HOW TO ASSESS NON-TARGET EFFECTS OF POLYPHAGOUS BIOLOGICAL CONTROL AGENTS: TRICHOGRAMMA BRASSICAE AS A CASE STUDY

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ABSTRACT

We show key elements of the risk assessment conducted for Trichogramma brassicae Bezdenko (Hymenoptera: Trichogrammatidae), an egg parasitoid which is successfully used for control of the European corn borer in European countries. The main factors that we addressed in this study were: the potential of establishment; acceptance and parasitism of non-target butterflies under laboratory, field-cage and field conditions; the searching efficiency in non-target habitats; the dispersal capacities; and the potential for effects on other natural enemies in maize.

Although high parasitism of non-target butterflies and other natural enemies were observed under laboratory conditions, very few eggs of the non-target species were attacked in the field. These findings may be explained by a low host searching efficiency and the observation that female T. brassicae do disperse only a few meters per day. We conclude that the possibility of using invertebrate agents with a broad host range in inundative biological control should not a priori be excluded, however, a thorough environmental risk assessment should be performed prior to release.

INTRODUCTION

Egg parasitoids of the genus Trichogramma are used for inundative biological control against a range of agricultural pests. In fact, Trichogramma spp. are the most widely used natural enemies in inundative biological control worldwide and both native and exotic species have been mass reared and released. The vast majority of Trichogramma species are known to be polyphagous attacking a wide range of lepidopterans as well as insects belonging to other orders (e.g., Thomson and Stinner 1989). Due to this wide host range concerns have been expressed already several years ago that mass released Trichogramma may threaten non-target species in natural habitats (Andow et al. 1995; Orr et al. 2000).
Concerns about detrimental effects of introduced species on the native fauna have been increasingly expressed over the last two decades. There is now general agreement that the potential for non-target effects has to be evaluated before releasing biological control agents. During the last 10 years, several guidelines addressing non-target effects have been developed. For instance, the Organisation for Economic Co-operation and Development (OECD) developed guidelines to provide ‘light regulation’ for invertebrates used in classical and inundative biological control (OECD 2004). Despite these initiatives which basically aim to provide guidance on what data should be considered for environmental risk assessment, there is still a debate on how these data can be obtained. Van Driesche and Reardon (2004) provided a ‘guide to best practice’ on how to conduct host specificity testing which generally forms an important part of the risk assessment. Babendreier et al. (2005) recently published a comprehensive review on the methods used to assess non-target effects in biological control and a book in which questions on environmental risk assessment of arthropod biological control are addressed will be published in the near future (Bigler et al. 2006).

In this paper we summarize key elements and results of an environmental risk assessment project conducted for Trichogramma brassicae Bezdenko (Hymenoptera: Trichogrammatidae) in Switzerland from 1998 to 2002 which was part of the EU funded project ‘Evaluating Environmental Risks of Biological Control Introductions into Europe’ (ERBIC).

**PRIME FACTORS FOR NON-TARGET EFFECTS**

Host specificity is one of the bottom lines in the assessment of non-target effects (Van Driesche and Reardon 2004; Van Lenteren et al. 2006) and hence, only agents with a narrow host range are considered for release in classical biological control. However, less specific agents are sometimes used in inundative biological control. One example is *T. brassicae* which is used since many years in European countries for control of the European corn borer, and it is known that this species attacks eggs of other lepidopterans and other non-target insects. We tested host acceptance and parasitism of *T. brassicae* on non-target butterflies and predators in maize fields under laboratory, semi-field and field conditions. Further we hypothesized that host searching efficiency in non-target habitats could be another important factor responsible for adverse effects on non-target butterflies.

In contrast to classical biological control, overwintering and establishment are negative properties of non-native agents if used for inundative biological control. If establishment does not occur, the risk for non-target species is limited to the period of release and possibly the following weeks if females can reproduce on target or non-target hosts in the crop or in other habitats. Therefore the risk of non-target impacts is spatially limited and of transient nature (Lynch et al. 2002). If *T. brassicae* would be able to survive the winters, reproduce on non-target host eggs in the area of introduction and disperse, there is potential for permanent effects on a large geographical scale.
OVERWINTERING

In order to test for the ability of *T. brassicae* to establish in Switzerland, two experiments were conducted. The first one was designed to study whether *T. brassicae* would survive outdoor winter conditions in Switzerland. Eggs of six non-target host species parasitized in the laboratory by *T. brassicae* were exposed under outdoor conditions (in Zurich) every two weeks between 26 September and 7 November. Control eggs were kept in an environmental chamber at 25 °C, 70% RH (for details see Babendreier et al. 2003a).

We found that *T. brassicae* is able to overwinter successfully on eggs of six lepidopteran species in the families Tortricidae, Noctuidae, Plutellidae, Pyralidae and Crambidae. Between 75% and 100% emergence was observed in the following spring for all of the six host species exposed on 26 September. On later exposure dates, spring emergence decreased significantly and no development of *T. brassicae* occurred from host eggs parasitized on 7 November.

In a second experiment, we evaluated at what time of the year diapause induction under field conditions occurs. Eggs of the flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were offered to *T. brassicae* females at five consecutive dates at weekly intervals from 27 August to 24 September. After parasitization in the laboratory, the eggs were exposed under outdoor conditions until emergence occurred. We found that the period of diapause induction is equal to the dates which allowed successful development and overwintering of *T. brassicae*. In order to evaluate the effect of overwintering on the fitness of females that had spent the winter in diapause inside the eggs of *E. kuehniella* under outdoor conditions from 17 September 1999 to beginning of May 2000, we measured the fecundity of 30 females. Fecundity of females that overwintered outdoors was not significantly different from the fecundity of females that were reared in the laboratory without diapause at 25 °C.

Our results demonstrate that the egg parasitoid *T. brassicae* is able to overwinter successfully in northern Switzerland and that it has the potential to establish if host eggs were available.

PARASITISM OF NON-TARGET BUTTERFLIES

Since *T. brassicae* was known to be polyphagous, we concentrated on butterflies because of the strong environmental concerns for this group of insects. We exposed eggs of 23 non-target lepidopteran species, including nine endangered species of Switzerland, to single *T. brassicae* females under no-choice conditions in the laboratory (Babendreier et al. 2003b). Most of the species were well accepted and parasitized at the same level as the target, *Ostrinia nubilalis* Hübn (Lepidoptera: Crambidae). In addition to oviposition, we also measured the number of times a female rejected a host egg before acceptance as well as the time from first host egg contact to acceptance.

In a next step, we investigated parasitism of six non-target butterfly species by *T. brassicae* in field cages of 2 x 2 x 2 m (Babendreier et al. 2003c). Eggs of the non-targets were glued on...
host plants together with E. kuehniella eggs (multiple choice) and exposed for 24 hours to the females. Parasitism of non-target species in field cages ranged between 2.5% and 18.7%. We found that parasitism was density dependent.

Field trials were then carried out in maize fields and adjacent meadows (Babendreier et al. 2003c). We released 30,000 female T. brassicae in a plot of 50 x 50 m. This corresponds to the number of females released in commercially treated maize fields. All release plots were situated inside the maize fields but bordering the meadows. We exposed eggs of two non-target hosts together with eggs of E. kuehniella as a control. Eggs were exposed for 3 days at 2 m distance inside the maize field and at 2 m and 20 m distance outside the maize field in the meadow. At each distance, we attached 30 single eggs of the non-tar\-gets and 30 egg masses of E. kuehniella (50 – 100 eggs each). As a control for natural occurrence of Trichogramma spp., we placed 30 egg masses of E. kuehniella on leaves of maize plants in two fields that were 1-2 km away from the treated fields.

Parasitism rates of E. kuehniella egg masses inside maize fields averaged 40% compared to significantly lower parasitism rates of 26.2% and 12.6% for eggs of the two non-targets. In the meadow, at 2 m distance from the maize field, parasitism rates decreased to 2.3% and 6.1% for the non-targets and 9.8% for E. kuehniella while no single egg was found parasitized in the meadow at 20 m distance from the maize field.

**HABITAT SPECIFICITY**

In order to evaluate whether the low parasitism in meadows can be generalized and to understand the underlying mechanisms, we studied the searching efficiency of T. brassicae in several non-target habitats such as meadows, flower strips and hedgerows. At the same time, T. brassicae was released at rates of 120,000 females/ha in plots of maize and one of the selected non-target habitats (plot size 24x24 m). Sentinel egg clusters of E. kuehniella were applied to the plants and recollected after 3 days. Parasitism of sentinel egg clusters was 1.6 - 3.6% in meadows and 2.0 - 4.0% in flower strips while the respective figures were 57.6% - 66.7% and 19.2% - 46.9% in maize (Babendreier et al. 2003d). Subsequent field cage experiments confirmed the higher parasitism rates in maize compared to meadows, flower strips and hedgerows.

To investigate the factors responsible for the low parasitism in non-target habitats, the behavior of individual T. brassicae females was observed on common meadow plants. Single females were directly observed on different plants and parameters such as mean walking speed, turning angles and number of wasps leaving the plants were measured (Babendreier et al. 2003d). Significant differences in these variables were found between maize and four meadow plants. The most pronounced effects were found between maize and red clover, a very common plant in meadows in Switzerland with very hairy leaf surface. In a laboratory choice experiment, carried out with all five host plant species together in cages, we obtained highest parasitism on maize and lowest on red clover, confirming the behavioral observations.
DISPERAL

While dispersal is a prerequisite of a successful classical biological control agent, it may be a negative feature in the context of non-target effects of inundatively released agents. The ultimate question is how many released biological control agents will enter a given non-target habitat or, more precisely, what densities of the agent can be found in certain distances from the release fields. To answer this question, experiments were carried out to investigate the dispersal behavior of \textit{T. brassicae} (Babendreier et al. 2002; Kuske et al. 2003; 2004; Mills et al. 2006). The first experiment aimed to establish the degree to which \textit{T. brassicae} will leave maize fields where they were released. Traps consisting of a plastic transparent sheet (30 x 21 cm), sprayed with glue on both sides, were placed at the edge of a maize field. These traps were mounted on wooden sticks at a height of 40-70 cm and positioned inside the field (0.8 m from the edge), at the edge of the field and outside the field (0.8 m from the edge). After one week the numbers of male and female \textit{T. brassicae} on each side of the trap were counted. The results indicated a strong decrease in numbers from inside to outside of the maize field.

Kuske et al. (2003) increased the scale of this experiment and placed traps of the same type at distances up to 40 m away from the edge of maize fields. Traps were placed directly above the vegetation and exposed for one week before and during the first and the second commercial release of \textit{T. brassicae} as well as for three weeks following the second release. A strong decrease in numbers with distance was observed and, altogether, it can be concluded from these experiments that a large fraction of \textit{T. brassicae} will not leave the field. Moreover, the experiments have shown that \textit{T. brassicae} will be present in non-target habitats close to the release field only for one or two weeks after releases.

In order to investigate the distance that individual \textit{T. brassicae} travelled in a given time period, about 100,000 wasps were released from parasitized eggs from a central release point in a meadow (Babendreier et al. 2002). Sticky traps that had been placed at distances of 2, 4, 8, 16, 32 and 64 m in four directions from this release site were changed daily and all \textit{T. brassicae} that had been collected were counted. This experiment revealed that \textit{T. brassicae} only flies a few meter per day (Mills et al. 2006). Finally, sticky traps were used to study whether hedgerows may act as a barrier for dispersing \textit{T. brassicae} (Babendreier et al. 2002).

INTRAGUILD PREDATION AND INDIRECT EFFECTS

After demonstrating that non-target effects will most likely be restricted in space and time, we decided to conduct a final experiment on potential effects on populations of other natural enemies in maize. In a tiered approach, experiments were conducted on the host acceptance of \textit{T. brassicae} towards eggs of \textit{Chrysoperla carnea} Stephens (Neuroptera: Chrysopidae), \textit{Episyrphus balteatus} (De Geer) (Diptera: Syrphidae), \textit{Coccinella septempunctata} L. and \textit{Adalia bipunctata} L. (both Coleoptera: Coccinellidae) under laboratory, greenhouse cages and field conditions (Babendreier et al. 2003e). While no offspring emerged from eggs of \textit{A. bipunctata}
and C. septempunctata, high parasitism rates were obtained for C. carnea and E. balteatus eggs in laboratory experiments. However, we observed significantly increased mortality on A. bipunctata eggs, compared to the control and also found young instars of T. brassicae inside A. bipunctata eggs. In a second experiment where the host acceptance behavior of the parasitoid female was directly observed for 10 min, 10% of T. brassicae females were found to oviposit in eggs of A. bipunctata but development of parasitoid offspring failed.

In greenhouse cages, parasitism rates of C. carnea eggs (7%) and E. balteatus eggs (0.4%) were significantly lower than parasitism of E. kuehniella eggs (21 and 27%, respectively) that were used as a control in the two experiments. In the field, only 3.1% of C. carnea eggs were parasitised by T. brassicae. This was significantly less than the observed parasitism rate of E. kuehniella egg clusters (64%). From direct observations of the parasitoids host acceptance behavior and the low parasitism rates observed in cages and under field conditions we conclude that ecologically relevant adverse effects of mass released T. brassicae on natural enemies in maize are unlikely to occur.

Finally, we aimed to assess the potential for negative effects on the native larval parasitoid Lydella thompsoni Hert. (Diptera: Tachinidae) (Kuske et al. 2004). In Switzerland, this tachinid was found to develop the first generation on the two non-target lepidopteran species Archanara geminipuncta Haworth (Lepidoptera: Noctuidae) and Chilo phragmitellus Hb. (Lepidoptera: Crambidae) living on common reed plants, Phragmites australis (Cav.), while subsequent generations attack the European corn borer in maize. Severe parasitism of the two non-target lepidopterans by T. brassicae, immigrating from maize fields into reed habitats could lead to negative effects on the tachinid due to competition. Under laboratory conditions, both non-targets were found to be suitable hosts for T. brassicae. However, parasitism rates were low, either because eggs are hidden between leaf sheaths and the stalk of the host plant or because of low attractiveness of the eggs. Field experiments and surveys of the two non-target lepidopteran species were conducted in common reed habitats located amongst maize fields with T. brassicae releases. No single egg of the two non-target species was found parasitized, indicating that negative effects on the native tachinid due to mass releases of T. brassicae are unlikely.

CONCLUSIONS

We have provided an example on how to conduct a full environmental risk assessment for a polyphagous biological control agent. The study on non-target effects of T. brassicae mass releases demonstrates that the final conclusion on environmental risks could be drawn only after investigating host range, establishment, dispersal and competition in laboratory and field experiments. We have evidenced that low dispersal capacities and low host searching efficiency in non-target habitats were the main determinants to explain the relatively low level of risk associated with this egg parasitoid. Our results indicate that the structural complexity of the plants and of the habitat play a role for the low searching efficiency. We conclude that the possibility of using agents with a broad host range in inundative biological control should not a priori be excluded, however, a thorough environmental risk assessment should be performed prior to release.
REFERENCES


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