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Bottom-up effects of *Brassica* genotypes on performance of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae)

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ABSTRACT

The adverse effects of excessive application of insecticides is causing a renewed interest to find resistant host plants to insect pest. Herein, we attempted to elucidate the bottom-up effects of five canola cultivars (Elite, SLM_{046}, Star, NSA2, and RGS_{003}), four cabbage cultivars (Glob-Master, Green-Cornet, Red-Rocky and Mikado) and one cauliflower cultivar (S-Mila) on the life table and biological parameters of diamondback moth, *Plutella xylostella* L., (Lepidoptera: Plutellidae). The age-stage, two-sex life table theory was used to unveil biological differences of *P. xylostella* on the *Brassica* genotypes. All experiments were carried out in a laboratory at 25 ± 1 °C, 65 ± 5% and a photoperiod of 16:8 (L: D) hours. The development time of *P. xylostella* varied from 13.0 days (on Elite) to 17.4 days (on Red-Rocky). The highest and lowest of net reproductive rate (R_0) were recorded in NSA2 (27.0 offspring/individual) and Red-Rocky (4.7 offspring/individual), respectively. The intrinsic rate of increase (r) ranged from 0.072 day^{-1} on Glob-Master to 0.169 day^{-1} on Star. The mean generation time (T) value varied between 18.1 days on RGS_{003} to 22.5 days on Red-Rocky. According to the results inferred from biological and demographical parameters studies, Glob-Master and SLM_{046} were found as the resistant and susceptible cultivars, respectively. Revealing the resistance range of the studied *Brassica* cultivars and life history traits of *P. xylostella* on these cultivars provide insight into the eco-friendly control strategies of the pest through decreasing its damage rate and improving the condition for activity of natural enemies.

1. Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) has been well known worldwide for its capacity to rapidly become resistant to different classes of insecticides (Talekar and Shelton, 1993; Sarfraz et al., 2006). It is likely that DBM is attracted to its brassicaceous host plants by chemical (olfactory/gustatory) and physical (tactile/visual) stimuli (Bukovinszky et al., 2005). In most crop fields worldwide, brassicaceous crops are cultivated as vegetables (like *Brassica oleracea* subsp. *acephala* and *B. oleracea* sub sp. *capitata*) and non-vegetables such as canola (*B. napus*). Therefore, not only is the evolution of resistance to pesticides a major problem, but also the harmful effects of the residual toxicity on both humans and the environment have been considered in chemical-based control programs of DBM (Shelton et al., 1993; Schellhorn et al., 2008).

Determining the life table parameters of a pest on different plants can deepen our understanding about pest-resistant varieties and facilitate efforts to decrease pesticide application. In addition, host plant resistance can be an effective approach for being replace broad-spectrum insecticides (Fathipour and Mirhosseini, 2017). The intrinsic rate of increase (r) of a pest species is a key growth parameter in evaluation of an insect's population under defined conditions such as climate and food resource (Southwood and Henderson, 2000; Soufbaf et al., 2012). Moreover, all of the biological events of insects can be summarized in r, which becomes it more suitable to unveil the differences of a host plant varieties. A tremendous body of knowledge is available about influencing of host plant on development, survival, reproduction and the life table parameters of insect pest (Ramachandran et al., 1998; Sarfraz

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et al., 2007; Golizadeh et al., 2009a; b; Soufbaf et al., 2010 a; b). The physical and volatile signals of host plants can influence the development and survival of larval stages as well as egg production of adults (Gols et al., 2009). So, by focusing on the different aspects of host plants, the suitable host plants can be recognized and exploited in a very safely manner in pest control programs.

Different phenotypes and cultivars of host plants may include different chemical and mechanical defenses, as well as avoidance/tolerance mechanisms affecting nourishing properties of them in facing with herbivorous insects. Therefore, the combination of these factors is an important approach that helps in the understanding of the performance of herbivore insects (Cornell and Hawkins, 2003; Agrawal, 2004). It seems that the defensive components (secondary compounds) referred to as Glucosinolates (hereafter GS) play a major role in brassicaceous plants (Fahey et al., 2001; Muller and Wittstock, 2005; Travers-Martin and Muller, 2008). In wild brassicaceous plants, the high level of GS affects negatively development time and adult body mass of insect pests (Gols et al., 2008). On the other hand, nitrogen as a primary plant metabolite can influence the biological parameters in a similar way to GS (Mattson, 1980; Johnson, 2008; Soufbaf et al., 2012). Knowing about influencing attributes of DBM host plants can provide a platform for designing a proper integrated crop management (ICM) program. Since DBM develops initial resistance to insecticides, this current study is an attempt toward finding eco-friendly approaches either to reduce or eliminate chemical control by providing non-chemical controlling approaches to be exploited. This study focuses on determining the life table parameters of DBM on the new cultivars of Brassicaceae including NSA2, Elite and Star, and their comparison with the other commercial cultivars.

2. Materials and methods

2.1. Plant and insect rearing

The *Brassica* genotypes used in the present study consisted of 10 *Brassica* genotypes including five cultivars of canola, *Brassica napus* (Elite, SLM_{046}, Star, NSA2, and RGS_{003}), four cultivars of cabbage, *Brassica oleracea* var. *capitata* (Glob-Master, Green-Cornet, Red-Rocky and Mikado), and one cultivar of cauliflower, *B. oleracea* var. *botrytis* (S-Mila). The canola cultivars were planted in the greenhouse of Tarbiat Modares University (35° 44′ N, 51° 09′ E, 1273.46 m), Tehran, Iran, and when they reached 10 to 12 leaves (about 6 weeks after planting), were included in the experiments. The cabbage and cauliflower seeds were sown in transparent boxes (30 × 50 × 30 cm) in the greenhouse and when the plants reached 5 to 8 leaves, each plant was transferred individually into a pot. All seeds were obtained from the Seed and Plant Improvement Institute, Karaj, Iran. During the experiments, neither fertilizers nor pesticides were applied.

The DBM larvae and pupae were collected from the cabbage field of the University of Tehran (located in Karaj, Iran). To obtain a pathogen and parasitoid free stock, the colony was established just with adult individuals. The DBMs were reared on Opera, a cultivar of canola, in a growth chamber set at 25 ± 1 °C, 65 ± 5% and a photoperiod of 16:8 (L: D) hours. The F2 generation of DBM was used in all experiments.

2.2. Experiment

Demographic parameters of DBM were studied on the 10 *Brassica* genotypes in a growth chamber set at 25 ± 1 °C, 65 ± 5% and a photoperiod of 16:8 (L: D) hours. The experiment was arranged in a complete randomized design (CRD). More than 100 pairs of *P. xylostella* were transferred into the oviposition cages (Plexiglas containers of 14 cm in diameter and 19 cm in depth, with the tops covered with a fine mesh net). In each container, one young leaf with a small cotton wick soaked in water was used as the oviposition substrate and changed daily. When DBM passed two generations on each cultivar, about 100 eggs of DBM were gathered randomly with a fine camel's hair brush. The eggs were individually transferred into plastic containers (diameter 8 cm, depth 1 cm) with a hole in the top covered by a fine mesh net for ventilation. There was a young leaf of each *Brassica* host plant inside the container, and end of its stem was covered with a small wet cotton, which was changed daily.

The eggs and other immature stages of the third laboratory generation were monitored daily for molting and mortality. The larval instars were identified by measuring the head capsules and their head color (Harcourt, 1957). The larval head capsule size increases with each molting, which can be used in larval instar determination. As which, color of the head varies in the different instars, exploiting profusely in differentiation of the larval stages (black, dark-brown, brown and green colors confirm the first, second, third and forth instars of *P. xylostella*, respectively (Talekar and Shelton, 1993).

Within 24 h of adult emergence, individuals were sexed. The males of *P. xylostella* have claspers at the end of abdomen and the females have some branches of hair and a tubular ovipositor (Harcourt, 1957). Then, a pair of *P. xylostella* was transferred into the new oviposition cage and kept until death. The adults were provided with water. The number of laid eggs and the rate of their mortality were recorded daily in all cages. If a male died, a newly emerged male (< 24 h old) was replaced. Accordingly, the pre-oviposition, oviposition and post-oviposition periods were determined.

2.3. Statistical analysis

All data were tested for normality using the Kolmogrov-Smirnov's test before they are analyzed using the analysis of variance (ANOVA) (PROC GLM, SAS Institute, 2003). Mean separations were conducted using the Duncan's Multiple Range test ($\alpha = 0.05$), if significance differences were detected. The life table parameters including the net reproductive rate (R_0), intrinsic rate of increase (r), finite rate of increase (λ) and mean generation time (T) were calculated using the formulae suggested by Carey (1993). The raw data of the life history of all individuals (males, females and those died before the adult stage) were analyzed by the age-stage, two-sex life table theory (Chi and Liu, 1985; Chi, 1988). Data analysis and population parameters (R_0, r, λ, T) data were calculated by using the TWOSEX-MSChart program (Chi, 2016). The standard errors of the population parameters were estimated by using the bootstrap procedure (Huang and Chi, 2013; Khanamani et al., 2013) and bootstrap values were compared using paired-bootstrap procedure (Riahi et al., 2016; Khanamani et al., 2017). The two-sex life table bootstrap-values of the *P. xylostella* on different host plants were compared using the paired-bootstrap procedure.

Nested analysis is useful as a mixed-method strategy for comparative research (Lieberman, 2005). A nested *t*-test is the appropriate hypothesis test when there is one measurement variable and two or more nominal variables (Here, there were two different groups including the canola group and the cabbage + cauliflower group) (Storm, 2010). Formerly, this analysis is the abundant application in genetic research; however there is no data on its application for comparing the biological parameters.

Biological characteristics of DBM such as immature stage, survival of immature stage, adult longevity, daily fecundity, total fecundity, oviposition period and demographic parameters including R_0, r, λ, T and sex ratio (female/female + male) were used for nested analysis for classification of the cultivars tested. The cultivars were classified into two groups, canola and cabbage + cauliflower. A normality test, ANOVA, and nested analysis were performed by SPSS software (SPSS 16, 2007).