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

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Abstract

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a cosmopolitan pest that causes leaf-area reduction in Brassicaceae plants. DBM populations have significant genetic variability that manifests in different feeding preferences and reproductive behaviors across generations. We evaluated the influence of Brassicaceae cultivars on biological and behavioral parameters across 18 generations of DBM populations that were separated and held on three varieties of Brassicaceae: *Brassica oleracea* var. *acephala* (kale), *Brassica oleracea* var. *italica* (broccoli), and *Brassica oleracea* var. *capitata* (cabbage). P, F6, F12, and F18 generations were evaluated, and biological aspects of young adulthood and fertility parameters of adults held on each host plant were examined over multiple generations. Additionally, larvae and adults were subjected to dual-choice and multiple-choice (feeding and oviposition) between cultivars, over generations. The results indicated that larvae of *P. xylostella* consumed greater quantities of kale and broccoli cultivars, on average (4.05 cm^2), than cabbage (2.7 cm^2). The number of eggs per female in F18 generation was 1.95 and 2.17 times higher than those in the parental (P) generation, when reared on kale and cabbage. The population reared exclusively on kale had higher net population growth rate (R_0), intrinsic rate (r_m), finite rate (λ) and generation time (T) than that reared on broccoli and cabbage. Last generations evaluated, the larval stage reared on cabbage showed feeding preference (F18) and oviposition preference (F12 and F18) for cabbage. Thus, we note the existence of learning, characterized as pre-imaginal conditioning to cabbage cultivars, over various DBM generations.

Keywords

Insect biology, feeding preference, oviposition preference, conditioning.

Introduction

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

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

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

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

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

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

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

Material and methods

The studies were conducted at Laboratory of Biology and Insects Rearing (LBIR), Department of Plant Protection, UNESP, Jaboticabal, Sao Paulo, Brazil. The insects used in the rearing experiments (~ 185 generations) were kept following a methodology adapted from Thuler (2009) on studies with *Brassica oleracea* var. *acephala*-kale HS20 (Horticeres®, Indaiatuba, Sao Paulo, Brazil) at LBIR. DBM populations were reared in a room at 25 ± 1°C, relative humidity of 70 ± 10% and photoperiod of 12 h light and 12 h dark.

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

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

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

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

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

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

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

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

Fertility life table

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

The biological data obtained permited estimates of the parameters needed for the construction of fertility life tables (Price, 1984), which are as follows: x = mean age of the female parental (seen as the emergence of the egg stage); lx = life expectancy to age x, expressed as a female; mx = specific fertility or number of offspring produced per female at age x that result in females; lx.mx = total number of females at age x. Growth parameters resulting from the life table were evaluated as follows: R_0 = net reproductive rate (considering the number of females produced by females in generation), r_m = intrinsic rate of increase in number, λ = finite rate of increase (the number of times the population multiplies in unit time), and T = average generation time in days. The growth parameters (R0, r_m, λ and T) were calculated using the following equations:

$R_0 = \sum (mx.lx)$

$r_m = \ln . R_0 / T$

188 $\lambda = e^{rm}$

189 $T = (\sum mx.lx.x) / (\sum mx.lx)$

190

191 *Feeding preference*

192

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

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

209

210 *Oviposition preference*

211

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

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

Data analysis

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

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

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

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

Results

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

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

broccoli (4.1 cm^2). However, when we evaluated the leaf consumption between generations (P, F6, F12, and F18), no significant differences were observed (kale, $F_{3, 16} = 0.31$, $P = 0.8203$, broccoli, $F_{3, 16} = 2.06$, $P = 0.1458$, and cabbage, $F_{3, 16} = 0.59$, $P = 0.6282$). When the caterpillars were reared on kale, consumption between generations ranged from 3.8 cm^2 to 4.8 cm^2. In broccoli, consumption was between 3.3 cm^2 and 5.1 cm^2, and in cabbage, consumption was 2.2 cm^2 3.8 cm^2 (Table 1).

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

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

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

shorter pupal period in the F6 generation (3.0 days) than in the P generation (5.6 days) ($F_{3, 16}$ = 55 87, $P < 0.0001$; Table 1).

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

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

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

Fertility life table

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

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

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

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

Feeding preference

In dual-choice tests, the DBM larvae of the P generation that were reared on kale preferred to feed on broccoli (68.7%) than cabbage ($\chi^2_{2,\ 3}$ = 4.08, *P* = 0, 0432). DBM reared on broccoli, in the same generation, preferred cabbage (68.6%) over kale ($\chi^2_{2,\ 3}$ = 4.87, *P* = 0.0273) (Fig 1A). In the F6 generation caterpillars reared on cabbage leaves preferred to feed on cabbage (67.6%) when presented broccoli ($\chi^2_{2,\ 3}$ = 4.12, *P* = 0.0422) (Fig 1B). The insects of the F12 generation held on broccoli leaves preferred to feed on cabbage (72.0%) when presented broccoli ($\chi^2_{2,\ 3}$ = 5.08, *P* = 0.0241) (Fig 1C). The larvae maintained on cabbage leaves in the F18 generation showed the same food substrate preference when presented with 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 1D).

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

361 DBM in the F12 and F18 generations held on each cultivar (kale, broccoli or cabbage)
362 showed no preference for feeding among other cultivars.
363
364 *Ovipositon preference*
365
366 In the dual-choice test, the DBM adult P generation originating from caterpillars reared
367 on broccoli preferred to lay eggs on broccoli (68.7%) when presented with cabbage ($\chi^2_{2,3}$ =
368 11.29, P = 0, 0008), but they preferred to oviposit on kale (59.8%) compared to broccoli ($\chi^2_{2,3}$
369 = 5.35, P = 0.0206) (Fig 2A). Adults originated the F6 generation reared on kale preferred to
370 lay eggs on cabbage (60.9%) when presented broccoli ($\chi^2_{2,3}$ = 15.53, P <0.0001). However,
371 when bred on broccoli, they displayed a preference for oviposition on kale (60.2%) ($\chi 2_{2,3}$ =
372 8.65, P = 0.0033) or cabbage (59.5%) ($\chi^2_{2,3}$ = 8.05, P = 0.0045) (Fig 2B).
373 In the F12 generation, the adults that originated from cabbage preferred to lay eggs on
374 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
375 broccoli. Adults that originated from kale preferred to oviposit on cabbage (62.1%) ($\chi^2_{2,3}$ =
376 14.21, P = 0.0002) (Fig 2C). In the F18 generation, the adults reared on cabbage preferred to
377 the oviposit on cabbage (71.5%) ($\chi^2_{2,3}$ = 11.04, P = 0.0009) rather than on broccoli and also
378 preferred cabbage (65.5%) than in kale ($\chi^2_{2,3}$ = 5.69, P = 0.0170). DBM reared in kale leaves
379 chose broccoli (54.0%) than cabbage ($\chi^2_{2,3}$ = 6.37, P = 0.0116) (Fig 2D).
380 In multiple-choice tests, the P generation reared on cabbage oviposited the highest
381 percentage of eggs on broccoli (40.8%) and kale (33.6%), as compared to cabbage leaves
382 (25.6%). In the F6 generation, adults originating from caterpillars fed on cabbage preferred to
383 lay eggs in broccoli (42.3%). However, when grown in kale, in the same generation, adults
384 preferred lay eggs on the same substrate, kale (52.6% of the eggs). In the F18 generation,

385 insects reared on cabbage prefer to lay eggs on cabbage (43.2%) and those originally reared
386 on broccoli sprouts preferred kale (41.7%) and cabbage (34.9%).
387
388 **Discussion**
389 Although several studies on the life-history and behavior of the DBM in cultivars have
390 already been conducted in different parts of the world, this is the first time this type of
391 research compared different generations of the population of DBM in cruciferous plants. The
392 results showed that the larval survival of the pest was similar in all Brassicaceae cultivars
393 tested along generations. Therefore, there was no increased mortality among the cultivars
394 examined. However, other biological aspects have changed in this study. During the larval
395 period, cultivars of kale and broccoli were more readily consumed than cabbage. This average
396 lower consumption of cabbage leaves was observed in all generations. Similar observations of
397 lower consumption of DBM were reported in Midori cabbage cultivars and hybrid TPC 681
398 when compared to cultivated kale (Volpe *et al* 2008). The lower consumption may be
399 explained by the plants' physical characteristics, such as a waxy leaf surface. It has been
400 reported that occurs less leaf consumption of DBM larvae in plants with the alkane content
401 where there are most waxy (Eigenbrode *et al* 1991; Ulmer *et al* 2002).
402 Besides the aforementioned physical characteristics, the existence of deterrent
403 chemicals, such as ethanol extracts, that are present in Brassicaceae cultivars can increase the
404 consumption of *P. xylostella*. The amount of sinigrin present in the leaves of each plant
405 species changes the feeding behavior of the DBM larvae. This was evidenced in studies by
406 Thuler *et al* (2007) reported the absence of sinigrin in six commercial varieties of
407 Brassicaceae (including cabbage) created through genetic processes aimed at improving the
408 cultivars. In another study, the improvement of crop plants caused a reduction in
409 glucosinolate levels in cruciferous crops (Bodnaryk 1997).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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621

622 Table 1 Biological parameters of the larval stage and the pupal stage of *P. xylostella* reared on

623 different cultivars of Brassicaceae over generations.

Parameters	Brassicaceae	Generations of *P. xylostella*			
		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

624 [1]Means ± (SE) followed by different letters in the column, for each parameter, differ by Tukey test ($P < 0.05$).

625

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

Parameters	Brassicaceae	Generations of *P. xylostella*			
		P	F_6	F_{12}	F_{18}
Male longevity (days)	Kale	24.7 ± 0.24 Aa[1]	12.2 ± 1.74 Bb	14.8 ± 1.71 Ba	16.3 ± 3.16 Ba
	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 longevity (days)	Kale	16.0 ± 1.51 Aa	11.4 ± 1.36 Aa	13.7 ± 1.44 Aa	14.1 ± 1.49 Aa
	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

628 [1]Means ± (SE) followed by different letters in the column, for each parameter, differ by Tukey test ($P < 0.05$).

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

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

R_0 = net reproductive rate (females/female); r_m = intrinsic rate of increase; λ = finite rate of increase (females/day); T = average generation time (days).

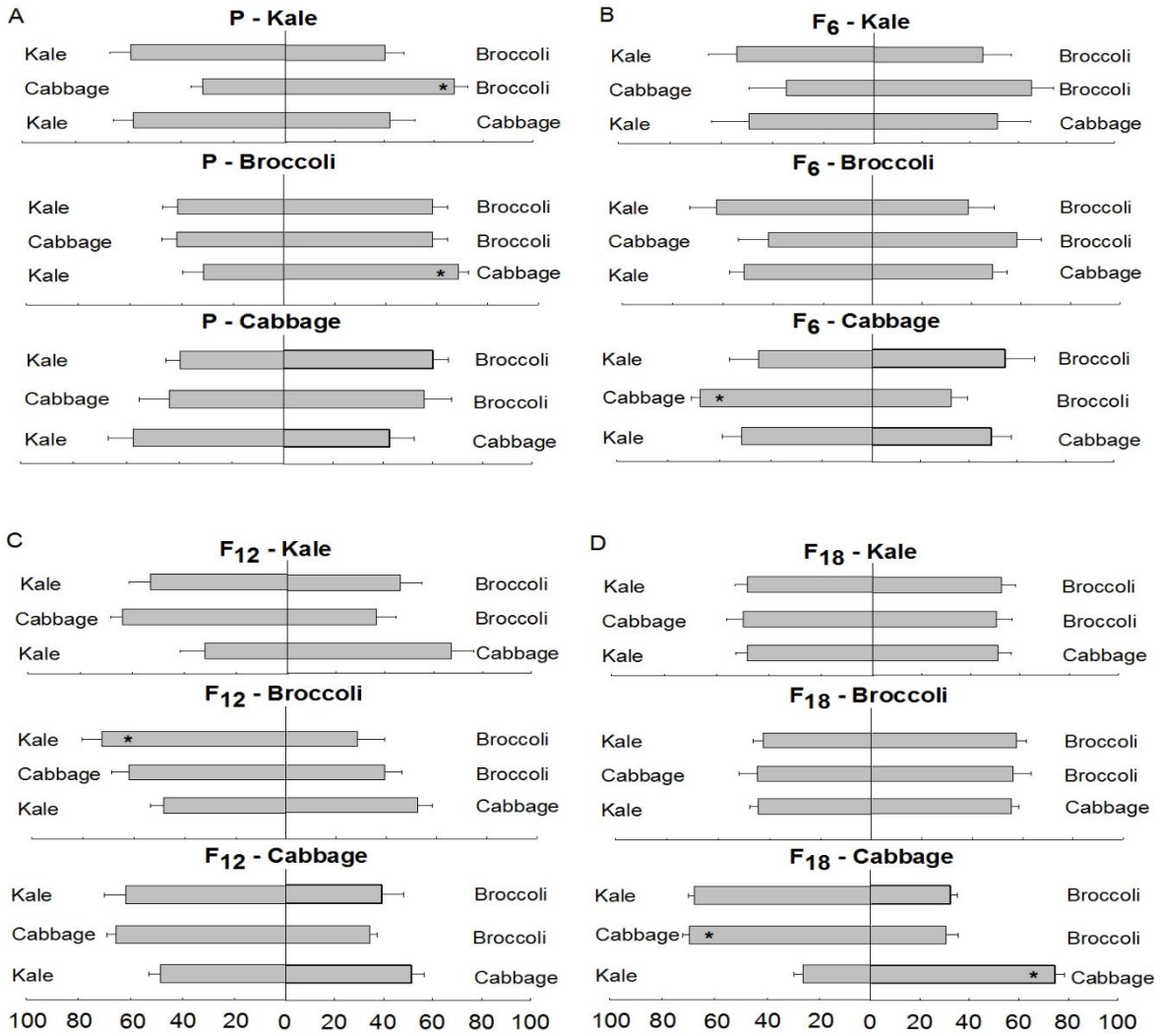

Fig 1 Percent (± SE) of *P. xylostella* larvae in double-choice testes, different generations and cultivars grown on Brassicaceae. *Indicates significant difference between treatments using the chi-square test at 0.05% probability.

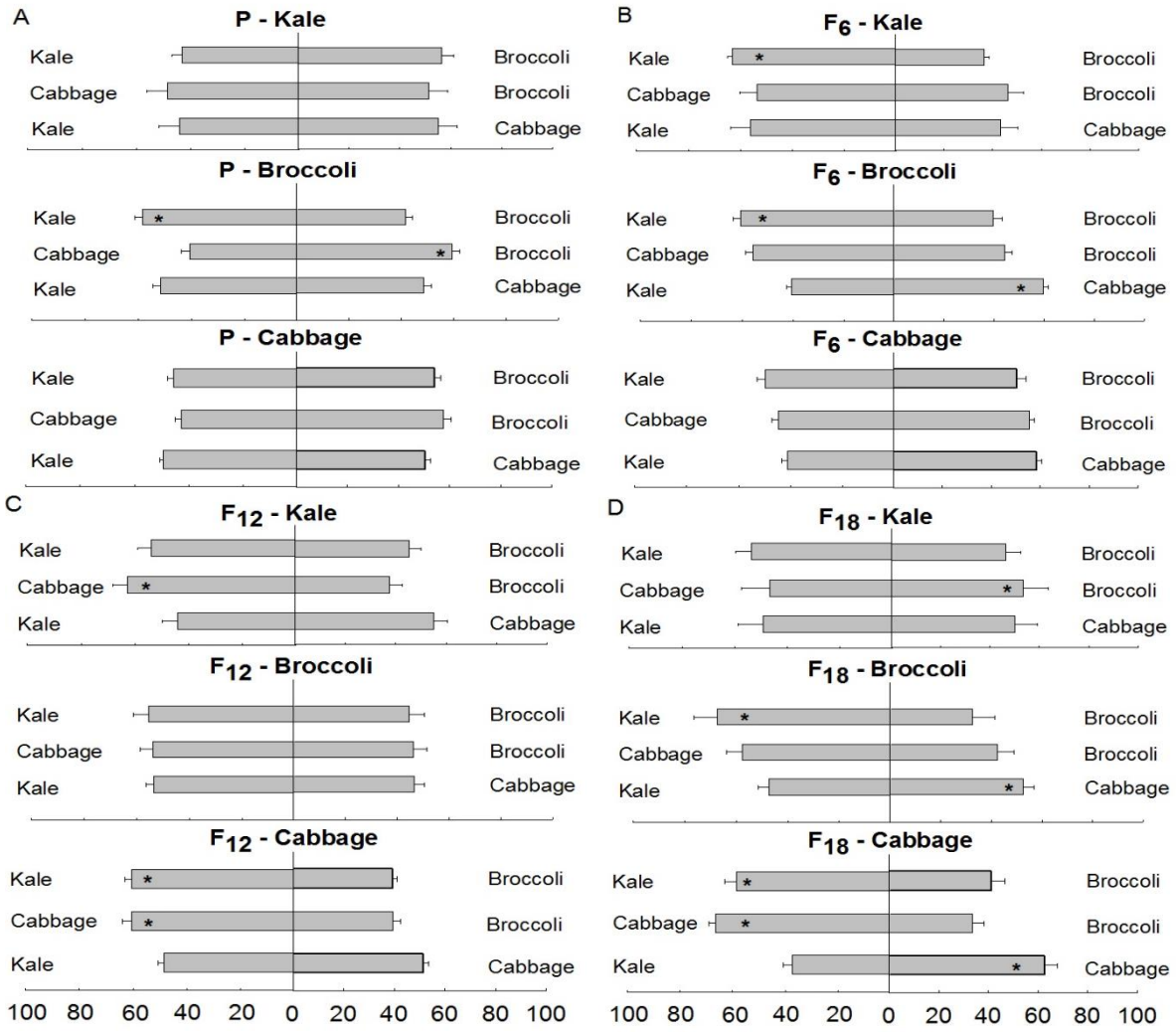

Fig 2 Percentage (± SE) oviposition of *P. xylostella* in double-choice testes, different generations and cultivars grown on Brassicaceae. *Indicates significant difference between treatments using the chi-square test at 0.05% probability.