

Starvation time and predatory efficiency of spider species on *Bemisia tabaci* (Homoptera: Aleyrodidae)

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Abstract

Four predatory spider species, *Leucauge venusta* (Orchard) (Araneae: Tetragnathidae), *Lycosa pseudoannulata* (Boeset) (Araneae: Lycosidae), *Larinioides cornutus* (Clerck) (Araneae: Araneidae), and *Tetragnatha shikokiana* (Yaginuma) (Araneae: Tetragnathidae), were used to control *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in the laboratory and greenhouse, and their longevity without food was observed. For the laboratory experiment, the spiders' feeding capabilities were checked at 1, 4, 8, and 16 h of exposure in a 10 mL vial containing 50 five-d-old whiteflies. For the greenhouse experiment, the preys' feeding ability was recorded at 24 and 48 h, with 100 five-d-old adult whiteflies in a screened cage. Individual spiders were kept in the lab in 10 mL vials, and their survival time was recorded every 5 h. Of the 4 spider species, *L. pseudoannulata* was the most active in the lab and consumed an average of 3.00 ± 0.22 , 6.17 ± 0.27 , 9.67 ± 0.43 , and 13.50 ± 0.49 at 1, 4, 8, and 16 h of the bioassay, followed by *L. venusta*, *L. cornutus*, and *T. shikokiana*. However, in the greenhouse experiment, *L. venusta* consumed the greatest number of whiteflies, with an average of 24.66 and 51.33 (out of 100) at 24 and 48 h, respectively, followed by *L. pseudoannulata*, *L. cornutus*, and *T. shikokiana*. The maximum longevity was recorded for *L. venusta* with 26.67 h, followed by *T. shikokiana*, *L. pseudoannulata*, and *L. cornutus* without prey. All spider species killed and consumed adult whiteflies in both experiments, which suggests that they are a controlling tool in the natural ecosystem. The results from our experiment will contribute to the biological control of whitefly.

Key Words: starvation; predation; spiders; whitefly

Resumen

Se utilizaron cuatro especies de arañas depredadoras, *Leucauge venusta* (Orchard) (Araneae: Tetragnathidae), *Lycosa pseudoannulata* (Boeset) (Araneae: Lycosidae), *Larinioides cornutus* (Clerck) (Araneae: Araneidae), y *Tetragnatha shikokiana* (Yaginuma) (Araneae: Tetragnathidae), contra *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) en el laboratorio y el invernadero, y se observó su longevidad sin alimentos. Para el experimento de laboratorio, la capacidad de alimentación de las arañas se verificaron a 1, 4, 8, y 16 h de exposición en un vial de 10 mL que contenía 50 moscas blancas que tenían cinco días de edad. Para el experimento de invernadero, la capacidad de alimentación de la presa se registró a las 24 y 48 h, con 100 moscas blancas adultas que tenían cinco días de edad en una jaula cribada. Las arañas se mantuvieron individualmente en el laboratorio en viales de 10 mL y su tiempo de sobrevivencia se registró cada 5 h. De las cuatro especies de arañas, *L. pseudoannulata* fue la más activa en el laboratorio y consumió un promedio de 3.00 ± 0.22 , 6.17 ± 0.27 , 9.67 ± 0.43 , y 13.50 ± 0.49 a 1, 4, 8, y 16 h del bioensayo, seguido por *L. venusta*, *L. cornutus*, y *T. shikokiana*. Sin embargo, en el experimento de invernadero, *L. venusta* consumió el mayor número de moscas blancas, con un promedio de 24.66 y 51.33 (de 100) a las 24 y 48 h, respectivamente, seguido de *L. pseudoannulata*, *L. cornutus*, y *T. shikokiana*. La longevidad máxima se registró para *L. venusta* con 26.67 h, seguida de *T. shikokiana*, *L. pseudoannulata*, y *L. cornutus* sin presa. Todas las especies de arañas mataron y consumieron adultos de la mosca blanca en ambos experimentos, lo que sugiere que son una herramienta de control en el ecosistema natural. Los resultados de nuestro experimento contribuirán al control biológico de la mosca blanca.

Palabras Clave: hambre; depredación; arañas; mosca blanca

Spiders are 8-legged predatory arthropods; they are widespread, and occupy many ecological environments throughout the world. There are > 45,700 spider species in 114 families that have been recognized by taxonomists throughout the world (World Spider Catalog 2015). They occur in the warm and dry regions of all continents (Lotz 1994; Levy 1998). Spiders are among the most abundant predatory groups on earth. They feed on small arthropods, especially insect pests, and play an important role in pest control. Spiders provide enormous pest control services to agriculture and urban areas, which is greatly appreciated. Spiders can change their behaviors in a range of habitat niches with correspondingly different prey preferences. Some spiders focus on ground-living prey, some roam on plants and trees, and some prefer to search in structures, such as buildings and fences

(David 2014). Spiders are beneficial because they control pests; they hunt, attack, kill, and consume most of the pests infesting ornamental plants, vegetables, fruits, and crops. Their prey usually is smaller than themselves. Natural ecosystems in the agricultural area are highly favorable to the population density and species abundance of spider communities (Riechert 1981; Tanaka 1989; Sunderland 1999; David 2014).

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is known as an economic insect pest that attacks agricultural crops and trees in the warmer regions of the world (Gerling & Mayer 1996; Barro et al. 1998). Their feeding habits damage the plants directly as they feed on the plant sap and indirectly by transmitting plant viruses (Lapidot & Polston 2006). To control the losses resulting from this insect

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pest, pesticides have been used in the past and still are used today. However, the continuous use of such chemicals leads to many side effects, including loss of biodiversity, the resurgence of both the targeted and secondary pests, insecticide resistance in the targeted pests, residual toxicity in the plants, and environmental pollution (Okonkwo & Okoye 1996; Shah et al. 2008). Therefore, it is necessary to develop alternative methods which can replace the use of toxic chemical pesticides. Recent trends in agriculture minimize the use of chemical pesticides, and have led to an increased interest in predators, including spiders and beetles, as potential biological control agents (Maloney et al. 2003).

The introduction of natural enemies is 1 approach to controlling pest populations (Smith & Capinera 2014). Spiders play a major role in controlling insect pests in the agricultural ecosystem, because they can consume large numbers of insects, either trapped in their webs or on the plant or soil surface, and they do not damage the plants or ecosystem structure; therefore, they have been considered important natural enemies (Duffey 1962; Fox & Dondale 1972; Tanaka 1989). There is limited information on the predatory efficiency of spiders, and on their ecological and predatory roles in pest control; therefore, they seldom have been treated as an important biological control agent (Turnbull 1973; Riechert & Lockley 1984). Until 1970, most of the research on spiders concentrated on their identification. Since the early 1970s, researchers have begun to study the basic biological and ecological characteristics of spiders as biological control agents. More research has been conducted on spiders in rice fields than with other crops (Okuma et al. 1978; Yoon & Namkung 1979; Yoon 1997; Kim 1998).

However, most of these studies have been limited to the predatory habits of spiders in the field. This study planned to rear spiders in the greenhouse and to conduct an experiment on the predatory efficacy of 4 spider species, *Leucauge venusta* (Orchard) (Araneae: Tetragnathidae) (web-spinner), *Lycosa pseudoannulata* (Boeset) (Araneae: Lycosidae) (forager), *Larinioides cornutus* (Clerck) (Araneae: Araneidae) (web-spinner), and *Tetragnatha shikokiana* (Yaginuma) (Araneae: Tetragnathidae) (web-spinner) to control the whitefly in both the laboratory and the greenhouse. The study also planned to observe the adult's longevity without prey or food, so that if the species can't find food in the field, it is known how long can they survive. It is hoped that the results from our experiments will be helpful for controlling insect pests through their natural enemies.

Materials and Methods

INSECT PEST CULTURE

The predatory efficiency of spiders controlling the whitefly was studied in the Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory of Huazhong Agricultural University, China, during Mar to Jun 2016. Seeds of the tomato variety "Xian Zao Hong" were sown in plastic pots 30 cm in width and 15 cm in height, which were filled with 3.5 kg of soil (1:1 soil and organic matter). After 40 d germination, when the tomato had up to 35 leaves, the potted plants were used for insect rearing. A whitefly colony was reared on each tomato plant in a screened cage (90 cm H × 80 cm W × 60 cm D) in a greenhouse at 28 ± 5 °C, 60% ± 10% RH, and a 10:14 h (L:D) photoperiod without the application of any chemical pesticides.

SPIDER REARING

Adults of 4 predatory spider species *L. venusta* (Orchard), *L. pseudoannulata* (Boeset), *L. cornutus* (Clerck), and *T. shikokiana* (Yaginuma)

were caught in the rice fields of the Huazhong Agriculture University, and reared on whiteflies in screened cages (90 × 80 × 60 cm) under the same environmental conditions as those in which the whiteflies were reared. Each cage contain 2 pots, a single plant in each pot with 500 to 600 adult whiteflies, and 80 to 100 new whitefly adults were released daily into the cage to maintain the food supply for the spiders. Colonies of the 4 spider species were reared in separate cages.

LABORATORY EXPERIMENT

Five-d-old single adult spiders of each species were placed separately in a 10 mL vial. Fifty 5-d-old whitefly adults were aspirated from a colony into each vial containing a single spider; 1 vial without spiders was used as control. The vials were covered with a lid and placed in the laboratory at 25 ± 2 °C, 60% ± 5% RH, and a 10:14 h (L:D) photoperiod. The total numbers of live and dead whiteflies were recorded after 1, 4, 8, and 16 h of exposure, and after each recording time, the whiteflies were replaced with fresh whiteflies to maintain live food for the spiders (Fig. 1). The experiment consisted of 8 replications.

GREENHOUSE EXPERIMENTS

A tomato plant with 30 to 35 leaves was placed in the center of a screened cage (80 × 90 × 60 cm). A single adult spider was placed in a vial and released near the plant. One-hundred 5-d-old whitefly adults were aspirated from the colony into a 10 mL vial and released into the cage containing the spider. After 24 and 48 h of bioassay, the predatory efficiency of the spiders was determined by counting the total number of live and dead whiteflies in each cage. The experiment consisted of 8 replications.

SURVIVAL WITHOUT FOOD

Five-d-old individual spiders were put into a 10 mL vial, covered with a lid, and placed in the same environment as the experiment in the laboratory. The survival of the individuals was recorded every 5 h, by shaking the vials to ensure whether the spiders were moving or dead.

ANALYSIS AND CALCULATION

The following equation was used to calculate the adjusted mortality rate (AMR) (Nakaji et al. 2004). This was calculated as $AMR = (T-C) / (1-C) \times 100\%$, where T stands for the total number of live whiteflies



Fig. 1. Feeding efficiency of different spider species in the laboratory experiment.

in the spider predation vial, and C stands for the total number of live whiteflies in the control vial. Analysis of variance (ANOVA) and Tukey's post hoc tests were used to compare the mean percentage of feeding efficiency between spider species. A statistical analysis was performed using SPSS, vers. 20 (IBM Corp., Armonk, New York, USA), with a significance level of $P < 0.05$ according to Wagan et al (2017). Sigma Plot, vers. 10.0 (Systat Software, San Jose, California, USA) was used to draw the figures according to Wagan et al (2017). The percentage data were arcsine square root transformed, and all count data were square root ($\times + 1$) or log 10 ($\times + 1$) transformed before being subjected to data analysis. The untransformed means are presented in the results.

Results

LABORATORY EXPERIMENT

The results of our experiment show that all the species of spiders effectively fed on silverleaf whitefly adults, but their feeding efficiency varied. *Lycosa pseudoannulata* was the most active predator during all the recorded h; it caught and consumed the most whitefly adults, with an average of 3.00 ± 0.22 , 6.17 ± 0.27 , 9.67 ± 0.43 , and 13.50 ± 0.49 flies at 1, 4, 8, and 16 h of predation (Fig. 2). *Leucauge venusta* was the second most active predator in the prey consump-

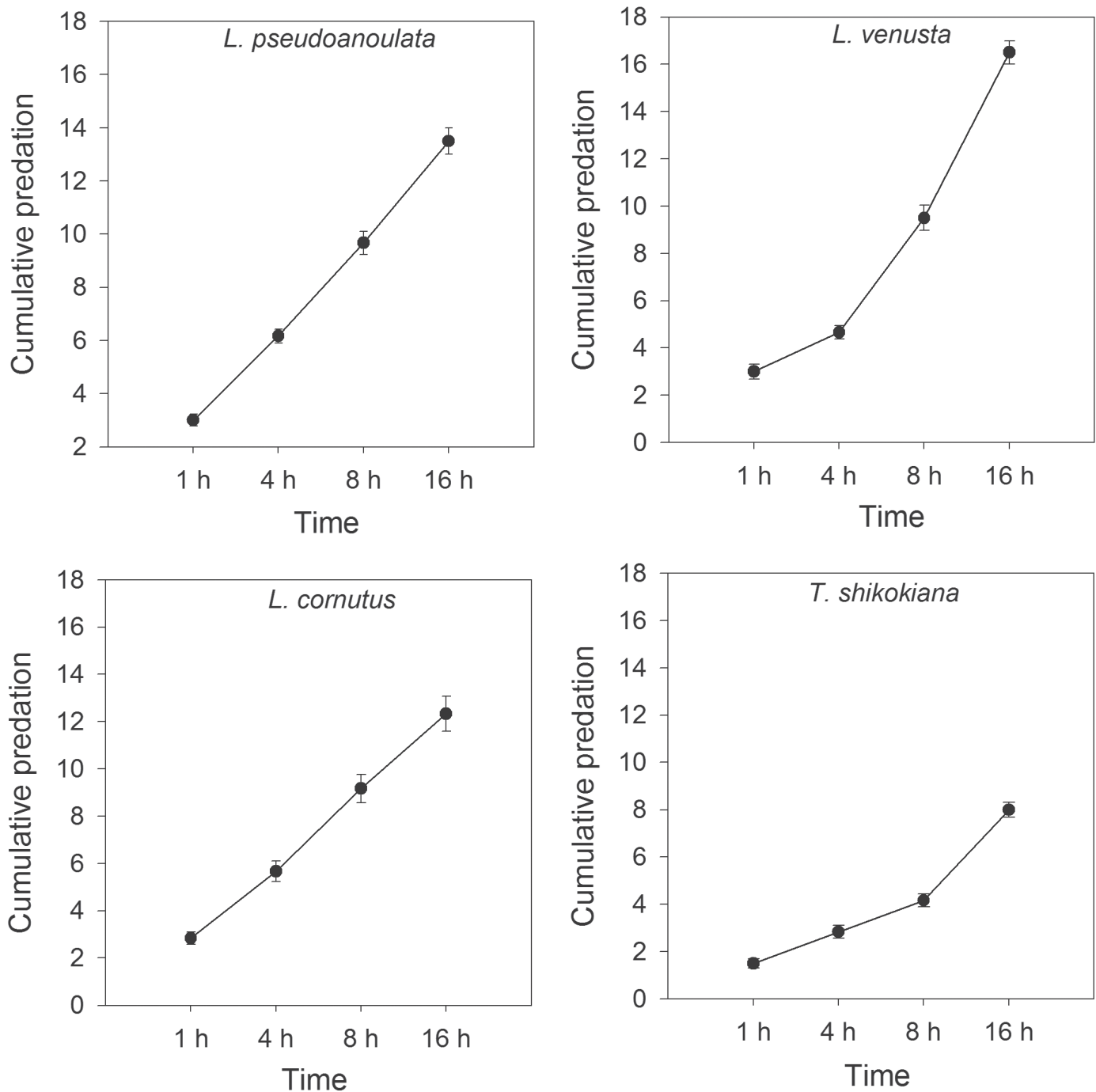


Fig. 2. Cumulative predation number (mean \pm SE) of each spider species at different times in the lab, at 1, 4, 8, and 16 h of bioassay. The differences were analyzed by 1-way ANOVA, using a Tukey HSD post-hoc test at a significance level of $P < 0.05$. Values are means of 8 replications.

tion index; like other species it also showed predatory response in all observations with an average consumption of 3.00 ± 0.32 , 4.67 ± 0.29 , 9.50 ± 0.54 , and 16.50 ± 0.49 flies at 1, 4, 8, and 16 h of predation (Fig. 2). *Larinioides cornutus* showed good predatory action, and was the third most active predator based on the feeding efficacy index; it consumed whitefly adults with an average of 2.83 ± 0.27 , 5.67 ± 0.43 , 9.17 ± 0.61 , and 12.33 ± 0.73 flies at 1, 4, 8, and 16 h of predation (Fig. 2). The results of the bioassay showed that *T. shikokiana* consumed fewer whitefly adults than the other spider species

for all time periods of the experiment, with an average consumption of 1.50 ± 0.19 , 2.83 ± 0.27 , 4.17 ± 0.27 , and 8.00 ± 0.32 flies at 1, 4, 8, and 16 h.

The results from the Analysis of Variance (ANOVA) analysis are presented in Figure 3. A significant difference in the feeding efficacy index was found between *T. shikokiana* and the other spider species at 1 h ($F = 18.11$; $df = 4$; $P < 0.05$), 4 h ($F = 48.18$; $df = 4$; $P < 0.00$), 8 h ($F = 68.98$; $df = 4$; $P < 0.00$), and 16 h ($F = 129.17$; $df = 4$; $P < 0.00$) of predation (Fig. 3). No significant difference was observed between *L. pseudoannulata*

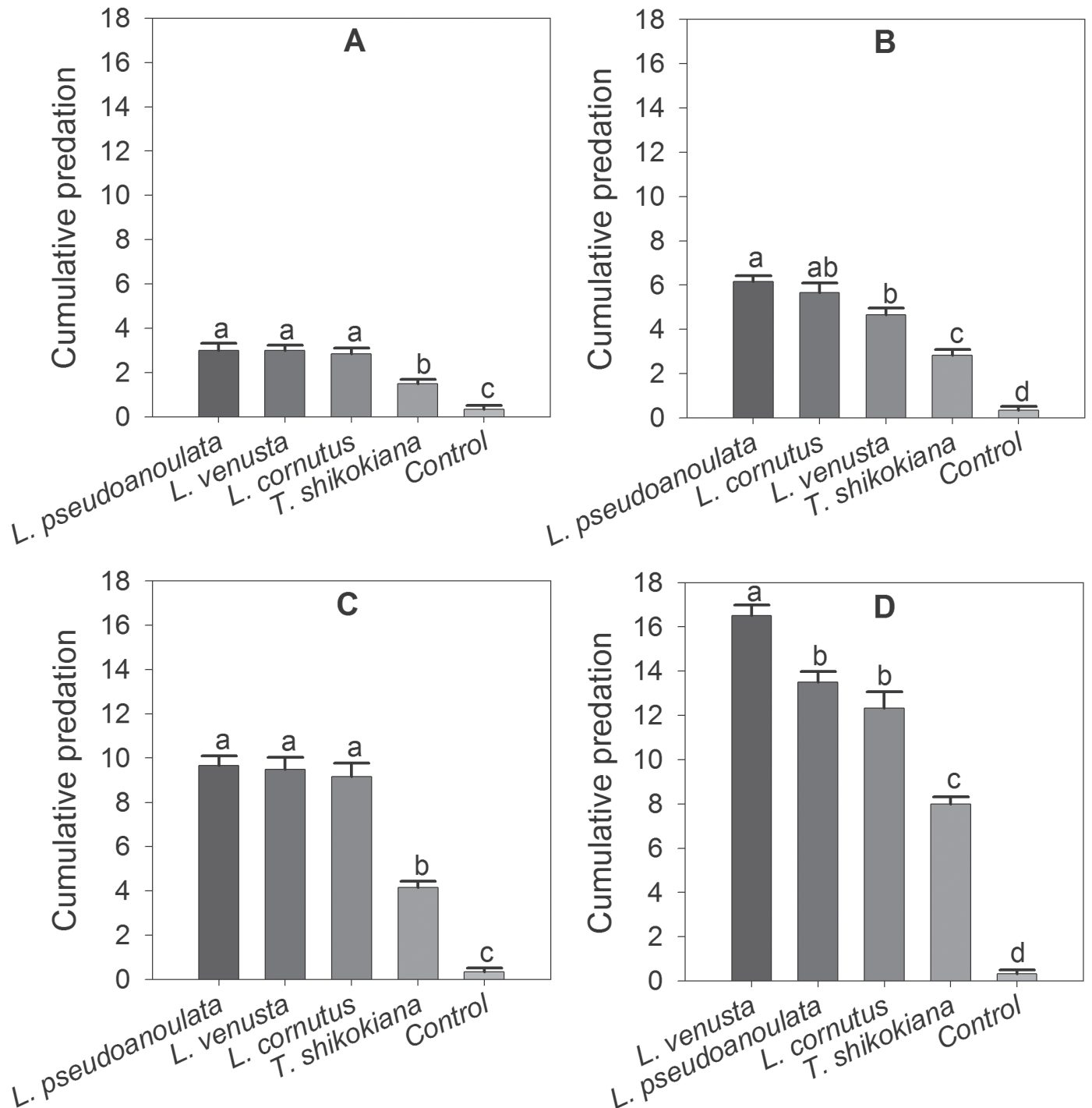


Fig. 3. Comparison of cumulative predation number (mean ± SE) of different spider species at the same times, at (A) 1 h; (B) 4 h; (C) 8 h; and (D) 16 h of bioassay. The differences were analysed by 1-way ANOVA, using a Tukey HSD post-hoc test at a significance level of $P < 0.05$. Values are means of 8 replications.

and *L. venusta* at 4 h or 8 h of predation, but a significant difference was found between these 2 species of spiders at 16 h of predation. No significant difference was found between *L. cornutus*, *L. pseudoannulata*, and *L. venusta* at 4 h or 8 h of predation, but a significant difference was found between *L. cornutus* and *L. venusta* at 16 h of predation.

GREENHOUSE EXPERIMENT

In the greenhouse experiment, the feeding efficacy of spiders controlling whiteflies was studied to understand the prey and predator relationship in the cage at 24 h and 48 h.

The ANOVA analysis results showed a significant difference between the spider species at both 24 h ($F = 79.66$; $df = 4$; $P < 0.00$) and 48 h ($F = 119.80$; $df = 4$; $P < 0.00$) of predation, except that no significant difference was found between *L. pseudoannulata* and *L. venusta* at 24 h of predation, or for *L. pseudoannulata* and *L. cornutus* at 48 h of predation. The spider species and whiteflies existed in the same cage, so the predators were feeding on whitefly both d and night, and the feeding rate was gradually increased over the 24 h to 48 h of exposure. It was observed that *L. venusta* consumed the most whiteflies (24.66 ± 1.26 and 51.33 ± 2.02 flies) in 24 h and 48 h, respectively, followed by *L. pseudoannulata*, *L. cornutus*, and *T. shikokiana* (23.33 ± 1.24, 17.33 ± 0.58, and 11.00 ± 0.55 flies, and 42.33 ± 1.56, 37.50 ± 1.50, and 24.50 ± 1.09 flies in 24 h and 48 h, respectively) (Fig. 4).

SURVIVAL WITHOUT FOOD

The longevity of the spiders without food (prey) also was evaluated. Different survival ratios were observed for the spider species examined in this study. A significant difference in longevity was observed between *L. venusta* and *T. shikokiana* in comparison to *L. pseudoannulata* and *L. cornutus* ($F = 19.95$; $df = 3$; $P < 0.00$). *Leucauge venusta* was found to have the highest rate of longevity, followed by *T. shikokiana*,

L. pseudoannulata, and *L. cornutus*, with a survival rate of 26.67 ± 0.91, 23.33 ± 0.91, 17.50 ± 0.97, and 16.67 ± 0.91 h, respectively (Fig. 5).

Discussion

All 4 species of spiders were found to continuously feed on prey; however, in both the laboratory experiment and the greenhouse experiment, the feeding efficiency was found to vary among the species of spiders. Spiders are predators, and they are the most important natural enemies that are capable of controlling insect pest populations, especially in rice fields (Yamano 1977). Spiders are especially known to prey upon soft-bodied sap sucking insects.

Among all the tested spider species, *L. pseudoannulata* consumed the most whiteflies in the laboratory and greenhouse experiments. Similar results were found in a study conducted by Mathirajan (2001). *Lycosa pseudoannulata* effectively controls sap sucking pests, such as *Sogatella furcifera* (Horváth) (Hemiptera: Delphacidae), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), and *Nephotettix virescens* (Distant) (Hemiptera: Cicadellidae) in rice fields. In addition, *L. pseudoannulata* and its predatory efficacy were recorded in both a pesticide-free area and in an area in Tamil Nadu, India, in which pesticides are frequently used to control pest infestations of brinjal and snake gourds (Sankari & Thiyagesan 2010). *Lycosa pseudoannulata* is more active in greenhouses, and this species of spider kills more whiteflies in that setting than in laboratory tests, because greenhouses are a natural environment. Jayakumar & Sankari (2010) also reported that *L. pseudoannulata* were found in rice ecosystems, from transplanting to harvest time, which showed that this spider species controlled the pest population in those environments.

The predatory potency of *L. venusta* was noticeable in both the laboratory and greenhouse experiments. *Leucauge venusta* was found to be the most active predator in the greenhouse experiment. It killed

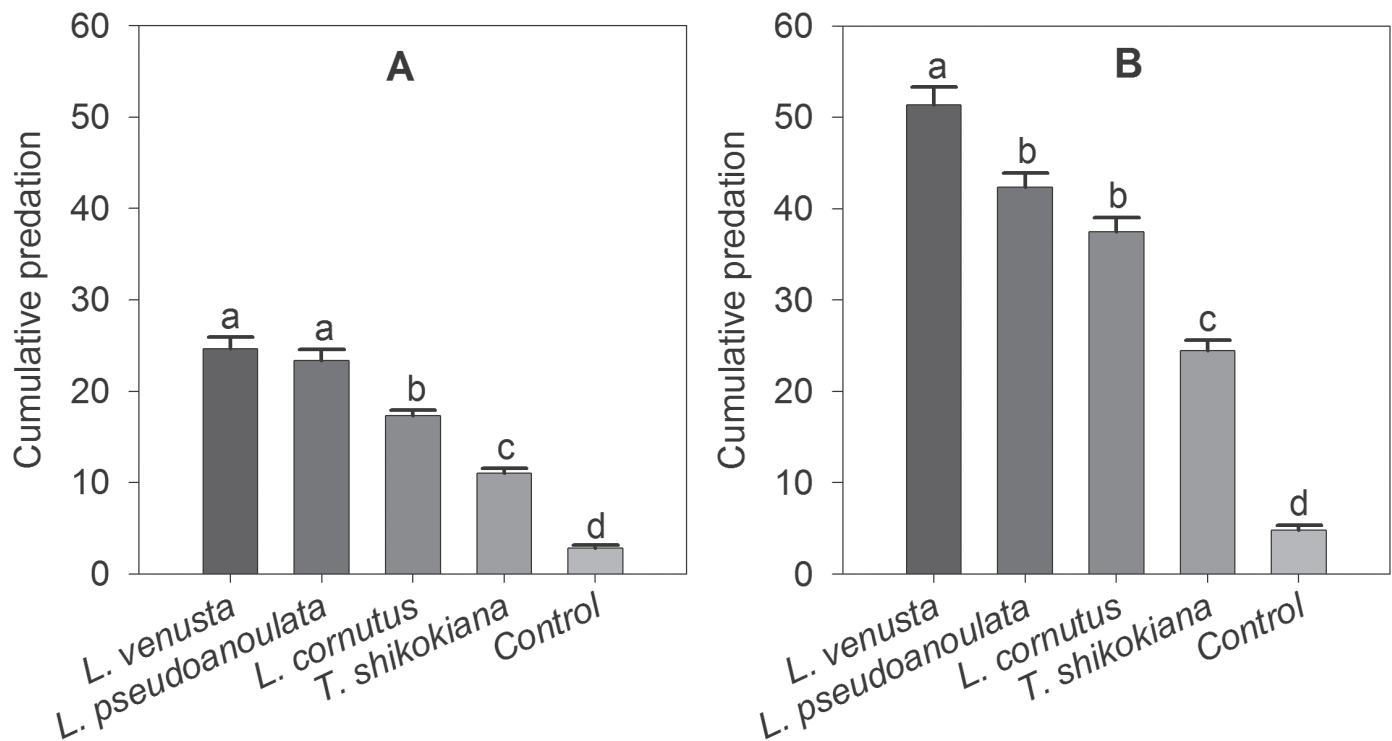


Fig. 4. Comparison of cumulative predation number (mean ± SE) of different spider species at the same times in a greenhouse. (A) 24 h and (B) 48 h of bioassay. The differences were analyzed by 1-way ANOVA, using a Tukey HSD post-hoc test at a significance level of $P < 0.05$. Values are means of 8 replications.

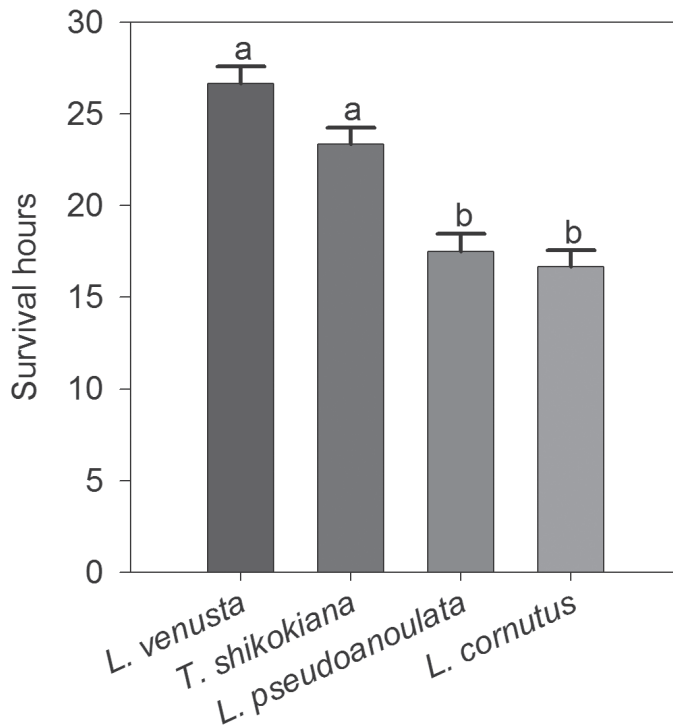


Fig. 5. Longevity of different spider species without prey (mean ± SE). The differences were analysed by 1-way ANOVA, using a Tukey HSD post-hoc test at a significance level of $P < 0.05$. Values are means of 8 replications.

and consumed the most whiteflies. In a previous study, Henaut et al. (2001) also reported on the predatory efficiency of *L. venusta* species. In that study, adult *L. venusta* captured and consumed 8 types of insects found in the Coleoptera, Hymenoptera, Diptera, Lepidoptera, and Homoptera coffee plantations in southern Mexico. Additionally, Zschokke et al. (2006) found that *L. venusta* captured prey more quickly than other species in the family (Araneidae, Nephilidae, Tetragnathidae, and Theridiidae) in an open field. *Leucauge venusta* is very sensitive to checking the presence of its prey. It reacts quickly, especially if the prey is inactive and small (Henaut et al. 2005; Henaut 2000). It has been observed that *L. venusta* is more active in a natural ecosystem against small, soft-bodied pests, including whitefly.

In this study, the prey and consumption behavior of *L. cornutus* controlling whitefly was proven. *Larinioides cornutus* actively hunted prey at every time interval in the experiment. Once the prey was captured, it injected venom into the pest and consumed the sap of the prey's body, leaving little waste for excretion. *Larinioides cornutus* spiders are predators of many insect species that are trapped in their webs, including common mosquitoes (*Culex pipiens* L.; Diptera: Culicidae), damselflies (*Platycnemis pennipes* Pallas; Odonata: Platycnemididae), and gnats (Diptera: Anisopodidae) (Foelix 2011; Prokop 2006). The predacious habitat of *L. cornutus* was praised in the previous research mentioned above, but unlike *L. pseudoannulata* and *L. venusta*, *L. cornutus* was not found to be more active controlling whitefly in either the laboratory or greenhouse experiments because this spider is nocturnal. It builds its web in the evening and actively hunts throughout the night, whereas it hides in the daytime (Bellmann 1997). *Larinioides cornutus* spiders are not social predators; they like to build their webs individually and locate them close to humid areas with ample vegetation or in sheltered areas that protect them from direct sunlight. *Larinioides cornutus* spiders are active during the night, and they rebuild their webs daily to repair damage (Nicholls 2010; Prokop 2006).

Tetragnatha shikokiana was less active controlling whitefly in both the laboratory and greenhouse experiments. This type of spider consumed fewer whiteflies than the other 3 species. *Tetragnatha shikokiana* spiders are found in early and late season rice fields; they help control leafhopper and planthopper populations (Kenmore et al. 1984; Zhang et al. 2013). While previous research studies have proven the predatory efficacy of *T. shikokiana*, in our experiments, this species was less active than the other species. Their low predatory habit could be due to the lack of availability of their desired pest species, or the life stage of the pest species. Disturbances in the surrounding environment also could make them uncomfortable, resulting in their low predatory habit.

The species survival ratio varied among the spider species. We observed that the species with a short abdomen or low body weight, such as *L. venusta* and *T. shikokiana*, can survive more than 1 d. In contrast, species with a long abdomen or more body weight, such as *L. pseudoannulata* and *L. cornutus*, can survive less than 1 d. It also seems that the variation in longevity depends upon whether or not the spiders' webs are threatened, or if they experience any disturbance in their living spaces (Ysnel 1993). In the field, after harvest of crops, the number of prey will accordingly decrease sharply or disappear; how long the predators can survive in the case of unavailability of prey is a very important characteristic as a biological control agent. So we investigated the longevity of the 4 spiders without prey, because this will afford a beneficial issue for the practical use of the spiders in the future.

This is the first study on spider rearing and is the model for release of spiders in natural ecosystems. Our study found that all 4 species of spiders are able to kill and consume adult whiteflies in a laboratory and in a greenhouse. It also observed that spiders are more active in a natural ecosystem than in an artificial environment, such as a laboratory; however, all 4 species controlled the whitefly population in both environments. The results of our experiment will be helpful for biological control of arthropods in open fields, and in specific locations with a controlled atmosphere. Future research is required to observe spider life cycle parameters, and to determine the activity of adult and young spiders controlling different arthropods in natural conditions.

Acknowledgments

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