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Intraspecific and Interstage Similarities in Host-Plant Preference in the Diamondback Moth (Lepidoptera: Plutellidae)

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Abstract: The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), is an important insect pest of cruciferous crops. Understanding its preference patterns can lead to more efficient management methods, such as trap crops. Several strains of *P. xylostella* were used to test whether there were differences in oviposition preference in a four-choice setting, on abaxial versus adaxial leaf surfaces in 28 different plant species, and on substrates with different concentrations of sinigrin (allylglucosinolate). Additionally, the larval preference of *P. xylostella* was studied with 17 plant species of known glucosinolate content that were compared to *Arabidopsis thaliana* L. in two-choice tests. Our research shows that the diet on which *P. xylostella* has fed hardly affects multiple-choice host-plant preference, abaxial and adaxial oviposition preference, or oviposition response to pure glucosinolates. Our study also shows that glucosinolate content affects larval preference, which together with the known correlation between glucosinolate content and *P. xylostella* oviposition, indicates that crops with high glucosinolate content could be more susceptible to damage by *P. xylostella* than crops with low glucosinolate content. These findings are discussed in regards to their significance in the management of *P. xylostella*.

Keywords: abaxial leaf side; adaxial leaf side; allylglucosinolate; glucosinolates; glucosinolate diversity; host-plant preference; larval preference; oviposition; *Plutella xylostella*; sinigrin

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1. Introduction

The diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) is considered one of the most damaging insect pests of cruciferous vegetables [1,2]. Management of *P. xylostella* is mostly through using insecticides [3–6], but the ability of *P. xylostella* to develop resistance to insecticides, combined with concerns on the negative effects of insecticide use, have stimulated interest in developing alternative management techniques such as trap crops and host-plant resistance [7–9].

The host range of *P. xylostella* includes mainly glucosinolate-containing plants in the family Brassicaceae [7,10,11]. Larvae of *P. xylostella* have sulfatases that allow them to desulphate glucosinolates [12], and glucosinolates and their hydrolysis products have been shown to be oviposition and feeding stimulants for adults and larvae, respectively [10,13–17]. However, different strains of *P. xylostella* show differences in adaptation to different diets and host-plants and, in several cases, it has been shown that *P. xylostella* can use plants outside the family Brassicaceae as host-plants [10,18–20]. For example, in Kenya, larvae of *P. xylostella* were found feeding on plants of the pea *Pisum sativum* L. (Fabaceae), adjacent to a cabbage field that had been heavily infested by the insect [21]. In a study conducted with 30 different plant species, plant glucosinolate content was shown to increase oviposition and larval survival in different *P. xylostella* strains reared on cabbage (DBM-C) and on two glucosinolate-free diets, either artificial wheat-casein diet (DBM-G88) or pea leaves (DBM-P) [10]. However, it is unknown if in *P. xylostella*, larval preference is equally influenced by glucosinolates as in the case of oviposition preference. In field experiments, densities

of larvae of *P. xylostella* were higher in lines of *Arabidopsis thaliana* L. and *Brassica oleracea* L. (Brassicaceae) with higher glucosinolate content [22,23]. However, on other studies conducted with *A. thaliana* and *B. oleracea*, the performance of *P. xylostella* larvae could not be explained by glucosinolate content [24–27], and one study conducted with *Brassica rapa* L. (Brassicaceae) found that herbivory by *P. xylostella* larvae increased with glucosinolate content up to an intermediate maximum, decreasing thereafter [28]. Larvae of *P. xylostella* can move among adjacent plants and some neonates can travel distances of more than 1 m [29].

Different lepidopteran populations of the same species can show different oviposition preferences [30,31]. In *P. xylostella*, host-plant use can also be affected by previous experience, which can be used to induce oviposition on non-host plants [32–35]. Two-choice tests conducted comparing 30 plant species to *A. thaliana* on the DBM-C, DBM-G88, and DBM-P strains of *P. xylostella* showed that, overall, they had similar oviposition preferences [10]. However, the response of different strains could differ when offered more than two host-plants in oviposition preference tests. Offering multiple host-plants to *P. xylostella* simultaneously can alter host-finding behavior and reduce oviposition [36]. Furthermore, it is unknown if there could be within-plant differences in oviposition preference, such as abaxial versus adaxial, among *P. xylostella* strains reared on very different diets.

The main goal of this research was to compare host-plant preference among different *P. xylostella* strains and between larvae and ovipositing adults. For this purpose, we collected abaxial and adaxial oviposition data on 28 plant species, using the *P. xylostella* strains DBM-C, DBM-G88, and DBM-P, to test if these could differ among the different strains. Furthermore, multi-preference oviposition tests were conducted with these and two additional *P. xylostella* strains reared on rape (DBM-W and DBM-NOQA). These multi-preference experiments were conducted to test if there were differences among the different strains in a setting with three different oviposition substrates besides the plant on which the insects were reared. As pure individual glucosinolates have also been shown to act as oviposition stimulants in *P. xylostella* [20,37–39], here we also compared the oviposition response of the strain DBM-C and the strain DBM-G88 (the strain that has been reared the longest without glucosinolates) to different concentrations of the pure glucosinolate sinigrin (allylglucosinolate). We hypothesized that *P. xylostella* could lose its ability to detect pure glucosinolates after many generations without glucosinolate exposure in its diet. Furthermore, using 18 different plant species of known glucosinolate content, we tested whether larval preference is influenced by glucosinolate content and if larval preference is correlated to oviposition preference in *P. xylostella*. Understanding the preference of *P. xylostella* may lead to more effective trap crops.

2. Materials and Methods

2.1. Plant Species and Plutella xylostella Strains Tested

The plants used in the experiments are shown on Table 1. Among the 28 plant species tested, 20 belong to 11 subfamilies in the family Brassicaceae, and 7 belong to the families Caricaceae, Cleomaceae, Gyrostemonaceae, Limnanthaceae, Moringaceae, Resedaceae, and Tropaeolaceae (order Brassicales) [40] (Table 1). Additionally, *P. sativum* was used as a control plant without glucosinolates. Seeds of *A. thaliana* Columbia-0 and G-type *Barbarea vulgaris* R.Br. were provided by the Nottingham Arabidopsis Stock Centre at Sutton Bonington, UK, and by Dr. Niels Agerbirk, respectively. Seeds of *Alyssum argenteum* All. were purchased from Jelitto (Schwarmstedt, Germany). Seeds of *Brassica napus* L. and *Nasturtium officinale* W. T. Aiton were purchased from Rieger-Hofmann GmbH (Blaufelden-Raboldshausen, Germany). Seeds of the collards *Brassica oleracea* var. *acephala*, cultivar Green Glaze, which produces glossy and waxy plants, were purchased from Pennington Seed (Madison, GA, USA). Seeds of *Cardamine pratensis* L. and *Iberis amara* L. were purchased from Rühlemann's (Horstedt, Germany). All other seeds, including also those of cabbage, *B. oleracea* var. *capitata* cultivar Gloria, and pea, *P. sativum* cultivar Oregon Sugar Pod, were purchased from B & T World Seeds (Aigues-Vives, France). *Arabidopsis thaliana* plants were

grown in a climate chamber (10:14 h light:dark, 21 ± 2 °C and 55 ± 5 RH). The other plant species were grown in a greenhouse (16:8 h light:dark, 25 ± 3 °C). Plants were grown in 7 × 7 × 8 cm pots containing a substrate of peat moss and clay, and fertilized every two weeks with an all-purpose fertilizer (Ferty® 3, Planta Düngemittel GmbH, Regenstauff, Germany). Plants were 5- to 6-weeks-old at the beginning of the experiments.

Table 1. Plants used in the different experiments conducted to determine abaxial vs. adaxial oviposition preference (AA) and larval preference (LP).

Family	Species	Common Name	Experiment
Brassicaceae	*Aethionema cordifolium* DC.	Lebanon stone cress	AA
Brassicaceae	*Alyssum argenteum* All.	Yellow tuft	AA, LP
Brassicaceae	*Arabidopsis thaliana* (L.) Heynh.	Thale cress	AA
Brassicaceae	*Arabis caucasica* Willd.	Mountain rock cress	AA, LP
Brassicaceae	*Barbarea vulgaris* R.Br.	Wintercress	AA
Brassicaceae	*Biscutella laevigata* L.	Buckler mustard	AA
Brassicaceae	*Brassica juncea* (L.) Czern.	Indian mustard	AA, LP
Brassicaceae	*Brassica napus* L.	Canola	AA
Brassicaceae	*Brassica oleracea* var. *capitata* L.	Cabbage	AA
Brassicaceae	*Brassica oleracea* var. *acephala* L.	Glossy collard greens	AA
Brassicaceae	*Brassica oleracea* var. *acephala* L.	Waxy collard greens	AA
Brassicaceae	*Bunias orientalis* L.	Turkish rocket	AA
Brassicaceae	*Capsella bursa-pastoris* (L.) Medik.	Shepherd's purse	AA, LP
Brassicaceae	*Cardamine pratensis* L.	Cuckoo flower	AA
Brassicaceae	*Diplotaxis muralis* (L.) DC.	Annual wall rocket	AA, LP
Brassicaceae	*Eruca sativa* Mill.	Arugula, rucola	AA, LP
Brassicaceae	*Erysimum cheiri* (L.) Crantz	Wallflower	AA, LP
Brassicaceae	*Iberis amara* L.	Bitter candytuft	AA, LP
Brassicaceae	*Lepidium sativum* L.	Garden cress	AA; LP
Brassicaceae	*Neslia paniculata* (L.) Desv.	Ball mustard	AA
Brassicaceae	*Nasturtium officinale* W. T. Aiton	Watercress	AA
Brassicaceae	*Sisymbrium officinale* (L.) Scop.	Hedge mustard	AA, LP
Caricaceae	*Carica papaya* L.	Papaya	AA, LP
Cleomaceae	*Cleome spinosa* L.	Spider flower	AA, LP
Fabaceae	*Pisum sativum* L.	Pea	AA, LP
Gyrostemonaceae	*Codonocarpus cotinifolius* (Desf.) F.Muell.	Bell-fruit tree	AA
Limnanthaceae	*Limnanthes douglasii* R. Br.	Douglas' meadowfoam	AA
Moringaceae	*Moringa oleifera* Lam.	Drumstick tree	AA, LP
Resedaceae	*Reseda odorata* L.	Common mignonette	AA, LP
Tropaeolaceae	*Tropaeolum majus* L.	Garden nasturtium	AA, LP

Five strains of *P. xylostella* were used in the experiments. Two strains came from Kenya, one (DBM-C) was collected in a cabbage field in 2002, and the other (DBM-P) was collected in a pea field in 2000, and since collection they were continually reared on cabbage and pea plants, respectively. Another strain (DBM-G88) was collected in 1988 in Geneva, NY, USA, and since then was reared on a wheat germ-casein artificial diet [41]. Two additional strains were reared on rape, a field selected strain resistant to *Bacillus thuringiensis* toxin Cry 1Ac (DBM-NOQA) [42] and a strain collected near Adelaide, Australia in 1987 and maintained in the laboratory feeding on rape at the Waite Agricultural Research Institute [43]. Insects of the strains DBM-C and DBM-P were donated to us by Dr. Bernhard Löhr, while insects of the strains DBM-G88 and DBM-NOQA were donated to us by Drs. Anthony Shelton and Bruce Tabashnik, respectively. Insects of the DBM-W strain were obtained from the Waite Institute at the University of Adelaide, Australia. Insects were reared in environmental growth chambers (16:8 h light:dark, 21 ± 2 °C and 55 ± 5 RH). Throughout the experiments, the number of individuals of each strain was always >250. Insects of these five *P. xylostella* strains completed at least 14 generations per year on the diet and conditions on which they were reared. Before carrying out the experiments described here, insects were continuously

and exclusively feeding on cabbage (DBM-C), on artificial diet (DBM-G88), on peas (DBM-P), and on *B. napus* (DBM-NOQA and DBM-Waite) for more than 100 generations.

2.2. Multiple-Choice Oviposition Preference Experiments

Multiple-choice oviposition preference experiments were conducted with one cabbage plant, one pea plant, one rape plant, and one strip of aluminium foil (approximately 2.5 × 12.0 cm). The strip of aluminium foil was placed in the center of a Petri dish (9 cm diameter), and it had previously been dipped into autoclaved cabbage juice (65 g of cabbage in 500 mL of water), as in Shelton et al. [41]. Three 1.5-mL samples of this autoclaved cabbage juice were analyzed to check if they contained any glucosinolates. Glucosinolate content of these cabbage juice samples was determined following the procedure described in Badenes-Pérez et al. [16] and no glucosinolates were detected. The multiple-choice oviposition experiments were conducted in screened cages 60 × 60 × 60 cm. Multiple cages were used, each of which was considered a replicate. In each cage, the four possible oviposition substrates (one potted plant of each cabbage, rape, and pea, and the Petri dish with aluminium foil), were placed at a distance of approximately 20 cm from the middle of the cage, leaving approximately 28 cm between adjacent oviposition substrates. Three pairs of *P. xylostella* moths (three females and three males, <3 days old) were released in each cage. To provide a food source for them, a small plastic cup with a 10% sugar solution on cotton was placed in the middle of each cage. The experiment was replicated five times for each of the insect strains. Two days after releasing the moths, the number of eggs on each plant was counted.

2.3. Abaxial vs. Adaxial Preference

Oviposition experiments to determine *P. xylostella* preference for abaxial vs. adaxial leaf surfaces in each plant species were conducted in two-choice experiments in comparison with *A. thaliana* (i.e., one plant of any of the tested types versus one plant of *A. thaliana*). The experimental arenas were screened cages 32.5 × 32.5 × 32.5 cm. Multiple cages were used for replication. Two pairs of moths (<3 days old) were released in each cage, which contained a small plastic cup with a 10% sugar solution on cotton to feed the moths. The experiment was replicated at least three times for each insect strain and plant comparison. Two days after releasing the insects, the number of eggs on each plant was counted and the location where eggs were found on the leaves (either abaxial or adaxial side) was recorded.

When moths approach a plant to oviposit, the adaxial side of the leaves is more exposed to the insects than the abaxial side, so an additional experiment was conducted with detached *A. thaliana* leaves to remove this effect. In this experiment, two leaves of similar size (approximately 4.5 cm long) were placed horizontally, parallelly aligned, and 2 cm apart in the center of a 9 cm Petri dish. Of these two leaves, one was placed with the abaxial side facing upwards and the other one was placed with the adaxial side facing upwards. Leaves were secured in this position by attaching them to the bottom of the Petri dish with transparent 1.9 cm wide Scotch™ tape (3M, Minneapolis, MN, USA) that covered approximately 1 mm of the two margins of the leaf at opposite sides of the midrib. The Petri dish was placed in the center of a screened cage (32.5 × 32.5 × 32.5 cm) and one pair of moths (<3 days old) of the strain DBM-C was released in each cage, which contained a small plastic cup with a 10% sugar solution on cotton to feed the moths. Multiple cages with each with one Petri dish each containing the two *A. thaliana* leaves were used, each of which was considered a replicate. The experiment was replicated 20 times. One day after releasing the insects, the number of eggs on each plant was counted.

2.4. Oviposition Preference Experiments with Sinigrin

Oviposition preference was also studied using different concentrations of sinigrin in pure form. These tests were conducted with DBM-C and DBM-G88, the latter being the *P. xylostella* strain that had been feeding on glucosinolate-free diet for the longest time. Pure sinigrin (Acros Organics, Geel, Belgium) was applied in 160 µL solutions in HPLC-

grade water to one fourth of a 90 mm diameter Whatman® filter paper disk (Whatman International Ltd., Maidstone, UK). The tip of each fourth filter paper section was cut by 0.5 cm to avoid contact between the two pieces of filter paper that were placed opposed to each other on a Petri dish (9 cm diameter). Treatments were randomly assigned, and multiple Petri dishes were used, each of which was considered a replicate. Concentrations of sinigrin were compared in the following two-choice comparisons: 10^{-7} vs. 10^{-6} M, 10^{-6} vs. 10^{-5} M, 10^{-5} vs. 10^{-4} M, 10^{-4} vs. 10^{-3} M, and 10^{-3} vs. 10^{-2} M. One pair of moths were placed in each Petri dish. The numbers of eggs laid on each of the filter paper sections were counted one day after releasing the moths. The experiment was replicated 3–17 times for each comparison of glucosinolate concentrations and only replicates in which two or more eggs were laid on the filter paper were considered.

2.5. Larval Preference Experiments

Two-choice experiments were conducted to compare preference of 3rd instar DBM-C larvae between *A. thaliana* and each of the 18 plant species tested. Plants were cut from the crown so only foliage was offered to the larvae. Plants were placed randomly on opposite sides of a plastic container that was used as experimental arena, at about 5 cm from the center of the container. A total of 5 larvae were placed in the center of the experimental arena and their preference was assessed after 24 h by recording on what plant type each larva was found. The experiment was replicated 4–10 times for each comparison between *A. thaliana* and the other plant species. The experimental arenas were 22.0 × 28.0 × 18.0 cm plastic boxes with a modified lid with a mesh to allow air into the box. Multiple boxes were used, each of which was considered a replicate. A larval preference index (LPI) was calculated as the number of larvae found on each individual plant plus one divided by the number of larvae found on the *A. thaliana* plant that it was compared with in the same box plus one. A LPI = 1 indicated no difference in larval preference between *A. thaliana* and the alternative plant species it was compared with; a LPI < 1 indicated that *A. thaliana* would tend to be preferred; and a LPI > 1 indicated that larvae of *P. xylostella* would tend to prefer the alternative plant species over *A. thaliana*. LPI data were compared to data on an oviposition preference index (OPI) that had been calculated in previous research with plants of the same species and age [10]. This allowed us to calculate the correlation between LPI and OPI. Data on glucosinolate content for plants of the same species and age were also taken from Badenes-Pérez et al. [10]. Glucosinolates were grouped as aliphatic with sulfur-containing side chains (AS), other aliphatic (AO), benzenic (BEN), and indolic (IN). In order to take into account the effect of the diversity of glucosinolates in each plant species, we used the number of different glucosinolates per plant species (glucosinolate richness, *S*) and a glucosinolate complexity index (GCI), similar to the previously proposed chemical complexity index [44,45]. The GCI was calculated as the sum of the Shannon's diversity index from the four chemical classes of glucosinolates (H_A) and the Shannon's diversity index from the relative concentrations of all individual glucosinolates (H_B) [10,44].

2.6. Statistical Analysis

For each *P. xylostella* strain, differences in oviposition preference in multi-choice tests were analyzed using a Kruskal–Wallis test ($p \leq 0.05$) with SPSS® version 24. The significance values of Kruskal–Wallis tests were adjusted by the Bonferroni correction for multiple tests. Data comparing larval preference, oviposition preference between abaxial and adaxial leaf surfaces, and oviposition preference between substrates with concentrations of sinigrin were analyzed using a one-tailed, two-sample test of proportions using STATA® version 15.1 with significance at $p \leq 0.05$. Kruskal–Wallis tests and tests of proportions were performed with untransformed data. Correlations between LPI and glucosinolate content and glucosinolate diversity as well as between LPI and OPI were performed using a two-tailed Spearman's correlation with SPSS®.

3. Results

3.1. Multiple-Choice Oviposition Preference Experiments

For the four *P. xylostella* strains tested (DBM-W, DBM-NOQA, DBM-C, and DBM-P) there were significant differences in oviposition preference among the four possible oviposition substrates (cabbage, rape, pea, and aluminum foil) ($p \leq 0.05$) and rape was the most preferred host-plant, while pea and aluminium foil were the least preferred oviposition substrates (Figure 1, Table S1). Unlike the other three *P. xylostella* strains tested, for the strain DBM-NOQA, oviposition on rape and on aluminium foil was not significantly different.

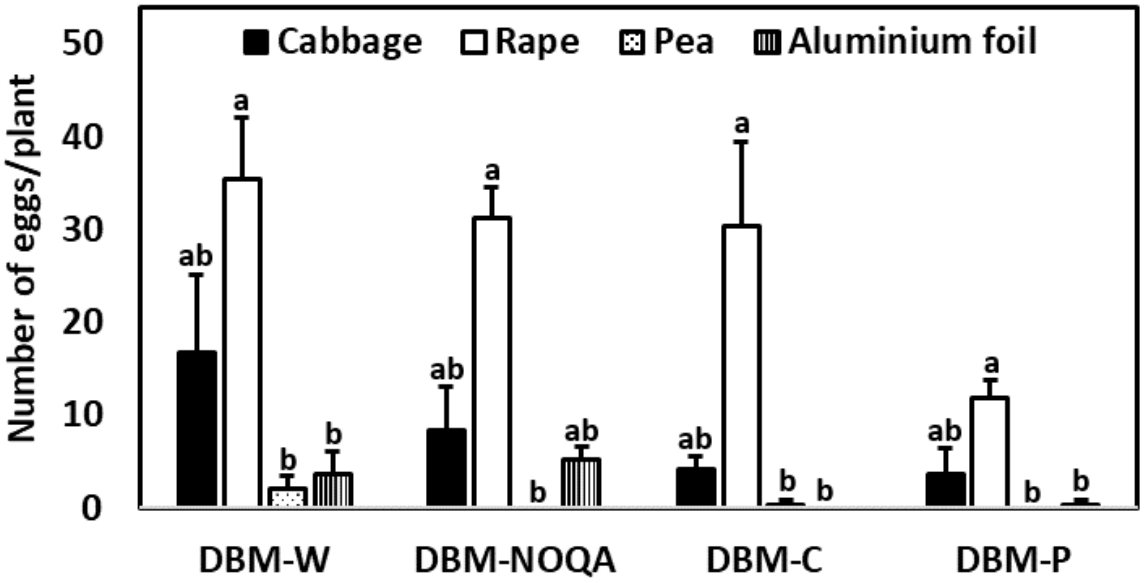

Figure 1. Mean ± SE eggs laid by *P. xylostella* strains DBM-W, DBM-NOQA, DBM-C, and DBM-P in a multi-choice setting consisting of cabbage, rape, pea, and aluminium foil. Significant differences ($p \leq 0.05$) in oviposition preference of each *P. xylostella* strain are shown with different lowercase letters.

3.2. Abaxial vs. Adaxial Preference

When comparing the three *P. xylostella* strains tested (DBM-C, DBM-G88, and DBM-P), there were no significant differences in oviposition preference in terms of abaxial versus adaxial oviposition in each of the plants tested ($p > 0.05$) (Table S2). When analyzing each strain separately for each plant type, preference for adaxial surfaces occurred in *A. argenteum*, waxy *B. oleracea* var. *acephala*, and *I. amara* in the three *P. xylostella* strains (Table 2). A preference for adaxial surfaces was also observed in glossy *B. oleracea* var. *acephala* for DBM-C and DBM-G88; in *L. douglasii* for DBM-C; in *S. officinale* for DBM-G88; and in *C. spinosa*, *E. cheiri*, *P. sativum*, and *R. odorata* for DBM-P. Preference for abaxial surfaces was only found in *C. bursa-pastoris* and *T. majus* for DBM-P. For the other plant species tested, differences in oviposition preference between abaxial and adaxial leaf surfaces were not statistically significant ($p > 0.05$). The experiment with detached leaves of *A. thaliana* showed no significant differences between abaxial and adaxial oviposition (Table 2).

Table 2. Two-choice preference between abaxial and adaxial leaf surfaces shown as the percentage of eggs laid abaxially (mean ± SE) in each of the tested plants in three *P. xylostella* strains reared on cabbage (DBM-C), artificial diet (DBM-G88), and pea (DBM-P). Data on the differences between the percentages of eggs laid abaxially and adaxially were analyzed using a one-tailed, two-sample test of proportions ($p \leq 0.05$). The test compared the percentages of eggs laid abaxially and adaxially for each plant type and *P. xylostella* strain (n = 3–96, except in the case of *C. papaya* and *M. oleifera*, and for DBM-C, *N. paniculata* for DBM-G88, and *R. odorata* for DBM-P in which n = 2). Significant *p*-values are shown in bold type. The experiment with detached leaves of *A. thaliana* is shown as "*A. thaliana*, detached".

	% Abaxial Oviposition as Mean ± SE, Test Statistic, and *p*-Value		
	DBM-C	**DBM-G88**	**DBM-P**
A. cordifolium	**11.15 ± 4.41, *z* = 2.70, *p* = 0.003** *	**11.98 ± 4.75, *z* = 2.63, *p* = 0.004** *	**8.71 ± 4.15, *z* = 2.84, *p* = 0.002** *
A. argenteum	**4.87 ± 2.23, *z* = 3.12, *p* ≤ 0.001** *	**4.11 ± 1.29, *z* = 3.19, *p* ≤ 0.001** *	**1.40 ± 0.95, *z* = 3.39, *p* ≤ 0.001** *
A. thaliana	53.41 ± 1.66, *z* = 0.95, *p* = 0.172	50.00 ± 1.25, *z* = 0.00, *p* = 0.500	50.33 ± 0.11, *z* = 0.09, *p* = 0.464
A. thaliana, detached	57.34 ± 4.47, *z* = 0.92, *p* = 0.178	-	-
A. caucasica	60.83 ± 4.67, *z* = 0.76, *p* = 0.223	52.72 ± 7.43, *z* = 0.21, *p* = 0.418	31.12 ± 8.16, *z* = 1.32, *p* = 0.094
B. vulgaris	50.38 ± 11.71, *z* = 0.00, *p* = 0.500	52.22 ± 3.68, *z* = 0.14, *p* = 0.445	58.84 ± 4.61, *z* = 0.62, *p* = 0.266
B. laevigata	56.33 ± 4.28, *z* = 0.34, *p* = 0.416	56.99 ± 3.04, *z* = 0.49, *p* = 0.314	43.55 ± 5.82, *z* = 0.42, *p* = 0.339
B. juncea	52.09 ± 6.11, *z* = 0.14, *p* = 0.445	64.76 ± 2.64, *z* = 0.24, *p* = 0.403	37.10 ± 3.20, *z* = 0.21, *p* = 0.418
B. napus	53.06 ± 8.35, *z* = 0.21, *p* = 0.418	44.99 ± 5.25, *z* = 0.35, *p* = 0.365	38.24 ± 8.10, *z* = 0.83, *p* = 0.203
B. oleracea (cabba.)	32.90 ± 11.86, *z* = 1.18, *p* = 0.119	35.33 ± 11.43, *z* = 1.04, *p* = 0.149	39.68 ± 15.71, *z* = 0.49, *p* = 0.312
B. oleracea (g. co.)	**17.62 ± 3.31, *z* = 2.22, *p* = 0.013** *	**21.20 ± 3.80, *z* = 2.01, *p* = 0.022** *	30.10 ± 5.39, *z* = 1.39, *p* = 0.083
B. oleracea (w. co.)	**4.17 ± 4.17, *z* = 3.19, *p* ≤ 0.001** *	**19.53 ± 9.99, *z* = 2.08, *p* = 0.019** *	**10.00 ± 6.12, *z* = 2.53, *p* = 0.006** *
B. orientalis	44.50 ± 9.38, *z* = 0.42, *p* = 0.339	31.49 ± 10.47, *z* = 1.32, *p* = 0.094	48.83 ± 6.77, *z* = 0.06, *p* = 0.475
C. bursa-pastoris	40.71 ± 16.36, *z* = 0.57, *p* = 0.285	n/a	**81.67 ± 8.22, *z* = 1.81, *p* = 0.035** **
C. pratensis	47.50 ± 4.61, *z* = 0.14, *p* = 0.445	45.37 ± 7.23, *z* = 0.24, *p* = 0.403	52.67 ± 3.60, *z* = 0.21, *p* = 0.418
C. papaya	55.88 ± 8.82, *z* = 0.24, *p* = 0.405	59.22 ± 7.30, *z* = 0.51, *p* = 0.305	34.77 ± 5.04, *z* = 0.73, *p* = 0.231
C. spinosa	33.46 ± 6.85, *z* = 1.08, *p* = 0.141	28.50 ± 7.69, *z* = 1.45, *p* = 0.073	**25.53 ± 9.47, *z* = 1.66, *p* = 0.048** *
C. cotinifolius	38.95 ± 8.37, *z* = 0.62, *p* = 0.267	56.02 ± 10.57, *z* = 0.34, *p* = 0.367	40.04 ± 8.81, *z* = 0.63, *p* = 0.263
D. muralis	49.53 ± 7.85, *z* = 0.00, *p* = 0.500	48.85 ± 3.11, *z* = 0.07, *p* = 0.472	41.46 ± 5.55, *z* = 0.62, *p* = 0.266
E. sativa	36.16 ± 3.12, *z* = 0.97, *p* = 0.166	32.24 ± 3.96, *z* = 1.25, *p* = 0.106	28.85 ± 6.63, *z* = 1.45, *p* = 0.073
E. cheiri	69.29 ± 2.96, *z* = 1.20, *p* = 0.115	50.46 ± 6.89, *z* = 0.00, *p* = 0.500	**23.80 ± 6.18, *z* = 1.80, *p* = 0.036** *
I. amara	**22.12 ± 4.74, *z* = 1.94, *p* = 0.020** *	**25.69 ± 6.98, *z* = 1.66, *p* = 0.048** *	**13.34 ± 5.97, *z* = 2.56, *p* = 0.005** *
L. sativum	71.84 ± 4.52, *z* = 1.52, *p* = 0.064	54.46 ± 2.66, *z* = 0.28, *p* = 0.391	26.93 ± 8.21, *z* = 1.59, *p* = 0.055
L. douglasii	**24.15 ± 1.33, *z* = 1.80, *p* = 0.036** *	36.00 ± 1.51, *z* = 0.97, *p* = 0.166	26.90 ± 5.39, *z* = 1.59, *p* = 0.055
M. oleifera	46.43 ± 3.57, *z* = 0.16, *p* = 0.436	n/a	n/a
N. officinale	58.54 ± 5.58, *z* = 0.39, *p* = 0.348	58.79 ± 1.83, *z* = 0.15, *p* = 0.442	47.10 ± 5.05, *z* = 0.34, *p* = 0.366
N. paniculata	60.00 ± 30.55, *z* = 0.62, *p* = 0.266	73.21 ± 1.79, *z* = 0.92, *p* = 0.179	n/a
p. sativum	n/a	n/a	**0.00 ± 0.00, *z* = 2.00, *p* = 0.023** *
R. odorata	48.33 ± 25.87, *z* = 0.10, *p* = 0.461	43.75 ± 25.77, *z* = 0.45, *p* = 0.325	**7.87 ± 4.56, *z* = 2.38, *p* = 0.009** *
S. officinale	35.31 ± 5.99, *z* = 1.04, *p* = 0.149	**25.45 ± 3.72, *z* = 1.66, *p* = 0.048** *	33.07 ± 6.74, *z* = 1.18, *p* = 0.119
T. majus	75.93 ± 14.46, *z* = 1.27, *p* = 0.100	n/a	**83.65 ± 12.71, *z* = 2.15, *p* = 0.016** **

* Adaxial leaf surface preferred. ** Abaxial leaf surface preferred.

3.3. Oviposition Preference Experiments with Sinigrin

For the two strains of *P. xylostella* tested, DBM-G88 and DBM-C, moths always laid more eggs on the filter paper with higher concentrations of sinigrin (Table 3).

Table 3. Oviposition preference of *P. xylostella* strains reared on cabbage (DBM-C) and on artificial diet (DBM-G88) on filter paper impregnated with different concentrations of sinigrin. Data were analyzed using a one-tailed, two-sample test of proportions ($p \leq 0.05$). Only the comparisons were more than one egg was laid on filter paper were considered (n = 3–17). For each comparison, means within a row followed by different superscript letters are significantly different.

	DBM-C			DBM-P		
Sinigrin Concentration (M)	**Lower Concentration**	**Higher Concentration**	**Test Statistic and *p*-Value**	**Lower Concentration**	**Higher Concentration**	**Test Statistic and *p*-Value**
10^{-3} vs. 10^{-2}	35.5 ± 9.0^{a}	64.5 ± 9.0^{b}	$z = 1.70$, $p = 0.045$	25.0 ± 5.5^{a}	75.0 ± 5.5^{b}	$z = 2.92$, $p = 0.002$
10^{-4} vs. 10^{-3}	26.6 ± 7.5^{a}	73.4 ± 1.7^{b}	$z = 2.29$, $p = 0.011$	15.5 ± 9.8^{a}	84.5 ± 9.8^{b}	$z = 3.09$, $p = 0.001$
10^{-5} vs. 10^{-4}	28.5 ± 7.5^{a}	71.5 ± 7.5^{b}	$z = 1.82$, $p = 0.034$	22.5 ± 11.1^{a}	77.5 ± 11.1^{b}	$z = 2.33$, $p = 0.010$
10^{-6} vs. 10^{-5}	15.4 ± 7.8^{a}	84.6 ± 7.8^{b}	$z = 1.70$, $p = 0.045$	26.8 ± 10.8^{a}	73.2 ± 10.8^{b}	$z = 1.74$, $p = 0.041$
10^{-7} vs. 10^{-6}	22.9 ± 11.1^{a}	77.1 ± 11.1^{b}	$z = 2.03$, $p = 0.021$	0.0 ± 0.0^{a}	100.0 ± 0.0^{b}	$z = 2.45$, $p = 0.007$

3.4. Larval Preference Experiments

Larvae of *P. xylostella* preferred *B. juncea* and *S. officinale* over *A. thaliana*, while *A. thaliana* was preferred over *A. argenteum*, *C. bursa-pastoris*, *M. oleifera*, *P. sativum*, and *R. odorata* (Table 4). In the set of plant species tested, LPI and OPI were positively correlated, and LPI was positively correlated with the glucosinolate diversity indexes S, H_A, and GCI, as well as to total content of glucosinolates and one type of aliphatic glucosinolates (AO) (Table 5 and Table S3). For these plant species, OPI was positively correlated to the same parameters as LPI and, additionally, it was positively correlated to IN and AS.

Table 4. Two-choice larval preference index (LPI) given as mean ± SE in larvae of *P. xylostella* (DBM-C). Data were analyzed using a one-tailed, two-sample test of proportions comparing the relative percentages of choices made between *A. thaliana* ($p \leq 0.05$) (n = 4–8). Significant differences are shown in bold type. Oviposition preference index (OPI) values taken from Badenes-Pérez et al. [10].

	LPI, Test Statistic, and *p*-Value	**OPI, Test Statistic, and *p*-Value**
A. argenteum	**0.31 ± 0.11, $z = 3.20$, $p \leq 0.001$ ***	**0.08 ± 0.02, $z = 2.11$, $p = 0.018$ ***
A. caucasica	0.87 ± 0.12, $z = 0.38$, $p = 0.352$	0.43 ± 0.05, $z = 0.98$, $p = 0.164$
B. juncea	**4.14 ± 0.69, $z = 2.52$, $p = 0.012$ ****	1.71 ± 0.25, $z = 0.59$, $p = 0.278$
C. bursa-pastoris	**0.17 ± 0.01, $z = 3.80$, $p \leq 0.001$ ***	**0.03 ± 0.03, $z = 2.30$, $p = 0.011$ ***
C. papaya	**0.23 ± 0.06, $z = 2.41$, $p = 0.008$ ***	**0.05 ± 0.05, $z = 2.25$, $p = 0.012$ ***
C. spinosa	**0.25 ± 0.05, $z = 2.15$, $p = 0.032$ ***	**0.09 ± 0.05, $z = 2.06$, $p = 0.020$ ***
D. muralis	1.71 ± 0.34, $z = 0.85$, $p = 0.197$	1.51 ± 0.17, $z = 0.49$, $p = 0.312$
E. sativa	1.42 ± 0.21, $z = 0.57$, $p = 0.285$	1.35 ± 0.25, $z = 0.29$, $p = 0.384$
E. cheiri	0.69 ± 0.10, $z = 1.34$, $p = 0.090$	**0.22 ± 0.18, $z = 1.71$, $p = 0.043$***
I. amara	0.49 ± 0.09, $z = 1.41$, $p = 0.079$	0.72 ± 0.46, $z = 0.78$, $p = 0.217$
L. sativum	1.69 ± 0.18, $z = 1.21$, $p = 0.114$	4.28 ± 1.74, $z = 1.18$, $p = 0.120$
M. oleifera	**0.22 ± 0.06, $z = 2.55$, $p = 0.005$ ***	**0 ± 0, $z = 2.45$, $p = 0.007$ ***
p. sativum	**0.17 ± 0.01, $z = 2.69$, $p = 0.004$ ***	**0 ± 0, $z = 2.45$, $p = 0.007$ ***
R. odorata	**0.24 ± 0.05, $z = 2.27$, $p = 0.011$ ***	0.36 ± 0.30, $z = 1.47$, $p = 0.071$
S. officinale	**3.10 ± 0.66, $z = 2.09$, $p = 0.018$ ****	**6.67 ± 2.04, $z = 1.67$, $p = 0.048$ ****
T. majus	1.33 ± 0.22, $z = 0.50$, $p = 0.308$	**0.04 ± 0.04, $z = 2.16$, $p = 0.016$ ***

* *A. thaliana* preferred, ** Plant species compared to *A. thaliana* preferred.

Table 5. Significance of correlations between plant glucosinolate content, larval preference index (LPI), and oviposition preference index (OPI) in the plants tested. Two-tailed Spearman's rho correlations (n = 16) were performed. The effect of the diversity of glucosinolates was analyzed taking into account the glucosinolate richness (*S*), Shannon's diversity index for the four glucosinolate classes (H_A), Shannon's diversity index for the relative concentrations of all individual glucosinolates (H_B), and glucosinolate complexity index (GCI) for each plant (**A**). Besides total glucosinolate content (TOT), four different classes of glucosinolates were distinguished, aliphatic with sulfur-containing side chains (AS), other aliphatic (AO), benzenic (BEN), and indolic (IN) (**B**). Significant *p*-values ($p \leq 0.05$) are shown in bold type.

A					
	***p*-Value and Correlation Coefficient of Spearman's Rho Correlation**				
	OPI	***S***	**H_A**	**H_B**	**GCI**
LPI	**$p \leq 0.001$; 0.864**	**$p = 0.007$; 0.641**	**$p = 0.010$; 0.624**	$p = 0.063$; 0.476	**$p = 0.010$; 0.623**
OPI	**-**	**$p = 0.011$; 0.614**	**$p = 0.002$; 0.723**	$p = 0.086$; 0.443	**$p = 0.005$; 0.668**
B					
	***p*-Value and Correlation Coefficient of Spearman's Rho Correlation**				
	TOT	**AO**	**BEN**	**IN**	**AS**
LPI	**$p = 0.015$; 0.594**	**$p = 0.016$; 0.590**	$p = 0.673$; −0.115	$p = 0.137$; 0.388	$p = 0.064$; 0.473
OPI	**$p = 0.002$; 0.716**	**$p = 0.024$; 0.561**	$p = 0.630$; −0.131	**$p = 0.033$; 0.533**	**$p = 0.049$; 0.498**

4. Discussion

The tested strains of *P. xylostella* had overall similar oviposition preferences in multi-choice tests and in abaxial/adaxial leaf surfaces. This and previous research [9] shows that, overall, the diet on which *P. xylostella* has fed hardly affects oviposition preference between plants. This indicates that in *P. xylostella*, preimaginal conditioning does not significantly affect oviposition preference in adults, as it has also been shown in other insects as opposed to what would be expected from the Hopkins' host-selection principle by which insects demonstrate a preference for the host species on which they have developed [46,47]. This also shows that in *P. xylostella* there seems to be a strong selection for following particular cues for ovipositing on plants and that these are not affected by long-term diet and host-plant exposure. Among these oviposition preference cues, glucosinolate content seems to be an important one, which is shown by the correlation found between host-plant glucosinolate content and oviposition [10] as well as by the preference for higher concentrations of sinigrin shown here with the strains DBM-C and DBM-G88. The resistance of DBM-NOQA to *B. thuringiensis* Cry 1Ac toxin also did not affect oviposition preference in the tests in which this *P. xylostella* strain was used.

Although there were no significant differences in abaxial and adaxial oviposition among the *P. xylostella* strains tested, oviposition occurred preferentially on the adaxial leaf surface, except in *C. bursa-pastoris* and *T. majus* in the case of the DBM-P strain, which mostly laid the eggs on the abaxial surface of these host-plants. Abaxial and adaxial preference can also influence management of *P. xylostella*. For example, some insecticide sprayers deposit more insecticide on the adaxial than on the abaxial leaf surface [48,49]. Rainfall and sprinkler irrigation can also wash off eggs and larvae of *P. xylostella* [11,50–53] and in cabbage, *P. xylostella* egg susceptibility to rainfall is higher on the adaxial than on the abaxial leaf surface [53]. It is unknown why *P. xylostella* shows these oviposition preferences for either adaxial or abaxial surfaces depending on the plant species. The plant cuticle can play an important role in insect-plant interactions [54]. Insects use plant secondary metabolites as 'fingerprints' to recognize host-plants and oviposit on them [55–57]. Although plants may contain sufficient amounts of glucosinolates on the leaf surface to be perceived by *P. xylostella*, higher concentrations (2- to 10-fold) of glucosinolates on the plants *Barbarea rupicola* Moris and *Barbarea verna* (Mill.) Asch. (Brassicaceae) were not correlated with

oviposition preference for either leaf surface [38]. Similarly, in *A. thaliana*, despite differences in glucosinolate content between abaxial and adaxial leaf surfaces [58], we found no oviposition preference differences in *P. xylostella* for any of the leaf sides. Glucosinolates are not the only plant compounds active as oviposition stimulants for *P. xylostella* and other specialists of Brassicaceae [7,14,59]. Waxes can also affect *P. xylostella* oviposition [60–62]. Trichome density, which has been shown to affect oviposition preference in *P. xylostella* [63], could also be different between the abaxial and adaxial of the plants tested. Moreover, the rugosity of the surface can increase the ovipositional preference of *P. xylostella* [20] and, in general, defense from natural enemies has also been shown to affect oviposition preference in insects [64–66]. The green leaf volatile (*Z*)-3-hexenyl acetate has also been shown to be an attractant to mated *P. xylostella* females [67], and this and other green leaf volatiles make *P. xylostella* oviposit preferentially on the adaxial leaf side of *Brassica rapa* var. *perviridis* L. (Brassicaceae) [68].

Pure glucosinolates have been shown to have a quantitative effect on *P. xylostella* oviposition, which oviposits more on substrates with higher concentrations of glucosinolates than on substrate with lower glucosinolate concentrations [16,38]. Here the DBM-C and DBM-G88 strains behaved similarly in preferring the higher concentrations of sinigrin. However, moths of the DBM-P did not oviposit at all on the substrate with the lowest concentration of sinigrin (10^{-7} M) and this could indicate that they have lost some sensitivity to detect glucosinolates after long-term rearing without glucosinolate exposure.

In our study there was a positive correlation between LPI and OPI. Besides being correlated, both OPI and LPI responded similarly to glucosinolate content and glucosinolate diversity in the plants tested, except for AS and IN content, which was significantly correlated with OPI but not with LPI. This could indicate that AS and IN content is less important for host-plant choice in *P. xylostella* larvae than for adults, at least when considering the set of plant species tested. Two isothiocyanates derived from glucoiberin and glucoraphanin (AS) have been shown to be oviposition stimulants for *P. xylostella* [14]. However, larvae of *P. xylostella* are also known to be stimulated by glucoiberin and glucobrassicin [13], which are AS and IN, respectively, and herbivory by *P. xylostella* decreased in *A. thaliana* mutants with reduced AS and IN content compared to wildtype *A. thaliana plants* with higher AS and IN content [25]. Besides glucosinolates, flavonoids have also been shown to act as feeding stimulants for larvae of *P. xylostella* [13].

Our results with *P. xylostella* larvae and adults, together with previously known oviposition and survival data [10], indicate that plants with higher glucosinolate content are likely to be more attractive to *P. xylostella*. Even though glucosinolates can provide some degree of resistance against generalist insects [69–72], in locations where *P. xylostella* is abundant, using crop varieties that have low glucosinolate content could reduce damage by this insect. On the other hand, the preference of *P. xylostella* for host-plants with high glucosinolate content should be taken into account in the choice of trap crops to be deployed for the management of this insect.

5. Conclusions

The tested strains of *P. xylostella* had, overall, very similar oviposition preferences in four-choice tests, in abaxial/adaxial leaf sides, and in substrates with different concentrations of sinigrin. In four-choice tests, the preferred host was *B. napus* and the least preferred host was *P. sativum*. Preference for adaxial leaf surfaces occurred in *A. argenteum*, waxy *B. oleracea* var. *acephala*, and *I. amara* in the three *P. xylostella* strains tested. Preference for adaxial leaf surfaces was also observed in glossy *B. oleracea* var. *acephala* for DBM-C and DBM-G88; in *L. douglasii* for DBM-C; in *S. officinale* for DBM-G88; and in *C. spinosa*, *E. cheiri*, *P. sativum*, and *R. odorata* for DBM-P. Preference for abaxial leaf surfaces was only found in *C. bursa-pastoris* and *T. majus* for DBM-P. For the other plant species tested, differences in oviposition preference between abaxial and adaxial leaf surfaces were not significant. The two *P. xylostella* strains tested with sinigrin, DBM-G88 and DBM-C, preferred to oviposit on the substrates with higher concentrations of sinigrin. Host-plant preference in *P. xylostella*

larvae and ovipositing adults were positively correlated and both larvae and ovipositing moths responded similarly to glucosinolate content and glucosinolate diversity, except for AS and IN content, which for the plants tested, was significantly correlated with oviposition preference, but not with larval preference.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9010039/s1, Table S1: Test statistic and *p*-values of Kruskall–Wallis tests comparing differences in oviposition on four different oviposition substrates (cabbage, rape, pea, and aluminium foil) for the strains DBM-W, DBM-NOQA, DBM-C, and DBM-P of *P. xylostella*. The significance values have been adjusted by the Bonferroni correction for multiple tests. Significant *p*-values ($p \leq 0.05$) are shown in bold type; Table S2: Comparison of the preference between abaxial and adaxial leaf surfaces among the three *P. xylostella* strains reared on cabbage (DBM-C), artificial diet (DBM-G88), and pea (DBM-P). Oviposition preference data were analyzed using a one-tailed, two-sample test of proportions ($p \leq 0.05$) comparing the percentages of the total number of eggs laid on the abaxial side of leaves (n = 3–96, except in the case of *C. papaya* for DBM-C, where n = 2).; and Table S3: Total glucosinolate content (TOT) and content of aliphatic glucosinolates with sulfur-containing side chains (AS), other aliphatic glucosinolates (AO), benzenic glucosinolates (BEN), and indolic glucosinolates (IN) for each of the plant types tested (A). Glucosinolate richness (*S*), Shannon's diversity index for the four glucosinolate classes (H_A), Shannon's diversity index for the relative concentrations of all individual glucosinolates (H_B), and chemical complexity index for glucosinolates (CCI) for each of the plant types tested (B). Values based on means across replicates taken from Badenes-Pérez et al. 2020 [10].

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