DETERMINATION OF NUMBER OF INSTARS OF *RHYSSOMATUS SUBTILIS* (COLEOPTERA: CURCULIONIDAE) BASED ON HEAD CAPSULE WIDTHS

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Abstract

Rhyssomatus subtilis Fiedler (Coleoptera: Curculionidae) is an important pest of the soybean crop in northwestern Argentina. Few studies have been made on specific parameters of its life history and ecology. The aim of this study was to determine the number of larval stages of *R. subtilis*. One thousand and eighteen larvae were collected from soybean plants during 2 yr (2011 and 2013), and head capsule width of each larva was measured. For analysis of data, the Hcap program and Dyar's rule were used. The Hcap program showed 4 different peaks in the frequency distribution of the head capsule widths. This result also agreed with Dyar's rule that revealed a perfect geometric larval growth pattern for each instar by regression analysis. The excellent fit to a linear model, indicates that no instar was overlooked. This research identified 4 instars for *R. subtilis*.

Key Words: black soybean weevil, Hcap program, Dyar's rule, pest, life cycle

Resumen

Rhyssomatus subtilis Fiedler (Coleoptera: Curculionidae) es una importante plaga del cultivo de soja en el Noroeste Argentino. Existen pocos estudios acerca de parámetros específicos del ciclo de vida y de comportamiento ecológico. El objetivo de este estudio fue determinar el número de instares larvales de *R. subtilis*. En total, 1018 larvas fueron colectadas del cultivo de soja durante 2011 y 2013. Se midió el ancho de la cápsula cefálica de cada larva. Para el análisis de los datos se utilizó el programa Hcap y la regla de Dyar. El programa Hcap mostró cuatro picos distintos en la distribución de frecuencias de la anchura de la cápsula cefálica. Estos resultados concuerdan con la regla de Dyar que muestra un perfecto crecimiento geométricos de las larvas para cada instar representada por la línea de regresión. El excelente ajuste del modelo lineal, indica que no hay instares solapados. Esta investigación identificó cuatro instares larvales para *R. subtilis*.

Palabras Clave: picudo negro de la soja, programa Hcap, regla de Dyar, plaga, ciclo de vida

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The black soybean weevil, *Rhyssomatus subtilis* Fiedler (Coleoptera: Curculionidae), is an important pest of soybean crops in northwestern Argentina (NWA), where it is expanding its range and causing economic losses through reduction of yields. It is important to mention that this species has been recorded only in the NWA region, and it has been detected more frequently in soybean cultures in different localities of Santiago del Estero, Salta, and Tucumán provinces (Cazado et al. 2013). Adults produce damage by their feeding and oviposition during the vegetative and reproductive stages of soybean. Female weevils deposited their eggs inside the pods, the eggs hatched and the larvae fed on the seed. The larvae developed inside the pod until the last instar, and then dropped to the soil. Within the soil, the individuals continued to develop through the winter as hibernating larvae, pupae or adults. Adult weevils emerged in spring as the rainfall increased (Socías et al. 2009; Cazado et al. 2013). The occurrence of *R. subtilis* causes significant soybean and dry bean production losses in the NWA region. The control of this pest is based mainly on the use of chemical insecticides, but when the number of the black soybean weevils is large, the entire soybean crop is lost and has to be reseeded (Casmuz et al. 2010; Cazado et al. 2013).

Little is known about this species' life history (durations of the egg, larval, pupal stages, instar numbers, adult longevity, etc.), because it is a new pest of soybean and dry bean crops in NWA (Cazado et al. 2013) and spends much of its life is hidden from view. To manage pests rationally, it is important to study their biology, and determination of the number of *R. subtilis* instars is basic for these studies.

As most of the instars of *R. subtilis* are concealed within the pod, it is difficult to distinguish between instars by larval molts, so head capsule width measurements were used to determine the number of instars (Dyar 1890; Daly 1985; Panzavolta 2007).

Several mathematical models have been used to determine the distinctions between instars. One of the best known models for distinguishing instars is Dyar's rule (Gaines & Campbell 1935), which is based on the fact that a sclerotized area of the insect body, such as the head capsule of an instar, remains more or less constant during a single stadium, but increases with a regular geometric progression from one molt to the next.

Other statistical methods used are based on the assumption that measurements of the head capsule are normally distributed for each instar and that each peak of the resulting multimodal curve, represents a different instar (Sokal & Rohlf 1995; Panzavolta 2007).

Computer programs have been developed to facilitate the analysis of these kinds of data and to determine overlapping dimensions of instars. The Hcap program determines an optimum separation rule based on the distribution of head capsule widths (Logan et al. 1998). Hcap generates optimum instar classification rules, mean and standard deviation estimates of head capsule widths for each instar, and misclassification probabilities.

The aim of this study was to determine the number of instars and the width of head capsules for each instar of *R. subtilis* using the Hcap program and Dyar's rule.

Materials and Methods

Sampling of larvae was carried out during 2 yr (2011 and 2013) from soybean crops located in Rosario de la Frontera (Salta province, Argentina) (S 25° 39' 57.6" -W 64° 56' 58.1"). Weekly from Feb to May, during the R4-R8 stages of the soybean (Fehr et al. 1971). Forty plants with damaged pods were selected randomly and carried to the laboratory. After 24 h, plant pods were extracted from collected plants and checked for presence of *R. subtilis* larvae. A total of 1,018 live larvae were collected, killed and preserved in 70% ethanol, and held for head capsule measurement. Dead larvae were not included in this study. The head capsule width of each larva was measured at the widest point according to the methodology described by Fox et al. (1972), McClellan & Logan (1994) and Panzavolta (2007), by a binocular microscope (Zeiss model Stemi DV 4) equipped with a 32X micrometer.

Head capsule width data of *R. subtilis* larvae were analyzed using the Hcap program developed by Logan et al. (1998), as a generalization of techniques developed by McClellan & Logan (1994) for the analysis of the gypsy moth (*Lymantria dispar* (L.): Lepidoptera: Lymantriidae) head capsule data. The program also calculated the mean, SD, and size range for each instar.

Dyar's ratios were then derived for all instars by taking the mean from one instar and dividing it by the mean of the previous instar. The strength of Dyar's rule was analyzed by the linear fit between number of instars and the natural log of the mean head capsule measurements, following the method proposed by Gaines & Campbell (1935). The significance of the regression line slope was tested with one-way ANOVA with StatSoft, Inc. 2007 as in Logan et al. (1998).

Results

The Hcap program analysis on the frequency distribution of head capsule widths indicated 4 distinct peaks, representing 4 instars (Fig. 1). Larval head capsule widths ranged from 0.280 to 1.332 mm and differences between mean head capsule widths for each instar were significant (Table 1). Estimated means, standard deviations, and number of individuals in each instar are presented in Table 1. The range of each instar and the probability of misclassifying instars illustrated that the chance of misclassification was low, with a maximum probability of misclassification of 2.1%. Dyar's ratio ranged from 1.32 to 1.53 (Table 1). The logarithm of head capsule widths plotted against the number of instars resulted in perfect geometric larval growth for each instar (Table 1; Fig. 2).

The linear regression equation for head capsule widths with successive instars was highly significant (P= <0.0001; R = 0.9882; R^2 =0.9764; RSS^2 = 186,341) (Fig. 2). The excellent fit to a linear model, indicated that no instar was overlooked. A significant difference in head capsule size between instars (F = 14,040.57; df = 3, 1015; P = < 0.001) (Table 1), confirmed the results observed by the linear relationship.

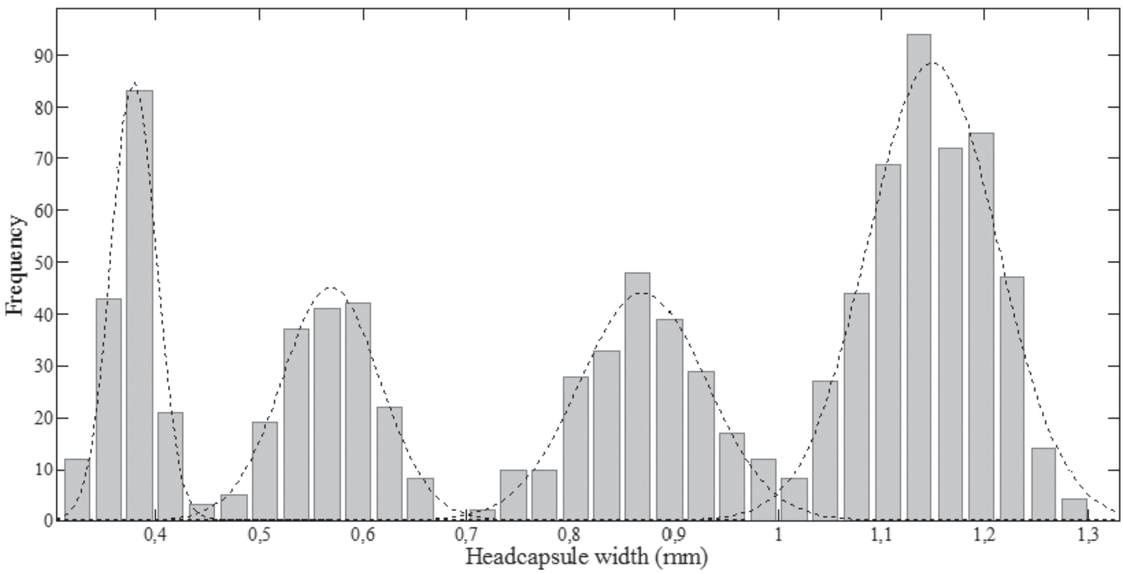

Fig. 1. Frequency distribution of observed head capsule widths (mm) of *Rhyssomatus subtilis* larvae. Graphs were produced by the Hcap program.

Discussion

Based on head capsule widths, this study determined that the number of instars of *R. subtilis* was 4. Previous studies also used this methodology to determine instars of various species (Fox et al. 1972; Mizell & Nebeker 1979; Caltagirone et al. 1983; Parra & Hadadd 1989; Hoffman-Campo et al. 1991; McClellan & Logan 1994; Logan et al. 1998; Panzavolta 2007, and others).

Morán Lemir et al. (1990), found 4 instars for *Rhyssomatus tomentosus* Fiedler, when the insect was reared on fruit of *Ipomoea purpurea* (L.) Roth (Convolvulaceae) and on artificial diet. O'Donnell (1967), Pantoja et al. (1999); Bailez et al. (2003) and Panzavolta (2007) identified 4 instars for the following species of Curculionoidea: *Sitophilus oryzae* (L.), *Lissorhoptrus kuscheli* O'Brien, *Conotrachelus psidii* Marshall and *Pissodes castaneus* De Geer.

For *R. subtilis*, Dyar's rule indicated a perfect geometrical progression of head capsule size with each successive instar. This observation agrees with previous studies for species of other weevil genera, e.g., *Sternechus subsignatus* Boheman (Hoffman-Campo et al. 1991), *Dendroctonus ponderosae* Hopkins (Logan et al. 1998), *C. psidii* (Bailez et al. 2003), and *P. castaneus* (Panzavolta 2007).

Head capsule width analyzed through Dyar's rule and the Hcap program reliably indicated the number of instars in field populations of the black soybean weevil in soybean. Results obtained in this work are important in building life tables and for use in other ecological studies to understand the behavior of this important pest, and to develop management programs in soybean crops in this region.

Endnotes

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Table 1. Head capsule means, ranges, and misclassification probabilities of the four instars of *Rhyssomatus subtilis* calculated by the Hcap program and Dyar's ratio.

				Probability of misclassification			
Instar (i)	*N*	Mean ± SD (mm)	Range (mm)	i as i - 1	i as i + 1	Total	Dyar's ratio
1	162	0.380 ± 0.022	0.280 - 0.443	0.0000	0.0018	0.0018	-
2	174	0.569 ± 0.048 *	0.443 - 0.701	0.0039	0.0027	0.0066	1.50
3	228	0.871 ± 0.061 **	0.701 - 0.998	0.0028	0.0183	0.0209	1.53
4	454	1.150 ± 0.063 ***	0.998 - 1.332	0.0080	0.000000	0.0080	1.32

An asterisk indicates significant differences between head capsule means ($P < 0.05$) (Tukey HSD test).

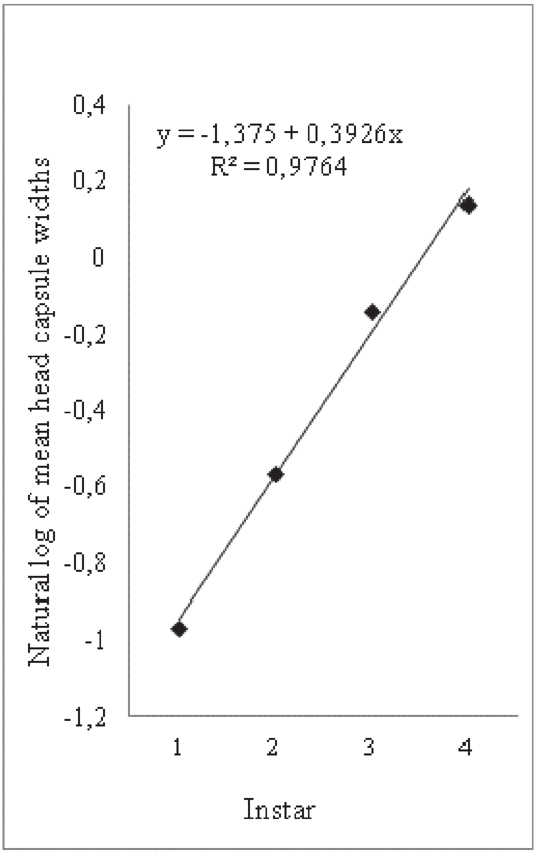

Fig. 2. Linear regression relationship between the natural logarithm of the mean larval head capsule widths and the number of the instars in the development of *Rhyssomatus subtilis* larvae.

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References Cited

BAILEZ, O. E., VIANA-BAILEZ, A. M., DE LIMA, J. O. G., AND MOREIRA, D. D. O. 2003. Life-history of the guava weevil, *Conotrachelus psidii* Marshall (Coleoptera: Curculionidae), under laboratory conditions. Neotrop. Entomol. 32: 203-207.

CALTAGIRONE, L. E., GETZ, W., AND MEALS, D. W. 1983. Head capsule width as an index of age in larvae of navel orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae). Environ. Entomol. 12: 219-221.

CASMUZ, A. S., CAZADO, L. E., SOCÍAS, M. G., ZAIA, D. G., ÁVILA, R., WILLINK, E., AND GASTAMINZA, G. 2010. Evaluación de la eficacia y persistencia de control de diferentes insecticidas sobre adultos de *Rhyssomatus subtilis* (Coleoptera: Curculionidae), pp. 171-175 *In* M. Devani, F. Ledesma y J. R. Sánchez [eds.], El cultivo de la soja en el Noroeste Argentino – Campaña 2009/2010, Publ. Especial EEAOC No. 41, Tucumán, Argentina.

CAZADO, L. E., MURÚA, M. G., CASMUZ, A. S., SOCÍAS, M. G., VERA, M. T., O'BRIEN, C. W., AND GASTAMINZA, G. 2013. Geographical distribution and new host associations of *Rhyssomatus subtilis* (Coleoptera: Curculionidae) in Argentina. Florida Entomol. 96: 663-669.

DALY, H. V. 1985. Insect morphometrics. Annu. Rev. Entomol. 30: 415-438.

DYAR, H. G. 1890. The number of molts of lepidopterous larvae. Psyche 5: 420-422.

FEHR, W., CAVINESS, C., BURMOOD, D., AND PENNINGTON, J. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci. 11: 929-931.

FOX, R. C., ANDERSON, N. H., GARNER, S. C., AND WALKER, A. I. 1972. Larval head capsules of the Nantucket pine tip moth. Ann. Entomol. Soc. America 65: 513-514.

GAINES, J. C., AND CAMPBELL, F. L. 1935. Dyar´s rule as related to the number of instars of the corn ear worm, *Heliothis obsoleta* (Fab.), collected in the field. Ann. Entomol. Soc. America 28: 445-461.

HOFFMAN-CAMPO, C. B., PARRA, J. R. P. AND MAZZARIN, R. M. 1991. Ciclo biológico, comportamiento e distribuição estacional de *Sternechus subsignatus* Boheman (Coleoptera: Curculionidae) em soja, no norte do Paraná. Rev. Brasileira Biol. 51: 615-621.

LOGAN, J. A., BENTZ, B. J., VANDYGRIFF, J. C., AND TURNER, D. L. 1998. General program for determining instar distributions from head capsule widths: Example analysis of mountain pine beetle (Coleoptera: Scolytidae) data. Environ. Entomol. 27: 555-563.

MCCLELLAN, Q. C., AND LOGAN, J. A. 1994. Instar determination for the gypsy moth (Lepidoptera: Lymantriidae) based on the frequency distribution of head capsule widths. Environ. Entomol. 23: 248-253.

MIZELL, R. F., AND NEBEKER, T. E. 1979. Number of instars of the southern pine beetle (Coleoptera: Scolytidae) and some comparisons of head capsule widths. Ann. Entomol. Soc. America 72: 313-322.

MORÁN LEMIR, A. H., FRÍAS, E., TERÁN, A., AND ZEN, S. R. 1990. Insectary rearing of *Rhyssomatus tomentosus* Fiedler (Coleoptera: Curculionidae), a weevil attacking fruits of "morning glory", *Ipomoea purpurea* (L.) Roth, (Convulvulaceae). CIRPON Rev. Invest. VIII: 23-35.

O'DONNELL, A. E. 1967. The determination of larval instars of the rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) in wheat. Proc. Arkansas Acad. Sci. 21: 36-38.

PANTOJA, A., RAMIREZ, L. M., ESCALONA, L. E., BASTIDAS, H., AND DUQUE, M. 1999. Number of instars of *Lissorhoptrus kuscheli* (Coleoptera: Curculionidae) from rice in Venezuela. Ann. Entomol. Soc. America 92(2): 236-238.

PANZAVOLTA, T. 2007. Instar determination for *Pissodes castaneus* (Coleoptera: Curculionidae) using head capsule widths and lengths. Environ. Entomol. 36: 1054-1058.

PARRA, J. R. P., AND HADADD, M. L. 1989. Determinação do número de ínstares de insetos. Fundação de Estudos Agrários-FEALQ, Piracicaba, SP, 49p.

STATSOFT, INC. (2007). STATISTICA (data analysis software system), version 8.0.

SOCÍAS, M. G., ROSADO-NETO, G. H., CASMUZ, A. S., ZAIA, D. G., AND WILLINK, E. 2009. *Rhyssomatus subtilis* Fiedler (Coleoptera: Curculionidae), primer registro para la Argentina y primera cita de planta hospedera, *Glycine max* (L) Merr. Revista Industrial y Agrícola de Tucumán 86: 43-46.

SOKAL, R. R., AND ROHLF, F. J. 1995. Biometry. 3rd ed. W. H. Freeman, New York, 887 pp.