Effect of Canola Physical Mutation on *Plutella xylostella* (L.) Life Table

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**ABSTRACT**

Life history of insect herbivores is unclear after feeding on mutant lines of many crops. To shed light, demographic parameters of *Plutella xylostella* on three canola cultivars (“Zar”, “RGS”, “Talaye”) and their physical mutation-derived lines (“Zar 9-9”, “RGS 8-1”, “RGS 10-2”, “RGS 8-13” and “Talaye 8-3”) were determined under greenhouse condition. Methods of life table including the female age specific life table and the age-stage, two-sex life table were applied. According to two-sex life table, there was no significant difference between demographic parameters of *P. xylostella* on “Talaye” and “Zar” in comparison with their mutant lines, but significant differences were observed between these parameters on mutant lines of “RGS”. Having suitable cultural traits, “RGS 8-1” was more susceptible than the other two mutant lines and its control cultivar “RGS” in terms of population growth of the pest. According to the two-sex life table, the net reproductive rate (*R₀*), intrinsic rate of increase (*r*), and finite rate of increase (*λ*) were the highest on RGS 8-1 (98.63 offspring per individual, 0.208 and 1.231 day⁻¹, respectively). Also, population projection showed the rapid growth of the pest on the latter line. There was a little difference between the same population parameters estimated by two methods of life table. Investigating some consequences of plant breeding using radiation techniques on insect fitness not only leads plant breeders to do more unfailing selections but also provides some enlightenment in pest management programs effectively when plantation of such crops is prioritized.

**Keywords:** Age-stage two-sex life table, Diamondback moth, Female age-specific life table, Gamma ray, Insect fitness.

**INTRODUCTION**

Canola, *Brassica napus* L., is an important economic oilseed crop highly impacted by one of its important pests, diamondback moth, *Plutella xylostella* L. (Lep.: Plutellidae) (Furlong *et al*., 2013) that causes more than 90% crop loss annually (Talekar and Shelton, 1993). Mutation breeding is an important branch of plant breeding that has been reported to improve self-pollinated crops such as canola. Producing plant varieties through this technique causes random, multiple genetic modifications in plants (Novak and Brunner, 1992). These changes can be within the gene, losses or additions of genes or groups of genes and, probably, changes in the relations of genes to each other (Ahloowalia and Maluszynski, 2001). Moreover, interaction of gamma rays with atoms or molecules of plants may produce free radicals in their cells; thereby cellular structure of plants and their metabolism are impacted depending on the irradiation level (Dhanavel *et al*., 2012). The effects of all these alterations on plant quality and higher trophic levels such as herbivores and their natural enemies, are unknown. To discover...
the effect of host plant quality on survival rate and reproduction of an arthropod population, calculation of demographic parameters can be applied as an important tool (Southwood and Henderson, 2000). Accordingly, evaluation of host plants antibiotic resistance to pest insects will be conceivable (Maia et al., 2000).

Chi and Liu (1985) pointed out that the traditional age-specific life-table does not consider male populations, the stage differentiation and the variable developmental rate among individuals. Disregarding these items in the calculations of demographic parameters may cause errors. These errors are related to errors in calculating the \( l_x \) and \( m_x \) (Huang and Chi, 2011). In other words, it is generally assumed that all female adults emerge at the same age. If \( m_x \) and \( l_x \) are constructed based on adult age and female individuals, they may also cause errors in population parameters (Chi and Su, 2006; Huang and Chi, 2011). Therefore, they constructed an age-stage, two-sex life table to consider the attributes in question.

To highlight the potential effects of physical mutation on fitness of insect pests, we aimed to study demographic parameters of \( P. \) xylostella as a model insect on some mutant lines of canola ("Zar 9-9", "RGS 8-1", "RGS 10-2", "RGS 8-13" and "Talaye 8-3") contrasting to their control cultivars ("Zar", "RGS", "Talaye"), and to analyze the data using age-stage, two-sex life table and the female age-specific life table procedures.

**MATERIALS AND METHODS**

**Modification of Seeds by Physical Mutation**

Gamma irradiation of canola seeds was carried out at Nuclear Science and Technology Research Institute (NSTRI), Karaj, Iran. The mutant canola lines, "RGS 8-1" and "RGS 8-13" were produced from cultivar "RGS" seeds irradiated at 800 Gy. "RGS 10-2" also resulted from the same cultivar but it was irradiated at 1000Gy. "Talaye 8-3" was obtained by irradiation of seeds from cultivar "Talaye" at 800Gy and "Zar 9-9" mutant line was derived from cultivar "Zar" seeds irradiated at 900 Gy. Physical mutation aimed at induction of high yield and early maturity in experimental varieties.

**Plants and Insects Rearing**

Seeds of canola cultivars and their mutant lines were sown in a 1:1:1 mix of peat moss: perlite: soil in 30 cm diameter plastic pots. Five-week-old plants were used in the present experiments. The colony of \( P. \) xylostella was established two months prior to initiation of the experiments with 30 adults collected from a cabbage field located in Karaj (35° 48' N, 51° 00' E), Iran. Colonies of \( P. \) xylostella were kept in ventilated cages (80×80×60 cm). Both plants and insects were reared under greenhouse condition (27±5°C, 65±10 RH, and a photoperiod of 16:8 L:D).

**Demographic Parameters**

All experiments were carried out under greenhouse conditions simultaneously. Thirty pairs of both sexes of \( P. \) xylostella were transferred from the colony to ventilated Plexiglas prepared as oviposition cages (28×23×15 cm) for 24 hours. Synchronized eggs with longevity of less than 24 hours were glued to the upper surface of a leaf of the potted plant individually; then, each leaf containing an egg was confined with a clip cage (9×7×2.5 cm). Fifty two to 81 clip cages were used in each treatment. All experimental units (clip cages) were observed daily until adults emerged. Then, newly emerged females were coupled with males from the same cohort in ventilated Plexiglas cylindrical cages (10 cm in diameter and 9 cm in height). The fecundity of females, longevity
and mortality of both sexes was determined daily during the mature stage. Experiments were continued until all individuals died.

**Statistical Analysis**

**Female Age-specific Life Table:** Age-specific fecundity \((m_x)\) and survivorship \((l_x)\) were determined for *P. xylostella* cohorts on each host plant based on female individuals. The iterative bisection method and the Euler–Lotka Equation with the age indexed from 0 to maximum age \((\omega)\) were applied to estimate the intrinsic rate of natural increase \((r)\).

\[
\sum_{x=0}^{\omega} e^{-r(x+1)} l_x m_x = 1
\]

Population growth parameters (net Reproductive rate \((R_0)\), mean generation Time \((T)\), finite rate of increase \((\lambda)\) and intrinsic rate of natural increase \((r)\) were estimated based on female population according to Carey (1993) and Maia et al. (2000). Data on “RGS” and its mutant lines were analyzed with unbalanced one-way ANOVA (Proc GLM, SAS Institute 2003 (ver. 9.1)) and their means were compared using the Tukey–Kramer procedure \((P<0.05)\) while the same values on two other cultivars in contrast with their mutant lines was compared using Student’s *t*-test \((P<0.05)\) (Minitab Inc., 2007).

**Age-stage, Two-sex Life Table:** The life history data of *P. xylostella* including males, females and immature individuals that died before the adult stage were analyzed according to the age-stage, two-sex life table (Chi and Liu, 1985; Chi, 1988) using the TWOSEX-MSChart (Chi, 2015). The age-specific survivorship \((l_x)\) including both male and female, the age-specific fecundity \((m_x)\), and \(R_0\) were calculated according to Chi and Liu (1985) as below:

\[
l_x = \sum_{j=1}^{\beta} s_{xj}
\]

\[
m_x = \frac{\sum_{j=1}^{\beta} s_{xj} f_{xj}}{\sum_{j=1}^{\beta} s_{xj}}
\]

\[
R_0 = \sum_{x=0}^{\infty} \sum_{j=1}^{\beta} s_{xj} f_{xj}
\]

Where, \(\beta\) is the number of stages. The intrinsic rate of increase was calculated by using the bisection method and Euler-Lotka Equation with age indexed from 0 (Goodman, 1982):

\[
\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1
\]

Moreover, \(T\) and \(\lambda\) were calculated as:

\[T = \frac{\ln R_0}{r}\]

\[\lambda = R_0\]

All parameters were estimated based on both sexes and the variable developmental rates among individuals (Naseri et al., 2014; Safuraie-Parizi et al., 2014; Mahmoudi et al., 2015). The variability of life table parameters was estimated in bootstrap procedure \((B=10,000)\). The bootstrap values on “RGS” and its mutant lines were compared using the paired bootstrap test \((P<0.05)\). The same values on “Talaye” and “Zar” as opposed to their mutant lines were compared using *t*-test \((P<0.05)\).

The program TIMING-MSChart was used to project population growth (Chi 1990, 2015). For the projection, we assumed an unlimited growth with an initial population of 10 newly laid eggs of *P. xylostella*.

**RESULTS**

**Female Age-Specific Life Table of *P. xylostella***

Survivorship \((l_x)\) and fecundity \((m_x)\) curves were demonstrated based on the female age-specific life table (Figure 1). The \(l_x\) schedule of the pest on all examined treatments showed a similar pattern in general. High mortality occurred during the first 10 days in periods of incubation and first-instar larvae. Age-specific survivorship of *P. xylostella* on “RGS 8-1”, “RGS 10-2”, “RGS 8-13”, “RGS”, “Talaye”, “Talaye 8-3”, “Zar” and “Zar 9-9” at the time of adult female emergence were 0.583, 0.471, 0.44,
According to the curve of $m_x$, the oviposition period of females began on days 17, 21, 23, 18, 21, 22, 16, 19 on the above mentioned varieties, respectively. Also, the highest daily fecundity of *P. xylostella* on the above mentioned varieties was 22.53, 10.19, 14.46, 14.31, 10.62, 11, 18.06 and 14.38 female offspring, respectively (Figure 1).

Based on the demographic parameters obtained from female age-specific life table, there were significant differences between $r$ values of *P. xylostella* on "RGS" and its mutant lines (Table 1). The cohort reared on "RGS 8-1" had the highest $r$ (F= 39.73; df= 3; P< 0.0001) and the lowest $T$ value (F= 19.74; df= 3; P< 0.0001). Also, there was no significant difference between the parameters on cultivar "RGS 8-13" and its mutant line "RGS 8-1" (Table 1). In addition, the $R_0$ value of *P. xylostella* on "Zar" was significantly higher (T= -2.57; df= 24; P= 0.017) compared to that on "Zar 9-9". Furthermore, there was no significant difference among other population parameters on "Zar 9-9" (Table 1). In addition, the $R_0$ value of *P. xylostella* on "Zar 9-9" was significantly higher (T= -2.57; df= 24; P= 0.017) compared to that on "Zar 9-9". Furthermore, there was no significant difference among other population parameters on "Zar 9-9" (Table 1).

The age-stage specific survival rates ($s_{xj}$) of *P. xylostella* on different treatments are shown in Figure 2. The curves of $s_{xj}$ demonstrated the survival rate, stage differentiation, and the variable developmental rate among individuals. The probability that a newborn egg survived to the adult female was 0.31, 0.21, 0.17, 0.26, 0.21, 0.11, 0.13, 0.29 and 0.32 on "RGS 8-1", "RGS 10-2", "RGS 8-13", "RGS", "Talaye 8-3", "Zar" and "Zar 9-9", respectively. The results revealed that the survival rate of a newborn egg to the adult female adult stage was related to *P. xylostella* on "Talaye 8-3". The age-stage, two-sex life table of *P. xylostella* on different cultivars and their mutant lines under greenhouse conditions, estimated by two methods of life table was obtained from female age-specific life table.
Figure 1. Age-specific survivorship ($l_x$) and fecundity ($m_x$) and age-specific maternity ($l_xm_x$) of *Plutella xylostella* on canola mutant lines and their controls, using female age-specific life table under greenhouse conditions.
Figure 2. Age-stage survival rate \( (s_{xj}) \) of *Plutella xylostella* on canola mutant lines and their controls under greenhouse conditions.
specific survivorship ($l_x$) and age-specific fecundity ($m_x$) and age-specific maternity ($l_xm_x$) of *P. xylostella* on mutant lines and their controls are shown in Figure 3. To calculate $l_x$, individuals of both sexes were considered. This curve is a simple form of the curves in Figure 2. Based on the curve of $f(x, \text{female})$, first oviposition of females on the above-mentioned varieties occurred at the age of 16.5, 19.5, 21.5, 17.5, 19.5, 20.5, 14.5 and 17.5 days, respectively. The peak of this curve depicting the highest daily fecundity of *P. xylostella* on the experimental varieties was 89.4, 59, 36.3, 38.7, 56, 37.7, 80 and 29.5 eggs, respectively. According to these results, the highest and the lowest daily fecundity was observed on “RGS 8-1” and “Talaye 8-3”, respectively (Figure 3). The age-stage reproductive value ($v_{0x}$) for a new egg ($v_{00}$) of *P. xylostella* on all mutant lines of canola and their controls is equal to the finite rate of increase ($\lambda$) on the respective varieties. The highest reproductive value of *P. xylostella* was at the age of 17.5, 20.5, 22.5, 21.5, 20.5, 21.5, 15.5 and 22.5 days on the aforementioned varieties, respectively. This indicates that, as opposed to other ages, female individuals of the above ages had the highest contribution to the future population on the experimental varieties, respectively (Figure 4).

Population parameters in this method of life table indicated a significant difference among population parameters of *P. xylostella* on cultivar “RGS” and its mutant lines (Table 1). The highest and lowest $r$, $R_0$ and $\lambda$ values were obtained on “RGS 8-1” and “RGS 8-13”, respectively. Furthermore, the lowest and highest $T$ values of *P. xylostella* were observed on two recent mentioned mutant lines, respectively. Moreover, no significant difference was observed among the population parameters of *P. xylostella* reared on “Zar” and “Talaye” in contrast with their mutant lines (Table 1).

Simulated population growth of *P. xylostella* fed on canola cultivars and their mutant lines demonstrated the best growth of population on mutant line, RGS 8-1 (Figure 5).

### DISCUSSION

Induction of desired plant attributes that have been lost through evolution is possible via mutation. There are random, multiple genetic modifications in plants which are produced through this option (Novak and Brunner, 1992). There is a noticeable variation in the parameters, particularly in the intrinsic rate of natural increase ($r$), among different studies. This parameter is known as the most valued item to evaluate the suitability of different host plants to an insect (Soufbaf et al., 2010; Goodarzi et al., 2015). Our obtained $r$ values for all three cultivars were much less than those reported by Soufbaf et al. (2010), but were more similar to the values obtained on different manipulated canola genotypes by Nikooei et al. (2015). Intrinsic rate of increase values obtained on canola by Golizadeh et al. (2009) were in the range of the current study. Also, our obtained $R_0$ values were higher than those reported by Soufbaf et al. (2010) and Nikooei et al. (2015). The differences among studies could be attributed to an array of allelochemical activity in excised leaves (dead plant) and in those that are still attached to the host plant (living plant) (Soufbaf et al., 2012). These dissimilarities may be explained by various qualities among host plants, genetic differences of the *P. xylostella* geographic populations, different rearing techniques, and differences in environmental factors (Awmack and Leather, 2002). This study revealed no significant difference between demographic parameters of *P. xylostella* on “Talaye” and “Zar” in comparison with those on their mutant lines but these parameters had significant difference on mutant lines of “RGS”. Intrinsic rate of increase, $R_0$ and $\lambda$ of *P. xylostella* were the highest on “RGS 8-1”, “RGS” and “RGS 10-2”, “RGS 8-13”, respectively. Furthermore, simulated population projection of *P. xylostella* fed on canola cultivars and their mutant lines confirms the susceptibility of mutant line, RGS 8-1 to population growth.
Figure 3. Age-specific survivorship ($l_x$), age-stage fecundity of female ($f_{xj}$) (offspring), age-specific fecundity ($m_x$) and age-specific maternity ($l_xm_x$) of Plutella xylostella on canola mutant lines and their controls using the age-stage, two-sex life table under greenhouse conditions.
Figure 4. Age-stage-specific reproductive value ($v_{xj}$) of *Plutella xylostella* on canola mutant lines and their controls under greenhouse conditions.
Figure 5. Simulated population growth of *Plutella xylostella* fed on canola mutant lines and their controls under greenhouse conditions.
of the pest. Hence, having suitable cultural traits, “RGS 8-1” was more susceptible compared with the control to population growth of \textit{P. xylostella} while the other two mutant lines i.e. “RGS 10-2” and “RGS 8-13” were more resistant to population growth of this pest. Similarly, Nikooei \textit{et al.} (2015) by using survival rate and \( r \) values suggested that canola mutant genotype (RGS003) was more resistant compared to its control cultivar. These results confirm findings of Ahloowalia and Maluszynski (2001), who stated that many factors such as species or genotypes of plants and irradiation doses can affect mutagenesis. In the debate upon two different methods of life table, Chi (1988) expressed that the variation in development rates, primary sex ratio, and pre-adult mortality can cause differences between the results of the age-stage two-sex life table and the female age-specific life table. In addition, this may be partly due to the difference between \( l_i \) and \( m_i \) in the two methods of life table. Also, Chi and Su (2006) discussed the possible problem of \( l_i \) and \( m_i \) based on adult age in female-based life table. Farhadi \textit{et al.} (2011) showed that there was no significant difference between \( r \), \( R_0 \), \( \lambda \) and \( T \) calculated by either female age-specific life table or age-stage two-sex life table. Their results showed that the male individuals and the variable developmental rate had no obvious effects on the population growth. They stated that this result was achieved because of the sex ratio (close to 1:1) and the stage overlapping which was short in comparison to the entire life span. Similar to our results, Khanamani \textit{et al.} (2013) used both methods of life table to analyze their data and showed little difference among the same life table parameters.

The achieved survival curves of two-sex and female-based life tables showed differences due to ignoring male individuals in \( l_i \) calculation in the female age-specific life table. Moreover, the daily fecundity is multiplied by the same sex ratio in calculating \( m_i \) in the method of female age-specific life table. Huang and Chi (2011) discussed the problem of applying female age-specific life table to insect populations and illustrated that when the age-specific female life table is applied to an age-stage-structured two-sex population, both survival and fecundity curves will be improperly manipulated because the variation in pre-adult development time among individuals is disregarded. Moreover, in this method of life table, male survival curve could not be calculated and the stage overlap would not be shown because of the utilization of the mean developmental time of each stage to partition the life history into different stages and it would ultimately result in errors in the survival curves (Chi, 1988). Since the mean duration of pre-adult stages is always greater than the first adult emergence, the \( m_i \) curve in female age specific life table starts later than the true one (Chi, 1988; Huang and Chi, 2011). Furthermore, if \( l_i \) and \( m_i \) are constructed based on adult age in age-specific female life table, they may also cause errors in population parameters (Chi and Su, 2006; Huang and Chi, 2011). Regarding the differences between the two methods of life table, it seems that age-stage, two-sex life table provides more data on the life history of pests theoretically. Although the method of female age specific life table is still applied in various studies, its application in population projection and biological control will be limited, because female life table cannot take male population and stage differentiation into consideration.

**CONCLUSIONS**

The present study showed that \textit{P. xylostella} can develop on mutant lines of three commonly grown canola cultivars. It revealed that population parameters of \textit{P. xylostella} were influenced by mutant varieties but mutation impact was dependent not only on plant cultivar but also different lines of one cultivar. The differences between the two methods of life table can be related to factors such as male population,
pre-adult mortality and variable developmental rate among individuals in age-stage, two-sex life table method. Besides, our results may provide considerable information to design a program for integrated management of *P. xylostella* in mutant canola fields. Nevertheless, related field studies should be conducted in future to attain more practical information successfully.

**ACKNOWLEDGEMENTS**

This research is a part of the PhD dissertation of first author that was funded by Shahid Chamran University.

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Canola Physical Mutation and Plutella xylostella


دموگرافیک بید کلم روی دو رقم “Talaye” و “Zar” در مقایسه با لایه‌های موتانت آنها مشاهده نشد ولی بین پارامترهای مذکور روی رقم “RGS” و لایه‌های موتانت آن تفاوت معنی‌دار وجود داشت. لایه موتانت “1-8” روی رقم داشت و دو رقم جمعیت آفت حساسیت بود به طوریکه بر اساس جدول زندگی دوجسنی بیشترین نرخ ذاک استفاده جمعیت (R0) و نرخ خالص نیروی (R) روی این لایه بود (به ترتیب ۲/۰۸ و ۸/۷۸ تخم به ازای هر روز و ۱/۰ روز). همچنین تصویر جمعیت، رشد بیش از این آفت را روی لایه مذکور نشان داد. بین پارامترهای رشد جمعیت آفت که به هر دو روش جدول زندگی محاسبه شد تفاوت‌های بسیار جزئی وجود داشت. بررسی پیامدهای اصلاح رقم گیاهی با استفاده از روش پروتئای بر سازگاری حشره گیاهخواره نتیجه پرشور منظمی در نتایج پذیرش گباشیه موفق تر می‌شود که به نحو مؤثر روشنگری‌هایی را در جوزه برنامه‌های مدیریت آفت در زمانی به این روش‌ها در اولویت کشته فارامند، در هر زمینه کن.