How host-specific is diamondback moth? A study on performance with brassicaceous and non-brassicaceous species

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ABSTRACT

Diamondback moth, Plutella xylostella (L.) (Lepidoptera: Plutellidae) is oligophagous on Brassicaceae, but is known to have occasionally switched to also feed upon hosts of other plant families. The spider flower, Cleome hassleriana Chodat (Capparaceae) and garden nasturtium Tropaeolum majus L. (Tropaeolaceae) are taxonomically distinct but chemically related to Brassicaceae by containing glucosinolates. Both of these plant species can occur sympatrically with wild crucifers, and so could potentially harbor P. xylostella populations, perhaps providing bridge hosts until crop plants are available. But these non-brassicaceous plants have never been reported to be infested with P. xylostella in the region. In this extensive laboratory and greenhouse study, we tested ovipositional preferences of P. xylostella in a free-choice situation using two Brassicaceae viz. conventional canola, Brassica napus L., flixweed, Descurainia sophia (L.) Webb ex Prantl, and two non-Brassicaceae viz. C. hassleriana and T. majus. No-choice tests were conducted to investigate key life history parameters i.e., survival, developmental time, herbivory, pupal weight, silk weight, adult weight, forewing wing area, and longevity (without food) for both female and male specimens when reared on all four plant species. Although several P. xylostella developmental parameters were similar on C. hassleriana and T. majus as on more preferred Brassicaceae, non-hosts lacked the ability to compensate as well for P. xylostella herbivory. Host shifting by P. xylostella is evidently a strategy for population survival when preferred hosts are not available, and is a strategy that may predispose new generation offspring for enhanced migratory capability.

INTRODUCTION

Despite a vast body of published research on diamondback moth, Plutella xylostella (L.) (Lepidoptera: Plutellidae) (Talekar and Shelton 1993, Sarfraz et al. 2006), its host range has not been well defined. Conventionally, P. xylostella is considered a specialist on Brassicaceae, but rigorous research on its developmental parameters in the context of insect-plant interactions is rare. Brassicaceae comprise a diverse group of 380 genera and over 3 000 species of cultivated and wild plants (Heywood 1993) that are characterized by secondary plant compounds, the glucosinolates (Kjaer 1974, Mithen 1992). Plutella xylostella relies on glucosinolates for host location, oviposition and feeding stimulation (Thorsteinson 1953, Gupta and Thorsteinson 1960b, Marazzi et al. 2004). Host plant cues stimulate the onset of its reproductive activities (Pittendrigh and Pivnick 1993), but the presence of non-host plants such as wheat, beans and
peas does not produce any such effect (Hillyer and Thorsteinson 1969, Renwick and Radke 1990).

Canola, *Brassica napus* L. (*Brassicaceae*), is a widely cultivated oilseed crop in western Canada (Canola Council of Canada, 2006). Flixweed, *Descurainia sophia* (L.) Webb ex Prantl, is one of the first wild *Brassicaceae* to appear in the spring (Morishita 1991, Mitich 1996), and is commonly found in canola fields. The spider flower, *Cleome hassleriana* Chodat (*Capparaceae*), and the garden nasturtium, *Tropaeolum majus* L. (*Tropaeolaceae*), are common ornamental herbs in North America (Foster 2001, Stephens 2003) and they readily escape gardens to invade roadsides and the shores of rivers and lakes. Glucocapparin (*aliphatic allylic glucosinolate*) and glucotropaeolin (*aromatic glucosinolate*) predominate in *C. hassleriana* and *T. majus*, respectively (Kjaer 1974, Renwick and Lopez 1999). Despite their close chemical relationship with *Brassicaceae* by containing glucosinolates, these non-Brassicaceae plants have never been reported to be infested with *P. xylostella* in the region and elsewhere.

*Cleome hassleriana* and *T. majus* can occur sympatriically with wild and cultivated crucifers, and so could potentially serve to harbor *P. xylostella* populations, perhaps providing bridge hosts until crop plants are available. Therefore, the present study was designed to address the following questions: Could *P. xylostella* females accept non-Brassicaceae species for their oviposition in the presence of natural host plants? What are the bottom-up effects of *Brassicaceae* and non-*Brassicaceae* on pre-imaginal and imaginal traits of female and male *P. xylostella*? What are the top-down effects of *P. xylostella* larval herbivory on belowground biomass of host and non-host plant species?

**MATERIALS AND METHODS**

**Insects and Plants**

The laboratory colony of *P. xylostella* was maintained on potted *B. napus* plants at 22 ± 0.5°C with 16 hours L:8 hours D under growth chamber conditions. Moths collected from different fields in Alberta, Canada were added to the culture every summer to maintain genetic diversity.

*Brassica napus* cv. Q2, *D. sophia*, *C. hassleriana* cv. Cherry Queen and *T. majus* cv. Golden Gleam were grown under greenhouse conditions. Plants were grown individually in 15.2 cm diameter pots using Metromix-220 as a potting medium fertilized with 20:20:20 (nitrogen: phosphorous: potassium) at 0.5 g/pot when plants were two to four weeks old. Five to seven-week-old plants were used for all experiments.

**Leaf Tissue Study**

This study was carried out in controlled environmental conditions (22 ± 0.5°C with 16 hours L:8 hours D) following the protocol described by Sarfraz et al. (2007).

**Pre-imaginal developmental parameters**

Excised leaves were placed on moist filter papers in 6 oz plastic containers; holes were poked in lids to ensure ventilation and to avoid condensation. For each plant species, 100 to 200 second-instar larvae (≈1 day old) taken from the laboratory colony were introduced into individual plastic containers (one larva per container); a total of 400 to 800 larvae were used. Larvae were provided with fresh leaf tissue every 24 hours until pupation. Developmental times from second-instar larva to pre-pupa and from pre-pupa to pupa were recorded. Pupae were harvested, weighed within 24 hours of pupation, returned to their respective containers and developmental times from pupa to adult emergence were recorded. After adult eclosion, the silk cocoons were weighed using a Sartorius Supermicro balance (Sartorius Inc., Edgewood, NY, USA).
To quantify levels of larval feeding, all leaves damaged by *P. xylostella* larvae were scanned daily into a digital format using a desktop scanner. Image J (National Institutes of Health, Bethesda, MD, USA) was used to quantify the amount of leaf area removed due to larval herbivory. Leaves of *D. sophia* were too small to be scanned. In this case, we had two sets of cups containing leaves: the first set received larvae whereas the second set served as uninfested controls. Both infested and uninfested leaves were weighed daily and the amount of foliage removed due to larval herbivory was quantified.

**Imaginal parameters**

Twenty pairs of moths reared from each plant species were used to determine their longevity without food. Moths were weighed within 24 hours of their death. Their forewings were removed, scanned using a desktop scanner, and their areas were measured using Image J.

Ten pairs of moths of almost the same age (= 1 day old) reared from each plant species were released in individual plastic containers (i.e., one pair per cup) containing *B. napus* cv. Q2 leaf discs (area = 52.8 cm²) placed on moist filter papers. A total of 40 cups were used following a completely randomized design and this study was conducted under growth chamber conditions (22 ± 0.5°C with 16L:8H). Every 24 hours, moths were transferred to new containers and provided with fresh leaf discs and food (10% sterile honey solution). This experiment was continued over an 8-day period and eggs found on the leaf disc, filter paper and container were recorded daily.

**Whole Plant Study**

This study was conducted in greenhouse following the protocol described by Sarfraz *et al.* (2007).

**Oviposition preference**

*Plutella xylostella* ovipositional preferences were investigated in a free-choice situation in ten screened cages (120 cm × 120 cm × 120 cm) using a completely randomized design and each cage was considered a replication. One plant from each species was placed randomly in each cage and a total of 40 plants were used in the experiment (i.e., four plants in each cage). Eight one-day-old adults (two moths per plant) were released in 1:1 (m: f) sex ratio and provided a 10% sterile honey solution for adult feeding.

**Survival from neonate to adult**

Survival from neonate to pupa was assessed in screened cages (40 cm × 40 cm × 80 cm), arranged on a greenhouse bench in a completely randomized design with each cage considered one replicate. Each cage contained a single plant; the entire experiment used 40 cages with 10 plants from each species. Five plants from each species were infested with first-instar larvae (at 10 larvae per plant) while the remaining plants served as uninfested controls. Insects were observed every 48 hours and the numbers of surviving individuals were recorded. Pupae were harvested, weighed and kept individually in transparent plastic cups until adult emergence.

**Root mass development in response to insect herbivory**

At the end of the experiment investigating *P. xylostella* survival on whole plants, infested and uninfested plants were uprooted; their roots were carefully washed, air-dried at room temperature and weighed to determine the effects of aboveground herbivory on root mass (mg dry weight basis) development.

**Statistical Analyses**

Analyses of variance (ANOVA) for a completely randomized design were performed to test the differences between treatments, and means were compared at the 5% level of significance using Tukey’s
studentized range test (SAS Institute 2004). Data on larval herbivory for *D. sophia* were not included in ANOVA owing to measurement units different from other tested plant species. T-tests were performed for each species for pair-wise comparison between root masses of infested and uninfested (control) plants.

## RESULTS

### Excised Leaf Tissue Study

**Pre-imaginal developmental parameters**

Female larval development was faster on *B. napus* than on *D. sophia* whereas male larvae developed faster on *B. napus* than on *T. majus*. Female pupae developed fastest on *B. napus* and *D. sophia* whereas male pupal development was fastest on *C. hassleriana* (Table 1). Both female and male larvae consumed similar leaf areas of *B. napus* and *C. hassleriana* whereas least foliage consumption occurred on *T. majus*. Female pupae were heaviest on *B. napus* and lightest on *D. sophia*; male pupae were significantly heavier on *B. napus* than on non-Brassicaceae. Female specimens reared on *D. sophia* produced least silk. Both females and males reared on *B. napus* and *T. majus* produced statistically similar amounts of silk (Table 1).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Developmental time (days)</th>
<th>Foliage consumed (cm²)</th>
<th>Pupal weight (mg)</th>
<th>Silk weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brassica napus</em></td>
<td>Larva to pre-pupa Female 6.40 (0.15)</td>
<td>Pre-pupa Female 5.60 (0.18)</td>
<td>Pupa to adult Female 1.00 (0.00)</td>
<td>4.30 (0.11)</td>
</tr>
<tr>
<td><em>Descurainia sophia</em></td>
<td>7.40 (0.17)</td>
<td>6.15 (0.22)</td>
<td>0.90 (0.05)</td>
<td>0.88 (0.11)</td>
</tr>
<tr>
<td><em>Cleome hassleriana</em></td>
<td>7.00 (0.24)</td>
<td>6.05 (0.28)</td>
<td>0.93 (0.04)</td>
<td>0.85 (0.05)</td>
</tr>
<tr>
<td><em>Tropaeolum majus</em></td>
<td>6.80 (0.20)</td>
<td>6.85 (0.21)</td>
<td>1.15 (0.08)</td>
<td>0.93 (0.04)</td>
</tr>
</tbody>
</table>

Means in a column followed by the same letter do not differ significantly (*P* = 0.05) using analysis of variance and Tukey’s studentized range test.

* Data are not included in analysis as amounts of herbivory are in grams.

### Imaginal parameters

Heaviest females were produced on *B. napus*; lightest females developed on *T. majus* and *D. sophia*. Female moths reared on *D. sophia* had the smallest forewings whereas forewing areas were similar for females on *B. napus*, *C. hassleriana* and *T. majus*. Male moths reared on *B. napus* and *C. hassleriana* had the largest forewings. Females reared on *B. napus* and *C. hassleriana* lived longer in the absence of food than those reared on either *D. sophia* or *T. majus* (Figure 1).
Figure 1  Mean (± S.E.) adult bodyweight, forewing area and longevity of female and male *Plutella xylostella* when reared as larvae on two Brassicaceae, one Capparaceae and one Tropaeolaceae

New generation adults reared on *D. sophia* deposited the fewest total eggs over the 8-day period whereas total oviposition did not differ for females raised on all other tested plant species. On day 1, females reared on *T. majus* and *B. napus* laid the most eggs whereas moths from *D. sophia* deposited fewest eggs. On days 2, 3 and 4, females raised on *B. napus*, *C. hassleriana* and *T. majus* deposited similar numbers of eggs but significantly more than those reared on *D. sophia*. Females from *D. sophia* and *C. hassleriana* had similar oviposition on days 5 and 7. Oviposition did not significantly differ on days 6 and 8 for females raised on the tested plant species (Table 2).

Table 2  Oviposition (± S.E.) by *Plutella xylostella* on and off the leaf discs of *Brassica napus* in no-choice situation when females were reared on *Brassica napus*, *Descurainia sophia*, *Cleome hassleriana* and *Tropaeolum majus*

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Total Oviposition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brassica napus</em></td>
<td>34.2a</td>
<td>30.00a</td>
<td>43.40a</td>
<td>30.40a</td>
<td>37.80a</td>
<td>19.90a</td>
<td>19.80a</td>
<td>14.00a</td>
<td>229.50a</td>
</tr>
<tr>
<td></td>
<td>(2.92)</td>
<td>(5.58)</td>
<td>(4.22)</td>
<td>(2.43)</td>
<td>(2.33)</td>
<td>(1.07)</td>
<td>(0.77)</td>
<td>(2.99)</td>
<td></td>
</tr>
<tr>
<td><em>Descurainia sophia</em></td>
<td>1.20c</td>
<td>2.80b</td>
<td>6.00b</td>
<td>12.40b</td>
<td>16.60b</td>
<td>15.20a</td>
<td>11.00ab</td>
<td>5.60a</td>
<td>70.80b</td>
</tr>
<tr>
<td></td>
<td>(0.8)</td>
<td>(1.22)</td>
<td>(1.87)</td>
<td>(3.46)</td>
<td>(2.58)</td>
<td>(1.51)</td>
<td>(1.35)</td>
<td>(1.05)</td>
<td></td>
</tr>
</tbody>
</table>
Whole Plant Study

Oviposition preference

Oviposition on *D. sophia* and *C. hassleriana* were statistically similar (83.40 ± 3.94 and 78.00 ± 8.21 eggs per plant respectively), but numbers of eggs deposited on *B. napus* exceeded all the tested plant species. Females laid 2.2-, 2.3-, and 15.1-fold more eggs on *B. napus* than on *D. sophia*, *C. hassleriana* and *T. majus* respectively.

Survival from neonate to adult

Survival from neonate to adult was highest on whole plants of *B. napus* (90%) followed by *T. majus*, *C. hassleriana* (88% and 75% respectively); least survival (55%) occurred on *D. sophia*.

Root mass development in response to insect herbivory

Pair-wise comparisons for each plant species indicated that infested *B. napus* had significantly more robust root systems than their uninfested counterparts (*t* = 14.71; *df* = 8; *P* < 0.001). The root masses of infested *C. hassleriana* and *T. majus* plants were significantly less than their uninfested counterparts (*t* = −8.87; *df* = 8; *P* < 0.001 and *t* = −8.06; *df* = 8; *P* < 0.001 respectively). However, the root masses of *D. sophia* did not differ significantly between infested and uninfested plants (*t* = −0.05; *df* = 8; *P* = 0.958) (Figure 2).

![Figure 2](image-url)  
**Figure 2** Root mass development (mean ± S.E.) of Brassicaceae, Capparaceae and Tropaeolaceae species when infested and non-infested by *Plutella xylostella*


DISCUSSION

Life History Traits

This is the first study of its kind to demonstrate that *P. xylostella* larvae can accept *C. hassleriana* and *T. majus* as food plants and perform equally well on these non-host plants. Our no-choice experiments ideally represent the natural situation where larvae would be reliant on their mothers for host selection owing to their limited mobility. Larvae need specific stimulants to accept a plant and initiate feeding (Gupta and Thorsteinson 1960a, van Loon *et al.* 2002) and may even starve to death on unsuitable plants (Sarfraz *et al.* 2006). For instance, larvae did not feed on non-crucciferous plants such as *Dalia* sp., *Gynura* sp., *Chrysanthemum* sp., *Cucumis* sp., *Euphorbia poinsettiana* Buist., *E. splendidus* Mill, *Abutilan* sp., *Maranta* sp., *Pepomia* sp., *Rhamnus* sp., *Clematis* sp. and *Rosa* sp., but they readily accepted leaf discs of these plants when treated with sinigrin (Gupta and Thorsteinson 1960a). Larval herbivory is a good indicator of food plant suitability. A similar amount of herbivory and larval development times on *B. napus* and *C. hassleriana* suggested that both plant species were equally suitable for *P. xylostella* larvae. Surprisingly, *P. xylostella* exhibited a better performance on *C. hassleriana* than on *D. sophia* in terms of overall survival, pupal weight, silk weight, adult weight, forewing area, longevity (without food) and oviposition of new generation adults.

Larval food plant significantly affected oviposition of new generation adults. Females reared on *B. napus*, *C. hassleriana* and *T. majus* deposited similar numbers of total eggs over the 8-day period. Oviposition peaked on day 1 for females raised on *T. majus* whereas most eggs were deposited on day 3 when females were raised on either *B. napus* or *C. hassleriana*. Females from *D. sophia* had the least overall oviposition and the most eggs were laid on day 5. To our knowledge, no previous study has described such oviposition behavior for *P. xylostella*. Our results indicate that *D. sophia* was the least suitable host plant among the tested brassicaceous and non-brassicaceous species as indicated by least survival, delayed larval development, lighter pupal weight, less silk, lower body mass, smaller forewings, reduced longevity without food, and reduced overall oviposition of new generation adults. The time lag between eclosion and peak oviposition when *P. xylostella* are reared on unfavorable host plants such as *D. sophia* may facilitate migration by *P. xylostella* to more favorable habitats. The latent period could be spent in migration and host plant location, with oviposition to follow after more optimal host plants are found. Alternatively, this strategy may reduce overall fitness of *P. xylostella*: moths would have less oviposition time if they spend more time in migration particularly when they are short-lived in the absence of food.

Oviposition Preference

*Plutella xylostella* laid similar numbers of eggs on *C. hassleriana* and *D. sophia*. The present study clearly demonstrated that females accept *C. hassleriana* even in the presence of favorable host plants such as *B. napus*. *Cleome hassleriana* is an annual herb that can serve as an efficient bridge host for *P. xylostella* populations when there are no Brassicaceae available, but to our knowledge, there is no published evidence to support or refute our findings. *Tropaeolum majus* received the fewest eggs among the tested plant species. In an earlier study, *T. majus* extracts provided only a limited enhancement effect for oviposition when applied to *Phaseolus vulgaris* L. (*Fabaceae*) (Renwick and Radke 1990) and this could perhaps explain why *P. xylostella* infestations have never been reported on this species under field conditions.
Root Mass Development in Response to Insect Herbivory

Plant species responded differently to aboveground herbivory in the form of root mass development. Our study indicated that defoliatiion can increase, decrease, or have no effect on belowground biomass. *Brassica napus* developed a more robust root system when infested, suggesting that these plants may replace tissues lost to herbivory by increased uptake of nutrients from soil. These findings are in accordance with our previous results (Sarfraz et al. 2007). Similarly, turnip plants (*Brassica rapa* L.) produced heavier roots when infested with *P. xylostella* larvae (Taylor and Bardner 1968). Aboveground herbivory had a non-significant effect on root growth of *D. sophia*. Root mass production in *C. hassleriana* and *T. majus* declined when infested, probably due to lack of evolutionary history with *P. xylostella*.

CONCLUSIONS

Our findings have important implications regarding adaptive behavior of a crucifer specialist and ecological and evolutionary interactions among herbivores and their host plants. Like other specialist herbivores, oligophagy serves *P. xylostella* well when brassicaceous plants are abundant in a habitat. However, in times of biotic and/or abiotic stress, it has the capability to survive on suitable non-Brassicaceae. Evidently the survival strategy of a species is that some segment of a given population seems to have fewer constraints on it (population heterogeneity), where selected females can oviposit on acceptable food plants where their offspring can survive and reproduce. Herbivores raised on unfavorable plants would be more adapted for emigration (Dent 2000) and our research suggests that *P. xylostella* reared on suboptimal food plants develop characteristics such as a latent oviposition period and smaller body mass that may facilitate their emigration.

There is considerable debate that insect herbivores and their host plants are in a co-evolutionary ‘arms race’ (Ehrlich and Raven 1964, Berenbaum 1983, Berenbaum and Zangerl 1998, Kareiva 1999, Zangerl and Berenbaum 2005). For instance, wild parsnip, *Pastinaca sativa* L. (Apiaceae), is defended against webworms, *Depressaria pastinacella* Duponchel (Oecophoridae), by the presence of furanocoumarins with heritabilities for individual compounds ranging from 0.54 to 0.62; the webworms can metabolize furanocoumarins with heritabilities for cytochrome P450 activity levels ranging from 0.33 to 0.46 (Berenbaum and Zangerl 1998). Similarly, brassicaceous plants possess the glucosinolate-myrosinase system for their defense, but crucifer specialists such as *Pieris rapae* (L.) (Pieridae) and *P. xylostella* have developed various mechanisms to disarm this ‘mustard oil bomb’ (Ratzka et al. 2002, Wittstock et al. 2004) and can even utilize some of these compounds as token stimuli for their host location. In addition to plant chemistry, if root growth in response to herbivory represents compensatory ability then we suspect that brassicaceous plants might have had better compensation (and even overcompensation) as a result of co-evolution, while their non-brassicaceous counterparts could not compensate due to lack of any evolutionary history with *P. xylostella*. Evidently host range shifts by insect herbivores can place substantial pressure on new host plant species to accommodate or compensate for loss of plant tissues, and represents an area in need of future research to uncover underlying mechanisms by which host plants respond to this herbivory.

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REFERENCES


Gupta, PD and Thorsteinson, AJ. (1960b) Food plant relationship of diamondback moth (Plutella maculipennis (Curt.)). II. Sensory regulation of oviposition of the adult female. Entomologia Experimentalis et Applicata 3: 305-314.


Hilleyer, RJ and Thorsteinson, AJ. (1969) The influence of the host plant or males on ovarian development or oviposition in the diamondback moth, Plutella maculipennis (Curt.). Canadian Journal of Zoology 47: 805-816.


Renwick, JAA and Lopez, K. (1999) Experience-based food consumption by larvae of Pieris rapae:


van Loon, JJA, Wang, CZ, Nielsen, JK, Gols, R and Qiu, YT. (2002) Flavonoids from cabbage are feeding stimulants for diamondback moth larvae additional to glucosinolates: Chemoreception and behaviour. Entomologia Experimentalis et Applicata 104: 27-34.
