WAGENINGEN UNIVERSITY
LABORATORY OF ENTOMOLOGY


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Effects of different prey-infested white cabbage cultivars on the performance and behaviour of the hoverfly *Episyrphus balteatus*

No: 09.19
Name: Rozemarijn Noordam
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Thesis ENT: ENT-80424
1e Examinator: Joop van Loon
2e Examinator: Martine Kos

Contents

Preface

In this report the results of my thesis project at the chairgroup of Entomology are presented and discussed. During four months I have studied the syrphid species *Episyrphus balteatus*, a biological control agent of aphids on white cabbage crops, through greenhouse experiments. The main focus of this thesis project is on the effects of intraspecific variation of the host-plant species on *Episyrphus balteatus* and its prey. This study is part of a larger project in which the effect of this intraspecific variation of white cabbage crops on a number of organisms is investigated, including various herbivorous, predacious and parasitoid insects. This research will than form a baseline to which the effects of genetically modified crops on these same organisms can be compared.

As this was my first thesis, it was the first time I worked on a research project for a longer period of time. During this project I gained more insight in the process of setting up and executing a scientific study and working within the framework of a larger project. The fact that this thesis was part of a larger project really motivated me and challenged me to study as many aspects of the behaviour of *Episyrphus balteatus* as possible.

Acknowledgements

I would like to thank the people at the chairgroup of Entomology for their warm welcome and support. Special thanks to Erik Poelman for providing me with the sleeves for the *Myzus persicae* performance experiment, to Koen Hendriks for helping me take care of the rearing and to Ana Pineda Gomez for being a very valuable source of information on *Episyrphus balteatus*. Finally, I would of course like to thank my supervisors Joop van Loon and Martine Kos for guiding me through this project and for providing the feedback I needed.

Abstract

In this study the effect of intraspecific variation in white cabbage crops on the performance and oviposition preference of *Episyrphus balteatus* (de Geer) and its prey, the aphids *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulzer), was investigated. *Myzus persicae*, like *Brevicoryne brassicae* in a previous study by Broekgaarden et al., 2008, was found to reach a faster population growth on the cultivars Christmas Drumhead and Badger Shipper than on Lennox and Rivera. *Episyrphus balteatus* was also found to be affected by the intraspecific variation of the cabbage crops, producing larger and heavier individuals when reared on aphids feeding on Christmas Drumhead than on Lennox. Next to effects of intraspecific host-plant variation, differences in *E. balteatus* performance were also found between diets on *M. persicae* and *B. brassicae*. When reared on *M. persicae, E. balteatus* individuals were found to have a higher survival rate and a faster development, whereas individuals reared on *B. brassicae* were found to be bigger and heavier as pupae and adults. Two-choice oviposition experiments showed some preferences for one cultivar over the other, though no clear performance-preference correlation was found.

Introduction

Tritrophic interactions

In agro-ecosystems the dynamics between the crop, its herbivores and their natural enemies are very important. None of these trophic levels can be studied without reviewing the tritrophic context. Through adaptations and co-evolutionary processes each of the trophic levels has developed defensive and offensive strategies to protect itself and, in case of the animals, searching techniques to locate their food sources as efficiently as possible and thus optimise their consumption rate *(Price et al., 1980; Ahmad et al., 2004; Vet & Dicke, 2007)*.

The crop

In order to establish themselves in any ecosystem, plants (first trophic level) have developed different ways to defend themselves against herbivores and pathogens. Plant defences can be categorised in two main strategies: resistance traits that aim to reduce herbivory and tolerance traits that aim to reduce the impact of herbivory on plant fitness *(Tiffin, P., 2000)*. To reduce the impact of herbivory on their fitness, plants may avoid herbivores, tolerate their presence by diverting them to eat on non-essential parts of the plant or fasten their own regeneration processes. In order to actively reduce herbivory, plants use direct or indirect defences to repel or kill their natural enemies *(Tiffin, P., 2000)*. Since damage to agricultural crops can not be tolerated and their density will prevent the plants from avoiding the herbivores, the crops have to rely on their direct and indirect defence mechanisms against herbivorous pests. These defences can be either constitutive, which means they are always present, or they can be induced by herbivore feeding *(Zangerl & Rutledge, 1996)*.

Direct defences can be mechanical by use of e.g. thorns, spikes, glandular hairs or trichomes, or chemical by use of so-called secondary metabolites. These secondary metabolites are produced by the plant to kill or repel the herbivores or to reduce the digestibility and are often released when the plant is damaged *(Wink, 1987)*.

Indirect defences are used to recruit natural enemies of the herbivore as the plant's bodyguards. Plants accomplish this by either providing food to these predators and parasitoids, or through the production of herbivore induced plant volatiles (HIPV's), which are emitted in large quantities and are often herbivore-specific *(Dicke, 2009)*.

Herbivores

Herbivores form the second trophic level and thus form the link between the tritrophic interactions. On the one hand they try to optimise their consumption rate and on the other hand they try to limit the encounters with predators and parasitoids *(Karban & Agrawal, 2002)*.

Driven by coevolution between herbivores and their host plants, herbivores have developed a wide array of offensive tactics to increase their feeding. Using both chemical and visual cues, they are capable to discriminate between host plants in order to find optimal feeding and oviposition sites. Furthermore, some species are able to manipulate the plant physiology to increase susceptibility, whereas others e.g.

have found symbionts to improve their feeding efficiency or are able to sequester the plants secondary metabolites to use for their own defence *(Karban & Agrawal, 2002)*.
Herbivores have developed defensive strategies against their natural enemies to avoid detection and to protect themselves from attacks. As their natural enemies can use any chemical information such as faeces, honeydew, pheromones, haemolymph, body scales and secretions of accessory glands to detect their prey *(Vet & Dicke, 1992)*, herbivores have to limit the production of these chemical cues to mask their presence. To avoid attack based on visual cues, herbivores may use mimicry or crypsis to protect themselves. Once under attack, herbivores may protect themselves by e.g. living close together in colonies or through chemical defences produced by themselves or derived from the host plant *(Ahmad et al., 2004)*.

Predators and parasitoids
At the third trophic level, predators and parasitoids mainly use chemical information to locate their prey. Usually, plant volatiles are used to detect the prey from a longer distance as they are emitted in large quantities and are usually easy to detect, while herbivore-derived cues, such as the presence of faeces or pheromones used for intraspecific communication, are used at a shorter distance to locate the prey-items more accurately *(Vet & Dicke, 1992)*.

An understanding of the tritrophic interactions can help to optimise strategies for integrated pest management (IPM), such as intercropping and the use of biological control agents for pest control. This study will therefore focus on such tritrophic interactions while investigating the use of *Episyrphus balteatus* as a biological control agent of two aphid pests on white cabbage crops (*Brassica oleracea convar. capitata var. alba*).

Brassica oleracea

In the family of the Brassicaceae (formerly Cruciferae) several economically important species occur. The species *Brassica oleracea* alone comprises a range of varieties of e.g. broccoli, cauliflower, Brussels sprouts and cabbage. The Brassicaceae are known for their ability to produce glucosinolates as a direct defence mechanism against herbivorous insects. Glucosinolates are plant secondary metabolites, of which at least 120 compounds are known *(Hopkins et al., 2009)*. The glucosinolates, which are normally stored in vacuoles, are released when the plant is attacked by a pest or pathogen *(Pratt et al., 2007)*. Upon release they come in contact with an enzyme called myrosinase that is normally stored in special myrosinase cells in every plant organ *(Hopkins et al., 2009)*. Under the influence of myrosinase, hydrolysis occurs and the glucosinolates are broken down releasing glucose, sulphate and several toxic and pungent compounds such as nitriles, isothiocyanates and oxazolidinethiones *(Hopkins et al., 2009)*. These latter chemicals are the ones that can affect herbivore performance and thus provide a defence mechanism for the plant.

Aphids

Even though the Brassicaceae family has this defence mechanism, the crops still suffer yield losses due to damage by herbivorous pests. One of the most important pests on *Brassica oleracea* crops are aphids. Aphid pests pose a unique problem for the plant as their populations can expand extremely rapidly and they feed through an intercellular route, which is harder for the plant to detect *(Goggin, 2007)*. They are known for their polymorphic lifecycles that can include both sexual and asexual forms as well as winged (alatae) and wingless (apterae) individuals. These different morphs give aphids the ability to adapt to seasonal changes, move to another host and escape overcrowded areas *(Dixon, 1977)*.

Aphids feed by use of stylets, piercing-sucking mouthparts with which they can penetrate the plant epidermis and follow an intercellular route to the sieve elements to feed on the phloem sap. Doing so, they secrete saliva to protect the stylet and prevent phloem clogging *(Goggin, 2007)*. Through manipulation of the plants' physiology, chlorosis, necrosis and leaf curling can occur *(Goggin, 2007)* and viral diseases may be transmitted *(Broekgaarden et al., 2008)*.

Myzus persicae

Based on their different coping mechanisms with respect to glucosinolates, two aphid species were selected for this study: *Myzus persicae* (Sulzer) (the green peach aphid) and *Brevicoryne brassicae* (L.) (the cabbage aphid). Both of these species are a common pest on white cabbage crops, but whereas *B. brassicae* is a specialist on cabbage crops, *M. persicae* is a generalist found on multiple host plant species. *Myzus persicae* is therefore not as well equipped to deal with glucosinolates and tries to avoid high concentrations by feeding on the older leaves of the plant, that have lower glucosinolate concentrations *(Ambrosino et al., 2007)*. Additionally, when it feeds on cabbage crops, *M. persicae* is found to spend a lot of time feeding on xylem, which is thought to be a way for this aphid to flush its system from glucosinolates *(Cole, 1997)*. The glucosinolates are than excreted along with the honeydew *(Cole, 1997; Bridges et al., 2001)*.

Brevicoryne brassicae

Being a specialist, *B. brassicae* has found a way to not only cope with the presence of glucosinolates, but to use them to its advantage by developing its own myrosinase-glucosinolate system. This specialist is able to produce its own myrosinase, which is stored in the head and thorax region *(Bridges et al., 2001)*. When feeding on cruciferous plants, it usually feeds on young tissues with high concentrations of glucosinolates and sequesters the glucosinolates, that are believed to be stored in the heamolymph *(Bridges et al., 2001)*. Thus the myrosinase and glucosinolates are kept separately, similarly to their storage within cruciferous plants. Upon attack by a natural enemy, *B. brassicae* is able to use the glucosinolates for both direct and indirect defences. Tissue damage caused by an attack will lead to hydrolysis of the glucosinolates, exposing the predator to the toxic hydrolytic products *(Francis et al, 2001)*. Additionally, the glucosinolates will act as synergists to the aphid alarm pheromone (*E*)-β-farnesene, alerting the rest of the colony to the presence of natural enemies *(Kazana et al., 2007)*.

Episyrphus balteatus

An important group of aphid natural enemies is the dipteran family Syrphidae. A field study by Nieto et al. (2006) showed that Syrphidae in particular were fast to respond to early stages of aphid colonisation by *B. brassicae* on broccoli corps. These predators are therefore very suitable biological control agents.

This study focusses on the syrphid species *Episyrphus balteatus* (de Geer), the most abundant syrphid species in western Europe *(Tenhumberg & Poehling, 1995)*. Like most other syrphid species, *E. balteatus* is an aphid-specific predator in its larval stages. First instar larvae will only eat 1 or 2 aphids a day, but once they reach the third instar, these larvae are able to consume up to 90 aphids a day, making them very efficient predators *(Tenhumberg & Poehling, 1995)*. These larvae, however, have a very limited mobility and are unable to travel great distances. The choice of a suitable oviposition site is therefore very important. Adult hoverflies use both visual and chemical cues to find a suitable oviposition site. Almohamad et al. (2007) state that females base their choice on several factors:

- the aphid species and its associated chemicals

  A number of studies have investigated the oviposition preference and subsequent larval performance of *E. balteatus* on different aphid species. A study by Putra & Yasuda (2006) showed that *E. balteatus* performance differs between different prey species. Next to this, a study by Almohamad et al. (2007) showed a clear correlation between performance on different aphid species and oviposition preference. One of the most important cues associated with aphid presence, is the aphid alarm pheromone (*E*)-β-farnesene. *Episyrphus balteatus* is known to use this chemical to detect aphid colonies *(Verheggen et al., 2008)*.
- the host plants chemical and physical characteristics associated with the aphid species

  According to the study by Almohamad, et al. (2007), performance of *E. balteatus* is not only affected by the aphid species, but also by the species of host-plant. Females were found to be able to differentiate between different host-plant species and a performance-preference correlation was found. A study by Van Haelen, et al. (2002) also suggests that the aphid-host plant combination may be an important factor. Specialist aphids on a host plant with high levels of glucosinolates were rejected for oviposition more often than a combination of the same aphid species on a host plant with lower glucosinolate levels or the same host plant with a more generalist aphid.
- the aphid colony size and density

  A study by Scholz & Poehling (2000) showed that females prefer to oviposit near larger aphid colonies. Even when the aphids were removed, *E. balteatus* was still attracted to the former aphid colonies by the honeydew that was left behind.
- the age of the female

  Discrimination amongst suitable oviposition sites decreases when the female gets older *(Sadeghi & Gilbert, 2000)*.

This study

Though *E. balteatus* is known to perform differently on different species of host plants and shows a different preference accordingly, the effect of intraspecific host plant variation is not clear. This study will therefore investigate the effect of intraspecific variation of white cabbage crops on the performance and behaviour of *E. balteatus*, using different white cabbage cultivars.

Cultivars

For this study four white cabbage cultivars were selected, namely Badger Shipper, Christmas Drumhead, Lennox and Rivera. These cultivars were previously used in a study by Broekgaarden et al. (2008) on the susceptibility of these cultivars for *B. brassicae*. Based on both field observations and a greenhouse experiment, it was concluded that *B. brassicae* populations were able to develop faster on Badger Shipper and Christmas Drumhead than on Lennox and Rivera. Additionally a genome-wide transcriptomic analysis was performed, through which small differences were found amongst the cultivars. It was concluded that the differences in susceptibility of the cultivars could partly be explained by differences in induced transcriptional changes.

Research questions and hypotheses

The prey performance on the four cultivars may be an important factor for the *E. balteatus* performance. The no-choice greenhouse experiment conducted with *B. brassicae* by Broekgaarden et al. (2008) was therefore repeated for *M. persicae*, to gain insight in the effect of the performance of both aphid species on the performance of *E. balteatus*. For this preliminary experiment, the following research question and hypothesis were formulated:

- Is there a difference in *Myzus persicae* performance between the cultivars?
  <u>Hypothesis:</u>
- Like *B. brassicae*, *M. persicae* will perform better on Christmas Drumhead and Badger Shipper than on Lennox and Rivera.

Next to this preliminary experiment, two experiments were designed to study the larval performance and oviposition preference of *E. balteatus* on these different cultivars. The following research questions and hypotheses were formulated:

1. Is there a difference in the performance of *E. balteatus* between different cultivars when feeding on either *B. brassicae* or *M. persicae*?
   <u>Hypotheses:</u>
- *Episyrphus balteatus* larvae will perform better on *M. persicae* than on *B. brassicae*.
- *Episyrphus balteatus* larvae will perform best on the cultivars that the aphids perform best on.

2. Is there a difference in oviposition preference between different cultivars when infested with *B. brassicae*?
   Hypothesis:
   - *Episyrphus balteatus* females will prefer the cultivars that the *E. balteatus* larvae performed best on.

3. Can a performance-preference correlation be found?
   Hypothesis:
   - A correlation will be found between larval performance and the oviposition behaviour.

Materials and methods

Plant cultivation and insect rearing

Brussels sprouts (*Brassica oleracea convar. oleracea var. gemmifera*) and white cabbage (*Brassica oleracea convar. capitata var. alba*) cultivars Rivera and Lennox (Bejo Zaden BV, Warmenhuizen, The Netherlands) and Christmas Drumhead and Badger Shipper (Centre of Genetic Resources, CGN, Wageningen, The Netherlands) were cultivated in a greenhouse compartment (22 ± 2 °C; 60-70% RH). The plants were provided with SON-T light (500 µmol m^{-2} s^{-1}) for a 16 h day and 8 h night period. The seeds were germinated on peat soil (Lentse potgrond, no. 4; Lent, The Netherlands) and after 10 days the individual seedlings were transferred to peat soil in 1.45-1 pots. All plants were watered daily and were fertilised by applying Kristalon Blauw (Hydro Agri, Rotterdam, The Netherlands) (N-P-K) 19-6-20-3 micro (2.5 mg L^{-1}) to the soil on a weekly basis from the age of 4 weeks onward.

Brevicoryne brassicae was reared on Brussels sprouts and on the four white cabbage cultivars in separate net cages in a greenhouse compartment (22 ± 2 °C; 60-70% RH; 16L:8D). Fresh plants were provided on a weekly basis and the plants were watered every other day.

Myzus persicae was reared on Brussels sprouts and on the four white cabbage cultivars in separate net cages in the same greenhouse compartment (22 ± 2 °C; 60-70% RH; 16L:8D). Fresh plants were provided on a weekly basis and the plants were watered every other day.

Episyrphus balteatus of which pupae were provided by Koppert B.V. (Berkel en Rodenrijs, the Netherlands), was reared in separate net cages in the greenhouse (22 ± 2 °C; 60-70% RH; 16L:8D). The adult hoverflies were provided with water to drink and biological sugar and pollen to feed on. The females were allowed to lay eggs on Brussels sprouts from the *B. brassicae* stock.

***Myzus persicae* performance**

To study the performance of *M. persicae* on the four cultivars a no-choice experiment was conducted based on the experimental set-up of the no-choice experiment on the performance of *B. brassicae* conducted by Broekgaarden et al. (2008). One newborn aphid nymph was placed on each of five older leaves of each 6-week-old white cabbage plant. The plants were set up in a greenhouse compartment (22 ± 2 °C; 60-70% RH; 16L:8D) with 15 biological replicates per cultivar following a randomised design and were watered every other day and fertilised on a weekly basis. Individual plants were covered with sleeves to prevent the aphids from escaping. The nymphs were monitored daily to estimate their development time (number of days from birth until reproduction) and on day 12, the day by which most of the individuals had reproduced, the nymph survival was scored. From day 12 onwards, population size was recorded twice a week.

The statistical analyses were performed using SPSS version 15.0.1. Nymph survival fractions were arcsine square root-transformed and development time data were log-transformed to obtain a normal distribution. The transformed data were analysed using a one-way ANOVA analysis of variance. If a significant difference amongst the cultivars was found, a post-hoc Student-Newman-Keuls test (α=0,05) was used to compare mean differences of the different cultivar treatments. Population size was corrected for the number of nymphs that started the population and the corrected data were log-transformed. A general linear model (GLM) repeated measures ANOVA was used to assess the impact of different cultivars on the number of *M. persicae* individuals over time. Day was considered a within-subjects factor and cultivar a between-subjects factor. If a significant cultivar effect was found, the mean differences were compared using a LSD test (α=0,05).

Episyrphus balteatus **performance**

Females in the stock were allowed to lay eggs on a fresh Brussels sprouts plant from the *B. brassicae* or *M. persicae* stock for 24 h. After hatching, individual larvae of approximately one day old were placed in a Petri-dish (ø 9 cm) in a greenhouse compartment (22 ± 2 °C; 60-70% RH; 16L:8D) with some fresh leaf fragments of the treatment-cultivar and filter paper on the bottom. The larvae were provided with either *B. brassicae* or *M. persicae* prey-items reared on one of the four cultivars, thus creating eight different treatments. All eight treatments consisted of 40 biological replicates which were set up in a randomised design. Every day new prey-items were added *ad libitum* and new leaves were provided as food for the prey and shelter for the larvae.

During the experiment the survival rate, development time from the time of hatching till pupation and adult-emergence, and the pupal weight were measured. After emergence the adults were collected and frozen, after which the gender, head width and wing length were measured using a light microscope with a camera and the computer program Image Focus (version 1.0). Lastly, the adults were kept in a stove at 80 ˚C for four days after which the dry weight was determined. This dry weight and the fresh weight of the pupae were used as indicators of female fecundity, as a study by Branquart and Hemptinne (2000) has shown a clear relationship between weight and lifetime fecundity in *E. balteatus*.

The survival data were analysed with a logistic regression analysis by use of GenStat the 11th edition. If a significant difference was found amongst the cultivars, a post-hoc Student-Newman-Keuls test was used to analyse the mean differences. All other data were analysed with a GLM univariate analysis of variance by use of SPSS version 15.0.1. The data on pupal weight were log-transformed to achieve normal distribution. As weight and sizes may differ between genders, sex was added to the model as a fixed factor. Any significant differences amongst cultivars were further analysed with a post-hoc Student-Newman-Keuls test.

Episyrphus balteatus **oviposition preference**

In two-choice experiments, approximately 2 to 3 week old females of *E. balteatus* were individually placed in net cages (67x50x75cm) in a greenhouse compartment (22 ± 2 °C; 60-70% RH; 16L:8D) with two seven week old plants of different cultivars. Four leaves of these plants were infested with 100 *B. brassicae* individuals four days prior to the experiment. Females were allowed to lay eggs for 24 hours, after which the number of eggs on each plant was counted. Next to this, the total number of aphids on the plant was estimated and the stem and leaf biomass were determined, to provide a rough insight in the morphological differences amongst the cultivars. As the experiment was conducted only with *B. brassicae*, a total number of 6 two-choice experiments were conducted, namely one experiment for each combination of cultivars. Each two-choice experiment was replicated 14 times. A control experiment was conducted 9 times using the cultivars Rivera and Christmas Drumhead. During this control experiment the females were given the choice between uninfested and infested plants. Again the total number of eggs after 24 hours was recorded.

For both the control and real experiment, the data on the number of eggs were analysed per combination with a Wilcoxon signed ranks test by use of SPSS version 15.0.1. For the real experiment the data on the number of aphids per plant per cultivar was analysed with a Kruskal-Wallis test and, if necessary, a post-hoc Student-Newman-Keuls test. Finally, the data on the stem and leaf biomass were log-transformed to achieve normal distribution and were compared amongst cultivars by use of a one-way ANOVA analysis with a post-hoc Student-Newman-Keuls test.

Results

Myzus persicae performance

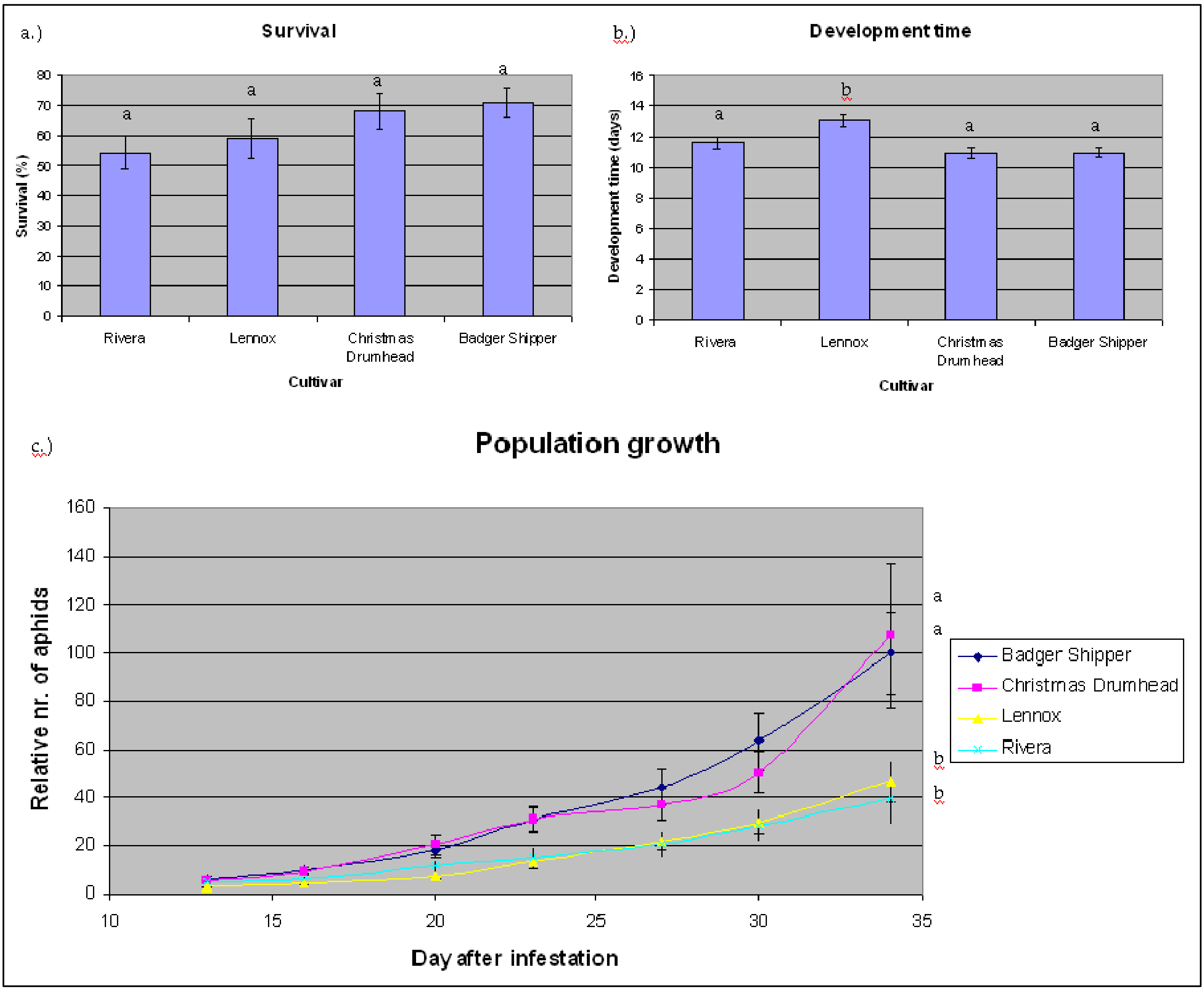


Figure 1: *Results of the no-choice experiment on the performance of Myzus persicae on the four different Brassica oleracea cultivars.*
a.) Nymph survival in percentages after 12 days of feeding.
b.) Nymph development time from birth till first reproduction in days.
c.) Relative aphid population growth per plant till 34 days after first infestation.
Error bars with the same letter represent no significant difference was found (α=0,05).

The results of the no-choice performance experiment of *M. persicae* on the four white cabbage cultivars are shown in Figure 1. The recorded nymph survival after 12 days was not found to differ significantly amongst the cultivars (ANOVA, F=1.886, P=0.142, Figure 1a), though the nymphs on Badger Shipper and Christmas Drumhead showed a slightly higher survival rate than those on Lennox and Rivera.

The development time (the time from birth till first reproduction) did differ significantly amongst the cultivars (ANOVA, F=6.921, P=0.000, Figure 1b). Significant (SNK, P<0.05) contrasts were found between nymphs reared on Lennox with a development time of 13.05±0.4 days and nymphs reared on Badger Shipper, Christmas Drumhead and Rivera with a development time of respectively 10.98±0.3, 10.94±0.4 and 11.58±0.4 days. Finally, the GLM based on repeated measures showed a significant increase in the number of aphids over time (F=337.197, P=0.000) and a significant difference in the relative population growth amongst the cultivars (F=5.553, P=0.002, Figure 1c). Significantly (LSD<0.05) faster growth rates were found on Badger Shipper and Christmas Drumhead compared to Lennox and Rivera. At the end of the experiment, the relative colony sizes per plant were about 2.4 times larger on Badger Shipper and Christmas Drumhead than on Lennox and Rivera.

Episyrphus balteatus performance

	Cultivar		*Aphid species*		*Interaction*		*Sex*	
Survival	*Dev.*	*P*	*Dev.*	*P*	*Dev.*	*P*	*Dev.*	*P*
Survival birth-pupation	2.745	0.455	35.406	**<0.001**	0.725	0.875	-	-
Survival birth-emergence	5.165	0.273	11.414	**0.004**	2.831	0.544	-	-
Development time	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Time birth-pupation	6.970	**0.000**	242.226	**0.000**	6.943	**0.000**	-	-
Time birth-emergence	12.082	**0.000**	267.870	**0.000**	5.972	**0.000**	-	-
Weight	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Pupa fresh weight	8.631	**0.000**	6.934	**0.009**	2.693	**0.047**	-	-
Adult dry weigh	17.218	**0.000**	18.218	**0.000**	4.057	**0.008**	17.277	**0.000**
Sizes	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Wing length	7.521	**0.000**	0.918	0.340	2.673	**0.049**	0.429	0.514
Head width	9.707	**0.000**	1.803	0.181	5.148	**0.002**	45.361	**0.000**

Tabel 1: *Results of the logistic regression and GLM univariate analyses of the Episyrphus balteatus performance data based on their survival, development time, weight and sizes.*
For the logistic regression analyses the deviance is shown to measure the fit of the overall model and for the GLM univariate analyses the F-value is displayed to show the ratio of variance between groups and variance within groups. The bold printed numbers show significant P-values (α≤0.05).

Survival

In table 1 an overview is given of the results from the statistical analyses of all parameters in the *E. balteatus* performance experiment. The survival analysis showed no significant differences amongst treatments with different cultivars, but did show a significant difference between the treatments with the two aphid species (Table 1; Appendix, Graph 1a & b). Both the survival analysis from birth till pupation and the analysis from birth till adult emergence showed a higher survival rate of larvae fed with *M. persicae* than those fed on *B. brassicae*. Of *E. balteatus* fed with *M. persicae* 88.8±2.7% survived till pupation and 81.6±3.2% survived till adult emergence, whereas of *E. balteatus* fed with *B. brassicae* only 57.8±4.3% were alive till pupation and 53.1±4.3% survived till adult emergence.

Development time

The development time of *E. balteatus* was found to differ significantly between the aphid species and amongst the cultivars (Table 1). It took the larvae fed with *M. persicae* 11.87±0.17 days to reach pupation and 18.45±0.18 days to reach adult emergence, whereas larvae fed with *B. brassicae* needed 15.82±0.23 days to reach pupation and 22.39±0.20 days to emerge as adults. The development time till pupation and the development time till adult emergence showed the same cultivar differences. In figure 2 the differences in development time till adult emergence amongst the cultivars are shown (for graphs development time till pupation, see Appendix, Graph 2a & b).

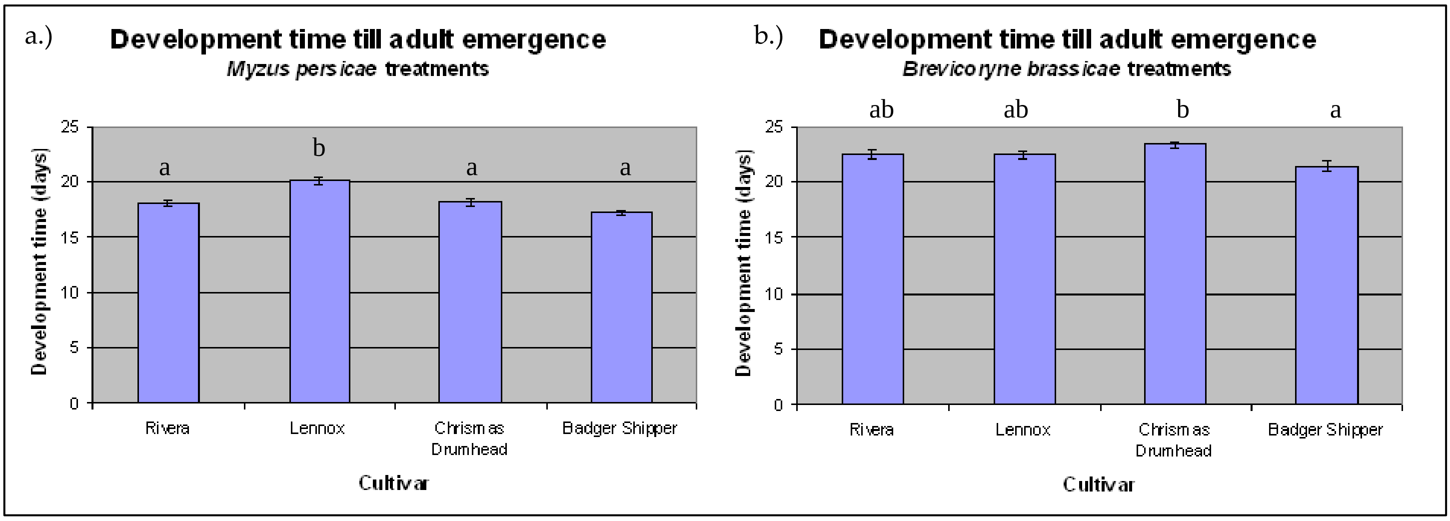


Figure 2: *Differences in the development time of E. balteatus amongst the cultivars.*
a.) Differences within the M. persicae treatments.
b.) Differences within the B. brassicae treatments.
Error bars with the same letter represent no significant difference was found (α=0.05).

Amongst the *M. persicae* treatments the larvae on Lennox developed significantly (SNK<0.05) slower than larvae on the other cultivars, needing 20.13±0.38 days to emerge as an adult in stead of 17.16±0.18, 18.13±0.32 and 18.07±0.27 days for Badger Shipper, Christmas Drumhead and Rivera respectively. The treatments with *B. brassicae* showed other cultivar differences. *Episyrphus balteatus* needed a longer development time of 23.33±0.32 days on Christmas Drumhead compared to only 21.42±0.45 days on Badger Shipper.

Weight

The analysis of the pupal fresh weight and adult dry weight again showed differences between the two aphid species as well as differences amongst the cultivars (Table 1). With adult dry weight, the sex of the animal was found to cause a significant difference as well (Table 1), which showed the males to be slightly heavier than the females. *Episyrphus balteatus* with a diet of *M. persicae* were found to be lighter than those with a diet of *B. brassicae*. The pupa weight was 20.95±0.38 mg and the adult dry weight was just 3.18±0.07 mg when reared on *M. persicae* compared to 22.28±0.48 and 3.50±0.10 mg on *B. brassicae*. The contrast in the cultivar differences was most explicit when analysing the adult dry weight (Figure 3). Data on pupa fresh weight showed the same cultivar differences, though slightly less significant (Appendix, Graph 3a & b).

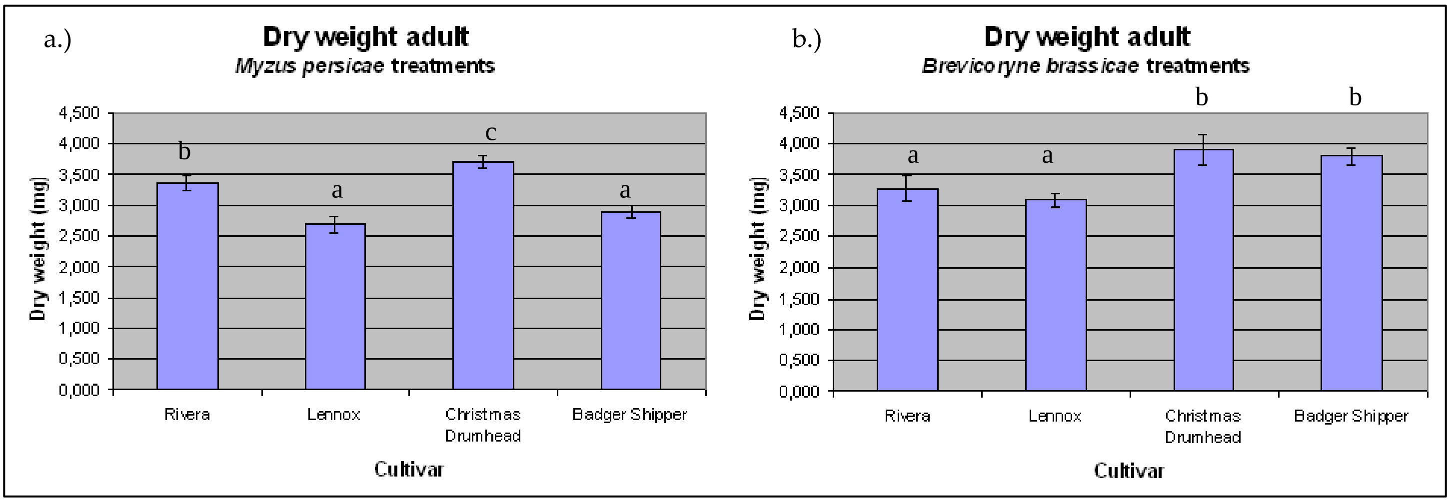


***Figure 3:** Differences in the adult dry weight of E. balteatus amongst the cultivars.*
a.) Differences within the M. persicae treatments.
b.) Differences within the B. brassicae treatments.
Error bars with the same letter represent no significant difference was found (α=0.05).

Within the *M. persicae* treatments *E. balteatus* individuals reared on Christmas Drumhead were found to be heaviest with an adult dry weight of 3.71±0.10 mg. Individuals reared on Rivera were found to be slightly lighter with a dry weight of 3.36±0.12 mg, and individuals reared on Badger Shipper and Lennox were found to be the lightest with weights of respectively 2.90±0.10 and 2.68±0.14 mg.

Amongst the *B. brassicae* treatments a contrast was found between individuals reared on Christmas Drumhead and Badger Shipper with dry weights of respectively 3.91±0.24 and 3.81±0.14 mg, and those reared on Lennox and Rivera with dry weights of 3.08±0.12 and 3.27±0.21 mg.

Size

Lastly, both the head width and wing length of the adult *E. balteatus* individuals showed significant differences amongst the cultivars (Table 1; Figure 4). Head width also seemed to differ among sexes, as the males had slightly larger head widths than the females.

The differences in head width and wing length amongst the cultivars were found to be similar. Within the *M. persicae* treatments, individuals reared on Christmas Drumhead and Rivera were found to have larger head widths (2811±23 and 2799±30 µm respectively) and wing lengths (6563±64 and 6563±83 µm) than those reared on Badger Shipper and Lennox (head width: 2643±27 and 2639±45 µm; wing length: 6249±66 and 6183±81 µm). Within the *B. brassicae*, individuals reared on Christmas Drumhead were found to have larger head widths (2807±41 µm) and wing lengths (6634±79 µm) than those reared on Lennox and Rivera (head width: 2670±27 and 2660±57 µm; wing length: 6302±62 and 6339±109 µm).

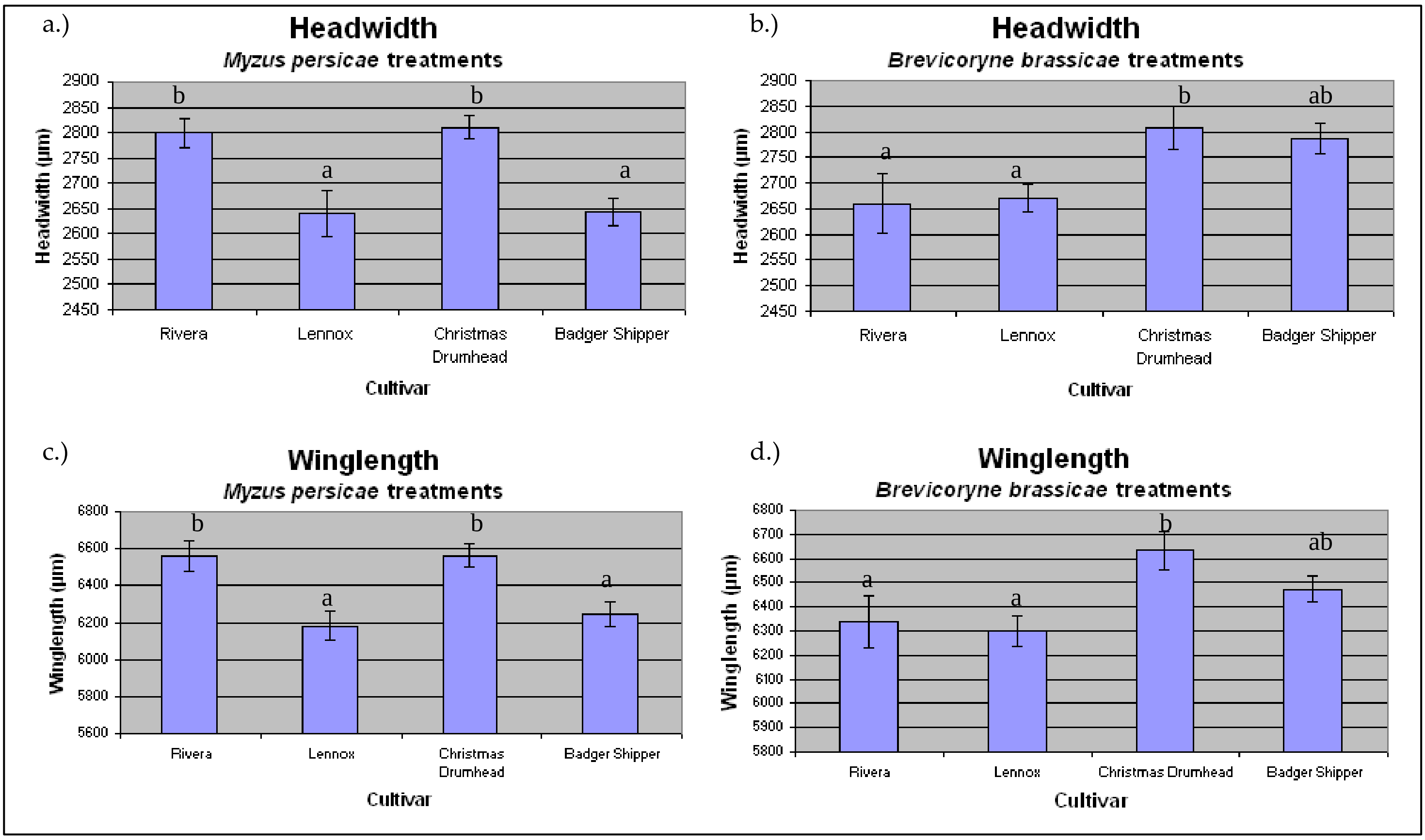


Figure 4: *Differences in the head width and wing length of E. balteatus amongst the cultivars.*
a.) Differences in head width within the M. persicae treatments.
b.) Differences in head width within the B. brassicae treatments.
c.) Differences in wing length within the M. persicae treatments.
d.) Differences in wing length within the B. brassicae treatments.
Error bars with the same letter represent no significant difference was found (α=0,05).

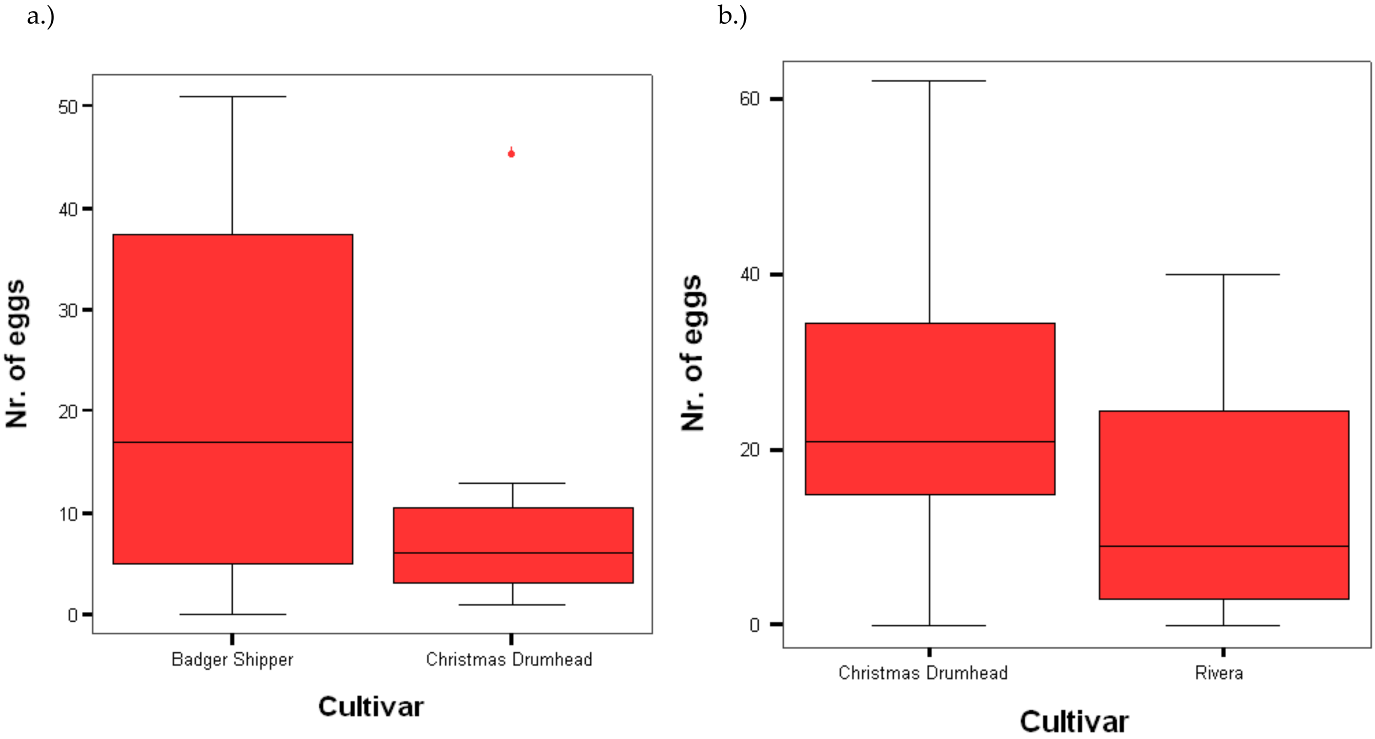
Episyrphus balteatus oviposition preference

The control experiment confirmed that *E. balteatus* was indeed attracted to the presence of aphids. Significantly more eggs were laid on the aphid-infested plants than on the non-infested plants when using both Christmas Drumhead (Wilcoxon, Z=-2.023, P=0.043) and Rivera (Wilcoxon, Z=-2.201, P=0.028) (Appendix, Graph 4a & b).

When testing oviposition preferences between cultivars, only the cultivar combinations Badger Shipper versus Christmas Drumhead and Christmas Drumhead versus Rivera showed a significant preference (Figure 5). Females seemed to prefer Badger Shipper over Christmas Drumhead (Wilcoxon, Z=-2.142, P=0.032) and seemed to prefer Christmas Drumhead over Rivera (Wilcoxon, Z=2.245, P=0.025). For none of the other cultivar combinations a preference was shown (Appendix, Graph 5).

The leaf and stem biomass of the plants used during the real oviposition experiment were also analysed. Both the weight of the stem and leaves were found to be significantly different amongst the cultivars (stem: ANOVA, F=11.247, P=0.000; leaf: ANOVA, F=17.737, P=0.000; Appendix, Graph 6a & b)). These data were than used to

Figure 5: *Results of the oviposition preference experiment for the cultivar combinations Badger Shipper versus Christmas Drumhead (a.) and Christmas Drumhead versus Rivera (b.), for which E. balteatus females were found to show a significant preference for on cultivar over the other.*



determine the ratio between leaf and stem biomass. A clear contrast (SNK<0.05) was found amongst the cultivars, as is presented in figure 6. Badger Shipper was found to have the largest leaf mass per unit of stem, followed by Christmas Drumhead, Rivera and Lennox.

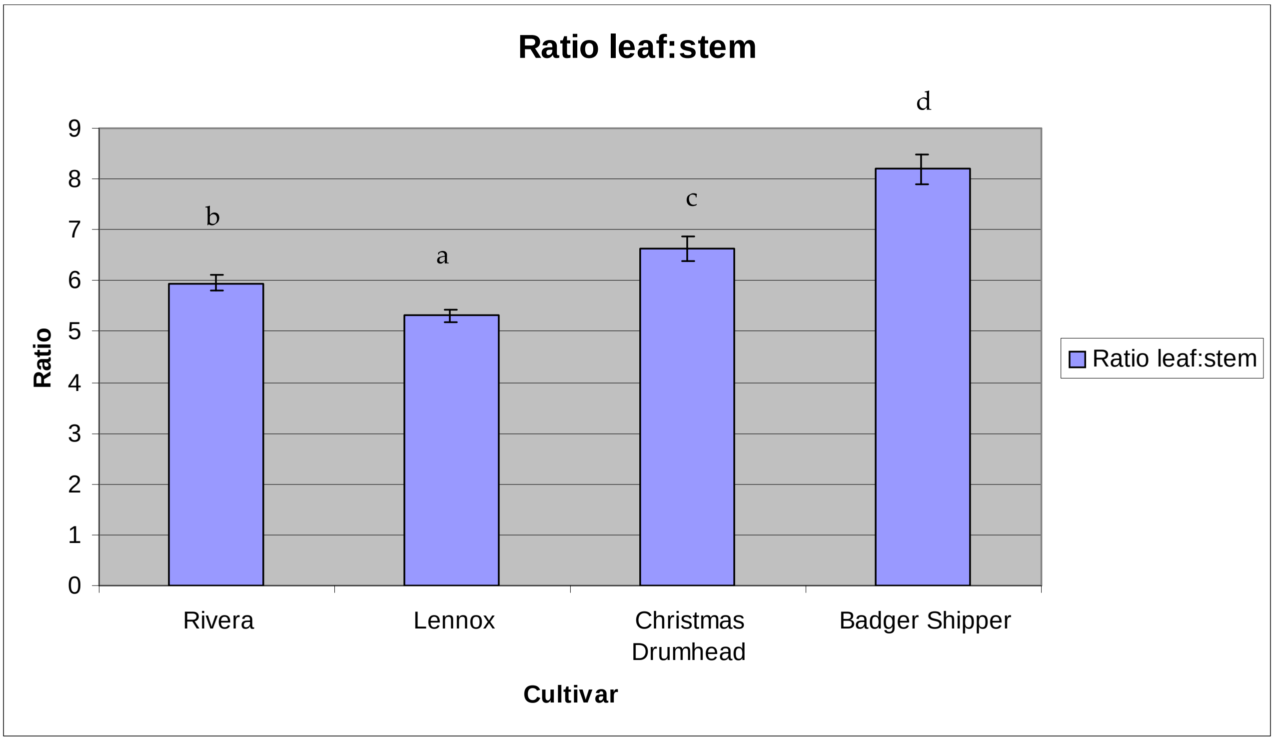


Figure 6: *Differences in leaf:stem ratio amongst the cultivars as determined during the oviposition experiments. Error bars with the same letter represent no significant difference was found (α=0.05).*

Discussion

As mentioned in the introduction, the behaviour of a predator like *E. balteatus* can only be explained by placing it in a tritrophic context. To answer the question on how intraspecific variation of the host-plant can affect the performance and behaviour of this predator, one should not only consider the direct effects of intraspecific host-plant variation on *E. balteatus* performance, but should also investigate the effect of this intraspecific variation on its prey, as the indirect effects on the quality of its prey will probably be even more important for the *E. balteatus* performance. The no-choice greenhouse experiment on the performance of *M. persicae* amongst different cultivars showed the same differences in cultivar susceptibility as were previously shown for the performance of *B. brassicae*. Both species reach a faster population growth on the cultivars Christmas Drumhead and Badger Shipper than they do on Lennox and Rivera.

Since the aphids perform better on Christmas Drumhead and Badger Shipper, one would expect that *E. balteatus* will perform better on these cultivars as well, as the quality of its prey is likely to be better. This was investigated in the *E. balteatus* performance experiment along with the hypothesis that *E. balteatus* will perform better on a diet of *M. persicae*, since this aphid is a generalist and does not posses the ability to sequester glucosinolates.

Indeed, clear differences were found in the performance of *E. balteatus* when comparing the diets on *M. persicae* and *B. brassicae*. *Episyrphus balteatus* had a higher survival rate when fed with *M. persicae* and needed less time to reach its pupa and adult stadium. Contrary to the hypothesis, however, *E. balteatus* individuals reared on *B. brassicae* were found to develop into heavier and larger adults, suggesting a higher fecundity potential *(Branquart & Hemptinne, 2000)*. These findings suggest that *E. balteatus* is susceptible to the glucosinolate defence mechanism of *B. brassicae* during its development. Due to the prolonged development time, however, *E. balteatus* individuals reared on *B. brassicae* may consume more aphids during their larval stages than those reared on *M. persicae*. This would explain the weight and size differences between the two treatments.

An effect of the intraspecific variation of the host-plant on the performance of *E. balteatus* was also found for the parameters of development time, weight and size. When looking at the difference in development time, no clear pattern is shown. Within the *M. persicae* treatments, *E. balteatus* was found to develop longer on Lennox compared to the other cultivars, whereas the *B. brassicae* treatment only shows a difference between individuals reared on Christmas Drumhead and Badger Shipper. The differences in weight and sizes however, show a clear contrast between individuals reared on Christmas Drumhead and those reared on Lennox. *Episyrphus balteatus* reared on Lennox is found to be light and small for all parameters, whereas the rearing on Christmas Drumhead produced heavy and large individuals. This pattern is consistent with the assumption that *E. balteatus* performs better on cultivars that are more susceptible to aphid infestation.

As the performance of *E. balteatus* on Christmas Drumhead and Lennox seems to be the most contrasting, one would suspect a clear oviposition preference of *E. balteatus* females for Christmas Drumhead. Unfortunately no such preference was found. In fact, the only preferences found were those of Christmas Drumhead over Rivera and Badger Shipper over Christmas Drumhead. Though these preferences suggest that Badger Shipper would be highly preferred over Rivera, only a slight and non-significant preference was found.

While the preference of Christmas Drumhead over Rivera could be explained by a better performance of *B. brassicae*, the preference for Badger Shipper over Christmas Drumhead can not be explained that way. Thus, no clear preference-performance correlation is found. The largest contrast between Badger Shipper and Christmas Drumhead was found in the leaf biomass. A possible explanation for the preference of Badger Shipper may therefore be that Badger Shipper provides a larger leaf surface area and thus more opportunities to lay eggs.

Overall, the oviposition experiments were only replicated 10 to 12 times if the times no eggs were laid are disregarded. This experiment should therefore be repeated in further research to determine whether these females do in fact prefer one cultivar over the other and why. This experiment should than also include a chemical analysis of the plant-volatiles and the chemicals produced by the aphid, as well as a further investigation in the morphological plant variables, such as the leaf surface area and the presence of a wax layer. Apart from the two-choice experiments, four-choice experiments could be conducted to gain more insight in the effect of intraspecific crop variation on the oviposition behaviour of *E. balteatus*.

Though further research is needed to explain the findings of this study, it is clear that intraspecific variation of host plants not only affects the performance of herbivores, but can even influence the performance of predators on the third trophic level. Especially generalist predators like *E. balteatus* may be affected by this intraspecific variation, as they are more vulnerable to host-plant and prey specific defence mechanisms than more specialised predators and parasitoids. The introduction of a new more aphid-resistant cabbage cultivar or a genetically modified crop will therefore not only affect the population dynamics of the aphids, but is also likely to affect the performance ánd the behaviour of *E. balteatus*.

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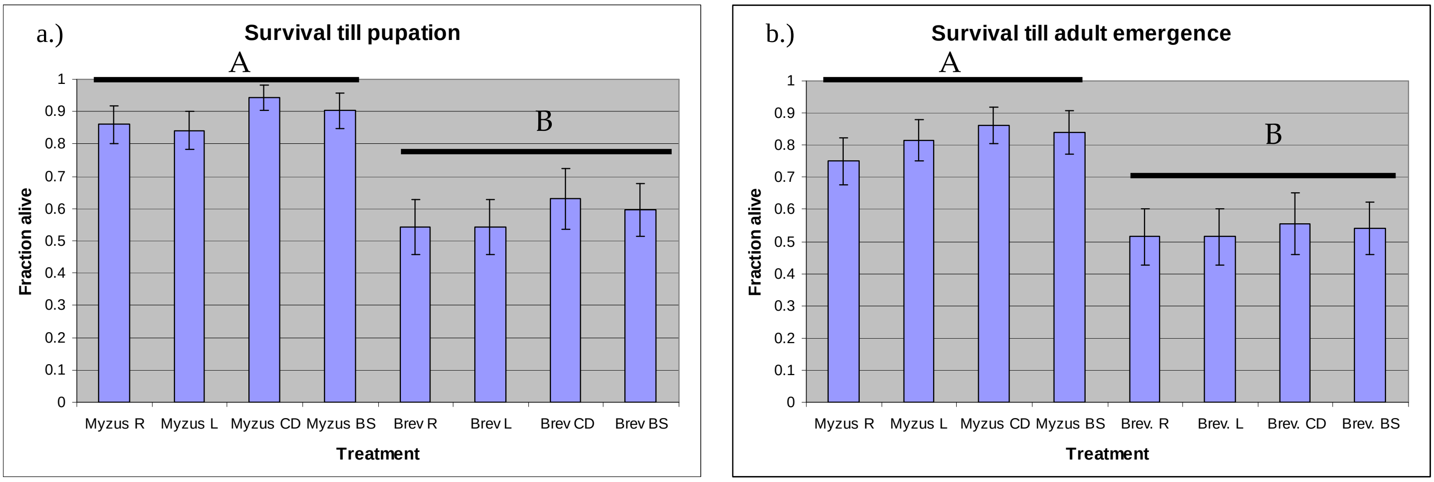
Appendix

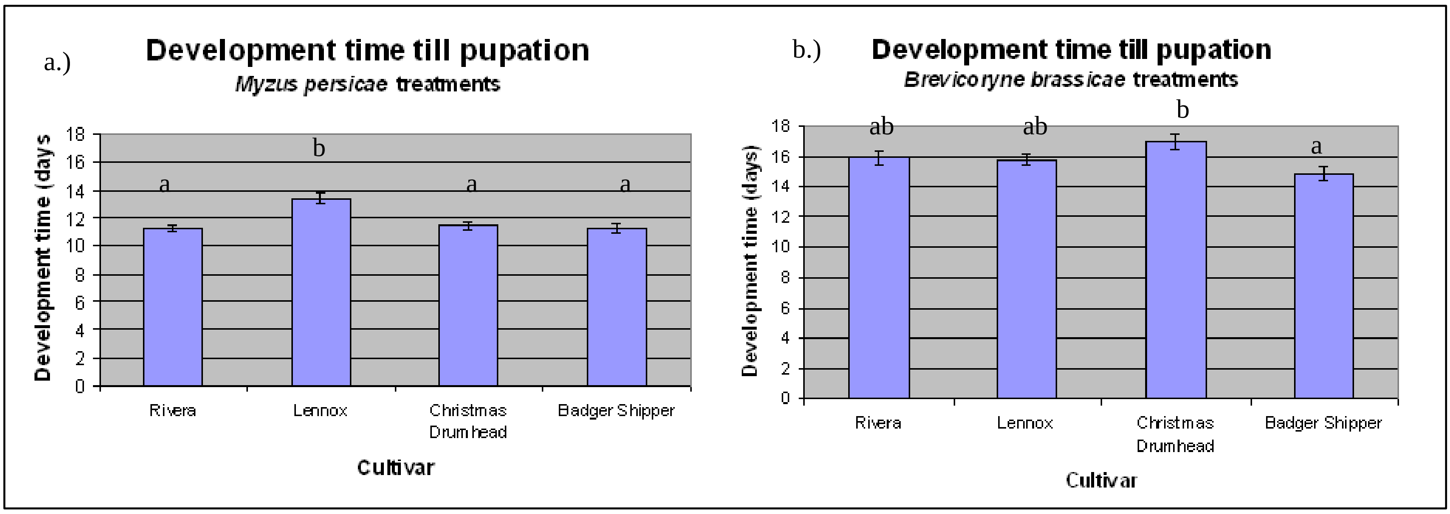
Graphs *Episyrphus balteatus* performance

Survival analysis:



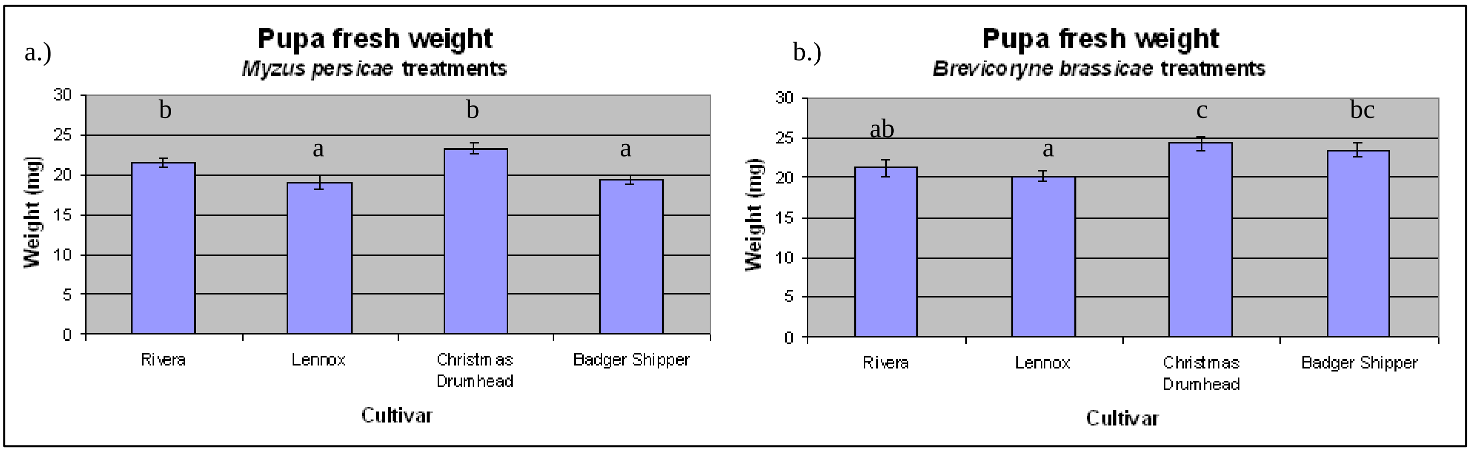
Graph 1: *Results survival analysis of E. balteatus reared on four different cultivars and two different aphid species.*
a.) Survival from birth till pupation
b.) Survival from birth till adult emergence
The capital letters represent the significant (α=0.05) difference in E. balteatus survival between the two aphid species.

Development time:



Graph 2: *Differences in the development time from birth till pupation of E. balteatus amongst the cultivars.*
a.) Differences within the M. persicae treatments.
b.) Differences within the B. brassicae treatments.
Error bars with the same letter represent no significant difference was found (α=0.05).

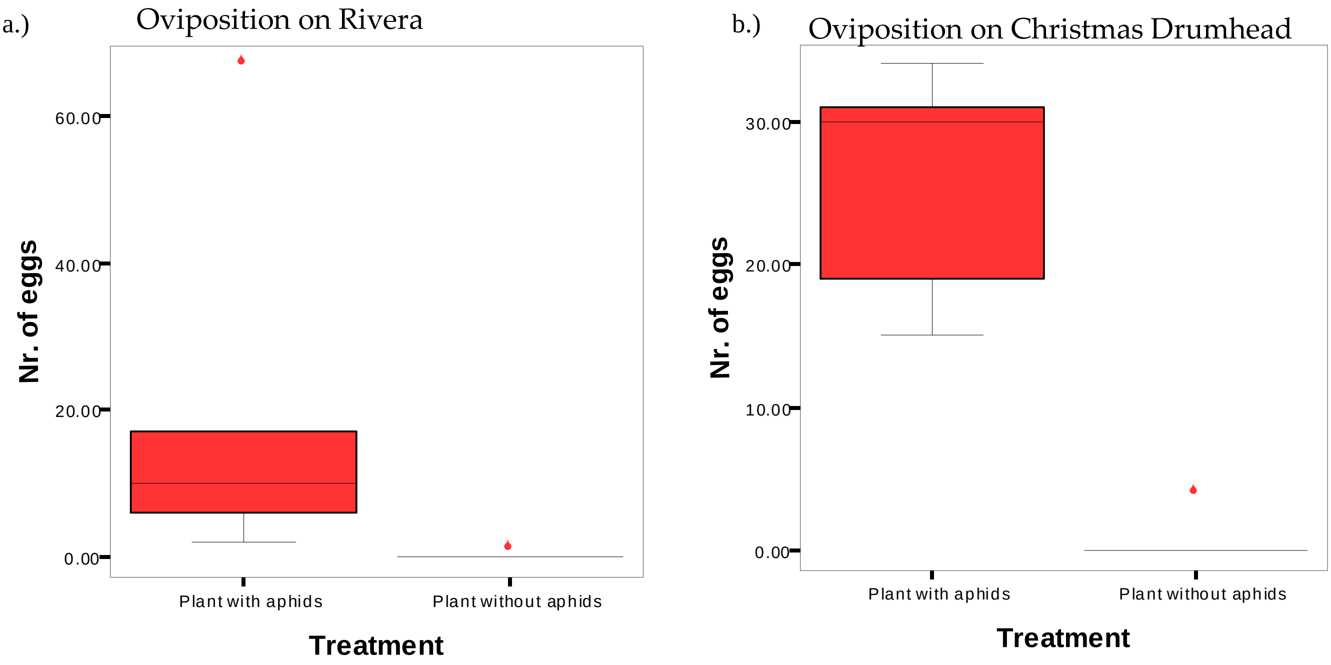
Weight:



Graph 3: *Differences in the pupa fresh weight of E. balteatus amongst the cultivars.*
a.) Differences within the M. persicae treatments.
b.) Differences within the B. brassicae treatments.
Error bars with the same letter represent no significant difference was found (α=0.05).

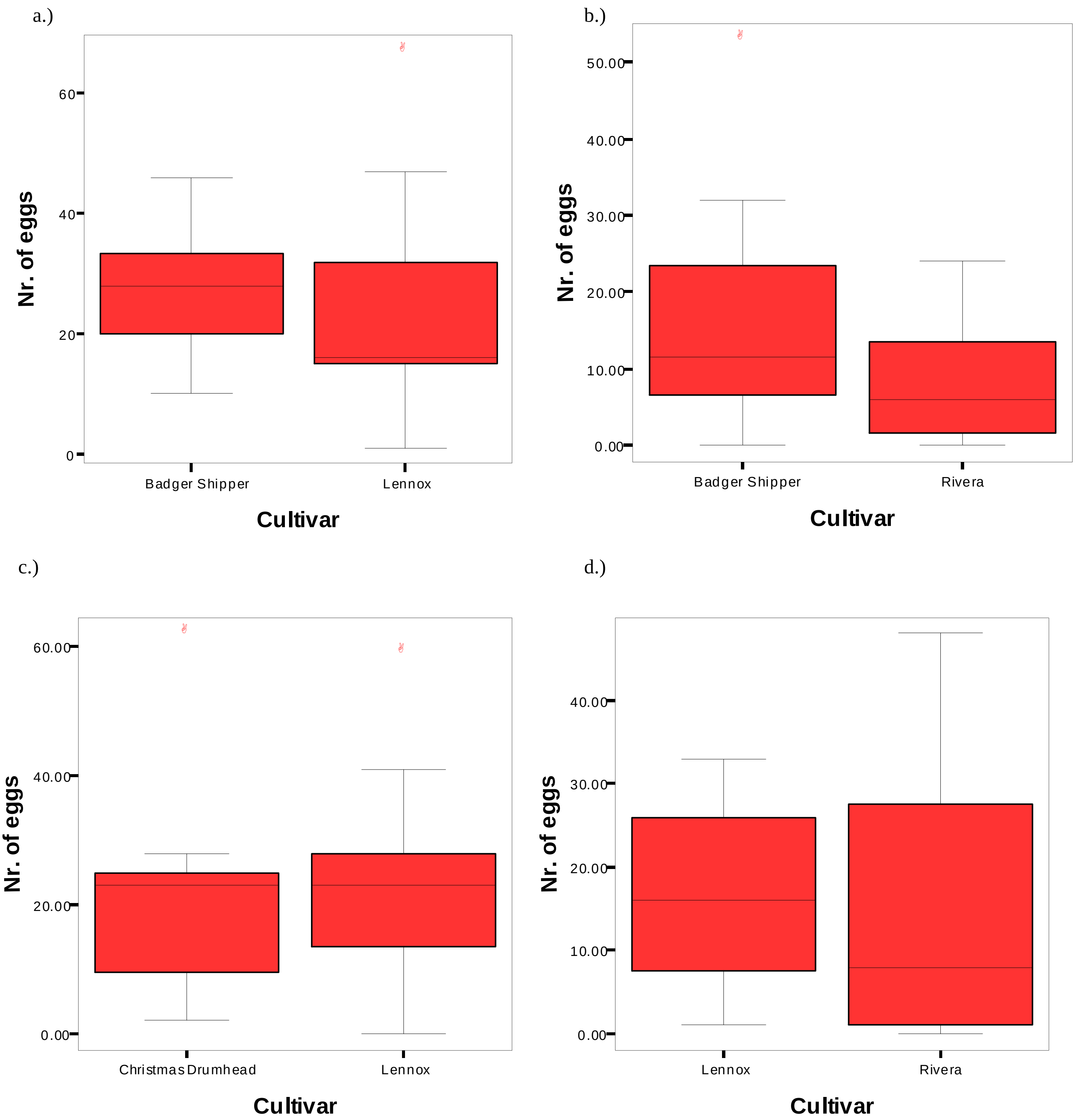
Graphs *Episyrphus balteatus* performance

Control experiment:



Graph 4: *Results of the control experiment on the oviposition preference of E. balteatus between infested and uninfested plants.*
a.) Results of the oviposition control on Rivera.
b.) Results of the oviposition control on Christmas Drumhead.

Real experiment:



Graph 5: *Results of the real experiment on the oviposition preference of E. balteatus between different cultivars.*

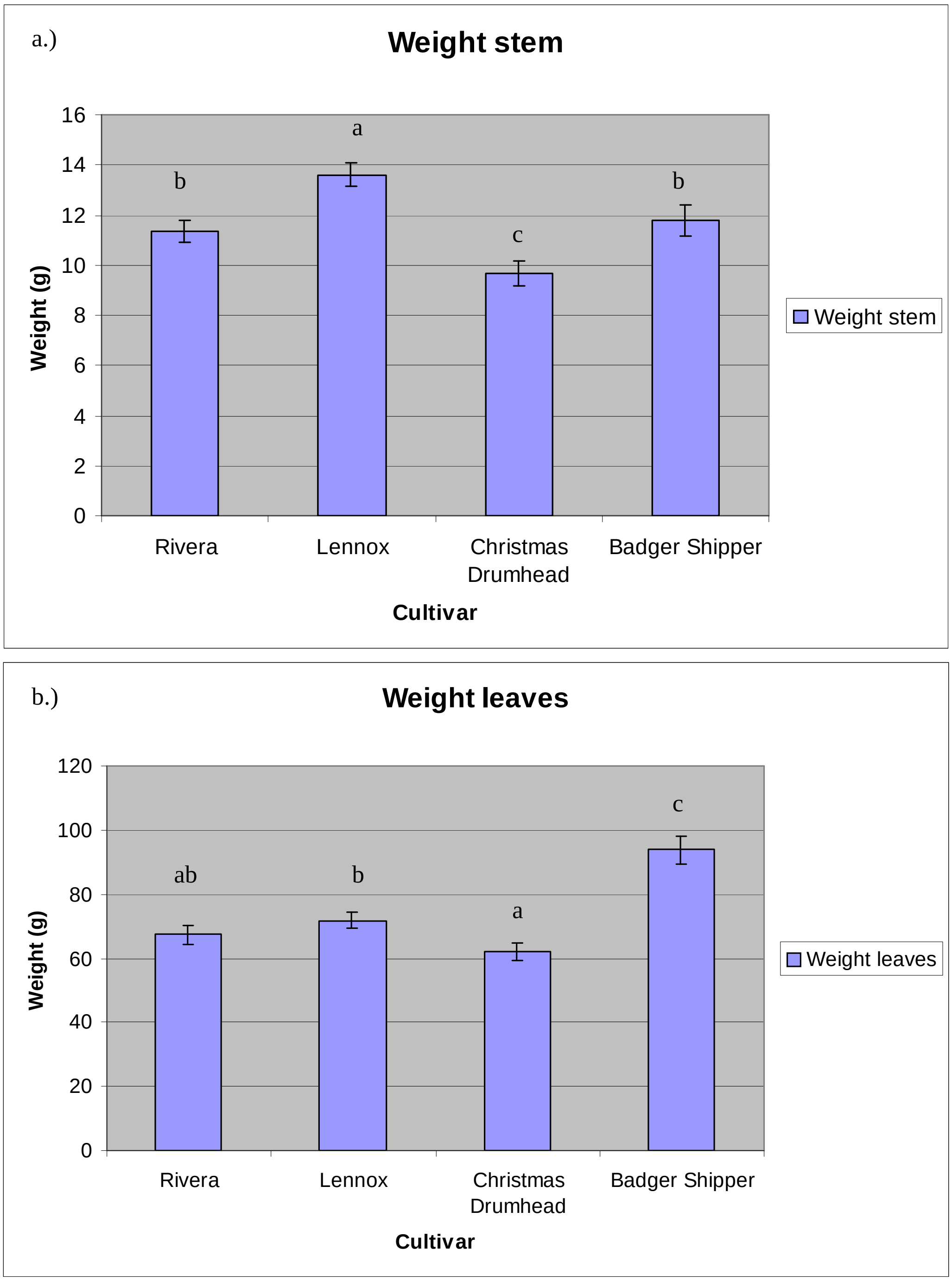
a.) Oviposition preference between Badger Shipper and Lennox (Wilcoxon, Z=-0.711, P=0.477)

b.) Oviposition preference between Badger Shipper and Rivera (Wilcoxon, Z=-1.611, P=0.107)

c.) Oviposition preference between Christmas Drumhead and Lennox (Wilcoxon, Z=-0.765, P=0.444)

d.) Oviposition preference between Lennox and Rivera (Wilcoxon, Z=-0.445, P=0.656)

Differences in stem and leaf biomass amongst the cultivars:



Graph 6: *Differences in stem and leaf biomass amongst the cultivars.*
a.) Differences in stem biomass.
b.) Differences in leaf biomass.
Error bars with the same letter represent no significant difference was found (α=0.05).