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10 Life-History and Behavior of the Diamondback Moth *Plutella xylostella* on Brassicaceae
11 Cultivars over Multiple Generations

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35 Running head: Life-History and Behavior of *Plutella xylostella*

36

37 **Abstract**

38 The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a
39 cosmopolitan pest that causes leaf-area reduction in Brassicaceae plants. DBM populations
40 have significant genetic variability that manifests in different feeding preferences and
41 reproductive behaviors across generations. We evaluated the influence of Brassicaceae
42 cultivars on biological and behavioral parameters across 18 generations of DBM populations
43 that were separated and held on three varieties of Brassicaceae: *Brassica oleracea* var.
44 *acephala* (kale), *Brassica oleracea* var. *italica* (broccoli), and *Brassica oleracea* var. *capitata*
45 (cabbage). P, F6, F12, and F18 generations were evaluated, and biological aspects of young
46 adulthood and fertility parameters of adults held on each host plant were examined over
47 multiple generations. Additionally, larvae and adults were subjected to dual-choice and
48 multiple-choice (feeding and oviposition) between cultivars, over generations. The results
49 indicated that larvae of *P. xylostella* consumed greater quantities of kale and broccoli
50 cultivars, on average (4.05 cm²), than cabbage (2.7 cm²). The number of eggs per female in
51 F18 generation was 1.95 and 2.17 times higher than those in the parental (P) generation, when
52 reared on kale and cabbage. The population reared exclusively on kale had higher net
53 population growth rate (R_0), intrinsic rate (r_m), finite rate (λ) and generation time (T) than that
54 reared on broccoli and cabbage. Last generations evaluated, the larval stage reared on cabbage
55 showed feeding preference (F18) and oviposition preference (F12 and F18) for cabbage.
56 Thus, we note the existence of learning, characterized as pre-imaginal conditioning to
57 cabbage cultivars, over various DBM generations.

58

59 **Keywords**

60 Insect biology, feeding preference, oviposition preference, conditioning.

61

62 **Introduction**

63 The diamondback moth (DBM) *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) is the
64 most detrimental pest of Brassicaceae worldwide, and is one of the main factors limiting the
65 cultivation of these plants. The DBM is widely distributed. Caterpillars that attack
66 Brassicaceae plants reduce leaf area, which interferes with plant growth, causes depreciation
67 of the product, and can lead to total loss of the crop (Castelo Branco & France 2001; Cheng *et*
68 *al* 2008). Plant losses can reach up to 80% defoliation and annual monetary losses are
69 approximately \$4 to \$5 billion USD (Zalucki *et al* 2012).

70 The significant genetic diversity of *P. xylostella* facilitates the development of
71 resistance to insecticides, which is a major pest control method (Ahmad *et al* 2012; Zago *et al*
72 2014). Thus, alternatives are being researched to optimize management of the pest. Studies
73 examining the life-history and behavior of DBM reared on different Brassicaceae cultivars are
74 important to determine cultivars that are more resistant and less conducive to the development
75 and reproduction of the pest (Badenes-Perez *et al* 2010).

76 Species of Brassicaceae have different types of defenses against herbivory, including
77 chemical and mechanical defenses (Travers-Martin *et al* 2008; Karowe & Grubb 2011).
78 However, selecting varieties appropriate for specific conditions can directly influence the
79 herbivory of insects (Louda & Collinge 1992). Pest insects prefer to feed on plants that offer
80 survivability for themselves and their descendants (Thompson 1988). Chemical defense
81 compounds formed in the Brassicaceae, such as sinigrin, undergo improved quantitative
82 variation between cultivars (Thuler *et al* 2007). However, this substance has great importance
83 in plant-insect interactions, and may positively affect the DBM by stimulating their feeding
84 and oviposition (Sarfraz *et al* 2006; Hopkins *et al* 2009).

85 Physical aspects also present in Brassicaceae plants provide mechanical protection
86 against insects, such as trichomes and leaf surface wax layers that may interfere with the

87 feeding and oviposition on *P. xylostella*. The DBM has been shown to have lower growth
88 rates in cultivars with more trichomes than in plants containing fewer mechanical defenses
89 (Mathur *et al* 2011).

90 However, in studies using plants rich in trichomes, such as black mustard (*Brassica*
91 *nigra*), was preferred over broccoli (*Brassica oleracea* var. *lieutenant*) and cabbage (*Brassica*
92 *oleracea* var. *acephala*) for oviposition (Newman 2014). These results suggest that the
93 presence of trichomes was not the main criterion influencing females' choice of oviposition
94 sites.

95 The ability of insect pests to satisfactorily feed and succeed in their life cycle covers a
96 sequence of behaviors (Karban & Agrawal 2002). Learning in holometabolous insects, such
97 as *P. xylostella*, occurs in both the larval and adult stages, and learning is retained after
98 metamorphosis of larvae up to pupae and to adult stages (Bernays & Chapman 1994). Host
99 location and host selection learned behavior has been extensively studied in the imaginal
100 stage, but no concrete evidence has shown that preimaginal conditioning influences
101 preferences in adults (Bernays 1995). The conditioning may influence the life-history (life
102 cycle or fecundity) and substrate preference for feeding and oviposition of Lepidoptera
103 (Bernays & Weiss 1996). Studies on multiple DBM generations reared on kale showed that
104 when DBM established in another Brassicaceae cultivar, such as cabbage, causes reduction in
105 biological parameters (De Bortoli *et al* 2011, 2013). Our hypothesis suggests that biological
106 and behavioral aspects of *P. xylostella* can be changed over generations when it is reared on
107 different cultivars of Brassicaceae. Therefore, to further examine our hypothesis, we
108 evaluated biological parameters of *P. xylostella* in three cultivars Brassicaceae, their
109 reproductive behavior, and the preference for feeding and oviposition over 18 generations.

110

111 **Material and methods**

112 The studies were conducted at Laboratory of Biology and Insects Rearing (LBIR),
113 Department of Plant Protection, UNESP, Jaboticabal, Sao Paulo, Brazil. The insects used in
114 the rearing experiments (~ 185 generations) were kept following a methodology adapted from
115 Thuler (2009) on studies with *Brassica oleracea* var. *acephala*-kale HS20 (Horticeres[®],
116 Indaiatuba, Sao Paulo, Brazil) at LBIR. DBM populations were reared in a room at $25 \pm 1^\circ\text{C}$,
117 relative humidity of $70 \pm 10\%$ and photoperiod of 12 h light and 12 h dark.

118

119 *Life-history of P. xylostella in kale, broccoli, and cabbage*

120

121 We evaluated the biological characteristics of DBM across various generations: P
122 (parental), F6, F12 and F18, reared on three varieties of Brassicaceae. The cultivars used
123 were: *Brassica oleracea* var. *acephala* - kale (HS20 cultivar; Horticeres), *Brassica oleracea*
124 var. *italica* - broccoli (Piracicaba cultivar; Feltrin[®], Farroupilha, Rio Grande do Sul, Brazil) e
125 *Brassica oleracea* var. *capitata* - cabbage (Bob Cat cultivar; Sakata[®], Sao Paulo, Sao Paulo,
126 Brazil). The host plants selected in this study are widely used by brassica producers in the São
127 Paulo state of Brazil. The seeds of each cultivar were planted in 10L pots containing a 3: 1: 1
128 substrate mixture of ravine of land, cattle manure, and rice husk. The potted plants were
129 maintained in a greenhouse designed for growing plants. After 60 days, only the formed
130 leaves (fully developed) were selected as substrate to avoid interference age.

131 To start the populations of *P. xylostella* on kale, broccoli and cabbage host plants, ~
132 3,000 pupae were removed from stock reared and divided into three cages until adult
133 emergence. Approximately 1,000 newly emerged adults were kept per cage with leaves of
134 each cultivar disc. Cages contained a circular plastic container (12-cm diameter × 15-cm
135 height) sealed with cling film (PVC). A foliar disc (8-cm diameter) of the substrate (kale,
136 broccoli or cabbage) and a filter paper disc (9-cm diameter) moistened with distilled water

137 were placed inside the cage. The leaf discs containing eggs were removed 24 hours after
138 introduction and transferred to Petri dishes (9-cm diameter) where they remained until larvae
139 hatched. Approximately 50 newly hatched larvae from each substrate were selected for the
140 study of the first generation (parental), and the surplus of the three populations were
141 maintained following the methodology designed by Thuler (2009) to repeat the study using
142 the F6, F12, and F18 generations.

143 Caterpillars in the first instar of the parental population were collected using a brush and
144 placed in Petri dishes (9-cm diameter) forming the following treatments: i) population on
145 kale, ii) population on broccoli, and iii) population on cabbage. In each dish, 10 larvae were
146 placed on each leaf disk substrate, totaling five Petri dishes for each population (5 replicates).
147 After transferring the caterpillars, the dishes were capped and sealed with plastic wrap (PVC)
148 in order to maintain moisture and prevent the escape of larvae.

149 The first evaluation was performed four days after the caterpillars were introduced to
150 the dishes that due to feeding habit of first instar are leaf mining. After the first evaluation, the
151 treatments were evaluated every three days, and leaf discs were replaced until the formation
152 of pupae. The assessed parameters were as follows: leaf consumption (using leaf area meter
153 Laser CID® CI-202 model, Washington, USA), survival (viability), and larval stage. Pupae
154 originating from each treatment were collected and individualized using the cell type ELISA®
155 96 well plates and sealed with plastic film (PVC). At this stage, we evaluated the viability
156 parameters and the pupal period, subject to the emergence of adults.

157 The newly emerged adults were removed from the cells of ELISA® plates with suction,
158 separated by gender, and transferred to the laying cages. Two couples from each of the
159 respective treatments were placed in cages, creating five replicates per treatment in total.
160 Inside each cage, leaf discs (8-cm diameter) were placed on paper moistened with distilled
161 water for oviposition. Adults were fed daily with 10% sucrose solution made with honey

162 dampened sponge, fixed to the top of the cage. Every day, the total egg count on the disk and
163 the survival of adults was recorded. At this stage, the following parameters were evaluated:
164 longevity of males, longevity of females, and number of eggs per female. All studied
165 parameters, including larval, pupal, and adult periods, were repeated in the F6, F12, and F18
166 generations of the three DBM populations.

167

168 *Fertility life table*

169

170 The study was conducted with three populations of *P. xylostella*, reared with kale
171 leaves, broccoli, and cabbage to determine the population growth characteristics of insects
172 over the P, F6, F12, and F18 generations. In this study, we used the previously obtained
173 information about survival and duration (both with respect to egg, larval, pupal, and adult
174 stages), sex ratio, and eggs per female per day.

175 The biological data obtained permitted estimates of the parameters needed for the
176 construction of fertility life tables (Price, 1984), which are as follows: x = mean age of the
177 female parental (seen as the emergence of the egg stage); l_x = life expectancy to age x ,
178 expressed as a female; m_x = specific fertility or number of offspring produced per female at
179 age x that result in females; $l_x.m_x$ = total number of females at age x . Growth parameters
180 resulting from the life table were evaluated as follows: R_0 = net reproductive rate (considering
181 the number of females produced by females in generation), r_m = intrinsic rate of increase in
182 number, λ = finite rate of increase (the number of times the population multiplies in unit
183 time), and T = average generation time in days. The growth parameters (R_0 , r_m , λ and T) were
184 calculated using the following equations:

$$185 R_0 = \sum (m_x.l_x)$$

$$186 r_m = \ln . R_0 / T$$

187 $\lambda = e^{rm}$

188 $T = (\sum mx.lx.x) / (\sum mx.lx)$

189

190 *Feeding preference*

191

192 The dual-choice feeding test was performed with *P. xylostella* caterpillars of the P, F6,
193 F12, and F18 generations derived from populations reared on kale, broccoli, and cabbage. The
194 arenas used in the feeding preference study consisted of Petri dishes (15-cm diameter) with
195 bottoms covered by filter paper, and were lightly moistened with distilled water to retain the
196 moisture of the leaf discs. In each arena, four leaf disks (2-cm diameter) were arranged
197 equidistantly and alternately, two cultivars per dish. Ten first instar caterpillars were then in
198 the center of each arena. In total, five replicates per treatment (three populations and four
199 generations) were assembled; each area was considered a replicate. The evaluations were
200 performed 24 hours after the release of caterpillars. Evaluations consisted of recording the
201 number of tracks present on the surface of each leaf disc.

202 In the multiple-choice test, three leaf discs (2-cm diameter) were placed in each arena
203 equidistant from each cultivar. In the center of the arena, 10 first instar caterpillars were
204 released. The experiment consisted of five replicates per treatment (three populations and four
205 generations); each area was considered a replicate. Evaluations were conducted 24 hours after
206 the release of the caterpillars and consisted of recording the number of tracks present on the
207 surface of each leaf disc.

208

209 *Oviposition preference*

210

211 The test dual-choice oviposition was performed with DBM adults in the P, F6, F12, and
212 F18 generations. Individuals from each of these generations were derived from caterpillars
213 reared on kale, broccoli, or cabbage. The cages used were made of transparent plastic
214 containers (13-cm diameter × 9-cm height). In each cage, two discs (8-cm diameter) from a
215 cultivar and two discs from other cultivar were arranged in an alternating fashion so that
216 DBM adults would encounter the two cultivars at the same time. The leaf disc containing two
217 host plants was placed on a Petri dish covered with filter paper. The filter paper was lightly
218 moistened with distilled water to maintain moisture. The two newly emerged couples were
219 then released inside the cage to copulate and oviposit. The experiment consisted of five
220 replicates per treatment (three populations and four generations); each cage was considered a
221 replicate. Evaluations were conducted 24 hours after the release of adults, and the number of
222 eggs present on the surface of each cultivar was recorded.

223 In the multiple-choice test, an 8-cm diameter leaf disc composed of one-third of each
224 cultivar (kale, broccoli, and cabbage) was placed in each cage. The two newly emerged
225 couples were then released inside the cage to copulate and oviposit. In total, five replicates
226 were assembled, and each cage was considered a replicate. Evaluations were conducted 24
227 hours after the release of adults, and the number of eggs present in each cultivar was recorded.

228

229 *Data analysis*

230

231 The effects of the different cultivars of Brassicaceae on the *P. xylostella* were analyzed
232 using the repeated measures procedure for an analysis of variance using the PROC MIXED of
233 SAS Institute software. Each biological characteristic was analyzed separately (independent
234 fixed variables: treatment and time, random variables, and replicates within treatment), and an
235 appropriate covariance structure for each characteristic was used (Littell *et al* 2006). As there

236 was a significant interaction between the main effects (cultivars and generations), an
237 additional analysis of variance was performed for each treatment. Assumptions of normality
238 and homogeneity of variance were checked using the Cramer-von Mises criterion and
239 Bartlett's test. If significant differences were found between the treatments, means were
240 compared using Tukey's test. All of the statistical analyses were conducted in SAS 9.1 (SAS
241 Software version 9.0, 2002; SAS Institute Inc, Cary, NC, USA).

242 The data on the fertility life table were analyzed according to the procedure described by
243 Maia et al. (2000) using the SAS GLM (SAS Institute, 2002) software. The proportion of
244 surviving adults between treatments was compared with the Kaplan-Meier method PROC
245 LIFETEST. The means and confidence intervals were compared using the Student t test for
246 paired groups generated by PROC LIFETEST (SAS Institute, 2002).

247 Collected data pertaining to the number of fed larvae and the number of eggs laid in
248 dual-choice and multiple choice tests were analyzed using Proc FREQ in SAS statistical
249 software (SAS Institute, 2002). The resulting analysis of the frequency of dual-choice and
250 multiple-choice were interpreted using the chi-square test (χ^2) using the ratio 50:50 as the
251 expected preference (i.e. no preference was displayed by test subjects) and was performed the
252 chi-square test using SAS software (SAS Institute, 2002).

253

254 **Results**

255

256 *Life-history of P. xylostella in kale, broccoli, and cabbage*

257

258 In the analysis of leaf consumption by DBM, significant differences were observed
259 between the kale, broccoli, and cabbage cultivars ($F_{2,57} = 8.44$, $P = 0.0006$). On average, less
260 consumption of cabbage leaves was observed (2.7 cm²) than kale leaves (4.0 cm²) and

261 broccoli (4.1 cm²). However, when we evaluated the leaf consumption between generations
262 (P, F6, F12, and F18), no significant differences were observed (kale, $F_{3, 16} = 0.31$, $P =$
263 0.8203, broccoli, $F_{3, 16} = 2.06$, $P = 0.1458$, and cabbage, $F_{3, 16} = 0.59$, $P = 0.6282$). When the
264 caterpillars were reared on kale, consumption between generations ranged from 3.8 cm² to 4.8
265 cm². In broccoli, consumption was between 3.3 cm² and 5.1 cm², and in cabbage,
266 consumption was 2.2 cm² 3.8 cm² (Table 1).

267 With regard to the duration of larval period, there was no significant difference between
268 cultivars ($F_{2,57} = 0.89$, $P = 0.4169$) with an average change from 6.7 to 7.1 days. In relation to
269 the generations, the larval period in kale was lower in P generation (6.0 days) ($F_{3,16} = 13.26$, P
270 <0.0001). In broccoli, there was a significant difference in the larval period of the P
271 generation (5.6 days), which displayed a shorter period than that displayed by the F18
272 generation (8.0 days) ($F_{3,16} = 5.40$, $P = 0.0093$). In cabbage, the shortest generation was
273 observed in the P generation (6.0 days), which differed from other generations ($F_{3,16} = 39.87$,
274 $P <0.0001$), with the largest period observed in the F18 generation (8.4 days) (Table 1).

275 In the larval stage, no significant differences were observed in the survival parameter
276 among cultivars ($F_{2, 57} = 0.17$, $P = 0.8459$) or between generations studied in: kale ($F_{3, 16} =$
277 1.73, $P = 0.2006$), broccoli ($F_{3, 16} = 0.78$, $P = 0.5230$), and cabbage ($F_{3, 16} = 1.65$, $P = 0.2185$).
278 The percentage of larval survival ranged from 56.0 to 90.0 among the three cultivars (Table
279 1).

280 When examining the pupal period, significant differences were observed among the
281 three cultivars ($F_{2, 57} = 7.28$, $P = 0.0015$). When the caterpillars were reared on cabbage, the
282 pupal period was shorter (3.2 days) than that on broccoli (3.9 days) and kale (3.6 days).
283 Significant differences were also observed between the generations reared on kale, with a
284 shorter pupal period observed in the P generation (2.4 days), and the longest observed in the
285 F18 generation (3.8 days) ($F_{3, 16} = 4, 44$, $P < 0.0188$). DBM reared on broccoli showed a

286 shorter pupal period in the F6 generation (3.0 days) than in the P generation (5.6 days) ($F_{3, 16}$
287 = 55.87, $P < 0.0001$; Table 1).

288 When examining the longevity of males, no differences were observed between the
289 three cultivars ($F_{2, 57} = 0.51$, $P = 0.6061$) with ranging from 15.4 to 17.0 days. However, there
290 was a significant difference between generations when the caterpillars were reared on kale
291 ($F_{3,16} = 5.23$, $P = 0.0010$) with a longer longevity of males observed in the P generation (24.7
292 days) (Table 2). There was no difference observed between the generations when the
293 caterpillars were reared on broccoli ($F_{3,16} = 0.67$, $P = 0.5837$) and cabbage ($F_{3,16} = 2.01$, $P =$
294 0.1525).

295 The longevity of females showed no differences among cultivars ($F_{2, 57} = 2.51$, $P =$
296 0.5274) and the average ranged from 13.8 to 15.4 days. However, there was a significant
297 difference between generations, when the caterpillars were reared on broccoli and cabbage
298 ($F_{3,16} = 6.98$, $P = 0.0032$) and ($F_{3,16} = 5.47$, $P = 0.0088$), respectively. In broccoli, there were
299 differences in the longevity of females; longevity was shorter in the F12 generation (10.5
300 days) than in the P generation (20.8 days). In cabbage, there was no difference between the
301 F12 generation (10.5 days) and P generation (20.8 days) ($F_{3,16} = 5.74$, $P = 0.0012$) (Table 2).

302 Regarding the average number of eggs per female, no difference was observed between
303 cultivars: kale, broccoli, and cabbage ($F_{2, 57} = 0.63$, $P = 0.5338$) with the average of 74.9 and
304 84.8 eggs. In the generations, a significant difference was observed between females reared
305 on kale, which had the lowest fertility in the P generation (60.8 eggs) and higher fertility in
306 the F18 generation (117.2 eggs) ($F_{3, 16} = 9.51$; $P = 0.0008$), and reared on cabbage, with lower
307 fecundity noted in the P generation (46.0 eggs) and higher in the F18 generation (100.2 eggs)
308 ($F_{3,16} = 4.84$, $P = 0.0140$) (Table 2).

309

310 *Fertility life table*

311

312 The net reproductive rate (R_0) was higher for the caterpillars reared on kale because
313 they produced more offspring per female than females reared on broccoli or cabbage in the
314 F6, F12, and F18 generations. Among the cultivars, the R_0 in kale was higher (64.8
315 females/female) than that in broccoli (57.1 females/female) and cabbage (53.0
316 females/female). Comparison of the R_0 females among generations showed that there was a
317 significant difference between F12 generation (83.3 females, which showed higher R_0 than P
318 generation (50.7 females) and F18 generation (53.9 females) (Table 3). This difference
319 between generations was also observed in broccoli, where the highest R_0 occurred in the P
320 generation (90.1) and the lowest in the F18 generation (30.1) (Table 3).

321 The intrinsic rate of increase (r_m) and finite rate of increase (λ) were higher for
322 caterpillars reared on kale compared with those reared on cabbage and broccoli, over two
323 generations. The developing kale sprouts generated an average of more descendants (0.37
324 females/female/day) than developed on broccoli (0.33 females/female/day) and cabbage (0.34
325 females/female/day). The λ was 1.43 female/female*day for kale, 1.40 females/female*day
326 for broccoli, and 1.39 females/female*day for cabbage. In comparisons between generations,
327 a significant difference was observed between the P and F18 generations for the two
328 parameters (r_m and λ) in broccoli. The r_m for the P generation was 0.39 females/female/day
329 compared to 0.27 females/female/day in the F18 generation. Since the λ in the P generation
330 was 1.48 female/female*day compared to 1.31 females/female*day in the F18 generation
331 (Table 3). A significant difference in parameters r_m and λ was also observed for larvae reared
332 on cabbage between P generations (1.32 females/female*day) and the other F6, F12, and F18
333 generations reared on cabbage (Table 3).

334 The generation time (T) was smaller for DBM reared on kale when compared to reared
335 on cabbage and broccoli. Thus, the T of cultivars was 11.6 days on kale, 11.9 days on

336 broccoli, and 12.0 days on cabbage. Comparing across generations, a significant difference
337 was observed between the F6 generation (13.1 days) and F12 generation (10.6 days) for the
338 larvae reared on broccoli. DBM showed longer T than P generation (13.4 days) than the other
339 generations reared on cabbage (Table 3).

340

341 *Feeding preference*

342

343 In dual-choice tests, the DBM larvae of the P generation that were reared on kale
344 preferred to feed on broccoli (68.7%) than cabbage ($\chi^2_{2,3} = 4.08$, $P = 0.0432$). DBM reared
345 on broccoli, in the same generation, preferred cabbage (68.6%) over kale ($\chi^2_{2,3} = 4.87$, $P =$
346 0.0273) (Fig 1A). In the F6 generation caterpillars reared on cabbage leaves preferred to feed
347 on cabbage (67.6%) when presented broccoli ($\chi^2_{2,3} = 4.12$, $P = 0.0422$) (Fig 1B). The insects
348 of the F12 generation held on broccoli leaves preferred to feed on cabbage (72.0%) when
349 presented broccoli ($\chi^2_{2,3} = 5.08$, $P = 0.0241$) (Fig 1C). The larvae maintained on cabbage
350 leaves in the F18 generation showed the same food substrate preference when presented with
351 broccoli (69.8%) ($\chi^2_{2,3} = 4.16$, $P = 0.0412$) and kale (74.0%) ($\chi^2_{2,3} = 6.11$, $P = 0.0134$) (Fig
352 1D).

353 In the multiple-choice tests of the P generation DBM reared on kale, the highest
354 percentage of caterpillars feeding was found on kale (46.0%) and broccoli (40.8%) compared
355 to feeding on cabbage leaves (13.2%). In the same generation, in caterpillars reared on
356 broccoli, a higher percentage of feeding was observed on broccoli (53.8%) and kale (41.8%)
357 compared to feeding on cabbage (4.4%). DBM reared on cabbage were found on broccoli
358 (52.7%) than on cabbage (34.5%). In the F6 generation, caterpillars grown on cabbage
359 preferred to feed again on cabbage (39.0%) and broccoli (44.0%), instead of kale (17%).

360 DBM in the F12 and F18 generations held on each cultivar (kale, broccoli or cabbage)
361 showed no preference for feeding among other cultivars.

362

363 *Oviposition preference*

364

365 In the dual-choice test, the DBM adult P generation originating from caterpillars reared
366 on broccoli preferred to lay eggs on broccoli (68.7%) when presented with cabbage ($\chi^2_{2,3} =$
367 11.29, $P = 0.0008$), but they preferred to oviposit on kale (59.8%) compared to broccoli ($\chi^2_{2,3}$
368 = 5.35, $P = 0.0206$) (Fig 2A). Adults originated the F6 generation reared on kale preferred to
369 lay eggs on cabbage (60.9%) when presented broccoli ($\chi^2_{2,3} = 15.53$, $P < 0.0001$). However,
370 when bred on broccoli, they displayed a preference for oviposition on kale (60.2%) ($\chi^2_{2,3} =$
371 8.65, $P = 0.0033$) or cabbage (59.5%) ($\chi^2_{2,3} = 8.05$, $P = 0.0045$) (Fig 2B).

372 In the F12 generation, the adults that originated from cabbage preferred to lay eggs on
373 cabbage (70.3%) ($\chi^2_{2,3} = 11.81$, $P = 0.0006$) and kale (69.0%) ($\chi^2_{2,3} = 12.12$, $P = 0.0005$) than
374 broccoli. Adults that originated from kale preferred to oviposit on cabbage (62.1%) ($\chi^2_{2,3} =$
375 14.21, $P = 0.0002$) (Fig 2C). In the F18 generation, the adults reared on cabbage preferred to
376 the oviposit on cabbage (71.5%) ($\chi^2_{2,3} = 11.04$, $P = 0.0009$) rather than on broccoli and also
377 preferred cabbage (65.5%) than in kale ($\chi^2_{2,3} = 5.69$, $P = 0.0170$). DBM reared in kale leaves
378 chose broccoli (54.0%) than cabbage ($\chi^2_{2,3} = 6.37$, $P = 0.0116$) (Fig 2D).

379 In multiple-choice tests, the P generation reared on cabbage oviposited the highest
380 percentage of eggs on broccoli (40.8%) and kale (33.6%), as compared to cabbage leaves
381 (25.6%). In the F6 generation, adults originating from caterpillars fed on cabbage preferred to
382 lay eggs in broccoli (42.3%). However, when grown in kale, in the same generation, adults
383 preferred lay eggs on the same substrate, kale (52.6% of the eggs). In the F18 generation,

384 insects reared on cabbage prefer to lay eggs on cabbage (43.2%) and those originally reared
385 on broccoli sprouts preferred kale (41.7%) and cabbage (34.9%).

386

387 **Discussion**

388 Although several studies on the life-history and behavior of the DBM in cultivars have
389 already been conducted in different parts of the world, this is the first time this type of
390 research compared different generations of the population of DBM in cruciferous plants. The
391 results showed that the larval survival of the pest was similar in all Brassicaceae cultivars
392 tested along generations. Therefore, there was no increased mortality among the cultivars
393 examined. However, other biological aspects have changed in this study. During the larval
394 period, cultivars of kale and broccoli were more readily consumed than cabbage. This average
395 lower consumption of cabbage leaves was observed in all generations. Similar observations of
396 lower consumption of DBM were reported in Midori cabbage cultivars and hybrid TPC 681
397 when compared to cultivated kale (Volpe *et al* 2008). The lower consumption may be
398 explained by the plants' physical characteristics, such as a waxy leaf surface. It has been
399 reported that occurs less leaf consumption of DBM larvae in plants with the alkane content
400 where there are most waxy (Eigenbrode *et al* 1991; Ulmer *et al* 2002).

401 Besides the aforementioned physical characteristics, the existence of deterrent
402 chemicals, such as ethanol extracts, that are present in Brassicaceae cultivars can increase the
403 consumption of *P. xylostella*. The amount of sinigrin present in the leaves of each plant
404 species changes the feeding behavior of the DBM larvae. This was evidenced in studies by
405 Thuler *et al* (2007) reported the absence of sinigrin in six commercial varieties of
406 Brassicaceae (including cabbage) created through genetic processes aimed at improving the
407 cultivars. In another study, the improvement of crop plants caused a reduction in
408 glucosinolate levels in cruciferous crops (Bodnaryk 1997).

409 The plants of the family Brassicaceae are characterized as containing glucosinolates in
410 different quantities. These compounds undergo hydrolysis via an endogenous myrosinase
411 enzyme when a tissue is damaged, resulting in the formation of substances toxic to insects,
412 such as thiocyanates, isothiocyanates, and nitriles epithionitriles (Halkier & Gershenzon 2006;
413 Hopkins *et al* 2009). When induced, glucosinolates are known to have a negative influence on
414 herbivores and play an important role in prevention of further damage to the plants (van Dam
415 & Raaijmakers 2006; Van Dam & Oomen 2008). However, populations of insects that
416 specialize in plants containing glucosinolates, such as DBM, can possess mechanisms to
417 overcome the toxicity of hosts (Halkier & Gershenzon 2006).

418 The larval development period was different across generations. When the larvae were
419 reared on kale, broccoli, and cabbage, the first generation (P) was completed in fewer days
420 than the other generations, especially the last generation (F18). This suggests that the
421 caterpillars anticipated the development stages for not being adapted cultivars (particularly
422 broccoli and cabbage), over the generations the larval period was longer lasting. Boiça Junior
423 *et al* (2011), working with kale genotypes, observed prolongation of the larval period in *P.*
424 *xylostella* for some genotypes for one generation of the pest. Other results were reported by
425 Veiga *et al* (2010) in kale, when compared to first generation DBM, which had lower larval
426 period (6.8 days) against population reared by 60 generations in the laboratory, which
427 presented longer larval period (9.1 days).

428 Our study also observed a lower pupal period for caterpillars reared on cabbage. The
429 average cabbage was higher due to compensation of the larval period in F18 generation of
430 cultivar, which was longer. Variations and compensation in the DBM development period
431 may exist between the larval and pupal when offered different cultivars. Sarfraz *et al* (2007)
432 observed a faster development of *P. xylostella* in cultivars of *Brassica juncea* var. Czern when
433 compared with *B. oleracea* var. Red Acre, *B. napus* var. Conquest and *B. napus* var. Liberty.

434 During adulthood, the average longevity of DBM males between cultivars and along 18
435 generations has not changed. Only occasional variation was found between generations for
436 insects reared on kale. On the kale cultivar, males survived 24 days, on average, to P
437 generation, surviving about 10 days longer than in broccoli and cabbage (Table 2).

438 In relation to female longevity, there was a change between the generations originating
439 from broccoli and cabbage. Females reared on both substrates survived for a longer period in
440 the P generation. However, the longer longevity did not correlate with the fertility parameter
441 presented in the study. For example, in the P generation, females survived for a longer period
442 in this generation, but the number of eggs deposited by females reared on cabbage was lower
443 in self-generation P (46.0 eggs) (Table 2). This difference observed in P generation
444 progressively disappeared in subsequent generations, showing that successive generations
445 have come to be beneficial for DBM.

446 The number of eggs deposited by females increased over generations in moths reared on
447 kale and cabbage (Table 2). Females deposited 1.95 and 2.17 times the eggs, respectively, in
448 the F18 generation compared to P generation. This increase in fertility not was observed when
449 studied up to the third successive generation of DBM. In other study, the number of eggs for
450 females reared on kale showed low variation, and was minimally affected along three
451 generations (Veiga *et al* 2010). The fecundity in insects of the Lepidoptera order is not altered
452 in adulthood (Leather 1988) because when the adult emerges, their eggs are already contained
453 in ovarioles. Then, changes in oviposit could be due to changes during the larval stage, as the
454 food substrate. Thus, the quality of the cultivar (host) during larval development would be the
455 most important step in reaching the maximum potential in fecundity of females (Awmack &
456 Leather 2002).

457 The life table parameters are the most important in estimating the success of a species
458 subjected to a particular environment, which in this case is the establishment of pest on

459 different host plants (Birch 1948). The population of *P. xylostella* reared on kale, in general,
460 showed the highest net reproduction rate (R_0) compared to those reared on broccoli and
461 cabbage. Therefore, populations reared on kale produced more offspring per female. The
462 evaluation of the chemical composition in cultivars is important for this parameter because,
463 according to Luengo (2011), kale, broccoli, and cabbage differ significantly in the amount of
464 nutrients and vitamin complexes they provide to feeding insects (Panizzi & Parra 2009).

465 Differences in net reproductive rate between the initial and final generations in kale and
466 broccoli were observed, but these variations were inconsistent in successive generations
467 (Table 3). It has been shown that variable and inadequate protein intake, as well as differences
468 in the quality of the different proteins and other nutrient levels, can generate a decrease or
469 increase in the growth rate of insects, occurring along the generations in each population
470 (Woods 1999; Sarfraz *et al* 2009).

471 The R_0 , r_m , and λ are important factors in the generation of descendants in a given
472 period, as indicated kale and cabbage best hosts. What about the comparisons between
473 generations, the P generation in broccoli was higher to r_m and λ parameters, indicating
474 increased production of offspring per female. However, in the F18 generation to increased
475 production of descendants it was obtained when insects were reared on kale and cabbage.

476 The results of the preference DBM between cultivars were different. In the double and
477 multiple-choice tests, caterpillars developed on kale and broccoli did not seek that substrate of
478 origin between generations. However, this occurrence was rarely observed with caterpillars
479 grown on cabbage, which demonstrated a feeding preference for discs containing the same
480 substrate (cabbage) in two of the four generations evaluated. This occurred in the F6, F12 and
481 F18 generations to feeding and oviposit, which may suggest a period of familiarization or
482 adjustment to the new substrate.

483 Although there are many studies of insect food preference tests, the analyses are based
484 on only one generation. In this study, differences between generations were analyzed, and
485 variations were found that do not indicate imaginal conditioning of *P. xylostella*. Insects
486 reared for several generations on a single substrate are influenced by a strong selection
487 pressure, causing a tendency to develop of insects, but this behavioral manifestation may take
488 many generations to occurred (Barros 1998).

489 Analyzing all generations until the last (F18), verified the existence of a pre-imaginal
490 conditioning for DBM on cabbage, which would support the argument for learning across
491 generations. This hypothesis is confirmed by the fact that in recent generations (F12 and F18),
492 females preferred the same substrate on which the insect was reared. Studies have shown that
493 in plants, sinigrin was related to the oviposition stimulation process in *P. xylostella* (Spencer
494 *et al* 1999). Structures such as trichomes and waxy present in small amounts in the leaves can
495 also encourage a choice for oviposition, as these leaves possess lower mechanical protection
496 (Hariprasad & Van Emden 2010). However, preference for oviposition of *P. xylostella* in
497 cabbage was probably triggered by a set of stimuli, both chemical and physical factors, and
498 sites with the most attractive stimuli were preferred for oviposition (Justus *et al* 2000).

499 This study evaluated the possible occurrence of conditioning over DBM generations and
500 contributes to the understanding of *P. xylostella* behavior in Brassicaceae cultivars. We
501 emphasize that our study was the first to consider the behavior of the pest over several
502 generations, thereby demonstrating that this insect behavior is complex and highly variable
503 over time. However, more research is needed on the approach conditioning so these
504 interactions can be better understood.

505

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620

621 Table 1 Biological parameters of the larval stage and the pupal stage of *P. xylostella* reared on
 622 different cultivars of Brassicaceae over generations.

Parameters	Brassicaceae	Generations of <i>P. xylostella</i>			
		P	F ₆	F ₁₂	F ₁₈
Leaf consumption (cm ²)	Kale	4.4 ± 0.37 Aa ¹	4.2 ± 0.59 Aab	3.8 ± 0.87 Aa	4.8 ± 0.96 Aa
	Broccoli	4.7 ± 0.34 Aa	5.9 ± 1.24 Aa	3.3 ± 0.65 Aa	5.1 ± 0.42 Aa
	Cabbage	2.9 ± 0.74 Ab	2.2 ± 0.40 Ab	2.5 ± 0.95 Aa	3.4 ± 0.69 Aa
Larval period (days)	Kale	6.0 ± 0.00 Ba	7.4 ± 0.24 Aa	7.6 ± 0.24 Aa	7.4 ± 0.24 Ab
	Broccoli	5.6 ± 2.24 Ba	7.0 ± 0.32 ABa	6.2 ± 0.80 ABa	8.0 ± 0.24 Aab
	Cabbage	6.0 ± 0.00 Ba	7.0 ± 0.00 ABa	6.8 ± 0.20 ABa	8.4 ± 0.00 Aa
Larval survival (%)	Kale	66.0 ± 10.29 Aa	70.0 ± 7.07 Aa	62.0 ± 11.13 Aa	88.0 ± 4.89 Aa
	Broccoli	82.0 ± 4.89 Aa	66.0 ± 11.66 Aa	70.0 ± 8.94 Aa	78.0 ± 5.83 Aa
	Cabbage	56.0 ± 16.00 Aa	90.0 ± 10.00 Aa	70.0 ± 7.07 Aa	64.0 ± 10.29 Aa
Pupal period (days)	Kale	2.4 ± 0.24 Bb	3.0 ± 0.35 ABa	3.6 ± 0.24 ABa	3.8 ± 0.48 Aa
	Broccoli	5.6 ± 0.24 Aa	3.0 ± 0.42 Ba	3.2 ± 0.20 ABa	4.0 ± 0.35 ABa
	Cabbage	2.8 ± 0.20 Ab	3.2 ± 0.20 Aa	3.2 ± 0.20 Aa	2.2 ± 0.49 Ab

623 ¹Means ± (SE) followed by different letters in the column, for each parameter, differ by Tukey test (P < 0.05).

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625 Table 2 Biological characteristics of the adult phase DBM reared on different cultivars of
 626 Brassicaceae over generations.

Parameters	Brassicaceae	Generations of <i>P. xylostella</i>			
		P	F ₆	F ₁₂	F ₁₈
Male	Kale	24.7 ± 0.24 Aa ¹	12.2 ± 1.74 Bb	14.8 ± 1.71 Ba	16.3 ± 3.16 Ba
longevity (days)	Broccoli	14.8 ± 2.21 Ab	18.1 ± 2.57 Aa	15.0 ± 2.03 Aa	13.6 ± 1.40 Aa
	Cabbage	14.0 ± 2.31 Ab	16.2 ± 1.28 Aab	17.6 ± 1.96 Aa	18.2 ± 2.57 Aa
Female	Kale	16.0 ± 1.51 Aa	11.4 ± 1.36 Aa	13.7 ± 1.44 Aa	14.1 ± 1.49 Aa
longevity (days)	Broccoli	20.8 ± 1.48 Aa	15.5 ± 2.11 ABa	10.5 ± 1.84 Ba	13.3 ± 1.24 ABa
	Cabbage	20.8 ± 1.86 Aa	16.0 ± 1.64 ABa	10.5 ± 2.25 Ba	14.9 ± 1.22 ABa
Number of eggs/female	Kale	60.8 ± 5.81 Bb	82.6 ± 7.17 ABa	79.0 ± 10.28 ABa	117.2 ± 6.52 Aa
	Broccoli	97.6 ± 8.97 Aa	75.8 ± 15.07 Aa	77.6 ± 16.06 Aa	56.4 ± 6.68 Ab
	Cabbage	46.0 ± 3.81 Bb	92.8 ± 5.86 ABa	60.6 ± 16.42 ABa	100.2 ± 15.27 Aa

627 ¹Means ± (SE) followed by different letters in the column, for each parameter, differ by Tukey test (P < 0.05).

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631 Table 3 Fertility life table parameters of *P. xylostella* after rearing on different cultivars of
 632 Brassicaceae.

Parameters	Brassicaceae	Generations			
		P	F ₆	F ₁₂	F ₁₈
R ₀	Kale	50.7 ± 13.61 Bb ¹	71.9 ± 24.93 ABa	83.3 ± 26.37 Aa	53.9 ± 16.08 Ba
	Broccoli	90.1 ± 13.08 Aa	60.6 ± 45.53 ABab	47.5 ± 32.40 ABb	30.1 ± 12.91 Bb
	Cabbage	42.3 ± 10.02 Ab	49.1 ± 15.59 Ab	66.6 ± 45.36 Aab	53.9 ± 22.81 Aa
r _m	Kale	0.35 ± 0.04 a	0.35 ± 0.04 a	0.38 ± 0.05 a	0.34 ± 0.03 a
	Broccoli	0.39 ± 0.08 Aa	0.31 ± 0.07 ABa	0.36 ± 0.13 ABa	0.27 ± 0.04 Bb
	Cabbage	0.28 ± 0.02 b	0.34 ± 0.02 a	0.39 ± 0.06 a	0.34 ± 0.04 a
λ	Kale	1.42 ± 0.06 Aa	1.42 ± 0.05 Aa	1.47 ± 0.07 Aa	1.40 ± 0.04 Aa
	Broccoli	1.48 ± 0.12 Aa	1.37 ± 0.09 ABa	1.44 ± 0.18 ABa	1.31 ± 0.06 Bb
	Cabbage	1.32 ± 0.03 Bb	1.40 ± 0.03 ABa	1.47 ± 0.09 Aa	1.39 ± 0.06 ABa
T	Kale	11.0 ± 0.78 Ab	12.1 ± 1.31 Aab	11.6 ± 1.27 Aa	11.7 ± 0.55 Aa
	Broccoli	11.4 ± 2.21 Bb	13.1 ± 1.03 Aa	10.6 ± 2.05 Ba	12.5 ± 1.51 Ba
	Cabbage	13.4 ± 0.68 Aa	11.6 ± 1.32 Bb	11.0 ± 0.33 Ba	11.9 ± 0.92 Ba

633 ¹Means ± (CI) followed by different letters in the column, for each parameter, differ by Student t test for paired
 634 groups.

635 R₀ = net reproductive rate (females/female); r_m = intrinsic rate of increase; λ = finite rate of increase
 636 (females/day); T = average generation time (days).

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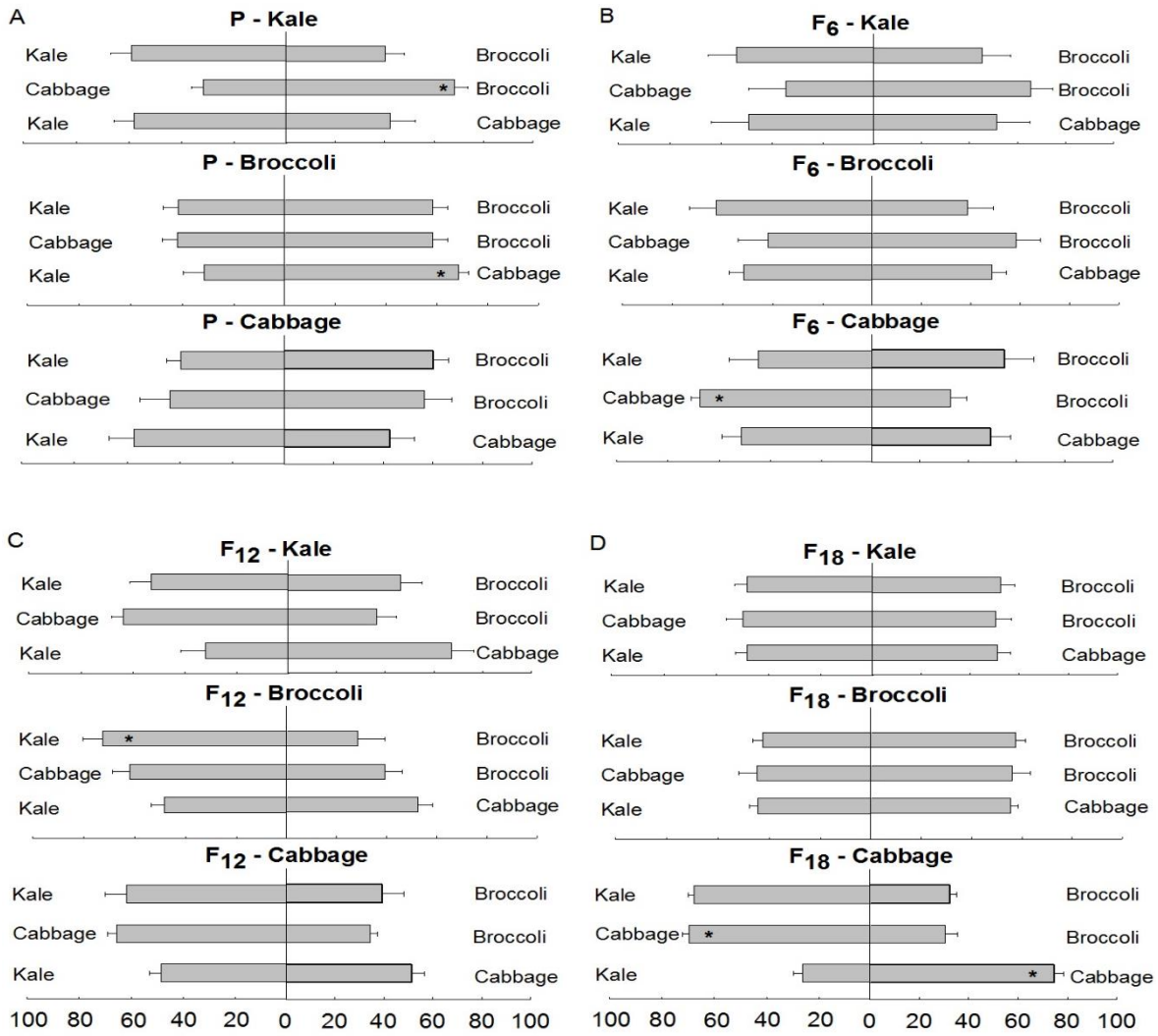


Fig 1 Percent (\pm SE) of *P. xylostella* larvae in double-choice testes, different generations and cultivars grown on Brassicaceae. *Indicates significant difference between treatments using the chi-square test at 0.05% probability.

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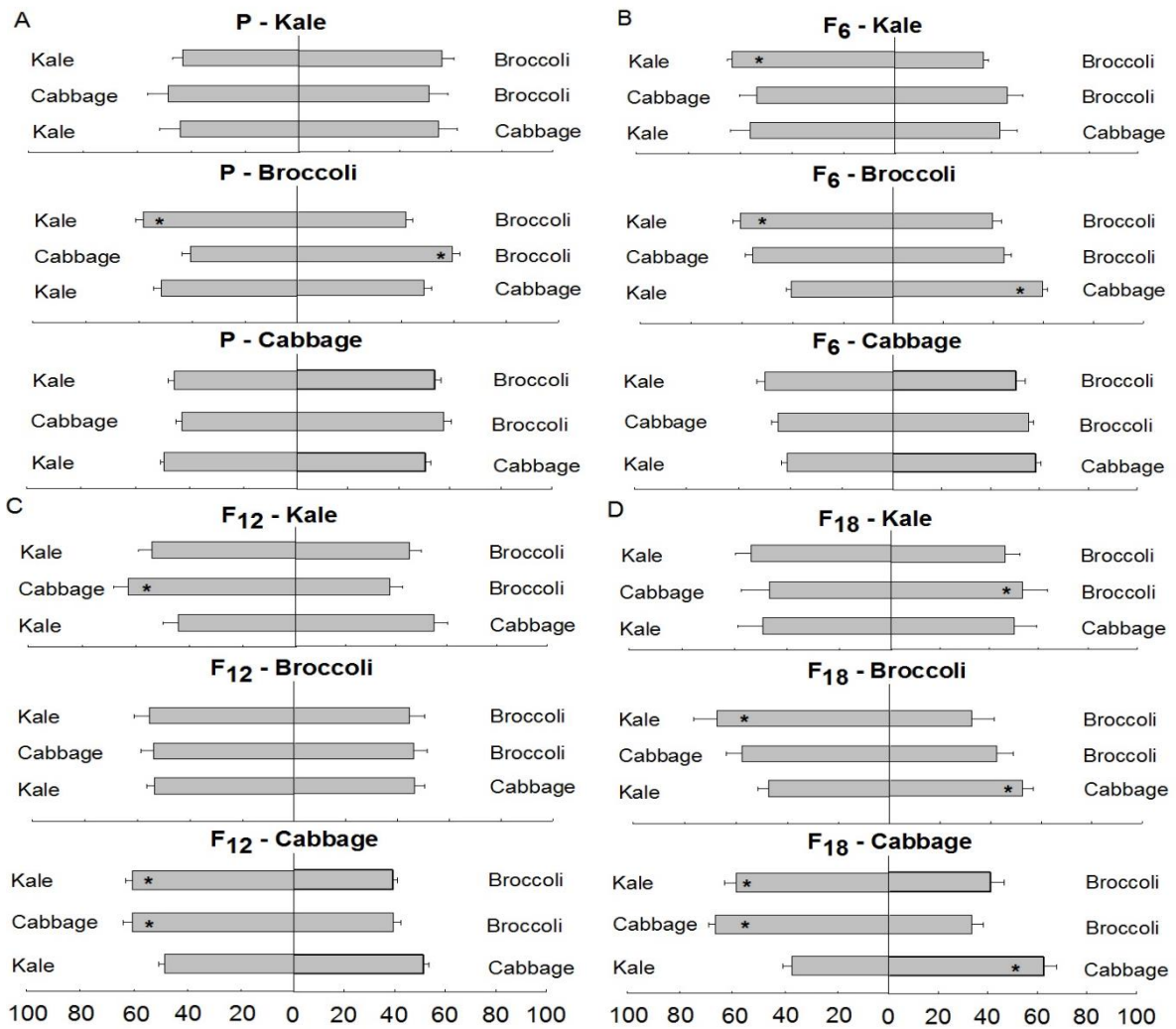


Fig 2 Percentage (\pm SE) oviposition of *P. xylostella* in double-choice testes, different generations and cultivars grown on Brassicaceae. *Indicates significant difference between treatments using the chi-square test at 0.05% probability.