Assessing the risks and benefits of flowering field edges

Strategic use of nectar sources to boost biological control

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ABSTRACT

The intensification of agricultural production systems during the last decades had a enormous impact on landscape structure in agro-ecosystems. Landscape elements like hedges and vegetational rich fieldmargins dissapeared and crops are cultivated in large monocultures. To let beneficial insects play a role in these 'ecological deserts' and to fullfill their food requirements in form of pollen and nectar the establishment of flowering field edges gets increasingly popular.

But not everything that flowers is exclusively positive for beneficial insects. Pest insects can profit from flowering field edges as well. In my PhD research, I analysed a number of nectar plants with respect to their potential benefit for cabbage pests and/or their natural enemies. In field studies, I observed the attractivity of nectar plants for pest insects and beneficial insects. In the laboratory, I studied to what extent the plant species differ in their nectar accessibility and their impact on insect longevity. In addition, I examined the impact of different nectar and honeydew sugars on the gustatory response and the longevity of the insects.

Based on the results that I found in the laboratory, I conducted field experiments with plants that provide food for either the herbivores or the beneficial insects. I collected individuals of the diamondback moth *Plutella xylostella* and its parasitoid *Diadegma semiclausum* and tested them for their sugar content. The results indicated food uptake in the field for both species. In a field experiment with standardized host density I could also show that suitable nectar plants, such as buckwheat, have an enormous positive impact on longevity and fecundity of the parasitoid *D. semiclausum*. In addition, I could demonstrate that nectar plants that are selectively used by herbivores, such as *Centaurea jacea* by the cabbage white *Pieris rapae*, can lead to higher pest densities in adjacent cabbage plantings. My most important conclusion is therefore that a selective approach and a careful choice of plant species are necessary to achieve improved biological control by flowering field edges.

To my parents

Chapter

1

General introduction

General introduction

From traditional farming to high tech production systems

During the last sixty years a tremendous change in agricultural production has taken place. Driven by the experience of hunger during the Second World War and by the continuously growing world population, the focus in agricultural policy, research and extension concentrated on the increase in agricultural production (Kluge, 2005). Within a few decades, traditional farming, based on mixed farming systems with the use of farm-produced organic fertilizer and an intelligent agro-ecosystem approach to prevent pests and diseases, changed into more intensive agricultural production, highly specialized and heavily depending on external inputs as mineral fertilizer and synthetic chemical pesticides (Buttel, 1990). With the increasing intensity of production, agricultural crops became more susceptible to attacks by pests and pathogens. Each level of the farming process, from the soil and plant level up to the level of animal husbandry and economic and social farm management, contributed in its own way to this vulnerability of modern agro-ecosystems.

Mineral fertilizers and pesticides enter agricultural production

Two events were essential for the current widespread use of mineral fertilizers in agricultural and horticultural production. In 1840, Justus Liebig (1803-1873) published his book "Die organische Chemie in ihrer Anwendung auf Agriculture und Physiologie", providing basic principles of plant nutritional chemistry (Klein, 1973). In 1910, Fritz Haber and Carl Bosch developed a method which allowed the extraction of nitrogen from the air (Klein, 1973). Aside from its importance for the arms industry, this discovery was a milestone in agricultural production. The availability of mineral nitrogen and other fertilizers demanded intensive research in the field of plant nutrition in order to exploit the full yield potential of a crop.

A comparable development to that in plant nutrition took place in the area of plant protection. After discovering the insecticidal effect of DDT, it first was used by the American troops in WW II for the control of insects that carried human diseases (Flint & Van den Bosch, 1981). Later, DDT became available for agricultural use. Besides the extensive use of DDT, numerous other synthetic organic insecticides, miticides, nematicides, herbicides and fungicides were developed (Crüger & Brammeier, 1998). Since the 1950's, pesticide application was no longer restricted to high value/small acreage crops such as fruits, vegetables and cotton, but were applied to large areas planted with corn and grain (Flint & Van den Bosch, 1981). Most of these insecticides had a broad spectrum activity, being effective not only against a great number of target species, but also against non-target species. In

1962, the negative effects of pesticide use were brought to public attention by the publication of 'Silent spring' (Carson, 1962). However, recognition of the off-target effects and limitations of pesticides did not reduce pesticide production, but shaped the future development and use of pesticides towards selective agents and application in integrated control programmes.

Mechanisation and the availability of fertilizers and chemical pesticides had a great impact on the structure of agricultural production (Buttel, 1990). However, the environmental costs related to this development were initially not fully recognised and accounted for.

Dramatic biodiversity losses due to large-scale plant production systems

The development of machinery, tractor driven instead of horse drawn, allowed the farmers to work on larger fields. In the sixties, whole landscapes changed their appearance, when hedgerows, ditches and other non-crop landscape elements were removed in order to enlarge and aggregate agricultural fields (Chapman & Sheail, 1994). Under the economic pressure to work effectively and cheaply, agricultural machinery steadily increased in size, working width, and, consequently, also in weight (Tijink & Spoor, 2004).

The availability of fertilizers and pesticides led to a focus in plant breeding on the development of high yielding varieties. These 'high yield - high input' varieties replaced numerous local varieties, which were adapted to the local production conditions and which were often (partially) resistant to pests and diseases (Soule et al., 1990). The loss of locally adapted species and cultivars could be observed in nearly all agricultural crops, in fruit and vine production, but also in animal husbandry. This development is not restricted to the industrialized countries of the developed world, but also took place in developing regions that were influenced by the 'green revolution'. It is therefore not surprising, that the decrease in total biodiversity is attributed for a great part to the changes in agricultural production systems (Stoate et al., 2001). First of all, a large reduction occurred in species and cultivars produced by farmers. Secondly, fertilizer and pesticide use resulted in environmental pollution, which, in its turn, led to a reduction of biodiversity in neighbouring ecosystems (De Snoo, 1999).

Another development was that due to the application of artificial fertilizers, crop production became independent of organic manure (Klein, 1973). Arable farms no longer needed to grow legumes as fodder. As a result, crop rotations became less diverse and consisted mainly of cash crops. For farmers focusing on meat or milk production, organic manure became a waste product rather than a valuable source of nutrients (Buttel, 1990).

Farmers become agro businessmen

The farmers of the last century initially worked on a mixed traditional farm. With increasing mechanization and external input they developed into bioindustry managers, often highly specialized in the production of either grain, vegetable, meat or milk (Buttel, 1990). The average farm size is still increasing and following the motto “Grow or Go” the number of farmers is decreasing steadily. Between 2000 and 2003, the number of farms in the Netherlands decreased by an average of 4.3% per year. In the European Union, the number of farms has decreased by 25-50% since 1980 (Berkhout & Van Bruchem, 2005). Nowadays the steadily decreasing prices for their products forces farmers to shift their focus from agricultural production towards the optimization of subsidy exploitation, provided by the national government or the European Union. As these subsidies are dependent on political decisions and the economic situation of the countries, they form a short term rather than a long term perspective for the farmers’ income. The proportion of the EU budget that is spent on agriculture has decreased from 87% in 1970 and 52% in 1993 to 46% in 2003 (Anonymous, 2005a). Recent discussions in the European parliament indicate that the amount of subsidies provided for agriculture most probably will further decrease.

Modern agriculture stimulates pest and disease development

The tremendous changes in crop production during the last decades contribute to a great extent to the vulnerability of modern agro-ecosystems (Buttel, 1990). The soil structure is, first of all, mechanically damaged by heavy machinery. Higher soil compaction has severe negative effects on plant root development, aeration and water management of the soil (Tijink & Spoor, 2004). Due to the replacement of organic manure by mineral fertilizers, biological activity and microbial diversity of the soil is low (Mader et al., 2002). High nitrogen inputs, which are more common in crops with mineral fertilisation, can lead to higher infestation rates and better development of herbivores (Scriber, 1984). Less diverse crop rotations increase the risk of soil-borne diseases (Thrupp, 1998). Large agricultural areas covered with the same crop, often even the same variety, favour the spread of airborne diseases and pests. The natural enemies that usually occur abundantly on small scale mixed farms, are negatively affected by large scale monocultures, where essential food sources as well as overwintering sites are often lacking (Gurr & Wratten, 1999, Van Lenteren, 1987). Due to the high degree of vagility in many pest species, they easily recolonise disturbed agro-ecosystems, often ahead of their natural enemies (Price, 1976).

Plants growing in agricultural production systems are frequently subject to insect attack, and their specialized herbivores usually exhibit higher abundance in monoculture than in diversified crop systems (Andow, 1983). Pest densities often exceed the economic threshold and the application of insecticides meant to solve a problem, often tends to increase it.

The development of resistance in insect pest populations is the main way in which pesticide use can lead to failures in pest control (Nicholls & Altieri, 2004, Van Lenteren, 1991). Another way in which pesticide use can foster outbreaks of pests is through the elimination of the natural enemies of the target pests. Beneficial insects of higher trophic levels are often more vulnerable to pesticides than their hosts. In addition, natural enemies appear to evolve resistance to insecticides much more slowly than herbivores (Nicholls & Altieri, 2004). The pest generally recovers quickly after insecticide application and, due to the suppression of their natural enemies, can reach much higher densities than before the application. Repeated application of pesticides is generally the result, making the situation worse instead of better. This phenomenon is often referred to as the "pesticide treadmill" (Van den Bosch, 1978) and illustrated in the box below. In addition, pesticides can also create new pest problems, so-called "secondary pests", when natural enemies of until then non-pest species are destroyed by chemicals, resulting in population explosions (Nicholls & Altieri, 2004, Van Lenteren, 1987).

The development of new pesticides has become increasingly difficult, while the rate at which insects are developing resistance to new and complex pesticides is not decreasing (Van Lenteren, 1991). Public concerns about contamination of the environment and the groundwater, awareness for the risks of pesticide residues in crops, have increased the need to look for alternative, more durable strategies of pest control in agricultural crops.

Control of the Diamondback Moth: example of a pesticide treadmill

Management of the diamondback moth, *Plutella xylostella,* forms an excellent example of the failure of the chemical pest control approach. *P. xylostella* is one of the most important pests in cabbage and can be found worldwide across all regions where cabbage is grown. Prior to the introduction of synthetic insecticides in the late 1940s, diamondback moth was not reported as major pest of crucifers (Takelar & Shelton, 1993). Since their development, synthetic insecticides have dominated attempts to control *P. xylostella*. The widespread and intensive use of insecticides has facilitated the development of insecticide resistance in *P. xylostella* populations (Cardona, 1997, Saucke, 1996). Additional negative effects of the ever increasing use of insecticide applications were unacceptable levels of residues in vegetables, poisoning of farmers and labourers, reduction of natural enemies and rising costs in vegetable production.

Alternative methods of pest control

There are many methods to prevent or reduce the development of pest outbreaks, e.g. resistance breeding, rotation strategies and mechanical, physical, genetic and biological control [for a complete overview see Van Lenteren (1991)]. Among the various ideas and approaches concerning the quest for better pest control strategies, two methods are of particular importance in the context of this thesis: biological control and integrated pest management (IPM).

Biological control

There are numerous definitions of biological control, and I will use the one by Eilenberg et al. (2001): “the use of living organisms to suppress the population of a specific pest organism, making it less abundant or less damaging than it would be otherwise”. *Classical biological control* involves the control of non-native pests by importing and releasing their natural enemies. This approach has been very successful in open field situations. One classical example is the control of the cottony cushion scale *Icerya purchasi* in Californian citrus orchards by importation and establishment of two of its natural enemies in 1889 (Sweetman, 1936).

When natural enemies are missing from an agricultural setting (e.g., greenhouses or row crops immediately after planting), or are too scarce to provide control, their numbers can be increased by either inoculative or inundative releases of mass-reared individuals, a method called *augmentative biological control.* This method is often used in greenhouse systems, for example for whitefly control through releases of the parasitoid *E. formosa* (Van Lenteren et al., 1997, Van Lenteren, 2000).

A third approach is *conservation biological control* where the impact of natural enemies is maximised by minimising pesticide-induced mortality and by providing key ecological resources (Gurr et al., 2000). Using selective instead of broad spectrum insecticides and by better targeting of the pest in time and space, negative effects of pesticides on beneficials can be reduced. Different techniques can be applied to provide key ecological resources, including provision of nesting and overwintering sites, supplementary food, alternative hosts and by improving pest-enemy synchrony (Van den Bosch & Telford, 1964).

Early examples of conservation biological control come from China, where for over 2000 years, farmers have purchased and placed nests of a predaceous ant in citrus trees to reduce the number of foliage feeding insects (DeBach, 1964). In England at the end of the 19th century, ladybird beetles were collected and released by hop growers for control of aphids (DeBach, 1964). Strip cropping and strip harvesting forms another strategy to reduce pest pressure in the target crop and conserve natural enemies (Van den Bosch & Telford, 1964). The establishment of beetle banks, conservation headlands and herbicide-free field margins are examples of strategies to increase structural and vegetational diversity within or near the crop (Thomas et al., 1992). Sowing flowering field edges and (re-) establishment of hedgerows and shrubs alongside agricultural areas increase vegetation diversity on farm scale and landscape scale.

Integrated pest management

Biological control is often used in combination with other non-chemical and selective chemical control programmes. The concept of ***integrated pest management*** (IPM) arose in the 1960s in response to concerns about impacts of pesticides on the environment (Stern et al., 1959). Over recent decades, IPM has emerged as the dominant paradigm in pest control (Gurr et al., 2004, Lewis et al., 1997). By implementing the full array of pest management practices such as mechanical, physical and cultural control, host plant resistance and biological control, one seeks to control pests in agricultural crops to below the economic threshold. The IPM approach has been applied successfully in almost all agricultural production systems. European examples are IPM of greenhouse vegetables, apple orchards and annual field crops such as corn. The incorporation of different pest management practices together with production objectives into a total system approach has led to remarkable reduction in pesticide use of up to 99% (Van Lenteren, 1993).

However, IPM has often been reduced to a monitoring program in which thresholds are established and chemicals are used only on an as-needed-basis (Lewis et al., 1997). A more appropriate translation for this kind of IPM would be ‘intelligent pesticide management’ (Nicholls & Altieri, 2004). When applied in a wrong way, IPM features the same shortcoming as the involved pest control practices as it does not fight the pest at its origin. But when applied in its original context, it is based on maximal use of non-chemical control. It is in this context that many countries are addressing pest control nowadays. An example of such an IPM approach is the recently published crop protection policy plan of the Dutch Ministry of Agriculture, Nature and Food Quality (Anonymous, 2005b). Dutch farmers will have to use all non-chemical methods for pest, disease and weed control, before being allowed to apply chemical pesticides.

To develop sustainable crop protection, the perspective should be changed from a reductionistic view to a system-oriented view. Instead of fighting a pest as an isolated phenomenon, one should ask the question ‘Why is a pest a pest?’ (Lewis et al., 1997), or even the more complex question: ‘What makes agro-ecosystems so vulnerable for pests?’ (Nicholls & Altieri, 2004).

From pest control to agro-ecosystem management

Agricultural ecosystems, though usually more simple than many natural ecosystems, are still rather complex. Their components interact in multiple ways and maintain through a set of feedback loops a ‘balance’ within functional fluctuating bounds (Lewis et al., 1997). Systems tend to respond to external therapeutic intervention with internal countermoves. As these countermoves neutralize the external interventions, the desired effect of the intervention is generally not long-lasting (Lewis et al., 1997).

In the interventionist pest control approach one observes the symptom ‘pest outbreak’ and treats it in order to eliminate the pest (Flint & Van den Bosch, 1981). In contrast, in the holistic system approach a pest outbreak is not seen as an isolated phenomenon, but as an indication for a weakness in the agro-ecosystem. Therefore the main issue in the agro-ecosystem approach is to maximize the build-in pest reduction features of the system (Lewis et al., 1997, Nicholls & Altieri, 2004, Pfiffner & Luka, 2003). The maintenance of the system’s function to compensate for external stress factors, also known as resilience, is a main goal in this approach. The emphasis lays on preventive plant protection measures to create a broad basis for the development of strong plants. Starting from soil fertility, with an intact structure and high microbial activity, this first broad ‘defence line’ also includes the selection of suitable plant varieties, wide crop rotation, etc., as an indispensable precondition for sustainable plant protection (Lewis et al., 1997).

In the second defence line, one makes use of plant characteristics hindering pest development, referred to as ‘bottom up control’ and to promote natural enemies inherent to the agro-ecosystem, referred to as ‘top down control’. The “bottom up control” is greatly determined by plant breeding, aiming to increase the resistance of plant varieties. But also cultivation methods, plant nutrition and other factors influence the defensiveness of a plant against herbivore attack. The “top down control” depends on both agricultural practice and landscape management aiming at promoting natural enemies**.** An important precondition in this approach is to reduce the mortality of beneficial organisms due to application of broad spectrum pesticides. Only after the elimination of the use of broad-spectrum pesticides does it make sense to create landscape elements to provide these beneficial species with other resources to support their successful development and reproduction.

Creating noncrop elements has also received attention in conventional, intensive farming systems. In the Netherlands, for example, preventing pesticide contamination of surface water by creating noncrop edges is an important issue (De Snoo & De Wit, 1998), while in other countries nature conservation, recreation and stimulating development of game for hunting form the main motivation to subsidize the establishment of noncrop field edges.

Even though not always intended to promote beneficial insects, these landscape structures still can contribute to improving their performance.

The requirements for survival and reproduction in beneficial insects may be different among species. Ladybird beetles, which are known to be effective aphid predators, depend on uncultivated habitats for hibernation such as hedgerows, forest edges, and grass tussocks (Fortmann, 1993). Beetle banks (grass strips present at certain distances in arable fields) provide shelter for overwintering carabid beetles (Gurr et al., 2004). While generalistic predators and parasitoids might profit from alternative prey or hosts, specialists would need alternative host plants on which their specific prey or hosts can be found.

One basic requirement for adults of nearly all beneficial insect species is food. (Extra-) Floral nectar and pollen is used by many species to fulfil their energetic needs and to gain nutrients for egg maturation. This aspect of food supply for beneficial insects, especially parasitoids, forms the main topic of this thesis and will be discussed in more detail below.

The two defence lines that were described earlier (bottom up and top down) form the basis for a more sustainable agro-ecosystem. Intervention with therapeutics, plant protection control agents with minimal interruption, is used as a last back-up**.** They are used in exceptional cases rather than as a standard response to pest outbreaks in unbalanced systems. A positive effect of the cautious use of these pesticides is that resistance develops much more slowly, and, thus, they can be used over a longer period. This reasoning holds for resistant plant varieties as well.

Establishment of flowering field edges to benefit parasitoids

Early studies have already pointed out that the lack of food in agricultural crops can be a potential impediment to the successful functioning of beneficial insects (Hocking, 1966, Illingworth, 1921, Wolcott, 1942). By providing food supplements, biological control workers seek to eliminate this obstacle and enhance the efficacy of biological control. To provide this food for beneficial insects, a number of seed mixtures with names such as “Good bug blend” or “Insectary blend” are commercially available (Gurr et al., 2003).

There are many examples showing enhanced longevity and lifetime fecundity of parasitoids under controlled conditions as a result of provided floral nectar (Baggen & Gurr, 1998, Idris & Grafius, 1995, Irvin et al., 1999, Johanowicz & Mitchell, 2000, Syme, 1974). In addition, parasitoids showed an increased overall activity and searched for prey or hosts in favour of food when sufficiently fed (Takasu & Lewis, 1994, Wäckers, 1994). But up to now, there is limited evidence to indicate that parasitoids actually feed on flowers in the field (Lee & Heimpel, 2003, Wäckers & Steppuhn, 2003) and that this feeding results in enhanced biological pest control (Heimpel & Jervis, 2005).

Observations of increased parasitism rates in the vicinity of flowering plants in the field go back to the first half of the 20th century (Allan & Smith, 1958, Leius, 1967, Wolcott, 1942). Since then, the potential role of nectar sources in biological control has been subject of numerous investigations, with mixed results (Heimpel & Jervis, 2005).

Besides beneficial insects, herbivores can also profit from floral nectar (Romeis et al., 2005), therefore the establishment of flowering field edges may inadvertently increase pest pressure when applied without caution (Baggen & Gurr, 1998, Van Emden, 1964, Zhao et al., 1992).

Research aim

Up to now, the composition of commercially available seed mixtures for the establishment of flowering field margins rarely takes the suitability of flower species as nectar sources for biological control agents into account (Wäckers, 2004). Surprisingly, the fact that also many pest insects visit and profit from nectar plants, is generally neglected. It is only recently that researchers are paying attention to the risk of stimulating herbivores in field margins, and thus the possibility of even increasing pest pressure in the target crop (Baggen et al., 1999, Wäckers, 1999). When aiming to design flowering field margins for pest reduction, the needs of beneficials as well as pest species must be taken into account. Flowering field edges should consist of 'selective food plants' (Baggen & Gurr, 1998) that mainly, if not exclusively, promote the performance of natural enemies without supporting the pest species.

In the present study the aim is to identify plant species that are selectively suitable for either herbivorous pest insects or beneficial insects. In this way I intend to gain the knowledge necessary to fine-tune the composition of vegetation in field edges in order to promote the beneficial species and not the herbivores.

The study system

As a study system I used Brussels sprouts (*Brasicca oleracea* L. gemmifera), representing the Brassica family, two lepidopteran key pests and their most prevalent natural enemies. The two lepidopteran pest species, the small cabbage white *Pieris rapae* and the diamondback moth *Plutella xylostella,* depend on sugar feeding during the adult stage. Their respective parasitoids, *Cotesia glomerata* and *Diadegma semiclausum* are also strongly dependent on carbohydrates during adult life. A number of nectar plants that are commonly used in commercially available seed mixtures for flowering field edges, were tested for their suitability to selectively promote the parasitoids in the field.

Outline of thesis

The exploitation of nectar sources differs among insect species. This selectivity can be based on various mechanisms. In **chapter 2**, I examine the abundance of the herbivores *Autographa gamma, P. rapae* and *P. xylostella,* as well as of the parasitoids *Cotesia glomerata* and *D. semiclausum* on a number of flower species commonly used in commercially available seed mixtures for flowering field edges.

To successfully exploit a nectar source, the insect, after having arrived on a plant, must be able to access the nectar. The accessibility of nectar is mainly determined by the floral architecture and the structure of the insect's mouthparts. While some flowers feature exposed nectaries, others have hidden nectaries in tubular shaped inflorescences. Adult Lepidoptera use a proboscis for food uptake, while parasitoids generally have less specialized mouthpart structures. In **chapter 3,** I first discuss the potential nectar accessibility for the four different insect species, based on corolla measurements of the different flower species and the proboscis length and head width of the herbivores and the parasitoids. Next, I investigate whether the different species actually reach and accept nectar from the various flower species. I test weight gain after short term exposure to different flowers and the impact of exposure to flowers on longevity of the insect species.

Instead of providing a flowering vegetation, sugar sprays can also be used to promote beneficial insects (Canas & O'Neil, 1998, Jacob & Evans, 1998, Lundgren et al., 2002, Rogers & Potter, 2004). As pest species may profit from this artificial food supply as well, knowledge on the responses of pests and beneficials can help to supply the right sugars (Rogers, 1985, Wäckers, 2001). In **chapter 4** I describe the gustatory and longevity response to single (nectar) sugars by *P. xylostella* and *D. semiclausum*.

Nectar consumption by beneficial insects is generally studied under controlled laboratory conditions. Field observations of flower visits by hymenopteran parasitoids are available but provide little information on the actual use of flowers as food source. In **chapter 5** I evaluate nectar consumption by the herbivore *P. xylostella* and its parasitoid *D. semiclausum* in cabbage fields bordered by four flower species that differ in their nectar accessibility.

In **chapter 6** I present an experiment in which I investigate the effect of nectar provision on the fecundity and life span of single *D. semiclausum* females under semi-field conditions. This study was performed to find out if results of laboratory experiments, showing increased reproducion and life span when nectar is available, would be supported by data from the field. Earlier laboratory research often demonstrated strong positive effects on fecundity of beneficials, but field data failed to indicate these effects.

Establishing flowering field edges is often propagated as a tool to support beneficial insects and thereby to enhance the efficacy of biological control of pests. In **chapter 7** I study the risks as well as the benefits that might be related to the various plant species used in field edges.

In **chapter 8** I review the most important results from my PhD research project and discuss the importance of the present results in the context of modern approaches in sustainable pest control.

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Chapter

2

Herbivores and their parasitoids show differences in abundance on eight different nectar producing plants

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Chapter 2

Herbivores and their parasitoids show differences in abundance on eight different nectar producing plants

Abstract

Flowering field margins can play an important role in enhancing biological control by providing food for beneficial insects. At the same time herbivorous insects might profit from the flowers, which unintentionally might result in higher pest numbers. Herbivorous and parasitic insects differ in their exploitation of nectar sources. Direct visual observation and sweep net sampling on eight plant species (*Anethum graveolens, Borago officinalis, Centaurea cyanus, Centaurea jacea, Fagopyrum esculentum, Lobularia maritima, Origanum vulgare, Tanacetum vulgare)* were used to identify flowers which are selectively visited by beneficial parasitoids, but not by their herbivorous hosts. On two plant species with either exposed nectaries (*A. graveolens*) or extrafloral nectaries (*C. cyanus*) the parasitoid *Diadegma semiclausum* was observed, but none of the herbivores in substantial numbers. Other plant species, like *O. vulgare*, were visited by at least one of the herbivore species, but not or in low numbers by *D. semiclausum.* These findings provide important information for fine-tuning the composition of flowering field edges in order to successfully boost biological control.

Introduction

Establishing flowering field margins to enhance the availability of nectar and pollen-rich plants for beneficial insects can be an element of agro-ecosystem diversification programs. Many beneficial insects, like for example hoverflies (MacLeod, 1999) and parasitoids (Heimpel et al., 1997, Leius, 1960, Syme, 1975) depend during their adult stage on food for maintenance, dispersal and reproduction. However, not only beneficial insects, but also herbivores may feed on floral nectar in their adult stage (Romeis et al., 2005). Indiscriminately adding nectar sources to agricultural cropping systems could result in higher pest numbers as pest insects may benefit from these food sources as well (Burleigh, 1972, Romeis & Wäckers, 2002, Zhao et al., 1992).

It is only recently that researchers are paying attention to this herbivore stimulating risk (Baggen et al., 1999). It means that with the design of flowering field margins, the needs of beneficial as well as pest species have to be taken into account. Flowering field edges should consist of "selective food plants" (Baggen & Gurr, 1998) that mainly, if not exclusively, promote the performance of natural enemies without supporting the pest species.

Herbivorous and parasitic insects differ in their exploitation of nectar sources. This selectivity can be based on various mechanisms, but generally visual or olfactorial attraction plays an important role (Patt et al., 1999, Proctor & Yeo, 1973, Wäckers, 2004). In the multitrophic system 'cabbage - herbivores - natural enemies' we investigated relative attractiveness of a number of flowering herbs for the most abundant cabbage pests and their associated natural enemies. Direct visual observation and sweep net sampling were used to identify flowers which are selectively visited by beneficial parasitoids, but not by their herbivorous hosts.

Material and methods

Plants

Fourteen flower species commonly used in commercially available seed mixtures were sown in three replicates at two experimental locations in the vicinity of Wageningen, the Netherlands. Plots were 3*3 m in size, arranged in three rows and separated with a 1 m wide grass strip within and between the rows. Grass was mown regularly and plots were hand weeded. As not all 14 plant species were flowering during the same period, the data presented here are restricted to those eight species that were flowering simultaneously (*Anethum graveolens, Borago officinalis, Centaurea cyanus, Centaurea jacae, Fagopyrum esculentum, Lobularia maritima, Origanum vulgare* and *Tanacetum vulgare*). For plant characteristics see Table 1.

Table 1: Species, families and food provision of flowers used in this study

Plant species	**Colour**	**Family**	**Provide**
Anethum graveolens	yellow	Apiaceae (Umbelliferae)	nectar
Borago officinalis	purple	Boraginaceae	nectar
Centaurea cyanus	blue	Asteraceae (Compositae)	nectar, extrafloral nectar
Centaurea jacae	purple	Asteracea (Compositae)	nectar
Fagopyrum esculentum	white	Polygonaceae	nectar
Lobularia maritima	white	Brassicaceae	nectar
Origanum vulgare	purple	Lamiaceae (Labitae)	nectar
Tanacetum vulgare	yellow	Asteracea (Compositae)	nectar

Monitoring insects

We walked around each plot once to count the larger lepidopteran species like *Autographa gamma* and *Pieris* spp in the flowering vegetation. Small species like *Plutella xylostella* and parasitoids were caught by sweep net sampling. A standardized number of 12 sweeps was used in each plot. To minimize disturbance in the plots, a neighbouring plot was not sampled immediately but later. Sampling was done from week 28 until week 32 on sunny and dry days between 10:00 and 15:00 h.

Statistical evaluation

The data for presence of insects on eight different flower species were pooled over weeks and locations. As data were not normally distributed, non-parametric statistics were used to check for significant differences between the groups. When the Kruskal-Wallis test indicated that there are differences among the eight flower species, the Mann-Whitney *U* test was used for pair wise comparison between flower species (α=0.05).

Results

Some insect species like *Mamestra brassicae*, *Cotesia glomerata* and *Microliptis* spp. were observed only in low numbers and were therefore excluded from statistical analysis. The average number of *Pieris* spp*, A. gamma, P. xylostella* and *Diadegma semiclausum* individuals observed per plant species is presented in Figure 1 a-d.

Pieris spp were observed on two plant species only, *O. vulgare* and *C. jacea*. On *O. vulgare* significantly more individuals were observed than on *C. jacea*. *A. gamma* was ob-

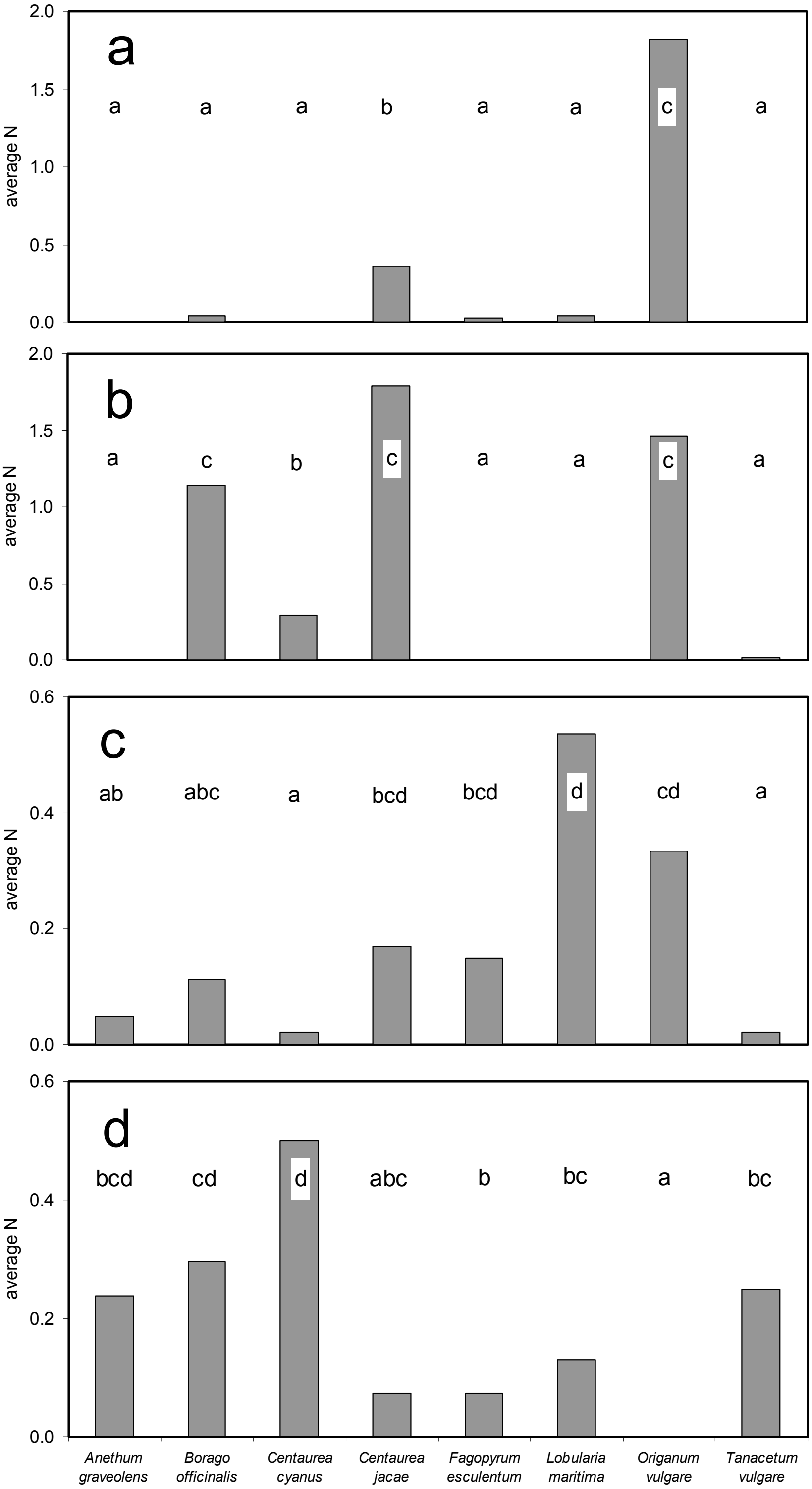

Figure 1: Average number of individuals visually observed [(a) *Pieris* spp. and (b) *Autographa gamma*)] and collected by sweep net sampling [(c) *Plutella xylostella* and (d) *Diadegma semiclausum*] per 3*3m plot on eight different nectar plants during 5 weeks. Different letters indicate significant differences between plant species (Mann-Whitney *U* test, α=0.05).

served on four out of the eight plants included in this study. Besides *O. vulgare* and *C. jacea* these were *B. officinalis* and, to a lower extent, *C. cyanus*. *P. xylostella* was observed in low numbers on any plant involved in this study. Highest numbers were caught on *L. maritima*, and this was significantly more than on *A. graveolens*, *B. officinalis*, *C. cyanus* and *T. vulgare*.

The parasitoid *D. semiclausum* was caught on any of the eight plant species except *O. vulgare*. Highest numbers were caught on *C. cyanus*, and this was significantly more than on *C. jacea*, *F. esculentum*, *L. maritima* and *T. vulgare*.

Discussion

Our observations indicate that herbivorous and parasitic insects differ in their relative attraction to nectar sources. There are three plant species (*A. graveolens, C. cyanus* and *T. vulgare*) on which *D. semiclausum* was observed, but none of the herbivores in substantial numbers. Other plant species, like *O. vulgare*, were visited by at least one of the herbivore species, but not or in low numbers by *D. semiclausum.* Even though additional aspects like nectar accessibility play a role as well, these findings provide important information for fine-tuning the composition of flowering field edges in order to successfully boost biological control.

D. semiclausum was caught in highest numbers on *C. cyanus*. Besides hidden floral nectar, this plant species also has extrafloral nectaries (Stettmer, 1993). In addition, *C. cyanus* plants were heavily invested with aphids during the sampling period. Like extrafloral nectar, aphid honeydew can be an important source of carbohydrates for parasitoids with short, unspecialized mouthparts. It is quite likely that this supply with easy accessible food caused high parasitoid catches on *C. cyanus*.

Earlier studies already stated the importance of Apiaceae as nectar source for hymenoptera (Kopvillem, 1960, Leius, 1960). *A. graveolens* provides nectar on exposed nectaries, which is in general more concentrated than hidden nectar (Kevan & Baker, 1983). While parasitoids can deal with a wide range of nectar concentrations (Siekmann et al., 2001), herbivores with their extended proboscis are restricted to less concentrated nectar (Daniel et al., 1989). This might explain why *D. semiclausum*, but none of the lepidopteran herbivores was found on *A. graveolens* in substantial numbers.

In case of *T. vulgare*, an *Asteraceae (Compositae)* species, nectar is hidden in small corolla tubes. As we could not observe individual parasitoids feeding on this plant species (in contrast to *C. cyanus* and *A. graveolens*), it might be that *D. semiclausum* was looking for something else than food in the *T. vulgare* plots, like a “comfortable” microclimate.

The herbivore species *Pieris* spp and *A. gamma* were found on *O. vulgare* and *C. jacea*, two plant species on which *D. semiclausum* was not present or caught in low numbers. As both plants have tubular flowers with hidden nectar, exploitation of the nectar is restricted to insects with longer tubular mouthparts. Exclusion of these plant species, which are selectively suitable for and visited by the herbivores, might reduce the risk of unintentionally increasing pest numbers.

The herbivore *P. xylostella* was caught in highest numbers on *L. maritima*. This plant species does not only provide accessible nectar (Chapter 3) but is also a host plant of *P. xylostella*. Its parasitoid *D. semiclausum* was found in only moderate numbers on this plant. While the suitability of this plant as nectar source for *D. semiclausum* is questionable (Chapter 3), recent studies indicate the potential of this plant species as a 'dead end trap crop' for *P. xylostella* (De Groot, 2005).

In order to shift insect composition and numbers to our benefit, we should particularly promote plant species like *A. graveolens* and *C. cyanus*, which were visited by the parasitoid but not by the herbivores. At the same time we should exclude plant species, which were selectively visited by herbivores. In this way the establishment of flowering field edges can be optimised in order to increase their impact on sustainable pest control in agricultural cropping systems.

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Chapter

3

Flowering herbs as food sources for herbivores and their parasitoids: nectar accessibility and impact on longevity

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Chapter 3

Flowering herbs as food sources for herbivores and their parasitoids: nectar accessibility and impact on longevity

Abstract

Nectar and pollen sources can be used to increase the effectiveness of biological control agents in agricultural cropping systems. However, introducing flowering plants could also have a negative impact when pest insects benefit from these same food sources. This drawback can be avoided when flowering field edges consist of 'selective' plants that provide suitable food sources for natural enemies without supporting pest species. Such selectivity can be based on the fact that herbivorous and parasitic insects often differ in their food preferences as well as in their exploitation of nectar sources.

Here I analysed the exploitation of 19 flowering plants by two important lepidopteran pests of cabbage, *Pieris rapae* and *Plutella xylostella*, and their hymenopteran parasitoids, *Cotesia glomerata* and *Diadegma semiclausum*. At 45±5% r.h., corresponding with field conditions at which *P. rapae* and its parasitoid *C. glomerata* are predominantly active, the pest and its natural enemy obtained nutritional benefit from different plants. Part of this selectivity was lost at high r.h. This indicates that selectivity of nectar accessibility can vary depending on environmental conditions. Whereas none of the plant species was selective towards *D. semiclausum* relative to its host *P. xylostella*, those plants that benefited both the parasitoid and the herbivore had a much stronger effect on the longevity of the parasitoid. Results underline the importance of fine-tuning the choice of nectar sources for field edges in order to improve biological control. Differences in flower suitability for herbivores and their parasitoids show the potential for application of selective food sources in diversification of agro-ecosystems.

Introduction

Numerous insect species visit flowers to gain food in the form of nectar and/or pollen. Access to nectar provides them with the necessary energy for maintenance, egg maturation and dispersal. Many researchers have demonstrated the importance of nectar and pollen sources for survival and reproduction of beneficial insects (Wäckers & Van Rijn, 2005). This includes many predators (Coll, 1998, Gilbert, 1986, Majerus & Kearns, 1989, Rousset, 1984) and parasitic wasps (Heimpel et al., 1997, Leius, 1960, Syme, 1975, Wäckers, 2001, Wäckers & Steppuhn, 2003). It has been suggested that food supplements can be used to increase the effectiveness of biological control agents in the open field (Gurr et al., 2005, Heimpel & Jervis, 2005, Johanowicz & Mitchell, 2000, Kopvillem, 1960, Leius, 1967, Wäckers, 2003, Wolcott, 1942), as well as in greenhouses (Van Lenteren et al., 1987, Van Lenteren, 1999). The establishment of flowering field margins is one option to enhance the availability of nectar for beneficial insects in agro-ecosystems.

However, in addition to possible positive effects on predators and parasitoids, introducing nectar sources to an agricultural cropping system could also have negative impacts as pest insects may benefit from these same food sources (Baggen et al., 1999, Romeis & Wäckers, 2002, Zhao et al., 1992). While nectar feeding is common among adult herbivores (Romeis et al., 2005), this potential risk factor has only been considered recently (Rogers, 1985; Baggen et al., 1999; Wäckers, 1999). When using flowering field margins, one should therefore account for the impact on beneficial as well as on pest species. Ideally, flowering field edges should consist of 'selective' plants that mainly, if not exclusively, promote the performance of natural enemies without supporting pest species.

Such selectivity is actually feasible as herbivorous and parasitic insects often differ in their exploitation of nectar sources. This selectivity can be based on various mechanisms (Wäckers, 2005). One important aspect in determining the suitability of a flower as a food source is the fit of floral architecture and insect mouthpart structure (Baggen et al., 1999, Idris & Grafius, 1995, Patt et al., 1997a). Differences in response to nectar composition and nectar viscosity can play an important role as well (Rogers, 1985, Wäckers et al., 1996).

In this chapter I analysed if two important lepidopteran pests of cabbage, *Pieris rapae* and *Plutella xylostella*, and their hymenopteran parasitoids, *Cotesia glomerata* and *Diadegma semiclausum,* can obtain nutritional benefits from 19 flowering plants. Based on the architecture of the flowers of the plant species, and the mouthpart structure of the pests and their parasitoids I studied theoretical nectar accessibility. Next, I tested the actual nectar accessibility in the flower species for the herbivores and parasitoids. Finally, I evaluated the impact of various nectar sources on longevity of the herbivores and parasitoids. Part of the latter experiment was conducted at different levels of relative humidity to investigate the impact of humidity on nectar exploitation.

Material and methods

Plants

Most of the plants were grown from seeds in 3x3 m plots in the field near the Laboratory of Entomology, Wageningen, the Netherlands. Some plant species were collected in the area around of the laboratory (see Table 1 for plant origin). Prior to access and longevity experiments, flowers were covered with thin gauze bags for 4 h between 8:00 and 13:00 hours to exclude flower-visiting insects and allow nectar accumulation. Only flowers without aphid infestation or obvious damage were chosen.

Floral architecture

Corolla opening and corolla depth of the flowers were measured with an ocular micrometer fitted to a microscope. Corolla depth was measured from the most proximal point of the corolla to the supposed location of the nectar at the corolla base. In Asteraceae, insects may take nectar from just above the stamen insertions, where traces of nectar sometimes can be found (Corbet, 2000). Therefore two measurements were done for *Centaurea cyanus* and *Centaurea jacea*, one from the deepest cleft in the corolla to the corolla base and the other from the deepest cleft in the corolla to the region where the stamens insert and the corolla flares out. For all Fabacea species I considered the actual opening as well as a potential aperture. This potential opening was measured by pushing the wing and the keel pedals down, resembling an insect trying to reach the nectar.

Insects

P. xylostella and *P. rapae* were reared on Brussels sprout plants (*Brassica oleracea* L. gemmifera) at 24±2 ºC, 35±10% r.h., L16:D8. *P. xylostella* pupae were collected and transferred to a climate chamber at 15±2°C, 60±10% r.h., L16:D8 for further development. Pupae of *P. rapae* remained in cages in the rearing room until emergence.

C. glomerata were reared on *P. brassicae* on *B. oleracea* L. at 21±2°C, 60±10% r.h. and 16L:8D. *D. semiclausum* were reared on *P. xylostella* at 23±2 ºC, 60±10% r.h. and 16L:8D. Parasitoid cocoons of both species were collected and transferred to a climate chamber (15±2°C, 60±10% r.h., 16L:8D) for further development.

Mouthpart structure

For *P. rapae* (N=10) the probosces of recently dead individuals were unrolled with a needle and measured from the tip to the front of the compound eye. *P. xylostella* individuals (N=5) were placed in alcohol to facilitate the extension of the proboscis. At it is difficult to straighten the proboscis of *P. xylostella* the length of the curved proboscis was measured by using the computer software LeicaQWIN. Like in many parasitic hymenoptera, elongated mouthparts are missing in *C. glomerata* and *D. semiclausum*. For these species, head width is the limiting factor in exploiting nectar from deep, narrow flower corollas. The proboscis length of *P. rapae* and the head width of *P. xylostella* and the parasitoids (N=10) was measured using the same optical set-up like for the flower measurements.

Actual nectar accessibility

Under dry, sunny and stable weather conditions individual insects were released into the gauze bags. Otherwise, flowers were cut, transferred to the laboratory (22±2ºC, 45±5% r.h.), put in a glass vial with water and placed in a cylindrical cage (14.5 cm diameter, 21 cm high, top covered with fine nylon netting for ventilation). For *D. semiclausum* and *P. xylostella*, access on *F. esculentum*, *A. graveolens* and *D. carota* was tested exclusively in the field. With *E. helioscopia*, *A. graveolens* and *H. sphondylium*, approximately half of the tests took place in the field and the other half in the laboratory. For the remaining plant species, 75% of the tests took place in the field and 25% in the laboratory. For *C. glomerata*, access to nectar was tested exclusively in the laboratory. Different plant species were tested on the same day and each of the plant species on different days to spread day effects over the different treatments. As control treatment (no nectar) I used, depending on their availability, leaves of *Daucus carota* for *P. xylostella* and *D. semiclausum*, and leaves of *Anthriscus sylvestris* for *C. glomerata*.

To test nectar accessibility I used the method described by Wäckers et al. (1996). Upon emergence of *P. xylostella*, *D. semiclausum* and *C. glomerata*, four to six individuals of both sexes were transferred to plastic tubes and provided with water only. For the experiment, two day-old food-deprived females were isolated individually in glass vials. Before exposure to the flowers they were weighed on a microbalance (C-33 Cahn, 1ug precision). Insects were anaesthetized with CO_2 to facilitate handling. Insects were exposed to the flowers for a period of one hour. Subsequently they were weighed again. As none of the tested species is known to feed on pollen and all individuals in the control treatment lost weight, nectar accessibility was assumed when insects gained weight during the experiment. Small quantities of nectar intake might have been missed due to weight loss during the exposure time. About ten females of *P. xylostella*, *D. semiclausum* and *C. glomerata* were tested for each flowering plant species. Each individual was tested only once. Due to problems with handling, *P. rapae* could not be tested using this experimental set-up.

Insect longevity

Newly emerged females of *P. xylostella*, *D. semiclausum* and *C. glomerata* (0-24 h old) were kept in groups of 4 to 7 individuals in cylindrical plastic cages as described above. Female *P. rapae* were kept in square wooden cages (25x25x40 cm, with three sides of fine nylon netting for ventilation). Insects were either kept with water only (control) or provided with water and one of the flower species, bagged prior to use like described above. To assure continuous nectar availability, flowers were exchanged every two to three days. Cages were placed at 22 ºC, 16L:8D and high r.h. (90±5% r.h.), and checked for survival on a daily basis.

In addition, *P. rapae* was tested for longevity with a range of flowers at 22 ºC, 16L:8D and low r.h. (45±5% r.h.) I focused on this species, as *P. rapae* forages primarily during sunny periods, which are often characterized by low relative humidity.

Data on longevity were first tested for normal distribution and consequently analysed with the Kruskal Wallis test followed by the Mann-Whitney U-test (SPSS 10.0). Pairwise comparisons were made between control and each plant species. Following the Bonferroni correction the significance level of $\alpha=0.05$ was adjusted for the number of comparisons.

Weather data

Data on relative humidity, temperature and sunshine duration were obtained from a local weather station located at 1 km from the department.

Results

Floral architecture

The results of the floral architecture measurements are listed in Table 1. Out of the 19 plant species that were used in the experiments, 11 are characterized by easily accessible nectar either due to flat corollas with exposed floral nectaries or extrafloral nectaries. Six plant species are characterized by a deep corolla and these are mainly pollinated by long tongued insects. Two plant species hold an intermediate position, as their nectaries are partially hidden in small, cup-shaped flowers.

Mouthpart structure

The measurements of the insect mouthparts show that *P. rapae* and *P. xylostella* have an average proboscis length of 11.58 mm (±2.02) and 3.22 mm (±0.01) respectively. The aver-

Table 1: Flower species used for this study, their common names, families, their origin, position of nectaries and floral architecture measurements (± s.e.). ([a] measurement from the deepest cleft of the corolla to the corolla base; [b] measurement from the deepest cleft of the corolla to the point that the stamens inserted; * potential corolla aperture)

Scientific name	**Common name**	**Family**	**Origin**	**Nectaries**	**Corolla depth (mm)**	**Corolla aperture (mm)**
Aegopodium podagraria L.	Groundelder	Apiaceae	plot	exposed		
Anethum graveolens L.	Dill	Apiaceae	plot	exposed	0.00 ± 0.00	2.63 ± 0.06
Anthriscus sylvestris (L.) Hoffm.	French Parsley	Apiaceae	wild	exposed		
Carum carvi L.	Caraway	Apiaceae	plot	exposed		
Daucus carota L.	Wild Carrot	Apiaceae	wild	exposed	0.00 ± 0.00	3.05 ± 0.13
Foeniculum vulgare Mill.	Fennel	Apiaceae	plot	exposed		
Heracleum sphondyllium L.	Hogweed	Apiaceae	wild	exposed	0.00 ± 0.00	4.91 ± 0.15
Centaurea cyanus L.	Cornflower	Asteraceae	plot	extrafloral, exposed	7.59 ± 0.15 [a] 2.57 ± 0.07 [b]	0.28 ± 0.02
Centaurea jacea L., s.l.	Brown Knapweed	Asteraceae	plot	hidden	10.11 ± 0.26 [a] 3.83 ± 0.09 [b]	0.44 ± 0.06
Euphorbia helioscopia L.	Sun Spurge	Euphorbiaceae	plot	extrafloral, exposed		
Fagopyrum esculentum Moench	Buckwheat	Polygonaceae	plot	exposed	0.23 ± 0.02	5.97 ± 0.16
Lobularia maritima (L.) Desv.	Sweet Alyssum	Brassicaceae	plot	partly hidden		
Medicago sativa L.	Alfalfa	Fabaceae	wild	hidden	5.63 ± 0.17	0.35 ± 0.04 1.15 ± 0.17*
Trifolium pratense L.	Red Clover	Fabaceae	plot	hidden	10.8 ± 0.32	0.05 ± 0.02 0.84 ± 0.05*
Trifolium repens L.	White Clover	Fabaceae	plot	hidden	7.29 ± 0.14	0.07 ± 0.02 0.80 ± 0.04*
Origanum vulgare L.	Oregano	Lamiaceae	plot	hidden	4.87 ± 0.09	1.43 ± 0.05
Ornithopus sativus Brot.	Pink Serradella	Fabaceae	plot	hidden	5.59 ± 0.21	0.15 ± 0.03 0.47 ± 0.04*
Sinapis alba L.	White Mustard	Brassicaceae	plot	partly hidden		
Vicia sepium L.	Bush Vetch	Fabaceae	wild	hidden		

age head width of *P. xylostella, C. glomerata* and *D. semiclausum* was 0.83 mm (±0.01), 0.68 mm (±0.01) and 0.77 (±0.01) respectively.

Nectar accessibility

The combination of measurements on floral architecture and mouthpart structure allows conclusions on the theoretical nectar accessibility for the four insect species (Table 2). Due to its long proboscis, *P. rapae* is theoretically able to make use of any of the tested plants, while *P. xylostella* cannot use flower species with deeply hidden nectar. The two parasitoid species can theoretically access the nectar of all Apiaceae species tested and of *F. esculentum*, as these flowers feature exposed nectaries. In some other plant species they might be able to reach the nectar by crawling into the umbel.

All individuals of the herbivores and parasitoids in the control treatment (without food) lost weight in the tests. *P. xylostella* lost on average 100 µg, which corresponds with 3.8% of the initial body weight (ibw). *D. semiclausum* lost 23 µg (1.8% ibw) and *C. glomerata* 26 µg (4.2% ibw) (Table 2).

P. xylostella did not gain weight when exposed to *E. helioscopia, O. sativus, T. pratense* and *T. repens*. For all other plant species tested, at least one female of *P. xylostella* gained weight. On *A. podagraria*, *C. cyanus, F. esculentum* and *L. maritima* more than 50% of the females gained weight (Table 2).

In *D. semiclausum,* more than 50% of the females gained weight on *F. esculentum, L. maritima* and *S. alba* and all Apiaceae species tested. None of the females gained weight when exposed to *M. sativa* and *T. pratense* (Table 2). When exposed to *C. cyanus, C. jacea, O. sativus and T. repens*, only 1-3 females gained some weight (3-50 µg). On *O. vulgare*, 4 females gained weight but only 10 µg on average. When *D. semiclausum* was exposed to *T. pratense* and *T. repens,* the weight loss was even significantly higher than in the control treatment (MW U-test on differences in % ibw, $\alpha=0.05$). *P. xylostella* also lost more weight when exposed to *T. pratense* then in the control ($P=0.055$).

More than 50% of *C. glomerata* females gained weight on *F. esculentum, L. maritima*, *S. alba* and all of the Apiaceae species tested. In case of exposure to *C. jacea*, none of the parasitoid females gained weight (Table 2). When exposed to O. *vulgare* and *C. cyanus,* weight gain was only recorded in one and two parasitoids, respectively. This weight gain was marginal (10 to 40 µg).

Table 2: Theoretical nectar accessibility (ta) and effect of nectar accessibility on the change in body weight. For each species the first column indicates wether it theoretically could acceess nectar of the plant species (Y=yes, N=no, * indicated access based on potential corolla aperture). The next collumn (except for *P. rapae*) shows the number of individuals with an absolute weight increase after the plant exposure period, while the second column shows the number of individuals with weight decrease. The third column shows the average weight gain/loss with the standard error.

	Pieris rapae	*Plutella xylostella*				*Diadegma semiclausum*				*Cotesia glomerata*			
Plant species	ta	ta	Individuals gaining/ loosing weight		Average weight gain/loss [mg] (± SE)	ta	Individuals gaining/ loosing weight		Average weight gain/loss [mg] (± SE)	ta	Individuals gaining/ loosing weight		Average weight gain/loss [mg] (± SE)
			+	-			+	-			+	-	
Control			0	10	-103 ± 24		0	10	-23 ± 2		0	10	-26 ± 4
Apiaceae													
Aegopodium podagraria			7	3	66 ± 88		5	3	51 ± 37		7	0	329 ± 30
Anethum graveolens	Y	Y	1	9	-121 ± 29	Y	8	2	69 ± 22	Y	8	2	153 ± 55
Anthriscus sylvestris			1	14	-79 ± 17		13	0	126 ± 23		12	1	119 ± 29
Carum carvi			3	8	-11 ± 78		11	1	165 ± 36		7	3	125 ± 43
Daucus carota	Y	Y	3	7	-120 ± 105	Y	9	4	70 ± 46	Y			
Foeniculum vulgare			5	13	215 ± 145		7	1	218 ± 48		13	1	189 ± 29
Heracleum sphondylium	Y	Y	5	8	-1 ± 68	Y	8	2	159 ± 42	Y	14	2	168 ± 34
Other plant species													
Centaurea cyanus	Y	Y[b]	5	4	281 ± 152	N	2	8	-7 ± 11	N	2	4	-22 ± 20
Centaurea jacea	Y	N	1	9	-47 ± 50	N	3	7	-11 ± 6	N	0	9	-56 ± 16
Euphorbia helioscopia			0	12	-86 ± 30		5	5	52 ± 40				
Fagopyrum esculentum	Y	Y	8	2	285 ± 91	Y	9	1	138 ± 36	Y	9	1	204 ± 38
Lobularia maritima			5	4	82 ± 106		6	4	20 ± 19		10	0	152 ± 20
Medicago sativa	Y	Y*	2	8	-13 ± 9	Y*	0	10	-15 ± 2	Y*			
Origanum vulgare	Y	Y	3	8	-134 ± 109	Y	4	6	-3 ± 3	Y	1	8	-36 ± 10
Ornithopus sativus	Y	N	0	10	-104 ± 23	N	3	7	45 ± 46	N			
Sinapis alba							9	3	114 ± 40		10	2	139 ± 40
Trifolium pratense	Y	Y*	0	10	-181 ± 31	Y*	0	10	-55 ± 9	Y*			
Trifolium repens	Y	Y*	0	10	-156 ± 30	Y*	1	9	-100 ± 30	Y*			
Vicia sepium			1	5	-22 ± 170		7	3	47 ± 20				

Longevity

P. xylostella lived 4.6±0.2 days in the control treatment. All flowering plant species, with the exception of *O. sativus* and *T. pratense,* extended longevity of this herbivore to a period of up to 14.8±0.8 days (Figure 1a).

In the control treatment *D. semiclausum* lived a mere 1.9±0.1 days. Longevity was significantly extended on *F. esculentum* (21±2.5 days) and Apiaceae species (Figure 1b). The other plant species did not increase longevity of this parasitoid.

In the control treatment *C. glomerata* survived an average of 2.1±0.1 days. Longevity increased significantly on all plant species, with the exception of *M. sativa* and *O. sativus* (Figure 1c). When exposed to *C. cyanus*, *C. jacea*, *O. vulgare* and *T. pratense*, longevity was only slightly higher than in the control group, while feeding on the Apiaceae species resulted in average longevities of 8 to 15 days. Longevity was highest on *F. esculentum* (16.7±1.4 days).

At low relative humidity (45±5% r.h.) *P. rapae* survived an average of 3.5±0.2 days in the control treatment. Longevity was significantly higher in the groups exposed to *C. jacea* (18.6±4 days)*, F. esculentum* (9.6±2.2 days) and *O. vulgare* (10.7±1.8 days) (Figure 1d). The other plant species did not enhance *P. rapae* survival.

At high relative humidity (90±5% r.h.) *P. rapae* survived an average of 5.4±0.1 days in the control treatment. With the exception of *D. carota* and *T. pratense*, all plant species resulted in a significant longevity increase relative to the control (Figure 1e). This included the species *A. podagraria, A. sylvestris, F. vulgare* and *H. sphondylium* that did not affect longevity at low relative humidity. Longevity was highest on *F. esculentum* (16.5±0.6 days).

In general, longevity of *P. rapae* was higher at 90±5% r.h. as compared to longevity at 45±5% r.h.

Discussion

Nectar accessibility and insect longevity

Plutella xylostella

Whether floral nectar is accessible for an insect depends on floral architecture as well as on the mouthpart morphology of the insect. The lepidopteran *P. xylostella* has a proboscis that is specifically suited for a suctorial way of feeding (Proctor & Yeo, 1973). Even though *P. xylostella* is theoretically able to access nectar in most of the flowers, often only a small fraction of the females of *P. xylostella* gained weight (Table 2). This may be due to two factors.

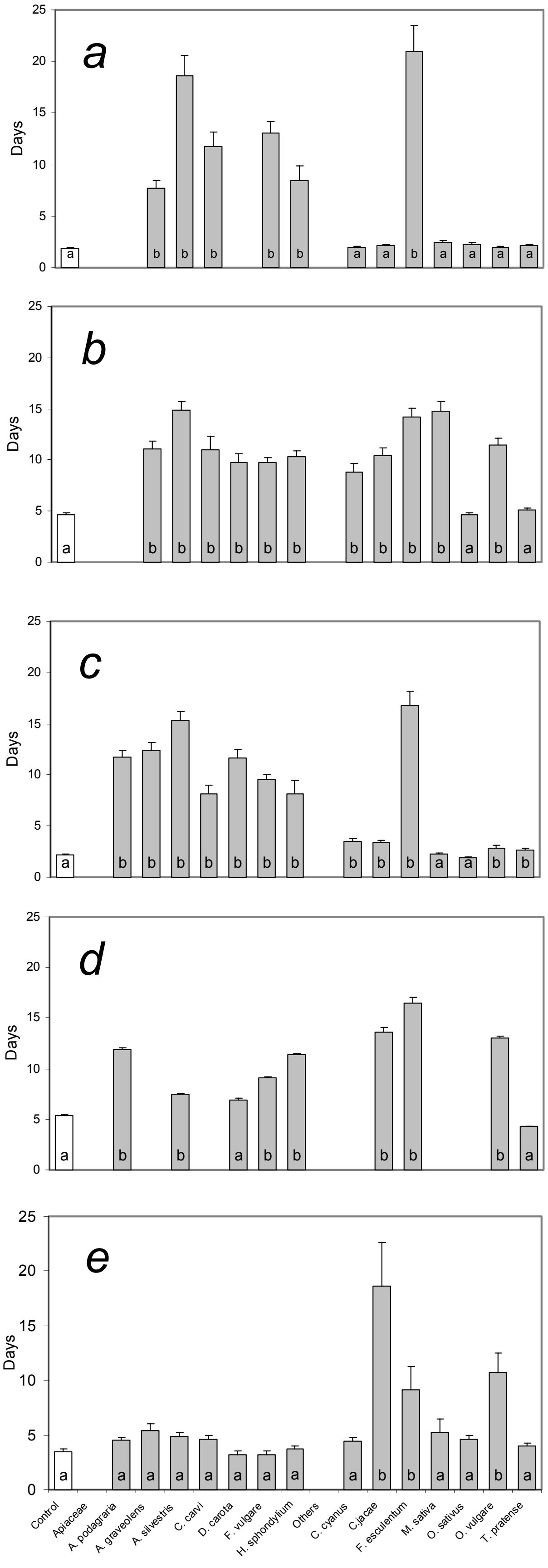

Figure 1a-e: Longevity of *Plutella xylostella* (a), *Diadegma semiclausum* (b), *Cotesia glomerata* (c) and *Pieris rapae* at low r.h. (d) and *Pieris rapae* at high r.h. (e) when exposed to water only (Control) or different flower species.

Treatments with the same letter are not significantly different at α=0.05.

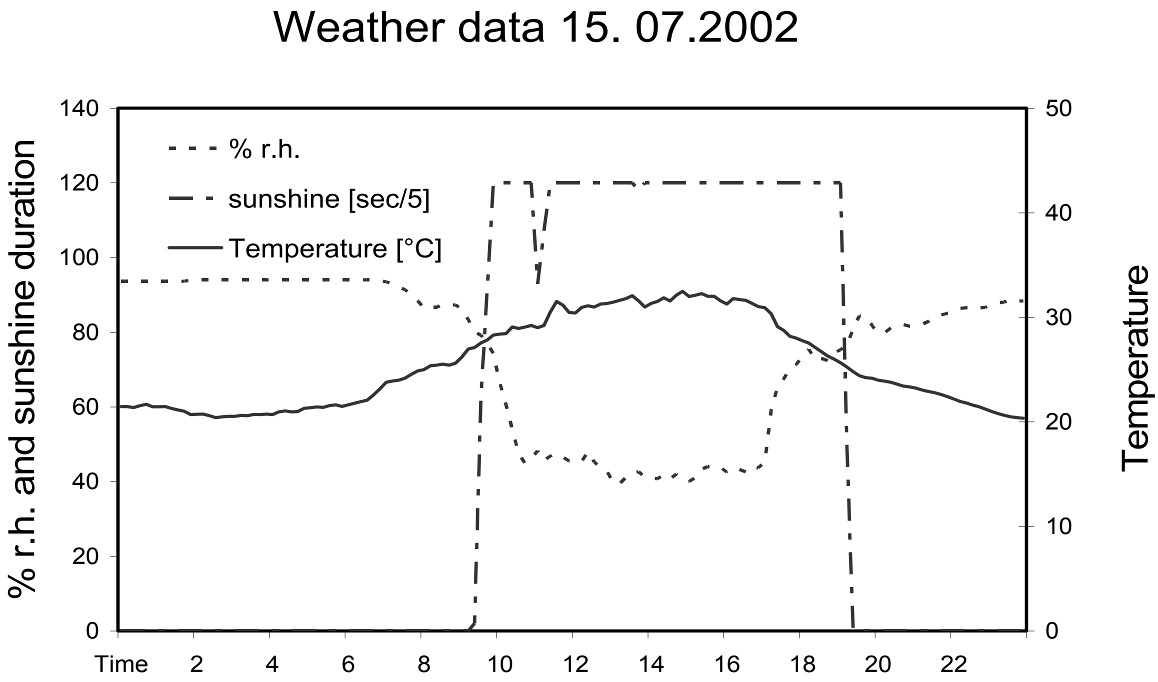

Figure 2: Sunshine duration, relative humidity and temperature on a representative sampling day when a high number of *Pieris* sp. was observed.

First, as a result of their proboscis morphology, Lepidoptera often have difficulties in imbibing nectars of high viscosity (Daniel et al., 1989). Due to the fact that secreted nectar is exposed to evaporation in the open corolla of Apiaceae species, high nectar sugar concentrations can be achieved in these species (Corbet, 1990). These high concentrations could have resulted in restricted food uptake by *P. xylostella* during the one hour of exposure at a low r.h. The longevity data recorded under high r.h. show that longevity increased significantly on all Apiaceae species. In the field, restrictions in nectar access due to low r.h. and corresponding high nectar concentration are unlikely for *P. xylostella*, as this species is mainly active during dusk, when r.h. is usually high (Fig 2).

Secondly, *P. xylostella* likely had to learn how to handle the floral architecture through operant conditioning (Lewis, 1986). This probably applies in particular for plant species with more hidden nectaries like *O. vulgare*, which theoretically provide accessible nectar. The fact that few *P. xylostella* reached and imbibed the nectar could indicate that the experimental period of one hour might have been too brief for operant conditioning to take place. On *C. jacea*, no access was expected based on floral architecture. Nevertheless *P. xylostella* gained weight. As the proboscis length and the corolla depth differ only 0.6 mm, individuals possibly managed to crawl a bit into the corolla and in this way reached traces of nectar. *P. xylostella* lived significantly longer on all plant species tested, including *C. jacea* and *O. vulgare* (Figure 1b).

The only two species that did not increase longevity, *O. sativus* and *T. pratense*, also did not allow unrestricted nectar access based on floral architecture and during short term exposure. Sweep net catches on flower plots confirmed that *P. xylostella* visits a broad range of flowering plants under field conditions (Chapter 2).

Parasitoids

Parasitoids can consume highly concentrated sugar sources such as undiluted honey (86% w/w) (Siekmann et al., 2001) or even crystalline sugar (Wäckers, 2000). On the other hand, they can be limited in accessing nectar sources by their short and less differentiated mouthparts. In our experiment all Apiaceae as well as *F. esculentum* provided accessible nectar for both parasitoid species. Our findings are in line with other reports that stress the importance of Apiaceae and other flower species with exposed nectaries as food source for parasitoids (Kevan, 1973, Leius, 1967, Patt et al., 1997b, Wäckers et al., 1996).

Besides having a positive or neutral effect on natural enemies, flowers can also have a negative impact on their performance. In the present study I found a significantly higher weight loss in *D. semiclausum* when exposed to *T. pratense* and *T. repens* as compared to the control. These plant species theoretically provide accessible nectar only in case the parasitoids are able to push parts of the corolla apart. Wäckers (2004) demonstrated that some plants are olfactory attractive for parasitoid species without providing accessible food. I speculate that *Trifolium* spp. may have stimulated foraging in *D. semiclausum*. By putting extra effort into entering the corolla of attractive flowers, the parasitoids lose more energy and, thus, weight than control females.

Parasitoid longevity increased when they could feed on either of the Apiaceae and on *F. esculentum*. These findings are in line with the findings from the accessibility study and with results from earlier studies with other parasitoid species (Baggen & Gurr, 1998, Foster & Ruesing, 1984, Ide & Lanfranco, 2001, Idris & Grafius, 1995, Irvin et al., 1999, Lee et al., 2004, Syme, 1975, Wratten et al., 2003). Parasitoids are diurnal and mainly active during sunny periods of the day. As pointed out earlier, restrictions in nectar access due to low r.h. and corresponding high nectar concentration are unlikely (Siekmann et al., 2001, Wäckers, 2000).

Four additional plant species *(C. cyanus, C. jacea, O. vulgare* and *T. pratense)* yielded only a marginal increase in *C. glomerata* longevity. However, these plants had no effect on *D. semiclausum* survival. Floral architecture and differences in insect size do not explain these differences. Patt et al. (1997a) described differences in searching behaviour among parasitoid species. One of the parasitoids showed lower propensity to search small openings for nectar and restricted itself to flowers with exposed nectaries. Direct observations on foraging behaviour are needed to evaluate whether this holds for *D. semiclausum* as well.

Even though they caused a significant increase in longevity for *C. glomerata*, the impact of *C. cyanus, C. jacea, O. vulgare* and *T. pratense* (concealed nectaries) was marginal when compared to the Apiaceae species and *F. esculentum* (exposed nectaries). Exploiting hidden nectar apparently costs more energy and searching effort than feeding from an exposed nectary. Depending on the abundance of food competitors on exposed nectaries (Lee & Heimpel, 2003); hidden nectar still might play an important role in food supply for parasitoids.

Pieris rapae

Based on the length of its proboscis, *P. rapae* should be able to access nectar on all plant species included in this study. I found a remarkable difference in the results depending on the relative humidity. At high r.h., *P. rapae* could extend its longevity on all plant species tested, with the exception of *D. carota* and *T. pratense* (Figure 1 d). At low relative humidity, this only applied to *C. jacea*, *F. esculentum* and *O. vulgare*, whereas the remaining 11 plants did not enhance butterfly survival (Figure 1 e). This shows that relative humidity can be an essential factor in nectar foraging and underlines the importance of studying nectar foraging under realistic climatic conditions.

There are two possible explanations for the low survival of *P. rapae* at low relative humidity: either *P. rapae* could not imbibe the concentrated nectar or plants did not secrete nectar at low r.h. *C. jacea* and *O. vulgare* bear flowers with hidden and therefore probably less concentrated nectar (Corbet, 1990). I hypothesize that under sunny field conditions with corresponding low r.h. (Figure 2), *P. rapae* can only access nectar from a restricted number of plant species. Field observations confirm that out of eight flowering plant species included in this study, *Pieris* spp exclusively visited and fed on *O. vulgare* and *C. jacea* (Chapter 2)

Flower selectivity

Especially the results for *P. rapae* show that floral architecture and insect mouthpart structure should not be used as the only tool to identify flowers selectively suitable for specific insects. Actual nectar accessibility under appropriate climatic conditions and the impact on longevity should be evaluated as well to get a reliable view on differences in flower suitability.

The herbivore *P. xylostella* exploits a much broader range of flowers than its parasitoid *D. semiclausum*. Plant species that may enhance pest fitness without benefiting its enemies (e.g., *C. jacea* or *O. vulgare*) could lead to higher pest pressure (Van Rijn & Sabelis, 2005). Consequently, when designing field edges one should select plant species that maximise the benefit to the parasitoid (e.g., *F. esculentum*), while having none or only a weak benefit to the pest species (Baggen et al., 1999, Wäckers & Van Rijn, 2005). Whereas none of the plant species was selective towards *D. semiclausum* relative to its host *P. xylostella*, those plants that benefitted both the parasitoid and the herbivore, had a much stronger effect on the longevity of the parasitoid. This differential benefit is reinforced by the different lifetime oviposition curves of *P. xylostella* and *D. semiclausum* in the presence of nectar. While *P. xylostella* lays 75% of its eggs within the first eight days after emergence (Pivnick et al., 1990), *D. semiclausum* has a one to two day pre-oviposition period, followed by a fairly constant oviposition rate over several weeks (Cardona, 1997, Winkler et al., in press, Yang et al., 1993). This means that the reproductive success of the parasitoid will benefit considerably more from lifetime extension as compared to its host. Even though *P. xylostella* benefits from these nectar sources, their inclusion in a field edge might still lead to a net benefit to pest control.

Taking into account that *P. rapae* is predominantly active during sunny periods with corresponding low r.h., I may conclude that *P. rapae* and its natural enemy *C. glomerata* obtain nutritional benefit from different plants. *D. carota* and *A. sylvestris*, for example, were successfully used by *C. glomerata* but not by *P. rapae*. Using these plants in a flowering field edge would provide suitable food for the parasitoid and might lead to increased parasitism levels as demonstrated by Leius (1967) and Topham & Beardsley (1975). In contrast, *C. jacea* could be used as food source by *P. rapae* and not by *C. glomerata*. Including this plant in a flowering field margin holds the risk of promoting *P. rapae* and increasing pest pressure in the crop as observed in Chapter 7. Also Zhao (1992) found higher numbers of *P. rapae* in broccoli interplanted with nectar producing plants than in a broccoli monoculture. In Zhao's study, the most prominent flowering plant was *Agastache foeniculum*, a purple flowering Labiatae species, which probably is especially attractive and suitable for *P. rapae* and other Lepidoptera.

In this study, insects were held under controlled climatic conditions in relatively small cages. Under field conditions insects are exposed to climatic and biotic stress factors, and they need to move in order to find food and hosts. Flight is energetically the most costly activity for parasitoids (Casas et al., 2005, Hoferer et al., 2000) as well as other insects (Kammer & Heinrich, 1978). Indeed, we found in a field study that the impact of food supply on longevity and fecundity of *D. semiclausum* was much more pronounced than in the laboratory (Chapter 6).

Results obtained in this study underline the importance of fine-tuning the selection of nectar sources for field edges in order to improve biological control. Differences in flower suitability for herbivores and their parasitoids show the potential for application of selective food sources. In case selective food plants cannot be identified, differences among flowers with respect to their impact on longevity and fecundity of the pest species and their parasitoids can be used as a selection criterion. With these strategies flowering field margins can be tailored to optimize the effect of biological control in agro-ecosystems.

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Chapter

4

Plutella xylostella (diamondback moth) and its parasitoid *Diadegma semiclausum* show different gustatory and longevity responses to a range of nectar and honeydew sugars

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Chapter 4

Plutella xylostella (diamondback moth) and its parasitoid *Diadegma semiclausum* show different gustatory and longevity responses to a range of nectar and honeydew sugars

Abstract

Parasitoids, as well as many of their herbivorous hosts, depend on carbohydrate-rich food during the adult stage. Different types of nectar and honeydew vary with regard to their sugar composition. In order to successfully exploit a food source, the insect has to show a positive gustatory response to its component sugars and be able to digest and metabolise them.

Here we test the herbivore *Plutella xylostella* L. (Lepidoptera: Plutellidae) and its parasitoid *Diadegma semiclausum* (Hellén) (Ichneumonidae: Campopleginae) with respect to their feeding response and longevity when provided with one out of nine sugars (fructose, glucose, lactose, maltose, melibiose, melezitose, raffinose, sucrose, trehalose). Both species respond to and show a prolonged life span on a broad range of sugars. The impact of food supply on life span was about six times higher for the parasitoid than for the herbivore. In general, there was a good fit between gustatory response and achieved longevity, with some outlyers. Both species showed only weak response to melibiose, which significantly prolonged lifespan. The parasitoid showed gustatory response to melezitose, which did not prolong life span. The parasitoid and its herbivorous host responded differently to trehalose. These differences in gustatory response and longevity show the potential for application of selective sugar sources in conversation biological control. At the same time it also reveals the risk that indiscriminate application of sugar sources may stimulate herbivory.

Introduction

Many insects depend in their adult stage on carbohydrate-rich food as the main source of energy for longevity, fecundity and mobility. This holds for many herbivorous species, including Lepidoptera (Romeis et al., 2004), as well as for their parasitoids (Jervis et al., 1996, Wäckers, 2003). An important aspect in nectar exploitation by insects is the morphological fit between their mouthparts and the floral architecture (Jervis, 1998). Floral nectar is generally thought to be the most important food source for Lepidoptera, which often feature highly specialised mouthparts (Boggs, 1987, Gilbert & Singer, 1975). Nevertheless, some species also feed on exposed sugar sources such as extrafloral nectar (Beach et al., 1985, Lukefahr, 1960) and homopteran honeydew. Parasitoids with their less specialised, short mouthparts (Jervis, 1998) primarily use easy accessible floral nectar (Idris & Grafius, 1995, Wäckers et al., 1996) as well as extrafloral nectar (Bugg et al., 1989) and honeydew (Zoebelein, 1955).

Acceptance of sugar sources such as (extrafloral) nectar and honeydew also depends on their chemical composition. Besides the three dominant sugars (sucrose, glucose and fructose) nectar and honeydew can contain a number of other saccharides, including maltose, melezitose, melibiose, raffinose and trehalose. Insects can vary in their gustatory responses to these sugars (Wäckers, 1999) as well as in their capacity to digest and metabolise them.

Most papers have addressed sugar suitability either from the point of the herbivore (J. Romeis & F.L. Wäckers, 2002), or the parasitoid (Beach et al., 2003, Jacob & Evans, 2004, Wäckers, 1999, Wäckers, 2001). The aspect of gustatory response and the impact of a sugar on longevity are also often investigated separately.

Here we investigate both the herbivore *Plutella xylostella* L. (Lepidoptera: Plutellidae) and its parasitoid *Diadegma semiclausum* (Hellén) (Ichneumonidae: Campopleginae) with respect to their gustatory response and longevity when provided with one out of nine sugars (fructose, glucose, lactose, maltose, melibiose, melezitose, raffinose, sucrose, trehalose). With the exception of lactose, all these sugars have been reported to occur in nectar or honeydew (Baker & Baker, 1983, Bentley, 1977, Kloft et al., 1985). This comprehensive approach allows us to investigate whether there are differences in sugar response and physiological use between the herbivore and its natural enemy. Such differences could be exploited in tailoring food sources to selectively support biological control agents, without benefiting herbivores (Wäckers, 1999).

Materials and Methods

P. xylostella was reared on Brussels sprout plants (*Brassica oleracea* L. gemmifera) at 24±2°C, 30±10% r.h. and L16:D8. Cages were 38*38 cm in size and 58 cm high, the ceiling and three side walls covered with fine metal mesh. The fourth wall was closed by a plexiglas door. For oviposition, adult *P. xylostella* were placed in a cage containing 4-5 weeks old plants. After 24h, plants were removed and placed into another cage. Plants were watered when necessary. In case the developing larvae depleted the leaf material, fresh plants were added to the cage. After completing the fourth larval stage, larvae pupated on the ceiling of the cage. Pupae were cut off with a razor-blade and either placed individually in glass vials (gustatory response) or in small groups in a separate cage (longevity) under the same climatic conditions.

D. semiclausum was reared on *P. xylostella* at 23±2°C and 60±10% r.h. and L16:D8. The cages were of the same type as described above for *P. xylostella*. About 30 females and 30 males of *D. semiclausum* were introduced into a cage containing Brussels sprout plants infested with L2 larvae of *P. xylostella*. In case the larvae depleted the leaf material, fresh plants were added to the cage. Parasitoid cocoons were collected and transferred to a climate chamber (21±2°C and 60±10% r.h., 16:8 L:D).

Upon emergence, individuals of both species were provided with water only until being used in experiments.

Gustatory response

Two-day old unfed *P. xylostella* females were used to test the gustatory response. To ensure that test insects were water-satiated at the time of the experiment, they were provided with a wet filter paper for a period of 30 minutes prior to the experiments. In the experiment, one end of a filter paper strip of about 5 * 70 mm was dipped in a sugar solution and inserted in the vial. Each of the sugars was tested at 1M, 0.5M, 0.25M and 0.125M. When the sugar solution was brought into contact with the antennae or tarsae of the moth, the insect either unrolled its proboscis and started feeding (feeding for more than 2 seconds was recorded as “acceptance”) or failed to do so (recorded as “non-acceptance”). Each individual was tested only once.

As *D. semiclausum* lives less than 2 days without provision of food, we tested one-day-old unfed parasitoid females. To ensure that parasitoid females were water-satiated, they were provided with a wet filter paper for a period of 30 minutes prior to the experiments. The remaining procedure was similar to the one described by Wäckers (1999) for *Cotesia glomerata.* Parasitoids were transferred to another vial, the bottom of which contained a 3 µl droplet of a sugar solution. The test vial was placed upside down on a wet filter paper in a

Petri-dish to avoid a concentration increase in the test solution due to evaporation. As soon as the parasitoid made contact with the sugar solution, its feeding response was recorded. The reaction was scored as acceptance (if feeding was for more than 5 sec) or rejection (contact for less than 5 sec).

Differences in feeding duration threshholds were chosen intentionally to identify selective sugars which are accepted by *D. semiclausum*, but not by *P. xylostella*. To be conservative, a shorter feeding time as acceptance threshold was chosen in the case of *P. xylostella*. For each species 30 females were tested per sugar concentration.

Longevity response

For the longevity experiment we used cylindrical cages of 21 cm height and 14.5 cm in diameter. The wall was made from a colourless acetate sheet and plastic Petri dishes formed the base and top. The top had an opening of 10 cm in diameter covered with nylon netting for ventilation.

For both, *P. xylostella* and *D. semiclausum,* 4-6 cages with 5-8 females each were prepared to test a minimum number of 30 individuals per sugar (0.5M in 2.5 ml Eppendorfs) and control (water only in 2.5 ml Eppendorfs). Females were 0-24 h old and presumably mated. A cotton wick, leading through a hole in the lid of the Eppendorf, provided access to the sugar solution or the water. Sugar solutions were exchanged every 2-3 days to avoid microbial growth and crystallisation due to evaporation.

Cages were placed in a climate chamber at 22 ºC and L16:D8 and checked for surviving individuals once a day. Individual females were considered as replicate in the statistical analysis. The effect of the tested sugars on longevity was analysed using one-way analysis of variance (SPSS 11.0). Any individual which escaped or was killed during the experiment was excluded from the analysis (n=4 for *D. semiclausum*). Multiple comparisons were performed with the Tukey's HSD test.

Results

Gustatory response

P. xylostella showed a high response to four out of the nine sugars (fructose, maltose, melezitose and sucrose) at all concentrations. In the case of glucose, melibiose and raffinose the response to the 1M solution was high, but the response dropped with decreasing concentration. In the case of lactose and trehalose the response was low, irrespective of the concentration (Table 1).

Table 1: Response of the herbivore *Plutella xylostella* and its parasitoid *Diadegma semiclausum* to a range of sugars in decreasing concentration. For each 'sugar*concentration' combination 30 individuals were tested; numbers indicate % positive response.

	Plutella xylostella				*Diadegma semiclausum*			
Sugar	1 M	0.5 M	0.25 M	0.125 M	1 M	0.5 M	0.25 M	0.125 M
Fructose	97	97	96	90	87	83	83	90
Glucose	73	71	40	10	100	90	63	33
Lactose	7	7	3	7	27	23	7	0
Maltose	100	97	97	75	83	73	93	66
Melezitose	100	100	97	95	87	87	90	97
Melibiose	73	7	0	0	33	17	17	10
Raffinose	97	60	17	0	70	23	10	27
Sucrose	97	100	100	90	90	90	70	83
Trehalose	0	17	20	10	80	70	77	50

D. semiclausum showed a high response to five out of the nine sugars (fructose, maltose, melezitose, sucrose and trehalose) irrespective of the concentration. In the case of glucose and raffinose the response was high to the 1M solution, but dropped with decreasing concentration. Lactose and melibiose elicited low responses, even at high concentrations (Table 1).

Longevity response

P. xylostella longevity differed distinctly between individuals provided with different sugar solutions (F=16.5, df=8, P<0.001). Individuals fed a trehalose diet did not show an increase in longevity relative to control individuals kept with water only. All other sugars significantly increased longevity compared to the control. Glucose and maltose yielded highest average longevity, but differed significantly only from the control, lactose, trehalose and raffinose (Figure 1).

Also *D. semiclausum* showed significant differences in average longevity when provided with different sugar solutions (F=65, df=9, P<0.001). Three sugars (lactose, raffinose and melezitose) failed to increase longevity over the water control. The other six sugars significantly increased longevity. Sucrose and maltose yielded highest average longevity, but did not differ significantly from melibiose and glucose (Figure 1).

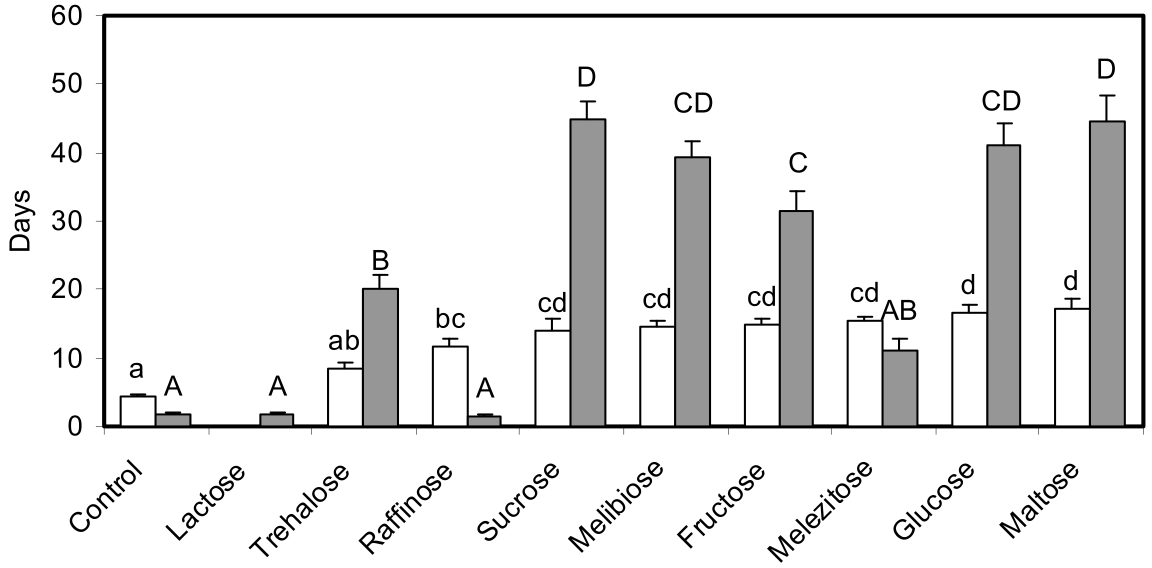

Figure 1: Longevity (mean±SE) of *Plutella xylostella* (white bars) and *Diadegma semiclausum* (grey bars) when provided with water only (control) or one of nine different sugars (0.5M). Treatments within insect species labeled with the same letter are not significantly different (ANOVA, Tukey's test: α = 0.05).

Discussion

Gustatory response

The herbivore *P. xylostella* accepts a wide range of sugars, in contrast to other Lepidopteran herbivores feeding on cabbage. In *Pieris brassicae* (Romeis, 2000) and *Pieris rapae crusivora* (Kusano & Sato, 1980) feeding is mainly, if not exclusively, elicited by sucrose and, to a lower extent, by fructose. Percival (1961) found that plant families with deep-tubed flowers tend to produce sucrose-rich nectar whereas those with open or shallow-tubed flowers tend to be hexose rich. The sugar acceptance of *P. xylostella* and *Pieris* spp on a gustatory level correlates with the flower visited by these herbivores in the field. Observations on standardised flower plots indicate that *P. xylostella* visits a broad range of flower species with exposed as well as with hidden nectar, whereas *P. rapae* and *P. brassicae* visit a restricted number of flower species with hidden nectar (Chapter 2). Choosing selective food plants, which promote the natural enemy but not the herbivore, is therefore more difficult for *P. xylostella* as compared to *P. rapae* and *P. brassicae*.

Also the parasitoid *D. semiclausum* responds to a wide range of sugars. In contrast to the findings for *P. xylostella*, the parasitoid showed a positive response to the honeydew sugar trehalose. This gustatory response might facilitate the exploitation of honeydew as a ubiquitous food source.

Compared with data from the parasitoid *C. glomerata* (Wäckers, 1999), *D. semiclausum* responds to a broader range of sugars. Sucrose, fructose and glucose elicited high responses in both species. The response to maltose, melezitose and trehalose was high in *D. semiclausum* but moderate in *C. glomerata*. *D. semiclausum* showed a moderate response to lactose, raffinose and melibiose, sugars to which *C. glomerata* did not respond at all. The egg parasitoid *Anaphes iole* showed strong responses to sucrose, maltose, glucose and melezitose. This species is less sensitive to fructose and responded poorly to trehalose (Beach et al., 2003).

Longevity response

Both the herbivore and the parasitoid showed a prolonged longevity on a wide range of sugars. This is in accordance with many reports stressing the importance of carbohydrate-rich foods for adult insects. However, in the current study, food supply has a far more pronounced effect on the life span of the parasitoid (up to 24-fold increase) as compared to the herbivore (up to 4-fold increase). A similar difference in effect was found for the herbivore *P. brassicae* (3-fold) and its parasitoid *C. glomerata* (15-fold) (Wäckers, 2001).

There is limited information available on the longevity of lepidopteran species exposed to sugars other than sucrose. Like *P. xylostella*, also *P. brassicae* lived significantly longer on sucrose, fructose, glucose and raffinose (Romeis, 2000, Romeis & Wäckers, 2000). While melibiose decreased life span of *P. brassicae* compared to the control, *P. xylostella* survived well on this sugar.

D. semiclausum did not survive long on raffinose and lactose. Inability to use these two sugars was also found for the parasitoid *C. glomerata* and honeybees (Wäckers (2001) and references therein). Longevity of *D. semiclausum* on melibiose was as good as on glucose and fructose. Although this sugar has been qualified as unsuitable for bees, it seems to be suitable for parasitoids like *C. glomerata* and for some Diptera (Wäckers (2001) and references therein).

Similar to the differences in the gustatory response, the honeydew sugar trehalose has a disparate effect on longevity of the two species tested here. While *P. xylostella* did not obtain a measurable longevity bonus from trehalose as compared to water, *D. semiclausum* showed a significantly longer life span on this sugar (20 days) than on water (1.8 days). Still, the increase in life span on trehalose was not as substantial as on fructose, glucose, sucrose, maltose or melibiose. Also the impact of melezitose differed between the two species. While *P. xylostella* survived on melezitose as long as on sucrose, this sugar did not extend *D. semiclausum* survival beyond control levels. The effect of trehalose and melezitose on longevity has been tested in two other parasitoids. *Bathyplectus curculionis* survival on trehalose and melezitose was as high as on sucrose and honey, but shorter than on glucose or fructose (Jacob & Evans, 2004). In *C. glomerata* the increase in life span was highest on sucrose, fructose and glucose, moderate on melezitose and lowest on trehalose, but still significantly higher than in the control (Wäckers, 2001). Our data confirm the overall pattern that honeydew-specific sugars appear to be less suitable as food sources, compared to the nectar sugars sucrose, fructose and glucose (Wäckers, 2000). However, the differences in sugar utilisation between species demonstrate that we can not implicitly generalise, even within an Order or Family.

Relationship between gustatory response and longevity

P. xylostella showed a good fit between the gustatory response and longevity, as it showed the highest responses to fructose, maltose, melezitose and sucrose, which are also suitable in increasing longevity. The unsuitable sugar trehalose, on the other hand, did not elicit a feeding response. In this case we do not know whether the short longevity is due to lack of sugar uptake or due to poor nutritional suitability of this sugar. Melibiose is somewhat exceptional, as it evoked only weak response (at lower concentrations), but appeared to be suitable to increase longevity.

Also *D. semiclausum* showed a general fit between gustatory response and longevity. In this species there are two clear outlyers. Like in *P. xylostella*, melibiose evoked only weak response, but appeared to be suitable to increase longevity. In contrast, the gustatory response to melezitose was as high as to sucrose, but survival was significantly poorer. The phenomenon that sugars elicit a feeding response without being nutritionally suitable has been reported for a number of other insects (Dethier et al., 1956, Dethier, 1968, Nettles & Burks, 1971, Romeis & Wäckers, 2002).

Providing food sources in the field to boost biocontrol

The limited ability of parasitoids to exploit flowers can restrict their longevity in nature as well as in agro-ecosystems. As *D. semiclausum* survives less than two days without food, it is fully dependent on carbohydrate feeding to achieve at least some of its reproductive potential. It is quite probable that these parasitoids feed on honeydew in the field (Wäckers & Steppuhn, 2003), even though this might be a suboptimal food (Elliott et al., 1987, Leius, 1961, Wäckers, 2001).

Providing natural sugar sources or applying sugar sprays to crops is one possible approach within conservation biological control to attract and/or retain beneficial insects (Cañas & O'Neil, 1998, McEwen & Liber, 1995). The differences in gustatory response between the herbivore and its parasitoid as reported in this study, show the potential for application of selective sugar sources. At the same time it also reveals the risk that indiscriminate use of sugar sources may result in a reverse selectivity: supporting herbivores without benefiting its antagonists. Raffinose, which evoked a gustatory response in both species but prolonged life span in the herbivore only, might be an example that may result in such a negative scenario. In contrast, trehalose, which evoked a gustatory response and prolonged life span in the parasitoid but not in the pest, would selectively promote the parasitoid. As parasitoid longevity on this sugar was considerably longer than on water, provision of this sugar in the field might result in strongly increased lifetime fecundity, and thus, in better pest reduction.

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Chapter

5

Do flowers make a difference? Nutritional state of *Diadegma semiclausum* and its host *Plutella xylostella* in the field

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Chapter 5

Do flowers make a difference? Nutritional state of *Diadegma semiclausum* and its host *Plutella xylostella* in the field

Abstract

The importance of food sources to the functioning of biological control in agroecosystems has been recognised by numerous biological workers. However, not all flower species are equally suitable to attract parasitoids and provide them with accessible nectar. Moreover, pest insects may exploit floral food sources as well. Here we investigate the effect of different flowering plant species on the nutritional state of the parasitoid *Diadegma semiclausum*, as well as on its host, the pest species *Plutella xylostella*. We tested fields bordered by flowering margins planted with one of three nectar plants that had shown to provide accessible nectar for the parasitoid and herbivore (*Anethum graveolens, Fagopyrum esculentum, Lobularia maritima*) or with *Centaurea jacea* that is only accessible to the herbivore. As a control we included margins planted with the grass *Lolium perenne*, which does not provide nectar. Insects collected in these fields were analyzed by HPLC to establish overall sugar content as a measure of their energy reserves.

P. xylostella showed increased numbers of fed individuals and higher levels of total sugar content in *A. graveolens* and *L. maritima*. The two other flower species, *C. jacea* and *F. esculentum*, did not alter the nutritional state of *P. xylostella*. To our knowledge this is the first time that it is shown that nectar plants enhance the nutritional state of a herbivore under field conditions. In three out of the four treatments individuals were shown to contain melezitose, indicating consumption of honeydew by the herbivore.

For *D. semiclausum*, the far majority of collected individuals in all five treatments had consumed sugars. Whereas the values of total sugar content were highest in the flower treatments, sugar levels did not differ significantly from the control. None of the flower species that provide accessible nectar under laboratory conditions yielded raised sugar levels among parasitoids collected. We propose that the effect of nectar sources on parasitoid nutritional state was superimposed by other factors like high mobility of the parasitoid and exploitation of alternative food sources.

Introduction

Many agro-ecosystem diversification programs aim at establishing flowering field margins as a tool to enhance the availability of nectar and pollen for beneficial insects. A great number of beneficial insects depend on plant-provided food for maintenance, dispersal and reproduction (Wäckers & Van Rijn, 2005). This includes a broad range of parasitoids (Heimpel et al., 1997, Jervis et al., 1993, Leius, 1960, Syme, 1975). Recent studies have used chemical analytical methods to provide unambiguous proof that parasitoids and predators actually make use of floral food sources (Lee & Heimpel, 2003, Wäckers & Steppuhn, 2003, Heimpel et al., 2004, Campan and Wäckers, unpublished).

Besides beneficial insects, also adult stages of insect herbivores depend on floral nectar (Kevan & Baker, 1983, Romeis et al., 2005). Access to suitable flowers can prolong lifespan and fecundity in herbivorous pests (Chapter 3; Baggen et al., 1999, Romeis & Wäckers, 2002). Moreover, the presence of flowering plants near crops might lead to increased pest pressure (Kienegger & Kromp, 2000, Latheef & Irwin, 1979, Zhao et al., 1992).

Herbivores and parasitoids may use different nectar sources. In a laboratory study we tested the nectar accessibility of 19 plant species with different flower structures for two cabbage pests and their parasitoids (Chapter 3). We identified flowers that were successfully exploited by the herbivore *Plutella xylostella* while being inaccessible to its parasitoid *Diadegma semiclausum.* In the present study we included field edges planted with one of these flower species, *C. jacea*, which selectively provides accessible food for the herbivore. In addition, we chose two plant species, *Fagopyrum esculentum* and *Anethum graveolens*, which provide food for the herbivore as well as for the parasitoid. The cruciferous plant *L. maritima* falls in the same category, but proved to be especially attractive for the herbivore *P. xylostella* (Chapter 2). As a control we used field edges with the grass *Lolium perenne*, which does not provide nectar.

We determined the nutritional state of field collected individuals and compared it with starved and ad libitum fed individuals from the laboratory. In this way we aimed to investigate whether *D. semiclausum* and *P. xylostella* actually use accessible nectar and whether herbivore and parasitoid differ in their exploitation of nectar sources under field conditions.

Besides floral nectar, insects may also feed on other sugar sources like honeydew. Honeydew often contains a number of sugars (melezitose, raffinose, erlose, trehalose) which are rarely or never found in nectar (Baker & Baker, 1982). In most insects, these sugars can be used as indicators of honeydew feeding (so-called 'signature sugars'). As melezitose is found in honeydew produced by the cabbage whitefly *Aleyrodes proletella* (Wäckers & Steppuhn, 2003) we used this 'signature sugar' to determine the frequency of honeydew feeding by *P.*

xylostella. As *D. semiclausum* is able to synthesise melezitose, erlose, trehalose and maltose when fed on sucrose, honeydew feeding can not be deduced in this species based on sugar content (Wäckers et al., unpublished).

Material and methods

Field sites

At two experimental locations in the vicinity of Wageningen, the Netherlands, 10 Brussels sprout (*Brassica oleracea)* fields were established. Fields were 8m*10m in size and consisted of 10 rows with 20 *B. oleracea* plants each. Each field was bordered by two 1m*10m strips of either grass (control), *Centaurea jacea, Anethum graveolens, Fagopyrum esculentum* or *Lobularia maritima*. The distance between the fields was 35 m, planted with grass, which was mown regularly. Weeds within the fields and the flower strips were removed by hand weeding. Throughout the whole field season no insecticides were used.

Sampling

During the period in which all bordering plants were in full bloom (wk 26 – wk 32) *D. semiclausum* and *P. xylostella* were collected by sweep net sampling in the Brussels sprout fields. Insects were placed individually in Eppendorf vials containing 200 ml of 70% ethanol. Sampling took place under dry weather conditions.

Samples were prepared by crushing the insects with a pestle and diluting 150 ml of the Eppendorf content ten-fold with Milli-Q water. 1 ml of the solution was filtered though a micro filter. 10 ml of the filtrate were injected in a Dionex Dx 500 HPLC-system, equipped with a GP 40 gradient pump, a Carbopac PA1guard, an analytical column (4x250 mm) and a ED 40 electrochemical detector for pulsed amperimetric detection (PAD). The column was eluded with 1 M NaOH and Milli-Q water and kept at 20ºC during the analysis. Curves of sugars were obtained by injecting calibration standards with different concentrations of sugars.

As controls, we also analysed ten newly emerged unfed individuals of *P. xylostella* and *D. semiclausum*, as well as ten individuals of both species which had been fed on sucrose for five days.

Interpretation of the data

The overall sugar content of an individual was calculated as the sum of the molar concentrations of the individual sugars. To test for differences between treatments including the unfed individuals from the laboratory we used the Kruskal-Wallis test (α=0.05). Subsequently, sugar levels of insects collected in the different treatments and from the unfed individuals were compared to levels in insects collected in the control fields (Mann-Whitney *U* test with Bonferroni correction, α=0.05/5=0.01).

Field collected individuals were classified as "fed" when their total sugar content exceeded the average sugar level of unfed individuals +2 times stdev. A chi-square test was used to compare the number of fed/non fed individuals in each flower treatment with the control (grass). When *P. xylostella* individuals contained any trace of melezitose they were classified as 'honeydew fed'.

Table 1: Overall sugar content of *Plutella xylostella* and *Diadegma semiclausum* collected in cabbage fields bordered by one of the five treatments and of number of individuals which were either not fed, or fed on sucrose for five days.

	Plutella xylostella			*Diadegma semiclausum*		
Treatment	Average ± S.E. [ppm]	N	%	Average ± S.E. [ppm]	N	%
Control (Grass)	8.0 ± 1.1	24	0.22	7.4 ± 1.3	25	0.13
Centaurea jacea	7.8 ± 1.2	23	0.21	12.5 ± 2.1	28	0.21
Anethum graceolens	16.9 ± 3.5	30	0.46	13.8 ± 2.6	25	0.23
Fagopyrum esculentum	8.3 ± 1.2	27	0.23	15.4 ± 5.3	26	0.26
Lobularia maritima	14.8 ± 3.8	30	0.40	12.3 ± 1.8	26	0.21
not fed	3.1 ± 0.4	10	0.08	1.5 ± 0.2	7	0.03
sucrose fed	36.6 ± 0.9	10	**1.00**	58.8 ± 9.2	10	**1.00**

Results

We collected a total of 23-30 individuals of *P. xylostella* and 25-28 of *D. semiclausum* in both the control and each of the four treatments.

Total sugar levels – absolute values

The average total sugar content of field collected *P. xylostella* was similar in the control (8.0±1.1 ppm) and the treatments *C. jacea* (7.8±1.2 ppm) and *F. esculentum* (8.3±1.2 ppm) (Table 1). The average total sugar content of *P. xylostella* collected in fields bordered by *A. graveolens* (16.9±3.5 ppm, MWU test p=0.03) and *L. maritima* (14.8±3.8 ppm, MWU test p=0.03) was about double the content in the control individuals.

The total sugar content was on average 3.1±0.4 ppm for unfed *P. xylostella* individuals and 36.6±0.9 ppm for sucrose fed individuals. *P. xylostella* individuals caught in the control treatment had a significantly higher total sugar content as compared to unfed individuals (MWU test P=0.008). Relative to 'ad libitum' fed *P. xylostella* individuals (100%), the total sugar content in unfed individuals was 8%, in individuals from control, *C. jacea* and *F. escu-*

lentum approximately 22% and in individuals from *L. maritima* and *A. graveolens* 40% and 46%, respectively (Table 1).

The total sugar content of *D. semiclausum* collected from the control was on average 7.4±1.3 ppm. The average sugar levels in parasitoids collected from flower margin fields was noticeably higher at 12-15 ppm (Table 1). Due to the high variation within the treatments this difference was not significant. The average total sugar content was 1.5±0.2 ppm for unfed individuals and 58.8±9.2 ppm for individuals with ad libitum access to sucrose. Parasitoids collected in the control field had a significantly higher total sugar content as compared to unfed individuals (MWU test $P<0.001$). When we put ad libitum fed individuals at 100%, the sugar level in unfed parasitoids was 3%, in control individuals this figure was 13% and in the treatments 21-26% (Table 1).

Total sugars - Classification fed/unfed

We found a significant higher proportion of fed *P. xylostella* individuals in cabbage fields bordered by *A. graveolens* (26 out of 30) and *L. maritima* (26/30) compared to the control (16/24). Cabbage fields bordered by *C. jacea* (14/23) and *F. esculentum* (17/27) did not show an elevated proportion of fed individuals (Figure 1a).

For *D. semiclausum* there was no significant difference between the control and any treatment regarding the feeding state of the collected wasps. The proportion of individuals with elevated sugar levels relative to newly emerged parasitoids was higher than 89% in all treatments, including the control (Figure 1b).

Melezitose in Plutella xylostella

From all field collected *P. xylostella* (N=134) only 11 individuals contained melezitose (Table 2). This included one individual (out of 24) in fields bordered by grass (control), two (out of 23) in fields bordered by *C. jacea*, three (out of 30) in fields bordered by *A. graveolens* and five (out of 30) in fields bordered by *L. maritima*. The average melezitose content in individuals containing this specific sugar ranged from 0.19 ppm in individuals collected in the control up to 1.48 ppm in individuals collected in *L. maritima* bordered fields.

Discussion

The potential importance of food sources for the functioning of biological control in agroecosystems has been recognized by numerous biological workers (Gurr et al., 2005, Illingworth, 1921, Landis et al., 2000, Wolcott, 1942). However, not all flower species are equally suitable as food source to parasitoids (Wäckers, 2004). Wäckers and Steppuhn (2003) could demonstrate that the availability of flowers in field borders enhances the nutritional state of the parasitoid *C. glomerata*. Here we investigate the effect of single plant species not only on the nutritional state of the parasitoid *D. semiclausum*, but also on its host, the pest species *P. xylostella*.

Under laboratory conditions, all four flower species used in this study provided accessible nectar and prolonged lifespan of the herbivore *P. xylostella* (Chapter 3). In the present field study, higher numbers of fed individuals and higher amounts of total sugar content were found only in two flower species, *A. graveolens* and *L. maritima,* as compared to the control. To our knowledge this is the first demonstration that nectar plants affect the sugar levels of a herbivore under field conditions. The two other flower species, *C. jacea* and *F. esculentum*, did not raise sugar levels in *P. xylostella*. This difference may indicate that the latter two spe-

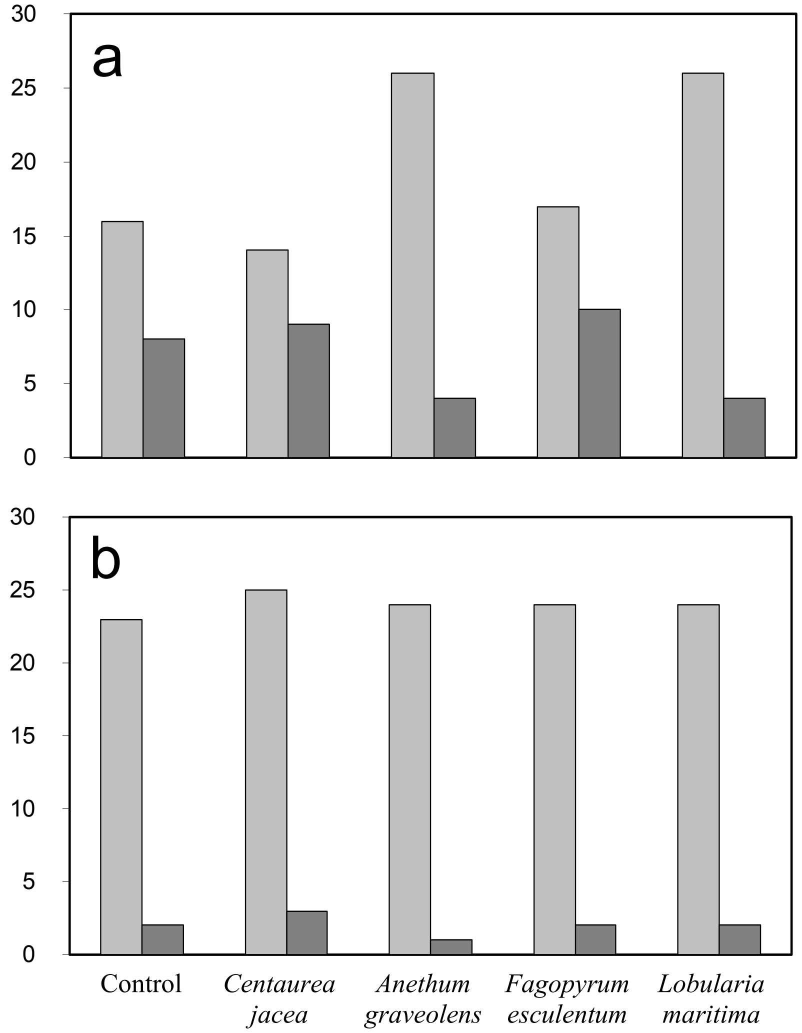

Figure 1: Number of *Plutella xylostella* (a) and *Diadegma semiclausum* (b) fed (light grey) and not fed (dark grey) on nectar sources in the field. Significant differences in the proportions fed/unfed individuals between the treatments as compared to the control are indicated with a * (Chi^2 test, P<0.05).

Table 2: Melezitose content of *Plutella xylostella*

Treatment	N melezitose fed (total)	Average fed [ppm]	Range [ppm]
Control (Grass)	1 (24)	0.19	-
Centaurea jacea	2 (23)	0.28	0.12-0.44
Anethum graceolens	3 (30)	0.58	0.18-1.35
Fagopyrum esculentum	0 (27)	-	-
Lobularia maritima	5 (30)	1.48	0.14-2.60

cies do not provide accessible nectar under field conditions. In our experiments *C. jacea* and *F. esculentum* were frequently visited by bees, bumblebees and hoverflies. This might have reduced the availability of floral nectar for *P. xylostella* on these flower species as a result of competition (Lee & Heimpel, 2003).

Individuals collected from the control treatment contained significantly higher sugar levels as compared to unfed individuals. *P. xylostella* obviously was able to locate and to take up sugars from other sources, like nectar from small weeds that may have been overlooked during weeding, or honeydew. In three out of the four treatments, some individuals were found to contain melezitose, indicating consumption of honeydew. I observed the cabbage whitefly *Aleyrodes proletella* in low densities throughout the season in all fields. Honeydew of this species contains significant levels of melezitose, in contrast to honeydew from the slightly more abundant cabbage aphid *Brevicoryne brassicae*, which does contain significant levels of trehalose and maltose, but not melezitose (Wäckers & Steppuhn, 2003). As *P. xylostella* is able to synthesise trehalose and maltose (Wäckers, in prep), the identification of aphid honeydew feeding by this herbivore remains difficult.

Despite variation in sugar levels among *P. xylostella* collected from the different treatments we found no differences in *P. xylostella* larval densities (Chapter 7). Under laboratory conditions, *P. xylostella* survives about 5 days without food supply (Chapter 3). Within this period, *P. xylostella* lays between 80% and 90% of the total number of eggs (Cardona, 1997, Harcout, 1954). Pivnick et al. (1990) found peak oviposition in *P. xylostella* on day 4 und 5. Food supply increases the longevity of *P. xylostella* under controlled conditions up to 15 days (Chapter 3). Under field conditions, non-starvation mortality by predation and exposure to unfavourable weather conditions might reduce life expectancy of *P. xylostella,* which, on its turn, limits the impact of food on lifetime fecundity. Under laboratory conditions, *F. esculentum* and *A. graveolens* were shown to provide accessible nectar for *D. semiclausum* and prolong the parasitoid's lifespan, whereas *C. jacea* failed to do so (Chapter 3). Unexpectedly we did not observe higher numbers of fed individuals and higher total sugar contents in flower treatments with accessible nectar as compared to the control and to flower treatments with un-accessible nectar. Instead, in all treatments the far majority of the collected individuals of *D. semiclausum* were qualified as ‘fed’. The values of total sugar content were higher, but not significantly, in individuals from all flower treatments as compared to the control. The fact that field collected parasitoids on average contained a mere 13-26% of the sugar levels found in ad libitum sucrose fed parasitoids under laboratory conditions indicates moderate feeding by parasitoids under field conditions. In contrast, field collected *P. xylostella* contained up to 46% of the sugar levels found in ad libitum sucrose fed individuals. These relatively high sugar levels under field conditions may reflect the relatively low mobility of *P. xylostella* as compared to its parasitoid *D. semiclausum*.

Our finding that differences in the nutritional state are lacking for *D. semiclausum*, corresponds well with the fact that we found similar rates of parasitism among the treatments (Chapter 7). Similar results have been reported for *Diadegma insulare*, for which Lee et al. (2003) found rates of parasitism of approximately 60 % in cabbage fields irrespective of the presence of *F. esculentum*. In contrast, Lavandero et al. (2005) reported higher parasitism rates in cabbage fields bordered by *F. esculentum*, relative to control fields.

There are two possible explanations for the lack of treatment effect on parasitization levels. Wasps may have exploited alternative food sources within the fields and/or they were highly mobile and obtained food outside the experimental field. As *D. semiclausum* emerges with a limited supply of energy and dies without food after less than two days, food uptake is of crucial importance for survival and reproduction (Chapter 6). We therefore expect that the wasps make use of honeydew and other carbohydrate sources (Lee et al., 2004, Wäckers & Swaans, 1993), even though honeydew might be difficult to detect and of lower nutritional value (Wäckers 2005). Wäckers and Steppuhn (2003) confirmed honeydew consumption in 80% of all *Cotesia glomerata* and 55% of all *Microplitis mediator* collected from unsprayed cabbage fields with and without flower margins. However, in the case of *D. semiclausum* we can not use the same methodology to identify honeydew consumption, as none of the sap-feeder synthesized honeydew sugars can be used as signature sugar in this particular species.

In their natural environment, the parasitoid *Venturia canescens* was able to maintain a nearly constant level of energy over an extended foraging period (Casas et al., 2003). Within one day, the wasps dispersed up to 60 m to the most distant traps. The parasitoid *Cotesia popularis* is able to disperse to the next host patch over distances of at least 80 m (Esch et al., 2005). Based on within field observations, Thompson (2002) predicted net displacement of 55 m within 12 h (total travel distance of around 400 m) for *D. semiclausum*. Lavandero (2005) observed that *D. semiclausum* can move 80 m in a four day period. High dispersal ability might therefore be responsible for a treatment dilution effect in our study.

We set out to investigate whether *P. xylostella* and *D. semiclausum* make use of accessible nectar and whether they differ in the exploitation of nectar sources under field conditions. The raised sugar levels in 74% of field collected *P. xylostella* and 92% of *D. semiclausum* indicates widespread food uptake by both species within all treatments.

Results from laboratory studies concerning food plant exploitation are not always valid under field conditions. Only two out of four flower species, which provide accessible nectar for *P. xylostella* under laboratory conditions, improved its nutritional state in the field. For the parasitoid *D. semiclausum*, none of the flower species, which provide accessible nectar under laboratory conditions, resulted in a significant improvement in nutritional state under field conditions.

In case of *P. xylostella*, enhanced nutritional state within the treatments *A. graveolens* and *L. maritima* did not translate in higher pest densities (Chapter 7). For *D. semiclausum*, the marked impact of suitable nectar sources on parasitoid fecundity could be demonstrated under controlled field conditions (Chapter 6). In the present experimental setup, high rates of parasitism were observed in all treatments (Chapter 7) and not only in the treatments with suitable nectar plants for *D. semiclausum*. We suppose that this is the result of a combination of high mobility of the parasitoid and the use of alternative food sources.

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Chapter

6

Nectar resources are vital for *Diadegma semiclausum* fecundity under field conditions

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Chapter 6

Nectar resources are vital for *Diadegma semiclausum* fecundity under field conditions

Abstract

Many insects, including parasitoids, depend in their adult stage on carbohydrate-rich food as their main source of energy for longevity, fecundity and mobility. The effect of food availability on parasitoid life table parameters is usually studied in laboratory experiments. However, these studies might be a poor representation of the field situation. Field experiments, on the other hand, are usually unsuitable to evaluate the impact of food availability on individual insects. To bridge this gap, we conducted a field experiment in which individual parasitoid wasps (*Diadegma semiclausum*) were released in large cages either containing spatially separated food and host (*Plutella xylostella*) sites, or host sites only (control). Out of the eleven wasps exposed to host larvae in the absence of a nectar source, only three were able to parasitize any larvae. Female wasps that had no access to nectar parasitized only 3.7±4.4 larvae. In contrast, all twelve wasps with nectar supply were able to parasitize more than 300 *P. xylostella*, with an average of 390±31 caterpillars parasitized per wasp. Nectar availability also increased the average reproductive lifespan of the parasitoids from 1.2 days (control) to 28 days. Surprisingly, the impact of food sources on *D. semiclausum* fecundity was more clear-cut than in previous laboratory studies with the same species, emphasizing the importance of studying life-table parameters under more natural conditions. These results also underline that access to carbohydrate-rich food can be indispensable to parasitoid fecundity and stress the importance of providing suitable nectar sources as an integral part of biological control programs.

Introduction

Most parasitoids depend on carbohydrate-rich food during the adult stage to cover their energetic needs. Early studies already pointed out that the lack of food in agricultural crops can be a potential impediment to the successful functioning of parasitoids and predators (Hocking, 1966, Illingworth, 1921, Wolcott, 1942). Providing food supplements, biological control workers seek to eliminate this obstacle and enhance the efficacy of biological control programs. Food supplements could be either provided by enhancing crop-produced nectar (Wäckers, 2005), adding plants producing floral and/or extrafloral nectar (Gurr et al., 1998, Landis et al., 2000, Powell, 1986), or by offering artificial sugar solutions (Canas & O'Neil, 1998, McEwen & Liber, 1995).

Parasitoids usually emerge with a limited supply of energy (Wäckers, 2003). Without access to food they may die within a few days. Experiments under controlled conditions underline the importance of food availability for parasitoid longevity (Idoine & Ferro, 1988, Idris & Grafius, 1995, Syme, 1975, Van Lenteren et al., 1987, Wäckers, 2001, Zoebelein, 1955). In addition, carbohydrate-rich food availability can increase the parasitoid's lifetime fecundity. This increase is either based on an extension of the reproductive life-span (Idris & Grafius, 1995, Irvin et al., 1999, McEwen & Liber, 1995), an increased rate of egg maturation (Schmale et al., 2001), or both (Baggen & Gurr, 1998, Hocking, 1966, Syme, 1975). The effect of food availability on longevity and fecundity is generally tested in the laboratory under standardized and unnatural conditions. In these experiments, parasitoids are usually severely restricted in their mobility. Moreover, they are often given unlimited access to hosts and food sources, thus precluding any searching and foraging behaviour. Finally, mortality factors such as predators or weather influences are also usually excluded.

There are a few field studies on the impact of flowering herbs. For example, studies on *Dolichogenidea tasmanica*, a parasitoid of leaf rollers and on parasitoids of the tent caterpillar *Malacosoma americanum* (F.) and the codling moth *Carpocapsa pomonella* L. revealed that the presence of nectar-providing herbs can lead to higher parasitism rates in orchards (Leius, 1967, Stephens et al., 1998). An increase in parasitism adjacent to nectar-producing plants could also be demonstrated in agricultural cropping systems for *Cotesia rubecula*, *Diaretiella rapae* and *Copidosoma koehleri* (Baggen & Gurr, 1998, Luna et al., 1999, Zhao et al., 1992). However, field studies are usually unsuitable to quantify fitness benefits of individual parasitoids. Moreover, there is the confounding effect that flowering vegetation can both attract additional natural enemies from adjacent areas (Patt et al., 1999, Wäckers & Lewis, 1994) and increase the retention of individuals within a given area (Stapel et al., 1997).

To examine the effect of food supply on parasitoid fecundity, we conducted a semi-field experiment in which individual parasitoids were released in large cages containing either spatially separated nectar and host sites, or host sites only (control). Consequently, parasitoids were forced to actively fly in search for food and hosts while being exposed to abiotic field conditions. We used flowering *Fagopyrum esculentum* as nectar source, as previous studies have shown that access to this plant increases longevity and fecundity in *Diadegma semiclausum* (Wratten et al., 2003). The exclusion of predators from the tents will reduce parasitoid mortality and thus may result in an overestimation of lifetime fecundity. Nevertheless, this experimental setup allowed us to address the question to what extent nectar plants increase a parasitoid's realized lifetime fecundity under more natural conditions.

Material and methods

Tents

Twelve tents, each 3m wide, 4m deep and 2m high, were placed in a field near the Laboratory of Entomology, Wageningen, The Netherlands. The metal tent frames were covered with insect netting, mesh size 0.6*0.6 mm (Mononet 600 NA, Rovero Systems). This material reduces light intensity by 48% (product information of Rovero Systems). Each tent could be entered by opening a zipper. On the inside of the tent, the zipper was covered by a fine-meshed sheet (3*2 m), which reduced the risk of insects escaping from or entering the tent. Tents were set up in a two row grid with 6 m distance between tents. An additional tent (width 3 m, length 12 m, height 1.2 m) was used to grow extra cabbage plants used in the experiment. Experiments were conducted in 2004 in two series during week 27-32 (June 26th – August 3rd; Experiment 1) and week 34-35 (August 17th - August 26th; Experiment 2), respectively.

Temperature

Temperature data were obtained from a weather station located at a distance of 500 m from the tents. In addition, temperature was measured with calibrated data loggers at about 1.5 m height in two of the tents and outside the tents during week 37 and 38.

Plants

Brussels sprout plants (*Brassica oleracea* L. gemmifera cv. Maximus) were grown from seeds and transplanted to the experimental tents on May 17, 2004. In each of the tents, twelve plants were planted in three rows with 0.8 m distance between and 0.7 m within the rows. Tents were hand-weeded to eliminate potential nectar-bearing plants, leaving bare soil between the Brussels sprout plants. In the extra tent four rows of 20 plants were planted.

Nectar producing buckwheat plants (*Fagopyrum esculentum*) were sown every fortnight in plastic pots (12 cm diameter) and also kept in the extra tent.

Insects

Plutella xylostella

Every day, two Brussels sprout plants were placed for 24 h in a cage containing adult *Plutella xylostella* (diamondback moth), for oviposition. Afterwards the plants were placed in a separate cage at 24±2°C, 40±10% r.h. and L16-D8. After two to three days caterpillars had developed to the L2 stage and were used to infest leaves from herbivore-free cabbage plants. Young cabbage leaves some 8-10 cm long were collected from the plants in the extra tent and individually placed in water-filled glass vials (6 cm high, 1 cm ∅). The opening of the vial was sealed with parafilm and fixed with a rubber band. Each leaf was subsequently infested with ten L2 *P. xylostella.* To prevent caterpillars from escaping, the glass vial was placed in a funnel constructed from transparent acetate sheet. The funnel was attached to a circular wire (Figure 1).

While Yang et al. (1993) found that *D. semiclausum* can parasitize up to 45 caterpillars per day, Cardona (1997) reports that daily oviposition does not exceed 20 eggs. In a four-day pilot study, using the experimental setup (with nectar supply) as described below, daily oviposition reached a maximum of 18 eggs per female. To assure a surplus of larvae in the experiments, we therefore decided to provide 30 caterpillars per wasp per day.

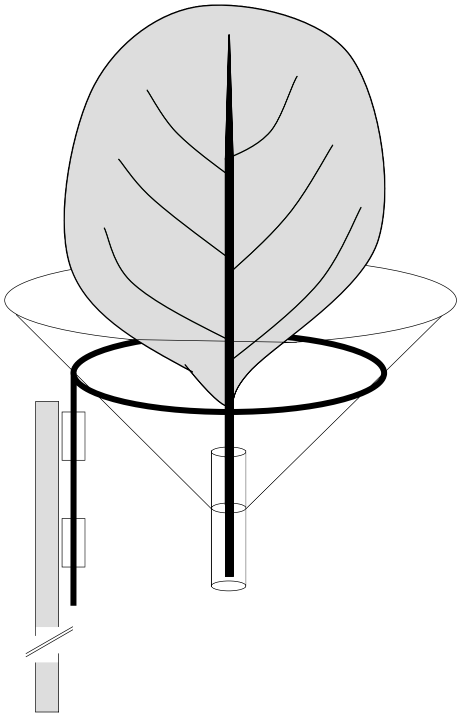

Figure 1: Funnel with Brussels sprout leaf, attached to a bamboo stick to expose *Plutella xylostella* larvae to *Diadegma semiclausum* parasitoids in the field-cages.

Diadegma semiclausum

The laboratory rearing of the parasitoid *D. semiclausum* was established from approximately 80 individuals that had been collected from Brussels sprout fields in the vicinity of Wageningen, The Netherlands, in 2003. Subsequently they had been reared for ten months on diamondback moth caterpillars feeding on Brussels sprout. The rearing was maintained in a climate room (20±2°C, 70±10% r.h. and L16-D8). The day before the start of the experiment, parasitoid cocoons were transferred to a clean cage without food or hosts. Moist cotton wool was provided as a source of water. On the first day of the experiment, newly emerged wasps (less than 24 h old) were collected individually in glass vials and in each tent one female was released together with one male.

Experimental procedure

The day before the start of an experiment, one pot with flowering *F. esculentum* was placed in six of the twelve tents. The other six tents were considered as control and did not receive a nectar source. At the start of the experiment, between 9:00 and 10:00 h, three funnels with infested leaves were introduced in each of the twelve tents. Each funnel was placed at the top of a bamboo stick. One bamboo stick with an infested cabbage leaf was placed in every cabbage row at the height of the canopy. Between 16:00 and 17:00 h the funnels with the infested leaves were recollected and taken into the laboratory. This procedure was repeated throughout the experiment, thereby providing the parasitoids with fresh hosts for a seven hour period every day. Due to problems in the *P. xylostella* supply on day 23 during the first experiment we could not offer hosts on this day to the wasps. When collecting caterpillars, the infested leaves and any caterpillars found on the funnel or vial were transferred to plastic Petri dishes (13 cm ∅) and stored in a refrigerator. The caterpillars were dissected and checked for the presence of *D. semiclausum* eggs during the following day.

The first experiment lasted until all released wasps were presumed dead, i.e. three days after the last oviposition was recorded. The duration of the second experiment was limited to ten days, because in the first series no ovipositions occured in the control treatment after day 7. Moreover, the first series had also provided data on the lifespan and lifetime fecundity of parasitoids in the buckwheat treatment.

As aphid colonies were observed in one of the control tents, all twelve tents were treated with Spruzit (36g/l pyrethrum; 144 g/l piperonyl butoxide) on august 10th, one week before the start of the second experiment. As the active compounds of this insecticide break down within two days, we decided to release the second batch of parasitoids seven days after insecticide application to exclude any potential negative effect on parasitoid performance. To avoid that the experiments would be affected by possible honeydew residues, the aphid infested tent was excluded in Experiment 2. Therefore we tested twelve replicates of the nectar treatment and eleven replicates of the control treatment.

Data analysis

To evaluate the effect of nectar supply on the number of parasitized caterpillars, an Univariate Analysis of Variance was used (SPSS 11.00). Lifetime fecundity and the number of days during which oviposition occurred, were analysed using Mann-Whitney-U test ($\alpha=0.05$) (SPSS 11.0). A χ2-test ($\alpha=0.05$) was used to check for differences between the treatments with and without nectar supply with respect to the proportion of ovipositing wasps.

Results

The number of parasitized caterpillars was similar among the replicates of the nectar supply experiment ($P=0.197$). Treatment, as well as the experimental day had a significant effect on the number of parasitized caterpillars. Within the first ten days, females with access to nectar parasitized significantly more caterpillars than females without access to nectar ($P<0.001$, Table 1 and 2, Figure 2). All twelve wasps with nectar supply parasitized *P. xylostella* caterpillars, with a fecundity ranging from 1 to 29 eggs during the first ten days. Out of the eleven wasps without access to nectar, only three were able to parasitize any *P. xylostella* caterpillars and only one managed to parasitize more than three larvae.

Parasitoids that had access to nectar realized a significantly higher fecundity compared to female parasitoids without access to nectar. This applied both to lifetime fecundity (first

Table 1: Effect of nectar supply on *Diadegma semiclausum*: average, SE and range of number of parasitized *Plutella xylostella* caterpillars during the first ten days, lifetime fecundity, number of days that wasps are ovipositing and number of wasps ovipositing. Averages within lines followed by different letters are significantly different (a= 0.05).

	Control (without nectar) Mean ± SE (range)	N	With nectar Mean ± SE (range)	N
Average number of parasitized caterpillars during the first 10 days of adult life	3.7±3.24 a (0-36)	11	124±10.42 b (66-172)	12
Number of wasps that oviposited	3 a	11	12 b	12
Lifetime fecundity	3.7±3.24 a (0-36)	11	390±30.08 b (301-493)	6
Number of days during which wasps oviposited	0.82±0.55 a (0-6)	11	27.8±2.06 b (22-34)	6

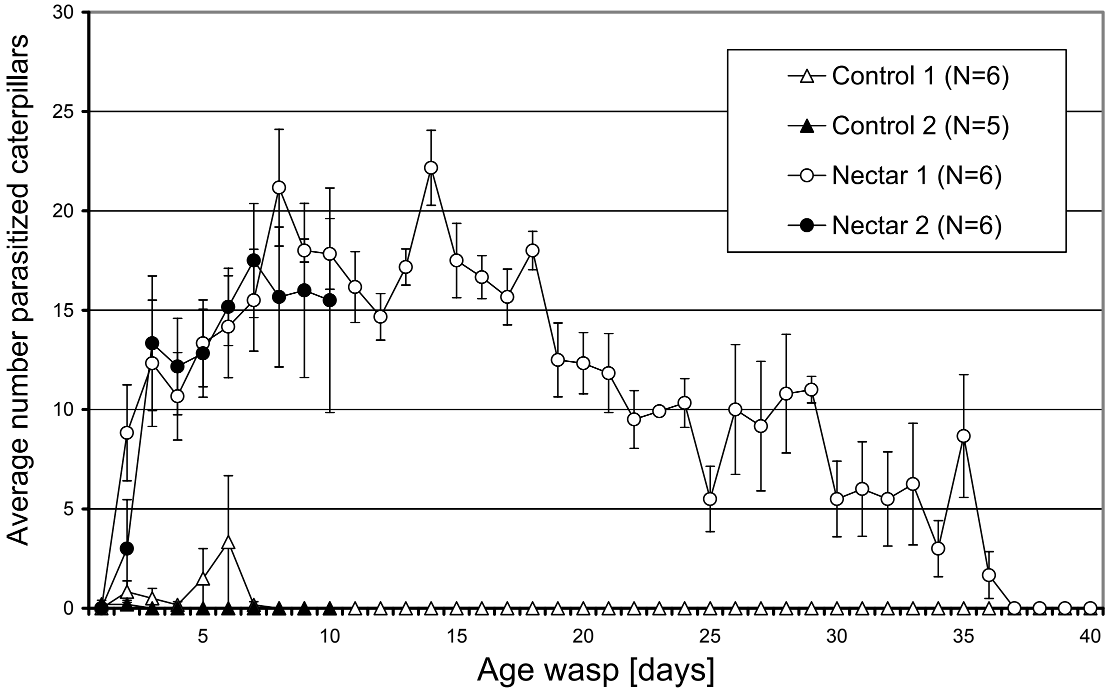

Figure 2: Effect of nectar supply on number of parasitized *Plutella xylostella* caterpillars. Experiment 1 lasted until all *Diadegma semiclausum* females had died, experiment 2 was terminated after 10 days. Error bars represent SE of average number parasitized caterpillars.

series) as well as to the fecundity achieved during the first ten days of adult life. Out of the eleven wasps exposed to hosts in the absence of a nectar source, only three were able to parasitize any larvae. All twelve female parasitoids with access to nectar managed to parasitize more than 65 caterpillars during the first ten days of adult life. The reproductive lifespan was much higher for parasitoids with access to nectar (27.8 days) as compared to the control (0.8 days) (Table 1).

Regardless of nectar supply, parasitoids started to parasitize on day two or three of the experiment, except for one female in the control treatment that parasitized one caterpillar on the first day. Out of in total 2340 parasitized caterpillars only 14 contained two *D. semiclausum* eggs. These cases of superparasitism occurred during the two oviposition peaks (day 6-10 and day 17-19; Figure 2).

Temperature

The average mean temperature recorded at the weather station was 15.9°C (range 14-18°C) during day 1-10 and 17.1°C (range 14-22°C) for the whole period of experiment 1. The average mean temperature recorded at the weather station during experiment 2 was 17.2°C

Table 2: Effect of nectar supply on *Diadegma semiclausum*: N parasitized caterpillars over life span resp. experimental period

Control	day1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
1	0	3	0	0	0	0	0	0	0	0																												
2	0	0	0	0	0	0	0	0	0	0																												
3	0	0	0	0	0	0	0	0	0	0																												
4	0	2	3	1	9	20	1	0	0	0																												
5	0	0	0	0	0	0	0	0	0	0																												
6	0	0	0	0	0	0	0	0	0	0																												
7	0	0	0	0	0	0	0	0	0	0																												
8	0	0	0	0	0	0	0	0	0	0																												
9	0	0	0	0	0	0	0	0	0	0																												
10	1	1	0	0	0	0	0	0	0	0																												
11	0	0	0	0	0	0	0	0	0	0																												
Nectar	day1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
1	0	17	13	11	12	20	25	29	20	21	12	17	19	28	19	18	11	17	12	12	15	12	-	11	0	0	0											
2	0	11	15	16	8	17	17	22	18	12	17	18	19	25	13	17	21	22	11	15	6	14	-	8	1	0	0	0										
3	0	8	17	15	21	16	13	29	18	21	15	10	17	18	16	20	15	18	8	7	12	10	-	14	8	14	18	18	11	7	11	11	10	6	14	5	0	0
4	0	5	22	9	16	16	14	14	19	13	20	13	18	21	12	16	14	15	8	9	7	4	-	6	10	12	15	16	11	4	2	0	0	0	0	0	0	
5	0	12	7	12	16	14	18	21	17	18	22	15	17	25	22	17	18	17	18	14	19	10	-	13	7	18	15	13	13	11	11	10	15	6	12	0	0	0
6	0	0	0	1	7	2	6	12	16	22	11	15	13	16	23	12	15	19	18	17	12	7	-	10	7	16	7	7	9	0	0	1	0	0				
7	0	3	14	16	14	16	23	0	6	0																												
8	0	1	0	3	14	15	23	18	2	0																												
9	0	14	22	17	17	23	14	20	20	25																												
10	0	0	12	12	5	14	7	16	18	16																												
11	0	0	19	9	9	13	16	19	24	24																												
12	0	0	13	16	18	10	22	21	26	28																												

(range 14-21°C). Simultaneous recordings at the weather station and the experimental location revealed great similarity in temperature fluctuations.

Discussion

Our studies show that nectar feeding is of crucial importance to parasitoid survival and fecundity under field conditions. While laboratory studies already indicated a great difference in fecundity in *D. semiclausum* with and without food supply (Cardona, 1997, Yang et al., 1993), they nevertheless showed comparable oviposition rates for females with and without food during the first three days. Contrary to these reports, nine out of eleven parasitoids in our control treatment failed to oviposit at all. The virtual absence of reproductive success in food-deprived individuals in our experiments can be explained by the fact that parasitoids in the field cages had to fly in search of hosts, food and mates. Energetically, flight is by far the most costly activity of parasitoid behaviour (Casas et al., 2003, Hoferer et al., 2000), and unfed parasitoids exhaust their energy reserves at considerably faster rates under field conditions as compared to individuals kept in confined conditions (Steppuhn & Wäckers, 2004). Surprisingly, one out of eleven wasps in the control treatment was able to survive for at least 7 days and to lay 36 eggs. Given that all weeds had been removed from the cage, we assume that this individual located and fed on another food source that had escaped our attention. Potential sugar sources in our experiment include plant sap exuding from caterpillar feeding sites, or sugars leaking through the undamaged epidermis (Wäckers, 2005). However, as the far majority of the wasps in the control treatment failed to oviposit, it is unlikely that these sources were involved.

Parasitoids often emerge with a limited supply of energy, allowing them to survive for only 1-2 days (Olson & Nechols, 1995, Steppuhn & Wäckers, 2004). Part of this time is not available for host search and reproduction (Wäckers, 2003), since many parasitoid species have a pre-oviposition period during which their eggs need to mature. Also, part of the initial period after emergence may be used for mate finding and for mating itself. The lack of experience in finding and handling the host during the initial period (Turlings et al., 1993, Vet et al., 1995) likely further limits the reproductive success of newly emerged parasitoids.

Given that we released newly emerged unfed parasitoids, they might have started foraging for food, rather than searching for hosts (Takasu & Lewis, 1994, Wäckers, 1994). Due to the absence of nectar sources in the control treatment, food foraging will have only expedited depletion of energy reserves, thereby further reducing oviposition opportunities. Thus, we can conclude that under field conditions *D. semiclausum* will usually hardly reproduce in the absence of sugar sources, and that its contribution to the biological control of *P. xylostella* will be negligible. In the buckwheat treatment, wasps apparently successfully located and accessed the nectar source. This allowed them to realize a long lifespan as well as a high daily parasitation activity.

Surprisingly, when provided with food parasitoids realized a much higher fecundity in our field experiment as compared to the laboratory studies mentioned earlier. Abbas (1988) recorded an average number of 164 eggs per female with a maximum of 223 eggs and an average longevity of 14.6 days under controlled laboratory conditions at 25°C. In greenhouse experiments with temperatures ranging between 11-22°C, Cardona (1997) reported an average lifetime fecundity of 179 eggs with a maximum of 194 eggs and an average longevity of 24 days. Both studies used diluted honey as food source. Temperatures in our experiment were within the range of those in the study of Cardona. Nevertheless, wasps in our study parasitized twice as many caterpillars (on average 390) and had longer reproductive life spans (on average 27.8 days). Combined with the reduced reproductive success by food-deprived parasitoids in our field cages, this suggests that laboratory studies might actually underestimate the effect of food provision and that the impact of food availability on parasitoid reproductive fitness can be even more pronounced in the field.

The higher reproductive lifespan in our experiments is surprising, as wasps had to fly in order to search for food and hosts. Given the energetic cost associated with flight (Hoferer et al., 2000) and the fact that high energy expenditure often reduces lifespan (Yan & Sohal, 2000) we would have expected a reduced lifespan relative to wasps kept in small cages.

Lee & Heimpel (Lee & Heimpel, 2004) reported that *Diadegma insulare* collected in cabbage fields with and without adjacent buckwheat strips showed a comparable incidence of sugar feeding. We found closely matching feeding frequencies in a study comparing cabbage plots bordered by grass (control), flowers with inaccessible nectar, and flowers with accessible nectar. Here, too, no significant effect on the occurrence of sugar-fed individuals nor on percentage parasitism was found (Winkler, unpubl.). In both studies, fields were kept free from flowering weeds. These findings therefore suggest that the wasps either exploited alternative food sources like honeydew (Heimpel et al., 2004, Wäckers & Steppuhn, 2003), or that wasps dispersed in excess of the 35 m (Winkler, unpubl.) or 67 m (Lee & Heimpel, 2003) that separated control from flower treatments. High parasitism rates of more than 65 % in the field (Winkler, unpubl.) indicate that wasps are able to realize at least part of their fecundity.

Semi-field experiments are suitable to bridge the gap between laboratory studies and the reality in the field (Jepson & Mead-Briggs, 1992). Our results prove that the impact of food availability on *D. semiclausum* fecundity is actually amplified under field conditions. This confirms the importance of complementing laboratory studies with (semi-) field experiments in order to fully appreciate the actual impact of food availability on fecundity of individual parasitoids. Our semi-field study also substantiates the conclusion from laboratory studies that access to food is essential to support *D. semiclausum* in its function as biocontrol agent.

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Chapter

7

Assessing the risks and benefits of flowering field edges

K. Winkler, F.L. Wäckers, A.J. Termorshuizen, J.C. van Lenteren

Chapter 7

Assessing the risks and benefits of flowering field edges

Abstract

The use of flowering field margins is often propagated as a method to support biological control in agro-ecosystems. Besides beneficial insects, many herbivores depend on floral food as well. The indiscriminate use of flowering species in field margins can therefore lead to higher pest numbers. Increased awareness of this risk resulted in a new approach in conservation biological control aiming at making strategic use of plant biodiversity. Based on results from field observations and laboratory experiments we designed a "worst-case" and a "best-case" scenario to assess potential risks as well as benefits associated with using nectar plants in field edges of a Brussels sprout production system.

Results confirm the risk that Brussels sprouts bordered by nectar plants that are especially attractive and suitable for the cabbage white *Pieris rapae* like *Centaurea jacea* and *Fagopyrum esculentum*, suffered from higher infestation levels by this herbivore. In contrast, Brussels sprouts bordered by nectar plants providing accessible nectar for the diamondback moth *Plutella xylostella,* did not show higher densities of *P. xylostella* larvae. Borders plants with *Anethum graveolens* significantly increased the number of adult *D. semiclausum* in the crop. Parasitism rates of *P. xylostella* by *D. semiclausum* were with more than 65% high in all treatments, irrespective of the plant in the field margin.

Introduction

Establishing flowering field margins is often propagated as tool to sustain parasitoids and predators and thereby enhance the efficacy of biological pest control. Under laboratory conditions, nectar supply often has a great impact on longevity (Idris & Grafius, 1995, Van Lenteren et al., 1987, Wäckers, 2001, Zoebelein, 1955), fecundity (Baggen & Gurr, 1998, Idris & Grafius, 1995, McEwen & Liber, 1995) and activity of parasitic wasps (Takasu & Lewis, 1994, Wäckers, 1994). Under field conditions, the presence of flowers may (locally) increase the abundance of beneficial insects (Patt et al., 1997, White et al., 1995) and rates of parasitism (Stephens et al., 1998).

By focusing on the nectar and pollen use by beneficial insects the fact that many herbivores depend on floral food as well is often ignored (Baggen & Gurr, 1998, McEwen & Liber, 1995, Romeis et al., 2005). The indiscriminate use of flowering species in field margins can lead to higher pest numbers (Latheef & Irwin, 1979, Romeis et al., 2005, Zhao et al., 1992). Herbivorous insects (often Lepidoptera) and beneficial insects (parasitoids and predators) differ in their exploitation of nectar sources. This variation can be exploited to identify selective food sources that are suitable for biological control agents without providing a nutritional benefit to pests (Baggen et al., 1999, Wäckers, 1999). Such selectivity can be based on different aspects, including floral attraction, nectar accessibility and nutritional suitability (Wäckers, 2005). The identification of ‘selective food plants’ that fulfil the needs of beneficial insects without supporting pests might increase the impact of biological control. At the same time, excluding plant species that primarily or exclusively attract and promote herbivores might help reduce the risk of pest outbreaks.

Our previous field observations on a range of plant species showed clear differences in flower visitation by a number of herbivorous cabbage pests and their parasitoids (Chapter 2). Laboratory studies indicated that nectar plants are not equally suitable in prolonging lifespan of cabbage herbivores and their parasitoids (Chapter 3). Based on these previous studies we designed a “worst-case” and a “best-case” scenario to assess potential risks as well as benefits associated with using flower margins in a Brussels sprout production system. The “worst-case scenario” consisted of a flower margin of *Centaurea jacea*. This species had proven to be attractive to herbivores like *Pieris rapae* and to provide them with accessible nectar, while being unsuitable for *Cotesia glomerata* as well as for *Diadegma semiclausum*, parasitoids of *Pieris* spp and *Plutella xylostella,* respectively. The “best-case scenario” consisted of a field margin containing *Anethum graveolens.* This plant species provides accessible nectar for the parasitoids *C. glomerata* and *D. semiclausum*, while being unsuitable for *P. rapae*. In addition, we also tested the plant species *Fagopyrum esculentum* which takes an intermediate position by supplying accessible nectar for both *P. rapae* and the parasitoids. All three plant species provide accessible nectar for *P. xylostella*, the host of *D. semiclausum*. As control we used field edges with the grass *Lolium perenne*, which does not provide nectar.

We hypothesised that 1) Brussels sprouts bordered by *C. jacea* and *F. esculentum* would be exploited by *Pieris rapae* adults which might result in a higher pest pressure by *P. rapae*; 2) Brussels sprouts bordered by any of the three nectar plant species would be exploited by *P. xylostella* adults, which might result in a higher pest pressure by *P. xylostella*; and 3) Brussels sprouts bordered by *A. graveolens* and *F. esculentum* would be exploited by parasitoids, which might result in higher rates of parasitism as compared to the control.

Material and methods

Experimental setup

Small fields (8*10 m in size) with Brussels sprouts (*Brassica oleracea* L. gemmifera variety Maximus) were established at Wageningen Hoog (WH) and Achterberg (AB) near Wageningen, The Netherlands. Brussels sprouts were transplanted as seedlings to the field in week 21 in 2002 and week 22 in 2003. Planting distance was 80 cm between the rows and 50 cm within the rows.

At two sides of each Brussels sprout field 1*10 m wide field edges were established. Field margins contained one of the following plants: *L. perenne* (grass, control), *C. jacea*, *A. graveolens*, or *F. esculentum* (Table 1). In 2002, *C. jacea* was sown in the greenhouse and planted into the field in week 18. Grass (week 20), *A. graveolens* (week 17 in WH and week 18 in AB), and *F. esculentum* (week 20) were sown directly into the field. *A. graveolens* was resown in week 20 to ensure good establishment of the plant. This resulted in flowering periods as indicated in Figure 1.

In 2003, grass and *F. esculentum* were sown directly into the field in week 20. *A. graveolens* was sown in the greenhouse and planted into the field in week 22. As *C. jacea* is a perennial plant it did not need to be replanted.

Fields and field margins were hand-weeded. The distance between the plots was 35 m. The area between the plots was sown with grass, which was mown regularly during the season.

Monitoring adult insects

In 2003 we monitored naturally occurring adult *Pieris* sp., *P. xylostella* and *D. semiclausum* both within the field margins and in row 3-4 and row 7-8 of each Brussels sprout field. To count the large *Pieris* spp. we walked once around each field margin as well as through the Brussels sprout rows. Small species like *P. xylostella* and *D. semiclausum* were sampled by sweep netting and checked for their sex. A standardized number of 12 sweeps was taken in each field margin as well as in each Brussels sprout row. Sampling was done only in 2003 from week 29 until week 32 on sunny and dry days between 10:00 and 15:00 h. Between 15 and 19 observations/sweep net samplings were done per treatment.

Monitoring eggs and caterpillars

Brussels sprout plants were randomly monitored in a non-destructive way for *P. rapae* eggs and caterpillars and for *P. xylostella* caterpillars, pupae and cocoons (parasitized pupae). Monitoring was done on a weekly basis. In 2002 sampling took place from week 26 until week 36. Sampling started as soon as the first flower species started blooming and lasted until two weeks after the first flower species terminated blooming. Sampling was done earlier in 2003, from week 24 until week 34, due to exceptionally warm weather and, thus, a faster development of the crop. With increasing plant size the number of monitored plants per field decreased. In 2002, 20 plants per field were sampled in week 26-28, 15 plants in week 29-30 and 10 plants in week 31-36. In 2003, 15 plants per field were sampled in week 24-25 and 10 plants in week 26-34.

Data evaluation

Adult insects

The number of observed individuals per species was summed up per plot and sample date to attain one figure for the field margins and one for the crop. To check for differences among treatments the data were summed up over sampling days and locations. As data were not normally distributed, non-parametric statistics were used. When the Kruskal-Wallis test indicated that there were differences among the treatments, the Mann-Whitney *U* test was used to compare the control treatment with each of the other treatments (α=0.05).

Table 1: Plant species used in the field edge: common name, floral colour, nectar position and nectar provision for *Pieris rapae* and parasitoids.

Plant species (Family)	Common name	Flower colour	Nectar position	Provide nectar for *Pieris rapae*/parasitoids
Lolium perenne (Poaceae)	Perennial Ryegrass	n.a.	no nectar	- / -
Centaurea jacea (Asteracea)	Brown knapweed	purple	hidden	+ / -
Anethum graveolens (Apiaceae)	Dille	yellow	exposed	- / +
Fagopyrum esculentum (Polygonaceae)	Buckwheat	white	exposed	+ / +

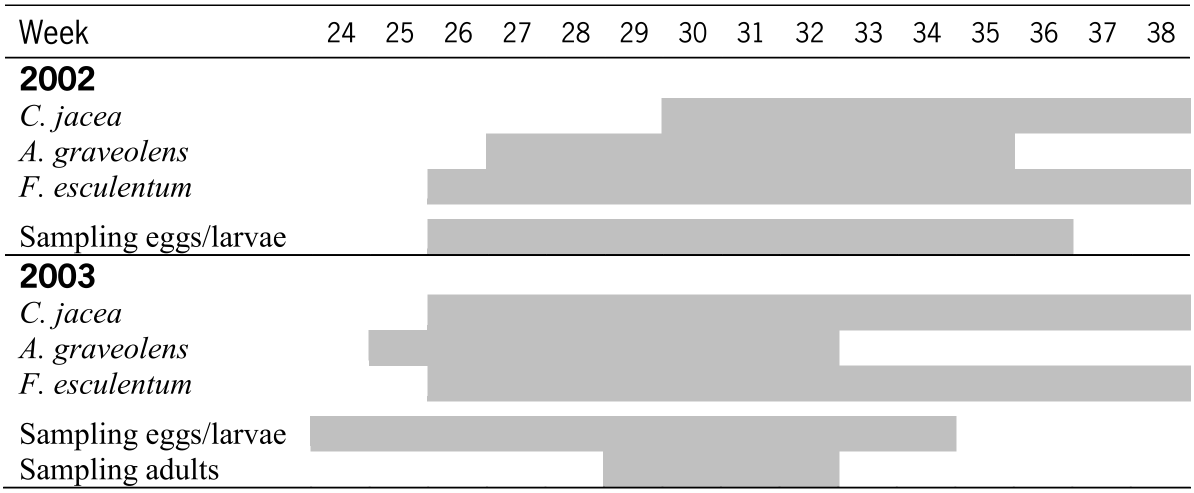

Figure 1: Flowering and sampling periods in the two experimental years

The proportions of *Pieris* spp. individuals observed in margins versus crop within the same plot were arcsine-root-transformed and subjected to a General Linear Model procedure (SAS 8e). Contrasts between treatments and control were tested for significance at $\alpha=0.05$.

Eggs and caterpillars

Per field and week the average number of eggs and caterpillars per plant was calculated. For each field we subsequently calculated the cumulative average number of eggs and caterpillars over the whole experimental period (integral of the curve).

Data were pooled over the factors year and location, resulting in 6 values per treatment. To check for the effect of the treatment on the number of eggs and caterpillars, data were analysed using a General Linear Model and consequently tested for contrasts of the treatments with the control (SAS 8e) ($\alpha=0.05$). We calculated the rate of parasitism by *D. semiclausum* as the proportion of pupae containing a parasitoid cocoon out of the total number of *P. xylostella* pupae. This may underestimate the rate of parasitism, as it ignores pre-pupal mortality of parasitoids. Average of parasitism per year and treatment were calculated from the rates of parasitism per plot and week.

Severe problems with *Delia radicum* in WH in 2002 resulted in extremely inhomogeneous plots and, therefore, no data were collected at this site.

Results

Presence of adult insects

For *Pieris* spp we observed 54 individuals in 68 samples. Numbers of adult *Pieris* spp in the margins and in the crop did not significantly differ between treatments. When the crop was bordered by *C. jacea*, the proportion of adults observed in the crop and in the margin was significantly different from the proportions of adults observed in the control (Generalized Linear Model, P=0.018) (Figure 2a).

For *D. semiclausum* we observed 136 females and 469 males in 78 samples. Significantly more *D. semiclausum* females and males were observed within the Brussels sprout fields bordered by *A. graveolens* as compared to the control (Mann-Whitney *U* test, females P=0.002, males P=0.008). The number of *D. semiclausum* males was significantly increased in fields bordered by *F. esculentum*. Numbers of *D. semiclausum* females and males observed in the field margins were low. (Figure 3 b, c).

Individuals of *P. xylostella* were caught in numbers too low for statistical evaluation (4 females and 17 males in 66 samples). P-values for all statistical tests performed on adult data are listed in Table 2.

Table 2: P-values received from statistical tests used for adult and offspring data. Treatments are indicated by 1=Control, 2=*Centaurea jacea*, 3=*Anethum graveolens*, 4=*Fagopyrum esculentum*. The asterisk (*) indicates statistical differences at α=0.05.

Kruskal Wallis	**margin**	**crop**	
Pieris rapae adults	0.063	0.465	
Diadegma semiclausum females	0.052	0.023 *	
Diadegma semiclausum males	0.028 *	0.020 *	
Mann Whitney U	**1 vs 2**	**1 vs3**	**1 vs4**
Diadegma semiclausum females crop	0.647	0.002 *	0.134
Diadegma semiclausum males crop	0.813	0.008 *	0.030 *
Diadegma semiclausum males margin	0.024 *	0.588	0.142
GLM			
Pieris rapae adult proportions	0.018 *	0.83	0.087
Plutella xylostella larvae (2002+2003)	0.6820	0.6299	0.2435
Plutella xylostella larvae (2003)	0.0705	0.8858	0.2498
Pieris rapae eggs (2002+2003)	0.1429	0.9630	0.0329 *
Pieris rapae eggs (2003)	0.0178 *	0.9027	0.2193
Pieris rapae larvae (2002+2003)	0.0017 *	0.7549	0.0472 *
Pieris rapae larvae (2003)	0.0353 *	0.4658	0.3660

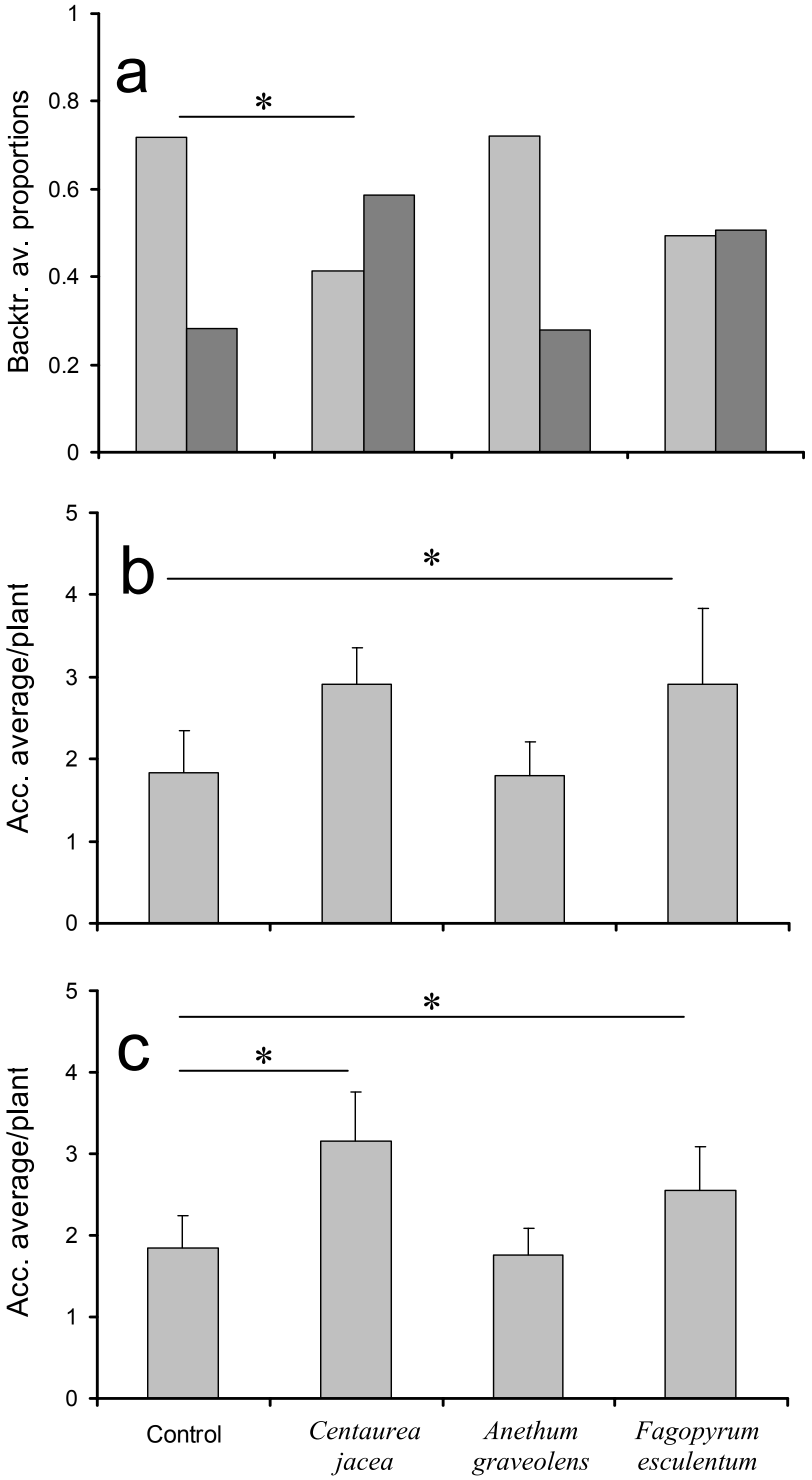

Figure 2: Backtransformed average proportions of (a) *Pieris* sp adults in crop (light grey) and edges (dark grey), and average number per Brussels sprout plant of (b) *Pieris rapae* eggs and (c) *P. rapae* caterpillars for the four different treatments. An asterix indicates significant differences at α=0.05 in the proportions of *Pieris* spp. adults and in the average number of *Pieris rapae* eggs and larvae between the Control and any treatment.

Presence of eggs and caterpillars

When data were pooled over years there were significantly higher numbers of *P. rapae* eggs per Brussels sprout plant in the field bordered by *F. esculentum* as compared to the control (GLM P=0.033, Figure 2b). When considering the data from 2003 only, there were significantly more eggs on Brussels sprout plants bordered by *C. jacea* (P = 0.0178) as compared to the control. Also the number of *P. rapae* larvae per Brussels sprout plant were higher in plots bordered by *C. jacea* and *F. esculentum* as compared to the control when data were pooled over years (GLM P=0.0017 and P= 0.0472, Figure 2b and c). In addition, in 2003 there were significantly more *P. rapae* caterpillars on Brussels sprout plants bordered by *C. jacea* as compared to the control (GLM P=0.0353).

We did not find significant differences in the number of *P. xylostella* caterpillars per Brussels sprout plant among treatments (Figure 3a). The percentages parasitism of *P. xylostella* by the parasitoid *D. semiclausum* did not differ among treatments and reached on average 78±3% in the year 2002 and 67±3% in the year 2003. For P-values of all comparisons made see Table 2.

Discussion

Our study indicates that the indiscriminate use of nectar plants in flowering field margins can enhance pest pressure. The herbivore *P. rapae* showed higher egg and larval densities in fields bordered by *C. jacea* and *F. esculentum* as compared to the control plots. This confirms our earlier finding that both flower species are attractive and suitable nectar sources for adults of this herbivore. In contrast, the herbivore *P. xylostella* did not show higher larval densities in fields bordered by *A. graveolens* and *F. esculentum*; even though these plant species provide accessible nectar for adults of this herbivore. The parasitoid *D. semiclausum* showed higher adult densities in *A. graveolens* bordered fields, a plant species providing accessible nectar to this parasitoid. However, rates of parasitism in Brussels sprouts were not affected by the composition of flowering field margins.

The number of *P. rapae* eggs (in 2003 only) and caterpillars (in 2002 and 2003) was significantly higher on Brussels sprout plants bordered by *C. jacea*, as compared to Brussels sprout plants bordered by grass and *A. graveolens*. This observation supports our first hypothesis. Zhao (1992) reported that *Pieris rapae* eggs and larvae were more abundant in crops interplanted with anise hyssop flowers (*Agastache foeniculum*) than in monocultures, but did not underpin this with statistics. Luna (personal comm.) reports a higher abundance of *P. rapae* larvae in broccoli due to *F. esculentum* borders, while Lee & Heimpel (2005) found no increase in *P. rapae* densities in cabbage adjacent to *F. esculentum*.

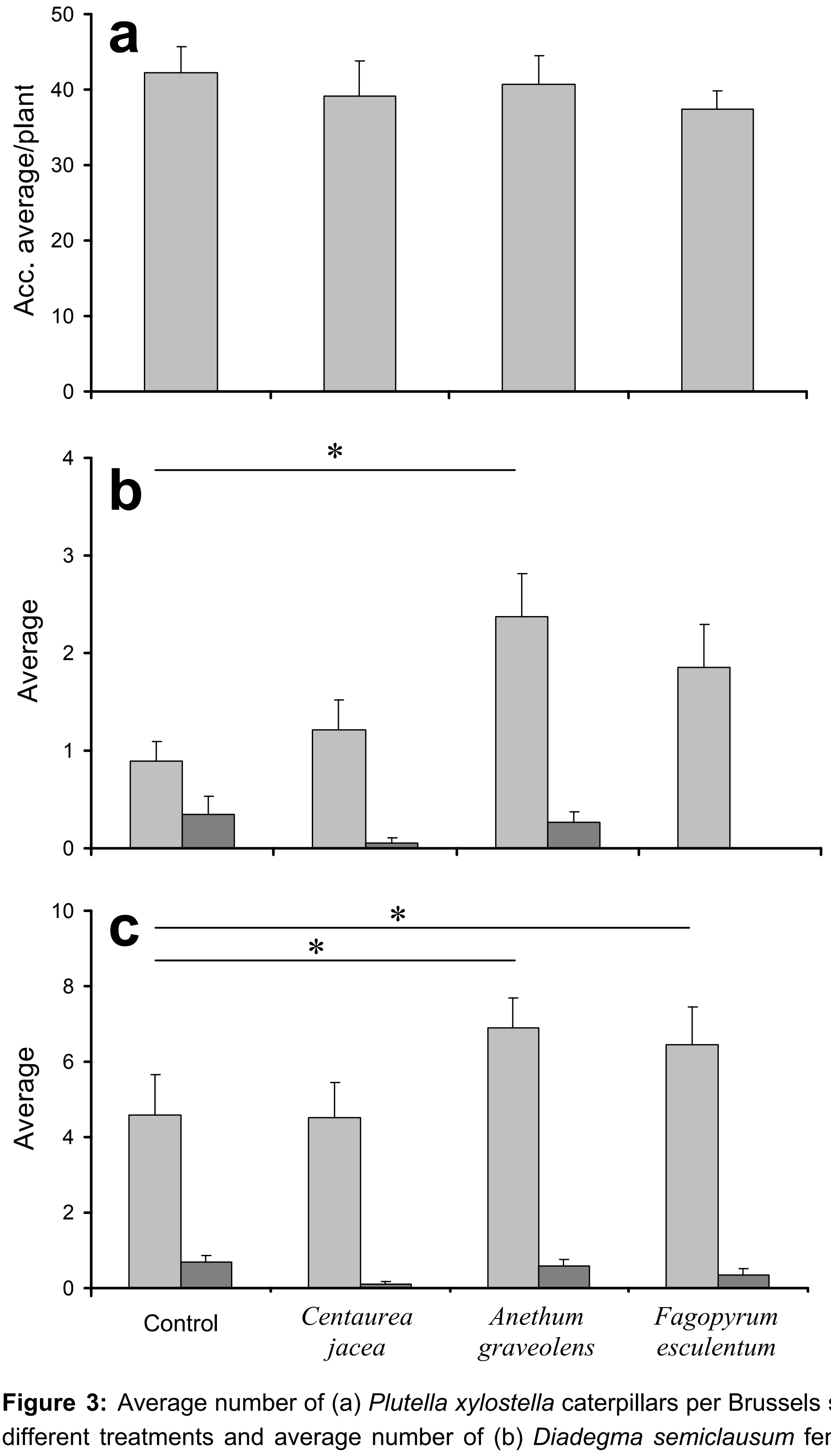

Figure 3: Average number of (a) *Plutella xylostella* caterpillars per Brussels sprout plant for the four different treatments and average number of (b) *Diadegma semiclausum* females and (c) males in crop (light grey) and edges (dark grey). Significant differences at α=0.05 between the Control and any treatment are indicated by an asterisk.

In case the crop was bordered by *C. jacea*; the proportion of adult *Pieris* spp. observed in the margins was significantly higher as compared to the margin of the control treatment planted with grass. This finding fits well with our prediction that *C. jacea* is especially attractive to *Pieris* spp. However, we found no statistical differences among treatments with respect to the average number of *Pieris* adults.

Larras (2002) and Winkler (Chapter 2) found significantly higher numbers of *Pieris* spp. visiting *C. jacea* and *Oregano vulgare* in comparison to *F. esculentum* and five other flowering plants. In these earlier experiments flowers were grown in square 9m^2 single species plots and the density of *Pieris* spp. butterflies was considerably higher than in the present study.

For the number of *P. rapae* eggs we found statistical differences among treatments when considering the data from 2003. The pooled data over years did not reveal differences among treatments. This may be explained by the fact that in 2002, *C. jacea* was newly established and started flowering four weeks later as compared to 2003 (see Figure 1). The oviposition behaviour of *P. rapae* is characterized by laying eggs singly on plants, followed by a linear flight pattern during which the butterfly passes over many suitable hosts (Root & Kareiva, 1984, Yamamura, 1999). In our experiments *P. rapae* females may have left a plot after two to three ovipositions. This means that in large commercial fields the impact of a field margin with suitable flowers may be at least equally marked.

Unlike the clear results with *P. rapae* we did not see any significant impact of flower margins on *P. xylostella*. Even though all of the flowers tested extended *P. xylostella* longevity under laboratory conditions (Chapter 3) neither adults nor caterpillars showed increased numbers in plots with flower margins, relative to the control. The result that we collected relatively few *P. xylostella* adults is probably due to the fact that this species is primarily active at dusk (Harcout, 1954). Using sticky traps, Bukovinszky (2003) found that field margins dominated by flowering *Sinapis alba*, increased pest densities in adjacent fields. De Groot (2005) found significantly more *P. xylostella* individuals on sticky traps in plots planted with *Lobularia maritima* than in Brussels sprout and *S. alba* plots. Both plant species, *L. maritima* and *S. alba*, are host plants for *P. xylostella*, which means that these effects need not have been food-mediated.

When host plants are abundant, *P. xylostella* does not fly far between ovipositions, but shows a strong arrestment response and stays on the plant or moves to directly neighbouring plants (Bukovinszky et al., 2005, Justus & Mitchell, 1996). In contrast to *P. rapae*, *P. xylostella* lays more than one egg on a host plant. Lifespan of *P. xylostella* was prolonged when given access to *C. jacea*, *A. graveolens* and *F. esculentum* under laboratory conditions (Chapter 3). Food supply (0.5M sucrose solution) increased longevity by factor 3 and lifetime fecundity by factor 2 to 3 (personal observation). The lack of significant differences among the treatments in the field leads us to conclude that food supply was not the limiting factor for the realized fecundity by *P. xylostella*. This assumption is supported by observations on the lifetime oviposition curve of *P. xylostella*. *P. xylostella* lays 90% of the total number of eggs within the first four days (Cardona, 1997, Harcout, 1954). Under laboratory conditions, *P. xylostella* lived on average five days without food (Chapter 3). In addition, *P. xylostella* might have utilized other food sources in the field like honeydew (Chapter 5).

We expected higher parasitoid numbers and, consequently, increased rates of parasitism in plots neighboured by *A. graveolens* and *F. esculentum*. Laboratory as well as semi-field experiments demonstrated the impact of *F. esculentum* and *A. graveolens* on the longevity of *D. semiclausum*, as well as a great impact of *F. esculentum* on fecundity of *D. semiclausum* (Wratten et al., 2003).

We did indeed find significantly higher numbers of *D. semiclausum* males and females in Brussels sprout fields bordered by *A. graveolens*. However, *F. esculentum* borders did not significantly increase the number of *D. semiclausum* individuals in Brussels sprout. During sampling we observed high number of bees and bumble bees on *F. esculentum*. Lee & Heimpel (2003) suggests that competition between bees and parasitoids on *F. esculentum* restricts nectar access to parasitoids, which might explain this result.

Independent from the treatment, we collected about three times more males than females. Under natural conditions the sex ratio is around 1:1. Bukovinszky (personal comm.) found approximately as many *D. semiclausum* females as males on sticky traps in cabbage fields. Differences in flight behaviour might account for our male-biased catches. Direct observations on individual wasps in the field confirmed that males tend to fly more and fly above the canopy, while females fly within the canopy, close to the plant, in order to find host larvae.

Regardless of the treatment, we found high rates of parasitism by *D. semiclausum*: 65% in 2002 and 80% in 2003. The density of *P. xylostella* larvae did not differ among the treatments. We did not find any effect of *A. graveolens* or *F. esculentum* field margins on rates of parasitism by *D. semiclausum* in adjacent Brussels sprout fields. Neither did we find an effect on sugar levels of field collected *D. semiclausum* (Chapter 5). Similarly Lee et al. (2003) found no effect of *F. esculentum* on *Diadegma insulare* parasitism in cabbage plots.

The “parasitoid nectar provision hypothesis” predicts that the presence of nectar-producing plants can improve biological control of pests (Heimpel & Jervis, 2005). A primary condition of this hypothesis is that parasitoids are sugar limited in the field. In our case *D. semiclausum* might have exploited alternative food sources like honeydew, produced by the cabbage aphid *Brevicoryne brassicae* or by the cabbage whitefly *Aleyrodes proletella*

(Wäckers & Steppuhn, 2003). These species were observed in (very) low densities throughout the season in any of the four treatments

High migration capacity of *D. semiclausum* might have diluted the effect of the different treatments. As alternative food sources were extremely rare in the studies by Lee & Heimpel (2003), they suggest between-treatment dispersal of a least 67m for *D. insulare*. Thompson (2002) predicts for *D. semiclausum* a travel distance of 409 m within 12 h, with a net replacement distance of 55m. Lavandero et al. (2005) found that *D. semiclausum* can move 80m within four days. Thus, the isolation distance of 35m between our plots might have been too small to prevent nectar-fed wasps from dispersing among the plots. Lavandero et al. (2005) used larger distances of 60 m between plots and could demonstrate differences in parasitism rates due to plantings of *F. esculentum*.

In summary: we could demonstrate that use of certain plant species in field margins can contribute to higher pest numbers, but we were unable to show a positive effect of field margins on rates of parasitism in adjacent crop plants. However, we suppose that the high migration capacity of the parasitoid *D. semiclausum* caused a “treatment dilution effect” in our experiments.

Acknowledgements

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Chapter

8

Summarizing discussion

Summarizing discussion

Promoting natural enemies by providing suitable nectar sources is considered to be an important tool within sustainable agro-ecosystem management (Gurr et al., 1998, Landis et al., 2000). Early studies on conservation biological control tended to adopt a simplistic "diversity is good" philosophy (Gurr et al., 2005). In this 'shotgun' approach floristically diverse plantings were used in the expectation that there will be some net benefit to pest management. In this study I applied a 'directed' approach following the idea that "appropriate diversity helps better" to manage agricultural pests. I aimed at the identification of plant species that are selectively suitable for key herbivorous pests in order to **ex**clude them in future field edge designs or for their most important natural enemies in order to **in**clude them in future field edge designs. By doing so, I intended to gain the necessary knowledge to fine-tune the composition of nectar sources in field edges, and promote the beneficial species, but not the herbivores. In addition, I aimed to get better insight in the mechanisms underlying successes and failures of floral diversification for improved biological pest control.

Results from this study

Within the study system Brussels sprouts (*Brassica oleracea* L. gemmifera) I investigated how the exploitation of nectar sources differs for two lepidopteran key pests and their most prevalent natural enemies. In **Chapter 2** I could demonstrate that the herbivores *Autographa gamma, Pieris rapae* and *Plutella xylostella* and the parasitoid *Diadegma semiclausum* differ in their abundance on a number of flowering plant species. There are three plant species (*A. graveolens, C. cyanus* and *T. vulgare*) on which *D. semiclausum* was observed, but none of the herbivores in substantial numbers. Other plant species, like *O. vulgare*, were visited by at least one of the herbivore species, but hardly by *D. semiclausum.* These results provided a first indication that selective exploitation of flowering plants by herbivores and their natural enemies does occur. Interestingly, highest numbers of *D. semiclausum* were caught on *C. cyanus*, a plant species providing extrafloral nectar (Stettmer, 1993). Due to aphid infestation during the sampling period, plants were also covered with carbohydrate-rich aphid honeydew. This observation indicated that other food sources than floral nectar might play an important role for this species.

Based on the architecture of the plant species and the mouthpart structure of the pests and their parasitoids, I studied theoretical nectar accessibility (**Chapter 3**). I also investigated whether the different species actually reach and accept nectar from the different flower species. Finally, I evaluated the impact of various nectar sources on longevity of the herbivores and parasitoids. Part of the latter experiment was conducted at high and low humidity levels to investigate the impact of relative humidity on nectar exploitation.

The insect species differed in their exploitation of the nectar plants, confirming the observations reported in Chapter 2. The herbivore *P. xylostella* was found on a broad range of flowers and could obtain a fitness benefit from them. The herbivore *P. rapae* exploited a restricted number of flower species, mostly with hidden nectar, when tested at low relative humidity (45±5%). Only at high r.h. (90±5%), *P. rapae* fed from a broader range of flowers. This species is mostly active on flowers during sunny and dry days when the r.h. is low.

For the parasitoids, the importance of Apiaceae species and *F. esculentum* as suitable food source was confirmed. The two parasitoids differed in their ability to exploit partly hidden nectar. Lifespan of *C. glomerata* was significantly extended when exposed to *C. jacea*, *C. cyanus*, *O. vulgare* and *T. pratense* compared to the lifespan of individuals exposed to water, whereas these plants did not enhance longevity of *D. semiclausum*. When competitors like bees or hoverflies are present on highly suitable nectar plants like *F. esculentum*, the parasitoid might escape to less suitable nectar plants, increasing their relative importance under field conditions.

Whereas none of the plant species was selectively providing nectar for *D. semiclausum*, those plants that benefitted both the parasitoid and the herbivore had a much stronger effect on the longevity of the parasitoid. This difference in benefit is magnified by the different impact of nectar on lifetime oviposition curves of *P. xylostella* and *D. semiclausum*. The pest *P. rapae* and its natural enemy *C. glomerata* obtained nutritional benefit from different plants at low r.h., corresponding with field conditions at which the herbivore and its parasitoid are predominantly active.

Different types of nectar and honeydew vary with regard to their sugar composition. In order to successfully exploit a food source, the insect has to show a positive gustatory response to its sugars components and be able to digest and metabolise them. In **Chapter 4** I tested the herbivore *P. xylostella* and its parasitoid *D. semiclausum* with respect to their feeding response and longevity when provided with nine different nectar and honeydew sugars. The herbivore *P. xylostella* accepted a wide range of sugars, in contrast to other Lepidopteran herbivores feeding on cabbage. Also the parasitoid *D. semiclausum* responded to a wide range of sugars. In contrast to the findings for *P. xylostella*, the parasitoid showed a positive response to the honeydew sugar trehalose. Honeydew is often difficult to detect (Wäckers, 2005). However, the parasitoid might encounter honeydew patches by chance while searching the leaf surface. Its gustatory response might then facilitate the exploitation of honeydew as a ubiquitous food source.

Both the herbivore and the parasitoid increased their longevity on a wide range of sugars. However, in my study, food supply had a far more pronounced effect on the life span of the parasitoid (an up to 24-fold increase) than on that of the herbivore (an up to 4-fold increase). While *P. xylostella* did not obtain a significant longevity increase from trehalose compared to water, *D. semiclausum* showed a significantly longer life span on this sugar (20 days) than on water (1.8 days). These differences in gustatory response and longevity showed the potential for application of selective sugar sources in conservation biological control. At the same time it also revealed the risk that indiscriminate application of sugar sources may stimulate herbivory.

In **Chapter 5** I investigated the effect of different flowering plant species on the energy level of field collected *D. semiclausum* and its host *P. xylostella*. The feeding status for *P. xylostella* and for *D. semiclausum* indicated that the majority of the individuals of both species within all treatments had consumed sugars. Two flower species, *A. graveolens* and *L. maritima,* were shown to increase the sugar levels of field collected P. xylostella, as well as the number of individuals with raised sugar levels relative to the control. The plant species *C. jacea* and *F. esculentum*, on the other hand, did not improve the nutritional state of *P. xylostella*. Here I showed for the first time that nectar plants can differ in their impact on the nutritional state of an herbivore under field conditions. In three out of the four treatments, one to five individuals of the herbivore contained melezitose, indicating consumption of honeydew. Differences in sugar levels among cabbage moths collected from the different treatments did not translate in differences in *P. xylostella* larval densities. I did not observe higher numbers of fed *D. semiclausum* individuals and higher total sugar contents in flower treatments with accessible nectar compared to the control and to flower treatments with inaccessible nectar. Instead, in all treatments the far majority of the collected individuals of *D. semiclausum* had raised sugar levels relative to unfed control specimen, indicating widespread feeding.

In **Chapter 6** I studied the importance of nectar feeding for parasitoid survival and fecundity under field conditions. In the absence of a nectar source, only three out of the eleven wasps were able to parasitize some larvae. In contrast, all twelve wasps with nectar supply were able to parasitize more than 300 *P. xylostella* caterpillars. Nectar availability also increased the average reproductive lifespan of the parasitoids from 1.2 days (control) to 28 days. The impact of food sources on *D. semiclausum* fecundity was more clear-cut than in previous laboratory studies with the same species.

Based on results from field observations and laboratory experiments I designed a "worst-case" and a "best-case" scenario to assess potential risks as well as benefits associated with using nectar plants in field edges of Brussels sprouts (**Chapter 7).** Results confirmed that Brussels sprouts in presence of nectar plants that are attractive and suitable for the cabbage white *P. rapae* suffered higher densities of this herbivore. In contrast, Brussels sprouts bordered by nectar plants providing accessible nectar for the diamondback moth *P. xylostella,* did not show higher densities of *P. xylostella* larvae. Surprisingly, rates of parasitism of *P. xylostella* by *D. semiclausum* did not increase when field edges consisted of plants providing accessible nectar for this parasitoid.

Conclusions in the light of the "nectar provision hypothesis"

This study is entitled "Assessing the risks and the benefits of flowering field edges". Concerning the potential benefits, Heimpel and Jervis (2005) formulated their "parasitoid nectar provision hypothesis" (PNPH) including the component 'improved biological control" as outcome and the component "nectar feeding" as mechanism. As herbivores might profit from floral nectar as well, I would like to add the "herbivore nectar provision hypothesis" (HNPH). Both hypotheses are based on the following questions: (1) Is the insect species sugar limited in the field? (2) Are nectar sources suitable to alleviate this sugar limitation? And (3) Does provision of suitable nectar sources for either herbivores or natural enemies translate in higher herbivore densities or improved biological control as a result of higher rates of parasitism? The experiments included in this study do not provide sufficient information for a comprehensive answer to all these questions. Still I will make an attempt to discuss my results in the light of these hypotheses, while at the same time indicating where essential information is lacking.

Only a few nectar sources, including *C. jacea*, resulted in a longer lifespan of *P. rapae* under laboratory conditions. The density of *P. rapae* eggs and larvae were significantly higher in Brussels sprout plots bordered by *C. jacea*, providing some support for the HNPH. Kusano and Adachi (1969) and Kusano and Nichide (1978) report 8.8 and 6.9 days for *P. rapae* survival on water only. How food deprivation influences P. rapae's life time fecundity under laboratory and natural conditions remains to be investigated.

For the herbivore *P. rapae* and its parasitoid *C. glomerata* I could demonstrate differences in abundance on flowers in the field (Chapter 2), which corresponded well with suitability of flower species as food source under laboratory conditions (Chapter 3). As a range of plants provided more benefits to the parasitoid than to the herbivore, selective provision of nectar plants seems possible. Due to low densities of *C. glomerata* in my field studies, it is not possible to conclude on the effect of nectar plants on *C. glomerata* densities in a neighbouring Brussels sprout crop.

In the field, *P. xylostella* was found on a broad range of flowers (Chapter 2). Nearly all nectar sources included in this study prolonged the lifespan of *P. xylostella* under laboratory conditions (Chapter 3). The feeding status of field collected individuals indicated food uptake within all treatments, including the control without nectar (Chapter 5). In two flower species, *A. graveolens* and *L. maritima*, higher numbers of fed individuals and higher amounts of total sugar content were found compared to the control. Interestingly these differences in sugar levels did not translate in differences in *P. xylostella* larval densities (Chapter 7). Here the HNPH seems not to apply. *P. xylostella* survives on average five days without food and lays 80-90% of its eggs within this period (Cardona, 1997, Harcourt, 1954). On suitable host plants, *P. xylostella* moves by walking and hopping, types of mobility which cost far less energy than flight. Under field conditions the impact of food on lifetime oviposition might be further limited by factors other than starvation.

In the field, also *D. semiclausum* was found on a broad range of flowers (Chapter 2). Unexpectedly I did not observe higher numbers of fed and higher total sugar contents in *D. semiclausum* in flower treatments with accessible nectar compared to the control and to flower treatments with inaccessible nectar. The feeding status of all field collected individuals was significantly better than that of starved individuals (Chapter 5). Plots in our experiment were separated by 35 m. As *D. semiclausum* is highly mobile (Lavandero et al., 2005, Thompson, 2002), individuals most probably were able to move between the experimental plots, thereby making it difficult to evaluate the effect of nectar plants on parasitism in the directly neighbouring Brussels sprout crop.

When food sources other than nectar were excluded, floral nectar had a clear impact on lifetime fecundity of *D. semiclausum*. This finding underlines that access to carbohydrate-rich food, which does not necessarily mean floral nectar, can be indispensable for high parasitoid fecundity. High numbers of *D. semiclausum* were observed on *C. cyanus* infested with aphids. In addition, the parasitoid showed a positive gustatory response to the honeydew sugar trehalose. These observations led to the assumption, that honeydew, even though it is generally of lesser nutritional value and more difficult to detect (Wäckers, 2005), might be an important food source for *D. semiclausum* in the field (Lavandero et al., 2005, Lee & Heimpel, 2003). As *D. semiclausum* is able to synthesise sugars, which are typically found in honeydew, it remains difficult to proof honeydew consumption in field collected individuals. Being able to (partly) feed and survive on honeydew makes *D. semiclausum* less dependent on floral nectar resources, and makes it difficult to test the PNPH under field conditions.

Results obtained in this study indicate the potentials of fine-tuning the selection of nectar sources for field edges in order to improve biological control. Differences in flower suitability for herbivores and their parasitoids show the potential for application of selective food sources. Where selective food plants cannot be identified, differences among flowers with respect to their impact on longevity and fecundity of pest species and parasitoids may be used as a selection criterion.

It is only recently that researchers include herbivores in their studies on nectar provision to obtain improved biological control. It is therefore not surprising that essential knowledge on the impact of nectar supply on herbivore longevity and lifetime fecundity is hardly available. A first study including the effect of nectar supply on both herbivores and parasitoids was done by Baggen and Gurr (1999). They identified selective food plants based on herbivore fecundity and parasitoid longevity. An ideal approach would include data of daily and lifetime fecundity of herbivores and parasitoids with and without nectar supply. In this way it would be possible to quantitatively compare the impact of nectar supply on the oviposition curves of herbivores and parasitoids. In addition, insect behaviour like mobility and possible exploitation of food sources other than floral nectar should be taken into account when testing HNPH and PNPH.

When testing the effect of nectar provision on herbivore-parasitoid dynamics, care must be taken in the interpretation of the response of parasitoids. In case the herbivore profits from the nectar in terms of increased density, a positive response of the parasitoid expressed in a higher number of parasitized hosts, might be due to the nectar provision, due to higher herbivore densities or to both.

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Samenvatting

Intensivering van de agrarische productie heeft het landschapsbeeld in de loop van de laatste decennia ingrijpend veranderd. Landschapselementen zoals heggen en kruidenrijke randstroken zijn grotendeels verdwenen en gewassen worden in grote monoculturen verbouwd. Om nuttige insecten in zulke ecologische woestijnen een rol te kunnen laten spelen en hun behoefte aan voedsel in de vorm van pollen en nectar te bevredigen, is het in toenemende mate populair geworden om bloeiende randstroken langs het gewas te zaaien.

Maar niet alles wat bloeit is alleen maar goed voor nuttige insecten. Ook schadelijke organismen kunnen van bloeiende randstroken profiteren. In mijn promotieonderzoek heb ik een aantal akkerrandplanten onderzocht op hun mogelijke nut voor plaaginsecten en/of natuurlijke vijanden in koolvelden. In veldproeven heb ik de attractiviteit van nectarplanten voor koolplagen en hun natuurlijke vijanden getoetst. In laboratoriumexperimenten heb ik bekeken in hoeverre de planten verschillen in de bereikbaarheid van hun nectar en hun invloed op de levensduur van insecten. Ook heb ik onderzocht hoe de verschillende suikers, die in nectar en honingdauw aanwezig zijn, de acceptatie door en de levensduur van insecten beïnvloeden.

Op basis van de laboratoriumresultaten heb ik in het veld experimenten uitgevoerd met planten die óf voor de herbivoren óf voor de sluipwespen geschikte voedselbronnen zijn. Ik heb individuen van de koolmot en diens sluipwesp verzameld en hun suikergehaltes geanalyseerd. De resultaten wijzen erop, dat beide soorten in het veld voedsel opgenomen hadden. Ook heb ik in een veldexperiment kunnen laten zien dat geschikte nectar planten, zoals bijvoorbeeld boekweit, een enorme positieve invloed hebben op de levensduur en de vruchtbaarheid van de sluipwesp *Diadegma semiclausum*. Verder heb ik kunnen aantonen dat planten die selectief gebruikt kunnen worden door herbivoren, zoals het knoopkruid *Centaurea jacea* door het koolwitje, tot hogere plaagdichtheiden in de aangrenzende kool kunnen leiden. Mijn belangrijkste conclusie is dan ook dat een doelgerichte aanpak en een nauwkeurige selectie van planten bij het inrichten van bloeiende randstroken noodzakelijk is.

Zusammenfassung

Die Intensivierung der Landwirtschaft hat im Verlauf der letzten Jahrzehnte das Landschaftsbild eingreifend verändert. Landschaftselemente wie Hecken, Knicks und krautreiche Randstreifen sind größtenteils der Flurbereinigung zum Opfer gefallen und die Äcker werden in großflächigen Monokulturen bestellt. Um nützliche Insekten in solchen ökologischen Wüsten eine Rolle spielen zu lassen und ihrem Bedarf an Nahrung in Form von Pollen und Nektar Rechnung zu tragen, ist es in zunehmendem Masse populär, blühende Randstreifen entlang der Äcker einzurichten.

Doch nicht alles, was blüht, ist ausschließlich gut für nützliche Insekten. Auch schädliche Insekten können von blühenden Randstreifen profitieren. In meiner Doktorarbeit habe ich eine Reihe von Nektarpflanzen untersucht in Hinblick auf ihren potentiellen Nutzen für Kohlschädlinge und/oder deren natürliche Feinde. In Feldversuchen habe ich die Attraktivität von Nektarpflanzen für die Schädlinge und Nützlinge untersucht. In Laborversuchen habe ich beobachtet, in wieweit sich die Pflanzen unterscheiden bezüglich der Nektarerreichbarkeit und ihrem Einfluss auf die Lebensdauer der Insekten. Zusätzlich habe ich die einzelnen Zucker, die in Nektar und Honigtau enthalten sein können, auf ihre geschmackliche Akzeptanz durch die Insekten und ihren Einfluss auf die Lebensdauer hin untersucht.

Auf Basis der gefundenen Resultate habe ich Feldexperimente durchgeführt mit Pflanzen, die geeignete Nahrungsquellen entweder für die Schädlinge oder die Nützlinge darstellen. Ich habe Individuen der Kohlmotte *Plutella xylostella* und ihrer parasitären Schlupfwespe *Diadegma semiclausum* gesammelt und auf ihren Zuckergehalt hin untersucht. Die Resultate weisen darauf hin, dass beide Insektenarten im Feld Nahrung aufgenommen haben. In einem Versuch mit standardisierter Schädlingsdichte konnte ich zeigen, das geeignete Nektarpflanzen, wie beispielsweise Buchweizen, einen enormen positiven Einfluss auf die Lebensdauer und die Fruchtbarkeit der parasitären Schlupfwespe *D. semiclausum* haben. Desweiteren konnte ich nachweisen, das Pflanzen, die selektiv von Herbivoren genutzt werden, so wie das Knopfkraut *Centaurea jacea* durch den Kohlweißling *Pieris rapae*, zu höheren Schädlingsdichten in benachbarten Kohlbeständen führen können. Die wichtigste Schlussfolgerung, die ich aus den gefundenen Resultaten ziehe, lautet dann auch das eine gezielte Herangehensweise und eine sorgfältige Selektion von Pflanzen bei der Einrichtung von blühenden Randstreifen zur Verbesserung des biologischen Pflanzenschutzes notwendig ist.

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In the past five years, I worked on my PhD project at the laboratory of Entomology in Wageningen. During that time, many people crossed my path or accompanied me for a while. It is most probable that not all persons, who have been important at a certain time, will be mentioned here. This is not due to ignorance or ill will, but simply due to the fact that five years is quite a long time, and with aging, my memory capacity decreases. But there are definitely a great number of people whom I will never forget....

First of all I would like to thank my supervisors, Joop C. van Lenteren and Felix L. Wäckers. The fact that I did not see you on a daily or weekly base was more than compensated by the input you gave at crucial moments of the project. Joop, aside from your scientific contributions, you organized the financial background of the project and somehow, somewhere, arranged additional financial means. This meant that I never felt severely restricted in my experimental work. Felix, any time I needed to discuss problems or results, you made it possible to drop by on your way to or from Heteren. In the writing phase, both of you did a perfect job in modifying my manuscripts from black to blue. Your constructive comments and concrete suggestions greatly improved my German-English written versions of the manuscripts.

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I cannot imagine the field work in Wageningen-Hoog and Achterberg without you, Tibor and Trefi. Under Dutch weather conditions field work is not always fun. As real muddy-boot entomologists you shared good times and bad times in the field and kept my motivation up at times when the energy level went down. With the other members of our biodiversity group, Gerbert Hiddink, Nick den Hollander and Felix Bianchi we had fruitful discussions on the complexity of biodiversity.

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The time of my PhD project is inseparably related to my being a mother. Six months after the start of the project, Kolja was born. Now, four years later, Kolja goes to school and I defend my PhD. This is possible only due to support from many sides.

Carsten, your presence in Wageningen in 2001 and 2002 allowed me to go back to work very soon after Kolja was born. You were amazingly patient in carrying around this "little crying creature" in the first weeks of its life. And in case I could not come home, you brought the hungry boy to all kinds of locations in order to get him fed. I am pretty sure that the good contact between the two of you at that time, but also presently, contributes greatly to the friendly character Kolja expresses. Thanks for your commitment!

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Liebe deutsche Mütter in Wageningen, was hätte ich bloß ohne Euch getan? Thanks to you, my German language is not yet totally degraded. You and your families opened the doors of your houses and your hearts whenever we dropped by. Thanks, Gudrun, for providing me with all kinds of non-entomological literature on career and kitchen management, for endless talks accompanied with coffee or wine and for numerous car transports when either Kolja or I was sick. Nadja, after completing our MSc study in 2000, we accompanied each other through bad and good times, through pregnancy and the exciting time of early motherhood. You did a great job in getting me back to reality in times when I felt bad and desperate. Conny, you did not only provide crucial information on how to organize child and job. The warm atmosphere in your family when we – once again and just accidentally – passed by around dinner time, felt like a safe harbour after crossing the rough sea. Pjotr and Jiri, you are two fantastic "big brothers" for Kolja, just as Catalina and Daniel are really good friends for him.

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My affection for the world of art and music is only one of the valuable things I received in my parents' house. My parents taught me and my two sisters reverence and respect for creation, and opened our eyes to the fascinating beauty of nature. Both my father and my mother supported me always in their best possible way. They imparted the importance of a certain amount of "Fleiss und Disziplin" (assiduity and discipline), which helped me now to complete this project nearly within the intended time. My mother was highly convinced that it is ones duty to do something with given gifts and often encouraged me when I had doubts about doing a PhD. It makes me unutterably sad that she passed away before the completion of my thesis. I am all the more happy and thankful that my father, and at his side Erika, joined me until today and will hopefully do so for many more month and years to come. Ursel and Sabine, even though we do not share a fascination for insects, you supported me in a number of other ways. In good and in bad times, it is valuable to know that there are two sisters on which I can count.

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Curriculum vitae

Karin Winkler was born in Bad Bentheim, Germany, on 30 January 1968. After graduating from secondary school in 1987, she volunteered for a year in Nes Amim, Israel. From 1988 until 1990, Karin trained as an agricultural technical assistant at the Justus Liebig University in Giessen, Germany. For about two years she worked at a seed breeding company in Rastatt, southwest Germany. A scholarship from the Carl Duisberg Association offered her the opportunity to do an internship at the plant protection service in Benin, West-Africa. The confrontation with severe problems in handling and application of pesticides reinforced her interest in biological pest management strategies. From 1993 until 1997 Karin followed a Bachelor degree programme in Ecological Agriculture at the Gesamthochschule Kassel-Witzenhausen, Germany. Subsequently, in 2000, she completed a Master study in Integrated Pest Management at the Wageningen Agricultural University. For her major thesis she investigated the learning ability of two hemipteran predators. In 2001, Karin was awarded a scholarship from the Bosch Foundation, Germany, to work on the strategic use of nectar sources in order to boost biological pest control. In July 2001, she gave birth to her son Kolja. He and his generation keep her motivation to work on biological pest control on a continuously high level.

List of Publications

Bukovinszky, T., M.J. Brewer, K. Winkler, H. Trefas, L.E.M. Vet & J.C. van Lenteren (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. In: *Integrated Control in Field Vegetable Crops, IOBC wprs Bulletin*, *26 (3)*, 307-314.

De Groot, M., K. Winkler & R. Potting (2005). Testing the potential of White mustard (*Sinapis alba*) and Sweet alyssum (*Lobularia maritima*) as trap crops for the diamondback moth *Plutella xylostella*. *Proceedings of the Netherlands Entomological Society Meeting*, *16*, 117-123.

Winkler, K., F.L. Wäckers, L. Valdivia, V. Larraz & J.C. van Lenteren (2003). Strategic use of nectar sources to boost biological control. *IOBC wprs Bulletin*, *26 (4)*, 209-214.

Winkler, K., F.L. Wäckers, L. Buitriago & J.C. van Lenteren (2005). Herbivores and their parasitoids show differences in abundance on eight different nectar producing plants. *Proceedings of the Netherlands Entomological Society Meeting*, *16*, 125-130.

Winkler, K., F.L. Wäckers, A. Stingli & J.C. van Lenteren (2005). *Plutella xylostella* (diamondback moth) and its parasitoid *Diadegma semiclausum* show different gustatory and longevity responses to a range of nectar and honeydew sugars. *Entomologica Experimentalis et Applicata*, 115, 187-192.

In press

Winkler, K., F.L. Wäckers, G. Bukovinszkine-Kiss & J.C. van Lenteren. Nectar resources are vital for *Diadegma semiclausum* fecundity under field conditions. *Basic and Applied Ecology*. doi: 10.1016/j.baae.2005.06.001

In preparation

Winkler, K., F.L. Wäckers, D. Pinto & L. Boitaud. Do flowers make a difference? Nutritional state of *Diadegma semiclausum* and its host *Plutella xylostella* in the field.

Winkler, K., F.L. Wäckers, L.V. Kaufman, V. Larraz & J.C. van Lenteren. Flowering herbs as food sources for herbivores and their parasitoids: nectar accessibility and impact on longevity.

Winkler, K., F.L. Wäckers & J.C. van Lenteren. Assessing the risks and benefits of flowering field edges.

Winkler, K., J.J.A. van Loon. & M. Dicke. Nymphal experience persists in the orientation behaviour of an adult polyphagous hemipteran predator.

Wäckers, F. L., J.C. Lee, G.E. Heimpel, K. Winkler & R. Wagenaar. Confusing signatures: parasitoids synthesize “honeydew-specific” signature sugars.

PE&RC PhD Education Statement Form

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 22 credits (= 32 ECTS = 22 weeks of activities)

Review of Literature (3 credits)

Flowering field edges in agricultural cropping systems (2001)

Writing of Project Proposal (4 credits)

Functional biodiversity: strategic use of nectar and pollen sources to boost biological control (2001)

Post-Graduate Courses (3 credits)

Autumn school: Interactions between plants and attacking organisms: mechanisms, genetics, ecology and evolution (2000)

Basic Statistics (2001)

Winter school: Functional biodiversity and agro-ecosystem management: implementing sustainable production (2001)

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PE&RC annual meeting: “Global climate change and biodiversity” (2003)

PE&RC annual meeting: “Biological disasters (2004)

International Symposia, Workshops and Conferences (4 credits)

Landscape management for functional biodiversity, IOBC Study group, Bologna, Italy (2003)

Symposium on insect plant interactions, Humboldt University Berlin, Germany (2004)

Multifunctionality of landscapes, Justus Liebig University, Giessen, Germany (2005)

International symposium on biological control of arthropods, Davos, Switzerland (2005)

Laboratory Training and Working Visits (1 credit)

Sugar analysis in nectar and honeydew (2002)

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