

Comparative Study of the effects of *Trichogramma pretiosum* (Hym., Trichogrammatidae) releases and Triflumuron Applications on *Epinotia aporema* (Lep., Tortricidae) in Birdsfoot Trefoil Seedbeds

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In Uruguay, the tortricid moth *Epinotia aporema* is a borer that causes substantial losses in birdsfoot trefoil crops (*Lotus corniculatus* L.). Conventional insecticides are toxic to beneficial organisms and are effective for only short periods. We know that the insect growth regulator Triflumuron is effective, but it is expensive. Studies were conducted to evaluate the effectiveness of inundative releases of *Trichogramma pretiosum* (800,000 parasitoids/hectare/release) against *E. aporema*. Four treatments were compared: 1) inundative releases of *T. pretiosum* (one to three releases were performed in 2002, 2003 and 2004); 2) application of Triflumuron; 3) control plots located near the *Trichogramma* release plots; 4) control plots located away from the *Trichogramma* release plots. In the year 2002, the number of *E. aporema* larvae in the *T. pretiosum* plots (two releases) were significantly lower than in the distant controls but not in the near controls. In the year 2003, Triflumuron treatment (not applied in 2002) resulted in significantly lower numbers of larvae than the *T. pretiosum* treatment (one release), which did not differ from either the near or distant controls. In the year 2004, numbers of larvae were not significantly different between the Triflumuron and *T. pretiosum* treatments (three releases), but these numbers were significantly lower than in both controls. Inundative releases of *T. pretiosum* can therefore be an effective alternative to traditional insecticides and to Triflumuron, and deserve further development.

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INTRODUCTION

The bud borer *Epinotia aporema* (Walsingham) (Lepidoptera, Tortricidae) is a significant insect pest in Uruguay, particularly in the case of leguminous plants. The larvae cause longitudinal galleries in the foliar and floral sprouts of plants, affecting flowers, inflorescences, and leaves. The damage may

extend to the fruits, resulting in perforated pods and seeds wholly or partially damaged. In recent years, the losses caused by this pest augmented because soybean and birdsfoot trefoil (*Lotus corniculatus* L.) suffered intensive attacks (ZERBINO and ALZUGARAY, 1994; BENTANCOURT and SCATONI, 1995). According to estimations, the damage in field conditions reaches 30 to 40% decrease in pro-

duction of birdsfoot trefoil seed, red clover and alfalfa (ALZUGARAY, 2004).

Due to the lack of a dormancy period, this tortricid remains active throughout the year by using several species of leguminous plants as host. It probably has five to six generations per year, with long lasting laying periods, and preferentially spring and summer population peaks (ZERBINO and ALZUGARAY, 1991; ALZUGARAY, 2004).

Traditional chemical insecticides are not a sustainable solution to the problem of controlling *E. aporema*, because re-infestations take place within 15 to 20 days after the applications. That also affects the entomophilous pollination of plants. The use of insect growth regulators has the advantage of its low toxicity for adult honeybees. However, its effect does not begin before 5 to 10 days following the application, and the pest continues to affect the crop during this period (ZERBINO and ALZUGARAY, 1991). These growth regulators interfere with the formation of chitin in the cuticle and induce larvicidal and ovicidal effects. In general, treated larvae appear normal until moulting. However, at that moment, the larvae are unable to break through the old cuticle, since the malformed cuticle of the new instar is fragile and permeable. They cannot withstand the internal pressure during ecdysis and/or cannot give sufficient support to the muscles involved. If the substances are applied to eggs, chitin is not produced during the embryo development. Then, the larva will not always emerge (PASQUALINI, 2000). Since, insect growth regulators treatment is expensive in Uruguay, it is not an economically alternative when compared to traditional insecticides.

Given this situation, inundatory releases of the egg parasitoid *Trichogramma pretiosum* Riley (Hymenoptera, Trichogrammatidae) could be a solution to the problem. A comparison was thus undertaken between the effects of growth regulator and parasitoids treatments. The *Trichogramma* genus parasitizes eggs of several insects, particularly those belonging to the order Lepi-

doptera, and prevents the egg hatching and the damage caused by the larvae on plants (PINTO and STOUTHAMER, 1994; PINTUREAU, 1994). Some *Trichogramma* species were already experimentally applied in different Uruguayan crops against different pests, and the results were encouraging (BASSO and PINTUREAU, 1998, 2004).

Trichogramma pretiosum is the most widely distributed species out of the five *Trichogramma* species reported in this country (BASSO and PINTUREAU, 2004). Preliminary field experiments showed that adults of this species, when released in 100 m² plots of a birdsfoot trefoil field, are capable of finding and parasitizing *E. aporema* eggs glued on paper cards distributed among the foliage (unpublished data). Experimental work with *T. pretiosum* was continued during the period 2002-2004 in birdsfoot trefoil seedbeds located nearby the city of Ombúes de Lavalle, Colonia (Uruguay).

MATERIAL AND METHODS

The experimental design was based on 100 m² plots (10 m x 10 m), laid in rows, at more than 20 m from the edge of plantation, in birdsfoot trefoil seedbeds close to blooming. Plots were randomly chosen for the release of *T. pretiosum*, for the application of a chemical insecticide, or as control plots. In order to allow the observation of a probably uneven distribution of the pest on the crop, and of the dispersion of *Trichogramma* wasp from the release plots to the remaining ones, a number of control plots were set close to the latter (near controls), and others at a distance of over 50 m from them (distant controls). Disturbance of the area around the plots was avoided by suppression of all "fauchage" operation.

To release the parasitoids, we used 8 cm x 4.5 cm paper envelopes containing small pieces of cardboard on which parasitized eggs of alternative host (*Ephestia kuehniella* Zeller, Lepidoptera: Pyralidae) are glued. The parasitoids are ready to emerge when they are released. These envelopes were per-

forated of small holes to allow the exit of the *Trichogramma* adults, and to avoid the access of predators that might cause damage to the parasitized eggs before their emergence. They were placed in the foliage with an even distribution along the plots.

The *Trichogramma pretiosum* released, collected in parasitized eggs of *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae) in a corn crop in Uruguay, were multiplied in the Entomology Laboratory of the School of Agronomy (Montevideo) following the method described by BASSO and GRILLE (2001). The dose used for each release was 800,000 parasitoids per hectare. The chemical treatment was performed using the inhibitor of the chitin synthesis Triflumuron (Bayer's "Alsystin 480 SC"), at a dose of 0.5 liter/hectare, according to recommendations (ZERBINO and ALZUGARAY, 1991).

Due to the difficulties implied in searching for eggs laid by *E. aporema* on the foliage and to properly compare the two kinds of treatments, their biological evaluation was carried out taking into consideration the larval stage. Each evaluation consisted of randomly picking and putting in bags 200 stems per plot; the material was then sent to INIA "La Estanzuela" Entomology Laboratory (Uruguay) to be observed under a stereoscopic microscope in order to count the number of *E. aporema* larvae present on them. The data obtained in the three tests were analyzed with a generalized linear model, assuming of a Poisson distribution for the number of larvae and a logarithmic (natural) link function, applying the GENMOD procedure of the SAS (1997) system. For each test, a comparison was made between the number of larvae per plot in each treatment by sampling date, and between the global averages per treatment accounting for all sampling dates corrected with the first date (covariance analysis) so as to eliminate any possible effects of the different initial populational abundance per plot.

Test during the year 2002. The experimental design included fifteen plots

arranged in a random plot design with 10 m distance between plots. Ten plots were randomly chosen for the release of *T. pretiosum*, and 5 plots were considered as near controls. Another five plots were considered as distant controls (Fig. 1A).

Two *T. pretiosum* releases were performed, the first at the start of the crop's flowering, and the second seven days later (respectively on February 15th and 22nd, 2002), for a total of 1.6 million parasitoids/ha. Thirty two envelopes containing parasitoids were distributed per plot. The evaluation of the number of live larvae was carried out through 8 successive weekly samplings starting before the first release of *T. pretiosum*.

Test during the year 2003. The experimental design included twelve plots from which six plots were randomly chosen for the *T. pretiosum* releases. Three other plots were selected for the application of Triflumuron, and the three remaining plots were considered as near controls. Another three plots were considered as distant controls (Fig. 1B).

Triflumuron was applied when the flowering period started (February 24th, 2003), and two days after this date, a unique release of *T. pretiosum* was performed (800,000 parasitoids/ha). The evaluation of the number of live larvae was carried out through 7 successive weekly samplings, the first of them taking place immediately before the application of the insecticide.

Test during the year 2004. The experimental design was identical to that of the year 2003. At the start of the crop flowering, one application of Triflumuron (March 4th, 2004) was performed on the respective plots, and two days after this date, a first release of *T. pretiosum* was carried out. A second and a third release were performed twelve and twenty-two days after this date, respectively; sixteen envelopes of parasitoids were used during each release, with a total of 2.4 million *Trichogramma*/ha. The evaluation of the

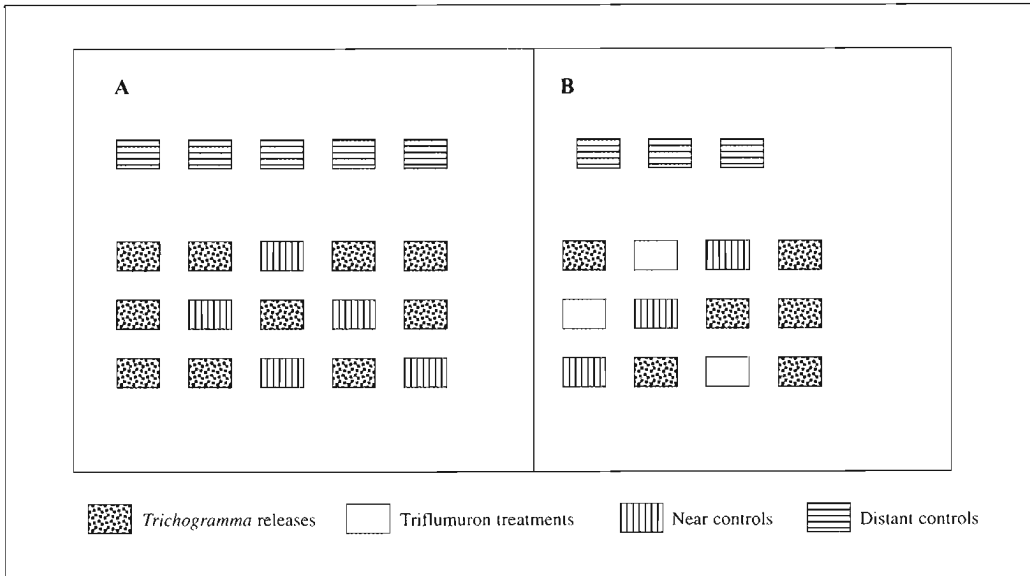


Figure 1. Spatial design of birdsfoot trefoil plots treated with *Trichogramma* or Triflumuron, or untreated. (A) Year 2002; (B) Years 2003 and 2004.

number of larvae was carried out through six successive weekly samplings, the first of them taking place immediately previous to the application of the insecticide.

RESULTS

Test during the year 2002. According to the larvae collected from the plots, the population of *E. aporema* completed two generations within the period studied. The first four sampling dates correspond to the first generation, and the following four dates to the second generation (Fig. 2A). Since the birdsfoot trefoil seedbed was not harvested due to the low number of pods on the plants, a long period of study was allowed.

Three days after the first release of *T. pretiosum* (sampling of week two), the larva population in *Trichogramma* plots was greater than both the larva population in distant controls and the larva population in the near controls (Table 1). *Trichogramma* plots had 40.5% more larvae than distant controls, and 48.6% more larvae than near controls.

The population in *Trichogramma* plots on week two was at its maximum for that generation. Ten days after the first release (sampling at week three), the parasitoid showed a significant effect on the *E. aporema* eggs since the population in distant controls showed an increase of 24.3%, the population in near controls an increase of 41.1%, and the population in *Trichogramma* plots a decrease of 33.4%, always in relation to week two.

The highest number of larvae in a plot was reached on week seven, corresponding to the second studied generation of the pest. The number of larvae in distant controls was significantly higher than the number of larvae in near controls and that in *Trichogramma* plots.

Considering all the sampling dates together, *Trichogramma* plots showed a lower number of larvae per plot than distant controls. Near controls showed an intermediate infestation, not different from the others. The correction by covariance with the values from the first date did not significantly modify the analysis.

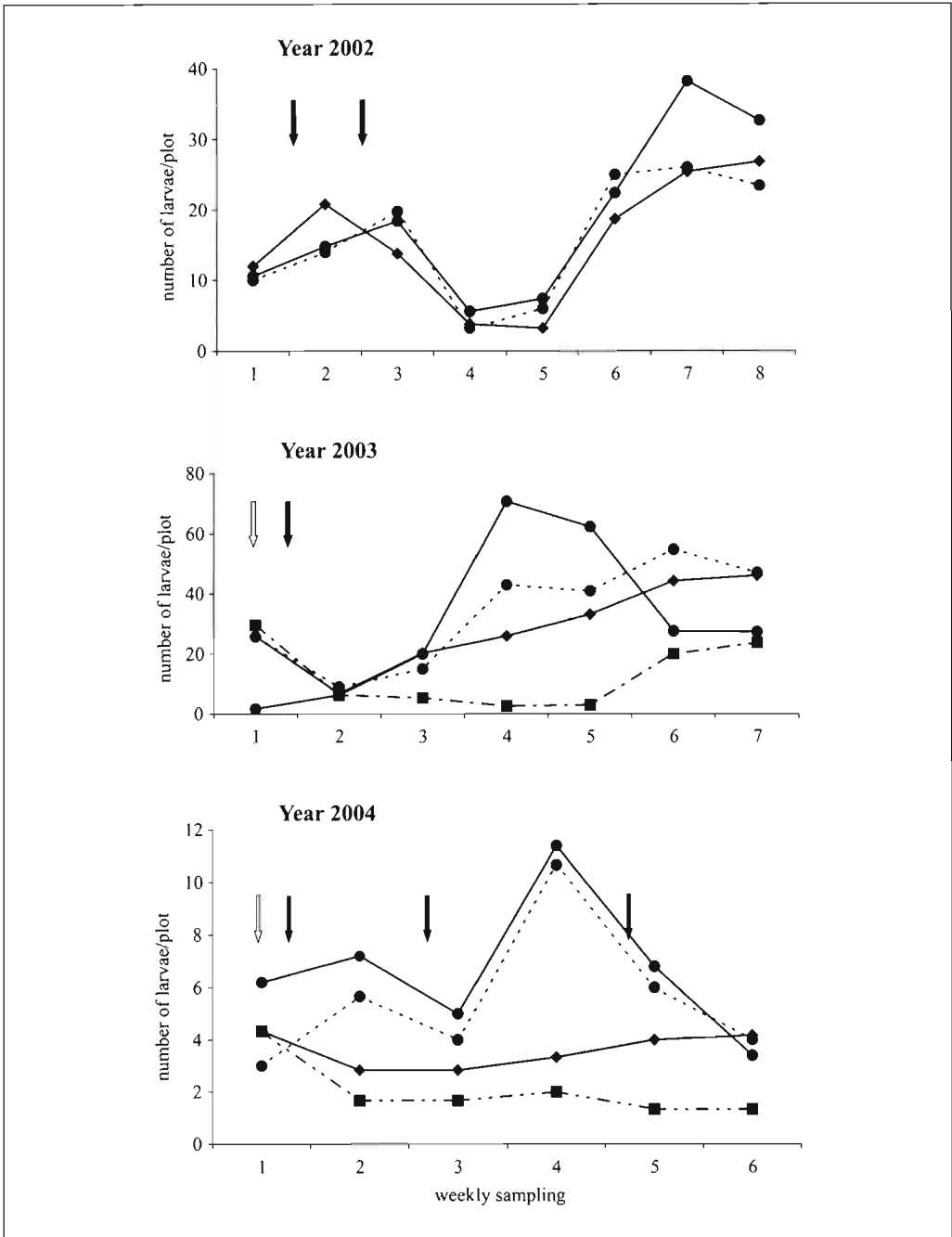


Figure 2. Weekly fluctuations in the average number of *E. aporema* larvae in birdsfoot trefoil plots: plots with *Trichogramma* releases are indicated by squares and unbroken lines, plots with Triflumuron treatment are indicated by squares and dashed lines, plots used as near controls are indicated by circles and unbroken lines, plots used as distant controls are indicated by circles and unbroken lines. *Trichogramma* releases are indicated by solid vertical arrows and Triflumuron treatments are indicated by open vertical arrows. Years: 2002, 2003, 2004.

Table 1. Estimated number of larvae of *E. aporema* per plot in each treatment during 8 weeks of the year 2002.

Treatment	1	2	3	4	5	6	7	8
DC 16.1a	10.6	14.8 a	18.4 a	5.6 a	7.4 a	22.4 a	38.2 a	32.6 a
NC 13.6ab	10.0	14.0 a	19.8 a	3.2 a	6.0 a	25.0 a	26.0 b	23.4 a
TR 12.9 b	12.0	20.8 b	13.8 a	3.8 a	3.2 a	18.7 a	25.4 b	26.8 6

DC: distant controls, NC: near controls, TR: *Trichogramma* releases.

1% level (treatments were applied after the week 1, not tested).

Means followed by the same letter do no differ at the 1% level.

Table 2. Estimated number of larvae of *E. aporema* per plot in each treatment during the year 2003.

Treatment	1	2	3	4	5	6	7
DC 36.3 a	1.7	6.3 a	20.0 ab	70.7 a	62.3 a	27.7 a	27.3 a
NC 29.0 a	25.7	9.0 a	15.0 ab	43.0 ab	41.0 a	54.7 a	47.0 a
TR 24.4 a	25.8	6.8 a	20.2 a	26.0 b	33.2 a	44.3 a	46.2 a
CH 8.2 b	29.7	6.3 a	5.3 b	2.7 c	3.0 b	20.0 a	23.7 a

DC: distant controls, NC: near controls, TR: *Trichogramma* releases, CH: Triflumuron treatments.

1% level (treatments were applied after the week 1, not tested).

Means followed by the same letter do no differ at the 1% level.

Table 3. Estimated number of larvae of *E. aporema* per plot in each treatment during 6 weeks of the year 2004.

Treatment	1	2	3	4	5	6
DC 6.4a	6.2	7.2 a	5.0 a	11.4 a	6.8 a	3.4 a
NC 6.0a	3.0	5.7 a	4.0 a	10.7 a	6.0 a	4.0 a
TR 3.3 b	4.3	2.8 a	2.8 a	3.3 b	4.0 a	4.2 a
CH 1.5 b	4.3	1.7 a	1.7 a	2.0 b	1.3 b	1.3 a

DC: distant controls, NC: near controls, TR: *Trichogramma* releases, CH: Triflumuron treatments.

1% level (treatments were applied after the week 1, not tested).

Means followed by the same letter do no differ at the 1% level.

Test during the year 2003. The unique release of *T. pretiosum* coincided with the ending of a generation in the field and the start of a new one, reflected by the decrease and the later increase in the number of larvae collected (Fig. 2B).

After the first sampling, Triflumuron plots always showed the lower number of larvae per plot, a number significantly different from numerous values calculated in other plots at each sampling week (Table 2). *Trichogramma* plots showed an intermediate number of larvae in the week 4 sampling in regards to the other treatments, when the effect of the *T. pretiosum* release was probably optimal. However, no significant difference was recorded in this sampling between *Trichogramma* plots and near controls.

Between weeks five and six, the number of larvae in *Trichogramma* plots and near controls increased following the same trend, and ended up in equivalent values on week seven.

Considering all the sampling weeks, Triflumuron plots showed a number of larvae lower than *Trichogramma* plots, near and distant controls. There was no difference between the three kinds of plots without insecticide. The correction by covariance with the values from the first week did not significantly modify the analysis (Table 2).

Test during the year 2004. The first release of *T. pretiosum* took place at the beginning of a generation of *E. aporema* in the field, reflected by the following increase in the number of larvae collected in the con-

trol plots (Fig. 2C). Triflumuron and *Trichogramma* plots showed a stable reduced number of larvae, while distant and near controls showed another dynamics, characterized by a slight decrease, followed by a strong increase and a subsequent decrease. Triflumuron plots were infested by the smallest population of larvae of all treatments, though no significant differences were often recorded with *Trichogramma* plots (Table 3).

Considering all the sampling weeks as a whole, Triflumuron and *Trichogramma* plots showed a similar number of larvae. Both treatments differed from the near and distant controls, which were not different from each other. The correction by covariance with the values from the first week did not significantly modify the analysis (Table 3).

DISCUSSION

Although the populational abundance of *E. aporema* varied during the three years under study, the *T. pretiosum* releases always showed more or less effectiveness as biological control agents. In year 2002, such effect was especially observed between weeks 2 and 3 samplings when the population of larvae in controls showed a linear growth trend and the population in *Trichogramma* plots showed a strong decrease. Even though some studies showed that *Trichogramma* dispersion is very limited (ANDOW and PROKRYM, 1991, MCDUGALL and MILLS, 1997), VOEGELÉ *et al.* (1975) point out that *T. brassicae* Bezdenko is able to parasitize *Ostrinia nubilalis* Hübner eggs in corn fields, as far as 78 and 147 m from the release point. Distances of 400 m and 700 m were even reported into the wind direction (STERN *et al.*, 1965; BIGLER *et al.*, 1990). Such a dispersion could explain the identical results obtained in the *Trichogramma* plots and the near controls during the latest samplings, and these controls have thus to be placed at a longer distance in following experiments.

In year 2003, between week three and week four samplings, the population of *E. aporema* larvae showed a very small increase

in *Trichogramma* plots as compared to controls. However, on the following weeks, the larva population in *Trichogramma* plots continued to grow, and reached the population recorded in near controls. This partial effect could be associated to the fact that only one release of *T. pretiosum* was carried out in 2003.

In year 2004, the effect of *T. pretiosum* on the *E. aporema* population was obvious. This effect lasted throughout the whole test, probably because three successive releases of parasitoids were carried out on the plots.

Moreover, our results confirmed the effectiveness of Triflumuron treatments on the *E. aporema* larva population (ZERBINO and ALZUGARAY, 1991). A single application of this insecticide was more effective than a single application of *T. pretiosum* in year 2003. However, this difference was reduced with the three releases of parasitoids performed in year 2004. The effectiveness of inundatory releases of *T. pretiosum* on the abundance of *E. aporema* populations provides a new tool to control this pest, and an alternative to the toxic effects of traditional insecticides and to the high cost of Triflumuron. The optimal number of *T. pretiosum* per hectare still needs some adjustments, but it seems necessary to make several successive releases in order to accurately cover the *E. aporema* extended laying period.

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RESUMEN

BASSO C., G. GRILLE, R. ALZUGARAY, B. PINTUREAU. 2006. Estudio comparativo de los efectos de liberaciones de *Trichogramma pretiosum* (Hym., Trichogrammatidae) y aplicaciones de Triflumuron sobre *Epinotia aporema* (Lep., Tortricidae) en semilleros de lotus. *Bol. San. Veg. Plagas*, **32**: 563-571.

En el Uruguay, el tortrícido *Epinotia aporema* es un insecto plaga que causa daños de importancia en semilleros de lotus (*Lotus corniculatus* L.). Los insecticidas convencionales son tóxicos para los organismos benéficos, y efectivos por cortos períodos, mientras que el regulador de crecimiento de insectos Triflumuron es efectivo pero costoso. Los estudios fueron conducidos para evaluar la eficacia de liberaciones inundativas de *Trichogramma pretiosum* (800,000 parasitoides/hectárea/liberación) contra *E. aporema*. Los tratamientos comparados fueron: 1) liberaciones inundativas de *T. pretiosum* (varió desde una a tres liberaciones en 2002, 2003 y 2004); 2) aplicaciones de Triflumuron; 3) testigos localizadas cerca de las parcelas liberadas con tricogramas; 4) testigos localizadas lejos de las parcelas liberadas con tricogramas. En el año 2002, el número de larvas de *E. aporema* en las parcelas con *T. pretiosum* (dos liberaciones) fue significativamente menor que en los testigos lejanos, pero no que en los testigos cercanos. En el año 2003, los tratamientos con Triflumuron (no aplicado en 2002) resultaron en un nivel de larvas significativamente menor que en las tratadas con tricogramas (una liberación), que no difirieron de los testigos cercanos y lejanos. En el año 2004, el número de larvas no fue significativamente diferente entre los tratamientos con Triflumuron y *T. pretiosum* (tres liberaciones), pero esos valores fueron significativamente más bajos que en las parcelas testigo. Los autores concluyen que las liberaciones inundativas con *T. pretiosum* pueden ser una alternativa efectiva a los insecticidas tradicionales y al Triflumuron, siendo estos resultados dignos de futuros desarrollos.

Palabras clave: control biológico, regulador de crecimiento, Tortricidae, leguminosae, Uruguay.

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