*Researches on ~\_QPULATION O~nal COLOGY pap* 

# **Statistical Study of** *Trichogramma brassicae* **Efficiency in Relation with Characteristics of the European Corn Borer Egg Masses**

N. HAWLITZKY, F. M. DORVILLE\* and J. VAILLANT\*

*INRA de Versailles, laboratoire de zoologie, F78026, France \*Universitd des Antilles-Guyane, UFR Sciences, F97167, Guadeloupe* 

**Abstract.** The efficiency of *Trichogramma brassicae* inundative releases in biological control of the European corn borer *Ostrinia nubilalis* was analyzed in seven plots of 504 plants, each situated in a corn field naturally infested by the European corn borer. Different strategies of *Trichogramrna*  releases were defined on the seven plots. These inundative releases were concluded to be highly efficient even on plots where there were either no parasitoid release or only one parasitoid release at the beginning of the egg mass laying period.

*Key words:* European corn borer, *Trichogramma brassicae,* biological control, inundative release, dispersion, parasitoid efficiency.

# **Introduction**

The European corn borer (ECB) has long been one of the major pests in French corn fields and many field studies have been carried out to acquire a useful knowledge of their damages caused to the corn plants (Stengel 1970; Stengel 1982) and the spatial and temporal distributions of this pest (Vaillant 1985; Hawlitzky 1986; Vaillant and Hawlitzky 1990). In order to control the ECB population levels, the egg parasitoid, *Trichogramma brassicae*  Bezdenko (Hym. Trichogrammatidae), presents many advantages as a means of biological control (Daumal et al. 1975; Voegele 1976; Gusev and Lebedev 1988). One can refer to Kabiri et al. (1990) and Hawlitzky and Voegele (1991) for relevant papers on the biology of this egg parasitoid, its mass production and field application against ECB.

This paper deals with the efficiency of *Trichogramma brassicae* in relation with the vertical, horizontal and temporal distribution of the ECB egg masses. From experiments conducted in naturally ECB-infested corn fields, several relevant variables concerning the ECB egg masses and the parasitoid were collected during the whole ECB egg mass laying period. Our primary goal is to know the parasitization behaviour of *Trichogramma* in relation with characteristics of ECB egg masses and leaf volume under conditions of inundative releases. It is also to provide further information about *Trichogramma* efficiency for

release-based strategies in ECB management (Hawlitzky et al. 1986).

# **Materials and Methods**

# *Experimental corn plots and sampling procedures*

Seven plots of 40 m  $\times$  40 m were defined in a 8 ha naturally ECB-infested corn field in the Paris area, France. The plots were at a minimal distance of 60 m from each other. In each plot, a permanent sample of 504 plants were examined twice a week during the whole ECB egg mass laying period. Thus, each plant was examined 16 times and the examination included the whole leaf strata. The experimental conditions of this study have been fully described by Hawlitzky (1986). When an egg mass was detected, its following variables were noted; the date of its first detection, its leaf position, and its size (number of eggs). When parasitized eggs were detected in this egg mass, the following variables were noted; the date of the first detection of the parasitoid, and the number of eggs parasitized. On the other hand, each plant could be associated with a nearest release point of *Trichogramma.* The trichogrammas were released once on plot 1 (at the first release date) and plot 3 (at the second release date), twice on plot 2 (at the first and second release dates) and three times on plot





**Fig. 1. Variance as a function of the mean for the number of European corn borer egg masses per plant. The dotted line corresponds to the pure random distribution case for which variance=**  mean.  $(\Box$  indicates a significant overdispersion;  $\cdot$ **indicates an insignificant overdispersion)** 

4, 5 and 6 (at the first, second and third release dates). No release was performed on plot 7. At each release date, 100,000 trichogrammas were released per ha from 147 release points (680 trichogrammas per release point). The spatial arrangement of the release-points was regular; every seven corn rows and every 14 m in the row direction.

## **Statistical analysis**

#### *Analysis of the egg mass dispersion*

The variance-mean ratio  $D$  for the cumulative number of egg masses per plant was calculated for each week, and the test of overdispersion was applied. Let  $n$  be the number of plants observed. Hoel (1943) has shown that  $(n-1)^{D}$  follows a chi-squared distribution with  $n-1$ degrees of freedom under the hypothesis of pure random distribution. By using the chi-square approximation to the normal distribution given by Johnson and Kotz (1970), Vaillant (1985) deduced the following result: when the number of plants *n* is large, the statistic  $(2(n-1)^{D})^{1/2}$ - $(2n-3)^{1/2}$  follows the unit normal distribution. Therefore, this statistic can be used to test the egg masses overdispersion when the number of egg masses detected is abundant enough (validity condition of Hoel's result). When it is not the case, a Monte-Carlo test (see Vaillant and Hawlitzky 1990) has to be applied. Drawing the variance as a function of the mean for the cumulative number of egg masses per plant gives also a rough idea of the dispersion of the egg masses over the oviposition period.

### *Analysis of correlation between egg mass characteristics and parasitism*

A table of correlation was built from the following variables observed for each detected egg mass; *PD* = nearest release point distance, *DD* = date of egg mass detection, *LP=* egg mass leaf position, *MS* = egg mass size, TP=time between *DD* and the first detection of parasitism, and  $PP =$ proportion of parasitized eggs at the date of first detection of parasitism. Tests of nullity of the correlation coefficient were performed using the transformed coefficient:

$$
z=\frac{1}{2}\log\left(\frac{1+\rho}{1-\rho}\right),\,
$$

where  $\rho$  is the classical correlation coefficient.

The significance of the test is based on  $z(N-3)^{1/2}$  which follows the unit normal distribution under the hypothesis of non-correlation.  $N$  is the number of egg masses involved in the calculation of  $\rho$ .

#### *Analysis of the time evolution of parasitism*

The proportion of parasitized egg masses  $\rho_t$  at a given date  $t$  was tested as an increasing function of time  $t$ 

through regression techniques (see for example Linhart and Zucchini 1986, chap. 6 and 7). Non-linear predictions of the  $\rho_t$  were calculated and plotted as a function of time t. The simple linear trend  $A + Bt$  was considered, and the positivity of coefficient  $B$  was tested by means of unilateral Student test.

# **Results and Discussion**

### *Analysis of the egg mass dispersion*

Figure 1 shows the relation between the mean and variance of the number of egg masses per plant. The variancemean ratio was close to unity at the beginning of the egg mass laying period and became significantly greater than unity, except for plots 2 and 7, when the mean increased with time. The more infested the plots were, the more overdispersed the egg masses were. It is worth noticing that the mean number of egg masses per plant varied a lot from one plot to another in the same field (see Fig. 2).

# *Analysis of correlation between egg mass characteristics and parasitism*

The two by two correlation coefficients are presented in Table 1. It can be seen that the nearest release point distance *PD* is not correlated with any of the other variables. This can be explained by the fact that the release was inundative. This also confirms that the radius of action by *Trichogramma* can be much greater than the maximal distance of 7 m between sampled plants and release points (Kabiri et al. 1990). The egg mass size *MS*  also was not correlated with any other variables. In particular, there was no preferential leaf strata for egg mass laying in relation with their sizes. Bigger egg masses were not more parasitized than smaller ones. It can be noticed that on plot 7, the correlation between *MS* and *DD* (first detection date) was significantly negative. This is due to the few small egg masses laid during the last dates on this plot. The egg mass leaf position *LP* was generally correlated with *DD* because the plant phenology evolved in time and the number of leaves per plant was larger at the end of the observation period. Almost 95% of egg masses were laid in the middle-plant stratum (Fig. 2). For a plant with 13 leaves, this stratum corresponds to leaf positions 4 to 10. *LP* was not correlated with *PP* (the observed proportion of eggs parasitized in an egg mass at the first date of detection of parasitism) except for plots 1 and 7 (Table 1). On these latter plots, it seems that the higher the egg masses were laid, the more severely parasitized they were. This was only the case for weeks 7 and 8. The correlation between *PP* and *DD* was significantly positive; the correlation between *PP* and *TP* was significantly



7 ~ek

**1 2 3 4 s s 7 s 9 10 11 12 13 Leaf number** 



		$\overline{PD}$	DD	LP	МS	TP	PP
Plot 1	PD	$\mathbf{1}$					
	DD	0.054	$\mathbf{1}$				
	$\cal LP$	$-0.112$	0.090	$\mathbf{1}$			
	MS	$-0.037$	$-0.035$	0.031	$\mathbf{1}$		
	TP	$-0.076$	$-0.197**$	$-0.155**$	0.005	1	
	$_{PP}$	0.041	$0.264**$	$0.134*$	$-0.007$	$-0.895**$	$\mathbf{1}$
Plot 2	PD	$\mathbf{1}$					
	DD	0.004	$\mathbf{1}$				
	LP	$-0.078$	$0.194**$	1			
	MS	0.125	0.065	$-0.067$	$\mathbf{1}$		
	$\boldsymbol{TP}$	0.053	$-0.110$	$-0.142*$	0.026	$\mathbf{1}$	
	PP	$-0.016$	$0.163*$	0.122	$-0.043$	$-0.932**$	$\mathbf{1}$
Plot 3	$\overline{PD}$	$\mathbf{1}$					
	$D\hspace{-.05cm}D$	0.105	$\mathbf{1}$				
	LP	$-0.021$	$0.159*$	$\mathbf{1}$			
	MS	0.029	$-0.087$	$-0.029$	$\mathbf{1}$		
	$\boldsymbol{TP}$	$-0.119$	$-0.228**$	$-0.089$	0.037	$\mathbf{1}$	
	PP	0.085	$0.304**$	0.050	$-0.078$	$-0.916**$	$\mathbf{1}$
Plot 4	${\cal PD}$	$\mathbf{1}$					
	DD	0.023	$\mathbf{1}$				
	LP	$-0.035$	$0.151**$	$\mathbf{I}$			
	MS	$-0.040$	$-0.031$	$-0.081$	$\mathbf{1}$		
	TP	0.012	$-0.120*$	$-0.121*$	$-0.025$	1	
	PP	$-0.005$	0.093	0.100	0.026	$-0.849**$	$\mathbf{1}$
Plot 5	$\overline{PD}$	$\mathbf{1}$					
	$D\hspace{-.05cm}D$	0.023	$\mathbf{1}$				
	LP	0.075	$0.121**$	$\mathbf{1}$			
	MS	0.035	0.036	0.007	$\mathbf{1}$		
	TP	0.072	$-0.077$	$-0.075$	$-0.067$	$\mathbf{1}$	
	PP	$-0.052$	$0.170**$	0.064	$-0.016$	$-0.796**$	$\mathbf{1}$
Plot 6	$\overline{PD}$	$\mathbf{1}$					
	$D\hspace{-0.1cm}D$	0.010	$\mathbf{1}$				
	LP	$-0.052$	$0.109*$	$\mathbf{1}$			
	MS	0.013	$-0.012$	0.052	$\mathbf{1}$		
	$\boldsymbol{TP}$	0.033	$-0.182**$	$-0.062$	0.018	1	
	PP	$-0.014$	$0.213**$	0.024	$-0.095$	$-0.785**$	$\mathbf{1}$
Plot 7	PD	$\mathbf{1}$					
	DD		1				
	$\cal LP$		$0.235**$	1			
	$\overline{MS}$		$-0.139*$	$-0.005$	$\mathbf{1}$		
	TP		$-0.314**$	$-0.162**$	0.064	1	
	PP		$0.337**$	$0.151**$	$-0.100$	$-0.950**$	$\mathbf{1}$

Table 1. Two by two correlation coefficients for the following variables observed for each detected egg mass:  $PD$ =nearest release point distance, DD=date of egg mass detection, LP=egg mass leaf position, MS=egg mass size, TP=time between *DD* and the first detection of parasitism, *PP=* proportion of parasitized eggs at the date of first detection of parasitism. \* and \*\* indicate that the test of nullity of the correlation coefficient was significant at 3% and 1% level, respectively.

negative; the correlation between *DD* and *TP* was also significantly negative. These relations mean that the parasitoid attack became more efficient with time. This increasing efficiency may be related with the increasing number of trichogrammas in the field due to new releases and the recruit of new generations of daughters. Also, during the first week of the study, the weather *conditions* 

were unfavourable for the activity of the parasitoid adults (low temperature and rainfall), but improved during the following weeks. Despite a slight variation from one plot to another concerning the significance of these correlations, the inundative release of *Trichogramma* was very efficient even on plot 7 where the parasitoid was not released (Fig. 3).



### Analysis of the time evolution of parasitism

We have seen above the relation between *PP* and *DD.* If we consider now for a given date t the ratio  $\rho_t$  of the cumulative number of parasitized egg masses (since the beginning of the oviposition period) to the cumulative number of egg masses, we get another aspect of the *Trichogramrna* efficiency: the proportion of egg masses reached by the parasitoid at a date  $t$ . Fig. 3 proves that there was a significant increase of  $\rho_t$  as a function of t except plots 2 and 5 for which the slope of the regression line was not significant; the value of  $\rho_t$  was already up to 50% and more at the beginning of the observation period on plots 2 and 5. On the other hand, although  $\rho_t$  can be considered as a non-decreasing function of  $t$ , the linear model  $\rho_t = A + Bt$  is not necessarily appropriate as shown by the non linear predictions of  $\rho_t$  in Fig. 3. The linear model has to be seen as an approximation model representing the linear trend of  $\rho_t$ .

In conclusion, we can underline the following results. Concerning the ECB distribution, (1) most of the egg masses (about 95%) were laid in the middle plant stratum, (2) the within-plant distribution of the egg masses was independent of the egg mass size, and (3) the egg masses were significantly overdispersed at the plant scale, when the infestation level was high enough.

Concerning the *Trichograrnma* distribution, (1) for inundative releases, there was no preferential attack on egg masses laid in particular leaf strata, (2) for inundative releases, the attack did not depend on the egg mass size, and (3) for inundative releases, the efficiency of the parasitoid was high even for one single release date at the beginning of the egg mass laying period. However, the highest values of efficiency were obtained for the plots where three releases were performed.

## **References**

Daumal, J., J. Voegele and P. Brun (1975) Les trichogrammes. II Unit6 de production massive et quotidienne d'un h6te de substitution *Ephestia kuehniella* Zell. *Ann. ZooL Ecol. Anita.* 7: 45-59.

- Gusev, G. V. and G. I. Lebedev (1988) Present state of *Trichogramma* application and research. *Les Colloques de I'INRA* 43: 477- 481.
- Hawlitzky, N. (1986) Etude de la biologie de la pyrale du maïis, Ostrinia nubilalis Hbn. en région parisienne durant quatre années et recherche d'616ments pr6visionnels du d6but de ponte. *Acta OecoL Appl.* 7: 47-68.
- Hawlitzky, N., M. Stengel, J. Voegele, B. Crouzet and B. Raynaud (1986) Strategy used in France in the biological control of the European corn borer *Ostrinia nubilalis* Hbn. by oophagous insects, *Trichogramma maidis* Voeg. et Pint. *Meded. Fac. Landbouww et Rijksuniv. Gent.* 51: 1029-1032.
- Hawlitzky, N. and J. Voegele (1991) Démarche utilisée pour 6laborer une strat6gie de lutte biologique par lachers inondatifs d'entomophages contre un ravageur du maïis. Problèmes apparus lors de la pratique et solutions apportées. *Bull. Soc. Zool. Fr.* 116: 319-329.
- Hoel, P. G. (1943) On indices of dispersion. *Ann. Math. Statis.* 14: 155-162.
- Johnson, N. L. and S. Kotz (1970) *Distributions in statistics, VoL 2.*  Houghton Mifflin, Boston.
- Kabiri, F., J. Frandon, J. Voegele, N. Hawlitzky and M. Stengel (1990) Strat6gie 6volutive des l~chers inondatifs de *Trichogramma brassicae* Bezd. (Hym. Trichogrammatidae) contre la Pyrale du ma'fis *Ostrinia nubilalis* Hbn. (Lep. Pyralidae). *Ann. ANPP* 3: *1225-1232.*
- Linhart, H. and W. Zucchini (1986) *Model selection.* Wiley, New York.
- Stengel, M. (1970) Une méthode de prévision des dégâts de la pyrale du maïis Ostrinia nubilalis. Mise au point de la lutte dans les cultures de la plaine d'Alsace. *Ann. Zool. Ecol. Anita.* 2: 309-325.
- Stengel, M. (1982) Essai de mise au point de la prévision des dégâts pour la lutte contre la pyrale du maïis Ostrinia nubilalis en Alsace (Est de la France). *Entornophaga* 27:105-114.
- Vaillant, J. (1985) Etude statistique des répartitions spatiales et temporelles des pontes de pyrale *(Ostrinia nubilalis)* dans le bassin parisien. Problèmes d'échantillonnage. Thèse de 3e cycle, Université Paul Sabatier, Toulouse.
- Vaillant, J. and N. Hawlitzky (1990) Statistical analysis of occupancy rates for overdispersed populations by redistribution procedures. Application to the European corn borer egg masses distribution. *Res. PopuL Ecol.* 32: 289-301.
- Voegele, J. (1976) La diapause et l'hétérogénéité du développement chez les Aelia et les trichogrammes. *Ann. Zool. Ecol. Anim.* 8: 367-371.

*Received 7 January 1994; Accepted 1 June 1994*