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Egg parasitoids of the genus *Trichogramma* (Hymenoptera, Trichogrammatidae) in olive groves of the Mediterranean region

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Abstract

A survey of egg parasitoids of the genus *Trichogramma* (Hymenoptera, Trichogrammatidae) was carried out in olive groves in Portugal, Greece, Egypt, and Tunisia during the years 2002–2004. Parasitoids were obtained either by exposing sentinel eggs (*Sitotroga cerealella* Olivier or *Ephestia kuehniella* Zeller) on olive trees or by collecting eggs of lepidopterous olive pests. Parasitized egg samples were reared separately in the laboratory for emergence of parasitoids. These were further reared in separate lines and processed by morphological and molecular biology techniques for species characterization. The recorded fauna of *Trichogramma* parasitoids in olive groves was species poor and consisted of species mainly known from the Mediterranean region. *Trichogramma bourarachae* Pintureau and Babault was found in Tunisia and Egypt, *T. cordubensis* Vargas and Cabello, and *T. euproctidis* Girault in Egypt, *Trichogramma cacoeciae* Marchal in Portugal, Greece, Egypt, Tunisia and *Trichogramma nerudai* Pintureau and Gerding in Portugal. Apart from that, *Trichogramma oleae* Voegelé and Pointel was collected in Tunisia. This species is probably not indigenous, but has established after several releases of a French strain were made in recent years. For selected strains, the sequence of the internal transcribed spacer 2 (ITS-2) region of rDNA was determined and deposited in the GenBank database. Differences in important biological attributes were found among collected strains of *T. bourarachae*, suggesting the existence of biotypes. The results contribute to the limited knowledge on distribution and biodiversity of the genus *Trichogramma* in the Mediterranean region. They can be helpful for the preservation and use of indigenous *Trichogramma* species in biological control of lepidopterous pests in olive and other local crops.

Keywords: *Trichogramma bourarachae*; *Trichogramma cordubensis*; *Trichogramma euproctidis*; *Trichogramma cacoeciae*; *Trichogramma nerudai*; *Trichogramma oleae*; ITS-2 region; Olive cultivation; Biodiversity; Biocontrol agent; *Prays oleae*; *Palpita unionalis*; Mediterranean region

1. Introduction

The cultivation of the olive tree, *Olea europea* L., is of socio-economic and ecological importance for the Mediterranean region since ancient times. Current olive growing

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spans from traditional techniques to intensively managed plantations and create a large diversity of ecological conditions. In several Mediterranean countries, e.g., Tunisia, Spain, or Greece, the olive tree is the landscape determining factor. Hence, the olive grove ecosystem is an important reservoir for biodiversity in this region (Rey, 1995; Cirio, 1997; Beaufoy, 2000, 2001).

Egg parasitoids of the genus *Trichogramma* are keystone natural enemies of many lepidopterous pests in agriculture and forestry (Li, 1994). More than 150 different species are known from various biotopes (Pinto and Stouthamer, 1994; Pinto, 1999). These minute parasitoids were considered excellent indicator species to study the side effects of pesticides on beneficial arthropods and the environment (Hassan, 1998). They are broadly distributed world wide, sensitive to pesticides compared to other hymenopterous parasitoids and they are effective natural enemies of various insect pests. Youssef et al. (2004) has recently published the results of testing the side effects of pesticides that are used against olive pests on *Trichogramma cacoeciae* Marchal. Due to the toxicity of a number of the agrochemicals tested, a negative impact on *Trichogramma* that naturally occur in olive groves can be expected if broad spectrum insecticides are repeatedly used. The particular *Trichogramma* fauna of the olive agroecosystem is not well known. Only few reports exist in literature (Pelekassis, 1962; Jardak, 1980) but they suggest an important role of these egg parasitoids for natural control of olive pests (Arambourg, 1986).

Several insect species within the order Lepidoptera damage flowers, fruits or leaves of the olive tree (Arambourg, 1986). The field release of mass-reared *Trichogramma* is considered as a promising approach to control the olive moth, *Prays oleae* Bernard (Lepidoptera, Yponomeutidae) and the jasmine moth, *Palpita unionalis* Hübner (Lepidoptera, Pyralidae) in order to reduce pesticide application in olive growing (Hegazi et al., 2004, 2005; Herz et al., 2005; Herz and Hassan, 2006). At present, the European Plant Protection Organization (EPPO) lists the species *Trichogramma brassicae* Bezdenko*, T. cacoeciae* Marchal, *Trichogramma dendrolimi* Matsamura, and *Trichogramma evanescens* Westwood for use in countries of the Euro-Mediterranean region (EPPO, 2002). But inundative releases of non-indigenous *Trichogramma* may have also adverse effects on non-target hosts and/or released parasitoids may compete with locally occurring *Trichogramma* (Louda et al., 2003). Preference should always be given to indigenous strains or species that are collected from the same region, when developing a new biocontrol program based on *Trichogramma* (Van Lenteren et al., 2003).

In the present study, a survey was carried out to record the local *Trichogramma*-fauna in olive groves in several countries of the Mediterranean region. One objective was to obtain essential data which allow to evaluate the risk for a potential shift in the egg parasitoid fauna after the application of plant protection measures. But most important was the aim to collect and characterize strains of *Trichogramma* for their potential use in biological control of olive pests (Herz et al., 2005). Native strains are more likely to be adapted to the olive tree environment, the target pests and the weather conditions of the region.

2. Materials and methods

2.1. Description of the study areas

Several olive groves located in various areas in Portugal, Tunisia, Greece, and Egypt were sampled during spring and summer of the years 2002, 2003, and 2004. The groves varied in age, environmental conditions and cultivation techniques, but represented typical olive growing conditions of the particular region (Table 1).

2.2. Field collection of Trichogramma

Recording of the *Trichogramma*-fauna was mainly done by exposure of sentinel eggs which were put in particular baiting devices. These consisted of small plastic frames (2 × 2 cm), covered from both sides with fine nylon or metal mesh to allow access of small *Trichogramma*-wasps, but

Table 1
Location and type of the olive groves included in the survey for *Trichogramma* species in four countries in the Mediterranean region during the years 2002–2004

	No. of olive groves surveyed	Region	Locations	Cultivation
Egypt	2 (2002) 2 (2003) 2 (2004)	Alexandria (coast) Cairo (desert)	Bourg-el-Arab Paradise Park	Traditional olive grove Young plantation
Greece	3 (2002) 3 (2003) 3 (2004)	Attikis region	Tanagra, Arma Viotias	IPM-cultivated olive groves
Portugal	7 (2002) 1 (2003)	Alto Douro region	Mirandela, Macedo de Cavaleiros, Vila Flor, Figueira de Castelo Rodrigo	Organic olive groves
Tunisia	1 (2002) 5 (2003) 1 (2004)	Sfax region	Siris, Taous, Bir Mellouli, Ketetna, Zarzis	IPM-cultivated olive groves

to prevent predation on the content inside. A small piece of paper with sentinel eggs (*Sitotroga cerealella* Olivier or *Ephestia kuehniella* Zeller depending on local availability) glued on was inserted in each device (Sakr et al., 2000). The device was fixed with a piece of wire close to branches or leaves of the olive tree (Hegazi et al., 2005). From 60 to 100 baiting devices were distributed on 6 to 10 randomly selected trees in each sampled olive grove. Also parasitized eggs of the olive moth and jasmine moth were collected in some cases. The survey started in early spring until late summer and sentinel eggs were replaced weekly. Collected samples were incubated separately in the laboratory in order to check for parasitism. Emerged parasitoids were reared in separate glass tubes and, after propagation, samples of parasitized eggs were sent for species characterization to the Institute for Biological Control in Darmstadt, Germany. Strains were propagated for at least one generation on *Sitotroga* eggs to obtain sufficient material for morphological and molecular biology studies. Vouchers of the original shipped samples were preserved in ethanol (70%) as reference and added to the collection of the Institute for Biological Control, Darmstadt, Germany.

2.3. Techniques for species characterization of collected strains

Morphological differentiation between *Trichogramma*-species is mainly based on structures of the male genitalia and antennae (Pinto, 1999). Whole body mounts of males from field collected arrhenotokous strains were prepared in Hoyer's medium for examination of morphological characters, using a Zeiss Axioplan Stereomicroscope at a magnification range of 200× to 640×. Examination of slides was done following descriptions of Sorokina (1993) and Pinto (1999). Specimens of selected strains were sent to Dr. Bernard Pintureau (Inst. Nat. Science Appl. de Lyon, Villeurbanne, France) for validation of results.

For molecular differentiation, size and sequence of the internal transcribed spacer 2 (ITS-2) region of rDNA of selected *Trichogramma* strains was examined following the procedures developed by Stouthamer et al. (1999) and Silva et al. (1999). For DNA-extraction, 5 specimens of each strain were ground in distilled water and incubated overnight at 56 °C after adding Proteinase K. PCR-amplification was done by using the QIAGEN Taq PCR Core Kit and the primers 5′ > TGTGAACTGCAGGACA CATG < 3′ (forward) and 5′ > GTCTTGCCTGCTCT GAG < 3′ (reverse) (Silva et al., 1999). PCR-Products were separated by electrophoresis in 2% agarose-gels, stained with ethidium bromide and photographed under UV-light for visualizing DNA-bands. The ITS-2 PCR products were analyzed by restriction enzyme digestion (Silva et al., 1999) and comparison of obtained restriction patterns of field collected strains with well defined reference strains of the strain collection at the Institute for Biological Control. For sequencing of the ITS-2-region, DNA of individual wasps of selected strains was first amplified by PCR. Cleanup of the PCR-products was performed with the QIAGEN MinElute™ PCR purification Kit according to the protocol of the supplier and the samples were dispatched for bidirectionally sequencing with the primers above. Comparison of the obtained sequences with those published in GenBank was done by BLAST analysis (BLAST 2.2.14, Altschul et al., 1997). Complete ITS-2 sequences of determined strains were deposited in the Genbank database and sent to Dr. Richard Stouthammer (Department of Entomology, University of California, Riverside, USA) for validation of results.

In selected cases, crossing experiments and partial life table studies were conducted in order to test sexual compatibility between strains and to study variation in important biological attributes (fecundity, longevity, sex ratio, and developmental time) of strains. The testing procedures followed standard protocols developed at the Institute for Biological Control in Darmstadt (Hassan and Zhang, 2001).

3. Results

3.1. Phenology and distribution of the Trichogramma-fauna in olive groves

Parasitized samples (i.e., parasitized eggs in baiting devices or eggs of the olive moth or jasmine moth) were found during the whole collection period in spring and early summer (Fig. 1) when activity of arthropods was high in the olive groves. In 2003, the baiting was started earlier in spring and continued to late summer. In Egypt, sentinel eggs were also parasitized during January/February (rainy season, mean temperature: 8 °C (min) to 19 °C (max)) and in July/August (dry season, mean temperature: 20 °C (min) to 35 °C (max)), indicating the activity of wasps during less favorable weather conditions. In Greece, parasitism was only detected in two samples in May and June 2004, although sampling was intense in several groves during the three years of the study (Table 1). In total, 170 samples that had positive parasitism were examined in the laboratory and resulted in the characterization of six *Trichogramma* species: *T. bourarachae* Pintureau and Babault, *T. cacoeciae* Marchal, *T. cordubensis* Vargas and Cabello, *T. euproctidis* Girault, *T. nerudai* Pintureau and Gerding, and *T. oleae* Voegelé and Pointel (Fig. 2). The characterization of the strains was considered as successful in the case of consistency of morphological and molecular data (except the record of *T. nerudai*, see below). *T. cacoeciae* was dominant in Portugal and Greece and *T. oleae* in Tunisia. Other species (*T. nerudai* in Portugal, *T. bourarachae* in Tunisia) occurred only sporadically. In Egypt, *T. bourarachae* was dominant at the grove in Bourg-el-Arab near Alexandria, whereas three other species (*T. cacoeciae*, *T. cordubensis*, and *T. euproctidis*) were collected at the grove in Paradise Park near Cairo.

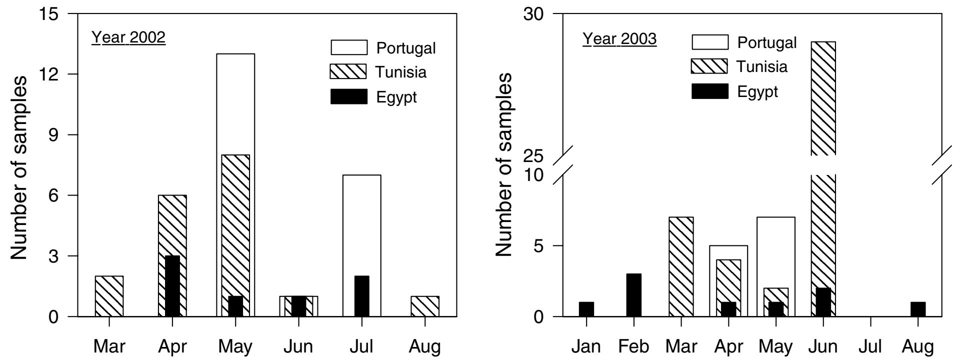

Fig. 1. Occurrence of *Trichogramma* egg parasitoids in olive groves in Portugal, Tunisia and Egypt in the years 2002 and 2003. Weekly sampling was done by exposing sentinel eggs (*S. cerealella* or *E. kuehniella*) or by collecting naturally occurring lepidopterous hosts (*P. oleae* and *P. unionalis*) in spring and summer.

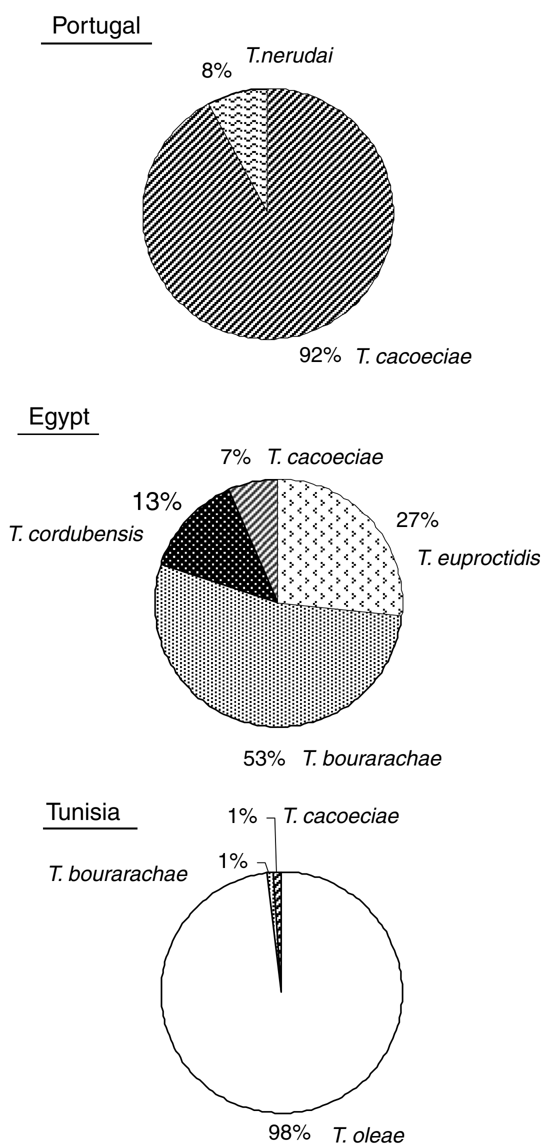

Fig. 2. Species diversity of *Trichogramma* egg parasitoids, collected in olive groves in Portugal, Tunisia and Egypt (samples obtained from 2002 to 2004). Weekly sampling was done by exposing sentinel eggs (*S. cerealella* or *E. kuehniella*) or by collecting naturally occurring lepidopterous hosts (*P. oleae* and *P. unionalis*) in spring and summer.

3.2. Description of *Trichogramma* species collected in olive groves

3.2.1. *Trichogramma bourarachae* Pintureau and Babault

Strains of this species were collected in Egypt as well as in Tunisia. In Egypt, this species was collected from one location (Bourg-el-Arab), but regularly in 2002 (5 samples) as well as in 2003 (4 samples) and 2004 (8 samples). In Tunisia, one sample was collected end of May 2002 at Siris and one sample in June 2003 at Zarzis, both located in the olive growing region around Sfax. A further sample was obtained from eggs of the carob moth (*Ectomyelois ceratoniae* Zeller) on pomegranate in a house garden at Sfax. The species has an arrhenotokous reproduction and both males and females are dark gray colored. ITS2-sequences of Egyptian and Tunisian strains were similar in size (555 to 559 bp, Table 2). Crossings between these strains (TP41EG × TP49TN) demonstrated sexual compatibility with 64% female progeny in the F1-generation and 41% female progeny in the F2-generation. But the strains differed in several biological attributes (Table 3). The Tunisian strain of *T. bourarachae* (”TP49TN”) developed within 17 days and thus 1 to 3 days faster than other collected strains of this species (strains “TP1EG”, “TP41EG” and “TP58EG” from Egypt). It had the highest fecundity with 17.9 eggs per female and day, but was short-lived. Samples obtained from Egypt (strains “TP1EG”, “TP41EG”, and “TP58EG”) were more similar to each other, especially regarding developmental time (around 18 days) and fecundity (10 to 13 eggs per female and day). Compared to these results, a Portuguese strain of *T. bourarachae* (”Tb26”) which had been collected from noctuid host eggs in tomato in 1992 (Silva et al. (1999)), needed longer for development (20 days), laid fewer eggs (6.7 eggs per female and day), but lived much longer. All strains of *T. bourarachae* produced about 40% males in the progeny at 25 °C.

3.2.2. *Trichogramma cacoeciae* Marchal

This species was collected in all four countries (Portugal: 22 samples, Tunisia: 2 samples, Egypt: 2 samples, and

Table 2
Analysis of the ITS-2-region of selected *Trichogramma*-strains collected in olive groves in four countries in the Mediterranean region 2002–2004

Species[a]	Strain ID[b]	Collection in	Size of ITS-2 [bp]	GenBank ID[c]	BLAST (version 2.2.14) alignment with sequence of species[d]
T. bourarachae	TP1EG	Egypt, 2002	557	DQ389071	AF043624 (*T. bourarachae*, sample Tb262)
T. bourarachae	TP41EG	Egypt, 2002	558	DQ389072	AF043624 (*T. bourarachae*, sample Tb262)
T. bourarachae	TP49TN	Tunisia, 2002	555	DQ389073	AF043624 (*T. bourarachae*, sample Tb262)
T. cacoeciae	TP97GR	Greece, 2004	466	DQ389075	AF408653 (*T. cacoeciae*, strain CACB)
T. cordubensis	TP52EG	Egypt, 2003	Partial	—	AF043619 (*T. cordubensis*, strain Tc14)
T. cordubensis	TP63EG	Egypt, 2004	408	DQ389074	AF043619 (*T. cordubensis*, strain Tc14)
T. euproctidis	TP42EG	Egypt, 2002	376	DQ389076	AF043614 (*T. turkestanica*, strain Tt2)
T. nerudai	TP23PT	Portugal, 2003	635	DQ872853	AY244467 (*T. nerudai*), strain "Chile"
T. oleae	IO47	Tunisia, 2003	403	DQ389070	U74601 (*T. oleae*)

[a] Species identification according to molecular and morphological data.
[b] Strain code in the strain collection at the Institute for Biological Control.
[c] Accession number of sequence in GenBank.
[d] Accession number of ITS2-sequences, published in GenBank, giving the highest identity score in a BLAST-alignment (E-value = 0).

Table 3
Biological attributes of different strains of *Trichogramma bourarachae* collected in Egypt (in olive), Tunisia (in olive), and Portugal (in tomato, Silva et al. (1999)) in the laboratory at 25 °C, 70% RH, L/D = 16/8 h

Strain ID[a]	Origin	Developmental time [days]	Daily number of eggs/female	Survival D7 [%][b]	Sex ratio [% males]
TP1EG	Egypt, 2002	18.1 ± 1.5	10.0 ± 3.0	60	No data
TP41EG	Egypt, 2003	18.4 ± 1.0	10.7 ± 5.2	49	41 ± 26
TP58EG	Egypt, 2004	18.8 ± 1.3	12.9 ± 9.6	69	40 ± 25
TP49TN	Tunisia, 2002	17.1 ± 1.5	17.9 ± 8.8	27	42 ± 27
Tb26	Portugal, 1992	20.3 ± 1.8	6.7 ± 4. 7	85	36 ± 27

Values are means ± SD.
[a] Strain code in the strain collection at the Institute for Biological Control.
[b] Survival in percentage of the initial number of females (n = 15 females, 3 replicates) until day seven of the experiment.

Greece: 2 samples). *T. cacoeciae* is a thelytokous species with a broad geographic distribution (Pinto, 1999). From literature, it is known that this species is mainly found in arboreal habitats like forests and orchards and the natural occurrence in olive groves is expected. The size of the ITS-2 PCR product of these strains was assessed as 580 bp in agarose gel electrophoresis. The sequence of the ITS-2 region of a Greek strain consisted of 466 bp (Table 2).

3.2.3. *Trichogramma cordubensis Vargas and Cabello*

Several samples of this species were collected in the young olive plantation at Paradise Park, Cairo, from eggs of the jasmine moth in January/February 2003 and in February 2004, respectively. *T. cordubensis* is a thelytokous species. Rearing of strains ("TP52EG", "TP63EG") at higher temperature (alternating regime of 25/35 °C) resulted in 5% of males and gynandropomorphs in the progeny. Examination of the male genital capsule, PCR studies as well as digests of the ITS-2 PCR product [size 520 bp] by the restriction enzymes *Eco*RI and *Mnl*I were performed in comparison to a reference strain (strain "Tc14", described in Silva et al. (1999)). These data as well as subsequent sequencing of the ITS-2 region and BLAST-analysis suggested the identity of these strains as *T. cordubensis* (Table 2).

3.2.4. *Trichogramma euproctidis Girault*

This arrhenotokous species was collected several times in the young olive plantation at Paradise Park, Cairo, during 2002 and 2003. It was first identified as *Trichogramma pretiosum* (Hegazi et al., 2005), but further work on morphological and molecular characters (color, kind of reproduction, size of the ITS-2 PCR product (490 bp, Silva et al., 1999)) suggested relationship to the species *Trichogramma turkestanica* Meyer, synonym of *Trichogramma meyeri* Sorokina. Whole body mounts of single specimen (strain "TP42EG") were identified as *T. euproctidis* Girauld (B. Pintureau, pers. communication) which is considered as synonym of *T. turkestanica* and *T. meyeri* according to Rohi and Pintureau (2003a). The BLAST-alignment of the ITS2-sequence suggested consistency with that of a Portuguese strain of *T. turkestanica*, studied by Silva et al. (1999).

3.2.5. *Trichogramma nerudai Pintureau and Gerding*

This species was collected in May 2002 and June 2003 at Figueira de Castelo Rodrigo, North-Eastern Portugal. It produced both males and females of dark gray coloring. Sex ratio was strongly female-biased (>80% females). The morphology of the male genital capsule allowed the assignment to the *parkeri* section described by Pinto (1999) and here to *T. bourarachae* (B. Pintureau, pers. communication). However, collected wasps (strains "TP23PT", "TP57PT") could be clearly separated by the size of the ITS-2 PCR product (730 bp) from the other strains obtained in the olive grove ecosystem. BLAST-analysis of the ITS-2 sequence (635 bp) resulted in the identification

Table 4
Offspring production per female and sex ratio in progeny (% females) in crossings between males and females of the *Trichogramma* strains "TP23PT" (*T. nerudai*, collected in olive in Portugal in 2002) and "NERCH99" (*T. nerudai*, collected in Chile in 1999) and between males and females of the F1-Generation. 10 replicates of each crossing were performed and eggs of *S. cereallela* were offered for seven days at 25 °C, 70% RH, (L/D: 16:8 h)

	TP23PT female x			NERCH99 female x	
	∑ Offspring	% Females		∑ Offspring	% Females
Without male	21 ± 25	0	Without male	55 ± 16	0
TP23PT male	33 ± 14	84.8	NERCH99 male	60 ± 30	56.3
NERCH99 male	41 ± 27	50.4	TP23PT male	41 ± 12	69.1
	F1 males × F1 females			F1 males × F1 females	
	145 ± 51	69.8		41 ± 11	87

as *T. nerudai* Pintureau and Gerding (Table 2), which is known only from Chile so far (Pintureau et al., 1999). A crossing experiment between the Portuguese strain "TP23PT" and a strain of *T. nerudai* (strain "NERCH99"), collected in Chile, resulted in fertile F1-females (50 and 70% of the progeny), indicating sexual compatibility between strains (Table 4). In contrast, crossings between "TP23PT" and a Portuguese strain of *T. bourarachae* ("Tb26") did not produce females in the F1-generation.

3.2.6. *Trichogramma oleae Voegelé and Pointel*

This species is not known to be indigenous to Tunisia, but was repeatedly released by the Institute de l'Olivier since 1986 in several olive groves in the Sfax region. During this study, parasitized sentinel eggs were obtained from sites where no releases had been made before. The size of the ITS-2 PCR product of examined wasps was estimated as 500 bp in agarose gel electrophoresis. The ITS-2 sequence of individuals from the field-collected strain "IO47" (403 bp)] was identical with that obtained from the original strain "2F", imported from France and reared at the Institute de l'Olivier in Tunisia, and revealed 98% identity with the already published sequence of *T. oleae* in GenBank (Schilthuizen and Stouthamer, 1997; Table 2). *T. oleae* was the most frequent species recorded in Tunisia in this survey, but it was not found in the other countries. *T. oleae* is known as thelytokous species.

4. Discussion

According to our results in the four countries surveyed, natural communities of *Trichogramma* parasitoids in olive groves are composed of only few species. Mainly species previously recorded from the Mediterranean region were found: *T. bourarachae*, *T. cordubensis*, *T. euproctidis* (=*turkestanica*), and *T. oleae*. Furthermore, the typical arboreal species *T. cacoeciae* as well as *T. nerudai* were collected. Low diversity of the *Trichogramma* fauna was observed in similar studies conducted in particular biotopes or agroecosystems. Three species (*Trichogramma fuentesi* Torre, *Trichogramma retorridum* Girault, and *T. pretiosum* Riley) were collected in a survey of naturally occurring egg parasitoids in sugarcane in Texas (Browning and Melton, 1987). In vineyards of Alsace, Barnay et al. (2001) found four native species (*T. cacoeciae*, *T. daumalae* Dugast and Voegelé, and *T. evanescens* Westwood, *T. principium* Sugonjaev and Sorokina). Glenn and Hoffmann (1997) recorded five species in vineyards in South Australia (*Trichogramma carverae* Oatmann and Pinto, *Trichogramma funiculatum* Carver, *T.* sp. *x*, *Trichogramma* nr. *brassicae*, *Trichogrammatoidea* sp. nr. *bactrae*). The occurrence of up to five species (*T. bourarachae*, *T. cordubensis*, *T. evanescens*, *Trichogramma pintoi* Voegelé, and *T. turkestanica*) in tomato fields of Portugal was confirmed by a recent study of Gonçalves et al. (2006). Two species (*T. cacoeciae*, *T. evanescens*) were found in vineyards in Rheingau/Germany by Ibrahim et al. (2004). Arboreal habitats are often dominated by one species, for instance *T. cacoeciae* in European forests (Walter, 1982) and apple orchards (Sakr, 2003) and *Trichogramma minutum* Riley in peach orchards (Atanassov et al., 2003) and spruce forests (Quayle et al., 2003) in North America. According to our results, also the olive grove ecosystem was dominated by one particular species of *Trichogramma* (*T. cacoeciae* in Portugal and Greece, *T. oleae* in Tunisia, and *T. bourarachae* in Egypt).

Natural egg parasitism of lepidopterous key olive pests like the olive moth or the jasmine moth was low in the olive groves surveyed (<17% in Egypt (Hegazi et al., 2005) and <1% in the other countries) and only 10% of the examined samples were obtained from naturally occurring host eggs. Pest control by naturally occurring *Trichogramma* egg parasitoids was insufficient and augmentative releases of reared wasps are needed. The strains described in this study could all be easily propagated on factitious hosts (*S. cereallela*, *E. kuehniella*), thus indicating their potential for mass production, one important prerequisite for their use as biological control agents.

In the present study, more *Trichogramma*-species were found in Egypt (4 species) compared to the other countries of the study, although only two locations were sampled. These two locations represented two distinct types of olive groves, situated in different regions (Table 1): the location "Bourg-el-Arab" represented a traditional grove with high vegetation diversity, where also apple, peach, guava, fig, and almond trees were grown. Insecticides were seldom applied in this grove. It was located at the coast and rainfed under semi-arid weather conditions. *T. bourarachae* was frequently collected in this biotope. In contrast, the grove "Paradise-Park" represented a young intensively managed plantation under arid weather conditions. *T. bourarachae* was not found there. Perhaps this species did not manage to spread into this recently established olive growing region because of the surrounding desert. Date palms as well as ornamental shrubs were also grown in Paradise Park, thus sustaining other potential lepidopterous host species as well as their specific natural enemies. The jasmine moth was common in higher densities and throughout the season on olive trees in Paradise Park, probably because young irrigated olive plantations are the preferred habitat of this pest. *T. cordubensis* was isolated several times from eggs of this host, indicating preference. In other studies, we found this species promising for use in the biological control of *P. unionalis*, supporting the idea of selecting indigenous biological control agents adapted to the pest/crop system (Herz and Hassan, 2006).

According to literature references, both *T. bourarachae* and *T. cordubensis* are known to be generally abundant and typical for the Mediterranean area (Table 5). *T. cordubensis* was described by Vargas and Cabello (1985) which obtained this species from eggs of *Helicoverpa armigera* Hübner in Spain. It was also found in Morocco and Algeria on eggs of the carob moth (*E. ceratoniae*) as well as of *H. armigera* (Pintureau and Babault, 1988) and was obtained in Portugal from noctuid eggs (Silva et al., 1999; Gonçalves et al., 2006). The first description of *T. bourarachae* was based on specimens who originated in Morocco and had been collected from eggs of *H. armigera* (Pintureau and Babault, 1988). It is also known from Portugal from noctuid eggs (Silva et al., 1999; Gonçalves et al., 2006). The results of our survey contributed to the knowledge of the geographic distribution of these both species as this was the first record of *T. bourarachae* in Tunisia and Egypt and of *T. cordubensis* in Egypt. According to literature and our observations, they were isolated from important target pests in the Mediterranean region (*E. ceratoniae*, *H. armigera*, and *P. unionalis*), indicating their potential for use in biocontrol programs. But for *T. bourarachae*, we also found differences in important biological attributes (fecundity, survival, developmental time) between strains from Egypt, Tunisia, and Portugal, showing the importance of selecting the appropriate biotype suitable for biological control (Unruh and Messing, 1993).

Trichogramma nerudai was recently discovered in Chile on eggs of the European pine shoot moth, *Rhyacionia buoliana* Denis and Schiffermüller (Pintureau et al., 1999). The species is currently under investigation for use in biological control of *R. buoliana* and other pests in South America (Gerding et al., 1996; Tezze and Botto, 2004). The reported record of this species in Portugal is the first one outside Chile. Further molecular studies are needed to prove the degree of relationship between the Portuguese and Chilean strain, also concerning a potential immigration of this species from South America to Europe or vice versa.

Based on our results, it is not possible to determine if *T. oleae* is native to Tunisia or not. Our data suggested successful dispersion and establishment of this species after several releases of a French strain were made in the past in Tunisia. However, natural egg parasitism of important olive pests was low and other hosts may sustain the existence of this species in the olive grove ecosystem. Our study points on the need for monitoring the existing *Trichogramma* fauna in a particular area before inundative releases of species are conducted for the first time. Only then it is possible to detect any shifts in the local egg parasitoid community after release of a potentially exotic species.

We are aware of the fact that the results of this survey may give an incomplete picture of the real existing *Trichogramma* fauna in the Mediterranean olive region. Most samples were obtained from sentinel eggs of the factitious hosts *S. cerealella* and *E. kuehniella.* There is the risk of sampling bias by excluding those species which did not accept these eggs. However, eggs of target pests like the olive moth and jasmine moth are quite small and are laid singly or in small clusters and thus they are not easy to collect. A high predation on egg cards within the baiting

Table 5
Host associations of *T. cordubensis* and *T. bourarachae* according to literature and collection data obtained in this study

Host	Crop	Collection	Reference
T. cordubensis Vargas and Cabello			
Helicoverpa armigera	Cotton	Spain, 1982	Vargas and Cabello (1985)
Helicoverpa armigera	No data	Morocco, 1983	Pintureau and Babault (1988)
Noctuidae	Tomato	Portugal, 1992	Silva et al. (1999)
Sphingidae and Noctuidae	No data	Azores, 1989	Pintureau et al. (1991)
Ectomyelois ceratoniae	No data	Algeria, 1979	Pintureau and Babault (1988)
Palpita unionalis	Olive	Egypt, 2003	This study, Hegazi et al. (2005)
T. bourarachae Pintureau and Babault			
Helicoverpa armigera	No data	Morocco, 1983	Pintureau and Babault (1988)
Noctuidae	Tomato	Portugal, 1992	Silva et al. (1999)
Vanessa cardui	*Malva sylvestris*	Morocco, 1992	Rohi and Pintureau (2003b)
Ectomyelois ceratoniae	Pomegranate	Tunisia, 2002	This study
Host bait (*Ephestia*)	Olive	Tunisia, 2002	This study
Host bait (*Sitotroga*), *Palpita unionalis*	Olive	Egypt, 2002, 2003, 2004	This study, Hegazi et al. (2005)

devices was observed, especially by ants in summer (Hegazi et al., 2005) and predators might have destroyed also parasitized eggs. Such positive samples were lost for further analysis. Nevertheless, the host exposure technique was shown to be a helpful tool to collect *Trichogramma* in the field. It can certainly be improved by using eggs of hosts known to occur in the sampling area and by a better protection from predators. The present work has clearly increased knowledge on the geographic distribution and species diversity of this important group of natural enemies and such surveys should be extended into other less studied areas of the world (Sithanantham et al., 2001).

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