To Combat Pests; a Study into the Ability of Cabbages to attract *Aphidius colemani* after Infestation by *Myzus persicae*

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Abbreviations

BWYV	Beet western yellows virus
C	Celsius
CaMV	Cauliflower mosaic virus
cm	centimeter
EU	European Union
FAO	Food and Agriculture Organization
g	gram
GPA	Green peach aphid
ha	hectare
hg	hectogram
HIPVs	herbivore-induced plant volatiles
HR	Croatia
intro	introduction
IT	Italy
l	liter
min	minute
ml	milliliter
NL	The Netherlands
Nr	Number
Prop	Proportion
PV	P-value
RCBD	Randomized Complete Block Design
Rep	Replicate
SCCM	Standard cubic centimeters per minute
SE	Standard Error
Sig	Significance
spp	several species
TuMV	Turnip mosaic virus
UA	Ukraine
UK	United Kingdom
var	variety
WUR	Wageningen University and Research

Abstract

Cabbage (*Brassica oleracea* var. *capitata* L.) is an important vegetable grown worldwide. *Myzus persicae* Sulzer, green peach aphid (GPA), feeds on a wide range of crops and is an important virus vector. *Aphidius colemani* Viereck, is a parasitoid wasp which is used in the biological control of GPA. *Aphidius colemani* uses herbivore-induced plant volatiles (HIPVs) for host-seeking. HIPVs are chemical compounds that differ between plants and are detected by natural enemies of herbivores. Breeders make selections among plants and improve crops in line with the objectives of breeding programs. However, some traits may be lost or ignored during selection. Some plants were found to lose their indirect insect resistance after domestication and breeding. In this study, we investigated the hypothesis that there is genetic variation between certain accessions of cabbage in the attraction of natural enemies and the consequential parasitization of aphids. To test this hypothesis, the following research question was answered; which of the studied accessions attract parasitoid wasps the most and is thus most suitable for plant breeding? This was researched by answering the following sub questions; which accessions contained the most parasitized aphids within the controlled environment of an insect rearing cage? And how do the level of attractiveness of infested and non-infested plants differ in an olfactometer assay? No statistically significant differences were found between infested and non-infested plants of each accession in the olfactometer assay. The cage experiment showed that three accessions, *B. incana* (Bwi2011-0023), *B. oleracea* var. *acephala* (Bol2011-0131) and *B. villosa* (Bwi2011-0050) had a higher parasitization rate than the others. The use of these accessions for parent line development in pre-breeding may increase the wasp attractiveness of hybrid cultivars, and therefore increase the effective use of the wasp *A. colemani,* in the control of the aphid *M. persicae.*

Acknowledgements

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Introduction

Cabbage (*Brassica oleracea* var. *capitata* L.) is an important vegetable grown worldwide. *Myzus persicae* Sulzer (Hemiptera: Aphididae), also known as green peach aphid (GPA) is a polyphagous aphid that quickly matures and reproduces as many other aphids (Dixon, 2012). It feeds on a wide range of crops and besides the cosmetic and mechanical damage it causes, it is an important virus vector for crops (Van Emden et al., 1969; Van Emden and Harrington, 2017). GPA is responsible for transmission of more than 100 viruses including major *Brassica* viruses such as turnip mosaic virus (TuMV), cauliflower mosaic virus (CaMV) and beet western yellows virus (BWYV) which lead to serious yield and quality loss in cabbage production (Bos, 1982; Kennedy et al., 1962; Kyriakou, 1984; Samara et al., 2021).

Chemical insecticides are mostly used in GPA management (Tial et al., 2022). However, insecticides and pesticides in general negatively affect human health and the environment (Keifer et al., 2007; Pimentel, 1995; Sharma et al., 2020). In addition, some chemical insecticides can be toxic to natural enemies of herbivorous pests (Haseeb et al., 2004). In particular, GPA has started to gain field resistance after insecticides were used to suppress populations in 1950s, and it has been noted that it is resistant to 84 active ingredients today (Anthon, 1955; Mota-Sanchez and Wise, 2023). Complimentary to chemical insecticides, biological control of GPA is also possible, especially parasitoid wasps, have potential in crop protection (Steenis, 1992; Van Lenteren, 2012; Wang et al., 2019). Parasitoid wasps use herbivore-induced plant volatiles (HIPVs) to locate aphids, oviposit their eggs by stinging their ovipositor into the aphid’s body, and their larvae kill aphids by feeding on them (Benelli et al., 2014). *Aphidius colemani* Viereck (Hymenoptera: Braconidae) is one of the species commercially produced for use in the biological control of GPA (Fernandez and Nentwig, 1997; Khatri et al., 2017).

Some plants have developed indirect defence mechanisms to resist herbivores (Dicke et al., 1990; Takabayashi and Dicke, 1996). HIPVs are species-specific chemical compounds that differ between plants and are detected by natural enemies of herbivores (Kessler and Baldwin, 2002; Takabayashi and Dicke, 1996). When attacked by herbivores, *Brassica* spp. emit HIPVs and attract natural enemies of herbivores that receive the cues (Potting et al., 1999; Takabayashi and Dicke, 1996; Uefune et al., 2017). These natural enemies then feed on the herbivores or cause their death by parasitization.

Breeders make selections among plants and improve crops in line with the objectives of breeding programs. These objectives can be yield, resistance to biotic and abiotic stress conditions, taste and many more (Miflin, 2000; Passioura, 2002). However, some traits may be lost or ignored during selection; for example, molecules that provide plant defense mechanisms may cause a bitter, undesired taste, but the same molecules may be involved in pest and disease resistance (Ku et al., 2020), and some plants were found to lose their indirect insect resistance after domestication and breeding (Chen et al., 2015; Moreira et al., 2018). This means that the genetic diversity of the population is reduced, which decreases the ability of the plant to produce compounds that can act as natural insecticides and other defense mechanisms (Mitchell et al., 2016). When indirect insect resistance is lost, the plant becomes more vulnerable to insect attack, leading to reduced crop yields and increased costs for farmers. Additionally, the lack of insect resistance can have an impact on the surrounding ecosystem, as it can lead to an increase in the number of insect pests and a decrease in the number of beneficial insects.

Restriction of pesticide use in agricultural products by official authorities has led to reduced crop yields (Handford et al., 2015; Popp et al., 2013), and therefore it is of great importance to consider alternatives in plant breeding to increase resistance against herbivores (Tamiru et al., 2015; Wink, 1988). One possible alternative is the use of the plants' natural defense system, through attracting the natural enemies of pests. This was previously researched by Caarls et al. in 2021, on the possibility of using the plants' ability to attract the natural enemies of its infestations in breeding programs to provide insect resistance. Eighteen accessions from *B. villosa*, *B. incana* and *B. oleracea* varieties were compared to the landrace Christmas Drumhead, which is known to attract natural enemies of *Pieris* spp. Caterpillars (Aartsma et al., 2019). In the experiment by Caarls et al, plants infested by *P. xylostella* were compared using an olfactometer on choices of *C. vestalis* between infested cabbage accessions. Among these accessions, accessions of *B. oleracea* var. *acephala*, *B. oleracea* var. *italica*, *B. incana* and *B. oleracea* var. *capitata* (landrace Christmas Drumhead) were determined to be attractive to the wasp, and the *B. oleracea* var. *capitata* (hybrid cultivar Rivera), *B. incana* (a different accession than the one just mentioned) and *B. villosa* were determined to not be attractive. However, it was not known whether the results obtained in the olfactometer assay would be the same under greenhouse conditions and more importantly, if they also have an effect on the parasitization of pest insects.

The development of parent lines which demonstratively have the ability to attract the natural enemies of their pests, and the consequential introduction of this trait through backcrossing into an already existing cabbage cultivar could result in a cabbage cultivar that will protect itself from severe infestation, thereby reducing the need for insecticides in cabbage production, improve biological control of pests, reduce inputs and more importantly it will positively contribute to human health and the environment. Thus this study examines which of the eight cabbage accessions attract *A. colemani* the most, so that they could be used in plant breeding.

The proposed hypothesis of this study is that there is genetic variation between certain accessions of cabbage in the attraction of natural enemies and the consequential parasitization of aphids. To test this hypothesis, the following research question was answered; which of the studied accessions attract parasitoid wasps the most and is thus most suitable for plant breeding? This was researched by answering the following sub questions; which accessions contained the most parasitized aphids within the controlled environment of a cage? And how do the level of attractiveness of infested and non-infested plants differ in an olfactometer assay?

Material and Methods

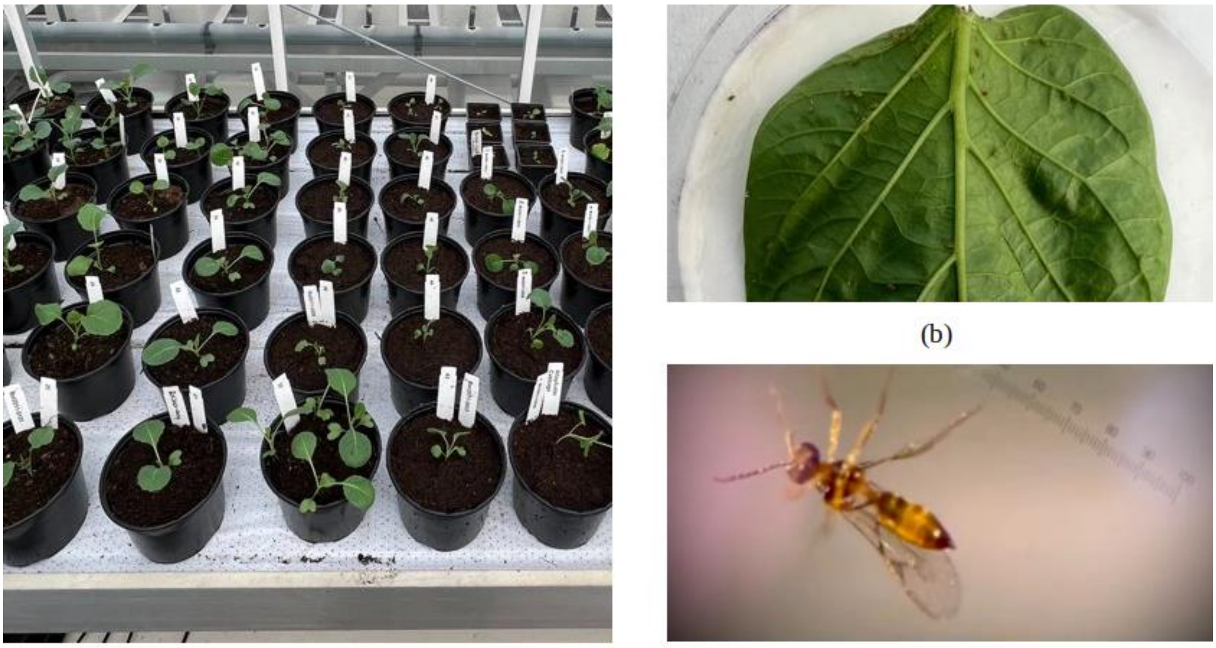
Seeds of the *B. oleracea* var. *capitata*, *B. oleracea* var. *acephala*, *B. oleracea* var. *italica*, *B. incana* and *B. villosa* (Table 1) were obtained from the Plant Breeding Department, Wageningen University and Research (WUR). It was observed in the test experiment that the growth rates of the accessions were different from each other, and therefore the seeds were sown at intervals in pots with potting soil. Plants were watered when needed and kept in the greenhouse at 19-21 °C with 70% relative humidity and 16:8 light and dark photoperiod.

Accession Name	Species	Description	Attractive*	Sown Date	Origin
Bol2012-0023	*B. oleracea* var. *capitata*	Christmas Drumhead; landrace of white cabbage	Yes	14.09.22	UK
Bol2021-0004	*B. oleracea* var. *capitata*	Rivera; hybrid cultivar of white cabbage	No	21.09.22	NL
Bol2011-0131	*B. oleracea* var. *acephala*	Kale; landrace	Yes	14.09.22	HR
Bol2011-0208	*B. oleracea* var. *italica*	Broccoli; landrace	Yes	21.09.22	IT
Bwi2011-0026	*B. incana*	Wild relative of *B. oleracea*	Yes	14.09.22	UA
Bwi2011-0023	*B. incana*	Wild relative of *B. oleracea*	No	21.09.22	IT
Bwi2011-0048	*B. villosa*	Wild relative of *B. oleracea*	No	31.08.22	IT
Bwi2011-0050	*B. villosa*	Wild relative of *B. oleracea*	No	31.08.22	IT

Table 1. Accessions selected from the study conducted by Caarls et al. in 2021.
* Attractiveness to *C. vestalis* after *P. xylostella* infestation on different *Brassica* plants (Caarls et al., 2021).

Another thing makes this study different than the previous is that a different parasitoid wasp and pest species with similar characteristics in terms of both insecticide resistance and ability to cause economic destruction was used. *Myzus persicae* aphids (Biofood population) were taken from a rearing on *Capsicum annuum* (pepper) of Plant Breeding - Insect Resistance Group, Wageningen University & Rearing. Approximately 100 adult aphids were collected, put in petri dishes (90 mm) with a piece of pepper leaf on moistened filter paper, sealed with parafilm and stored in the same greenhouse compartment at 23-25 °C with 60% relative humidity and 16:8 light and dark photoperiod. The first instar nymphs, which were born within 24 hours, were collected the petri dish and used in the cage experiment. For the olfactometer assay, the same method was applied to approximately 20 aphids. For each accession in the olfactometer assay, first instar nymphs they gave birth in every day were collected to be used.

Wasps were obtained in mummy formation, from a commercial product Aphipar (Koppert B.V.) and kept in a small mesh cage (50x50x50 cm) in the greenhouse at 21-23 °C with 60% relative humidity and 16:8 light and dark photoperiod. In three days, adult wasps emerged. A petri dish (50mm in diameter) containing 10 ml of 1:1 honey and water solution was put in the cage for wasps to feed on. They were caught one by one with a manual aspirator and kept in glass tubes. Female wasps were identified under a stereomicroscope (Stemi SV8, Zeiss) with light source (KL 1500 LCD, Zeiss) by checking their ovipositors without sedation. This process was repeated on different dates for both experiments.



(b)

(a) (c)

Figure 1. Pictures from the study.(a) cabbage accessions; (b) nymph synchronization; (c) wasp gender detection.

Choice in Cage Experiment

The experiment to study choice of *A. colemani* was designed as Randomized Complete Block Design (RCBD), consisting of 2 blocks with 2 replicates. Each block consisted of a 200 cm width, 100 cm depth and 100 cm height mesh insect rearing cage (BugDorm-6M1020); a total of 32 plants were randomly placed, 16 plants in each block (Figure 2). Plants were watered when needed and kept in the greenhouse at 21-23 °C with 60% relative humidity and 16:8 light and dark photoperiod. The plants were 4-7 weeks old and had 4-6 fully developed leaves at aphid introduction and 5-7 leaves at wasp introduction.

Aphids were placed in a clip cage (2 cm in diameter) on the youngest but fully developed leaf of the plants (Figure 2) as youngest leaves are more preferred by aphids (Cao et al., 2018), 5 aphids per clip cage. Aphids were given 6 days to feed. Subsequently, adult and naive *A. colemani* wasps were released into the cages, 16 per cage. Wasps stayed in the cages for 24 hours to parasitize the aphids. Afterwards, wasps were collected for extermination (freezing) and aphids for further examination. Number of the aphids on each plant before and after the wasp introduction was recorded. Before wasp introduction, some aphids were found to have died or escaped from pots, and this was recorded.

One petri dish was assigned for each plant, and the aphids were collected from the plants and placed in the petri dishes (Figure 2). Some leaves from the plants where the aphids were collected were placed in petri dishes for them to feed until the parasitization check. Petri dishes were sealed with parafilm and kept in the same greenhouse compartment during this time. Occasionally, excess moisture accumulated in the petri dish was removed with a napkin. Finally, 7 days after the collection each aphid was checked either visually or under the stereomicroscope (SZH, Olympus Europa SE & Co. KG) with light source (Highlight 2000, Olympus Europa SE & Co. KG) for the presence of *A. colemani* larvae (Figure 2). The quantity of parasitized aphids per plant was recorded. At the end of the experiment, all used plants and insects were disposed of and the tools and equipment used were cleaned.

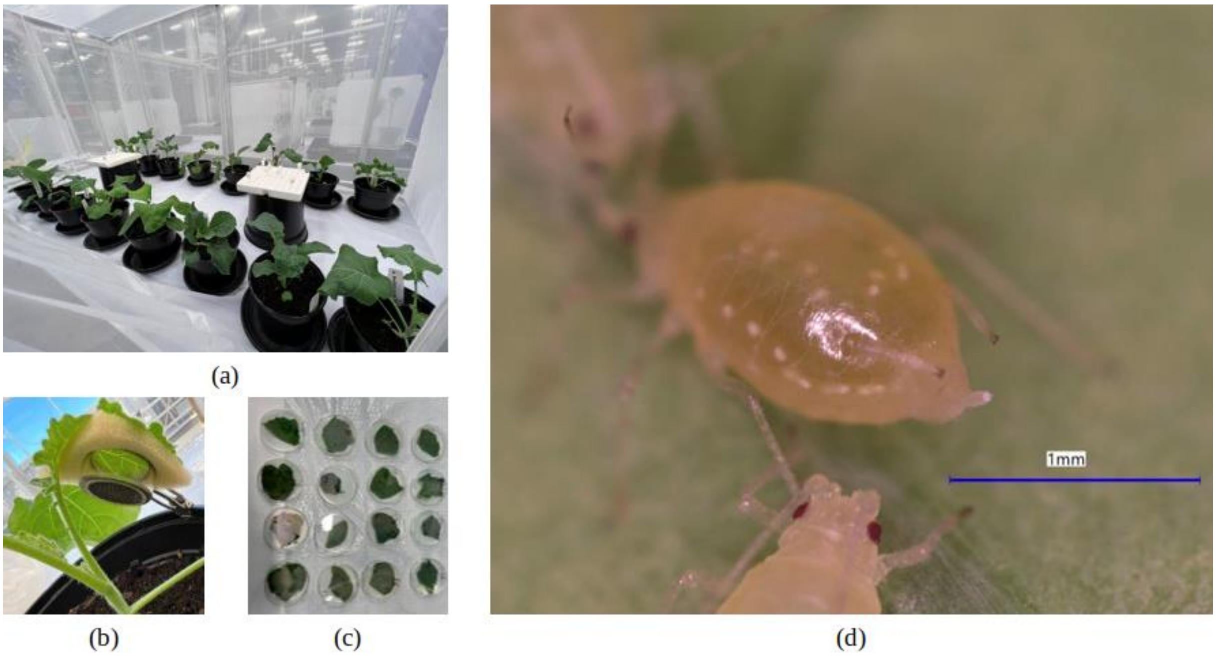


Figure 2. Pictures of cage experiment. (a) cage setup; (b) clip cage on the plant; (c) collected aphids after wasp release; (d) visible spiracles of *A. colemani* larvae inside the *M. persicae*

The total number of aphids differs for each accession, since in most of the accessions, a small number of aphids were seen to die or escape from the clip cages before wasp release. Since there were not equal numbers of aphids on all plants, parasitization ratios of accessions used in experiment evaluation. It was seen that the data obtained were not suitable for statistical analysis due to the complexity and small size of the experiment.

Olfactometer Assay

A total of 16 plants, sown on the same dates as the plants used in the cage experiment, were placed in insect-proof cages; 2 plants per accession. They were watered when needed and kept in the greenhouse at 19-21 °C with 60% relative humidity and 16:8 light and dark photoperiod. The plants were 8-11 weeks old and had 13-14 fully developed leaves when the assay was conducted.

Half of the plants were not infested. for the other half of the plants, aphids were placed in a clip cage (2 cm in diameter) on the youngest leaf of each plant, 5 aphids per clip cage and one clip cage per plant. They kept for 7 days on the plant. The clip cages were then collected and the infested plants were gently cleaned of aphids with a soft brush.

One infested and one non-infested plant of each accession were placed in glass chambers to observe for each accession whether wasps choose between infested and non-infested plants using a Y-tube olfactometer (Figure 3). Assay was conducted in a room which has only one entrance and does not have any windows or ventilation in 22 °C with 68% relative humidity.

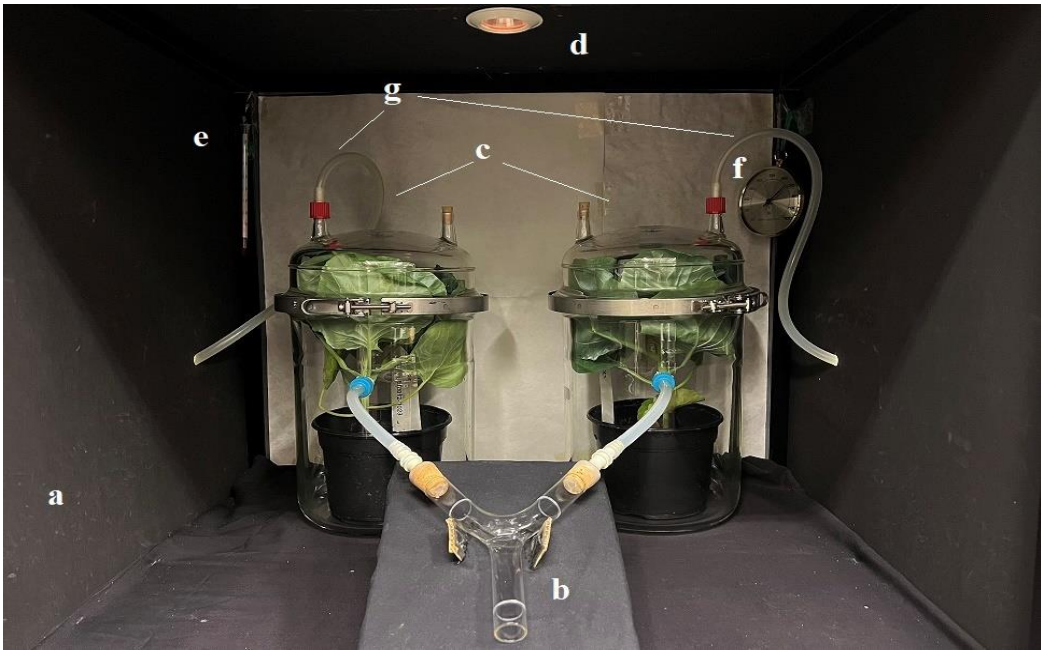


Figure 3. Olfactometer setup. The olfactometer setup consists of the following elements: (a) a plywood box reinforced with a metal frame forms the basis of the setup; (b) Y-tube and a stand carrying it; (c) two glass chambers (20 cm in diameter, 40 cm height) with in the one the left for the infested plants and the one on the right for the non-infested plants; (d) a lamp (20W 12V Halogen) which was placed right in the middle of chambers; (e) thermometer; (f) hygrometer; (g) silicone tubes (0.5 cm in diameter) that convey the pumped air from chambers to the Y-tube are inside the box.

Outside of the olfactometer, there is a flask of water filter (400 ml), a flask of charcoal filter (approximately 200 g) and a glass tube variable area flow meter (SHO-RATE 1355, Brooks Instrument B.V.), for each chamber. In addition to these, there is a blackout curtain and a mini diaphragm vacuum pump (LABOPORT N 86 KT.18, KNF Verder B.V.) which sucks the air through another water filter (400 ml) and conveys through the silicone tubes to the variable area flow meter. The variable area flow meter manually adjusts the air flow. The air is then sent directly to the chambers. The air supplied to the chambers was fixed manually at 300 SCCM (0.3 l/min), and controlled by measuring before each comparison with a digital air flow meter (Digital Flow Check, Alltech). During the experiment, the lamp was kept on to imitate sunlight and the laboratory lighting was blocked by the blackout curtain.

The Y-tube is a glass tube (2 cm in diameter) which has two arms (8 cm length each) that receive the airflow coming through plants and a stem (10 cm length) where the insect enters the experimental setup. A line was drawn in the middle of the tube arms (4 cm from the junction point) and another on the stem (2 cm from the entrance) to determine whether the wasp made a choice. By giving filtered air to the plants in the closed chambers, the plant odours reached the tube arms.

An adult but naive female wasp was released into the Y-tube in a small glass tube with the tube wrapped in a piece of cotton (to prevent the wasp from escaping) and aligned to the entrance line. Since it has been observed that wasps usually choose one of the plants within the first 5 minutes and want to move along the air tube or do not leave the Y-tube entrance, it was checked 5 minutes after the wasp's release whether it had made a choice. Wasps that choose any of the arms and cross the arm line in 10 minutes were considered to have made a choice. Wasps that did not choose any of the plants within 10 minutes were considered to not have made a choice.

After 10 wasps had made their choice, the plants were replaced by the next accession. Before replacement, both chambers were cleaned with 70% ethyl alcohol. At the end of the experiment, all used plants and insects were disposed of and the tools and equipment used were cleaned.

Eight pairwise comparisons (infested vs. non-infested plants for each accession) were planned, but due to disruptions in wasp supply, seven comparisons could be made, Bol2011-0131 was excluded from this experiment. For each comparison, 10 replicates were performed; the choice of a wasp was considered as a replicate. The responses from the wasps were received as either ‘has chosen the infested plant’ or ‘has chosen non-infested plant’, therefore they are converted to binary form as ‘1’ and ‘0’ respectively. Since this is a choice assay and binary comparisons were made, a two-tailed binomial test (test proportion=0.50) was performed for statistical analysis by using the software IBM SPSS Statistics 29.

Results

Cage Experiment

In this experiment, the attractiveness of *A. colemani* to some cabbage accessions was investigated in an insect cage. None of the 17 aphids placed on accession Bwi2011-0026 were parasitized. 2 out of 17 aphids placed on Bol2011-0208 were parasitized, 4 out of 19 placed on Bol2021-0004, 4 out of 18 placed on Bol2012-0023 and Bwi2011-0048, 13 out of 20 placed on Bwi2011-0023, 12 out of 18 placed on Bol2011-0131, and 14 out of 17 placed on Bwi2011-0050 were parasitized. The highest number of parasitizations were on these three accessions. See Appendix A for a table with detailed results.

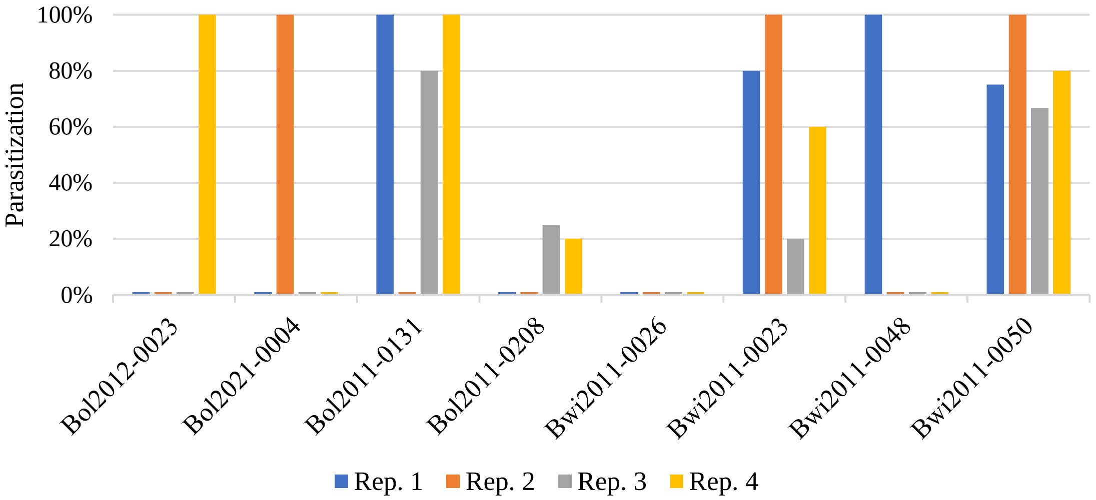


Figure 4. Parasitization ratios. No parasitization was observed in Bwi2011-0026. On Bol2012-0023, Bol2021-0004 and Bwi2011-0048 parasitization was observed only in one of the replicates, as 100%. Bol2011-0208 showed approximately 20% parasitization in two replicates. Parasitization was seen on three replicates of Bol2011-0131 and all of the four replicates of Bwi2011-0023 and Bwi2011-0050.

Olfactometer Assay

In this experiment, whether there is a difference in the choice of *A. colemani* between *M. persicae* infested and non-infested plants of some cabbage accessions was investigated in olfactometer. Since some wasps did not respond when they were released, 12-18 wasps were used for each comparison.

Accession		Category	N	Observed Prop.	Test Prop.	Exact Sig. (2-tailed)
Bol2012-0023	Group 1	Non-infested	6	.60	.50	.754
	Group 2	Infested	4	.40		
	Total		10	1.00		
Bwi2011-0048	Group 1	Non-infested	6	.60	.50	.754
	Group 2	Infested	4	.40		
	Total		10	1.00		
Bwi2011-0026	Group 1	Non-infested	5	.50	.50	1.000
	Group 2	Infested	5	.50		
	Total		10	1.00		
Bwi2011-0023	Group 1	Non-infested	4	.40	.50	.754
	Group 2	Infested	6	.60		
	Total		10	1.00		
Bol2011-0208	Group 1	Non-infested	5	.50	.50	1.000
	Group 2	Infested	5	.50		
	Total		10	1.00		
Bol2021-0004	Group 1	Non-infested	5	.50	.50	1.000
	Group 2	Infested	5	.50		
	Total		10	1.00		
Bwi2011-0050	Group 1	Non-infested	4	.40	.50	.754
	Group 2	Infested	6	.60		
	Total		10	1.00		

Table 2. *Results of olfactometer assay. Group 1 represents the choices of wasps for non-infested plants. Group 2 represents the choices of wasps for infested plants.*

No significant difference was found in the comparison of *M. persicae* infested and non-infested plant of each accession regarding choices of *A. colemani* (PV = .05). Both infested and non-infested plants were chosen around the same time in this experiment.

Discussion and Conclusion

In the choice in cage experiment, three cabbage accessions showed higher parasitization than the others. No significant difference was found between the infested and non-infested plants in the olfactometer assay. The experiments were expected to demonstrate that of the eight accessions, the four previously found to be most chosen in an olfactometer assay by Caarls et al. would also be the most attractive to the parasitoid wasp, *A. colemani*, when infested by the aphid *M. persicae*.

Although it was determined by Caarls et al. that they attract *C. vestalis* after a *P. xylostella* infestation, no parasitization by the *A. colemani* was observed in the cabbage wild relative *B. incana* (Ukraine) and the second lowest was observed at the accession of broccoli landrace (*B. oleracea* var. *italica*) with 11,8%. Cabbage hybrid cultivar Rivera (*B. oleracea* var. *capitata*) showed 21,1% parasitization. Slightly higher parasitization was seen in cabbage landrace Christmas Drumhead (*B. oleracea* var. *capitata*) and wild relative *B. villosa* (Bwi2011-0048, Italy) at 22,2%. However, another *B. villosa* accession Bwi2011-0050, which was also from Italy and determined as not-attractive by Caarls et al., showed the highest parasitization with 82,4%. Despite the fact that it was previously found to not be attractive for the *C. vestalis*, the other *B. incana* accession (Italy) showed 65,0% parasitization. An earlier thought attractive accession of kale (*B. oleracea* var. *acephala*) showed 66,7% parasitization.

The results show that some accessions determined to be attractive for the species in studied by Caarls et al., were not attractive for *A. colemani* in the cage experiment, and accessions found to be not-attractive by Caarls et al. were attractive for *A. colemani*. The results further revealed that, in some replicates, accessions that were deemed attractive showed no signs of parasitization when placed next to those deemed not-attractive (See Figures 5 and 6). Furthermore, the same accessions previously found to be attractive, exhibited no parasitization when placed side by side with each other (See Figures 5 and 6).

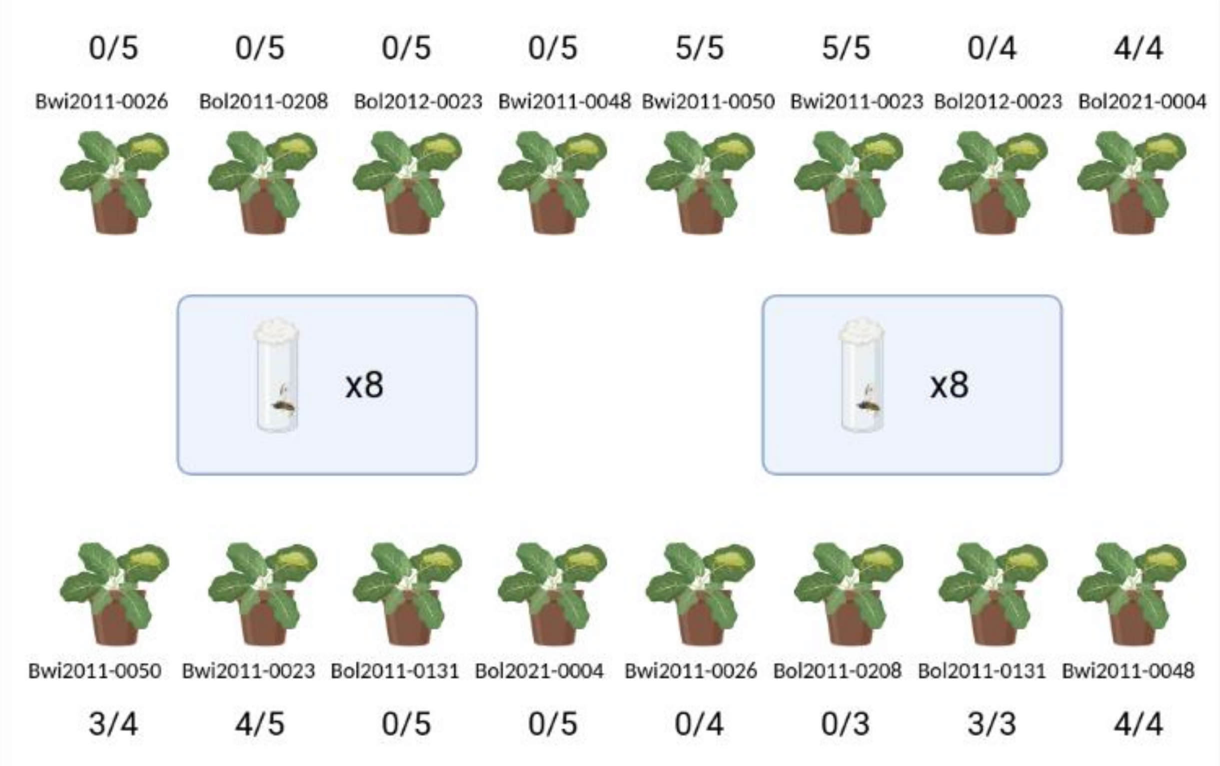


Figure 5. The placement of the plants in the first block. The bottom row represents the first replicate, while the top row represents the second replicate. The fractions below and above the plants indicate the results of the parasitization.

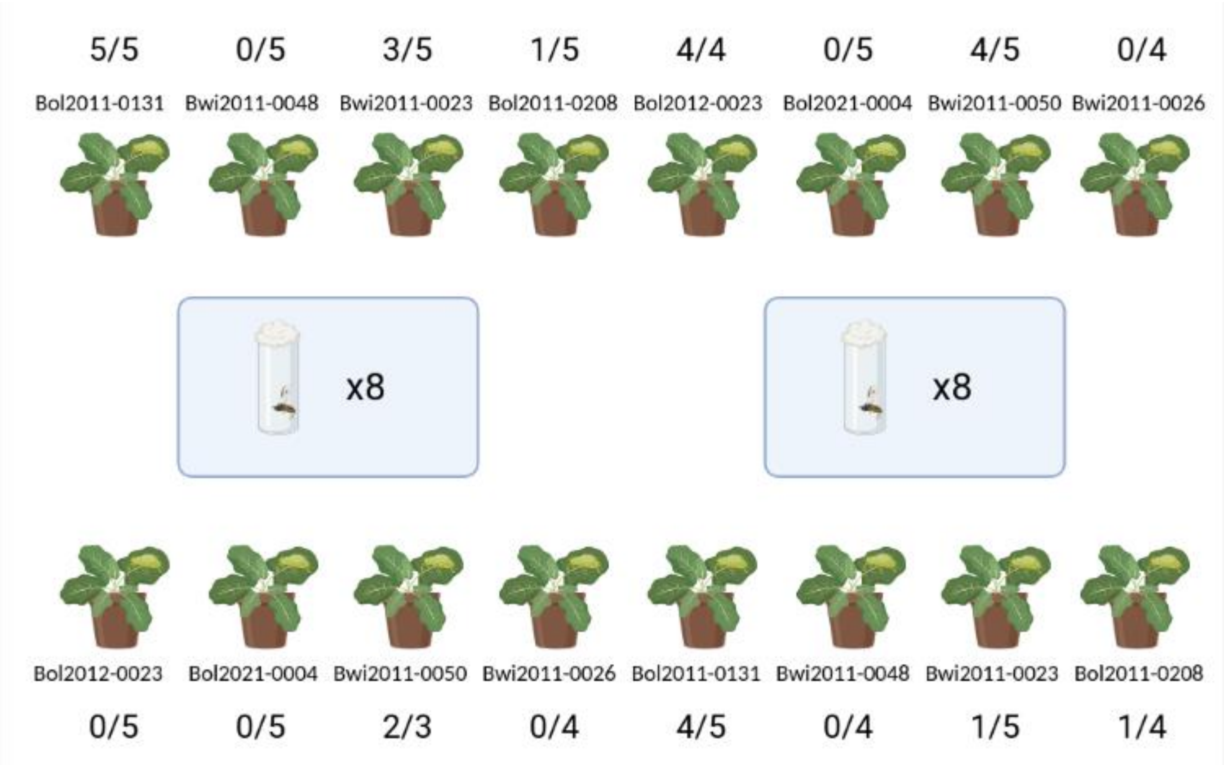


Figure 6. The placement of the plants in the second block. The bottom row represents the third replicate, while the top row represents the fourth replicate. The fractions below and above the plants indicate the results of the parasitization.

According to the review of Rehman and Powel (2010), wasps initially locate a suitable plant (habitat detection) and then search for a host on the same plant or on nearby ones. This explains that the accessions with high parasitization of this study are more suitable habitats for *A. colemani* than the others. On the other hand, this discrepancy between the Caarls et al. study might be due to the fact that different wasp species may react to different volatiles for host-seeking (Whitman and Eller, 1990). The close proximity of the plants to each other in the cage experiment may have had a small effect on the parasitization rate. For example, a wasp may have parasitized aphids on a plant and then taken a short flight to the adjacent plant to seek other hosts; or a wasp may have located one of the accessions which were deemed attractive, but not accepted by the hosts for parasitization and then took a flight to the other plants for seeking new hosts. Redesigning the experiment with a greater distance between plants, in an entire greenhouse compartment rather than a cage, could give more suitable data to statistically compare the differences between accessions.

On the other hand, in the olfactometer experiment, no significant difference was observed between the infested (cleaned of aphids prior to the assay) and non-infested plants of accessions. The reason for this may be that some plants emit cues, even when they are not infested, which makes it easier for natural enemies to detect suitable habitats (Lo Pinto et al., 2004). Interpreting the findings in this way can provide insight into how well they match up with the results of the cage experiment, as it allows for comparison of the accessions without infestation. Also, HIPVs are of great importance during the beginning processes of finding a host, however there is not much information available on their role in relation to *A. colemani* (Benelli et al., 2014). In addition, the inability to detect a difference in wasp attraction between infested plants and non-infested plants may also be due to the low number of replicates. Another possible explanation for the results might be that using only five aphids per plant may not have been enough to trigger the emission of HIPVs by the plants, as evidence suggests that HIPVs emission increases as the density of herbivores increases (Cai et al., 2014). Lastly, attraction of wasps might be negatively affected due to the modification of HIPVs blends by root colonizing bacteria (Pineda et al., 2013). Increasing the infestation density and the number of replicates, and assessing the microbial activity of the roots could improve the experiment.

The cage experiment showed that *B. incana* (Bwi2011-0023), *B. oleracea* var. *acephala* (Bol2011-0131) and *B. villosa* (Bwi2011-0050) had a higher parasitization rate than the others. The use of these accessions for parent line development in pre-breeding may increase the wasp attractiveness of hybrid cultivars, and therefore increase the effective use of the wasp *A. colemani*, in the control of the aphid *M. persicae*. It is rare to see such third trophic level interactions included in plant breeding programs. Unfortunately, pests' rapid increase in resistance to insecticide ingredients frequently causes pest outbreaks. HIPVs may contribute for protection against these types of devastation. The genes that produce these chemicals are the building blocks of the third trophic level interaction mechanism. The accessions in which these genes are found could be used in the development of hybrid cultivars that could help reduce pest outbreaks.

This study laid the groundwork for what could be done in future studies. It suggests that future research should use higher numbers of aphids in their experiment and to re-examine the cage set up. Furthermore, attractive accessions may also be effective in the attraction of other natural enemies. Thus, this study could be redesigned and implemented for other pests and natural enemies. Therefore, this master's thesis forms the basis for future studies.

Appendix A

Results of the Cage Experiment

Accession	Species	Nr. of Aphids	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Total
Bol2012-0023	B. oleracea var. capitata	Before wasp intro.	4	5	5	4	18
		Parasitized	0	0	0	4	4
		Parasitization Ratio	0,00	0,00	0,00	1,00	0,22
Bol2021-0004	B. oleracea var. capitata	Before wasp intro.	5	4	5	5	19
		Parasitized	0	4	0	0	4
		Parasitization Ratio	0	1	0	0	0,21
Bol2011-0131	B. oleracea var. acephala	Before wasp intro.	3	5	5	5	18
		Parasitized	3	0	4	5	12
		Parasitization Ratio	1,00	0,00	0,80	1,00	0,66
Bol2011-0208	B. oleracea var. italica	Before wasp intro.	3	5	4	5	17
		Parasitized	0	0	1	1	2
		Parasitization Ratio	0,00	0,00	0,25	0,20	0,11
Bwi2011-0026	B. incana	Before wasp intro.	4	5	4	4	17
		Parasitized	0	0	0	0	0
		Parasitization Ratio	0	0	0	0	0,00
Bwi2011-0023	B. incana	Before wasp intro.	5	5	5	5	20
		Parasitized	4	5	1	3	13
		Parasitization Ratio	0,80	1,00	0,20	0,60	0,65
Bwi2011-0048	B. villosa	Before wasp intro.	4	5	4	5	18
		Parasitized	4	0	0	0	4
		Parasitization Ratio	1,00	0,00	0,00	0,00	0,22
Bwi2011-0050	B. villosa	Before wasp intro.	4	5	3	5	17
		Parasitized	3	5	2	4	14
		Parasitization Ratio	0,75	1,00	0,67	0,80	0,82

Appendix A. Rep. 1 and Rep. 2 are replicates of first block, while Rep. 3 and Rep. 4 are replicates of second block.

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