Studies on biological control of *Ephestia kuehniella* Zeller (Lep.: Pyralidae) with *Trichogramma evanescens* Westwood (Hym.: Trichogrammatidae) — host-finding ability in wheat under laboratory conditions

M. Schöller*, C. Reichmuth* and S. A. Hassan†

Abstract

The ability of *Trichogramma evanescens* to locate and parasitise eggs of stored-products moths in grain was studied under laboratory conditions to evaluate the potential of this egg parasitoid as a possible biological control agent.

At 1, 2 and 5 cm depths, wire cages containing 100 host eggs were positioned in 3-litre jars containing wheat. Egg cards containing either 200, 500 or 1000 *T. evanescens*-parasitised *Sitotroga cerealella* eggs were placed on the surface of the grain. The experiments were conducted in climate chambers at 75%±5% r.h. To check the ability of parasitoids to penetrate deeper than 5 cm in bulk wheat, the distribution of *T. evanescens* in a plexiglass cylinder of 100 cm height and 20 cm diameter filled up to 90 cm with wheat was studied.

Females of *T. evanescens* were able to parasitise eggs of *E. kuehniella* at up to 55 cm depth in wheat. At 26°C, rates of parasitisation of *T. evanescens* at release rates of 200, 500 and 1000 eggs were 41, 45, and 78% respectively. At 17°C, a release rate of 1000 eggs yielded 67% parasitisation.

Introduction

The spread of insecticide resistant strains of stored-product pests and environmental problems connected with chemical control, like the effect of methyl bromide on the ozone layer, has led to intensified research into alternative control methods, for example biological control. Most insect species investigated for possible application against stored-product pests were parasitic hymenoptera; among them two genera of egg parasitoids belonging to the family Trichogrammatidae, *Uscana* against *Callosobruchus maculatus* (Fab.) (Pajni 1990) and *Trichogramma* against various pyralid stored-product moths (Brower and Press 1988; Hassan and Prozell 1992). Egg parasitoids complete their development inside the host egg, resulting in mortality of the host, and are therefore minute in size. The first records of natural occurrence of *Trichogramma* in warehouses were those of *T. pretiosum* Riley and *T. parkeri* Nagarkatti in peanut storages in Georgia (Brower 1984 a). Brower (1984b) suggested the application of *Trichogramma* in stored-product protection and conducted experiments on host-finding ability in bulked inshell peanuts (Brower 1990) and on suppression of stored-product moth populations in small inshell peanut storages (Brower and Press 1988, 1990). In this work, the host-finding ability of *Trichogramma evanescens* in bulk wheat under different temperatures was studied under laboratory conditions.

* Federal Biological Research Centre for Agriculture and Forestry, Institute for Stored Product Protection, Königin-Luise-Straße 19, 14195 Berlin-Dahlem, Germany.

† Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Pest Control, Heinrichstraße 243, 64287 Darmstadt, Germany.

Materials and Methods

Insects and wheat

The strain of *T. evanescens* Westwood was originally obtained from eggs of *Heliothis armigera* (Lep.: Noctuidae) in Egypt in 1981 and mass reared on the Angoumois grain moth, *Sitotroga cerealla* (Olivier), at the Institute for Biological Pest Control at Darmstadt, Germany. The strain is arrhenotokous, the sex ratio is 1.47:1 (25°C, 75 ± 5% r.h.). The strains of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, and the tobacco moth, *E. elutella* Hübner, were taken from the cultures of the Institute for Stored Product Protection at Berlin, Germany. The pyralids were reared in darkness at 25°C and 70% r.h.

For host-locating experiments, food-grain was used; corn lengths averaged 6.6 ± 0.5 mm and corn width averaged 3.2 ± 0.4 mm. Minimum length was 5.0 mm, maximum length 7.5 mm.

Minimum width was 2.1 mm, maximum width 4.3 mm. The interstitial space among the kernels averaged 637 cm^3/kg. Wheat was not treated with any pesticide, but heated to 100°C for 1.5 hours and moistened to 14% before use.

Distribution of *T. evanescens* in bulk wheat

Egg-cards (6 × 1 cm) with egg-groups of 50 host eggs each at both ends were placed in cages of metal-gauze (7.8 × 1.4 cm), which were closed with a rubber plug. The mesh width of the metal-gauze was 1.5 mm, so the wasps were able to enter the cages. The cages were inserted in 3-L jars with wheat in 1.0, 2.0 and 5.0 cm depth parallel to the bottom, so three cages, with 100 host eggs each, were offered simultaneously. Jars were covered with plastic lids. Egg-cards with 200, 500 or 1000 parasitised eggs of *S. cerealella*, with parasitoids at least 12 hours before emergence, were laid on the surface of the wheat. Jars were placed in climatic chambers; two temperature conditions, 26°C and 17°C, were tested. Relative humidity was 75 ± 5% in both cases. Light conditions were 30 lx in a 16:8 L:D cycle. Each test condition had 30 replications through time.

After a 6-day exposure, mortality and rate of *T. evanescens*-parasitisation of *E. kuehniella* eggs were determined. Mortality of *E. kuehniella* eggs consists of *Trichogramma*-parasitisation, infertility due to parasitoid activity like host feeding, and natural infertility. Parasitism is indicated by black host eggs. The percentage of eggs which were not black but which were incapable of hatching is referred to as infertileggs using the formula of Abbott (1925). Untreated eggs were handled in the same manner as in the experiments, except for the release of *Trichogramma*.

Data were analysed with an analysis of variance. Significant differences in mean parasitism at different cage depths within one test condition were calculated using the Wilcoxon matched-pairs signed-ranks test, significant differences in mean mortality at different conditions were calculated using the Mann-Whitney U-Wilcoxon rank sum test. Data were analysed for equality of variances using the test of Levene.

To check the ability of parasitoids to penetrate deeper than 5.0 cm into the bulk wheat, a plexiglass cylinder of 100 cm height and 20 cm diameter was filled up to 90 cm with wheat. The cylinder was covered with a glass plate. Cages with egg-cards as described above were placed at different levels in the cylinder. Egg cards with a large number of *T. evanescens*, about 30 × 3000, were placed at the surface of the wheat column. After 7 days, egg-cards were checked for parasitisation and cages for *Trichogramma* specimen.

Results

Distribution of *T. evanescens* in bulked wheat

Highest host-finding rates were achieved with 1000 *T. evanescens* at 17°C, where 98.3% of the egg-groups were found, as indicated by black host eggs. At 26°C, 96.1%, 90.6% and 81.1% of the egg-groups were found at release rates of 1000, 500 or 200 *T. evanescens*, respectively.

To estimate the number of female wasps who found single groups of host eggs, egg-groups were divided into two classes, those with less than 30 eggs parasitised (30) and those with more than 30 eggs parasitised (>30) (Figure 1). Classification was chosen because an unfed female parasitises on an average 25.13 eggs. At a release rate of 1000 *T. evanescens* more egg-groups rank with the class >30 at all depths of hosts. This is true only for host eggs at 1.0 cm depth at a release rate of 500 *T. evanescens*. At the 200 release rate, more egg-groups rank with the class 30 at all depths of hosts.

Rates of parasitisation

Depending on the position of host eggs in the wheat, temperature and release rate, the number of parasitised eggs differed significantly. Parasitism increased with release rate.

At 26°C, the rate of parasitisation at the 1000 release rate was 1.7 times the rate of parasitisation at the 200 release rate, and 22.4% more effective than the 500 release rate. At the 200, 500 and 1000 release rate, percentage of parasitisation of host eggs were recorded between the three depths of host eggs at the 1000 release rate. No interactions between release rate and depth of host eggs (analysis of variance, $p>0.05$) were detected.

At 17°C and the 1000 release rate, percentage of parasitism averaged 52.9%. Percentage of parasitism was significantly greater in 1.0 cm depth than in 2.0 cm ($p = 0.0005$) and 5.0 cm ($p = 0.0004$).

Comparing the two temperature conditions, average percentage of egg parasitism was significantly less at 2.0 cm depth of host eggs at 17°C ($p = 0.0057$) (Fig. 2), resulting in 9.6% more parasitised host eggs at 26°C.

Mortality of *Ephestia kuehniella*

Depending on depth of host eggs in the wheat, temperature and release rate, average percentage of eggs of *E. kuehniella* incapable in hatch differed significantly. Mortality increased with release rate and decreased with depth. No interactions between release rate and depth of host eggs ($p>0.05$) were detected (Table 1).

Mortality of *E. kuehniella* was significantly ($p<0.05$) higher at a release rate of 1000 parasitoids than at the 200 or 500 rate. The differences in average mortality between the 200 and the 500 release rate were not significant (Fig. 3).

Overall, 68.3% of the host eggs failed to hatch at 17°C compared to 79.7% at 26°C and a release rate of 1000. No interactions between temperature and depth of host eggs ($p>0.05$) were detected (Table 2).

Comparing the two temperature conditions, no significantly different average mortality was found between host eggs at 1.0 cm and 5.0 cm depth. At 2.0 cm depth, significantly ($p = 0.0005$) more egg larvae hatched at 17°C (Fig. 4).

Additional experiments with a 2000 release rate demonstrated the relationships of rate of parasitisation and infertility of host eggs to depth of host eggs (Fig. 5). Whereas the rate of parasitisation and the infertility decreased with depth of host eggs at the 200 release rate, parasitism increased and the infertility decreased with depth of host eggs at the 2000 release rate.

The effectiveness of *T. evanescens*-treatment decreased with depth of host eggs (Table 3). At 26°C at a release rate of 200, 500 or 1000 *T. evanescens*, it was 41.1% and 77.8%, respectively. At 17°C at a release rate of 1000, effectiveness was 66.96%.

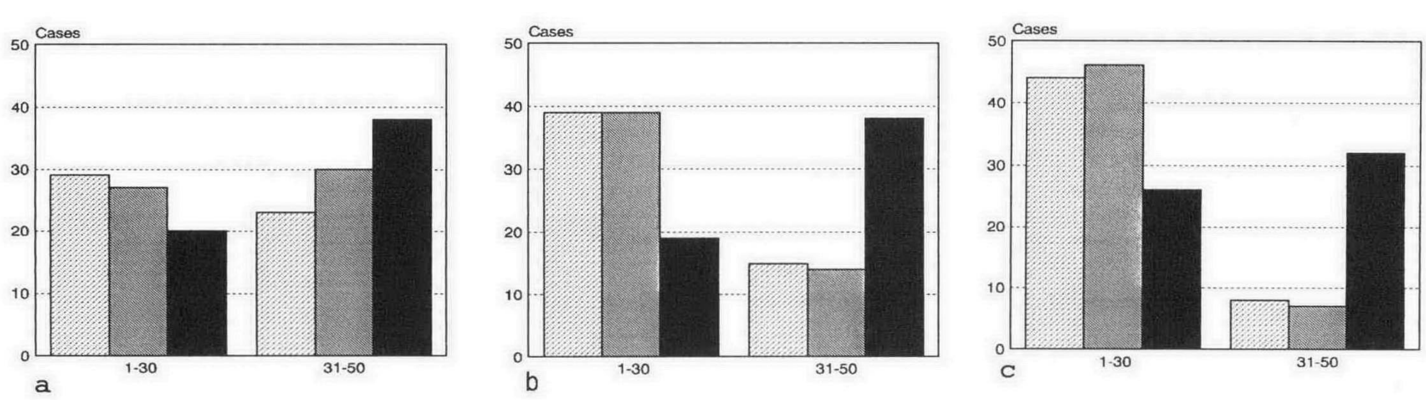

Fig. 1. Frequencies of parasitised eggs within egg-groups (50 eggs each). Egg-groups with 1–30 parasitised eggs and egg-groups with 31–50 parasitised eggs were summarised. 26°C, 75%±5% r.h.(a) Depth of host eggs 1.0 cm; (b) Depth of host eggs 2.0 cm; (c) Depth of host eggs 5.0 cm. 200 *T. evanescens*, 500 *T. evanescens*, 1000 *T. evanescens*.

Table 1. Analysis of variance for percentage of *E. kuehniella* mortality at 26°C at three parasitoid release rates (200, 500 or 1000 *T. evanescens*) and three host egg depths (1.0, 2.0 and 5.0 cm)

Source of variation	Sum of squares	df	Mean square	F	Sig. of F.	†
Release rate (R)	61395.34	2	30697.67	55.29	0.000	*
Position of host eggs (P)	14716.83	2	7358.42	13.25	0.000	*
2-way interactions R × P	2026.95	4	506.74	0.92	0.457	
Explained	78139.12	8	9767.39			
Residual	144922.07	261	555.26			
Total	223061.19	269	829.22			

† Level of significance * $P < 0.05$.

Table 2. Analysis of variance for percentage of *E. kuehniella* mortality at a release rate of 1000 *T. evanescens* at two temperatures (17°C or 26°C) and three host egg depths (1.0, 2.0 and 5.0 cm).

Source of variation	Sum of squares	df	Mean square	F	Sig. of F	†
Temperature (T)	5780.00	1	5780.00	11.86	0.001	*
Position of host eggs (P)	8296.13	2	4148.07	8.51	0.000	*
2-way interactions R × D	1887.60	2	943.80	1.94	0.147	
Explained	15963.73	5	3192.75			
Residual	84788.07	174	487.29			
Total	100751.80	179	562.90			

† Level of significance * $P < 0.05$

Table 3. The effectiveness of *Trichogramma evanescens* treatment, computed after Abbott (1925).

Egg-cage depth (cm)	200 *T. ev* 26°C n = 30	500 *T. ev.* 26°C n = 30	1000 *T. ev.* 26°C n = 30	1000 *T. ev.* 17°C n = 30
1	53.24	55.10	81.44	79.76
2	43.61	44.16	82.44	64.55
5	26.48	35.77	69.43	56.58

Experiments with a 100 cm cylinder

If cages of metal-gauze with host-eggs were displayed at 6.0 cm to 40 cm in the wheat column, parasitisation occurred at all depths. At the lower density of parasitoids, parasitised host-eggs were found only down to 25 cm depth. When cages were placed from 45 cm to 65 cm depth in steps of 5.0 cm, eggs were parasitised in 45 cm and at 55 cm depth. Host eggs were parasitised also in 55 cm, if the wheat column contained a cage only at this depth (Fig. 6). A male of *T. evanescens* was found in a cage at 55 cm depth.

Discussion

Trichogramma spp. are polyphagous egg parasitoids of lepidopteran species. In the field, females of *Trichogramma* search for host eggs on plants. They walk while host-seeking. The investigated strain of *T. evanescens* also penetrates into bulk wheat. Females are able to parasitise eggs of *E. kuehniella* at 55 cm depth in wheat. Parasitisation occurred both in wheat columns with egg-cards only in 55 cm depth and in those with several cages above this level simultaneously. If several cages were offered simultaneously, levels without parasitised host eggs were found between levels with parasitised eggs (Fig. 5). This could be due to the structure of bulk wheat, where the gaps between the kernels build channels. The wasps probably prefer walking through those channels while foraging. However, an accompanying study on the position of naturally laid eggs of *E. kuehniella* and *E. elutella* in bulk wheat and rye showed that the maximum depth of the pyralids eggs position is 8 cm, so the penetration ability of *T. evanescens* among bulk wheat is sufficient for a possible application as a biological control agent.

Parasitism averaged highest for both temperatures at the 1 cm depth, as expected. When the parasitoids were released at the surface of the bulk wheat, distance to these host eggs was shortest. Parasitism decreased with deeper position of host eggs. This was due to the number of egg-groups found by female parasitoids, but also to the number of females finding a single egg-group (Fig 1). Significantly higher parasitism at the 1000 release rate is attributed to parasitisation of single egg-groups by several females at the 2.0 cm and 5.0 cm depths. This is confirmed by the fact that at the 1000 release rate, in 77.8% of all egg-groups more than 48 eggs were parasitised, the observed maximum parasitisation by an unfed female of *T. evanescens*.

Brower (1990) found females of *T. pretiosum* Riley, a strain other than that occurring naturally in peanut warehouses, to penetrate at least 20 cm into inshell peanuts and locate and parasitise host eggs. Percent parasitism of 150 *Cadra cautella* (Walker) eggs averaged 28.3% at a 200 release rate and 46.9% at a 500 release rate.

At cool temperatures at the beginning of flight of the stored-product moths (Reichmuth et al. 1979), *T. evanescens* could be applied. It is known that *Trichogramma cacoeciae* (Quednau 1957) and *T. minutum* (Forsse, Smith and Bourchier 1992) don't fly at temperatures less than 20°C. If this applies also to *T. evanescens*, the already active parasitoids possibly remain in the warehouses.

The maximum average parasitism rate was 63.5% (1 cm cage depth, 1000 *T. evanescens*, 26°C). Brower (1990) found a maximum average parasitism rate of 63.3%. Also Quednau (1959) found in semi-field experiments, conducted with *Ephestia kuehniella* host eggs and one time inundative

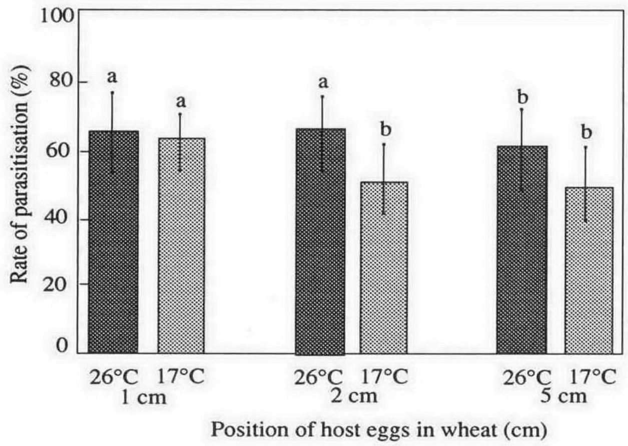

Fig. 2. Rates of parasitisation at a release rate of 1000 *Trichogramma evanescens* at 17°C or 26°C, 75%±5% r.h. Bars with the same letter are not significantly different; Mann-Whitney U-test (p = 0.05, 30 replicates).

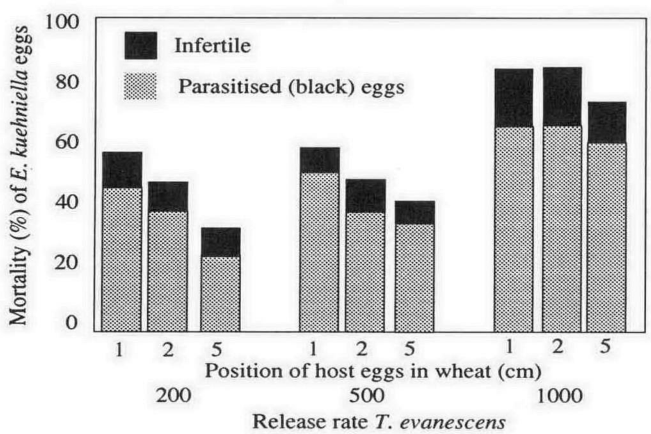

Fig. 3. Effect of *Trichogramma evanescens* on mortality of *Ephestia kuehniella* at three release rates, 26°C, 75%±5% r.h.

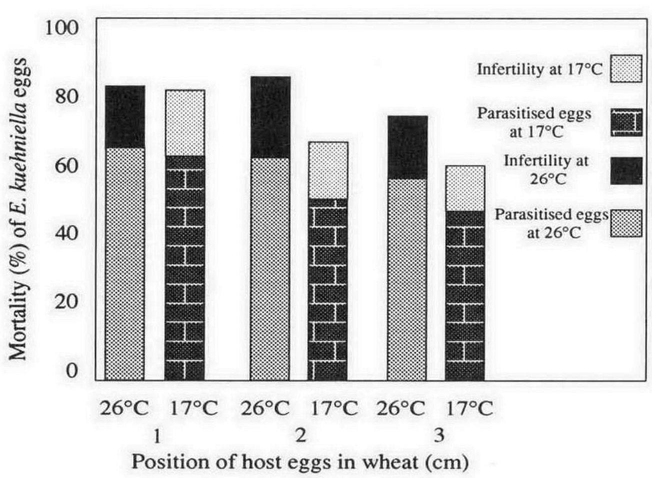

Fig. 4. Mortality of *Ephestia kuehniella* at a release rate of 1000 *Trichogramma evanescens* at 17°C or 26°C and 75%±5% r.h.

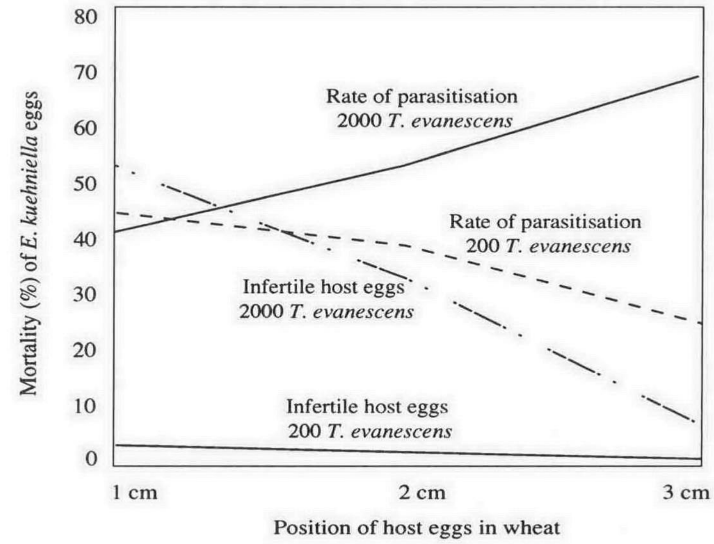

Fig. 5. Relation of rate of parasitisation and infertile host eggs corrected (Abbott 1925) for infertility of control eggs at release rates of 200 and 2000 *Trichogramma evanescens*, 26°C and 75%±5% r.h.

Depth of cage (cm)	Experiment 2.V.1993 Density 1	Experiment 27.V.1993 Density 2	Experiment 11.VI.1993 Density 1	Experiment 9.VII.1993 Density 2
6	●	●		
8	●	●		
10	●			
15	●	●		
20	●			
25	●	●		
30	○	●		
35	○	●		
40	○	●		
45			●	
50			○	
55			●	●
60			○	
65			○	

Parasitised eggs ●, Non-parasitised ○

Fig. 6. Parasitisation of host eggs at different cage depths. The depths indicated by frames contained cages with host eggs of *Ephestia kuehniella*. Density 1 = 30–3000 *T. evanescens*, Density 2 = 95–3000 *T. evanescens*.

releases of high *Trichogramma* rates, a maximum mortality of host eggs of 80%.

In this study, significantly higher mortality of *E. kuehniella* was found at the 1000 release rate at 1.0 cm and 2.0 cm compared to 5.0 cm depth, and compared to the 500 release rate and the 200 release rate. A possible reason for these limited rates of parasitisation indicated by black host eggs and increased mortality at 1.0 cm and 2.0 cm depth is superparasitism. In normally solitary egg parasitoids, superparasitism has been shown to result in neither development of the host nor of the parasitoid (Salt 1936). This is confirmed by some experiments conducted with a 2000 release rate (Fig. 4), where parasitism increased with depth of host eggs.

The higher mortality of *E. kuehniella* at 26°C compared to 17°C is due to the significantly higher rate of parasitisation at 26°C. Activity of females of *Trichogramma* is supposed to be higher at 26°C. Biever (1972) found all tested females of *T. evanescens* conducting host seeking behaviour at 25°C, 30°C and 35°C, but only 80% of them at 20°C. Pinnell (1970) found *T. evanescens* to be ineffective in field releases when daily minimum temperatures decreased below 15.5°C.

Trichogramma egg parasitoids hit the target pest at the egg stage, so ideally no frass damage caused by larvae of the stored-product moth occur. The mass release of *Trichogramma* is a preventative technique (Brower 1984b). The minute size of these wasps allow their separation from the stored products after the storage period together with dust and other particles. One could imagine, for example, a treatment of food grain, similar to the one used in this study, because the economic threshold level is higher in this product.

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