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Releasing number of *Telenomus remus* (Nixon) (Hymenoptera: *Platygastridae*) against *Spodoptera frugiperda* Smith (Lepidoptera: *Noctuidae*) in corn, cotton and soybean

Número de *Telenomus remus* Nixon (Hymenoptera: *Platygastridae*) a ser liberado para controle de *Spodoptera frugiperda* Smith (Lepidoptera: *Noctuidae*) em milho, algodão e soja

Aline Farhat Pomari[I] Adeney de Freitas Bueno[II] Regiane Cristina Oliveira de Freitas Bueno[III] Ayres de Oliveiras Menezes Junior[IV] Augusto Cesar Prado Fernandes Fonseca[V]

ABSTRACT

Telenomus remus *releasing numbers may vary depending on the crop, plant architecture and/or the plant phenological stage. Thus, we examined the number of parasitoids needed for effective pest control of* ***Spodoptera frugiperda*** *on corn, cotton and soybean. In all crops, the parasitism response in relation to increasing numbers of the parasitoids had a quadratic effect. In corn, the maximum parasitism observed was 99.8% and 96.8% at a parasitoid releasing number of 0.231 and 0.264* ***T. remus*** *females per* ***S. frugiperda*** *egg at phenological stages* V_4 *and* V_{10}*, respectively. Differently, in cotton and soybean, the highest parasitim were recorded using the highest tested* ***T. remus*** *releasing numbers (0.297 parasitoid per* ***S. frugiperda*** *egg). In cotton, it was 77.8% and 73.1% at the vegetative and reproductive stages, respectively and in soybean, it was 77.3% and 54.4% also at the vegetative and reproductive stages. Thus, the appropriated* ***T. remus*** *releasing number might vary accordingly to the crop and plant phenological stage, being higher for soybean and cotton and lower for corn.*

Key words: *biological control, parasitoid density, parasitism.*

RESUMO

O número de ***Telenomus remus*** *a ser liberado pode ser variável, dependendo de cada cultura, da arquitetura da planta e/ou do seu estágio fenológico. Assim, foi examinado o número de parasitoides necessários para obter o controle efetivo de* ***Spodoptera frugiperda*** *em milho, algodão e soja. Em todas as culturas, a resposta do parasitismo em relação ao número crescente de parasitoides teve um efeito quadrático. Em milho, o parasitismo máximo observado foi de 99,8% e 96,8% em um número de parasitoides liberados de 0,231 e 0,264 fêmeas de* ***T. remus*** *por ovo de* ***S. frugiperda*** *nos estádios fenológicos V4 e V10, respectivamente. Diferentemente, em algodão e soja, os maiores parasitismos foram verificados liberando o maior número de fêmeas de* ***T. remus*** *testados (0,297 fêmeas por ovos de* ***S. frugiperda****). Em algodão, foi 77,8% e 73,1% nos estágios vegetativo e reprodutivo, respectivamente e, em soja, foi 77,3% e 54,4% também nos estágios vegetativo e reprodutivo. Assim, o número apropriado de* ***T. remus*** *a ser liberado pode variar de acordo com a cultura e com o estágio fenológico da planta, sendo mais elevado para soja e algodão e mais baixo para milho.*

Palavras-chave: *controle biológico, densidade de parasitoides, parasitismo.*

INTRODUCTION

Spodoptera frugiperda Smith (Lepidoptera: ***Noctuidae***) is a polyphagous and voracious pest that cause damage to several crops such as corn, cotton and soybean (NAGOSHI, 2009; BUENO et al., 2011). Currently, the most common control measure to manage ***S. frugiperda*** is insecticides (van LENTEREN & BUENO, 2003). Although chemical control is an important tool against ***S. frugiperda***, its overuse may cause undesirable consequences such as the rapid selection of pest strains resistant to pesticides (DIEZ-

[I]Faculdade de Filosofia Ciências e Letras de Ribeirão Preto (FFCLRP), Universidade de São Paulo (USP), Ribeirão Preto, SP, Brasil.
[II]Embrapa Soja, CP 231, 86001-970, Londrina, PR, Brasil. E-mail: adeney@cnpso.embrapa.br. Autor para correspondência.
[III]Faculdade de Ciências Agronômicas (FCA), Universidade Paulista "Julio de Mesquita Filho" (Unesp), Botucatu, SP, Brasil.
[IV]Universidade Estadual de Londrina (UEL), Londrina, PR, Brasil.
[V]Centro de Ciências Agrárias (CCA), Universidade Federal de São Carlos (UFSCar), Araras, SP, Brasil.

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RODRIGUEZ & OMOTO, 2001), the elimination of beneficial arthropods, that can trigger secondary pest outbreaks or fast pest resurgence, for example. In addition, the use of insecticides is only a short-term solution to the pest problem (DESNEUX et al., 2007). Therefore, for the best management of those crops, the adoption of other strategy besides insecticides is crucial to the integrated pest management (IPM) success (PEDIGO et al., 1986). Even though of higher occurrence on corn, its importance on soybean and cotton has also increased, mainly in Brazil.

One of the tactics that have shown promissing results within the IPM strategy is biological control, especially for pests of the order Lepidoptera, through field releases of egg parasitoids (PARRA & ZUCCHI, 2004). Among the egg parasitoids, ***Telenomus remus*** Nixon (Hymenoptera: ***Platygastridae***) is especially noteworthy for its effective action on eggs of ***S. frugiperda*** which are oviposited in superposed layers (POMARI et al., 2012). ***T. remus*** is able to parasitize the entire egg mass, even the eggs located in the inner layers (FIGUEIREDO et al., 1999), a feature that is not observed in other egg parasitoids (BUENO et al., 2008). The success of ***T. remus*** field releases, however, depends upon different variables, such as the host, climatic conditions, number of insects to be released, pest density, parasitoid species or strain to be utilized, time and number of releases, parasitoid distribution method, and plant phenology influence on ***T. remus*** adult (HASSAN, 1994). Among these, ***T. remus*** searching capacity, in order to efficiently determine the amount of parasitoids to be released in a given field area is one of the most important issues. Therefore, the parasitism of ***T. remus*** after the release of different numbers of parasitoid mated females agaist fixed numbers of ***S. frugiperda*** was calculated in an attempt to better understand the releasing numbers of ***T. remus*** in different crops and crop stages. Even though, carried out in greenhouse conditions, where variables (climatic conditions, etc) are simplified, these preliminary trials will present parasitoid releasing numbers that should be further studied in the field.

MATERIALS AND METHODS

The experiments were performed under greenhouse conditions (25±5°C; 70±20% RU; 14/10 hours photoperiod L/D) during 2009 and 2010. The parasitism of different releasing numbers of ***T. remus*** on fixed number of ***S. frugiperda*** eggs was evaluated on cotton (cultivar IPR 114 - 3 plants per pot), corn (cultivar IPR 140 - 2 plants per pot), and soybean (cultivar BRS 260 - 5 plants per pot) plants. Each pot (40cm of diameter) was randomly distributed in the greenhouse, distanced 0,5 meter from each other. The tested number of plants per pot tried to represent the field adopted spacing between plants as close as possible. For each of these plants two trials were carried out independently in different dates. For corn the studied stages were V_4 and V_{10} (HANWAY, 1963) while for cotton they were V_5-V_6 (five or six with true leaves at its main ribs with a length of 2.5cm) and B_1 (first flower buds visible); and for soybean, V_4-V_6 and R_1-R_2 (FEHR et al., 1971). These stages tried to represent when usually ***Spodoptera*** spp attack those plants in the field.

Host and parasitoid colonies. The ***S. frugiperda*** eggs as well as the ***T. remus*** females used in the experiments were from insect colonies kept at controlled environmental conditions. ***S. frugiperda*** was originally collected on corn plants (***Zea mays*** L.) in RioVerde, State of Goiás, and was in around its 36th generation. Since its field collection, ***S. frugiperda*** was reared under laboratory controlled environmental conditions [25±2°C temperature, 70±10% RH, and a photoperiod of 12:12 (L: D) h] and fed on the artificial diet proposed by GREENE et al. (1976) and PARRA (2001). ***T. remus*** was originally collected in Ecuador and was multiplied at the parasitoid rearing facilities of ESALQ/USP (Luiz de Queiroz College of Agriculture/University of São Paulo), from where some specimens were transferred to Embrapa Soybean five years ago. Since then, ***T. remus*** has been reared in the laboratory using ***S. frugiperda*** egg masses (±150 eggs each) which were glued onto a cardboard sheet (2cmx8cm). Three of these sheets with the eggs were placed in a glass tube (8cm long and 2cm Ø) with eggs previously parasitized by ***T. remus***. Small drops of honey were placed in these tubes to feed the adults when they emerged. The tubes were then closed, and ***T. remus*** parasitism was allowed for 24h. Adults emerged from these eggs were used for trials or for colony maintenance.

Appropriate number of ***T. remus*** to release per host egg. The required density of ***T. remus*** per ***S. frugiperda*** egg was determined through the release of variable numbers of mated adult parasitoid females in relation to a given number of host eggs (BUENO et al., 2012). Pots from parasitoid colony were fed with honey for 24 hours after the first adults emerged. After that, wasps were individually placed in small vials for sex recognition under stereomicroscope and then the females later grouped in larger vials (8cm long and 2cm Ø) with other females in the appropriated number of parasitoids accordingly to each treatment. Therefore, parasitoids from 24 to 36 hours were used in the trials. An independent bioassay was carried out (in different dates) for each crop in each phenological stage of development under greenhouse conditions, using a fully randomized experimental design with ten treatments (0, 0.033, 0.066, 0.099, 0.132, 0.165, 0.198, 0.231, 0.264 and 0.297 ***T. remus*** females per ***S. frugiperda*** egg) and ten replications (a vase per replication). Those releasing numbers were obtained through different number of females (0, 5, 10, 15, 20, 25, 30, 35, 40 and 45) that were introduced per replication putting the vial containing the insects on the basis of each vase with the plants that had 3 eggs masses (up to 24-hour old) of 100 ***S. frugiperda*** eggs each.

Spodoptera frugiperda eggs of the pests were obtained from laboratory rearing and were exposed to the treatments inside iron-framed cages (50cmx50cmx120cm) covered with voile fabric. The ***S. frugiperda*** egg masses were fixed to the undersurface of the plant leaves by using a stapler, attaching 1 egg mass in each of the 3 thirds of the plants (lower, median, and upper) for cotton and soybean, and the 3 egg masses near the whorl for the corn plants to mimic the common egg distribution in those crops in field conditions. Furthermore, the research proceeded an attempt to understand the parasitoid capacity of locating the host eggs in different parts of the plant. Then, a variable number of 0, 5, 10, 15, 20, 25, 30, 35, 40 or 45 ***T. remus*** female parasitoids were released, representing exactly the proportion of 0, 0.033, 0.066, 0.099, 0.132, 0.165, 0.198, 0.231, 0.264 and 0.297 parasitoids per host egg. No parasitoid was released in the control treatment.

The parasitism was allowed to proceed for 24h, and the eggs were then collected and maintained in Petri dishes at 25ºC, until the eggs darkened and the parasitoids emerged, for subsequent evaluation. The parameters evaluated were: parasitism (%) per plant in each of the three studied crops. The data were submitted to regression analysis, relating the number of ***T. remus*** females per egg of each pest species and percentage of parasitism (SAS INSTITUTE, 2001).

RESULTS

Corn. The regression analysis between the percentage of parasitism and number of ***T. remus*** females released per host egg showed a quadratic effect for both studied phenological stages [stage V_4 ($y=-18.26+650.87x-789.82x^2$), and V_{10} ($y=-7.33+552.38x-689.43x^2$)], where "y" is the parasitism (%) and "x" is the parasitoid releasing ratio (Figures 1A and 1B). The parasitism (%) at the V_4 stage ranged from 18.6% to 99.8% for parasitoid females per pest egg ratios of 0.099 to 0.231 (Figure 1A). At the V_{10} stage, the corresponding values were 10.26% to 96.87% for females per pest egg ratios of 0.033 to 0.264 (Figure 1B). Parasitism levels above 70% were obtained at parasitoid ratios of 0.165 and 0.132 for stages V_4 and V_{10}, respectively (Figures 1A and 1B).

Cotton. Similar to corn, the regression analysis between the percentage of parasitism and number of ***T. remus*** females released per host egg showed a quadratic effect for both studied phenological stages [vegetative ($y=0.12+8.96x+6.10\,x^2$) and reproductive ($y=2.38-521.93x+12542x^2$)], where "y" is the parasitism (%) and "x" is the parasitoid releasing ratio (Figures 1C and 1D). The parasitism (%) at the vegetative stage ranged from 7.7% to 77.8% for the parasitoid females per pest egg ratios of 0.033 to 0.297 (Figure 1C). At the reproductive stage, that range was from 9.1% to 73.1% for the parasitoid female per pest egg ratios of 0.165 to 0.297 (Figure 1D). Parasitism above 70% was obtained at densities of 0.264 and 0.297 for the vegetative and reproductive stages, respectively (Figures 1C and 1D). It is importante to point out that there were no differences in the results comparing the parasitism in the whole plant with the parasitism of eggs placed on the bottom, median, or top of plant cannopy. Therefore, the results of parasitism from the whole plant was used to better represent the parasitoid efficacy and also due to the higher R^2 coeficient obtained from regression curves.

Soybean. As well as corn and cotton the regression analysis between the percentage of parasitism and number of ***T. remus*** females released per host egg also showed a quadratic effect for both studied phenological stages [vegetative ($y=-5.03-354.43x+5657.55x^2$), and reproductive ($y=0.41-213.12x+2936.45x^2$)], where "y" is the parasitism (%) and "x" is the parasitoid releasing ratio. ***T. remus*** parasitism (%) at the vegetative stage ranged from 0.9% to 77.3% for parasitoid females per pest egg ratios of 0.066 to 0.264 (Figure 1E). At the reproductive stage ***T. remus*** parasitism ranged was from 9.1% to 54.4% for the females per pest egg ratios of 0.066 to 0.297 (Figure 1F). Parasitism above 70% was obtained only at the vegetative stage, at minimum density of 0.264 females per pest egg (Figure 1E), indicating the need for further studies using higher parasitoid densities at the reproductive stage of the soybean. No differences in parasitism from whole plant compared to plant extracts (bottom, median or top of plant canopy) were also observed.

DISCUSSION

Among the three studied crops, ***T. remus*** had its best performance parasitizing ***S. frugiperda*** eggs located on corn, where parasitism reached more than

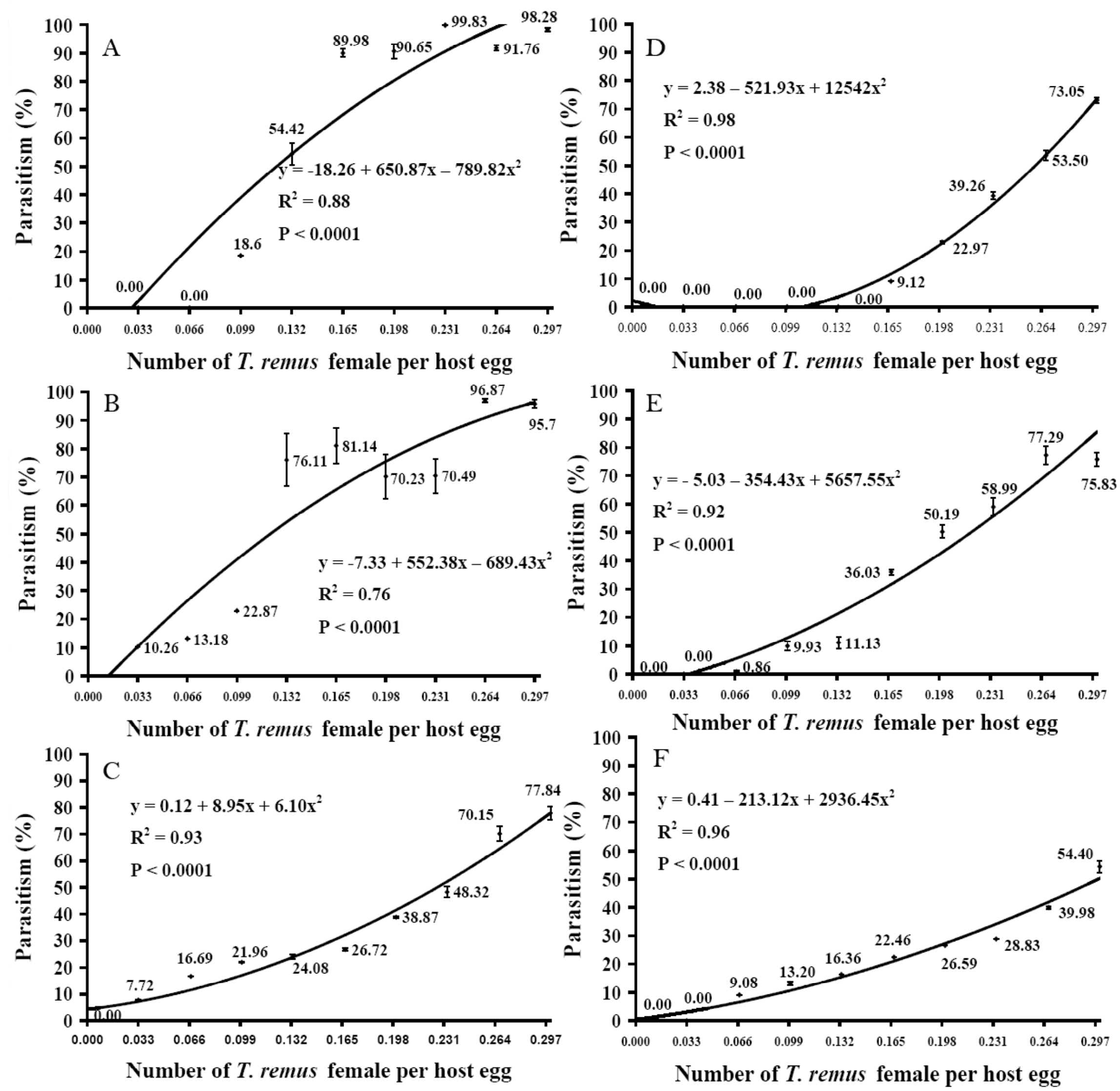

Figure 1 - Parasitism (%) (Mean ± SE) on eggs of ***Spodoptera frugiperda*** on corn plants at the V_4 (A) and V_{10} (B) stage; on cotton plants at the vegetative (C) and reprodutive (D) stage; and on soybean plants at the vegetative (E) and reprodutive (F) stage after the release of increasing numbers of ***Telenomus remus*** females.

90%, compared to the cotton and soybean, where the higher parasitism was below 80%. These differences noted among different crops and at their different developmental stages have been previously reported in the literature for other egg parasitoids (***Trichogramma*** spp.) (SÁ & PARRA, 1993). It had been demonstrated that the number of parasitoids to be released is variable as a function of different factors such as plant phenology, parasitoid species or strain, as well as of the oviposition dynamics of the host (SÁ & PARRA, 1993). Among such factors, ***S. frugiperda*** dynamic usually lay eggs concentrated close to the whorl on corn plants while it lays eggs evenly distributed on soybean and cotton plants. It might be important to explain the need to release a lower density of parasitoids on corn. It can happen because the parasitoid will have less work searching for their host eggs. On corn, once finding the eggs, it will be easier to have a higher efficacy on parasitizing all masses that are close to each other. Similarly, the more intensive parasitoid foraging required in order to find their host when plants are more developed, in older phenological stages, when compared to young plants with lesser foliar area, might help to explain the lower parasitism (%) obtained in the older plants for all three studied crops. The foliar area changes over the crop cycle reaching the highest values in the reproductive stage (BOARD, 2004). Consequently, the higher the foliar area is the higher is the work for the parasitoid to reach its host.

Telenomus remus ratio of 0.165 parasitoids per ***S. frugiperda*** egg is the appropriated number of parasitoid to be released in corn since it reached 80% parasitism or more at both the V_4 and V_{10} plant stages. On cotton, higher releasing numbers of parasitoid are needed and appropriated ***T. remus*** ratio is of 0.297 parasitoid females per pest egg that reached the highest recorded parasitism of 77.9% and 73.1% at vegetative and reproductive stage, respectively. Similarly, on soybean, 0.297 female parasitoids per pest egg also was the best studied ***T. remus*** releasing ratio which reached 75.8% of parasitism on the vegetative stage and only 54.4% of parasitism when soybean was at the reproductive stage. It is important to consider it is still necessary further study to evaluate the effect of higher ratios of ***T. remus*** females on the soybean crop in order to obtain a higher level of parasitism of ***S. frugiperda*** eggs. Moreover, not only do scout egg masses in the field is difficult but also inaccurate, therefore, further studies to adjust a sampling procedure (of moths and even of caterpillars) that present an acceptable correlation with the number of eggs is necessary to better determine the appropriated timing for parasitoid releasing to be performed.

Studies on the optimum density of parasitoid females to be used for effective ***T. remus*** parasitism on the eggs of ***S. frugiperda*** are scarce. In Venezuela, the release of 6 to 8,000 parasitoids ha^{-1} for the corn crop has been proposed (FERRER, 2001), starting from the time of crop germination, but the researchers also emphasized that the release frequency may be altered according to the levels of infestation of the pest in the field. In Brazil, only one field trial has reported the parasitoid density to be released on corn. The density of ***T. remus*** to be released for the control of ***S. frugiperda*** on corn at the V_6-V_8 growth stages in an experiment performed in a $16m^2$ area infested with a single egg mass per square meter was found to be 8 parasitoid females m^{-2}, to achieve a mean parasitism of 76.7%. This study proposed the release of 25 parasitoid females m^{-2} by using two ***S. frugiperda*** egg masses (FIGUEIREDO et al., 2002). The differences in the number of parasitoid females among the trials can be explained as a function of the density of the plants. In our study, the plant spacing used was the same as that used in the field for that crop. The appropriated number of parasitoids to be released may vary according to the density of the plants and the intensity of the pest infestation in the field, in addition to other intrinsic characteristics of each field crop (SÁ & PARRA, 1993).

It is important to consider that plant volatile might influence parasitoid capability of reaching the host egg (PEÑAFLOR et al., 2011). However, this is more evident when plant is under the stress of the pest attack and might respond differently accordingly to the plant species and pest that is occurring. However, the researchers believe this variable had little impact in the results since just the eggs were artificially infested on the plants that had zero defoliation or attack due to ***S. frugiperda***. Also, since the eggs used were originated from laboratory colony, no impact of parasitoid biology might be expected due to plant species differences, since the pest did not feed on those crops. In this context these work tried to focus on the impact of plant architecture on parasitoid ability to find its host. Therefore, in conclusion, it was estimate the appropriated number of ***T. remus*** to be released in corn, cotton and soybean, and noted that this releasing number may vary accordingly to the crop and plant phenological stage, being higher for soybean and cotton and lower for corn. Regarding to the phenological stage, it is necessary higher number of parasitoids in more developed plants as plants get bigger, with higher foliage area for ***T. remus*** searching. However, further studies are still needed to determine the methodology for the evaluation egg number in the field as well as studies to relate egg number and pest economic thresholds, in order to determine not only the optimal amount of parasitoids to be released but also the right timing for ***T. remus*** release.

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