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

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Abstract. The release of high numbers of the 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 and can achieve high parasitism rates. 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. Laboratory host specificity tests 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 parasitised 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.

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

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, and there are few 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 Paolo 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 into 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 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, where it shows parasitism rates that can exceed 70% (Kuske et al., submitted). Adults of the overwintering generation emerge too early to find ECB larvae in the field and must rely on alternative hosts in pristine habitats close to maize fields. Inundative releases of *T. brassicae* coincide with the oviposition period of these alternative hosts and *T. brassicae* moving out of release fields may attack and diminish populations of such hosts, creating a bottleneck situation for *L. thompsoni* in the subsequent spring.

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 world-wide 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, in the case of *Trichogramma*, 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 have shown that most of the non-target lepidoptera tested are parasitised 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 tested both direct and indirect effects of *T. brassicae* releases on non-target insects. Maize production in Switzerland suffers severely from the attack of the ECB and about 5000 hectares of maize fields are treated by releasing *T. brassicae* each year (Moos-Nuessli, 2001). However, despite *T. brassicae* being indigenous to some parts of Europe (Pintureau, 1990) naturally occurring populations of this parasitoid have never been reported from Switzerland in the past (Bigler et al., 1990). We therefore consider *T. brassicae* as a foreign species that is constantly added to the local arthropod fauna when mass released against the ECB. In southern Switzerland the ECB has two generations per year and 900,000 wasps per hectare are needed to control the pest in seed maize fields. This biological control approach has now been applied for more than ten years in the study area and has led to sufficient pest control. 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 in many maize fields the impact of *L. thompsoni* is therefore not sufficient to control the pest. The tachinid was, however, the most abundant and efficient native ECB parasitoid found in the study area during this project. ECB larvae experienced a mean parasitism by *L. thompsoni*, of about 20% in the first and around 10% in the second ECB generation, while in some maize fields parasitism exceeded 70%. These parasitism rates are similar to or even higher than, those found in other regions of Europe (Manojlovic, 1985; Cagan et al., 1999). In spring, *L. thompsoni* parasitise stalk borer larvae feeding on common reed plants (*Phragmites australis* (Cav.) Trin. ex Steud.) in natural habitats, where they complete their first generation. During this study, the most abundant species of such stalk borers in the study area, were *A. geminipuncta* and *C. phragmitellus* (Kuske, unpublished data). 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 parasitise 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 two important spring hosts of the tachinid. In addition, parasitism of these two non-target hosts 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 cm × 50 cm × 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 ± 1 °C, 75 ± 5% rh for up to 24 hours.

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 ± 1 °C, 80 ± 5% rh for up to 14 days. For the experiments sentinel egg-cards were used. These contained about 400 *E. kuehniella* eggs glued to white card-board strips (20 mm × 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 ± 1 °C, 70 ± 5% rh, L:D 16:8 hours.

Suitability and accessibility of Archanara geminipuncta *eggs*

Preliminary studies revealed that the host acceptance by *T. brassicae* is very high for *A. geminipuncta* (as well as for many other noctuid moths); therefore we mainly focussed on the suitability and accessibility of this host for *T. brassicae*.

Experiment 1 (Suitability): To test whether *A. geminipuncta* is a suitable host for *T. brassicae*, eggs of *A. geminipuncta* were offered to individual *T. brassicae* females within small plastic tubes (length: 50 mm, diameter: 10 mm) for 24 hours. *A. geminipuncta* lay their eggs inwardly of the leaf sheath of common reed plants, so that 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 parasitised at least one host egg, the average number of progeny per female, the average number of wasps emerging from parasitised eggs, and the sex ratio (percentage of females) were assessed. Differences in percentage of wasps parasitising host eggs between hosts and/or host egg age were analysed with Chi^2-test procedures. Differences in mean number of progeny per female between egg ages were analysed with ANOVA (Newman-Keuls).

Experiment 2 (Accessibility): 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: 90 mm, diameter: 13 mm) 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 parasitised at least one egg and the percentage of parasitised 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 and acceptance of Chilo phragmitellus *eggs*

Contrary to *A. geminipuncta* eggs, which are hidden underneath the leaf sheaths of common reed plants, eggs of *C. phragmitellus* are preferentially laid on the upper side of common reed leaves where they are easily accessible for *T. brassicae* females. We therefore restricted the investigations to suitability and acceptance of *C. phragmitellus* eggs. To test the suitability of this host for *T. brassicae*, reed leaves containing one day old egg masses of *C. phragmitellus* were cut into pieces (length: about 40 mm) 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: 80 mm, diameter: 25 mm) 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 parasitised at least one host egg, average number of parasitised 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). Differences in percentage of wasps parasitising host eggs between non-target host and control were analysed with Chi^2-tests. Differences in mean number of progeny per female between non-target and control host were analysed with Mann-Whitney-U-test.

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: 25 mm, height: 10 mm) 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; n = 35) or *O. nubilalis* (10–30 eggs; n = 32) were offered to individual *T. brassicae* females. 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 ± 1 °C, 50 ± 10% rh; the two hosts were alternated in the arena. We assessed the percentage of females that parasitised 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 parasitise the host, we assessed the average number of host contacts, drumming sequences, and the average time spent on the egg mass during the experiment. Differences in percentage of wasps parasitising host eggs between non-target and target were analysed with Chi^2-tests. Differences in means of all behavioural parameters per female between non-target and target were analysed with Mann-Whitney-U-tests.

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 1.2 million adults/ha. Females of the two non-target species (one to three days old) were allowed to

Table 1. Field exposure and parasitism of non-target lepidopteran eggs. Eggs were obtained through oviposition 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 eggs recovered	Percentage of parasitized[2] eggs (%)
Archanara geminipuncta 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
Chilo phragmitellus Hb. (Lep.: Crambidae)	1999	Plants (7 d; 30 m; 26)	23	327	0
		Stalks (7 d; 30 m; 14)	14	413	0
	2000	Plants (7 d; 30 m; 26)	61	221	0

[1] Numbers 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.

[2] Parasitoids belonged to *Telenomus* sp. (Hym., Scelionidae). *Trichogramma* did not attack non-targets.

oviposit on common reed plants that were enclosed in sleeve cages (length: 105 cm, diameter: 38 cm) for one day until the sleeves were withdrawn and the plants left unprotected. Under these conditions the oviposition of non-target host eggs was similar as found under natural conditions. Moths females were provided daily with water and honey.

Parasitism of Archanara geminipuncta

In 1998, *A. geminipuncta* females laid many eggs on common reed plants between 24 July and 10 August. 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 Tscharntke, 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 parasitised eggs. Healthy and parasitised 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 parasitised 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: 0.5 m) 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 at the species level either by PCR (only *Trichogramma*; according to Silva et al., 1999) or classical taxonomy.

Results

Laboratory no-choice experiments

Suitability and accessibility of Archanara geminipuncta *eggs*

Experiment 1 (Suitability): A. geminipuncta was found to be a suitable host for *T. brassicae.* The percentage of females that parasitised 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 parasitising females ($\chi^2 = 41.67$, df = 3, $p < 0.001$) and the number of progeny per female ($F_{3,76} = 26.7$, $p < 0.001$) decreased significantly with increasing egg age of *A. geminipuncta* (Figure 1). On the other hand, the mean number of offspring from parasitised 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.

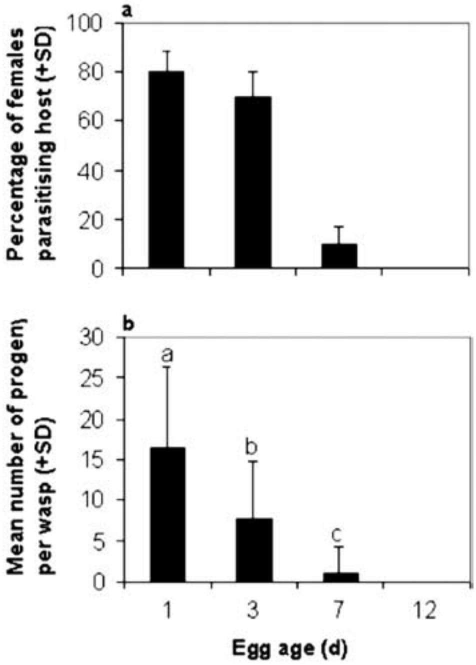

Figure 1. Percentage of *Trichogramma brassicae* (Hym., Trichogrammatidae) females parasitising *Archanara geminipuncta* Haw. (Lep., Noctuidae) eggs and mean number of progeny per wasp (+SD) in a 24 h no-choice laboratory experiment (24 ± 1 °C, 70 ± 5% rh, L:D 16:8). Eggs detached from stalk of host 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; $p < 0.001$).

Experiment 2 (Accessibility): 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 parasitise at least one non-target egg and to produce offspring, whereas in the control treatment 73.3% of the wasps (n = 60) parasitised on average 17.1 (2–35) *E. kuehniella* eggs. The percentage of *A. geminipuncta* eggs parasitised 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

C. phragmitellus proved to be a suitable host for *T. brassicae.* However, the percentage of *T. brassicae* females that parasitised *C. phragmitellus* was significantly lower than in the control treatments, both in 1999 ($\chi^2 = 31.89$, df = 1, $p < 0.001$, Figure 2a) and 2000 ($\chi^2 = 8.3$, df = 1, $p < 0.01$) (Figure 2a). The mean number of parasitised eggs per female was 0.9 (range: 0–9) in 1999

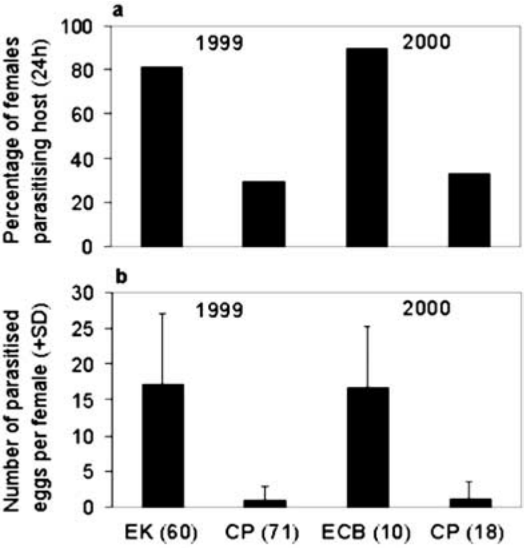

Figure 2. Parasitism of *Chilo phragmitellus* Hb. (CP), *Ostrinia nubilalis* Hb. (ECB), and *Ephestia kuehniella* Zeller (EK) by *Trichogramma brassicae* Bezd. in 24 h no-choice laboratory experiments. Freshly laid egg masses were offered to individual, one day old, honey fed *T. brassicae* females at 24 ± 1 °, 70 ± 5% 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; $^{***}p < 0.001$).

and 1.2 (0–8) in 2000 (Figure 2b). 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.* This non-target host was parasitised almost ten times less than the target ($\chi^2 = 35.97$, df = 1, $p < 0.001$) (Table 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 difference was not significant ($U_{3,26} =$

Table 2. Host acceptance behaviour of *Trichogramma brassicae* on egg masses of *Chilo phragmitellus* (n = 35) and *Ostrinia nubilalis* (n = 32) in direct observations (15 min per female). The average time used until acceptance or rejection is defined as the time a female spent on the 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)
O. nubilalis	81.3	87.4 (79.7) a	1.4 (0.8) a	2.7 (1.2) a
C. phragmitellus	8.6	7.3 (15.5) b	4.0 (2) b	1.3 (0.6) a

13.5, $p = 0.068$) (Table 2). Females that did not parasitise 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 (Table 1). The percentage of parasitised *A. geminipuncta* eggs was generally high on common reed plants, but very low (<1%) on stalk pieces (Table 1). However, none of the parasitised 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, pers. comm.).

The exposure of *C. phragmitellus* egg masses yielded no parasitised 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 parasitised. We found *Trichogramma evanescens* Westwood emerging from one of the parasitised egg-cards, whilst all others were parasitised 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 Archanara 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 parasitised 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, pers. comm.). Michel and Tscharntke (1993) found 28% of *A. geminipuncta* egg clusters parasitised by scelionids in a common reed habitat near Karlsruhe (Germany), whereas none of the eggs were parasitised by *Trichogramma*. However, they found the related noctuid, *A. dissoluta*, parasitised 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

Chilo species 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 and Hagen, 1964; David and 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 parasitised the ECB and the control host *E. kuehniella* in 24 h laboratory no-choice experiments, only one third of the females parasitised *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 parasitised *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 parasitised eggs per female and in the fecundity among strains. In his tests *T. brassicae* (= *T. maidis* Pintureau and Voegele) 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 parasitise *C. phragmitellus.* Thus, 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 parasitised. Unfortunately, in this field survey we did not succeed in proving the presence of *T. brassicae* in the common reed stand unequivocally, since none of the control egg cards harbouring *E: kuehni-*

ella eggs were parasitised either. However, in an additional two-years study (Kuske et al., unpublished) that was carried out at the same experimental site under similar conditions as those in the present study, we measured the emigration of mass released *T. brassicae* into surrounding non-target habitats using sticky traps and could clearly demonstrate that an immediate and quantitatively important transient inundation of such habitats takes place following inundative releases. It is therefore little reason to assume that *T. brassicae* did not disperse into the non-target habitats during this study. However, detrimental effects on *C. phragmitellus* due to *T. brassicae* inundative releases may not occur.

Impact on L. 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 *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 and Gauld, 1992; Unruh and Messing, 1993; Kruess and Tscharntke 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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