



Swedish University of Agricultural Sciences  
Faculty of Natural Resources and Agricultural Sciences  
Department of Ecology

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## Abstract

Insects search primarily to find food, egg-laying sites, resting sites or conspecifics. Understanding the search behaviour of insects at the point when they are not in contact with the host is relevant to for instance the control of pest species. There are many studies on how adult butterflies respond to different olfactory signals when distanced from the host, but less is known of how caterpillars use and respond to such signals. This study was conducted to investigate what olfactory signals attract gregarious *Pieris brassicae* caterpillars. Are damaged host plants more attractive than undamaged plants, or will signs of conspecifics such as frass attract the caterpillars? Furthermore, will caterpillars respond differently to different genotypes of the host?

Using four-way olfactometers, preference experiments were conducted on the genotypes broccoli (*Brassica oleracea* L. var. *cymosa*), cabbage (*Brassica oleracea* L. var. *capitata*) and cauliflower (*Brassica oleracea* L. var. *botrytis*) of the larval host *Brassica oleracea*. One preference experiment tested the caterpillars' response to undamaged plants of the genotypes. In the other three experiments the response of gregarious *P. brassicae* caterpillars to insect-induced damage (by conspecifics), no damage, and frass treatments of each of the genotypes were tested. The results showed no difference in preference for any of the undamaged genotypes. There was also no difference in the caterpillars' preference for any of the treatments of broccoli or cabbage, but a significant preference for damaged cauliflower over frass and the control (air). This could be explained by an attraction to the damaged plant tissue itself, or an attraction to conspecific presence because of the species-specific combination of compounds released by the plant. If the lack of difference between the undamaged genotypes is because the genotypes are equally preferred cannot be concluded before further studies have been made, comparing damaged treatments of the genotypes. It is clear from this study that caterpillars of *P. brassicae* respond to olfactory signals.



# Table of Contents

Abstract .....	3
1. Introduction .....	7
1.1. Sensory organs of lepidopteran larvae .....	9
1.2. Chemistry of the host species .....	9
1.3. The aim of the study .....	10
2. Method .....	10
2.1. The treatment preference experiments .....	10
2.1.1. Olfactometer .....	10
2.1.2. Test plants .....	11
2.1.3. Test species .....	11
2.1.4. Treatment of the plants .....	12
2.2. The genotype preference experiment .....	12
2.3. Conducting the experiments .....	12
2.4. Statistical analyses.....	12
3. Results .....	13
3.1. The treatment preference experiments .....	13
3.1.1. Broccoli .....	13
3.1.2. Cabbage .....	13
3.1.3. Cauliflower .....	13
3.2. The genotype preference experiment .....	14
4. Discussion .....	14
5. Acknowledgements .....	16
6. References .....	17



## 1. Introduction

Insects search for primarily food, resting sites, egg-laying sites and conspecifics such as mates. The extent of which they perform these behaviours depends on species, size and life stage. Considering the search for a host plant by a lepidopteran insect, many studies have been conducted about the signals used by adults when searching for food or for ovipositing sites. There are also many studies about caterpillars at the point when they are in contact with a potential host plant, where taste sensilla on the mouthparts of the caterpillar detect contact chemical signals (Miles et al, 2005). The way caterpillars search and locate an appropriate host when distanced from the plant, on the other hand, is less known. Understanding the search behaviour of caterpillars distanced from their hosts, however, is relevant as it may help in the control of pests as well as in the conservation of species.

When in search of suitable plants for food and egg laying sites, adult phytophagous insects can respond to different stimuli from a plant by using chemoreception to consider the chemistry of the plant or their optical senses to read visual cues such as the colour and shape of the plant. Contact-receptors test the plant's chemistry when the insect is in contact with the plant, but before contact has been made olfactory cues play a more important role (Schoonhoven et al, 2005). However, this is mainly a description of ways in which adult lepidopteran insects are directed towards their hosts and is not necessarily applicable to caterpillars since some sensory organs have not been fully developed in larvae.

Do caterpillars respond to stimuli in the same way that adult butterflies when distanced from the plant? It appears that although the visual organs of caterpillars are quite primitive in comparison with those of a fully-grown adult butterfly, they are nonetheless able to gather enough information to enable the caterpillar to distinguish between colours and shapes (see *The sensory organs of lepidopteran larvae* below for more details).

The olfactory perception of lepidopteran larvae is a lot less advanced than that of an adult and how well it works is unclear, since findings of previous studies examining this have been contradictory. On the one hand, there are reports such as the review by Schoonhoven (1968), where it is said that caterpillars of *Manduca sexta* are able to discriminate between hosts and non-hosts, indicating a relatively acute olfactory perception. This was also seen in another study where it was shown that caterpillars of *P. brassicae* were able to discriminate between macerated host plant tissue and intact host plants (Rather and Azim, 2009). On the other hand, there are studies showing limited abilities of caterpillars to discriminate between host and non-host (*Pieris napi*) or within a reasonable time-range (*P. brassicae*) if only able to use olfaction (e.g. Feltwell, 1982; Chew, 1980; also Visser, 1986). Considering this, it is unclear exactly how important olfactory stimuli are to the caterpillar when searching for an appropriate host and for this reason it is worthwhile to make further studies.

What makes caterpillars search for a host? For a caterpillar the optimal situation would be if the plant on which it hatched could fully support a successful development. That way it would not have to move to find a host, which is a risk. However, studies have shown that the precision with which the adult chooses a host for ovipositing appears in many cases to be only partially in line with the food preference of the caterpillar (Schoonhoven et al, 2005). This will result in the plant being either unable to support a successful larval development or only able to support a limited part of the larval development, either of which will eventually force

the caterpillar to find an alternative food source. Even with an ideal host, many other factors could also cause the caterpillar to search for an alternative plant. Predation or other environmental factors could cause the caterpillar to search for cover, or it may have simply fallen off the host plant. Another alternative is intra- or interspecific competition, leading to an insufficient food supply. However, even with competition, the presence of conspecific caterpillars could be also advantageous to the caterpillar. Gregarious insects find advantages in their large numbers through the protection from enemies because of dilution effect, group defence, increased chance of detecting predators. Many gregarious species are also aposematic in coloration and are found to be distasteful to predators, such as caterpillars of *P. brassicae* (Aplin et al, 1975). This could make it more advantageous for a gregarious caterpillar to search for a host plant with conspecifics. Alone it would be more vulnerable to predation, its aposematic coloration makes it clearly visible to predators and the defence a group offers has no effect alone. In other words, when searching for a host plant, factors other than simply finding the appropriate food could be involved.

If caterpillars have a way of picking up olfactory signals, what olfactory signals are the most attractive? Which signals that are the most attractive largely depends on the requirements of a specific species. Species that have received a lot of attention within this area of research are specialist herbivores like the Large White Butterfly, *Pieris brassicae*, which feeds on plants from the mustard family Brassicaceae (Aplin et al, 1975). Plants from this family contain a group of secondary metabolites called glucosinolates, compounds that in terms of chemical signals have been given a lot of attention in research over the past 30 years (Hopkins et al, 2009). When the cells of cruciferous plants are damaged the non-volatile glucosinolates are hydrolysed and more or less toxic volatile products are formed (Halkier and Gershenzon, 2006; Hopkins et al, 2009). The glucosinolates and its derivatives can have a repellent effect on some herbivores while attracting some specialists (e.g. Müller et al, 2003). The combination of glucosinolates may vary greatly between species as well as between different genotypes and for this reason the plant's chemical signals may differ significantly from one genotype or species to another (Hopkins et al, 2009). For a more detailed description of the glucosinolates, see *Chemistry of the host species* below.

The species *P. brassicae* was chosen to investigate what olfactory stimuli attract a caterpillar from a distance. *P. brassicae* is specialised on plants containing glucosinolates, which would enable an interesting comparison between the attractiveness of undamaged plants and plants with damaged tissue where higher concentrations of volatile hydrolysis products are found. Furthermore, because caterpillars of *P. brassicae* are aposematic in colouration and the species is gregarious in its first three instars, the possible influence of conspecific presence on what attracts the caterpillars could also be investigated. However, in a previous study by Collenette (1945) it was found that the scent of conspecific caterpillars did not appear to influence the behaviour of *P. brassicae*. That means that if conspecific presence attracts the caterpillars, the signals would have to be less conspicuous than the direct presence of individuals. One such signal could be the damage that chewing caterpillars inflict on the plant tissue, which releases volatile compounds. Mozūraitis et al (unpublished) found that the odour profiles of damaged cultivars of *B. oleracea* differed depending on the species of lepidopteran larvae (*Mamestra brassicae* and *Pieris rapae*) that had chewed on them. Knowing this, it is possible that the combinations of compounds released after damage caused



by *P. brassicae* caterpillars is species-specific and because of this it works as a signal of conspecific presence.

Another sign of conspecific presence could be from the frass excreted by *P. brassicae* caterpillars, which contains hydrolysed glucosinolate products among other compounds, possibly compounds specific to the species (see *Chemistry of the host species* below). Considering this, should plants with insect-induced damage be more attractive to the *P. brassicae* caterpillars than undamaged plants? Or would frass, even if it is not a direct indication of a host, be enough to attract caterpillars of *P. brassicae* to conspecifics? We know that the combination of glucosinolates can differ even between genotypes of a species. If there is a difference in preference for an intact host, damaged host tissue or frass, would this depend on the genotype tested or does the odour selection follow the same pattern no matter which host plant is tested? Furthermore, do *P. brassicae* caterpillars prefer some genotypes of a host plant more than other?

### **1.1. Sensory organs of lepidopteran larvae**

When locating and selecting hosts from a distance the signals picked up by a lepidopteran larva are visual or olfactory. For the visual organs of lepidopteran insects there is a clear difference between life stages, with caterpillars perceiving light through the use of ocelli while adults have compound eyes made up of ommatidia. Ommatidia are more complex structures than ocelli and are not developed until after the pupa has been formed (Feltwell, 1982). *Pieris brassicae* L. caterpillars have a total 12 ocelli on the head (Feltwell, 1982) and although the exact number of ommatidia of an adult butterfly is unclear, we know that the total number is considerably greater - according to Frantsevich and Pichka (1976) “Middle sized insects [have] from 2000-9000 ommatidia” (cited by Feltwell, 1982). However, Schoonhoven et al (2005) writes: “*Despite the fact that single rhabdome stemmata of caterpillars are very simple organs compared with the eye of the adult butterfly, caterpillars are able to discriminate object sizes and colours, enabling them to orient towards plant silhouettes after dropping to the ground.*”. Considering this it is clear that caterpillars have the ability to use the visual cues of a plant to guide them towards a host like adults do even if the degree, or even form, of vision appears to be considerably worse than that of an adult.

Olfactory signals are picked up by chemoreceptor cells that, according to Schoonhoven et al (2005), can be associated with the sensilla of the insect. These cells are considerably more numerous in adult lepidopteran insects than in caterpillars (between 6500 and 177 000 in adults and about 100 in caterpillars), but are nonetheless present (Schoonhoven et al, 2005).

### **1.2. Chemistry of the host species**

Secondary compounds are compounds that are not essential to the reproduction, growth or development of a plant. Crucifers contain a group of secondary compounds called glucosinolates. There are about 120 known compounds of glucosinolate, mainly associated with plants of the Brassicaceae family (Fahey et al, 2001). In intact leaves the glucosinolates are spatially separated from the enzyme myrosinase, but when the plant tissue has been damaged by e.g. chewing caterpillars, the substances come in contact with each other and hydrolysed products of the two are formed. These products, such as the volatile

isothiocyanates and nitriles, are often toxic in nature, and negative biological effects of glucosinolates have very often been associated with isothiocyanates (Halkier and Gershenzon, 2006).

For many specialists the glucosinolates act as feeding and oviposition stimulants whereas the volatile breakdown products can be feeding stimulants and attractants (Halkier and Gershenzon, 2006). This appears to be the case for *Pieris brassicae*. But for the caterpillars of *P. brassicae* to be able to feed on plant tissue containing toxic substances, strategies need to have evolved to cope with the toxicity. Studies have shown that in *Pieris* spp., the normal course of the glucosinolate hydrolysis is redirected by a gut protein called nitrile specifier protein (NSP) (Wittstock et al, 2004). Instead of producing the toxic isothiocyanates, the reaction produces the slightly less toxic nitriles. The nitriles are then excreted in the frass, amongst various other enzymes and compounds and so the toxic compounds never enter the system of the caterpillar (Wittstock et al, 2004).

### **1.3. The aim of the study**

The aim of this study is to investigate if caterpillars respond to olfactory signals. For gregarious *P. brassicae* caterpillars, what olfactory signals are the most attractive? Is there a difference in attraction to damaged or undamaged treatments of the host plant, or are other traces of conspecific presence such as frass attractive to the caterpillars? Furthermore, will *P. brassicae* caterpillars respond differently to different genotypes of the host plant?

## **2. Method**

The host plant chosen for this study is *Brassica oleracea*. To investigate what attracts gregarious *P. brassicae* caterpillars, this study will be divided into two main categories of experiments. In the first category, from now on referred to as *the treatment preference experiments*, three experiments will be conducted, one for each genotype of *B. oleracea* chosen (cauliflower, cabbage and broccoli). In each experiment the preference of *P. brassicae* caterpillars for the treatments *damaged*, *undamaged* and *frass* of each genotype of *B. oleracea* will be compared.

In the second category, referred to as *the genotype preference experiment*, the preference of *P. brassicae* caterpillars for undamaged treatments of the three genotypes of *B. oleracea* will be compared.

### **2.1. The treatment preference experiments**

#### *2.1.1. Olfactometer*

For all the bioassay experiments four-way olfactometers were used, the centre with a 4.5 cm radius and each arm with a length of 7.5 cm (Figure 1). The four treatments were 1) an undamaged plant, 2) a plant with larvae-induced damage, 3) frass from *P. brassicae* caterpillars fed with the genotype tested and 4) air as a control treatment. The bottom of the olfactometer was covered with a white piece of paper to create a suitable surface for the caterpillars to move across. The ends of the tubes connecting the chimneys with the olfactometer were covered with a fine mesh to stop the small caterpillar from leaving the choice-test arena. For each olfactometer three chimneys were used in the treatment preference

experiments, one for the control, one for the undamaged plant and one for the plant with larvae-induced damage. The fourth choice was frass, where 150-200 mg of frass saturated with 0.5 ml water was placed in a smaller chimney to allow the correct airflow. To avoid position effects and directional stimuli the four treatments were placed at random positions, and to exclude any visual stimuli the chimneys were all covered with white paper.

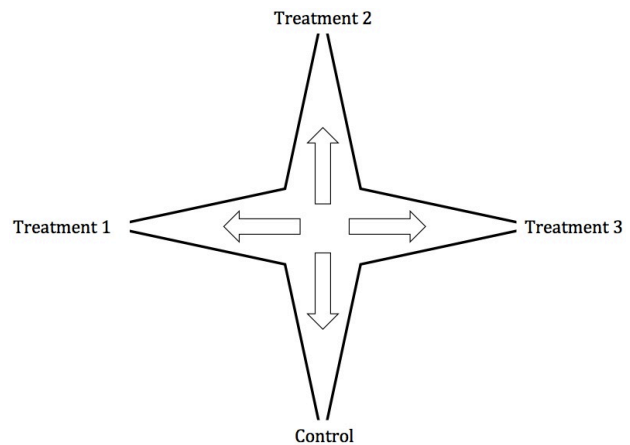


Figure 1. Sketch of the four-way olfactometer used in the experiments. Treatment 1-3 stands for the treatments or genotypes used in the different experiments. An empty chimney was used as control. The placement of the treatments and the control were chosen randomly.

### 2.1.2. Test plants

The following genotypes of the cabbage species *Brassica oleracea* L. were chosen: cabbage (*B. oleracea* L. var. *capitata* cv. Consul), cauliflower (*B. oleracea* L. var. *botrytis* cv. Nautilus) and broccoli (*B. oleracea* L. var. *cymosa* cv. Marathon). For ease of reading the different genotypes are referred to by their common names in this study. The plants were grown from seeds (provided by Olssons Frö AB) in 10x10x10 cm pots in a greenhouse with supplement lights (16L: 8D photoperiod), and were used in the experiments once they reached a height of approximately 25 cm. Three treatment preference experiments were conducted, one for each genotype of *B. oleracea*. In other words, the genotype was the unchanging variable for each experiment so that all the three different variables (treatments) were from that same genotype. Approximately 20 replicates were made for each genotype of *B. oleracea*.

### 2.1.3. Test species

Just like many crucifer specialists *P. brassicae* is considered a pest in many parts of the world because of the destructive way in which the caterpillars feed on their hosts, which can have a major negative impact on the crop yield in crucifer production.

The *P. brassicae* caterpillars used in this study were raised from specimens provided by the *Plant Sciences Group*, Wageningen University, Netherlands. They were reared on randomly chosen plants of the three genotypes of *B. oleracea* in cages in a climate room at the Ecology Department at SLU, Uppsala (18L: 6D photoperiod, 21°C, 75% humidity). Medium-sized caterpillars, late 2<sup>nd</sup> to early 4<sup>th</sup> instar were used in the experiments, once for each caterpillar to avoid pseudo replicate. This size of caterpillars was chosen in part because the caterpillars were of the optimal size for the olfactometers, in part because this size range exhibits among the highest activities. Late 4<sup>th</sup> instar to 5<sup>th</sup> instar caterpillars also exhibit among the highest food consumption proportionate to body mass (Feltwell, 1982), but were not suited for this type of experiment. This is because they are not gregarious (and therefore lacks one of the characteristics investigated in this bioassay) and are about to go through their last (and most difficult) ecdysis (i.e. molting) before diapause that causes a longer period of temporary inactivity (Feltwell, 1982).

#### 2.1.4. Treatment of the plants

Within 24 hours of to the start of the experiments 5-8 large caterpillars, late 4<sup>th</sup> to 5<sup>th</sup> instar caterpillars, were placed on a plant to feed. The pot of the plant had been covered with paper in order to prevent any frass contamination, and as a way of keeping the material from drying out the plant with the feeding caterpillars was placed in a plastic bag. Once an appropriate amount of frass had been produced the frass was collected and the plant was wiped from any larval or frass residue. The plants and the frass were then placed in the chimneys straight away to begin the experiment. Unlike in the actual bioassay experiments as a test species, the late 4<sup>th</sup> to 5<sup>th</sup> instar caterpillars were ultimate for the production of frass and damage because of the amount of frass they produce and their high consumption- and in turn high damage rate.

### 2.2. The genotype preference experiment

The genotype preference experiment was conducted in order to see if *P. brassicae* caterpillars showed a preference for undamaged plants of any of the three genotypes tested. The same set-up with the four-way olfactometers was used with the same size of caterpillars, but instead of three large chimneys and one small, four large chimneys were used. The four different treatments of the experiment were 1) cabbage (*B. oleracea* L. var. *capitata*), 2) cauliflower (*B. oleracea* L. var. *botrytis*), 3) broccoli (*B. oleracea* L. var. *cymosa*) and 4) air as a control treatment. Approximately 20 replicates were made.

### 2.3. Conducting the experiments

All bioassay experiments were conducted the same way, with approximately four olfactometers running simultaneously. One naïve caterpillar was placed in each olfactometer and was given 12 minutes to adapt to the new environment. Once 12 minutes had passed the exact position of the caterpillar was noted every three minutes until ten entries had been made. If the caterpillar was inactive (i.e. had not moved after three entries or not moved at all), that replicate was disregarded. Immediately after finishing, the olfactometers were cleaned with 70% ethanol and the papers were changed to new ones. The positions of the caterpillars were divided into five categories four of which were the choices (i.e. the treatments and control) of that particular experiment and the fifth was *undecided* (i.e. when the caterpillar remained in the middle of the olfactometer at the time when the note was made). After each session of replicates the caterpillars were replaced by naïve individuals.

### 2.4. Statistical analyses

Friedman ANOVA for matched pairs test was used to analyse the data from the four bioassays, in order to see if the *P. brassicae* caterpillars showed any preference for any of the four treatments at the  $p < 0.05$  significance level. For the treatment preference experiments a Wilcoxon's Matched Pairs test was subsequently performed to test pairs of treatments, also at a  $p < 0.05$  significance level. The number of visits to the treatment *damaged* by the caterpillars was compared with the number of visits to each of all the other treatments (*undamaged*, *frass* and *control*), in order to see if the preference for *damaged* was significantly higher than the preference for any of the other treatments. The data collected was analysed in STATISTICA 10 (StatSoft, Inc.).

### 3. Results

#### 3.1. The treatment preference experiments

##### 3.1.1. Broccoli

The results for broccoli (*B. oleracea* var. *cymosa*) showed no significant difference in preference by the *P. brassicae* caterpillars for the treatments (Friedman ANOVA,  $F=1.585$ ;  $p=0.6627$ ;  $N=22$ ;  $df=3$ ). The treatment *undamaged* appeared to be more preferred but this deviation was also not statistically significant (Figure 2).

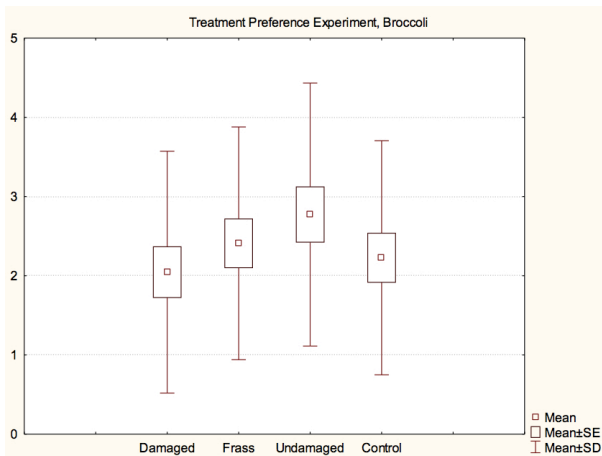


Figure 2. The average number of visits to each treatment of broccoli (var. *cymosa*) by *P. brassicae* caterpillar. No significant difference in preference found. Y-axis: number of visits.

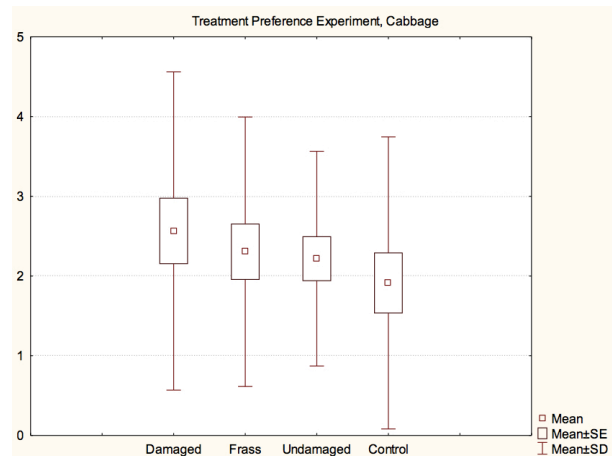


Figure 3. The average number of visits to each treatment of cabbage (var. *capitata*) by *P. brassicae* caterpillar. No significant difference in preference found. Y-axis: number of visits.

##### 3.1.2. Cabbage

The treatment preference experiment for cabbage (*B. oleracea* var. *capitata*) showed no significant preference by *P. brassicae* caterpillars for any of the treatments tested (Friedman ANOVA,  $F=1.200$   $p=0.7530$ ;  $N=23$ ;  $df=3$ ) (Figure 3).

##### 3.1.3. Cauliflower

The *P. brassicae* caterpillars showed no significant difference in preference for any of the treatments of cauliflower (*B. oleracea* var. *botrytis*) tested (Friedman ANOVA,  $F=4.804$ ;  $p=0.1867$ ;  $N=17$ ;  $df=3$ ). However, there were indications that there was a difference in preference for the treatment *damaged* of this genotype (Figure 4).

To examine these differences further a Wilcoxon's Matched Pairs test was performed, comparing the treatment *damaged* with each of the other treatments respectively. A significant difference between the treatments *damaged* and *frass* and the treatments *damaged* and *control* were found. An insignificant but nonetheless clear trend was also found in the preference for *damaged* compared with the treatment *undamaged* (Table 1).

Table 1. Results from the Wilcoxon's Matched Pairs test, showing the results of the comparison of the treatment damaged with each of the other treatments respectively. The treatment damaged is significantly more preferred by *P. brassicae* caterpillars than the treatments frass and control, respectively.

Pairs of Variables	N	Z	p-value
Damaged & Frass	14	2.040	<b>0.04133</b>
Damaged & Undamaged	16	1.758	0.07873
Damaged & Control	14	2.072	<b>0.03830</b>

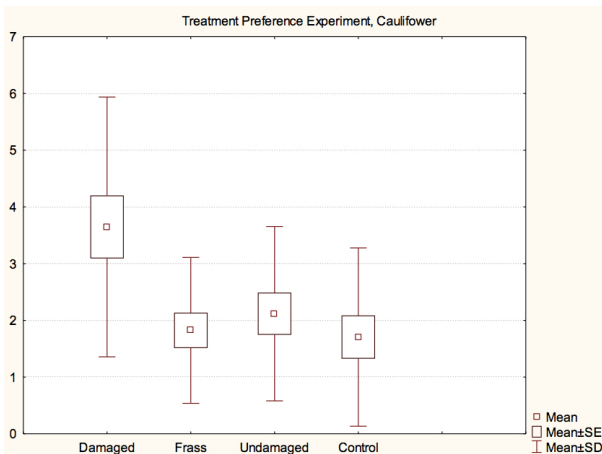


Figure 4. The average number of visits to each treatment of cauliflower (*var. botrytis*) by *P. brassicae* caterpillar. The treatment damaged was significantly favoured over the treatments frass and control. Y-axis: number of visits.

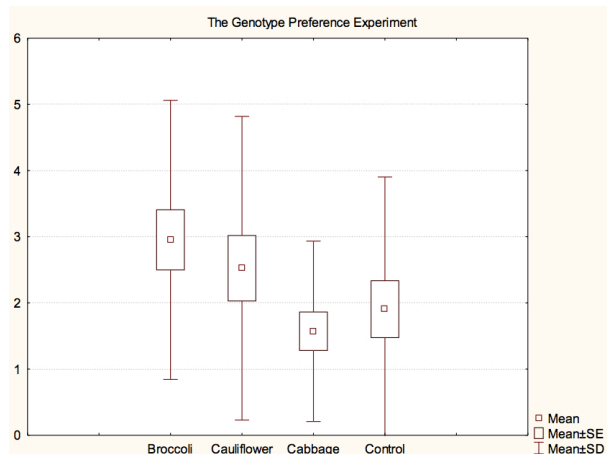


Figure 5. The average number of visits to each genotype of *B. oleracea* (including control treatment) by *P. brassicae* caterpillar. No significant difference in preference found. Y-axis: number of visits.

### 3.2. The genotype preference experiment

The genotype preference experiment showed no significant preference by *P. brassicae* caterpillars for either the control treatment or any of the genotypes of *B. oleracea* tested (Friedman ANOVA,  $F=5.537$ ;  $p=0.1364$ ;  $N=21$ ;  $df=3$ ) (Figure 5).

## 4. Discussion

In this study it was shown that caterpillars of *P. brassicae* favour damaged cauliflower over frass and the control treatment of that genotype. This finding contradicts in part those reports that claim that the olfactory perception of caterpillars is poor. For instance, Feltwell (1982) found that caterpillars of *P. brassicae* only respond to olfactory cues by finding their host after hours of search (Feltwell, 1982). This appears not to be the case since cauliflowers, although damaged, were significantly favoured over both frass and air (the control treatment).

The preference of caterpillars of *P. brassicae* for damaged cauliflower supports the results from the study by Rather and Azim (2009), who found damaged tissue of *B. oleracea* to be more favoured by *P. brassicae* than intact plant tissue. They suggest that this result could be explained by several different factors, one explanation being that as the tissue is damaged a higher concentration of preferred volatile substances is released (Rather and Azim, 2009), such as products of hydrolysed glucosinolates. With a higher concentration of substances released in combination with new hydrolysed products, this may make the damaged tissue more preferred than the other treatments of cauliflower tested. However, as there were only indications that damaged tissue was preferred by *P. brassicae* over intact plants (i.e.

undamaged), the option that the plant itself was the common denominator of attraction cannot be excluded.

Is the damage in itself (with the implications that follow) the attracting feature, or is it the strengthened signal of a cauliflower plant that made the damaged cauliflowers more preferred? The alternative that the damage strengthens the signal of a cauliflower is quite clear – compounds released by intact plants could have been released in higher concentrations as the plant tissue was damaged and so a more distinct signal was released by the plant. On the other hand, if the damage in itself is the attracting feature this should indicate that the compounds released from damaged tissue are what attract *P. brassicae* caterpillars. From a study by Mozūraitis et al (unpublished) we know that this is a possibility, since they found that the odour profile of intact cauliflower differed from the odour profile of cauliflower with insect-induced damage (caused by *M. brassicae* and *P. rapae* respectively). These “damage-specific” compounds could be an indication of either changes in the physiology of the host plant that might be preferential for *P. brassicae* caterpillars, or it could be an indication of for instance conspecific presence. Assuming that the combination of compounds released from damaged plant tissue is species-specific to *P. brassicae* caterpillars, it is possible that what appears to be a preference for damaged plant tissue is in reality an attraction to plants with conspecifics.

To check this with more certainty the difference in preference should be examined comparing mechanically damaged plants with plants damaged by chewing caterpillars of *P. brassicae*. This would show us if it is the damage in itself that attracts the caterpillar or if the attracting feature really is the conspecific presence. However, there are some crucial differences between mechanical damage and insect-induced damage that cannot be overlooked, in that they very often induce different responses in the plant (Pontoppidan et al, 2005). These responses, in turn, will most likely result in different olfactory signals being released by the plants, which may have an effect on the response of the caterpillar. Considering this, it would not be enough to just compare mechanical damage with damage caused by chewing *P. brassicae* caterpillars, it would also be necessary to compare these two forms of damage with insect-induced damage caused by another species of insect. This has been studied in relation to parasitoids and host search (Van Poecke and Dicke, 2002).

Following the same line of thought that conspecific presence is what ultimately attracts caterpillars, it was mentioned earlier that frass of *P. brassicae* caterpillars should be able to function as a sign of conspecific presence. If this is the case, however, frass does not appear to be a strong indicator of conspecific presence according to the results of this study. This treatment was favoured by *P. brassicae* caterpillars to the same degree as the other choices of treatments of the broccoli and cabbage genotypes. It is definitely possible that frass is unique to caterpillars of a certain species, considering its contents of for instance enzymes and derivatives of the plant, and it should therefore be able to work as a sign of conspecific presence. What might make it an inaccurate signal of conspecific presence is that frass mostly falls from the plant on which the caterpillars are feeding. Thus it should only be able to work as an attracting signal if the searching caterpillar is on the ground at a distance from the plant. However, if the caterpillar is sitting on a leaf of a host plant with no view of the group of feeding conspecifics found on another leaf, it would be “misleading” to follow the scent of frass as this might guide the caterpillar away from the group and not towards it. In other

words, frass as a guiding signal is quite unreliable compared to damaged plant tissue induced by conspecifics.

Considering the difference between the different genotypes, interestingly the caterpillars did not appear to respond to any specific treatment of any of the other genotypes besides cauliflower. One possible explanation for this is of course the way the experiments were set up, but there is also the possibility that the combination of compounds in the plants were so markedly different that this had a clear effect on the preference of the *P. brassicae* caterpillars. This was also shown by Mozūraitis et al (unpublished), who found several compounds to be unique to cauliflower compared with broccoli and cabbage cultivars. However, the theory that the specific compounds of the cauliflower cultivar made it more preferred by the caterpillars was contradicted by the results of the genotype preference experiment, which showed no difference in preference for any of the genotypes. Keep in mind though that this experiment was conducted on undamaged cultivars. It may therefore be possible that the caterpillars would react differently to a genotype preference experiment if the cultivars tested were all damaged. According to Mozūraitis et al (unpublished) similarities can be found between the broccoli, cabbage and cauliflower cultivars both before and after insect-induced damage, but there are also many differences between their odour profiles. These differences may have a greater impact on the response of *P. brassicae* caterpillars if damaged plants are compared. It could very well be that damaged cauliflowers are preferred over the other damaged genotypes since caterpillars of *P. brassicae* showed a preference for damaged cauliflower, which would support the findings in the treatment preference experiment. However, until more studies have been conducted on this it is difficult to draw any such conclusions.

Many studies have been conducted on how adult lepidopteran insects respond to different olfactory signals, but less is known of how caterpillars use and respond to this type of information. This study was conducted with the intention of bringing us one step closer to the understanding of what attracts specialised lepidopteran larvae to their host. From the experiments of this study it was shown that gregarious caterpillars of *P. brassicae* showed a preference for cauliflower with insect-induced damage. If this is because damaged plants are better hosts or if the indication of conspecific presence attracts the caterpillars remains to be seen, as more studies are required before any further conclusions should be drawn. However, it is clear from this study that caterpillars of *P. brassicae* respond to olfactory signals. This not only confirms findings in previous studies, it also constitutes a good foundation for further exploration of what attracts specialist insect pests.

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<sup>1</sup> Cited by Feltwell, 1982.