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1 **Title; Testing and improving the effectiveness of trap crops for management of the**
2 **diamondback moth *Plutella xylostella* (L.): A laboratory-based study**

3

4 **Running title; Trap crops for diamondback moth management**

5

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13

14 **ABSTRACT**

15

16 **BACKGROUND:** The aim of this study was to assess white mustard (*Sinapis alba* L.) as
17 a trap crop for diamondback moth (*Plutella xylostella* (L.)) on cauliflower (*Brassica*
18 *oleracea* (L.) var. Lateman). Moth behaviour upon these plants and the importance of
19 plant age and size in maintaining pest preference for trap crop plants was also
20 investigated.

21

22 **RESULTS:** Three times as many eggs were laid on cauliflower plants that were
23 'unprotected' compared with those 'protected' by a trap crop of white mustard. Moths

1 remained longer on the mustard plants as a result of a doubling in the mean duration of
2 information-providing behaviours. Plant age had little effect on *P. xylostella* host
3 preference. When plant age was constant, percentage oviposition on mustard was higher
4 when these were larger (93%) than co-presented cauliflower plants, compared with when
5 they were smaller (68%).

6

7 **CONCLUSION:** Trap cropping with white mustard may reduce the incidence of *P.*
8 *xylostella* in cauliflower crops. The pest management benefits of trap crops may be
9 maximised by using trap crop plants that are larger than the main crop plants, although
10 relatively smaller trap crop plants may still be preferred as hosts for *P. xylostella per se*.

11

12 **Keywords:** Trap crop, IPM, *Plutella xylostella*, host preference

13

14

15 **1 INTRODUCTION**

16 Trap crops are host plants of a particular pest which, when positioned near to a main
17 crop, intercept and retain the pest, thereby limiting their numbers on the main crop
18 plants.^{1,2} Scientific interest in trap cropping, as well as other integrated pest management
19 (IPM) strategies, has increased in recent years. A major driver for this is that stricter
20 pesticide safety standards have resulted in the withdrawal of many products used
21 previously in pest management. *Brassica* crops may be severely affected by product
22 withdrawals as there are often no alternative means of pest control.³ Furthermore, the use
23 of products still permitted for application may be hampered, as pest insects commonly

1 develop resistance to the pesticides used against them. Resistance to one or more
2 insecticides has now been reported in over 500 pest insect species.⁴ Some pests, such as
3 *Plutella xylostella* (L.), have even developed resistance to ‘bioinsecticides’ such as
4 Spinosad (*Saccharopolyspora spinosa* (Mertz & Yao)),⁵ as well as to a range of other
5 new generation insecticides.⁶

6 Trap cropping may offer a means of reducing reliance on chemical applications for
7 pest management, and it has been shown to have potential for the control of numerous
8 *Brassica* pests including *P. xylostella*.^{7,8,9,10} *Plutella xylostella* can be difficult to tackle
9 through the use of pesticides (due to the resistance issues described above) and responds
10 relatively weakly to some other IPM strategies such as the use of under-sowing with non-
11 host plants.¹¹ Thus, there is a need to investigate alternative methods of management for
12 this pest.

13 In a recent review, only ten examples of trap cropping systems that are being used
14 commercially worldwide were identified, despite numerous examples of experimental
15 trap crops being given.² More research is needed to understand how trap crops work and
16 how they can be most efficiently deployed before this method can be adopted more
17 widely in pest management. One way of maximising trap crop effectiveness is to ensure
18 that the trap crop species itself is optimally acceptable to the pest when compared with
19 the main crop.^{12,13,14,15} It may be possible, having selected a highly acceptable and
20 relatively preferred host plant as a trap crop, to optimise its effectiveness further by using
21 plants that are older, larger or at a more preferred growth stage than those of the main
22 crop. Studies using older or larger trap crop plants,^{16,17,18,19,20} or plants at a more
23 advanced growth stage,^{21,22} suggest that this is so. However, whilst the importance of the

1 relative growth stage of the trap crop has been well documented,^{21,22} little research has
2 been conducted with respect to plant age and size to show that older and/or larger plants
3 are completely necessary for trap crops to perform in pest management. It is also possible
4 that certain spatial arrangements of trap crop plants are better suited to managing pests
5 than others. *Plutella xylostella*, for example, are known to infest field edges, initially
6 tending to alight on the first plants they encounter.²³ It could therefore be expected that a
7 natural 'edge effect' might exist which could be used to the advantage of trap cropping if
8 spatial designs such as border, strip and block trap cropping were used.

9 The aim of this study was to assess whether white mustard (*Sinapis alba* L.) could
10 be used as a trap crop to reduce oviposition by *P. xylostella* on cauliflower (*Brassica*
11 *oleracea* (L.) var. Lateman), as suggested by earlier work on host preference with this
12 pest species.²⁴ The behavioural response of *P. xylostella* to white mustard and
13 cauliflower, once resident upon these plants, was also investigated. The importance of
14 any intercept or 'edge effect', as well as trap crop plant age and size, relative to that of
15 the main crop plants, in governing trap crop performance and pest preference for the
16 mustard was also considered.

17

18 **2 EXPERIMENTAL METHODS**

19 **2.1 Plant growth and *P. xylostella* culture**

20 All of the plants used in experiments were grown in 9 cm plant pots containing John
21 Innes No. 2 compost and remained in these pots during experiments. Unless otherwise
22 stated, all plants were 5 - 6 weeks old when used. All *P. xylostella* were from a culture
23 maintained at Newcastle University on Chinese cabbage (*Brassica pekinensis* (Lour.)) at

1 20°C ± 1°C. When used in experiments, adult *P. xylostella* were 1-3 days old, with no
2 adult experience of a host plant, and were at a 1:1 sex ratio. Plant growth parameters were
3 measured in all experiments. Leaf area was measured using a ‘Delta T Leaf Area Meter’
4 (Delta T Devices).

6 **2.2 Testing the effectiveness of a trap crop**

7 This experiment was done using cages (tunnels) covered in fine mesh netting
8 (‘Enviromesh®’ (‘Ultrafine’ from Agralan)) to model a ‘slice’ through a trap crop system
9 as it might be used in the field. The experimental tunnels were erected within a
10 greenhouse at Close House Field Station, Northumberland, UK. Within this greenhouse,
11 climatic conditions were partially controlled, so that the minimum daily temperature was
12 10°C and the maximum 30°C.

13 Within the greenhouse, three tunnels were erected on each of three benches. The
14 walls and ceilings of these cages consisted of ‘Enviromesh’, whereas the floor of each
15 cage consisted of the bench surface covered with brown/grey shingle. Each cage
16 measured 0.92 m x 0.80 m, was 8.50 m long and was supported by nine equidistant
17 bamboo canes along each side of the cage. The plants were placed in gravel trays (24 cm
18 x 37 cm x 5 cm deep), six per tray, and watered at the onset of each experimental run.

19 The three experimental treatments consisted of; (i) six ‘external’ trap crop and six
20 adjacent ‘internal’ cauliflower plants, (ii) 12 cauliflower plants (in two adjacent ‘external’
21 and ‘internal’ blocks of six plants each) and (iii) six ‘internal’ cauliflower plants, with no
22 ‘external’ plants. ‘External’ plants were those that were nearest the front of the cage and
23 were assumed to be protecting the ‘internal’ plants. ‘Internal’ plants were closer to the

1 rear of the cage. These treatments were positioned mid-way along the length of the cage.
2 Treatments were allocated to cages at random and the arrangement of plants within the
3 cage was always rotated by 180° between one run of the experiment and the next. Eight
4 runs were conducted over four weeks, where each treatment was tested eight times (once
5 in each run with two runs being done per week). *Plutella xylostella* adults were released
6 at the front of the cage (the end nearest to the trap crop plants), so that their passage
7 toward the trap crop and cauliflower plants would reflect that expected in practice with a
8 standard border trap crop and a pest that tends to infest a field area from the crop
9 periphery.²³ A 50 cm x 50 cm clear Perspex sticky trap was set at the end of the cage.
10 Thirty *P. xylostella* were released in each run of the experiment and oviposition on all
11 plants was assessed 48 h after release. Plant growth parameters are provided in Table 1.

12

13 **2.3 Investigating behaviour on trap crop and crop plants**

14 Ten moths were placed into a cage (75 x 50 x 50 cm with a fine mesh lid to provide
15 access) containing a single test plant of either white mustard or cauliflower. A food
16 source consisting of 10% sucrose solution absorbed on cotton wool was provided in the
17 cage. The moths were allowed to settle for 25 - 30 minutes, after which the first female
18 moth to land upon the plant was observed until it left the plant. The residence time and
19 behaviour of this female whilst upon the plant were recorded. A maximum period of 68
20 minutes was spent observing any moth on any replicate run of the experiment, where only
21 one moth was observed per replicate. On each of the plant species used, 12 replicates
22 were completed, using different moths and plants for each replicate on each run. The

1 moths were always observed between 0900 h and 1300 h. The order in which the plant
2 species were tested during each run of the experiment was alternated.

3 When a moth was resident on a plant, ‘Observer’ (version 5) software was used to
4 record its behaviour. The following activities were recorded; walking (moving across
5 plant surface at ‘normal’ speed), running (moving across plant surface at increased
6 speed), hopping (moving across plant by momentary loss of contact with plant surface),
7 resting (inactive upon plant surface), grooming and probing (stationary upon the host, but
8 assessing the plant surface or its immediate vicinity via antennal movement or tarsal
9 tapping). A female was deemed to have left a plant if contact was lost for more than a
10 second (whereas if contact was re-established within this time, a ‘hop’ was recorded).
11 Plant growth parameters are provided in Table 2.

12

13 **2.4 Improving pest preference for a trap crop by manipulating plant age**

14 Choice tests were used to investigate the oviposition preferences of *P. xylostella* when
15 female moths were presented with cauliflower and white mustard plants of different ages.
16 The plants were 4 - 7 weeks old when used in the experiments.

17 Two cauliflower plants and two white mustard plants of given ages were placed
18 into a wooden-framed cage (as in the previous experiment). Ten moths (1:1 sex ratio)
19 were used in each run of the experiment and in each run oviposition was recorded over 48
20 h. The moths were provided with food (10% sucrose solution absorbed on cotton wool)
21 during all experimental runs.

22 The treatment combinations used in the experiment are shown in Table 3. Each
23 treatment was repeated once in each of nine runs, hence there were nine replicates. Due to

1 restraints on resources all treatment combinations could not be processed simultaneously
2 in a run. Instead, each run of the experiment was conducted within one week, where the
3 order of treatments within a run was randomised. Plant growth parameters are provided in
4 Table 4.

5

6 **2.5 Improving pest preference for a trap crop by manipulating plant size**

7 The method used in this choice experiment was similar to that used in the previous one.
8 The cage was modified to allow the height of the mustard plants to be altered, whilst
9 keeping plant age constant. This modification involved cutting circular holes in the base
10 of the cage so that the mustard plants could be lowered through the cage floor. Their
11 height within the cage could then be varied. The cage stood on a wooden frame, to give
12 support to the plants once they had been lowered through the cage floor. Once lowered,
13 thin plywood base plates were fitted around the plant stems to seal the holes in the cage
14 floor. Plant pots were then fitted around the lower portion of the plant that was protruding
15 into the cage. Each of these pots was fitted with a cardboard support that rested on the lip
16 of the pot and allowed for a thin layer of sieved compost to be placed on the support
17 surface surrounding the plant stem. In this way all plants appeared to the moths to be
18 potted when used.

19 Three treatments were tested; white mustard plants were used when larger than the
20 cauliflower plants (normal size), or when their height within the cage was adjusted so
21 that, to the moths in the cage, they appeared similar to cauliflower plants in size, or
22 smaller than the cauliflower plants. There was one replicate of each treatment in each of
23 eight runs. Due to restraints on resources all treatment combinations could not be

1 processed simultaneously in a run. Instead, each run of the experiment was conducted
2 within one week, where the order of treatments within a run was randomised. The height
3 of the cauliflower plants was never manipulated. Ten moths (1:1 sex ratio) were used in
4 each run with access to sucrose as previously. Plant growth parameters are provided in
5 Table 5.

6

7 **2.6 Statistical analysis**

8 For the first experiment (section 2.2), data from the individual plants, whether trap crop
9 or ‘external’ or ‘internal’ cauliflower plants, were combined within any replicate. Paired
10 t-tests were used to assess oviposition within the treatments where ‘external’ and
11 ‘internal’ plants could be compared. A 2-way ANOVA was used to compare oviposition
12 by *P. xylostella* on mustard plants with that on ‘external’ cauliflower plants (considering
13 cage aspect as a factor in addition to treatment). Data were square root transformed
14 before analysis. The same analysis was used to investigate differences between
15 treatments in the number of eggs found on the ‘internal’ cauliflower.

16 For the second experiment (see section 2.3) all data were analysed by 1-way
17 ANOVA. Data on residence times were square-root transformed prior to analysis. To
18 compare the various moth behaviours between treatments, several of the behavioural
19 variables were combined with one another to identify types of behaviour classed as
20 ‘searching’ (walking and running), ‘not-searching’ (resting, probing and grooming),
21 ‘assessing’ (walking, running and probing) and ‘not-assessing’ (resting and grooming).
22 The numbers of these behaviours per 100 seconds were compared across treatments, as
23 was the mean duration of each of these behaviours, and the percentage of the overall

1 residence time spent engaged in these behaviours. Data for searching, assessing and not-
2 assessing per 100 seconds were square-root transformed (0.5 added to all data points for
3 searching and assessing) prior to analysis. Data for the mean duration of not-searching
4 and not-assessing behaviours were log transformed. All data expressed as percentages
5 were arcsin square-root transformed.

6 For the third and fourth experiments (sections 2.4 and 2.5), data from the two
7 mustard or two cauliflower plants in each replicate were combined to give one value for
8 mustard and one for cauliflower. Paired t-tests on square root transformed data were used
9 to determine whether there were oviposition preferences for any treatment in the 'effect
10 of plant age' experiment (where total egg laying differed between treatments). In the
11 'effect of plant size' experiment, a 2-way ANOVA was run on the square root
12 transformed data (with treatment and replicate as factors), as total egg laying did not vary
13 between treatments. The percentage of eggs laid on mustard was also calculated and
14 analysed (having first subjected the data to the arcsine square root transformation).
15 Spearman's Rank analysis was used to determine if there was any statistically significant
16 correlation between the percentage of eggs laid on plants and plant growth parameters
17 (i.e. the percentage of the total 'within-cage plant height', leaf number or leaf area taken
18 up by the plant species being considered).

19 Where ANOVA showed statistically significant effects, Tukey's tests were used to
20 identify differences between means if needed. Where transformed data were used for any
21 analysis, these data are back-transformed when presented.

22

23 **3 RESULTS**

1 **3.1 Testing the effectiveness of a trap crop**

2 There was a statistically significant difference between treatments in the numbers of eggs
3 laid on the ‘internal’ cauliflower plants ($F_{(2,14)} = 5.88, P < 0.05$) (Fig. 1). Almost three
4 times as many eggs were laid on ‘internal’ plants that were ‘unprotected’ compared with
5 those ‘protected’ by mustard plants as a trap crop ($P < 0.05$). Tukey’s testing showed no
6 other differences between pairs of means, including the numbers of eggs on ‘internal’
7 cauliflower plants protected by an ‘external’ trap crop (mustard) or ‘external’ cauliflower
8 plants ($P = 0.485$). Experimental run had no effect on the data ($F_{(7,14)} = 2.52, P = 0.067$).

9 When comparing the two treatments with ‘external’ plants, ‘external’ mustard
10 plants attracted higher levels of *P. xylostella* oviposition than the ‘internal’ cauliflower
11 plants they were protecting ($T_{(7)} = 10.71, P < 0.001$), but ‘external’ cauliflower plants did
12 not ($T_{(7)} = 0.46, P = 0.660$) (Fig. 1). More eggs were recovered from ‘external’ mustard
13 plants than ‘external’ cauliflower plants ($F_{(1,7)} = 92.67, P < 0.001$) (Fig. 1). Run number
14 did not affect these data in any instance.

15

16 **3.2 Investigating behaviour on trap crop and crop plants**

17 Female *P. xylostella* spent significantly longer on the white mustard than cauliflower
18 plants once they had landed ($F_{(1,22)} = 12.66, P < 0.01$) (Table 6).

19 For the various behaviours displayed by female *P. xylostella* on the different plants,
20 significant differences between treatments were found only when considering searching
21 and assessing behaviours, and only when the mean durations of these behaviours were
22 considered ($P < 0.05$ in all cases, where, respectively, $F_{(1,22)} = 5.93$ and 5.38) (Table 6).
23 In both cases the mean duration of these behaviours was increased on white mustard.

1

2 **3.3 Improving pest preference for a trap crop by manipulating plant age**

3 In all treatments, more eggs were laid on the mustard plants than on the cauliflower
4 plants ($T_{(8)} = 5.75 - 11.25$, $P < 0.001$ in all cases) (Fig. 2). The total numbers of eggs laid
5 differed ($F_{(8,64)} = 3.47$, $P < 0.01$). This was due to greater oviposition in the 5 week old
6 mustard vs 6 week old cauliflower treatment, compared with most other treatments (Fig.
7 2). The total numbers of eggs laid was affected by replicate run ($F_{(8,64)} = 6.44$, $P < 0.001$).

8 When considering the percentage of eggs laid on the mustard plants, there was no
9 difference between treatments ($F_{(8,64)} = 1.65$, $P = 0.128$) (Fig. 3). Run number had no
10 effect on these data.

11 The percentage of eggs laid on the mustard plants was positively and significantly
12 associated with the percentage of the total 'within-cage plant height' occupied by the
13 mustard plants (combined height of the mustard plants divided by the combined height of
14 all plants) ($R_{(81)} = 0.29$, $P < 0.01$).

15

16 **3.4 Improving pest preference for a trap crop by manipulating plant size**

17 In all treatments, *P. xylostella* females preferred to oviposit on the mustard plants rather
18 than the cauliflower (Fig. 4). This preference was most pronounced when the mustard
19 plants were larger than the cauliflower plants ($T_{(7)} = -8.14$, $P < 0.001$), but persisted when
20 the mustard was similar in size to the cauliflower ($T_{(7)} = -4.69$, $P < 0.01$) and when it was
21 smaller ($T_{(7)} = -2.66$, $P < 0.05$). There was no statistically significant difference between
22 treatments in the total number of eggs laid ($F_{(2,14)} = 1.48$, $P = 0.262$) and these data were
23 not affected by replicate ($F_{(7,14)} = 2.45$, $P = 0.072$).

1 When considering the numbers of eggs laid on the cauliflower, there was a
2 statistically significant difference between treatments ($F_{(2,14)} = 3.82, P < 0.05$). Tukey's
3 tests did not identify any statistically significant differences between pairs of means,
4 although more eggs were laid in the presence of the smallest mustard plants (Fig. 4). For
5 oviposition on the mustard, there were statistically significant differences between
6 treatments with regard to the numbers of eggs laid ($F_{(2,14)} = 5.48, P < 0.05$). This was
7 because more eggs were laid on the largest mustard plants when compared with the
8 smallest ($P < 0.05$). Run number had no effect on any of the oviposition data for
9 cauliflower, but did influence oviposition on mustard plants ($F_{(7,14)} = 3.95, P < 0.05$).

10 There was a statistically significant difference between treatments in the percentage
11 of eggs laid on mustard plants ($F_{(2,14)} = 6.43, P < 0.01$) (Fig. 5). More eggs were laid on
12 mustard plants that were larger than the presented cauliflower plants than on mustard
13 plants which were smaller ($P < 0.01$). Replicate did not influence the numbers of eggs
14 laid ($F_{(7,14)} = 1.36, P = 0.294$).

15 Correlation analysis showed that, in all treatments, the percentage of eggs laid on
16 mustard plants was positively and significantly associated with the percentage of the
17 'within-cage height', leaf number and leaf area occupied by the mustard plants ($R_{(24)} =$
18 $0.557, 0.467$ and 0.571 where $P < 0.01, 0.05$ and 0.01 , respectively).

19

20 **4 DISCUSSION**

21 Fewer eggs were laid on cauliflower plants 'protected' by a trap crop of mustard than on
22 'unprotected' cauliflower or cauliflower 'protected' by other cauliflower plants. These
23 observations are similar to those made in a number of other studies on *P. xylostella*. Trap

1 crops of Indian mustard (*Brassica juncea* (L.)),^{7,9} cabbage (*Brassica oleracea*) (different
2 variety to the main crop),⁷ rocket (*Barbarea vulgaris* (L.))¹⁰ and collard (*Brassica*
3 *oleracea*)⁸ have all been shown to reduce numbers of *P. xylostella* adults or eggs on the
4 main crop when they have been introduced into cabbage monocultures.

5 In the current study, the reduction in oviposition on ‘protected’ cauliflower
6 presented with a cauliflower ‘trap crop’ was around 27%, whereas that observed with a
7 mustard trap crop was nearer 66%. This suggests that there was an interception, or ‘edge
8 effect’ of both the mustard trap crop and the external cauliflower plants, but that this was
9 greater for mustard. By being more acceptable/stimulating, chemically, visually or both,
10 the mustard in the current study probably increased any ‘edge effect’ by encouraging
11 moths to land more readily upon these plants. Similar observations have been made
12 elsewhere with both *P. xylostella* and *Delia radicum* (L.).²⁵

13 In the present study, once they had landed moths remained upon the white mustard
14 for longer periods than the cauliflower, which would have maximised the trap crop effect.
15 There were no differences in observed behaviours on the white mustard and cauliflower
16 (i.e. searching, not-searching, assessing and not-assessing) when considering their
17 frequency in time, or in the percentage of the moths total residence time devoted to them.
18 However, the mean duration of some behaviours did differ significantly on different
19 plants, and were approximately doubled when the moths were on the potential trap crop
20 species, compared with when they were on cauliflower. This was true of both searching
21 and assessing behaviours. This might suggest that *P. xylostella* responded to the more
22 acceptable potential trap crop plants by increasing the duration of certain behaviours
23 when upon them, particularly those that provided females with information about their

1 host plant. Periods of non-information-providing behaviour (i.e. not-searching and not-
2 assessing) never differed between host plants in the current study, no matter how they
3 were measured (frequency over time, mean duration or percentage of total residence
4 time). More work is needed in this area of research, but it seems from the present study
5 that, in response to contact with more optimal hosts, pest insects increase their residence
6 times by increasing the length of each bout of information-providing behaviour, even
7 though both the frequency of these behaviours in time, and the percentage of the overall
8 residence time devoted to each behaviour, remains reasonably constant. This would be in
9 agreement with work that has found *D. radicum* activity rates to be comparable on
10 stimulating (real) and non-stimulating (artificial) host plants, despite these flies making
11 more exploratory flights and hops around the plant or to the soil from the former.²⁶
12 According to work on *D. radicum* host selection, such behaviours would have provided
13 the flies with increased information about the presumably more acceptable ‘real’ host
14 plant.²⁷

15 This study confirms that to function most effectively, trap crop plants should be
16 highly acceptable pest host plants. Where trap crops have consisted of relatively non-
17 preferred host plants, these have typically failed in pest management.^{28,29} The results also
18 suggest that where possible trap crop plants should be placed to intercept pests on their
19 route into the crop to take advantage of any interception effect. Unsurprisingly then,
20 where trap crops have been reported to be successful in managing *Brassica* pests, they
21 have been typically deployed as borders completely surrounding the main crop or as
22 strips running at right angles to the pests’ migration route into the crop.^{7,8,9,16,30,31,32}

23 In the second part of the study, *P. xylostella* females preferred mustard plants of all

1 ages as oviposition sites compared with cauliflower plants, regardless of whether the
2 mustard was older, younger or the same age as the cauliflower. In earlier work by the
3 authors and studies elsewhere, white mustard was a highly-preferred host plant for *P.*
4 *xylostella*,^{24,33} so such a preference in the present study was not unexpected. Several
5 authors have suggested seeding a mustard trap crop for *P. xylostella* 15 days before a
6 cabbage main crop,^{16,17} so what was more unexpected was that *P. xylostella* did not
7 display a greater preference for relatively older mustard plants in the current study.
8 Nevertheless, the importance of the relative size of host plants in dictating the host
9 preferences of phytophagous insects has been highlighted elsewhere,^{18,19} where
10 preferences for larger host plants tend to be the norm, including by *P. xylostella*.²⁰ Had a
11 wider range of plant ages (and hence sizes) been used in the current study, different
12 results might have been obtained, especially if younger mustard plants were then smaller
13 in size than older cauliflower plants. Indeed, even with this not being the case in the
14 current study, a positive correlation was still seen between percentage oviposition of *P.*
15 *xylostella* on the trap crop plants, and the percentage of the within-cage plant height taken
16 up by these plants. Had a wider range of plant ages been used, this would also have
17 allowed a range of plant growth stages to be tested. As growth stage has been shown
18 previously to influence the preference of *Brassica* pests for trap crop over crop plants of
19 the same age, this could have yielded some interesting data.^{21,22}

20 When plant size (height, leaf number and leaf area) was varied in the current study,
21 regardless of whether the mustard plants were larger, smaller or similar in size to the
22 cauliflower plants, they were always preferred by *P. xylostella*. However, whilst mustard
23 plants of all sizes were preferred to cauliflower plants *per se*, this preference varied

1 across treatments. Mustard plants that were larger than the cauliflower plants were
2 relatively more preferred than smaller mustard plants. It was also apparent that
3 oviposition on the cauliflower plants increased as their relative size was increased. This
4 suggests that the size of trap crop plants is important in ensuring that *P. xylostella* is
5 attracted and/or arrested to the greatest extent even where the trap crop plants are always
6 preferred. In support, positive correlations were found between oviposition preference of
7 *P. xylostella* for the trap crop plants, and the percentage of within-cage plant material
8 (height, leaf number and leaf area) taken up by these plants.

9 In conclusion, it appears from the data presented here that a white mustard trap crop
10 could be of use in limiting pest attack by *P. xylostella* on cauliflower by utilising the
11 pests' preference for these plants and tendency to remain upon them for long periods with
12 a naturally occurring 'edge effect'. This study also suggests that there may be a benefit in
13 using larger plants in trap crops as they are relatively more preferred and should therefore
14 attract/arrest and retain pests more efficiently than smaller plants. Mustard species might
15 make for attractive trap crops on this basis as they are fast growing and could achieve
16 such large relative sizes, even if sown at the same time as main crops such as cauliflower.
17 Nevertheless, the present study also suggests that trap crop plants can still be preferred by
18 pests when smaller or similar in size to main crop plants. This too may benefit the use of
19 mustards in trap cropping as these plants may need re-sowing if used with longer-lived
20 brassicas like cauliflower, and may thus need to be temporarily present at a relatively
21 small size. Further study will nevertheless be required to confirm these results under field
22 conditions where *P. xylostella* population densities and the relative density of the trap
23 crop to crop plants would be expected to be lower than those used in the current study.

1 Such study will also need to confirm that any ‘self-seeding’ that might occur with the use
2 of relatively fast growing, short-lived trap crops would not pose a weed problem for the
3 main crop, or subsequent crops grown in the same field. It is likely that trap crops for *P.*
4 *xylostella* are best planted as borders surrounding the main crop to take advantage of this
5 pests’ tendency to invade crops from field peripheries.²³ Maintaining trap crops as
6 discrete borders in this way would hopefully minimise the spread of trap crop plants into
7 the main crop area.

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23

1 **Table 1.** Physical characteristics of plants used: height (cm); leaf number; leaf area
 2 (mm²) in the experiment on the effectiveness of a trap crop (Section 2.2). n = 18.

Plant type	Treatment type		
	Trap crop of white mustard	Double cauliflower	Single cauliflower
External plant	49.8; 13.1; 38238.9	21.3; 7.4; 18906.6	NA
Internal plant	21.9; 7.8; 19701.1	22.1; 7.9; 20024.2	21.6; 7.7; 20236.3

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7 **Table 2.** Physical characteristics of plants used; height (cm), leaf number, leaf area
 8 (mm²) in experiment on moth residence time and behaviour (Section 2.3). n = 12.

Plant species	Growth parameter		
	Height	Leaf number	Leaf area
Cauliflower	20.4	6.6	15155.3
White mustard	48.2	10.9	41194.6

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Table 3. Treatments used for oviposition preference experiments using plants of varying ages (weeks).

	White mustard			
	Age	4-5	5-6	6-7
Cauliflower	4-5	Treatment 1	Treatment 2	Treatment 3
	5-6	Treatment 4	Treatment 5	Treatment 6
	6-7	Treatment 7	Treatment 8	Treatment 9

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Table 4. Physical characteristics of plants used: height (cm); leaf number; leaf area (mm²) in the experiment on the age (weeks) of trap crop plants (Section 2.4). n = 18.

Age of mustard: cauliflower	Plant species	
	Cauliflower	Mustard
4:4	15.9; 5.0; 5057.1	30.3; 9.6; 25990.8
5:5	18.8; 5.3; 8520.6	39.7; 10.2; 30472.5
6:6	20.8; 6.2; 14075.9	50.9; 11.2; 39227.7
4:5	14.7; 5.1; 4783.1	27.6; 9.2; 22806.4
4:6	21.7; 6.2; 14544.5	22.5; 7.9; 16396.8
5:4	11.1; 4.1; 2088.7	30.5; 9.5; 27138.9
5:6	22.3; 6.8; 17312.8	43.5; 10.6; 28402.0
6:4	11.9; 4.4; 2704.6	59.8; 11.5; 36401.4
6:5	16.6; 5.6; 6213.4	55.4; 11.5; 38512.7

1 **Table 5.** Physical characteristics of plants used; height (cm), leaf number, leaf area
 2 (mm²) in experiment on plant size (Section 2.5). n = 18.
 3

Plant species	Relative size of mustard to cauliflower		
	Smaller	Similar	Larger
Cauliflower	18.1; 5.5; 8227.6	16.8; 5.4; 6890.9	16.8; 5.4; 6994.1
Mustard	11.4; 2.8; 3222.8	16.9; 3.8; 7717.6	36.7; 10.4; 33482.8

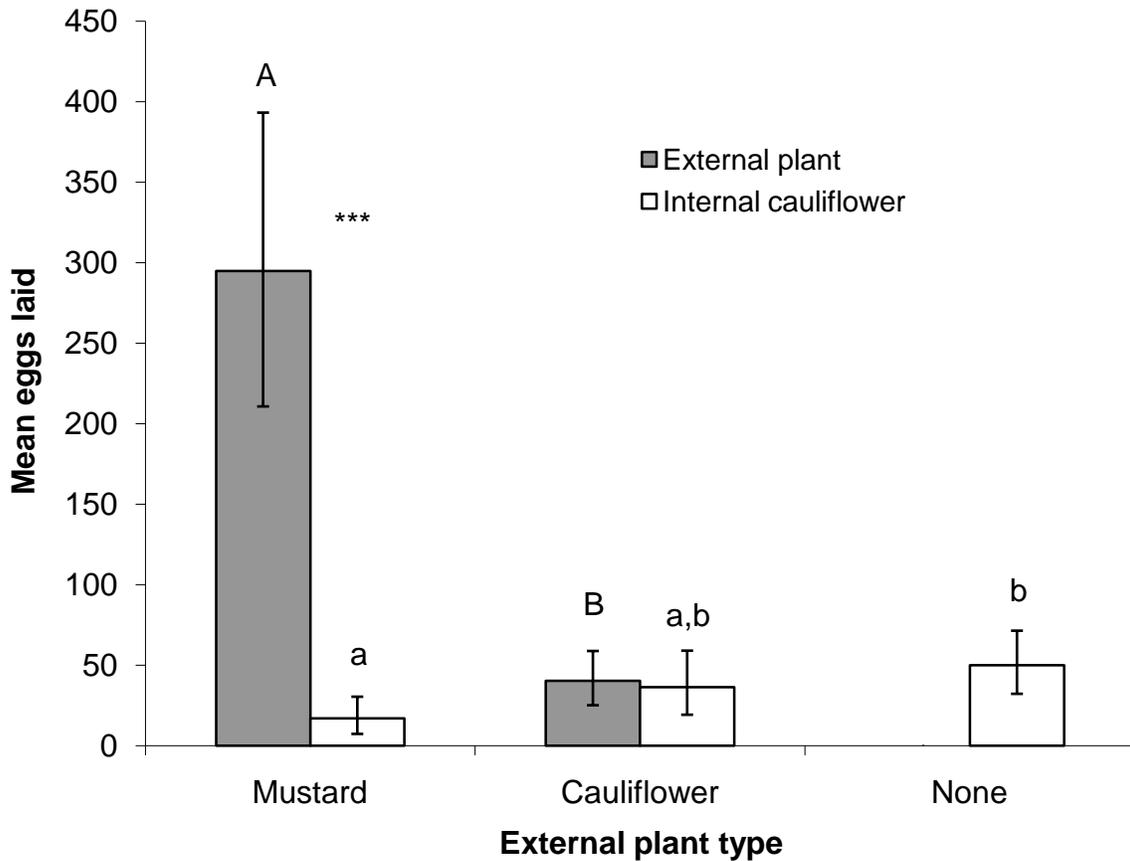
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1 **Table 6.** Mean frequency of, durations of and percentage of residence times spent engaging in various behaviours on white mustard
2 and cauliflower plants in no-choice tests. Frequencies are expressed as the mean number of occurrences of a given behaviour per 100
3 seconds of residence time. \pm values displayed in parenthesis are 95% confidence limits. Data that were transformed for ANOVA have
4 been back-transformed. Data presented in bold typeface are significantly different within a row. n = 12.
5

		Plant species			
		Cauliflower	Mustard	F _(1,22)	P
Total residence	Per plant visit	575.8 (+740.8, -438.8)	2859.6 (+1247.6, -1022.3)	12.66	< 0.01
Searching	Frequency	1.1 (+1.0, -0.8)	0.9 (+0.5, -0.4)	0.16	0.694
	Duration (sec)	6.2 (\pm3.1)	12.2 (\pm3.8)	5.93	< 0.05
	% of time	8.6 (+13.9, -7.6)	11.9 (+9.3, -6.9)	0.22	0.646
Not-searching	Frequency	3.0 (\pm 1.4)	1.8 (\pm 0.8)	1.83	0.190
	Duration (sec)	58.0 (+101.7, -36.9)	65.3 (+51.3, -28.7)	0.04	0.843
	% of time	93.0 (+6.1, -1.1)	88.3 (+6.9, -9.2)	0.56	0.463
Assessing	Frequency	1.7 (+1.5, -1.1)	1.4 (+0.7, -0.6)	0.17	0.685
	Duration (sec)	4.8 (\pm2.1)	9.1 (\pm6.2)	5.38	< 0.05
	% of time	11.5 (+17.9, -10.1)	13.2 (+10.0, -7.5)	0.04	0.845
Not-assessing	Frequency	1.7 (+1.3, -0.9)	1.1 (+0.7, -0.5)	1.16	0.293
	Duration (sec)	76.5 (+139.5, -49.4)	96.5 (+77.6, -43.0)	0.15	0.706
	% of time	90.7 (+7.8, -13.4)	87.0 (+7.4, -10.0)	0.24	0.627

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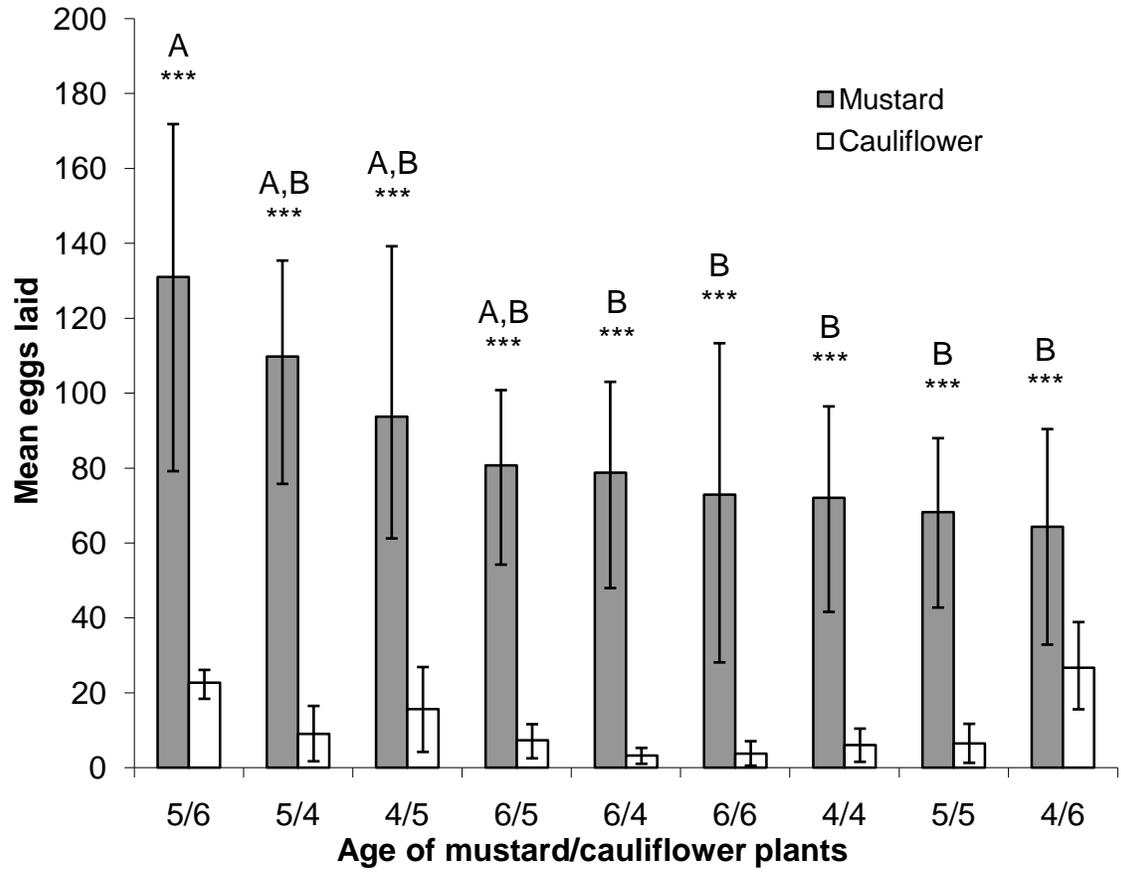
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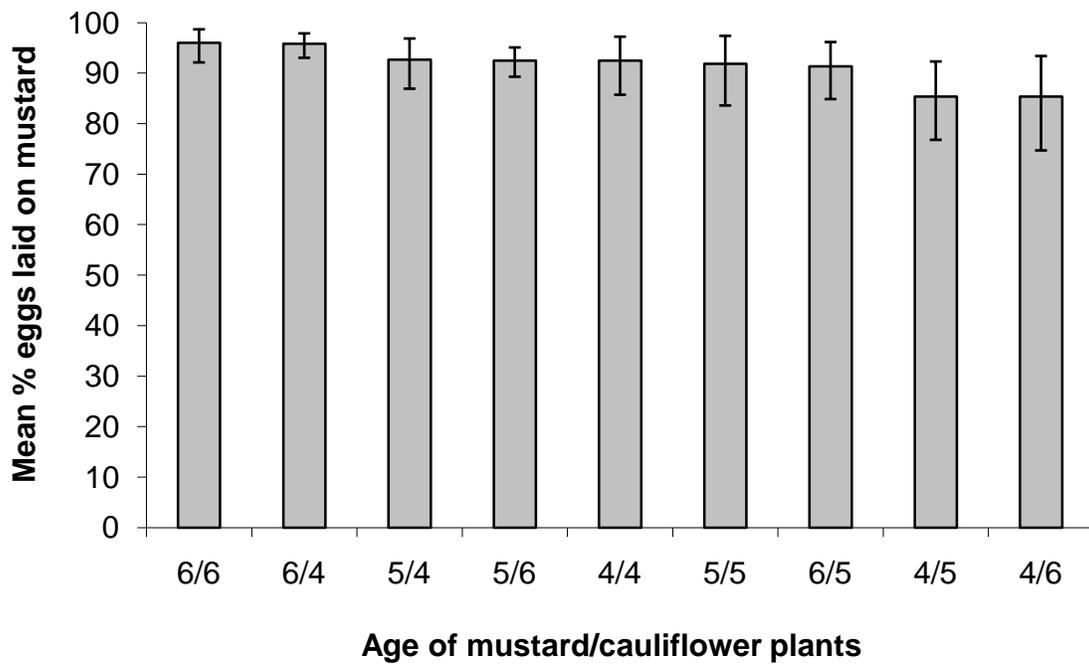
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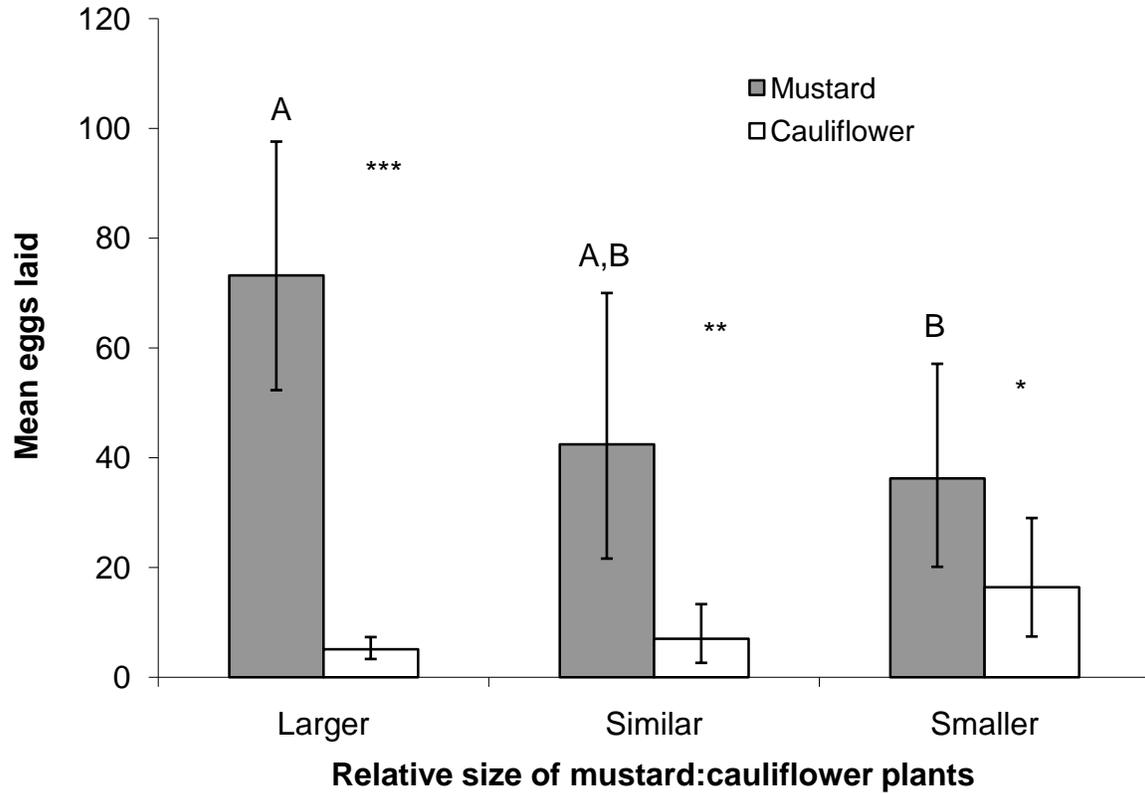
Figure 1. Mean number of eggs laid over 48 hours on cauliflower plants with different trap crop (external plant) types (error bars show 95% confidence limits). All data were back-transformed from ANOVA. Bars with different letters denote significant differences “between main treatments” (Tukey’s Test $P < 0.05$), where treatments accompanied by letters of the same case were compared statistically. $n = 8$. Asterisks denote significant differences between “within treatment” means; (paired t-test, *** $P < 0.001$). $n = 8$.



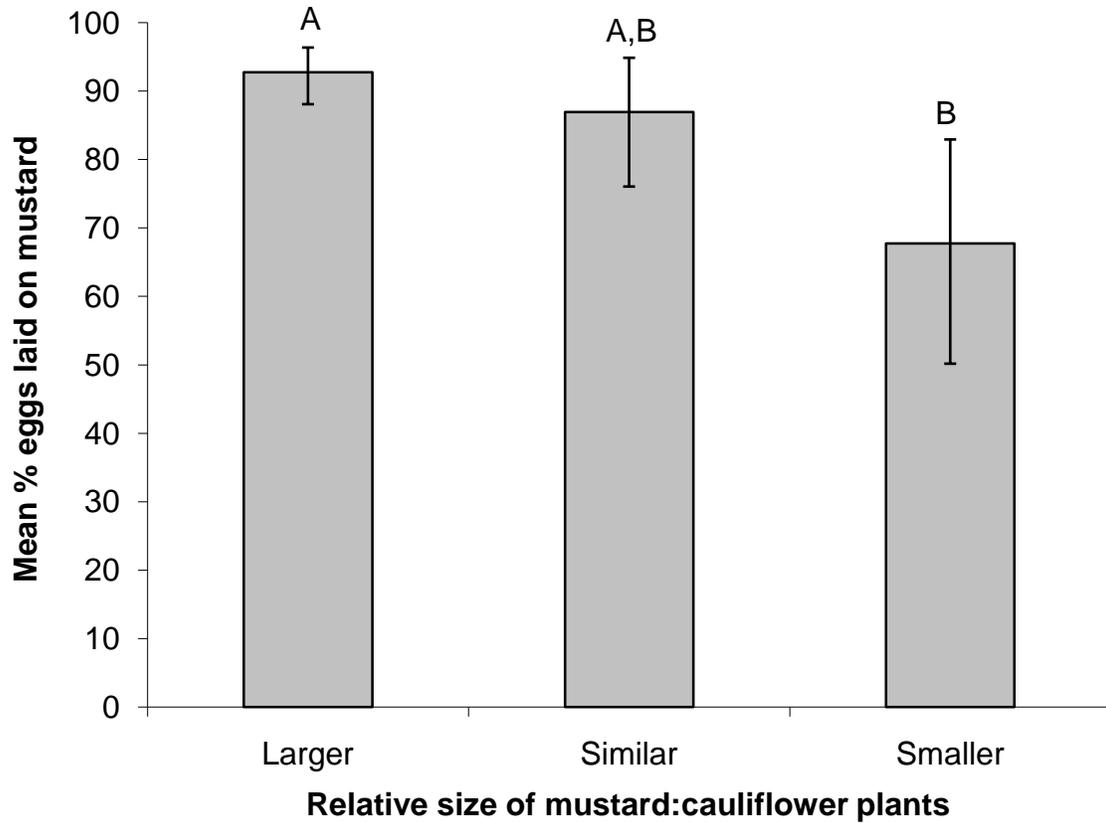
1
2 **Figure 2.** Mean number of eggs laid over 48 hours on mustard and cauliflower plants of
3 various ages (error bars show 95% confidence limits). All data were back-transformed
4 from ANOVA. Treatments not sharing a common letter denote statistically significant
5 differences ($P < 0.05$) between treatments in total oviposition on both mustard and
6 cauliflower plants. Asterisks denote significant differences between “within treatment”
7 means; (paired t-test, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). $n = 9$.
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 2 **Figure 3.** Mean percentage of eggs laid on mustard plants of various ages presented
 3 together with cauliflower plants of various ages (error bars show 95% confidence limits).
 4 All data were back-transformed from ANOVA. n = 9.
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2 **Figure 4.** Mean number of eggs laid over 48 hours on mustard and cauliflower plants of
3 various sizes relative to one another (error bars show 95% confidence limits). All data
4 were back-transformed from ANOVA. Within each figure, bars not sharing a common
5 letter denote statistically significant differences ($P < 0.05$) between size groups in the
6 total number of eggs laid on mustard. Asterisks denote statistically significant differences
7 between “within treatment” means; (paired t-test, * $P < 0.05$; ** $P < 0.01$; *** $P <$
8 0.001), $n = 8$.
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2 **Figure 5.** Mean percentage of eggs laid on mustard plants of varying size compared with
3 cauliflower plants of a constant size (error bars show 95% confidence limits). All data
4 were back-transformed from ANOVA. For particular oviposition targets, bars not sharing
5 a common letter denote statistically significant differences ($P < 0.05$) between the sizes
6 of mustard plants. $n = 8$.
7