Laboratory rearing of *Sycanus annulicornis* (Hemiptera: Reduviidae) on two species of prey: Differences in its biology and efficiency as a predator of the nettle caterpillar pest *Setothosea asigna* (Lepidoptera: Limacodidae)

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Abstract. *Setothosea asigna* van Eecke is a dominant defoliator pest in oil palm plantations. To control this pest, a generalist predatory bug, *Sycanus annulicornis* Dohrn, was used as it is easy to rear on several different species of prey. In this study, we evaluated the influence of different prey on the biology and the ability of *S. annulicornis* to attack and kill the nettle caterpillar pest *S. asigna*. Based on laboratory rearing, the larvae of *Crocidolomia pavonana* F. (Lepidoptera: Crambidae) is a suitable prey for both the growth and development of *S. annulicornis*, as its nymphal development is shorter (74.0 ± 7.3 days) and adult longevity longer (81.0 ± 9.0 days for male and 64.8 ± 12.4 days for female, respectively) than when reared on the larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (44.0 ± 16.7 days for male and 52.6 ± 14.4 days for female). However, *S. annulicornis* reared on *T. molitor* larvae attacked 2.0–2.2 larvae of *S. asigna* per day, which is more than the 1.6–1.7 larvae per day of those reared on *C. pavonana*, which indicates that the larvae of *T. molitor* are a more effective diet for rearing *S. annulicornis* as biocontrol agent for the *S. asigna*.

INTRODUCTION

Oil palm, *Elaeis guineensis* Jacq, is an important oil crop introduced to meet the vegetable oil needs of Indonesia. The main factor limiting the oil palm productivity in Indonesia is existence of the nettle caterpillar, which defoliates oil palm (Kalidas, 2012). Outbreaks of nettle caterpillars cause serious damage to oil palm plantations in Indonesia and Malaysia (Kalshoven, 1981). The nettle caterpillar pest most commonly found in oil palm plantations, particularly in East Kalimantan and generally in Southeast Asia, is *Setothosea asigna* (Lepidoptera: Limacodidae) (Cock et al., 1987; Lay, 1996). *S. asigna* attacks both young and old oil palm plantations with heavy infestation causing yield losses of 70% in the first year after defoliation and 90% when the attack continues for a second year (Sipayung et al., 1989; Sudharto, 1998). Heavy incidence of infestation leads to complete defoliation, resulting in a short period of time in the burnt appearance of the palms (Kochu Babu & Kalidas, 2004) and a decrease in the production of up to 93% (Pahan, 2006). Currently, the major tool for controlling this pest in oil palm plantations are environmentally unfriendly broad-spectrum insecticides. Intensive use of insecticides has a negative effect on environmental sustainability, pest resistance, resurgence, outbreaks of secondary pests and extinction of non-target insects, such as natural enemies and pollinating insects (Aktar et al., 2009; Noor Farehan et al., 2013; Becker & Liess, 2015). Therefore, several methods of biological control focusing on the rearing and releasing of natural enemies for suppressing defoliator pests are regarded as more efficient than using insecticides as it costs less and there are fewer social and ecological problems (Garcia & Ricalde, 2013; van Zyl & Malan, 2014; Tomson et al., 2017).

Reduviid predators are polyphagous and important natural enemies of several defoliating pests (Sahayaraj, 2014). *Sycanus* is a predacious reduviid that has a wide prey diet including several orders of insects, such as both the larvae and pupae of Lepidoptera, Coleoptera and Diptera (Sahayaraj & Balasubramanian, 2016) and is considered to have the potential to be a biological control agent. Several species of *Sycanus* have been studied as biological control agents of the defoliator pests in oil palm plantations

(Kalshoven, 1981), such as *S. dichotomus* Stål (Singh, 1992; Zulkefli et al., 2004; Syari et al., 2010; Jamian et al., 2016), *S. macracanthus* Stål (Tiong, 1996) and *S. collaris* (Jamjanya et al., 2014).

S. annulicornis Dohrn (Hemiptera: Reduviidae) is one of the *Sycanus* species commonly found in several agro-ecosystems in Indonesia, such as soybean and rice ecosystems (Kalshoven, 1981) and in oil palm plantations (pers. observ.), but is not reported as a biological control agent of the nettle caterpillar, either in the laboratory or the field. Kalshoven (1981) mentions that *S. annulicornis* attacks large caterpillars and *Helopeltis* bugs. This accords with our observation in the laboratory that *S. annulicornis* can attack the last instar of *S. asigna*, which has a body length of up to 3.5 cm (1 cm longer than *S. annulicornis* adults) and other caterpillars with body lengths up to 5.0 cm (twice the longth of *S. annulicornis* adults) (Fig. S5). This indicates that this predatory insect has the potential to be used as a biological control agent to control *S. asigna*.

Due to the intensive use of insecticides for controlling the *S. asigna* pests, predatory insects, including *S. annulicornis*, are rarely found in the field. The scarcity of predatory insects, especially when outbreaks of the nettle caterpillar occur, means pest control based solely on the natural abundance of predatory insects cannot be relied upon. Hence, predators must be mass reared in large quantities if biological control is to be successful. One of the problems in the mass production of this predatory insect is an inherent problem associated with producing sufficient prey for the predator. Different species of prey can influence the biology of the predatory insects reared on them (George et al., 1998; Mendes et al., 2002; Zulkefli et al., 2004; Chandral & Sinazer, 2011; Syari et al., 2011; Ahmad & Kamarudin, 2016) and their predatory ability in terms of the target pest (van Alphen & Jervis, 1996; Lv et al., 2016). Currently, there is little information on an appropriate species of prey for rearing of *S. annulicornis* in the laboratory and the ability of *S. annulicornis* to attack and kill *S. asigna* is unknown. Therefore, in order to find a suitable prey for rearing *S. annulicornis* with a strong tendency to attack and kill *S. asigna*, we evaluated the biological characteristics and predatory ability of *S. annulicornis* in terms of *S. asigna* when reared on different species of prey, i.e., *Tenebrio molitor* L. larvae (Coleoptera: Tenebrionidae) and *Crocidolomia pavonana* F. larvae (Lepidoptera: Crambidae).

MATERIAL AND METHODS

Source and rearing of prey

T. molitor

T. molitor larvae used in this study were obtained from a traditional bird market in Bandung, West Java Province, Indonesia. The larvae were reared in the laboratory using a plastic container (width: 30 cm, length: 50 cm, height: 30 cm) using bran as the source of food and a medium. A cabbage leaf was placed on top of the bran as a source of moisture. The pupae were transferred into a new plastic container to avoid cannibalism of pupae by the larvae and kept until beetle emergence. Once a mating couple of beetles were observed, they were moved to a new container

containing bran. Eggs with hardened shells were obtained after 3–4 d. Eggs laid by female beetles were collected. After the eggs hatched into larvae, the larvae were reared to the 11th – 12th instar, and then used in this study as prey for *S. annulicornis*.

C. pavonana

C. pavonana larvae used in this study were collected from cabbage crops in Balai Penelitian Tanaman Sayuran (Balitsa), Lembang, West Java Province, Indonesia. The larvae were reared in the laboratory until they developed into pupae in a plastic container (width: 30 cm, length: 50 cm, height: 30 cm) using cabbage leaves as a source of food. The pupae were collected and placed in a new plastic container until the moths emerged. A 10% (v/v) honey solution in small cotton wool balls was provided as a supplementary food for the moths. Egg batches laid by the moths on cabbage leaves were collected daily and transferred to a new container where they were kept until they hatched. Newly emerged larvae were reared on a diet of cabbage leaves to the 3rd instar and then used as a prey for *S. annulicornis*.

Rearing / maintenance of *S. annulicornis*

The adult males and females of *S. annulicornis* were collected from soybean plantations at Bogor, West Java Province, Indonesia (6°33′6″S and 106°44′44″E). They were individually reared in plastic containers (diameter 5 cm and height 6.5 cm) on 11th–12th instar larvae of the mealworm, *T. molitor*, under laboratory conditions (temp. 26 ± 2°C, 65 ± 5% rh, and 12L : 12D photoperiod. The males and females were allowed to mate in the rearing containers. The eggs laid by the females were collected, separated and placed in new containers with wet cotton swabs for maintaining optimum humidity (75%) and a 10% (v/v) of honey solution in cotton wool was provided as a supplementary food. The nymphs that hatched from these eggs were the 1st generation (F1) nymphs used in this experiment.

Biological parameters of *S. annulicornis* reared on different type of prey

The newly emerged 1st generation (F1) nymphs were gently separated soon after eclosion and reared individually in new rearing containers (diameter 5 cm and height 6.5 cm), in order to avoid cannibalism, on either a diet of *T. molitor* larvae (the 11th to 12th instar) or *C. pavonana* larvae (the 3rd instar). Five prey per nymph were provided daily and the prey consumed were replaced daily with new larvae. The containers with the nymphs were provided with wet balls of cotton wool for maintaining an optimum humidity (75%) and a 10% (v/v) of honey solution in a small cotton wool ball as a supplementary food. The cotton swabs were changed periodically in order to prevent fungal attack. The nymphs were reared under the same laboratory conditions as described above until adult emergence. Six days after adult emergence, the males and females were allowed to mate. Mated females were subsequently separated and maintained in a rearing container until they laid eggs. Egg batches laid by 1st generation females reared on *T. molitor* and *C. pavonana* were collected and used in the experiment. Three batches of eggs from each dietary treatment (*T. molitor* larvae or *C. pavonana* larvae) were used. Each batch of eggs was kept individually in a container along with wet cotton wool swabs and monitored daily. Newly hatched 1st instar nymphs from each batch of eggs were the 2nd generation nymphs and were individually reared on the same prey diet as the 1st generation. The biological parameters recorded during the growth and development of the 2nd generation nymphs were: (a) the development time of each nymphal stage, (b) adult longevity, (c) sex ratio and (d) egg development (fecundity, fertility and egg incubation period). Twenty males and females from each treatment were randomly selected to be kept until they died in order to

record adult longevity. Sex ratio was the average of the total number of females to the total number of males that emerged from the same egg batches. To obtain the data on egg development, virgin males and females were allowed to mate in the rearing containers 6 days after emergence. The length of time for which they copulated was recorded. After copulation, mated females were kept individually in order to record the number of egg batches laid during their lifespan. The number of eggs in each batch (fecundity) was carefully counted under a stereomicroscope at a magnification of 40×. The incubation period was the time from egg laying to egg hatch. The fertility of the eggs was determined by counting the number of nymphs emerging from each batch of eggs.

Consumption of prey and growth of *S. annulicornis*

The daily consumption of each species of prey was evaluated for all the nymphal stages and adults (male and female) of *S. annulicornis* over a period of 7 days, each based on 13 replicates. Observations were made daily by counting the number of prey consumed and replacing them with fresh specimens from the stock cultures. The daily consumption of prey was determined by dividing the average total number of prey consumed by 7. The growth of *S. annulicornis* was recorded by weighing the nymphs and adults on an analytical balance (OHAUS PA244) 10 days after emergence.

Ability of *S. annulicornis* as a predator of *S. asigna*

The predatory ability of both males and females of *S. annulicornis* reared on different species of prey (*T. molitor* and *C. pavonana*) was tested by providing them with 4th instar larvae of *S. asigna* in the laboratory. Each of eight of the predators reared on *T. molitor* larvae and *C. pavonana* larvae, respectively, were randomly selected and starved for 24 h. These predators were individually placed in a plastic rearing container (diameter of 11 cm and height of 6 cm) and allowed to move undisturbed for 10 min before prey were provided. Each of the predators were provided with three larvae of *S. asigna* over a period of 5 days. The number of *S. asigna* larvae killed was recorded and then replaced with living larvae every 24 h.

Data analysis

Individual nymphal development times, adult longevities and body weights of *S. annulicornis*, reared on different species of prey, were compared using one-way analysis of variance (ANOVA), and the differences among the treatments tested using Duncan's multiple range test (DMRT). The numbers of prey consumed by the nymphs and adults of *S. annulicornis* and the total number of *S. asigna* killed by male and female *S. annulicornis*, reared on different species of prey, were also analyzed using one-way ANOVA and DMRT. Data on the sex ratio, egg development (fecundity, fertility and egg incubation period), and duration of copulation of *S. annulicornis*, reared on different species of prey, were compared for the 2 treatments using an independent two-sample *t* test. All analyses were performed using IBM SPSS statistics software version 16.0 for windows (SPSS Inc., Chicago, USA). All statistical analyses were carried out at 5% level of significance ($\alpha = 0.05$).

RESULTS

Biological parameters

Results revealed that the different species of prey differently affected the nymphal development time, adult longevity, sex ratio, and egg development of *S. annulicornis* reared in the laboratory. The details of the developmental times of each of the nymphal stages, adult longevity, sex

ratio, egg incubation period, fecundity and fertility are presented in Table 1. The nymphs reared on larvae of *C. pavonana* throughout their development had a mean total nymphal period of 74.0 ± 7.3 days, which was significantly shorter ($t = 3.05$, $df = 35$, $P < 0.01$) than those reared on the larvae of *T. molitor* (80.1 ± 5.3 days), except for the 1st instar, which had a significantly longer developmental time ($F = 8.205$, $df = 199$, $P < 0.01$). *S. annulicornis* adults reared on *C. pavonana* lived significantly longer than the adults reared on *T. molitor* ($F = 28.791$, $df = 79$, $P < 0.01$). The males reared on *C. pavonana* lived longer (81.0 ± 9.0 days) than the females (64.8 ± 12.4 days) and vice versa as the males reared on *T. molitor* lived for a shorter period (44.0 ± 16.7 days) than the females (52.6 ± 14.4 days). The sex ratio of *S. annulicornis* reared on both species of prey was female biased (1 : 1.2 and 1 : 1.9 reared on *T. molitor* and *C. pavonana*, respectively) and the different species of prey significantly influenced the sex ratio of *S. annulicornis* ($t = 9.73$, $df = 4$, $P < 0.01$).

The males and females were allowed to mate 6 days after emergence and 7 days after mating, females started laying batches of eggs on the gauze sides of the rearing container. There were no significant difference between the two diets in terms of either the number of egg batches laid per female, which ranged from 1–4 or mean fecundity with an average of 105 ± 38 eggs per batch ($t = 1.63$, $df = 49$, $P < 0.5$). Each egg was glued vertically to the substratum and covered by a white glue-like substance that protected the eggs from dehydration (Fig. S1). The fertility of eggs laid by females reared on *T. molitor* was significantly higher (70.2 ± 21.3%) and they developed faster (14.1 ± 3.2 days incubation) compared to those laid by females reared on *C. pavonana*, which had a fertility of only 48.5 ± 20.1% ($t = 2.74$, $df = 26$, $P < 0.05$) and incubation period of 20.3 ± 1.7 days ($t = 5.74$, $df = 18$, $P < 0.01$). As shown in Fig. 1, duration of copulation of *S. annulicornis* adults reared on *C. pavonana* (7.3 ± 4.1 min) was significantly shorter than

Table 1. Biological parameters, nymphal development time, adult longevity, sex ratio, egg incubation period, fecundity and fertility of *S. annulicornis* reared on different species of prey in the laboratory.

Biological parameter	Prey	
	T. molitor	*C. pavonana*
Nymph (development time, day)**:		
1st instar	14.3 ± 1.6[bc]	18.5 ± 4.5[de]
2nd instar	15.8 ± 2.5[c]	12.5 ± 4.3[ab]
3rd instar	15.0 ± 4.0[c]	15.3 ± 4.9[c]
4th instar	15.7 ± 3.2[c]	11.3 ± 3.1[a]
5th instar	19.3 ± 2.5[e]	16.4 ± 5.4[cd]
Total nymphal period (day)*	80.1 ± 5.3[a]	74.0 ± 7.3[b]
Adult:		
Male longevity (day)**	44.0 ± 16.7[a]	81.0 ± 9.0[c]
Female longevity (day)**	52.6 ± 14.4[b]	64.8 ± 12.4[d]
Sex ratio (male : female)*	1 : 1.2[a]	1 : 1.9[b]
Egg incubation period (day)*	14.1 ± 3.2[a]	20.3 ± 1.7[b]
Fecundity (egg)*	97.1 ± 38.8[a]	114.4 ± 36.4[a]
Fertility (%)*	70.2 ± 21.3[a]	48.5 ± 20.1[b]

Means ± SD followed by a different letter for each biological parameter, are significantly different (*P* < 0.05). *Data was analyzed using independent *t*-tests. **Data was analyzed using one-way ANOVA, followed by DMRT analysis.
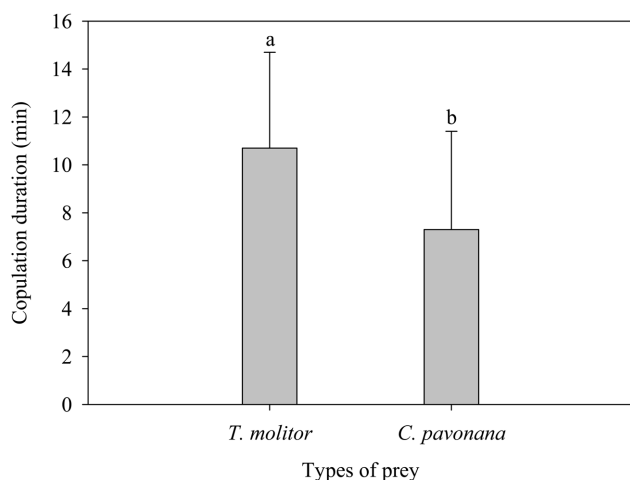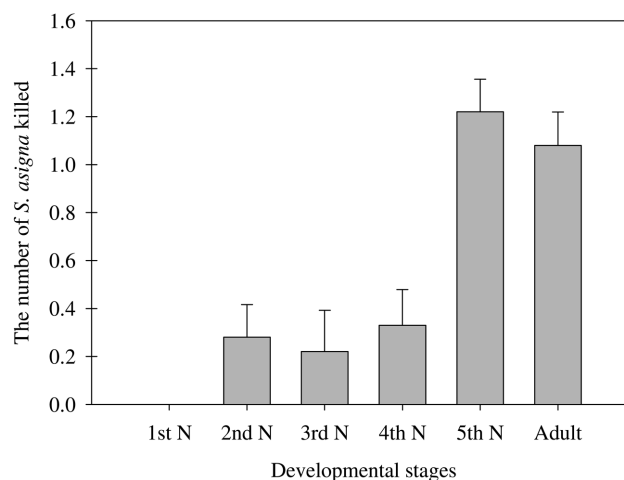
Fig. 1. Mean (± S.D.) duration of copulation of *S. annulicornis* depending on the prey they were reared on. Bars with different letters are significantly different (Indifferent two sample *t*-test, P < 0.05).

Fig. 2. Mean (± S.D.) number of *S. asigna* larvae killed in 24 h by nymphs (N) and adults of *S. annulicornis*.

that recorded for adults reared on *T. molitor* (10.7 ± 4.0 min) ($t = 1.88$, $df = 18$, $P < 0.05$).

Prey consumption and growth of *S. annulicornis*

The daily consumption of prey by nymphs and adults of *S. annulicornis* is summarized in Table 2. The 1st to 3rd instar nymphs of *S. annulicornis* consumed more larvae of *C. pavonana* than of *T. molitor* ($F = 174.506$; $df = 181$; $P < 0.01$), but the consumption of the larvae of the two species of prey by 4th and 5th nymphs of *S. annulicornis* did not differ significantly. The mean number of prey consumed by males (1.91 ± 0.64 larvae and 1.80 ± 0.32 larvae for *T. molitor* and *C. pavonana*, respectively) and females (3.29 ± 0.22 larvae and 2.91 ± 0.29 larvae for *T. molitor* and *C. pavonana*, respectively) differed significantly in both treatments ($P < 0.01$). The numbers of the different species of prey consumed by males did not differ significantly, but the females of *S. annulicornis* consumed more larvae of *T. molitor* than *C. pavonana*. In this study, we also recorded a difference in the trend in the consumption of prey by *S. annulicornis* fed on different prey species. *S. annulicornis* fed *T. molitor* (Fig. S3) exhibited a gradual increase in the total number of prey consumed during each nymphal stage from

the 1st instar to the 5th instar. The 1st and 2nd instar nymphs fed on *T. molitor* consumed up to 1–4 larvae per nymph over 7 days, then increased up to 2–5 larvae per nymph during the 3rd and 4th instars, and increased up to 3–10 larvae per nymph during the 5th instar over 7 days (Table S1). Conversely, *S. annulicornis* fed on *C. pavonana* (Fig. S2) showed a constant prey consumption rate (2–7 larvae per nymph) in all nymphal stages (Table S2). A significant increase in the number of prey consumed was recorded also in the adult stage (Table 2). To understand the correlation between the number of prey consumed and the growth of this predatory insect, we measured the increase in body weight of *S. annulicornis* at each developmental stage as presented in Table 3. No significant difference was recorded in the body weight of the 1st to 3rd instar nymphs fed on *T. molitor* and the body weight of the 1st and 2nd instar nymphs fed on *C. pavonana*, but significant increases in the body weight were recorded in the 4rd instar nymphs fed on *T. molitor* and the 3rd instar nymphs fed on *C. pavonana*. The increases in body weights of the 3rd to 5th instar nymphs and adults of *S. annulicornis* fed on *C. pavonana* were significantly faster than those fed on *T. molitor* ($F = 528.158$; $df = 279$; $P < 0.01$).

**Table 2.** Daily consumption of prey by the nymphs and adults of *S. annulicornis* (n = 13).

Developmental stages	Prey	
	T. molitor	*C. pavonana*
Nymph:		
1st instar	0.28 ± 0.11[a]	0.62 ± 0.22[bc]
2nd instar	0.35 ± 0.16[a]	0.61 ± 0.13[bc]
3rd instar	0.46 ± 0.1[ab]	0.63 ± 0.15[bc]
4th instar	0.60 ± 0.10[bc]	0.65 ± 0.15[bc]
5th instar	0.90 ± 0.32[d]	0.81 ± 0.21[cd]
Adult:		
Male	1.91 ± 0.64[e]	1.80 ± 0.32[e]
Female	3.29 ± 0.22[g]	2.91 ± 0.29[f]

Means ± SD followed by the same letter are not significantly different (*P* < 0.05) based on a one-way ANOVA followed by DMRT analysis.

Table 3. Body weights (mg) of the nymphs and adults of *S. annulicornis* reared on different species of prey in the laboratory (n = 20).

Developmental stages	Prey	
	T. molitor	*C. pavonana*
Nymph:		
1st instar	1.3 ± 0.4[a]	1.1 ± 0.4[a]
2nd instar	3.3 ± 1.0[a]	2.7 ± 0.9[a]
3rd instar	12.1 ± 2.7[a]	34.5 ± 9.0[b]
4th instar	38.6 ± 6.1[b]	84.5 ± 31.0[c]
5th instar	81.3 ± 19.1[c]	114.4 ± 23.3[d]
Adult:		
Male	122.5 ± 11.0[d]	140.6 ± 15.7[f]
Female	251.8 ± 27.9[e]	309.9 ± 42.5[g]

Means ± SD followed by the same letter are not significantly different (*P* < 0.05) based on a one-way ANOVA followed by DMRT analysis.
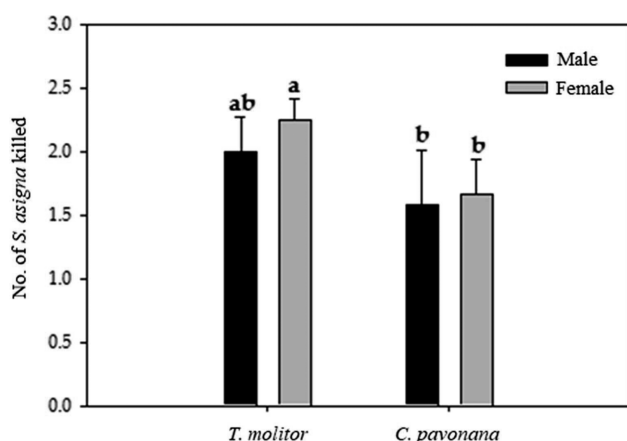
Fig. 3. Mean (± S.D.) numbers of *S. asigna* larvae killed in 24 h by adults (male and female) of *S. annulicornis* reared on different types of prey. Bars with the same letter are not significantly different (one-way ANOVA followed by DMRT analysis, *P* < 0.05).

Ability of *S. annulicornis* as a predator of larvae of *S. asigna*

The results of the preliminary study evaluating the ability of all nymphal stages and adults of *S. annulicornis* to attack and kill 4th instar larvae of *S. asigna* are shown in Fig. 2. Data shows that the 1st instar of *S. annulicornis* did not prey on *S. asigna* during the experiment, but the 2nd and 3rd instar were able to prey on *S. asigna* after 4 days exposure to the prey (Table S3). The 4th instar was able to prey on *S. asigna* after 1–3 days exposure to the prey, whereas the 5th instar and adults preyed on *S. asigna* within a day (Table S3, Fig. S4 and video in Suppl. S2). The number of *S. asigna* killed by 5th instar nymphs was higher than that killed by adults (i.e., 1–3 larvae per day for the 5th instar and 1–2 larvae per day for adults). The evaluation of the predatory ability of adults of *S. annulicornis* reared on different species of prey revealed no significant difference in the predatory ability of males and females (*F* = 4.278; *df* = 15; *P* < 0.05). *S. annulicornis* reared on *T. molitor* can attack as many as 2.2 larvae of *S. asigna* per day, which is more than those reared on *C. pavonana*, which only attack 1.7 larvae of *S. asigna* per day (Fig. 3).

DISCUSSION

S. annulicornis was capable of completing its nymphal development when fed both species of prey. In this study, the species of prey influenced several biological parameters of *S. annulicornis*, such as nymphal developmental time, adult longevity and sex ratio, which are the relevant biological parameters for the mass production of a predatory insect. The species of prey had a differential effect on the developmental time of each nymphal stage of *S. annulicornis*. The 1st instar nymphs fed on *T. molitor* developed faster than those fed on *C. pavonana*, but in the following instars those fed on *C. pavonana* developed significantly faster. This was most likely due to differences in the nutrient requirements of each nymphal stage, particularly for survival during the 1st instar stage and for growth and development in subsequent nymphal stages. Morales-Ramos

et al. (2014) show that different species of arthropods, including *T. molitor* and *C. pavonana*, may have different nutritional compositions, in particular, in terms of the ratio of carbohydrate, lipid and protein, which can affect the development of their insect predators.

Ahmad & Kamarudin (2016) also report that *Sycanus dichotomus* (Hemiptera: Reduviidae) reared on the larvae of *Corcyra cephalonica* Stainton (Lepidoptera) have a shorter developmental period than when reared on larvae of *T. molitor* (Coleoptera). Differences in the nutrient composition of each species of prey not only affects the development of insect predators, but also their survival and reproduction (Huang et al., 2013; Bong et al., 2014; Lv et al., 2016). We recorded that males and females of *S. annulicornis* reared on *C. pavonana* lived longer than those reared on *T. molitor*. These results are consistent with those of previous studies that report that lepidopteran prey can increase survival and shorten the development of generalist predators (Ambrose et al., 1990; Eubanks & Denno, 2000). Insect predators with a long adult survival and short developmental time are more suitable for mass rearing (Sahayaraj, 2004) and releasing as biological control agents for suppressing pest populations in the field.

Srikumar et al. (2014) report a significant difference in the longevity of males and females, but in the present study, there were no consistent differences in the longevity of the male and female reduviids reared on the different species of prey. Males of *S. annulicornis* reared on the lepidopteran *C. pavonana* lived longer than the females, conversely, females of *S. annulicornis* reared on the coleopteran *T. molitor* lived longer than the males. Females living longer than males is a common feature of most reduviids (Ambrose et al., 1990, 2007; Saharayaj & Paulraj, 2001; Saharayaj, 2002; Srikumar et al., 2014; Shanker et al., 2016; Nitin et al., 2017), but males living longer than females is also reported for the reduviid *Sycanus aurantiacus* reared on the coleopteran *Tenebrio molitor* (Yuliadhi et al., 2015) and *Amphibolus venator* Klug reared on the coleopteran *Tribolium confusum* (Youssef & Abd-Elgayed, 2015). The longevity of adult predatory insects may be significantly affected by the species of prey they feed on as adults (Jervis & Copland, 1996). A different effect was recorded for the sex ratio. Adults reared on both species of prey (*T. molitor* and *C. pavonana*) had a female-biased sex ratio. Female-biased sex ratios are reported in the Reduviidae (Ambrose & Claver, 1997). The highly female-biased sex ratio and long longevity of adult *S. annulicornis* makes this species more suitable for mass culture because female-biased sex ratio can result in multiple mating's by males and an enhanced fecundity. Van den Assem (1996) assumes that the longer a male can live, the more likely it is that females will be inseminated by males and more eggs fertilized and the longer a female lives the more eggs she will lay.

No significant difference was recorded in the number of eggs laid by *S. annulicornis* reared on the different species of prey. Mated females of *S. annulicornis* reared on both species of prey can lay 1–4 egg batches throughout their

lifespan, which is similar to that reported for *Sycanus dichotomus* by Zulkefli et al. (2004), but the number of eggs per batch (fecundity) was higher (105 ± 38 eggs per batch) compared to that for *S. dichotomus*, which only ranges from 15 to 119 eggs per batch (Syari et al., 2011). *Sycanus aurantiacus* lays more batches of egg, ranging between 5–11 batches of egg, during its lifespan, but its fecundity is much lower at only 35–73 eggs with an incubation period of 12–14 days (Yuliadhi et al., 2015). Ambrose et al. (2006) reports that the Reduviidae that lay a high number of egg batches have significantly lower fecundities and fertilities. Even though, in this study, the fecundity of *S. annulicornis* was not affected by the species of prey, the fertility of the eggs was significantly affected. Fertility is the more important parameter in mass rearing of predatory insects, as it determines the number of individuals in the next generation. Both male and female contribute to the production of each offspring. The sperm transmitted from the male to the female during mating, is stored by females in a spermathecae to be used for fertilization of the eggs at the time of oviposition (van den Assem, 1996). Thus, the amount of sperm in the spermathecae can affect the fertility of the eggs laid by females. The fertility of eggs laid by females reared on *C. pavonana* was significantly lower and had a longer incubation period than those laid by females reared on *T. molitor*. We suggest that this is related to the shorter duration of copulation recorded for *S. annulicornis* reared on *C. pavonana*, which resulted in less sperm being transferred to the females. Fricke et al. (2015) state that duration of copulation is affected by the diet of males during their immature stages. Males with a good prospect of multiple mating, particularly in situations in which the sex ratio is highly female biased, might adjust the allocation of their ejaculates (sperm) over time and between females (Fricke et al., 2015). However, in this study, each male and female was only allowed to mate once, so the potential for multiple mating by males of *S. annulicornis* reared on *C. pavonana* should be further evaluated as it may enhance the fertility of the eggs laid by their mates. Li et al. (2014) report that mating dramatically reduces male longevity in *Echinothrips americanus*. Therefore, we suggest that a short duration of copulation and its associated lower energy costs will result in the males living longer.

The energy source for survival, growth, development and reproduction of *S. annulicornis* was the prey it consumed. We recorded that the number of prey consumed and the body weight gained by *S. annulicornis* reared on two species of prey differed significantly depending on the species of prey and the developmental stage. The 1st and 2nd instar nymphs consumed over 7 days more *C. pavonana* (2–6 larvae) than *T. molitor* (1–4 larvae). This was due to the 1st and 2nd instar nymphs have difficulty in paralyzing *T. molitor* larvae that have harder cuticles and move faster compared to the slow moving soft-bodied lepidopteran larvae. Interestingly, we did not find similar differences in the weight gains of the nymphs indicating that the nymphs were able to channel the energy they acquired mainly into survival and nymphal development rather than into growth

(body weight), which is expensive in terms of energy. Coleopteran larvae contain a higher proportion of carbohydrate, which is important for the survival and development of an insect at the beginning of each stadium (Bong et al., 2014; Morales-Ramos et al., 2014), so, even though few larvae of *T. molitor* were consumed by the 1st and 2nd instar nymphs, they were able to survive and develop into the next nymphal stage. We also recorded that although the 1st and 2nd instar nymphs killed more larvae of *C. pavonana* they only partially consumed them. Therefore, we assume that this insect can adjust its nutritional needs depending on the nutritional characteristics of their prey.

A significant increase was recorded in the number of prey consumed by the 5th instar nymphs and adults due to their greater energy requirement for growth and the subsequent reproduction of the adults. *S. annulicornis* gained more body weight when reared on larvae of *C. pavonana*, most likely because they are rich in the proteins required for their growth. Saharayaj et al. (2016) report that the total protein content of *Rhynocoris marginatus* (Heteroptera: Reduviidae) was higher when it was reared on lepidopteran larvae, which indicates they are a better quality food for Reduviidae. Females of *S. annulicornis* consumed more prey than males, which is presumably to meet their reproductive needs for egg production. This is supported by a previous study on another species of Reduviidae showing that female *R. marginatus* consume more food than males (Saharayaj, 2008; Saharayaj et al., 2016). Duran Prieto et al. (2016) and Lv et al. (2016) suggest that female predatory insects consume more prey to produce more offspring. However, the greater consumption of prey did not affect the longevity of the predators. Surprisingly, even though the females *S. annulicornis* reared on *T. molitor* consumed more prey than those reared on *C. pavonana*, the gain in body weight of the former was significantly lower. Mendes et al. (2002) report that *Orius insidiosus* (Hemiptera: Anthocoridae) consumes more nymphs of *Aphis gossypii* than eggs of *Anagasta kuehniella*, due to the lower nutritional quality of the former, so the increase in the consumption of low quality of prey might compensate for their poor quality. It is reasonable to postulate that the greater consumption of prey by females is associated with the need to support reproduction, which is a major factor determining their fitness. Thus, it also makes sense if female *S. annulicornis* consume more *T. molitor* larvae in order to obtain sufficient energy for egg production and survival when reared on this poor quality prey.

To be an effective biological control agent, *S. annulicornis* must be an effective predator of the main target pest, *S. asigna*. Inability of a 1st instar nymph to kill *S. asigna* was most likely due to the relative size of *S. asigna*, which is much larger. The 2nd, 3rd and 4th instar nymphs were able to attack the larvae of *S. asigna*, although they needed more time to recognize and accept *S. asigna* as prey. We found that 5th instar nymphs were better predators than adults, however, they are less effective in the field as they lack wings, which makes it difficult for them to reach pests on the high leaves of oil palm. Consequently, we used adults

of *S. annulicornis* when evaluating its predatory ability in the laboratory. The species of prey supplied as food during the growth and development of *S. annulicornis* influenced its ability as a predator of the main target, *S. asigna*. The *S. annulicornis* adults reared on *T. molitor* killed more *S. asigna* per 24 h than those reared on *C. pavonana*. We suggest that this could be due to an habituation to the prey supplied during the growth and development of the predator. Due to the difference in the characteristics of the prey, especially its speed of movement and the hardness of its cuticle, *S. annulicornis* exhibited different behaviours when paralyzing the two species of prey. We frequently observed that *S. annulicornis* that fed on *T. molitor* often left this prey after piercing, because it induced the prey to make rapid movements as a form of self-defence, and subsequently the predator re-pierced and sucked the paralyzed prey or attacked another larva. In contrast, *S. annulicornis* can more easily pierce and paralyze the larvae of *C. pavonana* as they remain motionless and are soft-bodied. This is similar to the results of Saharayaj et al. (2016) for the reduviid *Rhynocoris marginatus*, which is also generally faster in capturing lepidopteran larvae than coleopteran larvae. Therefore, the consumption of *T. molitor* larvae by *S. annulicornis* in which they leave the prey they kill unconsumed or partially consumed was assumed to affect its ability as a predator of *S. asigna*. So, *T. molitor* larvae are considered to be a potential prey for producing this predatory insect as biocontrol agent of *S. asigna*.

This study demonstrates that it is possible to rear successfully the predatory bug, *S. annulicornis*, on both the larvae of *C. pavonana* (Lepidoptera) and *T. molitor* (Coleoptera), but that it has different biological parameters when reared on these two species of prey. The present study revealed that rearing a generalist insect predator on only one species of prey results in a predator that does not have all the requisites for the successful mass production of a biological control agent, including high reproductive rate, long survival, strong predatory ability and synchronization with the prey (pest). Therefore, we recommend rearing a generalist predator, such as *S. annulicornis*, on a combination of different species of prey consisting of both a lepidopteran and a coleopteran so as to meet all the nutritional needs for the development and survival of *S. annulicornis* as well as it resulting in the development of a strong predator of the target pest, *S. asigna*. This recommendation is based on the finding that the growth and development of *Sycanus dichotomus* is enhanced if reared on a combination of both lepidopteran and coleopteran larvae rather than a single species of prey (Ahmad et al., 2011). Grundy et al. (2000) also report that the assassin bug *Pristhesancus plaginnis* develops and reproduces better when reared on a combination of prey consisting of suitable proportions of a lepidopteran (*Helicoverpa armigea* Hubner) and coleopteran (*Tenebrio molitor* L.). In addition, the evaluation of their ability as a predator of the main target pest, *S. asigna*, in the laboratory also revealed that *S. annulicornis* is a potential biological control agent of this oil palm pest, however, this needs to be evaluated under greenhouse conditions and in the field before it is utilized in either inundative or augmentative release programmes. In summary, the present study could serve as a guideline for the mass rearing of the predatory bug *S. annulicornis* for use in biological control of the nettle caterpillar pest in oil palm plantations.

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Supplementary files:

S1 (http://www.eje.cz/2018/019/S01.pdf). Figs S1–S5 and Tables S1–S3.

S2 (http://www.eje.cz/2018/019/S02.3GP). Video sequence showing *Sycanus annulicornis* feeding on the caterpillar of *Setothosea asigna*.