New species and new methods of application – a new chance for *Trichogramma* in Codling Moth Control?

J. Kienzle,[1] O. Zimmermann[2], B. Wührer[2], P. Triloff [3]; J. Morhard[4]; E. Landsgesell[4]; C.P.W. Zebitz[5]

Abstract

Diversification of methods of codling moth control is urgently needed in organic fruit growing. Since the application of sulphur products in late summer is not necessary on scab-resistant varieties, augmentative releases of the sulphur-sensitive beneficial Trichogramma *species are possible in commercial growing.* Trichogramma evanescens*, occurring naturally in the orchards, showed promising capacities in codling moth control, as revealed in field trials with infestation simulated using "bait apples" with natural codling moth eggs deposited by females obtained from a laboratory rearing. Furthermore, an application method of spraying commercially produced parasitized eggs was developed using large nozzles, low pressure and a hydrocolloid based on 2 % Xanthan and 0,01 % Tween. Field tests with natural infestation are ongoing.*

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Introduction

The utilization of *Trichogramma* species for codling moth control is known since more than twenty years (HASSAN et al., 1988). Up to now, the species *Trichogramma dendrolimi* and *T. cacoeciae* were mixed to assure an effective short and long term control. The method has not been implemented in practical use on larger areas because of high costs and erratic efficacy. Furthermore, *Trichogramma* species are sensitive to sulphur application used in scab control, which is the dominating fungicide in organic fruit growing. Problems arising from *Cp*GV- and insecticide resistance of codling moth in integrated fruit growing, the interest in using *Trichogramma*-egg parasitoids increased. From 2007 til 2011, a project coordinated by AMW Nuetzlinge in cooperation with Marktgemeinschaft Bodenseeobst, the Institute for Biological Plant Protection of the JKI and the University of Hohenheim was established to improve the application techniques for *Trichogramma*-releases. Different strains and species of *Trichogramma* have been tested on their efficacy in codling moth control. Furthermore, a new application system by spraying the beneficials in a liquid formulation was developed, based on results from Moser (1980).

Material and Methods

In 2007 and 2008, first field trials to ***compare the efficacy of different strains and species*** of *Trichogramma* have been conducted. In a randomized block design 4 trees per plot with 4 replications on four varieties (*viz.* "Topaz", "Melrose", "Idared", "Berlepsch")

[1] Apfelblütenweg 28, D-71394 Kernen, jutta@jutta-kienzle.de
[2] AMW Nützlinge GmbH, Außerhalb 54, D-64319 Pfungstadt
[3] Marktgemeinschaft Bodenseeobst eG, Albert Maier Str. 6, D-88045 Friedrichshafen
[4] University of Hohenheim, Institute of Agricultural Engineering, Process Engineering in Plant Production (440d), Garbenstr. 9, D-70599 Stuttgart
[5] University of Hohenheim, Institute of Phytomedicine, Dep. of Applied Entomology, Otto Sander Str. 5, D-70599 Stuttgart

were treated. Between the plots, 7 trees and between the tree rows, one “untreated” tree row were left as a barrier. On each treated tree, one cardboard with 500 *Trichogramma* pupae obtained from AMW (shortly before adult emergence) and 6 “bait apples” were fixed (max. 24 apples per replicate / 96 apples per variant). To obtain “bait apples”, the fruits were picked from the trees and exposed to *Cydia pomonella* gravid females for oviposition overnight. Until the next morning, usually 3 to 10 eggs were laid per fruit. Subsequently after oviposition, the apples were fixed on the trees with florist wire and marked with a clothespin.

At the “red ring” developmental stage of the eggs, the apples were removed and incubated in the laboratory until larval hatch of *C. pomonella*. When parasitization was observed (blackened eggs) the respective part of the fruit skin was removed carefully, put into a glass tube and closed with cotton until the *Trichogramma* emerged. The *Trichogramma* specimens have been determined by electrophoresis of PCR-amplified fragments of the ribosomal DNA internal transcribed spacer 2 (ITS-2) (Silva et al. 1999, Li 2007; and own protocols). Baiting was done three times after *Trichogramma* release replicates in 5 days intervals not only the number of apples found by the different species but also to observe the efficiency as mediated by time.

To observe the distribution of the beneficials, cards with ca. 1,500 pupae of *Trichogramma* (shortly before emergence) were fixed at every 13th tree in four rows. In each of the two middle rows, in 13 adjacent trees two “bait apples” per tree were placed (see Fig. 2).

To develop a spray-application method**,** it was necessary to obtain a steady distribution of *Trichogramma* pupae in a liquid gel formulation (Zimmermann, 2010a, 2010b). On pure water as carrier, pupae were floating. Thus, a solution with the optimum viscosity had to be found, which would also not affect the emergence of *Trichogramma*. Furthermore, spraying pressure had to be optimized because a too high pressure may also reduce the emergence rate of the *Trichogramma* pupae.

In cooperation with the Institute fof Agricultural Engineering of the University of Hohenheim, an optimal solution for the carrier liquid and spray pressure should be found. Gel solutions with *Trichogramma* pupae were sprayed at different pressures and the sprayed pupae were collected using a mesh covered by a card web. The emergence rate was determined and compared with the emergence rate of “dry pupae” and of pupae that had just been dropped in pure water were taken as control.

Results

After the determination of the baited *Trichogramma* species, it became obvious, that one species found, has not yet been released: *Trichogramma evanescens* occurred naturally. Because of the high natural density and the regularly observed abundance in vineyards, this species was included into the trials.

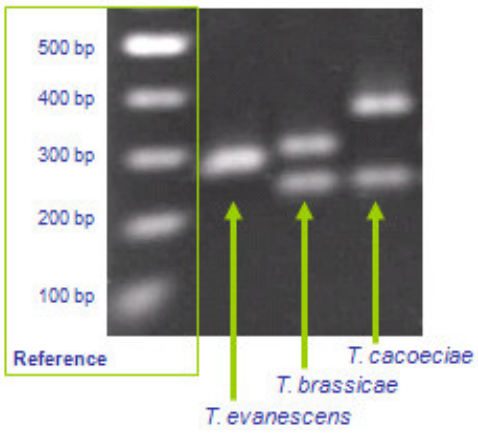

Figure 1: Gel electrophoresis of ITS-2 PCR products

In laboratory tests, this species showed best results in parasitization of codling moth eggs of all the 26 species and strains of *Trichogramma* tested.

In 2008, *T. evanescens* showed best results of all tested strains and species in the field. During the first exposure of “bait apples”, this species and one strain of *T. cacoeciae* showed a significantly better finding rate (as indicated by blackened =

parasitized eggs). Also in the second and third repeat of “bait apples”, *T. evanescens* showed a higher efficacy than the other species (Table 1). These results were confirmed by other trials in 2009. Even in the control, some eggs were parasitized (species not determined by PCR analysis).

Table 1: Percentage of “bait apples” found by different species/strains of *Trichogramma* (as indicated by any blackened eggs) during three periods starting 2 days after releasing *Trichogramma* pupae on cardboards. (Same indices in a column indicate no significant differences; oneway-ANOVA, followed by TUKEY-KRAMER HSD-test)

Trichogramma species/strain	20. - 25.7.2008	25. – 30.7.2008	30.7. - 6.8.2008
T. evanescens DE 07 W	100,0 a	82,3 a	41,7 a
T. brassicae	46,9 b	47,2 a	25,4 a
T. cacoeciae DE 90 O	46,6 b	50,0 a	33,3 a
T. cacoeciae DE 07 CAC W	93,8 a	40,6 a	18,8 a

Due to these observations, in the end of 2008 and in 2009 field trials were designed to observe the distribution of *T. evanescens* in the rows, if released with cardboard cards.

It could be observed, that *Trichogramma* dispersed rather good within the tree row, but also in the parallel tree row directly opposite to the trees with cards, parasitized eggs were found (figure 2).

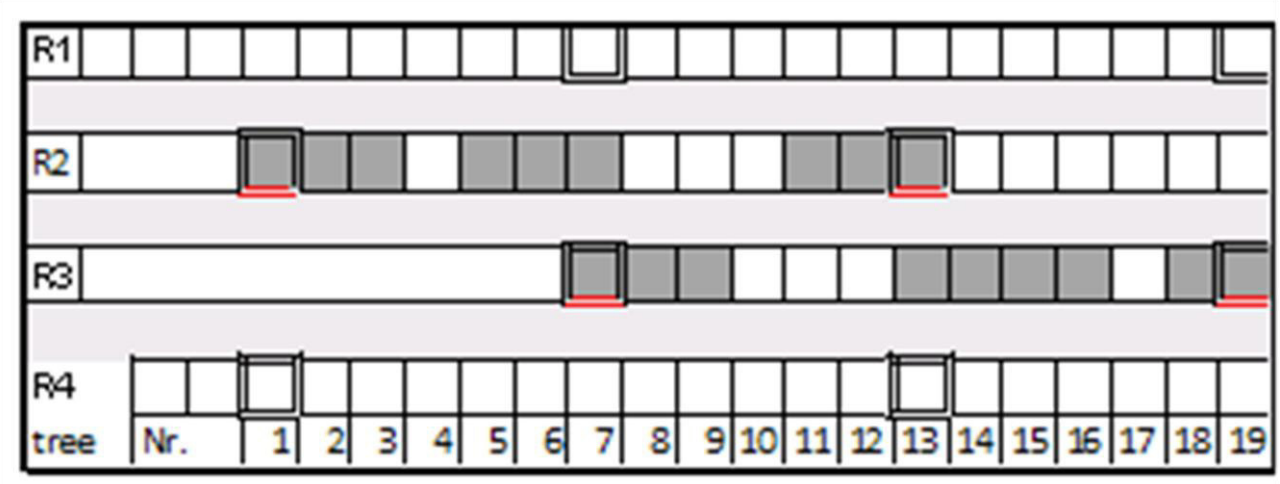

Figure 2: Trial to observe the distribution of *Trichogramma evanescens* in an orchard, variety Jonagold (cards with ca. 1,800 pupae per 13th tree): The double prime frame marks the trees with a *Trichogramma* card, dark colour marks trees where the “bait apples” were found.

Application technique

The comparison of three different hydrocolloids showed no significant difference. Because guar bean flour (E 412, from *Cyamopsis tetragonolobus*) may exert allergic reactions, Xanthan, (E 415, bacterial fermentation from *Xanthomonas campestris*) was preferred. The addition of Tween to Xanthan hydrocolloid resulted in smaller droplets and a better distribution of the spray plume. Since large droplets dry off very slowly and therefore might compromise the emergence rate, the addition of Tween seemed advisable.

In field trials with this hydrocolloid, the distribution of the droplets in the canopy was assessed. Using one TEEJET 11015 flat fan nozzle with a large opening, mounted in an approx. 45° upward facing angle at each side of a standard axial fan orchard sprayer,

operated at 3 bar spray liquid pressure, an air flow rate of approx. 25.000 $m^3 h^{-1}$ at 400 min^{-1} PTO and a forward speed of 5 km h^{-1}, most of the hydrocolloid droplets have been deposited at the lower leaf surface. This is important to reduce losses from rainfall and predators since in trials not presented here, predation of the pupae was much lower when deposited at the lower leaf surface. The position of the nozzle depends on the canopy characteristics. Usually it should be mounted at the 4th or 5th nozzle position from the bottom.

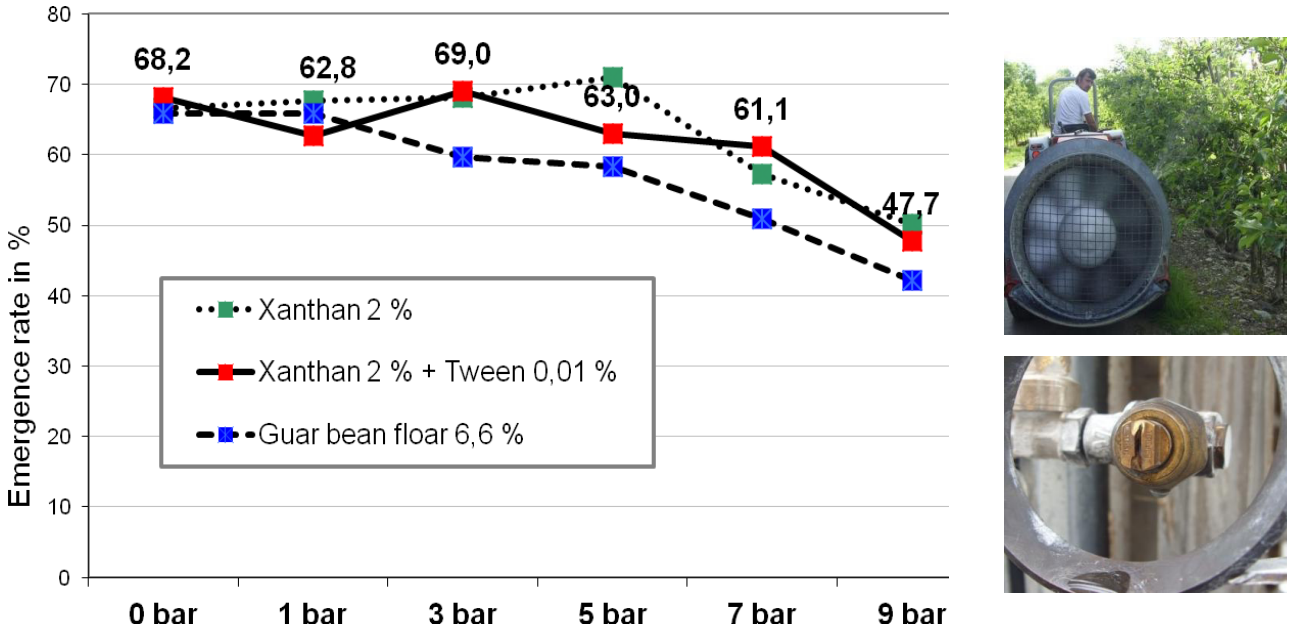

Figure 3: Emergence rate of *Trichogramma* (in %) with different hydrocolloids and pressure levels (in bar). The emergence rate out of dry eggs (control 1) was 88,2 %, out of eggs dipped in water (control 2) was 79,3 %. At the lower right side a picture of the nozzle (TEEJET 11015) used for the experiments. At the upper right side a picture of the application with orchard sprayer.

Discussion

Trichogramma evanescens showed a promising potential for codling moth control in the field. Although this species was not mass released in orchards before these studies, it occurs frequently in apple orchards and vineyards. It was more effective than *T. cacoeciae*, although they reproduce sexually (only 50 % females released, compared to 100% in parthenogenetic species) and exhibit a shorter lifespan. Nevertheless, in the field tests, *T. evanescens* showed a better parasitization of the “bait apples” exposed at a certain distance from the point of release. In all trials, the weather conditions were not favourable with periods of rain. The better efficacy of *T. evanescens* in our tests might also be due to a better tolerance of this species to unfavourable weather conditions.

Generally, the persistence of *Trichogramma* was not longer than 14 days (stage of released pupae was shortly before adult emergence to reduce loss by predation).

Due to the potential of distribution observed for *T. evanescens*, which seems to be much larger than the distribution of other species (Wetzel et al., 1995; Sakr, 2002), *Trichogramma* cards seem an interesting option again. In this case, a mixture of different developmental stages is advisable to expand the persistence and compensate possible losses by adverse climatic conditions. For spraying application, the first trials have started with material with different development stages, a definitive conclusion, however, is actually not possible.

Generally, it seems that *Trichogramma* cards are more appropriate when the canopy is small and rainfall is to be expected. The spraying application seems best when the canopy is large, perhaps due to a better distribution of the parasitoids.. It is also more suitable for large areas and growers that have few attitude to manual work.

In organic fruit growing, especially on scab resistant varieties other products than sulphur can be used for disease control at the end of summer. Thus, actually, in the framework of the BOELN-project 2809OE098 the application of *T. evanescens* in *Trichogramma* cards and with spray application is tested for the control of fruit damage in the second generation of codling moth. Since the infestation level of codling moth in most orchards in 2011 was very low, definitive results are not yet available.

Acknowledgements

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