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Oviposition preferences of *Plutella xylostella* (L.) (Plutellidae: Lepidoptera) on water-stressed and non-stressed plants of *Brassica napus* L.

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Abstract
Water stress influences the plant growth and quality, which in turn, alters the suitability of the host plant for insect herbivores. We investigated the suitability of four and six-week-old water-stressed host plants versus non-stressed plants in terms of oviposition preferences of the diamondback moth, *Plutella xylostella* (L.), on *Brassica napus* L. (Brassicaceae).
Ovipositional preferences were significantly affected by the water treatment, plant age, and site for oviposition. Female moths preferred to oviposit on six-week-old, non-stressed plants of *B. napus* compared to the water-stressed counterparts. On average, 2.15 times more eggs were laid on non-stressed plants than the water-stressed ones. Similarly, 1.58 times more eggs were laid on host plant upper leaf surface than on the lower leaf surface. This study determined that *P. xylostella* females preferred to deposit eggs on six-week-old vigorous plants not under water stress to ensure the successful development and survival of their offspring.

Keywords: Brassicaceae, damondback moth, oviposition, moisture stress

1. Introduction
Plants experience several environmental biotic and abiotic stresses that not only impact their growth, development, and productivity [1] but also their fitness and interaction with herbivores [2, 3]. Water deficit stress is perhaps the most important abiotic stress to which plants are exposed [4, 5]. The alteration in host plant quality due to water/drought stress often influences the response of insect herbivores, including their biological and life history traits [6-9].
The effects of plant water stress on the insect attraction, oviposition and development can be very complex, uncertain and variable [10-12]. The overall performance of insect herbivores can be altered on host plants, experiencing a water deficit relative to plants not under stress [13]. Oviposition of lepidopteran pests can be enhanced [14-12], reduced [16] or not affected [17], in plants under water deficit stress. In addition to stress patterns, several other factors can determine female oviposition behavior, such as the quality of the host plant [18-20], preference as to where on an individual plant to lay eggs, leaf age [21, 17], leaf size, internode length [22], leaf and root-damaged plants [23], presence of previously laid eggs on the plant, leaf shape, and secondary plant compounds [24-27]. Survival of immature stages in Lepidoptera is greatly influenced by oviposition choices of adult females [28].
The impact and importance of stressed host plants in mediating plant-herbivore interactions are unclear. Stress may induce contrasting patterns in oviposition, development and feeding preferences among herbivore species due to the change in plant suitability and attractiveness [29, 30]. The diamondback moth is a serious, worldwide pest of brassicaceous crops [31], and its extensive geographic distribution encompasses regions and seasons where its host plants may develop under moisture deficit stress. So far, only one previous research has been conducted to determine the responses of *P. xylostella* to water stressed and non-stressed plants of cabbage (*Brassica oleracea* L.) [17]. However, the present study is focussed on canola (*Brassica napus* L.), a major economically important global oilseed crop in Canada [32]. The objective of this research was to investigate if diamondback moth, *Plutella xylostella* L., responds differently between the water-stressed and non-stressed plants regarding ovipositional preferences.

2. Materials and methods

2.1 Experimental plants and insects

Four and six-week-old *Brassica napus* L. var. Q2 plants were grown individually in the plastic pots (15 cm diameter) using Metromix-220 (W.R. Grace & Co., Ajax, Ontario, Canada) as a potting medium. Pots were thoroughly watered and placed in a growth chamber at a constant temperature of 21 ± 0.5°C, 50-70% relative humidity and 16L: 8D photoperiod.

Plutella xylostella adults originated from a laboratory colony maintained on *B. napus* plants. *Plutella xylostella* were obtained from the different commercial fields of *B. napus*, *Brassica juncea* (L.) Czern. and *Sinapis alba* L. throughout Alberta, Canada and were periodically added to the laboratory colony to maintain the genetic diversity.

2.2 Imposition of water stress

All pots were watered daily to saturation until they reached the desired age of four and six-week. Plants were randomly allocated to two alternative treatments: water-stressed or non-stressed (control). Plants for the control treatment were watered at 88 ml/day/plant throughout the experiment. For the water-stressed plants, irrigation was reduced to 30 mL/d/plant for four days and finally withheld for two to three days before the beginning of the experiment. All tests were initiated approximately 48 h after these conditions were imposed.

2.3 Assessment of leaf water potential

At the beginning of water stress treatment, the leaf water potential (bar) of one randomly selected, fully expanded leaf from the center of each plant was measured using a pressure bomb (Model-610; PMS Instrument Co., Corvallis, OR).

2.4 Oviposition choice test

Ovipositional preference of *P. xylostella* on water-stressed and non-stressed four- and six-week-old *B. napus* was determined under the greenhouse conditions, using a complete factorial design in a choice arena. The wooden screened cages were arranged on a greenhouse bench at 22 ± 0.5ºC, 50-70% relative humidity and a photoperiod of 16 L: 8 D. Each cage was assembled with two plants of *B. napus,* one for each treatment (water-stressed and non-stressed) of the same age. Each treatment was replicated four times. Six pairs of newly eclosed *P. xylostella* adults were released in each cage and provided with 10% honey water on cotton wicks immersed in 20-mL plastic cups. After 48 h exposure to both treatments, adults were removed and plants were examined for eggs. Total numbers of eggs deposited on stressed and non-stressed plants were counted. Moreover, the number of eggs deposited on different plant parts like upper and lower leaf surfaces, stem, and flowers, were calculated separately in the laboratory using a dissecting microscope.

2.5 Plant parameters

Plant stem height and stem diameter were measured at the end of the oviposition choice experiments. Stem diameter was measured using vernier calipers (Electronic Caliper, Mastercraft) at 10 cm above the root/shoot junction.

2.6 Statistical analysis

Variables were tested for normality and homoscedasticity, before subjecting them to the analysis. A two-way analysis of variance (ANOVA) (PROC MIXED) was performed to determine the effects of water treatments on oviposition preferences of four or six-week-old plants. Similarly, a three-way analysis of variance (ANOVA) (PROC MIXED) was also performed to evaluate the oviposition preferences of *P. xylostella* on upper or lower leaf surfaces of stressed and non-stressed four and six-week-old plants. If significant treatment effects were indicated, means were compared at the 5% level of significance using Fisher's Least Significant Difference (LSD) test [33]. PROC TTEST for leaf water potential was performed. Correlations (PROC CORR) were determined between plant height and egg deposition, and plant stem diameter and egg deposition [33].

3. Results

Leaf water potential of the water-stressed plants was significantly more negative (indicating a low leaf water potential) than the leaf water potential of non-stressed *B. napus* ($t = 9.64$, $df = 6$, $P < 0.0001$). Water stress affected the oviposition decisions of *P. xylostella* females. On average, 2.15 times more eggs were laid on non-stressed than the stressed plants ($F = 8.88$, $df = 1, 12$, $P = 0.0115$) (Fig. 1). Plant age also had a significant effect on ovipositional preference. The mean number of eggs laid on six-week-old *B. napus* plants was three times greater than on four-week-old non-stressed plants regardless of water treatment ($F = 19.43$, $df = 1, 12$, $P = 0.0009$) (Fig. 2). In general, 2.34 and 1.65 times more eggs were laid on six- and four-week-old unstressed *B. napus* plants than the stressed plants, respectively (Fig. 3). The proportion of eggs laid by *P. xylostella* females differed significantly among the different plant parts. Egg laying was 1.58 times greater on upper than the lower leaf surfaces ($F = 6.23$, $df = 1, 24$, $P = 0.0199$). In general, 2.73 and 2.0 times more eggs were laid on the non-stressed upper leaf surface of six- and four-week-old non-stressed *B. napus* plants than on stressed plants, respectively. There was a significant interaction between water treatment and plant age ($F = 10.63$, $df = 1, 24$, $P = 0.0033$), but no significant interactions between the water treatment and plant leaf surfaces ($F = 1.36$, $df = 1, 24$, $P = 0.2557$), or between plant age and leaf surfaces ($F = 0.11$, $df = 1, 24$, $P = 0.7423$). A significant positive correlation was found between oviposition and plant height ($r = 0.64$, $P = 0.0069$), but no correlation existed between oviposition and plant stem diameter ($r = -0.164$, $P = 0.54$).

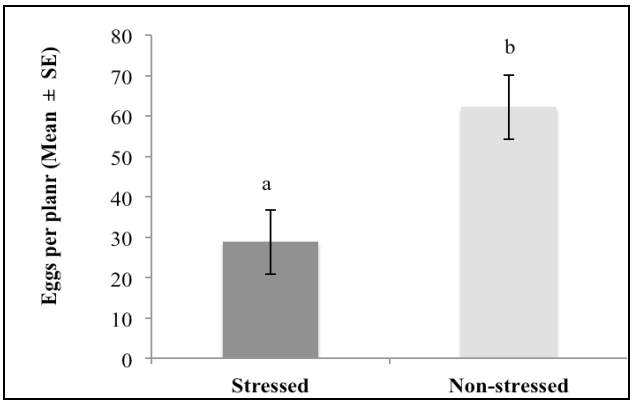

Fig 1: Mean eggs of *Plutella xylostella* (± SE) deposited on water stressed and non-stressed plants

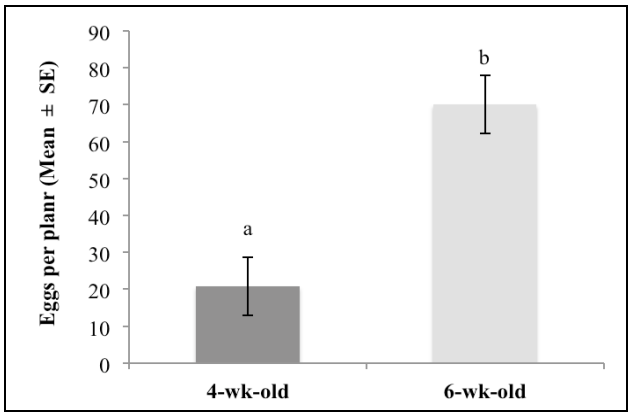

Fig 2: Mean eggs of *Plutella xylostella* (± SE) deposited on 4- and 6-week-old plants

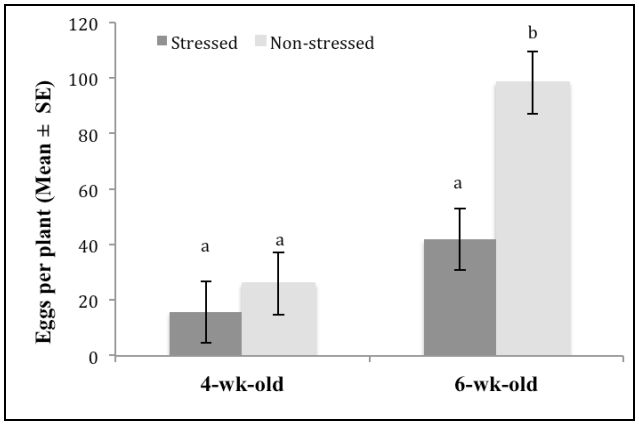

Fig 3: Mean eggs of *Plutella xylostella* (± SE) deposited on 4- and 6-week-old stressed and non-stressed *Brassica napus*. Means followed by the same letter are not significantly different at 5% level (Fisher's PLSD test following ANOVA)

4. Discussion

Greater ovipositional preference by insect herbivores for water-stressed host plants has been observed in many earlier studies [14, 12, 34, 35]. Insect herbivores are reported to perform differently when host plants are under moister stress depends on plant species, duration, and intensity of stress [36]. Furthermore, the effects of plant water stress on insect attraction can be inconstant, unpredictable and complex [10, 11]. For instance, no significant differences in *P. xylostella* ovipositional preferences were found between the water-stressed and non-stressed cabbage (*Brassica oleracea* L.) and yellow rocket (*Barbarea vulgaris* R. Br.) plants. However, oviposition on non-stressed cabbage and the yellow rocket were 2.5 and 1.3 times greater respectively, as compared to their water-stressed counterparts [17].

In the present study, a clear ovipositional preference for non-stressed plants of *B. napus* was observed for *P. xylostella*. On average, 1.65 and 2.34 times more eggs were laid on four- and six-week-old non-stressed *B. napus* plants than on water-stressed ones. These contradictory results relative to previous study might reflect differences in host plant species used in different experiments. Female *P. xylostella* might evaluate host plant quality (regarding the plant water status and nutritional value) and preferred suitable hosts for oviposition by morphological and physical characteristics, or might prefer to oviposit on plant tissues that are high in water content [35]. Furthermore, moths may choose oviposition host that meets more than just nutritional needs of their offsprings, hence maximizing the chances of their progeny survival and growth [37].

Ovipositional preference can be driven by plant age. A positive correlation between oviposition by *P. xylostella* and plant age has been observed in plant species like cabbage and yellow rocket [17]. In this study, a significant positive correlation between oviposition and increasing plant height was detected. Female moths may receive more stimulating visual and olfactory cues for oviposition as a result of the greater total leaf area in older plants. For example, three times more eggs were laid on six-week-old, as compared to four-week-old plants. Older plants may also offer more oviposition sites, the potential for shelter from natural enemies and more rich resources, for larval development [17].

Oviposition site selection (different plant parts) is likely to be constrained and influenced by both abiotic and biotic factors [23]. Various studies have demonstrated a mixed pattern of oviposition site preference by females of *P. xylostella*. For instance, selection of lower regions of the plant stem over leaves [38, 39], the upper leaf surface [40, 41], the lower leaf surface [42, 43], or neither surface [44]. However, in this study, results of oviposition preference on the upper leaf surface are consistent with those of Harcourt and Justus *et al*. [40, 41].

We observed that water stress caused a significant reduction in the shoot/stem growth. Reduced growth is one of the most evident and consistent responses of plants to the water deficit [45, 46]. The strong significant positive relationship between a total number of eggs laid and plant stem height indicating that females select more healthy plants, on which their offspring can develop. In general, the research findings support the preference-performance hypothesis. According to that oviposition preference should correspond with host suitability. Thus, female phytophagous insects select to oviposit on host plants that optimize the fitness of their progeny [47]. The study results confirmed that *P. xylostella* females preferred to deposit eggs on six-week-old vigorous plants, not under water stress to ensure the successful development and survival of their offspring.

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