

Foraging decision of aphidophagous predators and conservation biological control: A case study with syrphid antagonists

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DEDICATION

This thesis is dedicated to my sons Lance Calvin Kahato and Galvin Kahato.

‘The greatest part of our happiness depends on our dispositions, not our circumstances’- Martha
Washington

ABSTRACT

The foraging behaviour of aphidophagous predators plays a key role in conservation biological control. However for understanding the foraging behaviour of syrphids most investigations have focussed on prey-predator interactions using larvae while overlooking the important aspect of foraging of adult hoverflies. The larvae of the hoverfly *Episyrphus balteatus* (DeGeer) (Diptera: Syrphidae) are aphid predators while the adults depend on pollen and nectar for reproduction and longevity, hence they depend on availability of convenient flowering plants. However, in agroecosystems, the spatial and temporal patterns of crops as well as accompanying flowering plants change. This leads to a fluctuation in food resources which may limit syrphid predatory performance. Thus it is important to understand how food utilization affects the fitness of adult hoverflies and how this affects conservation biological control.

In the first part of the thesis the impact of resource availability on the fitness of *E. balteatus* was investigated using oilseed rape plants infested with *Brevicoryne brassicae* L. (Hemiptera: Aphididae) while pollen and sugar supply was manipulated to simulate limitation in food resources. Oviposition was evaluated while foraging patterns were video recorded. During pre-oviposition period a lack of pollen and sugar feeding alone led to a complete absence of egg laying. *Episyrphus balteatus* lived 2 fold longer when pollen and sugar were available throughout compared to feeding on sugar only. Feeding on pollen alone resulted in similar longevity as when feeding on pollen and sugar throughout. Female *E. balteatus* visited food patches more frequently during pre-oviposition period than during oviposition period. A similar trend was observed for the residence time on food patches during both periods. These results demonstrate that pollen availability is not only essential for oviposition but also for enhanced survival.

In the second part of this study, the effects of pollen feeding frequency on the fitness of *E. balteatus* were investigated. Pollen feeding intervals were varied from 10 to 60 minutes per day, every 2nd, 3rd, 5th day and throughout as control. A broad bean (*Vicia faba*. L) infested with *Aphis*

faba Scopoli (Hemiptera: Aphididae) was used to stimulate oviposition. From all the frequencies tested the control and pollen availability every 2nd day resulted in the shortest pre-oviposition period, the highest fecundity and the longest oviposition duration. Hoverflies in those treatments lived longest and had the highest dry weight. On the contrary, the lowest longevity was with pollen every 5th day. These results demonstrate the importance of daily access to pollen or every 2nd day feeding.

In the third part, the influence of selected flowering plants on the fitness of *E. balteatus* was determined. Based on previous studies, seven flower species were chosen: phacelia (*Phacelia tanacetifolia*, Hydrophyllaceae), buckwheat (*Fagopyrum esculentum*, Polygonaceae), cornflower (*Centaurea cyanus*, Asteraceae), chamomile (*Matricaria recutita*, Asteraceae), white mustard (*Sinapis alba*, Brassicaceae), nettle (*Urtica pilulifera*, Urticaceae) and perennial ryegrass (*Lolium perenne*, Poaceae). A broad bean plant infested with *A. fabae* was used to stimulate oviposition. Among the plants tested phacelia led to the shortest pre-oviposition period and highest fecundity. The longevity of hoverflies was enhanced the most by cornflower and buckwheat. The size of female hoverflies was not influenced by the different flower species, whereas feeding on phacelia led to the highest dry weight. An analysis of pollen protein revealed that phacelia had the highest protein content being 3 fold more than in nettle.

In conclusion, this study has demonstrated that lack of pollen sources during pre-oviposition has a negative impact on egg output and longevity. Furthermore syrphids benefit from daily or at least every second day access to pollen resources to ensure maximum fitness. In improving biological control, the use of phacelia, buckwheat and cornflower to provide food resources is highly recommended for enhanced predatory efficiency.

Key words: foraging behaviour, hoverflies, fitness, biological control.

ZUSAMMENFASSUNG

Das Futtersuchverhalten von aphidophagen Prädatoren nimmt eine Schlüsselrolle in der „conservation biological control“ ein. Jedoch hatten die meisten Untersuchungen zum Futtersuchverhalten von Schwebfliegen Räuber-Beute-Interaktionen der Larven als Schwerpunkt, während das Verhalten der adulten Syrphiden, die Blüten benötigen, vernachlässigt wurde. Die Larven von *Episyrphus balteatus* (DeGeer) (Diptera: Syrphidae) sind Blattlausräuber, während die Adulten von Nektar und Pollen für Reproduktion und Lebensdauer abhängen. Allerdings ist in Agrarökosystemen das Angebot an Kultur- und blühenden Begleitpflanzen zeitlichen und räumlichen Schwankungen unterworfen, was die Leistung der Schwebfliegen als Räuber begrenzen kann. Daher ist es notwendig zu verstehen, wie die Nutzung von Nahrungsquellen die Fitness adulter Schwebfliegen und die conservation biological control“ beeinflusst.

Im ersten Teil der Arbeit wurden die Auswirkungen der Verfügbarkeit von Nahrungsressourcen auf die die Fitness von *E. balteatus* untersucht, wobei Raps der mit *Brevicoryne brassicae* L. (Hemiptera: Aphididae) befallen war verwendet wurde, während das Pollen- und Zuckerangebot verändert wurde, um Einschränkungen zu simulieren. Die Eiablage wurde erfasst und das Futtersuchverhalten mit Videoaufzeichnungen analysiert. Während der Präovipositionsperiode führte eine Fütterung ohne Pollen (ausschließlich Zucker) zum kompletten Ausfall der Eiablage. *Episyrphus balteatus* lebte, im Vergleich zur Fütterung ausschließlich mit Zucker, doppelt solange, wenn Pollen und Zucker während der gesamten Zeit vorhanden waren. Fütterung mit ausschließlich Pollen führte zu einem ähnlichen Ergebnis. Weibliche *E. balteatus* besuchten Futterplätze häufiger während der Präovipositionsperiode als während der Eiablagephase. Ein ähnlicher Trend wurde für die Aufenthaltszeit auf Futterplätzen beobachtet. Diese Ergebnisse zeigen, dass Pollenverfügbarkeit nicht nur für die Eiablage, sondern auch für eine erhöhte Überlebensrate essentiell ist.

Im zweiten Teil der Arbeit wurden die Auswirkungen der Frequenz von Pollengaben auf die Fitness von *E. balteatus* untersucht. Die Intervalle des Pollenangebotes dauerten 10 bis 60 Minuten pro Tag, jeweils am zweiten, dritten und fünften Tag, sowie durchgängig als Kontrolle. Mit *Aphis fabae* Scopoli (Hemiptera: Aphididae) befallene Saubohnen (*Vicia faba*. L) dienten zur Stimulation der Eiablage. Von allen getesteten Intervallen führten die Kontrolle und die Pollengabe jeden zweiten Tag zu der kürzesten Präovipositionsperiode, der höchsten Fekundität und der längsten Eiablagedauer. Außerdem hatten die Schwebfliegen bei diesen Intervallen die höchste Lebensdauer und das höchste Trockengewicht. Im Gegensatz dazu führte die Pollengabe an jeden fünften Tag zu der kürzesten Lebensdauer. Diese Ergebnisse zeigen die Wichtigkeit einer täglichen oder zweitäglichen Verfügbarkeit von Pollen.

Im dritten Teil der Arbeit wurde der Einfluss von verschiedenen Blütenpflanzen auf die Fitness von *E. balteatus* untersucht. Ausgehend von früheren Studien wurden sieben Arten ausgewählt: Phazelia (*Phacelia tanacetifolia*, Hydrophyllaceae), Buchweizen (*Fagopyrum esculentum*, Polygonaceae), Kornblume (*Centaurea cyanus*, Asteraceae), Kamille (*Matricaria recutita*, Asteraceae), Weißer Senf (*Sinapis alba*, Brassicaceae), Pillen-Brennnessel (*Urtica pilulifera*, Urticaceae) und Deutsches Weidelgras (*Lolium perenne*, Poaceae). Mit *Aphis fabae* Scopoli (Hemiptera: Aphididae) befallene Saubohnen (*Vicia faba*. L) dienten zur Stimulation der Eiablage. Phazelia führte zur kürzesten Präovipositionsperiode und höchster Fekundität. Die Lebensdauer der Schwebfliegen wurde am meisten durch Kornblume und Buchweizen gefördert. Die Größe der weiblichen Schwebfliegen wurde nicht durch die verschiedenen Pflanzenarten beeinflusst, wohingegen das Trockengewicht bei Aufnahme von Phazelia-Pollen am höchsten war. Eine Analyse der Proteingehalte zeigte bei Phazelia die höchsten Gehalte, die bis zu dreimal höher waren als bei Brennnesselpollen.

Diese Arbeit hat gezeigt, dass das Fehlen von Pollen während der Präovipositionsperiode negative Auswirkungen auf die Eiablage und Lebensdauer von *E. balteatus* hat. Außerdem profitieren die

Schwebfliegen von der Möglichkeit, häufig auf Pollenquellen zugreifen zu können, was eine maximale Fitness gewährleistet. Für die Optimierung der biologischen Schädlingsbekämpfung wird die Verwendung von Phazelia, Buchweizen und Kornblume als Futterquellen empfohlen, um die Effizienz der Antagonisten zu steigern.

Schlagworte: Nahrungssuchverhalten, Schwebfliegen, Fitness, Biologische Schädlingsbekämpfung

ABBREVIATIONS

ANOVA	Analysis of variance
CBC	Conservation biological control
HSD	Highest Significant Difference
L	Linnaeus
L: D	Light: Darkness photoperiod
SAS	Statistical Analysis System
SE	Standard Error

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CHAPTER 1

GENERAL INTRODUCTION

Natural enemies of pest species play an important role in population regulation of pest insects in the field as well as in the greenhouse (Schoenly, 1990). Natural enemies are promoted using different approaches and key among them is conservation biological control (CBC). This approach focuses on the modification of the environment to protect and enhance specific natural enemies by improving their fecundity and longevity (Eilenberg et al., 2001). However, successful CBC requires a detailed knowledge of the biology and ecology of the antagonist (Lewis et al., 1997) including the specific resources that antagonists need in the environment. Among aphidophagous antagonists the syrphid *Episyrphus balteatus* (DeGeer) (Diptera: Syrphidae) is a potential agent in CBC. It is one of the most abundant species on arable land in Europe (Dean, 1982). Their voracious aphidophagous larvae have been identified as economically important owing to their capability to halt aphid population growth in both greenhouses and the agricultural fields (Chamber & Adams, 1986). The adult syrphids feed on pollen and nectar from flowers. Additionally, they supplement their diet using honey dew from aphids (van Rijn et al., 2006). Non prey food such as pollen and sugar can have a dramatic effect on longevity and fecundity of predators and parasitoids. Therefore the availability of pollen and sugar is crucial to the efficacy of syrphids with the absence of food resources translating to dismal performance. However, little information exists on the effects of pollen and sugar on the survival and egg laying in *E. balteatus*. In adult syrphids pollen provides proteins and amino acid (Rasmont et al., 2005) both necessary to mature the ovaries of hoverflies since they hatch with an immature reproductive system, while nectar and honeydew provide carbohydrates for movement and survival (Jervis et al., 1993). The effectiveness of hoverflies as aphid predators depends on the quality and availability of pollen and nectar (van Rijn & Tanagoshi 1999; Winkler et al., 2006) but most investigations on their foraging behaviour have focussed on the interaction between the prey and the predator, while overlooking the relative

importance of pollen and nectar during the different physiological periods (Jervis & Kidd 1996). A detailed understanding of how food supply affects the fitness of syrphids is needed. However, studies on the food requirements during different physiological period are scarce. Furthermore, pollen supply is likely to affect egg laying in a field situation (Schneider, 1948) and especially in areas with intensive agriculture which are characterized by scarcity of suitable flowering plants (Winkler et al., 2006). Therefore providing pollen resources to hoverflies in the field or green houses may be helpful in improving conservation biological control. However, it is important to understand how often syrphids need access to pollen and sugar to achieve maximum efficiency. Recently the feeding frequency and lifespan gain has been examined for various parasitoids (Azzouz et al., 2004; Wu et al., 2008) with positive results. From the parasitoids studies, continuous access to sugar resulted in more progenies and longer lifespan. While some studies have been carried out on pollen and nectar feeding in syrphids (van Rijn et al., 2006), the role of feeding frequency on the fitness has not been examined. Understanding the feeding frequency requirements of syrphids may provide information to in improving the efficiency of syrphids in greenhouse.

The current conversion of natural habitats to agricultural monoculture has resulted in landscapes that are depleted in flowering plants (Carreck & Williams, 2002). Consequently, hoverflies have to fly large distances between food resources and oviposition sites. This phenomenon leads to energy and time wastage that could otherwise have been used in pest suppression (Tylianakis et al., 2004). Planting flower resources near agricultural fields may provide a possible solution to this problem (Gurr et al., 2003). The flowering plants provide pollen, nectar, alternative prey and shelter for the predators (Landis et al., 2000) this in return may lead to an enhanced survival, development and reproduction of natural enemies (Heimpel & Jervis, 2005; Berndt & Wratten, 2005). Such studies are however rare particularly for predators such as hoverflies. Based on this knowledge of flowers attracting natural enemies, CBC strategies are currently focusing on the use of flowers to boost the population of natural enemies in the agricultural fields and in greenhouses (Haenke et al., 2009).

The selection of these flowering plants has largely been based on the attractiveness of different flowers to hoverflies through human observation or using pan traps (Colley & Luna, 2000; Ambrosino et al., 2006; Hogg et al., 2011). However, these criteria of floral resource selection does not address whether the attractiveness of the selected flowers lead to an improved fitness of the hoverflies that feed on them. Furthermore without laboratory tests it is difficult to say which pollen type will lead to maximum egg output and enhanced longevity or if all flower pollen types are suitable to syrphids in equal measures (Hickman et al., 1995).

This study generally focussed on the foraging decisions of syrphids and conservation biological control. Specifically experiments were undertaken to investigate how a limitation in pollen supply affects the fecundity and survival of aphidophagous syrphid. Additionally the effect of feeding frequency on pollen diet on the fitness of *E. balteatus* was investigated in the laboratory. Further experiments on improving biological control were conducted in the laboratory to investigate how a range of selected flower species affects the fitness of *E. balteatus*.

CHAPTER 2

THE IMPACT OF FOOD RESOURCES ON FITNESS OF THE SYRPHID *EPISYRPHUS BALTEATUS*

Abstract

Syrphids effectiveness as predators of aphids depends on the supply and quality of pollen and nectar. Pollen is required for reproduction and nectar for survival. Most investigations have focused on the interaction between prey and the predator while overlooking the importance of food foraging and supply during the different physiological phases. It is important to know how pollen and nectar supply affects the overall fitness of hoverflies. We investigated the impact of resource availability on the fitness of *E. balteatus*. Individual mated female syrphids were released in net cages with rape plant infested with *Brevicoryne brassicae*. Pollen and sugar supply was manipulated to simulate limitation in food resources. As a control, pollen and sugar were provided throughout the lifespan while the treatments included: pollen only, sugar only, withdraw of pollen during pre-oviposition and oviposition period. Oviposited eggs were counted daily and foraging patterns recorded with a video observation system. Feeding on pollen and sugar throughout resulted in a 2 fold the amount of eggs compared to different shortage treatments. Lack of pollen during pre - oviposition and feeding on sugar only lead to a complete lack of egg laying. The highest longevity occurred if pollen and sugar were available throughout being twice as long as if feeding on sugar only. Feeding on pollen alone resulted in same longevity as when feeding pollen and sugar throughout. Syrphids contacted the food patch containing pollen quite often and for longer durations during pre-oviposition period. The number of visits and durations then decreased during oviposition period. These results demonstrate that pollen availability is not only essential for oviposition but also enhanced survival in hoverflies. Consequently, lack of pollen sources during pre-oviposition period has a negative impact on egg output and longevity.

Key words: Syrphidae, foraging behavior, pollen, nectar, oviposition, fitness

2.1 Introduction

In the agricultural landscape *Episyrphus balteatus* is one of the most efficient aphid specific predators and the most common hoverfly in central Europe (Tenhumberg & Poehling, 1995). Like many other natural enemies adult hoverflies require pollen and nectar for development (Wäckers et al., 2007) while it's larvae feed on aphids and other soft bodied prey (Sadeghi & Gilbert, 2000). Since larvae have only limited movement capacities the survival of hoverflies in the agricultural landscape depends largely on the female hoverflies foraging behavior, i.e. the location of food and oviposition sites (Chandler, 1969). In general a female lifespan can be characterized by three physiological periods: A pre-oviposition period which lasts between a week (Geusen-Pfister, 1987) to 10 days (Almohamad et al., 2007), a peak oviposition period lasting about 10 to 14 days (Almohamad et al., 2007) and later a slow decrease until death (Branquart & Hemptinne, 2000). In total, adult hoverflies can survive for more than 1 month (Kan, 1988). In the absence of pollen hoverflies are not able to produce any eggs (Van Rijn et al., 2006) however without any food shortage females can lay up to 4500 eggs during the entire lifespan (Branquart & Hemptinne, 2000). Literature on the food requirements during different physiological period is scarce. For adult syrphids pollen provides proteins and amino acid (Rasmont et al., 2005) both necessary for sexual maturation, while nectar and honeydew provide carbohydrates for energy provision, i.e. locomotory activities (Jervis et al., 1993). Syrphids effectiveness as aphid predators depends on the supply and quality of pollen and nectar (van Rijn & Tanagoshi, 1999; Winkler et al., 2006) but most investigations on their foraging behavior have been concerned with the interaction between the prey and the predator, while overlooking the relative importance of pollen and nectar during the different physiological periods (Jervis & Kidd, 1996). A detailed understanding of how food supply affects the fitness of syrphids is needed. The objective of this work was therefore to investigate how a limitation in pollen supply affects the fecundity and survival of aphidophagous syrphid *E. balteatus*.

2.2 Materials and methods

2.2.1 Plant and insect culture

Broad beans (*Vicia faba*. L., Fabaceae; Hangdown) and rape plants (*Brassica napus* L., Brassicaceae) were grown in plastic pots inside a nursery. A stock rearing of adult *E. balteatus* hoverflies was kept in flight cages (52 x 40 x 60 cm). They were fed ad libitum on grounded bee pollen (Supplied by Imkerei Hohmann, Germany) and crystalline sugar bought from supermarket. Water was provided in Petri dishes on moist tissue paper. Broad bean plants infested with *Aphis fabae* Scopoli (Homoptera: Aphididae) were placed in the cages for 8 hours every day to stimulate oviposition. Frequently eggs were transferred to plastic boxes and larvae were fed on *Megoura viciae* (Harris) (Homoptera: Aphidae). Emerging adults were used in the experiments.

2.2.2 Experimental setup

The experiments were conducted in 25 large net cages (2 x 2 x 2m) that were placed inside a green house. The temperature ranged between 20 – 28 °C while the relative humidity was between 65 - 85 %. On each net cage a single 3 days old mated naive female was released. Inside the experimental cages, pollen and sugar availability were manipulated to simulate a limitation of food resources. In the control, pollen and sugar were provided throughout the lifespan while the treatment involved: sugar alone, pollen alone, withdrawal of pollen during the pre-oviposition period and withdrawal of pollen during the oviposition period (Table 1).

Table 1. The summary of food regimes adopted to simulate limited food resources. Unshaded regions indicate absence of the corresponding food source during that period.

		Pre- oviposition period (1-11)	Oviposition period (11-25)	Senescence period (> 25)
Control	Sugar			
	Pollen			
Treatment 1	Sugar			
	Pollen			
Treatment 2	Sugar			
	Pollen			
Treatment 3	Sugar			
	Pollen			
Treatment 4	Sugar			
	Pollen			

Water was provided in a Petri dish on moist tissue paper. The bee pollen (supplied Imkerei Hohmann, Germany) was crushed using a grinder (Model A10; IKA, Germany) while sugar was added in crystalline form. Five milligram of each food resource in a plastic Petri dish was placed inside the net cages on the ground. Both food resources were changed daily. Each of the five food manipulations was simultaneously replicated in five cages. To monitor the oviposition activity, each net cage contained two rape plants (20 cm high) that were each infested with 50 cabbage aphids *Brevicoryne brassicae*. After 24 hours, laid eggs were counted and plants on which eggs had been laid were removed and replaced. A video observation system equipped with video cameras (Meyhöfer, 2001) was used to monitor the feeding behavior on pollen and sugar patches in all cages for the entire hoverfly life span. The computer program Ethovision®XT (version 7.0, 2009, Noldus, Wageningen, The Netherlands) was used to analyse video recordings. Mean number of eggs laid per day for the entire life span, survival rates, feeding frequencies and total feeding time (pollen and sugar) were analyzed using analysis of variance (ANOVA, SAS® software Version 9.2, SAS Institute, 2008). Prior to analysis the data was tested for normality and homoscedasticity (Sokal & Rolf, 1995). When F-values were significant at the 5 % level, Tukey's test for multiple comparisons was carried out.

2.3 Results

2.3.1 The effects of food resources on longevity of *E. balteatus*

Analysis of food impact on the longevity of female *E. balteatus* showed a significant difference among the treatments ($F = 22.24$; $df = 4$; $P < 0.001$) (Fig. 1). Feeding throughout the life span on sugar only (16 ± 2.11 d) reduced the longevity significantly by more than half compared to provision of sugar + pollen in the control (43 ± 2.07 d). However there was no significant difference in longevity between *E. balteatus* feeding on pollen only and pollen + sugar (control) throughout the life span ($F = 3.83$; $df = 4$; $P = 0.114$). In contrast withdrawal of pollen only during the pre-oviposition period reduced longevity significantly by half (22 ± 1.10 d) ($F = 3.09$; $df = 4$; $P = 0.006$) compared to the control, while withdrawal only during the oviposition period had no influence (39 ± 2.03 / d) ($F = 4.01$; $df = 4$; $P = 0.106$) (Fig. 1).

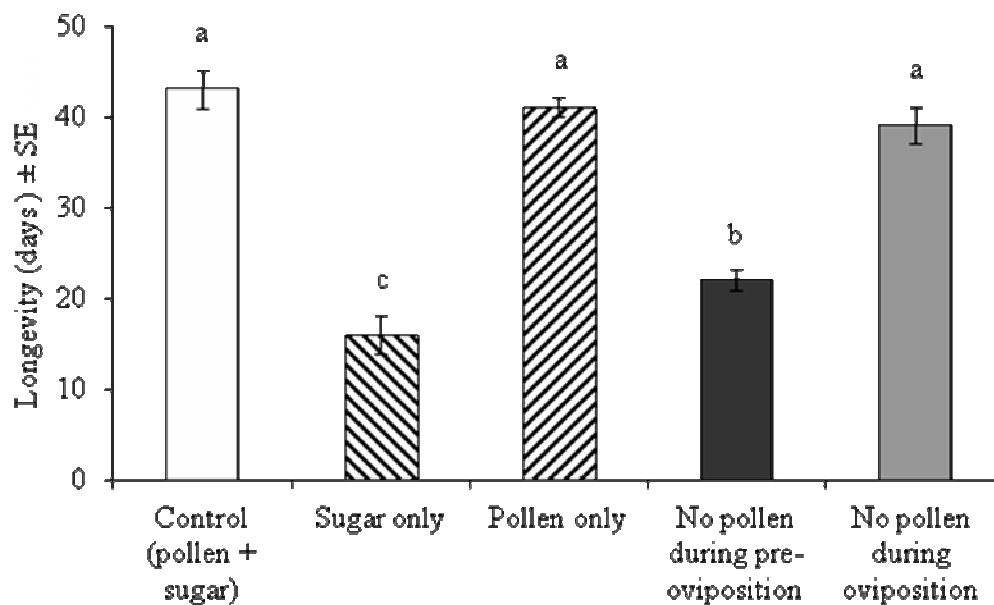


Figure 1. The impact of food resources on longevity of *E. balteatus*. Bars indicate the standard error. Different lower-case letters above columns indicate significant differences (Tukey HSD, $P < 0.05$)

2.3.2 The impact of food resources on egg laying

Egg laying of *E. balteatus* started earliest after 11 days in the control treatment and two days later in the treatments with access to pollen during the pre-oviposition period. Life time fecundity of females was significantly influenced by the different food treatments ($F = 5.10$; $df = 4$; $P = 0.008$). No eggs were laid if *E. balteatus* was allowed to feed on sugar only throughout the lifespan span. The same effect was noticed during the pre-oviposition period if only pollen was withdrawn (Fig. 2). Life time fecundity was similar to the control (sugar + pollen) (103.27 ± 8.15 eggs / d) if *E. balteatus* had access to pollen only throughout the entire life span (95.14 ± 7.13 eggs / d) ($F = 2.47$; $df = 4$; $P = 0.274$). In contrast life time fecundity was significantly reduced by half if only pollen was withdrawn during the oviposition period (55.46 ± 5.14 eggs / d) compared to the control (103.27 ± 8.15 eggs / d) ($F = 5.89$; $df = 4$; $P < 0.001$).

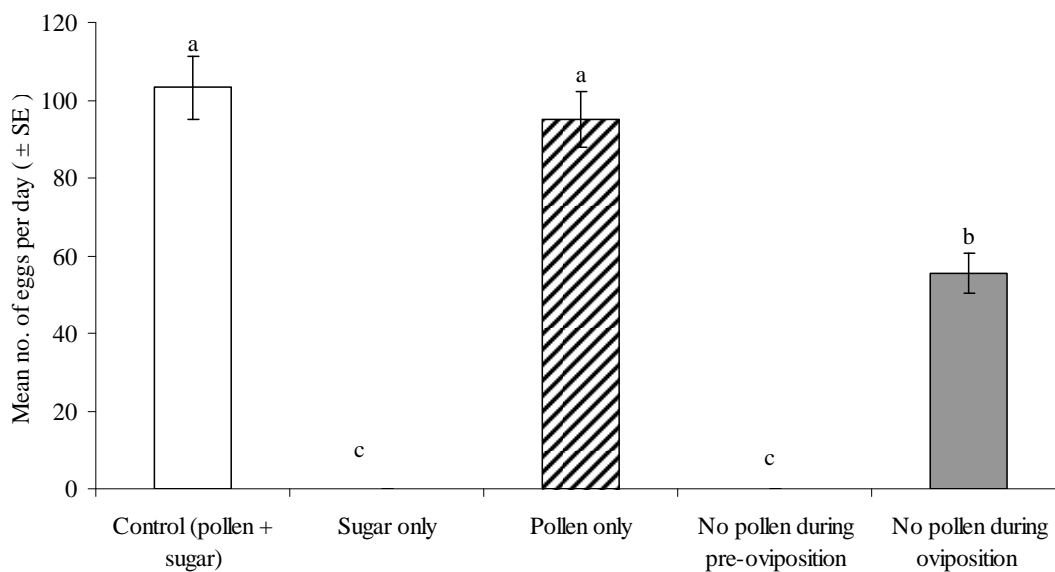


Figure 2. Mean number of eggs laid by *E. balteatus* when food resources were varied. Bars indicate the standard error. Different lower-case letters above columns indicate significant differences (Tukey HSD, $P < 0.05$)

2.3.3 The activities of *E. balteatus* on food patch

The video recordings of the behavior on the food patch were analyzed for the pre-oviposition and oviposition period separately with an aim to relate the allocation of female hoverfly activities to the different food treatments. In general female *E. balteatus* contacted the food patch more frequently during the pre-oviposition period (6.36 ± 0.66 visits / d) than during the oviposition period (4.07 ± 0.60 visits / d). The same trend could be observed for the residence time on the food patch (Fig. 4 AB). During both periods, i.e. pre-oviposition and oviposition, the food patch was visited at similar frequencies (Fig. 3 AB) and residence times (Fig. 4 AB) if pollen only was provided as compared to the control (pollen + sugar). In contrast, visiting frequencies of hoverflies and residence time on the patch were significantly reduced by approximately 50 % if sugar only was provided compared to the control during both physiological periods. For the analysis of visiting frequencies and residence time during the pre-oviposition period withdrawal of pollen during pre-oviposition led as expected to the same results as for the sugar only and the control treatment ($F = 2.94$ $df = 4$; $P = 0.072$) because food provision was identical (Fig. 3A, Fig. 4A). Withdrawal of pollen during the oviposition period reduced landing frequencies and residence time on the food patch by 50 % compared to the control and to the same level as in the sugar only treatment. Similar differences could be observed if pollen was provided during the oviposition period for the first time (withdrawal of pollen during the pre-oviposition period): while food patch visiting frequencies remained at the level of sugar only (Fig. 3 A) the food patch residence times increased to the level of pollen only.

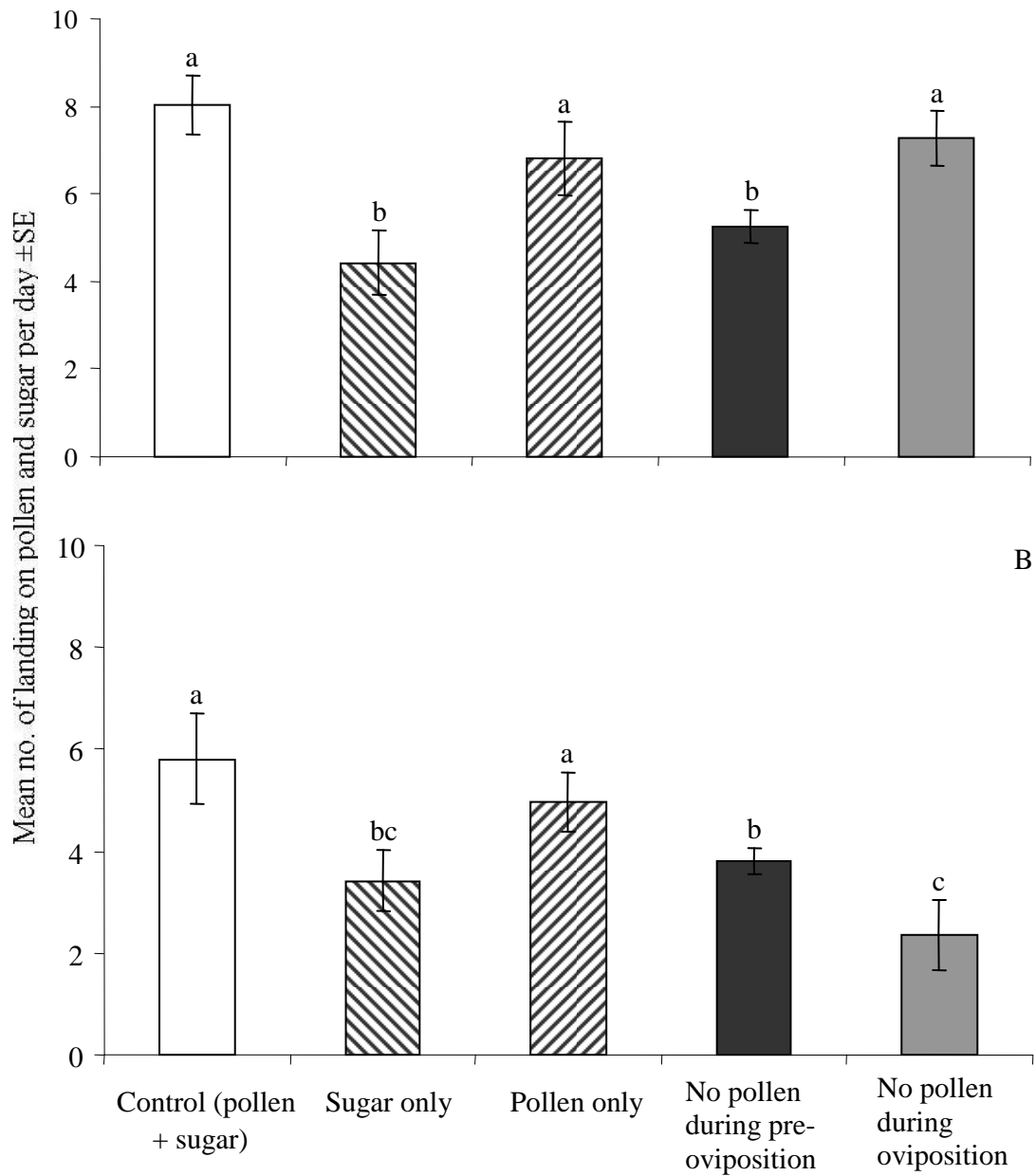


Figure 3. The frequency of *E. balteatus* landing on sugar and pollen during pre-oviposition period (A) and oviposition period (B). Bars indicate the standard error. Different lower-case letters above columns indicate significant differences (Tukey HSD, P < 0.05)

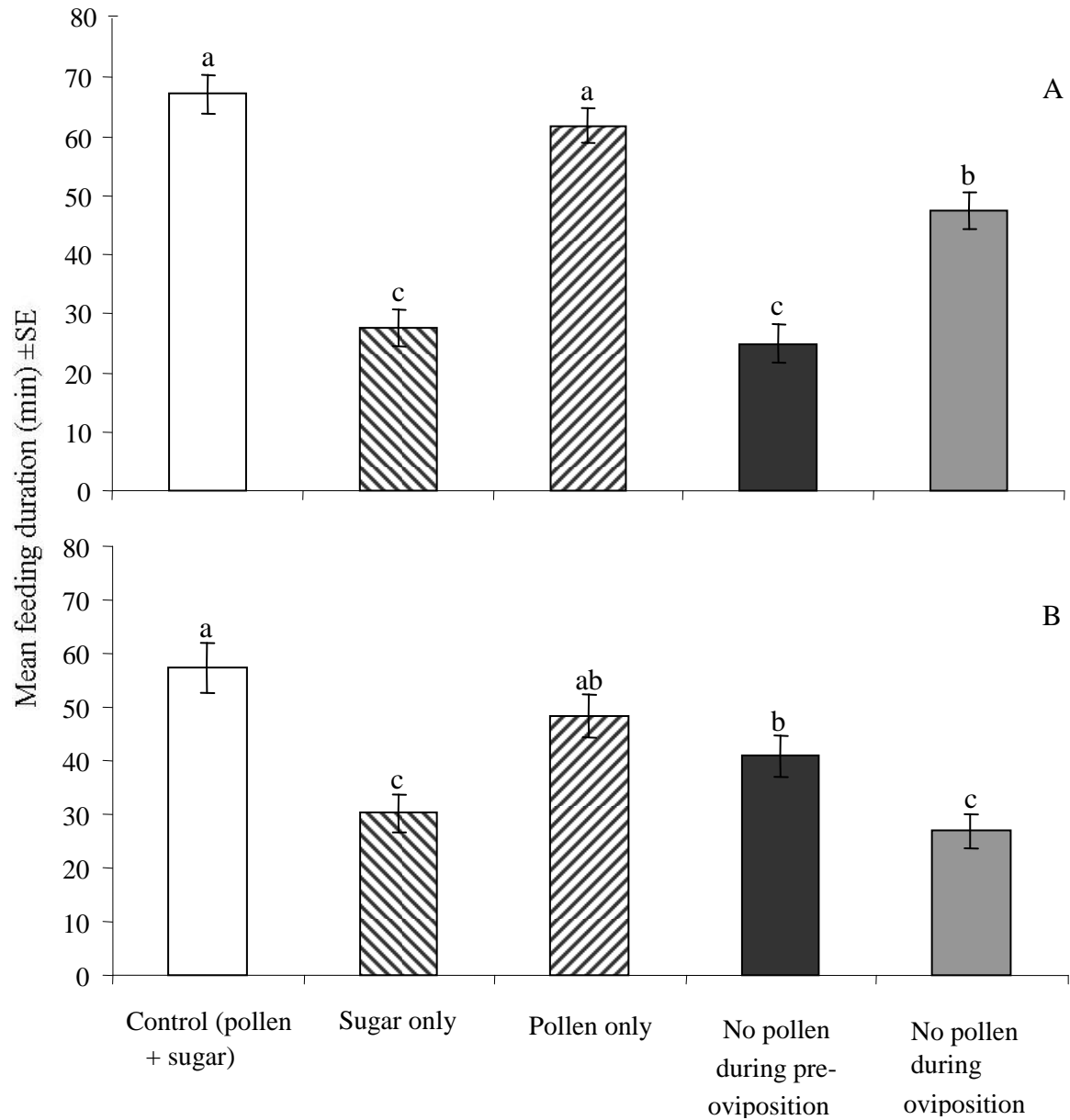


Figure 4. The mean feeding durations of *E. balteatus* on sugar and pollen during pre-oviposition (A) and oviposition period (B). Bars indicate the standard error. Different lower-case letters above means indicate significant differences (Tukey HSD, P < 0.05)

2.4 Discussion

Our results show that the availability of proteins and carbohydrates in the diet of adult *E. balteatus* during certain physiological periods influence survival and lifetime fecundity. Feeding on pollen only throughout an individual life-time resulted in equal longevity and fecundity as compared to the provision of both pollen and sugar (control). Lack of access to pollen during the pre-oviposition period or feeding on sugar as the only food source completely impaired the egg laying and considerably reduced longevity. The results of the present study suggest that changes in the availability of food resources has a far reaching effect on egg laying behavior and survival of syrphids and are in agreement with the findings of Whittingham (1991) and van Rijn et al. (2006) that the reproductive capacity and longevity of predators such as syrphids is dependent on availability of suitable food resources. While using flowers as a nectar and pollen source (van Rijn et al., 2006) found that when flowers are available during the pre-oviposition period only, females produced eggs in the first week of reproduction. However, in the following two weeks, almost no eggs are produced by these females anymore. On the contrary, those with continuous flower access continued to lay eggs. Similarly, in our findings when *E. balteatus* were allowed access to pollen during pre-oviposition and denied the same thereafter, they still laid eggs albeit the numbers were slightly lower. Lower oviposition rate may be an indication that bee collected pollen is not optimal in fecundity compared to flower pollen. When honey bees are transporting pollen on their legs they add nectar. Later the liquid portion of the nectar evaporates but the sugar portion remains. Therefore it is probable that bee pollen contains large amount of sugar than proteins. Most pollen ingestion period by females occurs during the pre-oviposition period and this activity coincides with the time of yolk deposition in the eggs (Haslett, 1989). Pollen provides syrphid with nitrogen needed for maturation of the ovaries and also to sustain the production of eggs, hence pollen is an important food resource which should be supplied throughout the lifespan of a syrphid for continuous oviposition and high efficiency in biological control. In hoverflies, reproduction only occurs when

pollen is available. For example females of *Sphaerophoria scripta* laid no eggs in the absence of pollen food sources (Nengel & Drescher, 1991). Nutrition has been shown to affect egg development in many insects (Rivero et al., 2001) and in our study when *E. balteatus* had no access to pollen during pre-oviposition period they did not lay any eggs and were unable to compensate even after they accessed it later, although residence times and presumably food intake were increased. Presumably if syrphids are denied protein during vitellogenesis, their egg chambers are degenerated by apoptosis like it is the case in drosophila (Bownes & Terashima, 2004). Complete lack of egg output was also observed when *E. balteatus* fed on sugar only throughout their lifespan corroborating the findings of (Schneider, 1969) who found that syrphids were unable to oviposit any eggs when they were reared on honeydew or sucrose alone. In hoverflies it is believed that most pollen ingestion period by females coincide with the time of yolk deposition in the eggs until the oocytes occupies up to 90 % of the follicle (Haslett, 1989) however continuous pollen supply is necessary to ensure sustained egg production (Branquart & Hemptinne, 2000). In this study, the longevity of *E. balteatus* was hypothesized to be affected by food resource supply. We found that the highest survival was noted when *E. balteatus* was supplied with pollen and sugar throughout the lifespan. There was no significant difference when *E. balteatus* was supplied with pollen only or when pollen was withdrawn during oviposition period. On the contrary, a complete withdraw of pollen during the pre-oviposition period significantly reduced the longevity. In addition, feeding on sugar only elicited the lowest survival rates. Other findings with *S. scripta* indicate that when females had pollen as an additive, they lived 3 fold more than those without it. In the absence of any food (water only) adult female *S. scripta* only survived for 1.9 days (Nengel & Drescher, 1991). A prolongation of adult lifespan has been documented after providing syrphids with pollen or a mixture of honey and 10 % pollen (Hong & Hung, 2010). These findings agreed with our results whereby those female who fed on pollen and sugar throughout their lifespan had the highest longevity while those that fed on sugar only had the lowest. From our study a comparison of landing on the food patch has revealed higher

landing rates on food patch containing pollen during the pre-oviposition period. Sugar only as a food resource was half fold visited by the syrphids. This is probably because eggs are maturing at this stage and therefore there are higher requirements for protein sources. For example in syrphid *Rhingia campestris* pollen requirements are normally high while yolk deposits are being laid down in eggs (Haslet, 1989). Although carbohydrates are needed at all stages, during pre-oviposition its role is not as important as proteins. On the contrary, landing rates on food patch went down during oviposition period. Presumably this is owing to the low food requirements and a priority shift to oviposition of the already mature eggs. Our results show higher feeding duration during pre-oviposition compared to oviposition period. Our findings supports the previous findings (van Rijn et al., 2006) which suggest that female syrphids most probably focus on food foraging during the first week after emergence, and thereafter they focus more on oviposition sites. From our study, little amount of time was invested in visiting a food source with sugar only during pre-oviposition and oviposition period. Female hoverflies need sugar but the peak is at the beginning and end of yolk deposition (Haslet, 1989). A decrease in the feeding time even on food patch containing pollen was evident during oviposition period. In *Eupeodes corollae* pollen consumption is higher before any egg laying and then a decrease occurs (Whittingham, 1991). In conclusion this study has demonstrated differences in food requirements during different physiological periods of hoverflies. Most importantly, we have shown the essence of pollen availability during pre-oviposition period. Furthermore, pollen should be available throughout the diet of hoverflies to ensure sustained fecundity and prolonged longevity. Other studies (Roulston & Cane, 2000) have indicated that pollen also contains carbohydrates thus implying its dual use to provide proteins and energy sources to syrphids. However further studies should be undertaken to determine the fitness of the resulting offspring after the realized fecundity from the different food manipulations.

CHAPTER 3

RELATIONSHIP BETWEEN FEEDING FREQUENCY AND FITNESS OF HOVERFLIES. DOES TIME INVESTED MAKE A DIFFERENCE?

Abstract

Hoverflies are important predators worldwide. They also play an important role of aphid control in greenhouses. Larvae and adults feed on different food sources. While the larvae feed on aphids and other soft-bodied insects their adults rely on pollen and nectar for reproduction and survival. In the field suitable pollen for syrphids has been documented to be a scarce resource in certain periods of the year and especially in the agricultural areas that have limited available flowers. Therefore, the role of pollen cannot be underestimated in seeking a successful biological control. Furthermore a detailed understanding of how pollen availability affects the performance of hoverflies is necessary. This study examined how pollen frequency of availability affects the fitness of hoverfly *Episyrphus balteatus*. In a laboratory set up, pollen as a food resource was provided in varying frequencies. The various treatments were: pollen was provided everyday for 10, 30 or 60 minutes. Also pollen was provided every 2nd, 3rd, or 5th day for a period of 8 hours and throughout the lifespan (control). A broad bean (*Vicia faba*. L) infested with aphid *Aphis fabae* Scopoli (Hemiptera: Aphididae) was used to stimulate oviposition while water was provided on a Petri dish on moist tissue paper. From all the frequencies of pollen availability tested the treatments with continuous pollen supply (control) and every 2nd day resulted in the shortest pre-oviposition period. Females with fewer feeding frequencies produced less eggs compared to those who had continuous access to pollen. The highest mean number of eggs was laid when hoverflies had access on every day and every 2nd day while the lowest was on every 5th day pollen access. Hoverflies egg deposition period was longer upon accessing pollen on every 2nd day and throughout compared to the other frequencies. The highest longevity was achieved by hoverflies which were fed continuously (control) and on every 2nd or every 3rd day. On the contrary, hoverflies lived the

shortest when they had pollen on every 5th day or 10 minutes per day. Hoverflies had the highest dry weight after accessing pollen on every 2nd day and throughout (control). The dry weight was however similar between every 2nd day and every 3rd day. On the contrary the dry weight was 2 fold less when they had pollen for 10 min and on every 5th day. These results provide an insight into the nutritional requirements of *E. balteatus* under laboratory conditions. Furthermore to improve the efficiency of *E. balteatus* in the greenhouse, a continuous supply of pollen and sugar sources is necessary, as infrequent feeding opportunities leads to limited benefits.

Keywords: Hoverflies, feeding frequency, pollen, fitness

3.1 Introduction

Hoverflies are important natural enemies of aphids in open field crops such as cereals. They can also play an important role in controlling aphids in the green houses. For instance *E. balteatus* is one of the most abundant species in Spain's sweet pepper greenhouses (Pineda & Marcos-Garcia, 2006). While larvae of these predators are exclusively feeding on animal prey and in particular on aphids (Rojo et al., 1996) their adults need pollen and sugar (nectar) for their survival and reproductive success. From previous studies (Kahato & Meyhöfer 2012, see chapter 2.....) pollen is the most important ingredient to a female hoverfly in promoting egg laying and also longevity. Furthermore other studies have found similar findings for instance almost three times as many hoverflies were seen taking pollen as compared to nectar implying that the former is quantitatively the more important resource for the syrphid family as a whole (Gilbert, 1985). Female hoverflies hatch with an immature reproductive system and must access proteins from pollen to mature their ovaries (Chambers, 1988). In a field situation pollen supply is likely to affect egg laying (Schneider, 1948) and especially in areas with intensive agriculture which are characterized by flower scarcity (Hickman & Wratten, 1996). Providing pollen resources to hoverflies in the field or green houses may be helpful in improving conservation biological control. This however requires an understanding of how food availability or lack of it affects syrphid fitness. In parasitoids, higher feeding frequency on sugar has been shown to increase their longevity and reproduction (Azzouz et al., 2004; Li et al., 2008). While some studies have been carried out on pollen and nectar feeding in syrphids (van Rijn et al., 2006), the role of feeding frequency on their fitness has not been examined. Understanding the feeding frequency requirements of a syrphid may be helpful in controlling aphids in the greenhouses. In the present study the effects of feeding frequency on pollen diet on the fitness of *E. balteatus* were investigated in the laboratory.

3.2 Materials and methods

3.2.1 Plants and insects rearing

Broad beans (*Vicia faba*. L) were grown in plastic pots inside a nursery. A stock rearing of adult hoverflies (*Episyrphus balteatus*) was kept in flight cages (52 x 40 x 60 cm). Pupae were supplied by Katz, Germany. After emergence hoverflies were fed on grounded bee pollen (supplied by Imkerei Hohmann, Germany) and crystalline sugar bought from supermarket. Water was provided in Petri dishes on moist tissue paper. Broad bean plants (*Vicia faba* L.) infested with *Aphis fabae* Scopoli (Homoptera: Aphididae) were placed in the cages for 8 hours every day to stimulate oviposition. Frequently eggs were transferred to plastic boxes and larvae were fed on *Megoura viciae* (Harris) (Homoptera: Aphidae). This rearing provided adult for experiments.

3.2.2 Experimental set up

The experiments were conducted in 35 net cages that were placed inside a climate room. The temperature ranged between 20°-25°C while the relative humidity was between 65-85%. In each net cage a single 3 days old mated naive female was released. Inside the experimental cages, pollen availability was provided in varying frequencies. Pollen was provided every day for a period of 10 min, 30 min, 60 min, in the other treatments pollen was provided every 2nd day, every 3rd day, every 5th day and a positive control (pollen and sugar throughout) for a period of 8 hours. Water was provided in a Petri dish on moist tissue paper. The bee pollen (supplied Imkerei Hohmann, Germany) was crushed using a grinder (Model A10) while sugar was in crystalline form (Supplied by supermarket). Five milligram of pollen in a plastic Petri dish was placed inside the net cages. Each of the different treatments was conducted simultaneously in five cages. To monitor the oviposition activity, each cage was provided with a broad bean plant infested with 100 *Aphis faba* aphids. After 24 hours, laid eggs were counted and plants on which eggs had been laid were removed and replaced. In order to determine the female dry weight a similar set up like previously described (see 3.2.2) was used. On the 15th day hoverfly dry mass was recorded using a micro

balance (Sartorius MC5) after drying the specimen in oven at 40° for 72 hours. Pre-oviposition period, mean number of eggs laid per day for the entire life span, oviposition period, longevity and dry weight were analyzed using analysis of variance (ANOVA, SAS® software Version 9.2, SAS Institute, 2008). When F-values were significant at the 5 % level, Tukey's test for multiple comparisons was carried out.

3.3 Results

3.3.1 Influence of feeding frequency on the pre-oviposition period

From this study the feeding frequency significantly affected the pre-oviposition period ($F= 3.41$, $df= 6$, $P= 0.003$). Feeding on pollen for only 10 min, 30 min and every 5th day led to the longest pre-oviposition period compared to the control (9.5 ± 0.19 d). However there were no significant differences in pre-oviposition period between syrphids who had access to pollen every second day (10 ± 0.49 d) compared to the control neither there were differences between those who fed every second day compared to those who fed on every 3rd day ($10.5 \pm d$) (Fig. 1).

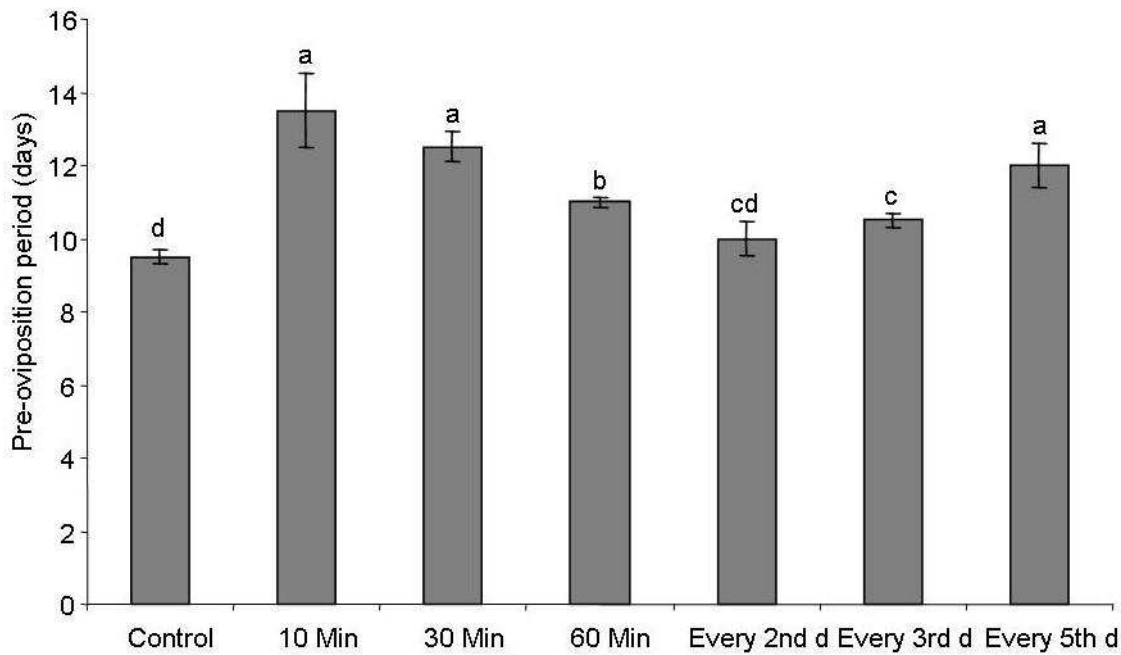


Figure 1. The effect of feeding frequency on the pre-oviposition period of *E. balteatus*. Error bars represent standard error (SE). Different letters indicate that treatments were significantly different at the 95% confidence level

3.3.2 Effect of food frequency on the mean fecundity

An analysis of the hoverfly fecundity showed that food frequency influenced the egg laying of *E. balteatus* ($F = 5.89$, $df = 6$, $P = 0.041$). Feeding on every 5th d led to the lowest fecundity being more than 4 fold less compared to the control (92 ± 8.2 eggs / d). Ten min (25 ± 7.5 eggs / d) and 30 min (32 ± 3.8 eggs / d) feeding frequency elicited lower fecundity being almost 3 fold less compared to the control. On the contrary, feeding every second day (78 ± 8 eggs / d) did not significantly lower the number of eggs laid compared to the control ($F= 1.83$, $df= 6$, $P= 0.710$). Access to pollen for 60 minutes per day led to a half fold decrease (45 ± 6.5 eggs / d) in the number of eggs laid by hoverflies compared to the control Fig. 2

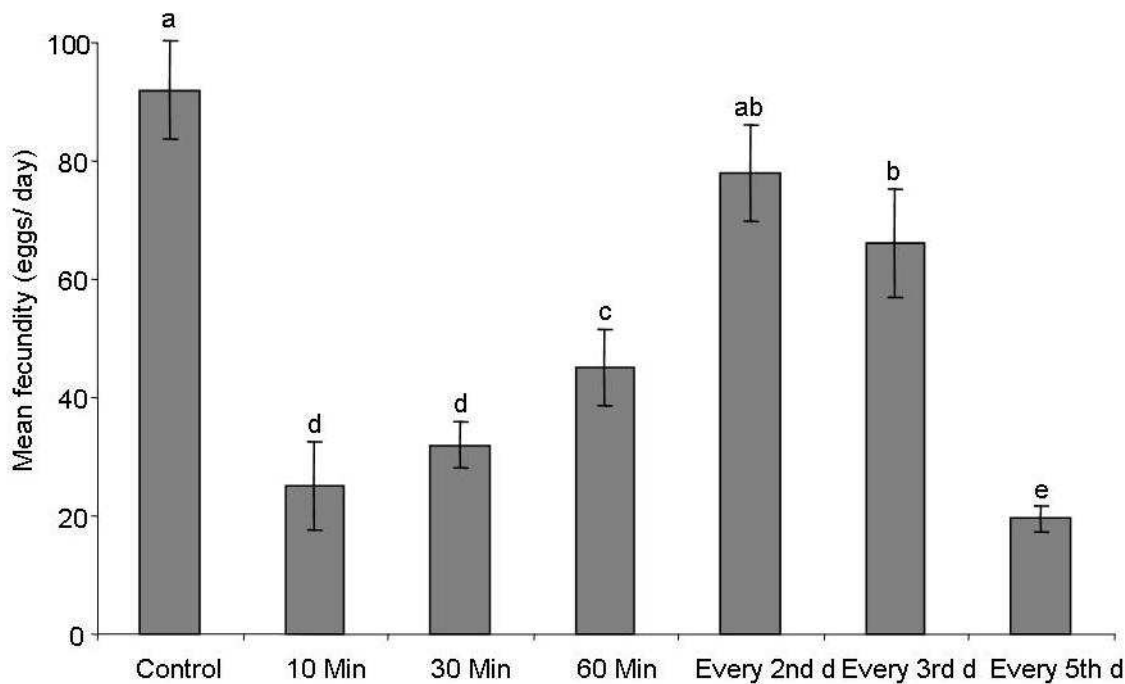


Figure 2. Mean fecundity of *E. balteatus* fed on different frequencies of pollen and sugar. Error bars represent standard error (SE). Different letters indicate that treatments were significantly different at the 95% confidence level

3.3.3 Effect of feeding frequency on oviposition duration

The oviposition duration was significantly influenced by the feeding frequency ($F = 9.46$, $df = 6$, $P = 0.025$). Feeding for 10 min every day and on every 5th day reduced the oviposition duration to almost 3 fold compared to the control (42.8 ± 3.6 d). The oviposition duration was 2 fold less when hoverflies fed on pollen resources for 30 and 60 min (20.5 ± 1.5 d and 24.5 ± 4.5 d respectively) in comparison to the control. However, feeding on every 2nd and 3rd day (34.5 ± 2.6 and 37 ± 3.1 d) did not lower oviposition duration significantly compared to the control ($F = 6.19$ $df = 6$, $P = 0.261$) (Fig. 3)

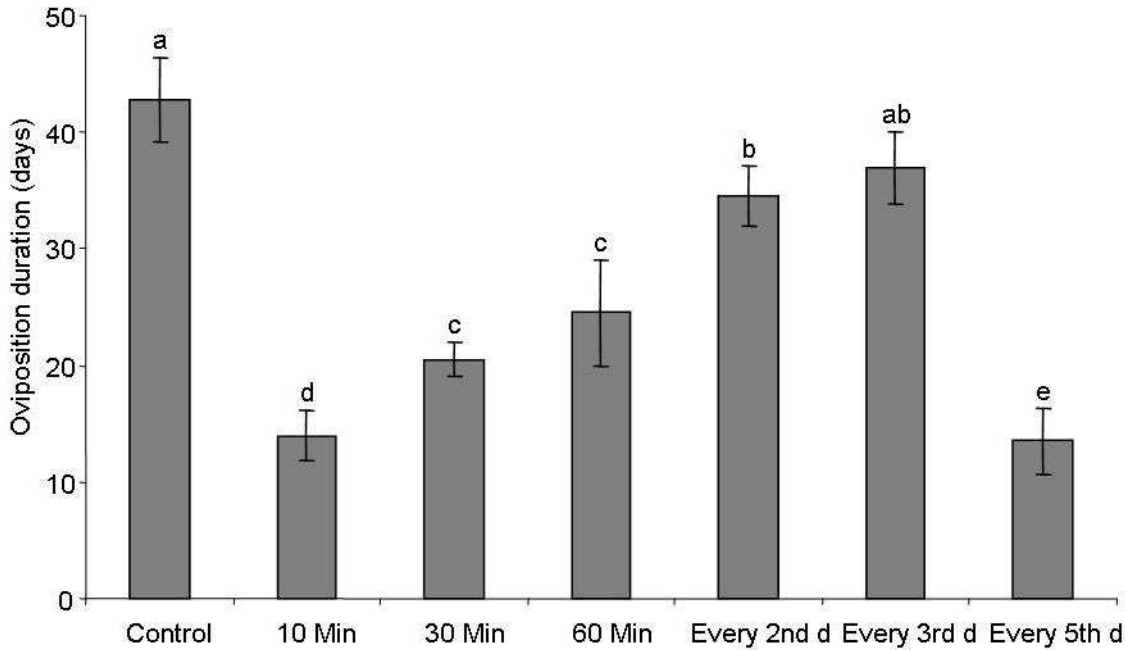


Figure 3. The effect of feeding frequency on oviposition duration. Error bars represent standard error (SE). Different letters indicate that treatments were significantly different at the 95% confidence level

3. 3.4 Effect of feeding frequency on longevity of *E. balteatus*

Analysis of feeding frequency on the longevity of female *E. balteatus* showed a significant difference among the treatments ($F = 2.72$, $df = 6$, $P = 0.004$). Feeding on every 5th day reduced the longevity by half fold (26 ± 1.5 d) compared to the control (56.5 ± 6.5 d). 10 min per day feeding reduced longevity to almost half compared to the control while those who fed 30 and 60 min per day lived 35 ± 4 and 37.5 ± 4.5 d respectively. On the contrary, feeding on every 2nd and 3rd day did not affect the longevity significantly compared to the control ($F = 10.71$, $df = 6$, $P = 0.061$), the hoverflies lived 49.5 ± 3.5 d and 52 ± 5 d respectively (Fig. 4).

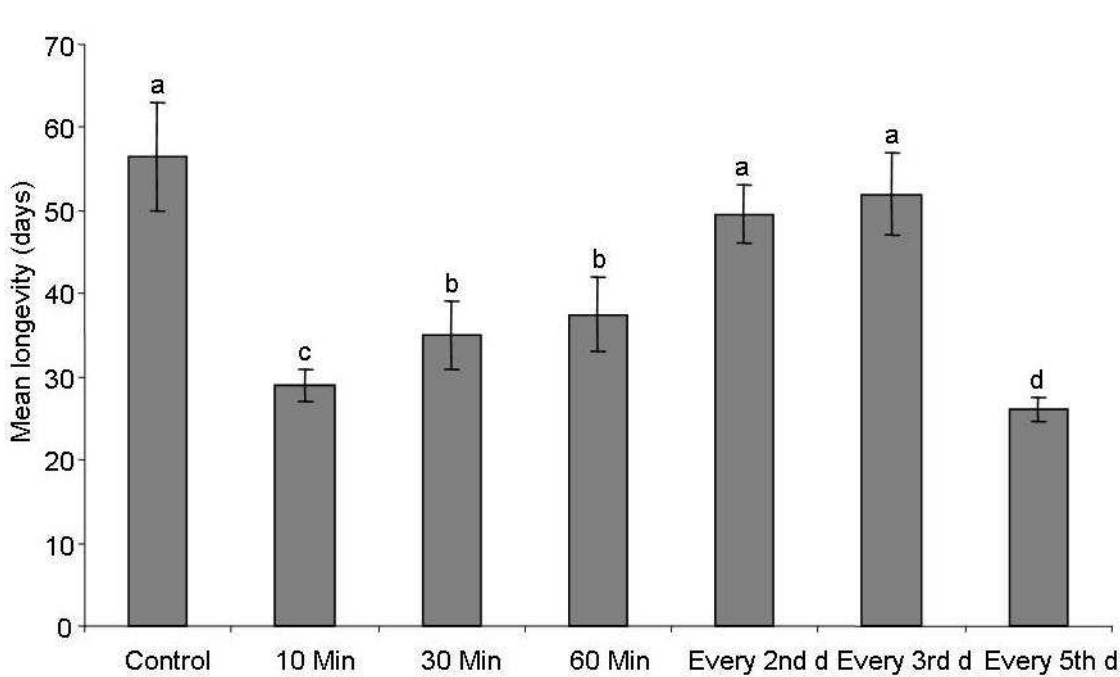


Figure 4. Average lifetime (mean \pm SE) of *E. balteatus* fed different feeding frequencies. Each individual was fed on pollen and sugar. Different letters indicate significant differences ($P < 0.05$)

3.3.5 Effect of food frequencies on the dry weight of *E. balteatus*

The dry weight of female hoverflies was affected by the feeding frequency ($F = 5.20$, $df = 6$, $P = 0.006$). Feeding every 10 min per day and on every 5th day reduced the dry weight by half fold compared to the control (5.84 ± 0.11 g). Feeding for 30 and 60 min resulted in lower dry weight (3.02 ± 0.26 and 4.05 ± 0.12 respectively) of the hoverflies. Every 2nd day feeding resulted in significantly similar dry weight (5.63 ± 0.24 g) compared to the control (Fig. 5).

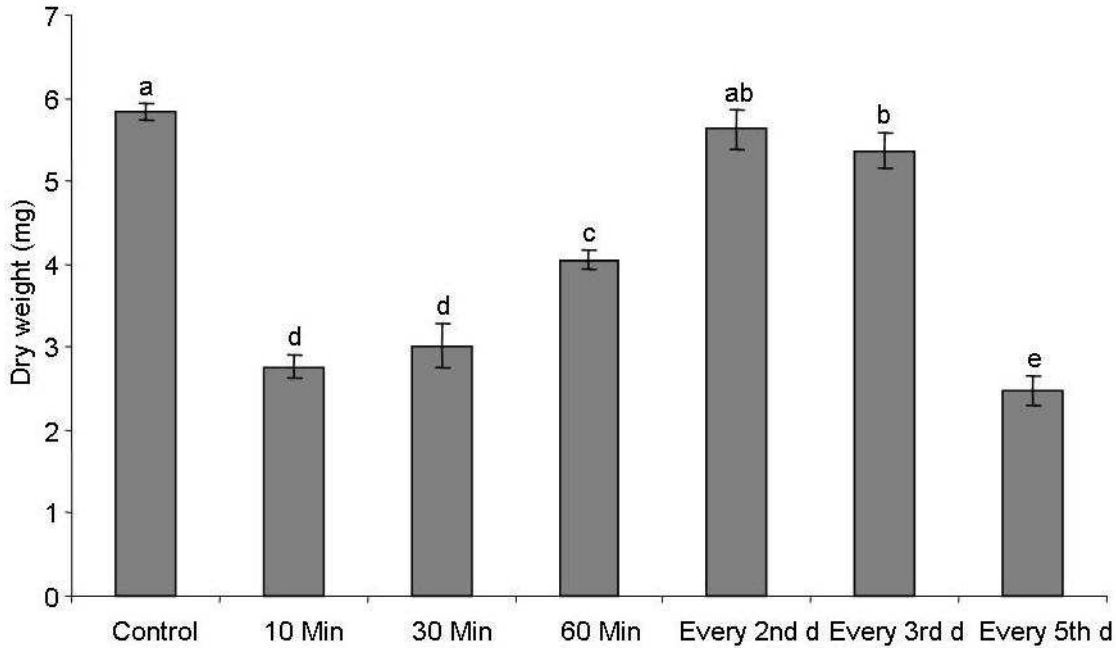


Figure 5. The effect of feeding frequency on the dry weight of *E. balteatus*. Each individual was fed on pollen and sugar. Different letters indicate significant differences ($P < 0.05$)

3.4 Discussion

The hoverfly *E. balteatus* needs different diets in the larval and adult stages. The larvae are aphidophagous predators of aphids. The adults feed on nectar and pollen from different flowers (Gilbert, 1980). Non prey food (pollen and nectar) plays a key role in enhancing the reproduction success of the adults and hence the predatory efficiency of hoverflies. Hoverflies only mature eggs after adult emergence and therefore they need protein sources in ensuring egg maturation, high egg load and continued egg laying (Chambers, 1988). Pollen has been shown to be a major factor in affecting the number of eggs laid (Schneider, 1948). From previous studies it has been shown that without pollen access, female *E. balteatus* do not lay any eggs, furthermore their longevity reduces drastically (Kahato & Meyhöfer, 2012). Without any food shortage female hoverflies can lay eggs from the onset of oviposition to death (Branquart & Hemptinne, 2000) though the number reduces in old age. *Episyrphus balteatus* can lay between 2000-4500 eggs in its lifetime (Branquart &

Hemptinne, 2000). Unlike parasitoids the energy requirements of hoverflies are not adequately documented despite their role in conservation biological control. In our study we investigated the fitness of *E. balteatus* by varying the pollen frequency of availability. Our study demonstrates the importance of feeding frequency for hoverflies fitness. We show that pollen access on every 2nd or every day lead to the best fitness that was characterized by a higher fecundity and a shorter pre-oviposition duration. Additionally these two treatments resulted in prolonged oviposition duration. On the contrary, feeding on pollen for 10 minutes per day and on every 5th day resulted to the lower fecundity and longer pre-oviposition period. Similarly the duration of egg laying (oviposition) was shorter in these treatments. Pollen requirements are greatest during pre-oviposition while yolk deposits are laid down in the eggs (Haslet, 1989). Pre-oviposition is therefore likely to be influenced by the availability of protein. Probably from our results access to pollen for only 10 minutes does not give the hoverflies enough amount time to ingest adequate pollen that can result in high fecundity. Consequently, lower feeding frequency may lead to inadequate ingestion of pollen therefore resulting in longer pre-oviposition. However further studies on the amount of proteins needed by hoverflies should be undertaken.

In hoverflies egg production depends on the availability of nutrients that limit oogenesis (Wheeler, 1996). Furthermore hoverflies need continuous pollen access for continued egg laying (Schneider, 1948). In *Meteorus pulchricornis* (Hymenoptera: Braconidae) a life time production of progeny increased with feeding frequency. An every day feeding resulted in 50 % more progenies than those with less frequent feeding (Wu et al., 2008). From our results the highest fecundity was as a result of continuing availability of protein sources or at least on every 2nd day. The length of oviposition duration is crucial in determining the overall fecundity. Mostly longer oviposition results in increased egg laying. From this study feeding on pollen throughout and on every 2nd day resulted in the longest oviposition duration. This was probably due to hoverflies living longer compared to the other treatments.

In studies with parasitoids, the longevity of *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) fed on flowers every other day was not significantly differently than those fed on flowers every day (Lee & Heimpel, 2008). In our study, the lifetime of female *E. balteatus* depended in part on the quantitative variation of pollen supply. When pollen frequency of availability was on every 2nd or daily this yielded the highest longevity values, prolonging the average lifetime of females hoverflies 2 fold compared to the other treatments. Unlike in *C. glomerata* studies where survival was poor when they were fed on flowers every 3rd day, in hoverflies when they had pollen on every 3rd day they were able to survive for a similar period like the control. This may indicate that hoverflies are able to store some food reserve for survival albeit at low quantities. On the contrary, feeding on pollen for 10 minutes per day and on every 5th day greatly reduced the longevity of *E. balteatus*. Therefore from these results it is probable that hoverflies longevity and egg laying will be limited in part by the quantity of available pollen resources. However in the field other parameters like host searching flight and oviposition activity should be taken into account to get a more realistic approximation of energy requirements. One probable reason is that pollen also provides hoverflies with carbohydrates that enables a prolonged lifetime. This contradicts the commonly held observation that hoverflies acquire energy from nectar and honeydew only (Faegri & Pijl, 1979) while pollen provides nitrogen, amino acids and lipids. Dry weight can be used as an indicator for potential fecundity. In our results hoverflies who had daily access to pollen and on every 2nd day had the highest dry weight. The dry weight was however low when hoverflies had pollen access for 10 minutes and on every 5th day. Higher dry weight may have been due to higher amount of eggs that had matured after acquiring enough protein from pollen. In previous studies heavier hoverflies also had higher fecundity than lighter hoverflies (Branquart & Hemptinne, 2000). Thus the frequency of pollen availability is necessary in ensuring maximum fecundity of hoverflies. Knowledge on food requirements has resulted in the application of artificial food sprays in different formulations to act as food supplements for natural enemies (Wade et al., 2008). From

our study, daily provision of pollen as a food supplement in the field might have a potential in conservation biological control. This may increase the level of natural enemies either by attracting them to or aggregating around the pollen sources. This may be more important in mono cultures that are flowers depleted. Earlier studies (McEwen & Kidd, 1995) have advocated for frequent replenishment of food resources to increase the longevity and fecundity of lacewings *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). From our results the best replacement interval for pollen sources would be every second day since this frequency produced similar results with pollen provision throughout the lifespan (control) as infrequent feeding opportunities leads to minimal benefits.

In conclusion our results provide insight into the nutritional requirements of *E. balteatus* under laboratory conditions. Such information can be a basis to improving the longevity and fecundity of *E. balteatus* in the field and the greenhouses. Furthermore provision of pollen sources in agricultural fields that have limited flower species may help in improving biological control of aphids. This might be achieved by providing pollen sources near crops on every second day.

CHAPTER 4

THE INFLUENCE OF SELECTED FLOWERING PLANTS ON THE FITNESS OF THE APHIDOPHAGOUS SYRPHID, *EPISYRPHUS BALTEATUS* (DEGEER) (DIPTERA: SYRPHIDAE)

Abstract

Hoverflies depend on different food sources as adults and larvae. While adults need pollen and nectar their larval stages forage on aphids. Providing floral resources in vicinity to the fields have been documented to improve pest suppression. However floral resources need a careful selection to avoid promoting pest species. Mostly, selection of flowers for use in conservation biological control has used attractiveness based on human observation such as visit frequencies. However, studies on the effect of different flower species on *E. balteatus* fitness are rare. This study aimed at investigating how fitness is influenced by selected flower species. Using cages/indoor experiments, female hoverflies were offered the flowers of phacelia, buckwheat, cornflower, chamomile, mustard, stinging nettle or perennial rye grass. A broad bean plant infested with *Aphis fabae* was placed in the cage to stimulate oviposition.

Our results revealed that phacelia promoted the shortest pre-oviposition period while feeding on nettle elevated the pre-oviposition period. Also, hoverflies who fed on phacelia had the highest fecundity while perennial rye grass resulted in the lowest. Cornflower best enhanced longevity of hoverflies as well as buckwheat while individuals feeding on grass had the shortest. The size of female hoverflies was not influenced by the different flower feeding. Feeding on phacelia resulted in the heaviest hoverflies on the contrary flies on perennial rye grass had the lowest weight. An analysis of pollen protein revealed phacelia had the highest protein content being 3 fold more than in nettle. Phacelia, cornflower and buckwheat improved the most important fitness parameters and their use in biological control is highly recommended. A mixture of flowers with different

phenologies such as phacelia, buckwheat and cornflower should be evaluated to determine how they influence fitness.

Keywords: Hoverflies, floral resources, longevity, fecundity, Syrphidae, pollen, protein content, fitness

4.1 Introduction

In agricultural fields hoverflies are common natural enemies against aphids and other soft bodied prey. In central Europe *Episyrphus balteatus* is one of the most common and economically significant hoverfly species. The larvae of *E. balteatus* are predators to more than 100 species of aphids worldwide (Sadeghi & Gilbert, 2000). Their adults however, need proteins for egg maturation while sugar is essential for survival and flight activities (Lavandero et al., 2005) that are provided by pollen and nectar. Today agricultural landscapes are often characterized by lack of suitable flowering plants and hoverflies have to fly large distances between food resources and oviposition sites. This phenomenon leads to energy and time wastage which could otherwise have been used in pest suppression (Tylianakis et al., 2004). Planting flower resources near agricultural fields may minimize this problem (Gurr et al., 2003). Through consideration of the floral features such as colour, the depth of corolla and nutritional value of pollen as important factors in determining preferences of different flowers by hoverflies (Gilbert, 1981) it is possible to select suitable flowers. Based on this knowledge, conservation biological control strategies are focusing on the use of flowers to increase the population of natural enemies in the fields (Haenke et al., 2009). However, the selection of these flowering plants has largely been based on the attractiveness of different flowers to hoverflies through human observation or using pan traps (Colley & Luna, 2000; Ambrosino et al., 2006; Hogg et al., 2011 a). Furthermore harvestable floral resources have been exploited in CBC with the dual benefits of economic gains such as cut flowers and also in attracting hoverflies (Hogg et al., 2011 a). These strategies however do not address whether the attractiveness of the selected flowers leads to an improved fecundity and longevity of the hoverflies that feed on them. Some studies have shown that an increase in suitable flowering plants can enhance pest suppression by improving different fitness components such as fecundity and longevity of natural enemies (Heimpel & Jervis, 2005; Berndt & Wratten, 2005; Hogg et al., 2011 b). For instance females of *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae)

lived 7 times longer with flowers than those without while the males lived 3 times longer (Berndt & Wratten, 2005). With alyssum flowers the mean lifetime fecundity was 8 folds more than without. In addition flowering crops in the vicinity of agricultural fields can moderate environmental conditions for instance humidity and temperature and also provide alternative habitats for natural enemies (Thomas et al., 1991). However such studies are rare for hoverflies (Laubertie et al., 2012), furthermore without laboratory tests it is difficult to say which pollen type will led to maximum egg output or if all pollen types are suitable to syrphids in equal measures (Hickman et al., 1995). In this study we conducted laboratory studies to investigate how a range of selected flower species affects the fitness of *E. balteatus*. The selection of these flower species was based on the results of earlier work (Hickman & Wratten 1996; Colley & Luna 2000; Carreck & Williams 2002; Hogg et al., 2011). Phacelia has flowers that are in flat-topped clusters in shades of purple or occasionally white. Phacelia flowers 6-8 weeks after germination while the flowering continues for 6 to 8 weeks. In the field the plant flowers in mid summer to late summer (July to September). It is widely thought that hoverflies forage for phacelia pollen because they have a short proboscis which is not long enough to remove nectar from the relatively deep corollae, therefore the plant is most likely a pollen source only to syrphids (White et al., 1995). Buckwheat has 5 petaled flowers which are arranged in a compound raceme that produces laterally flowered cymose clusters. Its flowers are white to pink in color (Cawoy et al., 2009). Flowering starts 4-6 weeks after sowing and goes on during 4 to 15 weeks (Halbrecq et al., 2005). In the field, buckwheat blooms in July to August. Buckwheat provides pollen, nectar and extra floral nectaries to syrphids. The cornflower has blue flowers that are produced in flower heads with a ring of a few large spreading ray floret that surround a central cluster of disc florets. Cornflower takes 10 to 12 weeks to produce flowers and stay in bloom for 4 weeks. In the field, cornflower blooms from June to August. Cornflower has readily accessible pollen to the syrphids.

The German Chamomile flower head has a hollow conical center covered with tiny yellow florets surrounded by white to cream colored florets. It takes 2 weeks for chamomile to germinate. Its flowering season is May to August (Kuberappa et al., 2007). German chamomile readily provides syrphids with pollen. *Sinapis alba* is an annual plant that is grown as green manure or as fodder crop. White mustard has yellow flowers. Flowering begins 5 weeks after planting. In the field white mustard blooms from June to August. Furthermore Roman nettle (*U. pilulifera*) and Perennial Rye Grass (*L. perene*) were included in the experiment as non preferred flowering plants (Alhmedi et al., 2007). Roman nettle has green flowers and blooms from May to August. *Urtica pilulifera* is able to produce flowers faster than the common nettle. However the food resources it provides to hoverflies are not well documented. Perennial ryegrass has unbranched inflorescence with spikelets on alternating sides edgewise on the stem. The plant blooms from May to November (Shen et al., 2007). The flowers are pale yellow. In previous study some syrphid species such as *Melanostoma fasciatum* (Macquart) entirely fed on anemophilous pollen from grass families (Hickman et al., 1995). However preference of grass pollen in *E. balteatus* is not well documented. It is probable that perennial ryegrasses provide pollen to hoverflies when other flowering plants are not blooming. Additionally, apart from phacelia, cornflower and perennial rye grass all the other flowering plants were white or yellow and these colors have been shown to be highly attractive to hoverflies (Cowgill et al., 1993). From this study it was expected that hoverflies will feed on flower species that increase their fitness most, as optimal foraging theory predicts (Pyke, 1984). Fitness data were further facilitated by analyzing the protein content of the pollen from the selected flower species.

4.2 Materials and methods

4.2.1 Insect stock culture

Pupae of *E. balteatus* were supplied by Katz Biotech AG, Germany. They were then placed on Petri dishes inside net cages (24°C and 65-80% rh). After emergence adult hoverflies (*E. balteatus*) were kept in flight cages (52 x40x 60 cm). They were fed on grounded bee pollen (supplied by Imkerei Hohmann, Germany) and crystalline sugar bought from supermarket. Water was provided in Petri dishes on moist tissue paper. Broad bean plants (*Vicia faba* L.) infested with *Aphis fabae* Scopoli (Homoptera: Aphididae) were placed in the cages for 8 hours every day to stimulate oviposition. Frequently eggs were transferred to plastic boxes and larvae were fed on *Megoura viciae* (Harris) (Homoptera: Aphidae). This stock provided adults for the experiments.

4.2.2 Experimental setup

Seven flower species were evaluated in this study: phacelia (*Phacelia tanacetifolia* Benth., Hydrophyllaceae Var. *Angelia*), buckwheat (*Fagopyrum esculentum*., Polygonaceae), cornflower (*Centaurea cyanus*., Asteraceae), chamomile (*Matricaria recutita*., Asteraceae), white mustard (*Sinapis alba* L., Brassicaceae) nettle (*Urtica pilulifera* L., Urticaceae) and perennial ryegrass (*Lolium perenne* L., Poaceae). Flowering plants were grown from seeds and maintained in a green house until they bloomed. The experiments were conducted in large net (2 x 2 x 2 m) cages that were placed inside a greenhouse. The temperature ranged between 20 - 28 °C while the relative humidity was between 65 – 85 %. In each net cage a single 3 days old mated naive *E. balteatus* that had been maintained on a similar flower species like the test candidate was released. Inside the experimental cages 6 plants of an individual candidate flower were placed. A broad bean plant (*Vicia faba*) infested with 100 *Aphis fabae* aphids was used to stimulate oviposition in each cage and was replaced daily while the flowering plants were replaced appropriately to provide fresh flowers. In each cage water was provided in a Petri dish on moist tissue paper. The pre-oviposition

duration, female longevity, number of eggs laid and oviposition duration were measured. To determine the size and dry mass of hoverflies a similar set up like previously described was used on the 15th day flower feeding was stopped for 8 hours to avoid having hoverflies with pollen in their crops. The hoverflies were then killed using carbon dioxide and their length was immediately measured from the head to the tip of the abdomen using a microscope (KEYENCE VHX-500FD). The dry mass was recorded using a micro balance (Sartorius MC 5) after drying the specimen in oven at 40 °C for 72 hours. Test on each candidate flower was conducted simultaneously in five cages. Data were analysed using a one way Analysis of Variance (ANOVA) with SAS software version 9.2 (SAS Institute, 2008). Tukey's HSD test was carried out for multiple comparisons.

4.2.3 Chemical analysis of pollen from different flowers

Pollen was collected manually by tapping the flowers and the resulting pollen was collected in a Petri dish. The pollen was then dried immediately in an oven at 30 °C for 72 hours. Pollen from the selected flowers was analysed for protein using the dye- binding method (Bradford, 1976). One mg of oven dried pollen samples was ground using a mortar and a pestle. Nine sample of each flower species were used in this experiment. To facilitate grinding 1 mg of aluminium powder was added in each sample. Each of the samples was moistened with two drops of 0.1 mol / L NaOH. After grinding each pollen sample was retrieved in 1.5 mL of 0.1 mol / L NaOH. The samples were then kept in a refrigerator for a period of 24 hours after which it was then placed in boiling water for 5 minutes and centrifuged for 15 minutes. The solid was separated with supernatant by pipetting. The assay reagent was made by dissolving 100 mg of coomassie Brilliant Blue G250 (supplied by Sigma, Germany) in 50 mL of 95 % ethanol. The solution was then mixed with 100 mL of 85 % (w / v) phosphoric acid. The resulting solution was diluted using distilled water to a final volume of 1 liter. The reagent were then filtered through whatman no. 1 filter paper and then stored in amber bottle at room temperature. Bovine serum albumin (BSA) (supplied by Sigma, Germany) at

a concentration of 1mg/mL (50 mg / 50 mL) in distilled water was used as a stock solution. To make a calibration curve, duplicate volumes of 1, 5, 10, 15, 20, 25, 30, 40 and 50 μ L of 1mg / mL BSA standard solution were pipetted into 100 mm test tubes and made each up to 50 μ L with distilled water. Fifty microlitre of distilled water was pipetted into a further tube to provide the reagent blank. 1, 45 ml of the protein reagent was added to each tube and the contents were then mixed using vortex machine (Vortex genie 2, Scientific industries Inc., USA). Five minutes after mixing, the absorption readings of samples and standards were measured against the blank in 2.5 ml plastic cuvettes at 595 nm on a Beckman Du - 640 spectrophotometer (Ippendorf, Germany). The average weight of the protein was plotted against the corresponding absorbance resulting in a standard curve that was used to determine the protein in unknown samples.

4.3 Results

4.3.1 Effects of different flower species on the fitness parameters of *E. balteatus*

The tested flowers species differently affected the longevity of *E. baltaetus* ($F = 7.24$, $df = 6$, $P = 0.019$). Cornflower greatly enhanced longevity (54 ± 5.5 d) but was not different compared to buckwheat (47 ± 3.5 d). Longevity of hoverflies was 2 fold shorter when they fed on perennial rye grass (19 ± 2.5 d) or nettle (21 ± 1.0 d) compared to cornflower feeding (Figure 1). There was a weak positive correlation between longevity of the hoverflies and the protein content of pollen from different flowers ($r = 0.111118$) (Figure 2).

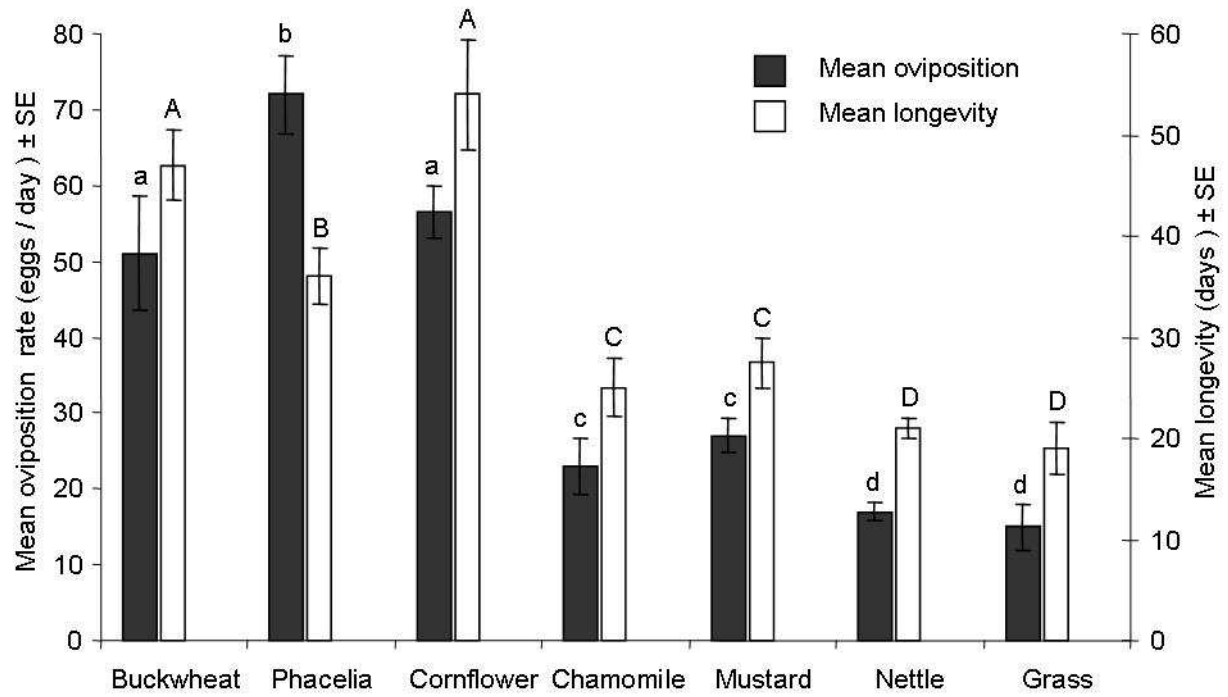


Figure 1. The effect of different flowers on the mean fecundity and longevity of *E. balteatus*. Treatments labeled with same letters are not significantly different (ANOVA, Tukey HSD: $P < 0.05$)

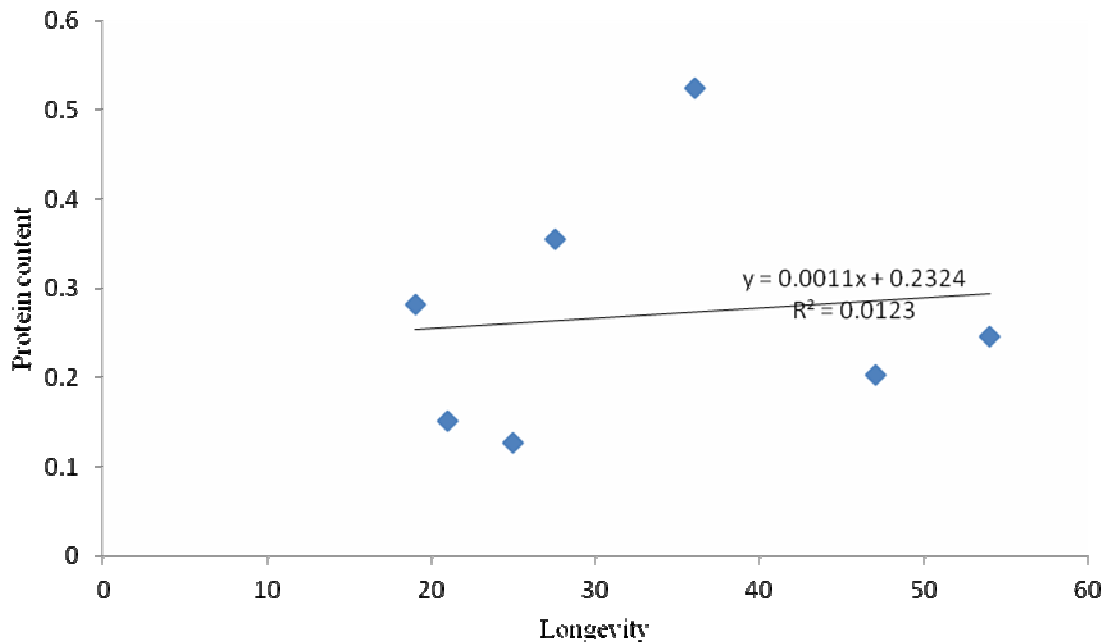


Figure 2. Correlation between the longevity of *E. balteatus* and the pollen protein content of buckwheat, phacelia, cornflower, chamomile, mustard, nettle and rye grass

Overall fecundity was significantly influenced by the flower species ($F = 2.16$, $df = 6$, $P = 0.004$). The highest mean fecundity was realized when hoverflies fed on phacelia flowers (72 ± 5.2 eggs / d) laying more than 4 fold number of eggs compared to nettle (17 ± 1.2 eggs / d) and perennial rye grass (15 ± 2.5 eggs / d) (Figure 1). Fecundity did not differ when hoverflies fed on either buckwheat or cornflower. There was a strong positive correlation between the number of eggs laid and the longevity of hoverflies ($r = 0.993844$) (Figure 3).

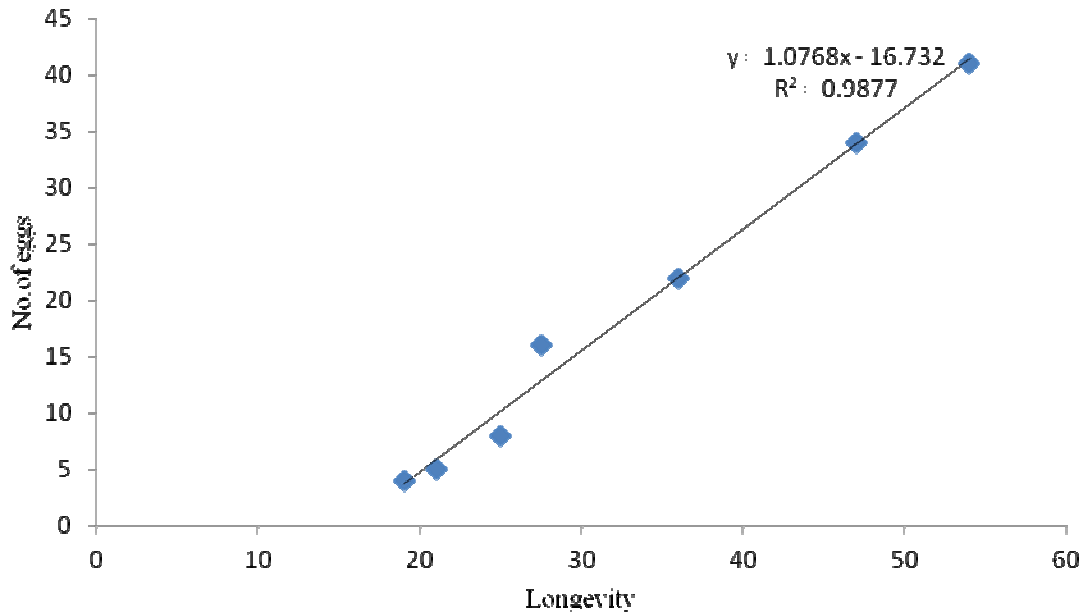


Figure 3. Correlation between the number of eggs laid and the longevity of female *E. balteatus*

Different flower species had a significant effect on pre-oviposition period of *Episyrphus balteatus* ($F = 7.46$, $df = 6$, $P = 0.027$). The shortest pre-oviposition period was recorded when hoverflies fed on phacelia (8 ± 1 d) or mustard (9 ± 0.5 d) being almost 2 fold lower than when hoverflies fed on nettle. Buckwheat, cornflower and perennial rye grass lead to a similar pre-oviposition period (Figure 4). The duration of oviposition was longest when hoverflies fed on cornflower ($F = 1.08$, $df = 6$, $P = 0.036$) while the lowest duration of oviposition occurred when hoverflies fed on perennial rye grass (4 ± 2.2 d) or nettle (5 ± 1.25 d) being more than 8 fold lower than on cornflower (41 ± 5.8 d) (Figure 4).

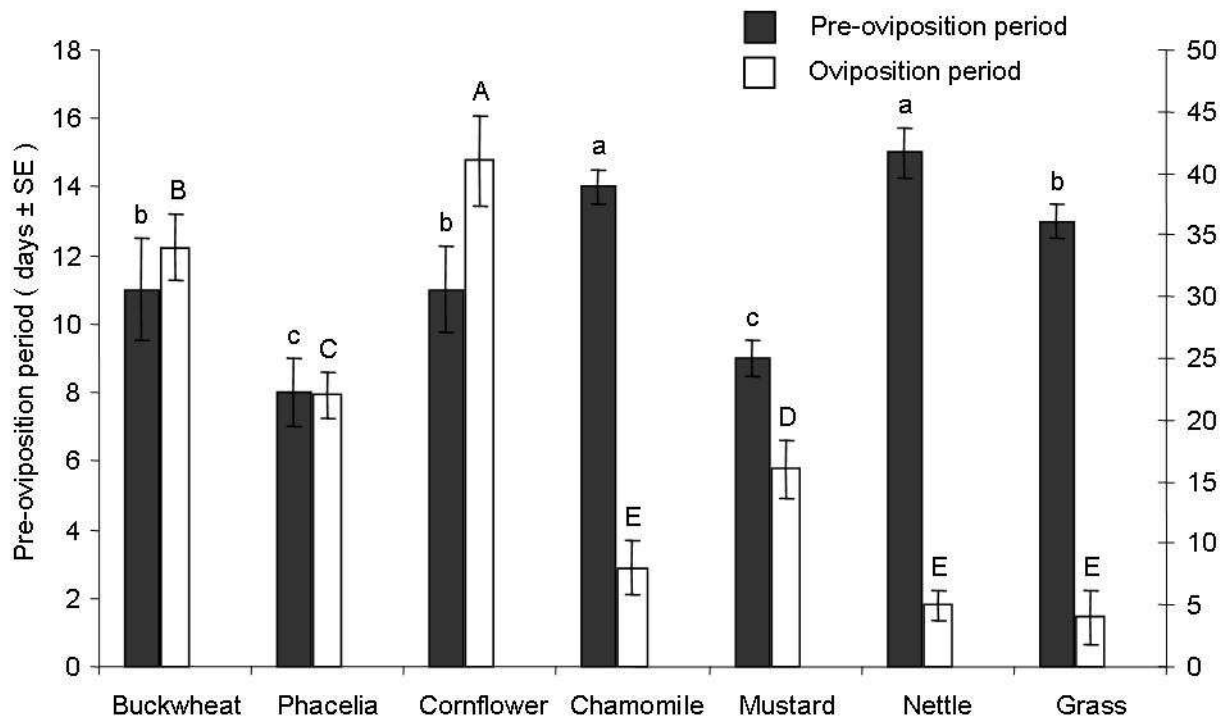


Figure 4. The effect of different flowers on the pre-oviposition and oviposition period of *E. balteatus*. Treatments labeled with same letters are not significantly different (ANOVA, Tukey HDS: $P < 0.05$)

Flower species significantly affected the dry weight of hoverflies ($F = 5.19$, $df = 6$, $P = 0.008$). Feeding on phacelia flowers led to the highest mass (5.84 ± 0.33 g) and was 2 fold more than in rye grass (2.55 ± 0.31 g) or in nettle (2.31 ± 0.11 g). The dry weight of females who fed on buckwheat and cornflower was similar (Figure 5). There was a positive correlation between the dry weight and the number of eggs laid by *E. balteatus* ($r = 0.644404$, Figure 6).

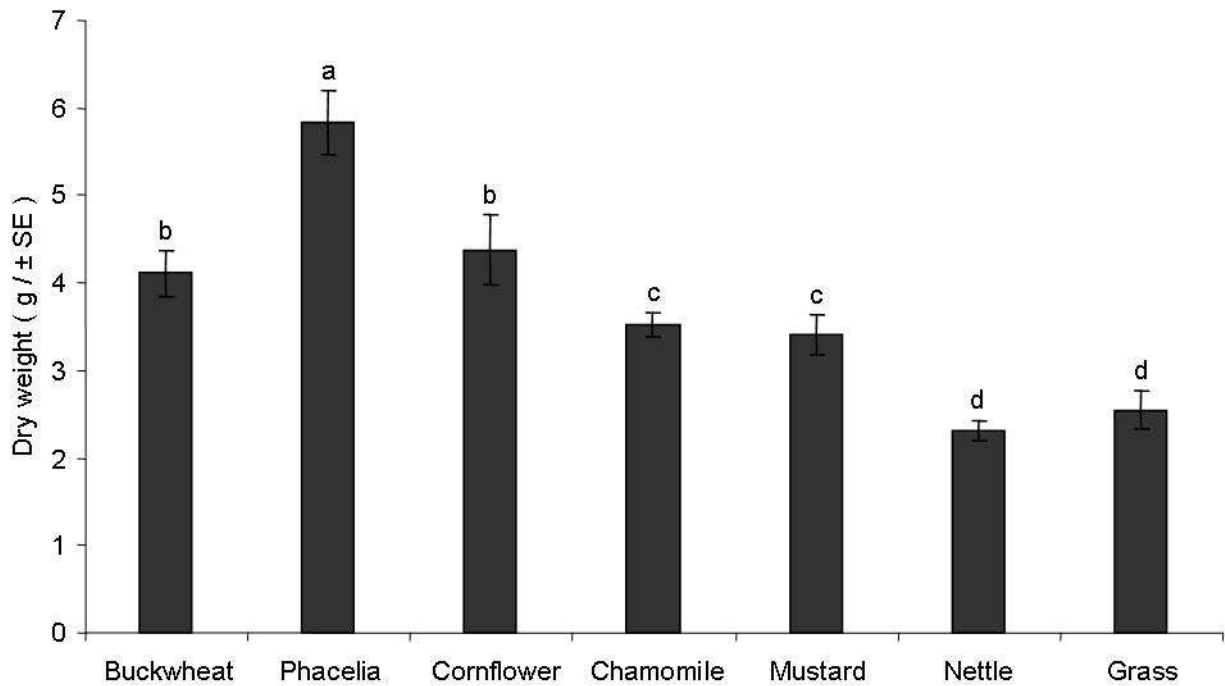


Figure 5. The effect of different flowers on the dry weight of *E. balteatus*. Treatments labeled with same letters are not significantly different (ANOVA, Tukey HSD: $P < 0.05$)

However, female size of the adult hoverflies did not significantly differ between the flower species ($F = 4.62$, $df = 6$, $P = 0.814$). From this study phacelia pollen had the highest protein content ($0.524 \mu\text{g}/\text{mL}$) being 2 fold more than in all the other flower species apart from mustard and grass. Although mustard and rye grass are not highly preferred plants they had higher protein content $0.355 \mu\text{g}/\text{mL}$ and $0.282 \mu\text{g}/\text{mL}$ respectively than the highly attractive buckwheat and cornflower had low protein content (Figure 7). However, there was a weak positive correlation between the number of eggs laid and the protein contents from different flowers ($r = 0.167559$, Figure 8).

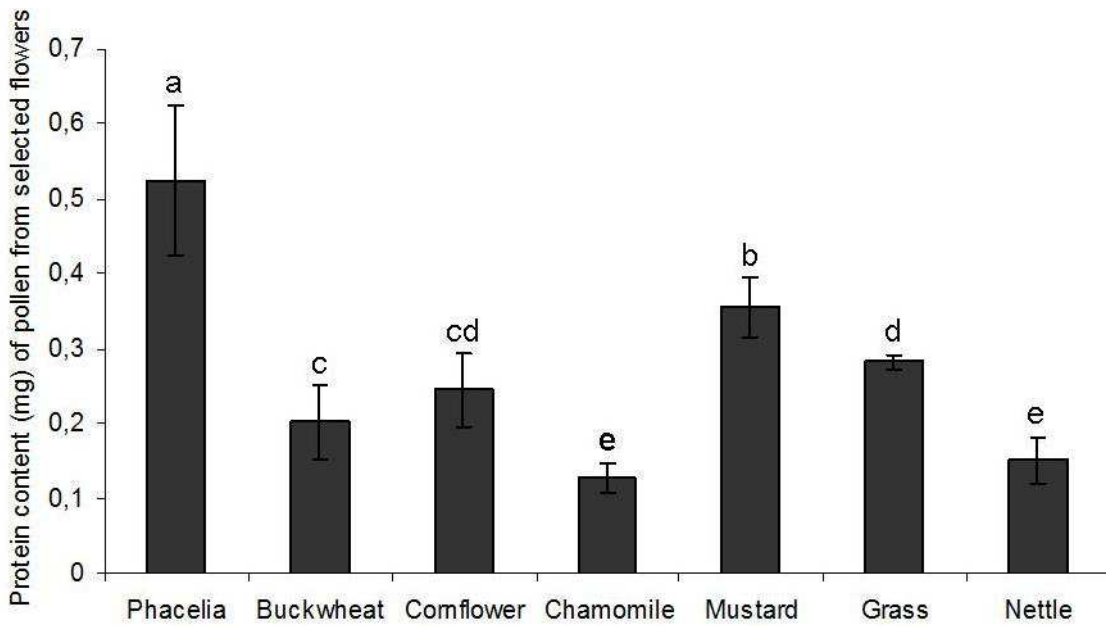


Figure 7. The average protein content of pollen from different flowers species after Bradford analysis. Columns labeled with same letters are not significantly different (ANOVA, Tukey HSD: $P < 0.05$).

n = 9

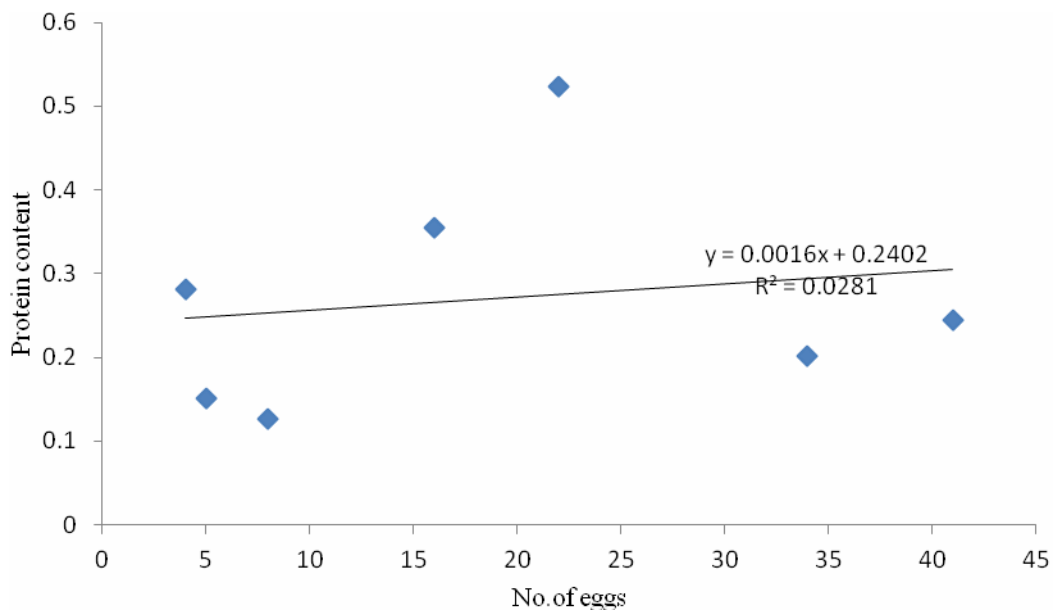


Figure 8. Correlation between the number of eggs laid by *E. balteatus* and the pollen protein content of buckwheat, phacelia, cornflower, chamomile, mustard, nettle and rye grass

4.4 Discussion

Female hoverflies hatch with an immature reproductive system. Therefore during the pre-oviposition period hoverflies need pollen and amino acids for sexual maturation and sustained egg production (Chambers, 1988). Moreover most of the yolk deposition is believed to occur during pre-oviposition period hence the high protein requirements (Haslet, 1989). Studies with bee pollen on the fecundity of hoverflies showed a pre-oviposition period of 11 days (Kahato & Meyhöfer in press). In this study the fastest sexual maturation was realized when hoverflies fed on the flowers of phacelia and mustard. Our findings are in agreement with those from Hickman & Wratten (1996) who concluded that phacelia is an important pollen source for egg maturation of aphidophagous species. Furthermore our pollen protein analysis revealed that phacelia and mustard had the highest level of protein contents. It is probable that access to higher protein content was a contributing factor for the shortened pre-oviposition period in hoverflies. The amount of nectar and pollen availability to adult hoverflies can have a significant effect on egg load (Whittingham, 1991). Phacelia produces pollen that is readily accessible hence hoverflies are able to take large amounts. In contrast, access to pollen with lower protein content as for example nettle and chamomile led to a longer pre-oviposition period, this may be due to slow sexual maturation following lack of enough proteins in hoverflies diet.

From the different plants tested the highest mean fecundity was realized when hoverflies fed on phacelia while perennial rye grass led to the lowest. Phacelia has been documented to be an attractive plant to hoverflies that feed on its pollen (Hickman & Wratten, 1996; White et al., 1995) resulting in maximum and continued egg output (Hogg et al., 2011 a). Other study with flowers phacelia led to more than 2 fold mean oviposition rate than buckwheat (Laubertie et al. (2012)). On the converse, nettle led to lower fecundity and longevity and these results are comparable with the finding of Hickman et al. (1995) who found nettle to be less preferred by syrphids and Alhmedi et al. (2007) who found a weak presence of predatory hoverflies in all plots with nettle strips of

Urtica dioica L species. This may be due to the relatively low nutritional value exhibited by nettle since it has low protein content. Additionally the shape of nettle flowers may have contributed to lower fecundity since it has tubular flowers with the stamens hidden by hooded upper lip thus making pollen access impossible to the short proboscis of *E. balteatus*.

Oviposition duration was highest in hoverflies who fed on cornflower while nettle and perennial rye grass had the lowest. In our study buckwheat promoted longer oviposition duration than phacelia and this agrees with the findings of Laubertie et al. (2012) who also found buckwheat to have almost 2 fold longer oviposition duration than phacelia flowers. Oviposition duration is likely linked to longevity of female hoverflies (Scott & Barlow 1984) and since cornflower has pollen and floral nectar that are accessible to hoverflies this may have given them a higher longevity potential compared to the other flowering plants.

Episyrphus balteatus that fed on cornflower lived the longest being 2 fold more than in chamomile, mustard, nettle and rye grass. However longevity did not differ when hoverflies fed on either buckwheat or cornflower. Both of these plants have been documented to be highly attractive to syrphids (Stephens et al., 1998; Colley & Luna, 2000) and have readily accessible pollen. Morphologically buckwheat has short corollae (Vattala et al., 2006) thus readily available pollen and nectar to the short tongued *E. balteatus* (Gilbert, 1981). Additionally cornflower have floral and extra floral nectaries (Stettmer, 1993) thus providing more nectar compared to other flower species. Richness in sugar content and considerable amount of proteins found in these two flowering plants may account for the higher longevity upon feeding on them. In studies with bees longevity increased also when protein concentration of the pollen increased (Schmidt et al., 1987). From our study though phacelia led to lower longevity than buckwheat and cornflower, this presumably was due to the deep corolla of phacelia (Baggen et al., 1999) thus their nectaries are inaccessible to *E. balteatus*.

In our findings the size of hoverflies was not affected by the tested flower species. Presumably the size of adult hoverflies is determined by the level of feeding during the larval stages. It would be expected that larger larvae result in larger adult syrphids. It was evident from this present study that feeding on pollen and or nectar as an adult does not improve the size of female hoverflies. From this study the dry weight of *E. balteatus* differed according to the flower species. Feeding on phacelia led to the highest dry weight of hoverflies while cornflower and buckwheat had similar effect on dry weight. Phacelia feeding had resulted in the highest fecundity and this may explain the higher dry weight. This finding agrees with Branquart & Hemptinne (2000) who found heavier hoverflies had higher fecundity. On the converse, nettle and perennial rye grass had the lowest dry weight owing to the low egg numbers as a result of poor nutritional value from these two flower species.

Pollen protein contents

Although some authors have refuted the use of crude pollen proteins estimate to measure pollen quality (Todd & Bretherick, 1942), it remains the only chemical component of pollen that influences most of the pollen consumer performance (Roulston & Cane, 2000). Previous analysis of pollen has shown a protein range of 2.5 to 61 % (Buchmann, 1986). In our study phacelia had the highest protein (0.524 $\mu\text{g}/\text{mL}$) content being 3 fold more than in nettle and chamomile. Our findings corroborates those of Torchio et al. (1967) who found phacelia to be rich in protein content (58.9%) though our figures were slightly lower. From this study cornflower had 0.245 $\mu\text{g}/\text{mL}$ protein and are consistent with the findings of Svensson & Wigren (1985) who found levels of 26.2 % protein content in cornflower. Flowers that have been documented to be highly visited such as phacelia buckwheat and cornflower also showed higher protein content level than the non preferred species. Furthermore they have greater impact on important fitness parameters such as survival and fecundity of hoverflies. On the contrary, plants that are not highly visited such as

perennial rye grass and nettle also had lower protein content and consequently led to lower fitness. The optimal foraging theory predicts that organisms must forage in a way that maximizes return. Variances in protein reward may partly explain why phacelia, buckwheat and cornflower are highly preferred flowers by *E. balteatus*. However other factors such as flower morphology have a role to play. Although nettle and grass didn't promote the best fitness, it is imperative that their presence is important in the agricultural fields. The roman nettle is able to bloom in May, a period when flowers are not readily available. Perennial ryegrass is on the other hand blooming from May to November. Most of the suitable flowers are already out of bloom in November. Therefore it is probable that these two plants are able to provide alternative flowers sources when the other suitable and preferred plants are not flowering. Furthermore in agricultural landscapes there is less competition of hoverflies with other insects such as bees to forage on nettle and perennial rye grass. From the flowering plants tested in this study, we conclude that phacelia, buckwheat and cornflower have shown the best potential to increase the fecundity and longevity of *E. balteatus* and their use in conservation biological control target to this species of hoverfly is recommended. In the field, cornflower blooms from June to August. Therefore should be sowed in April since it requires 10 weeks to start flowering so as to provide flowers in June. Buckwheat on the other hand requires 6 weeks to produce flowers. Its blooming period is in July to August and therefore should be sowed in May to provide flowers in July when the corn flowers availability start decline. Last to be sowed should be phacelia since it flowers from July to September and it is therefore able to provide suitable flowers when the other 2 plants are out of season in September. However, testing of flower mixture (Pontin et al., 2006) for example phacelia and buckwheat or with cornflower is highly recommended from this study. This can be done for example by synchronizing the flowering period taking into account their different blooming period.

CHAPTER 5

GENERAL DISCUSSION

Episyrphus balteatus is one of the most specific aphid predators. Their larvae feed on different aphid spp and other soft bodied insects, while adults depend on pollen for reproduction and on nectar as energy source. Unlike this study, most of the investigations have dwelt on the pest-predator relations while ignoring the more important aspect of non prey food. Furthermore, there are temporal changes of the flowering plants throughout the year hence there are fluctuations of the food resources. In some instances such as agricultural monocultures that are characterized by impoverished flower sources, pollen may be a limiting factor in the efficiency of syrphids. A detailed understanding of the foraging decisions and the resulting effects on the fitness of the hoverflies is therefore important in improving the conservation biological control. Such information can be exploited to maximize the fitness of aphidophagous syrphids in the greenhouse and mass rearing programs as well as in the field conditions.

Therefore to study the impact of food resources on the fitness of *E. balteatus*, laboratory experiments were carried out. Pollen as a food resource was manipulated to simulate a limitation and the effects examined. To study the effects of feeding frequency on the fitness of hoverflies experiments with varying frequency on the availability of pollen were undertaken. In addition the effects of different flowers on the fitness of *E. balteatus* were investigated in the laboratory using phacelia (*Phacelia tanacetifolia*), buckwheat (*Fagopyrum esculentu*), cornflower (*Centaurea cyanus*), chamomile (*Matricaria recutita*), white mustard (*Sinapis alba*), nettle (*Urtica Pilulifera*) and perennial ryegrass (*Lolium perenne*).

Our results show that the availability of proteins and carbohydrates in the diet of adult *E. balteatus* during certain physiological periods influence survival and lifetime fecundity. Feeding on pollen only throughout an individual life-time resulted in similar longevity and fecundity as compared to the provision of both pollen and sugar (control). Lack of access to pollen during the pre-oviposition

period or feeding on sugar as the only food source completely impaired the egg laying and considerably reduced longevity. The results of the present study suggest that changes in the availability of food resources have a far reaching effect on egg laying behaviour and survival of syrphids. This is consistent with the findings of (Whittingham, 1991; van Rijn et al., 2006) that the reproductive capacity and longevity of predators such as syrphids is dependent on availability of suitable food resources. In our study when *E. balteatus* had no access to pollen during pre-oviposition period they did not lay any eggs and were unable to compensate even after they accessed it later, although residence times and presumably food intake were increased. In this study, the longevity of *E. balteatus* was hypothesized to be affected by food resource supply. We found that the highest survival was noted when *E. balteatus* was supplied with pollen and sugar throughout the lifespan. There was no significant difference when *E. balteatus* was supplied with pollen only or when pollen was withdrawn during oviposition period. On the contrary, a complete withdraw of pollen during the pre-oviposition period significantly reduced the longevity. In addition, feeding on sugar only elicited the lowest survival rates. From our study a comparison of landing on the food patch has revealed higher landing rates on food patch containing pollen during the pre-oviposition period. Sugar only as a food resource was half fold visited by the syrphids. This is probably because eggs are maturing at this stage and therefore there are higher requirements for protein sources. For example in syrphid *Rhingia campestris* pollen requirements are normally high while yolk deposits are being laid down in eggs (Haslet, 1989). Although carbohydrates are needed at all stages, during pre-oviposition its role is not as important as proteins. On the contrary, landing rates on food patch went down during oviposition period. These results imply that in CBC programs pollen availability should be observed. For instance, in green houses pollen should be provided to hoverflies immediately after hatching or release since they hatch with an immature ovary. This would lead to a continuous maturation of eggs batches therefore to leading to high fecundity and survival.

In studies looking at the effects of feeding frequency on the fitness of hoverflies the control and every 2nd day pollen feeding resulted in the shortest oviposition, the highest fecundity and the longest oviposition duration. Hoverflies lived the longest and had the highest dry weight upon feeding on pollen throughout and on every 2nd day. On the contrary, longevity was lowest when they fed every 5th day. These results suggest the importance of providing pollen to hoverflies throughout its lifespan or alternatively every second day. In greenhouses and in mass rearing programs, these results indicate that ad libitum provision of pollen is not necessary since the same can be provided on every 2nd day with maximum fecundity and longevity.

Among the flowers tested in this study phacelia and mustard led to the shortest pre-oviposition period. The highest mean fecundity was realized when hoverflies fed on phacelia while perennial rye grass led to the lowest. Phacelia has been documented to be an attractive plant to hoverflies that feed on its pollen (White et al., 1995) resulting in maximum and continued egg output. Oviposition duration was highest in hoverflies who fed on cornflower while nettle and perennial rye grass had the lowest. *Episyrphus balteatus* that fed on cornflower lived the longest being 2 fold more than in chamomile, mustard, nettle and rye grass. However longevity did not differ when hoverflies fed on either buckwheat or cornflower. Cornflower has pollen and sugar sources that are easily accessible to hoverflies this may have given them a higher longevity potential compared to the other flowering plants. Feeding on phacelia led to the highest dry weight of hoverflies while cornflower and buckwheat had similar effect on dry weight. Phacelia feeding had resulted in the highest fecundity and this may explain the higher dry weight. This finding agrees with Branquart & Hemptinne (2000) who found heavier hoverflies had higher fecundity. On the protein analysis of pollen, phacelia had the highest protein content being 3 fold more than in nettle and chamomile. Flowers that have been documented to be highly visited such as phacelia buckwheat and cornflower also showed higher protein content level than the non preferred species. Furthermore they have greater impact on important fitness parameters such as survival and fecundity of

hoverflies. Thus for purposes of conservation biological control the utilization of phacelia, buckwheat and cornflower is highly encouraged. This can be done by planting flowering strips from the tested plants next to the crop or in close proximity to agricultural fields to attract hoverflies. From our experimental plants phacelia has already been tested in the field with some studies reporting success (Hickman & Wratten, 1996) thus confirming our results. The other two plants: cornflower and buckwheat should also be tested in field to confirm our results. It would however be interesting to test flower mixtures among them phacelia, buckwheat and cornflower. This would check the existence of a complementary effect in different flowers as also observed by Pontin et al., 2006 while using other flower spp.

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Erklärung

Hierdurch erkläre ich, dass ich meine Dissertation mit dem Titel 'Foraging decision of aphidophagous predators and conservation biological control: A case study with syrphids antagonists' selbständig verfasst und die benutzten Hilfsmittel und Quellen sowie gegebenenfalls die zu Hilfeleistungen herangezogenen Institutionen vollständig angegeben habe.

Die Dissertation wurde nicht schon als Masterarbeit, Diplomarbeit oder andere Prüfungsarbeit verwendet.

Declaration

I Michael Kahato Ngugi declare that this thesis, entitled 'Foraging decision of aphidophagous predators and conservation biological control: A case study with syrphids antagonists' is an original piece of my work conducted by myself and has not been submitted for a degree in any other university.

Michael Kahato Ngugi

Hannover, 31.01.2013

Gottfried Wilhem Leibniz Universität Hannover