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Original Article

The life cycle of Neotropical ground beetle, *Abaris basistriata* (Coleoptera: Carabidae) reared on different substrates

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Abstract

Carabids are recognized worldwide as biological control agents of agricultural pests. The objective was to compare the life cycle of *Abaris basistriata* Chaudoir (Coleoptera: Carabidae) on three substrates: soil, fine vermiculite, or paper napkins. The biological cycle of *A. basistriata* presented different durations in soil and paper. The viability of eggs and larvae survival of the first and second instars were similar on all three substrates, while the third instar and pupa in the soil presented higher survival when compared with vermiculite and paper. The soil substrate was more favorable for the longevity of the carabid beetle. *Abaris basistriata* showed a shorter pre-oviposition period and a higher oviposition and post-oviposition period in the soil. Fecundity and fertility were higher when *A. basistriata* was reared on soil. The soil was most favorable substrate for rearing of *A. basistriata* in the laboratory. This information may make this species useful for the biological control.

Keywords: biological cycle, biological control, laboratory rearing, predation, prey.

Ciclo de vida do besouro de solo Neotropical, *Abaris basistriata* (Coleoptera: Carabidae) criado em diferentes substratos

Resumo

Os carabídeos são reconhecidos mundialmente como agentes de controle biológico de pragas agrícolas. O objetivo foi comparar o ciclo de vida de *Abaris basistriata* Chaudoir (Coleoptera: Carabidae) em três substratos: solo, vermiculita fina, ou guardanapos de papel. O ciclo biológico de *A. basistriata* apresentou diferentes durações no solo e no papel. A viabilidade dos ovos e a sobrevivência das larvas do primeiro e segundo instares foram semelhantes nos três substratos, enquanto que o terceiro instar e a pupa apresentaram maior sobrevivência no solo quando comparados com vermiculita e papel. O substrato solo foi mais favorável à longevidade do carabídeo. *Abaris basistriata* mostrou menor período de pré-oviposição e maior período de oviposição e pós-oviposição no solo. A fecundidade e a fertilidade foram maiores quando *A. basistriata* foi criado no solo. O solo foi o substrato mais favorável para a criação de *A. basistriata* no laboratório. Estas informações são importantes e podem tornar essa espécie útil para o controlo biológico.

Palavras-chave: ciclo biológico, controle biológico, criação em laboratório, predação, presa.

1. Introduction

The expansion and evolution of Integrated Pest Management programs have required the refinement of techniques for insect rearing in order to implement new technologies for pest control, such as biological control with predators and parasitoids (Cônsoli and Parra, 1997). Furthermore, it can permit the introduction of individuals in the field for biological control (Symondson et al., 2002).

Beetles of the family Carabidae are considered dominant predators because of their role in food chain dynamics and pest control (Toft and Bilde, 2002). In order to develop and implement biological control programs, it is essential to have information about the biology of the potential biological control species to understand their reproductive capacity, feeding habits, voracity, and predatory potential if they are to be used as agents of agricultural pests (Suenaga and Hamamura, 1998; Snyder and Ives, 2001; Fawki and Toft, 2005; Saska, 2008).

The number of predatory insects per generation in the laboratory can be increased from a small number of adults in the field (Chambers, 1977), but only if the rearing conditions provide the proper environment for their development (Goulet, 1976; Huk and Kühne, 1999). To determine these conditions, observations and tests are required, which can be difficult to perform if the species is rare or poorly studied (Gwiazdowski et al., 2011). Moreover, larval development requires very specific

conditions, mainly related to the structure of the substrate on which they are reared (Petersen, 1998). Several studies have demonstrated the importance of substrate choice for insect breeding in the laboratory (Van Dijk and Den Boer, 1992; Huk and Kühne, 1999; Ávila et al., 2000). According to Lundgren et al. (2005), some substrates do not adequately retain water, which affects larval development and the stages larval. Furthermore, some substrates types can hinder the movement of larvae.

In Brazil, there is little information on the biology and rearing techniques of carabid species found in agro-ecosystems. The carabid beetle genus *Abaris* Dejean occurs only in the Americas (Will, 2002). *Abaris basistriata* Chaudoir (Coleoptera: Carabidae) is a specie widely distributed in South America agro-ecosystems (Will, 2002; Cividanes et al., 2009). However, there are no studies on the life cycle of *A. basistriata* in the laboratory.

Thus, the objective of this study was to investigate the life cycle of the carabid beetle *A. basistriata* reared on three different types of substrate.

2. Material and Methods

The study was conducted at the Ecology Laboratory of the College of Agricultural and Veterinary Sciences, State University of São Paulo (UNESP/FCAV). The beetle colony and all experiments were maintained in a climatic chamber at 26 ± 1 °C, 70 ± 10% relative humidity, and L14:D10 h photoperiod. Adults of *A. basistriata* were collected from soils planted with maize crop at Jaboticabal city, São Paulo, Brazil (21°15'22"S, 48°18'58"W, at 595 m asl). The insects were collected by hand or caught in pitfall traps on December 2012 to January 2013 (Clark et al., 1994; Barbosa et al., 2012).

Beetles were identified by comparison with specimens of the collection at the Ecology Laboratory (UNESP/FCAV). The sex of specie was determined by examining the shape of the protarsi, with the aid of a magnifying lens. The protarsi of male are dilated and pronounced than in the females (Barbosa et al., 2012).

2.1. Carabid adults colonies and egg collection

Carabid beetle eggs were obtained based on the methodology reported by Barbosa et al. (2012). Adults of *A. basistriata* collected from the field were separated into 30 pairs of males and females and confined in plastic containers (11 × 11 × 3.5 cm). In each plastic container was kept a carabid couple. These containers were divided into two halves with a 1.0-cm high silicone barrier. One half was filled with a soil layer of 1.0 cm and served as an oviposition substrate for females, while the other half was lined with filter paper for food supply. Black pieces of Ethyl Vinyl Acetate rubber (3.0 cm^2) were used as shelter for the insects. Water was provided through moistened wads of absorbent cotton. The adult pairs were fed daily with *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae ad libitum and the soil of containers was sieved through to collect eggs and then transferred to life cycle experiments (see below).

For feeding carabids, we used larvae of yellow mealworm beetles *T. molitor* as alternative prey as they are easily cultured in the laboratory (Zanuncio et al., 2008).

2.2. The life cycle of Abaris basistriata reared on different substrates

The substrates for rearing assessed were as follows: 1) soil (Saska, 2008; Saska and Honek, 2008); 2) vermiculite (Lundgren et al., 2005; Weber et al., 2006); and 3) paper (Jorgensen and Toft, 1997).

The soil was (same soil type used on adults colonies and egg collection) collected from a layer (0-20 cm) of red eutrophic latosol of clay texture (Centurion et al., 1995), located at Jaboticabal, São Paulo, Brazil. The soil contained 62% clay, 16% silt, 22% sand; 14 g dm^{-3} of organic matter, 20 mg dm^{-3} of P, 8 mg dm^{-3} of S, 28 mmol dm^{-3} of Ca, 11 mmol dm^{-3} of Mg, 3 mmol dm^{-3} of K, 1 mmol dm^{-3} of Al, with a pH ($CaCl_2$) of 5.5. We used vermiculite Refratil® with a granulometry of 1.00-2.00 mm and paper napkin Scott® with 80% cellulose and 20% secondary fiber. Before being placed in the containers the paper was lightly kneaded with the hands. Soil and vermiculite were sterilized by an autoclave at 121 °C for 30 min. Substrates were kept at 50% humidity using the gravimetric method to determine the amount of water required. The soil was chosen because it is the natural substrate carabids, and fine vermiculite and paper napkins because they are easy to handle substrates.

Initially, the eggs were transferred to Petri dishes 9.0 × 1.5 cm containing one of three substrates (see above) to observe the development of *A. basistriata*. The eggs were observed daily and hatched larvae were counted.

The assessment of the larval and pupal stages was carried out using 140 mL plastic pots, filled to a height of 3 cm with their respective substrates of origin. The pots were closed with synthetic polyester fabric (0.03 × 0.03 mm mesh size) and substrate moisture maintained at 50%. The newly hatched first instar larva of carabid was placed individually in these pots to avoid cannibalism. *Tenebrio molitor* larvae (3.0 mm in length) were provided ad libitum as food for developing carabids. The pots were evaluated daily to determine the presence of dead larvae and pupae, and to check the substrate moisture and renew the food.

After emergence, adults were left undisturbed for 24 h to complete integument sclerotization. Subsequently, they were separated by sex, with one pair per plastic container, similar to those used to maintain carabids collected in the field. The pairs were fed ad libitum with *T. molitor* larvae, 5.0 mm in length. The containers were checked daily to verify female oviposition. All eggs laid were removed and transferred to Petri dishes 9.0 × 1.5 cm containing the respective substrates used by the preceding larval and pupal stages (i.e., treatments were continuous throughout the lifecycle).

2.3. Statistical analyses

The biological parameters evaluated included: eggs incubation period, larval instar duration, period of pupation, adult longevity, pre-oviposition, oviposition,

and post-oviposition, viability of stages, and duration of egg-to-adult development. The sex ratio was calculated by formula: Sex ratio = number of females/total number of individuals (females + males). The experiment was conducted using a completely randomized design of the three substrate treatments (soil, vermiculite, or paper), with 120 replicates per treatment (n = 360). Data were analyzed using analysis of variance (ANOVA) and means were compared using Tukey's test at a 5% probability level with software Assistat (Silva and Azevedo, 2002). Before being analyzed statistically, the data recording the numbers of eggs were log transformed (× +5). Data expressed as percentages were transformed into arc sine. The presented data values are not transformed.

3. Results

3.1. The life cycle of Abaris basistriata reared on different substrates

The biological cycle duration of *A. basistriata* from egg to adult ranged from 35.8 to 38.5 days, with a significant difference between the substrates. Eggs held in soil, vermiculite, or paper presented an incubation period of 6.0, 5.9, and 5.5 days, respectively, which did not differ significantly (Table 1). There are three larval instars. Larvae of first and second instars showed similar duration in the three substrates (Table 1). Third instar larvae and pupae developed faster in the soil. The soil also led to a shorter biological cycle (egg to adult).

Beginning of the experiment biological cycle of *A. basistriata*, eggs viability was similar between substrates. The larval survival the first and second instar was similar. In the third instar, and in pupa, this biological parameter was significantly higher when the carabid beetle was kept in soil. The survival of the biological cycle (egg-adult) differed significantly, with the soil as the best substrate for rearing the carabid beetle. The percentages of insects reaching adult stages reared in soil, vermiculite, and paper, were 57.5, 37.5, and 32.5%, respectively (Table 2).

Female longevity in the soil, vermiculite, and paper ranged from 226, 168.6, and 143.4 days, respectively, whereas males showed a longevity of 192.7 days (soil), 147.7 days (vermiculite), and 124.3 days (paper). Soil was the most favorable substrate for longevity since the carabid beetle survived about 1.4 times longer than when kept in vermiculite or paper. It is noteworthy that female longevity was significantly higher than male longevity (born in the

Table 1. Mean duration days (± SE) of different stages of development of *Abaris basistriata* and egg to adult (Biological cycle).

Substrate	Egg[1]	Instar			Pupa	Total (Biological cycle)
		1°	2°	3°		
Soil	6.0 ± 0.12a[2] (*n=101*)	4.5 ± 0.12a[2] (*n=91*)	5.4 ± 0.12a[2] (*n=82*)	13.1 ± 0.35b[2] (*n=75*)	6.0 ± 0.10b[2] (*n=69*)	35.8 ± 0.44b[2] (*n=69*)
Vermiculite	5.9 ± 0.11a (*n=100*)	4.6 ± 0.10a (*n=90*)	5.5 ± 0.13a (*n=81*)	14.4 ± 0.39a (*n=62*)	7.0 ± 0.13a (*n=45*)	37.3 ± 0.87ab (*n=45*)
Paper	5.5 ± 0.12a (*n=99*)	4.8 ± 0.11a (*n=90*)	5.8 ± 0.16a (*n=81*)	15.1 ± 0.33a (*n=55*)	7.3 ± 0.17a (*n=39*)	38.4 ± 0.51a (*n=39*)
Statistics	F=0.78[ns] df=2; P>0.05	F=1.40[ns] df=2; P >0.05	F=2.03[ns] df=2; P >0.05	F=8.16** df=2; P <0.001	F=24.89** df=2; P<0.001	F=5.68** df=2; P<0.01

Carabids reared at 26 ± 1 °C, 70 ± 10% RH and 14:10 (L:D) h. [1]The experiment started with 120 eggs in all treatments; [2]Data are mean ± SE. Different letters in each row indicate ignificant differences between substrates by Tukey test (analysis of variance, F-test, P < 0.05); **Differ significantly P< 0.01; [ns]: Not differ significantly P > 0.05.

Table 2. Egg viability (%) and survival (%) of *Abaris basistriata* from first instar larvae to adults in different substrate types.

Substrate	Egg[2] viability	Instar			Pupa	Total (Biological cycle)
		1°	2°	3°		
Soil	84.2 ± 4.56a[1] (*n=101*)	90.6 ± 2.30a[1] (*n=91*)	89.9 ± 2.51a[1] (*n=82*)	91.5 ± 2.65a[1] (*n=75*)	91.6 ± 3.79a[1] (*n=69*)	57.5 ± 4.72a[1] (*n=69*)
Vermiculite	83.3 ± 4.31a (*n=100*)	90.3 ± 2.05a (*n=90*)	89.5 ± 2.11a (*n=81*)	76.4 ± 3.22b (*n=62*)	73.0 ± 1.84b (*n=45*)	37.5 ± 3.93b (*n=45*)
Paper	82.5 ± 4.39a (*n=99*)	90.1 ± 2.50a (*n=90*)	88.8 ± 2.67a (*n=81*)	66.7 ± 2.57b (*n=55*)	69.3 ± 4.23b (*n=39*)	32.5 ± 4.35b (*n=39*)
Statistics	F=0.01[ns] df=2; P>0.05	F=0.04[ns] df=2; P>0.05	F=0.01[ns] df=2; P>0.05	F=19.42** df=2; P<0.0001	F=3.67* df=2; P<0.05	F=5.90** df=2; P<0.01

Carabids reared at 26 ± 1 °C, 70 ± 10% RH and 14:10 (L:D) h. [1]Data are mean ± SE. Different letters in each row indicate significant differences between substrates by Tukey test (analysis of variance, F -test, P < 0.05); [2]The experiment started with 120 eggs in all treatments; n = number of egg to adult. *Differ significantly P < 0.05; **Differ significantly P < 0.01; [ns]: not differ significantly P > 0.05; Original egg viability (%) and survival (%) data were transformed into arc sine.

laboratory belonging to the biological cycle experiment) (Table 3).

The total number of adults that emerged in the soil, vermiculite, and paper was 69, 45, and 39 individuals, respectively, of which only 2.2% had wing deformities when reared in the soil, 4.4% in vermiculite, and 9.6% on paper. Regardless of the rearing substrate, there was no difference in the percentage of deformed adults, soil 2.2 ± 2.2, vermiculite 4.4 ± 2.5, and paper 9.6 ± 2.9 (F=1.11; df=2; P>0.05). All adults with deformed wings did not mate and died within 2 to 25 days. The average carabid beetle sex ratio reared in the three substrates was 0.56 and there was no difference between the different conditions, soil 0.55 ± 0.03, vermiculite 0.56 ± 0.04, and paper 0.56 ± 0.05 (F=0.96; df=2; P >0.05).

The pre-oviposition period was shorter for *A. basistriata* maintained in the soil than in vermiculite or paper. Moreover, the periods of oviposition and post-oviposition were significantly longer for females kept in soil (Table 4).

The reproduction of *A. basistriata* females born in the laboratory (belonging to the biological cycle experiment) was differed between substrates. The overall egg production was higher in soil than in vermiculite and paper substrates (Table 5), with differences between substrates were significant. On average, egg production was lowest on paper substrate and better on soil substrate. In soil, there were 85.5 eggs per female, which was significantly higher than that obtained in vermiculite and paper. Considering the number of eggs produced by *A. basistriata* in the three substrates, it was 5.2 times higher in the soil than that observed in vermiculite and 9.5 times higher than in paper (Table 5). Thus, the least favorable substrate for oviposition was paper, while soil was the preferred option.

Among the substrates that allowed *A. basistriata* females born in the laboratory to reached oviposition, we observed that the fertility of eggs varied significantly, ranging from 69.9 to 86.3%. The highest fertility was observed for females reared in the soil substrate. The fertility of eggs

Table 3. Mean longevity days (± SE) of females and males of *Abaris basistriata*.

Adult	Substrate			Statistics
	Soil	Vermiculite	Paper	
Females	226.0 ± 14.45 a[1]A[2] (*n=38*)	168.6 ± 10.57 bA (*n=25*)	143.4 ± 6.27 bA (*n=22*)	*F*=14.96** df=2; *P*<0.001
Males	192.7 ± 9.16 aB (*n=31*)	147.7 ± 9.11 bB (*n=20*)	124.3 ± 6.11 bB (*n=17*)	*F*=14.56** df=2; *P*<0.001
Statistics	*F*=5.52* df=1; *P*<0.05	*F*=5.76* df=1; *P*<0.05	*F*=4.76* df=1; *P*<0.05	

Beetles reared at 26 ± 1 °C, 70 ± 10% RH and 14:10 (L:D) h, born in the laboratory (belonging to the biological cycle experiment). [1]Data are mean ± SE. Different letters in each row indicate significant differences between substrates by Tukey test (analysis of variance, F -test, P < 0.05); [2]Data are mean ± SE. Different letters in each column indicate significant differences between substrates by Tukey test (analysis of variance, F -test, P < 0.05); n = numbers of adults. *Differ significantly P < 0.05; **Differ significantly P < 0.01.

Table 4. Mean duration days (± SE) of the periods of pre-oviposition, oviposition and post-oviposition females of *Abaris basistriata*.

Substrate	Pre-oviposition	Oviposition	Post-oviposition
Soil[2]	5.1 ± 0.38 b[1]	90.2 ± 6.40 a[1]	130.8 ± 8.96 a[1]
Vermiculite[3]	7.0 ± 0.44 a	60.6 ± 5.10 b	101.0 ± 7.70 b
Paper[4]	7.8 ± 0.62 a	61.8 ± 4.70 b	73.9 ± 4.57 c
Statistics	*F*=7.78** df=2; *P*<0.01	*F*=9.44** df=2; *P*<0.001	*F*=15.14** df=2; *P*<0.001

Egg-laying at 26 ± 1 °C, 70 ± 10% RH and 14:10 (L:D) h, belonging to the biological cycle experiment. [1]Data are mean ± SE. Different letters in each row indicate significant differences between substrates by Tukey test (analysis of variance, F-test, P < 0.05); [2]number of female = 38; [3]number of female = 25; [4]number of female = 22; **Differ significantly P < 0.01.

Table 5. Effect of substrate on the fecundity (eggs per female) and egg fertility (%) of *Abaris basistriata*.

Substrate	Fecundity	Fertility (%)
Soil[2]	85.5 ± 0.07 a[1]	86.3 ± 1.17 a[1]
Vermiculite[3]	16.6 ± 0.03 b	69.9 ± 1.32 b
Paper[4]	9.0 ± 0.01 c	70.0 ± 1.94 b
Statistics	*F*=26.83** df=2; *P*<0.0001	*F*=36.78** df=2; *P*<0.0001

Eggs incubated at 26 ± 1 °C, 70 ± 10% RH and 14:10 (L:D) h, belonging to the biological cycle experiment. [1]Data are mean ± SE. Different letters in each row indicate significant differences between substrates by Tukey test (analysis of variance, F -test, P < 0.05); [2]number of female = 38; [3]number of female = 25; [4]number of female = 22; **Differ significantly P < 0.01. Original fecundity data were transformed into log (x + 5). Egg fertility (%) data were transformed into arc sine.

was similar when *A. basistriata* was kept on paper (70.0%) and vermiculite (69.9%) substrates (Table 5).

4. Discussion

In this study, the duration of the complete life cycle of a species of the genus *Abaris* was determined, to our knowledge, for the first time. In Brazil, life cycle information of carabid beetles is scarce, particularly on the Pterostichini tribe that embraces the genus *Abaris* (Will, 2002). The biological cycle duration of *A. basistriata* (35.8-38.5 days) was higher than that reported for some other species of carabid beetles present in Brazilian agro-ecosystems (Pegoraro and Foerster, 1985; Correa-Ferreira and Pollato, 1989).

The selection of suitable substrate is a critical step for determining the duration of the biological cycle of *A. basistriata*. Intrinsic factors of each substrate play an important role in the success or failure of carabid beetle development. Petersen (1998) noted that larval development of carabid beetles requires very specific conditions, especially related to substrate structure. According to Lundgren et al. (2005), substrates that are more abrasive can affect the larvae cuticle and/or interfere with instar transition, whilst the consistency of some substrates may restrict movement of carabid beetle larvae. Furthermore, some substrates do not adequately retain water, adversely influencing larval development.

Soil enabled tunneling and the formation of pupal chambers by *A. basistriata* larvae, considerably increasing the survival of third instar larvae. On the other hand, paper and vermiculite substrates did not permit tunneling or the formation of pupal chambers. The formation of a pupal chamber by carabid beetles is very common. Goulet (1976) noted that 80 species of carabid beetles built chambers before the pupal period.

Cornelisse and Hafernik (2009) and Gwiazdowski et al. (2011) point out that each species of Carabidae requires a specific substrate, justifying the search for substrates that are easy to handle and more suitable for the insect. The high survival of *A. basistriata* pupae in soil is probably due to the presence of the pupal chamber, which provides adequate moisture for development.

In general, the results obtained from rearing carabid beetles on filter paper were not satisfactory (Kirk, 1971), as there was no larval development (Lundgren et al., 2005). However, it was found that paper type could directly influence carabid beetle development. In our study, the *A. basistriata* biological cycle was completed on the paper napkin. It is likely that the texture of the paper used was critical for survival of *A. basistriata*. The texture of the paper probably allowed enough water retention to maintain each biological stage of *A. basistriata*.

The longevity of *A. basistriata* observed in this study is consistent with the reports of Barbosa et al. (2012). These authors found that *A. basistriata* adults collected in the field, fed *T. molitor,* and kept in soil, survived for up to 225 days. According to Ball and Bousquet (2000), the life cycle of carabid beetles is long; a year for most species, but up to four years for others.

Abaris basistriata deposited individual eggs into small holes in the soil, a fact noted by Huk and Kühne (1999) and Ball and Bousquet (2000). Differences in fecundity and fertility of *A. basistriata* can be attributed to types of substrate used; soil provided higher reproductive capacity and higher egg fertility compared to vermiculite and paper. Goulet (1976) observed egg fertility between 70-90% for various species of carabid beetles kept in soil under laboratory conditions.

Although *A. basistriata* completed their biological cycle in soil, vermiculite, and paper, the results for the latter two substrates showed that they had an adverse effect on the biology of the species when compared to soil. In the vermiculite and paper substrates, some pupae were found on the surface, while others were observed in contact with the bottom of rearing pots, apparently in an attempt to form a pupal chamber. Vermiculite and paper substrates did not provide adequate conditions for *A. basistriata* pupal chamber formation. It is probable that the absence of a pupal chamber led to pupae exposure to adverse microclimate conditions, related to humidity, temperature, and light. So it is so important to study the choice of substrate for the creation carabids. Other carabids created in the laboratory also had interference of the substrates on the development, mainly in the stage of pupa (Luff, 1973; Symondson, 1994; Lundgren et al., 2005).

The present study showed that soil, fine vermiculite, and paper napkins can be used to rear *A. basistriata*, but soil provides the most favorable conditions for rearing this species in the laboratory. Thus, the use of soil in rearing laboratory *A. basistriata* a reduced mortality of larvae and pupae. Moreover, in this substrate the reproductive period and fecundity were higher compared to those on other studied substrates. It is noteworthy that our experimental conditions permit the continuous rearing of *A. basistriata* in the laboratory and hence are an indication that this species can be used for inoculative release in biological pest control programs.

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