or Brussels sprout (monotypic set-up); Spacing of plants and the release platform was identical as described in Chapter 6.

2.4. Behavioural observations

In order to increase the motivation of females to search for hosts (Geervliet, 1997, Potting et al. 1999), a Brussels sprout leaflet, damaged by the host but not containing the host or its products was placed in a vial together with a female wasp. The vial was placed on the release station, a glass cylinder (length 30 cm, ø 15 cm) situated at a height of 10 cm and at a distance of 90 cm from the first target plant. To record foraging behaviour and parasitoid position, we used The Observer software 3.0 (Noldus Information Technology 1993©). The observation started when the female left the release platform and ended when it left the set-up, i.e. it flew to the fabric of the tent and stayed there for more than a minute. In addition, if a wasp returned in the set-up within a minute and then landed on the fabric for the second time, independent of the duration, the observation was ended. If the wasp left the set-up for more than a minute before finding a target plant, it was discarded from the data analysis. While foraging in the set-up, wasps could either stay on the uninfested Brussels sprout, on the companion plants, on one of the two target plants or fly. We recorded three behaviours: flying, searching (including walking and grooming) and standing still. Flights included both hovering around a plant and travelling between plants. When a wasp found the host and attempted to oviposit, it was considered a host encounter. By numbering the position of leaves on the target plants, the number of encounters with each host could be followed. When a host dropped on the table, it was removed to avoid herbivore damage on the uninfested Brussels sprout and the mustard plants. The time that wasps spent on the table, on the release station, or on the fabric of the tent was not considered in the analysis. A wasp was used only once and for each observation, a new set of target plants was used. Twenty-five observations were carried out in each set-up.

2.5. Data analysis

To evaluate the effect of plant species composition of the set-up on behavioural elements such as flying and visiting the different plants in the set-up as well as encounter rates with the hosts, the proportional hazards regression model was used (Cox, 1972, Kalbfleisch and Prentice, 1980, PHREG, ©SAS, 1999-2001). It is described by the hazard formula that models the effect of p time-independent covariates (explanatory variables) z_i ($i=1\ldots,p$) on the hazard rate for the event performed by an individual at time t. For those comparisons where it was assumed that for different values of the covariate z_i, the hazard rates $h(t;z)$ were proportional, we used the general form of the proportional hazards model (eqn 1):

eqn 1

$$h(t;z) = h_0(t)\exp\left\{\sum_{i=1}^{p} \beta_i z_i\right\}$$

where $h(t;z)$ is the probability per second that the event occurs and h_0 is the baseline hazard rate. The z_i are the fixed (time-independent) covariates that may affect the baseline probability. The β_i are coefficients representing the relative strength of covariate z_i. The hazard at time t is the product of the baseline hazard $h_0(t)$ and the cumulative effect of the covariates. Some covariates may be time-dependent, that is the hazard rate at time t will depend on the value $z_j(t)$. The effects of these covariates were tested by the extended proportional hazards model (Haccou and Hemerik, 1985, Kleinbaum 1996, eqn 2).

eqn 2

$$h(t;z(t)) = h_0(t)\exp\left\{\sum_{i=1}^{p1} \beta_i z_i + \sum_{j=1}^{p2} \delta_j z_j(t)\right\}$$

where δ_j are coefficients representing the relative strength of the p_2 time-dependent covariates $z_j(t)$.

2.5.1. The events of interest and the selection of covariates

The times to events analysed by the Cox's model are defined and explained in fig. 2 and table 1. For the evaluation of foraging behaviour before and after a female has located a target plant, each observation was split into two phases. The first phase started when the foraging initiated (i.e. departure from release station) and ended when the wasp landed on a target plant for the first time. The second phase started with the arrival to one of the target plants and ended when the wasp left the set-up. With respect to the first phase, the first occurrence of two events was investigated, (1) landing in the set-up (on any plant) and (2) arrival at one of the target plants (fig. 2). With respect to the second phase, the time was measured until the wasp (3) flew to the second target plant. The time was measured (4) between subsequent encounters with the hosts and the observation ended when (5) the wasp left the set-up. For these five events hazard rates were quantified and inferences on the effects of several covariates were made.

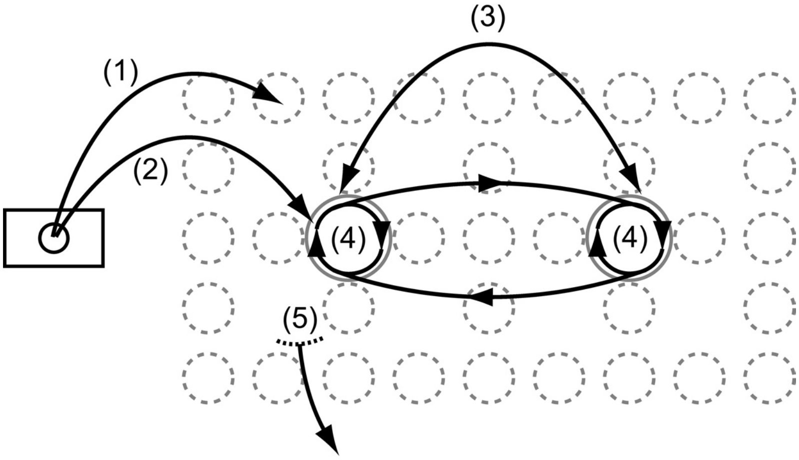

Figure 2. The variables describing the time- allocation of *D. semiclausum* females were (1) arrival at the set-up and at (2) the target plant, encountering (3) target plants and (4) subsequent hosts and (5) departure from the set-up (see also table 1).

Table 1. Events (variables) recorded while observing foraging behaviour of *D. semiclausum* and their description. These variables were analysed with the proportional hazards regression model.

ID	Variable	Description
1.	Arrival in the set-up	Time from leaving release station until first landing on any of the plants
2.	Arrival at the target plant	Time from leaving release station until first landing on one of the target plants
3.	Leaving set-up	Time from first landing on the first target plant until leaving the set-up
4.	Encounter 2nd target plant	Time between arrivals on the first and the second target plant
5.	Encounters with hosts	Time between two host-encounters

The time-independent covariates tested for their effects on the tendency (hazard rate) of all of the five events to occur were the three set-ups, with the monotypic set-up considered as the baseline hazard (table 2). Several time-dependent covariates that were assumed to be influential on leaving tendencies from the set-up were selected *a priori*. It has been shown that encountering unparasitised hosts may decrease or increase leaving tendencies from the host-patch (Waage, 1979, Van Roermund et al., 1994, Wang and Keller, 2003). As host encounters may affect leaving tendencies from the set-up, the number of hosts found and the number of visits on target plants were included as covariates. Similar to arrival tendencies to the target plants, the percent of visits to the companion plants can potentially influence leaving tendencies from set-ups (Chapter 6.). Therefore we included the visits that wasps made on companion plants as the percentage of total landings and the number of take-offs until departure from the set-up. The number of flights was also included as a covariate.

Table 2. The covariates and their coding tested for their effect on leaving tendencies from the set-up.

Type	Covariate	Coding
Time independent	Companion plant species (Z_1)	*0*-B. sprouts, mustard, *1*-barley;
	Companion plant species (Z_2)	*0*-B. sprouts, barley, *1*-mustard;
Time-dependent	Number of hosts encountered in the set-up (Z_5)	*0,1,2,3,…*
	Number of visits on target plants (Z_6)	*1,2,3,…*
	Number of flights (Z_4)	*0,1,2,3,…*
	Percent of visits on companion plant (Z_3)	$(c(t)/c(t)+h(t))*100^{§}$

§- where c(t) is the cumulative number of visits on companion plants and uninfested plants together, and h(t) the cumulative number of visits on host-infested (target) plants between time 0 and t.

To include covariates in the final models on leaving tendencies we used a stepwise selection procedure. Variables were entered into and removed from the model in such a way that each forward selection step could be followed by one or more backward elimination steps, where the significance level for entry and removal of a new covariate was $\alpha=0.05$. The proportionality assumption was visually inspected by comparing the residuals against the observed –log survivors (Chapter 6) or by plotting the deviance residuals (©SAS, 8.02.).

2.5.2. *Time-budget of parasitoids*

From the moment that the female arrived at the first target plant, the time spent flying, the time spent on the target, the uninfested Brussels sprout and the companion plants were extracted from the dataset, and these variables were log (x+1) transformed. For the analyses of the number of flights, the time spent flying and the time spent on uninfested Brussels sprout and companion plants, General Linear Model (GLM) procedures were used. As the time spent on target plants was dependent on the previous visits to them, the visits were considered as repeated measurement on independent subjects (i.e. parasitoids) within the set-ups. To compare the time spent on target plants over successive visits in the treatments, we used a Mixed Model with random coefficients (PROC MIXED, SAS®, Littell et al., 1996, eqn 3). As each individual parasitoid visited the same target plant more than once, the times spent on the target plants were considered to be repeated measurements on the independent subjects. We assumed that the time spent on target plants was linearly related to the number of previous visits to them, and that each parasitoid followed its own regression line (in that way allowing for correlations between observations on the same parasitoid):

$$y_{ijk} = \alpha_i + a_{ij} + \beta_i x_{ijk} + b_{ij} x_{ijk} + e_{ijk} \qquad \text{eqn 3}$$

where y_{ijk} is the time interval of the k^{th} visit of parasitoid j at set-up i, x_{ijk} is the number of the visit minus 1 (which is equal to k-1); $\alpha_i + \beta_i x_{ijk}$ is the fixed part of the model with separate intercepts (expected time spent at the first visit) and slopes (change in expected time spent per extra visit) for the three set-ups, whereas $a_{ij}+b_{ij}x_{ijk}+e_{ijk}$ is the random part of the model (Littell et al., 1996). The parasitoid specific deviations for the intercept a_{ij} and slope b_{ij} are assumed to be random, possibly correlated, drawn from gaussian distributions. In all the comparisons, the significance level was α=0.05. All analyses were carried out by SAS 8.02 (1999-2001 ©SAS Institute Inc) and SPSS 11.5 (1989-2002 ©SPSS Inc).

3. Results

3.1. *Arrival tendencies to the set-up and to the first target plants*

Parasitoids released in the different set-ups had different tendencies to enter the vegetation and start searching. When compared to the tendency of entering the monotypic set-up (average time±SE: 67±15s, fig. 3a), wasps in the mustard entered significantly earlier (23±4s, PHREG, P=0.015, expβ=2.43). Wasps released in the barley set-up entered later (111±27s) than those in the mustard (PHREG, P<0.001, expβ=0.263) and in the monotypic set-ups, but the latter was not significantly different (PHREG, P=0.308). Females arrived at the first target plant in the monotypic set-up the fastest (114±11s, fig. 3b), followed by arrival at the target plant in the barley set-up (192±30s, PHREG, P=0.06, expβ=0.49), while it took longest for wasps to locate the target plant in the mustard set-up (547±83s, PHREG, P<0.001, expβ=0.13).

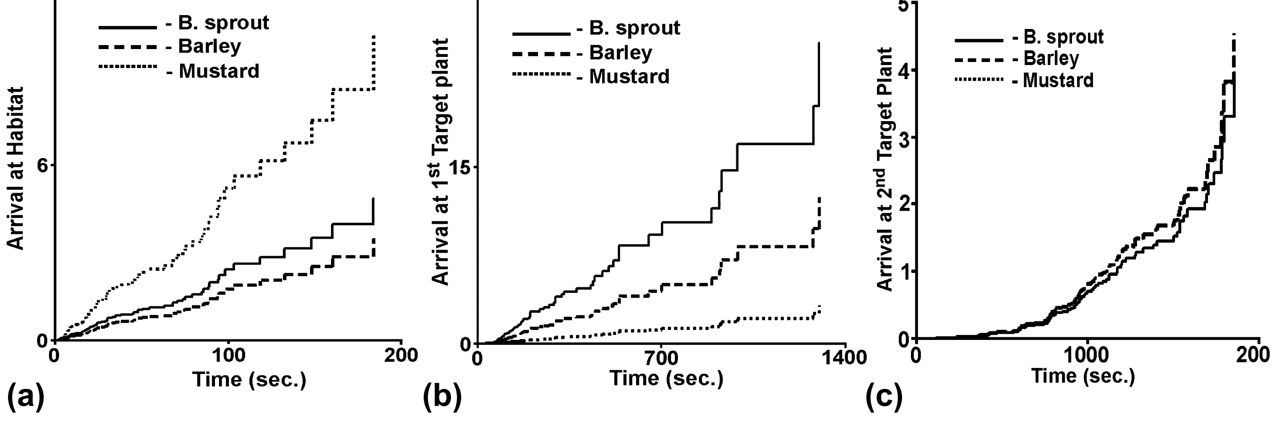

Figure 3. Arrival tendency of foraging wasps at the **(a)** set-up, at the **(b)** first and **(c)** at the 2nd target plant in the monotypic (solid line), in the barley (dashed line) and in the mustard (dotted line) set-ups.

Analysis with the random coefficient model showed that the time spent by females during their first visit on the target plant in the monotypic set-up (579±74s, fig. 4a) was not different from the barley (614±50s, MIXED, P=0.23, fig. 4b) or the mustard set-ups (583±50s, MIXED, P= 0.75, fig.4c).

3.2. Locating the second target plant

Except for one specimen out of twenty-five foraging in the barley set-up, all the wasps that located the first target plant, also managed to locate the second one. When compared to the baseline hazard in the monotypic stand, the tendencies of wasps to arrive at the second target plant in the barley (PHREG, P=0.88, expβ=1.16,fig.3c) and in the mustard (PHREG, P=0.89, expβ=1.16) set-ups were not different, in contrast to the different arrival tendencies at the first target plant.

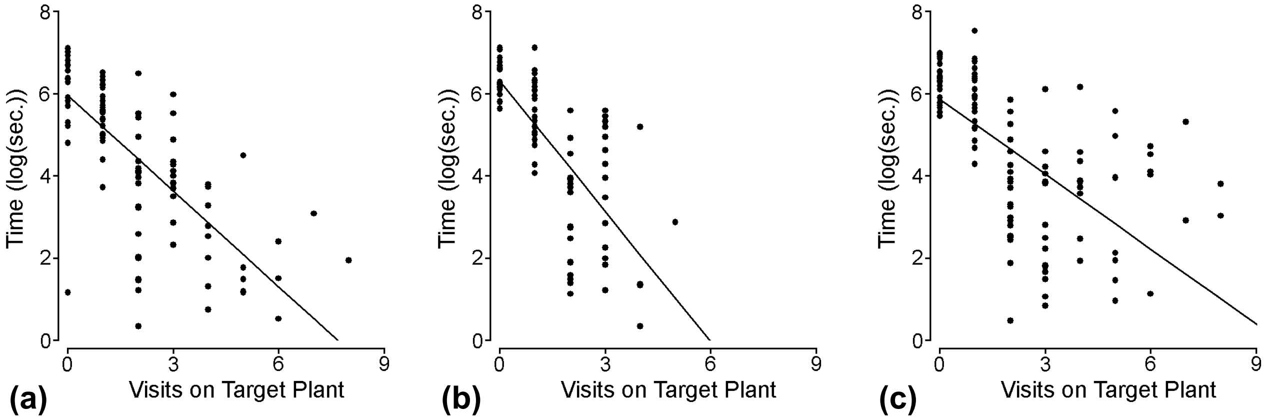

Figure 4. Time spent by foraging wasps on target (host-infested) plants over successive visits in the **(a)** monotypic ((log)time= 5.96-0.78*visits), **(b)** barley ((log)time= 6.31-1.06*visits) and the **(c)** mustard ((log)time= 5.87-0.61*visits) set-ups. The intercept of the regression lines corresponds with the first arrival at one of the two target plants.

As wasps spent the same time on the target plant at the first encounter (fig. 4), this meant that the travel times to the second target plant were not different in the three set-ups. When females arrived at the second target plant, they stayed shorter than on the first target plant and further visits significantly decreased the time spent on the two target plants (fig. 4., MIXED, P<0.001). Although the total time spent on the two target plants was not significantly different between the set-ups (MIXED, P=0.28), an interaction between the number of visits and the type of set-up (MIXED, P=0.004) indicated that the time over visits did not decrease in the different set-ups in a similar fashion. Females in the barley spent significantly less time on target plants during successive relocations than females in the monotypic (MIXED, P=0.04, fig. 4ab) and the mustard set-ups (MIXED, P=0.001, fig 4c). Wasps in the mustard set-up spent the most time on infested plants during successive relocations, however this was not significantly different from the monotypic stand (MIXED, P=0.14).

3.3. *Host-encounters*

The average number of hosts encountered on the two target plants was between 6.2 and 6.5, and did not differ between the three set-ups (GLM, P=0.89). The average percent of multiple encounters was 12.3±1.5% and it was not different between the three set-ups (GLM, P=0.89).

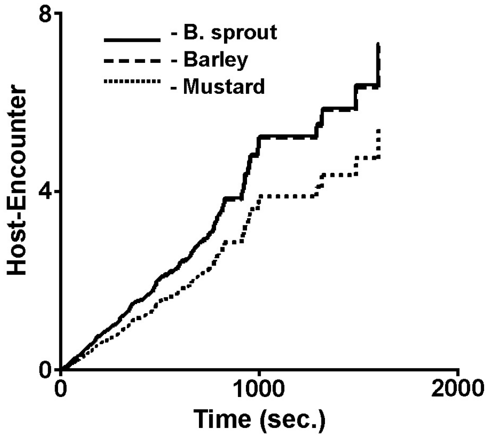

Figure 5. Tendency of foraging wasps to encounter subsequent hosts in the monotypic (solid line), the barley (dashed line) and in the mustard (dotted line) set-ups.

Most hosts were encountered during the first visits on the two target plants. In the mustard set-up, it took the females more time to encounter the same number of hosts than in the monotypic set-up. Compared to the monotypic set-up, the mustard set-up significantly decreased the tendency to encounter hosts (PHREG, P=0.01, expβ=0.75, fig. 5), whereas barley had no effect (PHREG, P=0.97).

3.4. *Time spent searching the set-up*

After finding the first target plant, wasps spent on average 48% of the observation time away from target plants, mostly flying (45%) or by staying on uninfested Brussels sprout and companion plants (3%). In the mustard set-up, females spent significantly more time flying (1248±64s) than either in the barley (958±79s, GLM, P=0.006) or in the monotypic stands (914±63s, GLM, P=0.004). The average time parasitoids spent on companion and uninfested Brussels sprout plants was not different between the different set-ups (GLM, P>0.05). Parasitoids had a characteristic behaviour just before leaving the mustard set-up. After they visited a target plant for the last time and before leaving the set-up, they often flew around several minutes inspecting mustard and uninfested Brussels sprout plants. Their flight was fast and "frantic", and they rarely landed on a plant. When the time from the last visit on the target plant until leaving the set-up was compared, wasps left the mustard set-up later (158s) than either the monotypic (77s, PHREG, P=0.002, expβ=0.338) or the barley (97s, PHREG, P=0.06, expβ=0.492) set-ups, however the latter was only tending to significance.

3.5. Leaving tendency from the set-ups

We compared the time from arrival at the first target plant until the set-up was left. Parasitoids started to leave the set-up after ±1000 seconds after they landed on one of the host-infested plants (fig. 6). The mustard set-up and the number of visits to target plants were the only significant covariates that were used in the final model (table 3.). Wasps had an increasing tendency to leave the set-up when successively visiting target plants (note the increasing slope in fig. 6). While the barley set-up had no effect, females left later from the mustard than from the other set-ups (table 3). The interaction between the set-up and number of visits on target plants in the mustard was not significant (PHREG, P=0.86), indicating that the number of visits to target plants had an equally increasing effect on leaving tendencies in the three set-ups.

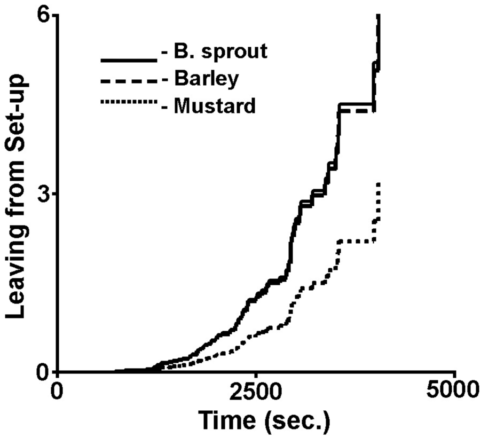

Figure 6. Tendency of foraging wasps to leave from the monotypic (solid line), the barley (dashed line) and from the mustard (dotted line) set-ups.

Table 3. Estimated effects (expβ) of time-independent (plant species) and time-dependent covariates (# Host, # Flights, % Visits on companion plant, # Visit target plant) in the full and the final models for leaving tendencies of wasps from the set-up.

	Covariates	**Chi^2 (df)**	**Estimated effect (exp β)**	**Effect on leaving**
Full model	Barley	0.18(1)ns	1.15	
	Mustard	7.3(1)**	0.37	
	% visits on companion	3.02(1)ns	1.03	
	# flights	2.72(1)ns	0.99	
	# host-encounters	0.00(1)ns	1.00	
	# visits target plants	19.15(1)**	1.47	
Final model	Mustard	8.65(2)*	0.45	↓
	# visits target plant	20.27(2)**	1.44	↑

ns –not significant, * -P<0.05, ** -P<<0.05;

4. Discussion

4.1. Time- allocation and habitat type

We demonstrated a differential effect of species composition of plants neighbouring the host-infested plants on time- allocation of *D. semiclausum*, which are summarised in figure 7. The findings that females in the mustard set-up started searching the earliest and arrived at the target plant latest followed by those in the barley set-up were in line with our earlier findings (Chapter 6). When finding the first target plant, wasps became equally efficient in locating the second target plant in the three set-ups. Yet, females spent more time in the mustard until the same number of hosts were encountered than in the other set-ups. After females finally gave-up on the target plants, those in the mustard habitat stayed and searched uninfested plants longer than those in the other set-ups.

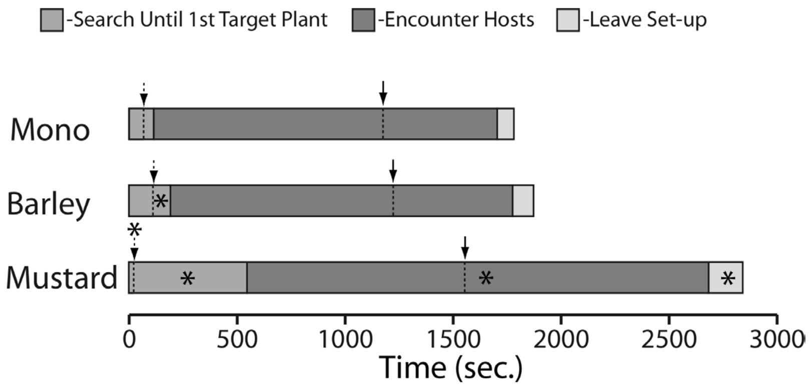

Figure 7. Summary of the time- allocation of *D. semiclausum* females foraging in the different set-ups. The length of the bars is based on the average time spent on the different sections of the foraging process. Dashed arrows denote when wasps entered the set-up, solid arrows denote when the second target plant was found. Asterisks indicate where significant differences were found compared to the monotypic set-up.

Previous studies (Chapter 5) showed a higher preference of inexperienced *D. semiclausum* for white mustard odour compared to Brussels sprout odours. Data suggest that this preference may be related to a possibly higher encounter probability and better quality of the host on this plant species in nature (K. Winkler and R. Gols, unpublished). *D. semiclausum* has a narrow host range, but its host *P. xylostella* is known to feed on several cruciferous plant species and is considered a generalist among the herbivores specialised on Cruciferae (Yano, 1994, Bigger and Fox,1997). *P. xylostella* prefers volatiles from white mustard over Brussels sprout (K. Winkler, unpublished), and attains greater body weight and faster development on the former plant species (R. Gols, unpublished).

Through oviposition experiences on host-infested plants, parasitoids can learn to discriminate subtle quantitative differences between cues, increasing their success of locating further host-patches (Vet et al., 1998, Geervliet et al., 1997). Indeed, after locating the first target plant, the ability of *D. semiclausum* females improved: it took them less time to find the next target plant. Based on our previous olfactometer study, we expect that without oviposition experience, females will already discriminate between cues from the barley, the host-infested and the uninfested Brussels sprout plants, but not between host-infested plants and mustard, leading to a lower efficiency in finding the first target plant in the mustard set-up (Chapters 5 and 6). When encountering hosts on the Brussels sprout, the response of females to host-infested Brussels sprout plants was enhanced and foraging success was increased in locating the second target plant. The findings that females in the mustard set-up flew longest and were retained longer in the set-up after leaving the target plants, indicates that mustard was not readily “neglected” in further foraging. Probably as a result of a higher overall responsiveness, it took longer for females to encounter the same number of hosts in the mustard than in the monotypic set-up.

4.2. Host-patch quality and habitat leaving

In order to increase their foraging success, parasitoids use cues directly associated with the host’s presence and acquire information about the size of the host patch, host density and the quality of the patch (Waage, 1979, Godfray, 1994, Geervliet et al., 1998, Vos et al., 1998, Driessen and Bernstein, 1999, Wajnberg et al., 2000, Van Alphen et al., 2003). Parasitoids may use infochemical cues to discriminate between exploited and unexploited host patches (Waage, 1979, Godfray, 1994), but other mechanisms like learning the spatial location of encountered patches may also play a role (Papaj et al., 1994). Host discrimination is probably important in patch leaving decisions of *D. semiclausum* (Legaspi, 1984, 1986, Wang, 2001, Wang and Keller, 2003) as it is in other parasitoids (van Lenteren, 1991). A decrease in residence times on target plants was observed over repeated visits to these plants, indicating that *D. semiclausum* obtained information about the decreasing quality of patches. However, further studies are needed to explain what experiences at the level of host-patches will also affect leaving tendencies between plant patches.

Most of the hosts were encountered during the first visits on the two target plants, yet females kept on travelling between them. This might have been due to the confined experimental design. However, should leaving tendencies be influenced by the experimental design, since the spatial arrangement of the set-ups was the same, the observations were expected to be equally biased in the different set-ups. Therefore, any observed differences in leaving tendencies between the set-ups, would be the effect of treatment. Besides, parasitisation rates observed in this study were between 70-75%, which were comparable to the few observed parasitisation rates by *D. semiclausum* obtained by independent field observations (e.g. Waage, 1983, L. Hemerik pers. observation).

4.3. Habitat composition and host-parasitoid interactions

In this and in a previous study (Chapter 6), the enhancement of responsiveness of searching parasitoids led to a decrease in host encounter rates in sprouts in the mustard set-up. Other studies found an increase in initial encounter rates with an increase in general responsiveness (Papaj and Vet, 1990, Perfecto and Vet, 2003). However, when *P. xylostella* is present on Brussels sprout in the field, mustard would certainly also harbour hosts, so a higher overall encounter tendency with the host and a greater net foraging efficiency may be expected in a mustard habitat than in the other habitats types. Because of the higher arrival tendencies and lower leaving tendencies, a larger number of females is expected to forage in the mustard than in the other habitat types leading to higher parasitism rates on mustard. Differential attractivity of *Cotesia plutellae* to volatile blends between common and Chinese cabbage resulted in differential parasitism rates of *P. xylostella* (Liu and Jiang, 2003). When exposing Brussels sprout plants infested by *P. xylostella* to *D. semiclausum* in different habitat types, Legaspi (1984) observed a strong effect of habitat type on levels of parasitism, with highest parasitism observed in cruciferous habitats. However, to see how exactly host-encounters and parasitism rates on Brussels sprout are influenced under field conditions when an attractive plant like mustard is present, an additional experiment would be needed, where both Brussels sprout and mustard would harbour hosts. The outcome would than depend on the relative reward rate of the parasitoid on the two different plant species. Experience with hosts on mustard could either lead to a limited focus of the parasitoid to mustard, leading to an enemy-free space for *P. xylostella* on Brussels sprout plants, or parasitoids would forage indiscriminately on both plants.

The "enemies hypothesis", formulated more than 30 years ago, predicted a generally greater success of natural enemies in vegetationally diverse than in pure stands (Root, 1973). However, this hypothesis did not consider that infochemicals may mediate trophic interactions via their influence on foraging behaviour (Sheehan, 1986, Perfecto and Vet, 2003). Perfecto and Vet (2003) demonstrated the importance of differences in foraging behaviour in the responses of parasitoids to changing plant composition of the habitat.

The predictions on how resource subsidy (i.e. food, prey, host) of natural enemies affects biological control in diversified agro-ecosystems strongly depends on how searching behaviour of natural enemies is influenced by these subsidies (Kean et al., 2003). Our results show that plant species composition in a habitat may influence time- allocation of the same parasitoid species in different ways. Plant species mixtures may act as a source and increase populations of parasitoid by attracting and retaining them longer (i.e. mustard), others may decrease their populations by acting as sinks (i.e. barley). This shows that simple generalisations, like dietary breadth alone, may be insufficient predictors of how vegetation diversity affects foraging efficiency of parasitoids. It is more likely that foraging efficiency of a parasitoid in diverse habitats will depend on how the informational value of plant volatiles is perceived and used.

Acknowledgements

This study is part of the project “Enhanced biodiversity” funded by the section Earth and Life Sciences of the Dutch Scientific Organisation (NWO-ALW, project number: 014-22.031). We thank Wouter Tigges for maintenance of the parasitoid rearing and Gerrit Gort for his help on the analysis with the random coefficients model.

Chapter

8

General discussion

T. Bukovinszky

General discussion

1. Introduction

Intercropping is a habitat management practice that aims at the local reduction of herbivore populations and the selective augmentation of natural enemies in crops. It is hypothesised that increasing vegetation diversity suppresses herbivore populations by a reduction in the apparency and quality of crop plants in species mixtures compared with those in monotypic stands. The host-plant quality hypothesis and the resource concentration hypothesis have been formulated to explain pest-suppressive effects of diverse vegetation (e.g. Root, 1973, Bach, 1981). The enemies hypothesis predicts that success of natural enemies is greater in diverse systems because of the increased subsidy of resources (i.e. prey, host, pollen, nectar) and because host-parasitoid populations fluctuate less widely (through greater availability of refuges and prey) (Root, 1973). However, the highly variable responses of both herbivores and natural enemies to intercropping provide insufficient information to make generalisations (Russell, 1989, Andow, 1991, Smith and McSorley, 2000, Hooks and Johnson, 2003). An explanation for this lack of information is that the predictions of some hypotheses (i.e. resource concentration, and enemies hypothesis) are relevant at the population and species levels, while the underlying mechanisms at the behavioural (i.e. functional) level remain largely untested (but see Khan et al., 1997, Finch and Collier, 2000, Gohole, 2003, Perfecto and Vet, 2003). Hence, the legacy of some of the hypotheses (i.e. host plant quality) is either unjustified or unaccounted for. The aim of my research was (1) to understand the cause(s) of lower herbivore numbers in vegetationally more diverse cropping systems compared to monocultures and (2) to study the behavioural bases of the responses of natural enemies to vegetation diversity. The studied tritrophic system consisted of Brussels sprout (*Brassica oleracea* gemmifera), several specialist herbivore species of crucifers, and *Diadegma semiclausum*, a larval parasitoid of the diamondback moth. Vegetational diversity was characterised by mixing the crop plant with so-called companion plant species. The responses of herbivores were studied when the companion plant was malting barley (*Hordeum vulgare*). The responses of *D. semiclausum*, parasitoid of the diamondback moth (*Plutella xylostella*), were studied when the companion plants were barley or white mustard (*Sinapis alba*).

In this chapter, I summarise and discuss the findings of the thesis concerning effects of vegetation diversification on herbivores and parasitoids, respectively. I describe how the information collected here may be used for further research by specifying the ecological conditions for pest-suppression and natural enemy-augmentation through vegetation diversification.

2. Responses of herbivores to habitat heterogeneity

In the study described in **chapter 2**, I found that populations of several herbivore species were reduced by intercropping Brussels sprout with barley. The numbers of both immigrant (winged) and apterous forms of aphids (*Brevicoryne brassicae, Myzus persicae*), eggs and larvae of the cabbage moth (*Mamestra brassicae*) were lower in the inter- than in the monocrop. Diamondback moth (*Plutella xylostella*) gave a variable response: early in the season numbers of larvae and pupae were lower in the intercrop, but they reached higher numbers later in the season. Intercropping did not influence levels of parasitism by the aphid parasitoid *Diaeretiella rapae* and by *D. semiclausum.*

2.1. Role of plant quality and foraging behaviour

Changes in host- plant quality as a result of plant competition could influence responses of herbivores to intercropping (**Chapter 3**). When compared to the monocrop, the size of Brussels sprout was strongly reduced by competition with barley in the intercrop. As a result, the significantly lower numbers of *P. xylostella* larvae and pupae per plant represented higher densities at a leaf area basis in the inter- than in the monocrop. The delayed phenology and reduced quality of plants in the intercrop were probable causes of the variable responses of the cabbage aphid to intercropping: aphid densities were lower in the inter- than in the monocrop in most part of the season, but reached higher densities in the intercrop at the end of the season. Intercropping positively influenced nutritional quality of Brussels sprout for the large cabbage butterfly (*Pieris brassicae*). Larvae utilised stressed intercropped plants better and attained higher growth rates on inter- than on monocropped plants.

In a combination of field and simulation studies described in **chapter 4,** I demonstrated that herbivore responses to vegetation diversity could be influenced by interspecific differences in stimulus modality in foraging behaviour (i.e. contact, visual and olfactorial) and in travelling behaviour between patches. In the field experiment, the three observed specialist species, the cabbage aphid (*B. brassicae*), the cabbage butterfly (*P. rapae*) and the diamondback moth (*P. xylostella*) responded to changing size of host plant patches in different ways. When numbers in host- plant patches surrounded by barley were compared with those surrounded by mown grass, numbers of colonising cabbage aphids and diamondback moth were lower, with lowest densities observed in small patches in barley background. Densities of the cabbage butterfly were not influenced by background vegetation. When simulating basic differences in foraging strategies of the three herbivores in an individual-based simulation framework, similar patch size-population abundance relationships were found as observed in the field.

2.2. Concluding remarks on herbivore responses

In this study, intercropping led to a general reduction in herbivore numbers when compared with monotypic stands. Evidence on effects of altered plant quality and plant apparency on herbivore population responses has been found. Yet, the responses to plant quality and spatial habitat heterogeneity varied with the herbivore species studied. Since competitive relationships are different in every plant combination and different feeding guilds of herbivores may respond to changes in plant quality in different ways, these results may account for part of the variation in herbivore responses to intercropping found in earlier field trials. As stress-induced changes in plant vigour may even influence performance of parasitoids (Teder and Tammaru, 2002), this could further complicate any mechanistic studies. Therefore, when different plant species mixtures are compared, more adequate experimental approaches (e.g. like that used in chapter 4) are needed where the direct effects of vegetation diversity may be studied without the confounding effects of plant stress.

The spatial dynamics of herbivores were influenced by species-related differences in the relative importance of stimulus modality (i.e. visual, olfactory). Although more research is required to determine how vegetation diversity is likely to interact with searching herbivores (e.g. Finch and Collier, 2000), I conclude that basic knowledge on searching strategies and resource exploitation may help us explain the spatial dynamics of herbivores in diverse agro-ecosystems. A possibility to make generalisations could be to define “behavioural groups” that show similarities in their foraging behaviour and thereby similar spatial dynamics.

3. Responses of parasitoids to habitat heterogeneity

3.1. Behavioural responses of *D. semiclausum* to plant species mixtures

In **chapter 5,** a differential attractivity of *D. semiclausum* females in relation to inter- and intraspecific variation in plant volatiles was demonstrated. Odour blends of all tested plants were preferred to clean air. When uninfested plants of different species were tested against each other for their attractivity in a Y-tube olfactometer, females clearly preferred volatile blends of crucifers to barley. Within crucifers, odour blends of uninfested mustard and the feral *Brassica* were both more attractive to *D. semiclausum* than Brussels sprout. The bioassay combined with the chemical analysis of headspace volatiles suggested that the attraction of females towards plants was not the result of a greater amount of total plant- volatiles produced: despite of the much lower amount of volatiles produced, females found the odour blend of uninfested mustard plants more attractive than that of uninfested Brussels sprout plants. Wasps were able to discriminate between odour blends based on subtle differences. For example discrimination was observed when odour blends of host-infested and uninfested Brussels sprout were compared or those of uninfested feral *B. oleracea* and

Brussels sprout. In both of these cases composition of headspace volatiles were similar. Despite of the greater differences in volatile blend composition, wasps were equally attracted to infested Brussels sprout and uninfested mustard. In **chapter 6**, the efficiency of *D. semiclausum* females to locate a host-infested (i.e. target) Brussels sprout plant was analysed in different set-ups, where treatments were the changing density, species composition (barley or mustard as companion plants) and configuration of plants surrounding the target plant. Compared with the monotypic set-up, mustard reduced searching efficiency of females by a combination of high volatiles attractivity and possibly structural camouflage. Females started searching fastest in the mustard set-up, but as they searched the highly preferred mustard plants first, compared to the other set-ups, they located the target plant later. When tall mustard plants surrounded the target plant, females searched mustard plants longer and found the target plant later than in the control set-up, where short mustard plants neighboured the target plant. Females in the barley set-up needed more time to orientate before starting to search the set-up and arrived later to the target plant than females in the other set-ups. Therefore, based on the result of the present and previous study (Chapter 5), it is likely that structural properties of the barley set-up, rather than volatile masking, were responsible for the reduction in the efficiency of females to locate the target plant.

The analysis of the time-allocation of *D. semiclausum* in a set-up with different plant species composition (same as in Chapter 6) and two target plants was presented in **chapter 7**. Differences in initial foraging efficiency of females in the different set-ups were in line with the earlier findings described in chapter 6. However, after finding the first target plant, females became equally efficient in locating the second target plant in all set-ups. Although they spent more time in the mustard than in the other set-ups, they encountered the same number of hosts. Furthermore, after leaving the target plants, females were retained longer in the mustard than in the other set-ups.

3.2. Behavioural dynamics of D. semiclausum in plant- species mixtures

To illustrate the behaviour of a *D. semiclausum* female searching for hosts in a vegetationally diverse habitat, the results described in chapters 5,6 and 7 are incorporated in a conceptual “variable response model”. This model illustrates how experiences modify behaviour (Vet et al., 1990, 2003, fig. 1). The model assumes that (1) for each stimulus that the parasitoid perceives, there is a corresponding response potential, (2) parasitoids do not respond to every perceived stimulus in the same way and (3) strong responses are less variable than weak ones. Furthermore, (4) learning can change response levels of stimuli, where (5) the change in the response level due to experience will depend on the original level of the stimulus and that (6) learning increases weak responses more than strong ones (Vet et al., 1990, 2003).

In addition, the physiological state of the parasitoid (e.g. egg limitation, hunger) can influence the oviposition strategy and patch-time allocation in the field (Driessen and Hemerik, 1992, Godfray, 1994). Parasitoids do not only forage for hosts but also for food (Casas et al., 2003, Wäckers, 2003, Kean et al., 2003), so depending on the types of resources present in the habitat, a change from a hunger to a food saturated state could make the difference between acceptance and avoidance of a habitat. Let us assume that there is a *threshold responsiveness*, set by internal variables like egg limitation or hunger, which determines if a female actually responds to host-related stimuli in a local environment; e.g. stay and commence with searching, or moves to another plant patch. Therefore, if responsiveness to a stimulus is high above the threshold, there is also a greater likelihood that it will elicit a behavioural response locally, e.g. the parasitoid enters the plant patch and commences searching for hosts. Also, if the responsiveness to cues from all the subsets (i.e. plants) of a plant patch fall below the threshold, the tendency that the host-searching behavioural response is elicited is minimal; e.g. the parasitoid does not enter the plant patch, or if entered, leaves it. The time when the threshold responsiveness is reached by a stimulus, will depend on the types of experience and the initial responsiveness to that stimulus. Positive experiences with a stimulus (i.e. oviposition) increase, whereas negative experiences (i.e. encountering parasitised hosts) decrease the female's responsiveness. The rate of change in responsiveness due to positive or negative rewarding experiences is greater than due to unrewarding experiences, because rewarding experiences are more directly related to key stimuli, like the host itself (Vet et al., 1990).

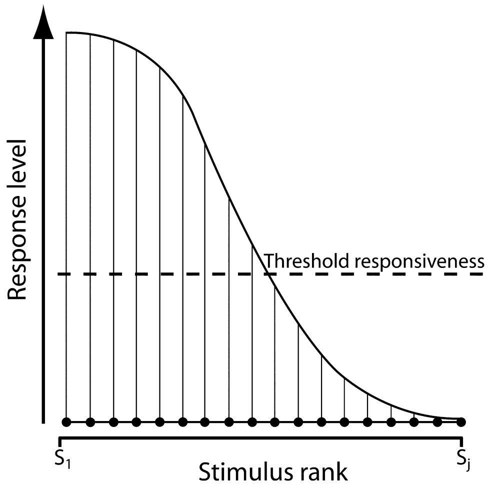

Figure 1. Diagram of a female parasitoid's potential behavioural response to a variety of environmental stimuli. All stimuli perceived by the insect are ranked according to their response potential in the naïve insect. Stimuli beyond S_j are outside the range of sensory perception of the animal (from Vet et al., 1990, 2003). Threshold responsiveness is set by internal variables (i.e. egg limitation, energy state) and affects whether a stimulus with a given response level will likely to elicit a behavioural response. Stimuli below the threshold responsiveness do not elicit behavioural response (e.g. arrestment) in the local environment.

Parasitoids arriving in a habitat to search for hosts perceive a limited volume of their environment. Within this volume they may receive information from different habitat subsets that may or may not harbour hosts (i.e. undamaged plants, exploited and unexploited host-infested plants). For simplicity, we assume that females discriminate perfectly between odour blends of different plants in the habitat, so a stimulus may correspond to a complex odour blend. Figure 2 illustrates the behavioural dynamics of a *D. semiclausum* female searching in a habitat composed of host-infested Brussels sprout plants surrounded by uninfested mustard and barley. An inexperienced female has an innate ranking of stimuli that will determine her responsiveness to different cues when arriving at a habitat (Chapters 5 and 6). Until finding a host-infested Brussels sprout, unrewarding experiences with uninfested plants result in a decay in her responsiveness to these plant stimuli. When a host patch is located on a sprout plant, oviposition rewards increase her responsiveness to infested Brussels sprout to a higher level than those of the uninfested plants. This increases the female's success to locate further host patches (Chapter 7).

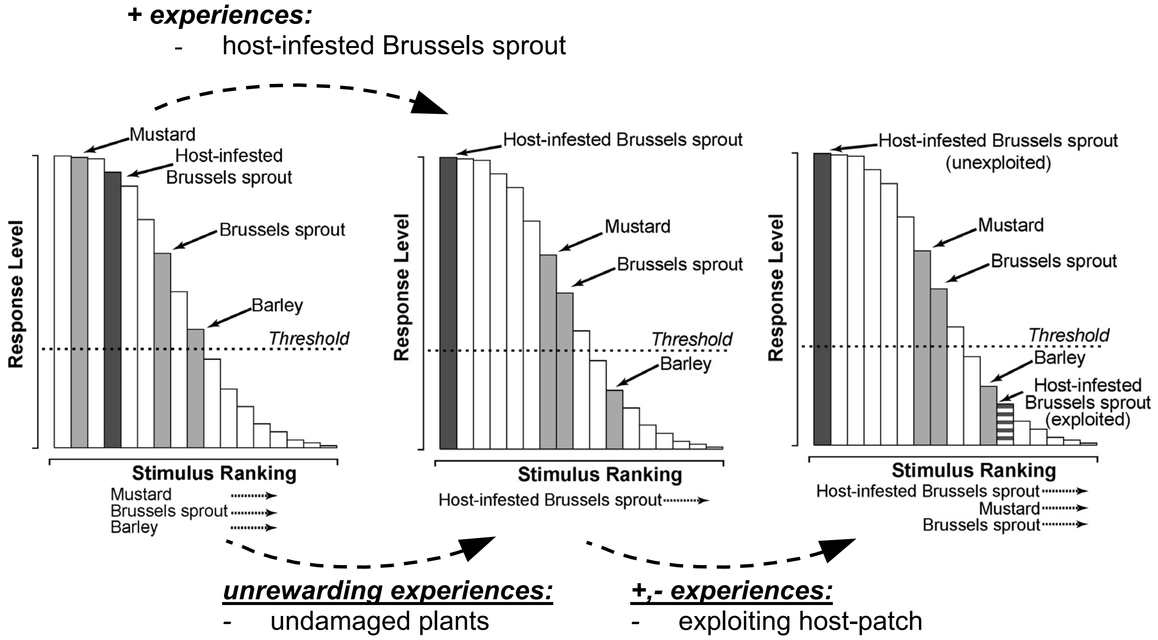

Figure 2. A variable response model for tendency to leave a vegetationally diverse plant patch by *Diadegma semiclausum*. Names with arrows under abscissa are directional changes in stimulus ranking due to unrewarding experiences. Unrewarding experiences with uninfested plants slowly reduce the response level to these plants. Preference ranking of stimuli from infested Brussels sprout increases upon first encounter with the host patch. Negative experiences with exploited host patches reduce response levels to visited plant-host complexes. Dotted line represents the threshold response to stay in the plant patch. The plant patch is examined till the threshold responsiveness is reached, after that the female moves to another plant patch.

Within the host patch, giving-up rules may operate for optimal patch-exploitation. Similar to other parasitoids (van Lenteren, 1991), successful, unsuccessful and multiple encounters with hosts are important factors influencing patch leaving decisions in *D. semiclausum* (Wang, 2001, Wang and Keller, 2003). I found that repeated encounters with the target plants (i.e. exploited patches) were negative experiences and increased the tendency of females to leave the plant patch (Chapter 7, fig. 2). There was a lower tendency to leave the mustard than the other habitats, but that was not explained by encounters with the target plants. Yet, after giving up in the host patches, females were "retained" longer in the mustard set-up before leaving (Chapter 7). A high initial responsiveness towards mustard could explain this: after the target plants were abandoned, mustard still evoked a higher response level, which fell below the threshold later than those of other plants (fig. 2).

For a comprehensive picture on the behavioural dynamics of *D. semiclausum* in plant species mixtures in the field, further research is needed. When a response is not reinforced, it will return to the initial response potential, so parasitoids return to their naïve preference (Vet et al., 1995, Geervliet et al., 1998). (1) We need to know how fast the experienced response potential of a stimulus, when not reinforced, gradually returns to the initial response potential. (2) We have no information on how foraging behaviour of *D. semiclausum* would be influenced if more than one plant species would contain hosts. For example, differences in reward rates (i.e. encounter probability, host quality) on different plant species would probably influence host searching. Hence, initial experience with hosts on particular plant species could either lead to a specialisation of the parasitoid to search on that plant only, or parasitoids could forage on all infested plant species. (3) Finally, plant species may influence the female's ability to discriminate between volatiles emitted upon damage by different herbivore species (Geervliet et al., 1996). Therefore, we need to know how females discriminate in vegetationally diverse habitats between blends of host and non-host infested plants of different species.

3.3. Concluding remarks on responses of parasitoids to habitat heterogeneity

D. semiclausum extensively used plant-related volatiles and was highly efficient in locating hosts in all habitat types, but the different habitat types interacted with its foraging behaviour in different ways. I conclude that the individual responses of parasitoids to vegetation diversity will depend on how the informational value of plant volatiles is perceived and used in a given multitrophic context and how that use is modified by experiences. The efficiency to find the host will depend on the capability of the parasitoid to discriminate between signal (i.e. host-infested plants) and "background noise" (i.e. uninfested plants). For example, it can be very difficult for the female to find the host already in a monoculture, if the host-infested plant does not give specific information compared to uninfested plants. If signals from the infested plants can be easily distinguished from uninfested plants, the searching efficiency of the female is expected to be high in the monotypic habitat and its searching efficiency in the diverse stand will depend on the level of "background noise" that heterospecific plants represent. If the female can ignore non-relevant information in the diverse stand easier than in the monoculture, e.g. the parasitoid has a very low response level to the companion plant, females may be more efficient in the diverse than in the monotypic stand, whereas the opposite tendency is to be expected if the background noise is higher in the vegetationally diverse than in the monotypic stand.

Since structural complexity and leaf area index are often higher in vegetationally diverse than in simple habitats (Sheehan, 1986), one could say that vegetation diversity may reduce foraging success of parasitoids by structural interference. Although structural properties of barley and mustard probably contributed to the lower initial foraging success of *D. semiclausum*, following oviposition experiences, parasitoids improved their foraging efficiency irrespective of the plant-species mixture where they were tested. It is more likely that a negative effect of greater structural complexity in diverse vegetation is to be expected, where complexity interferes with the mobility of the parasitoid within the vegetation, like in case of some egg parasitoids (Andow and Prokrym,, 1990, Gingras et al., 2002).

Because parasitism rates in a habitat are also determined by the number of parasitoids present, the findings that time allocation (i.e. searching, arrival and leaving tendencies) of parasitoids were differentially influenced by barley or mustard as companion plants has implications for population-level interactions. Plant -species mixtures may act as sources and increase populations of parasitoids by attracting and retaining individuals longer (i.e. mustard), whereas other mixtures may function as sinks and decrease populations because of reduced immigration (i.e. barley). To know how a given plant species mixture would influence parasitism rates in the field, a link between the behavioural (i.e. functional) and population (i.e. numerical) level responses needs to be established.

4. Future work

4.1. Linking individual behaviour to processes at the population level

The apparency of crop plants for herbivores is best camouflaged by using taller, structurally complex companion plants in the close proximity of crop plants (Finch and Kienegger, 1997, Finch and Collier, 2000, Åsman et al., 2001, Finch et al., 2003, chapter 4). Depending on (1) the plant-species mixture, (2) the foraging behaviour of the herbivore, (3) the foraging behaviour of the parasitoid and (4) the capacity of the regional parasitoid species-pool to provide local populations with sufficient immigrants, a *complementarity* between reduced host-plant apparency for herbivores and increased parasitism rates may exist. For example, a lower individual foraging efficiency on Brussels sprout in a mustard intercrop may still lead to higher parasitism rates compared with the monotypic stand, if population sizes are much larger in mustard. Individual-based simulation frameworks could be used to predict population-level responses of both herbivores and their natural enemies (e.g. Potting et al., 2002, Chapter 4). An example for such a framework that simulates foraging behaviour of *D. semiclausum* in a spatially explicit vegetationally diverse habitat is presented in figure 3. The flowchart is similar to the one used for herbivore foraging in chapter 4, but it incorporates differences in information use from the plant- and from the host-patch levels.

The preference ranking of the female parasitoid determines priorities in olfactorial orientation towards different plant species and the tendency to stop for searching (see section 3.2.), whereas an arrestment response would determine the tendency to stay and search longer in the vicinity of a plant (e.g. Chapter 4). Positive and negative experiences may influence preference ranking and arrestment responses to stimuli. When a host on a plant is found, giving-up rules within a patch will determine when the host patch will be abandoned (Wang, 2001, Wang and Keller, 2003).

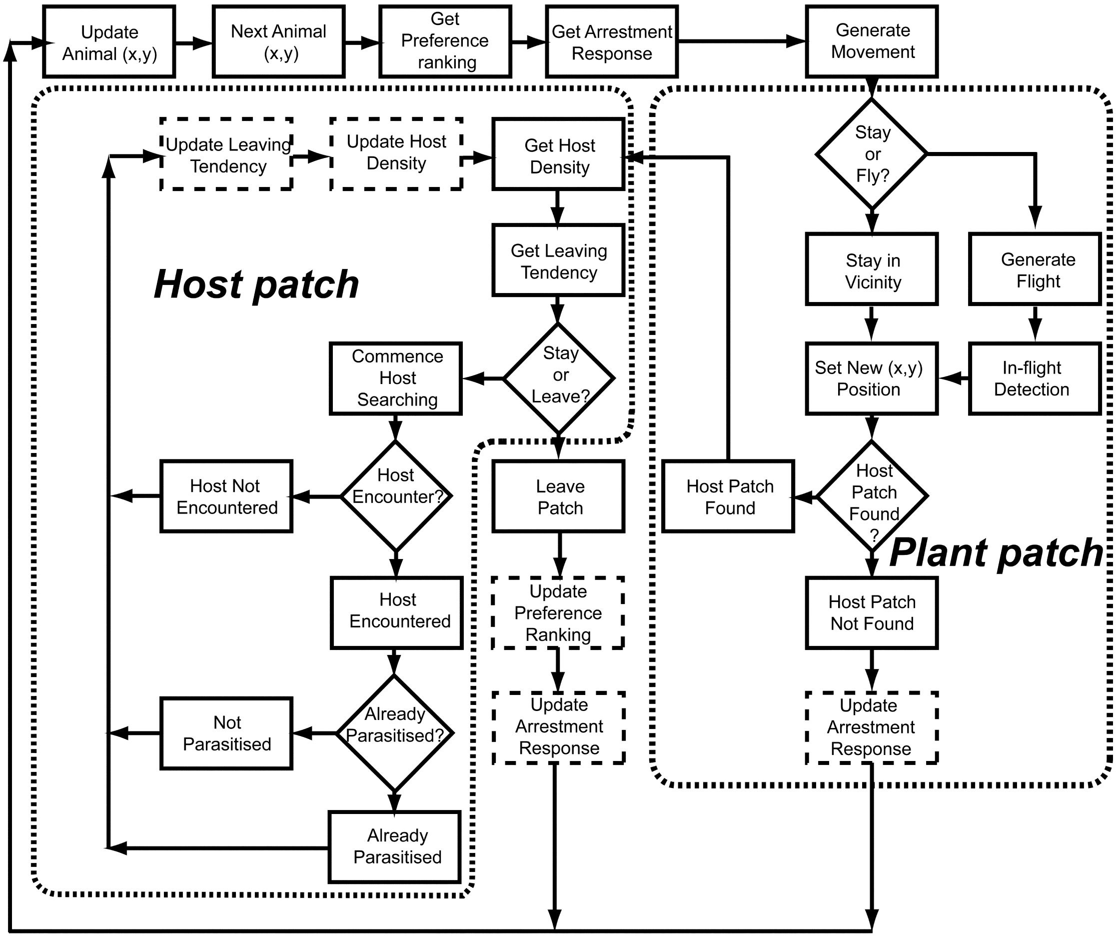

Figure 3. Relational diagram representing habitat and host selection behaviour of *D. semiclausum* in a spatially explicit vegetationally diverse environment. Foraging decisions take place at the between plant- and host- patch levels. The way rewarding and unrewarding experiences will influence detection probability and arrestment response to different plants is described in the variable response model (section 3.2). At the host-patch level, patch leaving decisions are mediated by optimal patch-time allocation mechanisms. Boxes with dashed frames are dynamic-state variables.

By using behavioural information on the responses of herbivores and their parasitoids to vegetation diversity, we may be able to select plant-species mixtures and specify the scale of arrangement, so that herbivore populations are suppressed both by reduced host-plant apparency and higher mortality through the greater success of natural enemies.

4.2. Linking individual behaviour to processes at the community level

Biological control traditionally focused on maximising mortality of a target pest organism by a single natural enemy species, but how interactions with other natural enemies and herbivores in the food-web influence mortality of the target pest is little understood. It may be that constraints in functioning by one species would reduce the chance for competitive exclusion, so other species may also exist and exploit the same resource. This enhanced species coexistence could influence net biological control in either way, but conscious selection of natural enemies may lead to *synergism* in the control of a pest species (Thomas, 1999, Cardinale et al., 2003). The generally higher amount of resource subsidies, like prey, hosts, pollen, nectar sources and microclimate (e.g. Dyer and Landis, 1997), all favour survival of natural enemies leading to higher species richness in vegetationally diverse systems. Therefore, the opportunity for synergistic interactions is inherently higher in vegetationally diverse than in monotypic systems. An important quest for conservation biological control is to link function of individual species to coexistence of competitors in multitrophic contexts (Chapter 1), so synergism between species may be harvested to reinforce biological control in local habitats in agro-ecosystems.

Acknowledgements

The helpful suggestions of Joop C. van Lenteren and Lousie E.M. Vet greatly improved earlier versions of this chapter.

References

Agelopoulos, N.G. and Keller, M.A. 1994. Plant-natural enemy association in the tritrophic system, *Cotesia rubecula-Pieris rapae-Brassiceae* (Cruciferae): III. Collection and identification of plant and frass volatiles. Journal of Chemical Ecology. 20: 1955-1967.

Agelopoulos, N.G., Dicke, M. and Posthumus, M.A. 1995. Role of volatile infochemicals emitted by feces of larvae in host-searching behavior of parasitoid *Cotesia rubecula* (Hymenoptera: Braconidae): a behavioural and chemical study. Journal of Chemical Ecology. 21: 1789-1811.

Altieri, M.A. 1994. Biodiversity and pest management in agroecosystems. Food Products Press, New York.

Andow, D. 1991. Vegetation Diversity and Arthropod Population Response. Annual Review of Entomology. 36: 561-586.

Andow, D.A. and Prokrym D.R. 1990. Plant structural complexity and host-finding by a parasitoid. Oecologia. 82: 162-165.

Ankersmit, G.W., Dijkman, H., Keuning, N.J., Mertens, H., Sins, A. and Tacoma, H.M. 1986. *Episyrphus balteatus* as a predator of the aphid *Sitobion avenae* on winter wheat. Entomologia Experimentalis et Applicata. 42: 271-277.

Åsman, K. 2002. Trap cropping effect on oviposition behaviour of the leek moth A*crolepiopsis assectella* and the diamondback moth *Plutella xylostella*. Entomologia Experimentalis et Applicata. 105: 153-164.

Åsman, K., Ekbom, B. and Rämert, B. 2001. Effect of intercropping on oviposition and emigration behaviour of the leek moth (Lepidoptera: Acrolepiidae) and the diamondback moth (Lepidoptera: Plutellidae). Environmental Entomology 30: 288-294.

Bach, C.E. 1981. Host plant growth form and diversity: effects on abundance and feeding preference of a specialist herbivore, *Acalymma vittata* (Coleoptera, Chrysomelidae). Oecologia. 50: 370-375.

Bach, C.E. 1986. A comparison of the responses of two tropical specialist herbivores to host plant patch size. Oecologia. 68: 580-584.

Bach, C.E. 1988a. Effects of host plant patch size on herbivore density: patterns. Ecology. 64: 1090-1102.

Bach, C.E. 1988b. Effects of Host Plant Patch Size on Herbivore Density: Underlying Mechanisms. Ecology. 69:1103-1117.

Banks, J.E., 1998. The scale of landscape fragmentation affects herbivore response to vegetation heterogeneity. Oecologia. 117: 239-246.

Barbosa, P. and Benrey, B. 1998. Plant Influences on Parasitoids. In. Barbosa, P. (ed.) Conservation Biological Control. pp. 396. San Diego Academic Press.

Bender, D.J., Contreras, T.A. and Fahrig, L. 1998. Habitat loss and population decline: a meta-analysis of the patch size effect. Ecology. 79: 517-533.

Benrey, B., Callejas, A., Rios, L., Oyama, K. and Denno, R.F. 1998. The effects of domestication of *Brassica* and *Phaseolus* on the interaction between phytophagous insects and parasitoids. Biological Control. 11: 130-140.

Benrey, B. and Denno, R.F. 1997. The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. Ecology. 78: 987-999.

Bergelson, J. and Kareiva, P. 1987. Barriers to movement and the response of herbivores to alternative cropping patterns. Oecologia. 71: 457-460.

Berlow, E.L. 1999. Strong effects of weak interactions in ecological communities. Nature. 398: 330-334.

Bianchi, F.J.J.A. & van der Werf, W. 2003. The effect of the area and configuration of hibernation sites on the control of aphids by *Coccinella septempunctata* (Coleoptera: Coccinellidae) in agricultural landscapes: A simulation study. Environmental Entomology 32: 1290-1304.

Bierzychudek, P. 1988. Can Patchiness Promote Prey Outbreaks? Trends in Ecology and Evolution. 3: 62-63.

Bigger, D.S. and Fox, L.R. 1997. High-density populations of diamondback moth have broader host-plant diets. Oecologia. 112: 179-186.

Björkman, C. and Larsson, S. 1999. Insects in drought-stressed trees: four feeding guilds in one experiment. Physiology and genetics of tree-phytophage interactions. Proceedings International Symposium, Gujan, France. 90: 323-335.

Blaakmeer, A., Geervliet, J.B.F., van Loon, J.J.A., Posthumus, M.A., van Beek, T.A. and de Groot, Ae. 1994. Comparative headspace analysis of cabbage plants damaged by two species of *Pieris* caterpillars: consequences for in-flight host location by *Cotesia* parasitoids. Entomologia Experimentalis et Applicata. 73: 175-182.

Bommarco, R. and Banks, J.E. 2003. Scale as modifier in vegetation diversity experiments: effects on herbivores and predators. Oikos. 102: 440-448.

Bottrell, D.G., Barbosa, P. and Gould, F. 1998. Manipulating natural enemies by plant variety selection and modification: a realistic strategy? Annual Review of Entomology. 43: 347-367.

Bowman, J. Cappuccino, N. and Fahrig, L. 2002. Patch Size and Population Density: The Effect of Immigration Behaviour. Conservation Ecology. 6: 9. [online] URL: http://www.consecol.org/vol3/iss2/art11

Bradburne, R.P. and Mithen, R. 2000. Glucosinolate genetics and the attraction of the aphid parasitoid *Diaeretiella rapae* to *Brassica*. Proc. R. Soc. Lond. 267: 89-95.

Brodeur, J., Geervliet, J.B.F. and Vet, L.E.M. 1998. Effects of *Pieris* host species on life history parameters in a solitary specialist and gregarious generalist parasitoid (*Cotesia* species). Entomologia Experimentalis et Applicata. 86: 145-152.

Bukovinszky, T., Rasztik, V., van Lenteren, J.C., Vet, L.E.M. and Bujáki, G. 2003. The effects of undersowing (Brussels sprouts-black mustard) on population density of *Brevicoryne brassicae* and natural enemies of aphids. Integrated Control in Field Vegetable Crops. IOBC/WPRS Bulletin. 26: 167-176.

Bukovinszky, T., Tréfás, H., van Lenteren, J.C., Vet, L.E.M. and Fremont, J. 2004. Plant competition in pest-suppressive intercropping systems complicates evaluation of herbivore responses. Agriculture Ecosystems and Environment. 102: 185-196. (Chapter 3. in this thesis).

Burgio, G., Ferrari, R. and Boriani, L. 2000. Spazi preziosi ai margini del campo. il Divulgatore Agricoltura Alimentazione Ambiente. 23: 13-22.

Casas J. and Djemai I. 2002. Canopy architecture and multitrophic interactions. In: Tscharntke T. and Hawkins B.A. (eds.) Multitrophic Level Interactions. Cambridge University Press, Cambridge.

Casas, J., Driessen, G., Mandon, N., Wielaard, S., Desouhant, E., van Alphen, J., Lapchin, L., Rivero, A., Christides, J.P. and Bernstein, C. 2003. Energy dynamics in a parasitoid foraging in the wild. Journal of Animal Ecology. 72: 691-697.

Cardinale, B.J., Harvey, C.T., Gross, K. and Ives, A.R. 2003. Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. Ecology Letters. 6: 857-865.

Charleston, D.S. 2004. Integrating biological control and botanical pesticides for management of *Plutella xylostella*. Ph.D thesis. Wageningen University, Wageningen. Ponsen & Looijen bv, The Netherlands.

Charleston, D.S. and Kfir, R. 2000. The possibility of using Indian Mustard, *Brassica juncea*, as a trap crop for the diamondback moth, *Plutella xylostella*, in South Africa. Crop Protection. 19: 455-460.

Clough, Y., Bukovinszky, T., van Lenteren, J.C. and Vet, L.E.M. 2002. Evaluating pest pressure in the Brussels sprout-barley intercropping system: influence of host plant patch size and surrounding vegetation. Proceedings Experimental and Applied Entomology. NEV Amsterdam. 13: 123-126.

Compton, S.G. 2002. Sailing with the wind: dispersal by small flying insects. In Bullock, M.M., Kenward, R.E. and Hails, R.S. (eds.) Dispersal Ecology. Blackwell Publishing. Oxford.

Connor, E.F., Courtney, A.C. and Yoder, J.M. 2000. Individuals-area relationships: the relationship between animal population density and area. Ecology. 81: 734-748.

Cortesero, A.M., Stapel, J.O. and Lewis, W.J. 2000. Understanding and manipulating plant attributes to enhance biological control. Biological Control. 17: 35-49.

Costello, M.J. and Altieri, M.A. 1995. Abundance, growth rate and parasitism of *Brevicoryne brassicae* and *Myzus persicae* (Homoptera, Aphididae) on broccoli grown with living mulches. Agriculture Ecosystems and Environment. 52: 187-196.

Cox, D.R. 1972. Regression models and life tables. Journal of the Royal Statistical Society. 34B: 187-220.

Cromartie, W.J. 1975. The effect of stand size and vegetational background on the colonization of cruciferous plants by herbivorous insects. Journal of Applied Ecology. 12: 517-533.

Cromartie, W.J. 1981. The environmental control of insects using crop diversity. – In: Pimentel, D. (ed.), Handbook of pest management in agriculture, vol. 1. CRC, Boca Raton, Florida.

Cuddington, K. and Yodzis, P. 2002. Predator-Prey Dynamics and Movement in Fractal Environments. The American Naturalist. 160: 119-134.

Dale, D. 1988. Plant-mediated effects of soil mineral stresses on insects. – In: Heinrichs, E.A. (ed.) Plant stress-insect interactions. John Wiley & Sons.

Davis, A.J. 1987. Effects of experience and kairomones on searching behaviour of *Diadegma eucerophaga* (Ichneumonidae) for *Plutella xylostella* (Plutellidae) on *Brassica* plants. Medelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit, Gent, 52: 403-411.

Daza-Bustamante, P., Fuentes-Contreras, E., Rodríguez,L.C.,Figueroa, C.C. and Niemeyer, H.M. 2002. Behavioural differences between *Aphidius ervi* populations from two tritrophic systems are due to phenotypic plasticity. Entomologia Experimentalis et Applicata. 104: 321-328.

De Moraes, C.M., Lewis, W.J., Paré, P.W., Alborn, H.T. and Tumlinson, J.H. 1998. Herbivore-infested plants selectively attract parasitoids. Nature. 393: 570-573.

Den Belder, E., J. Elderson, van den Brink, W.J. and Schelling, G. 2002. Effect of woodlots on thrips density in leek fields: a landscape analysis. Agriculture Ecosystems and Environment. 91: 139-145.

Dicke, M. 1999a. Direct and indirect effects of plants on performance of beneficial organisms. In: Ruberson, J.R. (ed.) Handbook of Pest Management. Marcel Dekker Inc., New York.

Dicke, M. 1999b. Specificity of herbivore-induced plant defences. – In: Insect-plant interactions and induced plant defence. Novartis foundation.

Dicke, M. and van Loon, J.J.A. 2000. Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. Entomologia Experimentalis et Applicata. 97: 237-249.

Dicke, M., de Boer, J.G., Höfte, M. and Rocha-Granados, M.C. 2003. Mixed blends of herbivore-induced plant volatiles and foraging success of carnivorous arthropods. Oikos. 101: 38-48.

Dover, J. 1986. The effect of labiate herbs and white clover on *Plutella xylostella* oviposition. Entomologia Experimentalis et Applicata. 42: 243-247.

Driessen, G. and Bernstein, C. 1999. Patch departure mechanisms and optimal host exploitation in an insect parasitoid. Journal of Animal Ecology. 68: 445-459.

Driessen, G. and Hemerik, L. 1992. The time and egg budget of *Leptopilina clavipes*, a parasiotid of larval *Drosophila*. Ecological Entomology. 17. 17-27.

Duelli, P., Obrist,M.K. and Schmatz, D.R. 1999. Biodiversity evaluation in agricultural landscapes: above-ground insects. Agriculture Ecosystems and Environment. 74: 33-64.

Dyer, L.E. and Landis, D.A. 1997. Influence of noncrop habitats on the distribution of *Eriborus tenebrans* (Hymenoptera: Ichneumonidae)) in cornfields. Environmental Entomology 26: 924-932.

Ehrlich, P.R. and Ehrlich, A.H. 1981. Extinction: the causes and consequences of the disappearance of species. New York: Random House.

Ellner, S.P., McCauley, E. Kendall, B.E., Briggs, C.J., Hosseini, P.R., Wood, S.N., Janssen, A.,Sabelis, M.W., Turchin, P., Nisbet, R.M. and Murdoch, W.W. 2001. Habitat structure and population persistence in an experimental community. Nature. 412: 538-543.

Elton, C.S. 1958. Ecology of Invasions by Animals and Plants. Chapman & Hall, London.

Elzen, G.W., Williams, H.J., and Vinson, S.B. 1983. Response by the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) to synomones in plants: implications for host habitat location. Environmental Entomology 12: 1873-1877.

Elzen, G.W., Williams, H.J., and Vinson, S.B. 1984. Isolation and identification of cotton synomones mediating searching behaviour by the parasitoid *Campoletis sonorensis*. Journal of Chemical Ecology. 10: 1251-1264.

Elzen, G.W., Williams, H.J., and Vinson, S.B. 1986. Wind tunnel flight response by hymenopterous parasitoid *Campoletis sonorensis* to cotton cultivars and lines. Entomologia Experimentalis et Applicata. 42: 285-289.

Estes, J.A. and Palmisano, J.F. 1974. Sea otters: their role in structuring near-shore communities. Science. 185: 1058-1060.

Ewel, J.J 1986. Designing Agricultural Ecosystems For The Humid Tropics. Annual Review of Ecology and Systematics. 17: 245-271.

Fahrig, L. and Paloheimo, J. 1988. Effect of spatial arrangement of habitat patches on local population size. Ecology. 69: 468-475.

Finch, S., Billiald, H. and Collier, R.H. 2003. Companion planting - do aromatic plants disrupt host-plant finding by the cabbage root fly and the onion fly more effectively than non-aromatic plants? Entomologia Experimentalis et Applicata. 109: 183-195.

Finch, S. and Collier, R.H. 2000. Host plant selection by insects-a theory based on appropriate/inappropriate landings by pest insects in cruciferous plants. Entomologia Experimentalis et Applicata. 96: 91-102.

Finch, S. and Kienegger, M. 1997. A behavioural study to help clarify how undersowing with clover affects host-plant selection by pest insects of *Brassica* crops. Entomologia Experimentalis et Applicata. 84: 165-72.

Fox, L.R. and Eisenbach, J. 1992. Contrary choices: possible exploitation of enemy-free space by herbivorous insects in cultivated vs. wild crucifers. Oecologia. 89: 574-579.

Fox, L.R. and Morrow, P.A. 1981. Specialization: species property or local phenomenon? Science. 211: 887-893.

Garcia, A.A. and Altieri, M.A. 1992. Explaining differences in flea beetle *Phyllotreta cruciferae* Goeze densities in simple and mixed broccoli systems as a function of individual behaviour. Entomologia Experimentalis et Applicata. 62: 201-209.

Geervliet, J.B.F. 1997. Infochemical use by insect parasitoids in a tritrophic context: comparison of a generalist and a specialist. PhD Thesis. Wageningen University. Wageningen, The Netherlands.

Geervliet, J.B.F., Ariëns, S., Dicke, M. and Vet, L.E.M. 1998. Long distance assessment of patch profitability through volatile infochemicals by the parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae). Biological Control. 11: 113-121.

Geervliet, J.B.F., Posthumus, M.A., Vet, L.E.M. and Dicke, M. 1997. Comparative analysis of headspace from different caterpillar-infested or uninfested food-plants of *Pieris* species. Journal of Chemical Ecology. 23: 2935-2954.

Geervliet, J.B.F., Verdel, M.S.W., Schaub, J. Snellen, H., Dicke, M. and Vet, L.E.M. 2000. Coexistence and niche segregation by field populations of the parasitoid *Cotesia glomerata* and *C. rubecula* in the Netherlands: predicting field performance from laboratory data. Oecologia 124: 55-63.

Geervliet, J.B.F., Vet, L.E.M. and Dicke, M. 1996. Innate responses of the parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae) to volatiles from different plant-herbivore complexes. Journal of Insect Behavior. 9: 525-538.

Gingras, D. and Boivin, G. 2002. Effect of Plant Structure, Host Density and Foraging Duration On Host Finding By *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae). Environmental Entomology 31: 1153-1157.

Gingras, D., Dutilleul, P. and Boivin, G. 2002. Modeling the impact of plant structure on host-finding behaviour of parasitoids. Oecologia. 130: 396-402.

Godfray, H.C.J. 1994. Parasitoids Behavioural and Evolutionary Ecology. Princeton University Press.

Gohole, L.S. 2003. Enhancing foraging behaviour of stemborer parasitoids: role of a non-host plant *Melinis minutiflora.* Ph.D. Thesis. Wageningen University & Research Centre. pp. 140. Ponsen & Looijen bv, The Netherlands.

Gold, C.S., Altieri, M.A. and Bellotti, A.C. 1990. Direct and residual effects of short duration intercrops on the cassava whiteflies *Aleurotrachelus socialis* and *Trialeurodes variabilis* (Homoptera: Aleyrodidae) in Colombia. Agriculture Ecosystems and Environment. 32: 57-67.

Gonring, A.H.R., Picano, M. and Guedes, R.N.C. 2000. Effect of larvae presence and defoliation rate in the oviposition of *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) in cabbage. Acta Scientiarum. 22: 951-953.

Goodman, L.A. 1968. The analysis of cross-classified data: independence, quasi-independence, and interactions in contingency tables with or without missing entries. Journal of the American Statistical Association. 63: 1091-1131.

Gratton, C. and Welter, S.C. 1999. Does "enemy-free space" exist? Experimental host shifts of an herbivorous fly. Ecology. 80: 773-785.

Grez, A.A. and Gonzalez, R.H. 1995. Resource concentration hypothesis: effect of host plant patch size on density of herbivorous insects. Oecologia. 103: 471-474.

Grez, A.A. and Prado, E. 2000. Effect of plant patch shape and surrounding vegetation on the dynamics of predatory coccinellids and their prey *Brevicoryne brassicae* (Hemiptera: Aphididae). Environmental Entomology 29: 1244-1250.

Groeters, F.R., Tabashnik, B.E., Finson, N. and Johnson, M.W. 1992. Oviposition preference of the diamondback moth (*Plutella xylostella*) unaffected by the presence of conspecific eggs or *Bacillus thuringiensis*. Journal of Chemical Ecology. 18: 2353-2362.

Haccou, P. and Hemerik, L. 1985. The influence of larval dispersal in the cinnabar moth (*Tyria jacobaeae*) on predation by the red wood ant (*Formica polyctena*): an analysis based on the proportional hazards model. Journal of Animal Ecology. 54: 755-769.

Hagen, K.S. Tassan, R.L. 1970. The influence of food wheast and related *Saccharomyces fragilis* yeast products on the fecundity of *Chrysoperla carnea* (Neuroptera, Chrysopidae). Canadian Entomologist. 102: 806-811.

Harvey, J.A., van Dam, N.M. and Gols, R. 2003. Interactions over four trophic levels: foodplant quality affects development of a hyperparasitoid as mediated through a herbivoore and its primary parasitoid. Journal of Animal Ecology. 72: 520-531.

Hemerik, L., Driessen, G. and Haccou, P.1993. Effects of intra-patch experiences on patch time, search time and searching efficiency of the parasitoid *Leptopilina clavipes.* Journal of Animal Ecology. 62: 33-44.

Hern, A., Jones, E.G. and McKinlay, R.G. 1996. A review of the pre-oviposition behaviour of the small cabbage white butterfly, *Pieris rapae* (Lepidoptera: Pieridae). Annals of Applied Biology. 128: 349-371.

Hokkanen, H.M.T. 1991. Trap Cropping in Pest Management. Annual Review of Entomology. 36: 119-138.

Holling, C.S. 2001. Understanding the Complexity of Economic, Ecological, and Social Systems. Ecosystems. 4: 390-405.

Holling, C.S., Gunderson, L.H. and Ludwig, D. 2002. In Search of a Theory of Adaptive Change. In Gunderson L.H. and Holling C.S. Panarchy: Understanding Transformations in Human and Natural Systems. Island Press, Washington.

Holtzer, T.O., Archer, T.L. and Norman, J.M. 1988. Host plant suitability in relation to water stress. – In: Heinrichs, E.A. (ed.) Plant stress-insect interactions. John Wiley & Sons. pp.

Hooks, C.R.R. and Johnson, M.W. 2003. Impact of agricultural diversification on the insect community of cruciferous crops. Crop Protection. 22: 223-238.

Hooper, D.U. and Vitousek, P.M. 1997. The Effects of Plant Composition and Diversity on Ecosystem Processes. Science. 1302-1305.

Hopkins, R.J., Ekbom, B. and Henkow, L. 1998. Glucosinolate content and susceptibility for insect attack of three populations of *Sinapis alba*. Journal of Chemical Ecology. 24: 1203-1216.

Huffaker, C.B. 1958. Experimental studies on predation: dispersion factors and predator-prey oscillations. Hilgardia. 27: 343-383.

Huffaker, C.B., Shea, K.P. and Herman, S.G. 1963. Experimental studies on predation: complex dispersion and levels of food in an Acarine predator-prey interaction. Hilgardia. 34: 305-330.

Huisman, J. and Weissing, F.J. 1999. Biodiversity of plankton by species oscillations and chaos. Nature. 402: 407-410.

Hunter, M.D. 2002a. Landscape structure, habitat fragmentation and the ecology of insects. Agricultural and Forest Entomology. 4: 159-166.

Hunter, M.D. 2002b. A breath of fresh air: beyond laboratory studies of plant volatile – natural enemy interactions. Agricultural and Forest Entomology. 4: 81-86.

Ives, P.M. 1978. How discriminating are cabbage butterflies? Australian Journal of Ecology. 3: 261-276.

Jones, C.G. 1989. Measuring plant protein with the Bradford assay. 1. Evaluation and standard method. Journal of Chemical Ecology. 15: 1989.

Jones, R.E. 1977. Movement patterns and egg distribution in cabbage butterflies. Journal of Animal Ecology. 46: 195-212.

Jones, R.E. and Ives, P.M. 1979. The adaptiveness of searching and host selection behaviour in *Pieris rapae* L. Australian Journal of Ecology. 4: 75-86.

Justus, K.A. and Mitchell, B.K. 1996. Oviposition site selection by the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Journal of Insect Behavior. 9: 887-898.

Kalbfleisch, J.D. and Prentice, R.L. 1980. The statistical analysis of failure time data. Wiley New York.

Kareiva, P. 1985. Finding and loosing host plants by *Phyllotreta*: Patch size and surrounding habitat. Ecology. 66: 1809-1816.

Kareiva, P. 1987. Habitat fragmentation and the stability of predator-prey interactions. Nature. 326: 388-390.

Kareiva, P. and Odell, G.M. 1987. Swarms of predators exhibit 'preytaxis' if individual predators use area-restricted search. American Naturalist. 130: 233-270.

Karowe, D.N. and Schoonhoven, L.M. 1992. Interactions among three trophic levels: the influence of host plant on performance of *Pieris brassicae* and its parasitoid, *Cotesia glomerata*. Entomologia Experimentalis et Applicata. 62: 241-251.

Kean, J., Wratten, S., Tylianakis, J. and Barlow, N. 2003. The population consequences of natural enemy enhancement, and implications for conservation biological control. Ecology Letters. 6: 604-612.

Kennedy, J.S. Booth, CO. and Kershaw, W.J.S. (1959). Host finding by aphids in the field II. *Aphis fabae* Scop. gynoparae and *Brevicoryne brassicae* L.; with a re-appraisal of the role of host-finding behaviour in virus spread. Annals of Applied Biology. 47, 424-444.

Kennedy, J.S., Booth, C.O. and Kershaw, W.J.S. 1961. Host finding by aphids in the field. III. Visual attraction. Annals of Applied Biology. 49:1-21.

Kessler, A. and Baldwin, I.T. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. Science. 291: 2141-2144.

Khan, Z.R., Ampong-Nyarko,K., Chiliswa, P., Hassanali, Kimani, S., Lwande, W., Overholt, W.A., Pickett, J.A., Smart, L.E., Wadhams, L.J. and Woodcock, C.M. 1997. Intercropping increases parasitism of pests. Nature. 388: 631-632.

Kleinbaum, D.G. 1996. Survival analysis: a self-learning text. Springer-Verlag.

Kostal, V. 1993. Physical and chemical factors influencing landing and oviposition by the cabbage root fly on host-plant model. Entomologia Experimentalis et Applicata. 66: 109-118.

Krawchuk, M.A. and Taylor, P.D. 2003. Changing importance of habitat structure across multiple spatial scales for three species of insects. Oikos. 103: 153-161.

Krebs, C.J. 1989. Niche overlap and diet analysis. – In: Krebs, C.J. (ed.) Ecological Methodology. Harper Collins Publishers, Inc. pp. 654.

Kruess, A. and Tscharntke, T. 1994. Habitat fragmentation, species loss, and biological control. Science. 264: 1581-1584.

Landis, D.A. and Marino, P.C. 1999. Landscape Structure and Extra-Field Processes: Impact on Management of Pests and Beneficials. In Ruberson J.R. (ed.) Handbook of Pest Management. New York.

Landis, D.A., Wratten, S.D. and Gurr, G.M. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annual Review of Entomology. 45: 175-201.

Lawton, J.H. 1994. What do species do in ecosystems? Oikos. 71: 364-374.

Leather, S.R., Walters, K.F.A and Bale, J.S. 1993. The Ecology of Insect Overwintering. Cambridge University Press. pp. 255.

Legaspi Jr. B.A.C. 1984. A comparative study of the foraging behaviour of two species of *Diadegma* (Hymenoptera: Ichneumonidae) attacking the diamond-back moth. Ph.D. Thesis. University of London, Imperial College Ascot.

Legaspi, B.A.C. 1986. Host discrimination in two species of ichneumonid wasps, *Diadegma spp*., attacking larvae of *Plutella xylostella*. Entomologia Experimentalis et Applicata. 41: 79-82.

Lehmus, J., Hommes, M., Vidal, S. 1996. Unterschiedliche auswirkungen einer antersaat auf die populationsdynamik von herbivoren und ihren gegelspiern in weisskohl. Mitteilungen der Deutchen Gesellschaft fur allgemeine und angewandte Entomologie. 10: 265-268.

Letourneau, D.K. and Goldstein, B. 2001. Pest damage and arthropod community structure in organic vs. conventional tomato production in California. Journal of Applied Ecology. 38: 557-570.

Levin, S.A. 1992. The Problem of Pattern and Scale in Ecology. Ecology. 73: 1943-1967.

Levin, S.A. 1999. Fragile Dominion. Perseus Books, Reading (MA).

Lewis, W.J., Lenteren, J.C. van, Phatak, S.C. and Tumlinson, J.H. 1997. A total system approach to sustainable pest management. Proceedings of the National Academy of Sciences of the USA. 94: 12243-12248.

Littell, R.C., Milliken, G.A., Stroup, W.W. and Wolfinger, R. 1996. SAS System for Mixed Models. SAS Institute Inc.

Liu, S.S. and Jiang, L.H. 2003. Differential parasitism of *Plutella xylostella* (Lepidoptera: Plutellidae) larvae by the parasitoid *Cotesia plutellae* (Hymenoptera: Braconidae) on two host plant species. Bulletin of Entomological Research. 93: 65-72.

Loughrin, J.H., Manukian, A., Heath, R.R. and Tumlinson, J.K. 1995. Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. Journal of Chemical Ecology. 21: 1217-1227.

MacArthur, R.H. 1955. Fluctuations of animal populations and a measure of community stability. Ecology. 36: 533-536.

MacArthur, R.H. and Wilson, E.O. 1967. The Theory of Island Biogeography. Princeton University Press, Princeton.

Maguire, L. 1983. Influence of collard patch size on population densities of lepidopteran pests (Lepidoptera: Pieridae, Plutellidae). Environmental Entomology 12:1415-1419.

Maguire, L.A. 1984. Influence of Surrounding Plants on Densities of *Pieris rapae* (L.) Eggs and Larvae (Lepidoptera: Pieridae) on Collards. Environmental Entomology 13: 464-468.

Maron, J.L. and Harrison, S. 1997. Spatial Pattern Formation in an Insect Host-Parasitoid System. Science. 278: 1619-1623.

Mattiacci, L., Dicke, M. and Posthumus, M.A. 1994. Induction of parasitoid attracting synomone in Brussels sprouts plants by feeding of *Pieris brassicae* larvae: Role of mechanical damage and herbivore elicitor. Journal of Chemical Ecology. 20: 2229-2247.

McCann, K., Hastings, A. and Huxel, G.R. 1998. Weak trophic interactions and the balance of nature. Nature. 395: 794-798.

McCann, K.S. 2000. The diversity-stability debate. Nature. 405: 228-233.

McCloskey, C. and Isman, M.B. 1993. Influence of foliar glucosinolates in oilseed rape and mustard on feeding and growth of the bertha armyworm, *Mamestra configurata* Walker. Journal of Chemical Ecology. 19: 249-266.

Mewis, I., Ulrich, Ch. And Schnitzler, W.H. 2002. The role of glucosinolates and their hydrolysis products in oviposition and host-plant finding by cabbage webworm, *Hellula undalis*. Entomologia Experimentalis et Applicata. 105: 129-139.

Miles, P.W., Aspinall, D. and Rosenberg, L. 1982. Performance of the cabbage aphid, *Brevicoryne brassicae* (L.), on water-stressed rape plants, in relation to changes in their chemical composition. Australian Journal of Zoology. 30: 337-345.

Mithen, R. 2001. Glucosinolates – biochemistry, genetics and biological activity. Plant Growth Regulation. 34: 91-103.

Moericke, V, 1955, Uber die lebensgewohnheiten der geflugelten blattlause (Aphidina) unter besonderer berucksichtigung des verhaltens beim landen. Zeitschrift fur Angewandte Entomologie 37: 29-91.

Moericke, V. 1969. Host-plant specific colour behaviour by *Hyalopterus pruni* (Aphididae). Entomologia Experimentalis et Applicata. 12: 524-534.

Monteith, L.G. 1960. Influence of plants other than the food plants of their host on host-finding by Tachinid parasites. Canadian Entomologist. 92: 641-652.

Montoya, J.M., Rodríguez, M.A. and Hawkins, B.A. 2003. Food web complexity and higher-level ecosystem services. Ecology Letters. 6: 587-593.

Muriel, S.B. and Grez, A.A. 2002. Effect of plant patch shape on the distribution and abundance of three lepidopteran species associated with *Brassica oleracea*. Agricultural and Forest Entomology. 4: 179-185.

Need, J.T. and Burbutis, P.P. 1979. Searching efficiency of *Trichogramma nubilale*. Environmental Entomology 8: 224-227.

Nordlund, D.A., Lewis, W.J. and Altieri, M.A. 1988. Influence of plant-produced allelochemicals on the host/prey selection behavior of entomophagous insects. In: Barbosa, P. and Letourneau, D. (eds). Novel Aspects of Insect-Plant Interactions. Wiley, New York.

Nottingham, S.F., Hardie, J. 1993. Flight behaviour of the black bean aphid, *Aphis fabae*, and the cabbage aphid, *Brevicoryne brassicae*, in host and non-host plant odour. Physiological Entomology. 18: 389-394.

Nottingham, S.F., Hardie, J., Dawson, G.W., Hick, A.J., Pickett, J.A., Wadhams, L.J. and Woodcock, C.M. 1991. Behavioural and electrophysiological responses of aphids to host and non-host plant volatiles. Journal of Chemical Ecology. 17: 1231-1242.

Ode, P.J., Berenbaum, M.R., Zangerl, A.R., Hardy, C.W. 2004. Host plant, host plant chemistry and the polyembryonic parasitoid *Copidosoma sosares*: indirect effects in a tritrophic interaction. Oikos. 104: 388-400.

Odum., E.P. 1953. Fundamentals of ecology. Saunders, Philadelphia.

Ohara, Y., Takafuji, A. and Takabayashi, J. 2003. Response to host-infested plants in females of *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae). Applied. Entomology and Zoology. 38: 157-162.

Oppenheim, S.J. and Gould, F. 2002. Behavioural adaptations increase the value of enemy-free space for *Heliothis subflexa*, a specialist herbivore. Evolution. 56: 679-689.

Ormel, G.J., Gort, G. and van Alebeek, F.A.N. 1995. Analysing host location in *Uscana lariophaga* (Hymenoptera: Trichogrammatidae), an egg parasitoid of bruchids (Coleoptera: Bruchidae), using Cox's proportional hazards model. Bulletin of Entomological Research. 85: 113-123.

Paine, T.R. 1966. Food web complexity and species diversity. American Naturalist. 100: 65-75.

Palaniswamy, P., Gillot, C. and Slater, G.P. 1986. Attraction of diamondback moths, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), by volatile compounds of canola, white mustard, and faba bean. Canadian Entomologist. 118:1279 - 1285.

Papaj, D.R. and Vet, L.E.M. 1990. Odor learning and foraging success in the parasitoid *Leptopilina heterotoma*. Journal of Chemical Ecology. 16: 3137-3150.

Papaj, D.R., Snellen, H., Swaans, K. and Vet, L.E.M. 1994. Unrewarding experiences and their effect of Foraging in the parasitic wasp *Leptopilina heterotoma* (Hymenopter: Eucoilidae). Journal of Insect Behavior. 7: 465-481.

Perfecto, I. and Vet, L.E.M. 2003. Effect of a Nonhost Plant on the Location Behavior of Two Parasitoids: The Tritrophic System of *Cotesia* spp. (Hymenoptera: Braconidae), *Pieris rapae* (Lepidoptera: Pieridae), and *Brassica oleraceae*. Environmental Entomology. 32: 163-174.

Peterson, G., Allen, C.R. and Holling, C.S. 1998. Ecological Resilience, Biodiversity, and Scale. Ecosystems. 1: 6-18.

Peterson, G.D. 2002. Estimating resilience across landscapes. Conservation Ecology. 6: 17. [online] URL: http://www.consecol.org/vol6/iss1/art17

Petterson, J. 1973. Olfactory reactions of *Brevicoryne brassicae* (L.). Swedish Journal of Agricultural Research. 3: 95-103.

Pfisterer, A. B. and Schmid, B. 2002. Diversity-dependent production can decrease the stability of ecosystem functioning. Nature. 416: 84-86.

Pickett, J.A., Wadhams, L.J., Woodcock, C.M. and Hardie, J. 1992.The chemical ecology of aphids. Annual Review of Entomology. 37, 67-90.

Pimentel, D. 1961. Species diversity and insect population outbreaks. Annals of the Entomological Society of America. 54: 76-86.

Pimentel, D. 1991. Diversification of biological control strategies in agriculture. Crop Protection. 10: 243-253.

Plotnick, R.E and Gardner, R.H. 2002. A general model for simulating the effects of landscape heterogeneity and disturbance on community patterns. Ecological Modelling. 147: 171-197.

Podani, J. 1997. Bevezetès a többváltozós biológiai adatfeltárás rejtelmeibe. [Introduction to the multivariate analysis of biological data.] Scientia Kiadó, Budapest.

Polis, G.A. and Strong, D.R. 1996. Food web complexity and community dynamics. The American Naturalist. 147: 813-846.

Polis, G.A. and Winemiller, K.O. 1996. Food Webs: Integration of Patterns and Dynamics. Chapman and Hall. New York.

Potting, R.P.J., Perry, J.N. & W. Powell. 2002. Possibilities and constraints of agro-ecosystem diversification as a pest management strategy: a simulation approach. Proceedings BCPC Conference, Pests and Diseases. 2002: 91-96.

Potting, R.P.J., Poppy, G.M. and Schuler, T.H. 1999. The role of volatiles from cruciferous plants and pre-flight experience in the foraging behaviour of the specialist parasitoid *Cotesia plutellae*. Entomologia Experimentalis et Applicata. 93: 87-95.

Potting, R.P.J., Vet, L.E.M. and Dicke, M. 1995. Host microhabitat location by stem-borer parasitoid *Cotesia flavipes*: the role of herbivore volatiles and locally and systematically induced plant volatiles. Journal of Chemical Ecology. 21: 525-539.

Powell, G. and Hardie, J. 2001. The chemical ecology of aphid host alternation: How do return migrants find the primary host plant? Applied Entomology and Zoology. 36: 259-267.

Power, M. 1992. Top-down and bottom-up forces in food-webs: Do plants have primacy? Ecology. 73: 733-746.

Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N. and Weis, A.E. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annual Review of Ecology and Systematics. 11: 41-65.

Price, P.W. 1981. Semio-chemicals in evolutionary time. In: A.A. Nordlund, R.L. Jones and W.J. Lewis (eds.), Semiochemicals, their role in pest control. Wiley & Sons, New York, pp. 251-271.

Price, P.W. 1988. Inversely density-dependent parasitism: the role of plant refuges for hosts. Journal of Animal Ecology. 57: 89-96.

Price, P.W.1991. The plant vigor hypothesis and herbivore attack. Oikos. 62: 244-251.

Read, D.P., Feeny, P.P. and Root, R.B. 1970. Habitat selection by the aphid parasite *Diaeretiella rapae* (Hymenoptera: Braconidae) and hyperparasite *Charips brassicae* (Hymenoptera: Cynipidae). Canadian Entomologist. 102: 1567-1578.

Reddy, G.V.P. and Guerrero, A. 2000. Behavioural responses of the diamondback moth to green leaf volatiles of *Brassica oleracea* subsp. *capitata*. Journal of Agricultural and Food Chemistry. 48: 6025-6029.

Reddy, G.V.P., Holopainen, J.K. and Guerrero, A. 2002. Olfactory responses of *Plutella xylostella* natural enemies to host pheromone, larval frass, and green leaf cabbage volatiles. Journal of Chemical Ecology. 28: 131-143.

Reed, H.C., Tan, S.H., Haapenen, K., Killmon, M., Reed, D.K. and Elliot, N.C. 1985. Olfactory responses of the parasitoid *Diaeretiella rapae* (Hymenoptera: Aphididae) to odour of plants, aphids, and aphid-plant complexes. Journal of Chemical Ecology. 21: 407-418.

Rice, W.R. 1989. Analyzing Tables of Statistical Tests. Evolution. 43: 223-225.

Risch, S.J., Andow, D. and Altieri, M. 1983. Agroecosystem diversity and pest control: data, tentative conclusions, and new research directions. Environmental Entomology. 12: 625-629.

Ritchie, M.E. and Olff, H. 1999. Spatial scaling laws yield a synthetic theory of biodiversity. Nature. 400: 557-560.

Rodríguez, M.A. and Hawkins, B.A. 2000. Diversity, function and stability in parasitoid communities. Ecology Letters. 3: 35-40.

Rojas, J.C., Wyatt, T.D. 1999. Role of visual cues and interaction with host odour during the host-finding behaviour of the cabbage moth. Entomologia Experimentalis et Applicata. 91: 59-65.

Roland, J. and Taylor, P.D. 1997. Insect parasitoid species respond to forest structure at different spatial scales. Nature. 386: 710-713.

Romeis, J., Shanower, T.G. and Zebitz, C.P.W. 1998. Physical and chemical plant characteristics inhibiting the searching behaviour of *Trichogramma chilonis*. Entomologia Experimentalis et Applicata. 87: 275-284.

Root, R.B. 1973. Organization of a plant-arthropod association in simple and diverse habitats: The fauna of collards (*Brassica oleracea*). Ecological Monographs. 43: 95-124.

Root, R.B. and Kareiva, P.M. 1984. The search for resources by cabbage butterflies (*Pieris rapae*): ecological consequences and adaptive significance of markovian movements in a patchy environment. Ecology. 65: 147 – 165.

Rosenheim, J.A. 1998. Higher-order predators and the regulation of insect herbivore populations. Annual Review of Entomology. 43: 421-447.

Russell, E.P. 1989. Enemies Hypothesis: A Review of the Effect of Vegetational Diversity on Predatory Insects and Parasitoids. Environmental Entomology 18: 590-599.

Saucke, H., Dori, F. and Schmutterer, H. 2000. Biological and integrated control of *Plutella xylostella* (Lep., Yponomeutidae) and *Crocidolomia pavonana* (Lep., Pyralidae) in *Brassica* crops in Papua New Guinea. Biocontrol Science and Technology. 10: 595-606.

Scheffer, M., Carpenter, S. Foley, J.A., Folke, C. and Walker, B. 2001. Catastrophic shifts in ecosystems. Nature. 413: 591-596.

Scheffer, M. 2002. Searching Explanations of nature in the mirror world of math. Conservation Ecology. 3: 11. [online] URL: http://www.consecol.org/vol3/iss2/art11

Schellhorn, N.A. and Sork, V.L. 1997. The impact of weed diversity on insect population dynamics and crop yield in collards, *Brassica oleraceae* (Brassicaceae). Oecologia. 111: 233-240.

Sheehan, W. 1986. Response by Specialist and Generalist Natural Enemies to Agroecosystem Diversification: A Selective Review. Environmental Entomology 15: 456-461.

Shiojiri, K. Takabayashi, J. Yano, S. and Takafuji, A. 2001. Infochemically mediated tritrophic interaction webs on cabbage plants. Population Ecology. 43: 23-29.

Shiojiri, K. Takabayashi, J. Yano, S. and Takafuji, A. 2000. Flight response of parasitoids toward plant-herbivore complexes: A comparative study of two parasitoid-herbivore systems on cabbage plants. Applied Entomology and Zoology. 35: 87-92.

Simpson, S.J. and Raubenheimer, D. 2001. The geometric analysis of nutrient-allelochemical interactions: a case study using locusts. Ecology. 82: 422-439.

Smid, H.M., van Loon, J.J.A., Posthumus, M.A. and Vet, L.E.M. 2002. GC-EAG-analysis of volatiles from Brussels sprouts plants damaged by two species of *Pieris* caterpillars: olfactory receptive range of a specialist and a generalist parasitoid wasp species. Chemoecology. 12: 169-176.

Smith, H.A. and McSorley, R. 2000. Intercropping and pest management: a review of major concepts. The American Entomologist. 46: 154-161.

Sokal, R.R. and Rohlf, F.J. 1995. Biometry. W. Freeman Publishing.

Stanton, M.L. 1983. Spatial Patterns in the Plant Community and Their Effects upon Insect Search. In Ahmad, S. (ed.) Herbivorous Insects: Host-Seeking Behaviour and Mechanisms. Academic Press, New York.

Steinberg, S., Dicke, M. and Vet, L.E.M. 1993. Relative importance of infochemicals from first and second trophic level in long-range host location by the larval parasitoid *Cotesia glomerata*. Journal of Chemical Ecology. 19: 47-60.

Steinberg, S., Dicke, M., Vet, L.E.M. and Wanningen, R. 1992. Response of the braconid parasitoid *Cotesia* (=*Apanteles*) *glomerata* to volatile infochemicals: effect of bioassay set-up, parasitoid age and experience and barometric flux. Entomologia Experimentalis et Applicata. 63: 163-175.

Takabayashi, J., Dicke, M. 1992. Response of predatory mites with differing rearing histories to volatiles of uninfested plants. Entomologia Experimentalis et Applicata. 64: 187-193.

Takabayashi, J., Noda, T., and Takahashi, S, 1991, Plants produce attractants for *Apanteles kariyai*, a parasitoid of *Pseudaletia separate*: cases of "communication" and "misunderstanding" in parasitoid-plant interactions. Applied Entomology and Zoology. 26: 237-243.

Takabayashi, J., Sato, Y., Horikoshi, M., Yamaoka, R., Yano, S., Oshaki, N. and Dicke, M. 1998. Plant effects on parasitoid foraging: differences between two trophic systems. Biological Control. 11:97-103.

Talekar, N.S. and Shelton, A.M. 1993. Biology, ecology and management of the diamondback moth. Annual Review of Entomology. 38: 275-301.

Teder, T. and Tammaru, T. 2002. Cascading effects of variation in plant vigour on the relative performance of insect herbivores and their parasitoids. Ecological Entomology. 27: 94-107.

Tenhumberg, B. and Poehling, H.A. 1995. Syrphids as natural enemies of cereal aphids in Germany: Aspects of their biology and efficacy in different years and regions. Agriculture Ecosystems and Environment. 52: 39-43.

Theunissen, J. and Den Ouden, H. 1980. Effects of intercropping with *Spergula arvensis* on pests of Brussels sprout. Entomologia Experimentalis et Applicata. 27: 260-268.

Theunissen, J. and Schelling, G. 1996. Undersowing crops of white cabbage with strawberry clover and spurrey. Bulletin-IOBC-WPRS 19: 128-135.

Thomas, M.B. 1999. Ecological approaches and the development of "truly integrated" pest management. Proceedings of the National Academy of Sciences of the USA. 96: 5944-5951.

Thompson, J.N. 2001. The geographic dynamics of coevolution. -In: Fox and Roff (eds) Evolutionary Ecology. pp. 331-343.

Tilman, D. and Downing, J.A, 1994. Biodiversity and stability in grasslands. Nature. 367: 363-365.

Tilman, D., Lehman, C.L. and Kareiva, P. 1997. Population Dynamics in Spatial Habitats. In Tilman, D. and Kareiva, P. (eds.). Spatial Ecology. The Role of Space in Population Dynamics and Interspecific Interactions. Princeton University Press, Princeton. pp. 3-20.

Tilman, D., Wedin, D. and Knops, J.M.H. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature. 379: 718-720.

Tollsten, L. and Bergström, G. 1988. Headspace volatiles of whole plants and macerated plant parts of *Brassica* and *Sinapis*. Phytochemistry. 27: 4013-4018.

Traynier, R.M.M. 1984. Associative learning in the ovipositional behaviour of the cabbage butterfly, *Pieris rapae*, induced by contact with plants. Physiological Entomology. 4: 87-96.

Traynier, R.M.M. 1986. Visual learning in assays of sinigrin solution as an oviposition releaser for the cabbage butterfly, *Pieris rapae*. Entomologia Experimentalis et Applicata. 40: 25-33.

Tscharntke, T. and Brandl, R. 2003. Plant-insect interactions in fragmented landscapes. Annual Review of Entomology. 405-430.

Tscharntke, T., Dewenter, I.S., Kruess, A. and Thies, C. 2002. Characteristics of insect populations on habitat fragments: A mini review. Ecological Research. 17: 229-239.

Tumlinson, J.H., Turlings, T.C.J. and Lewis, W.J. 1993. Semiochemically mediated foraging behavior in beneficial parasitic insects. Archives of Insect Biochemistry and Physiology. 22: 385-391.

Turlings, T.C.J., Tumlinson, J.H. and Lewis, W.J. 1990. Exploitation of herbivore-induced plant odors by host seeking parasitic wasps. Science. 250:1251-1253.

van Alphen, J.J.M., Bernstein, C. and Driessen, G. 2003. Information acquisition and time allocation in insect parasitoids. TRENDS in Ecology and Evolution. 18: 81-87.

van der Werf, W., Evans, E.W. and Powell, J. 2000. Measuring and modelling the dispersal of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in alfalfa fields. European Journal of Entomology. 97: 487-493;

van Lenteren, J.C., 1980. Evaluation of control capabilities of natural enemies: does art have to become science? Netherlands Journal of Zoology. 30: 369-381.

van Lenteren, J.C., 1991. Encounters with parasitised hosts: to leave or not to leave a patch? Netherlands Journal of Zoology. 41: 144-157.

van Lenteren, J.C. 1998. Sustainable and safe crop protection: a reality? Proceedings 50th International Symposium on Crop Protection. Vol. 63(2b), Gent, pp. 409-413.

van Lenteren, J.C. and de Ponti, O.M.B. 1990. Plant-leaf morphology, host-plant resistance and biological control. Proceedings of the 7th International Symposium on Insect-Plant Relationships. 39: 365-386.

van Lenteren, J.C., Zhao, L.H., Kamerman, J.W. and Xu, R. 1995. The parasite-host relationship between *Encarsia formosa* (Hym., Aphelinidae) and *Trialeurodes vaporariorum* (Hom., Aleyrodidae).26. Leaf hairs reduce the capacity of *Encarsia* to control greenhouse whitefly on cucumber. Journal of Applied Entomology. 119: 553-559.

van Loon, J.J.A., Everaarts, T.C. and Smallegange, R. 1992. Associative learning in host-finding by female *Pieris brassicae* butterflies: relearning preferences. - In: Menken, S.B.J., Visser, J.H. and Harrewijn, P. (eds.) Proceedings of the 8th International Symposium on Insect-Plant Relationships. Kluwer Academic Publisher, Dordrecht.

Vandermeer, J. 1992. The Ecology of Intercropping. Cambridge University Press.

Van Roermund, H.J.W, Hemerik, L. and Lenteren, J.C. van. 1994. Influence of intrapatch experiences and temperature on the time allocation of the whitefly parasitoid *Encarsia formosa* (Hymenoptera: Aphelinidae). Journal of Insect Behavior. 7: 483-501.

Verkerk, R.H.J. and Wright, D.J. 1996. Multitrophic interactions and managements of the diamondback moth: a review. Bulletin of Entomological Research. 86: 205-216.

Vet, L.E.M. 1999. Evolutionary aspects of plant-carnivore interactions. - In. Insect-plant interactions and induced plant defence. Novartis foundation.

Vet, L.E.M. 2001. Parasitoid searching efficiency links behaviour to population processes. Applied Entomology and Zoology. 36: 399-408.

Vet, L.E.M. and Dicke, M. 1992. Ecology of Infochemical Use by Natural Enemies in a Tritrophic Context. Annual Review of Entomology. 37: 141-172.

Vet, L.E.M., Hemerik, L., Visser, M.E. and Wäckers, F.L. 2002. Flexibility in host-search and patch-use strategies of insect parasitoids. – In: Lewis, E.E., Campbell, J.F. and M.V.K. Sukhdeo (eds.) The behavioural ecology of parasites. CABI Publishing.

Vet, L.E.M., de Jong, A.G., Franchi, E., and Papaj, D.R. 1998. The effect of complete vs incomplete information on odour discrimination in a parasitic wasp. Animal Behaviour. 55: 1271-1279.

Vet, L.E.M., Lewis, W.J. and Cardé, R.T. 1995. Parasitoid foraging and learning. - In: Bell, W. & Cardé, R.T. (eds.) Chemical Ecology of Insects, 2nd ed. Chapman & Hall, London, pp. 65-101.

Vet, L.E.M., Lewis, W.J., Papaj, D.R. and van Lenteren, J.C. 1990. A variable-response model for parasitoid foraging behaviour. Journal of Insect Behavior. 3: 471-490.

Vet, L.E.M., Lewis, W.J., Papaj, D.R. and van Lenteren, J.C. 2003. A variable-response model for parasitoid foraging behaviour. – In: van Lenteren, J.C. (ed.) Quality control and production of biological control agents: Theory and testing procedures. CABI Publishing.

Vet, L.E.M., Wäckers F. and Dicke, M. 1991. How to hunt for hiding hosts: the reliability-detectability problem in foraging parasitoids. Netherlands Journal of Zoology. 41: 202-213.

Vidal, S. 1997. Factors influencing the population dynamics of *Brevicoryne brassicae* in undersown Brussels sprout. Biological Agriculture and Horticulture. 15: 285-295.

Vinson, S.B. 1976. Host Selection by Insect Parasitoids. Annual Review of Entomology. 21: 109-133.

Vinson, S.B. 1998. The General Host Selection Behavior of Parasitoid Hymenoptera and a Comparison of Initial Strategies Utilized by Larvaphagous and Oophagous Species. Biological Control. 11:79-96.

Völkl, W. and Sullivan, D.J. 2000. Foraging behaviour, host plant and host location in the aphid hyperparasitoid *Euneura augarus*. Entomologia Experimentalis et Applicata. 97: 47-56.

Vos, M., Berrocal, S.M., Karamaouna, F., Hemerik, L. and Vet, L.E.M. 2001. Plant-mediated indirect effects and the persistence of parasitoid-herbivore communities. Ecology Letters. 4: 38-45.

Vos, M., Hemerik, L. 2003. Linking foraging behavior to lifetime reproductive success for an insect parasitoid: adaptation to host distributions. Behavioral Ecology. 14: 236-245.

Vos, M., Hemerik, L. and Vet, L.E.M. 1998. Patch exploitation by the parasitoids *Cotesia rubecula* and *Cotesia glomerata* in multi-patch environments with different host distributions. Journal of Animal Ecology. 67: 774-783.

Waage, J.K. 1979. Foraging for patchily distributed hosts by the parasitoid *Nemeritis canescens*. Journal of Animal Ecology. 48: 353-371.

Waage, J.K. 1983. Aggregation in field parasitoid populations: Foraging time allocation by a population of *Diadegma* (Hymenoptera, Ichneumonidae). Ecological Entomology. 8: 447-453.

Wäckers, F. 1994. Multisensory Foraging by Hymenopterous Parasitoids. PhD Thesis, Wageningen University. pp. 155.

Wäckers, F.L. 2001. A comparison of nectar- and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. Journal of Insect Physiology. 47: 1077-1084.

Wäckers, F.L. 2003. The parasitoids' need for sweets: sugars in mass rearing and biological control. – In: van Lenteren, J.C. (ed.) Quality control and production of biological control agents: Theory and testing procedures. CABI Publishing.

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

Wäckers, F.L. and Lewis, W.J. 1994. Olfactory and visual learning and their combined influence on host site location by the parasitoid *Microplitis croceipes* (Cresson). Biological Control. 4: 105-112.

Wäckers, F.L. and Steppuhn, A. 2003. Characterizing nutritional state and food source use of parasitoids collected in fields with high and low nectar availability. Landscape Management and Functional Biodiversity. IOBC/WPRS Bulletin. 26: 209-213.

Wahbi, A.A. 1967. Untersuchungen über den Einfluß der Temperatur und der relativen Luftfeuchtigkeit auf das Fraßvermögen von Syrphidenlarven (Diptera, Syrphidae). Ph.D. Thesis, University of Göttingen.

Wajnberg, E., Fauvergue, X. and Pons, O. 2000. Patch leaving decision rules and the Marginal Value Theorem: an experimental analysis and a simulation model. Behavioral Ecology. 11: 577-586.

Walker, B. 1995. Conserving biological diversity through ecosystem resilience. Conservation Biology. 9: 747-52.

Wang, X. 2001. Patch exploitation by the parasitoids of *Plutella xylostella* (L.): from individual to population dynamics. PhD Thesis, University of Adelaide, Adelaide, Australia, pp. 230.

Wang, X.G. and Keller M. 2002. A comparison of the host-searching efficiency of two larval parasitoids of *Plutella xylostella*. Ecological Entomology. 27: 105-114.

Wang, X.G. and Keller, M.A. 2003. Patch time allocation by the parasitoid *Diadegma semiclausum* (Hymenoptera: Ichneumonidae). I. Effect of interpatch distance. Journal of Insect Behavior. 16: 279-293.

Ward, S.A., Leather, S.R., Pickup, J. and Harrington, R. 1998. Mortality during dispersal and the cost of host-specificity in parasites: how many aphids find hosts? Journal of Animal Ecology. 67: 763-773.

Wearing, C.H. (1967). Studies on the relations of insect and host plant. II. Effects of water stress in host plants on the fecundity of *Myzus persicae* (Sulz.) and *Brevicoryne brassicae* (L.). Nature. 213: 1052-1053.

Wearing, C.H. and Van Emden, H.F. (1967). Studies on the relations of insect and host plant. I. Effects of Water Stress in Host Plants on Infestation by *Aphis fabae* Scop., *Myzus persicae* (Sulz.) and *Brevicoryne brassicae* (L.). Nature. 213: 1051-1052.

White, T.C.R. 1974. A hypothesis to explain outbreaks of looper caterpillars, with special reference to populations of *Selidosema suavis* in a plantation of *Pinus radiata* in New Zealand. Oecologia. 16: 279-301.

Wilby, A. and Thomas, M.B. 2002. Are the ecological concepts of assembly and function of biodiversity useful frameworks for understanding natural pest control? Agricultural and Forest Entomology. 4: 237-243.

Wilkinson, T.K. and Landis, D.A. in press. Habitat diversification in biological control: the role of plant resources. In. F.L. Wäckers, P.C.J. van Rijn & J. Bruin (eds.). Plant Provided Food and Plant-Carnivore Mutualism. Cambridge University Press

Winkler, K., Wäckers, F.L., Valdivia, L., Larraz, V. and Lenteren, J.C. 2003. Strategic use of nectar sources to boost biological control. Landscape Management and Functional Biodiversity. IOBC/WPRS Bulletin. 26: 209-213.

Wiskerke, J.S.C. and Vet L.E.M. 1994. Foraging for solitarily and gregariously feeding caterpillars: a comparison of two related parasitoid species. Journal of Insect Behavior. 7: 585-603.

Wissinger, S.A. 1997. Cyclic Colonisation in Predictably Ephemeral Habitats: Template for Biological Control in Annual Crop Systems. Biological Control. 10: 4-15.

With, K.A. and Crist, T.O. 1995. Critical thresholds in species` responses to landscape structure. Ecology. 76: 2446-2459.

With, K.A., Pavuk, D.M., Worchuck, J.L. Oates R.K. and Fisher J.L. 2002. Threshold effects of landscape structure on biological control in agroecosystems. Ecological Applications. 12: 52-65.

Wolfson, J.L. 1980. Oviposition response of *Pieris rapae* L. to environmentally induced variation in *Brassica nigra*. Entomologia Experimentalis et Applicata. 27: 223-232.

Yamamura, K. 1999. Relation between plant density and arthropod density in cabbage fields. Researches on Population Ecology. 41: 177-182.

Yano, S. 1994. Ecological and evolutionary interactions between wild crucifers and their herbivorous insects. Plant Species Biology. 9: 137-143.

Samenvatting

Gediversificeerde ecosystemen bezitten vaak positieve eigenschappen, zoals bescherming tegen de uitbraak van plagen. Een afname in herbivore populaties wordt vaker gevonden in vegetatie-diverse dan in eenvoudige agro-ecosystemen. Mengsels van plantensoorten (m.a.w. mengteelt) kunnen derhalve worden gebruikt om problemen met plagen in agro-ecosystemen te beheersen, daarbij meewerkend aan de ontwikkeling van een ecologisch duurzame gewasproductie. In vergelijking met een monocultuur kan een toename in vegetatiediversiteit herbivore populaties onderdrukken door een afname in de zichtbaarheid en kwaliteit van de planten in een cultuurgewas en door het grotere success van natuurlijke vijanden. Helaas biedt de zeer variabele respons van zowel de herbivoren als de natuurlijke vijanden op mengteelt onvoldoende informatie om generalisaties te maken voor het selecteren van plaag-onderdrukkende plantenmengsels. Een verklaring voor het ontbreken van informatie is dat voorspellingen van de hypothesen die de onderdrukking van de plaag verklaren relevant zijn op het populatieniveau, terwijl de onderliggende mechanismen op gedrags- (m.a.w. functionele) niveau nog hoofdzakelijk ongetoetst zijn.

Het doel van mijn promotieonderzoek was het specificeren van de ecologische condities die nodig zijn voor het onderdrukken van herbivoren en het vergroten van het succes van natuurlijke vijanden, daarbij gebruik makend van vegetatiediversiteit in agro-ecosystemen. Het experimentele werk richtte zich op het toelichten van de interacties tussen diverse habitats (m.a.w. mengsels van plantensoorten), herbivore insecten en hun natuurlijke vijanden. De specifieke doelen waren (1) het begrijpen van de oorzaken van lagere aantallen herbivoren in diverse gewassystemen i.v.m. monoculturen en (2) het bestuderen van de gedragsrespons van natuurlijke vijanden op vegetatiediversiteit. De experimentele systemen omvatten spruitjeskool (*Brassica oleracea* gemmifera), haar herbivoren, en *Diadegma semiclausum*, een sluipwesp van de koolmot. Vegetatiediversiteit werd gekarakteriseerd d.m.v. het mengen van spruitjeskool met gerst (*Hordeum vulgare*) of witte mosterd (*Sinapis alba*).

In de veldobservaties die zijn beschreven in **hoofdstuk 2** werd onderzocht of het mengen van spruitjeskool met gerst de herbivore populaties en natuurlijke vijanden beïnvloedde. Het aantal herbivore soorten werd gereduceerd als spruitjeskool werd gemengd met gerst. In **hoofdstuk 3** bestudeerden we of competitie tussen planten de respons van herbivoren beïnvloedde door de plantgrootte en kwaliteit te veranderen in de intercrop vergeleken met de monocrop van spruitjeskool. Hier werd bewijs gevonden voor effecten van veranderde plant kwaliteit door interspecieke competitie tussen planten op de respons van de herbivore populatie. Herbivore respons op plant kwaliteit varieerde met de bestudeerde insectensoort. In een combinatie van veldonderzoek met simulatiestudies in **hoofdstuk 4** werd basale kennis toegepast van de fourageerstrategiën van drie specialistische herbivoren, de melige koolluis (*Brevicoryne brassicae*), het koolwitje (*Pieris rapae*) en de koolmot (*Plutella xylostella*), om de verschillen in hun respons op “small-scale” fragmentatie van hun habitat te verklaren. We vonden dat de ruimtelijke dynamiek van herbivoren beïnvloed kon worden door soort-gerelateerde verschillen in de relatieve belangrijkheid van stimulus modaliteit (m.a.w. contact, visueel, olfactorisch) die de locale aanwezigheid van herbivoren in gastheerplant “patches” van verschillende grootte beïnvloedt. In **hoofdstuk 5** werd de respons van *D. semiclausum* op inter- and intraspecifieke variatie in geurprofielen van cruciferen en een niet-crucifere soort beschreven d.m.v. het gebruik van een combinatie van Y-buis olfactometer biotoetsen en een chemische analyse van “headspaces” van vluchtige plantenstoffen. Gedrags- en analytische studies benadrukten de importantie van inter- en intraspecifieke variatie in vluchtige stoffen van zowel beschadigde als onbeschadigde planten voor de aantrekking van de sluipwesp *D. semiclausum*. De aantrekkelijkheid van planten voor vrouwtjes was niet het resultaat van een grotere hoeveelheid geproduceerde vluchtige plantenstoffen: ondanks de lagere hoeveelheid geproduceerde vluchtige stoffen vonden vrouwtjes het geurmengsel van ongeïnfecteerde witte mosterd planten aantrekkelijker dan dat van ongeïnfecteerde spruitjeskool planten. De effecten van dichtheid, compositie van de soorten en configuratie van planten op de zoekefficiëntie van *D. semiclausum* om gastheer “patches” te localiseren werd bestudeerd in verschillende semi-veld opstellingen in **hoofdstuk 6**. Ook hebben we in een soortgelijke opstellingen in **hoofdstuk 7** onderzocht hoe ervaringen met het ontmoeten van gastheer “patches” het daaropvolgende fourageersuccess en de tijd-allocatie van *D. semiclausum* in verschillende composities van plantensoorten beïnvloedde. Er bleek op verschillende manieren een interactie te bestaan tussen de mengsels van planten en het zoekgedrag en de tijd-allocatie van de wespen. Vergeleken met de pure spruitjeskool “patches” trok witte mosterd meer individuen aan en hield ze ook langer vast terwijl gerst de tendens om een patch met planten te betreden en het starten met zoeken verminderde. Alhoewel zowel witte mosterd en gerst de tendens van wespen om gastheren op spruitjeskool te vinden verminderden, verhoogden sluipwespen hun fourageerefficiëntie door eilegervaringen en werden even efficiënt in het vinden van volgende gastheren in alle habitattypen. In **hoofdstuk 8** worden de in dit proefschrift beschreven ontdekkingen bediscussieerd met een nadruk op hoe de resultaten gebruikt kunnen worden om de ecologische condities te specificeren voor beheer en het vegroten van het succesvan natuurlijke vijanden bij diversificatie van agro-ecosystemen.

In conclusie kan worden gezegd dat dit proefschrift verschillende mogelijke mechanismen heeft geïdentificeerd die de variabele respons van herbivoren en sluipwespen op mengsels van plantensoorten verklaren, en die ook bruikbaar zijn voor toekomstig onderzoek. Competitie tussen planten in mengteelt systemen kan verschillende effecten hebben op herbivore populaties. Daarmee kunnen zij verantwoordelijk zijn voor een deel van de variatie in herbivore respons op mengteelt die zijn gevonden in eerdere veldproeven. Omdat competitieve relaties verschillen tussen combinaties van planten, is een mechanistische evaluatie van verschillende mengteelt systemen in conventionele veldproeven practisch onmogelijk. Er bestaat een noodzaak om veldwerk te combineren met, uit gedrags- en fysiologische studies bestaande, laboratoriumproeven om de directe effecten van een toename in vegetatiediversiteit te onderscheiden van de effecten van plantenstress. Verschillen in fourageergedrag van herbivoren kunnen de locale populatiegrootte van herbivoren beïnvloeden en hun variabele respons op mengsels van plantensoorten verklaren. Alhoewel meer onderzoek nodig is om te bepalen hoe vegetatiediversiteit kan interacteren met het zoekgedrag van herbivoren, kan een basale kennis van hun zoekgedrag ons helpen om hun ruimtelijke dynamiek in diverse agro-ecosystemen te begrijpen.

De sluipwesp *D. semiclausum* maakt extensief gebruik van plant-gerelateerde vluchtige stoffen en bleek zeer efficiënt in het localiseren van gastheren in alle habitattypen. Het resultaat dat er, op verschillende wijzen, een interactie bestaat tussen verschillende mengsels van plantensoorten en het fourageergedrag van deze sluipwesp toont aan dat eenvoudige generalisaties van de effecten van mengsels van plantensoorten op één enkele natuurlijke vijand niet mogelijk kan zijn. De individuele respons van sluipwespen op vegetatiediversiteit is waarschijnlijk afhankelijk van hoe de informatieve waarde van plantenstoffen wordt waargenomen en gebruikt in een gegeven multitrofe context en hoe dat gebruik wordt veranderd door ervaringen. Omdat parasiteringsgraden in een habitat ook worden bepaald door het aantal aanwezige sluipwespen, heeft de vondst dat tijd-allocatie (m.a.w. zoek-, aankomst- en vertrekneigingen) van sluipwespen op verschillende manieren wordt beïnvloed door de compositie van mengsels van plantensoorten implicaties voor interacties op het populatieniveau. Mengsels van plantensoorten kunnen namelijk dienen als bronnen en een toename in sluipwespen populaties veroorzaken d.m.v. het aantrekken, en langer vasthouden, van individuen (bv. witte mosterd), terwijl andere mengsels als een afvoer kunnen dienen en een afname in populaties kunnen veroorzaken d.m.v. een verminderde immigratie (bv. gerst). Om de plaag-onderdrukkende waarde van een bepaald mengsel van planten te voorspellen moet er daarom een link tot stand worden gebracht tussen de respons van herbivoren en natuurlijke vijanden op gedrags- en populatieniveau.

Összefoglalás

A természetesen diverz ökoszisztémák gyakran rendelkeznek pozitiv tulajdonságokkal, mint például, a kártevő-populációk felszaporodásának csökkentésével. Következésképpen, agro-ökoszisztémákban gyakran alkalmaznak egyes élőhely rendezési eljárásokat, például növénytársításokat (pl. köztesvetést, kevert vetést, alávetést), hogy a térbeli monokultúrákban megfigyelt szintekhez képest a rovarkártevők egyedszámát csökkentsék. A növénytársításokban megfigyelt alacsonyabb kártevő populációszintek kialakításában számos tényező játszhat szerepet. A feltételezett mechanizmusok egyike például az, hogy a diverz rendszerekben annak valószínűsége, hogy a kártevők megtalálják tápnövényüket, alacsonyabb mint az egynövényes rendszerekben. Továbbá a tápnövények minősége diverz rendszerekben csökkenhet, illetve a kártevők természetes ellenségeinek előfordulása és azok eredményessége a kártevő populációk csökkentésében is gyakran növekedhet a monokulturákhoz hasonlítva. Sajnos, mind a kártevők, mind a természetes ellenségeik populációi igen változó szintet mutatnak köztestermesztési rendszerekben a monokulturákhoz hasonlítva. Így nem rendelkezünk elég információval ahhoz, hogy olyan kritériumokat állíthatnánk fel, melyek segítségünkre lehetnének a kártevő-visszaszorító növénytársítások tudatos kiválasztásában. Ezen információhiánynak a fő oka az, hogy az egyes hipotézisek a diverz rendszerek kártevő-visszaszorító hatását populáció szinten tárgyalják, míg az azokat magyarázó mechanizmusok, melyek az egyedi viselkedés szintjén működnek, nagyrészt feltáratlanok maradnak. A tézisben leírt kísérleteken keresztül megpróbáltam a vegetáció diverzitásnak a kártevők visszaszorításáért felelős mechanismusokról rendelkezésünkre álló tudásanyagot bővíteni. A célkitűzés az volt, hogy magyarázatot találjak arra, hogy (1) a monokultúrás rendszerekhez képest, a diverz rendszerekben miért alacsonyabbak a specialista kártevők populációszintjei. (2) Másrészt, a vegetáció diverzitás növekedésének a természetes ellenségek gazda/préda állat keresési viselkedésére gyakorolt hatását vizsgáltam. A tanulmányozott modellrendszer a bimbóskelt (*Brassica oleracea* gemmifera), annak növényevő rovarfajait, és a *Diadegma semiclausum*-ot, a káposztamolynak egyik parazitoidját foglalta magába. A vegetáció diverzitásának növelésekor a bimbóskelt tavaszi árpával (*Hordeum vulgare*), illetve fehér mustárral (*Sinapis alba*) társítottuk.

A tézisben leírt kísérletek számos, eltérő mechanizmust azonosítottak, melyeken keresztül a növénytársítások befolyásolhatják a herbivórok populációinak egyedszámát. Az egynövényes bimbóskel kultúrához képest, a bimbóskel-árpa növénytársítás több növényevő faj populációinak szintjét csökkentette. Az ezt követő tanulmány bizonyította, hogy a társításban részt vevő növényfajok közötti versengés befolyásolhatja a herbivórok fejlődését a tápnövény minőség megváltoztatásával. Ezáltal a versengésnek a növény minőségére, méretére és fenológiájára gyakorolt hatása együttesen bonyolíthatja a vegetáció diverzitásnak a felsőbb trofikus szintekre gyakorolt hatásainak egyértelmű kiértékelését. Ezáltal a növényfajok közötti versengés felelőssé tehető a kártevő populációknak, a korábbi összehasonlítások egy részében megfigyelt változó reakcióiért. Szabadföldi és számítógépes szimulácós tanulmányok bizonyították, hogy az egyes rovarfajok által használt tápnövénykeresési viselkedésbeni különbségek befolyásolhatják azok helyi populációinak méretét térbelileg heterogén habitatokban. Így a fajok között fellépő viselkedésbeni különbségek, részben felelőssé tehetők a diverzitás fokozásának függvényében mefigyelt változékony populációszintekért. Az eredmények azt mutatják, hogy már az általános információ a herbivórok tápnövény keresési és kihasználási viselkedéséről is hasznos lehet, azok heterogén térben megfigyelt dinamikájának jellemzésében. Viselkedési bioesszéket, és kémiai analitikai módszereket használva demonstráltuk a növényi illatanyagok fajok közötti és fajokon belüli különbségeinek fontosságát mind károsított és intakt növényeknél, a *D. semiclausum* növények felé történő vonzódásában. Az ezt követő kísérletek kimutatták, hogy a különböző növénytársítások a parazitoidok gazdaállat keresési viselkedését és keresési időbeosztását különbözőképpen befolyásolták. Az egynövényes bimbóskel habitathoz képest, a mustár az egyedeket erősebben vonzotta és hosszabb ideig tartotta vissza; míg az árpa mint társnövény az egyedek azon tendenciáját hogy a habitatba behatoljanak és keresni kezdjenek, csökkentette. Mind a mustár és az árpa mint társnövények, az első gazdaállat- csoport megtalálásának hatékonyságát csökkentették. A parazitoidok a tojásrakások során gyorsan tanultak, ezáltal a további gazdaállatok megtalálásában, a növénytársítástól mint kezeléstől függetlenül, egyformán hatékonyakká váltak. A *D. semiclausum*-nak különböző növénytársításokra adott eltérő viselkedési reakciója azt mutatja, hogy a természetes ellenségek vegetáció diverzitásra adott reakcióira vonatkozóan egyszerű általánosítások nem fogalmazhatóak meg. A parazitoidnak az adott növénytársításra vonatkozó egyedszintű reakciói valószínűleg azon múlhatnak, hogy a növényi illatanyagnak milyen az információs értéke az adott multi-trofikus összefüggésben, és azt a tapasztalatok, mint például a tanulás, hogyan befolyásolhatják. Mivel azonban a parazitáltsági százalék az élőhelyen belül függeni fog a jelen lévő parazitoidok számától, az egyedek időbeosztásában a növénytársítástól függően megfigyelt különbségek (pl. érkezési, keresési és távozási tendenciák a habitatban) jelentősek lehetnek a populácók szintjén megfigyelt reakciókban is. Így a monokultúrákhoz képest egyes növénytársítások „forrásként” (pl. mustár) hathatnak, és a parazitoidok vonzásával és visszatartásával magasabb populációszintek kialakulásához vezethetnek. Míg mások „nyelőként” (pl. árpa) funkcionálhatnak, mert a immigráció csökkentésével alacsonyabb populációszintek alakulhatnak ki. Így aztán ahhoz, hogy egy adott növényfaj kártevő-visszaszorító hatását meghatározzuk, szükség van a herbivórok és a természetes ellenségek populáció-szintjein megfigyelt jelenségeknek az egyedi viselkedés szintjén történő magyarázatára.

Acknowledgements

The past four years that I spent as a Ph.D student at the Laboratory of Entomology was very rich both in challenges and in pleasant experiences. When I started this project, my experience was restricted mainly to do fieldwork and taxonomy. Hence, it was quite a change to start- up with laboratory experiments and to get into research related to behavioural ecology. It turned out to be a wonderful experience! It was due to the help of several individuals and the good working atmosphere that surrounded me, that made it possible to carry out and finalise this research.

I would like to thank my promotors, Joop van Lenteren and Louise Vet, for providing me excellent guidance and support throughout this project. Joop and Louise, I greatly value the independence that I was given when planning and carrying out experiments and the inspiring discussions we had at our meetings. When needed, we always discussed problems and found the necessary solutions in time. In the writing-up phase, when timing was important, I always received comments in time, so I never felt stuck with the work. Louise, your enthusiastic help when discussing parasitoid behaviour, inspired me to look at the complex results from different angles. Joop, you did not only handle most of the administrative work related to the project with great efficiency, but thanks to you, I had the chance to work together with several researchers and students. I am grateful for the possibility to work together with members of our “biodiversity-gang” and to learn from them. Namely, Michael Brewer, who spent a sabbatical at our lab, and several students: Julien Fremont, Yann Clough, Tatiana Estay, Regina Csorba, Ágnes Spáda and Jacek Twardovski.

Not only students helped me in setting-up the experiments and collecting data. Thank you for your contribution Rieta, without it, a great part of this thesis would be missing. You helped me to carry out experiments with parasitoids and to discuss data, you collected and together with Maarten Posthumus analysed headspace samples. I am grateful for the statistical help of Lia Hemerik, Gerrit Gort and Joep Burger. Thank you Lia that you were always available, armed with the necessary amount of coffee, to discuss data for long hours and taught me several ways how survival analysis can be applied in behavioural studies. Roel, with your expertise in individual-based simulations and programming, you did not only contribute to this work, but thanks to you, I look at insect behaviour in a more mechanistic way. Tréfi and Karin, I am grateful for the good discussions and for the valuable scientific and not-scientific times that we spent together. Members of our functional diversity group; Felix Bianchi, Felix Wäckers, Nick, Gerbert and those of the Ph.D. discussion group at our laboratory, often helped in debating new ideas or solving the actual problems. Joop van Loon, Marcel Dicke, Jeff Harvey and Matthijs Vos, your valuable and constructive comments greatly improved several chapters of this thesis. Antoon, I am happy for the good discussions we had, whenever I visited your office. Yde, you were always ready to answer taxonomic questions. I thank Ties for translating the summary into Dutch. Many administrative problems that were related to the project, were handled with great efficiency by Ineke Kok and Sabine Meijerink.

Several colleagues provided the necessary conditions and materials for experimental work. Thank you Wouter for maintaining and preparing high-quality *Diadegma* wasps for experimental work. André, Frans and Leo, you took excellent care of the diamondback moth and the cabbage butterfly rearing. Leo, however urgent my requests were for plants or greenhouse space, you always provided the quick and permanent solution, often followed by Dutch expressions and idioms that still constitutes the basis of my “spreekwoord” collection. Herman Dijkman and Wouter provided space in the thermostats and climate rooms. I thank Herman Masselink, John van der Lippe and the crew of Unifarm for the preparation of the layout of the field experiments.

Friends make the staying in abroad easier and pleasant! Wouter, Aafke, Pentcho, Sandra, Karin, Trefi and Viktor, you did not only help us in problematic situations, like moving from one accommodation to another, but we also had great times talking, going to exhibitions, movies or parties together, some of these activities we also enjoyed with Nina, Ties and Clemens. Yde, we organised many BBQ’s together, a pleasant activity that I hope we continue in the future. Thanks to my roommates Gladys and Raul, our office was a place where I liked to spent the working hours.

And at last, I would like to thank my Family. My dear parents, without your help this all would not have been possible. You always supported me every ways you could and motivated me to find a profession that is also my hobby. And you tolerated my enthusiasm really well too: I never forget how shocked my mother was, when she found one of my first pitfall -trap materials among the canned fruits in the cellar… Miklós, it is fantastic to have such a big-hearted and open-minded brother like you. We have had an adventurous childhood, and you still manage to surprise me every time we meet...

And of course, this work would not have been possible without the help of Gabriella. You are a true partner in life whom I can always count on. Thank you for providing the unique atmosphere at home and helping me in every possible ways you could, whether it was fieldwork, labwork or “just” listening. In times when I was disappointed and desperate, you always managed to take my mind off the problems for a moment and cheer me up. I hope that I can be as much help to you as you are to me.

Tibor
Wageningen, May, 2004

Curriculum vitae

I, Tibor Bukovinszky, was born in Hatvan (Hungary) on the 11th of March 1977. I lived in Százhalombatta, where I studied in the local primary school. When finishing my secondary grammar school education at the biology class of the Mihály Vörösmarty Gimnázium (Èrd), I continued my studies at the Agricultural University at Gödöllő, with the specialistaion in Crop Protection and Entomology. In the second and the third years of my studies (1997 and 1998), I visited the Laboratory of Entomology of Wageningen University for two summer seasons to participate in field experiments. This was followed by a study for an M.Sc. in Integrated Pest Management that I received in 1999. For a major thesis, I studied the effects of intercropping on aphid populations and their natural enemies. For a minor thesis, I studied the infochemical use of hyperparasitoids of aphids. Between 1 April, 2000 and 1 April, 2004, I worked as a Ph.D. student at the Laboratory of Entomology of the Wageningen University. The aim of my thesis project was to understand the cause(s) of lower herbivore numbers in vegetationally diversified cropping systems compared with monocultures and to study the behavioural responses of natural enemies to vegetation diversity. The work was supervised by Joop C. van Lenteren and Louise E.M. Vet. The project was financed by the section Earth and Life Sciences of the Dutch Scientific Organisation (NWO-ALW). The results of this research is written in this thesis. Since 1st of May 2004, I am appointed as a post-doc at the Laboratory of Entomology of Wageningen University. My task is to study the role of induced plant defences in food-web dynamics.

List of publications

Published

Clough, Y., Bukovinszky, T., van Lenteren, J.C. and Vet, L.E.M. 2002. Evaluating pest pressure in the Brussels sprouts-barley intercropping system: influence of host plant patch size and surrounding vegetation. *NEV. Proceedings Experimental and Applied Entomology*. 13: 123-126.

Bukovinszky, T., Rasztik, V., van Lenteren, J.C., Vet, L.E.M. and Bujáki, G. 2003.The effects of undersowing (Brussels sprouts – black mustard) on population density of *Brevicoryne brassicae* and natural enemies of aphids. Integrated Control in Field Vegetable Crops. *IOBC/WPRS Bulletin*. 26: 307-314.

Bukovinszky, T., Brewer, M.J., Winkler, K., Tréfás, H., Vet, L.E.M. and van Lenteren, J.C. 2003. Effects of flowering field margins on flight activity of the diamondback moth (*Plutella xylostella* L.) and its parasitoids *Diadegma* spp., and observations on distance from field edge and vertical position of traps. Integrated Control in Field Vegetable Crops. *IOBC/WPRS Bulletin*. 26: 307-314.

Bukovinszky, T., Potting, R.P.J., Clough, Y., van Lenteren, J.C. and Vet, L.E.M. 2003. Responses of insect herbivores to habitat texture: the role of foraging strategy. Landscape Management and Functional Biodiversity (2003, Bologna, Italy). *IOBC/WPRS Bulletin*. 26: 25-29.

Bukovinszky, T., Tréfás, H., van Lenteren,J.C., Vet, L.E.M. and Fremont, J. 2004. Plant competition in pest-suppressive intercropping systems complicates evaluation of herbivore responses. *Agriculture, Ecosystems and Environment*. 102: 185-196.

Submitted

Bukovinszky, T., Potting, R.P.J., Clough, Y., van Lenteren, J.C. and Vet, L.E.M. The role of foraging behaviour in the spatial dynamics of herbivores in heterogeneous habitats. (to *Oikos*).

Bukovinszky, T., van Lenteren, J.C. and Vet, L.E.M. Functioning of natural enemies in mixed cropping systems. – In: Pimentel, D. (ed.) *Encyclopedia of Pest Management*. Marcel Dekker Inc.

Bukovinszky, T., Gols, R., Posthumus, M.A., Vet, L.E.M. and van Lenteren, J.C. Variation in plant volatiles and the attraction of the parasitoid *Diadegma semiclausum* (Hellén). (to *Journal of Chemical Ecology*).

The research described in this thesis was carried out at the Laboratory of Entomology of Wageningen University. The project was supported by the Council for Earth and Life Sciences (ALW), which is subsidised by the Netherlands Organisation of Scientific Research (NWO), under project number 014-22.031.

Printed by Ponsen en Looijen BV, The Netherlands
Cover design: Ecosystem stability and conservation biological control by Tibor Bukovinszky