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Biological Control of *Spodoptera frugiperda* Eggs Using *Telenomus remus* Nixon in Maize-Bean-Squash Polyculture

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ABSTRACT

The maize earworm, *Spodoptera Frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is an important pest in maize. *Telenomus remus* Nixon (Hymenoptera: Scelionidae) is an important control agent of this pest due to its capacity to invade the whole egg mass. The percentage of parasitism by *Telenomus remus* Nixon (Hymenoptera: Scelionidae) on *Spodoptera Frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) eggs was evaluated in maize-bean, maize-squash and maize-bean-squash polyculture and maize monoculture systems. Data were analyzed statistically by using a Poisson regression (log-linear model). The analysis showed highly significant differences in the percentage of parasitism of *S. frugiperda* eggs by *T. remus* in plots with *jarocho crema* maize in polyculture systems (91.00±1.42%) compared to the yellow maize genotype (68.90±3.10%). Parasitism percentages increased in the *jarocho crema* maize genotype in maize-bean, maize-squash, maize-bean-squash polycultures and maize monoculture by 87.88±3.27%, 89.75±1.99, 99.50±0.19 and 86.88±2.66%, respectively and in the yellow maize genotype they dropped by 70.00±7.05, 64.50±5.63, 77.88±6.51 and 63.25±5.20%, respectively. The percentage of *T. remus* parasitism on *S. frugiperda* eggs was found to be affected by the genotype of maize, bean and squash, polyculture system, weeds, densities of the host eggs and numbers and quality of egg masses.

Keywords: International Institute of Biological Control (IBCI), Polyculture System, Analyzed Statistically, Causing Severe Damage, Important Lepidoptera Pests, Temperature Variation

1. INTRODUCTION

The maize earworm, *Spodoptera Frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is an important pest in maize and other crops in neotropical and subtropical regions of America (Bennett, 1994). When there are heavy outbreaks of this blight, the losses and cost of control on the American Continent may surpass $300 million (Gross and Pair, 1986). It is the most important maize pest in Mexico, Central America, South America and the Pacific Basin (Ashley *et al*., 1989; Mitchell *et al*., 1984).

In Mexico, *S. frugiperda* is present in all of the regions where maize is grown, causing severe damage in the tropical and subtropical climate regions (Sifuentes, 1967). In Chiapas it causes losses between 53 and 60% (Silva, 1977). The maize earworm mainly attacks the leaves and tender shoots of maize, one of the basic grains in the local diet (Marenco *et al*., 1992; Kumar and Mihn, 2002). This pest has been the subject of many studies, but most of them focus on chemical control. To date, no plant protection program has included biological control as its cornerstone.

On the other hand, Ashley (1979) reported 53 species of parasitoids of the maize earworm belonging to 43 genera and 10 families, of which the family Tachinidae comprises 53% of the species. Molina-Ochoa *et al*. (2003) reported 150 species of parasitoids and parasites of *S. frugiperda* in the Americas and the Caribbean basin belonging to 14 families, nine in Hymenoptera, four in Diptera and one in Nematoda. The family Tachinidae is not only the most diverse of the Diptera, but overall as well (55 species). To date none of these species of parasitoids has had a controlling effect on the maize earworm, because their reproduction in the laboratory is not easy. However, *Telenomus remus* Nixon (Hymenoptera: Scelionidae) can be grown relatively easily under laboratory conditions. It is an important control agent due to its capacity to invade the whole egg mass (Figueiredo *et al*., 2002). This primary parasitoid of insect eggs has been used in biological control programs of important Lepidoptera pests with excellent results (Alam, 1974; Dass and Parshad, 1984). It has been estimated that the release of 5000-8000 parasitoids per hectare can cause up to 90% parasitism of *S. frugiperda* eggs (Hernández *et al*., 1989; González and Zocco, 1996; Cave and Acosta, 1999; Cave, 2000). It has been reproduced in the laboratory on the eggs of *S. littoralis* Boisduval (Lepidoptera: Noctuidae) (Gerling, 1972), *S. litura* F. (Gautam, 1986), *S. frugiperda* and other Lepidoptera (Wojcik *et al*., 1976).

Telenomus remus was first introduced in India in 1963 from New Guinea to control *Achaea janata* (Linneus) (Lepidoptera: Noctuidae) and *S. litura* (Sankaran, 1974). It was later introduced in Israel from India in 1969, in an attempt to control *S. littoralis* (Schwartz and Gerling, 1974). *T. remus* comes from Sarawak and New Guinea, where it is found in nature (Rothschild, 1970) and has become established in some regions where it has been released (Gross and Pair, 1986).

The International Institute of Biological Control (IBCI) in England has provided *T. remus* for control of *S. frugiperda* in the United States, the Caribbean and South and Central America (Yassen *et al*., 1981; Bennett, 1994). It was first introduced in Mexico in 1979 by the *Secretaría de Agricultura y Recursos Hidráulicos* (Secretariat for Agriculture and Hydraulic Resources) (Bennett, 1979; Cock, 1985). *T. remus* has been successfully established on eggs of *S. frugiperda* in (Alam, 1974; 1979; Wojcik *et al*., 1976) with parasitism levels varying from 47-90% (CIBC, 1980; Bennett 1994), in Venezuela (Hernández *et al*., 1989; Hernández and Díaz, 1995; 1996) with parasitism levels of 60-100% (Lacayo, 1978; González and Zocco, 1996) and Ferrer, 2001) in Honduras (Cortés and Andrews, 1979; Cave, 1995). But it failed to become established in Trinidad (Bennett, 1981) or in Florida (Waddill and Whitcomb, 1982). In Mexico, Montoya-Burgos (1979), Morales-Pérez (1982), Canseco-Román (1988), García-Lagunas (1988) and Barilla-Vera (1989) have reported the presence of *T. remus* in maize crops as a parasitoid of *S. frugiperda* eggs.

The purpose of this study was to evaluate *T. remus* parasitism of *S. frugiperda* eggs in maize-bean, maize-squash, maize-bean-squash polyculture and monoculture maize systems to determine what variables favor or affect its establishment.

2. MATERIALS AND METHODS

2.1. Study Area

This study was done under seasonal conditions of the spring-summer cycle, in Predio Santa Elena, located 7 kilometers from the seat of municipal government, Villa Flores, Chiapas, Mexico. It is located in the Central Depression at 16ºC14′ latitude north and 93ºC 16′ longitude west, at an altitude of 610 m a.s.l., with a total area of 1 232 km^2 (Gomez, 1987). The study area has the driest of the warm sub-humid climates Aw_1 (w)(i)g (García, 1973), with rain in summer, a dry winter and a temperature variation of less than 5-7°C. Annual mean rainfall is 1198.2 mm, with mid-summer drought in August. The mean annual temperature is 24.3°C and the minimum is 21.6°C, with a well-defined dry season from November to May.

2.2. Treatments and Experimental Design

A complete random block design was used with two repetitions and 64 experimental units in an 8 * 2^3 factor array. The experimental plots were 11 * 11 m = 121 m^2. *Jarocho crema* (J) and yellow (A) Creole genotypes of maize (*Zea mays* L.) were used in this study as well as *jamapa* beans (*Phaseolus vulgaris* L.) and seasonal squash (*Cucúrbita maxima* Duch. *C. moschata* L.).

The culture systems were maize Monoculture (M), Maize-Bean polyculture (MB), Maize-Squash polyculture (MS) and Maize-Bean-Squash polyculture (MBS). The genotype and culture system combinations resulted in eight different treatments, *jarocho crema* maize monoculture (M-J), *jarocho crema* Maize-Bean polyculture (MB-J), *jarocho crema* Maize-Squash polyculture (MS-J), *jarocho crema* Maize-Bean-Squash polyculture (MBS-J), yellow maize monoculture (M-A), yellow Maize-Bean polyculture (MB-A), yellow Maize-Squash polyculture (MS-A) and yellow Maize-Bean-Squash polyculture (MBS-A).

Soil preparation was begun by collecting the residue from the previous harvest to allow free movement of the tiller and uniform tillage. Then the soil was plowed up so any weeds that had sprouted would dry out and die, for which the land was left in that condition for six days. After that the ground was harrowed to loosen and level the soil completely. The ground was left that way for three days so the loose soil could take on good consistency with the rain and achieve more uniform germination.

Maize, beans and squash were sown in alternating rows. This was done manually at the same time for all four crops. For the two types of maize, two seeds (20 000 plants h^{-1}) and four seeds (40 000 plants h^{-1}) were deposited per point, at a distance of 1 m between furrows and plants. For beans, from four to five seeds were deposited per point at a separation of 1 m between furrows and 25 cm between plants, for a density of 200 000 plants h^{-1}. For squash, three to four seeds per point were deposited at a distance of 3.67 m between furrows and plants for a density of 2 352 plants h^{-1}.

2.3. Release of Parasitoids

The parasitoids were donated by Dr. Ronald D. Cave of the Pan-American School of Agriculture Center for Biological Control in Central America, El Zamorano, Honduras. They were taken to the field for release in the plots when the maize, bean and squash plants were 12 days old. Before that, *S. frugiperda* egg masses were evaluated to be sure there were no natural biological controls present and parasitoids were found to be absent.

For the first inoculation, about 15 000 adult *T. remus* were released. Five hundred parasitoids were released around the edges and in the center of each plot assigned parasitoids. The sex ratio was 2:1. The first release took place when the maize, bean and squash plants were 12 days old (following emergence in the first growth stage). The procedure for release consisted of uncovering containers with the parasitoids, which left slowly and dispersed onto the maize leaves. Release was done in this way to facilitate dispersion in the crop. Later at 20 days in the active growth stage of maize, 12 000 *T. remus* were released in four consecutive releases (every eight days). About 100 parasitoids were released in each plot, by the same method described above.

One hundred plants/plot of maize sampled at random were inspected every eight days. The *S. frugiperda* egg masses collected were taken to the entomology laboratory at the Chiapas Autonomous University School of Agricultural Science, Campus V in Villa Flores. The egg masses were separated in plastic jars by treatment and repetition to determine the percentage of egg masses parasitized. The control plots were also sampled to quantify the number of egg masses parasitized by *T. remus*.

2.4. Statistical Analysis

A Poisson regression-Log-linear model ($p<0.05$), taking the percentage of parasitism as the dependent variable and the maize genotype, the culture system and the treatment as fixed factors, was applied to determine whether the polyculture system and variety of maize influenced the amount of *T. remus* parasitism on *S. frugiperda* eggs. The data were analyzed using the general linear model procedure available in PASW Statistics 2009.

3. RESULTS

The percentages of *S. frugiperda* eggs parasitized by *T. remus* varied from 36 to 100% (**Table 1**). Highly significant statistical differences in parasitism were observed among maize genotype (R^2 = 0.11%, $F_{1,126}$ = 17.42, P = 0.00005), culture system (R^2 = 0.11%, $F_{3,124}$ = 6.21, P = 0. 001) and treatments (R^2 = 0.10%, $F_{7,120}$ = 3.04, *P* = 0. 006). More parasitism was observed in the *jarocho crema* maize genotype and in both MBS treatments (**Fig. 1**).

Significant differences were observed among treatments with the *jarocho crema* maize genotype (R^2 = 0.33%, $F_{3,28}$ = 6.16, P = 0.02), which had 91.0±1.4% (mean±SE) parasitism. In the yellow maize genotype, the percentage of parasitism was 68.9±3.1% and no significant differences were observed among treatments (R^2 = 0.17%, $F_{3,28}$ = 1.18, P = 0.34). The percentages of parasitism in the *jarocho crema* maize genotype varied from 87.88±3.27-99.50±0.19% compared to the yellow maize genotype where they were 63.25±5.20-77.88±6.51%.

Higher percentages of parasitism were observed in the polyculture systems, but no statistically significant differences were found among them (R^2 = 0.05%, $F_{3, 63}$ = 1.99, P = 0.13). Parasitism was 75.06±4.15% in M, 78.94±4.41% in MB, 77.13±4.35% in MS and 88.69±4.21% in MBS.

Significant differences (R^2 = 0.44%, $F_{3,63}$ = 8.01, P = 0.000001) were also observed between treatments. The widest differences observed were between treatments M-J and MS-J with MBS-A (**Table 2**). Only MS-A was not statistically different from any of the other study treatments. The parasitism percentages were higher in the polyculture system with the *jarocho crema* maize genotype 87.88±3.27% was recorded in MB-J, 89.75±1.99% in MS-J and 99.50±0.19% in MBS-J compared to 86.88±2.66% in M-J. Furthermore, average percentages of parasitism were lower in the yellow maize genotype. In the MB-A it was 70.00±7.05%, MS-A 64.50±5.63%, MBS-A 77.88±6.51% and in M-A 63.25±5.20%.

Table 1. Percentage parasitism of *Spodoptera Frugiperda* eggs by *Telenomus remus*

Treatment	%	Treatment	%	Treatment	%	Treatment	%
M-J	87	MS-J	91	M-A	57	MS-A	74
M-J	82	MS-J	97	M-A	42	MS-A	68
M-J	94	MS-J	87	M-A	84	MS-A	36
M-J	100	MS-J	94	M-A	57	MS-A	66
M-J	77	MS-J	83	M-A	70	MS-A	47
M-J	80	MS-J	94	M-A	60	MS-A	86
M-J	86	MS-J	81	M-A	83	MS-A	74
M-J	89	MS-J	91	M-A	53	MS-A	65
MB-J	98	MBS-J	99	MB-A	57	MBS-A	87
MB-J	90	MBS-J	99	MB-A	59	MBS-A	66
MB-J	83	MBS-J	99	MB-A	50	MBS-A	52
MB-J	72	MBS-J	100	MB-A	53	MBS-A	58
MB-J	82	MBS-J	100	MB-A	100	MBS-A	100
MB-J	100	MBS-J	100	MB-A	69	MBS-A	100
MB-J	93	MBS-J	100	MB-A	100	MBS-A	73
MB-J	85	MBS-J	99	MB-A	72	MBS-A	87

Table 2. P values of statistical differences according to Tukey's test

	M-J	MB-J	MS-J	MBS-J	M-A	MB-A	MS-A	MBS-A
M-J	-	0.015	1.000	0.004	0.968	0.009	0.352	0.00002
MB-J		-	0.025	1.000	0.189	1.000	0.867	0.54200
MS-J			-	0.007	0.990	0.016	0.468	0.00005
MBS-J				-	0.071	1.000	0.618	0.81200
M-A					-	0.138	0.929	0.00100
MB-A						-	0.792	0.64200
MS-A							-	0.03400
MBS-A								-

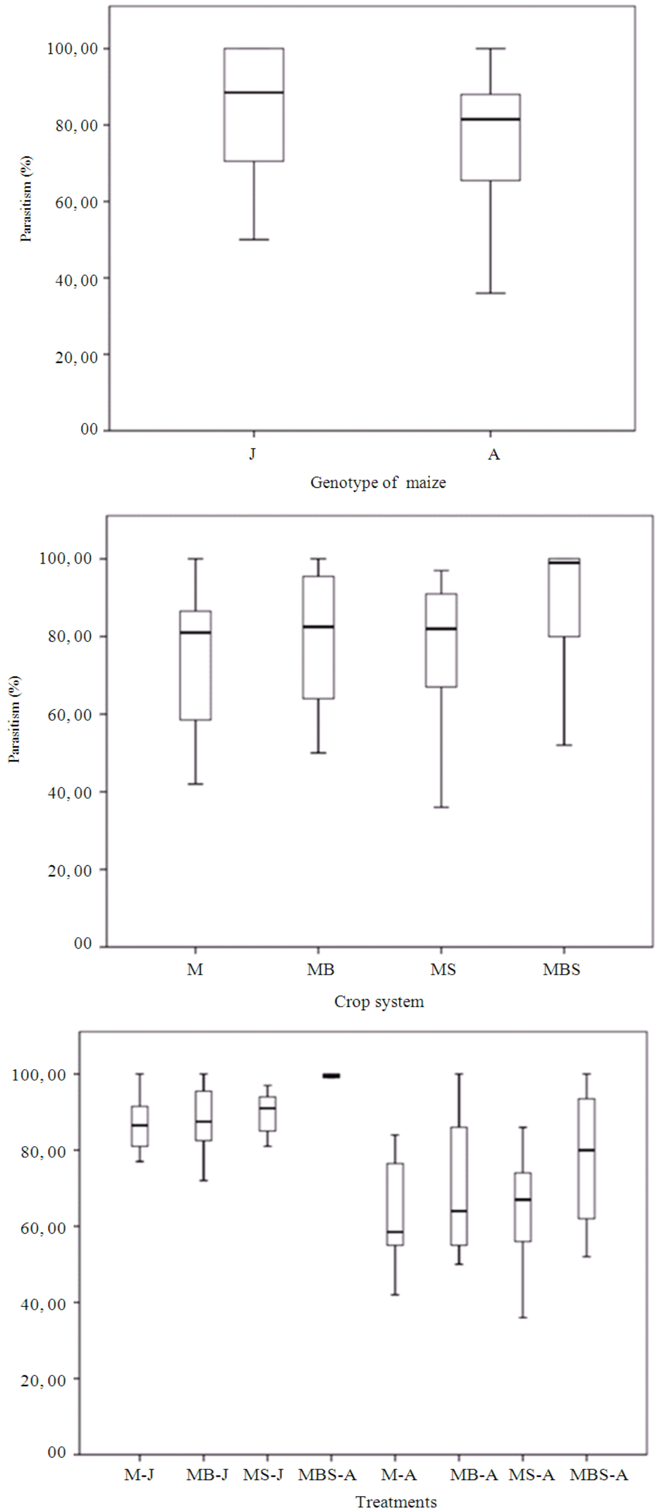

Fig. 1. Box diagrams showing levels of parasitism in maize genotypes, culture systems and treatments

4. DISCUSSION

High percentages of *T. remus* parasitism on *S. frugiperda* eggs in the *jarocho crema* maize genotype have also been reported in studies by Wojcik *et al*. (1976) in Florida, CIBC (1980), Rojas and García (1995) in Colombia and Hernández *et al*. (1989), Hernández and Díaz (1995; 1996), González and Zocco (1996), Morales *et al*. (2000; 2001) and Ferrer (2001)in Venezuela, where percentages were 65-100%. This parasitoid control on the number of eggs per egg mass was mainly due to the increase in eggs laid by the female *S. frugiperda* which coincided with the time of release of this parasitoid and with the maize genotype (the *jarocho crema* genotype is more attractive than yellow maize) (Hernández and Díaz, 1995; 1996; Morales *et al*., 2000; 2001). This increase observed in the number of eggs invaded by *T. remus* when the density of the host was increased corresponds to the results of similar studies done with other species of parasitoids and predators (Holling, 1959; 1961; Messenger, 1968; Hull *et al*., 1977; Morales and Burandt, 1985; Cave and Gaylor, 1989; Morales, 1991).

The percentages found for parasitism by polyculture system with *jarocho* crema genotype coincide with those recorded in Ithaca, New York (Root 1973), in Costa Rica (Risch, 1981), in Philippines (Hasse, 1981) and in Minnesota (Andow, 1991), where it was shown that parasitism is higher in polycultures than in monocultures.

The lower percentages of parasitism in the polyculture systems with the yellow maize genotype are due to *T. remus* having more difficulty in locating *S. frugiperda* eggs in them. One of the factors that influenced these results was masking by volatile chemicals released by the maize, bean, squash crops and eggs (Root, 1973; Risch, 1981; Hasse, 1981; Altieri, 1980; Andow, 1991).

The evidence of parasitism in maize-bean, maize-squash, maize-bean-squash polyculture and maize monoculture systems in both genotypes of maize suggests that the *T. remus* parasitoid has great potential as a biological control agent for *S. frugiperda* eggs. Studies by Schwartz and Gerling (1974), Yassen *et al*. (1981), Hernández *et al*. (1989), Gomez (1987), Corrêa-Figueiredo *et al*. (1999), Morales *et al*. (2000; 2001), Oliveira-de-Freitas-Bueno *et al*. (2008) also show the high potential of *T. remus* as a biological control. Furthermore, *T. remus* can develop throughout the year under field conditions (Oliveira-de-Freitas-Bueno *et al*., 2008), has been demonstrated to adapt well to the absence of the host (*S. frugiperda*) and can be kept for several days in the laboratory when release is not possible (Carneiro *et al*., 2009). Carmo *et al.* (2010) point out that in integrated pest management including *T. remus,* it should be noted that it is not compatible with the use of pyrethroids and organophosphates and would be an alternative to insect growth regulators as they are less harmful to beneficial arthropods. De-Souza-Tavares *et al*. (2009) found that Asteraceae family (*Eremanthus elaeagnu* and *Lychnophora ericoides*) extracts are more selective for *T. remus*, so their use is not recommended in combination with biological control of *S. frugiperda*.

5. CONCLUSION

According to the results of this study, the percentage of *T. remus* parasitism on *S. frugiperda* eggs was affected by maize genotype, bean, squash and polyculture system. Other factors that influenced the percentage of parasitism were the presence of weeds, densities of host eggs, numbers of masses and quality of eggs, temperature and kairomones in eggs of female *S. frugiperda* (Altieri and Letournneau, 1982; Powell, 1986; Gazit *et al*., 1996; Altieri and Nicholls, 2010; Oliveira-de-Freitas-Bueno *et al*. 2010). Furthermore, it is highly probable that it may already have become established in the region of Frailesca, Chiapas, Mexico.

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