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Abstract

The plant family of Brassicaceae is widespread around the world and many members of the family are important food and oil-seed crops. There are many insect pests specialized on brassicaceous crops and interactions between crop and pest are well studied. Many studies concern *Brassicas* as hosts in mixed cropping systems with non-host species but very few use a mix of host-plants. Intercropping or mixed cropping can be seen as a way to manipulate patch heterogeneity. In this project I investigate if patch heterogeneity on a small scale has an effect on the egg distribution of two *Brassica* pests; one butterfly, *Pieris brassicae* (Lepidoptera: Pieridae) and one moth, *Plutella xylostella* (Lepidoptera: Plutellidae). Three *Brassica oleracea* genotypes were used; cabbage (*B. oleracea* subs. *capitata*, cv. Consul), cauliflower (*B. oleracea* subs. *botrytis*, cv. Nautilus) and broccoli (*B. oleracea* subs. *cymosa*, cv. Marathon). Experiments were carried out in cages and turntable using two arrangements; complex (all three genotypes in every patch) and simple (every patch consisting of one genotype). Results differed between species. *P. brassicae* responded significantly to arrangement. Eggs numbers differed much more between genotypes in the complex patches than in the simple patches. *P. xylostella* on the other hand were not significantly affected by the arrangement. The two species differ in the way they find their host plants and how they utilize a patch of host plants once found, these differences may explain their different response to small scale patch heterogeneity.

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Introduction

The plant family of Brassicaceae is widespread around the world and occurs in tropical and temperate regions. Many members of the family are important food and oil-seed crops such as *Brassica oleracea* (eg. cabbage, broccoli, cauliflower) *Brassica napus* (eg. Chinese cabbage, turnip, rapeseed) and *Brassica rapa* (eg. rapeseed). Brassicaceous crops are cultivated around the world in scales ranging from large-scale agricultural fields to small plots in gardens and allotments. In temperate regions there are over 20 major pests of cultivated Brassicaceae (Finch and Thompson 1992). It has been shown that patch scale and heterogeneity has an effect on the colonization by pests (eg. Cromartie 1975, Bukovinszky et al. 2005 & 2010). Many studies have been done, but most mixed host plants with non-host plants, very few have been done mixing host plants (but see eg. Hambäck et al. 2009). The study Hambäck et al. (2009) was done at field scale but what is happening at a really fine scale? Gaining more knowledge about how pest insects find their host-plants is a way to find more efficient ways to reduce the damage to crops.

In this project I investigate if patch heterogeneity on a fine scale has an effect on the egg distribution of two *Brassica* pests; one butterfly, *Pieris brassicae* and one moth, *Plutella xylostella*.

Pieris brassicae

Pieris brassicae (Lepidoptera: Pieridae) is a creamy white to pale yellow butterfly with a wingspan of 50-64 mm (Eliasson 2005) (figure 1a). The forewings have black tips and on females two black dots. Both sexes have a little black dot on the front edge of the hindwing and two black dots on the underside of the forewing that are hidden by the hindwing when resting with their wings closed. Eggs are bright yellow and bottle shaped with 16 longitudinal ridges (figure 1b). The caterpillar is pale blue green with three yellow stripes along the body (figure 1c). Between the stripes are a lot of black spots and the head and body are covered with fine hairs. When the caterpillars are fully grown they are 25-40 mm long (Finch & Thompson 1992).

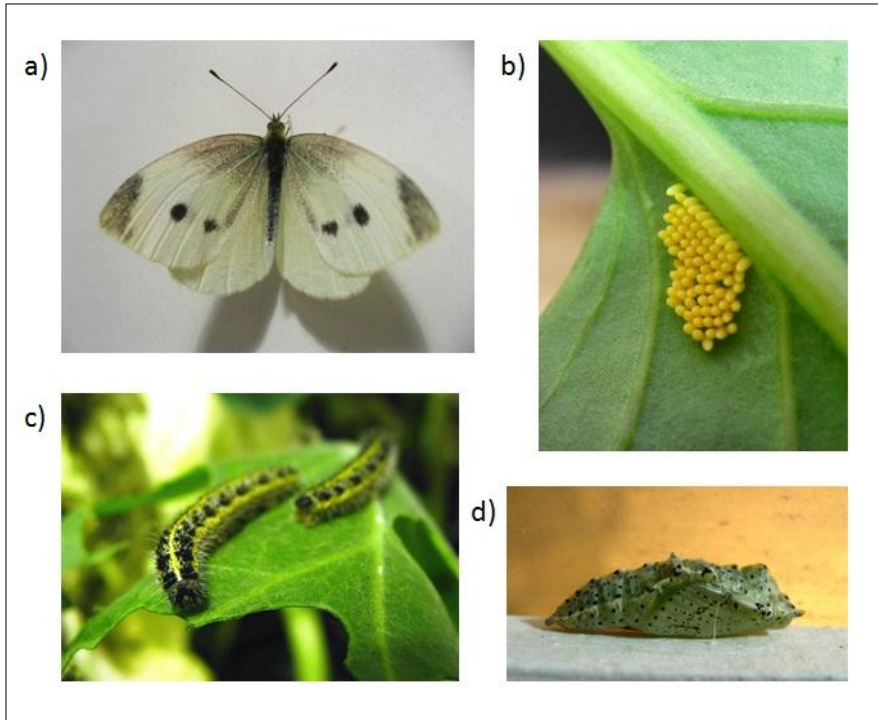


Figure 1 *Pieris brassicae* female (a), egg clutch (b), caterpillar (c) and chrysalis (d)

Pieris brassicae is migratory and a strong flyer found in agricultural areas and in other places where the ground is disturbed (Eliasson 2005). In warm weather the larval development is fast and survival is high. Females lay their eggs in groups of up to 150 on the underside of leaves of host plants (Le Masurier 1994). Eggs hatch in 4-14 days (Eliasson 2005, Finch & Thompson 1992, Jones & Jones 1984) and caterpillars start feeding on the egg case before feeding on the leaf. They continue feeding together until the third moult when they separate and crawl to new leaves (Jones & Jones 1984). Pupation takes place after 2-4 weeks and the pupal stage lasts for 1-3 weeks in summer (figure 1c) (Eliasson 2005; Jones & Jones 1984).

The number of generations per year varies with climate, usually two in mid Sweden where the first generation emerges from overwintering as chrysalis in mid May (Eliasson 2005). In warmer parts of Europe there are three to four generations per year (Eliasson 2005, Finch & Thompson 1992). *P. brassicae* is common in all European countries and is widespread from Northern Africa and Europe through Asia (Eliasson 2005). It has been introduced to South America.

Caterpillars of *P. brassicae* are well known for causing damage to cabbage, most is done by the second generation (Eliasson 2005, Finch & Thompson 1992, Jones & Jones 1984). Damage is limited in agricultural fields since pesticides can be used with good results, the majority of damage occurs in the field margins. A lot of damage is done where pesticides are not used such as in gardens and allotments. If caterpillars remain unchecked they can easily skeletonise entire plants.

Plutella xylostella

Plutella xylostella (Lepidoptera: Plutellidae) is a small brownish moth about 6 mm long and with average wingspan of 15 mm (Finch & Thompson 1992) (figure 2). It is commonly known as the Diamond-back moth because of the light brown to white triangular markings along the inner margins of the forewings, that come together to form a diamond pattern when the moth is at rest. The hindwing is grey with a fringe of long hairs, females are usually lighter coloured than the males. The caterpillars grow to about 12 mm long and widest in the middle, the colour is light green (figure 2d) (Jones & Jones 1984).

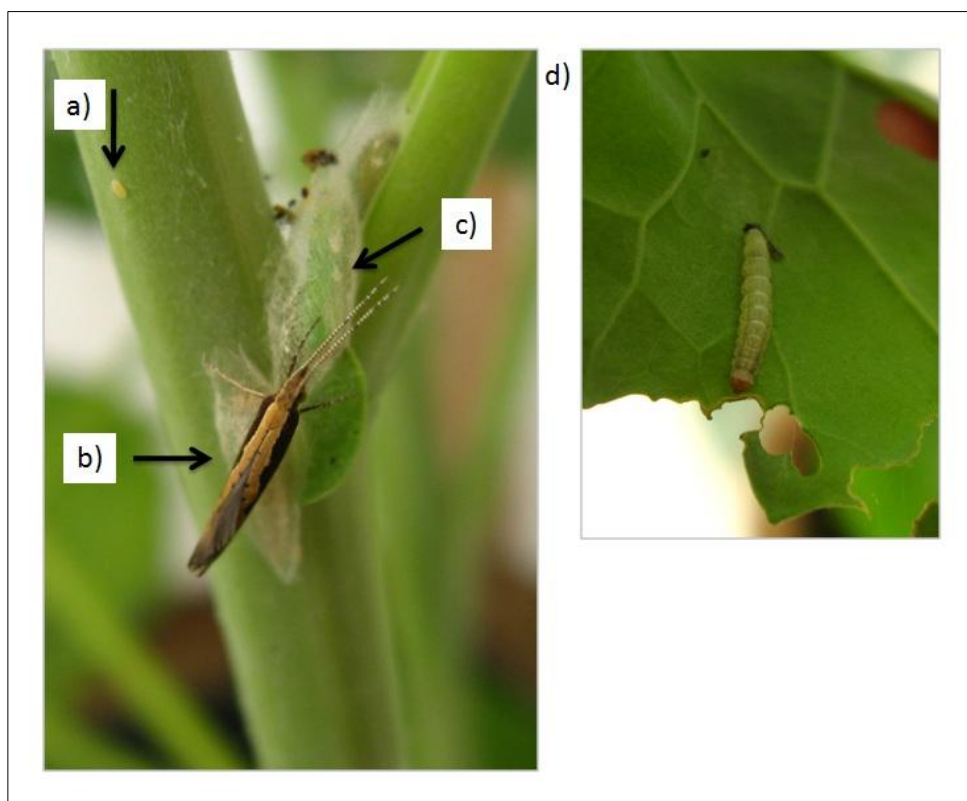


Figure 2 *Plutella xylostella* egg (a), male resting on cocoon (b), chrysalis inside cocoon (c) and caterpillar (d).

Plutella xylostella is a cosmopolitan pest known to migrate over large distances with the wind (Talekar & Shelton 1993). Mass migrations are an important factor in causing infestations in areas with poor winter survival such as Sweden. The adults are mainly active at dusk and into the night (Jones & Jones 1984). Soon after emerging in spring they mate and start laying eggs (Finch & Thompson 1992). During an oviposition period of 4 days 11-188 small yellow eggs are laid (figure 2a) (Harcourt 1954 in Talekar & Shelton 1993), singly or in groups of 2-3 (Finch & Thompson 1992). Females prefer to lay their eggs in concavities and along veins of leaves of brassicaceous plants rather than on a smooth surface (Talekar & Shelton 1993). The eggs hatch after 5-6 days depending mainly on temperature and the newly hatched caterpillars crawl to the underside of the leaf and start feeding, often they eat everything except the waxy top layer thus creating “windows” in the leaf (Talekar & Shelton 1993). The caterpillar moults in total 4 times; two of the instars have black heads and the other two yellow heads (figure 2d) (Finch & Thompson 1992). Apart from temperature, host crop can have an influence on the development rate (Talekar & Shelton 1993). If caterpillars are disturbed they move violently and often drop from the leaf hanging by a silk thread (Jones & Jones 1984). When the fourth instar caterpillar have finished eating they make an open-network cocoon on the leaf surface or a protected place nearby (Talekar & Shelton 1993). They stay in quiescent prepupal stage for 1-2 days before forming green or light yellow 9 mm chrysalis (figure 2c) (Finch & Thompson 1992). The pupal and prepupal stage is about 1/3 of the total generation time. There can be as many as 15 generations per year in the tropics, in northern Europe there are usually 1-2 overlapping generations (Finch & Thompson 1992).

P. xylostella is a major pest of brassicaceous crops in America, South-east Asia, Australia, Canada, Europe and New Zealand (Finch & Thompson 1992). It has developed resistance to virtually all chemical control measures. *P. xylostella* caterpillars are able to feed on a wide variety of brassicaceous crops and weeds containing mustard oils and their glucosides (Talekar & Shelton 1993). Being active early in the year they can cause severe damage on newly transplanted plants that result in lower crop yield with headless or skeletonised plants (Finch & Thompson 1992). Later damage may have little effect on the yield but lowers the quality and value of the crop due to holes in the wrapper leaves. In broccoli the silk threads and frass contaminates the crop and caterpillars may remain after the harvest (Jansson 2001).

Utilization of patches

A patch is defined by Forman (1995) as “a relatively homogenous nonlinear area that differs from its surroundings.” Patches can be of different scales and shapes can for example consist of a forest, an agricultural field or a pool of water. There are different hypotheses on how herbivorous insects utilize patches. Root (1973) proposed in the “resource concentration hypothesis” that herbivorous insects with a narrow host range are more likely to find and remain in a concentrated stand of host plants. In a study on collards (*Brassica oleracea*) he found that the plants growing in pure stands had a higher herbivore load than those growing in rows surrounded by meadow vegetation. The reverse pattern is found in some species, including many butterflies, and is called “Edge effect” (Jones 1992). Plants at the edge of a stand receive more eggs per plant than plants in the middle and more eggs per plant are found in sparse patches than in dense, isolated plants receive more eggs than plants in groups. Cromartie (1975) showed that this is the case for *Pieris rapae*, a close relative to *P. brassicae*.

Many plants have a patchy distribution by nature; this is true for wild cabbage, *Brassica oleracea* L. var. *oleracea* (Wichmann et al. 2008). In agricultural landscapes and gardens people control the size, distribution and heterogeneity of patches. Several studies have been done on intercropping *Brassic*as with other species to try different mechanisms to reduce the attack rate of *Brassica* pests. Root & Kareiva (1984) interplanted collards with potatoes and found no effect on the oviposition of *P. rapae* but the numbers of flea beetles was significantly lower than in pure collard stands. When undersowing *B. oleracea* and *B. rapa* with clover Finch & Kienegger (1997) found that the effect varied between different pest species. The largest effect was found on species that lay their eggs in large clutches, making relatively few selections (eg. *P. brassicae*) and on species that rarely leave once a host is found. Badenes – Perez et al. (2004) evaluated potential trap crops for *P. xylostella*. They concluded that the differences found were likely due to volatiles, colour, morphology of the leaves or a combination and that volatiles are a major factor influencing host preferences. Broad et al. (2008) tried to add non-host crops to broccoli and as in many other studies results differed between pest species. They suggest that diversity itself is less important than the scale of diversity and the ability of the target pest to locate their host-plants. To my knowledge the only recent study truly intercropping different *Brassica* genotypes is done by

Hambäck et al. (2009). Three different *Brassica oleracea* genotypes were used to create monoculture and biculture plots with high and low contrast and different spatial heterogeneity. In that system attack rates of three cabbage herbivores were studied. The results differed between species; *P. rapae* had a higher difference in egg load between genotypes in the biculture plots than between plants in monoculture plots suggesting that *P. rapae* can detect the difference in genotypes from a small distance but not at a larger spatial scale. The present study focuses on oviposition choices and how they are affected by patch heterogeneity at a very fine scale.

Finding a host

The choices made by females when finding a host for egg-laying are very important since the newly hatched caterpillars of *P. brassicae* and *P. xylostella* are relatively immobile and their survival depends on those choices. Justus & Mitchell (1996) observed that the leaf mining first instar of *P. xylostella* desiccated rapidly if unable to burrow under the protective plant cuticle. Jones (1992) states that for flying insects it is useful to divide the way they find their way to a host for feeding or oviposition into pre-alighting and post-alighting responses.

Pre-alighting

At this stage of host finding the insect use visual or olfactional cues to search for a potential host plant, for *P. brassicae* and *P. xylostella* a rule might be formulated like this: “If you have mature eggs, its light/dark and you smell cabbage – fly against the wind” (modified from a rule for Colorado potato beetle in Jones 1992). Renwick & Chew (1994) in their review on oviposition behaviour in Lepidoptera stress the importance of vision at this stage of host-finding, hosts being located on the basis of their shape and colour. Colour can to some extent depend on plant chemistry and that can in turn often be related to the physical condition of the plant (Renwick & Chew 1994). When it comes to nocturnal lepidoptera they write “*several studies using laboratory bioassays have demonstrated the role of plant volatiles in the orientation of various moths to their host plants.*” Finch & Collier (2000) discuss and dismiss seven hypotheses (including resource concentration) on host finding in diverse habitats and propose a theory about host finding in cabbage pests based on what they call ‘appropriate/inappropriate landings’. An insect flying over a host plant is

stimulated to land by the volatiles that the plant gives off. The odour may be enough to arrest the insect but will rarely be enough to give sufficient directions to the plant. If the plant is clearly visible against a background of soil the insect will land on the host plant, an “appropriate” landing. But if the host plant is surrounded by other green plants the insect cannot discriminate between the green surfaces and may land on a non-host plant, an “inappropriate” landing. The theory is based on day flying insects and Couty et al. (2006) showed that plant odour plays a major role in host-finding by the night flying *P. xylostella* and provide enough direction for landing, although visual cues may play some role. But when plants in the mini crop were intermingled in a checkerboard design there was no difference in the number of landings between Chinese cabbage and lettuce and when the first three rows were lettuce *P. xylostella* landed preferentially on those. This can be seen as an edge effect but also fits the ‘appropriate/inappropriate landings’ theory. The final step in the pre-alighting process is when the insect lands on a plant. Often landing is triggered by a combination of physical and chemical cues (Renwick & Chew 1994). The role of vision is well documented, particularly important is the shape and colour of the leaves. In a study by Bukovinszky et al. (2005) *P. xylostella* show a strong arrestment response once a host plant is found, the female stays on the plant or hops around to neighbouring plants. *P. brassicae* are known to fly distances of 5-20 meters, making frequent changes of direction, between alightments (Nikolaus 1974 in Feltwell 1982)

When different genotypes of a host plant are mixed as in the present study the insect searching for a suitable host may be confused by the mix of volatile compounds emitted by the plants.

Post-alighting

After landing on a potential host plant the gravid female must assess its suitability using a mixture of physical and chemical cues (eg. Renwick & Chew 1994). In lepidopterans the involved sensory receptors are present on the tarsi, antennae, proboscis and ovipositor. After landing *Pieris* butterflies are known to drum their front legs against the leaf surface (eg. Terofal 1965 in Städler & Reifenrath 2009. Original paper in German) fluttering of the wings is also commonly reported (and observed by me). *P. xylostella* rotate their antennae and use them to repeatedly strike the plant surface and also feel the surface with the ovipositor (Justus & Mitchell 1996). The family of Brassicaceae is known to contain a class

of plant secondary metabolites called glucosinolates or mustard glucosides, consisting of three functional groups; a β -thioglucose group, a sulfonated oxime group and a variable side chain (Hopkins et al. 2009). More than 120 different glucosinolates have been identified and glucosinolate profile can differ between *Brassica oleracea* genotypes. When the plant tissue is damaged glucosinolates can be converted into volatile compounds such as isothiocyanates, nitriles, and oxazolidinethiones, by the enzyme myrosinase stored in specialized plant cells. The first experimental evidence of a link between *P. brassicae* and glucosinolates in their host plants was presented by Verschaffelt in 1910 (Renwick 2002) and it has been long established that tarsi of female *P. brassicae* are sensitive to mustard oil glycosides (Ma & Schoonhoven 1973). Renwick et al. (2006) isolated two isothiocyanates that acted as effective oviposition stimulants on *P. xylostella*. *Brassica* leaves are covered by a layer of wax and several studies (reviewed by Städler & Reifenrath 2009) show no traces of glucosinolates in that layer. However for many Brassicaceae herbivores there is plenty of evidence that they can perceive glucosinolates by contact with the leaf surface, how they do this is still unknown though several possibilities have been proposed (Städler & Reifenrath 2009). A decision to lay eggs on a potential host not only depends on oviposition stimulants but also on plant deterrents, plant condition, presence of conspecific and heterospecific eggs and condition and age of the female (Renwick & Chew 1994). If the host plant is accepted eggs are laid.

Material and methods

Plants

Seeds were provided by Olssons Frö AB. Three genotypes of *Brassica oleracea* (figure 3) were used: cabbage (*B. oleracea* subs. *capitata*, cv. Consul), cauliflower (*B. oleracea* subs. *botrytis*, cv. Nautilus) and broccoli (*B. oleracea* subs. *cymosa*, cv. Marathon). Seeds were planted in 10x10x10 cm pots with soil from Hasselfors Garden (SLU mix) and grown in glasshouses with supplement lights. Nutrients were provided with the water. Nutrients from Cederroth International AB (Wallco växtnäring 51-10-43 + micro) were diluted with de-ionized water to a conductivity of 1.0 mS/cm which corresponds to a nitrogen concentration of 100 mg/l.



Figure 3 Leaf shape of cabbage, broccoli and cauliflower

Insects

P. brassicae were shipped as chrysalis from *Plant Sciences Group*, Wageningen University, Netherlands. They were then kept in culture in a climate chamber set to 21°C, 80% humidity and 16L: 8D photoperiod. Caterpillars were raised on a mix of the three *Brassica oleracea* genotypes and on occasion some store bought cabbage. Adult butterflies were fed sugar water (1 lump of sugar, approx. 3.6 grams, dissolved in 30 ml of water). The *P. xylostella* were from a culture kept in a glasshouse at the Department of Ecology, SLU. The culture is based on wild caught specimens and has been kept for more than 10 years.

Experimental setup

In the experimental setup a patch consisted of three plants. Two arrangements were used; homogenous (simple) with all plants in the patch being the same genotype, one patch of each genotype; and heterogeneous (complex) with all the three patches having all three genotypes (figure 4). Plants were chosen with the aim to have as similar size as possible. In general cabbage had 1-2 more leaves than broccoli and cauliflower.

Pieris brassicae

Glasshouse

The first four replicates had to be done in 160 by 60 by 110 cm cages in a glasshouse with supplement lighting. Two cages were used, ten butterflies were kept in the cages for the duration of all four replicates and arrangements were alternated between the cages (placement). In the homogenous arrangement the position of the patches within the cage were also alternated. Replicates lasted for four hours in the afternoon. Then plants were taken out and all egg groups were photographed. The photos of the eggs were then magnified in Microsoft Paint and the eggs manually counted. At the start of a replicate dead or flightless butterflies were replaced and food removed.

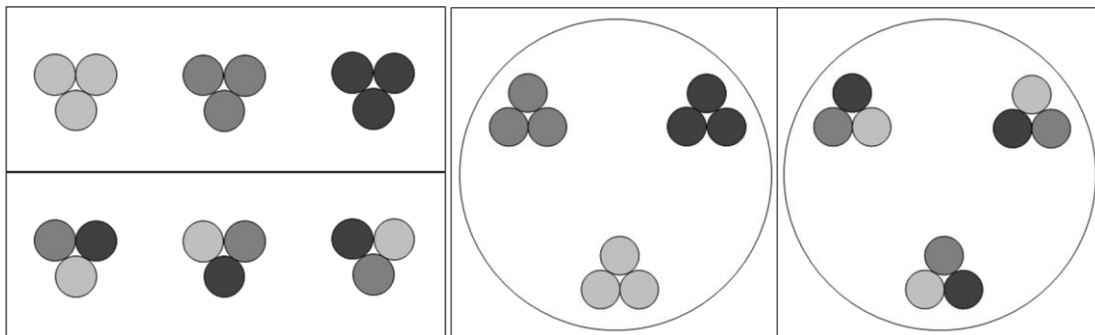


Figure 4 Experimental setup in the glasshouse cages and turntable.

Turntable

The rest of the replicates were done in a turntable. The turntable consists of a cage with two levels, each with a disk turning once every 3 minutes (figure 5). A fine mesh fabric hangs inside all of the doors to prevent escapes when the doors were open to release butterflies or to remove plants. The turntable were lit with three 36 W fluorescent lamps in addition to

those providing the light in the room. The temperature was kept at 20-24°C with the aid of a 3 kW heating fan. Before the start of a replicate the plants were arranged in complex and simple patches on the turning disks (figure 4), arrangement were alternated between the two levels (placement). At the start of a replicate ten butterflies were placed on the mesh fabric inside the doors at each level and checked for ability to fly away. Replicates started at approx. 11:00 am and lasted four hours. At the end of a replicate first the plants and then the butterflies were removed. Eggs were photographed and counted as in the glasshouse replicates.

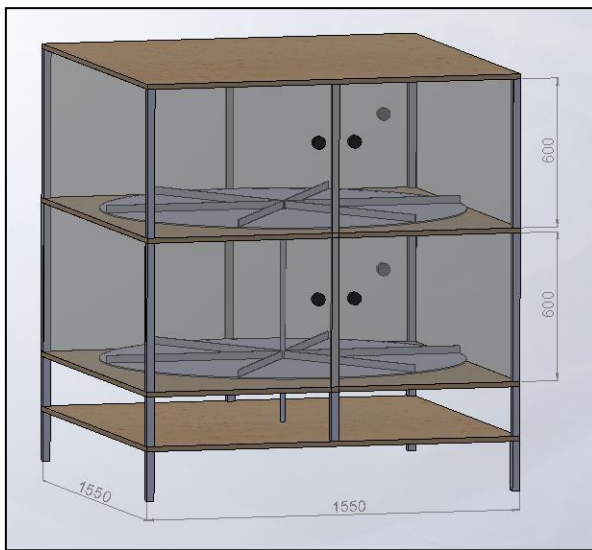


Figure 5 Sketch of the turntable used, measurements in millimeters.

Plutella xylostella

Plants were arranged in the turntable the same way as for *Pieris brassicae*. At the start of the first replicate 30 adults were added to each level. Replicates started in the evening and lasted for 16 hours during night. Every evening before the start of the next replicate five females were released to replace possible deaths and escapes. The lights of the turntable were turned off but a 40W light were kept on out of direct view to somewhat mimic the light summer nights. Temperature was 17-20 °C. At the end of a replicate the plants were removed and the eggs counted using magnifying glass. After the first replicate I discovered that most eggs were laid on the cotyledons and in the following replicates smaller/younger plants were used to avoid bias due to wilted cotyledons. In total eight replicates were made.

Statistical analyses

Egg numbers were modelled in R (R Development Core Team 2007) with a Generalized linear mixed model with Poisson error distribution (lme4-package). Explanatory variables in the analyses were arrangement, genotype and the genotype-by-arrangement interaction, and random factors were patch nested within placement. To test the significance of the interaction, models with and without the interaction were tested with an ANOVA.

Results

Pieris brassicae and *Plutella xylostella* were differently affected by the arrangement. The genotype-by-arrangement interaction were significant for *P. brassicae* (Table 1a; $P < 0.0001$) but not for *P. xylostella* (Table 1b; $P = 0.056$).

Table 1 Statistical model describing egg numbers of (a) *Pieris brassicae* and (b) *Plutella xylostella* without (M1) and with (M2) the interaction and the P-value of the ANOVA of the two models.

(a) <i>Pieris brassicae</i>			
M1	Estimate	S.E.	P
Intercept (Broccoli, Complex)	3.60	0.28	<0.0001
Simple	-0.21	0.025	<0.0001
Cabbage	0.015	0.036	0.67
Cauliflower	0.40	0.033	<0.0001
M2			
Intercept (Broccoli, Complex)	3.44	0.28	<0.0001
Simple	0.18	0.054	0.0006
Cabbage	0.42	0.042	<0.0001
Cauliflower	0.38	0.043	<0.0001
Simple x Cabbage	-1.36	0.078	<0.0001
Simple x Cauliflower	0.10	0.075	0.16
ANOVA			
M1, M2			<0.0001
(b) <i>Plutella xylostella</i>			
M1	Estimate	S.E.	P
Intercept (Broccoli, Complex)	2.97	0.082	<0.0001
Simple	0.091	0.038	0.017
Cabbage	-0.39	0.057	<0.0001
Cauliflower	0.082	0.051	0.11
M2			
Intercept (Broccoli, Complex)	2.92	0.086	<0.0001
Simple	0.23	0.075	0.002
Cabbage	-0.34	0.073	<0.0001
Cauliflower	0.17	0.064	0.008
Simple x Cabbage	-0.13	0.12	0.28
Simple x Cauliflower	-0.27	0.11	0.012
ANOVA			
M1, M2			0.056

For *P. brassicae* the egg distribution differs between the complex and simple patches. The graph (figure 6a) show a visible difference in egg means between the *B. oleracea* genotypes when they were kept separate in simple patches, cauliflower received more eggs than broccoli which in turn received more eggs than cabbage. When the genotypes were intermingled in complex patches the visual difference in egg numbers are much smaller. For *P. xylostella* the egg distribution between genotypes is very similar in the complex and simple patches (figure 6b). The ranking of the genotypes is the same as for *P. brassicae*; cauliflower > broccoli > cabbage, although the differences between the genotypes is smaller than for *P. brassicae* in the simple arrangement.

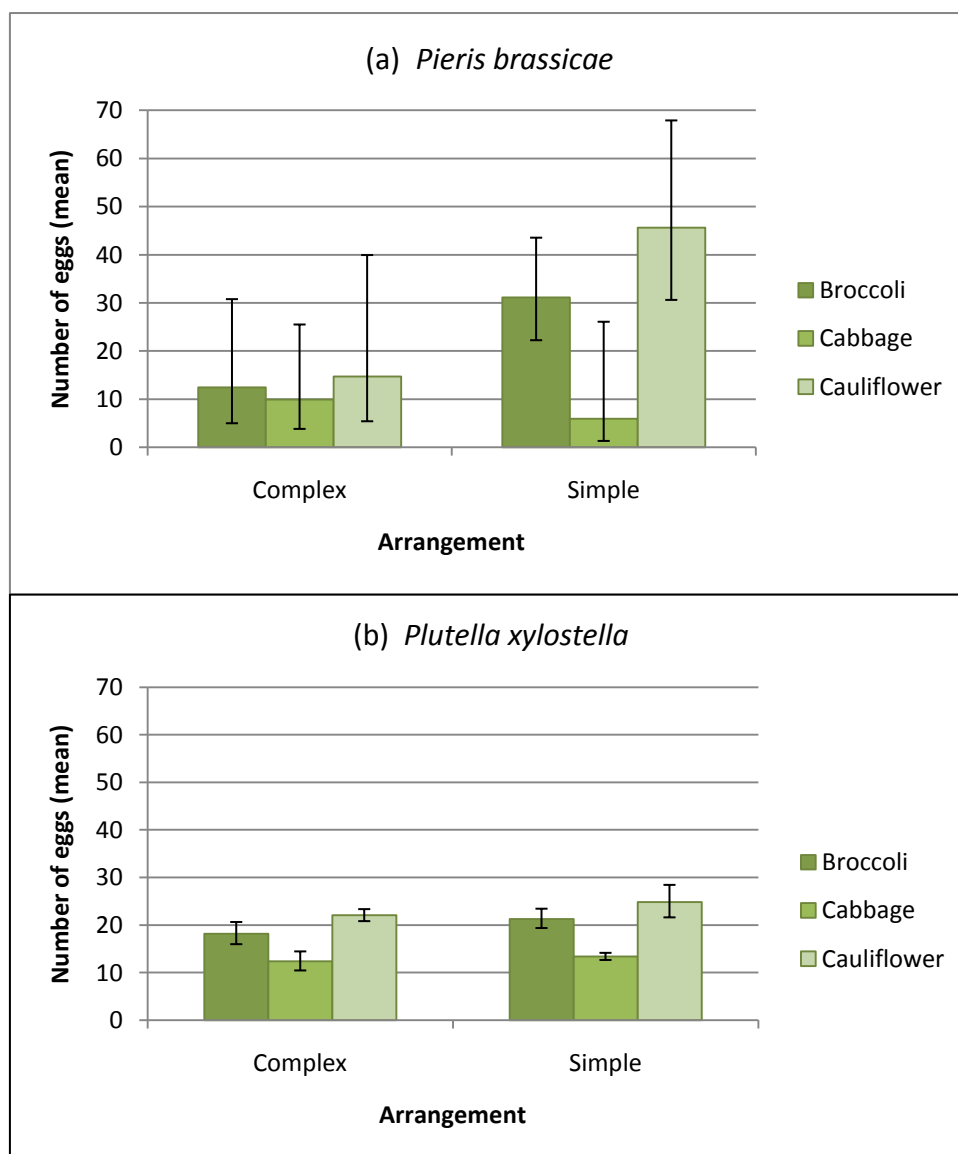


Figure 6 Number of eggs (mean \pm SE) of (a) *Pieris brassicae* and (b) *Plutella xylostella* on broccoli, cabbage and cauliflower in complex and simple patches.

Discussion

Arrangement has clear and significant effect on the oviposition of *Pieris brassicae* whereas for *Plutella xylostella* there is not a clear and significant effect of arrangement. For *P. brassicae* the egg distribution between genotypes clearly differs with arrangement thus patch heterogeneity has an effect. In the arrangement with simple patches there is a visible difference between the genotypes and cabbage seems to be the least preferred genotype for oviposition. In the arrangement with complex patches eggs are much more evenly distributed between the genotypes although the trend is the same. *P. xylostella* is not affected in the same way by the arrangement. The difference between genotypes is smaller than for *P. brassicae* in the simple patches and the pattern is the same regardless of arrangement. Both species in this study are oligophagous lepidoptera specialized on brassicaceous plants containing glucosinolates and thus have a similar host range. Their difference in response to arrangement in this study cannot be explained by differences in host range. Can the difference be explained by how *P. brassicae* and *P. xylostella* locate and utilize host plants?

Vision is the major sense when *P. brassicae* is trying to locate a host plant while *P. xylostella* use olfaction which may lead them to perceive the "landscape" in the cage in different ways. The leaves of the plants differ in shape and colour (figure 3) which should affect the visually oriented *P. brassicae* (Renwick & Chew 1994). In the simple arrangement it seems *P. brassicae* can tell the different genotypes apart, a patch consisting of only one genotype may reinforce what is attracting or deterring them. In the complex arrangement the egg distribution might be explained by confusion or an inability to tell the genotypes apart.

Although vision may play some role for *P. xylostella* and they had enough light in this setup to see the plants, olfaction is still the principal sensory modality (Couty et al. 2006). A chemical analysis of the genotypes done by Mozūraitis et al. (in prep) show that the plants differ in both composition and quantity of volatile chemicals. The odours from the plants mix in the cage and it is possible that the rotation of the turntable create a mixed odour trail that made it hard for *P. xylostella* to tell the genotypes apart before landing regardless of

arrangement. Looking at figure 6b it seems they can distinguish somewhat between the genotypes in both arrangements and, as for *P. brassicae*, cabbage is less preferred. If this is an effect of contact chemoreception or visual differences cannot be discerned but cabbage is the (to me) most “visually different” genotype and the leaves are smoother which may have an effect since *P. xylostella* prefer to lay their eggs in concavities. However most of the eggs were laid on the cotyledons so maybe the structure of the leaves were of less importance.

P. brassicae and *P. xylostella* utilize patches, once a host plant is found, in different ways. *P. brassicae* finds an acceptable host plant and lay eggs and then fly 5-20 m before laying more eggs (Nikolaus 1974 in Feltwell 1982). If patches are small this would result in only one clutch/patch. *P. xylostella* show a strong arrestment response once a host plant is found, the female stays on the plant or hops around to neighbouring plants (Bukovinszky et al. 2005) and stays within the patch for “some time”. During this time they may lay eggs on more than one plant.

P. brassicae lay eggs in clutches of up to 150 eggs (Le Masurier 1994) (my average is 40) while *P. xylostella* lay their eggs singly or in groups of up to three eggs (Finch & Thompson 1992). When Finch & Kienegger (1997) did a study on undersowing Brassicas with clover the largest effects were found on insects that rarely leave once a host-plant is found and on *P. brassicae* and *Mamestra brassicae* both making relatively few decisions and both laying many eggs once a host-plant is selected. *P. brassicae* depositing a large number of eggs on one plant may be more selective (Le Masurier 1994) when choosing a host plant due to the large investment while *P. xylostella* laying only one egg at a time might spread their risks by ovipositing on a number of plants within the same patch even if some of them are less preferred. *P. brassicae* caterpillars being “group feeders” staying together for the first three instars (Jones & Jones 1984) feed on more than one plant during their development and it is suggested by Le Masurier (1994) that the female might treat clumps of host plants rather than individual plants as the unit of resource over which oviposition decisions should be made. This might be an alternative explanation to the eggs being more evenly distributed between the genotypes in the complex arrangement. *P. xylostella* caterpillars can stay on the same host plant during their whole development providing the plant is big enough. The choice of host-plant may be important to the individual caterpillar (eg. Justus & Mitchell

1996) but since the female can spread their offspring over multiple host plants it may have less importance for the reproductive success of the female.

In most studies regarding egg-laying single plants or even single leaves are used. It has been shown by eg. Bukovinszky et al. (2005) that different insects treat groups of plants differently and that scale is an important factor. When studies are done at larger scale most studies use mixtures of different plant species, hosts and non-hosts, and very little is done on mixing plants within host-range. Hambäck et al. (2009) showed that spatial heterogeneity was a very important factor for host selection by *Pieris rapae*. Contrasting plants that were grown close to each other in mixed patches showed a larger difference in egg density than when plants were grown in monoculture. It was suggested that *P. rapae* can “differentiate among genotypes from a small distance, while selection is compromised at a larger scale.” This might be true for *P. brassicae* as well but selection might not only be compromised at a larger scale but also at a very fine scale. In my study the patches are less than 0.5 meters apart which is the distance between plants in the study by Hambäck et al. (2009). When insects are searching for host-plants they make their decisions on an increasingly fine spatial scale. The ‘appropriate/inappropriate landings’ theory proposed by Finch & Collier (2000) can be applied to insects landing in a patch. According to the theory an insect locates a host and is stimulated to land by olfactory cues but olfactory doesn’t give sufficient direction for landing and the insect lands on any green object using visual cues. If the landing is on a host plant it is considered an ‘appropriate’ landing. In this scenario all plants are host plants and thus all landings on a plant could be considered ‘appropriate’, although a preference hierarchy exists. But on the other hand landing on the less preferred genotypes could also be considered an ‘inappropriate’ landing. According to Couty et al. (2006) olfaction gives sufficient direction for *P. xylostella* to land but they used a mix of hosts and non-hosts and even so when using a checkerboard design the number of landings didn’t differ significantly between Chinese cabbage and lettuce indicating a difficulty to tell them apart prior to landing.

P. brassicae can assess the number of eggs laid on a plant using visual or chemical cues and can switch to a previously less acceptable host plant (Rothschild & Schoonhoven 1977 in Stamp 1980). It is possible that the egg distribution between the genotypes in the simple arrangement may have been even more different had there been fewer females in the cage.

The confinement of the cage may also have had an effect on *P. brassicae* since they typically fly several meters between ovipositions and the cage walls may have affected their natural searching pattern. No heating fan was used during the *P. xylostella* experiments and the air might have moved relatively little in the room during the night creating a scenario with a more mixed, and non directional, odour trail than would be seen in more natural conditions.

In order to find new ways to deal with pests it is important to “know your enemy”, finding how insect pests find and utilize their host plants is one central piece of the puzzle. The difference in reaction to patch heterogeneity found even with two species that have similar host range and are both oligophagous lepidoptera highlights what is found in so many other studies; pest insects respond differently to heterogeneity at different scales depending on a multitude of factors. Some important factors are how they localize their host-plants, how they utilize that plant or patch once found and oviposition or reproductive strategy. It will be virtually impossible to find a strategy working against all pest species. Both species in this study seem to have had their selection compromised but at slightly different scales. Finding out the scale on which decisions are made are important to succeed with mixed cropping systems and more experiments are still needed. Sometime the goal might be to confuse the insect but sometimes also the opposite, when for example using trap crops it is essential to have the trap crop separated just enough from the crop so that the target insect can tell them apart.

Conclusion

At the scale used in this study patch heterogeneity has an effect on the oviposition of *P. brassicae* but not *P. xylostella*, as discussed this can be for a number of reasons. The species being both oligophagous lepidoptera still differ in the way they find their host plants, how they utilize patches once found and oviposition strategy where *P. brassicae* lay eggs in clutches and *P. xylostella* singly. These are all factors that could influence how they respond to patch heterogeneity.

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