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JOURNAL OF PLANT PROTECTION RESEARCH Vol. 56, No. 1 (2016)

DOI: 10.1515/jppr-2016-0007

Resistance of canola cultivars affect life table parameters of *Nysius cymoides* (Spinola) (Hemiptera: Lygaeidae)

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Received: August 11, 2015
Accepted: February 3, 2016

Abstract: A life table can be used as an important and appropriate tool to evaluate the susceptibility or resistance level of different host plant cultivars to insect pests**.** In the current study, we determined the suitability or inferiority of five different canola cultivars (Hayula420, Hayula401, Hayula50, Hayula60, RGS) to *Nysius cymoides*, under laboratory conditions. Data were analysed based on the age-stage, two-sex life table theory. *Nysius cymoides* which fed on Hayula420 had the longest nymphal period, while those which fed on Hayula50 had the shortest nymphal period. Developmental times (sum of incubation and nymphal periods) was longest for those which fe d on Hayula420 and the shortest for those which fed on Hayula50. The adult pre-oviposition period (APOP), total pre-oviposition period (TPOP), mean fecundity, and adult longevity of adults reared on different canola cultivars showed significant differences. The highest and lowest net reproductive rates (R_0) were obtained for those which fed on Hayula420 (11.40 offspring per individual) and Hayula401 (5.47 offspring per individual), respectively. The highest value (0.0395 d^{-1}) for the intrinsic rate of increase (r) was obtained for those which fed on Hayula 60 cultivar and the lowest value (0.0261 d^{-1}) for those which fed on Hayula401 cultivar. The shortest and longest mean generation times (T) were obtained for those which fed on RGS and H401 cultivars, respectively. The lowest and highest values of life expectancy (e_{xj}) were obtained for those which fed on RGS and Hayula420 cultivars, respectively. The results showed that Hayula401 and RGS were not susceptible cultivars to *N. cymoides*. These cultivars showed higher resistance to *N. cymoides,* while Hayula60, Hayula420, and Hayula50 were found to be suitable cultivars but with lower resistance to *N. cymoides,* respectively.

Key words: canola, *Nysius cymoides*, resistance, two-sex life table

Introduction

Canola (*Brassica napus* L.) is one of the most important sources of edible oils in the world (Scarisbrick and Daniels 1986; Shahidi 1990; Raymer 2002; Karaosmanoğlu 2004). About 62 million tons were produced in 2011 (FAO 2013). New canola cultivars contain less than 2% erucic acid in the oil and are recognised as high quality, healthy, edible oils (Gunasekera *et al.* 2006).

Different canola cultivars which have the same nutrient requirements, are planted in Iran (Mostafavi Rad *et al.* 2011), but they have a different susceptibility to pests (Fathi *et al.* 2010; Fathi *et al.* 2011a, b; Fallahpour *et al.* 2015).

Resistance of arthropods to insecticides and residue of conventional pesticides in agricultural crops has convinced managers and scientists to find alternative strategies for controlling pests. Selecting host plants with partial resistance to herbivorous insects is a major method for controlling insect pests in Integrated Pest Management (IPM) programs (Goodarzi *et al.* 2015).

The species *Nysius cymoides* (Spinola) (Lygaeidae) is of economic importance in Iran because of their outstanding damage to alfalfa (Yasunaga 1990; Wheeler 2001; Mirabbalou *et al.* 2008), cotton (Behdad 2002), clover (Wipfli *et al.* 1990), canola (Heidary Alizadeh *et al.* 2009; Sarafrazi *et al.* 2009), almond, and apple (Ghauri 1977). Despite using chemical and biological control measures against these insect pests, their damage is still outstanding. The continuation of the damage could partly be related to the lack of information about some biological aspects and the impact of different climates on their performance (Solhjouy-Fard *et al.* 2013).

The geographic distribution map in Iran shows the presence of *N. cymoides* in most of the areas (68.78%), except for central and eastern Iran. In general, the central part of Iran was predicted to be the least suitable area, and the northern part was predicted to be the most suitable one. The pest has been recorded from most fields, semi-desert areas, and steppic areas of the Palaearctic region (Péricart 1999; Linnavuori 2007; Solhjouy-Fard *et al.* 2013).

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It is important to understand the basic biology and population parameters of arthropod herbivores to assess the resistance of host plants, so that convenient control strategies can be conducted. Careful consideration is needed to make a correct decision in selecting the appropriate cultivar to cultivate (Sedaratian *et al.* 2011; Goodarzi *et al.* 2015).

The intrinsic rate of increase is the most appropriate biological index for evaluating the suitability of different host plants and for assessing the plant resistance level to an insect herbivore (Southwood and Henderson 2000).

The false chinch bug, *N. cymoides*, has threatened canola farm fields since a crowd of bugs swarmed out onto adjacent fields at harvest time (Mohaghegh-Neyshabouri 2008).

Different canola cultivars have different primary and secondary metabolites (Soufbaf *et al.* 2012). The metabolites are expected to cause different effects on the performance of *N. cymoides*, but no study has been done regarding the effect of canola cultivars on the life table parameters of *N. cymoides*. It is necessary to make a correct decision when selecting a cultivar to cultivate. The first objective of this study was to determine the suitability or inferiority of different canola cultivars to *N. cymoides*. The second objective was to find out the effect of different canola cultivars on the biological and life table parameters of *N. cymoides* which would be an initial step toward substituting non-chemical methods in an IPM program.

Materials and Methods

Insect rearing

The initial population was originally collected from canola fields in Gonbad e Qabus, Iran, in late July 2014. The seeds of five canola cultivars (Hayula420, Hayula50, Hayula60, Hayula401, RGS) as host plants were obtained from the Gonbad Agriculture Research Station.

The bugs were reared on five canola cultivars separately for three generations to adapt with new host plants, in growth chambers at 24±1°C, 65±5% relative humidity (RH), and 16 : 8 (L : D) h.

Age-stage, two-sex life table

At least 100 eggs of *N. cymides* laid on cotton within 24 h by females were used to start the experiments on each host plant cultivar. The newly emerged nymphs were placed individually in Petri dishes (6 cm in diameter × 1.5 cm in height) with a hole in the lid. The dishes were covered with a fine mesh net for ventilation. The newly emerged nymphs fed on different freshly germinated seeds of the canola cultivars. The eggs and nymphs were checked daily and their developmental times were recorded. The egg incubation periods, developmental times of immature stages, and their mortality, were recorded daily. Upon emergence, the adult females were paired with males and transferred to transparent plastic containers (30 cm in diameter × 5 cm in height) for oviposition. The containers were covered with fine mesh for ventilation. The adults also fed on freshly germinated seeds of different canola cultivars. The number of eggs laid and the adult longevity were also recorded daily. The observation continued until the death of the last individual in the cohort.

The life-history raw data were analyzed according to the age-stage, two-sex life table theory (Chi and Liu 1985; Chi 1988). The age-stage specific survival rate (s_{xj}) (where x = age in days and j = stage); the age-stage specific fecundity (f_{xj}) (daily number of eggs produced per female of age x); the age-specific survival rate (l_x); the age-specific fecundity (m_x) [daily number of eggs divided by all individuals (male and female) of age x]; and the population growth parameters [the intrinsic rate of increase (r)]; the finite rate of increase (λ); the net reproductive rate (R_0), and the mean generation time (T) were calculated by using the TWOSEX-MS Chart programme (Chi 2015). The TWOSEX-MS Chart is available at http://140.120.197.173/Ecology/prod02.htm (Chung Hsing University) and http://nhsbig.inhs.uiuc.edu/wes/chi.html (Illinois Natural History Survey).

The standard errors of the population parameters were estimated using the Bootstrap procedure (Efron and Tibshirani 1993; Huang and Chi 2013). In the bootstrap procedure, a sample of n individuals was randomly taken from the cohort with replacement and the $r_{i\text{-}boot}$ for this bootstrap sample was calculated as follows.

$$\sum_{x=0}^{\omega} e^{-r_{i\text{-}boot}(x+1)}\, l_x m_x = 1,$$

where: *i-boot* – the *i* bootstrap, l_x and m_x – are calculated from the n individuals selected randomly with replacement. Generally, the data on the same individual are repeatedly selected. We repeated this procedure m times (m = 10,000).

The same methods were used for the corresponding estimates of the λ, R_0, and T. The two-sex life table bootstrap-values of the *N. cymoides* on different canola cultivars were compared using the Paired Bootstrap Test procedure (TWOSEX-MS Chart programme, Chi 2015). Drawings were done using SigmaPlot software (SigmaPlot 2011).

Results

Biological parameters

The results showed significant differences in the incubation period of *N. cymoides* reared on different canola cultivars ($p < 0.05$). The longest period was 8.48 days on the Hayula420 cultivar, and the shortest period was 7.02 days on the Hayula50 cultivar. The nymphal period of *N. cymoides* which fed on Hayula420 was the longest, while those fed on Hayula50 had the shortest nymphal period. The nymphal periods varied significantly among canola cultivars. Developmental times (the sum of the incubation and nymphal periods) were the longest for those individuals which fed on Hayula420, and the shortest for those individuals which fed on Hayula50. Adult longevity for adults reared on different canola cultivars showed significant differences ($p < 0.05$). The longest and shortest female and male longevities were for those which fed on Hayula 401, Hayula 420, and RGS, respectively (Table 1).

Table 1. Biological parameters of *Nysius cymoides* on five canola cultivars

Culti-vars	Stages (days)							Adult longevity	
	egg	N1	N2	N3	N4	N5	pre-adult development time	female	male
H420	8.48±0.49 a	7.94±0.22 a	6±8.03 a	5.94±0.2 a	5.97±0.15 a	7.99±0.09 a	44.31±0.79 a	71.15±2.32 a	77.7±2.29 a
H401	8.27±0.44 a	7±4.12 b	6±2.99 a	5.14±0.3 a	5.02±0.16 b	7.14±0.34 b	42.28±0.89 a	74.62±2.42 a	69.26±2.56 b
H50	7.02±0.16 b	5.67±0.47 c	4.05±0.23 c	3.9±0.02 b	4.21±0.08 c	4.9±0.29 c	31.94±0.65 c	63.61±2.28 bc	59.38±2.07 c
H60	7.98±0.14 a	5.99±2.23 c	4.99±1.99 b	4 b	4 c	4.53±0.49 c	34.96±0.76 b	64.83±1.69 b	67±1.93 b
RGS	7.13±0.34 b	5.99±2.64 c	5 b	4±2.99b	4 c	5.07±0.26 c	34.84±0.71 b	58.74±1.91 c	58.41±2.25 c

The means followed by the same letters in each column are not significantly different ($p > 0.05$)

Table 2. The means and standard errors of the adult pre-oviposition period, total pre-oviposition period, female longevity (days), and mean fecundity (eggs) of *Nysius cymoides* on five canola cultivars

Cultivars	Adult pre-reproductive period (APOP)	Total pre-reproductive period (TPOP)	Female longevity	Fecundity
H420	6.02±0.23 b	50.08±1.18 a	71.15±2.32 a	41.77±4.07 a
H401	8.92±0.84 a	52.65±1.62 a	74.62±2.42 a	20.56±2.61 c
H50	5.94±0.28 b	38.88±0.96 b	63.61±2.28 bc	27.19±3.06 bc
H60	5.02±0.17 c	40.28±0.95 b	64.83±1.69 b	31.16±2.99 b
RGS	5.81±0.48 bc	41.87±1.19 b	58.74±1.91 c	22.44±2.88 c

The means followed by the same letters in each column are not significantly different ($p > 0.05$)

The adult pre-oviposition period (APOP), total pre-oviposition period (TPOP), female longevity and fecundity are listed in table 2. There were significant differences among APOP, TPOP, female longevity, and fecundity for the individuals which fed on different canola cultivars (Table 2). The results showed the highest fecundity (41.77 eggs) for those individuals which fed on Hayula420, and the longest female longevity (74.62 days) and lowest fecundity (20.56 eggs) for those individuals which fed on Hayula 401 (Table 2).

Population growth parameters

The population parameters such as the r, λ, R_0, T and gross reproductive rate (*GRR*) and their standard errors are shown in table 3. There were significant differences for the effect of different canola cultivars on population parameters (Table 3). The highest value (0.0395 d^{-1}) for r was found for those individuals which fed on Hayula 60 cultivar and the lowest value (0.0261 d^{-1}) was recorded for those individuals which fed on Hayula 401 cultivar. The shortest and longest T was obtained for those individuals which fed on RGS and H401 cultivars, respectively (Table 3). The highest and lowest R_0 were obtained for those individuals which fed on Hayula420 (11.40 offspring) and Hayula401 (5.47 offspring), respectively. The shortest and longest T was obtained for those individuals which fed on RGS and H401 cultivars, respectively (Table 3).

The age-stage, two-sex life table revealed the overlap of the s_{xj} curve of the cohort of *N. cymoides* reared on five canola cultivars (Fig. 1). This curve shows the probability that a fertile *N. cymoides* egg will survive to age x and stage j. The survival rates of *N. cymoides* reared on cultivars Hayula50, Hayula60, and RGS were lower than the survival rates of *N. cymoides* reared on Hayula420 and Hayula401 cultivars. This was due to the higher mortalities of immature stages that occurred of *N. cymoides* reared on these three cultivars.

Table 3. Two-sex life table parameters (means±SE) of *Nysius cymoides* on five canola cultivars

Cultivars	R_0	r	λ	T
H420	11.41±2.12 a	0.0390±0.003 a	1.0398±0.0032 a	62.32±1.41 a
H401	5.48±1.13 b	0.0265±0.003 a	1.0268±0.0033 a	64.15±1.52 a
H50	7.47±1.49 ab	0.0377±0.003 a	1.0384±0.0040 a	53.26±1.40 b
H60	8.53±1.63 ab	0.0399±0.003 a	1.0407±0038 a	53.70±1.09 b
RGS	5.96±1.25 b	0.0343±0.004 a	1.0349±0.0044 a	51.97±1.19 b

The means followed by the same letters in each column are not significantly different ($p > 0.05$)
R_0 – the net reproductive rate; r – the intrinsic rate of increase; λ – the finite rate of increase; T – mean generation time

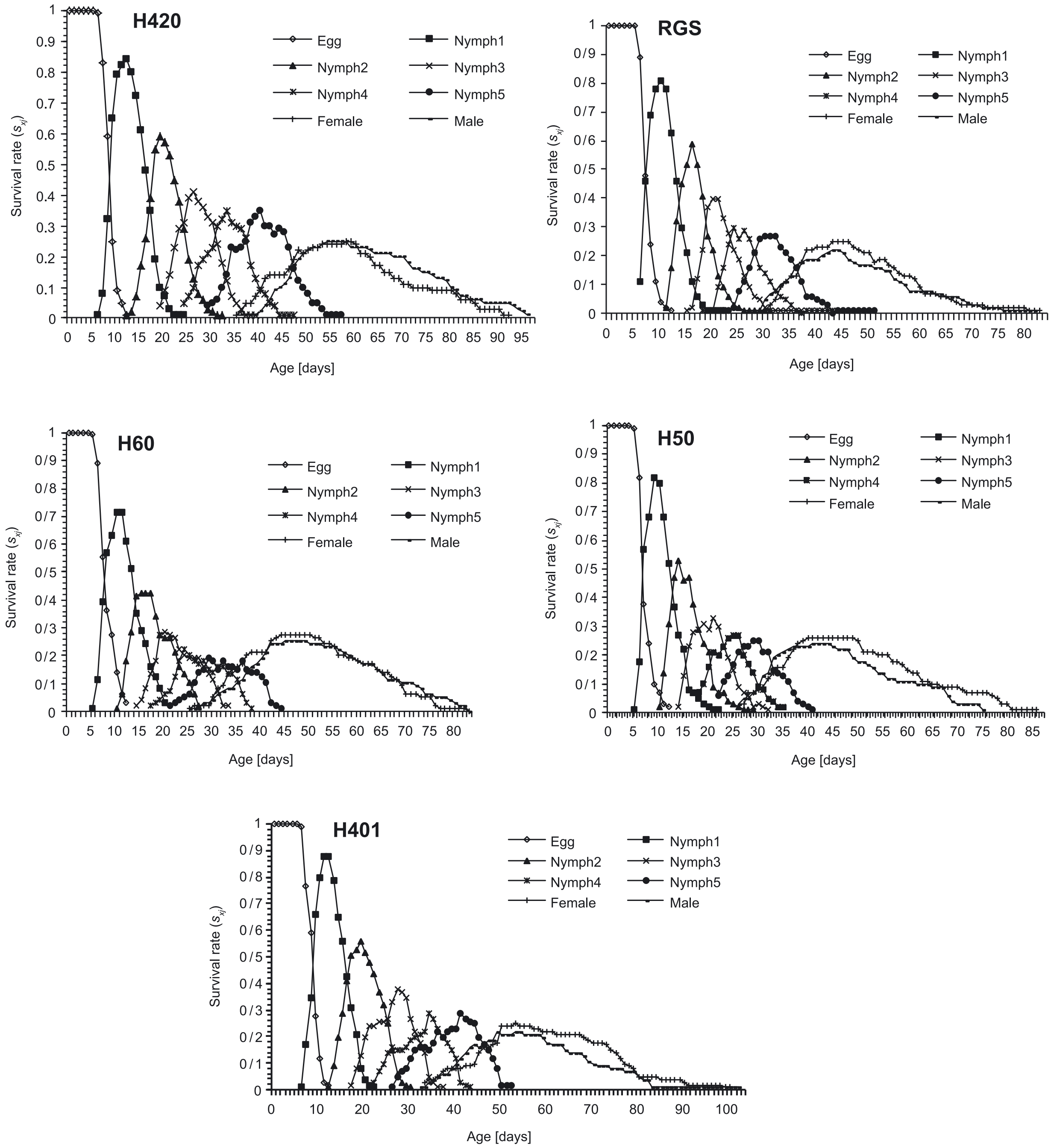

Fig. 1. Survival rate to each age-stage interval (s_{xj}) of *Nysius cymoides* on five canola cultivars

There were prominent overlaps in survival curves of different life stages. Adult females had better survival rates than males concerning those individuals reared on Hayula401, Hayula50, and RGS cultivars and the maximum lifespan (121 days) was observed for adult males reared on Hayula420 (Fig. 1). This s_{xj} curve can be simplified to the l_x curve (Fig. 2) by ignoring the stage differentiation: including all individuals in the cohort. The result showed that these insects had been reared on different canola cultivars and had a high ability to survive, and that mortality does not follow the same trend in all canola cultivars (Fig. 2).

The female age-specific fecundity (f_{xj}), the age-specific fecundity of the total population (m_x), the age-specific maternity ($l_x m_x$), and the cumulative reproductive rate (R_x) (Jha *et al.* 2014):

$$R_x = \sum l_y m_y,$$

of *N. cymoides* are also shown in figure 2.

The curve of f_{xj} shows the mean number of fertile eggs produced by a female adult at age *x*, while the curve of m_x includes all individuals of age *x*. The cumulative reproductive rate value in Hayula420, Hayula60, Hayula50, RGS, and Hayula401 was 11.41, 8.53, 7.47, 5.96, and 5.48, respectively (Fig. 2).

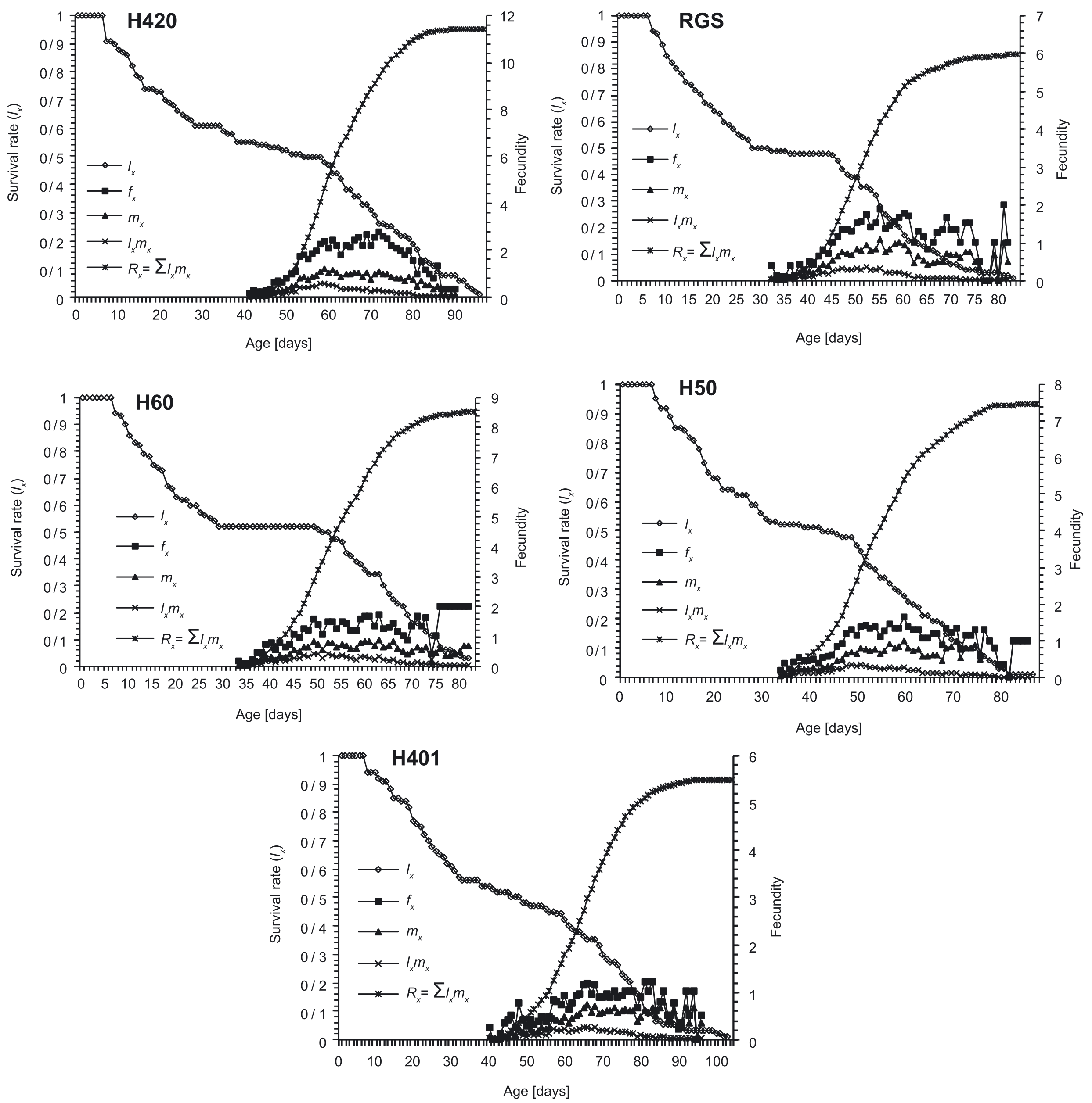

Fig. 2. Age-specific survival rate (l_x), female age-specific fecundity (f_x), age-specific fecundity of the total population (m_x), age-specific maternity (l_xm_x), and cumulative reproductive rate (R_x) of *Nysius cymoides* on five canola cultivars

The age-stage life expectancy (e_{xj}) shows the total time that an individual of age x and stage j is expected to live (Fig. 3). Life expectancy shows a gradual decrease as aging occurs. Changes in life expectancy almost showed an inverse relationship to the mortality rate so that in the first days of life, the mortality rate is lowest and life expectancy is the highest. The highest life expectancies were obtained for individuals reared on Hayula 420 and Hayula401 cultivars and the lowest life expectancies were for individuals reared on Hayula50 and RGS cultivars, respectively (Fig. 3).

Discussion

The three mechanisms of resistance (antixenosis, antibiosis, and tolerance cultivar) will influence the population dynamics of a pest insect throughout a season by their action on the life history parameters: initial colony size, duration of larval period, fecundity of adults, mortality of larvae and adults. The last four of these parameters are used to determine the intrinsic rate of increase (r) (Dent 2000). Thomas and Waage (1996) investigated the effect of each of the four parameters on the rate of increase, assuming that host plant resistance seeks to reduce r below zero, the point at which the pest population will decline over time. The analysis revealed that not all the components of antibiotic resistance have equivalent effects. The individual mechanisms depend on the life history of the pests. With a slow growing population, increased development time or increased juvenile mortality have roughly the same effects on reducing r.

Host plants with deleterious compounds may reduce the survival rate, size or weight, longevity, and reproduction of herbivorous arthropods or they may have an indi-

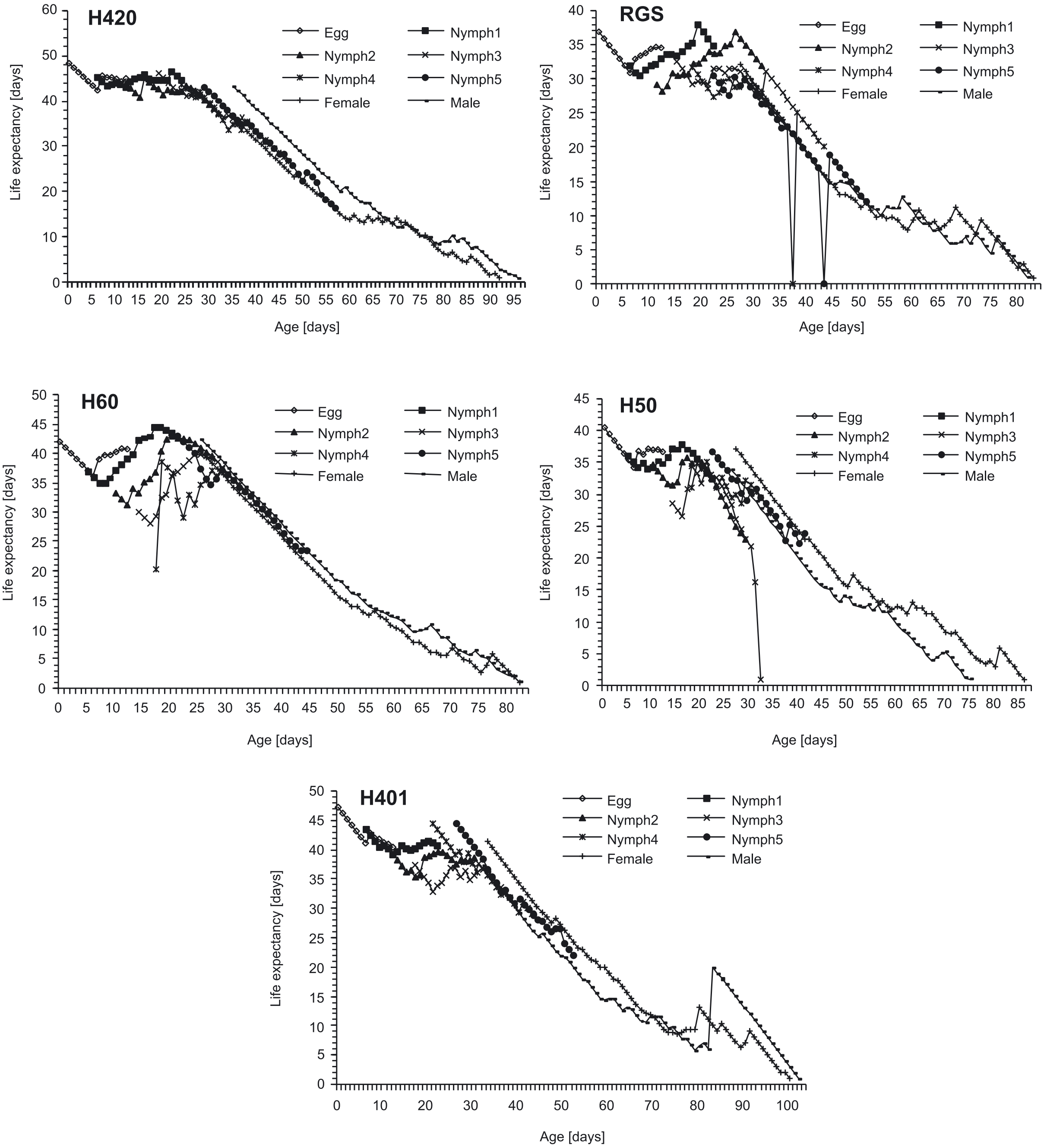

Fig. 3. Age-stage life expectancy (e_{xj}) of *Nysius cymoides* on five canola cultivars

rect effect by increasing the exposure of the arthropod to its natural enemies as a result of prolonged development time (Ali and Gaylor 1992; Greenberg *et al.* 2001; Chen *et al.* 2008; Goodarzi *et al.* 2015).

Host plant availability and quality may have a role in pest population dynamics by affecting immature as well as adult performance (Golizadeh *et al.* 2009). No studies have examined the effect of host plants on the developmental stages and life table parameters of *N. cymoides*. In the present study, there were significant differences in developmental times and adult longevity, for individuals reared on different canola cultivars. The immature period of individuals reared on the Hayula 420 cultivar was higher than that of individuals reared on other cultivars, which was similar to other research results (Vattanakul and Rose 1982; Mohaghegh-Neyshabouri 2008). Although this period in *Nysius inconspicuus* Distant, at 30°C, of individuals reared on sunflower, was much lower than our results (Kakakhel and Amjad 1997). This difference could be related to the temperature, species, and host plant. Our results showed that canola cultivars affected the development time, adult longevity, APOP, TPOP, and fecundity, significantly.

A slower developmental time of a herbivore on a particular host plant means a longer life span, usually a lower reproductive ability, slower population growth, and increased exposure to natural enemies. A faster developmental time on a particular host plant may allow a shorter life cycle and more rapid population growth and may also reduce generation time (Goodarzi *et al.*

2015). We found that the Hayula50 cultivar with the individuals having the shortest developmental time (31.94 days) was the most suitable host plant and the Hayula420 cultivar with *N. cymoides* individuals having the longest developmental time (44.31 days) was considered as the least suitable host for *N. cymoides*.

A life table provides the most comprehensive description of a cohort of individuals or of a typical individual from a given population in terms of survival, development, and reproduction (Price 1997; Yu *et al.* 2005; Yang and Chi 2006). The age-stage, two-sex life table theory helps construct a comprehensive life table describing the demographic characteristics of insect and mite populations (Chi and Su 2006). A correct understanding of a pest's life table is essential for implementing an ecology oriented management program (Zalucki *et al.* 1986). The intrinsic rate of increase is the most useful and appropriate life-table parameter for comparing the fitness of populations across diverse climatic and food-related conditions (such as certain glucosinolate, cardenolids, plant volatiles, waxes, leaf morphology as well as host plant nutritional quality or a combination of these factors) (Smith 1991; Soufbaf *et al.* 2010a, b; 2012; Goodarzi *et al.* 2015).

The comparison of the intrinsic rate of increase often provides considerable insight for evaluating host plant suitability to herbivorous arthropods in integrated pest management programs (Fathipour and Sedaratian 2013; Rezaie *et al.* 2013; Ahmad and Shafiq Ansari 2014; Goodarzi *et al.* 2015). The value of r determines whether a population increases exponentially ($r > 0$), remains constant in size ($r = 0$), or declines to extinction ($r < 0$). Our results showed that canola cultivars affected the r value of *N. cymoides.*

In this study, the r value obtained for those individuals reared on the Hayula 60 cultivar (0.0395±0.003) was higher than other canola cultivars and the R_0 value for those individuals reared on Hayula420 (11.4±2.12) was highest but because of its generation time (62.31±1.4 days), the r value for those individuals reared on Hayula420 was lower than for those individuals reared on Hayula60. However, Mohaghegh-Neyshabouri (2008) obtained the r value (0.0717), R_0 (32.86) and generation time (48.69 days) for *N. cymoides* feeding on the Option canola cultivar. They also obtained life table parameters on *Nysius wekiuicola* (while they were fed daily with *Drosophila melanogaster*), such as, the net reproductive rate: 15.4, the gross reproductive rate: 38.15, the mean generation time: 39.7 days, intrinsic rate of increase: 0.069, and finally, the doubling time of the population assuming overlapping generations: 10.1 days (Eiben 2012). Their findings differed from our results. This difference may be due to the host and geographical area. In conclusion, the high r value for those individuals reared on Hayula60 indicated that *N. cymoides* had a greater reproductive potential and it was presumably a more suitable host plant than other canola cultivars.

The results showed that Hayula401 and RGS were, in effect, unsuitable cultivars to *N. cymoides* and showed higher resistance. Hayula60, Hayula420, and Hayula50 were found to be suitable cultivars with lower resistance to *N. cymoides,* respectively. Plant nutritional quality referencing to primary and secondary metabolites in many plants may considerably affect herbivore biology and ecology (Soufbaf *et al.* 2012; Goodarzi *et al.* 2015). The type of chemicals responsible for insect resistance are numerous but the major classes include the terpenoids, flavonoids, quinines, alkaloids, and the glucosinolates. These are all secondary metabolites. They are chemicals that are not required for the general growth and maintenance of the plant but which serve as plant-defence products. The organic isothiocyanates are the main biologically active catabolites from the glucosinolate components of crucifers (e.g. canola), which like other glucosinolates defend plants against generalised insects (Panda and Khush 1995) but can also act as phagostimulants and as kairomones for crucifer specific pests (Dawson *et al.* 1993). Some primary and intermediate metabolites, such as citric acid and cysteine, can also act in plant defence chemistry (Jager *et al.* 1996). Twelve glucosinolates have been detected in canola leaves (Velasco *et al.* 2008; Goodarzi *et al.* 2015). These chemicals and other metabolites might reflect the reason for the different effects of canola cultivars on the life table parameters of *N. cymoides.*

The impact of antixenosis on the population dynamics is no less complex, with some of the effects paralleling those of antibiosis. For instance, reduced oviposition through non-preference is equivalent to reduced fecundity, and it can also increase larval movement, thereby slowing development time or increasing juvenile mortality (Thomas and Waage 1996).

Clearly an evaluation of host plant resistance should look further than just assessing damage or numbers of insects to gain a full appreciation of the ways in which resistance will have an impact on pest population. Therefore, the two-sex life table parameters of *N. cymoides* on five canola cultivar provided useful information for the establishment of integrated pest management in canola fields.

Acknowledgements

We wish to thank the authorities of the College of Agriculture, University of Guilan for their financial support. We would also like to say that this research was supported by the University of Gonbad e Qabus and we wish to thank the University for providing us with research facilities and Mr. Salahi Farahi from the Agriculture Research Station of Gonbad e Qabus provided us with canola seeds, which we greatly appreciated.

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