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LABORATORY OF ENTOMOLOGY

Development and attraction of *Diadegma semiclausum*; a comparison between four *Brassica oleracea* cultivars

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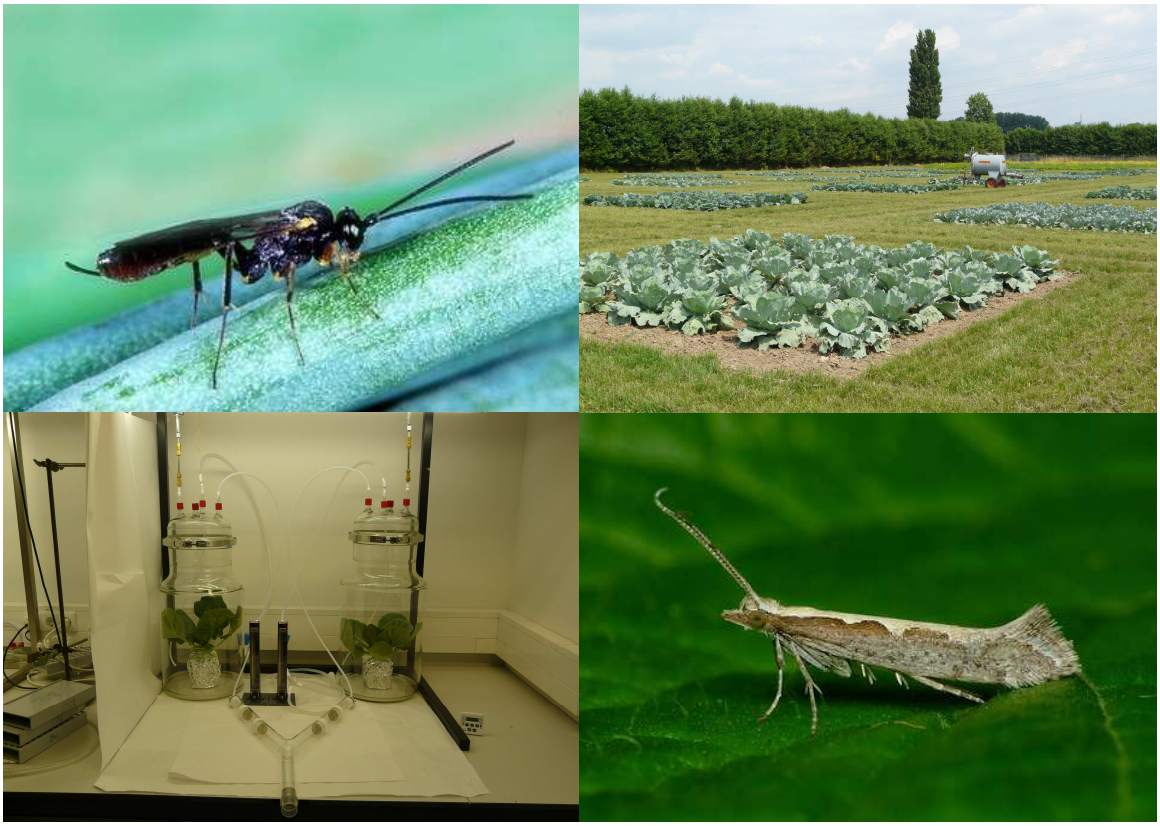

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Preface

Biological control and functional biodiversity; these were the topics that I had in mind for this thesis. Working with insects, performance of greenhouse- and field experiments; these were the activities that I had in mind for this thesis. Indeed, the topic and the activities fully corresponded to these pre-defined conditions, but the real key-characteristic for this thesis at the Laboratory of Entomology might be actually ‘patience’. Being patient; that is the characteristic I really had to make my own, as a consequence of insects playing the leading part.

Next to the functional use of insects, one of the things that actually caught my attention is the passion of entomologists for the intrinsic value of the little creatures and the attention they pay to it. I guess it has to do with my background in agriculture that I look at them in a more functional or problematic way than the people with a background in biology. Anyhow, one thing is for sure; nobody leaves the entomology department without getting affected by the ‘insect-virus’.

For now, I gladly would like to make use of this preface to thank the people that helped me during my thesis. Of course, to start with, I like to thank Ir. Martine Kos. I really much appreciated her way of supervision, which was straightforward, easy-going and very much accessible. I also would like to thank Dr. Roland Mumm, who, although not an employee at the Laboratory of Entomology anymore, was still very helpful in the process of volatile trapping. For the same reason I would like to thank Danielle Lucas Barbosa for sharing her findings with me during volatile trapping. Furthermore, I would like to thank Tjeerd Snoeren for sharing his experiences with the Y-tube testing and for providing his help when technical problems occurred. Thanks also to Léon Westerd and Andre Gidding for their efforts they have put in the rearing of the insects I used. Lastly, I would like to thank Dr. Joop van Loon and Prof. Marcel Dicke for their role as supervisor and examiner.

Abstract

Plutella xylostella is the most destructive pest in crucifers and is distributed worldwide. Synthetic pesticides are used to control the pest, of which pesticide resistance, environmental problems and elimination of natural enemies are important negative side-effects. A natural enemy of *P. xylostella,* that can play an important role in the control of this pest, is the specialist parasitoid *Diadegma semiclausum*. In this study the attraction of *D. semiclausum* to four white cabbage cultivars (*Brassica oleracea* convariety *capitata* variety *alba L.*), named Badger Shipper, Christmas Drumhead, Lennox and Rivera, and its performance in *P. xylostella* hosts reared on these cultivars was tested. The objectives of this thesis were: (1) to assess which of the four selected white cabbage cultivars, when fed upon by *P. xylostella,* is most attractive to *D. semiclausum* in the laboratory; (2) to assess which of the four selected white cabbage cultivars, when fed upon by *P. xylostella,* is most favourable for the development of *D. semiclausum*; (3) to assess which of the four selected white cabbage cultivars supports the highest percentages of parasitism by *D. semiclausum* in the field. For the three objectives respectively a preference experiment, a performance experiment and a field experiment were performed.

For the preference experiment a Y-tube olfactometer was used for performing two-choice behavioural tests with *D. semiclausum* for the six combinations of the four cultivars. However, due to problems with the rearing of *D. semiclausum*, this experiment could not be completed. Development of *D. semiclausum* was determined in *P. xylostella* larvae reared on the four *B. oleracea* cultivars and took place in the greenhouse. Survival rate of *D. semiclausum* until pupation on Badger Shipper was significant lower than on Lennox and on Rivera. Egg-to-adult development time was not significantly different between *D. semiclausum* wasps that had developed in *P. xylostella* feeding upon Badger Shipper, Christmas Drumhead, Lennox or Rivera. Significant differences were found in adult dry weight, with a significant higher adult dry weight in case development took place in *P. xylostella* larvae feeding upon Christmas Drumhead in comparison with Lennox and Rivera. A significant higher adult dry weight of *D. semiclausum* was also found on Badger Shipper when compared to Lennox. A striking result was the male-biased sex ratio, with approximately 88% of the adult wasps being male. The fraction of dead cocoons and larvae of *D. semiclausum* was not significantly different between the cultivars. Attractiveness of the four cultivars to *D. semiclausum* in the field was evaluated by comparing parasitisation rates of released *P. xylostella* larvae in an experimental field. The average parasitisation rate appeared to be highest on Rivera plants and lowest on Lennox plants, but differences were not significant. All together, not one of the four cultivars appeared to be optimal for the development of *D. semiclausum* while at the same time most attractive to *D. semiclausum*.

Contents

1. Introduction

Plutella xylostella (L.) (Lepidoptera: Yponomeutidae) is the most destructive pest in crucifers and is distributed worldwide (Saucke et al., 2000; Reddy et al., 2004; Golizadeh, 2009). The insect, also known as diamondback moth, knows four developmental stages; egg stage, larval stage, pupal stage and adult stage. The larval stage, which is the destructive phase, comprises of four instars and a pre-pupal phase, and can last fourteen days under greenhouse conditions (Cardona, 1997). To control *P. xylostella*, synthetic pesticides are used, but this brings along negative side-effects, such as environmental problems (Macharia et al., 2005) and resistance against a broad range of pesticides (Cardona, 1997; Tonnang et al., 2009). Moreover, natural enemies of *P. xylostella* often get eliminated by the pesticides, leading to increased pesticide use (Sarfraz et al., 2005), thereby speeding up the process of pesticide resistance. To improve the control strategy, integrated pest management (IPM) programs have been developed worldwide with as main focus the manipulation of natural enemies (Sarfraz et al., 2005).

Diadegma semiclausum Hellén (Hymenoptera: Ichneumonidae) is a specialist parasitoid of larvae of *P. xylostella* and can play an important role as natural enemy in the control of this pest (Poelking, 1986; Cardona, 1997; Khatri et al., 2008). Adult *D. semiclausum* wasps normally oviposit single eggs in *P. xylostella* larvae from which parasitoid larvae emerge (Cardona, 1997). The parasitoid larvae first consume the tissues near the integument of the host and as soon they reach the pupal stage, they start consuming the organs of the host, resulting in the host's death. The females that oviposit eggs in hosts are easily distinguished from the males by their long ovipositors. *D. semiclausum* knows an egg-stage, a larval stage, a pupal stage and an adult stage. The pupae of *D. semiclausum* are enclosed by a cocoon of silk that the larvae spin themselves as soon as they are going to pupate. To prevent confusion with *P. xylostella* pupae during the rest of the text, *D. semiclausum* pupae are called cocoons.

Use of *D. semiclausum* to control *P. xylostella* might be beneficial because parasitized larvae will not develop into moths, potentially leading to a decrease in offspring and possibly smaller crop losses. To increase the efficiency of *D. semiclausum* as a biocontrol agent of *P. xylostella*, crop characteristics become important. Plants release volatile organic compounds in response to feeding damage by the herbivore; so-called herbivore induced-plant volatiles (HIPVs) (Turlings and Ton, 2006; Gols and Harvey, 2009). The release of HIPVs is an indirect defence strategy of the plant to attract parasitoids and predators and to enhance their ability of locating herbivores on the plant (Poelman et al., 2008c; Gols and Harvey, 2009). It might therefore be attractive to engineer crop plants in such a way that the release of these HIPVs becomes stronger and/or that the chemical composition will be improved. In fact, already several studies have shown a successful introduction/increase of terpenoids, which are compounds playing a dominant role in HIPV blends, in crop plants by genetic engineering (Aharoni et al., 2005; Kappers et al., 2005).

Although pesticide use is still the major control method (Macharia et al., 2005), IPM programs gain influence. The idea behind IPM is that a single management technique, like spraying pesticides, is usually not a sustainable and successful management tactic in the long run (Pedigo and Rice, 2009). The integration of preventive and therapeutic practices on the other hand can be effective. A way to reduce pesticide use in management programs for controlling *P. xylostella* is to integrate the use of biological agents, such as *D. semiclausum*, and to grow cabbage cultivars that are partially resistant to *P. xylostella* and attractive to *D. semiclausum* (Sarfraz et al., 2005). At the most, a selective insecticide can be used, but only based on pest scouting, to keep the pest population on a minimum acceptable pest infestation level for *D. semiclausum*. Whatever measures are taken, the use of biocontrol agents within

IPM programs, such as *D. semiclausum*, can only lead to effective pest control when it is well co-ordinated with other management decisions.

During this thesis, the attractiveness of the four white cabbage (*Brassica oleracea* convariety *capitata* variety *alba* L.) cultivars Badger Shipper, Christmas Drumhead, Lennox and Rivera to *D. semiclausum* were tested. Previous field and laboratory experiments were performed with the same cultivars by Poelman et al. (2008b). These authors have shown that intraspecific variation in HIPVs of plants results in variable parasitisation rates of *Pieris rapae* and *P. brassicae* by *Cotesia glomerata* and *C. rubecula* in the field. Christmas Drumhead was preferred by parasitoids over Badger Shipper and Rivera in a wind tunnel assay. This correlated with the ranking order of degree of parasitism by *D. semiclausum* in the field, suggesting that laboratory assays on HIPV-preferences of parasitoids provide reliable data about differential parasitisation rates in the field. In this study comparable experiments were performed, but then with *P. xylostella* and *D. semiclausum*.

To attract natural enemies of a pest like *P. xylostella*, cultivating a cabbage cultivar with good indirect resistance traits can be effective. However, a cultivar that is attractive to natural enemies is not necessarily a cultivar optimal for the fitness of natural enemies (Gols et al., 2008b). Direct resistance traits of a plant that have an adverse effect on the development and behaviour of the herbivore can also negatively affect the herbivore's natural enemy. A study by Poelman et al. (2008a) has shown that the larval and pupal mass of *P. xylostella* was significant higher on Badger Shipper than on the other three cultivars while the development time until pupation was lowest on Badger Shipper. Furthermore, the larval mass of *P. xylostella* on Christmas Drumhead was significant higher than on Lennox and Rivera while the development time until pupation was lower. Lennox and Rivera therefore seem interesting cultivars, as they seem to be less favourable for *P. xylostella* development, but the question remains whether they are favourable for the development of *D. semiclausum*.

The aim of this study was to test four different white cabbage cultivars with regard to the attraction and performance of *D. semiclausum*. Field and laboratory experiments have been performed to acquire data under controlled and simplified conditions and in a complex environment. The objectives of the research were:

(1) to assess which of the four selected white cabbage cultivars, when fed upon by *P. xylostella*, is most attractive to *D. semiclausum* in the laboratory.

(2) to assess which of the four selected white cabbage cultivars, when fed upon by *P. xylostella*, is most favourable for the development of *D. semiclausum*.

(3) to assess which of the four selected white cabbage cultivars supports the highest percentages of parasitism by *D. semiclausum* in the field.

2. Materials and Methods

2.1. Plants and insects

The following four white cabbage (*Brassica oleracea* convariety *capitata* variety *alba* L.) cultivars were used for the study: Badger Shipper, Christmas Drumhead (Centre for Genetic Resources, Wageningen, The Netherlands), Lennox and Rivera (Bejo Zaden BV, Warmenhuizen, The Netherlands).

Plutella xylostella and *D. semiclausum* were acquired from the Laboratory of Entomology, Wageningen University and Research Centre (WUR). The rearing of *P.*

xylostella and *D. semiclausum* took place in a climate room and greenhouse compartment respectively at 20 ± 2°C, 50-70% relative humidity and a photoperiod of 16 hours light and eight hours dark. *Plutella xylostella* was reared on Brussels sprouts (*B. oleracea* variety *gemmifera* L. cultivar Cyrus). *Diadegma semiclausum* was reared on the same Brussels sprout variety infested with *P. xylostella* larvae.

2.2. Preference experiment

The response of *D. semiclausum* to volatiles released from the four different *B. oleracea* cultivars, infested by *P. xylostella* larvae, was analyzed. A Y-tube olfactometer was used for performing two-choice behavioural tests with *D. semiclausum* for the six combinations of the four cultivars. Prior to testing, a pilot experiment was performed with *P. xylostella* infested plants against non-infested control plants to check whether wasps were reacting at all to the volatile compounds from the infested plants. For the pilot experiment, plants of the cultivars Christmas Drumhead and Rivera were used.

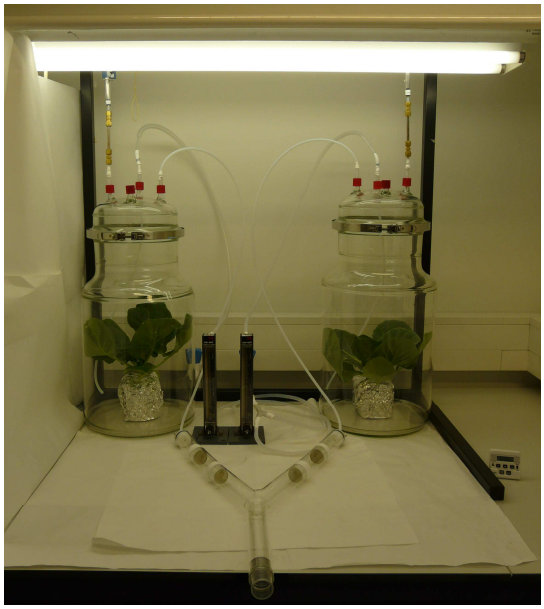

Figure 1. Set-up of the preference experiment.

For both the main and pilot experiment, a single seven week old plant was placed in both jars connected to the olfactometer (Figure 1). For the main experiment, each plant was infested with 20 third instar larvae of *P. xylostella* for 48 hours prior to testing. For the pilot experiment one of the two plants was infested with 20 third instar larvae and infestation time took 24 hours. The jars had a volume of 30 litres. Via fluoropolymer hoses (Teflon, DuPont, USA) air was led into the jars, each with a flow of 3.50 l/min. Consequently, air was able to leave the jars and was led into the two olfactometer arms through which the odours reached the inlet of the Y-tube where the wasps were released individually.

At the start and at the end of both olfactometer arms a line was drawn (Figure 2). As soon as wasps were passing the first line in one of the olfactometer arms, the cultivar that was responsible for the odour source in that particular arm was written down as a ‘first choice’. A ‘final choice’ was considered to be made when wasps were passing the second line, but since the wasps were allowed to move backwards, a ‘final choice’ was only valid when they stayed behind the first line for a minimum of 15 seconds (Bukovinszky et al., 2005). Behind the second line, wire gauze was positioned to prevent the wasps from going any further. In case wasps did not make a final choice within 10 minutes after their release, they were removed from the Y-tube and noted as ‘no choice’. The same notation was made for wasps that still had not passed the first line within five minutes after release.

In total, six sets of new plants were used for each of the six combinations in the main experiment, and eight sets of new plants for both Christmas Drumhead and Rivera were used for the pilot experiment. The plastic pots around the plants were removed and the soil was wrapped into aluminium foil. The age of the female wasps was approximately five to seven days and for each repetition 10 wasps were used. The wasps did not have any oviposition experience. To prevent that any dissimilarity between the two jars would have an influence on the wasp’s choice, the odour sources were switched to the other arm of the Y-tube after five

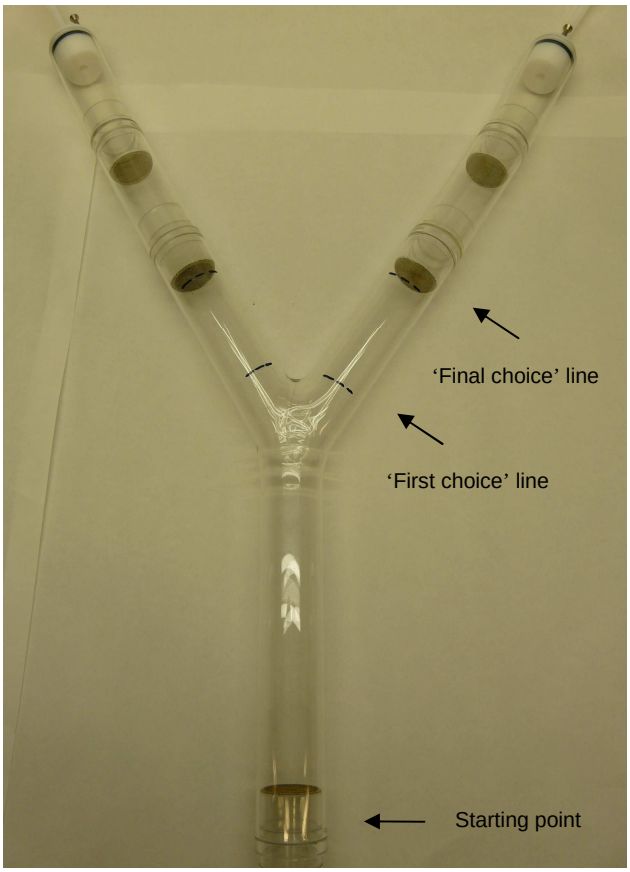

Figure 2. The Y-tube set-up, with the starting point, the 'first choice' line and the 'final choice' line indicated.

wasps were tested. The experiment took place in a climate room at 22°C and the set-up was lighted with two fluorescent tube lights (FTD 32 W/84 HF, Pope, The Netherlands) that were positioned 90 cm above the set-up.

During the first and third repetition, plant volatiles were collected. Tubes filled with an adsorbent resin (Tenax, Scientific Instrument Services, USA) were connected to a small pump and attached to an opening in the lid of the jars (Figure 1). The flow from the pump was adjusted to 200 ml/min and the duration of trapping was two hours. In case trapping took place, the air flow through each jar was adjusted to 3.70 l/min. Collected volatiles were analyzed by gas chromatography / mass spectrometry (GC-MS).

2.3. Performance experiment

Performance of *D. semiclausum* was determined in *P. xylostella* hosts reared on the four *B. oleracea* cultivars. *P. xylostella* moths were released in four rearing cages (40.5 x 29.5 x 61 cm), each containing plants of one of the four cultivars, and were allowed to oviposit on the plants. After 24 hours, the moths were removed and the eggs were allowed to develop into larvae. When necessary, extra plants were added to sustain larval growth. When the larvae reached the right age (second instar) they were individually parasitized by *D. semiclausum*. A vial with a single mated female was placed over a leaf with a single *P. xylostella* larva until the larva was seen to be parasitized. Each female wasp was used to parasitize up to ten individual host larvae. With help of a fine paintbrush, parasitized larvae were transferred to plants of the same cultivar on which they hatched, after which the plants were placed in gauze nets (65 x 95 cm; Figure 3). Every net


Figure 3. The plants of the performance experiment.

contained one plant and there were 15 nets per cultivar. On every plant five parasitized larvae were released. The average parasitisation time of the larvae was noted per plant. The experiment was performed in a greenhouse compartment at 22 ± 2°C, 60% relative humidity and a photoperiod of 16 hours light and eight hours dark. In case light intensity was lower than 500 µmol photons/m²/s during the 16 hours light, cages were illuminated by high-pressure mercury lamps (Gols et al., 2008a).

After the parasitoid larvae had emerged from their hosts and had spun cocoons, they were transferred singly to tubes after which they were checked for emergence every two hours during the day. Time of emergence was noted and egg-to-adult development time and gender were determined. Immediately after emergence, wasps were put in a freezer after which they were dried for 72 hours at 80°C and weighed on a microbalance (Cahn C-33, Cahn instruments, USA; Gols et al., 2008a).

2.4. Field experiment

The experimental field is situated just outside Wageningen in The Netherlands (Figure 4). There were 32 plots that were used for the experiment; each planted with a monoculture of one of the four white cabbage cultivars (Figure 5). The cultivars were randomly assigned to the 32 plots. The plots were 6 by 6 m and in every plot there were 7 x 7 plants with a distance of approximately 0.75 m between the plants (Figure 6). The distance between the plots was 6 m and this soil was covered by a grass mixture of *Lolium* and *Poa* species. The crosses in the green area in Figure 6 represent the plants that were used for this experiment.

Five-week-old plants were transplanted to plots in the experimental field in the first week of May. From the second week of June 2009 till the third week of August 2009, 20 second instar larvae were put weekly on one plant in every plot. Second instar larvae were obtained by releasing adult *P. xylostella* in rearing cages (40.5 x 29.5 x 61 cm) for oviposition on Brussels sprout plants. After 24 hours, the moths were removed and eggs developed into larvae. Larvae with the age of approximately four days (second instar) were used for the field experiment. Every week, a new plant was used in a consistent pattern for all the 32 plots. After 72 hours, larvae were recollected from the field and put in plastic trays. The trays contained a single leaf of Brussels sprouts and were closed with gauze lids. The trays were stored in a climate room, where further development of the larvae was allowed. Meanwhile, fresh leaves of Brussels sprouts were added regularly to the trays. The conditions in the climate room were the same as described for the rearing of *D. semiclausum*. Larvae that yielded parasitoid cocoons were transferred singly to glass tubes. After emergence of *D. semiclausum*, the gender was determined. Meanwhile the number of adult moths, dead *P. xylostella* larvae, dead pre-pupae and dead pupae was counted.

Figure 4. The experimental field with the cabbage plots.

Finally, the average parasitisation rates were calculated for the four cultivars

by dividing the sum of cocoons and dead *D. semiclausum* larvae by the sum of cocoons, dead *D. semiclausum* larvae, moths and pupae. Dead *P. xylostella* larvae and pre-pupae that died after re-collection were excluded. The reason for excluding dead pre-pupae is because at that stage it is uncertain whether they would transform into pupae or cocoons. *D. semiclausum* is a tissue feeder and consumes the complete host as soon as *P. xylostella* is going to pupate. At the moment pre-pupae develop into pupae, it is for sure that moths are formed, and therefore larvae that turned into pupae were included in the calculations as non-parasitized larvae.

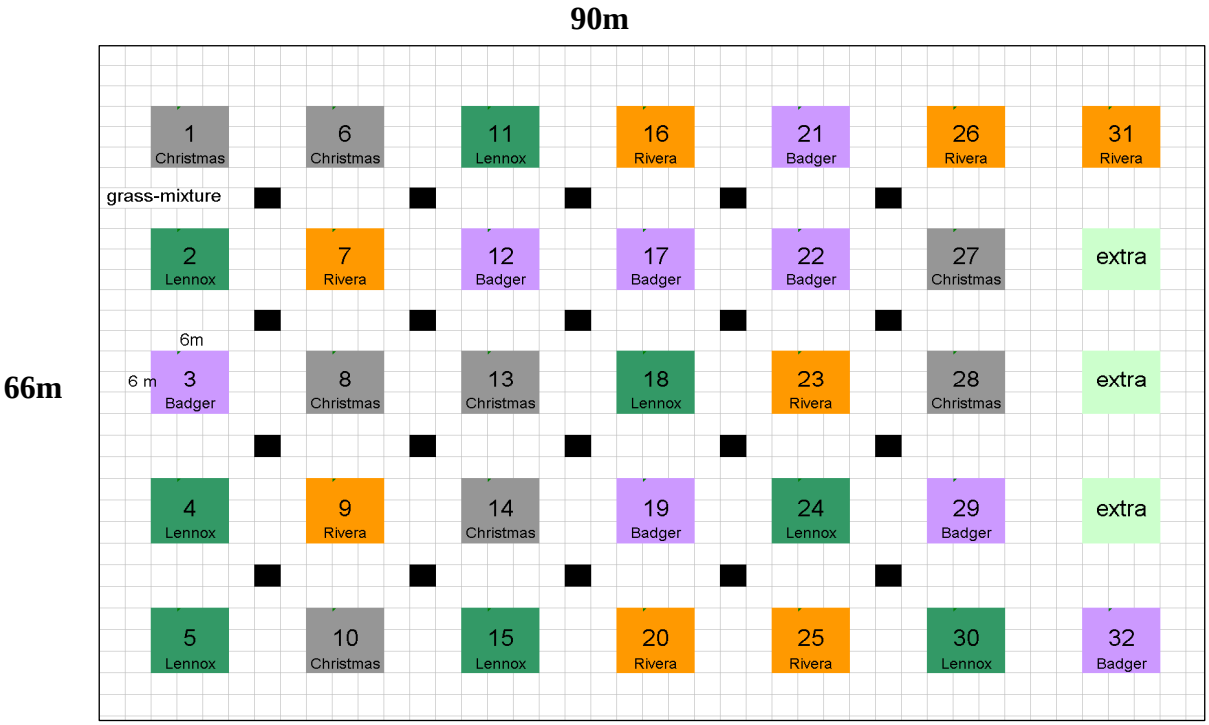

Figure 5. Map of the experimental field, consisting of 32 plots that were randomly assigned to one of the four cultivars, resulting in eight plots with a monoculture of one of the four cultivars Christmas Drumhead (Christmas), Badger Shipper (Badger), Lennox and Rivera. In the 'extra' plots, plants of the four white cabbage cultivars were grown and served as back-up in case plants in the 32 plots needed to be replaced because of plant damage.

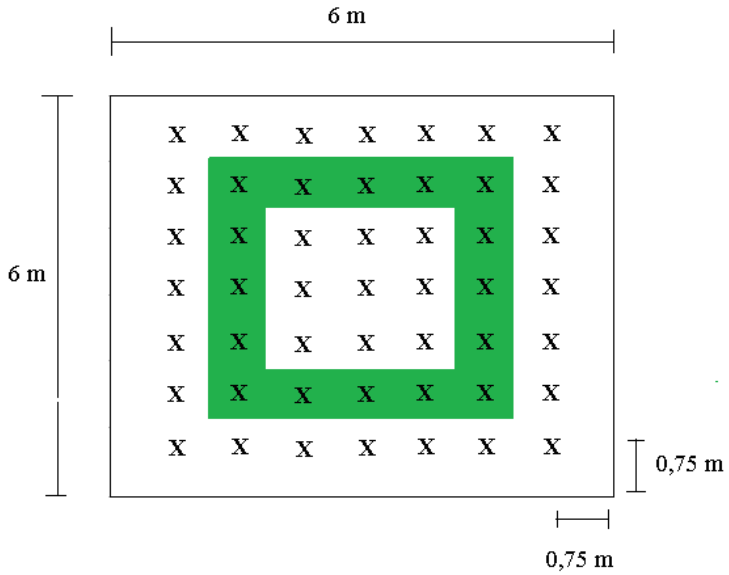

Figure 6. A single plot of 6 by 6 m with the crosses representing the 49 plants. The plants in the green area are the ones that were used for inoculation of the second instar larvae of *P. xylostella*.

2.5. Statistical analysis

For the preference experiment a chi-square test was performed to indicate whether *D. semiclausum* had a statistically significant preference for one of the cultivars that was offered. For the pilot experiment the same test was used for checking preference for the infested plants with respect to the control plants. Chi-square tests were performed with Microsoft Office Excel 2003.

For analyzing data from the performance experiment, several Generalized Linear Models (GLIMs) were used. First, a GLIM was used to analyze whether the fraction of recollected larvae was significantly different between the four cultivars. The number of recollected larvae was included in the model as a binomially distributed dependent variable with as fixed binomial totals the number of larvae that were released on the plants (Poelman, 2008b). In some cases *P. xylostella* larvae had succeeded in developing into pupae. Therefore, to check whether survival rate of *D. semiclausum* until pupation was different between the cultivars, a GLIM was used with as dependent variable the sum of cocoons and dead parasitoid larvae, and as fixed binomial totals the number of recollected larvae. A comparable GLIM was performed to check differences in survival rate of *D. semiclausum* until emergence between the four cultivars, with as difference the number of adult wasps as a binomially distributed dependent variable. In case significant differences were found in survival rate, a post-hoc analysis was used to see which individual differences between the cultivars were responsible for those findings. Furthermore, a GLIM was used to analyze whether there were differences in *D. semiclausum* mortality between the cultivars. In this model, the sum of dead cocoons and dead parasitoid larvae was included as dependent variable with as fixed totals the sum of cocoons and dead parasitoid larvae. Last, a GLIM was used to check whether there were differences in the fraction of females between the four cultivars. The dependent variable and fixed totals in this case were respectively the number of female wasps and the number of adult wasps. For the GLIMs, Genstat 12th edition was used.

Data about the egg-to-adult development time and adult dry weight, gathered during the performance experiment did not fulfil the preconditions for an Analysis of Variance (ANOVA). Therefore the data were subjected to a Kruskal-Wallis test, with as goal to

determine whether egg-to-adult development time and adult dry weight were significantly different between wasps that had developed in larvae feeding upon the four different cabbage cultivars. For checking individual differences between the cultivars, separate Mann-Whitney U tests were performed. Furthermore, correlation between adult dry weight and development time was checked by calculating Pearson's correlation coefficient. Tests were performed with SPSS 16.0.

The experimental design of the field experiment was a completely randomized design. There were four treatments (four cultivars) and eight repetitions of each cultivar. First, a GLIM was used to test whether the fraction of recollected larvae from the field was different for the four cultivars. The binomially distributed dependent variable in the model was the number of recollected larvae. In some cases more larvae were recollected than the 20 larvae that were released, probably as a result of collection of naturally occurring larvae. The number of released larvae, which was included in the model as fixed binomial totals, was therefore set on the highest number (35) of larvae that was recollected once during the 10 weeks. Second, to check statistically significant differences between the cultivars concerning the fraction of recollected larvae that appeared to be parasitized, a GLIM was used, with as dependent variable the sum of cocoons and dead parasitoid larvae, and as fixed binomial totals the sum of moths, pupae, cocoons and dead parasitoid larvae. Third, a GLIM was used to reveal whether the fraction of dead cocoons and dead parasitoid larvae was significant different between the four cultivars. The binomially distributed dependent variable was the sum of dead cocoons and dead parasitoid larvae, while the sum of cocoons and dead parasitoid larvae represented the fixed totals. Last, a GLIM was used to analyze whether there were differences in the fraction of female wasps between the cultivars. Dependent variable and fixed totals were respectively the number of female wasps and the number of adult wasps.

3. Results

3.1. Preference experiment

Results of the pilot experiment are presented in Figure 7. From the 80 wasps that were tested during the pilot with Christmas Drumhead plants, approximately 46% chose infested plants over control plants, 5% chose control plants over infested plants and 49% did not make a choice. From the 80 wasps that were tested during the pilot with Rivera plants, approximately 58% chose infested plants over control plants, 1% chose control plants over infested plants and 41% made no choice. Preference for the infested plants was significant ($P < 0.001$) in case of both cultivars.

Due to problems with the rearing of *D. semiclausum*, the final experiment could not be performed as planned. Eventually, only one repetition was feasible, which was too few to draw any conclusions from. During the few bioassays that were performed, the percentage of wasps not responding was much lower than during the pilot experiment. From all wasps that were tested during the main experiment approximately 17% did not respond, while this rate was 45% during the pilot experiment.

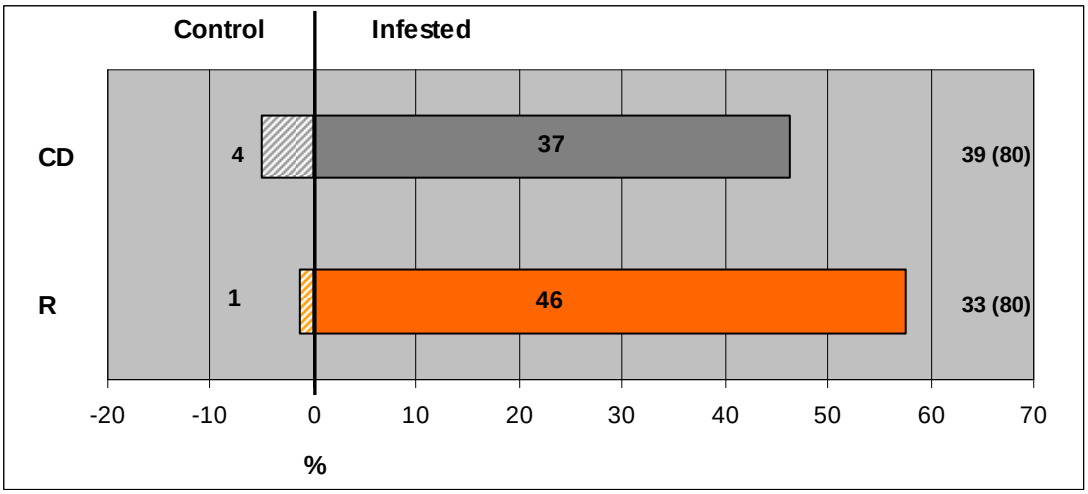

Figure 7. Percentage of *D. semiclausum* wasps that chose for volatiles released from infested and non-infested (control) plants of Christmas Drumhead (CD) and Rivera (R). Numbers left from the bars and in the bars, represent respectively the number of wasps that made a choice for the control plants and the number of wasps that made a choice for the infested plants. Numbers at the right of the bars represent the number of wasps that did not make a choice, with in between brackets, the total number of wasps that were tested.

3.2. Performance experiment

Regarding the number of recollected larvae, there was no significant difference (P = 0.592) between the four cultivars. A significant difference ($P < 0.05$) was found for the survival rate until pupation between the cultivars and a post-hoc analysis showed that survival rate until pupation on Badger Shipper was significant lower than on Lennox ($P < 0.05$) and on Rivera ($P < 0.05$). No significant difference (P = 0.616) was found in the survival rate until emergence between the cultivars. Finally, regarding *D. semiclausum* mortality (dead cocoons and larvae) and the fraction of female wasps no significant differences (respectively P = 0.606 and P = 0.344) were found between the cultivars. However, the number of females that emerged on the four cultivars was low compared to the number of males. The total number of wasps that emerged from Badger Shipper, Christmas Drumhead, Lennox and Rivera was respectively 61, 66, 61 and 62, from which respectively about 11%, 17%, 5% and 16% were females.

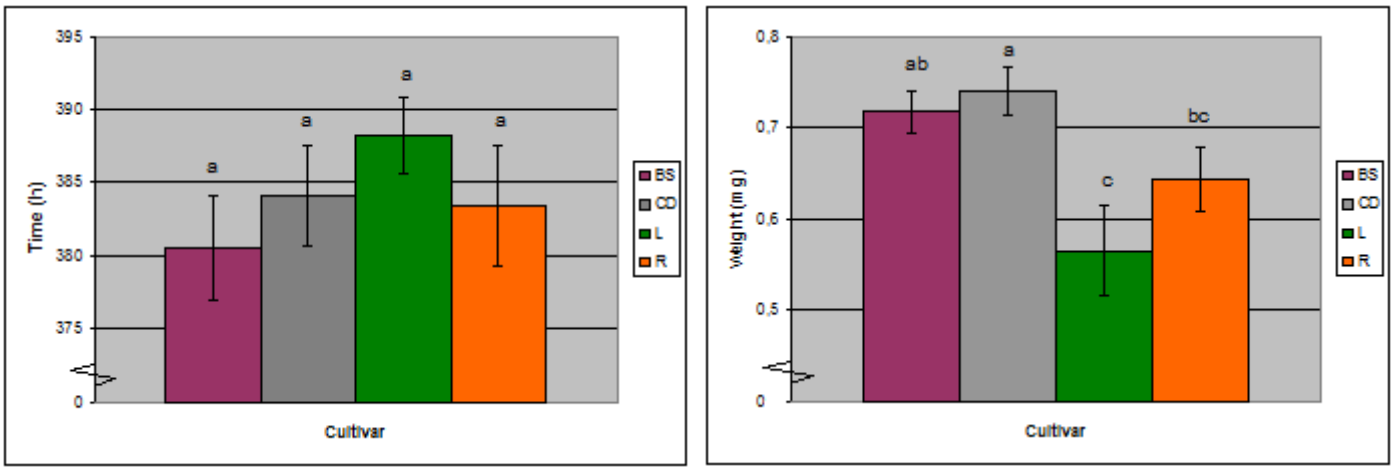

Figure 8. Average egg-to-adult development time (left) and dry weight (right) of *D. semiclausum* wasps that have developed in *P. xylostella* larvae feeding from Badger Shipper (BS), Christmas Drumhead (CD), Lennox (L) and Rivera (R). The bars, with standard error (SE) of the mean, are significant different ($P < 0.05$) in case different letters are used.

The average egg-to-adult development time and adult dry weight of *D. semiclausum* wasps that had developed in *P. xylostella* larvae feeding from the four white cabbage cultivars Badger Shipper, Christmas Drumhead, Lennox or Rivera is presented in Figure 8. Egg-to-adult development time of *D. semiclausum* took longest when parasitoid larvae developed in *P. xylostella* that had been feeding upon Lennox (388 hours and 20 minutes) and shortest when they had been feeding upon Badger Shipper (380 hours and 56 minutes). A Kruskal-Wallis test (SPSS) reveals that differences between the cultivars were not statistically significant ($P = 0.334$; Table 1).

Table 1. Results of the Kruskal-Wallis test which was used for data analysis of the performance experiment. The mean rank is shown of egg-to-adult development time and dry weight of *D. semiclausum* wasps that have developed in *P. xylostella* larvae, feeding from Badger Shipper (BS), Christmas Drumhead (CD), Lennox (L) or Rivera (R). The chi-square and *P*-value show that differences between cultivars were only significant with regard to dry weight of *D. semiclausum* wasps.

	Cultivar	Repetitions	Mean Rank	Chi-square	d.f.	*P*
Egg-to-adult development time	BS	15	28.40	3.397	3	0.334
	CD	15	28.93			
	L	15	37.60			
	R	15	27.07			
Adult dry weight	BS	15	36.27	11.248	3	0.010
	CD	15	39.13			
	L	15	20.37			
	R	15	26.23			

Significant ($P < 0.05$) differences were found when comparing adult dry weight of *D. semiclausum* (Table 1). Separate Mann-Whitney tests show that differences are only

significant between the cultivars Badger Shipper and Lennox ($P < 0.05$), Christmas Drumhead and Lennox ($P < 0.01$), and between Christmas Drumhead and Rivera ($P < 0.05$). Another finding is the negative correlation ($P < 0.01$) between the variables development time and adult dry weight (Figure 9). A clear example is the cultivar Lennox, on which *D. semiclausum* had the lowest average weight, while the average development time took longest on this cultivar.

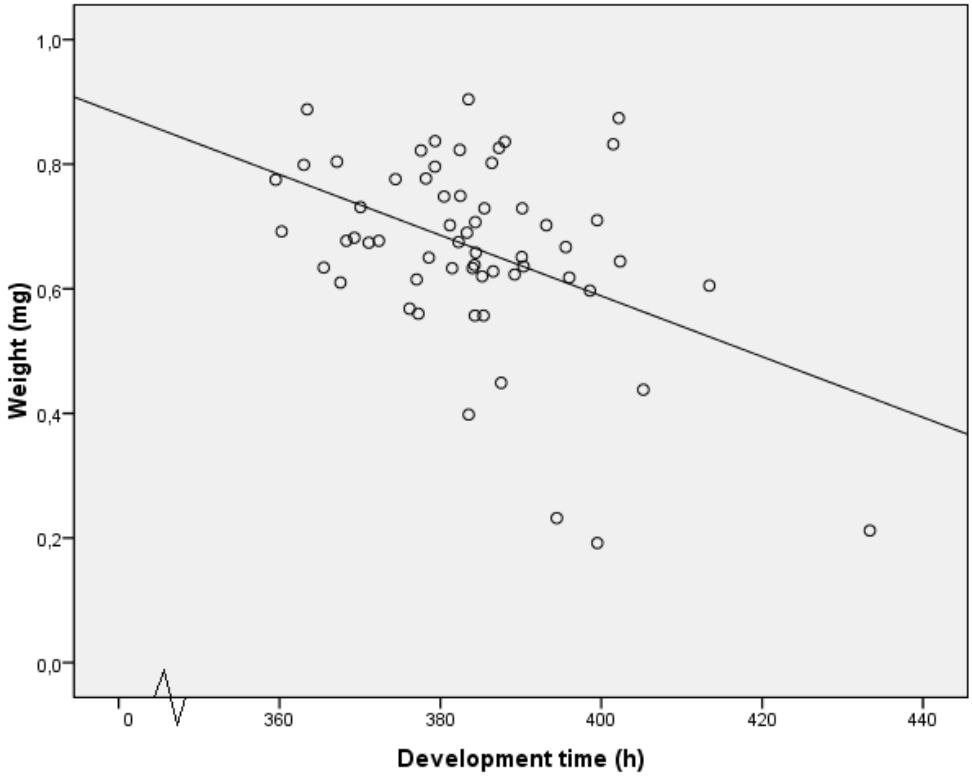

Figure 9. Negative correlation between the variables egg-to-adult development time and dry weight of *D. semiclausum* wasps.

3.3. Field experiment

Plutella xylostella larvae were released and recollected 10 times. However, from week seven onwards, the number of recollected larvae was very low, which resulted frequently in 0% or 100% parasitisation. These extreme parasitisation rates give no realistic view and therefore data are presented and analyzed for only the first six weeks. There was no significant difference ($P = 0.816$) in the number of recollected larvae between the four cultivars.

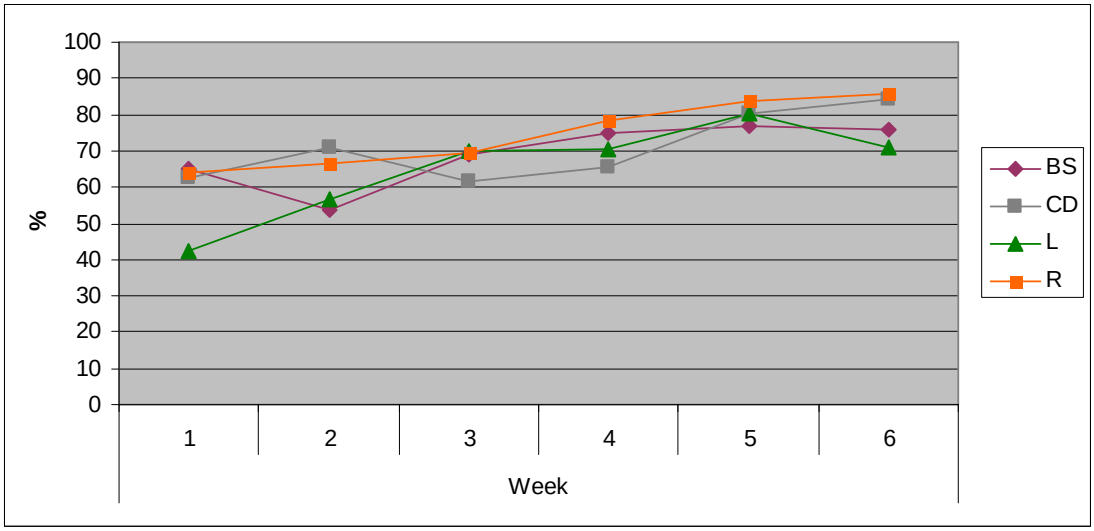

Figure 10. Percentage of *P. xylostella* larvae that was parasitized on the four cultivars Badger Shipper (BS), Christmas Drumhead (CD), Lennox (L) and Rivera (R) during the first six weeks of the field experiment.

Figure 10 shows the average parasitisation rate per cultivar per week. No significant difference (P = 0.134) in parasitisation rate was found between the cultivars (Table 2). Furthermore, no significant differences (respectively P = 0.147 and P = 0.076) were found between the cultivars regarding *D. semiclausum* mortality and the fraction of females. The total number of wasps that had emerged during the first six weeks amounted 1480, from which exactly 50% was male and 50% was female.

Table 2. Average parasitisation rate per cultivar over the first six weeks of the field experiment and results of the GLIM. The *P*-value shows there was no significant difference between the four cultivars Badger Shipper (BS), Christmas Drumhead (CD), Lennox (L) and Rivera (R).

Cultivar	Average parasitisation rate	Deviance ratio	d.f.	*P*
BS	69.30	1.88	3	0.134
CD	70.85			
L	65.10			
R	74.67			

4. Discussion

4.1. Preference experiment

The results of the pilot preference experiment show clear attraction of *D. semiclausum* to *P. xylostella* infested plants when exposed to odours from both infested and non-infested plants. Apparently the wasp's behaviour was mediated by the volatile organic compounds, released by the plant upon herbivory. That, at least seems plausible, since plants do not release these volatiles in such significant amounts when artificially damaged or non-injured (Turlings et al., 1990). Next to volatile release, another difference between the control and infested plants was the presence of the herbivores and their excreta. However, compared to plant volatiles,

herbivore derived stimuli generally play a less important role in the attraction of natural enemies (Vet and Dicke, 1992). Whether or not the larvae and their products would have had an influence on the wasp's behaviour, the choice for leaving the larvae on the plants is because the two are simply not inseparable in agro-ecosystems. What does become important is the use of naïve wasps, especially when evaluating the function of these volatile compounds (Allison and Hare, 2009). Naïve wasps have no oviposition experience. In case naïve wasps prefer infested plants above non-infested plants, they apparently respond innately to the plant volatile compounds, and are not biased due to previous oviposition experiences. Although the wasps used during this preference experiment had no oviposition experience, they had been in contact with previously infested plants, due to the rearing of *D. semiclausum* on Brussels sprout plants infested with *P. xylostella* larvae. At the time *D. semiclausum* wasps emerged, pupae and cocoons were still present on the plants. It is therefore conceivable that this previous experience with the host-plant complex had an influence on their behaviour during the y-tube bioassays. However, time of contact was kept as short as possible by regularly transferring the wasps from the rearing cages into empty cages. And besides, apart from practical reasons for doing it this way, natural enemies in agro-ecosystems have been in contact with the host-plant complex anyway, and thus also in the experimental field.

The significant preference of *D. semiclausum* for the infested plants over the control plants made performing the main experiment with combinations of the four cultivars certainly worthwhile. However, due to problems with the rearing of *D. semiclausum*, not enough female wasps were available for the planned repetitions. Moreover, the chromatograms showed very high contamination peaks which would be undesirable during volatile analysis. An explanation for the increase in response during the main experiment compared to the pilot experiment could be the longer infestation time prior to testing. Allison and Hare (2009) mention some studies in which natural enemies became increasingly attracted to herbivore infested plants as soon as infestation length increased. In this case plant infestation length during the pilot and main experiment was increased from 24 hours to 48 hours. Furthermore, it is also imaginable that the high contamination present in the system during the pilot experiment had an influence on the olfactory receptor neurones of the wasps, making the wasps indecisive. Before the start of the main experiment, several operations were carried out to reduce the contamination, and indeed the most recent chromatograms that were made during the main experiment showed substantial improvements compared to the chromatograms made during the pilot experiments.

4.2. Performance experiment

The results from the performance experiment showed differences in parasitoid survival rate until pupation between the four cultivars. Of course it is possible that some oviposition attempts where observed as successful, while they were not. However, since the survival rate until pupation on Badger Shipper was even significant lower than on Lennox and Rivera, another explanation is conceivable. Perhaps the larvae developing upon Badger Shipper were stronger and better capable in defending themselves against parasitisation. In a study by Poelman et al. (2008a) a significant higher weight was found for *P. xylostella* larvae that had been feeding upon Badger Shipper in comparison with larvae that had been feeding upon Rivera and Lennox. These heavier larvae might have had a more efficient immune system through which they were capable in encapsulating and killing the eggs of *D. semiclausum* (Sarfraz et al., 2007; Bukovinszky et al., 2009). Benrey and Denno (1997) found during experiments with *P. rapae* and *C. glomerata* on *B. oleracea* plants that encapsulation rates were higher for fast-developing larvae than for slow-developing larvae. Indeed, the *P.*

xylostella larvae in the study of Poelman et al. (2008a) had by far the shortest development time until pupation on Badger Shipper compared with larvae on Lennox and Rivera.

A male-biased sex ratio, as was the result from the performance experiment, is common during laboratory experiments with *D. semiclausum* and other parasitoids of the genus *Diadegma* (Yang et al., 1993; Butcher et al., 2000; Gols et al., 2007; Khatri et al., 2008). *Diadegma semiclausum* is a haplodiploid species and more specific arrhenotokous, which means that fertilized eggs develop into females and unfertilized eggs into males (Heimpel and de Boer, 2008). Since females have precise control over depositing fertilized or unfertilized eggs into their hosts (Steiner and Ruther, 2009), the sex ratio that was found during the performance experiment, could be the result of this phenomenon, called sex determination. Several studies showed a higher number of female wasps when *P. xylostella* larvae were parasitized as fourth instar, while parasitisation of younger instars resulted in a majority of males (Yang et al., 1993, Cardona, 1997; Gols et al., 2007). Size of the host is considered an important aspect of host quality (Harvey and Strand, 2003) and is usually strongly correlated with the size of adult wasps (Godfray, 1994). Larger adult wasps often turn out to have a higher fitness in terms of fecundity and longevity (Godfray, 1994; Harvey and Strand, 2003), and it has been argued that females might benefit more from large hosts for oviposition than males for mating (Godfray, 1994). That we used second instar larvae during the performance experiment might thus explain the high number of male wasps. Moreover, a relatively high weight was found for female wasps. Average weight of all males was approximately 0.69 mg, and from the 31 females that had emerged, 27 females had a higher weight than this average.

In contrast to egg-to-adult development time, for which no significant difference was found, a significant difference was found between dry weight of the wasps that had developed in larvae feeding upon the four different cultivars. More specific, a significant higher dry weight of *D. semiclausum* was found on Christmas Drumhead when compared to Lennox and Rivera, and on Badger Shipper when compared to Lennox. Again, the findings of Poelman et al. (2008a) about the performance of *P. xylostella* on the four cabbage cultivars might explain the weight variability between the wasps in this experiment. As mentioned already, usually a strong correlation exists between host size and parasitoid size of a solitary wasp (Godfray, 1994). Since weight of *P. xylostella* larvae appeared highest on Badger Shipper and Christmas Drumhead and lowest on Lennox and Rivera, it is imaginable that more nutrients were available for *D. semiclausum* larvae that developed on Badger Shipper and Christmas Drumhead than for *D. semiclausum* larvae that developed on the other two cultivars.

Another interesting finding is the negative correlation between adult dry weight and egg-to-adult development time of *D. semiclausum*. Several studies have described a longer development time of parasitoids in smaller hosts than in larger hosts (Harvey and Strand, 2002; Harvey and Strand, 2003; Khatri et al., 2008). Assuming there was a positive correlation between host size and parasitoid size in this experiment, then what could be the explanation for the longer egg-to-adult development time of *D. semiclausum* in smaller *P. xylostella* larvae and vice versa? Harvey and Strand (2003) argue that parasitoid progeny in small hosts postpone their development until their hosts increase in weight. So although *P. xylostella* larvae on the four cultivars were parasitized at the same age in this experiment, it could be that larvae on Badger Shipper had developed faster and thus obtained quicker a higher larval mass. As result, parasitoid progeny on Badger Shipper might have started consuming host tissues immediately, while parasitoid progeny on for example Lennox could have delayed their development.

4.3. Field experiment

Parasitisation rates of *P. xylostella* larvae in the field were not significantly different for the four cultivars. Poelman et al. (2008b), who performed a similar field experiment with *P. rapae* and *P. brassicae* larvae and its parasitoids *C. glomerata* and *C. rubecula* found significant higher parasitisation rates on Christmas Drumhead compared to the other three cultivars. Furthermore, the results from their laboratory assays demonstrated that parasitoids were attracted most to the same cultivar on which highest parasitisation rates were found in the field. Although not significantly different in this field experiment, parasitisation rate was highest on Rivera, followed by Christmas Drumhead. Unfortunately, due to problems with the Y-tube bioassay, results of the field experiment could not be compared with findings from the preference experiment.

To avoid collection of *P. xylostella* larvae naturally occurring in the field, second instar larvae were released in the field and only second or third instar larvae were recollected. However, especially at the end of June and early in July more than 20 larvae were recollected from one plant, making it impossible to determine the actual rate of the released larvae that were parasitized within the three days between release and recollection. In contrary to the beginning of the field experiment, the number of larvae recollected during the four last times of release was very low, which was also the reason for leaving out these data. Probably a majority of larvae died in the field, since from that moment onwards problems occurred with the rearing of *P. xylostella* in the laboratory, whereby larvae massively died.

Where survival rate until pupation and survival rate until emergence was calculated for the performance experiment, parasitisation rate was calculated for the field experiment. The objective of the performance experiment was to focus on the development of *D. semiclausum* in *P. xylostella* hosts feeding upon the four cabbage cultivars. For this objective, all larvae were parasitized, and therefore parasitisation rate was not determined, but the number of parasitized larvae in which *D. semiclausum* had successfully developed until pupation and until emergence. In contrast to the performance experiment, the objective of the field experiment was to focus on the attraction of *D. semiclausum* to the four cabbage cultivars in the field, and not to focus on the development of *D. semiclausum* on the four cultivars in the field. For this objective, parasitisation rates become interesting, also if that includes dead cocoons and dead parasitoid larvae. After all, whether or not adult wasps would have emerged from the cocoons, in any case no *P. xylostella* larvae would have emerged from the parasitized larvae, which would still lead to a benefit in pest control. Nevertheless, a separate GLIM was used for checking differences in *D. semiclausum* mortality, because reduced offspring threatens continuous establishment of *D. semiclausum* on the four cultivars, but significant differences were not found.

In contrast to the findings of the performance experiment, the number of males and females turned out to be exactly the same; 740 males and 740 females had emerged during the first six weeks of the field experiment. Apparently differences in sex ratio, as a result of complementary sex determination, did not appear in the field. Among the females, some *D. fenestrale* were noticed and were included in the parasitisation rate, since *D. fenestrale* males are hard to distinguish from *D. semiclausum* males and it otherwise would have led to overestimation of males. However, the percentage of *D. fenestrale* females was very low, making up not more than 3% of the total number of females.

5. Conclusion

Summarizing the results, not one of the cultivars Badger Shipper, Christmas Drumhead, Lennox or Rivera appeared to be optimal for the development of *D. semiclausum* while at the same time most attractive to *D. semiclausum*. First of all, no significant differences were

found in parasitisation rate between the four cultivars in the field. Furthermore, not one cultivar was outstanding for the performance of *D. semiclausum*, since no significant differences were found in egg-to-adult development time between the cultivars, while only a significant higher dry weight of *D. semiclausum* was found on Christmas Drumhead when compared to Lennox and Rivera, and on Badger Shipper when compared to Lennox. Moreover, including results of other studies, it seems as if there is a positive correlation between development of *P. xylostella* and development of *D. semiclausum* on the four cultivars. Where Badger Shipper seems an interesting cultivar for the development of *D. semiclausum*, same conclusions could be drawn for the development of *P. xylostella* on this cultivar. That also holds for Lennox, on which development of both *P. xylostella* and *D. semiclausum* was least favourable compared to the other cultivars. The lower survival rate until pupation of *D. semiclausum* that was found on Badger Shipper during the performance experiment might have been the result of stronger *P. xylostella* larvae that were better capable in encapsulating eggs of *D. semiclausum*. However, *D. semiclausum* seems to have compensated this lower rate with a relatively high number of viable adults, since survival rate until emergence on Badger Shipper was not significantly lower than on the other three cultivars. Nevertheless it is important to realize that although a cultivar is good for the development of a parasitoid like *D. semiclausum*, it does not necessarily mean that it leads to a benefit in pest control in case its host is also developing well on that cultivar. Concerning attractiveness of the four cultivars to *D. semiclausum*, it would have been favourable if more data were obtained during the field experiment and that findings of the field experiment could have been compared with preference tests in the laboratory. Therefore the preference experiment still needs to be performed and a new field experiment with more successful repetitions would be strongly recommended.

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