Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biological Control 31 (2004) 306–310
Biological Control
www.elsevier.com/locate/ybcon

Functional response of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) to *Aphis gossypii* Glover (Homoptera: Aphididae) in the Laboratory

Joon-Ho Lee* and Taek-Jun Kang

Entomology Program, School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Republic of Korea

Received 13 January 2004; accepted 19 April 2004
Available online 19 May 2004

Abstract

Stage specific functional response of *Harmonia axyridis* (Pallas) to varying densities of *Aphis gossypii* Glover was examined in a simplified cucumber leaf arena under laboratory conditions. All stages of *H. axyridis* were isolated individually for 24 h with different prey densities at 25 °C and a photoperiod of 16:8 (L:D) h. The number of prey consumed by the predator was checked at 3, 6, 12, and 24 h. All stages of *H. axyridis* showed a Type II functional response. Based on the random predator equation, estimated attack rates of *H. axyridis* at 24 h were 0.0037, 0.0442, 0.3590, 0.3228, and 0.1456, and estimated handling times were 4.1001, 2.4575, 0.7500, 0.2132, and 0.1708 h for the first, second, third, and fourth instars, and female adult, respectively.

Keywords: *Harmonia axyridis*; *Aphis gossypii*; Functional response

1. Introduction

The cotton aphid, *Aphis gossypii* Glover, is one of the major insect pests of cucumber, *Cucumis sativus* L (Parrella et al., 1999). Generally, chemical control has been the major tool for the control of aphids in cucumber. However, insecticide resistance and growing concerns on environmental hazard due to frequent use of insecticides prompted development of biological control of aphids. Biological control of aphids is being increasingly applied in the greenhouse crops (Parrella et al., 1999; Van Lenteren and Woets, 1988). For example, parasitoid, *Aphidius colemani* Vireck, and the predatory gall midge, *Aphidoletes aphidimyza* Rondani have been employed successfully in aphid control in greenhouse, and commercialized in many countries (Bennison and Corless, 1993; Mulder et al., 1999). However, studies are needed for evaluating more aphidiphagous insects because the availability of additional natural enemies of aphids would lead to an increase in successful biological control of aphids under various situations.

The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), has been considered a good candidate for biological control of aphids such as *A. gossypii* (Choi and Kim, 1985; Hodek and Honek, 1996; Hong, 1996; Mogi, 1969; McClure, 1987; Seo and Youn, 2000), and its biology and uses in biological control was extensively reviewed recently (Koch, 2003). Quantification of the aphidiphagous response of *H. axyridis* is one of many research prerequisites for establishing biological control of aphids in cucumber using *H. axyridis*. Several studies have been conducted on the aphidiphagous activity of *H. axyridis* (Choi and Kim, 1985; He et al., 1994; Hu et al., 1989; Lou, 1987; Mogi, 1969; Seo and Youn, 2000) but no detailed study has been conducted on its functional response to *A. gossypii*. The functional response of a predator to changing densities of its prey is determined by the length of prey exposure to the predator, the rate of a successful predator attack, and the prey handling time exhibited by the predator (Hassell et al., 1976). Information on these variables will provide insights into the predator–prey interaction

* Corresponding author. Fax: +82-2-880-4705.
E-mail address: jh7lee@snu.ac.kr (J.-H. Lee).

1049-9644/$ - see front matter
doi:10.1016/j.biocontrol.2004.04.011

between *H. axyridis* and *A. gossypii*, which could lead to the development of a better strategy for the biological control of *A. gossypii* using *H. axyridis*. Thus, the objective of this study was to determine the functional response of *H. axyridis* to *A. gossypii* under laboratory conditions.

2. Materials and methods

2.1. Insect culture

Stock colonies of *H. axyridis* and *A. gossypii* were maintained in the laboratory and a glasshouse at the experimental farm of the Seoul National University, respectively. *A. gossypii* were reared on the leaves of cucumber plants, *C. sativus*, at 20–32 °C, 50–80% RH, and a photoperiod of 16:8 (L:D) h. However, during summer a small colony of *A. gossypii* was maintained on cucumber seedlings in the laboratory at 23–30 °C, 60–80% RH, and a photoperiod of 16:8 (L:D) h. *H. axyridis* were reared on *A. gossypii* at 23–30 °C, 60–80% RH, and a photoperiod of 16:8 (L:D) h. However, during summer *H. axyridis* were reared on artificial diet (Hong, 1996) because of limited availability of *A. gossypii*. Colonies of *H. axyridis* and *A. gossypii* were replenished with field collected individuals in spring and fall.

2.2. Functional response study

Prior to the functional response study, adult *H. axyridis* were transferred from the stock culture into Petri dishes (6 cm diameter) containing 6 cm cucumber leaf disks infested with *A. gossypii*. The dishes were maintained in an incubator at 25 °C, 60–70% RH, and a photoperiod of 16:8 (L:D) h. In each dish, one female and male adult were enclosed, and approximately seventy aphids were supplied in 12-h interval. A sufficient number of sets were prepared to ensure an ample *H. axyridis* population. After one full generation cycle, *H. axyridis* at each growth stage were used for the study. Adults were allowed to oviposit for <12 h in the Petri dishes, and removed. Then, the Petri dishes were placed in an incubator at 25 °C, 60–70% RH, and a photoperiod of 16:8 (L:D) h. Observations were made at 6-h intervals and within 6 h after molting to the desired stage (first, second, third, and fourth instar larvae, and adults), individuals were each transferred to separate 6-cm cucumber leaf disks in 6-cm Petri dishes and starved for 18–24 h. For adults, only mated females were used.

The experimental arena consisted of a 6-cm cucumber leaf disk placed ventral-side-up (avoiding the major veins) on water-saturated cotton in a Petri dish (6 cm diameter). After allocating adult *A. gossypii* at different densities, *H. axyridis* were placed individually into each arena. Prey densities were 3, 5, 10, 15, 20, 25, 30, and 35 aphids for first instar *H. axyridis*; 2, 4, 8, 10, 15, 20, 25, 30, and 35 aphids for second instar *H. axyridis*; 2, 4, 8, 10, 15, 20, 25, 30, 35, and 40 aphids for third instar *H. axyridis*; 5, 10, 15, 20, 30, 60, 80, 90, 100, and 130 aphids for fourth instar *H. axyridis*; and 5, 10, 20, 30, 60, 70, 80, 90, and 100 aphids for adult *H. axyridis*. The Petri dishes were held in an incubator at 25 °C, 60–70% RH, and a photoperiod of 16:8 (L:D) h. The experimental arenas were examined after 3, 6, 12, and 24 h to record the number of aphids consumed. The experiment was replicated 10–16 times simultaneously for each stage. *A. gossypii* consumed by *H. axyridis* were not replaced during the experimental periods, and *A. gossypii* nymphs produced by the adult *A. gossypii* were removed from the arena using a smooth brush at 3–4 h intervals.

2.3. Data analysis

Analysis of variance was performed to test differences in daily prey consumption at high prey densities (first instar: $\geqslant$25 aphids; second instar: $\geqslant$25 aphids; third instar: $\geqslant$30 aphids; fourth instar: $\geqslant$90 aphids; adult: $\geqslant$80 aphids) among different predator stages using PROC GLM in SAS (SAS Institute, 1999), and means were separated using the Tukey studentized range test (SAS Institute, 1999).

The functional response was analyzed using the predation data for 24 h. A cubic logistic regression between proportion of prey consumed and prey density was performed to determine the shape (e.g., Type II or Type III) of functional response:

$$N_e/N_0 = \frac{\exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}{1 + \exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}, \tag{1}$$

where N_e is the number of prey eaten; N_0 is the initial number of preys. P_0, P_1, P_2, and P_3 are parameters to be estimated. Fitting was performed using a SAS program given by Juliano (2001). Significant negative or positive linear coefficients (i.e., P_1) from the regression indicate Type II or Type III, respectively (Juliano, 2001).

Because logistic regression analysis indicated that our data generally fit the Type II response, further analysis was restricted to the Type II response. The random predator equation (Rogers, 1972) was used to describe the functional responses because it allows for prey depletion during the course of the experiment. The form of the equation is as follows:

$$N_e = N_0[1 - \exp(aT_hN_e - aT)], \tag{2}$$

where N_e and N_0 are described in Eq. (1); T is the total time which in this case is 24 h; a is the attack rate; T_h is the handling time. Fitting was performed using a SAS program given by Juliano (2001).

3. Results

3.1. Predation ability

The number of *A. gossypii* consumed increased significantly as their densities increased (Fig. 1). Aphid consumption by *H. axyridis* at high prey densities (first instar: ⩾25 aphids; second instar: ⩾25 aphids; third instar: ⩾30 aphids; fourth instar: ⩾90 aphids; adult: ⩾80 aphids) during 24-h period was significantly different among different growth stages ($F = 1231.54$; $df = 4, 177$; $P < 0.0001$; Table 1). The first and second instars consumed least amount of aphids. The fourth instar consumed most, followed by female adults.

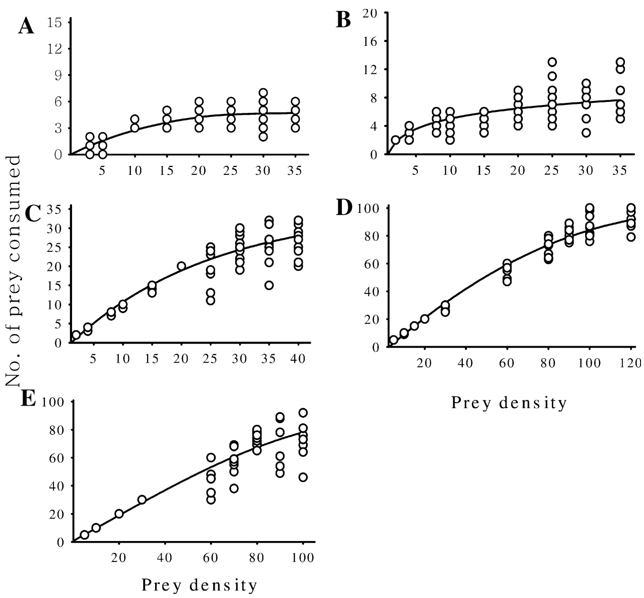

Fig. 1. Functional response of *H. axyridis* to adult *A. gossypii* on cucumber leaf disks (6 cm diameter) over 24 h-period. (A) first instar larva, (B) second instar larva, (C) third instar larva, (D) fourth instar larva, and (E) female adult. Each data point represents the observed number of *A. gossypii* consumed. Curve was fitted using the random predator equation (Eq. (2)).

3.2. Functional response

Parameter estimates from the logistic model (Eq. (1)) of proportion of *A. gossypyi* consumed by *H. axyridis* over a 24-h period versus *A. gossypyi* density are presented in Table 2. Except for the first and third instars, estimates of the linear parameter, P_1, were significantly negative. The estimates of linear and quadratic coefficients for the first instar larva were positive and negative, respectively. However, these values were not statistically different from 0. Overall, the logistic model analysis indicated a Type II response.

The functional response data for *H. axyridis* preying on *A. gossypyi* over a 24-h period fit the random predator equation (Rogers, 1972) well, confirming a Type II response for all growth stages (Fig. 1). Predation efficiency increased as *H. axyridis* developed from one growth stage to another. The parameters of attack rate (a) and handling time (T_h) illustrate this relationship numerically (Table 3). The attack rate was highest in the third and fourth instars, followed by female adult. Handling times of the fourth instar and female adult, 0.21 and 0.17 h, respectively, were much shorter than the handling times of other stages.

Table 1
Mean predation amount (±SE) of *H. axyridis* on cucumber leaf disks (6 cm diameter) at high adult *A. gossypii* densities (first instar: ⩾25 aphids; second instar: ⩾25 aphids; third instar: ⩾30 aphids; fourth instar: ⩾90 aphids; and adult ⩾ 80 aphids) during 24 h at 25 °C

Stage	First instar	Second instar	Third instar	Fourth instar	Female adult
	4.5 ± 0.17d (41)	7.3 ± 0.35d (44)	26.7 ± 0.66c (43)	86.4 ± 1.52a (27)	74.8 ± 2.41b (27)

Means followed by the same letters within a row are not statistically different ($P > 0.05$).
Numbers in parentheses are sample size.

Table 2
Maximum likelihood estimates (±SE) for parameters of the logistic model fit to proportion of prey consumed versus initial prey density

Parameter	Larva				Female adult
	First instar	Second instar	Third instar	Fourth instar	
P_0	−1.0602*	2.6273**	4.7735**	7.3186**	29.794**
	(0.4920)	(0.5030)	(1.5448)	(1.0444)	(6.1697)
P_1	0.1011	−0.4499**	−0.0104	−0.1498**	−1.2478**
	(0.0906)	(0.0887)	(0.1942)	(0.0425)	(0.2429)
P_2	−0.0082	0.0190**	−0.00516	0.00161**	0.0179**
	(0.0049)	(0.0046)	(0.00771)	(0.00054)	(0.00315)
P_3	0.000134	−0.00027**	0.000073	-6.5E^{-6}**	−0.00008**
	(0.00008)	(0.00007)	(0.000096)	(2.125E^{-6})	(0.000013)

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

Table 3
Parameter estimates (±SE) of the random predator equation (Rogers, 1972) for *H. axyridis* preying on adult *A. gossypii*

Stage	a	T_h (in hour)	r^2
First instar	0.0037 ± 0.00617	4.1001 ± 0.3046	0.94
Second instar	0.0442 ± 0.00834	2.4575 ± 0.2304	0.92
Third instar	0.3590 ± 0.1261	0.7500 ± 0.0597	0.97
Fourth instar	0.3228 ± 0.0581	0.2132 ± 0.0125	0.99
Female adult	0.1456 ± 0.0506	0.1708 ± 0.0580	0.97

a is the attack rate.
T_h is the handling time.

4. Discussion

4.1. Predation ability

In coccinellids, the fourth instar is generally responsible for approximately 60–80% of total prey consumption by larvae (Hodek and Honek, 1996). A similar trend was observed in our study. Over 24 h, the fourth instar accounted for 69.2% of the total *A. gossypii* intake by larvae, followed by the third (21.4%), second (5.8%), and first (3.6%) instars. These proportions are similar to those of *H. axyridis* preying on *Myzus persicae* (Sulzer) (Hodek and Honek, 1996). *A. gossypii* consumption by female adult *H. axyridis* was 86.6% of that by the fourth instars per day. It indicates that the fourth instar and adult stages are most important for biological control.

4.2. Functional response

Harmonia axyridis exhibited a Type II functional response at all growth stages against *A. gossypii* though Type I (Lou, 1987) or Type III (Hu et al., 1989) was reported for adult *H. axyridis* against different prey species (*Rhopalosiphum prunifoliae* (= *padi*) and *Cinara* sp., respectively). However, Lou (1987) also fit a Type II and data of Hu et al. (1989) lacked the characteristic sigmoidal shape of a Type III response (Koch, 2003). Type II functional response curves have been reported in other coccinellids (Dixon, 2000; Hodek and Honek, 1996), including *H. axyridis* larvae against *Aphis craccivora* Koch (Mogi, 1969), and *Lipaphis erysimi* (Kaltenbach) (He et al., 1994), larval and adult *H. axyridis* against eggs and larvae of *Danaus plexippus* (Linnaus) (Koch et al., 2003), *Coccinella septempunctata* L. against *L. erysimi* (Sinha et al., 1982), male adults of *Cheilomenes sulphurea* against *Aphis fabae* Scopoli (Hodek et al., 1984), and the fourth instars and adults of *Coleophora inaequalis* (F.) against *Toxoptera citricida* (Kirkaldy) (Wang and Tsai, 2001). The low predation rates observed in the first and second instars of *H. axyridis* are likely due to their low attack rates and longer prey handling times (Table 3). This is understandable because the first and second instar larval sizes are small and their movements are slow. Although third instars showed the highest attack rate, their maximum mean aphid consumption was much lower than those of the fourth instars and female adults, probably due to third instars having a longer handling time (Table 3). Furthermore, low variation in prey consumption, high attack rate, and short handling time in fourth instars indicate that the fourth instar is the most voracious stage.

Functional response experiments conducted under laboratory conditions may have limited value for determining characteristics of predation under field conditions. O'Neil (1997) described the discrepancy between the laboratory and field functional responses of the pentatomid, *Podisus maculiventris* (Say), based on a series of experiments (O'Neil, 1988a,b, 1990; Wiedenmann and O'Neil, 1991a,b, 1992). Some criticisms are: the small size of arenas used in laboratory predation experiments may be not representative of the natural searching efficiency of a predator (Murdoch, 1983); spatial complexity is also important in nature, but cannot be represented through a simple experimental arena (Kareiva, 1990); and plant characteristics also affect the predation response of a predator (Hodek and Honek, 1996).

Our laboratory-measured functional response of *H. axyridis* may not exactly correspond to the field situation. However, our study has value as a first step in evaluating *H. axyridis* as a biological control agent of *A. gossypii*. We speculate that our results may describe the functional relationship between prey density and predation rate when *H. axyridis* is on cucumber leaves where *A. gossypii* is relatively abundant because coccinellids generally aggregate where prey are abundant and once prey are captured, coccinellids conduct area-restricted searches (Dixon, 2000) as also observed in *H. axyridis* (Kawai, 1976; Koch, 2003; Lee and Kang, unpublished observation). To develop a biological control program for *A. gossypii* using *H. axyridis*, further studies for *H. axyridis* should be conducted under both laboratory and field conditions on the foraging behavior, dispersal within and among plants, and population dynamics, among others.

Acknowledgment

This research was supported by the Brain Korea 21 project.

References

Bennison, J.A., Corless, S.P., 1993. Biological control of aphids on cucumbers: further development of open rearing units or banker plants to aid establishment of aphid natural enemies. Bull. IOBC/WPRS 16, 5–8.

Choi, S.Y., Kim, G.H., 1985. Aphidvorous activity of a Coccinellid beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). Kor. J. Plant Prot. 24, 11–14 (in Korean with an English abstract).

Dixon, A.F.G., 2000. Insect predator–prey dynamics: ladybird beetles and biological control. Cambridge university press, New York. p. 257.

Hassell, M.P., Lawton, J.P., Beddington, J.R., 1976. The components of arthropod predation. I. The prey death rate. J. Anim. Ecol. 45, 135–164.

He, J.L., Ma, E.P., Shen, Y.C., Chen, W.L., Sun, X.Q., 1994. Observations of the biological characteristics of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). J. Shanghai Agric. Coll. 12, 119–124 (in Chinese with an English abstract).

Hodek, I., Chakrabarti, S., Rejmanek, M., 1984. The effect of prey density on food intake by adult *Cheilomenes sulphurea* (Col., Coccinellidae). Entomophaga 29, 179–184.

Hodek, I., Honek, A., 1996. Ecology of Coccinellidae. Kluwer Academic Publishers, Dordrecht. p. 464.

Hong, O.K., 1996. Studies on the reproductive physiology and laboratory rearing of the aphidophagous ladybeetle *Harmonia axyridis* Pallas. Ph.D. Dissertation, Kangwon National University, 118 p. (in Korean with an English abstract).

Hu, Y.S., Wang, Z.M., Ning C.L., Pi, Z.Q., Gao, G.Q., 1989. The functional response of *Harmonia* (*Leis*) *axyridis* to their prey of *Cinara* sp. Nat. Enem. Ins. 11, 164–168 (in Chinese with an English abstract).

Juliano, S.A., 2001. Nonlinear curve-fitting: predation and functional response curves. In: Scheiner, S.M., Gurevitch, J. (Eds.), Design and Analysis of Ecological Experiments, second ed. Oxford university press, New York, pp. 178–196.

Kareiva, P., 1990. The spatial dimension in pest–enemy interaction. In: Mackauer, M., Ehler, L.E., Roland, J. (Eds.), Critical Issues in Biological Control. Intercept. Anover, Hants. pp. 213–227.

Kawai, A., 1976. Analysis of the aggregation behavior in the larvae of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) to prey colony. Res. Popul. Ecol. 18, 123–134.

Koch, R.L., 2003. The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. J. Insect Sci. 3 (32), 16. Available from insect-science.org/3.32.

Koch, R.L., Hutchison, W.D., Venette, R.C., Heimpel, G.E., 2003. Susceptibility of immature monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by *Harmonia axyridis* (Coleoptera: Coccinellidae). Biol. Control. 28, 265–270.

Lou, H.H., 1987. Functional response of *Harmonia axyridis* to the density of *Rhopalosiphum prunifoliae*. Nat. Enemies Insects. 9, 84–87 (In Chinese).

McClure, M.S., 1987. Potential of the Asian predator, *Harmonia axyridis* Pallas (Coccinellidae), to control *Matsucoccus resinosae* Bean and Godwin (Homoptera: Margarodidae) in the United States. Environ. Entomol. 16, 224–230.

Mogi, M., 1969. Predation response of the larvae of *Harmonia axyridis* Pallas (Coccinellidae) to the different prey density. Jap. J. Appl. Zool. 13, 9–16 (in Japanese with an English abstract).

Mulder, S., Hoogerbrugge, H., Altena, K., Bolckmans, K., 1999. Biological pest control in cucumbers in the Netherlands. IOBC Bull. 22, 177–180.

Murdoch, W.W., 1983. The functional response of predators. J. Appl. Ecol. 10, 335–342.

O'Neil, R.J., 1988a. Predation by *Podisus maculiventris* on Mexican bean beetle, *Epilachna varivestis*, in Indiana soybeans. Can. Entomol. 120, 161–166.

O'Neil, R.J., 1988b. A model of predation by *Podisus maculiventris* on Mexican bean beetle, *Epilachna varivestis*, in soybeans. Can. Entomol. 120, 601–608.

O'Neil, R.J., 1990. Functional response of arthropod predators and its role in the biological control of insect pests in agricultural systems. In: Baker, R.R., Dunn, P.E. (Eds.), New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases. Alan R. Liss, New York, pp. 83–96.

O'Neil, R.J., 1997. Functional response and search strategy of *Podisus maculiventris* (Heteroptera: Pentatomidae) attacking Colorado potato beetle (Coleoptera: Chrysomelidae). Environ. Entomol. 26, 1183–1190.

Parrella, M.P., Hansen, L.S., van Lenteren, J.C., 1999. Glasshouse environments. In: Fisher, T.W., Bellows, T.S., Caltagirone, L.E., Dahlstein, D.L., Huffaker, C.B., Gordh, G. (Eds.), Handbook of Biological Control. Academic Press, New York, pp. 819–839.

Rogers, D., 1972. Random search and insect population models. J. Ainm. Ecol. 41, 369–383.

SAS Institute, 1999. SAS Online®, SAS Institute, Cary, NC.

Seo, M.J., Youn, Y.N., 2000. The Asian ladybird, *Harmonia axyridis*, as biological control agents: 1. Predacious behavior and feeding ability. Kor. J. Appl. Entomol. 39, 59–71 (In Korean with an English abstract).

Sinha, T.B., Pandey, R.K., Singh, R., Tripathi, C.P.M., Kumar, A., 1982. The functional response of *Coccinella septempunctata* Linn., a coccinellid predator of mustard aphid, *Lipaphis erysimi* Kalt. Entomology 7, 7–10.

Van Lenteren, J.C., Woets, J., 1988. Biological and integrated pest control in greenhouses. Annu. Rev. Entomol. 33, 239–369.

Wang, J.-J., Tsai, J.H., 2001. Development and functional response of *Coleophora inaequalis* (Coleoptera: Coccinellidae) feeding on brown citrus aphid, *Toxoptera citricida* (Homoptera: Aphididae). Agric. Forest Entomol. 3, 65–69.

Wiedenmann, R.N., O'Neil, R.J., 1991a. Laboratory measurement of the functional response of *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae). Environ. Entomol. 20, 610–614.

Wiedenmann, R.N., O'Neil, R.J., 1991b. Searching behavior and time budgets of the predator *Podisus maculiventris*. Entomol. Exp. Appl. 60, 83–93.

Wiedenmann, R.N., O'Neil, R.J., 1992. Searching strategy of the predator *Podisus maculiventris* (Say) (Hetroptera: Pentatomidae). Environ. Entomol. 21, 1–9.