

LIFE, SEASONAL CYCLES, AND POPULATION FLUCTUATION OF *Hippodamia variegata* (GOEZE) (COLEOPTERA: COCCINELLIDAE), IN THE CENTRAL PLAIN OF LA ARAUCANÍA REGION, CHILE

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ABSTRACT

This study was performed on an alfalfa crop located on the central plain of La Araucanía Region, Chile and in the Laboratorio de Entomología Aplicada de la Facultad de Ciencias Agropecuarias y Forestales at the Universidad de La Frontera. Certain aspects of the biology of *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) were determined, more specifically in relation to its life cycle, seasonality, and population fluctuation. It was established that this coccinellid requires 190.32 ± 10.2 degree-days to complete a generation under laboratory conditions. This information along with the field samplings made it possible to calculate that *H. variegata* completes four generations per season in the alfalfa crop (*Medicago sativa* L.).

Key words: life cycle, seasonal cycle, population fluctuation, *Hippodamia variegata*, *Medicago sativa*.

INTRODUCTION

Hippodamia variegata (Goeze) is an active aphid predator used in the biological control of plant lice in cereals and oil plants in diverse countries (Linskii, 1984; Zúñiga, 1985; Zúñiga *et al.*, 1986; Obrycki and Orr, 1990; Shing and Shing, 1994; El-Hag and Zaitoon, 1996; Obrycki, 1998; González, 2006). Its origin is Palearctic, with a cosmopolitan distribution (Krafsur *et al.*, 1996; Franzmann, 2002), and is found in Asia (Kim *et al.*, 1968; Butani, 1972; Hameed *et al.*, 1977; Wu, 1986), Africa (Badawy, 1969; Haile and Megenasa, 1987; Aalbersberg *et al.*, 1988; Saharaoui and Gourreau, 1998), and Europe (Pruszyński and Lipa, 1971; Natskova, 1973; Radwan and Lovei, 1982; García and Ribeiro, 1983; Plaza, 1987; Ferran *et al.*, 1989; Nicoli *et al.*, 1995; Pekín, 1996; Burgio *et al.*, 2006). It was first introduced in Chile in 1967 as a result of the manifestation of the pale green louse of *Metopolophium dirhodum* (Walk.) gramineae and the dark ear louse of *Sitobion avenae* (Fabricius) (Rojas, 1980 a; 1980b). *H. variegata* is found in Chile from the Arica and Parinacota Region to the Los Lagos Region (González, 2006). According to Aguilera *et al.* (2005, 2006) and Rebolledo *et al.* (2007), its occurrence is notable in La Araucanía Region and is very abundant. Grigorov

(1977), Honek (1985) and Rebolledo *et al.* (2007) state that *H. variegata* prefers herbaceous plants. Nevertheless, Rebolledo *et al.* (2007) point out that it is possible to find this species in shrubby and arboreous plants.

Hagen (1962) affirms that coccinellids determine their conduct through four fundamental actions: voltinism, dormancy or diapause, migrations, and formation of aggregates (Hagen, 1962; Hodek, 1967). Voltinism (the number of generations per year) varies according to latitude. Hagen (1962) recognizes four types of voltinism: I = one generation; II = two generations; III or IV = three or more generations; IA = one generation whose adults migrate to hibernate.

Diapause is intimately related to voltinism given that the latter is a consequence of the former (Nieto y Mier, 1985). Hagen (1962) states that there are three types of dormancy depending on the season: (1) hibernation (Types with voltinism I, II, and III), (2) estivation and hibernation (Type IIA), (3) estivo-hibernation (Type IA). With regards to the formation of aggregates, this same author points out that this is perhaps the most fascinating phenomenon of the coccinellids. The majority of them have an instinctive tendency to hibernate socially, just as it occurs with established tribes such as Hippodamini and Anisocictini. *H. variegata* prefer an aggregation site on mountain tops or close to these (Khan *et al.*, 2007). With respect to the migration phenomenon, Hagen (1962) indicates that the long migratory flights are related to the search for dormancy sites, and that these are associated at the same time with the formation of aggregates. This coccinellid has been studied in the country as regards

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distribution (Arias, 2000) and predatory activity (Grez and Prado, 2000; Grez and Villagrán, 2000). However, there are no data about voltinism and population fluctuation. In La Araucanía Region, *H. variegata* is an abundant species and especially in alfalfa (*Medicago sativa* L.) (Rebolledo *et al.*, 2007).

In order to complement the previous studies and increase knowledge about its behavior in La Araucanía, it was proposed to determine its life and seasonal (voltinism) cycles, fluctuation, relative abundance, and its possible natural entomophagous enemies.

MATERIALS AND METHODS

Field work

Two seasons (1999-2000 and 2000-2001) were required to determine fluctuation and population density of *H. variegata* (in an alfalfa field located in the former Estación Experimental Maipo belonging to the Facultad de Ciencias Agropecuarias y Forestales of the Universidad de La Frontera, located in the urban radius of the city of Temuco (38°44' S, 72°35' W, 100 m.a.s.l.). Breeding of *H. variegata* adults was done with the alfalfa greenbug *Acyrtosiphon pisum* (Harris).

H. variegata adults were periodically collected with entomological nets during October and March in an alfalfa field. Adults were collected in December and were placed in plastic containers measuring 6.5 cm in height, 5.0 cm diameter, and covered with tulle.

To determine the relative abundance and population fluctuation between October 1999 and March 2001, the alfalfa field was visited 46 times. The sampling was carried out periodically every 10 ± 1 day during the spring, summer, and autumn months, and every 20 ± 1 day during the winter months. Each sample consisted in passing 20 times with a standard 30 cm diameter entomological net over the foliage in a 180° range, at a regular pace following the methodology proposed by Metcalf and Luckman (1990) and Apablaza and Stevenson (1995). The sampled area was divided into four 1 ha⁻¹ quadrants, numbered clockwise to facilitate sampling. Three samples were taken in each quadrant by leaving a minimum distance of 25 m between each replicate, checking, and registering the collected material. To determine the number of individuals per m², considering that the width was the amplitude that the net covered in a 180° horizontal movement and the length as the distance covered in the sample.

Laboratory work

H. variegata adults collected in the field were moved to a germination chamber (Archiclíma, Temuco, Chile) with controlled humidity conditions ($70 \pm 8\%$), temperature (21 ± 2 °C), and photoperiod (16:8 light:darkness) to observe

their behavior. Following copulation, males and females were separated. The confined adults were controlled daily to register and eliminate the parasitoids from the breeding.

The laboratory study of the *H. variegata* life cycle was initiated by obtaining eggs from the adults collected in December. These were deposited on Petri dishes and were incubated in the germination chamber in the above-mentioned conditions. When the eggs hatched, 80 larvae were separated, individually placed on numbered plastic dishes, and named initial breeding or group A. From this initial breeding, 57 adults were obtained from which 28 couples were formed at the beginning of January and separated into two groups of 14. A subgroup called AI was made up of 14 females that were permanently maintained with a male, and the second subgroup (AII) was formed by the remaining couples, but maintained with the male only during 48 h. Ten eggs were taken from each female which were bred in isolation in order to determine the influence of the male in the oviposition.

To measure the duration of the life cycle during the month of January, 48 individualized larvae were used as group B and obtained from the 10 isolated eggs of each couple. A new group of 40 larvae were chosen randomly from a single emergence date to determine the growth of each larval stage. The measurements were taken and registered every 24 h. Seven larvae from each larval stage were placed in glass containers with 75% alcohol to subsequently measure the length and width of each one with the help of graph paper. The widest sector of the thorax was used to measure the width, and the length was considered from the top of the head to where the abdomen ends. Furthermore, the width and length of seven pupas, also chosen randomly from groups A and B, were measured.

From the individuals used previously, 22 adults were taken to determine their longevity (50% males and 50% females), were fed daily with *A. pisum* and observed until the moment of their natural death.

The summation of degree-days required for a generation was established to determine the seasonal cycle of *H. variegata*. The formula proposed by Dinelli (1999) was used to calculate this summation. This value was contrasted with the results obtained during the months of field study allowing to determine the degree-days required for the activation of the adults following their hibernation, and the number of generations that theoretically occurred in the study zone.

The reason to use two groups (A and B) was the fact that these coccinellids were collected in different periods, and hence separate statistical analyses were done. An experimental randomized complete block design was used where each individual corresponded to one replicate.

The data of the specimens that completed the life cycle (groups A and B) were compared with variance analysis. Then these results were analyzed using appropriate tests to compare the means of two independent samples, whether parametric (t-Student) or nonparametric (U - Mann-Whitney) (Visauta, 1997; 1998).

RESULTS AND DISCUSSION

Population fluctuation of *H. variegata*

The first adults were examined during October and their number varied between seasons for what seemed to be a clear dependence on the particular yearly environmental conditions. They were completely absent during the winter which accounts for their natural behavior to enter diapause or hibernation. The fluctuation during the period under study showed two annual maxima, one on 10 December 1999 (15 specimens) and the second on 6 February 2001 (13 specimens) (Figure 1).

A noticeable increase in population levels occurred at the beginning of autumn, possibly related with the generation that spent the winter in diapause, results which coincided with those obtained by Apablaza and Stevenson (1995) who pointed out that the annual population maxima for coccinellids in alfalfa in the Metropolitana Region took place at the end of March.

Relative abundance of *H. variegata*

The relative abundance of this insect was 0.17 ± 0.18 adult specimens m^{-2} with a variation of 0 to 0.68 individuals m^{-2} . The larvae showed a mean relative abundance of 0.16 ± 0.25 specimens m^{-2} fluctuating between 0 and 1 individuals m^{-2} .

Volitinism of *H. variegata*

H. variegata required 190.32 ± 10.2 degree-days to complete a generation considering $10\text{ }^{\circ}\text{C}$ as the threshold temperature, the one recommended for the majority of coccinellids. During the first sampling season (spring 1999-summer 2000), it was difficult to estimate the number of generations due to a generational overlap since larvae and adults were found in the crop at the beginning of the measurements. Taking into account only the degree-days accumulated during October and November, it was estimated that the necessary degrees would be on 28 November to complete a first generation. The second generation would be obtained at the end of December, a third on the first days of February, and a fourth and final generation during the first week of March, going through the winter in diapause as an adult.

In the second season (spring 2000-summer 2001), the exact date was registered when adults first appeared coming out of their diapause. It was estimated that soon after the appearance of posthibernational adults, at the end of September, the first generation in the crop was completed around 10 December, obtaining the second generation during the second half of January, and the third generation at the end of the first half of February (Figure 2).

These results differed from those informed by Kontodimas and Stathas (2005) who indicate that a study carried out in Greece using *H. variegata* as a food source for *D. crataegi*, completed a total of seven generations between April and November.

H. variegata completed four generations during this study under the environmental conditions of the central plain of La Araucanía Region, the first of them with a longer duration with two to two and one half months, whereas

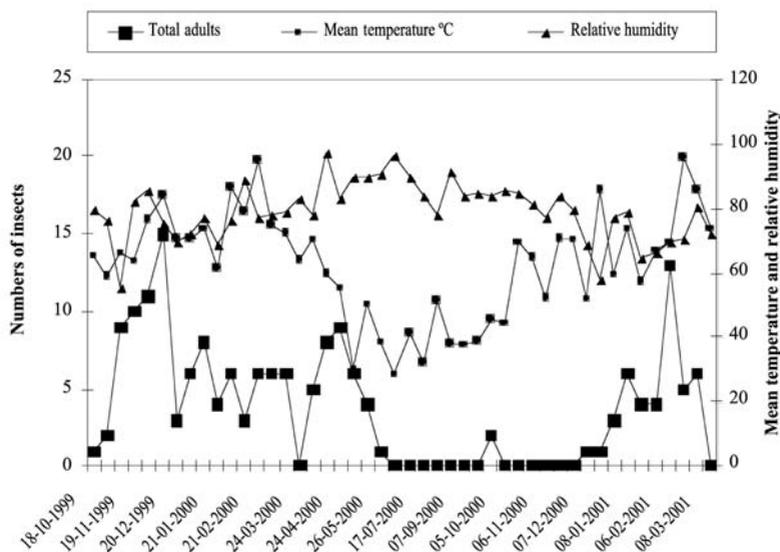


Figure 1. Population fluctuation of *Hippodamia variegata* adults in the central plain of La Araucanía Region.

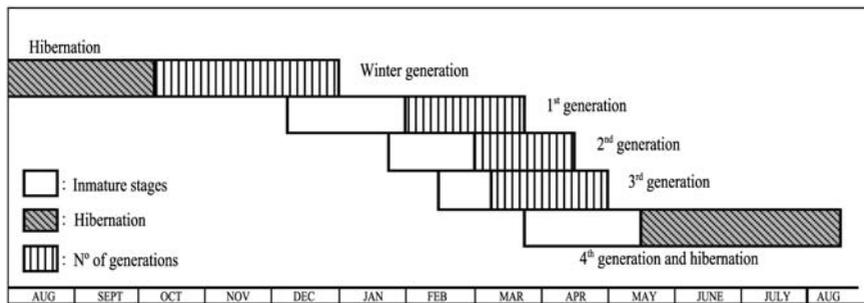


Figure 2. Diagram of *Hippodamia variegata* voltinism in the central plain of La Araucanía Region (2000-2001).

the rest only took one month that is, they showed a type III voltinism in accordance with that proposed by Hagen (1962). The fact that there was a high overlap between one generation and another is emphasized, fact essentially due to the extensive duration of the adult stage in comparison to the rest of the stages of the life cycle. The polyvoltinism observed concurred with Hagen (1962) who pointed out that the environmental conditions are what determine the number of possible generations for coccinellids.

Life cycle of *H. variegata*

H. variegata showed a life cycle of 17.3 ± 0.93 days varying in a range of 16 to 21 days. The pupa stage showed the longest duration with 32% of the life cycle total time, followed by the egg stage and fourth larval stage with 17% each. There is a discrepancy between this result and the one reported by Badawy (1969) who indicates a mean duration of 10.7 days for the life cycle. Breeding temperature would be a fundamental factor in the rate of preimaginal development according to Hagen (1962), Mitchels and Bateman (1986) who mention a duration of 15.1 days at 25 °C for the *H. variegata* life cycle, whereas the life cycle decreased to 7.8 days at 30 °C. The difference between this study and those stated by Hagen (1962) and Badawy (1969) can be attributed to the use of other breeding temperatures.

Oviposition, hatching, and incubation period of *H. variegata* eggs

Mating occurred between 2 and 5 days of life, registering the first ovipositions two days later, which is in accordance with that pointed out by Hodek (1967) and Badawy (1969). The oviposition and hatching of the *H. variegata* eggs are shown in Table 1. With respect to incubation time, it was three days for both groups.

The mean monthly oviposition of *H. variegata* was 223 ± 103.9 eggs and its frequency reached 4.37 ± 5.82 days, a contrast with that reported by Kontodimas and Stathas (2005) who obtained a mean of 956.6 eggs in one breeding of *H. variegata* carried out in Greece. However, that study

used *Dysaphis crataegi* (Kaltenbach) as a food source at a temperature of 25 °C, while the temperature in the present research was 21 °C and the food was *Acyrtosiphon pisum* (Harris). The mean of eggs per day of oviposition in the AI subgroup reached 17.58 ± 6.73 , whereas the groups of eggs attained a mean of 1.59 ± 0.41 per day of oviposition. At the same time, subgroup AII showed values similar to those in the previous subgroup with a mean oviposition of 18.73 ± 6.33 eggs per day of oviposition, a mean of 1.61 ± 0.27 groups per day of oviposition. Differences were not significant between the two subgroups.

Duration of *H. variegata* larval stages

In group A, made up of larvae emerged from the AI and AII subgroups, the mean duration of their development was 7.98 ± 0.73 , days with a range that fluctuated between 7 and 10 days, with the first and fourth stages having the longest duration with 26 and 32%, respectively, considering the total development of this stage of the insect, and coinciding with that reported by Badawy (1969). However, it must be pointed out that this author used the aphid *Aphis gossypii* Glover as food for the *H. variegata* larvae.

The larval stage in group B developed in 10.67 ± 1.62 days with a range that varied between 9 and 15 days, coinciding with that found by Mitchels and Flanders (1992), El-Hag and Zaitoon (1996) who determined a mean duration of the larval stage of 11 days using *Diuraphis noxia* (Mordvilko) and *Brevicoryne brassicae* (L.) + *Rhopalosiphum padi* (L.) as food, respectively. This would indicate that the aphid species used did not determine a difference in the duration of the larval stage. The difference in the duration of the larval period between groups A and B would be due to the capture period of the adults (December for group A and January for group B) in which breeding was initiated (Figure 3). The results of the growth of the randomly chosen larval stages are shown in Table 2.

Duration of *H. variegata* pupa stage

The duration of the pupa stage showed significant differences depending on its origin as group A or B.

Table 1. Oviposition and egg hatching percentage of *Hippodamia variegata*.

Subgroups	Eggs day ⁻¹	Hatching mean (%)	Groups of eggs day ⁻¹	variation (%)
AI ¹	17.58 ± 6.73	87.81 ± 5.76	1.59 ± 0.41	80-100
AII ²	18.73 ± 6.33	81.96 ± 7.27	1.61 ± 0.27	72-100

¹Permanent couple. ²Couple with male only 48 h.

Table 2. Larval instar size of *Hippodamia variegata*.

State	Mean length (mm)	Range	Mean width (mm)	Range
1	1.64 ± 0.56	1-1.15	0.5	0
2	2.28 ± 0.27	2-2.25	1	0
3	4.07 ± 0.45	3.5-4.5	2.28 ± 0.27	2-2.25
4	6.57 ± 0.44	6-7	2	2.25

Group A had a mean duration of 6.27 ± 0.45 days with a range of 6 to 7 days, while group B reached a mean of 4.28 ± 0.61 days and a variation between 3 and 5 days. Badawy (1969) points out a smaller mean duration value of 2.61 days than the one found in this study, difference based on a higher breeding temperature. The mean size of the randomly chosen pupas was a length of 4.57 ± 0.45 mm varying between 4 and 5 mm, and an observed width of 2.57 ± 0.45 with a range of 2 to 3 mm.

Longevity of *H. variegata*

The longevity mean of the adults in both groups was 53.27 ± 11.93 days. The females showed longevity of 55.09 ± 10.85 days and the males 51.45 ± 13.03 days. However, these numbers did not show any significant differences. The mean longevity attained in this study was less than the one cited by El-Hag and Zaitoon (1996) who

fed *H. variegata* with *B. brassicae* and *R. padi* at 25 ± 2 °C, and observed a mean adult longevity of 70 days.

Natural enemies of *H. variegata*

The only species of parasitoid found was *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) which affected 30% of the adults collected in the field according to the breeding mortality register of adults collected in alfalfa.

CONCLUSIONS

The life cycle of *H. variegata* had a mean duration of 17.3 ± 0.93 days, with a mean adult longevity of 53.27 ± 10.82 days. Both measurements did not differ statistically when comparing both groups grupos ($P = 0.377$ and $P = 0.485$ for the life cycle and longevity, respectively).

It was determined that *H. variegata* required 190.32 ± 10.2 degree-days to complete a generation, signifying that under the existing environmental conditions in the central plain of La Araucanía Region, this coccinellid can complete up to four generations per season. However, based on the population fluctuation and relative abundance records, it is concluded that this insect is not abundant as a natural control agent in the zone under study.

The population fluctuation of coccinellid was markedly seasonal with a complete absence of specimens during the winter and progressive population increases up to an annual maximum at the beginning and middle of the summer, during the first and second year, respectively. The relative abundance of this insect in alfalfa was 0.17 ± 0.18 specimens m⁻² for adults and 0.16 ± 0.25 specimens m⁻² for larvae.

The presence of the hymenoptera parasitoid *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) was confirmed in 30% of the adults collected in the alfalfa crop.

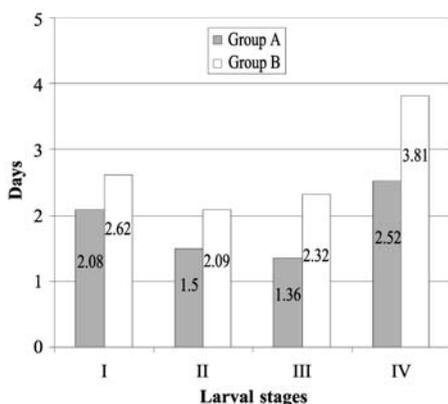


Figure 3. Duration (days) of the *Hippodamia variegata* larval stages under artificial breeding conditions. Group A (December) and Group B (January).

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RESUMEN

Ciclo vital, estacional y fluctuación poblacional de *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), en el llano central de La Araucanía, Chile. El presente estudio fue llevado a cabo en un cultivo de alfalfa ubicado en el llano central de la Región de La Araucanía, Chile, y en el laboratorio de Entomología Aplicada de la Facultad de Ciencias Agropecuarias y Forestales de la Universidad de La Frontera, donde se determinaron aspectos de la biología de *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), específicamente en relación a su ciclo vital, estacional y fluctuación poblacional. Se determinó que en condiciones de laboratorio este coccinélido requiere $190,32 \pm 10,2$ grados días para completar una generación, antecedente que sumado a los muestreos de campo permitió estimar que *H. variegata* completa cuatro generaciones por temporada en el cultivo de alfalfa (*Medicago sativa* L.).

Palabras clave: ciclo vital, ciclo estacional, fluctuación poblacional, *Hippodamia variegata*, *Medicago sativa*.

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