

Density and spatial distribution of different development stages of *Sternechus subsignatus* Boheman (Coleoptera: Curculionidae) in soybean crops



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ARTICLE INFO

Article history:

Received 17 March 2014

Received in revised form

14 June 2014

Accepted 23 June 2014

Available online

Keywords:

Soybean stalk weevil

Spatial pattern

Density

Plant protection

ABSTRACT

The population recruitment and the spatial distribution of eggs, larvae, overwintering stages and adults of the soybean stalk weevil, *Sternechus subsignatus* Boheman, on soybean were estimated in two commercial farms of the Tucumán province, Argentina, during three consecutive productive cycles. At weekly intervals 30 sampling units were taken in a random distribution from each farm, and the number of adults as well as the number of “rings” and gall-like structures around stems and/or branches recorded, from which the number of eggs and larvae, respectively, were inferred. At fortnightly intervals 20 sampling units of soil were taken at random and the number of overwintering stages recorded. The recruited number of the different developmental stages was analyzed by nested ANOVA, and the spatial distribution was estimated by the Taylor's Power law and Iwao's regression methods. Adults were recorded in all samples while eggs and larvae were found from mid-January to late April. The recruited number of the different stages was low and did not differ between stages or farms, and the spatial disposition of all developmental stages was at random. This study constitutes the first of its kind for this pest, and provides information that will be useful for the purposes of monitoring for biological studies and for insect pest control in the field.

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1. Introduction

The spatial distribution of the members of a local population, particularly insects, depends on the spatial scale considered, characteristics of the habitat, the life cycle and population density as well as interspecific interactions (Benard and McCauley, 2008; Coll and Yuval, 2004; Slansky and Feeny, 1977; Schowalter, 2006). The spatial distribution affects the efficiency of sampling plans as well as the analysis and interpretation of data, and if it is a pest, knowledge of the distribution allows a selective approach in implementing control measures in time and space in the framework of integrated pest management (IPM) (Bechinski and Pedigo, 1981; Nestel et al., 2004).

The soybean stalk weevil, *Sternechus subsignatus* Boheman, 1836 (Coleoptera: Curculionidae: Sternechini), is one of the most important pests on soybean crops in Bolivia, Brazil, Paraguay, and in the northwestern of Argentina (Hoffmann-Campo et al., 1991; Silva, 1999; Socías et al., 2011). This weevil is a Neotropical univoltine species restricted to certain herbaceous leguminous plants among which soybean, *Glycine max* Merrill (Fabaceae), is the preferred host (Silva, 1997). The life cycle comprises the egg, larval (five instars), pupa and adult stages. Eggs develop in five days approximately, with extreme values of three to 11 days (Silva, 1999). Eggs are laid individually, mainly in the middle portion of the stem and principal branches of the soybean plant, and each oviposition point is surrounded by a characteristic “ring” produced with plant tissues and is visible from the outside. Larvae are sedentary and larval feeding produces a characteristic gall-like structure also easily visible from the outside (Hoffmann-Campo et al., 1991). They remain in the plant for around 44 days, when in mid-autumn (end of April) the fully grown larvae leave the plant and burrow into the ground to hibernate. They pupate in early spring (by the end of September),

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reach the adult stage by October, and emerge from late November to early December coinciding with the onset of rains (Socías, 2012). Late instar larvae, pupae and pre-emergent adults are the non-active overwintering stages in the ground (Socías et al., 2011). Adults are long-lived, can disperse over considerable distances and extreme estimates of maximum fecundity are 79–445 eggs per female. Due to an oviposition period longer than 120 days (Hoffmann-Campo et al., 1991; Lorini et al., 1997; Silva, 1999) different developmental stages overlap widely (Socías et al., 2011).

The soybean stalk weevil reduces soybean productivity and it is highly harmful since both larvae and adults damage soybean (Silva et al., 1998). The adult produces a longitudinally fraying in the main stem and branches of the plant due to feeding and females in the form of a “ring” for egg oviposition (Casmuz et al., 2009b) while larval feeding, due to the destruction of the vascular system, reduces the flow of sap in the main stem completely preventing it (Silva, 1999, 2000). Presumably, the status of *S. subsignatus* as a serious pest on soybean was due to the expansion of this culture in the mentioned countries during the last decades, facilitated by non-tillage and minimum tillage systems (Casmuz et al., 2009a; Costilla and Venditti, 1990; Hoffmann-Campo et al., 1999; Panizzi et al., 1977; Pruett et al., 1996). In effect, associated with its high fecundity and a relatively low or null impact of natural enemies of eggs and larvae (Lorini et al., 1990), no-tillage or minimum tillage systems facilitate survival of hibernating stages (Hoffmann-Campo et al., 1991; Socías, 2012), enhancing the numerical response of the local population.

The control of *S. subsignatus* is currently based on cultural and chemical strategies such as crop rotation with grasses, trap crops, delayed planting dates, seed treatment with chemical insecticide and foliar insecticide applications (Casmuz et al., 2009a; Hoffmann-Campo et al., 1999). To optimize the results of such practices, it is necessary to have all available information about the pest in order to realize an efficient management within the guidelines of IPM program, and to our knowledge, information about the spatial distribution of *S. subsignatus* in soybean cultures is lacking. The objective of this work was to analyze the density and spatial distribution of the different developmental stages of *S. subsignatus* in soybean crops cultivated under conventional pest management.

2. Materials and methods

2.1. Sampling sites

Two soybean farms located in Tucumán province, northwestern of Argentina, were used to carry out this study during three consecutive productive cycles: 2007/2008, 2008/2009 and 2009/2010. One farm was located in La Cocha County (farm 1), representative of the southern productive zone, while the second farm, was in Cruz Alta County (farm 2), was representative of the eastern productive zone of Tucumán. Table 1 contains additional information related to each farm under study.

The climate in Tucumán province is subtropical (25 °C mean annual temperature) and characterized by a quasi monsoon

weather: dry winters are followed by rainy summers: 1100 mm of cumulated annual rainfall is distributed from October, early spring (October) to early autumn (March) (Minetti et al., 2005). The main crops are sugarcane (*Saccharum officinarum* L), lemon (*Citrus limon* (L) Burm), corn (*Zea mays* L), and soybean.

The soybean crops were kept under conventional management practices: weed management was performed by one glyphosate application prior to seeding and another application between the V6 to R1 phenological stages (Fehr and Caviness, 1977). For weevil control during the first vegetative stages (V1–V3), soybean seeds were treated with thiamethoxam and during older vegetative stages, 1–3 applications of pyrethroids were made according to weevil density. During the initial reproductive stages of the crop (R1 to R3) one insect growth regulator (IGR) application was made for the control of defoliator caterpillars, and during R4 to R6 one application of an organophosphate + pyrethroid was made for stink bug control. For control of diseases, one application of strobilurin + triazol was made at the R2 to R5 stages. In this research, the plots under study were in a soybean monoculture: soybean (summer) – wheat (winter) – soybean (summer).

2.2. Insect sampling

In each farm, sampling of *S. subsignatus* was done during three consecutive soybean productive cycles: 2007/2008, 2008/2009 and 2009/2010.

The size of study areas or plots was 2 ha in each farm. On each plot and approximately at weekly intervals and during sunshine hours, from the V3 phenological stage up to senescence, we randomly took 30 sampling units of 1 lineal meter (the spatial scale used by technicians and farmers to monitor this pest in the field) which include a mean number of 18 plants per sampling unit. The total number of adult weevils as well as the total number of “rings” around stems and/or branches and the total number of galls were recorded. Previous studies showed that each ring generally corresponded to only one egg, and each gall to only one larva (Hoffmann-Campo et al., 1991; Silva et al., 1998), so the number of eggs and larvae were inferred from the number of rings and gall-like structures, respectively, in all plants situated in the linear meter. Due to time restrictions, sampling of the number of overwintering stages (late instar larvae, pupae and pre-emergent adults) was estimated by means of 4–6 ground samples randomly distributed from early autumn up to early spring on a fortnightly basis. Each sample was 1 m length, 0.3 m wide (0.15 m at each side of the row of plants), and 0.2 m depth. The ground was sifted *in situ* with a 20-mesh metallic sieve and the total number of overwintering non-active forms registered (Socías et al., 2011).

2.3. Data analysis

2.3.1. Recruitment

We considered that the number of “rings” and gall-like structures placed in the main stems and lateral branches of the soybean plants represented the number of eggs and larvae, respectively.

Table 1
Consecutive productive cycles (soybean seasons), coordinates of each one of the farms located in the two areas (South and East), soybean variety, maturity groups and growth habit of soybean together with the corresponding sowing date.

Soybean season	Area	Farm	Soybean variety	Maturity group	Growth habit	Sowing date
2007/2008	South	1 (27° 46' S, 65° 30' W)	A 8100 RG	8.1	determinate	29/12/2007
	East	2 (26° 49' S, 64° 51' W)	A 7636 RG	7.6	determinate	21/12/2007
2008/2009	South	1 (27° 46' S, 65° 30' W)	A 8000 RG	8.0	determinate	28/12/2008
	East	2 (26° 49' S, 64° 51' W)	Munasqa RR	8.3	determinate	17/12/2008
2009/2010	South	1 (27° 46' S, 65° 30' W)	A 7636 RG	7.6	determinate	14/12/2009
	East	2 (26° 49' S, 64° 51' W)	Munasqa RR	8.3	determinate	23/12/2009

However, eggs appear on plants from mid-January to the end of March, and the larval period lasts from the end of January to the end of April (Socías et al., 2011). Thereby, rings and gall-like structures lasted throughout the entire sampling period of a given cycle, and in order to prevent counting more than once the same structures in successive date samplings, we considered that the recruited number of eggs per linear meter was the absolute maximum number of “rings” plus the number of gall-like structures (larvae) estimated in a given sampling date. The same criterion was applied to the adults which are long-lived insects and the only stage which can actively enter or leave the study area. However, in the case of adults, field sampling was conducted during sunshine hours despite adult activity is nocturnal. With respect to the overwintering stages, due to the lower number of sampling units per sampling date, recruitment was estimated as the mean number of all overwintering stages from the successive ground samplings which were made once all grown larvae had left their galls and were buried into the ground. We assumed that there was no mortality. We analyzed the recruited number of insects by nested ANOVA with developmental stage as the fixed variable with three levels (egg, adult and overwintering stages), and farm as the random variable with two levels (farm 1 and farm 2) nested within each level of the fixed variable, and the three soybean productive cycles as repetitions, given that weather conditions and crop management were similar. Homoscedasticity was evaluated by the Cochran C test ($C = 0.74$; $df = 5$; $p = 0.07$), and normality by the Kolmogorov-Smirnoff D test ($D = 0.14$; $p < n. s.$), and no transformation of the data was necessary. Differences between recruitments were analyzed by Tukey HSD test and all data were analyzed using the statistical package STATISTICA (StatSoft Inc., 2007).

2.3.2. Spatial distribution

The spatial distribution of the different developmental stages of *S. subsignatus* in each farm was determined by Taylor's power law and Iwao's patchiness regression methods. In order to consider the maximum number of points in the regressions (each sampling date provided one point) we pooled data from all samplings in the 2007/2008, 2008/2009, and 2009/2010 productive cycles, given that weather conditions and crop management were similar, as mentioned.

The Taylor's (1984) power law is a consistent empirical relationship between the variance (s^2) and the mean (m) of the number of counts per sampling, given by: $s^2 = a m^b$. In practice b is often regarded as a species-specific measure of dispersion of sampling counts, with $b > 1$, $b = 1$, and $b < 1$ indicating aggregated, random, and regular spatial patterns, respectively, and a is a sampling factor which has less interest to define the aggregation pattern. Taylor's coefficients can be estimated according to the given equation, after the logarithmic transformation: $\log_{10}(s^2) = \log_{10} a + b \log_{10}(m)$, which yields the least-squares regression parameters: $\log_{10} a$ (the ordinate) and b (the slope) (Kuno, 1991; Taylor, 1984).

The Iwao's method is a deductive one which regressed the mean crowding m^* of Loyd (1967) on m in a series of population samplings, which proved to be useful in detecting the duality in the dispersion pattern characteristic of the species. The mean crowding m^* provides the degree of crowding experimented by the individuals and was defined as the mean number per individual of other individuals in the same sampling unit, $m^* = m + [(S^2/m) - 1]$ (Loyd, 1967). The relation of m^* on m can be expressed by: $m^* = a + bm$, the constant a reflects the degree of contagiousness and may be considered as the index of basic contagion. The slope b of the regression describes the manner in which individuals or groups of individuals distribute themselves in their habitat with changing mean density, and may be called the density-

contagiousness coefficient. If the distribution of isolated individuals was at random, the regression line passes through the origin ($a = 0$) and its slope $b = 1$ (Iwao, 1968).

Student's t -test was used to determine significant differences between a (the ordinate) and b (the slope) with respect to 0 and 1, respectively.

3. Results

3.1. Recruitment

In both farms, adults were recorded in all sampling dates and attained higher densities during early January to mid-February (V1 to R1). Eggs were recorded from mid-January to late March (V5 to R8) attaining highest densities in mid-February, while larvae were registered from mid-February to late April (V5 to R8) and higher densities occurred in March. Overwintering stages were recorded from late April to early December.

The estimated recruited number of adults, eggs and overwintering stages in both farms are shown in Fig. 1, and nested ANOVA showed that recruitment did not differ significantly between farms ($F = 0.318$, $df = 3, 12$, $p = 0.812$) nor between developmental stages ($F = 1.429$, $df = 2, 12$, $p = 0.278$).

3.2. Spatial distribution of adults, eggs and larvae and overwintering forms in the ground

For the weevil density recorded in this study, Taylor's power law indicated that the spatial distribution of adults, eggs, larvae and overwintering stages in both farms were basically at random, except overwintering stages in farm 2 which tended to be aggregated. The intercept (a), the slope (b), and the coefficient of determination (r^2) of each equation in farms, as well as the t test value and the corresponding probability for the slope which differed significantly from the value 1.0 are shown in Table 2.

Iwao's patchiness regression indicated that the spatial distribution of adults, eggs, larvae and overwintering stages in both farms was at random. The intercept (a) was equal to 0, indicating no tendency to crowding or neutral interaction between individuals. The b values of the Iwao model were equal to 1 indicating a random spatial pattern. As above, the intercept (a), the slope (b), the coefficient of determination (r^2), and the value of the t test and the corresponding probability for the slope differed significantly from 1.0, are shown in Table 3.

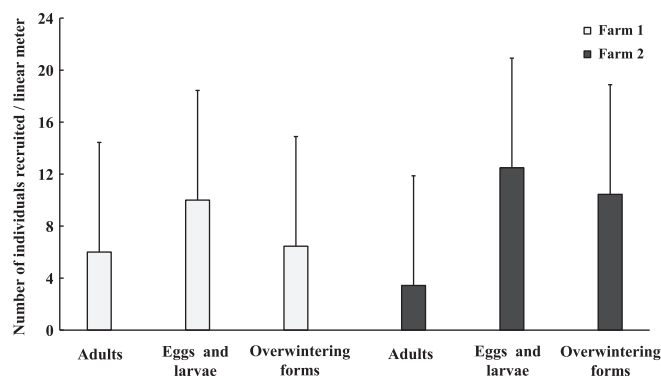


Fig. 1. Recruited number of different developmental stages of *S. subsignatus* in Farm 1 (South zone) and Farm 2 (East zone). Vertical bars denote 0.95 confidence intervals.

Table 2
Spatial pattern of *S. subsignatus* on soybean using Taylor's power law analysis.

Stage	Taylor's power law							
	Farm	n	a	b	r ²	T	P value	Distribution pattern
Adult	1	26	1.28*	1.06*	0.95	0.25	0.801	Random
	2	22	1.58*	1.16*	0.93	0.6	0.554	Random
Egg and larvae	1	45	1.023	0.99*	0.92	−0.003	0.997	Random
	2	36	1.25*	1*	0.9	0	>0.999	Random
Overwintering forms	1	29	0.89	1.15*	0.61	−0.7	0.488	Random
	2	20	0.2	1.58	0.18	−12.99	<0.0001	Aggregated

*P value <0.05.

4. Discussion

Habitat selection is an important feature of animal behavior and population dynamics (Morris, 1987; Orians and Wittenberger, 1991). Theories of habitat selection assume that for a given species, favorable habitats will more often be occupied than less favorable ones, however, where the habitats are more homogeneous and all are rather favorable, the spatial distribution of individuals can be more stochastic (Åberg et al., 2000; Rotenberg and Wiens, 1980). The spatial distribution of the members of a local population, in addition to habitat selection and habitat heterogeneity, also depend on characteristics of the life cycle and population density as well as on the selected spatial scale (Coll and Yuval, 2004; Iwao, 1968; Slansky and Feeny, 1977; Schowalter, 2006).

S. subsignatus is currently a serious pest on soybean, and only the adults can disperse considerable distances (Casmuz et al., 2010), whereas individuals in pre-imaginal stages (eggs, larvae and pupa) are almost sedentary (Hoffmann-Campo et al., 1991; Silva, 1999). At the spatial scale of a single soybean plant, an adult female selects certain sites for oviposition: almost 80% of eggs, laid individually, occurred in the middle third of the plant, with 90% of them on the main stems and only 9% on secondary side branches. The sedentary larvae were recorded at the same sites: 87% of them developed in the main stems and 12% in the secondary side branches, and only one was found per oviposition "ring". These sites are considered more suitable for oviposition because of an increased food supply which in turn may ensure higher larval survival (Silva et al., 1998).

From the viewpoint of the insect and the availability of resources, the soybean crop is a homogeneous habitat; however, the adults may concentrate on certain plants due to aggregation pheromones produced by the *S. subsignatus* adult males that attract both sexes, acting as mediators in the search for food and the meeting of the sexes (Ambrogi and Zarbin, 2008). Despite this behavior, the maximum number of eggs recorded at a relatively high *S. subsignatus* adult density was 11 eggs per plant (Lorini et al., 2000), which represents only a small fraction of its potential fecundity (Hoffmann-Campo et al., 1991; Lorini et al., 1997; Silva,

1999). Aggregation pheromones only mediate the sexes meeting and feeding; subsequently, individuals tend to randomness because of its high fecundity and their preferences for oviposition sites, avoiding competition from females for the resource.

Aggregation has been pointed out as a common pattern present in weevil's species (Christensen et al., 1977; Faleiro et al., 2002; Jiménez et al., 2008; Latheef and Pass, 1974; Miller et al., 1972; Salama and Abd-Elgawad, 2010). Moradi-Vajargah et al. (2011) observed an aggregated distribution pattern for all life stages of the alfalfa weevil *Hypera postica* Gyllenhal (Coleoptera: Curculionidae) except pupae, which exhibited a random pattern. They observed an aggregated disposition when a spatial scale of 0.25 m² was considered. The same pattern was observed by Latheef and Pass (1974) who considered 0.28 m² of an alfalfa crop as the sampling unit. These authors suggested that the basic spatial distribution pattern was aggregated only when the *H. postica* population density was low, dispersing in the direction of randomness with increasing density. Similarly, the early and late larval stages of the Egyptian alfalfa weevil, *Hypera brunneipennis* Boheman, are most aggregated when the population density is relatively low, indicating that the sample unit considered for this analysis, the individual alfalfa stem, represented an "effective habit unit" where all intraspecific interactions may occur (Christensen et al., 1977). Metzler (2004) mentioned that such aggregation patterns provide some benefits in mating, feeding and defense against predators. Shibuya and Ouchi (1955) found that the distribution of the gall-midge, *Asphondylia*, on soybean beans was contagious if the plant was taken as the sampling unit, but random if the numbers per pod were considered. The contagion appeared to be due to there being more eggs on those plants with more pods. Burrage and Gyrisco (1954) demonstrated the effect of the size of the sampling unit on the apparent distribution for the chafer beetle larvae. Therefore, a careful testing of the size and pattern of sampling is necessary to discover such an artifact (Southwood, 1966).

The spatial distribution of a local population was not only density-dependent but also scale-dependent. Analysis of the spatial pattern is an essential basis for understanding the scales at which organisms interact with one another or with their environment. Some processes can only act at small scales and others only at large scales, and the scale of investigation may have profound effects on the patterns one finds (Underwood and Chapman, 1996).

This present work was also made in a soybean crop and the selected spatial scale for the sampling unit was a linear meter, approximately 17–20 soybean plants, which is the one used in field sampling for pest monitoring and control purpose (Bimboni, 1985; Iannone and Leiva, 1993; Mazzaro, 2010a, 2010b; Salas and Costilla, 1996). Our results showed that the density of *S. subsignatus* was low: the minimum and maximum average numbers of recruited adults during all cycles and in both farms were 0.3 and 8.0 adults per linear meter that represented only 0.02 and 0.44 recruited adults per plant, respectively. The estimated minimum and maximum recruited number of eggs were 0.45 and 28 per linear

Table 3
Spatial pattern of *S. subsignatus* on soybean using Iwao's patchiness regression method.

Stage	Iwao regression method							
	Zone	n	a	b	r ²	T	P value	Distribution pattern
Adult	1	26	1.41	1.19*	0.73	−0.48	0.634	Random
	2	22	1.23	1.79*	0.36	−1.65	0.1079	Random
Egg and larvae	1	45	1.47	0.99*	0.92	0.05	0.9616	Random
	2	36	1.94	1.07*	0.91	−0.25	0.803	Random
Overwintering forms	1	29	2.13	1.05*	0.95	−0.21	0.8354	Random
	2	20	0.95	1.01*	0.91	−0.09	0.9257	Random

*P value <0.05.

meter, equivalent to 0.03 and 1.56 recruited eggs per plant. Concerning overwintering stages, *S. subsignatus* adults have a prolonged emergence from the ground with peaks in mid-December and January (Socías et al., 2011; Socías, 2012) and the estimated recruitment values were 2.64 and 13.6 equivalent to 0.15 and 0.76 recruited stages per plant. Due to the potential damage severity on soybean plants in its early vegetative stages (Casmuz et al., 2009a, 2009b) as well as during vegetative and early reproductive stages (Socías et al., 2011), numerous insecticide applications took place to control this pest. This fact could explain the low densities recorded in this study. The reduction of adult density in the field seems to occur to decrease their potential future offspring because, once eggs are laid, immature stages exhibit high survival. The spatial distribution of the eggs, and hence of the larvae, is suspected to be a consequence of adult behavior: adult females lay their eggs on the middle portion of the main stems of the soybean plant enhancing in this way larval survival (Silva et al., 1998). Thus, the availability of oviposition sites is not a limiting factor for the species at low and medium adult density.

Even at this relatively low density, the spatial pattern of the soybean stalk weevil followed a random distribution for adults as well as for the other developmental stages at least at the selected spatial scale. The combination of cultural and chemical alternatives are used for the management and control of this species, such as rotation with grasses, trap crops and delayed sowing dates between the first, and seed treatment and foliar applications to chemical alternatives. Their combination has allowed a reduction in the insect population densities in recent years, reaching low levels presented in this paper. This result will be useful to optimize the design of monitoring plans of *S. subsignatus* in soybean cultures, improving the set of alternatives existing management and currently in practice for the pest. Besides, aggregation pheromones identified by Ambrogi and Zarbin (2008) related to food sources finding, especially in the early stages of adult life, provide a solid foundation for the adoption of trap crops as alternative to exert a localized insect control on the first adults emerged from the soil. The spatial pattern will allow the number of samples needed for both the estimation of population size to insect monitoring according to their densities and the phenological stage of the crop to be calculated. From the results of this study and previous studies of the species, it is possible to propose an integrated and successful management based on biological and behavioral knowledge, reducing the number of insecticide applications and combined with cultural and/or mechanical alternatives.

Acknowledgments

We thank Lic. Eduardo Willink (EEAOC) for constructive comments on an earlier draft of the manuscript. This study was supported by Estación Experimental Agroindustrial Obispo Colombres (EEAOC) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

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