Host range expansion of diamondback moth, *Plutella xylostella* to peas: Effects on its parasitoids in Kenya

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ABSTRACT

A population of the diamondback moth (DBM), *Plutella xylostella* L., was recently found on sugar snap peas and snowpeas (*Pisum sativum* L.) in the Rift Valley in Kenya, causing heavy damage. The impact of this host range expansion on the interaction with associated parasitoids was investigated. In laboratory experiments, we studied the role of host plants in host location of the indigenous *Diadegma mollipa* (Holmgren), regarded as a generalist, and the exotic *Diadegma semiclauaum* (Hellen), which is considered a specialist. Host location was tested in a Y-tube olfactometer. Results showed that the specialisation of *D. semiclauaum* is mediated by host plant signals associated with crucifers, which are not encountered in peas. In contrast, *D. mollipa* does not use crucifer volatiles as primary cues for host location. In field studies, peas provided an enemy-free space for DBM. Local parasitoids occurred only sporadically and in very low numbers on DBM on peas. The release of *D. semiclauaum* into the study site led to a reduction of DBM only in kales. With strong competition on kales, *D. mollipa* was assumed to find a niche in peas. However, it did not occur in larger numbers in peas. In conclusion, we demonstrated that the host plant had a strong influence on host location of the specialist parasitoid. The inclusion of a new host plant into its feeding repertoire enables the herbivore host to avoid enemy attack.

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is considered stenophagous and limited to crucifers (Talekar and Shelton 1993). Despite this common belief, the species has occasionally been found on other host plants. It has been reported on chickpea (*Cicer arietinum* L.) and a chenopodaceous vegetable *Salsola kali* in Russia (Reichert 1919, in Talekar et al. 1985) and on okra in Ghana (FAO 1971). On peas, occasional occurrences of DBM have been reported from Taiwan province in China and the Philippines (Talekar pers. comm.), but no published information exists. Recently a diamondback moth population in central Kenya, in the area of Lake Naivasha, expanded their host range to sugarsnap and snowpea (*Pisum sativum* L.), causing heavy damage (Löhr 2001). This population turned out to be a biologically different strain with the ability to develop equally well on both host plants (Löhr and Gathu 2002).

This phenomenon was an opportunity to investigate the influence of a host range expansion of a herbivore on associated parasitoids. Historically, plant chemistry and physiology were seen as the determinant for host
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plant specificity in phytophagous insects (Ehrlich and Raven 1964). The hypothesis of enemy-free space by Jeffries and Lawton (1984) drew the attention to the importance of host plant selection by herbivores on natural enemies. One way to escape natural enemies would be the utilization of novel host plants that provide an ecological refuge from enemies. Several recent studies have confirmed the validity of the hypothesis of enemy-free space through the acquisition of a new host plant (Brown et al. 1995, Feder 1995, Gratton and Welter 1999, Gross et al. 2004, Mulatu et al. 2004). Herbivores seem to be able to overcome physiological trade-offs on novel host plants in order to avoid parasitoid attack.

So how does a host range expansion affect parasitoids? Chemical and morphological plant attributes can influence the foraging success of parasitoids as well as the role of plants as host finding cues (Cortesero et al. 2000). The shift to a novel host plant can result in a loss of plant-related cues and to a reduced searching efficiency, particularly in the case of specialist parasitoids (Brown et al. 1995). When feeding on different food plants, physiology and size of the herbivore can change and this indirectly influences the development of the immature parasitoid (Godfray 1994). Plants also affect the level of parasitism due to food plant quality (Price et al. 1980, Fox et al. 1996) and host plants can provide shelter for the herbivore and thus reduce the efficiency of natural enemies (Sznajder and Harvey, 2003).

The effect of the host range expansion of DBM was studied using two parasitoid species: *Diadegma mollipla* (Holmgren, 1868) (Hymenoptera: Ichneumonidae), the most important solitary endoparasitoid of DBM in Kenya, and *Diadegma semiclausum* (Hellen), an exotic parasitoid of European origin that was introduced to Kenya as part of a biocontrol programme for DBM. The local *D. mollipla* is considered a relative generalist. This species is also known as a parasitoid of the potato tuber moth, *Phthorimaea operculella* (Zeller) (Gelechiidae) and is reported to be indigenous to eastern and southern Africa. The original host is unknown (Broody 1971, Gupta 1974, Azidah et al. 2000). *Diadegma semiclausum* has been widely used for biological control of DBM in various countries (Talekar and Shelton 1993) and is known as a DBM specialist (Abbas 1988).

The host location behaviour of the two species was investigated in olfactometer experiments in the laboratory. The influence of DBM on peas on the entire parasitoid guild associated with DBM was investigated under natural conditions in the field.

≡ MATERIAL AND METHODS ≡

**Laboratory Experiments**

*Host larvae*

The cabbage strain of the diamondback moth originated from cabbage fields in Wundanyi Division, Taita Taveta District of Kenya and was reared in the laboratory (T = 23 ± 2°C) on potted cabbage plants (*Brassica oleracea* L. var. *capitata* (Copenhagen Market)). Rearing procedures were as described in Löhr and Gathu (2002). Diamondback moth larvae reared on cabbage are referred to as c-DBM. Larvae of the DBM pea strain were collected from a sugar snap pea field (*Pisum sativum*, var. Oregon sugar pod) near Naivasha in Nakuru District, Kenya. Since 1999, the colony has been maintained in the laboratory on potted snowpeas (*Pisum sativum*, var. Oregon sugar pod). Diamondback moth larvae from peas are referred to as p-DBM.

*Parasitoids*

*Diadegma mollipla* was obtained from cabbage fields in Wundanyi Division and Maragua in Muranga
District, central Kenya. Cultures were started in 2001 and newly collected individuals from the field were frequently added to the colonies. A colony of *D. semiclaustrum* was started in 2001 from pupae from a laboratory culture imported from the Asian Vegetable Research and Development Center (AVRDC) in Taiwan province in China. Both parasitoid species were reared on second and third instar DBM larvae on cabbage in perex cages. Before the experiments, adults remained for at least 2 days in a cage to ensure mating.

**Behaviour assays**

The attractiveness of odours emitted by DBM larvae, larval fecal material and host plants with feeding larvae to *D. molipla* and *D. semiclaustrum* females was studied in a Y-tube olfactometer. Two to six day old mated female parasitoids without previous exposure to host larvae were introduced individually in the stem of the Y-olfactometer. Parasitoids that crossed a line 0.5 cm beyond the intersection and remained in one arm for at least 20 sec were recorded as having made a choice. To avoid bias the odour sources were switched between the left and the right arm of the Y-tube after every 10 parasitoids. At least 60 parasitoids were tested for each treatment (detailed description see Rossbach *et al.* 2005).

The following odour sources were tested for both parasitoids:

1. c-DBM larvae vs clean air: Twenty 2\textsuperscript{nd} to 3\textsuperscript{rd} instar DBM larvae that fed on cabbage or pea respectively before the experiment were placed in one of the glass tubes (2.5 cm in diameter, length 10 cm). The other tube of the same size remained empty. (2) c-DBM faeces vs clean air: Fresh faeces produced by 30 DBM larvae (L2-L3) during 24h was placed in the glass tube. Every 30 min the faeces was changed to ensure a consistent odour. (3) c-DBM infested cabbage plant vs clean air. (4) c-DBM infested cabbage plant vs p-DBM infested pea plant. (5) uninfested cabbage plant vs c-DBM infested pea plant (only *D. semiclaustrum*): An entire potted plant (4 weeks old) with 20 feeding larvae was placed in a glass jar of 2 L capacity. The other glass jar of the same size remained empty. DBM larvae were allowed to feed for 20 hours before the experiment. (6) PTM infested potato vs c-DBM infested cabbage. (7) PTM infested potato vs p-DBM infested pea (only *D. molipla*): Two potatoes, infested with five PTM larvae each, were placed in a glass jar (as described under 5) and tested against potted cabbage or pea plants infested with 20 DBM larvae.

**Field Study**

**Experimental fields**

The field study took place between 25 September 2002 and 6 July 2004. The experimental site was a field of 800 m\textsuperscript{2} located on a horticultural farm in Naivasha, Nakuru District in the Rift Valley in Kenya [Altitude: 1 500 m, 00\textdegree44'98S, 036\textdegree26'27E]. Eight plots of 10 m x 5 m were established and planted simultaneously with kale (*Brassica oleracea* acephala L. var. Thousandheaded) and snowpea (*Pisum sativum* var. Oregon Sugarpod) on four plots each.

During the whole period, both crops were constantly in the field. Because of differences in the duration of the crop cycle, six crops of kale and eight of snowpea were planted. New transplants and seeds were brought into the field before the old crop was harvested in order to ensure a quick infestation of the new crop with DBM. Plots were rotated within the area and half of the field was always left uncultivated for one growing period (approx. three months). New crops were transplanted or sown alternately on the plots, kale on former pea plots and vice versa. During the first crop natural infestation of snowpea with DBM was extremely low and only five larvae were found in the entire period. In order to investigate the impact on natural enemies, a higher infestation level was required and eggs of the pea strain of DBM were brought from a laboratory culture into the field. From February 2003, pea plots were infested with approximately 500 p-DBM
eggs/plot every week until the end of the study period. The eggs were produced in the laboratory and were laid on aluminum foils (see Rossbach et al. 2005). Strips of foil containing 50 to 100 eggs were distributed randomly and fixed to pea plants.

Sampling

Twenty randomly selected plants were sampled per plot weekly or bi-weekly. Larvae and pupae of DBM were counted, recorded, collected and reared in the laboratory until the adult stage. Total number of emerging parasitoids was determined and species were identified. Unknown species were labelled and sent for identification to Georg Goergen at IITA (International Institute of Tropical Agriculture) in Benin. After a sampling period of nine months, five pairs of *D. semiclausum* were released in each snowpea plot in November 2003. Sampling continued as described above.

Statistical Analysis

The results of the choice tests between odour sources in the Y-tube olfactometer were analysed with the Yates-corrected Chi-Square Test. Parasitoids that made no choice were excluded from the analyses.

RESULTS

Host Location

Choice test with one odour

*D. mollipla* did not show preference for either c-DBM larvae, their faeces or DBM-infested cabbage plant when tested against clean air (Figure 1A). In contrast, *D. semiclausum* responded clearly to cabbage-related odour sources (Figure 1B). The preference was strongest for larvae-infested cabbage plants. Even c-DBM faeces as well as larvae alone were significantly preferred over clean air, although larvae alone elicited the weakest response.

Choice test with two odours

*D. mollipla* females chose the larvae-infested pea plant slightly more often than the infested cabbage plant (Figure 2A). However, this difference was not significant. When DBM-infested cabbage was tested against PTM-infested potatoes, *D. mollipla* was significantly more attracted to the infested potatoes (Figure 2A). A similar result, although not significant, was obtained in a test involving a choice of PTM-infested-potato...
Figure 1  Responses of naïve parasitoid females to cabbage-related odours tested against clean air (c-DBM larvae = c-larvae; c-DBM faeces = c-faeces; c-DBM-infested cabbage plant = inf cabbage). A. *Diadegma molipla*

B. *Diadegma semieclausum*. N = number of females. Number of parasitoids that did not respond are indicated in brackets. Asterisks indicate statistically significant preferences within tests (Yates corrected Chi$^2$-Test ("$p<0.05$, **$p<0.01$, n.s. = not significant")

Figure 2  A. Responses of naïve *Diadegma molipla* females to infested plants (c-DBM-infested cabbage plant = inf cabbage, p-DBM-infested pea plant = inf pea, PTM-infested potatoes = inf potato). B. Responses of naïve *Diadegma semieclausum* females to infested and uninfested plants (c-DBM-infested cabbage plant = inf cabbage, p-DBM-infested pea plant = inf pea, uninfested cabbage plant = uninf cabbage). N = number of females. Number of parasitoids that did not respond are indicated in brackets. Asterisks indicate statistically significant preferences within tests (Yates corrected Chi$^2$-Test ("$p<0.05$, **$p<0.01$, n.s. = not significant")
and DBM-infested pea plant (Figure 2A). *D. semiclaustrum* was clearly attracted to the cabbage plant (Figure 2B). When an uninfested cabbage plant was tested against DBM-infested pea plant. The response to cabbage was even stronger when the cabbage plant was infested with DBM.

Field Parasitism

Seasonal abundance of DBM and the number of parasitized larvae on kale are presented in Figure 3. The population peaked between the end of June and end of August 2003, with up to 235 larvae per 20 plants

![Graph A](image)

kale before release of *D. semiclaustrum*

![Graph B](image)

kale after release of *D. semiclaustrum*

Figure 3  Seasonal distribution of total and parasitized DBM larvae on kale
The number of parasitized larvae fluctuated between 2 and 42 per 20 plants. After the release of *D. semiclaustrum* the DBM population on kale decreased continuously from 43 larvae per 20 plants at the end of November 2003 to below 5 larvae per 20 plants from mid-February to the end of the study period in May 2004 (Figure 3B). After an initial increase, the number of parasitized larvae decreased simultaneously with the host decline.

Peak numbers of DBM on snowpea occurred before the release of *D. semiclaustrum* at the beginning of June until mid-July 2003 (21 to 58 larvae per 20 plants) (Figure 4A) and after release from early January

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**Figure 4** Seasonal distribution of total DBM larvae and parasitized DBM larvae on snowpea
2004 until February 2004 (14 to 50 larvae per 20 plants) (Figure 4B). The number of parasitized DBM on snowpea was very low throughout the study period (0 to 3.5 larvae per 20 plants), irrespective of the number of DBM in the field. With the constant infestation of snowpea with DBM eggs, the DBM population on snowpea was kept artificially high. Main causes of the fluctuations of the DBM population were heavy rains on the one hand, washing away foils with eggs or newly hatched larvae, and Ascochyta disease on the other. The infection caused the plant to dry up rapidly and prematurely. DBM larvae were not able to develop on dying plants.

Seven parasitoid species were reared from DBM on snowpea (Table 1). Four species are parasitoids commonly found on DBM in crucifers in Kenya. The two braconids Apanteles sp. Cameron (Braconidae) and Distatix sp. Mason (Microgastrinae), reared from DBM on snowpea, were not recovered from DBM on kale. The eulophid wasp Oomyzus sokolowskii Kurdjumov was the species most frequently found on the new host plant. The parasitism rate on snowpea before the release of Diabrotica speciosa was 2.3%. After the release it rose to 4.0%, due to a slight increase of numbers of O. sokolowskii and Cotesia sp. The exotic parasitoid only occurred sporadically on snowpea. Diamondback moth on kale yielded six parasitoid species. After its release, Diabrotica speciosa established quickly on DBM on kale (Table 1). Numbers of DBM decreased from a total of 7571 collected over nine months before the release to 1136 collected over seven months after the release and the parasitism rate increased from an average of 25.6% to 75.7%. Parasitism by Diabrotica speciosa in our plots in June 2003, prior to our release of this parasitoid in the experiment, resulted from immigration from a release on a horticultural farm approximately three kilometers distant from the study plots.

Table 1 Parasitoid guild of the diamondback moth, on kale and snowpea in Naivasha, Rift Valley, Kenya. For gregarious Oomyzus sokolowskii the number of parasitized DBM larvae is given and total number of emerged parasitoids is shown in brackets. Diadegma cocoons where the parasitoid failed to emerge are listed as Diadegma sp.

<table>
<thead>
<tr>
<th></th>
<th>Kale Total number</th>
<th>total parasitism (%)</th>
<th>Snowpea Total number</th>
<th>total parasitism (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oomyzus sokolowskii</td>
<td>1332 (9128)</td>
<td>68.6</td>
<td>Oomyzus sokolowskii</td>
<td>17 (147)</td>
</tr>
<tr>
<td>Diadegma malipla</td>
<td>400</td>
<td>20.6</td>
<td>Apanteles sp.</td>
<td>11</td>
</tr>
<tr>
<td>Apanteles sp.</td>
<td>77</td>
<td>4.0</td>
<td>Cotesia sp.</td>
<td>3</td>
</tr>
<tr>
<td>Diadegma sp.</td>
<td>60</td>
<td>3.1</td>
<td>Brachymeria sp.</td>
<td>2</td>
</tr>
<tr>
<td>Diadegma semiclausum</td>
<td>52</td>
<td>2.7</td>
<td>Diadegma malipla</td>
<td>1</td>
</tr>
<tr>
<td>Itoplectis sp.</td>
<td>19</td>
<td>1.0</td>
<td>Diadegma semiclausum</td>
<td>1</td>
</tr>
<tr>
<td>Brachymeria sp.</td>
<td>2</td>
<td>0.1</td>
<td>Distatix sp.</td>
<td>1</td>
</tr>
<tr>
<td>No. parasitized DBM</td>
<td>1942</td>
<td></td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>Total no. DBM</td>
<td>7571</td>
<td></td>
<td></td>
<td>1425</td>
</tr>
<tr>
<td>Total parasitism (%)</td>
<td>25.6</td>
<td></td>
<td></td>
<td>2.3</td>
</tr>
</tbody>
</table>

After the release of Diabrotica speciosa

<table>
<thead>
<tr>
<th></th>
<th>Kale Total number</th>
<th>total parasitism (%)</th>
<th>Snowpea Total number</th>
<th>total parasitism (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oomyzus sokolowskii</td>
<td>472(3117)</td>
<td>54.9</td>
<td>Oomyzus sokolowskii</td>
<td>29(179)</td>
</tr>
<tr>
<td>Diadegma semiclausum</td>
<td>197</td>
<td>22.9</td>
<td>Cotesia sp.</td>
<td>13</td>
</tr>
<tr>
<td>Diadegma sp.</td>
<td>135</td>
<td>15.7</td>
<td>Apanteles sp.</td>
<td>10</td>
</tr>
<tr>
<td>Diadegma malipla</td>
<td>31</td>
<td>3.6</td>
<td>Diadegma semiclausum</td>
<td>6</td>
</tr>
<tr>
<td>Itoplectis sp.</td>
<td>27</td>
<td>3.1</td>
<td>Diadegma malipla</td>
<td>4</td>
</tr>
<tr>
<td>Apanteles sp.</td>
<td>6</td>
<td>0.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Brachymeria sp.</td>
<td>1</td>
<td>0.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>No. parasitized DBM</td>
<td>860</td>
<td></td>
<td></td>
<td>62</td>
</tr>
<tr>
<td>Total no. DBM</td>
<td>1136</td>
<td></td>
<td></td>
<td>1560</td>
</tr>
<tr>
<td>Total parasitism (%)</td>
<td>75.7</td>
<td></td>
<td></td>
<td>4.0</td>
</tr>
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**DISCUSSION**

The use of a new host plant of the diamondback moth affected the searching behaviour of the two investigated parasitoids. The DBM-crucifer complex did not seem to be the preferred host-plant association for *D. mollipila*. Host location studies in the Y-tube olfactometer showed that *D. mollipila* females walked randomly when they had a choice between cabbage-related odours and clean air, whereas DBM-infested snowpea, as well as PTM-infested potatoes were preferred over clean air, indicating that *D. mollipila* has a broader host range on a variety of plants, and cabbage ranks low on the list. Accordingly, *D. mollipila* showed higher parasitism levels on the pea strain of DBM than on the original host (Rossbach et al., 2006). It is assumed that generalist natural enemies use general chemical cues that are present in all hosts or their respective food plants ( Vet and Dicke 1992, Godfray 1994). Therefore, *D. mollipila* is either attracted to a non-specific blend of volatiles from a variety of host-infested plants, or it uses other shared cues present in several hosts or host-plant complexes. Since *D. mollipila* is less dependent on plant volatiles for its host location, DBM feeding on a different plant does not necessarily mean a loss of cues, although the nature of cues that mediate in its host location process remain unknown. On the other hand, *D. semiclaustrum* relies on volatiles associated with cabbage, the original host of DBM. The relative indifference towards DBM-infested snowpea implies lack of attractive volatiles and their precursors from this plant. Ohara et al. (2003) had already demonstrated that the specialisation of *D. semiclaustrum* on DBM is due to its recognition of crucifer-typical volatiles (perhaps derives of glucosinolate). Therefore a host expansion to a different plant family leads to a loss of host location cues for *D. semiclaustrum*, and this seriously affects its searching efficiency. Consequently, on a new host plant, efficiency of *D. semiclaustrum* as a biocontrol agent is greatly reduced.

As a conclusion from the laboratory studies on the two *Diadegma* species, their differential degree of specialization and behavioural differences in host location, the host plant expansion of DBM was expected to have different effects on members of its entire parasitoid guild. The number of parasitoids attacking DBM in Kenya is small and most species are considered relative generalists. However, most local parasitoids did not seem to recognize the novel host plant as a potential site for the occurrence of their host. They attacked DBM on snowpeas only sporadically and in very low numbers. The most frequent species was *Oomyzus sokolowskii* Kurdjumov, a cosmopolitan DBM parasitoid (Liu et al. 2000). The inclusion of snowpea into its host plant range provided enemy-free space for the diamondback moth. Reduction of natural enemy attack through acquisition of a new host plant has been demonstrated by various authors (Feder 1995, Gratton and Welter 1999, Gross et al. 2004, Mulatu et al. 2004). *Diadegma mollipila* was expected to occur more frequently in snowpeas, but it was hardly found on the novel host plant. It was gradually displaced from kales by *D. semiclaustrum*, but seemed to move to other hosts rather than to DBM on snowpeas. The field studies were conducted within a very short time after DBM was first found on snowpeas. After the outbreak in 1999, DBM has not occurred in such high numbers on snowpeas anymore. That might have given parasitoids little chance to adapt to the new host plant.

**Acknowledgements**

The study was conducted under the ICIPE-led project entitled (*Biocontrol of Diamondback Moth in Eastern Africa*), financed by the German Federal Ministry of Economic Cooperation and Development (BMZ). The first author and the research were funded by the German Research Foundation (DFG). We thank Hortitec Seeds Ltd. in Naivasha for providing us with land and facilities. Special thanks are due to Livingston Mkwata for the management of the study site. The *Apanteles* sp. was identified as an undescribed
species by G. Delvare, CIRAD, Montpellier, France. Voucher specimens of the parasitoid species listed have been deposited at the ICIPE, Nairobi, Kenya.

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