

## Research Article

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# Biological response of *Rhopalosiphum padi* and *Sipha flava* (Hemiptera: Aphididae) changes over generations

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**Abstract:** Multigenerational effects on biological aspects and on the life table of *Rhopalosiphum padi* (Linnaeus) (Hemiptera: Aphididae) and *Sipha flava* (Forbes) (Hemiptera: Aphididae) are not known. These studies can help further our understanding of the reasons for the conflicting results of the biological performance of aphids determined under laboratory conditions. Hence, the objective of the present work was to evaluate the biological aspects and life table of *R. padi* in brachiaria (*Urochloa ruziziensis* (R.Germ. & C.M.Evrard) Crins Poaceae) and *S. flava* in elephant grass (*Pennisetum purpureum* Schumach. Poaceae) during five consecutive generations, in the laboratory. The insects were observed daily under a stereoscopic microscope, recording: number of instars, duration and survival of each instar, duration and survival of the nymphal period, longevity of the adult, biological cycle (nymph + adult) and daily and total production of nymphs. During the five successive generations, the insects were subjected to the same climatic conditions and host plants. A joint analysis of the data obtained allowed us to record that the characteristics of the original infestation (first generation) influenced the results of research carried out in the laboratory with *S. flava* and *R. padi*, with significant differences in the biological parameters and life table of the aphids. Based on biological data, it was observed that *S. flava* showed greater adaptation,

with faster development and greater fertility in the fifth generation, and *R. padi* was negatively affected, showing lower longevity, nymphal survival and fecundity in the second, fourth and fifth generations. Thus, the standardization of the generation used and the use of multigeneration studies are important tools for biological studies of these aphid species and the application of these studies to forage improvement programs.

**Keywords:** multigenerations; aphids; forages; *Urochloa ruziziensis*; *Pennisetum purpureum*

**Resumo:** Os efeitos multigeracionais sobre os aspectos biológicos e sobre a tabela de vida de *Rhopalosiphum padi* (Linnaeus) (Hemiptera: Aphididae) e *Sipha flava* (Forbes) (Hemiptera: Aphididae) não são conhecidos. Esses estudos podem ajudar a entender as razões dos resultados conflitantes do desempenho biológico dos pulgões determinados em condições de laboratório. Assim, o objetivo do presente trabalho foi avaliar os aspectos biológicos e tabela de vida de *R. padi* em braquiária (*Urochloa ruziziensis* (R.Germ. & C.M.Evrard) Crins Poaceae) e *S. flava* em capim elefante (*Pennisetum purpureum* Schumach. Poaceae) durante cinco gerações consecutivas, em laboratório. Os insetos foram observados diariamente sob um microscópio estereoscópico, registrando-se: número de instares, duração e sobrevivência de cada instar, duração e sobrevivência do período ninfal, longevidade do adulto, ciclo biológico (ninfa + adulto) e produção diária e total de ninfas. Durante as cinco gerações sucessivas os insetos foram submetidos as mesmas condições climáticas e plantas hospedeiras. Uma análise conjunta dos dados obtidos permitiu registrar que as características da população natural (primeira geração), influenciam os resultados das pesquisas realizadas em laboratório com *S. flava* e *R. padi*, havendo diferenças significativas nos parâmetros biológicos e tabela de fertilidade dos afídeos. Com base nos dados biológicos observou-se que *S. flava* mostrou maior adaptação, com um desenvolvimento mais rápido e maior fertilidade na quinta geração e, *R. padi* foi negativamente afetado, apresentando longevidade,

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sobrevivência ninfal e fecundidade menores na segunda, quarta e quinta gerações. Assim, a padronização da geração utilizada e o uso de estudos de multigerações são ferramentas importantes para os estudos biológicos destas espécies de afídeos e da aplicação destes estudos em programas de melhoramento de forrageiras.

**Palavras-chave:** multigerações; afídeos; forrageiras; *Urochloa ruziziensis*; *Pennisetum purpureum*

## 1 Introduction

Livestock farming is socioeconomically important for Brazil. The sector employs more than 1.5 million people and generated approximately R\$357.4 billion in 2022 (CNA 2023). An important characteristic of Brazilian livestock farming, which guarantees low production costs, is that most herds are raised in pastures (Dias-Filho 2016) without supplemental feed. However, it is estimated that 80 % of the cultivated pasture areas in Central Brazil are in some state of degradation (Carvalho et al. 2017).

Pasture degradation refers to a process of sharp and continuous drop in productivity (Dias-Filho 2017), caused by the biotic and/or abiotic disturbances that interrupt normal growth of a forage crop (Batunacun et al. 2021), such as attack by insect pests (Dias-Filho 2011; Fonseca et al. 2016). Insect pests are considered the main biotic problem of pastures, considerably reducing the quantity and quality of forage, interfering with plant persistence, and consequently accelerating degradation processes (Congio et al. 2020; Valério and Nakano 1987).

The main groups of insect pests in pastures in Brazil are spittlebugs (Hemiptera: Cercopidae), stink bugs (Hemiptera: Miridae) and aphids (Hemiptera: Aphididae) (Auad et al. 2012; Fonseca et al. 2014, 2016; Paiva et al. 2020; Silva et al. 2019). Aphids damage plants by sucking sap, injecting toxic substances and acting as virus vectors (Sturza et al. 2012). *Rhopalosiphum padi* (Linnaeus) and *Sipha flava* (Forbes) are species widely distributed in tropical and subtropical regions, associated with different species of the Poaceae (Auad et al. 2009; Hentz and Nuessly 2004; Lumbierres et al. 2004; Oliveira et al. 2009a; Parchen and Auad 2016; Sturza et al. 2012; Wilson 2019).

Some studies of the biological aspects of *R. padi* and *S. flava* were conducted in Brazil, investigating, for example, the life expectancy and fertility parameters of *S. flava* in *Pennisetum purpureum* Schumacher. (Poaceae) (Oliveira et al. 2009b; Parchen and Auad 2016), and of *R. padi* in *Urochloa ruziziensis* (R.Germ. & C.M.Evrard) Crins (Poaceae) (Auad et al. 2009). These studies brought significant results and contributions to the study area of aphids in pastures, helping

to guide management strategies. However, the assessment and interpretation of the effects of pest outbreaks on the persistence of forages in pastures is still limited due to the limited knowledge of biology, life cycles and mobility of pests in different forages (Zydenbos et al. 2011), which includes the effect of successive generations of insects on their biological and behavioral aspects, as most research on insect responses has referred to a single generation (Moura et al. 2022; Seo et al. 2020).

Multiple generational effects in insect life table parameters sometimes are used to evaluate the nutritional components of host plants in artificial diets, resistance of transgenic plants, effects of elevated CO<sub>2</sub> and temperature, and effects on biological control agents in mass rearing (Chen et al. 2011; Gao et al. 2014; Guo et al. 2013; Li et al. 2022; Smith et al. 2007). For *R. padi* and *S. flava* multigenerational effects on biological parameters, including the life table, are not known. These studies could help in understanding, at least in part, the reasons for the conflicting results of the biological performance of aphids determined under laboratory conditions.

The objective of the present work was to evaluate the biological aspects and life table of *R. padi* in brachiaria (*U. ruziziensis*) and *S. flava* in elephant grass (*P. purpureum*) during five consecutive generations in the laboratory.

## 2 Materials and methods

### 2.1 Obtaining and maintaining plants and insects

The studies were carried out in the laboratory. The forage species, *U. ruziziensis* (brachiaria) and *P. purpureum* (elephant grass), were obtained from Embrapa Gado de Leite (Juiz de Fora, Minas Gerais, Brazil). The plants were kept in 1.5 L plastic pots. The soil was fertilized with urea (0.09 g/planter), simple superphosphate (0.04 g/planter), and potassium chloride (0.04 g/planter) based on soil analysis.

The aphid species *S. flava* and *R. padi* were obtained from a source population maintained on elephant grass and brachiaria, respectively, in a greenhouse (Embrapa Gado de Leite). One hundred adults of each aphid species were transferred, with fine brushes, to Petri dishes (100 × 15 mm) containing a 1 cm layer of 1 % agar, on which leaf sections from each forage were placed, covering the entire Petri dish. After aphid transfer, the Petri dishes were covered with voile fabric held by elastic bands and kept in a Fitotron-type climate chamber (25 ± 1 °C, 70 ± 10 % relative humidity and 12:12 h L:D). To prevent potential issues with fungi, all Petri dishes, leaf sections, and tools used in the process were properly sterilized before use. No fungal contamination

occurred during the experiment. Aphid voucher specimens were deposited in the Entomology Laboratory of Embrapa Gado de Leite (Juiz de Fora, Minas Gerais, Brazil). The elephant grass and brachiaria used as feeding substrate for *S. flava* and *R. padi*, respectively, as well as the climatic factors used were selected because they are the most suitable for the development of the species in the laboratory (Auad et al. 2009; Oliveira et al. 2009a).

## 2.2 Biological aspects and life tables

Nymphs of *S. flava* and *R. padi*, up to 12 h old, from our stock colony, were placed individually in rearing units (2.5 cm in diameter and 2.5 cm in height), containing a leaf disc (2.4 cm in diameter) of elephant grass and brachiaria used as feeding substrate for *S. flava* and *R. padi*, respectively, which was placed on a 1.0 cm layer of agar:water (1:1) to maintain the turgidity of the plant material. The breeding units were covered with voile fabric fixed with elastic and placed in BOD-type (biochemical oxygen demand-type) air-conditioned chambers (25 °C and 14 h photophase). The leaf discs were replaced when they showed signs of yellowing and drying. These individuals represented the first generation. The four successive generations were subjected to the same climatic conditions and host plants described previously. The studies were conducted in a completely randomized design with five treatments (generations) and 150 replications per treatment for each aphid species.

The insects were observed daily under a stereoscopic microscope, recording the following variables: number of instars, duration and survival of each instar, duration and survival of the nymphal period, longevity of the adult, the biological cycle, which includes both the nymph and adult stages, along with the daily production and total number of nymphs.

The life table was based on the parameters and equations proposed by Silveira-Neto et al. (1976) which include: net reproductive rate ( $R_0$ ), time interval between generations ( $T$ ), intrinsic rate of population increase ( $rm$ ), finite rate of increase ( $\lambda$ ) and time required for the population to double in size ( $DT$ ), the average age in the interval ( $x$ ), the specific fertility ( $m_x$ ) and the probability of survival ( $l_x$ ). The calculations were carried out using the equations  $R_0 = \sum (m_x \cdot l_x)$ ;  $T = \sum (m_x \cdot l_x \cdot x) / \sum (m_x \cdot l_x)$ ;  $rm = \log_e R_0 / T = \ln R_0 / T$ ;  $\lambda = e^{rm}$ ; and  $DT = \ln(2) / rm$ .

## 2.3 Statistical analysis

The data met the assumptions of normality for residue and homogeneity of variance (Shapiro–Wilk test and Bartlett

test,  $p < 0.01$ ). The variables: duration, survival and fecundity were compared between the five generations within each aphid species. For survival analysis, 10 individuals were grouped together, representing a repetition. The biological parameters evaluated were subjected to analysis of variance (ANOVA), and the means were compared using the Scott Knott test, with a significance of 5 %, using the Sisvar (2010) 5.1 software (Lavras, Minas Gerais, Brazil). The parameters of the life tables were analyzed using the Jackknife technique. The means were compared through the  $t$ -test at 5 % probability, using the software package Lifetable SAS (Maia et al. 2000).

## 3 Results

### 3.1 *Sipha flava*: biological aspects and life table

Significant differences were observed in the duration of each instar and the complete nymphal stage between the five generations of *S. flava* (Table 1). Individuals from the first generation took a significantly longer time to reach adulthood compared to subsequent generations, with an increase of 20 % when compared to the fifth generation. The longevity of adults and the biological cycle (nymph + adult) were observed to be significantly greater in the first generation (Table 1).

There was no significant difference in the total survival of the nymphs among the generations. However, for 1st instar nymphs, survival was significantly higher in the third generation, for 2nd instars it was higher in the fifth generation and for 4th instars it was higher in the first and fifth generations (Table 1). The average total number of nymphs produced by each female was significantly different among generations, with fifth generation females producing the highest number of nymphs (Table 1).

Significant differences were observed between generations in all life table parameters (Table 2). The time interval between generations and the time to double the *S. flava* population were significantly shorter in the fifth generation and the life table parameters  $rm$ ,  $R_0$  and  $\lambda$  were significantly higher. These results were related to greater survival, shorter time interval between the generations and greater number of *S. flava* nymphs produced in the fifth generation. Between the second and third generations there were no significant differences for any of the life table parameters. For the fourth generation, the values found were unfavorable for the population increase of *S. flava* (Table 2).

**Table 1:** Duration (days) and survival (%) of 1st, 2nd, 3rd and 4th instar nymphs, daily and total fertility and longevity of *Sipha flava* nymphs and adults on elephant grass, followed for five generations (G1 to G5) in the laboratory. Juiz de Fora, Minas Gerais, Brazil.

	Duration (days)					F	p
	G1	G2	G3	G4	G5		
1st instar	3.83 c ± 0.10	2.46 a ± 0.09	2.64 a ± 0.07	3.59 c ± 0.11	3.08 b ± 0.09	40.61	0.0000
2nd instar	2.41 b ± 0.13	2.08 a ± 0.09	2.17 a ± 0.09	2.65 b ± 0.09	2.70 b ± 0.11	7.80	0.0000
3rd instar	3.25 c ± 0.13	2.70 b ± 0.09	2.78 b ± 0.08	2.21 a ± 0.15	2.24 a ± 0.14	12.54	0.0000
4th instar	3.64 b ± 0.17	2.90 a ± 0.10	3.12 a ± 0.12	3.33 b ± 0.23	2.65 a ± 0.11	7.20	0.0000
Nymph	13.24 c ± 0.30	9.96 a ± 0.18	10.46 a ± 0.17	12.03 b ± 0.29	10.56 a ± 0.21	34.34	0.0000
Adult	12.03 b ± 0.65	8.56 a ± 0.76	7.19 a ± 0.64	8.88 a ± 1.09	9.92 a ± 0.75	6.16	0.0001
Biological cycle	25.27 c ± 0.71	18.52 a ± 0.74	17.65 a ± 0.66	20.90 b ± 1.17	20.48 b ± 0.74	15.54	0.0000

  

	Survival (%)					F	p
	G1	G2	G3	G4	G5		
1st instar	71.33 a ± 4.01	74.67 a ± 2.91	88.67 b ± 2.74	72.00 a ± 3.93	77.50 a ± 5.24	3.68	0.0091
2nd instar	67.98 a ± 4.29	75.61 a ± 4.10	89.72 a ± 3.05	78.16 a ± 5.40	83.10 b ± 5.54	3.68	0.0014
3rd instar	80.38 a ± 4.61	79.20 a ± 5.37	73.85 a ± 4.85	73.64 a ± 4.53	79.86 a ± 5.18	0.47	0.7574
4th instar	96.11 b ± 2.68	64.94 a ± 8.18	58.43 a ± 6.57	66.08 a ± 5.81	84.44 b ± 8.27	5.94	0.0004
Nymph	39.33 a ± 5.11	33.33 a ± 5.04	34.67 a ± 4.24	26.67 a ± 3.33	47.08 a ± 5.85	2.40	0.0590

  

	Number of nymphs produced per female					F	p
	G1	G2	G3	G4	G5		
	16.48 a ± 1.26	14.83 a ± 1.12	12.08 a ± 1.06	15.65 a ± 1.70	19.33 b ± 1.70	3.89	0.0045

Means ( $\pm$ SE) of duration and survival followed by the same letter in the rows did not differ according to the Scott-Knott test ( $p > 0.05$ ).

### 3.2 *Rhopalosiphum padi*: biological aspects and life table

All biological variables of *R. padi* showed significant differences between generations (Table 3). The nymphal period, longevity and biological cycle were significantly shorter for the second and fifth generations (Table 3).

Significantly higher survival was observed for 1st instar *R. padi* in the first generation, whereas significantly lower survival was observed for 4th instars in the fifth generation. The 2nd and 3rd instars showed no significant differences in

survival between generations. For the entire nymphal stage, survival was significantly higher in the 1st and 3rd generations (Table 3). The average total number of nymphs produced by each female was significantly higher for the third generation and significantly lower for the second and fifth generations.

There were also significant differences between generations in all life table parameters (Table 4). The third generation of *R. padi* showed a significantly shorter time to double the population along with significantly high  $rm$ ,  $R_0$  and  $\lambda$  values than the other generations. The second and fifth generations presented significantly lower reproductive parameters ( $rm$ ,  $R_0$  and  $\lambda$ ) and significantly longer times to double the population. The time between generations was observed to be significantly shorter for the second generation than for other generations (Table 4).

**Table 2:** Life table parameters for *Sipha flava* on elephant grass over five generations (G1 to G5) under laboratory conditions.

Generation	Parameters				
	T	$R_0$	$rm$	$\lambda$	DT
G1	19.43 a	2.80 b	0.053 b	1.055 b	12.99 b
G2	14.85 b	2.08 c	0.049 b	1.051 b	13.87 b
G3	14.97 b	2.06 c	0.049 b	1.050 b	13.98 b
G4	17.91 a	1.25 d	0.013 c	1.013 c	39.27 a
G5	13.95 c	3.45 a	0.089 a	1.093 a	7.75 c

T, time interval between each generation;  $R_0$ , net reproductive rate;  $rm$ , intrinsic rate of population increase;  $\lambda$ , finite rate of increase; DT, time necessary to double in population size (days).

## 4 Discussion

There are different variables that influence the development, longevity and fertility of aphids, including temperature and type of food (Hong et al. 2019; Park et al. 2017; Seo et al. 2020). However, most studies do not take into account that the influence of these variables may be different throughout the

**Table 3:** Duration (days) and survival (%) of nymphs of the 1st, 2nd, 3rd and 4th instars, daily and total fertility and longevity of nymphs and adults of *Rhopalosiphum padi* on brachiaria, followed for five generations in the laboratory.

	Duration (days)						<i>p</i>
	G1	G2	G3	G4	G5	<i>F</i>	
1st instar	2.52 b ± 0.08	2.15 a ± 0.07	2.55 b ± 0.08	2.58 b ± 0.09	2.49 b ± 0.06	4.68	0.0001
2nd instar	2.06 b ± 0.08	1.64 a ± 0.08	1.94 b ± 0.09	2.09 b ± 0.08	1.75 a ± 0.06	6.05	0.0001
3rd instar	1.85 b ± 0.10	1.60 a ± 0.08	1.93 b ± 0.07	1.86 b ± 0.07	1.86 b ± 0.06	2.29	0.0580
4th instar	2.24 b ± 0.13	2.04 b ± 0.09	1.90 a ± 0.07	1.82 a ± 0.09	1.92 a ± 0.08	2.98	0.1930
Nymph	8.58 b ± 0.18	7.35 a ± 0.18	8.19 b ± 0.16	8.25 b ± 0.15	7.68 a ± 0.13	8.84	0.0000
Adult	6.18 b ± 0.46	4.17 a ± 0.40	8.94 c ± 0.54	6.21 b ± 0.56	4.20 a ± 0.32	17.48	0.0000
Biological cycle	14.75 b ± 0.50	11.52 a ± 0.42	17.13 c ± 0.56	14.46 b ± 0.59	11.88 a ± 0.36	20.89	0.0000

  

	Survival (%)						<i>p</i>
	G1	G2	G3	G4	G5	<i>F</i>	
1st instar	96.67 c ± 1.26	80.00 a ± 3.09	82.07 a ± 3.39	88.00 b ± 2.79	90.00 b ± 2.18	6.26	0.0002
2nd instar	91.00 a ± 3.33	82.93 a ± 3.65	89.13 a ± 2.28	82.93 a ± 3.40	90.07 a ± 2.67	1.63	0.1754
3rd instar	82.67 a ± 3.38	82.27 a ± 3.54	89.07 a ± 2.73	77.07 a ± 3.94	81.93 a ± 4.15	1.43	0.2325
4th instar	84.73 b ± 4.38	82.80 b ± 3.44	88.80 b ± 3.65	73.47 b ± 6.14	55.93 a ± 6.71	6.78	0.0001
Nymph	62.00 b ± 4.60	46.00 a ± 4.45	58.47 b ± 4.77	40.67 a ± 4.31	40.00 a ± 6.09	4.37	0.0033

  

	Number of nymphs produced per female						<i>p</i>
	G1	G2	G3	G4	G5	<i>F</i>	
	15.76 b ± 1.51	7.26 c ± 0.96	24.71 a ± 1.71	14.35 b ± 1.62	8.20 c ± 1.01	23.46	0.0000

Means (±SE) by the same letter in the rows did not differ according to the Scott-Knott test ( $p > 0.05$ ).

**Table 4:** Life table parameters for *Rhopalosiphum padi* on brachiaria over five generations (G1 to G5) under laboratory conditions.

Generation	Parameters				
	<i>T</i>	<i>R</i> <sub>0</sub>	<i>rm</i>	$\lambda$	<i>DT</i>
G1	14.78 a	4.14 b	0.096 b	1.101 b	7.16 b
G2	9.36 c	1.27 d	0.026 e	1.027 d	15.76 a
G3	11.55 b	7.76 a	0.178 a	1.194 a	3.89 c
G4	14.97 a	3.09 c	0.076 c	1.079 c	9.02 b
G5	14.69 a	1.89 d	0.043 d	1.044 d	15.29 a

*T*, time interval between each generation; *R*<sub>0</sub>, net reproductive rate; *rm*, intrinsic rate of population increase;  $\lambda$ , finite rate of increase; *DT*, time necessary to double in population size (days).

generations of the insect. Biological studies applied over generations of target organisms allow a better understanding of the adaptation of aphid species, for example, to their host plants and to abiotic conditions.

In the present research it was shown that *S. flava* and *R. padi* demonstrated significant differences in performance depending on the generation, even under ideal host plant and climatic conditions, as determined in previous studies (Auaad et al. 2009; Oliveira et al. 2009a). These results contrast with those found in previous studies with other insect species, for example, in biological studies of *Mahanarva fimbriolata* (Stål

(Hemiptera: Cercopidae) for three generations in the laboratory, the results were similar for all generations (Garcia et al. 2006), which, in our case, gives a reliable support to validate the adaptation of the insect to the technique used to rear the species in the laboratory. Similarly, the life table parameters of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) remained relatively stable over five successive generations, between temperatures of 27–31 °C, indicating the insect's adaptation to these temperatures across generations (Guo et al. 2013).

Our results suggest that *S. flava* undergoes changes in biological variables across generations. In our study, the first generation exhibited a longer nymphal period and increased longevity, resulting in a longer biological cycle compared to subsequent generations. In contrast, previous studies on this species reported a shorter life cycle (from birth to death) and higher fertility in the first generation (Oliveira et al. 2009b; Parchen and Auaad 2016) when compared with the current research.

From the second generation onwards, the nymphal development of *S. flava* accelerated. The shorter feeding time of nymphs, and consequently less nutrient accumulation, may have led to shorter adult longevity. However, the shortening of the nymphal period and longevity over generations appears to be a positive adaptation for the species as



there are no significant effects on nymphal survival. Furthermore, in the fifth generation, high production of nymphs and high values in the life table parameters ( $rm$ ,  $R_0$  and  $\lambda$ ) were observed with shorter time between the generations and population doubling time. The duration of biological development in phytophagous insects is an important factor to help evaluate host quality, which over the course of development may or may not indicate low nutritional quality (Oliveira 2012; Parchen and Auad 2016). Furthermore, the action of abiotic factors can act in conjunction with nutrition, influencing the duration of development periods of the insects. Here we observed that over five generations in the laboratory, the biotic potential of *S. flava* increased.

*R. padi*, in general, presented unfavorable biological characteristics (longevity, nymphal survival, and fecundity) for the second, fourth and fifth generations, as well as a lower biotic potential for these generations. It suggests that the development of *R. padi* on brachiaria may be negatively affected over generations. In other studies, with this species, with the same host plant and under the same climatic conditions (Auad et al. 2009; Oliveira et al. 2009a; Parchen and Auad 2016) a shorter duration of the nymphal phase, a faster population doubling time, and higher nymphal survival were reported for the first generation compared to our findings.

Comparing some biological characteristics of the two aphid species, it was observed, for example, that the number of nymphs produced per female were approximately 15 % higher for the fifth generation of *S. flava*, in relation to the first, and about 90 % lower for the fifth generation of *R. padi*. Variations in life table parameters also were recorded with clear and specific variations among generations between the two species studied. The innate capacity to increase in number followed the following order for *S. flava*  $rm$  (generation = G):  $G1 = G2 = G3 < G4 + G5$  and for *R. padi*  $rm$ :  $G1 > G2 < G3 > G4 > G5$ , suggesting a greater adaptation for *S. flava* and an unfavorable relationship for *R. padi*, respectively, across the generations. These differences observed in the biology of *S. flava* and *R. padi* can be explained, in part, by the characteristics of each aphid species. Aphids are organisms sensitive to changes in temperature, humidity, quality of the host plant and each species can present different responses in relation to the biotic and abiotic factors and the intrinsic characteristics of the insect.

Our results are different from the results obtained for the first generation of these same species in other studies conducted under similar conditions (climatic conditions and host plant) (Auad et al. 2009; Oliveira et al. 2009a; Parchen and Auad 2016). We attribute these differences to the intrinsic genetic characteristics of the first generation collected in the original infestation, as in the other studies, the first generation was obtained from aphid colonies raised under

controlled conditions. This suggests the need for standardization of the individuals to be used in aphid biological studies.

A joint analysis of the data presented here allows us to hypothesize that the characteristics of populations collected in the original infestation (first generation) influence the results of the biological studies carried out in the laboratory with *S. flava* and *R. padi*, as significant differences were observed in the biological parameters and life table parameters of aphids over multiple generations. Thus, the standardization of the generation to be used and the origin of the insects to be used in the studies (from the field or from laboratory colonies) are important factors in obtaining more reliable and comparable biological information between the studies carried out by the different research groups of insects with the same aphid species. Finally, multigeneration studies are important tools for understanding the responses of *S. flava* and *R. padi*, especially in relation to the adaptation of these species to host plants over multiple generations. Such studies can be informative in genetic improvement programs of forages, for example those programs aimed at enhancing genetic resistance to aphid species.

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