Screening of North American species of *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) for control of the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)[1].

Matthias Schöller[2] & Paul Fields[3]

Abstract

Information on Nearctic *Trichogramma* species was compiled in order to find candidates for the biological control of stored-product moths, especially the Indian meal moth *Plodia interpunctella*. We evaluated experimentally four species of *Trichogramma* native to North America: *T. deion*, *T. minutum* (2 strains), *T. pretiosum* (2 strains), *T. sibericum*, and the palaearctic introduction *T. brassicae* as candidates. Previous studies indicated that the Indian meal moth *P. interpunctella* is least preferred among the stored-product moths, so we used this species as target host. We performed the indirect Hassan-test for host preference between the mass-rearing host *E. kuehniella* and the target host *P. interpunctella*, and the performance at 15°C. The preparation of *Trichogramma* spp. in Canada balsam is described.

Keywords: *Ephestia kuehniella*, stored products, voucher specimens, parasitism

1. Introduction

Stored-product moths are among the major stored-product pests, infesting a wide variety of cereals and cereal based products. An alternative control method to synthetic chemical insecticide is to release parasitoid wasps of the genus *Trichogramma* Westwood into the stored-product environment (Brower, 1984b; Prozell & Schöller, 1998; Schöller & Flinn, 2000; Steidle et al., 2001). These chalcid wasps lay their eggs into the moth eggs, thus killing the eggs and preventing the development of the pest. World-wide, *Trichogramma* species are the most frequently used natural enemies for the control of lepidopteran pests of fruits and cereals in agriculture in the field (Li, 1994). Some species of *Trichogramma* are known to accept all stored-product moths of economic importance (Schöller & Hassan, 2001). In Germany and Austria, the control of the Indian meal moth, *Plodia interpunctella* (Hübner), and the Mediterranean flour moth, *Ephestia kuehniella* (Zeller), in food processing facilities is achieved by releasing large quantities of *Trichogramma evanescens* Westwood using the inundative release strategy (Schöller, 2001). In Canada, *Trichogramma* species are commercially reared, and used to control field and glass house insect pests (Shipp et al., 1998). Despite the wide-spread use of parasitoids in field and glass house settings, this biological control has not been used commercially in North America to control warehouse and food processing insect pests.

The genus *Trichogramma* is taxonomically complex (Pinto, 1998), and both species and strains differ significantly in biological characteristics (Hassan, 1994). There is currently no standard

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[1] Draft version submitted to editors.

[2] Biological Consultance, Hosemannstr. 8, 10409 Berlin, Germany, bip@biologische-beratung,de, Fax 0049 30 42 800 841

[3] Agriculture and Agri-Food Canada, Cereal Research Centre, 195 Dafoe Rd., Winnipeg, Manitoba, Canada, R3T 2M9, pfields@agr.gc.ca, Fax: 204 983-4604

method to prepare microscopic slides of *Trichogramma* (Platner et al., 1999). The present study aimed (1) to compile information on Nearctic *Trichogramma* species, (2) to develop a method for preparation of voucher specimens to deposit them in accessible insect collections and (3) to access and assess candidate species for the use as natural enemies in biocontrol measures against stored-product pest moths.

2. Materials and methods

2.1. Preparation and deposition of voucher specimens of Trichogramma *spp.*

We fixed the *Trichogramma* in Canada balsam, because this preparation was shown to be stable for a long time. The *Trichogramma* spp. were collected from the rearing and stored in 70% ethyl alcohol . These specimens were transferred to lactophenol and stored for 24h. After moving them to 10% KOH, they were heated to 60°C. Then the specimens were transferred to a mixture of glacial acetic acid and oil of wintergreen (1:1) and stored for 60 min. Finally, they were moved to Richards solution, composed from oil of cedar, oil of lilac, oil of wintergreen and glacial acetic acid, until mounting in Canada Balsam. Specimens were mounted whole. Prior to mounting, the abdomen of the specimens had to be flattened dorso-ventrally using a splinter of a cover-slide. Otherwise, aedeagus and ovipositor cannot be studied (see Platner et al., 1999). The slides were dried at 40°C for 10 days.

The slide-mounted specimens were deposited in the following collections: JBWM = Canada, Manitoba, Winnipeg, University of Manitoba, J.B. Wallis Museum of Entomology; MESC = Matthias Schöller personal collection; SMFD = Germany, Frankfurt am Main, Forschungsinstitut und Naturmuseum Senckenberg; the specimens received the following collection codes from SMFD: *T. deion* (SNGH 2495), *T. sibericum* (SNGH 2496), *T. minutum* (SNGH 2497), *T. minutum* strain12 (SNGH 2498), *T. pretiosum* strain5 (SNGH 2499), *T. pretiosum* strainUSA (SNGH 2500), *T. brassicae* (SNGH 2501). The codens of collection depositories used are according to the insect and spider collections of the world web site (http://www.bishopmuseum.org/bishop/ento/codens-r-us.html).

2.2. Insect rearing

Moths: The Indian meal moth, *P. interpunctella,* was reared on a diet consisting of 3 kg whole wheat, 50 ml glycerol, 50 ml honey and 60 g milled almonds, at 25°C and 70% RH and 8h dark : 16h light in a climatic chamber. *Ephestia kuehniella* pupae were obtained from Beneficial Insects Inc., California, USA. Emerged moths were allowed to mate and oviposit through mesh gauze. Eggs were collected daily and were used as hosts for the experiments and for the maintenance of the *Trichogramma* populations. Parasitoids: *Trichogramma* spp. were reared on UV-sterilised eggs of *E. kuehniella* at 25°C.

2.3. Performance at 15°C.

Eggs of *P. interpunctella* were exposed to *Trichogramma* spp. at 25°C. After five days, they were transferred to 15°C and checked for emergence daily. In the case of emergence, emerged wasps were provided with fresh host eggs for 7 days at 15°C, and the exposed eggs were transferred to 25°C. The development of adult progeny was recorded.

2.4. Host-preference tests

The preference test developed by Hassan (1989, 1994) was applied. The trials were performed in glass vials (50 mm length and 9 mm diameter). Eggs of *P. interpunctella* were introduced into the vials as well as eggs of the mass-rearing host, *E. kuehniella*, the latter served as a standard. Sixty eggs of each host species were glued on a small piece of paper card stock (20 x 20 mm) with the help of Tragant glue. Thirty eggs each were placed diagonally on every edge of the paper (Figure 1). The eggs were sterilised by exposing them to a UV light (General Electric 15 Watt G15T8 UV-lamp) for 2h. The eggs were 30 cm from the lamp. There was one female per vial. We used mated females that were 0-24 h old with no oviposition experience prior to the trials, they had contact to conspecific females and were mated.. First contact is defined as walking over the host egg and drumming (Schmidt & Smith, 1985) as first step of parasitisation. Five days after the exposure, the eggs of the two hosts were separated and transferred to new vials. After the filial generation died, the number of black host eggs (successful parasitism) and the number and sex ratio of the emerged adults were recorded. The experimental conditions were 25.4 ± 0.2°C, 60 ± 5% r.h. and 16L:8D. For every species/strain there were 20 replications. As a control, the strain of *Trichogramma evanescens* used commercially in Germany (Schöller & Hassan, 2001) was also tested

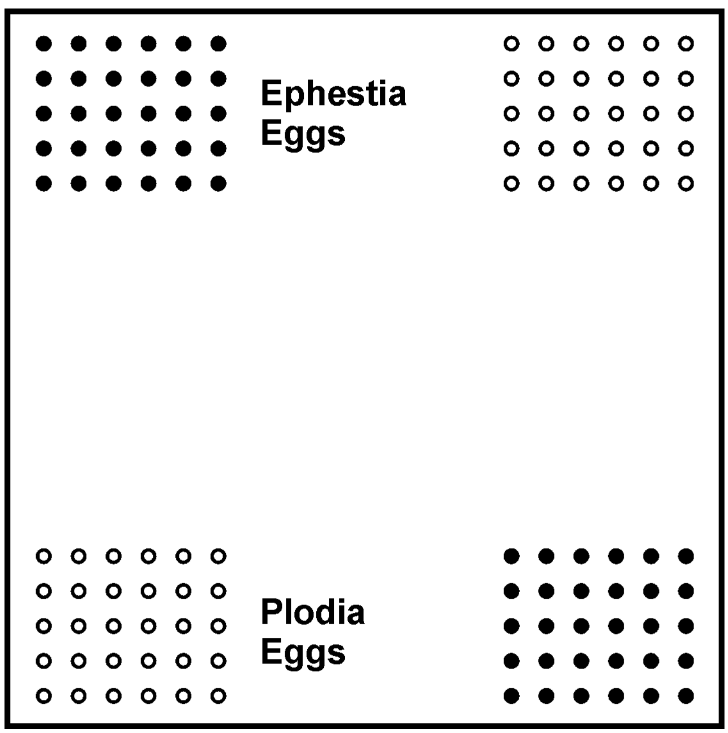

Figure 1. The Hassan preference test: The 60 eggs of *P. interpunctella* and 60 eggs of *E. kuehniella* are glued to a card and presented to one female *Trichogramma*. The precent emergence of the parasite was recorded for each moth.

3. Results

3.1. Trichogramma *spp. in North America*

Sixty-eight species of *Trichogramma* are recognised from North America, 27 of which were described as late as 1998. From Canada, 23 species were recorded. Sixteen species are commonly associated with agriculture and disturbed habitats in Northern Mexico, the United States and Canada (Pinto, 1998): *Trichogramma atopovirilia, Trichogramma brassicae, Trichogramma brevicapillum, Trichogramma cacoeciae, Trichogramma deion, Trichogramma exiguum*, *Trichogramma Fuentes, Trichogramma minutum, Trichogramma ostriniae, Trichogramma parker, Trichogramma pintoi* , *Trichogramma platneri,Trichogramma pretiosum, Trichogramma maltbyi, Trichogramma nubilale, Trichogramma retorridum, Trichogramma thalense* Most species used for biological pest control are from this group. *Trichogramma ostriniae* and *T. brassicae* are introductions from the Palaearctic.

3.2. Species of Trichogramma *studied experimentally*

Information on the origin of the species and strains of *Trichogramma* studied are given in Table 1, followed by general information on biogeography and biology of the species.

Trichogramma brassicae Bezdenko, 1968 was originally described from Belorussia, and was recently introduced into North America from the Palaearctic. It is the most important species for biological control of *Ostrinia nubilale*, the European corn borer (Bigler, 1986). The species now is readily available commercially and presumably is being released repeatedly throughout much of North America. However, it is not yet known if it has established in North America (Pinto, 1998).

Trichogramma deion Pinto and Oatman, 1986 was collected in western North America. It is the most widespread and commonly collected species, and it was found in a variety of both natural and disturbed habitats, at elevations ranging from below sea level to at least 2700m. In Canada, it was found in British Columbia (Pinto, 1998). *Trichogramma evanescens* Westwood, 1833 was originally described from the United Kingdom. *Trichogramma evanescens* is a Palaearctic species currently thought to have a wide distribution ranging from Northern Africa to Turkestan. However, the *evanescens* species-group was never revised and cryptic species might be present. Two strains were studied, the eva 57 strain was evaluated for the control of cruciferous crops in the Netherlands (Pak, 1988) and the Lager strain is currently applied commercially to control stored-product moths in Germany and Austria (Schöller, 2001). *Trichogramma minutum* Riley, 1871 is distributed in Eastern Canada and the United States of America east of 110 W longitude. It is commonly collected both in natural and disturbed habitats. *Trichogrammae minutum* appears frequently to attack host eggs on trees, but was also found on herbaceous plants (Pinto, 1998). We studied a strain which was applied to control the spruce budworm, *Choristoneura fumiferana* in Canada's Eastern boreal forest in the past (Smith et al., 1986) and a strain collected from blueberry in Canada.

In the New World, *Trichogramma pretiosum* Riley, 1879 is the most widespread species, and one of the most commonly collected. It is especially abundant in agricultural and other disturbed habitats, occurring from southern Canada to Argentina. It is also known from the Hawaiian Islands and Australia. *Trichogramma pretiosum* was released against *Helicoverpa* sp. in cotton (Smith, 1996). Hosts in the field reduce the number of pyralid Lepidoptera, including *Amyelois transitella* (Walker). Although relatively uniform morphologically, certain collections are reproductively incompatible, suggesting the presence of cryptic species (Pinto, 1998). We studied two strains of this species, a thelytokous strain from the U.S.A, and an arrhenotokous strain collected from corn in Canada.

Trichogramma sibericum Sorokina, 1981 is a Holarctic species, in North America known only from the north-western United States and British Columbia. Natural hosts are in the order Hymenoptera, Tenthredinoidea, and Lepidoptera, Torticidae and Notodontidae. It is produced commercially for the control of *Rhopobota naevana* in cranberry.

3.2. Performance at 15°C.

Of the 9 species/strains studied, all emerged and parasitised at 15°C. The minimum time required varied from 10 d in *T. evanescens* Lager to 27 d in *T. sibericum* (Table 2).

3.3. Host-preference tests (Hassan-test)

Looking at the total number of *P. intepunctella* and *E. kuehniella* eggs combined, *T. sibericum* and *T. minutum* parasitised fewer eggs than the other species/strains (Table 2). *Trichogramma pretiosum* USA and *T. pretiosum* 5 parasitised more than *T. minutum* and *T. brassicae*.

Four host-patches were offered to females *Trichogramma*. All females of all species/strains parasitised at least one patch with *E. kuehniella* eggs. The *P. interpunctella* eggs had lower percent parasitism compared to *E. kuehniella* (Table 2).

4. Discussion

The importance of systematics in taxa of economic significance being obvious, this is particularly true in the genus *Trichogramma* because of their considerable morphological homogeneity and the fact that almost all sampled terrestrial habitats harbour native species. Consequently, any biological control programme must identify this fauna, distinguish the various species from one another and from introduced exotics (Pinto, 1998). An appreciation of native species may actually preclude the need to introduce exotics in the first place. The introduction of exotics implies the risk of establishment of these species resulting in a threat for native species. This is particularly true for introductions from the Palaearctic into the Nearctic or vice versa, because these species are often adapted to comparable climatic conditions. This is also the case if parasitoids are released to control stored-product pests, because the storage structures usually do not prevent insects from moving outside, e.g. into natural habitats. For this reason, we preferred to screen the Nearctic fauna for suitable species to control stored-product moths instead of risking the introduction of the commercial available, Palaearctic *T. evanescens*.

Curiously, *Trichogramma* spp. are very rare in insect collections, there is currently no standard method to prepare microscopic slides of *Trichogramma,* and only one technical publication on preparation technique is available (Platner et al., 1999). Consequently we decided to describe the preparation technique used, and to deposit specimens in public collections to allow the future taxonomic study of the species/strains tested here.

Trichogramma spp. are not typical elements of stored-product habitats. Unfortunately, the only strains collected in an stored-product environment by Brower (1984a) are lost (Brower, pers. com). On the other hand, many species are reared in the laboratory on eggs of stored-product moths, and are consequently potential candidates for the biological control of these moths (Brower, 1984b). Certain species, especially those common to agricultural and other disturbed habitats, are widely distributed. For example, *T. evanescens* is reported throughout the Palaearctic. *Trichogramma pretiosum* is distributed throughout much of the New World (Pinto & Stouthamer, 1994). This distribution pattern facilitates the commercial application in cases where the native status has to be proven on a more local level.

The successful utilisation of *Trichogramma* spp. in biological control systems rests on the proper selection of *Trichogramma* species and populations (Hassan, 1994). Laboratory evaluations usually compare different traits between populations, which serve as indices and are (occasionally) followed by field evaluations to assess searching efficiency (Pak, 1988; Hassan, 1994; Silva, 1999). The performance at low temperatures was thought to be important for two reasons. Parasitism in *Trichogramma* spp. is known to be strongly dependant on temperature. (Russo & Voegelé, 1982). Generally, biological control has been shown to be more effective in situations where pest abundance is low. Consequently, control should start early in the season to prevent build-up of the pest population. *Ephestia kuehniella* starts oviposition at 15°C, the low temperature tested in this study. A species of *Trichogramma* which is capable of parasitising at 15°C can be released early in spring, but also in cooled warehouses. Secondly, the duration of suitability of the host-eggs for parasitism by *Trichogramma* spp. is strongly dependant on temperature. Typically, after oviposition host suitability is decreasing dramatically after day 3 within the range of 22-28°C (Ruberson & Kring, 1993). At 15°C, host suitability is thought to be extended, and longevity of *Trichogramma* spp. is increased (Schöller & Hassan, 2001), resulting in an increase of probability of host-finding. Finally, emergence of the parasitoids from the release units at low temperatures is important, too. The release units contain pre-imaginal stages of the parasitoids, to guarantee emergence within the stored-product environment.

Performance at low temperatures is only one of many potential parameters to select a suitable species of *Trichogramma* (Hassan, 1994). A list of criteria to select natural enemies for inundative biological control of stored-product moths was suggested by Schöller (2000). Basic parameters are fecundity and host preference. The three species with the highest mean total fecundity, i.e. *T. deion* and the two strains of *T. pretiosum*, parasitised a large percentage of *P. interpunctella* patches, too. This is indicating the suitability of both species of stored-product moths for these *Trichogramma* species.

Both the results of development at 15°C and the fecundity data suggest that *T. deion*, the two strains of *T. pretiosum* and *T. minutum* 12 are candidates for further studies. There seems to be no need to apply the exotic *T. brassicae*. The question often arises as to whether laboratory tests for monitoring parasitoid potential can adequately assess traits important under field conditions (Laing & Bigler, 1991). We found at least three promising candidates for further studies. A procedure has to be developed including additional laboratory tests and the evaluation of searching efficiency under semi-field conditions to choose among these candidates the most promising one for field applications.

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Table 1. Origin of the species and strains of *Trichogramma* studied. The mass rearing host was *E. kuehniella.*

Information	*Trichogramma* species and strains							
	T. evanescens Westwood[1]	*T. pretiosum* Riley	*T. pretiosum* Riley	*T. deion* Pinto and Oatman	*T. minutum* Riley	*T. brassicae* Bezdenko	*T. sibericum* Sorokina	*T. minutum* Riley
Strain	Lager	5	USA		12			
Original host	*Heliothis armigera*	unknown	unknown	unknown	unknown	unknown	*Rhopobota naevana*	*Choristoneura fumiferana*
Plant	unknown	sweat corn	unknown	cranberry	blueberry	unknown	cranberry	spruce
Country	Egypt	Canada	USA	Canada	Canada	Moldavia	Canada	Canada
Location		QC	CA	QC	QC		BC	ON
Collector		F. Fournier		F. Fournier	F. Fournier	F. Bigler	D. Henderson	S. Smith

1. *T. evanescens* is used in Germany for biological control of stored-product moths

Table 2. The comparison of several species of *Trichogramma* species in various laboratory tests to evaluate their capacity to control *P. interpunctella*..

Test	*Trichogramma* species and strains							
	evanescens [1]	*pretiosum* 5	*pretiosum* USA	*deion*	*minutum* 12	*brassicae*	*sibericum*	*minutum*
Time to emerge at 15EC (days)	10	12	14	11	12	11	27	21
Number of eggs parasitised in Hassan test [2]	42 ± 7	30 ± 6 cd	40 ± 11 d	28 ± 7 bc	25 ± 9 b	27 ± 8 b	19 ± 5 a	19 ± 7 a
Patch 1 of *E. kuehniella* that had some parasitism (%)		100	100	100	100	100	100	100
Patch 2 of *E. kuehniella* that had some parasitism (%)		89	82	72	91	59	95	40
Patch 1 of *P. interpunctella* that had some parasitism (%)		39	94	72	91	71	60	50
Patch 2 of *P. interpunctella* that had some parasitism (%)		33	88	38	82	47	45	0
Females that parasitized only one patch (%)		6	0	14	0	6	0	15
Ranking		1	1	1	2	2	3	4

1. *T. evanescens* is used in Germany for biological control of stored-product moths

2. Means (± SEM) with different letters differ significantly ($p<0.05$), t-test or Mann-Whitney Rank Sum Test.