

Non-target effects of mass releasing *Trichogramma brassicae* (Hym., Trichogrammatidae) against the European corn borer

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Summary

Inundative releases of *Trichogramma brassicae* against the European corn borer (ECB) in maize in southern Switzerland were evaluated for potential detrimental effects on the native non-target arthropod fauna. It was found that a considerable number of *T. brassicae* move out of the crop following such releases, which leads to a transient inundation of non-target habitats. We further demonstrated that these parasitoids can overwinter in southern Switzerland and persist in non-target habitats, even though they were introduced more than ten years ago without the intention of establishing resident populations. However, we found that the native common reed stalk borers *Archanara geminipuncta* and *Chilo phragmitellus* in natural wetlands near to maize fields are not threatened by invasive *T. brassicae*. The eggs of *A. geminipuncta* are so well hidden between leaf sheaths and the stalk of their host plants that they are not accessible for *T. brassicae*, whereas the eggs of *C. phragmitellus* were not attractive. In addition, we found no harmful effects for the indigenous parasitic fly *Lydella thompsoni*, which is an important natural enemy of the ECB throughout southern Europe. In southern Switzerland, this larval parasitoid relies upon the presence of these two stalk borers, which occur in pristine reed habitats, for its first annual generation. Our results imply that the pest control potential of *L. thompsoni* has been largely underestimated and can be enhanced if the number of habitats with alternative hosts of this parasitoid is increased and if a favourable spatial arrangement of these habitats in the agro-ecosystem is implemented. Landscape management that favours *L. thompsoni* combined with inundative releases of *T. brassicae* is therefore considered a highly effective and environmentally sound strategy for the biological control of the ECB and may further reduce the need for pesticides against this important pest.

Zusammenfassung

Massenfreilassungen von *Trichogramma brassicae* gegen den Maiszünsler im Tessin wurden auf mögliche Gefahren für einheimische Nicht-Zielorganismen untersucht. Ein beachtlicher Anteil der ausgebrachten Nützlinge verliess nach den Freilassungen die Maisfelder und hielt sich vorübergehend in grosser Zahl in angrenzenden Nicht-Zielhabitaten auf. Eine kleine Zahl der Nützlinge konnte dort überwintern und im Folgejahr erneut nachgewiesen werden. Es ist anzunehmen, dass sich *T. brassicae* im Untersuchungsgebiet seit der ersten Freisetzung vor mehr als zehn Jahren angesiedelt hat, obschon ihre dauerhafte Ansiedlung damals nicht angestrebt wurde. Die Auswanderung von freigesetzten *T. brassicae* in angrenzende Feuchthabitate stellte allerdings keine Gefährdung dar für die dort lebenden einheimischen Schilfstengelbohrer *Archanara geminipuncta* und *Chilo phragmitellus*. *A. geminipuncta* legt die Eier versteckt unter die Blattscheiden von Schilf ab, wo sie für *T. brassicae* fast unzugänglich sind. Eigelege von *C. phragmitellus* sind wohl ähnlich wie die des Maiszünslers, wurden von *T. brassicae* in der Regel aber schon beim ersten physischen Kontakt abgelehnt. Es konnten zudem keine unerwünschten Auswirkungen von *T. brassicae* Massenfreisetzungen auf die einheimische Raupenfliege *Lydella thompsoni* festgestellt werden. Diese Larvalparasitoide ist in ganz Südeuropa ein wichtiger natürlicher Feind des Maiszünslers, wobei sie für die erste Generation im Frühling zwingend auf die Raupen der Schilfstengelbohrer angewiesen ist. Unsere Resultate deuten an, dass das Potenzial von *L. thompsoni* für die biologische Kontrolle des Maiszünslers bisher deutlich unterschätzt wurde und dass eine Steigerung der Maiszünslerkontrolle erreicht werden könnte, indem die Zahl der Habitate mit Alternativwirten erhöht und ihre räumliche Verteilung im Agrarökosystem optimiert würde. Eine gezielte Förderung von *L. thompsoni* in Kombination mit *T. brassicae* Massenfreisetzung darf als äusserst effiziente und umweltfreundliche Strategie bei der biologischen Kontrolle des Maiszünslers angesehen werden und könnte vielerorts zur Reduktion des Pestizideinsatzes gegen diesen wichtigen Schädling beitragen.

Riassunto

Il presente lavoro ha avuto lo scopo di valutare i potenziali effetti negativi che i lanci inondativi di *Trichogramma brassicae* contro la piralide del mais possono esercitare sull'artropodofauna utile nel sud della Svizzera. E' stato dimostrato come un elevato numero di parassitoidi sia in grado di migrare dalla coltura dopo i lanci, causando una temporanea invasione degli ambienti *non-target*. E' stato inoltre evidenziato che questi parassitoidi possono svernare in Ticino e sono in grado di persistere negli ambienti *non-target*, benché la loro acclimatazione non fosse stata prevista al momento dei primi lanci, più di dieci anni fa. Comunque, le nostre ricerche hanno evidenziato come le specie native *Archanara geminipuncta* e *Chilo phragmitellus* non siano attaccate dagli individui di *T. brassicae*. Le uova di *A. geminipuncta* risultano essere nascoste talmente bene fra le foglie e il culmo delle loro piante ospiti, da risultare inaccessibile per *T. brassicae*, mentre le uova di *C. phragmitellus* sono risultate non attrattive. Inoltre non sono stati trovati effetti dannosi nei confronti del parassitoide indigeno *Lydella thompsoni*, un importante antagonista della piralide in Europa meridionale. Nel sud della Svizzera, questo parassitoide larvale dipende da questi due fitofagi endofiti che vivono nei canneti naturali, per completare la prima generazione. I nostri risultati dimostrano come la capacità di controllo esercitata da *L. thompsoni*, finora largamente sottostimata, possa essere potenziata nel caso siano aumentati gli ambienti adatti per gli ospiti alternativi di questo parassitoide, e sia migliorata la gestione di tali aree. Una gestione del paesaggio che può favorire le popolazioni di *L. thompsoni*, integrando i lanci inondativi di *T. brassicae*, è perciò considerata una strategia per il controllo della piralide altamente efficace e in armonia con l'ambiente, che può inoltre ridurre il fabbisogno di insetticidi contro questo fitofago.

General introduction

Trichogramma egg parasitoids have been used successfully for many decades as inundative biological control agents against a wide range of economically important agricultural and forest pests; they are currently the most widely produced and released natural enemies in biological control throughout the world (Li 1994; van Lenteren 2000). Their use is favoured by simple mass rearing systems, persistent economic efficiency of the parasitoids and the suitable technologies for their commercial use (Li 1994; Smith 1996). Populations of released natural enemies are often self-perpetuating and self-dispersing. As a result, biological control measures are often irreversible, although this is usually not the case with inundative types of biological control (Lynch *et al.* 2001). These characteristics are exactly the reasons why biological control programmes are generally considered sustainable, environmentally benign and highly economic compared to other control methods (Bellows & Fisher 1999; van Lenteren 2001).

Although biological control is recognized to be environmentally safe (Lai 1988; McEvoy 1996; Onstad & Manaus 1996; Jervis 1997), all pest control methods have inherent risks (Howarth 2000). Evidence of environmental impacts of introduced biocontrol agents was already available early in the history of biological control (Perkins 1897; Tothill *et al.* 1930), but it has been largely ignored. Only a few *Trichogramma* species have been used in biological control programmes against a small number of pests, of which the European corn borer (ECB), *Ostrinia nubilalis* Hb. (Lepidoptera, Crambidae), is one of the most successful examples. In many places where *Trichogramma* were released as non-native species they may have become permanent additions to the local fauna. In recent years, concerns about harmful effects of such invaders upon indigenous non-target species have been raised by several authors (Howarth 1991; Simberloff & Stiling 1996; Stiling & Simberloff 2000). The discussion has mainly focussed on risks caused by the import and release of non-indigenous natural enemies against exotic pests (classical biological control), while far less attention has been paid to non-target effects from inundative releases of natural enemies (which is the common practice with *Trichogramma*). Up to now, only a small number of studies has strictly addressed non-target risks of biological control (Lynch & Thomas 2000). However, any effects on non-target species, whether direct or indirect, may have been largely overlooked (Lynch & Thomas 2000; Lynch *et al.* 2001).

An EU-funded 4-year research project on "*Evaluating Environmental Risks of Biological Control Introductions into Europe*" (ERBIC) was conducted between 1998 and 2002 with the aim of facilitating the development of sustainable agricultural production systems and of reducing the use of chemical pesticides. The objectives of this project were, first, to review and examine current and past biological control practices against arthropod pests to assess their environmental impact, and, second, to develop appropriate methodologies and guidelines to allow the assessment of biological control agents in the future (Lynch *et al.* 2001). Four European biological control systems in different agro-ecosystems were investigated and analysed with regard to their potential for non-target impacts. The mass releases of *Trichogramma brassicae* Bezd. (Hym., Trichogrammatidae) against the ECB in maize and its risks for native insect species was one of the topics selected. This study deals explicitly with the risks for both non-target moths in pristine habitats and *Lydella thompsoni* Herting (Dipt.: Tachinidae), an important native natural enemy of the ECB in southern Europe.

Most *Trichogramma* species show a broad host range that includes hosts from several insect orders (Clausen 1940; Fulmek 1955; Quednau 1960). For example, *T. brassicae* is known to parasitize more than 45 lepidopteran species under laboratory conditions (Orr 2000; Babendreier *et al.* 2002 (in press)), and also attacks beneficial insects such as *Chrysoperla carnea* Stephens (Neuroptera, Chrysopidae), and *Episyrphus balteatus* (Dipt.: Syrphidae) (Silva & Stouthamer 1999; Babendreier *et al.*, (submitted)). Despite the known polyphagy, most *Trichogramma* species exhibit strong preferences for certain hosts and habitats (Hassan & Guo 1991; Pinto & Stouthamer 1994). These preferences will certainly reduce the realised host range of a *Trichogramma* species in a given environment. The first studies addressing the potential of *Trichogramma* spp. to cause non-target impacts have only been performed within the past few years (Andow *et al.* 1995; Mansfield 2000; Orr *et al.* 2000). Nevertheless, the potential of these parasitoids to affect non-target hosts inside and outside the crop, as well as native natural enemies of target and non-target hosts has rarely been investigated and further studies are clearly needed.

Trichogramma brassicae was introduced into Western Europe about 30 years ago from the Black Sea region (Moldavia, former Soviet Union) to control the ECB, and has led to a strong reduction in the quantities of pesticides applied to maize (Bigler 1986; Hassan 1988). In Switzerland, the first commercial releases of *T. brassicae* were made in 1978, but with no intention of establishing resident populations. Today these parasitoids are released on about 5.000 hectares of maize each year (Moos-Nuessli 2001). The number of adults applied per hectare and season is 120.000 in regions where only one pest generation occurs. However, in Ticino (southern Switzerland) the pest has two generations per year and 900.000 parasitoids are needed to successfully control the ECB. This biological control approach has now been applied successfully for more than ten years.

In Ticino, the native tachinid fly *Lydella thompsoni* is an important parasitoid of the ECB, and regularly shows high parasitism rates that can reach up to 70%. This parasitic fly is known for its important role in regulating populations of the ECB and the pink stalk borer, *Sesamia nonagrioides* Lefebvre (Lep.: Noctuidae), throughout southern Europe (Guennelon & Audemard 1960; Hsiao & Holdaway 1966; Grenier *et al.* 1990). *L. thompsoni* was introduced as a biological control agent into the United States shortly after the accidental introduction of the ECB in the early twentieth century (Thompson & Parker 1928; Baker *et al.* 1949; Brindley *et al.* 1975). In Ticino, the parasitic fly emerges too early in spring to find pest larvae in maize fields and must rely on alternative hosts in natural wetlands. The reed stalk borers *Archana geminipuncta* Haworth (1809) (Lep.: Noctuidae) and *Chilo phragmitellus* Hübner (1805) (Lep.: Crambidae) were found to be the most frequent and abundant species among alternative hosts of *L. thompsoni* in the study area. Mass releases of *T. brassicae* coincide with the oviposition period of these two moths, and it was suggested that *T. brassicae* moving out of release fields could parasitize eggs of these species. If this is indeed the case, larval populations would be diminished and fewer hosts would be available for *L. thompsoni*. Such a competitive situation could result in reduced *L. thompsoni* population build up in reed habitats. Moreover, the dispersal ability of *T. brassicae*, combined with its polyphagy, could mean that inundative releases have negative effects on populations of native hosts. Previous studies carried out by Bigler *et al.* (1990) showed that a considerable number of *T. brassicae* do move into adjacent maize fields following inundative releases. Although they did not assess this aspect, it is likely that a considerable amount of

T. brassicae also entered adjacent non-target habitats, where they could parasitize alternative hosts of *L. thompsoni*.

Mass releases of *T. brassicae* such as those practised in Ticino raise a number of questions on possible non-target effects. In this study we tried to answer, firstly, whether direct effects such as parasitism of non-target lepidopteran populations in natural wetlands can be found following mass releases and, secondly, whether there are indirect effects on native parasitoids (e. g. native *Trichogramma* species, *L. thompsoni*) due to competition for the same hosts in non-target habitats. In order to answer these questions we performed experiments to evaluate the dispersal ability of *T. brassicae*, their persistence in non-target habitats, and their impact on native *Trichogramma* species in the field (Chapter 1). We also carried out no-choice laboratory and field tests to investigate parasitism of alternative hosts of *L. thompsoni* by *T. brassicae* (Chapter 2). We tested the hypothesis of whether the presence of alternative hosts and habitats in the agricultural landscape is of major importance for *L. thompsoni*, and whether changes in the management of non-target habitats could enhance the effectiveness of biological control (Chapter 3).

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if that's how it is, that's how it is & apparently, that's just how it is

Robert Lax (*1915)

Chapter 1

Dispersal and persistence of mass released *Trichogramma brassicae* (Hym., Trichogrammatidae) in non-target habitats

Stefan Kuske, Franco Widmer, Peter J. Edwards, Ted C. J. Turlings, Dirk Babendreier and Franz Bigler

Abstract

Field studies were carried out to check whether inundative releases of *Trichogramma brassicae* Bezd. (Hym., Trichogrammatidae) against the European corn borer, *Ostrinia nubilalis* Hb. (Lep., Crambidae), can have negative effects on the indigenous arthropod fauna in adjacent non-target habitats. Emigration of released *T. brassicae* from maize fields into non-target habitats was monitored and its persistence in non-target habitats was assessed throughout the season. For this purpose, sticky traps were installed on metal sticks along transects from the inside of *T. brassicae* treated maize fields into sown wildflower strips and a natural common reed stand. In addition, cards with host eggs were used to monitor *Trichogramma* within potential overwintering sites. Although highest numbers of released parasitoids were trapped within maize, a considerable number of *T. brassicae* moved into non-target habitats. We found a significant transient increase of *T. brassicae* in both wildflower strips and reeds and no consistent decrease in numbers of trapped wasps up to 40 meters from the maize field borders, when monitored directly following mass releases. Indigenous *Trichogramma* species were present both in the wildflower strip and reeds, and *T. brassicae* represented only a minor part of the overall *Trichogramma* population therein, except few days after release. However, few *T. brassicae* persisted in non-target habitats throughout the season and very few were still present prior to releases in the subsequent year. These low numbers are not expected to seriously affect populations of native *Trichogramma* or non-target host species.

Key words: *Trichogramma brassicae*; *T. evanescens*; *T. cacoeciae*; *T. semblidis*; *Ostrinia nubilalis*; biological control; inundative release; dispersal; emigration; establishment; interspecific competition; risk assessment.

Introduction

Trichogramma egg parasitoids are important biological control agents and have been successfully used against a wide range of agricultural and forest pests for more than 70 years (Li, 1994). World-wide, approximately 15 million ha of agricultural crops and forests are treated annually with large numbers of *Trichogramma* (van Lenteren, 2000). The widespread use of large numbers of *Trichogramma* and their dispersal ability have recently raised concerns about possible non-target effects (Andow *et al.*, 1995; Mansfield, 2000; Orr *et al.*, 2000). Nevertheless, their potential to affect non-target hosts outside the crop has rarely been investigated.

Most *Trichogramma* species are generalist egg parasitoids showing a broad potential host range including Lepidoptera, Diptera, Coleoptera, Hymenoptera, Neuroptera and Megaloptera (Principi, 1948; Fulmek, 1955; Quednau, 1960; Thomson and Stinner, 1989; Pintureau and Keita, 1990; Orr *et al.*, 2000). Although *Trichogramma* are polyphagous they exhibit habitat and/or niche preferences (Hassan and Guo, 1991; Pinto and Stouthamer, 1994). Host habitat selection, host location, and host acceptance behaviour play a major role in determining their potential to cause non-target effects. Another factor mediating potential non-target effects is their ability to disperse from release fields into non-target habitats. Some studies show that *Trichogramma* dispersal is quite limited (Yu *et al.*, 1984; Heiningen *et al.* 1985; Andow and Prokrym, 1991; McDougall & Mills, 1997). However, Voegelé *et al.* (1975) showed that *T. brassicae* migrate out of a release fields into fields without release within few days and can parasitize eggs of the European corn borer (ECB), *Ostrinia nubilalis*, at 78 m and 147 m distance from the closest release point. Bigler *et al.* (1990) made similar observations in southern Switzerland, where *T. brassicae* dispersed into fields without release and parasitized egg masses of the ECB up to 400 m away in downwind direction. Stern *et al.* (1965) recaptured marked females of *T. buesi* 17 hours after release at a distance of 700 m.

In Switzerland, *T. brassicae* has been used against the ECB since 1978, but with no intention of establishing resident populations. At present, *T. brassicae* is used on approximately 5.000 hectares of maize each year (Moos-Nuessli, 2001). Naturally occurring populations of *T. brassicae* have never been reported for Switzerland in the past (Bigler *et al.*, 1990), though this parasitoid is indigenous to parts of Europe (Pintureau, 1990). In southern Switzerland, the ECB has two generations a year and reaches much higher infestations than it does north of the Alps where it develops one generation per year. To successfully suppress the pest in seed maize fields the release of 0.9 million wasps ha⁻¹ is needed against the second pest generation. Previous studies carried out by Bigler *et al.* (1990) showed that a considerable number of *T. brassicae* moved into adjacent maize fields following inundative releases. These findings suggest that *T. brassicae* may also enter adjacent non-target habitats where they might parasitize non-target hosts. *T. brassicae* is polyphagous and known to parasitize more than 45 lepidopteran species under laboratory conditions (Orr *et al.*, 2000; Babendreier *et al.*, 2002 (in press)), as well as beneficial natural enemies, such as *Chrysoperla carnea* Stephens (Neuroptera, Chrysopidae) (Silva and Stouthamer, 1999). Therefore its release could have negative effects to the native arthropod fauna, such as a decrease in population density, displacement, or, in a worst case, the extinction of non-target species.

The objectives of the present study were to quantify the emigration of *T. brassicae* from release fields into non-target habitats and to assess their persistence in these habitats. Thus, we

aimed to evaluate the risk of mass released *T. brassicae* to affect non-target arthropods outside the release habitats.

Materials and Methods

Site description

The persistence of mass released *T. brassicae* in non-target habitats was investigated on four different farms in southern Switzerland during 1998-2001. Surveys were performed in maize fields and adjacent non-target habitats. The four sites taken into consideration can be characterised as follows: Site 1 was selected in the Magadino Plain South-west of Bellinzona. The selected area consisted of two seed maize fields of 4 ha and 11 ha that were surrounded by herbaceous field edges, hedgerows, sown wildflower strips, and reeds (Figure 1). The site was located in an area of seed maize production where high numbers of *T. brassicae* have been released against the ECB for the past decade. Site 2 was also located in the Magadino Plain, about 4.5 km west of Site 1. The non-target habitats adjacent to the selected maize field (5 ha) consisted of herbaceous field edges and a low intensity meadow. The maize field was not treated with *T. brassicae* during this study. Site 3 was located in the region of Lugano, where a seed maize field of about 6 ha was selected, and the non-target habitats comprised herbaceous field edges. At this site, mass releases had been frequent, but were stopped in 1997. Thus, at the time of the survey, no release had been carried out for at least two years. Site 4 was located on the farm of the Cantonal Agricultural Institute of Mezzana (IACM) at Balerna. The selected maize field (2 ha) was surrounded by herbaceous field edges and low intensity meadows. *T. brassicae* has never been released in this region.

Parasitoids and inundative releases

All *T. brassicae* that were used in this study originated from a laboratory colony reared by Biotop (Valbonne, France) and marketed as Trichocap®. In southern Switzerland, inundative releases are carried out against the second generation of the ECB by the end of July (0.4 million wasps ha⁻¹) and the beginning of August (0.5 million wasps ha⁻¹) respectively. In 1999, an additional release (0.3 million wasps ha⁻¹) was carried out for experimental purposes at mid July.

Sampling procedures

The dispersal of released *T. brassicae* from maize fields into non-crop habitats, and the persistence of these parasitoids in non-target habitats was investigated using a set of different sampling methods. An overview of all sampling procedures is given in Table 1.

Sticky trap survey. To quantify the emigration of *T. brassicae* from maize fields sticky traps were placed along transects leading from the inside of a release field into adjacent non-target habitats in 2001. Traps consisted of transparent plastic sheets (230 x 305 mm) that were sprayed with insect glue (souveurode® aerosol, Siegfried Agro Zofingen, Switzerland) on both sides. They were attached to metal sticks and placed directly above the vegetation. Exposure periods of the traps were the week

preceding the first release (only in 2001), as well as the week following the first and the second *T. brassicae* release, and three weeks after the second release. The layout of the experiment is shown in Figure 1. Distance between transects was 15 m and first day of sticky trap exposure coincided with the first emergence of released *T. brassicae* in the crop. The total number of *Trichogramma* adults per trap was counted under a dissecting microscope in the laboratory and the mean number of parasitoids per date and distance was calculated. The differences in the mean number of caught *Trichogramma* wasps per sticky trap during our survey were analyzed with a double factored ANOVA for each sampling period, including the factors species - *T. brassicae* vs. native *Trichogramma* species (pooled) - and habitat into the model. Species identification of individual *Trichogramma* was done by PCR. An additional survey aimed at determining the occurrence of *T. brassicae* in potential overwintering habitats and at quantifying their contribution to the overall *Trichogramma* population therein (Table 1). Two different methods using either sticky traps or egg-cards were applied. The sticky trap survey was carried out from July to the end of September 2000 in biweekly intervals. Traps were placed in the surroundings of a release field at 25 m distance from the field borders (Figure 1). The distance between traps was 50 m. Non-target habitats considered were field edges and hedgerow borders. We assessed the mean number of *Trichogramma* wasps caught per trap and date.

Egg-card survey. Cards containing lepidopteran eggs were used to monitor *Trichogramma* from 1998-2000. Two types of egg-cards were used. The first type consisted of 400-500 UV-irradiated eggs of *Ephestia kuehniella* Zeller (Lep., Pyralidae), glued on white cardboard strips (20 x 70mm). These eggs were provided by Biotop (Valbonne, France), and stored at 5°C (90% rh) for maximal two weeks, if not used immediately for the survey. The second type of egg-cards were white wax paper strips (about 10 cm²) containing 50-100 eggs of *Mamestra brassicae* L. (Lep., Noctuidae), that were purchased from Pierrick Aupinel (INRA Magneraud, Surgères, France), and were less than 3 days old when used in the survey. The surveys were carried out in relatively undisturbed field boundaries, which were expected to provide food, alternative hosts, shelter, and overwintering sites for emigrating *T. brassicae*. Sampling procedures are listed in Table 1. Single egg-cards were attached to leaves of wild plants at 0,2-1,8 m above ground level. Egg-cards were exposed for two days, then collected and kept individually in plastic vials (25 x 80mm) at 24±1°C, 70±5 % RH, L:D (16:8). Mean parasitism and egg loss was assessed for each survey date. Egg-cards were regarded as parasitized if at least one egg was parasitized. Egg loss on cards was scored according to five categories: 100 %, 99-75 %, 74-50 %, 49-25 %, and < 25 % egg loss. To identify *Trichogramma* spp., two different methods were applied: In 1998 male adult wasps that emerged from parasitized egg-cards were identified by B. Pintureau (INSA Villeurbanne, Cedex, France) based on morphological characters. In 1999 and 2000 we reared isofemale lines from up to ten emerging females from each parasitized egg-card. Two or three specimens of each isofemale line were then identified by PCR (see below).

Collection of naturally laid eggs of the ECB. To investigate whether *T. brassicae* can be found in maize fields without release, a survey was carried out in late summer 2000 at all four experimental sites (see site description). Instead of Site 1 a field without release 4 km distance East was selected, since most of the maize fields in the surroundings of Site 1 had been treated with *T. brassicae*. At all sites random samples of naturally laid egg masses were collected from maize plants and incubated under laboratory conditions at 24 ± 1°C, 70 ± 5 % RH, L:D (16:8) until young larvae hatched or

parasitized eggs turned black and offspring emerged. Isofemale lines were reared from emerging females of each parasitized egg mass. Identification of *Trichogramma* was done by PCR.

***Trichogramma* identification by PCR**

Identification of *Trichogramma* at the species level was based on genetic analyses. DNA from individuals was extracted using the method of Edwards & Hoy (1993), with some modifications described below. Single wasps were placed in 2 ml reaction tubes containing 3-8 glass beads (1 mm diameter) covered with smaller glass beads (0.1 mm diameter). Wasps were ground for 60 sec in a mixer mill (type MM300, Retsch, Haan, Germany) at 25 beats/sec. Then 100 μ l Chelex-100 (5%) (Bio-Rad Laboratories, Glattbrugg, Switzerland) and 4 μ l proteinase K (20 mg/ml) were added. Mixtures were incubated overnight at 56°C, followed by denaturation for 10 min at 95°C. PCR was performed in 50 μ l using a PTC-200 thermocycler (MJ Research, Watertown, Mass.). Reaction mixtures contained 4 μ l template DNA, 5 μ l (10x) PCR-buffer, 2 μ l $MgCl_2$ (conc.), 1 μ l dNTPs (10 mM each), 0.25 μ l forward and reverse primer (20 pmol/ μ l), 0.2 μ l TAQ polymerase (5 U/ μ l) (Life Technologies, Basel, Switzerland). Primers used to amplify the ITS-2 region were ITS2-forward and ITS2rev-Trich (Stouthamer et al. 1999). The PCR cycling program was 3 min initial denaturation at 94°C followed by 36 cycles of 40 sec at 94°C, 45 sec at 53°C and 45 sec at 72°C, with final extension for 3 min at 72°C after the last cycle. PCR products were sized by electrophoreses in 2 % agarose gels (Sigma Chemical Co., St. Louis, MO) containing ethidium bromide. Molecular weight standards were run along with the samples for reference. Restriction analyses of PCR products were performed according to Silva (1999), by using the restriction enzymes *Dra*I, *Mse*I or *Eco*RI. Restriction fragment length polymorphism (RFLP) was analysed on 1.5-2% agarose gels. RFLP-based *Trichogramma* species identification was performed according to Stouthamer *et al.* (1999).

Results

Dispersal into non-target habitats

Prior to inundative releases we found significantly fewer *T. brassicae* per sticky trap than specimens of native *Trichogramma* species (summarized) ($F_{1,54} = 21.1$; $p < 0.001$). *T. brassicae* was virtually absent, while native *Trichogramma* were distributed throughout maize, wildflower strip (WFS), and reeds (Figure 3). The mean number of wasps per sticky trap was not significantly different between habitats ($F_{2,54} = 1.9$; $p > 0.05$). Following the first inundative release in maize an immediate increase in numbers of trapped *T. brassicae* was measured in maize and non-target habitats. The mean number of wasps per sticky trap was not significantly different between species ($F_{1,53} = 2.1$; $p > 0.05$), but between habitats ($F_{1,53} = 3.2$; $p < 0.05$). Native *Trichogramma* species were distributed throughout target and non-target habitats. After the second inundative release, the mean number of wasps per sticky trap was significantly higher for *T. brassicae* than for native *Trichogramma* ($F_{1,54} = 14.5$; $p < 0.001$) and also significantly different between habitats ($F_{2,54} = 4.3$; $p < 0.05$). In addition, we found an interaction between species and habitat ($F_{2,54} = 4.4$; $p < 0.05$), indicating that one species

dominates the other species in particular habitats. We found *T. brassicae* to be predominant in maize, the WFS, and in the reeds. Native *Trichogramma* were trapped in similar numbers as following the first release, both in maize and non-target habitats (Figure 3). Three weeks after the second release, the contribution of *T. brassicae* to the native *Trichogramma* population had strongly decreased both in maize, WFS and reeds (Figure 3). Significant differences in mean number of wasps per sticky trap were found between *T. brassicae* and native *Trichogramma* species (summarized) ($F_{1,54} = 18.4$; $p < 0.001$) and also between habitats ($F_{2,54} = 3.6$; $p < 0.05$). In addition, we found an interaction between species and habitat ($F_{2,54} = 5.3$; $p < 0.01$), indicating that one species dominates the other in particular habitats. The majority of trapped *Trichogramma* during this last experiment belonged to native species in all investigated habitats.

***Trichogramma* species composition in maize and non-target habitats**

The distribution of *Trichogramma* species at the species level in target and non-target habitats is shown in Figure 4 for all survey periods. Prior to releases, *T. semblidis* Aurivilius (1897) was predominant in maize, but also caught in the WFS and reeds. *T. evanescens* Westwood (1833) occurred only in the WFS while some other *Trichogramma* species - including *T. sp.* sequence close to north American *aurosum* (Pinto *et al.*, 2002), and individuals that belonged to *Trichogramma* species whose identification on species level failed - were found in the two non-target habitats. *T. brassicae* was trapped in the WFS, but represented only a minor part of the overall *Trichogramma* population present therein (Figure 4). Following the first release, *T. brassicae* occurred in maize and both non-target habitats. *T. semblidis*, however, was not caught in reeds, *T. evanescens* not in maize, while some other species (see above) occurred both in maize, the WFS and reeds (Figure 4). After the second release, mean number of *T. brassicae* per sticky trap (over all habitats) was 2.6 fold higher than after the first release, though no *T. brassicae* were trapped in reeds at 40m from the maize field border. *T. brassicae* was predominant in all investigated habitats. *T. semblidis*, *T. evanescens*, and individuals of the unidentified species (see above) were found in maize, WFS, and reeds in small numbers (Figure 4). Three weeks after the second release, *T. brassicae* was found in maize in similar numbers as *T. semblidis* and *T. evanescens* (Figure 4). Very small numbers of *T. brassicae* were caught in the WFS and in the reeds, and no *T. brassicae* were caught in reeds at 40 m. *T. semblidis* were found in small numbers throughout the investigated habitats. *T. evanescens* was predominant in the WFS and also found in maize and reeds. Specimens belonging to unidentified *Trichogramma* species (see above) were trapped in maize, the WFS, and reeds, showing highest numbers in the WFS (Figure 4). The records of *T. evanescens*, *T. semblidis*, and *T. sp.* sequence close to North American *aurosum* are new for southern Switzerland.

Survival and persistence in non-target habitats

Sticky trap survey. The sticky traps placed in field edges and hedgerow borders at 25 m from release field borders in 2000 yielded useful information on the presence of *Trichogramma* in these non-target habitats. The mean number of adults caught per sticky trap ranged between 0.9 and 4.1 and was significantly different between survey periods (ANOVA, Newman-Keuls; $F_{5,114} = 5.2$; $p < 0.001$).

There was a slight increase in numbers caught following mass releases, but means were significantly higher only for the survey period from 23-30 August, compared to the survey periods prior to release (Figure 5). In September mean number of wasps per trap decreased to levels similar as found earlier in the season (Figure 5).

Egg-card survey. In 1998 we found no parasitism until August, when few parasitized egg-cards were obtained from Site 1, 3 and 4 (Table 2), but not from Site 2. The *Trichogramma* belonged mainly to *T. evanescens* and in one case to *T. semblidis*. *T. brassicae* was not found at any of the four sites. In 1999 and 2000 the percentage of parasitized egg-cards per survey ranged between 0 and 4.6 (Table 3 & 4). In 1999 a total of 40 egg-cards with 439 eggs were parasitized from May to October. In 2000, 31 egg-cards with 461 eggs were parasitized from May to the end of September. In 1999 *T. evanescens* was reared from 52.5 % of the parasitized egg-cards, *T. brassicae* from 25 %, and *T. cacoeciae* Marchal (1927) from 7.5 %, whereas in 2000 *T. evanescens* was reared from 45.2 %, *T. cacoeciae* from 25.8 %, *T. semblidis* from 16.1 %, and *T. brassicae* from 9.7 % of the parasitized egg-cards. The species identification failed for 15 % and 3.2 % of the parasitized egg-cards in 1999 and 2000 respectively, since offspring did not emerge, or samples were lost or damaged. *Trichogramma* parasitized egg-cards over the whole season and we never found more than one species per egg-card. These are the first records for *T. cacoeciae* in southern Switzerland. Species composition over the season is shown in Figure 6 for 1999 and 2000. In both years *T. brassicae* was already present prior to inundative releases, and repeatedly found throughout the season. However, the number of parasitized egg-cards by *T. brassicae* did not increase following commercial releases.

The use of *Mamestra brassicae* eggs yielded similar results to those found with *E. kuehniella* egg-cards. However, on 23-24 May 2000 neither *Mamestra* nor *Ephestia* eggs were parasitized, whereas on 8-9 August 5.9 % of the *M. brassicae* but only 1.5 % of the *E. kuehniella* egg-cards were parasitized. 84.6% of *M. brassicae* egg-cards were parasitized by *T. evanescens* and each 7.7 % by *T. brassicae* and *T. sp.* close to north American *aurosom* sequence. In addition, three *M. brassicae* egg-cards were parasitized by scelionids (species not determined).

At all monitoring dates, predation seemed to be a main reason for high egg loss on the egg-cards. Total egg loss ranged between 31 and 90 % in 1998-2000 (Tables 3 and 4). The predators responsible were not investigated, though the following organisms were repeatedly found on egg-cards: slugs, ants, predatory bugs, spiders, and larvae of syrphids, chrysopids, and coccinellids. Weather conditions were good for all survey periods (mean temperature: 20°C ; no or very little precipitation; mean wind speed: 1.4 m/s (range: 0.6-2.7m/s)).

Occurrence of natural *Trichogramma* populations in maize

Parasitized egg masses of the ECB in fields without release of *T. brassicae* were obtained from three of the four sites. 8.9 % of the egg masses were parasitized at Site 1 (n=56), and 5.9 % at Site 2 (n=51). Hardly any ECB egg masses were observed at Site 3, and only six egg masses were collected. Two of them were parasitized. Egg mass density of the ECB was also low at Site 4, and no parasitism by *Trichogramma* was detected (n=23). All specimen from Site 1, and 3 were identified as

T. brassicae. At Site 2 *T. evanescens* was found together with *T. brassicae*. It is the first record of native *T. evanescens* reared from ECB for Switzerland.

Discussion

Dispersal into non-target habitats

Our surveys clearly demonstrate that high numbers of *T. brassicae* can leave release fields and move into non-target habitats. Directly after the first and second release an immediate and distinct increase of *T. brassicae* was observed in the wildflower strip and the natural common reed stand up to 40 m distance from maize field borders. No consistent decrease in numbers of trapped adult wasps was found within this distance. The number of trapped *T. brassicae* was in relation to the number of released parasitoids in maize and was 2.6 fold higher after the second (0,5 Mio wasps ha⁻¹) then after the first release (0,2 Mio wasps ha⁻¹). Following second release the relative increase in numbers of trapped *T. brassicae* was even higher in non-target habitats than in maize. This indicates a quick and quantitatively important dispersal into adjacent habitats. However, the number of *T. brassicae* trapped outside maize quickly decreased within three weeks after the second release. Thereafter, *T. brassicae* represented only a minor part of the overall *Trichogramma* population in non-target habitats and even in maize. The additional sticky trap survey that was carried out in relatively undisturbed field boundaries, along hedgerows, low intensity meadows and forest borders up to 30 m away from maize fields with release, did not show a consistent increase in numbers of trapped *Trichogramma* following inundation. It may be true that higher numbers of trapped individuals at the end of August were a consequence of the *T. brassicae* releases but could be due to increased population densities of native *Trichogramma* species as well, as was shown for *T. evanescens* in the WFS at the end of August.

These results confirm our initial hypothesis that a considerable number of released *Trichogramma* can move into non-target habitats where they may affect non-target hosts. The findings concerning dispersal capacity are in line with previous studies carried out by Bigler *et al.* (1990) within the same study area, who found that a high percentage of *T. brassicae* spread into adjacent maize fields without release, and many parasitized eggs of the ECB could be found up to 400 m distance in downwind direction. On the contrary, studies from other areas show rather limited dispersal capacity of *T. brassicae* (Bigler *et al.*, 1989, Maini *et al.* 1991, Greatti and Zandigiacomo, 1995). However, all these studies focussed on dispersal within maize fields and did not go beyond crop borders, while Orr *et al.* (2000) investigated downwind dispersal of *T. brassicae* from maize into five different types of surrounding non-target habitats. He found no parasitism of sentinel egg masses of three non-target hosts in four of these habitats. In the fifth habitat two sentinel egg masses were parasitized, one at about 20 m and the second at 32 m away from the release plot. The fact that many eggs parasitized by *T. brassicae* may be found downwind at greater distances in maize fields without release, but not in non-target habitats, indicates that wind can support passive dispersal considerably, whereas the potential of *T. brassicae* to migrate actively out of release fields is limited to rather short distances and may depend on the habitat preferences of the parasitoid.

Survival and persistence in non-target habitats

Following inundative releases in maize, cardboard strips containing lepidopteran eggs that were placed in non-target habitats up to 25 m away from release fields, were parasitized by *T. brassicae*. Although the percentage of parasitized egg-cards was generally low, parasitism was repeatedly found until the end of the season and again prior to inundative releases in the subsequent year. Thus, *T. brassicae* survived and overwintered in non-target habitats, where they obviously find alternative hosts. Successful overwintering of *T. brassicae* has also been shown in southern France (Voegelé *et al.*, 1988) and in Switzerland north of the Alps (Babendreier *et al.*, in press). Since we found parasitized ECB eggs in maize fields without release at more than 3 km distance from closest release fields in late summer, it is evident that *T. brassicae* populations that were established through previous releases can be found in the study area. Earlier surveys carried out by Bigler and Brunetti (1981, unpublished data) before the first releases of *T. brassicae* in southern Switzerland, where several hundreds of natural and bait egg masses of the ECB were checked from May until September, indicated absence of *T. brassicae* or other *Trichogramma* species in maize fields (Bigler *et al.*, 1990). Similar observations have been reported by Voegelé *et al.* (1975) for the Alsace (France). Both studies concluded that natural parasitism of the ECB by *Trichogramma* would not occur in the respective study areas. This contrasts with a study carried out by Maini *et al.* (1982), who found 13-96 % of naturally laid ECB egg masses parasitized by indigenous *T. brassicae* (Syn.: *T. maidis* Pintureau & Voegelé) in late summer in maize fields in northern Italy.

Interactions with indigenous *Trichogramma* species

In general we might expect interspecific competition among *Trichogramma* species to be relatively rare under natural conditions, existing only when one species is present in very large numbers relative to the number of available hosts, and exploiting the same habitat (Tavares, 1991). These conditions are more likely to occur after an inundative release. In our surveys *T. brassicae* did enter non-target habitats that were exploited by several native *Trichogramma* species, such as *T. evanescens*, *T. cacoeciae*, *T. semblidis*, *T. sp.* sequence close to north American *aurosum*, and potentially other *Trichogramma* species, that have yet to be identified. The temporal and spatial overlap of *T. brassicae* with various indigenous *Trichogramma* species may potentially lead to competition with the native species. However, although *T. brassicae* was repeatedly trapped, it represented only a minor part of the overall *Trichogramma* records in the two investigated non-target habitats, except during the week after inundative release respectively. The sticky trap survey further revealed that native *Trichogramma* species can be also trapped in maize, but ECB egg masses were never found parasitized by native *Trichogramma*, except those parasitized by *T. evanescens*.

Habitat and host preferences are known for *T. evanescens*, *T. semblidis*, and *T. cacoeciae* and will certainly influence competition interactions. In western Europe *T. evanescens* are common natural enemies of cabbage pests, showing a preference for noctuid moths (e.g. *M. brassicae* and *Pieris* spp.), whereas *T. cacoeciae* normally exploit arboreal habitats and usually parasitize *Tortricidae* (Baggiolini, 1956, 1958; Geier, 1956; Quednau, 1960; Neuffer, 1988; Pintureau and Keita, 1990). *T. semblidis* is reported from marshes but also occurs in vineyards and field crops and is known to parasitize hosts in

several insect orders, including Lepidoptera, Diptera, Coleoptera, Neuroptera and Megaloptera (Flanders and Quednau, 1960; Nagarkatti, 1971; Franz and Voegelé, 1974; Sengonca and Leisse, 1987; Pintureau, 1990). According to their niche preferences, *T. evanescens*, *T. cacoeciae* and *T. semblidis* can be readily assigned to field, arboreal and marsh types, as proposed by Flanders (1937). The finding of these three species in the same habitat may be explained by the fact that our surveys were carried out in a heterogeneous environment containing small patches of many habitats, such as field edges, shrubs, hedgerows, common reed stands, low intensity meadows and cropland. Although competition with *T. brassicae* cannot be excluded for any of the native *Trichogramma* species found, it is likely that differences in habitat and host preferences tend to minimise such interactions (Hirose *et al.*, 1988). Data from our sticky trap surveys clearly demonstrate that despite transient inundation of non-target habitats after mass releases in maize (which occurs annually since more than ten years now), *T. brassicae*'s population density in non-target habitats is low and hardly reaches similar levels as found for indigenous *Trichogramma* species. Egg-card surveys confirmed these findings and therefore we conclude that inundative releases are unlikely to have severe population level impacts on other *Trichogramma* species.

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Captions for figures

Figure 1 Schematic diagram of Site 1 showing maize fields, non-target habitats, and locations of egg-cards and sticky-traps that were used to monitor *Trichogramma*.

Figure 2 Layout of emigration experiment 2001. Continuous black lines show transects leading from maize fields with *Trichogramma brassicae* releases into non-target habitats. Position of sticky-traps along transects are indicated by bold black lines.

Figure 3 Emigration of *Trichogramma brassicae* from maize into non-target habitats and presence of native *Trichogramma* in these habitats in 2001. Parasitoids were caught on sticky traps along transects leading from maize (M) into wildflower strips (F), and reeds (R) (n=5). Survey periods lasted one week and included the week prior to first inundative release (0,2 Mio wasps ha⁻¹) from 23-30 July, the weeks following first and second release (0,5 Mio wasps ha⁻¹) from 30 July to 6 August and 9 to 16 August respectively, and the week from 27 August to 3 September.

Figure 4 Occurrence of *Trichogramma brassicae* and native *Trichogramma* species in target and non-target habitats. Parasitoids were caught on sticky traps in maize (M), wildflower strips (F), and reeds (R) (n=5). Survey periods lasted one week including the week prior to first inundative release (0,2 Mio wasps ha⁻¹) from 23-30 July, the weeks following first and second release (0,5 Mio wasps ha⁻¹) from 30 July – 6 August and 9 August –16 August respectively, and the week from 27 August – 3 September. Total number of *Trichogramma* adults on traps was assessed for each species and data pooled for each habitat separately. Identification of wasps was done by PCR.

Figure 5 Mean number of *Trichogramma* wasps caught on sticky traps in non-target habitats in southern Switzerland in the year 2000. Sticky traps were installed in non-target habitats bordering seed maize fields that were treated with *Trichogramma brassicae* Bezd. releases against the European corn borer. Sticky traps were placed at a distance of about 25 m from the field borders and were exposed for one week. Different letters above bars indicate significant differences in means (ANOVA, Newman-Keuls-Test; p<0.05).

Figure 6 Seasonal distribution of *Trichogramma* species in non-target habitats in southern Switzerland. *Trichogramma* were monitored by using egg-cards. The survey was carried out from mid May to the beginning of October in 1999 and to the end of September in 2000. Inundative releases of *T. brassicae* were carried out in adjacent maize fields on 13 and 27 July, and 4 August in 1999 and on 25 July and 3 August in 2000 (indicated with arrows).

Figure 1.

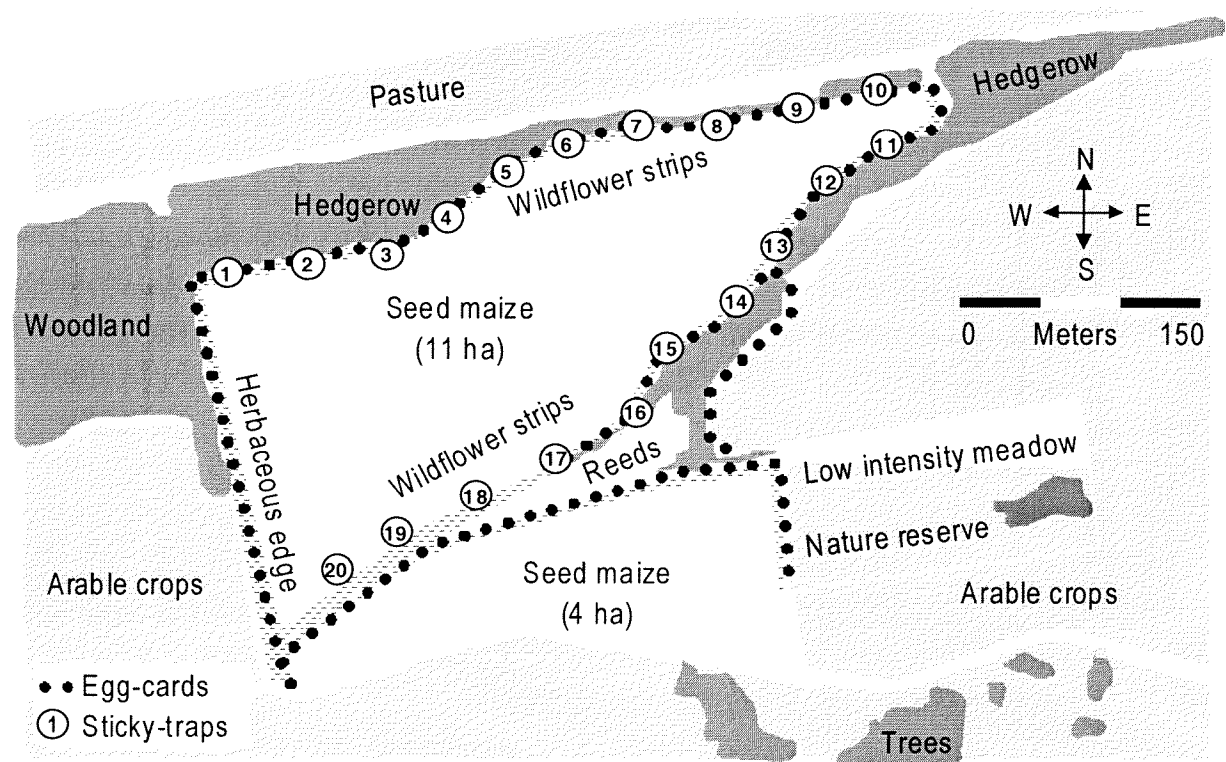


Figure 2.

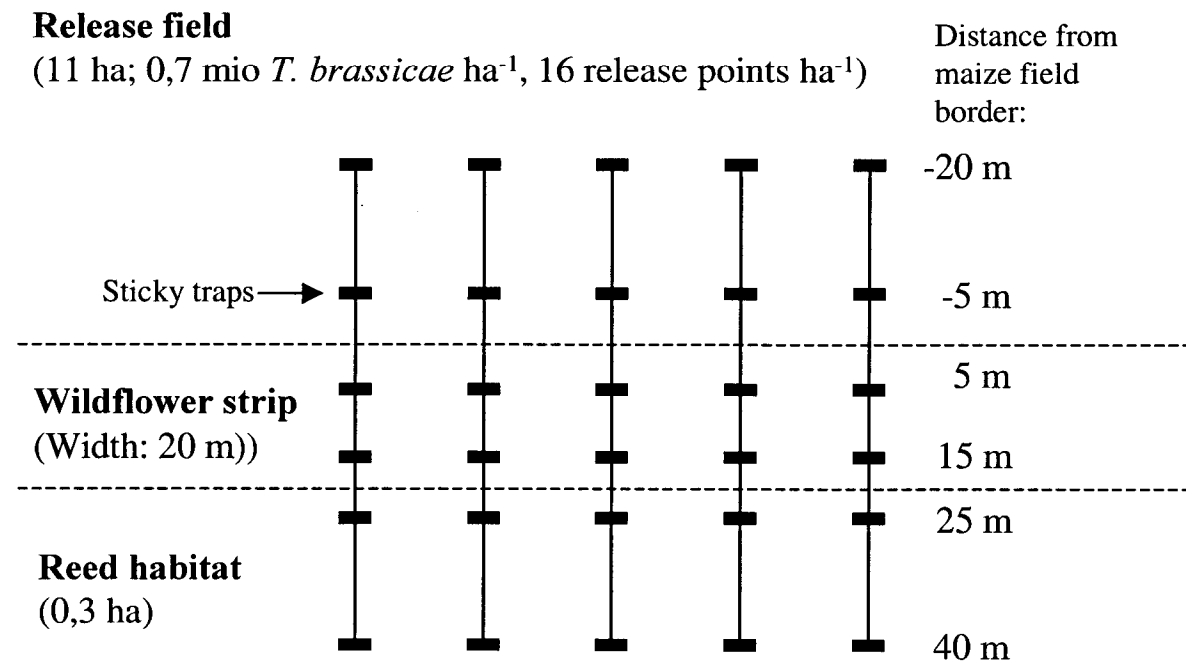


Figure 3.

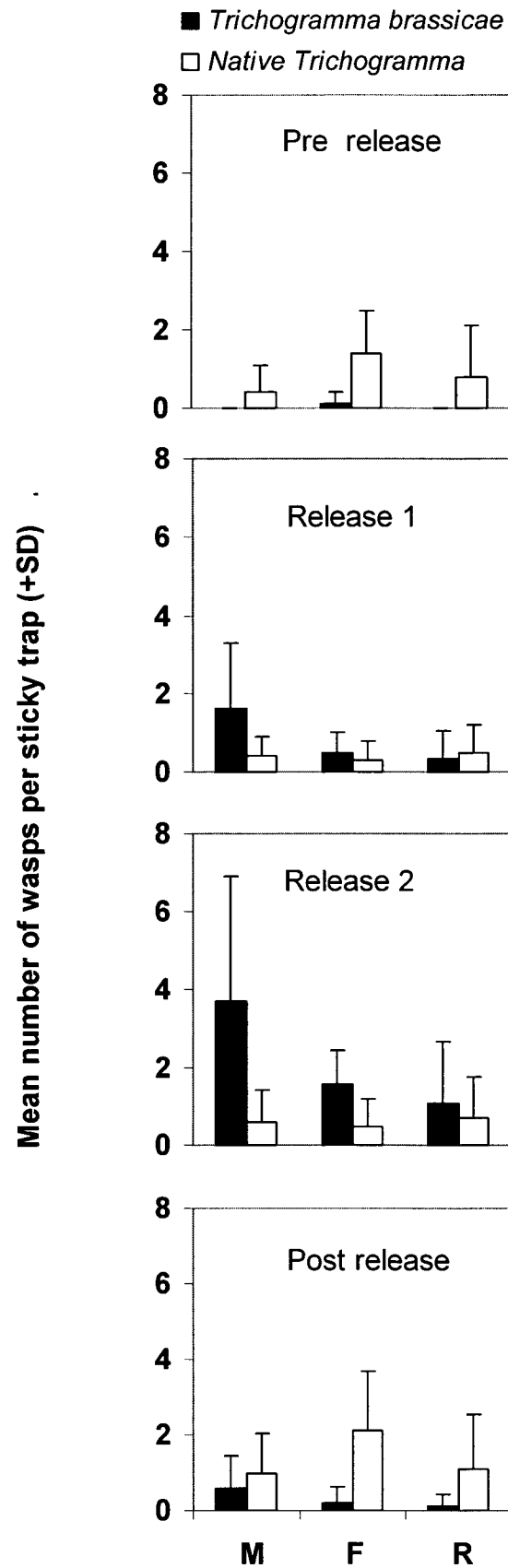


Figure 4.

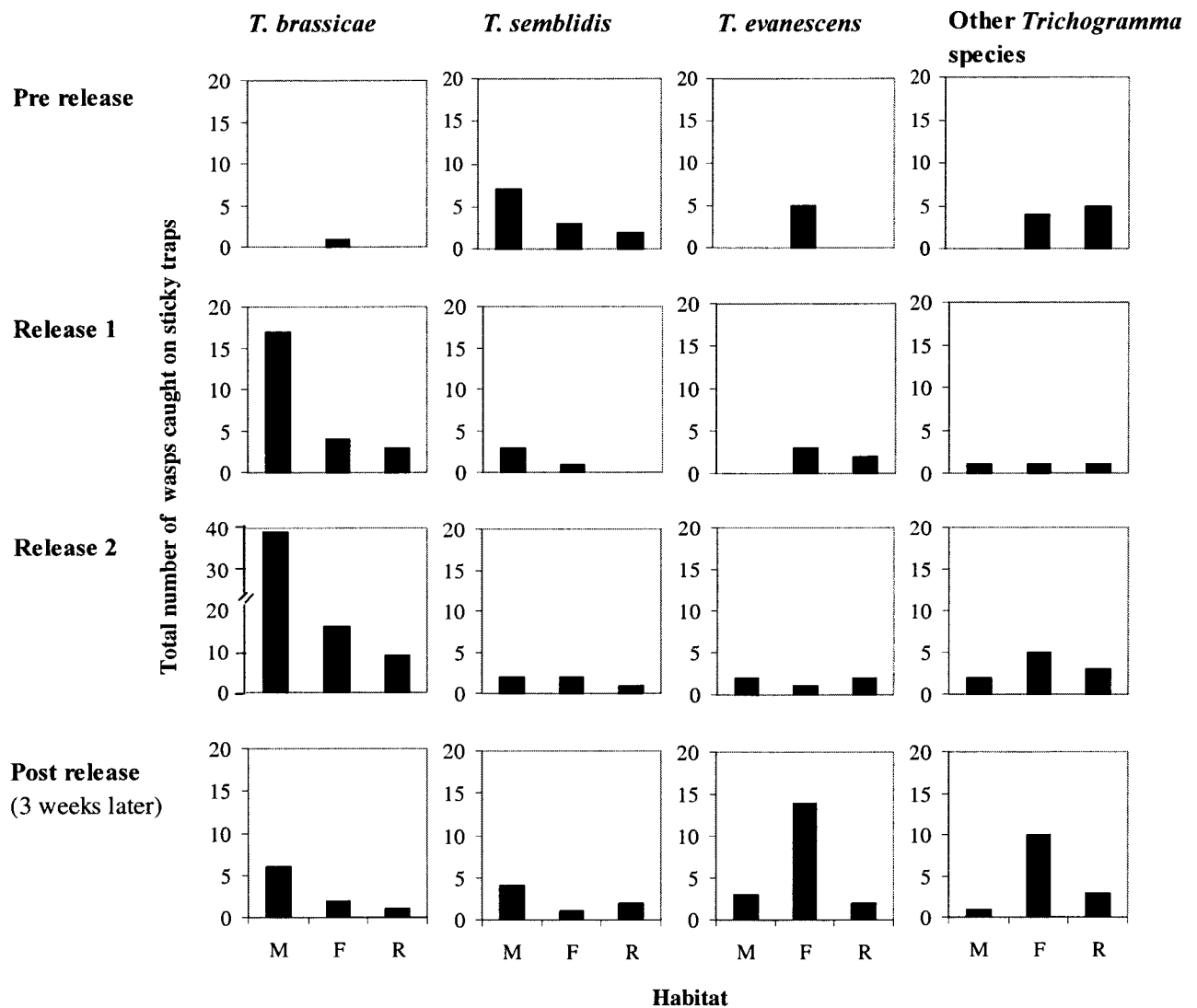


Figure 5.

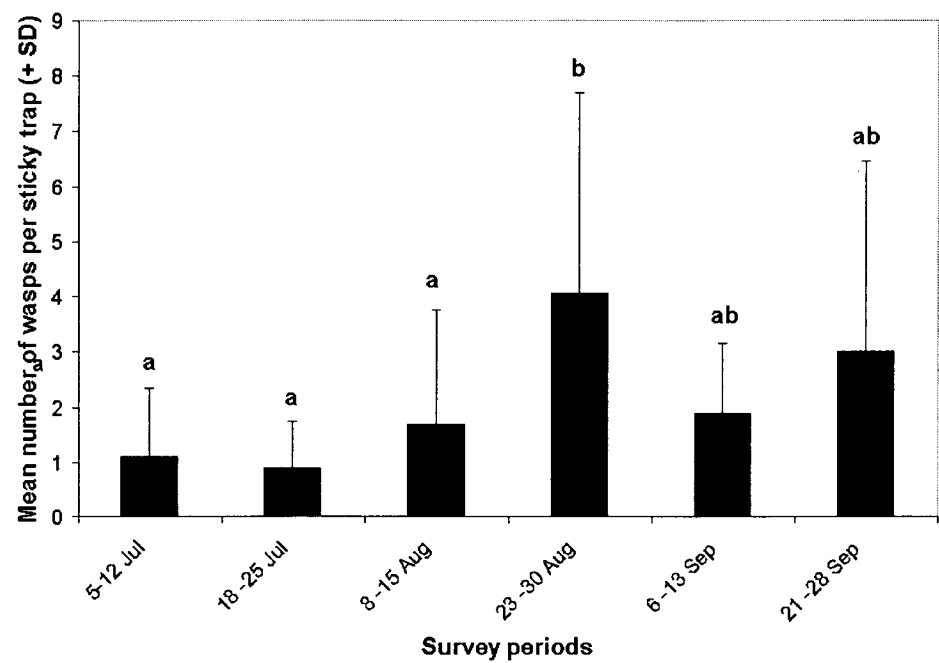


Figure 6.

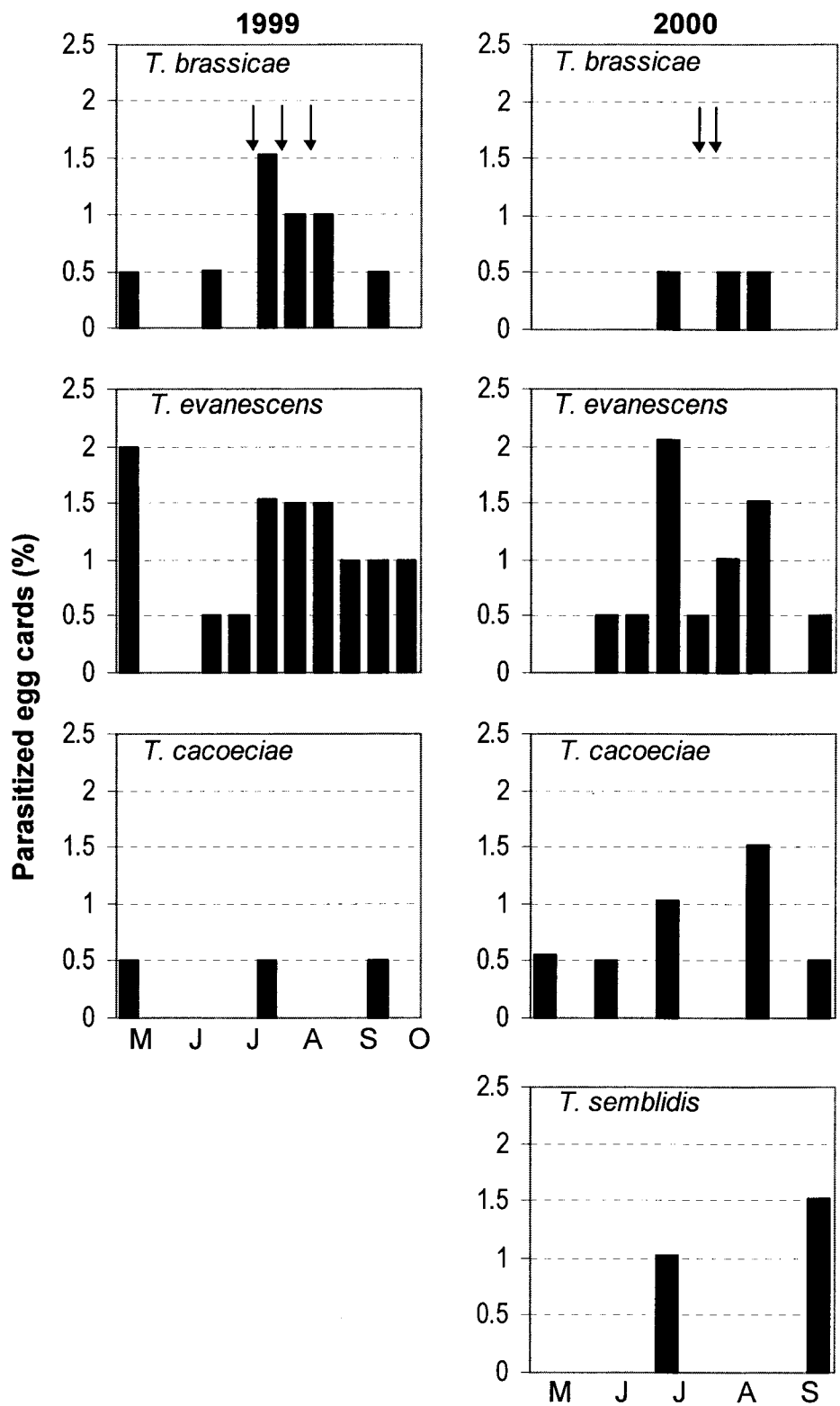


TABLE 1 Overview of *Trichogramma* sampling procedures

Trap type	Aim of survey	Sites	Year	Number of traps of per date	Number of exposure periods	Distance of maize field borders (m)	Notes
Sticky traps							
	Quantify emigration into non-target habitats and identification of species composition	1	2001	30	4	-20, -5, 5, 15, 25, 40	Similar experiment was carried out in 2000, when PCR identification failed.
	Assess species composition in non-target habitats (overwintering sites)	1	2000	20	4	25	PCR identification failed
Egg cards							
Host: <i>Ephestia kuehniella</i>	Assess dispersal and persistence in non-target habitats	1-4 1 1	1998 1999 2000	50 200 200	3 ^a 11 10	2-5 2-5 or 25 2-5 or 25	In 1998, only few egg-cards were parasitized. Therefore, monitoring frequency and egg-card number was increased in 1999 and 2000, and the survey restricted to Site 1. Half the cards were placed 2-5 m and half 25 m away from release fields.
Host: <i>Mamestra brassicae</i>	Assess dispersal and persistence in non-target habitats	1	2000	220	2	2-5 or 25	
Naturally laid <i>Ostrinia nubilalis</i> eggs	Prove occurrence of established populations	1-4	2000	136	1		Survey carried out in maize fields without release (>3 years).

^a At Site 1, *Trichogramma* were surveyed during 4 exposure periods

TABLE 2 Identify of *Trichogramma* species. Species identification based on genetic analyses. individual wasps were identified by amplification of their internally transcribed spacer (ITS2) with general primers, determining the size of the PCR product using standard agarose electrophoresis, followed by DNA-digestion with restriction enzymes. Origin, pattern types, typing, sequences and GenBank accession numbers are given for representative clones.

Pattern Type	Origin ^a	Clone	Typing ^b	Sequence	GenBank accession number
I	C	SK09	<i>T. brassicae</i>	<i>T. brassicae</i>	AY146635
	C	SK10	<i>T. brassicae</i>	<i>T. brassicae</i>	AY146636
	C	SK18	<i>T. cacoeciae</i>	<i>T. cacoeciae</i>	AY146642
II	C	SK19	<i>T. cacoeciae</i>	<i>T. cacoeciae</i>	AY146643
	C	SK20	<i>T. cacoeciae</i>	<i>T. cacoeciae</i>	AY146644
	B	SK12	<i>T. evanescens</i>	<i>T. evanescens</i>	AY146637
III	C	SK14	<i>T. evanescens</i>	<i>T. evanescens</i>	AY146638
	L	SK15	<i>T. evanescens</i>	<i>T. evanescens</i>	AY146639
	C	SK16	<i>T. evanescens</i>	<i>T. evanescens</i>	AY146640
IV	C	SK17	<i>T. evanescens</i>	<i>T. evanescens</i>	AY146641
	C	SK21	<i>T. evanescens</i>	<i>T. evanescens</i>	AY146645
	C	SK22	<i>T. semblidis</i>	<i>T. semblidis</i>	AY146646
V	C	SK24	<i>not possible</i>	<i>T. species close to evanescens</i> sequence	AY146647
VI	C	SK13	<i>not possible</i>	<i>T. species close to north-american aurosum</i> sequence	AY146648
		SK25	<i>not possible</i>	<i>T. species close to north-american aurosum</i> sequence	AY146649

^a B = Breganzona, C = Cadenazzo, L = Locarno-Magadino

^b Typing was done according to an identification key for European *Trichogramma* species which is in preparation by Stouthamer (personal communication)

TABLE 3 Number of sentinel egg-cards parasitized by *Trichogramma* in non-target habitats in southern Switzerland in 1998

Monitoring sites	Releases in past	Distance to closest release site (km)	15-17 June	Number of parasitized egg-cards (N = 50)	22-24 July ^a	5-7 August	Parasitoid species
Site 1	Yes	< 0.01	0	0	0	2	<i>Trichogramma evanescens</i>
Site 2	Yes	1.50	0	0	-	0	-
Site 3	Yes	>15.00	0	0	-	1	<i>T. evanescens</i>
Site 4	No	>30.00	0	0	-	3	<i>T. evanescens</i> , <i>T. semblidis</i>

^a Survey was restricted to Site 1

TABLE 4 Egg loss and parasitism of sentinel egg-cards during *Trichogramma* survey 1999 and 2000. Cards were attached to wild plants in the surroundings of release fields for 2 d and considered parasitized if at least one egg was parasitized. Number of evaluated cards ranged between 182-200.

Survey year	Exposure dates	Parasitized egg cards (%)	Total number of parasitized eggs	Mean parasitized eggs	number of parasitized eggs (Range)	of Mean egg loss (%)
1999	May 10-11	4.5	169	18.8 (1-60)		44
	May 25-26	0	0	0		82
	June 9-10	0	0	0		68
	June 23-24	1.5	38	12.7 (1-21)		52
	July 4-5	1.0	3	1.5 (1-2)		70
	July 21-22	3.6	59	8.4 (1-18)		52
	August 2-3	2.0	41	10.3 (1-18)		45
	August 18-19	3.0	21	3.5 (1-8)		62
	September 1-2	1.0	11	5.5 (2-9)		58
	September 10-11	2.5	79	15.8 (6-47)		46
	October 7-8	1.0	18	9 (5-13)		31
	May 16-17	0.5	4	4		54
	May 23-24	0	0	0		50
	June 8-9	1.0	20	10.6 (6-14)		40
2000	June 19-20	0.5	10	10		46
	July 5-6	4.6	103	11.4 (3-39)		64
	July 18-19	0.5	5	5		67
	August 8-9	1.5	71	23.7 (13-34)		66
	August 23-24	3.5	187	26.7 (10-65)		61
	September 6-7	0	0	0		72
	September 21-22	3.5	60	8.6 (1-18)		67

Chapter 2

Parasitism of non-target lepidoptera by mass released *Trichogramma brassicae* and its implication for the larval parasitoid *Lydella thompsoni*

Stefan Kuske, Dirk Babendreier, Peter J. Edwards, Ted C.J. Turlings and Franz Bigler

Abstract

We investigated whether inundative releases of *Trichogramma brassicae* (Hym.: Trichogrammatidae) against the European corn borer, *Ostrinia nubilalis* Hb. (Lep.: Crambidae), pose a risk for two non-target lepidopteran species - *Archana geminipuncta* Haworth (Lep.: Noctuidae) and *Chilo phragmitellus* Hb. (Lep.: Crambidae) - and the native larval parasitoid *Lydella thompsoni* Hert. (Dipt.: Tachinidae). In laboratory trials, host acceptance by *T. brassicae* of the two most abundant spring hosts of the tachinid was assessed. In addition, parasitism of these two non-target species was investigated with extensive field surveys in a common reed habitat located amongst maize fields with *T. brassicae* releases. Both non-targets were found to be suitable hosts for *T. brassicae* under laboratory conditions. However, parasitism rates were significantly lower than for the target and/or the factitious host *Ephestia kuehniella* (Lep.: Pyralidae), either because their eggs are hidden between leaf sheaths and the stalk of the host plant or because of low attractiveness of the eggs. Under field conditions both hosts escaped parasitism by *T. brassicae* and we found no evidence of risk for both the non-target populations and the population of the native tachinid. We conclude that negative effects due to mass releases of exotic *T. brassicae* are unlikely to occur in this system.

Key words: *Lydella thompsoni*; *Ostrinia nubilalis*; *Trichogramma brassicae*; biological control; displacement; exotic parasitoids; host acceptance; inundative releases; native parasitoids; non-target effects

Introduction

Indirect interactions among species are pervasive in natural communities and are often quantitatively as important as direct interactions (Bender *et al.*, 1984; Polis and Holt, 1992; Menge, 1995). Nevertheless, they are rarely considered in ecological risk evaluation. Yet there are several examples of the displacement of a native parasitoid by an introduced one. Flanders (1958) found that the imported black scale parasitoid *Scutellista cyanea* Motschulsky largely replaced the indigenous black scale parasitoid *Moranila californica* Howard in California. The braconid *Cotesia flavipes* Cameron was introduced against the stalk borer *Diatrea lineolata* Wlk. in maize in Trinidad, and led to a strong reduction of the dominant native parasitoid *Apanteles diatraeae* Muesebeck (Bennett, 1993). After the introduction of a cool-weather strain of *C. flavipes* against *D. saccharalis* (F.) and *D. flavipennella* Box in sugarcane in Sao Paulo state in Brasil, the braconid became the dominant species, whilst the native tachinids *Metagonistylum minense* Townsend and *Paratheresia claripalpis* Wulp. have become scarce (Botelho, 1992). In Florida, the introduction of the aphelinid *Aphythis holoxanthus* DeBach led to successful control of the Florida red scale *Chrysomphalus aonidum* L., but almost displaced the encyrtid *Pseudohomalopoda prima* Girault, which was the main control agent before *A. holoxanthus* was introduced (Selhime *et al.*, 1969). Following the success of *A. holoxanthus* in Florida, this parasitoid was also introduced in Brasil for the control of the Florida red scale (Rosen and DeBach, 1979) and it seems that this introduction led to complete displacement of the native aphelinid *A. costalimai* Gomes, which previously had been the most common parasitoid there (Bennett, 1993). Although these examples have not resulted in extinction, they suggest that competitive suppression of native parasitoids does occur.

The release of high numbers of the exotic egg parasitoid *Trichogramma brassicae* Bezd. (Hym.: Trichogrammatidae) to control the European corn borer (ECB), *Ostrinia nubilalis* Hb. (Lep.: Crambidae) in maize has raised concerns about potential negative effects on native natural enemies. The native larval parasitoid *Lydella thompsoni* Herting (Dipt.: Tachinidae) is the most frequent and important ECB parasitoid in southern Switzerland. Its first generation emerges too early to find ECB larvae and must rely on alternative hosts living in natural habitats close to maize fields. Inundative releases of *T. brassicae* coincide with the oviposition period of the alternative hosts of the tachinid. *T. brassicae* moving out of release fields may attack and diminish the population of these hosts, creating a bottleneck situation for *L. thompsoni* in the subsequent spring. Host specificity tests in the laboratory showed that the tachinid's two most abundant spring hosts, *Archanara geminipuncta* Haworth (1809) (Lep.: Noctuidae) and *Chilo phragmitellus* Hübner (1805) (Lep.: Crambidae), are successfully parasitized by *T. brassicae* females in no-choice situations. Our extensive field surveys, however, showed that the two tested spring hosts escape parasitism since their eggs are well hidden or not attractive. Negative effects of inundative releases of *T. brassicae* on the native tachinid fly *L. thompsoni*, such as population density reduction, displacement, or local extinction, are very unlikely.

The work described in this paper concerns the impact of mass releases of *T. brassicae*. Inundative releases of *Trichogramma* are carried out against a wide range of lepidopteran pests worldwide and can be an effective and ecologically sound approach to deal with serious agricultural pest problems (Li, 1994; van Lenteren, 2000). However, large scale releases of these generalist egg

parasitoids may result in the attack of non-target hosts, both within and outside the crop. Consequently, direct interactions between agent and non-targets may influence non-target populations through population reduction, displacement or local extinction. Whilst pre-release studies and, in particular, host range testing has a long-standing tradition in weed biological control, it has only recently been taken into consideration in biological control programs against insect pests. Since testing of the host range of insect natural enemies is often relatively easy to assess and can be done both in laboratory and field trials, it should become a common feature in future ecological risk evaluation programs, even if the interpretation of host range tests is not always easy (Barratt *et al.*, 1999; Orr *et al.*, 2000; Schaffner, 2001). Recent studies evaluating the risk posed by *Trichogramma* for non-target insect species have paid a lot of attention to host-specificity and shown that most of the non-target lepidopterans tested are parasitized in the laboratory, and some of them even under field conditions (Andow *et al.*, 1995; Orr *et al.*, 2000). These findings call for further studies that assess the indirect impact of *Trichogramma* releases on native natural enemies of target and non-target hosts.

With the present study we simultaneously tested direct and indirect effects of *T. brassicae* releases on non-target insects. Maize production in southern Switzerland suffers severely from the attack of the ECB. For that reason, commercial inundative releases of 900,000 *T. brassicae* per hectare are carried out annually against the second generation of the ECB in most of the seed maize fields in Ticino. This biological control approach has now been applied for more than ten years and has led to successful control of the pest. The indigenous larval parasitoid *L. thompsoni* was selected to evaluate the potential for undesirable effects of released *T. brassicae* upon native natural enemies. This tachinid is known for its important role throughout southern Europe in regulating populations of the ECB and the pink stalk borer, *Sesamia nonagrioides* Lefebvre (Lep.: Noctuidae) (Guennelon, 1960; Hsiao, 1966; Grenier *et al.*, 1990). In the United States, *L. thompsoni* was introduced between 1920 and 1940, shortly after the ECB was brought into America (Thompson, 1928; Baker *et al.*, 1949; Brindley *et al.*, 1975). In the early 1960s it disappeared from North America, but was reintroduced in the late 1970s (Jarvis and Guthrie, 1987), and nowadays is established in several regions where it contributes to the natural control of the pest (Mason *et al.*, 1994).

In southern Switzerland, where *L. thompsoni* is native, parasitism rates are highly variable both among maize plots and years, and the impact of *L. thompsoni* is not sufficient to control the pest. However, the tachinid is the most abundant and efficient native ECB parasitoid in the study area. ECB larvae experience a mean parasitism by *L. thompsoni*, of about 20 % in the first and around 10 % in the second ECB generation, and in some maize fields up to 50 % or more. These parasitism rates are similar to or even higher than, those found in other regions of Europe (Cagan *et al.*, 1999; Manojlovic, 1985). In spring, *L. thompsoni* parasitizes stalk borer larvae feeding on common reed plants (*Phragmites australis* (Cav.) Trin. ex Steud.) in natural habitats, where they complete their first generation. Afterwards, adults emerging in reed habitats move into maize fields to complete two subsequent generations on the ECB (Galichet *et al.*, 1985; Eizaguirre *et al.*, 1990; Grenier *et al.*, 1990). It is likely that *T. brassicae* adults disperse from release fields in summer and enter adjacent non-target habitats where they could parasitize eggs of native hosts of *L. thompsoni*. Since the tachinid

relies on the larvae of these hosts in spring of the next year, egg parasitism by *T. brassicae* could have undesirable effects on the population of *L. thompsoni*.

The aim of this study was to determine whether potential spring hosts of the native larval parasitoid *L. thompsoni* are threatened by inundative releases of *T. brassicae* in seed maize fields in Ticino. In laboratory trials, we investigated host acceptance and suitability of the two most abundant spring hosts of the tachinid. In addition, parasitism of these two non-targets was investigated with extensive field surveys in a common reed habitat located amongst maize fields with *T. brassicae* releases. Finally, we analysed whether the impact of *Trichogramma* can reach across habitat boundaries and lead to the displacement of *L. thompsoni*.

Materials and Methods

Parasitoids

All *T. brassicae* used in this study originated from a laboratory colony reared by Biotop (Valbonne, France) and marketed as Trichocap®. Inexperienced, mated, one day old *T. brassicae* females, which had been fed with honey were used in all laboratory experiments. The field observations were conducted in seed maize fields following commercial inundative releases of *T. brassicae* against the ECB.

Hosts

The non-target hosts used in this study were the twin-spotted wainscot *A. geminipuncta* and the common reed borer *C. phragmitellus*. Both species were collected as late instar larvae from common reed plants in their natural habitats in the Magadino Plain, and reared in the laboratory. Adult moths were allowed to emerge in cages (30 x 50 x 60 cm) and freshly cut stalk pieces of reed (50 cm length, > 5 mm diameter) were offered for oviposition. *A. geminipuncta* females stick their eggs in a line underneath the leaf sheaths, whereas *C. phragmitellus* females oviposit on the leaf surface. Stalk pieces containing freshly laid eggs were changed daily and, if not used immediately for the experiments, stored at $14\pm1^{\circ}\text{C}$, $75\pm5\%$ rh.

Ephestia kuehniella Zeller (Lep.: Pyralidae) and the ECB were used as hosts in the control treatments. Freshly laid ultraviolet-irradiated eggs of *E. kuehniella* were provided by Biotop (Valbonne, France). If not used immediately, the eggs were stored at $5\pm1^{\circ}\text{C}$, $80\pm5\%$ rh. For the experiments sentinel egg-cards were used. These contained about 400 *E. kuehniella* eggs glued to white cardboard strips (20 x 70 mm) using a water soluble glue (Planatol®, HW 730, dilution 1:10).

ECB eggs were obtained from moths that were collected as late instar larvae or pupae in maize fields in the Magadino plain and reared in the laboratory. Adult moths were allowed to oviposit on the leaves of maize plants in sleeve cages. Egg masses were not older than 24 hours when used in the experiments.

Laboratory no-choice experiments

All laboratory experiments with *T. brassicae* and their hosts were performed, if not otherwise stated, at $24\pm1^{\circ}\text{C}$, $70\pm5\%$ rh, L:D 16:8 hours.

Accessibility and suitability of *Archanara geminipuncta*.

Experiment 1: To test whether *A. geminipuncta* is accepted and successfully parasitized by *T. brassicae*, eggs of *A. geminipuncta* were offered to individual *T. brassicae* females within small plastic tubes (length: 50mm, diameter: 10mm) for 24 hours. *A. geminipuncta* lay their eggs inwardly of the leaf sheath of common reed plants, such as they stick between the leaf sheath and the stalk. Thus, leaf sheaths were first detached from the stalks and then cut into small pieces containing five eggs each. Individual females were offered a piece with either one, three, seven, or twelve day old eggs. There were 20 females tested per age class. The control treatment consisted of the sentinel egg cards (n=20). Parasitism was checked after one week, and eggs were reared until offspring emerged. The percentage of females that parasitized at least one host egg, the average number of progeny per female, the average number of wasps emerging from parasitized eggs, and the sex ratio (percentage of females) were assessed.

Experiment 2: To test whether the hidden position of naturally laid *A. geminipuncta* eggs provides protection against parasitism by *T. brassicae*, the following experiment was set up: Pieces of reed stalk (length: 85-90 mm) containing 12-36 h old *A. geminipuncta* eggs were offered to individual *T. brassicae* females (n=51) within plastic tubes (length: 90mm, diameter: 13mm) for 24 hours. Adhesive tape was fixed on both ends of the stalks before the experiment was started, to prevent the leaf sheaths from detaching. The total number of eggs per stalk was counted, and the percentage of females that parasitized at least one egg and the percentage of parasitized eggs were assessed. In a control treatment 60 individual *T. brassicae* females (of the same *Trichogramma* patch as used above) were offered an egg-card containing *E. kuehniella* eggs in plastic vials (length: 80 mm, diameter: 25 mm) for 24 hours.

Suitability of *Chilo phragmitellus*.

We investigated whether *T. brassicae* is able to parasitize egg masses of *C. phragmitellus* successfully. Reed leaves containing one day old egg masses of *C. phragmitellus* were cut into pieces about 40 mm long and fixed onto cardboard strips (length: 70 mm, width: 20 mm) with adhesive tape to prevent them from curling up. The egg masses were offered to individual *T. brassicae* females within plastic tubes (length: 80mm, diameter: 25mm) for 24 hours. Altogether 71 and 18 females were tested in 1999 and 2000 respectively. The mean number of eggs per egg mass was 28 (3-89) in 1999 and 27 (2-85) in 2000. The following parameters were assessed: percentage of females that parasitized at least one host egg, average number of parasitized eggs, and total number of offspring per female. Hosts used in the control treatments were *E. kuehniella* on sentinel egg-cards in 1999 (n=60), and ECB on maize leaves in 2000 (n=10).

Acceptance of *Chilo phragmitellus*.

The host acceptance behaviour of individual *T. brassicae* females was studied directly under a dissecting microscope after releasing an inexperienced female into a plastic arena (diameter: 25mm, height: 10mm) covered with a microscope slide. Host contact (female touches host with antennae), drumming on host eggs, drilling into host, probing host contents with ovipositor, and the final acceptance of the host (oviposition) were recorded as behavioural events. Individual 12-36 h old egg masses of *C. phragmitellus* (mean: 31 eggs, range: 5-102) or *O. nubilalis* (10-30 eggs) were offered to individual *T. brassicae* females. The experiments were conducted with 35 females on *C. phragmitellus*, and 32 females on *O. nubilalis*. Observations started after the female had been introduced into the arena and lasted 15 min. If no host contact was observed within the first ten minutes the female was replaced. All experiments were conducted between 8 and 12 a.m. at $24 \pm 1^\circ\text{C}$, $50 \pm 10\%$ rh; the two hosts were alternated in the arena. We assessed the percentage of females that parasitized at least one host egg during the experiment. As behavioural parameters, we measured the time from first host contact until a female either penetrated the egg shell with the ovipositor (followed by egg laying) or left the egg mass and searched the arena for at least five seconds. We also counted the number of egg mass contacts until final acceptance, and the average number of ovipositions during the experiment. For those females who did not parasitize the host, we assessed the average number of host contacts, drumming sequences, and the average time spent on the egg mass during the experiment.

Field experiments

Site description and inundative releases.

To check whether *A. geminipuncta* and *C. phragmitellus* are attacked by *T. brassicae* under field conditions, egg masses of both species were exposed in a common reed stand (0.3 ha) at Gudo (Ticino). The selected non-target habitat was located between seed maize fields that were commercially treated with *T. brassicae*. The treatment dates were: 24 July, and 3 August in 1998, 13 July, 27 July and 4 August in 1999, 25 July and 3 August in 2000. In 1998 and 2000, 900.000 adults/ha were released, and in 1999 1.200.000 adults/ha. Females of the two one to three days old non-target species were enclosed in sleeve cages (length: 105 cm, diameter: 38 cm) for up to two days to oviposit on reed plants (three plants per cage); they were provided daily with water and honey. The sleeves then were withdrawn and the plants left unprotected.

Parasitism of *Archanara geminipuncta*.

Between 24 July and 10 August 1998 eggs of *A. geminipuncta* were obtained from reed plants. It was not possible to count the total number of eggs laid without damaging the leaf sheaths of the host plants; this parameter was therefore assessed when the host plants were dissected in the laboratory. Twenty host plants per date (one plant per sleeve cage and date) were collected after one, three, and eight months respectively. These exposure periods include the egg stage of *A. geminipuncta*. Eggs are laid in July and August and last in a dormant stage until early spring of the subsequent year when embryonic development is completed (Michel and Tscharnkte, 1993). Exposure time, distance to the closest treated maize field, and the number of reed plants offered for oviposition are shown in Table 1. We determined the total number of host plants from which eggs were recovered; we also assessed the total number of eggs recovered and assessed the percentage of parasitized eggs. Healthy and parasitized eggs were incubated under outdoor conditions until a parasitoid or a caterpillar emerged, or until it was clear that neither would develop. If parasitoids had already emerged when the plants were collected, the family of the parasitoid could be identified from the position and shape of the emergence holes; in addition some insects were found which had died prior to emergence.

In addition to the eggs laid on the plants, pieces of reed stalk (length: 25 cm) containing eggs of *A. geminipuncta* (obtained from ovipositions in the laboratory) were placed 10 m apart in the vegetation at the edge of a stand of reeds. Detailed information concerning the exposure of stalk pieces is shown in Table 1. The first day of exposure coincided with the emergence of the *T. brassicae* released in the adjacent maize field. After collection they were immediately dissected in the laboratory. Additional pieces of stalk containing *A. geminipuncta* eggs were exposed simultaneously in a natural reed stand 3 km to the west of the first site. In contrast to the first site, no *T. brassicae* had been released into the surrounding maize fields at this location. For each of the pieces of stalk the total number of eggs recovered and the percentage of parasitized eggs were assessed.

Parasitism of *Chilo phragmitellus*.

In order to determine levels of parasitism in the field *C. phragmitellus* eggs on reed plants were checked between 3 and 10 August in 1999 (n=23), and between 31 July and 12 August in 2000 (n=61). In addition, 14 pieces of reed stalk (length: 0.5m) containing *C. phragmitellus* eggs were obtained from the laboratory ovipositions and exposed 10 m apart in the vegetation at the edge of the reed stand in 1999. The number of exposed eggs, the exposure time and the distance to the closest maize field with *T. brassicae* releases are shown in Table 1. After collection the eggs were incubated in plastic vials in the laboratory to observe whether a parasitoid or a caterpillar emerged.

Parasitism of control hosts.

For each reed plant or piece of stalk containing eggs of either *A. geminipuncta* or *C. phragmitellus*, one sentinel egg-card, containing 400-500 UV-irradiated *E. kuehniella* eggs was exposed as a control. In addition, we exposed in the reed stand egg-cards (n=26) containing 50-100 eggs (< 3 days old) of *Mamestra brassicae* L. (Lep.: Noctuidae) from 9-11 August in 2000. The sentinel egg-cards were attached to neighbouring plants during the first day of exposure of the non-target eggs,

and were collected after two days and incubated in the laboratory. Parasitism was determined one week later. Parasitoids that emerged from exposed eggs in each of the field experiments were identified either by PCR (only *Trichogramma*) or classical taxonomy.

Results

Laboratory no-choice experiments

Accessibility and suitability of *Archanara geminipuncta*.

Experiment 1: *A. geminipuncta* was found to be a suitable host for *T. brassicae*. The percentage of females that parasitized at least one host egg and the mean number of progeny per female were similar for one day old *A. geminipuncta* eggs (parasitism: 80%, progeny per female: 16.4 ± 10.5) and *E. kuehniella* eggs (parasitism: 95%, progeny per female: 15.8 ± 8.9). However, both the number of parasitizing females ($\chi^2=41.67$, $df=3$, $p<0.001$) and the number of progeny per female (Mann-Whitney-U-test, $U_{3,76}=26.7$, $p<0.001$) decreased significantly with increasing egg age of *A. geminipuncta* (Figure 1). On the other hand, the mean number of offspring from parasitized eggs was similar for eggs which were one (6.3; range: 3-10), three (6.1 range: 3-11), and seven (6.7 range: 6-7) days old; the sex ratios were 0.50, 0.60, and 0.65 respectively.

Experiment 2: The mean number of *A. geminipuncta* eggs exposed to individual *T. brassicae* females was 18.9 (2-82) per stalk piece. Only two out of 51 females tested (3.9%) were able to parasitize at least one non-target egg and to produce offspring, whereas in the control treatment 73.3% of the wasps ($n=60$) parasitized on average 17.1 (2-35) *E. kuehniella* eggs. The percentage of *A. geminipuncta* eggs parasitized was 0.21% and the total number of offspring per female was in one case eight (6 females, 2 males) and in the other five (4 females, 1 male) individuals.

Suitability of *Chilo phragmitellus*.

Chilo phragmitellus proved to be a suitable host for *T. brassicae*. However, the percentage of *T. brassicae* females that parasitized *C. phragmitellus* was significantly lower than in the control treatments, both in 1999 (χ^2 : 31.89, $df=1$, $p<0.001$, Fig. 2) and 2000 (χ^2 : 8.3, $df=1$, $p<0.01$) (Figure 2). The mean number of parasitized eggs per female was 0.9 (range: 0-9) in 1999 and 1.2 (0-8) in 2000 (Figure 3). The numbers of progeny per female were 2.2 (0-27) and 2.3 (0-15) respectively; these values were significantly lower than the mean of 17.3 (0-32) on *E. kuehniella* ($U_{71,60}=586.5$, $p<0.001$), and of 16.8 (0-27) ($U_{18,10}=16.5$, $p<0.001$) on *O. nubilalis*.

Acceptance of *Chilo phragmitellus*.

Direct observations (15 min) of the host acceptance behaviour showed that *C. phragmitellus* was much less attractive than *O. nubilalis*. The non-target *C. phragmitellus* was parasitized almost ten times less than the target ($\chi^2 = 35.97$, $df = 1$, $p < 0.001$, Tab. 2). Following the first host contact, *T. brassicae* females spent significantly less time on *C. phragmitellus* before deciding whether to penetrate an egg with the ovipositor and to lay eggs or to leave the egg mass for at least 5 s ($U_{35,32} = 42.0$, $p < 0.001$, Table 2). The average number of egg mass contacts until final acceptance was significantly higher ($U_{3,26} = 5.5$, $p = 0.016$), with *C. phragmitellus* than with *O. nubilalis*; the mean number of ovipositions was also lower, though the differences not significant ($U_{3,26} = 13.5$, $p = 0.068$) (Tab. 2). Females that did not parasitize the host showed similar numbers of host contacts and of drumming sequences upon either the target or the non-target ($U_{32,6} = 78.5$, $p = 0.48$); however, they spent only 40 ± 52 s on *C. phragmitellus* eggs, whereas they stayed on *O. nubilalis* egg masses for 159 ± 170 s ($U_{32,6} = 33$, $p = 0.12$).

Field experiments

The total numbers of *A. geminipuncta* eggs that were laid on common reed plants and on stalk pieces were assessed in the laboratory after dissection of the plants. We recovered 788 eggs from 23 common reed plants and 4842 eggs from 61 stalk pieces (Tab. 1). The percentage of parasitized *A. geminipuncta* eggs was generally high on common reed plants, but very low ($< 1\%$) on stalk pieces (Tab. 1). However, none of the parasitized eggs were attacked by *Trichogramma* parasitoids. All parasitoids found in this experiment belonged to the genus *Telenomus* (Hym.: Scelionidae), and were assigned to the *busseolae* species-complex (F. Bin, personal communication).

The exposure of *C. phragmitellus* egg masses yielded no parasitized egg masses at all, either in 1999 or in 2000. However, eggs were fertile and larvae hatched from all exposed egg-masses. Moreover, none of the egg-cards containing *E. kuehniella* eggs but 23 % of those containing *M. brassicae* eggs were parasitized. We found *Trichogramma evanescens* Westwood emerging from one of the parasitized egg-cards, whilst all others were parasitized by an unidentified scelionid.

Discussion

The present study addresses the question of whether inundative releases of *T. brassicae* in maize may affect the population of the native larval parasitoid *L. thompsoni* by exploiting the same hosts in reed habitats. The hosts in question are *A. geminipuncta* and *C. phragmitellus*, and we investigated whether their eggs are attacked by *T. brassicae* and if so, whether their population is affected.

Impact on *Archana geminipuncta*

A. geminipuncta escaped parasitism by *T. brassicae* because its eggs are laid hidden between leaf sheath and reed stalk. Under field conditions, none of 5630 *A. geminipuncta* eggs were attacked. In the laboratory, the effective protection reduced *T. brassicae* attacks to less than 10 %. Eggs that were removed from the usual position and presented in the lab without protection, however, are suitable hosts for *T. brassicae*. Production of *T. brassicae* offspring from one day old *A. geminipuncta* eggs was similar to that from the factitious host *E. kuehniella*, but decreased significantly with increasing egg age. Decreasing acceptance of older host eggs, however, is widespread in *Trichogramma* (Pak *et al.*, 1986). In this case, the combination of both mechanical protection and decreasing suitability of host eggs reduce almost to zero the risk of *A. geminipuncta* being affected by *Trichogramma* parasitism. Lu (1991) made similar observations for the African maize stemborer *Busseola fusca* (Lep., Noctuidae) in Kenya, which was shown in the laboratory to be a suitable host of *Trichogramma* sp. (near *T. mwanzai* Schultien and Feijen); because the eggs are laid underneath the leaf sheaths, *B. fusca* was not attacked in fields when *T. sp.* (near *T. mwanzai*) was released to control *Chilo partellus* Swinhoe.

Although *A. geminipuncta* was not attacked by *Trichogramma* it was parasitized by scelionids. Unlike *T. brassicae*, scelionids can overcome the mechanical protection provided by the leaf sheath by crawling between the stalk and the leaf sheath (F. Bin, personal communication). Michel and Tscharnkte (1993) found 28 % of *A. geminipuncta* egg clusters parasitized by scelionids in a common reed habitat near Karlsruhe (Germany), whereas none of the eggs were parasitized by *Trichogramma*. However, they found the related noctuid, *A. dissoluta*, parasitized by an unidentified *Trichogramma* species. Since all noctuid spring hosts of *L. thompsoni* that are known to occur in the study area lay their eggs in a similar position on the plant, it seems likely that they all escape parasitism by *T. brassicae*; their populations are probably not affected by inundative releases of this parasitoid in Ticino.

Impact on *Chilo phragmitellus*

Many species of the genus *Chilo* are agricultural pests of several important crops and have been successfully controlled by *Trichogramma* in Asia, Africa, USA, South America, Hawaii, and other places (DeBach & Hagen, 1964; David & Easwaramoorthy, 1990; Li, 1994). *C. phragmitellus* egg masses are similar both to those of other *Chilo* species and to the ECB, and we expected them to be attractive hosts for *T. brassicae*. Contrary to our expectations *C. phragmitellus* was strongly rejected. While most *T. brassicae* females parasitized the ECB and the control host *E. kuehniella* in 24 h laboratory no-choice experiments, only one third of the females parasitized *C. phragmitellus*. Direct observations (15 min) of *T. brassicae*'s host acceptance behaviour on *C. phragmitellus* showed that its acceptance was only 10% of that of the ECB. Following the first egg mass contact *T. brassicae* showed only little interest in *C. phragmitellus* eggs. Within few seconds they made a decision whether to reject or accept the host, whereas ECB egg masses were in general drummed for about one and a half minutes until they were either rejected or - as was usually the case - accepted. Even those

T. brassicae females that finally parasitized *C. phragmitellus* still contacted the eggs significantly more often until first oviposition, and the total number of ovipositions was lower than with the ECB.

Bournier (1982) investigated the performance of ten *Trichogramma* strains (including *T. brassicae*) on *C. partellus* and *Heliothis armigera* Hb. (Lep., Noctuidae) and found big differences in the number of parasitized eggs per female and in the fecundity among strains. In his tests *T. brassicae* (= *T. maidis* Pintureau & Voegelé) showed best results on *C. partellus*. Explanations for these findings, however, were not given.

Lu and Oloo (1990) found that 0-2 d old eggs of *C. partellus* were the most preferred by *T. sp. nr. mwanzai* while 4 d old eggs escaped parasitism. Similar observations are known for *T. perkinsi* and *T. australicum*, that prefer 1-3 d old eggs of *C. partellus* (Somchoudhury and Dutt, 1989). However, the age of eggs cannot explain the low parasitism in our experiments, since one day old eggs were offered to *T. brassicae* in the laboratory. Although the reasons for the low attractiveness of *C. phragmitellus* eggs to *T. brassicae* remained unclear, we conclude that under natural conditions *T. brassicae* is unlikely to successfully parasitize *C. phragmitellus*. Populations of *C. phragmitellus* are probably not affected by *T. brassicae* moving into common reed habitats. This conclusion, which is in contradiction to our initial hypotheses was confirmed in our field survey, where none of the *C. phragmitellus* egg masses exposed were parasitized. Detrimental effects on *C. phragmitellus* due to *T. brassicae* inundative releases therefore may not occur.

Impact on *Lydella thompsoni*

Inundative releases of *T. brassicae* against the ECB have been carried out in southern Switzerland for more than 10 years without leading to displacement of *L. thompsoni* in maize. Contrary to our initial hypothesis the impact of *T. brassicae* mass releases did not reach across habitat boundaries, and populations of the tachinid's spring hosts appear not to have been reduced. Potential negative effects on the native tachinid fly *L. thompsoni* are restricted to maize fields with *T. brassicae* releases, where the tachinid is placed at a disadvantage, in as much as it depends on the larval stage of the ECB. Since the first ECB generation is not treated with *Trichogramma*, only the *L. thompsoni* generation that attacks the second ECB generation will be affected. Moreover, in the study area *T. brassicae* is released in only about 10% of the maize fields, making it even less likely that *L. thompsoni* populations are affected. The risk that *L. thompsoni* is excluded from maize, however, may increase if both the percentage of *Trichogramma* treated maize fields and the degree of isolation of untreated fields increases. However, even if *L. thompsoni* were to disappear completely from maize fields, (which has not yet occurred even in maize fields that have been treated annually with *T. brassicae* for more than ten years) it could still persist in native habitats. We conclude that negative effects due to mass releases of exotic *T. brassicae* are unlikely.

Although we found no evidence of risk, either for the tested non-target hosts or the native tachinid fly *L. thompsoni*, further studies are needed which analyse the effects of parasitoid introductions on non-target hosts and native natural enemies in other systems. Some have argued that indirect impacts of arthropod introductions on parasitoids of non-target hosts may be even larger than the impacts on the hosts themselves, since parasitoids may be more liable to extinction than their

hosts (LaSalle & Gauld, 1992; Unruh & Messing, 1993; Kruess and Tscharncke 1994, 2000). Hence, both direct and indirect effects on native natural enemies should be considered in future ecological risk evaluations. The present study may give some impulse to how this could be done for other systems as well.

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Captions for figures

Figure 1. (a) Percentage of *Trichogramma brassicae* (Hym., Trichogrammatidae) females parasitising *Archana geminipuncta* Haw. (Lep., Noctuidae) eggs and (b) mean number of progeny per wasp (+SD) in a 24 h no-choice laboratory experiment ($24\pm1^{\circ}\text{C}$, $70\pm5\%$ rh, L:D 16:8). Eggs detached from stalk of host plants were offered to individual, one day old, honey fed *T. brassicae* females (n=20). Bars marked with different letters indicate significant differences in offspring number between host eggs of different age (ANOVA, Newman-Keuls test; $p<0.001$).

Figure 2. Parasitism of *Chilo phragmitellus* Hb. (CP), *Ostrinia nubilalis* Hb. (ECB), and *Ephesia kuehniella* Zeller (EK) by *Trichogramma brassicae* Bezd. in 24 h no-choice laboratory experiments. Freshly laid egg masses were offered to individual, one day old, honey fed *T. brassicae* females at $24\pm1^{\circ}\text{C}$, $70\pm5\%$ rh, L:D 16:8. Number of tested females is given in brackets. (a) Percentage of *T. brassicae* females that parasitised the host. Bars marked with asterisks indicate significant difference between parasitism rate of non-target host and respective control (χ^2 -test procedure; ** $p<0.01$; *** $p<0.001$). (b) Mean number of parasitised host eggs per female (+SD). Bars marked with asterisks indicate significant difference between number of parasitised non-target host eggs and both target and control host eggs respectively (t-test procedure; *** $p<0.001$).

Figure 1

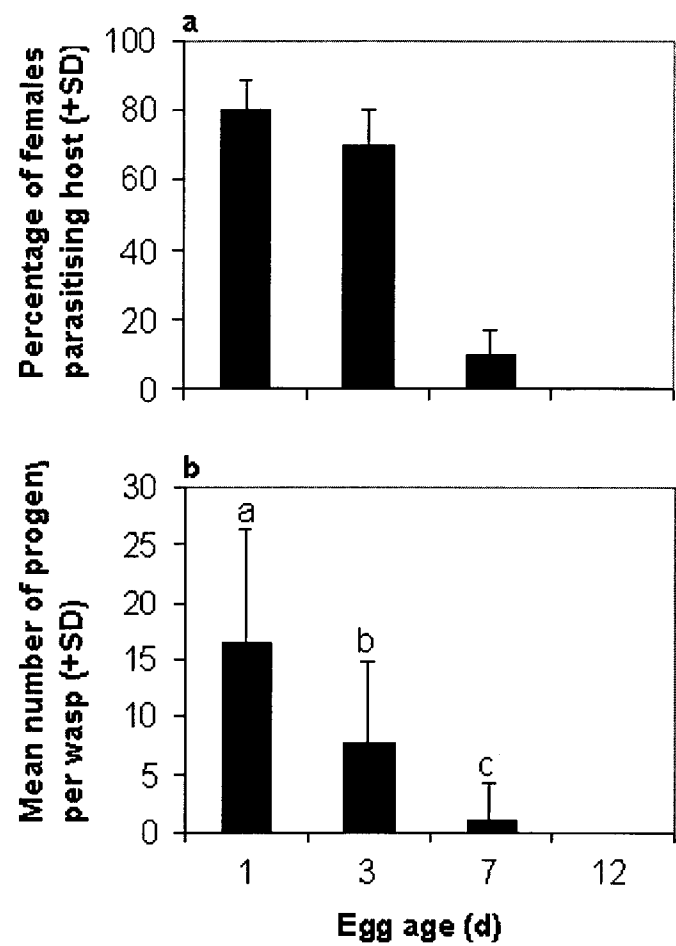


Figure 2

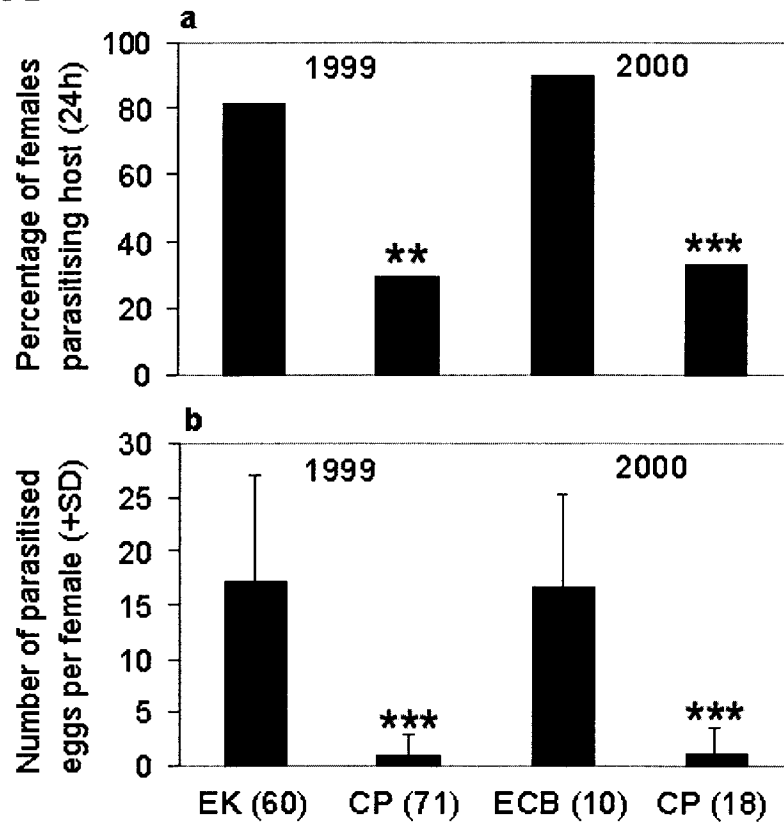


TABLE 1 Field exposure and parasitism of non-target lepidopteran eggs. Eggs were obtained through ovipositions by non-target adult moths in sleeve cages in a natural common reed habitat adjacent to maize fields that were commercially treated with *Trichogramma brassicae* against the European corn borer.

Host	Study year	Experimental conditions ¹	Total number of host plants on which eggs were recovered	Total number of recovered eggs	Percentage of parasitized ² eggs (%)
<i>Archanara geminipuncta</i> Haw. (Lep., Noctuidae)	1998	Plants (30 d; 10 m; 20)	7	320	65.3
		Plants (83 d; 10 m; 20)	10	367	68.4
		Plants (237 d; 10 m; 20)	6	101	81.2
		Stalks (8 d; 5 m; 20)	20	1929	0.8
		Stalks (8 d; 1500 m; 20)	19	1641	0
		Stalks (8 d; 5 m; 19)	11	738	0.05
		Stalks (8 d; 1500 m; 20)	11	534	0.2
<i>Chilo phragmitellus</i> Hb. (Lep., Crambidae)	1999		23		0
			14		0
	2000		61		0

¹Numbers in brackets show mean exposure period of eggs (d), distance to closest treated maize fields (m), and total number of plants or stalks offered for oviposition

²Parasitoids found belonged to *Telenomus* sp. (Hym., Scelionidae); *Trichogramma* did not parasitize non-targets.

TABLE 2 Host acceptance behaviour of *Trichogramma brassicae* on egg masses of *Chilo phragmitellus* (n=35), and *Ostrinia nubilalis* (n=32). The average time used until acceptance or rejection was defined as the time a female spent on a host egg mass after first contact until either the egg was penetrated with the ovipositor (followed by egg laying) or the female left the host and searched the arena for at least 5 s. Numbers followed by different letters in the same column indicate significant differences in means (Mann-Whitney-U test procedure, $p < 0.05$).

Host	Percentage of females that parasitized host	Average time used until acceptance or rejection in s (+SD)	Average number of egg-mass contacts until oviposition (+SD)	Average number of ovipositions per accepting female (+SD)
<i>O. nubilalis</i>	81.3	87.4 (79.7) a	1.4 (0.8) a	2.7 (1.2) a
<i>C. phragmitellus</i>	8.6	7.3 (15.5) b	4 (2) b	1.3 (0.6) a

Chapter 3

Bridging the generation gap between a major insect pest and its natural enemy

Stefan Kuske, Ted C.J. Turlings, Peter J. Edwards, Dirk Babendreier & Franz Bigler

Although it is generally accepted that increasing structural diversity in agro-ecosystems favours predatory and parasitic insects, we know little about exactly how habitat diversification affects the control of insect pests by native natural enemies. Here we present a study that clearly shows that the effectiveness of an important parasitic fly in controlling the European corn borer (ECB), *Ostrinia nubilalis*, is strongly increased in the vicinity of common reed stands, and that simple changes in agricultural practices could significantly improve biological control of this important pest.

Preserving natural habitats to enhance biodiversity in the vicinity of crop fields is recognised to be a key measure in promoting insect pest control by native natural enemies¹⁻³. However, for sustainable effectiveness of natural enemies, their specific requirements, for example alternative food sources, shelter or overwintering sites⁴⁻⁷, must be met by these non-crop habitats. Several authors have stressed the need for field studies of sufficiently large scale to investigate the response of natural enemies to disturbance and disappearance of essential resources in the agro-ecosystem, and to make predictions of their pest control potential⁸⁻¹¹. Here we present a two-year field study that reveals the mechanism by which the proximity of maize fields to stands of common reed determines the efficiency of an important larval parasitoid in controlling the ECB. The spring generation of the tachinid parasitoid *Lydella thompsoni* utilises non-pest hosts in reeds and builds up a population which attacks ECB larvae that hatch around six weeks later in nearby maize fields. Thus, the reed habitat allows the parasitoid to overcome the gap between parasitoid emergence and the availability of pest larvae in maize. The seasonal dynamics of these metapopulations imply that simple, non-invasive management of otherwise unexploitable wetlands could significantly enhance the effectiveness of biological control of ECB.

The ECB - one of the most destructive pests of maize in Europe and North America - is often controlled by the release of *Trichogramma* egg parasitoids¹²⁻¹³. Although this form of biological control can be successful, it is not always cost effective. A complementary method, applicable in southern Europe, would be to enhance the effectiveness of the parasitic fly *Lydella thompsoni*, a native natural enemy of ECB that can reach control levels of up to 70% and more. Parasitisation rates by this parasitoid are, however, extremely variable¹⁴. *L. thompsoni* initially showed promising results in controlling the ECB when introduced from Europe into the USA, but it disappeared almost completely after only a few years; most subsequent introductions have also been unsuccessful¹⁵⁻¹⁶. One reason for the decline of *L. thompsoni* in the USA may be its competitive replacement by the microsporidium *Nosema pyrausta*¹⁷. Another explanation, however, could be the poor phenological synchronisation between pest and parasitoid, although this aspect has never been investigated. In the southern maize growing regions of Europe, the pest has two generations per year, while *L. thompsoni* has three^{14,18-19}. This means that in spring the parasitoid usually emerges too early to find ECB larvae, and must rely on alternative hosts¹⁹. This is the case in southern Switzerland, where the majority of *L. thompsoni* adults emerge about six weeks earlier than those of ECB and attack reed stalk borers in natural wetlands. The two most important are *Archanara geminipuncta* and *Chilo phragmitellus*, of which the former species appears to be the preferred spring host of *L. thompsoni* and a key species in its life-cycle (Fig. 1). Here we test the hypothesis that the ECB control potential of *L. thompsoni* depends on the presence of this early season host.

During this study *Trichogramma* releases were minimal in the study area on the Magadino Plain and ECB infestation was high compared to that reported from other regions in Europe^{14,20-22}. Pest densities, parasitism rates, and parasitoid population density in maize fields are shown in Table 1. Population densities of *L. thompsoni* in the alternative host habitats investigated in this study were 7.791 ± 5.647 flies per ha in 1999 and 13.997 ± 11.516 flies per ha in 2000. We found no significant correlation between the population density of *L. thompsoni* in a reed habitat and parasitism of ECB in surrounding maize fields. Moreover, neither pest population density nor the prevailing winds in the Magadino Plain could explain differences in parasitism rates found in different maize fields. However, in both years there was a very strong negative correlation between parasitism of first generation pest larvae by *L. thompsoni* and distance from a natural reed stand ($p < 0.01$ in 1999 and $p < 0.001$ in 2000; Fig. 2). The spatial pattern of parasitism around each of the selected alternative host habitats clearly shows that parasitism by *L. thompsoni* decreased with increasing distance from a reed stand (Fig. 3). In the second pest generation, the effect of distance from a stand of reeds upon ECB parasitism by *L. thompsoni* in maize fields was significant in 1999 ($Y = 69.74 - 9.2\text{Log}(x)$, $t(16) = -3.21$, $r^2 = 0.392$, $p < 0.01$) but not in 2000 ($Y = 40.90 - 2.51\text{Log}(x)$, $t(11) = -1.63$, $r^2 = 0.195$, $p = 0.131$). Thus, the strength of the distance effect was reduced in the second pest generation, presumably because maize fields are supplied with *L. thompsoni* adults originating from reeds only during the first pest generation, whereas later in the season parasitoids are distributed throughout the area and every maize field is a potential source of the parasitic fly.

These findings indicate that the presence and spatial arrangement of common reed stands in the agricultural landscape have a strong influence on the effectiveness of biological control of the ECB

by *L. thompsoni*. It is also clear that the availability of suitable reed habitats within the study area is a limiting factor for this complex system. The Magadino Plain is typical of many parts of Europe that have experienced drastic changes in land use as a result of agricultural intensification. The proportion of the study area covered by maize fields increased five-fold between 1929 and 1985²³. The remaining wetlands occur as scattered fragments within the large maize-dominated areas, representing relicts of a habitat type that has decreased to 0.1-1% of its former extent²³⁻²⁵. Such habitat fragmentation is generally considered to affect the abundance of specialised natural enemies such as *L. thompsoni* more severely than that of their phytophagous hosts²⁶⁻²⁸. It is surprising, therefore, that *L. thompsoni* is still a frequent and effective parasitoid of the ECB not only in southern Switzerland, but also in other parts of southern Europe^{15,18-20,29}. The complex relationship between the parasitoid and its hosts explain the dramatic fluctuations in parasitism between sites, hosts and seasons, but it seems that in the study area the remaining natural habitat is sufficient to maintain a certain balance in this particular system.

Metapopulation studies focus on the dynamics of populations that occupy discrete patches in fragmented habitats. The *Lydella*-ECB system seems a useful model to add a new dimension to such studies of habitat fragmentation by including the consequences of seasonal changes. In our study the distribution of suitable host habitats was extremely fragmented in spring and highly uniform over a large area later in the season. Our results show the importance of this seasonal change in patchiness of suitable host habitats for the build-up of the *L. thompsoni* population.

We suspect that the pest control potential of *L. thompsoni* has been greatly underestimated because suitable habitats for its early season hosts are absent in many agricultural areas. One way to increase populations of these hosts would be to ensure that patches of their habitat are distributed throughout the agricultural landscape, and that these patches are managed appropriately. Although reed is common along irrigation and drainage ditches, streams and canals in arable areas throughout southern Europe, these stands are often unsuitable for reed stalk borers such as *A. geminipuncta* because of mowing and other forms of disturbance. To complete its univoltine life-cycle, *A. geminipuncta* requires reed stalks of adequate diameter and minimal disturbance. The stems of frequently cut reeds are usually too thin³⁰. Hence, simple changes in the management of reed vegetation could significantly enhance the effectiveness of biological control of the ECB and may further reduce the need for pesticides against this highly destructive pest.

Material and methods

Alternative host and habitat selection

On the Magadino Plain *Lydella thompsoni* parasitises the European corn borer and the four non-pest hosts *Archanara geminipuncta*, *A. dissoluta*, *Nonagria typhae*, and *Chilo phragmitellus*. During this study, only the monophagous species *A. geminipuncta* and *C. phragmitellus* on common reed (*Phragmites australis*) were frequently found, and only the former was heavily parasitized. We therefore focussed on *A. geminipuncta*. In 1998 we recorded its abundance in all reed sites > 0.1 ha on the Magadino Plain. The fading colour of the top leaf is a good indicator of reed stalk borer attack¹⁹ and was used to obtain a rapid estimate of the abundance of stalk borers within these habitats. For

subsequent study, we selected patches of reeds ≥ 0.3 ha in which $> 5\%$ of shoots were attacked by *A. geminipuncta*. In addition, we selected three (1999) or four (2000) maize fields at increasing distances up to 1130 m away from the reeds. Distances were measured from the edge of a reed stand to the centre of a maize field. Thus, a study site consisted of one reed stand and 3-4 maize fields at different distances from the reeds. Developmental stage of maize plants was similar in all fields selected. Reed habitats (0.3-1.5 ha in size) were sufficiently separated from each other to minimise colonisation of the maize fields investigated from other study sites. There were six focal patches of reeds in 1999 of which only four were used in 2000. The field study was done without any habitat manipulation.

Estimates of host density, parasitism, and parasitoid population density

Stalk borer density in reeds was estimated by dissecting reed plants from 12 randomly selected sample squares of 0.25 m^2 per reed stand and counting the number of late-instar larvae. Parasitism of *A. geminipuncta* by the parasitic fly was estimated by collecting 30-50 late-instar larvae in each reed habitat and assessing parasitoid emergence. Population density of *L. thompsoni* was estimated for each reed habitat. In maize fields, ECB attack was estimated by assessing the percentage of damaged plants out of random samples of 200 plants. ECB density was estimated by dissecting 50 randomly selected plants from each field in both pest generations and counting the number of late-instar larvae. Parasitism was estimated by collecting at least 50 late-instar larvae per field of each pest generations and assessing parasitoid emergence.

Influence of wind

The Magadino Plain lies between two mountain ranges, one to the north and one to the south; the wind usually blows in an easterly direction during the first half of the day, and in westerly direction in the second half. To investigate whether prevailing winds could affect parasitoid movement and the pattern of parasitism in maize fields, we assessed the angular deviation of each maize field from the East-West wind axis that crossed the reed habitat, which was considered the parasitoids starting point.

Statistical methods

We used multiple linear regression models to test for significant correlations between parasitism rates in maize and potential explanatory variables, such as the distance between a reed stand and a maize field, the population size of the parasitic fly in the reeds, the pest density in maize, and the angular deviation of the field from the prevailing wind direction. All four explanatory variables were tested together in the models. Models were run separately for both pest generations and each study year and fit using STATISTICA software (StatSoft, Inc., Tulsa).

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Captions for figures

Figure 1 Life-cycle of the parasitic fly *Lydella thompsoni* in southern Switzerland. Numbers indicate the start of each generation. Parasitoids overwinter within fully-grown European corn borer (ECB) larvae in corn stalks and other plant residues. In early spring they emerge before ECB larvae are available and rely upon alternative hosts in natural wetlands. In early summer, the fly moves back into maize fields, where it completes two generations on the pest. The inner circle shows the ECB life-cycle. The exterior circle shows the life-cycle of the univoltine reed stalk borer *Archanara geminipuncta*. The central circle shows the parasitic fly and its host habitats throughout the year.

Figure 2 Parasitism of first generation European corn borer (ECB) larvae by *Lydella thompsoni* in maize fields in the Magadino Plain (southern Switzerland) in 1999 and 2000. The x-axis denotes the distance between a maize field and the border of the closest alternative host habitat of the parasitoid. Parasitism (%) is the arcsin-squareroot transformed percentage of mean parasitism in a field. (a) Parasitism of ECB (1999): $Y = 120.93 - 10.8\ln(x)$, $t(16)=-3.66$, $r^2=0.455$, $n = 18$, $p < 0.01$. (b) Parasitism of ECB (2000): $Y = 134.35 - 12.5\ln(x)$, $t(14)=-5.19$, $r^2=0.659$, $n = 16$, $p < 0.001$. Same symbols indicate data points from bordering maize fields for which it is expected that the parasitoids originated from the same common reed (*Phragmites australis*) stand.

Figure 3 Spatial distribution of important alternative host habitats of the parasitic fly *Lydella thompsoni* in the Magadino Plain (southern Switzerland) and parasitism of first generation European corn borer (ECB) larvae in maize fields in 1999-2000. Alternative host habitats (circles) were natural common reed (*Phragmites australis*) stands of >0,3 ha surface and >5 % of reed plants damaged by *Archanara geminipuncta*, the most frequent and abundant alternative host of *L. thompsoni* in this area. In both study years three to four maize fields were selected around each alternative host habitats in different distances up 1130 m away from the edge of the reeds. Level of parasitism (%) is indicated with grey colour gradation. Data from 1999 and 2000 were pooled.

TABLE 1 Pest densities, parasitism, and parasitoid population size. European corn borer (ECB) densities were assessed in maize fields in the first (ECB1) and second (ECB2) pest generation in 1999 and 2000. Mean parasitism by *Lydella thompsoni* was assessed in each maize field and separately for each pest generation; parasitoid population density in a maize field is given as well. n = number of maize fields sampled.

Year	Pest larval densities/m ² (means + sd)		Parasitism % (means + sd)		Parasitoid population density/m ² (means + sd)		Number of samples (n)
	ECB1	ECB2	ECB1	ECB2	ECB1	ECB2	
1999	3.4±2.2	26.8±12.1	17.5±16.6	6.0±6.7	0.6±0.4	1.6±0.8	18
2000	7.4±3.3	47.8±29.0	13.7±18.0	7.4±3.4	1.0±0.6	3.6±1.0	16

Figure 1

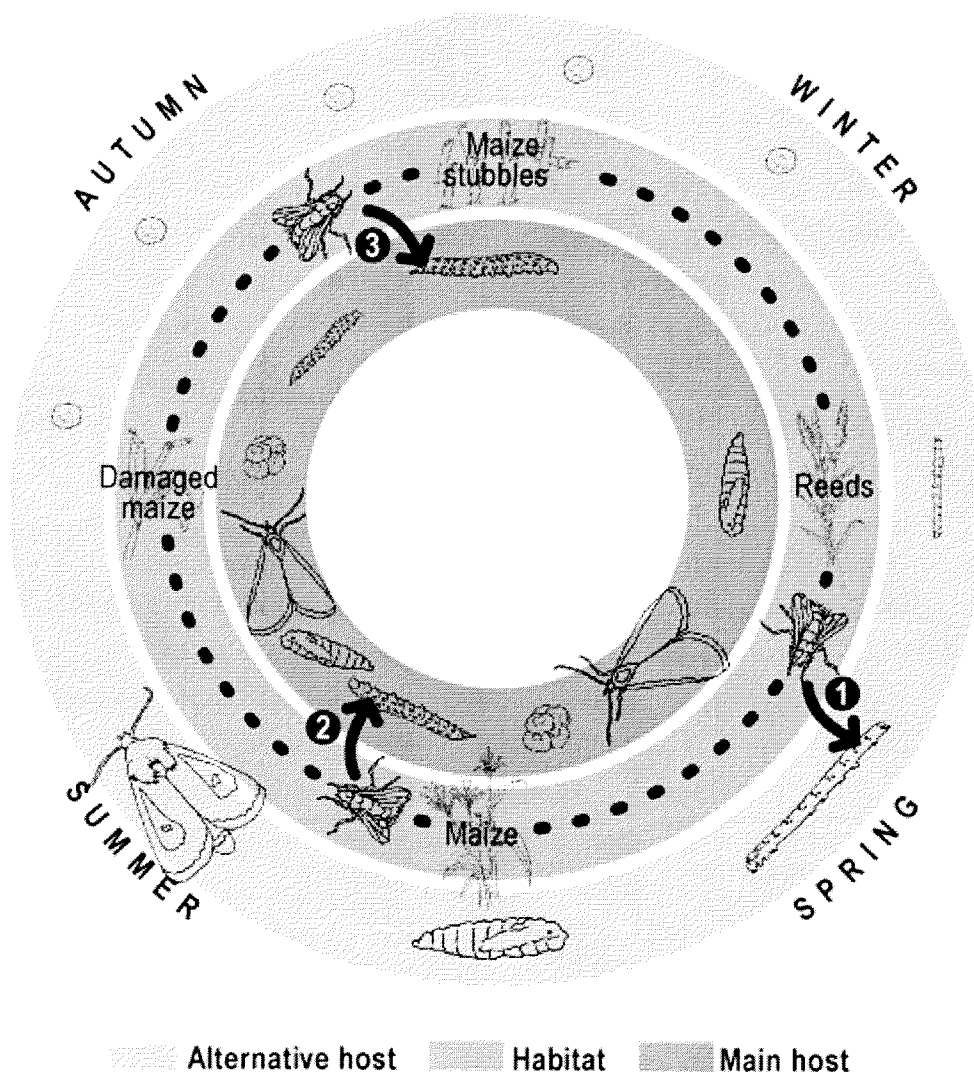


Figure 2

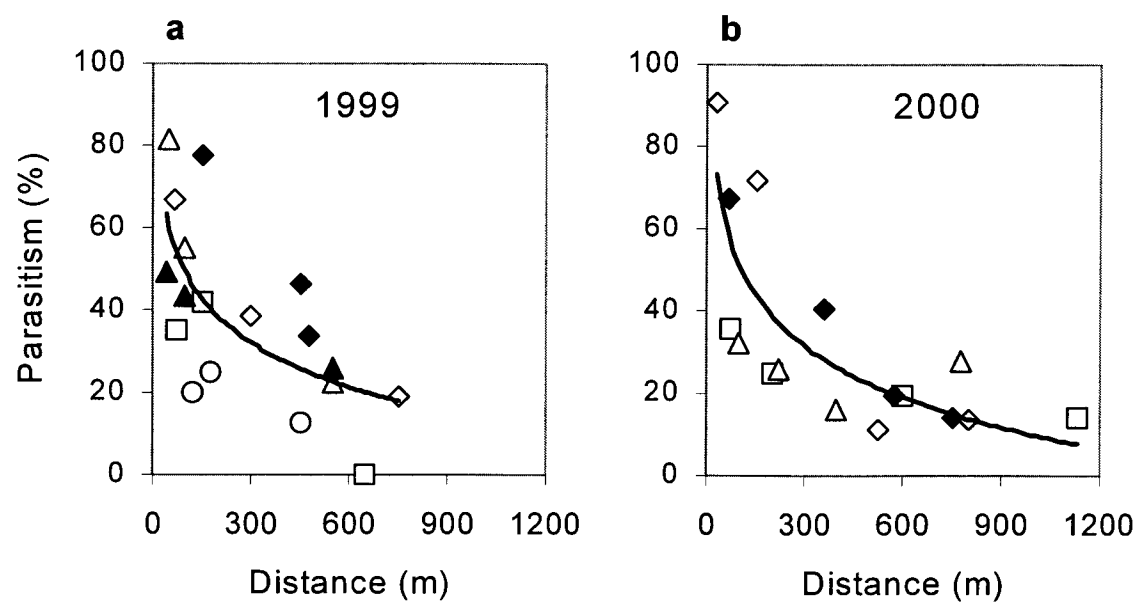
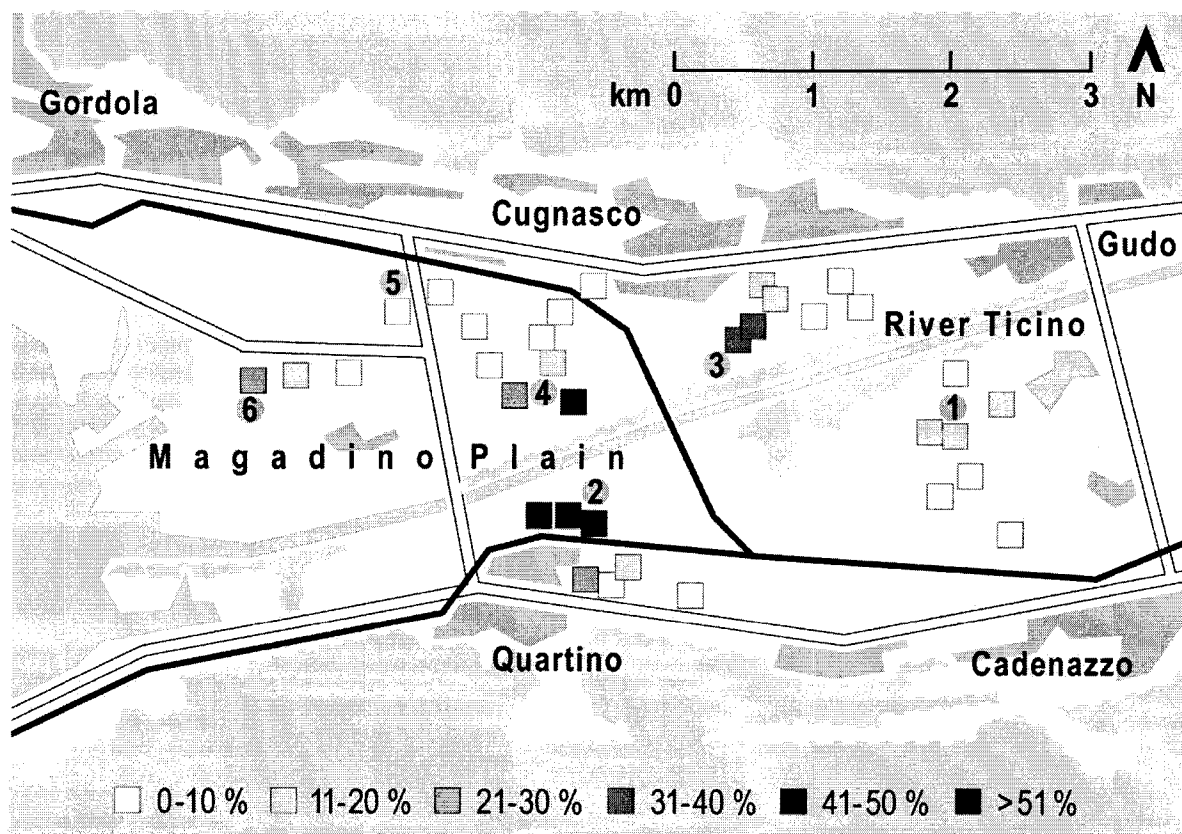


Figure 3



General conclusions

1. Persistence of *Trichogramma brassicae* in non-target habitats (Chapter 1)

This thesis provides evidence that *Trichogramma brassicae*, which was introduced to control the European corn borer without the intention of establishing resident populations may have become a permanent addition to the local fauna. Following commercial inundative releases in maize, a considerable number of the parasitoids disperse into the surroundings and can lead to a transient increase of *T. brassicae* in non-target habitats. Extensive field surveys revealed that *T. brassicae* exploits non-target hosts outside release fields which allow them to develop multiple generations and to overwinter in non-target habitats. However, the contribution of *T. brassicae* to the local *Trichogramma* fauna was found to be generally minor. Native species dominate the *Trichogramma* species complex in non-target habitats throughout the year, except for about four weeks immediately following mass releases. The potential for non-target effects is highest during this short period, when also competition with indigenous *Trichogramma* species for the same hosts may occur. Population reduction or displacement of indigenous *Trichogramma* species are, however, very unlikely.

2. Impact of *Trichogramma brassicae* mass releases on non-target moths (Chapter 2)

Non-target moths in pristine habitats were not negatively affected by mass released *T. brassicae*. Host specificity tests in the laboratory showed that populations of the reed stalk borers *Archanara geminipuncta* and *Chilo phragmitellus* were at most parasitized to a very small extent. These two species were selected for study because their oviposition period coincides closely with *T. brassicae* releases in maize, and because they are alternative hosts of *L. thompsoni*. *Archanara geminipuncta* escaped parasitism by *T. brassicae* because it lays its eggs between leaf sheaths and stalk of reed plants, where *T. brassicae* has no access. *Chilo phragmitellus* lays eggs that are similar to those of the target pest, but direct observation of the egg laying behaviour of *T. brassicae* showed that most parasitoid females rejected the eggs after the first physical contact. These findings suggest that under natural conditions there is little risk to either non-target species from *T. brassicae*; this conclusion was confirmed in an extensive field experiment under realistic "worst case conditions". More than 5000 *A. geminipuncta* eggs and about 100 egg masses of *C. phragmitellus* – exposed in a common reed stand in-between maize fields with *T. brassicae* releases (0.9-1.2 mio wasps ha⁻¹) – were checked on parasitism; no single egg was found parasitized by *T. brassicae*. As a consequence, even indirect effects of *T. brassicae* mass releases on the native larval parasitoid *L. thompsoni*, through deprivation of alternative hosts, can be excluded.

3. Biological control potential of *Lydella thompsoni* (Chapter 3)

In this study we could demonstrate that the pest control potential of the native larval parasitoid *Lydella thompsoni* has been largely underestimated since the parasitic fly is rendered ineffective in many places due to the fact that suitable habitats for early season hosts are lacking. Strategies to enhance biological control of the European corn borer should include increasing the number of alternative host habitats for *L. thompsoni* in a favourable spatial arrangement. Simple, non-invasive

management of reeds and nearby wetlands could significantly increase the effectiveness of *L. thompsoni* and thus complement the commercial use of *T. brassicae* against the European corn borer in maize. We believe that changes in the management of non-crop habitats in order to enhance populations of *L. thompsoni* within the agro-ecosystem could be achieved relatively easily and would not conflict with the interests of either pest managers or conservationists. Current agricultural policy in Switzerland requires that if a farmer is to receive government subsidies 8% of the area of any farm must be managed as ecological compensation areas aimed at enhancing biodiversity. Similar schemes exist in other parts of Europe where there is overproduction of agricultural products. In some countries land taken out of production is used to re-establish natural habitats. This study has shown that by promoting populations of natural enemies, such changes in land-use, can be of significant benefit to agriculture.

4. General implications for future biological control programmes

Although this post-release study may be suitable to show that the commercial use of *T. brassicae* is an efficient and environmentally sound method to control the ECB in southern Switzerland, future risk assessments of biological control agent introductions should evaluate more thoroughly the potential risks of biological control agents prior to their release. This important first step clearly failed when *T. brassicae* was brought into Switzerland about 25 years ago. *Trichogramma* egg parasitoids - as well as many other biocontrol agents - clearly have biological characteristics that may cause non-target effects to the local fauna. A thorough risk assessment procedure should therefore include investigations on the biological control agents physical requirements for overwintering, its host and habitat specificity, the temporal availability of non-target hosts in the release area, as well as the agents dispersal capacity. Attention should also be paid to possible indirect effects upon non-target hosts of introducing biological control agents. In addition, efforts should be made to identify promising native natural enemies prior to any introduction of foreign biocontrol agents; it may be possible to enhance the pest control potential of these native species by optimising local agricultural practices. Quantitative data gained from pre-release studies should be used to define the non-target risk potential (e.g. low, intermediate, high) of a biocontrol agent by combining likelihood of non-target effects to occur and magnitude (e.g. persistent population level impacts) that such effects could achieve. These quantitative data should then be used to decide whether a particular biological control agent is authorized for release or not.

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Die Natur ist doch das einzige Buch, das auf allen Blättern grossen Gehalt bietet.

Johann Wolfgang von Goethe (1749-1832)

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