Research

Phenological stages of a soybean crop affect the number of mating pairs and egg load in *Rhyssomatus nigerrimus* (Coleoptera: Curculionidae) females under natural conditions

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Abstract

The soybean weevil, *Rhyssomatus nigerrimus* Fahraus (Coleoptera: Curculionidae), is an economically important pest that attacks soybean (*Glycine max* [L.] Merrill) crops in northern and southern Mexico. Knowledge of bioecological and behavioral aspects of this insect can contribute to developing integrated management strategies for its control. This study aimed to register the number of mating *R. nigerrimus* pairs during different phenological stages of the soybean crop and at different times of day, as well as to determine the egg load in *R. nigerrimus* females during different phenological stages of the crop. During the soybean vegetative stages (VE to V5) and reproductive stages (R1 to R3), no copulating *R. nigerrimus* pairs were observed. The first mating pairs were found from phenological stage R4 to phenological stage R9. *Rhyssomatus nigerrimus* pairs were observed mating from 7:00 AM to 5:30 PM, with the largest number of mating pairs between 11:30 AM to 2:30 PM. The number of copulating *R. nigerrimus* pairs correlated positively with the temperature and negatively with relative humidity. In the ovaries of female *R. nigerrimus* collected in the field at different phenological stages, eggs started appearing at the phenological stage R5. The largest egg load was found in females collected during the phenological stage R7.1.

Key Words: *Glycine max*; the soybean weevil; soybean pest; mating behavior; copulate

Resumen

El picudo de la soya, *Rhyssomatus nigerrimus* Fahraus (Coleoptera: Curculionidae), es una plaga de importancia económica que ataca cultivos de soya (*Glycine max* [L.] Merrill) del norte y sur de México. Conocer aspectos sobre la bioecología y comportamiento de este insecto puede ayudar a desarrollar estrategias de manejo integrado para su control. El objetivo del presente fue registrar el número de parejas de *R. nigerrimus* en cópula en diferentes etapas fenológicas del cultivo de soya y en diferentes horas del día; así como determinar carga de huevos corionados en las hembras de *R. nigerrimus* en diferentes etapas fenológicas del cultivo de soya. Durante las etapas vegetativas del cultivo de soya (VE a V5) y reproductivas (R1 a R3), no se observaron parejas de *R. nigerrimus* copulando. Las primeras parejas en cópula, se encontraron a partir de la etapa fenológica R4 hasta la etapa fenológica R9. Se observaron parejas de *R. nigerrimus* en cópula desde las 7:00 a. m. a 5:30 p. m., con la mayor cantidad de parejas en cópula entre 11:30 a. m. y 2:30 p. m. La cantidad de parejas de *R. nigerrimus* en cópula se correlacionó de manera positiva con la temperatura y de manera negativa con humedad relativa. En los ovarios de las hembras de *R. nigerrimus* recolectadas en campo en diferentes etapas fenológicas, se comenzaron a encontrar huevos corionados a partir de la etapa fenológica R5. La mayor carga de huevos fue encontrada en hembras recolectadas en la etapa fenológica R7.1.

Palabras Clave: *Glycine max*; el picudo de la soya; plagas de la soya; comportamiento de apareamiento; cópulas

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Mating affects a series of physiological processes during insect reproduction. It is considered the main stimulus of oogenesis, vitellogenesis, egg maturation in females, and other processes (Engelmann 1970; Raabe 1986; Wheeler 1996). During the process of copulation, male sperm is transferred to the female. This has implications for egg production and fertilization, oviposition, and adult longevity (Simmons et al. 2000). In some insect species, recognition and attraction of the opposite sex is immediately followed by copulation, while in other species it is preceded by more or less elaborate forms of courting (Manning 1966). In the Curculionidae, mating has stereotyped precopulatory, copulatory, and post-copulatory behavior (Walgenbach & Burkholder 1987; Polak & Brown 1995; Vanderbilt et al. 1998; Sirot & Lapointe 2008; Kamiya et al. 2015), in which copulation can last a few minutes or hours, but characteristics of mating behavior in the field are little known. Understanding the copulatory process and the factors that intervene will permit designing management strategies aimed to interfere with reproduction of the pest insect (Unnithan & Paye 1991).

The soybean weevil *Rhyssomatus nigerrimus* (Fahraeus) (Coleoptera: Curculionidae) is considered an economically important soybean (*Glycine max* [L.] Merrill) pest in Mexico (López-Guillén et al. 2012; Terán-Vargas & López-Guillén 2014). It is distributed in the states of Chiapas, Tamaulipas, San Luis Potosí, and Veracruz (Terán-Vargas & López-Guillén 2014). Adult male and female weevils feed on both vegetative and reproductive parts of the soybean plant. However, it is the larvae developing inside the beans that cause the most damage, diminishing grain yield and seed viability (López-Guillén et al. 2012).

Adult *R. nigerrrimus* emerge when the rainy season begins in soybean-producing regions and infest newly established seedlings (Terán-Vargas & López- Guillén 2014). When the soybean crop reaches the R5 phenological stage, the female weevils begin depositing their eggs on the pods and the larvae that hatch feed on the seeds (Lopez-Guillén et al. 2012); they pass their final life cycle stage in the soil where *R. nigerrimus* pupae and, finally, adults develop to emerge in the following crop cycle (Martínez-Zarate 2015). To date, it is unknown at which phenological stages of the soybean crop *R. nigerrimus* form pairs and copulate, and which phenological stages are associated with females having mature eggs, which could be successfully fertilized at copulation.

Application of selective methods for integrated management of insect pests demands precise knowledge of the insect's biology, including physiological, ecological, and ethological aspects (Unnithan & Paye 1991; Sirot & Lapointe 2008). Therefore, understanding the biology, physiology, and sexual behavior of *R. nigerrimus* in the field can contribute to development of effective sampling and control methods relative to soybean crop phenology. The number of copulating *R. nigerrimus* pairs associated with different phenological stages of the crop is part of the mating behavior of this pest in the field and has thus far not been studied. The objective of this study was to record the number of *R. nigerrimus* pairs copulating during different phenological stages of the soybean crop at different hours of the day. The *R. nigerrimus* female egg load at different phenological stages of the soybean crop also will be determined. The results of this study will contribute to making recommendations on when it is most effective to apply control measures for *R. nigerrimus* adults.

Materials and Methods

STUDY LOCATION

The study was conducted in a soybean field located in the ejido Zapata Las Varillitas (14.8194444 °N; 92.4261111 °W), in the municipality of Tapachula, Chiapas, Mexico, with an area of approximately 20 ha planted with the variety 'Luziania' (SNICS 2022) in the spring-summer crop cycle (rainy season), between Jul and Nov 2020.

NUMBER OF *R. NIGERRIMUS* PAIRS COPULATING AT DIFFERENT PHENOLOGICAL STAGES OF THE SOYBEAN CROP

Samples were taken at each crop phenological stage, described by Fehr et al. (1971), in both the vegetative (VE to V5) and reproductive stages (R1 to R9). The reproductive stage R7 has 2 substages (R7.1 and R7.2), which were sampled. During each sampling, with a different phenological plant stage, 100 randomly selected 1 m transect sections with soybean plants were examined; each 1 m transect had 10 to 12 plants. Sampling each crop phenological stage was conducted between 12 PM and 3 PM. The 1 m transect with soybean plants was carefully observed for at least 1 min, without moving the plants, to look for mating *R. nigerrimus* pairs. Mating occurs when the male mounts the female to introduce its aedeagus into the genital chamber of the female and can last several minutes. The number of mating pairs in a 1 m transect with soybean plants was recorded. In total, 100 samples (each a 1 m transect with soybean plants) were obtained for each phenological stage.

NUMBER OF *R. NIGERRIMUS* PAIRS COPULATING AT DIFFERENT TIMES OF THE DAY ON R7.1 SOYBEANS

The number of *R. nigerrimus* pairs that were mating at different times of the day was determined. Samples were taken between 7:00 AM and 5:30 PM during the R7.1 phenological stage of the soybean crop because in this stage more matings were observed in the previously described experiment. Every 1.5 h the number of *R. nigerrimus* pairs that were mating in 1 m of soybean plants was recorded in the first 10 mins of each sampling. A total of 100 randomly selected linear meters of soybean plants were inspected. The number of *R. nigerrimus* pairs mating in 1 linear meter of soybean plants was recorded. Hourly data on relative humidity and temperature during the experiments were recorded with a USB-502-LCD (Longicbus, Guadalajara, México). The recorded data was used to detect correlations of copulation frequency with temperature and relative humidity.

EGG LOAD OF MATING FEMALE *R. NIGERRIMUS* DURING DIFFERENT PHENOLOGICAL STAGES OF THE SOYBEAN CROP

Adult insects were collected carefully both before they mated and when pairs of weevils were mating in the field during each phenological stage of the soybean crop. The specimens were kept individually in 2.5 mL plastic jars with 70% alcohol until they were processed. The sex of the insects was determined following the description by López-Guillén et al. (2016). The females were dissected in the laboratory under a Nikon stereoscopic microscope (Nikon Instruments Inc., Tokyo, Japan) using entomological forceps and a Petri dish that contained distilled water. Once the *R. nigerrimus* females were dissected, the number of eggs found in each was recorded. At least 20 females, collected during each phenological stage of the crop, were dissected.

STATISTICAL ANALYSIS

The number of mating pairs and the number of eggs present in the females during the different phenological stages, as well as the data on the number of *R. nigerrimus* pairs copulating at different times of the day, were analyzed using generalized linear models (GLM) with Poisson distribution. The females, collected during some phenological stages of the soybean crop, with no egg load were not included in the

GLM (all vegetative stages, and R1, R2, R3 and R4 reproductive stages). The comparison of means of the data on number of pairs mating in different soybean phenological stages and at different times of the day, was performed with intervals at 95% confidence of the value estimated relative to the number of mating pairs per phenological stage and time of the day, respectively. Multiple comparison, post-hoc tests of female egg load were performed using contrasts with $\alpha = 0.05$. The correlation between the frequency of copulations at different times of the day with temperature and relative humidity was carried out using the non-parametric Spearman test because there was no linearity. The GLM and the Spearman correlation were performed with R software (Development Core Team 2022).

Results

NUMBER OF *R. NIGERRIMUS* PAIRS COPULATING AT DIFFERENT PHENOLOGICAL STAGES OF THE SOYBEAN CROP

The phenological stage of the soybean crop significantly affected the number of *R. nigerrimus* copulating pairs ($\chi^2 = 41.75$; df = 5; $P = 0.001$) (Fig. 1). During all the vegetative stages of the soybean crop (VE to V5), no *R. nigerrimus* pairs were observed copulating, nor in the reproductive stages R1 to R3 (complete flowering to the end of flowering). The first copulating pairs were found during the R4 phenological stage (completely developed pods) up to the R9 phenological stage (harvest maturity). The highest number of mating pairs was observed in the R7 phenological stage (beginning of maturation), followed by the phenological stages R8 (initial leaf drop to preharvest) and R6 (seed completely developed). Mating *R. nigerrimus* pairs were found on the upper part of the plants, between the pods and branches.

NUMBER OF *R. NIGERRIMUS* PAIRS COPULATING AT DIFFERENT TIMES OF THE DAY ON R7.1 SOYBEANS

The time of day significantly affected the number of *R. nigerrimus* pairs in copulation ($\chi^2 = 362.24$; df = 7; $P = 0.001$). We observed *R. nigerrimus* pairs copulating from 7:00 AM to 5:30 PM. The largest number of mating pairs was observed between 11:30 AM and 2:30 PM, followed by 10:00 AM, 4:30 PM, and 5:30 PM. We found the lowest number of mating pairs at 7:00 AM and 8:30 AM (Fig. 2).

During the period of observation, the mean temperature was 33.29 °C (range 24–44 °C), and relative humidity was 73.86% (range 51–96%) in the sun. The number of copulating pairs correlated positively with temperature (*Rho* = 0.88; *p* = 0.007) and negatively with relative humidity (*Rho* = −0.86; *p* = 0.01). With increasing temperature and decreasing relative humidity during the day, a larger number of copulating pairs was observed. The highest mean temperature (between 32.41 and 42.68 °C) occurred between 10 AM and 4 PM, while the lowest relative humidity was recorded between 11 AM and 4 PM (53.58 to 61.86%) (Table 1).

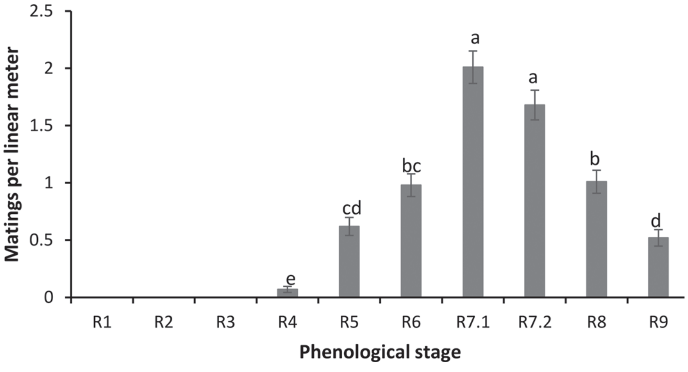

Fig. 1. Effect of soybean crop phenological stages on matings per linear meter of *Rhyssomatus nigerrimus* pairs. Estimated values are 95% confidence intervals ±SE.

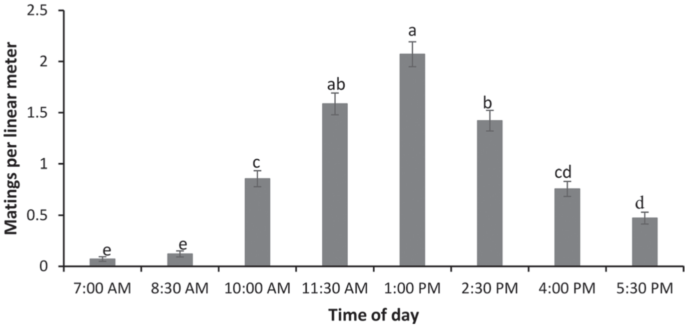

Fig. 2. Effect of time of day on matings per linear meter of *Rhyssomatus nigerrimus* copulating in the R7 phenological stage in a soybean crop. Estimated values are 95% confidence intervals ±SE.

EGG LOAD OF MATING FEMALE *R. NIGERRIMUS* DURING DIFFERENT PHENOLOGICAL STAGES OF THE SOYBEAN CROP

The egg load of *R. nigerrimus* females differed significantly among the different phenological stages of the soybean crop ($\chi^2 = 122.59$; df = 5; $P = 0.001$). Before the R5 phenological stage, no eggs were found in the collected females. However, as of the R5 phenological stage (initial seed formation), eggs were found in the ovaries of dissected adult *R. nigerrimus* females. The largest egg load was found in females collected during the R7.1 phenological stage, followed by those collected in the R6 and R7.2 phenological stages. The lowest egg load was found in females collected in the R5 phenological stage, and intermediate egg loads were found in females collected during the R9 and R8 phenological stages (Table 2).

Discussion

Here, we report for the first time the influence of soybean growth stage on the copulation behavior of *R. nigerrimus* pairs, and egg load of *R. nigerrimus* females in natural conditions. The first copulating *R. nigerrimus* pairs were found as of the R4 phenological stage of the soybean crop (completely developed pods) and the highest number of copulating pairs was observed during the R7 stage (beginning of maturation). Similarly, Polak and Brown (1995) reported that most of the mating pairs of *Cleogonus rubetra* (Fabricius) (Coleoptera: Curculionidae) oc-

Table 1. Mean temperature and relative humidity at different times of day during matings of *Rhyssomatus nigerrimus* in a soybean field.

Time of day	Temperature (°C)	Relative humidity (%)
07:00 AM	23.86	95.50
08:30 AM	24.71	95.13
10:00 AM	32.42	80.16
11:30 AM	42.68	53.58
01:00 PM	37.18	63.77
02:30 PM	36.64	65.07
04:00 PM	37.88	61.86
05:30 PM	30.99	75.79

Table 2. Egg load (mean ± SE) per dissected female *Rhyssomatus nigerrrimus* at different phenological stages of the soybean crop.

Phenological stage	Number of females dissected	Number of females with eggs	Egg load per female[a]
R5	20	14	5.30 ± 0.52a
R6	22	22	12.50 ± 0.75cd
R7.1	22	22	14.55 ± 0.81d
R7.2	22	22	11.05 ± 0.71c
R8	22	21	8.41 ± 0.62b
R9	22	20	8.09 ± 0.61b

[a]Means in a column followed by different lowercase letters were significantly different ($P \leq 0.05$; GLM and orthogonal contrasts test).

curred when the *Andira inermis* (W. Wright) DC (Fabaceae) plants were in the fruiting stage. Moreover, Calyecac-Cortero et al. (2004) observed that *Trichobaris championi* Barber (Coleoptera: Curculionidae) copulated more when the *Physalis ixocarpa* Brot (Solanaceae) crop was in the phenological stage of flower and fruit development. According to Stadler & Buteler (2007), adult boll weevils *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) do not begin to copulate until they have fed on the pollen of cotton flowers, while oviposition does not occur until cotton fruits are present. We also observed that the first *R. nigerrimus* matings occurred after the soybean plants flowered and oviposition occurred when the pods were developing. Likely, *R. nigerrimus* females need to ingest proteins, which they obtain from pollen and pods, to be receptive and develop eggs, such as was suggested in *A. grandis*, *Listronotus bonariensis* (Kuschel), and *Sitona lineatus* L. (Coleoptera: Curculionidae) (Evans & Barratt 1995; Landon et al. 1995; Stadler & Buteler 2007).

In the vegetative period and first reproductive stages of soybean, we did not find eggs in the females; it was not until the R4 reproductive stage that eggs were observed in the female ovaries. Similarly, Greenberg et al. (2007) reported that when *A. grandis* females were collected during the cotton-free period in the Lower Rio Grande Valley and were fed daily cotton fruits cultivated in a greenhouse, oviposition occurred 7 d after feeding. However, when the *A. grandis* females did not have access to cotton fruits, as occurs naturally in the Lower Rio Grande Valley, the weevils begin to reabsorb their egg load and enter diapause (Summy et al. 1993). We hypothesized that during the vegetative stages and the first reproductive stages of the soybean crop, *R. nigerrimus* females do not have an egg load in their ovaries because they have not yet had access to sources of protein, which they cannot obtain until there is pollen in the flowers and soybeans in the pods.

The largest number of copulating *R. nigerrimus* pairs was between 11:30 AM and 4:00 PM. Likewise, Kamiya et al. (2015) reported that *Cyrtomon luridus* (Boheman) (Coleoptera: Curculionidae) mate during the photophase, with a peak between 11:00 AM and 1:00 PM. Sirot & Lapointe (2008) found that *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) had the highest mating activity between 11:00 AM and 2:00 PM under field conditions. We suggest that more pairs of *R. nigerrimus* were observed between 11:30 AM and 4:00 PM because mating behavior is linked to the weevil's circadian rhythm, which helps not only to evade natural enemies, but also regulates intraspecific communication between adults of the weevil through the pheromonal compounds that are released by the insects during this time. Likewise, we suggest that mating behavior is linked to the quantity and quality of volatiles emitted by soybean pods during these hours of the day. However, more studies should be done to clarify why more copulations of *R. nigerrimus* occur at this time of day.

Environmental temperature and relative humidity affect the number of *R. nigerrimus* pairs copulating during the day. We observed that when the temperature increased, there were more copulating pairs. Katsuki & Miyatake (2009) also observed that pairs of *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) copulated more at temperatures between 25 and 33 °C, compared with 17 °C. We found that when relative humidity decreased (between 53.58–61.86%), the number of copulating *R. nigerrimus* pairs increased. Krüger et al. (2021) also reported that the number of copulating pairs of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) was higher with relative humidity above 61%, compared with lower relative humidity (between 0–40%). The weather data collected in our study showed that as temperature increased, relative humidity decreased, and the number of copulating *R. nigerrimus* pairs increased. In this respect, Kanno & Sato (1980) observed that the number of copulations of *Chilo suppressalis* Walker (Lepidoptera: Pyralidae) was lower when relative humidity decreased, and temperature increased. In insects, response to conditions of humidity and atmospheric pressure have been associated with a hygroreceptive sensilla located in the antennae, which detects changes in atmospheric conditions (Tichy & Kallina 2010). The presence of thermoreceptive sensilla in non-articulated walls of the bodies of some insect species also has been reported (Gillott 2005). It is thus likely that adult *R. nigerrimus* also have these receptors that allow them to detect the conditions of temperature and relative humidity favorable for mating.

These results will contribute to decision-making for sampling and control of *R. nigerrimus*. It is known that adult *R. nigerrimus* begin to emerge with the first rains of the season and after the soil has been plowed (Terán-Vargas & López-Guillén 2014). Once the first soybean seedlings have emerged, populations of the weevil increase as additional adults emerge and cause significant damage when the crop reaches the R5 phenological stage (López-Guillén et al. 2012). We can thus suggest that, according to the results of this study, sampling and control measures should intensify in the R3 phenological stage to prevent serious damage to the beans in later phenological stages, and at the same time, to avoid the application of insecticides during the R1 stage so as not to eliminate pollinating insects. Insecticides should be applied in the early morning to prevent *R. nigerrimus* pairs from mating, which is more common in mid-day.

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