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Biological control of grape berry moths *Eupoecilia ambiguella* Hb. and *Lobesia botrana* Schiff. (Lepidoptera: Tortricidae) by using egg parasitoids of the genus *Trichogramma*

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DEDICATION

I dedicate this PhD dissertation to both souls of my Mother and my Father

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1 INTRODUCTION

The concept of biological control of insect pests is known since more than a century. During this period considerable efforts have been given to develop this method of insect pests control (HOFFMANN *et al*. 1994). Natural enemies were first used to control insect pests when farmers in ancient China and Yemen moved colonies of predaceous ants to control pests of tree crops (HAJEK, 2002). Parasitic wasps are already well-known since a long time as a limiting factor for the grape berry moths (GBM) (RÜBSAAMEN 1909). In the context of integrated plant protection in the viticulture as an additional control method, the application of *Bacillus thuringiensis* is recommended. However, in practice the temporary efficacy (HOLST 1986), the relatively high price (HAUB 1981), the necessity for the correct time (WOHLFARTH and SCHRUFT 1986), the relatively low effectiveness with high infestation strength (BOLLER and REMUND 1981) as well as strong weather dependence have been considered as unfavorable. A very specifically biotechnical procedure represents the use of pheromones in the confusion method. This method is certified from the Federal Biological Research Center for Agriculture and Forestry (BBA) since March 1986 (SCHRUFT 1987). The confusion method can be applied successfully only on a large scale (LOUIS 1987). Increasing problems of pesticide application, let biological control arise again with more interest in the context of integrated plant protection (FRANZ and KRIEG 1982). An important contribution to control methods of GBM described above, is by the expansion and intensification of the biological control agents. A crucial condition for the implementation of this alternative is the development of a procedure line with standard usage. However, a conscious utilization can hold in the frame of integrated pest management of the GBM, hence it keeps its population under the economical damage level. Under the various already well-known beneficials, the egg parasitoids of the genus *Trichogramma* are the most important and worldwide distributed. As the native populations of *Trichogramma* develop itself only in the field (SCHRUFT 1978), their contribution was not sufficiently regarded as control agents to prevent the pest from reaching damaging levels (SMITH 1996, KNUTSON 1998). However, by means of useful promotion and selection of suitable *Trichogramma*, there are promising species for controlling the GBM (KAST and HASSAN 1986, REMUND and BIGLER 1986, LEISSE 1988). *Trichogramma* controls different lepidopteran pests. Annually about 32 ha of agriculture and forestry are treated with it worldwide (LI 1994). Parasitoid releases in Canada, China, Switzerland and the former USSR have shown consistent high levels of parasitism (60% to 80%) with reduction in pest damage by 77% to 92% on such crops as sugarcane, wheat and corn (LI 1994). The successful application of a biological control program of GBM in the viticulture with *Trichogramma* is based on several conditions: (1) more information about GBM and *Trichogramma,* (2) survey of the naturally existing *Trichogramma* population, (3) selection of suitable species and/or strains, (4) development of a mass production and release procedure and (5) evaluation of that procedure (CASTANEDA 1990, SMITH 1996).

The promotion of beneficial organisms as well as their periodic release are two options, which are available in the biological control of pests (FRANZ and KRIEG 1982). The selection of suitable species and/or strains represents the critical phase in the development of a biological control program. Thereby, the consideration of different criteria is essential (van LENTEREN 1986, PAK 1988, KNUTSON 1998): (1) adjustment of the beneficials to climatic conditions and cultivated plants, (2) coincidence of the beneficial insects and insect pests, (3) host selection, (4) natural reproduction potential and (5) suitability to the mass production. Another source of error in the biological control with *Trichogramma* is a wrong evaluation of the host-parasite relationship. The evaluation of the suitability of a parasite for the application against insect pests is strongly dependent on more knowledge about its behavior in the field (MAYER 1955). In the laboratory many reared beneficial insects behave as natural enemies for some insect pests. However, in the field sometimes an unexpected behavior can be observed, because there are changes in some conditions such as temperature or humidity (HASSAN *et al*. 1984). Hence, an inventory of locally occurring egg parasitoids should be established first and introduction of exotic species should be considered only if there are no promising locally occurring species or if pre-introductory evaluations indicate advantages of exotic over local species (SMITH 1996). On the other hand, the released exotic parasitoids may pose a risk to non-target lepidopterous species, for example the endangered butterflies (BABENDREIER *et al.* 1999).

Despite all of the positive experience with biological procedures either in the laboratory or in the insectarium, success in the practical application is not yet guaranteed. Therefore, trials under field conditions are important, whereby additional factors have to be considered. In this study, the natural occurrence of *Trichogramma* species was accomplished in the vineyards (Rheingau/ Hessen) and their contribution to suppress the grape berry moths population was examined. In the laboratory and vineyards, experiments were carried out with various species of this genus to control grape berry moths. This work was aimed to collect more information about the native *Trichogramma* species in the Rheingau area, their ecology and abundance across the season in the vineyards. Also, this was aimed to understand interactions of species between vineyard and surrounding biotopes and why natural *Trichogramma* has only partially control GBM populations. There are few previous studies which had been carried out on the annual activity of *Trichogramma* and their biotopes (KOT 1964, CHEPETILNIKOVA 1970, HUNG *et al*. 1985, SENGONCA and LEISSE 1987, PINTUREAU and KEITA 1990, SCHADE 1990, BARNAY *et al*. 2001, SAKR 2003). This work is also aimed to compile the basis for the development of a procedure for the biological control of GBM.

The main objectives of this study are:

1. Determination of the efficiency of various *Trichogramma* spp. to control the grape berry moths *Eupoecilia ambiguella* Hb. and *Lobesia botrana* Schiff. under laboratory conditions.
2. Efficiency of some *Trichogramma* spp. to control the grape berry moths *Eupoecilia ambiguella* and *Lobesia botrana* under vineyard conditions.
3. Estimation of the dispersal potential of some *Trichogramma* spp. in vineyards.
4. Surveying the natural occurrence and distribution of *Trichogramma* in vineyards and surrounding biotopes.

2 GENERAL ASPECTS

2.1 Biology and behavior of the grape berry moths (GBM)

In Germany, viticulture areas cover about 101,000 ha. Economically, vine production lies in fourth place of general plant products, e.g. it constitutes 9.1% (1.2 billion €) of all plant products (ANONYM 2001). The grape berry moths (GBM) *Eupoecilia ambiguella* Hb. and *Lobesia botrana* Schiff. are the economically most important pests for the German vineyards. They cause considerable losses in both quality and yield of grapevine (KAST 1990). In many of the German vine growing areas a striking shift of the two species has been recorded. Over the past years, *L. botrana* populations increased while those of *E. ambiguella* have decreased. In some areas, for example the Palatinate, there are regions where currently only *L. botrana* occurs. This species shows a higher flight activity and a greater reproduction potential, which results in a more serious injury to grape clusters than in the case of *E. ambiguella* (LOUIS and SCHIRRA 2001). In addition to the genus *Vitis,* there are other numerous host plants / green shrubs for *E. ambiguella* and *L. botrana* outside of the vineyards in its proximity. These host plants are: *Acer campestris, Clematis vitalba, Cornus alba, Cornus mas, Cornus sanguinea, Crataegus pyracantha, Daphne gnidium, Galium mollugo, Hedera helix, Ligustrum vulgare, Lonicera spp., Mahonia aquifolium*, *Medicago sativa, Prunus spinosa, Rhus glabra, Ribes rubrum, Rubus fructicosus, Rubus ideaus, Sambucus racemosa, Silene inflata, Trifolium pratense,* and *Viburnum lantana* (STELLWAAG 1928).

The economic importance of GBM depends strongly on the developmental stage of the grapevine. Before and during flowering, the larvae at first penetrate single flower buds and later on they start to tie together several flower buds, building glomerules in which they stay and continue their feeding activities. In this stage, the tolerance level for grape berry moth infestation is relatively high and depends on the ability of the grape variety to compensate the damage (ROEHRICH and SCHMID 1979). Laboratory and field experiments showed that *L. botrana* is mainly active during the evening, whereas *E. ambiguella* feeds in the evening as well as early in the morning. Mating activities begin after midnight until early morning, and eggs are laid in the afternoon and evening.

Single eggs are laid on or near the food source of the neonate larvae, in spring on bracts, petals and stems of the flower clusters and in summer on the berries. After hatching, larvae penetrate the flower buds or berries. The mature larva leaves the clusters and weaves their cocoons either on the edge of the leaves or on the trunks (GÖTZ 1943). Every year there are two generations of both species of GBM, whereby the damage caused by larvae is different in each case. In warm years with long vegetation period, a third generation can be registered for *L. botrana*. Larvae of the first generation are named “hay worm“ generation, because they appear in the time of the hay harvest. The second generation shows up at the beginning of July and is well-known under the name “sourly worm‘‘ generation, however the larvae infest still sourly berries and the infestation prevent their maturation. The third generation strikes the maturated clusters and therefore named “sweet worm“ (HILLEBRAND and EICHHORN 1988). The optimum climatic conditions are not the same for the two species. Activity and oviposition in both species are high above 20°C, however the range of optimum RH% is 40-70% for *L. botrana* and above 70% for *E. ambiguella* (SPRENGEL 1931). *E. ambiguella* occurs in all palaearctic vine-growing areas but is the predominant species in the north, whereas *L. botrana* dominates in southern areas. Their abundance in the various viticulture areas is not uniform and can change within relatively short distances. In certain places they can cause heavy damage every year, in other areas the populations are always low, and there are also areas where the abundance changes from year to year according to the local climatic conditions. In areas where both species occur together, *L. botrana* can be found in higher densities at sunny exposures and during hotter seasons (ROEHRICH and BOLLER 1991). Both GBM species overwinter as diapausing pupae. Prepupae diapause in *L. botrana* is controlled by photoperiodism and induced in the young larvae. The mature larvae develop into pupae as soon as they have woven their cocoons. Diapause in *E. ambiguella* is induced also by photoperiodism, whereas the mature larvae stay for several months in a prepupal stage (LEHOCZKY and REICHART 1968). The economic threshold depends on various aspects, such as whether the grapes are produced as table fruit or for vinification and the level of precipitations (higher or lower risk of *Botrytis* infestation) (REMUND and SIGFRIED 1982, SCHRUFT 1983). Generally, for the first generation of GBM the economic threshold was recommended as 20 larvae/100 flower cluster. Whereas, 2 to 5 larvae /100 flower clusters for the second generation can be only tolerated as a result of the infection pressure by *B. cinerea* (BOURQUIN 1987).

2.2 Biology and behavior of *Trichogramma*

The family Trichogrammatidae includes 620 species and 80 genera. The genus *Trichogramma* has received the most attention because of its importance in biological control. *Trichogramma* is worldwide distributed and consists of 145 described species (PINTO and STOUTHAMMER 1994). According to HOFFMANN and FRODSHAM (1993), no other parasitoids have been used worldwide as extensively as *Trichogramma* for direct control of pests. The representatives of this cosmopolitan genus are egg parasitoids. Investigations on the population dynamic of pests showed that *Trichogramma* represents a considerable natural mortality factor. The first fundamental study was reported by SALT (1934, 1935, 1937 and 1940), which accomplished detailed investigations about various aspects of parasitization, such as host-selection and host acceptance. The first biological control studies with *Trichogramma* were conducted in the USSR and in the USA at the beginning of the 20th century (SCHIEFERDECKER 1970). The development of a rational and most economical mass rearing method by means of *Sitotroga cerealella as* an alternative host, led to an intensification of the field use of these parasitic wasps in numerous crops (FLANDERS 1930). *Trichogramma* are solitary endoparasitoids, they exploit eggs of more than 400 hosts (SILVA 1999), mostly Lepidopteran species on a large variety of plants, from herbs to large trees (SUVERKROPP 1997). *Trichogramma* are used against various lepidopterans in North and South America, South East Asia, Middle Asia, Middle East countries and Australia (FLANDERS 1929, STSCHEPETILNIKOWA 1976, HASSAN 1993). *Trichogramma* species have a short generation time and can easily be mass-produced. They kill the lepidopteran pests during the egg stage before caterpillars can emerge and damage the crop (KING *et al*. 1986, HASSAN 1990 &1993). At a constant temperature of 27°C it takes about 10 days from the start of parasitism to the emergence of wasps (HOFFMANN *et al*. 1995). Female parasitization follows a sequence in five phases: the female contact the host egg, drumming, drilling, oviposition and host feeding (KLOMP *et al*.1980, PAK 1988). It was reported that the average duration of the individual phases at 20°C were 5 s (seconds) contact, 30 s drumming, 60 s boring and 300 s oviposition, it depends on the temperature, *Trichogramma* strain and the host species (PAK 1988). *Contact* is the physical touching of host eggs by the female; *drumming* means waving the antenna over host eggs; *drilling* is insertion of the females ovipositor through the egg shell; *oviposition* means the laying egg(s) into the host egg. RUBERSON and KRING (1993) summarized the process of parasitism that once a female finds a host egg, it drills a hole through the chorion and inserts an egg (s) into the host egg. The internal pressure of the host egg forces a small drop of yolk out of the oviposition hole. Females feed on this yolk *(host feedin*g), which increases their longevity. According to RUBERSON and KRING (1993) a female *Trichogramma* parasitizes from 10 to 190 eggs during its life. Sometimes, more than one egg is inserted into each host egg and this depends on the respective species and the female body size. *Trichogramma* females that were provided with honey lived longer (on average 11 days) and parasitized more eggs than those without honey (average only 3 days). *Trichogramma* females prefer younger eggs for parasitism rather than older ones. For instance, *T. pretiosum* (Riley) and *T. galloi* (Zucchi) parasitized younger eggs of *Diatraea rufescens* Box and *D. saccharalis* F. better than old ones. Five-day old eggs were never parasitized (MONJE *et al*. 1999). *Trichogramma* eggs hatch in ca. 24 hours after oviposition and the parasitoid larvae develop very quickly. There are 3 larval instars in *Trichogramma*. Larvae then transform to the inactive pupal stage (STRAND 1986). The host egg turns black during the third instars (3 to 4 days after parasitism) as a result of dark melanin granules deposited on the inner surface of the egg chorion. The black layer inside the chorion and the exit hole are evidence of parasitism by *Trichogramma* (STRAND 1986). The adult wasps emerge (after ca. 4 to 5 days) and escape from the host egg by chewing a circular hole in the egg-shell (STRAND 1986). The parasitoids pupate within the host eggs. Few hours after emergence and mating, *Trichogramma* females begin with the oviposition (PAK and OATMAN 1982, WAAGE and MING 1984, KNUTSON 1998). Arrhenotoky is the common mode of reproduction in *Trichogramma* where unfertilized eggs produce haploid males and fertilized eggs produce diploid females. Whereas, thelytokous populations consist of only females that produce female offspring without mating (STOUTHAMER *et al*. 1990). Some *Trichogramma* species are entirely thelytokous while others consist of both thelyokous and arrhenotokous populations (PINTO and STOUTHAMER 1994). There are two forms of thelytoky observed in *Trichogramma* revertible and non-reversible thelytoky. Bacteria of the genus *Wolbachia* cause revertible thelytoky, whereas there are no microbes in the non-reversible thelytoky (STOUTHAMER and KAZMER 1994). The latter can be reverted to arrhenotoky by treatment with several specific antibiotics (STOUTHAMER *et al*. 1990). *Wolbachia* is widespread in the phylum arthropoda, it can modify the reproductive phenotype of its host (WERREN and O'NEIL 1997). *Wolbachia*-induced thelytoky is a form of parthenogenesis that presently has only been found in various species of order Hymenoptera (ROUSSET *et al*. 1992, STOUTHAMER *et al*. 1993, van MEER *et al*. 1995, ZCHORI-FEIN *et al*. 1995). STOUTHAMER and KAZMER (1994) reported that the presence of *Wolbachia* in eggs of thelytokous females causes a disruption of the chromosome segregation in the first mitotic division (anaphase) of the haploid egg. As a result, haploid eggs become diploid and develop into thelytokous females.

The successful use of egg parasitoids of the genus *Trichogramma* in biological control is greatly dependent on the suitability of the chosen *Trichogramma* species (WÜHRER 1996). It is clearly proven that the causes of the low effectiveness of some *Trichogramma* applications are due to the choice of unsuitable species and the application under unfavorable ecological conditions (MAYER 1960). Thereby, local species are generally preferred because they are likely to be adapted to the ecological conditions such as climate, habitat and host conditions than exotic species (VOEGELÈ *et al*. 1988, HASSAN 1994, SMITH 1996). Therefore, selection of suitable species/strains represents the critical phase for the guarantee of the success of a biological control program with *Trichogramma*.

3 MATERIAL AND METHODS

3.1 The used *Trichogramma* species/strains

In both laboratory and in the vineyard, 17 species/strains of *Trichogramma* were tested against grape berry moths (GBM) *Eupoecilia ambiguella* Hb. and *Lobesia botrana* Schiff. The *Trichogramma* species/strains used are listed in Table 1 and include 6 thelytokous and 11 arrehenotokous strains.

Tab. 1: *Trichogramma* species/strains which were tested against the GBM.

Trichogramma species	abbrv.	Origin (host or bait)	Origin (culture)	Origin (country)	Year	Sexmodus
T. cacoeciae Ea-st	Cac-Ea	*Sitotroga cerealella*	Plum	Germany	1990	T
T. cacoeciae Lb-st	Cac-Lb	*S. cerealella*	Plum	Germany	1990	T
T. cacoeciae Sit-st	Cac-sit	*S. cerealella*	Plum	Germany	1990	T
T. cacoeciae-01	Cac-01	*S. cerealella*	Vine	Germany	2001	T
T. cacoeciae -94	Cac-94	*S. cerealella*	Vine	Germany	1994	T
T. cacoeciae-com	Cac-com	*Cydia pomonella*	Apple	Germany	2000	T
T. minutum	Min	*S. cerealella*	Apple	Germany	1992	A
T. evanescens-01	Eva-01	*S. cerealella*	Vine	Germany	2001	A
T.evanescens-com	Eva-com	*Mamestra brascicae*	Cabbage	Germany	1996	A
T. dendrolimi- com	Den-com	*S. cerealella*	Apple	Germany	1990	A
T. exiguum	Exi	Nd	Nd	USA	Nd	A
T. principium	Pri	*Archips rosana*	Nd	Kirgisistan	1988	A
T. piceum	Pic	Nd	Nd	Moldavia	Nd	A
T. pretiosum	Pre	Nd	Nd	Egypt	Nd	A
T. japonicum	Jap	Nd	Nd	Thailand	Nd	A
T. bourarachae	Bou	Nd	Nd	France	1992	A
T. semblidis	Sem	*Lymantria monarcha*	Forestry	Germany	1994	A

T= thelytokous; A = arrhenotokous; Nd = No data

3.2 Rearing method of *Trichogramma* species

T. cacoeciae (Cac-01), and *T. evanescens* (Eva-01) were caught from vineyards in the Rheingau area in 2001. *T. cacoeciae* (Cac-Ea) and *T. cacoeciae* (Cac-Lb) (generation no. 98) were provided by the Department of Phytomedicine, State Research Institute Geisenheim. These strains are further reared on GBM eggs. *T. cacoeciae* (Cac-com), *T. evanescens* (Eva-com) and *T. dendrolimi* (Den-com) were provided by AMW-Nützlinge Company. The remaining *Trichogramma* species were provided by the Federal Biological Research Center for Agriculture and Forestry (BBA), Darmstadt. *Trichogramma* strains were reared on eggs of the angoumois grain moth *S. cerealella* in an environmental cabinet at 27±1 °C, 16 h Light and 60 to 70 % RH. The different strains were kept separately in glass tubes (24.5 cm long x 2 cm Ø) closed with musselin. Two tubes for each strain were put together in plastic containers (18 x 13 x 6 cm). By means of black paper, one half of the container was darkened in order to keep the adults away from the lid. About 3,000 adult *Trichogramma* per tube were supplied with ca. 10,000 host eggs. After three to four days, when the eggs started to turn black, the tubes were placed in another environmental cabinet at 18 °C to slow down their development. Two days later, when all adults were dead, the egg-cards were removed from the cabinet, reduced to one third and placed into a new tube. New *Sitotroga* egg-cards were supplied when the adults started to emerge, they were placed again into the warm chamber.

3.3 Rearing method of the grape berry moths (GBM)

Several hundred pupae of the GBM were collected from the field and kept in a climatic cabinet at a temperature of 24°C. Emerged adults were kept separately according to species in plexi-glass cylinders with 25 cm length and 15 cm in diameter. The inner walls of these cylinders were lined with PE foil, where the females lay their eggs. The nutrition of the adults took place via soaked cotton wool with 10% saccharose solution. For the withdrawal of the freshly laid eggs of GBM, adults were stupefied by CO_2 and converted into new plexi- glass cylinders. Dead adults were segregated. The foil with the GBM eggs was cut in ca. 3 cm broad strips and put on medium in a 8 cm high plastic box. The medium consisted of 17 ingredients (Tab. 2). Before the completion of the larval development, corrugated paper was settled, in whose curvatures the developed larvae could pupate. Briefly before emergence, the pupated larvae in the corrugated paper were moved in another clean plastic box.

Tab. 2: Components of the rearing media of the GBM :

Components	Quantity
Water	750 ml
Wheat germs	93.5 g
Alfalfa “seeds”	25 g
Agar	30 g
Yeast	20 g
Sugar	40 g
Wesson salt*	12.5 g
Casein	45 g
Cholesterol	1.25 g
Sun flower oil	10 ml
Sorbic acid	2 g
Nipagin	1.25 g
Vitamin C	0.20 g
Multivitamin solution	1.6 ml = (50 Drops)
Propionic acid	2.5 ml
Formaldehyde	0.4 ml
Aureomycin	0.0025 mg (solved in 12.5ml alcohol)

3.4 Laboratory Experiments

Parasitism of various *Trichogramma* species/strains was examined first by introducing eggs of the GBM (acceptance test). Then simultaneous offer of GBM & *Sitotroga* eggs were examined whether a host preference is present among these species (preference test). Furthermore, generation time, longevity, parasitism potential and reproduction potential of various *Trichogramma* species during its entire life span in GBM eggs were examined (Analysis of various life history parameters in the course of the life span). All laboratory tests were accomplished in climatic cabinet at 25±1 °C, 70 – 80 % RH and 16 h light.

* Wesson salt: a mixture of: $CaCo_3$ (42g); $MgSo_4$ (18g); KCl (24g); NaCl (20.1g); KH_2Po_4 (62g) and $Ca_5(Po_4)_3$ OH (30.2g).

3.4.1 Host acceptance by *Trichogramma* species/strains

In order to determine whether or not the various *Trichogramma* strains accept eggs of GBM, 11 *Trichogramma* strains which include Cac-01, Cac-sit, Eva-01, Exi, Min, Pri, Jap, Pic, Bou, Pre and Sem were tested. In order to investigate the host acceptance, a PE-strip with ca. 70 eggs of the GBM (at maximum one day old) was placed in each Petri dish (5 cm Ø). A freshly hatched female *Trichogramma* (at maximum 24 h old) was added. To separate single females, *Trichogramma* were scattered on a white paper and captured again by placing small tubes (4 x 1 cm Ø), open end down to cover only one parasitoid. The parasitoid moved up in the tube and was easily examined by using a binocular. A drop of honey/agar was added in the petri dish. By shaking the vials, single females were released into the Petri dishes and left with the host eggs for its entire life. The number of parasitized eggs per female was determined. The test was repeated 20 times. The experiments were conducted in two separate lines in the same time for *E. ambiguella* and for *L. botrana*. Experiments were carried out under 25±1 °C, 70 – 80 % RH and 16 h light.

3.4.2 Host preference of *Trichogramma* species/strains

In Laboratory, eleven *Trichogramma* species/strains (see 3.4.1) were compared for their suitability to control grape berry moths (GBM) *E. ambiguella* and *L. botrana*. To test the host preference, *Trichogramma* females were offered simultaneously the choice among GBM eggs and eggs of the mass rearing host *Sitotroga cerealella* Oliv. A single *Trichogramma* female (12 to 24 h after emergence) was released in a Petri dish (5 cm Ø) (see 3.4.1). Eggs of GBM were already laid on foil, then counted (each 50), cut by cork drill (1.2 cm Ø), and glued of a larger foil (4 cm Ø). Eggs of *S. cerealella* (50 eggs) were glued on a paper disc (1.2 cm Ø) (Fig. 1). A small drop of honey/agar was added in the center. The female was left in the Petri dish for 5 days with the eggs. The test consisted of 30 replicates. Experiments were carried out at 25±1 °C, 70 – 80 % RH and

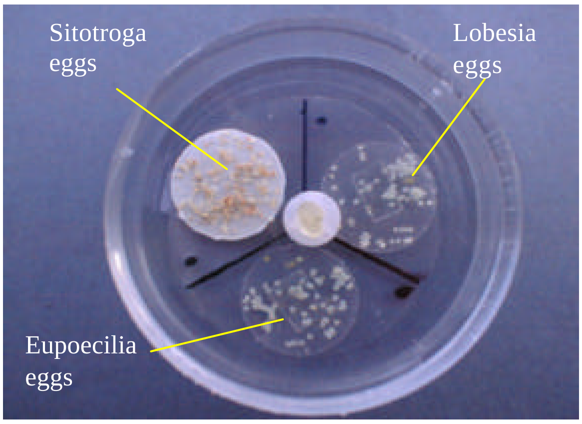

Fig. 1: Preference test

16 h light. After 5 days, eggs of GBM and *Sitotroga* were separated each alone. After hatching and death of the imagines, parasitized eggs as well as the hatched females and males were counted. Parasitism, emergence rate and sex ratio for arrhenotokous species were determined.

3.4.3 Analysis of various life history parameters in the course of the life span of *Trichogramma* strains in grape berry moths

Life activity parameters of 14 various *Trichogramma* species/strains (Cac-01, Cac-Ea, Cac-Lb, Cac-94, Cac-sit, Eva-01, Exi, Min, Pri, Jap, Pic, Bou, Pre and Sem) (see Tab. 1) were tested in the whole life of the parasitoids in both GBM eggs. In order to determine these life activities of *Trichogramma* strains, 25 females were individually released into petri dish (see 3.4.1). Each female was offered ca. 70 GBM eggs on PE-strips which were one day old. A small drop of honey/agar was added. Both the GBM egg strips and the honey/agar drop remained in the petri dish three days, then replaced by fresh GBM egg strips and a drop of honey/agar. Each experiment consisted of 25 replicates. Every female was supplied with fresh GBM eggs during its entire life span. The time when a female died was recorded. The number of parasitized eggs by the female every three days was recorded. The parasitized GBM eggs were kept 10 days later under the same conditions. The experiments were conducted in two separate lines in the same time for *E. ambiguella* and for *L. botrana*. Generation time, longevity, parasitism potential and reproduction potential were determined for each *Trichogramma* strain. Experiments were carried out at 25±1 °C, 70 – 80% RH and 16 h light.

3.5 Field Experiments

3.5.1 Description of the vineyards

Field experiments were conducted in 4 vineyards in the Rheingau area. The main characteristics of these experimental sites are summarized in tab. (3):

Tab. 3: An overview of the vineyards.

	Vineyards			
	Sand	Fuchsberg	Mäuerchen	Berg Rottland
Planting year	1971	1981	1982	1965
Rows direction	North-South	North-South	North-South	North-South
No. of rows	31	21	58	24
Vineyard area (m^2)	3000	2700	ca. 5000	2743
Row spacing (m)	2.5	2.6	1.8	1.4
Vine spacing (m)	1.4	1.5	1.3	1.2
Vine plants / m^2	2.1	2.7	2.3	1.68
Grape variety	Riesling	Riesling	Riesling	Riesling
Rootstock	5C	5C	5C	5BB
Training system	guyot	guyot	guyot	guyot
Height of canopy	2.00	2.00	2.00	1.8
Form of management	“Integrated”	“Integrated”	“Ecological”	“Integrated”

Cultural and Plant protection measures

The cultural measures corresponded to usual practice in the enterprise. In all vineyards, the distance in between each second vine row was covered with wild plants. Both the vineyard Sand and Berg Rottland were surrounded by hedge plants at 3 sides, however the last is slopey. Only the vineyard Mäuerchen, was ecologically managed (but without hedges), whereas the remaining vineyards were managed according to the principles of “integrated pest management” (IPM) (BRADER 1979).

Hedge plants

As mentioned above, only two vineyards (Sand and Berg Rottland) were surrounded by hedge plants at three sides. There were various plant species belonging to different families. Hedge plants were identified* and classified into 3 groups according to its location around the vineyard.

Tab. 4: Hedge plant combinations.

Hedge Plants	Vineyards	
	Sand	Berg Rottland
Group A	Dominated by: *Acer campestris, Cornus sanguinea, Crataegus monogyna, Ligustrum vulgare, Rubus idaeus, Sambucus nigra*	Fallow vineyard dominated by: *Achillea millefolium, Artemisia vulgaris, Cotoneaster integerrimus, Ligustrum vulgare*, *Prunus spinosa, Rosa canina, Rubus fruticosus, Sambucus nigra,Tanacetum vulgare*
Group B	*Clematis vitalba, Crataegus monogyna, Juglans regia, Ligustrum vulgare, Prunus avium, Prunus spinosa, Rosa canina, Rubus fruticosus, R. idaeus*	Dominated by: *Cirsium vulgare, Cornus sanguinea, Hedera helix, Prunus spinosa*
Group C	*Coryllus avellana, Crataegus monogyna, Ligustrum vulgare, Prunus domestica,* house garden	*Cichorium intybus, Prunus arvium, P. spinosa, Sonchus arvensis*

Chemical preparations

The preparations used, active ingredients, concentrations and its effect grades on *Trichogramma* are listed in Tables 5, 6, 7 and 8. No sulfur was applied at Sand site, but one fungicide harmful to *Trichogramma* (Tab. 5). The site Berg Rottland was IPM - managed (Tab. 6). There were neither insecticides nor sulfur applied at Fuchsberg location (Tab. 7). The biggest amount of sulfur was applied at Mäuerchen (Tab. 8). So, in all pesticide use was at a rather low level, but the use of sulfur was partly very high.

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* Identification was conducted by Dr. V. Behrens, Department of ornamental plants, State Research Institute Geisenheim. Also identified according to SCHMEIL and FITSCHEN (1960), FITTER (1987) and ZANDER *et. al*. (2000)

Tab. 5: An overview of the preparations used and its effect grades on *Trichogramma* (Sand).

Trade name	Active ingredient	Quantity / ha 1)	E. 2)
Insecticides :			
Kiron	Fenpyroximate	0.6 kg	*)
Fungicides :			
Ridomil combi	Folpet & Metalaxyl	0.6 kg	1
Polyram WG	Metiram	0.8 kg	4
Melody multi	Tolyfluanid+Iprovalicarb	0.8 kg	*)
Forum	Dimethomorph	0.48 L	*)
Vento	Fenarimol & Quinoxyfen	0.4 L	*)
Topas	Penconazole	0.06 L	1
Flint	Trifloxystrobin	0.06 kg	*)

1) based on 400 l/ha water *) No data
2) Effect grade 1 = harmless, 2 = slightly harmful, 3 = moderately harmful, 4 = harmful (from HASSAN *et al*. 1983, 1988 and 1994 and STERK *et al*. 1999)

Tab. 6: An overview of the preparations used and its effect grades on *Trichogramma* (Berg Rottland).

Trade name	Active ingredient	Quantity / ha 1)	E. 2)
Insecticides			
Steward	Indoxacarb	0.05 kg	*)
Fungicides			
Netzschwefel	Sulfur 3)	0.8 - 2.4 kg	3
Vento	Fenarimol & Quinoxyfen	0.4 L	*)
Ridomil combi	Folpet & Metalaxyl-M	0.6 kg	1
Prosper	Spiroxamine	0.2 L	*)
Forum star	Dimethomorph & Folpet	0.48 L	*)
Aktuan	Cymoxanil & Dithianon	0.5 kg	1
Topas	Penconazole	0.06 L	1
Funguran	Copper oxychloride	1 kg	1
Switch	Cyprodinil & Fludioxonil	0.24 kg	*)

1) based on 400 l/ha water *) No data
2) Effect grade 1 = harmless, 2 = slightly harmful, 3 = moderately harmful, 4 = harmful (from HASSAN *et al.* 1983, 1988 and 1994 and STERK *et al*. 1999)
3) Sulfur: was sprayed 5 times in total quantity : 9.7 kg/ha

Tab. 7: An overview of the preparations used and its effect grades on *Trichogramma* (Fuchsberg).

Trade name	Active ingredient	Quantity / ha [1)]	E. [2)]
Fungicides			
Aktuan	Cymoxanil &Dithianon	0.5 kg	1
Vento	Fenarimol & Quinoxyfen	0.4 L	*)
Funguran	Copper oxychlorid	1 kg	1
Ridomil combi	Folpet & Metalaxyl-M	0.6 kg	*)
Scala	Pyrimethanil	0.5 L	*)
Forum star	Dimethomorph & Folpet	0.6 kg	*)
Switch	Cyprodinil & Fludioxonil	0.24 kg	*)
Topas	Penconazole	0.06 L	1
Melody multi	Tolyfluanid+Iprovalicarb	0.8 kg	*)
Flint	Trifloxystrobin	0.06 kg	*)

1) based on 400 l/ha water *) No data
2) Effect grade 1 = harmless, 2 = slightly harmful, 3 = moderately harmful, 4 = harmful (from HASSAN *et al*. 1983, 1988 and 1994 and STERK et al. 1999)

Tab. 8: An overview of the preparations used and its effect grades on *Trichogramma* (Mäuerchen).

Trade name	Active ingredient	Quantity / ha [1)]	E. [2)]
Insecticides			
XenTari	*Bacillus thuringiensis*	0.4 kg	1
Fungicides :			
Netzschwefel	Sulfur [3)]	0.8 – 2.4 kg	3
Mycosin	Sulfuric acid + alumina + *Equisetum* sp. extract	4.8 -12 kg	*)
Funguran	Copper oxychloride	1 kg	1
Steinhauer-Mehltauschreck	Sodium hydrogencarbonate	15 kg	*)
Robus	Phosfit+Lecithin+soap	15 L	*)
Kaliwasserglas	Potassium silicate	2 kg	*)
Kupferkalk	Copper oxychloride	3.5 kg	1

1) based on 400 l/ha water *) No data
2) Effect grade 1 = harmless, 2 = slightly harmful, 3 = moderately harmful, 4 = harmful (from HASSAN *et al*. 1983, 1988 and 1994 and STERK et al.1999)
3) Sulfur: was sprayed 8 times in total quantity : 23.8 kg/ha

3.5.2 Surveying the natural occurrence of *Trichogramma* spp. in both vineyards and surrounding biotopes

Experiments were conducted for 2 successive years to survey the native populations of *Trichogramma* in the vineyards of Rheingau area (Hessia/ Germany). The investigations for surveying *Trichogramma* species were accomplished in 4 different vineyards (Tab. 3) in addition to 6 glass houses. The glass houses were planted with cucumber, spinach, tomatoes and the remaining 3 with various ornamental plants. The device which has been used in that survey is a small plastic card with the dimensions: 25 x 20 x 2 mm. From this card, a hole was punched out (1 cm Ø). On both sides of the card gauze (0.7 mesh) was fastened, which makes it possible only for *Trichogramma* to move in and out of the baiting device, however not the other predators. By means of a vibrating spatula, fresh eggs of *S. cerealella* were scattered and glued on self-adhesive points (Fig. 2 a) and used as bait. The gauze on the front side had an opening, through it the baiting eggs could be pushed in. The opening was closed with a paper clip after the baiting eggs had been inserted. By means of a fine wire, the device can be fastened easily to all vine structures (Fig. 2 b). The devices were placed both on the leaf lower surface (LLS) and on the leaf upper surface (LUS) and on the flower clusters (FC) and berries (Fig. 2 c & d). The devices were hung also in three different canopy levels on the grapevines (80-120, 120-150 and >150 cm) and on hedge plants. The device units were left one week outside, then recollected again and incubated with 25 °C, 70 - 80 % RH and 16 h light for 5 days (Fig. 2 e). The number of parasitized eggs/device, the number of units with parasitism and the preferred habitat for the parasitoids on the vine canopy were evaluated. In order to evaluate the flying periods of GBM, pheromone traps (type Biotrap, Manufacturer: Temmen GmbH Hattterersheim) were placed in the vineyards. These traps were controlled three times weekly.

Fig. 2: Scattering of *S. cerealella* eggs on self-adhesive points (a), the trap card parts (b), trap cards hung on leaf upper surface (c) and on flower cluster (d) and incubation of *Sitotroga* eggs (e).

3.5.3 Dispersal behavior of *Trichogramma* in vineyards

Horizontal and vertical dispersal behavior of *Trichogramma cacoeciae* (Cac-01) and *T. evanescens* (Eva-01) were tested to determine its dispersal capacity in the vineyard. Release cards of the same material and features of the trap cards (see 3.5.2) however with other dimensions: 55 x 20 x 2mm were used (Fig. 3). From this frame, a hole (3.5 x 1.7 cm) was punched out. A self-adhesive label (3 x 1.2 cm) with ca. 3000 parasitized *Sitotroga* eggs were introduced into this frame. A releasing card was hung in the middle level of the canopy in the center of each plot. Each plot consisted of 5 vine rows, releasing row and the 2 neighbouring rows from each direction (Fig. 4). Trap cards (the new device which were used in the survey study), were hung at 0,5m intervals in two directions from the releasing point, till 9 m, 7.5 m and 5 m in the releasing row, direct neighbour row (DNR) and in the secondary neighbour row (SNR) respectively. The trap cards were hung at the first height level of the canopy (80-120 cm). In order to monitor the vertical dispersal activity of *Trichogramma* in the canopy, trap cards were distributed at 3 various levels of the canopy (08-120, 120-150 and > 150 cm) only at the releasing row (Fig. 5). Three days later, the trap cards were recollected and replaced by the same number of trap cards with fresh *Sitotroga* eggs. The recollected trap cards were kept by 25±1 °C and 70-80 % RH and 5 days later the percentage of parasitism was determined. The experiment was repeated 3 times per *Trichogramma* species.

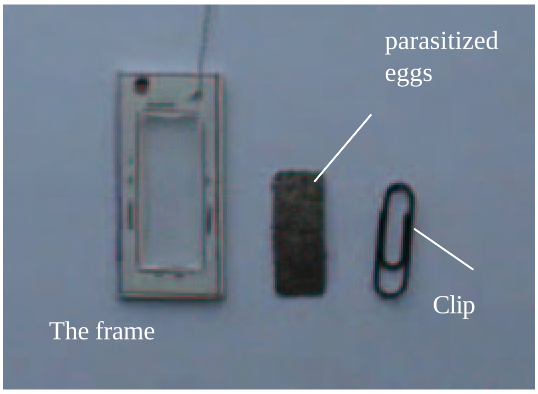

Fig. 3: The releasing card

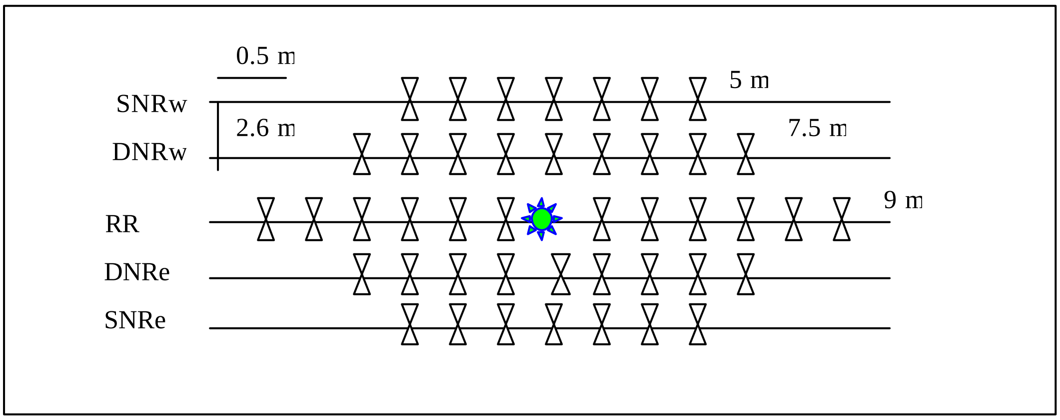

Fig. 4: An overview of the horizontal dispersal of the trap cards in the vineyard. (RR = releasing row, DNRw = direct neighbor rows (west), DNRe = direct neighbour rows (east), SNRw = secondary neighbour rows (west), SNRe = secondary neighbor rows (east), ⧗ = Trap cards ✹ = Release card

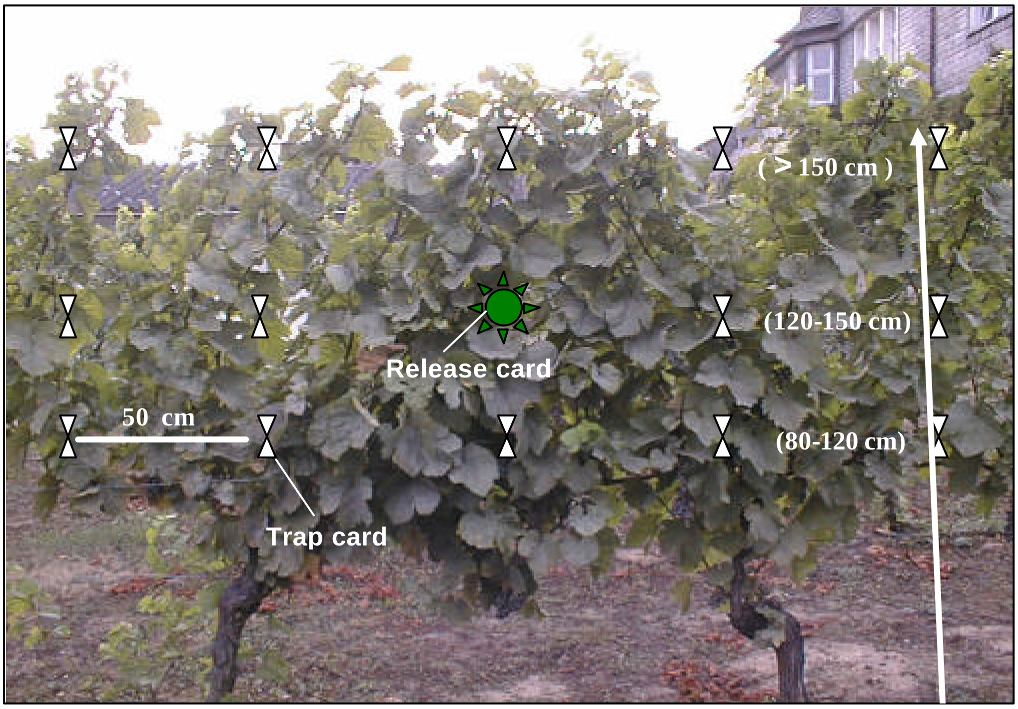

Fig. 5: Arrangement of the vertical dispersal of trap cards on the vine canopy.

3.5.4 Biological control of grape berry moths (GBM) by releasing *Trichogramma* spp. in vineyards

The field experiments were carried out in Fuchsberg site for two years (2002 and 2003). In 2002, *Trichogramma* species/strains were released to control only the second generation of the GBM. Whereas, in 2003 releases took place during both the second and the third generation. In 2002, these tests were conducted by releasing *T. cacoeciae* (Cac-com) (commercial strain), *T. evanescens* (Eva-01) (vineyard strain 2001) and *T. cacoeciae* (Cac-94) (vineyard strain 1994). In 2003, *T. cacoeciae* (Cac-com) (commercial strain), *T. evanescens* (Eva-com) (commercial strain) and *T. dendrolimi* (Den-com) (commercial strain) were used. In order to cover the whole flight period of the GBM, releasing dates of *Trichogramma* were chosen according to the flight period of the GBM. Two releases of each *Trichogramma* strain were used to correspond with the egg laying period of GBM. But only one release was used against the third generation of *Lobesia* in 2003. Release cards (AMW Nützlinge Company) each with ca. 3000 parasitized *Sitotroga* eggs were used (Fig. 6 a). Releasing cards were hung in the middle level of the canopy at a rate of one card each 5 m within the vine row of ca. 32 m length. The frst release of *Trichogramma* was carried out few days after trapping of GBM. Flight activity of GBM was monitored by means of pheromone traps, which were controlled 3 times weekly. The vineyard was divided into plots. Each *Trichogramma* species/strain was released in 3 adjacent vine rows (each row served as a replicate). Three vine rows were leaved as barrier (untreated) between the treatments. As control served the first 6 vine rows of the site. After 4 weeks from the last releasing date, 800 cluster/treatment were examined for tortricid damage. The same number of clusters were also examined in the control plot. Both infestation rate and efficacy rate* (infestation reduction) of each *Trichogramma* strain were determined.

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* Efficacy rate: according to ABBOTT 1925.

Emergence indicator tube

To determine the appropriate time (begin of hatching, hatching period and the total number of hatched parasitoids per releasing card) for these treatments, samples of the released parasites cards were kept in an emergence indicator under field conditions. The openings of two vials (9.5 long x 2.5 cm Ø) were connected together by para-film. About $^{3}/_{4}$ of the lower vial was covered with black paper. A releasing card was cut and put in the darkened tube (Fig. 6 c).
The emerged parasitoids moved up to the upper vial towards the source of light. From the beginning, the upper vial was changed and the parasitoids were counted daily. Under the vine leaf shadow, emergence indicator tubes were hung by a wire on vine branches in the same time with the releasing cards (Fig. 6 b). This method insured the continuous presence of *Trichogramma* in the release plots. Emergence period of each *Trichogramma* strain was monitored by three emergence indicator tubes.

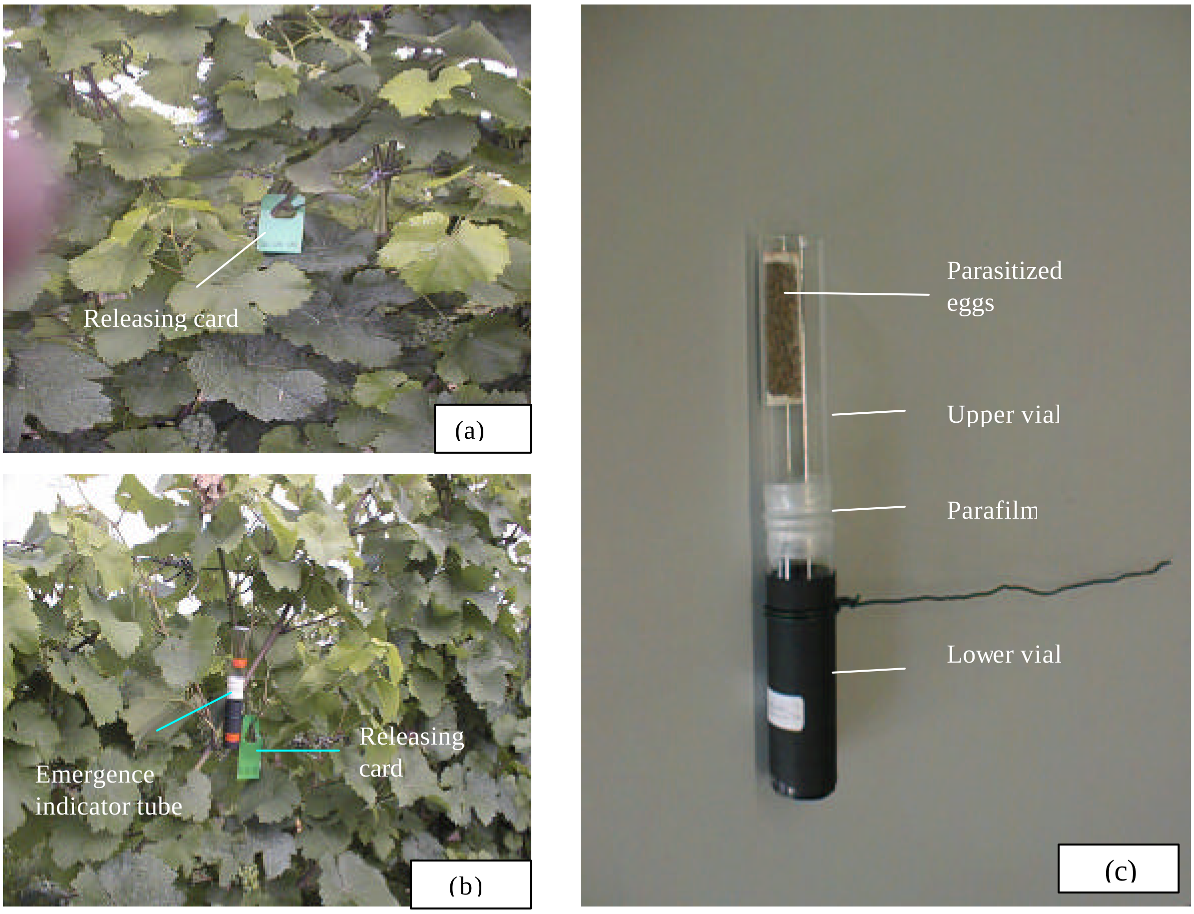


Fig. 6: Releasing card (a & b) and emergence indicator tube (c).

3.6 Statistical analysis

Statistical analysis was performed by using the statistical software *STATISTICA* V6.0 (StatSoft, 2001). After ANOVA multiple mean comparisons were made by the Tukey-HSD-Test ($P < 0.05$). Percentage data were arcsine transformed prior to analysis.

4 RESULTS

4.1 Laboratory experiments:

4.1.1 Host acceptance by *Trichogramma* species/strains

Figure1 shows the results of host acceptance of various *Trichogramma* strains, to parasitize eggs of grape berry moths (GBM) *E. ambiguella* and *L. botrana*. The results showed clearly that all *Trichogramma* species accepted GBM eggs as host, but they varied greatly in their egg laying capacity. The mean numbers of parasitized *Lobesia* eggs ranged from 17.3 to 43.2 for *T. piceum* (Pic) and *T. evanescens* (Eva-01), respectively. Whereas, in *Eupoecilia* eggs it ranged from 14.9 to 27.4 for *T. principium* (Pri) and *T. evanescens* (Eva-01), respectively (Fig. 1). Field *Trichogramma* species were more fecund and active than other laboratory species. Eggs of *L. botrana* were more attractive for almost all *Trichogramma* species/strains than those of *E. ambiguella*. Statistical analysis showed significant differences in the mean numbers of parasitized eggs per female between field species (Eva-01 and Cac-01) and other laboratory *Trichogramma* species.

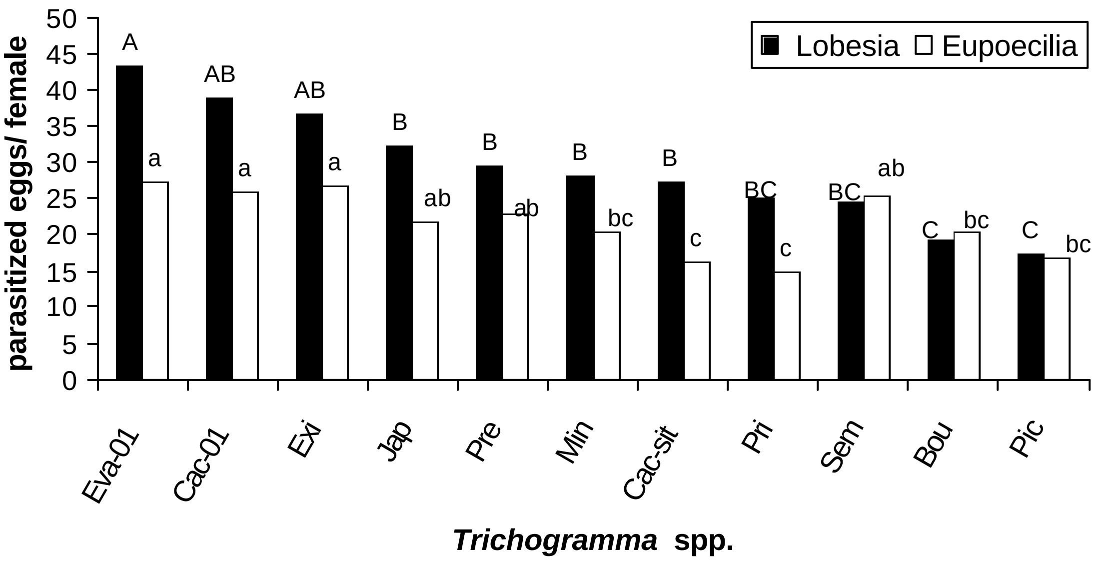


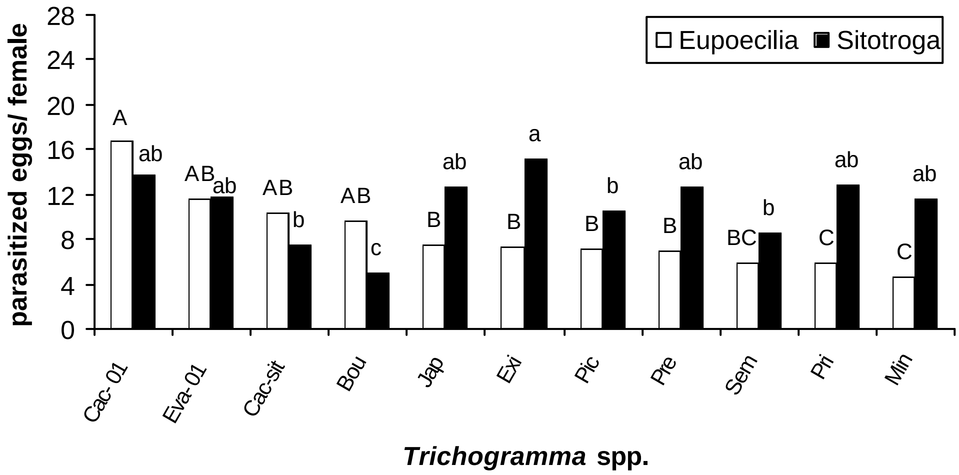
Fig. 1: Mean number of parasitized *L. botrana and E. ambiguella* eggs by 11 *Trichogramma* strains. Different letters (upper case for *Lobesia* and lower case for *Eupoecilia*) indicate significant differences (P< 0.05, Tukey, HSD-Test).

4.1.2 Host preference of *Trichogramma* species/strains

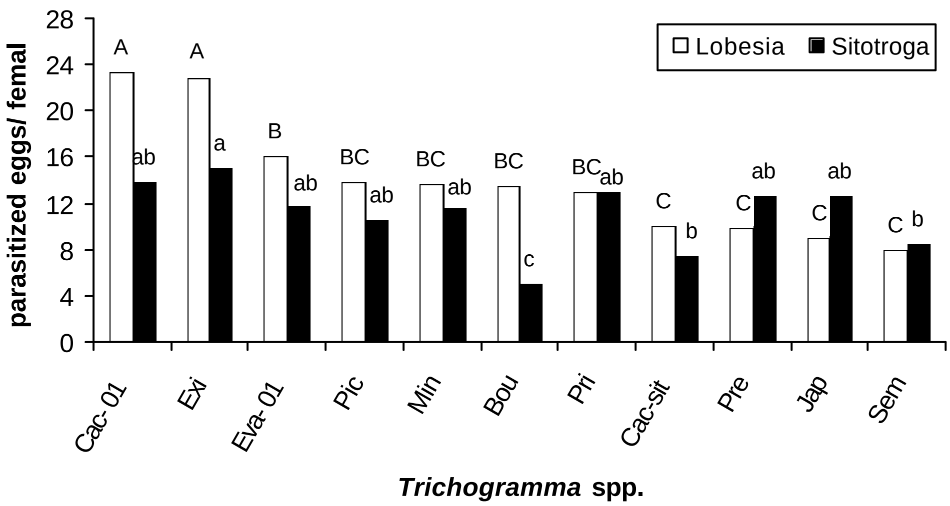
a) Parasitism

In figure 2 a-c, *Trichogramma* strains were arranged according to their decreasing preference for the parasitism of *Eupoecilia* and *Lobesia* eggs. Among the eleven species of *Trichogramma* tested, field species (Cac-01 and Eva-01) were more fecund and highly efficient than other laboratory reared species. *T. exiguum* was the most efficient candidate among all laboratory strains. *T. cacoeciae* (Cac-01), *T. cacoeciae* (Cac-sit) and *T. bourarachae* (Bou) preferred eggs of *Eupoecilia* rather than *Sitotroga* eggs. Mean numbers of parasitized eggs per female for these species (Cac-01, Cac-sit and Bou) were 16.8, 10.4 and 9.6 in *Eupoecilia* eggs, whereas in *Sitotroga* 13.8, 7.5 and 5 eggs per female, respectively. The remaining *Trichogramma* strains preferred *Sitotroga* eggs (Fig. 2 a). By contrast, almost *Trichogramma* species strongly preferred eggs of *L. botrana* compared to *Sitotroga* and *Eupoecilia* eggs (Fig. 2 b & c). The lower parasitization recorded for *T. semblidis* (Sem) 8.1, *T. minutum* (Min) 4.6 and *T. bourarachae* (Bou) 5.1 in *Lobesia, Eupoecilia* and *Sitotroga* eggs, respectively. The differences in parasitization between the 11 *Trichogramma* strains with various host eggs were significant.

(a)



(b)



(c)

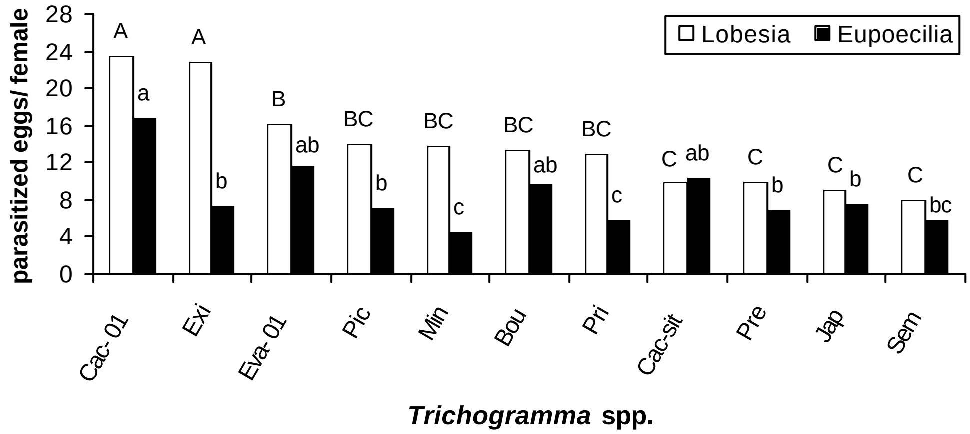


Fig. 2: Comparison of the preference of 11 *Trichogramma* spp. when offered simultaneously the choice among grape berry moths and Sitotroga eggs, a) *E. ambiguella* eggs vs. *Sitotroga* eggs, b) *L. botrana* eggs vs. *Sitotroga* eggs and c) *L*. *botrana* vs. *E. ambiguella* eggs*.* Different letters (upper case for *Lobesia* and lower case for *Eupoecilia* or *Sitotroga*) indicate significant differences (P< 0.05, Tukey HSD-Test).

b) Emergence rate

Emergence rates differed significantly among various *Trichogramma* strains (P < 0.05, Tukey HSD-test). The rate of emergence from *Sitotroga* eggs was significantly higher in comparison to *Eupoecilia* and *Lobesia* eggs. The rate of adult emergence from *Sitotroga* ranged from 77.7 to 97.4 % (Fig. 3 a & b). Whereas from *Lobesia* eggs it varied from 67 to 89.5 %, thereby it was significantly higher than in *Eupoecilia* eggs (Fig. 3 c). The rate of adult emergence from *Eupoecilia* eggs ranged from 52.4 to 86.5 % (Fig. 3 a & c).

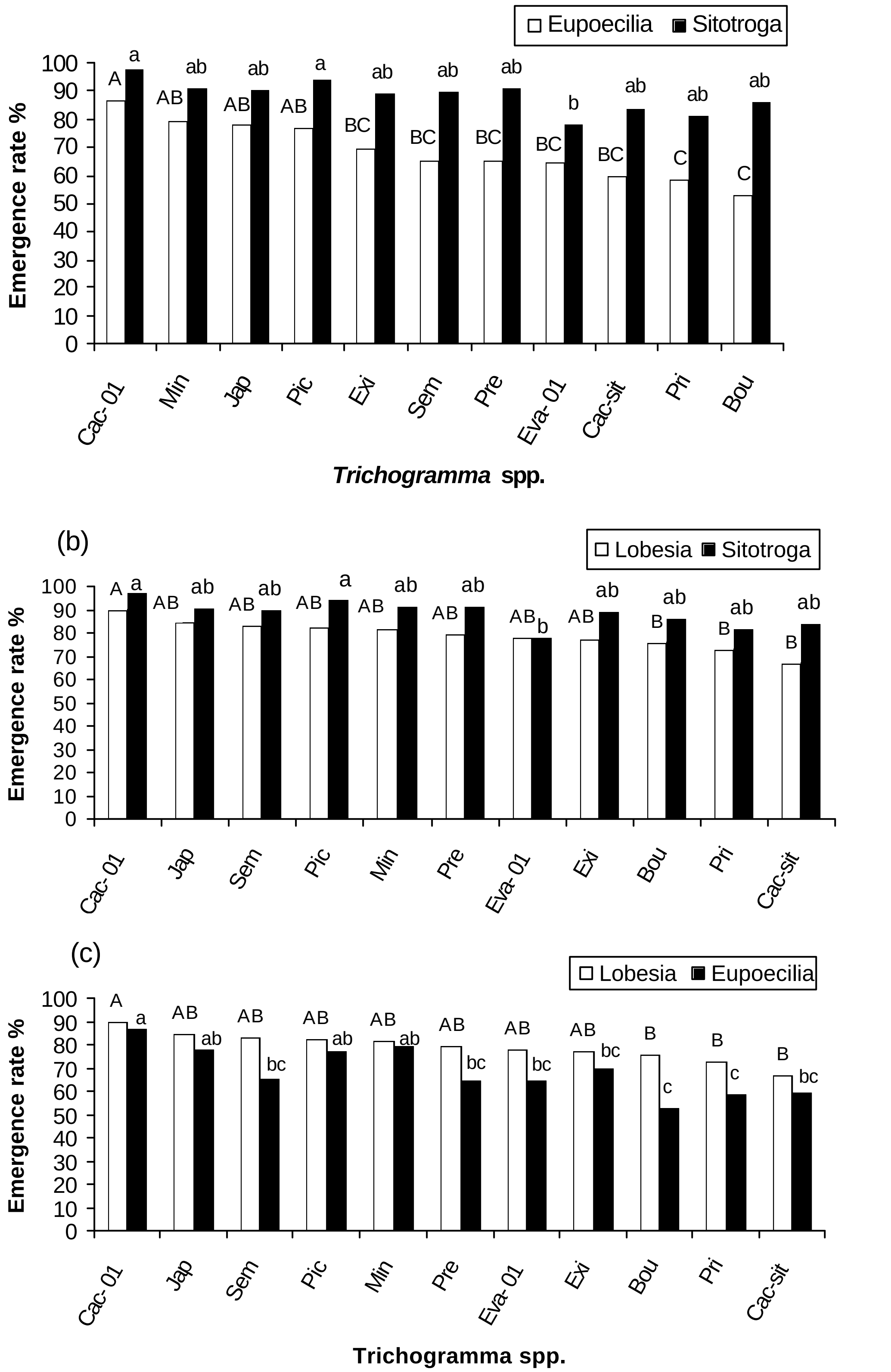


Fig. 3: Emergence rates of 11 *Trichogramma* spp. when offered simultaneously the choice among grape berry moths and *Sitotroga* eggs, a) *E. ambiguella* vs. *Sitotroga* eggs, b) *L. botrana* vs. *Sitotroga* eggs and c) *L. botrana* vs. *E. ambiguella* eggs. Different letters (upper case for *Lobesia* and lower case for *Eupoecilia* or *Sitotroga*) indicate significant differences (P< 0.05, Tukey, HSD-Test).

c) Sex ratio

Trichogramma cacoeciae was the only uniparental species. All other *Trichogramma* species were arrhenotokous. Except the thelytokous species, female portions were significantly higher (P <0.05, Tukey´s HSD) than males and ranged from 41.8 % (*T. minutum* in *Eupoecilia*) to 91.2% (*T. semblidis* in *Sitotroga*). Male portion was predominant only for *T. minutum* in *Eupoecilia* eggs and reached 58.2 % (Tab.1). The female portion of *T. minutum* was significantly lower than that of all other *Trichogramma* strains. Averages of female offspring were 76.5, 73.2 and 70.2% from the parasitized eggs of *Sitotroga*, *Lobesia* and *Eupoecilia*, respectively.

Tab.1: Sex ratio of 11 *Trichogramma* species/strains, when developed in grape berry moths and *Sitotroga* eggs (F: Female, M: Male).

Trichogramma Species/strain	abbrev.	*E. ambiguella* F : M	*L. botrana* F : M	*S. cerealella* F : M
T. cacoeciae-sit	Cac-sit	100 : 0	100 : 0	100 : 0
T.cacoeciae-01	Cac-01	100 : 0	100 : 0	100 : 0
T. principium	Pri	80.2 : 15.8	73.2 : 26.8	76.2 : 23.8
T.evanescens-01	Eva-01	72.9: 27.1	77: 23	60 : 40
T. minutum	Min	41.8 : 58.2	55.8 : 44.2	68 : 32
T. exiguum	Exi	74.8 : 25.2	62.4 : 37.6	77.5 : 22.5
T. piceum	Pic	71.2 : 28.8	74.1 : 25.9	82.1 : 17.9
T. pretiosum	Pre	54.4 : 45.6	60.5 : 39.5	72.2 : 27.8
T. japonicum	Jap	88.1 : 11.9	87.7 : 12.3	87.9 : 12.1
T. bourarachae	Bou	61.6 : 38.4	80.3 : 19.7	73.2 : 26.8
T. semblidis	Sem	86.6 : 13.4	87.4 : 12.6	91.2 : 8.8

4.1.3 Analysis of various life history parameters in the course of the life span of *Trichogramma* strains in grape berry moths

a) Generation time

Figure 4 shows mean generation time by days of various *Trichogramma* species/strains, which were developed in both *Eupoecilia* and *Lobesia* eggs. Generation time of *T. cacoeciae* was significantly longer than in all other

Trichogramma strains in both GBM and lasted 12.7 ± 0.71 and 13 ± 0.63 days (means ± SE) in *Eupoecilia* and *Lobesia* eggs, respectively (Fig. 4). There were no significant differences between eggs of both GBM on the generation time of various *Trichogramma* strains. The lowest generation time was recorded for *T. exiguum* and varied from 7.6 ± 0.47 to 8 ± 0.32 days in *Eupoecilia* and *Lobesia,* respectively.

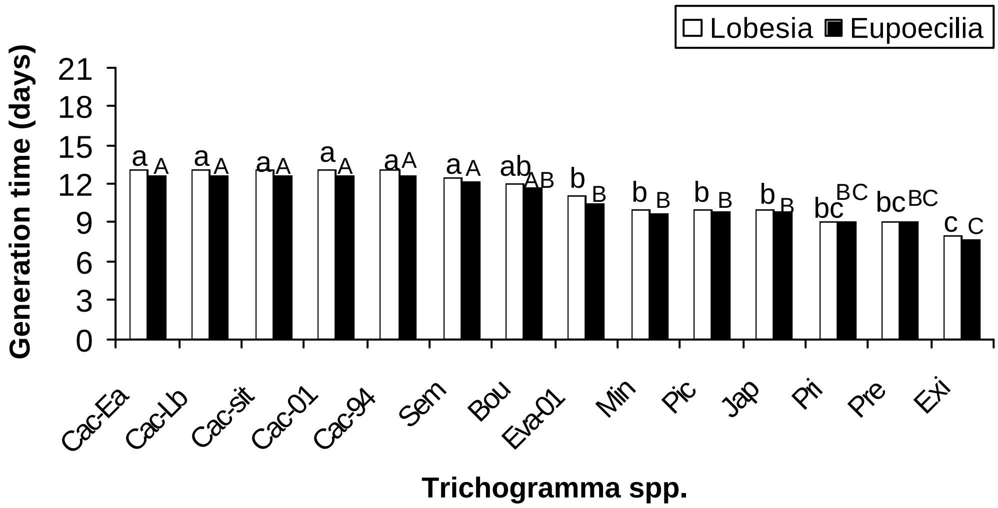


Fig. 4: Mean generation time of various *Trichogramma* species/strains when developed in both grape berry moths' eggs. Different letters (upper case for *Lobesia* and lower case for *Eupoecilia*) indicate significant differences (P < 0.05, Tukey HSD-test).

b) Longevity

The longevity of the various *Trichogramma* strains differed significantly depending on the rearing host. Longevity of *Trichogramma* varied significantly from 5.3 ±1.1 to 20.4 ± 0.6 and from 7.5 ± 0.9 to 27.1 ± 0.43 days for females which were reared on *Eupoecilia* and *Lobesia* eggs, respectively (Fig. 5 a & b). The longevity was significantly longer for *Trichogramma* females reared on *Lobesia* than those ones reared on *Eupoecilia* eggs. The shortest longevity was for *T. piceum* (Pic) and recorded 5.3 and 7.5 days in *Eupoecilia* and *Lobesia* eggs, respectively. The highest longevity (27.1 days) was achieved by *T. cacoeciae* (Cac-Ea) in *Lobesia* eggs.

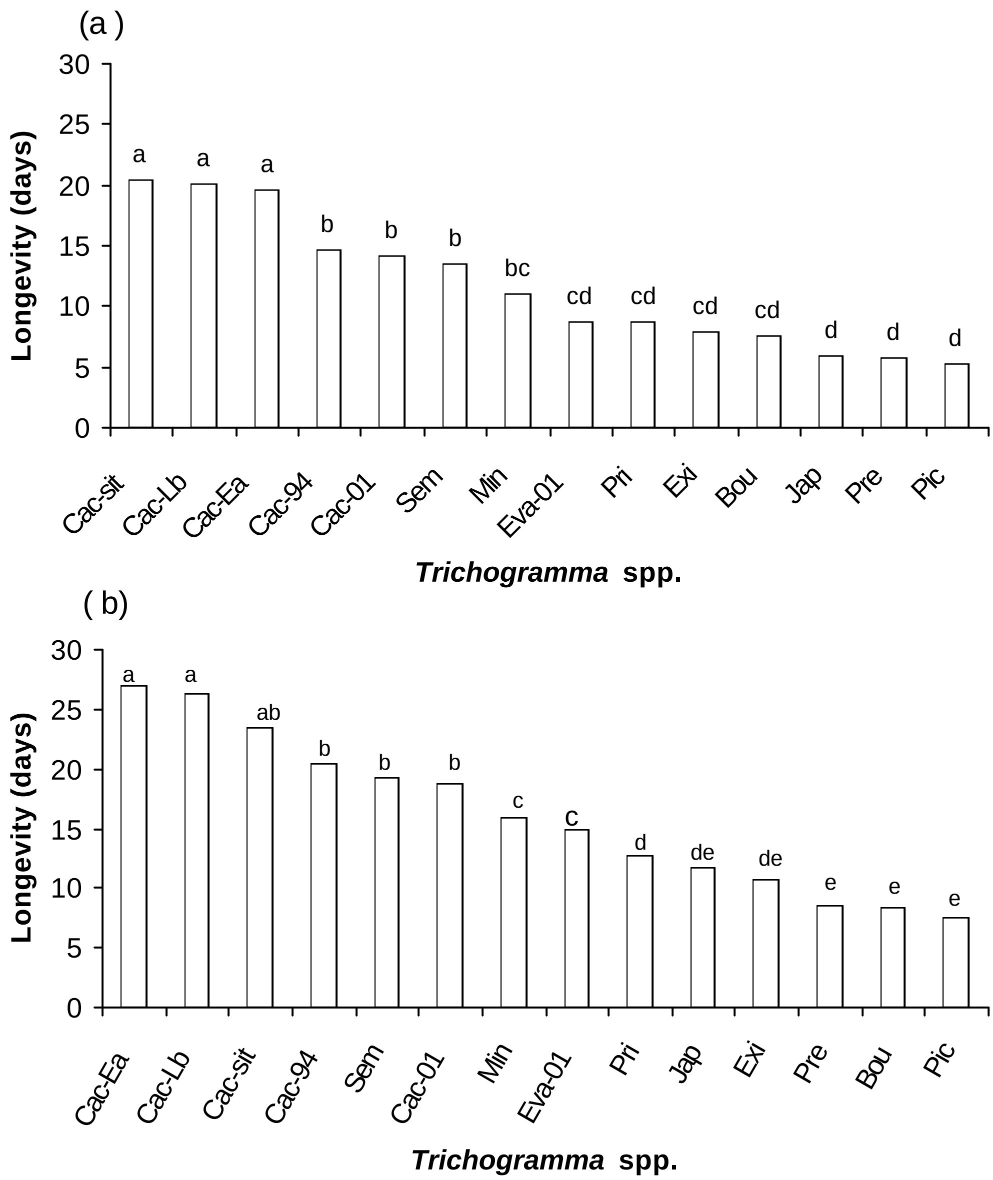


Fig. 5: Longevity of the tested females of various *Trichogramma* species/strains, (a) in *Eupoecilia* eggs, (b) in *Lobesia* eggs. Different letters indicate significant differences ($P < 0.05$, Tukey, HSD-test).

c) Parasitism potential

The parasitism potential (mean number of parasitized eggs per female during its life span) of the tested *Trichogramma* females was significantly higher in *Lobesia* eggs than *Eupoecilia* (Fig. 6 a & b). Parasitism potential of various *T. cacoeciae* strains ranged from 59.9 ± 1.6 (Cac-Lb) to 64.6 ± 0.7 (Cac-94) and 85.6 ± 5.2 (Cac-Lb) to 104.2 ± 3.6 (Cac-01) eggs per female (Mean ± SE, Tukey´s, HSD), in *Eupoecilia* and *Lobesia* eggs, respectively. The parasitism potential of other *Trichogramma* females differed from 25.1 ± 3.8 (*T. piceum*) to 49.5 ± 5.7 (*T. minutum)* eggs per female in *Eupoecilia* eggs (Fig. 6 a). In contrast, in *Lobesia* eggs parasitism of other *Trichogramma* females varied between 20.8 ± 6.1 (*T. bourarachae*) to 81.4 ± 3.1 (*T. evanescens* Eva-01) (Fig. 6 b). Thereby, females of the various *T. cacoeciae* strains had the highest parasitism potential.

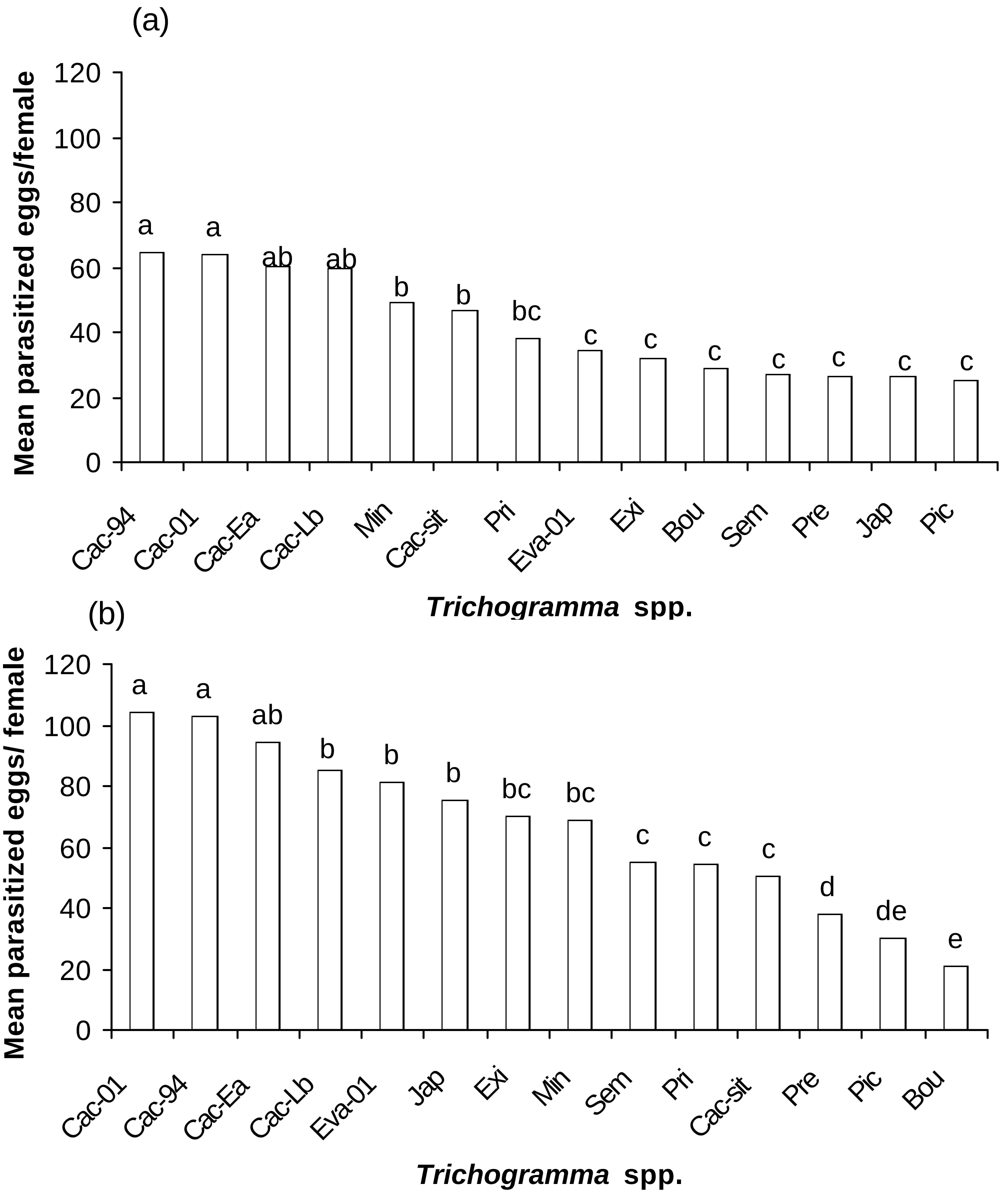


Fig. 6: Parasitism potential in the course of life span of the tested females of various *Trichogramma* species/strains, (a) in *Eupoecilia* eggs, (b) in *Lobesia* eggs. Different letters indicate significant differences (P <0.05, Tukey, HSD-test).

d) Reproduction potential and female offspring/ Female

The reproduction potential (mean number of total progeny (?+?) per female in the course of the life span) of females of various *T. cacoeciae* strains was significantly higher in *Lobesia* eggs than that in *Eupoecilia* (Fig. 7 a & b). In *Eupoecilia* eggs, reproduction rate of females of *T. cacoeciae* (Cac-01) was the highest (43.3) (Fig. 7 a), whereas it was the lowest for *T. exiguum* (Exi)(13.5). The reproductive potential of various *Trichogramma* strains reared on *Lobesia* eggs varied significantly between 13.3 and 74.6 individual per female for

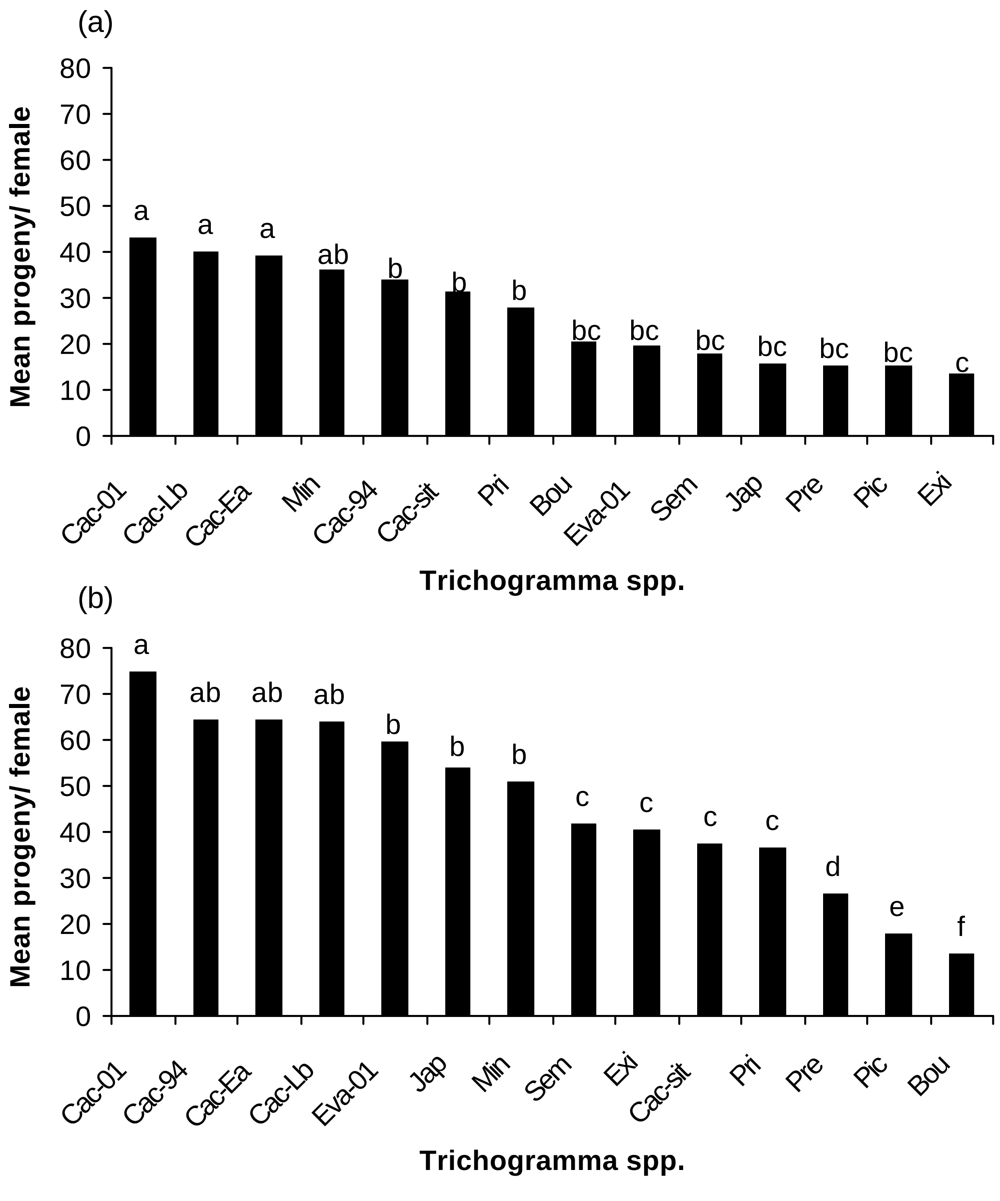


Fig. 7: Reproduction potential of the tested females of various *Trichogramma* species/strains in the course of life span, (a) in *Eupoecilia* eggs, (b) in *Lobesia* eggs. Different letters indicate significant differences (P < 0.05, Tukey, HSD-test).

T. bourarachae (Bou) and *T. cacoeciae* (Cac-01), respectively. As expected, offspring of the uniparental *T. cacoeciae* strains produced 100% females (Fig. 8 a & b). Reproduction of daughter offspring of various *Trichogramma* females reared in *Eupoecilia* eggs ranged from 4.6 to 43.3 daughters per female for *T. exiguum* (Exi) and *T. cacoeciae* (Cac-01), respectively (Fig. 8 a). However, in *Lobesia* eggs it ranged from 9.1 to 74.6 daughters per female for both *T. bourarachae* (Bou) and *T. cacoeciae* (Cac-01), respectively (Fig. 8 b).

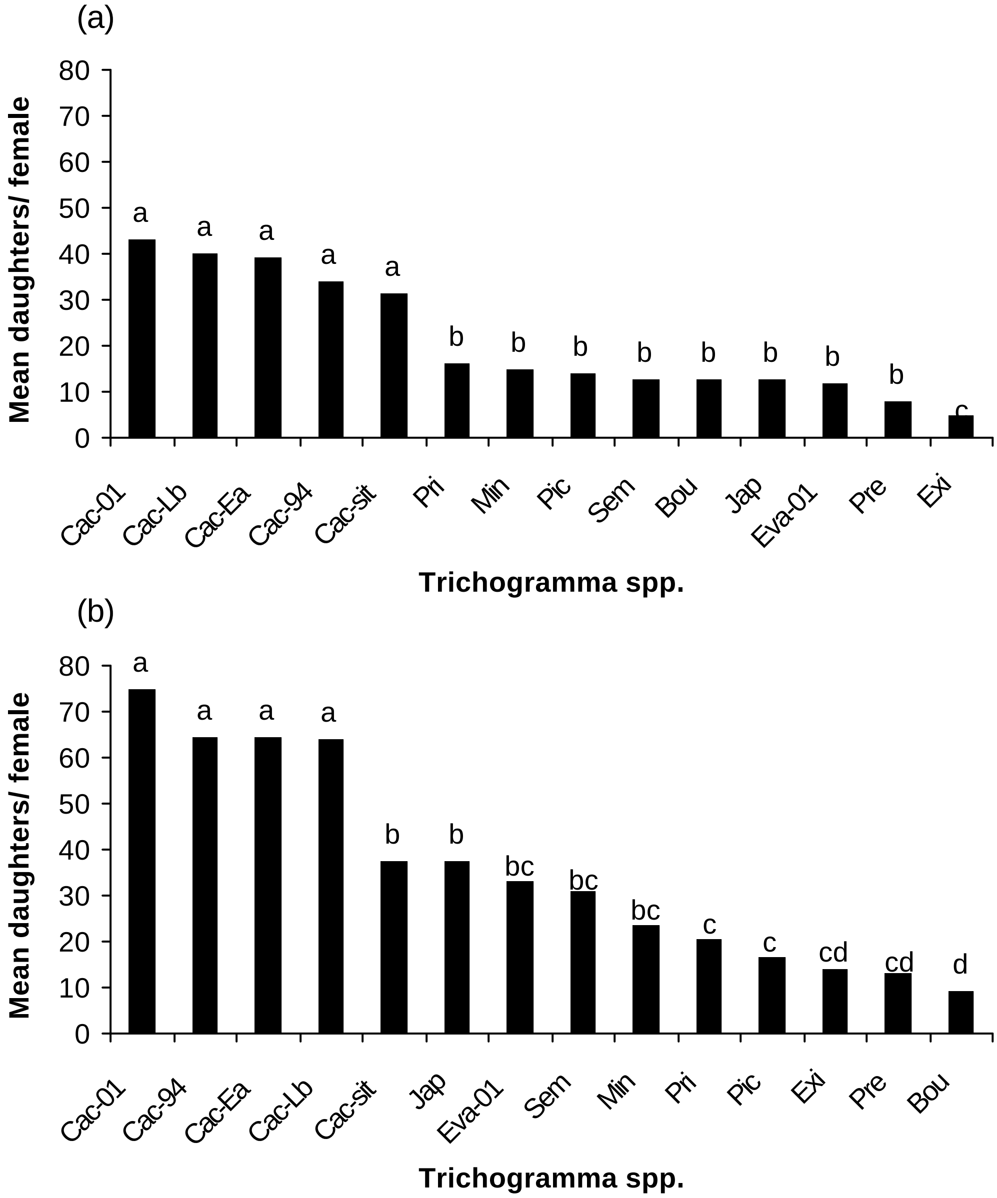


Fig. 8: Daughters offspring of the tested females of various *Trichogramma* species/strains in the course of life span, (a) in *Eupoecilia* eggs, (b) in *Lobesia* eggs. Different letters indicate significant differences (P < 0.05, Tukey, HSD-test).

4.1.4 Conclusions of the laboratory experiments

It must be stated here that *Lobesia botrana* proved to be the better host for most of *Trichogramma* species/strains tested than *Eupoecilia ambiguella*. This concerns host acceptance and preference, prevalence of females in the offspring, longevity and parasitism potential. Considerable differences were observed among *Trichogramma* species/strains, concerning their suitability as biological control agents of GBM. Newly collected *Trichogramma* strains from the field were more effective against *E. ambiguella* and *L. botrana* than strains reared in the laboratory for a long time.

4.2 Field Experiments

4.2.1 Survey of *Trichogramma* in the vineyards and surrounding biotopes

a) Natural occurrence of *Trichogramma* and flying activities of grape berry moths (GBM)

Sand site

The flight activities of GBM in 2001 showed peaks, from 10 to 27 May and form 17 May to 7 June during the first generation, from 12 to 23 July and from 15 to 27 July during the second generation for *Eupoecilia* and *Lobesia*, respectively (Fig.9 a). A third generation was observed for *Lobesia*. Whereas in 2002, flights of both GBM began earlier than in 2001, without a third generation for *Lobesia* (Fig.10 a). In the same year, *Trichogramma* showed three peaks of activity (Fig.9 b). At the beginning of the season, parasitism activity of *Trichogramma* was higher in hedge strips (23.7%) than in vine (15.8 %). The second activity period of *Trichogramma* was detected during the second generation of GBM, whereupon parasitism activity was higher in vine (11.8%) than in hedges (7.8%). A third peak of activity was detected only in hedges, from end August till September (Fig.9 b). In 2002, *Trichogramma* was detected earlier than in 2001 and showed also three peaks of activity (Fig.10 b). In the vineyard, parasitism activity ranged from 0.9% to 11.7%, whereas in hedge was varied between 0.8% to 6.3%. At both the flight of first and second generation of tortorcids, *Trichogramma* occurred more often in the vineyard than in hedges. Parasitism was detected in hedge strips one week earlier (on 2 April) than in vine (on 9 April). In both years, the dynamics of parasitism in the vineyard was corresponding with the flight activity of the GBM.

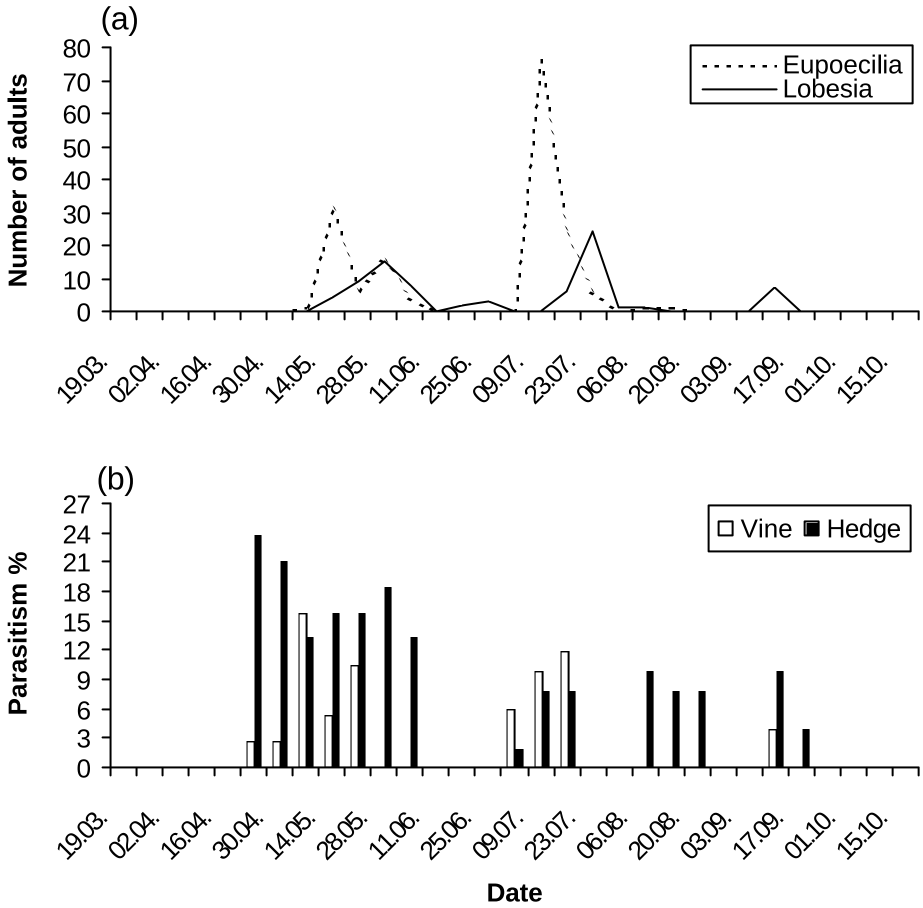


Fig. 9: Flights of grape berry moths (a) and parasitism activity of *Trichogramma* in the vineyard and surrounding biotopes (b) (Sand, 2001).

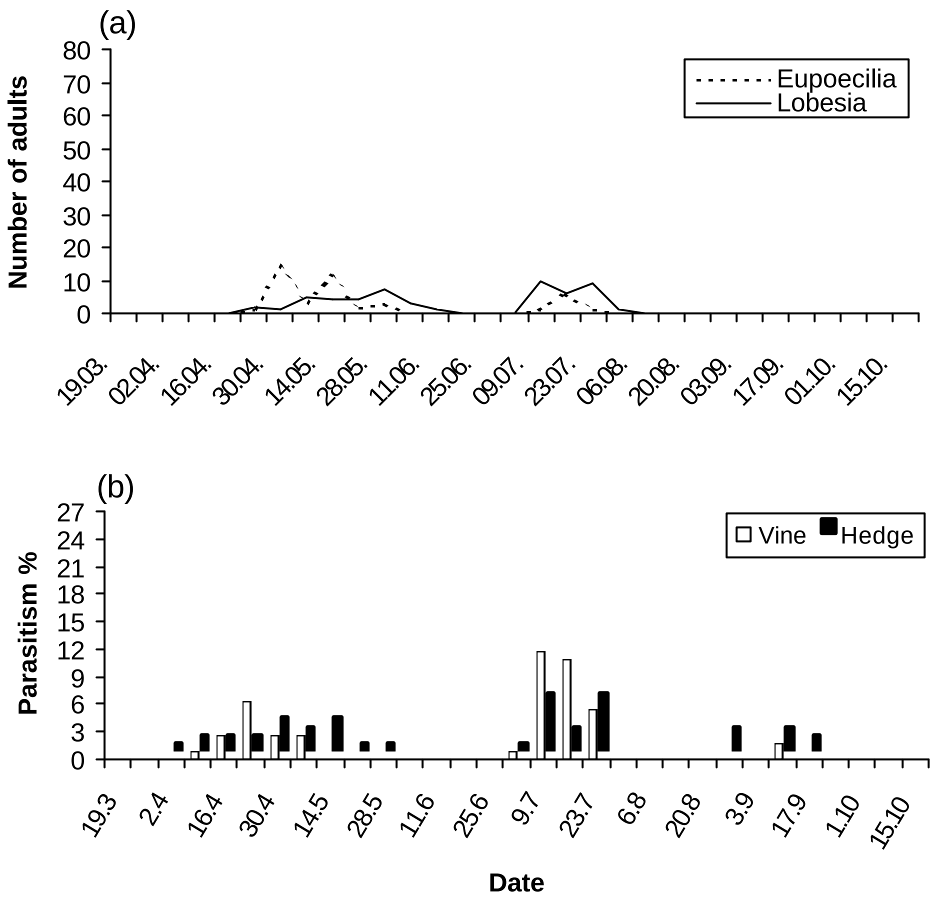


Fig. 10: Flights of grape berry moths (a) and parasitism activity of *Trichogramma* in the vineyard and surrounding biotopes (b) (Sand, 2002).

Berg Rottland site

During first and second generation of GBM, flight activities of *Lobesia* were higher than that of *Eupoecilia,* in 2002 (Fig.11 a). A third generation was observed for *Lobesia*. Population dynamics of *Trichogramma* was synchronized with the flight periods of both GBM. During flights of both GBM, *Trichogramma* was significantly more active in the vineyard than in the hedge (Fig.11 b). Parasitism activity during the first period varied between 1.6 to 19.8 % und 0.9 to 7.4 % for vineyard and hedge, respectively. However, the highest parasitism (22.3%) was during the second activity period of *Trichogramma* in the vineyard. At this site, *Trichogramma* showed also a third activity peak and parasitism at hedge was higher (4.1 %) than that in vineyard (1.6 %) (Fig.11 b).

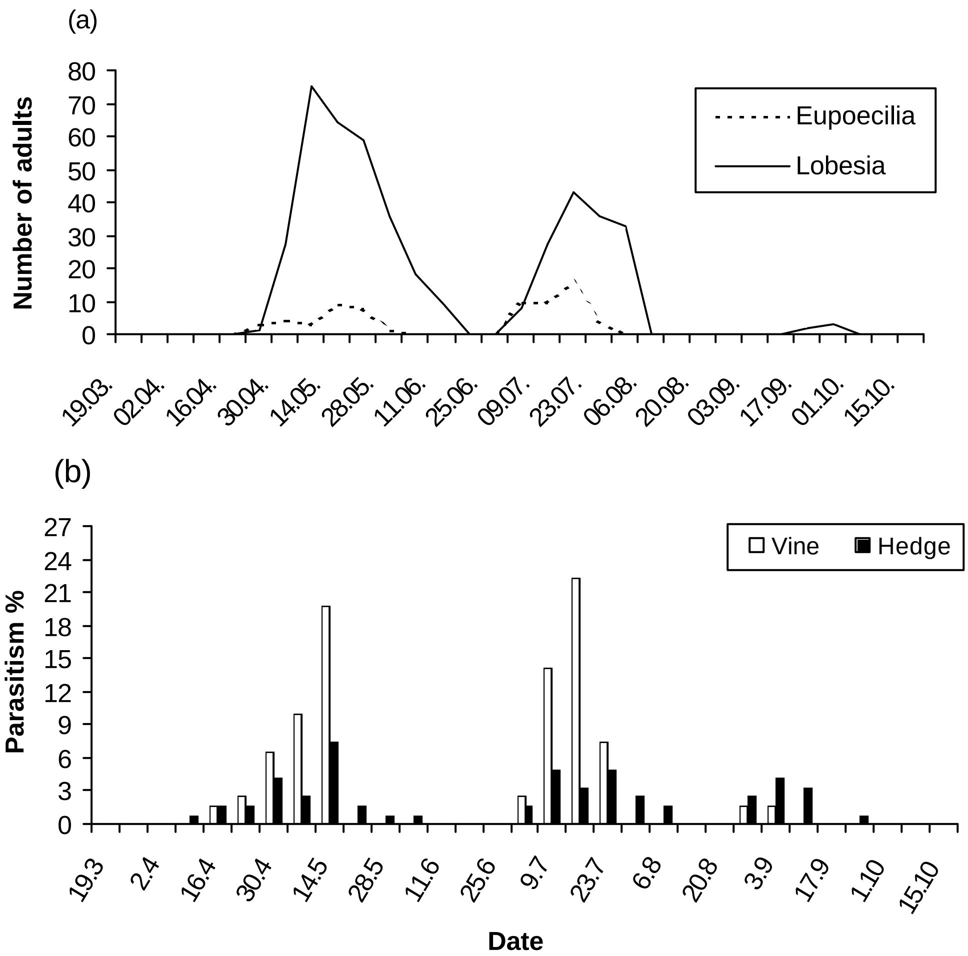


Fig. 11: Flights of grape berry moths (a) parasitism activity of *Trichogramma* in the vineyard and surrounding biotopes (b) (Berg Rottland, 2002).

Fuchsberg site

The Fuchsberg location was not surrounded by hedges. Flight dynamics of *Eupoecilia* was higher than that of *Lobesia*. However, a third generation was observed for *Lobesia* (Fig. 12 a). Only two parasitism peaks of *Trichogramma* were detected which ranged from 3.2 to 11.4 % and from 1.9 to 7.1% during the first and second activity periods of *Trichogramma*, respectively (Fig. 12 b). These activity periods corresponded with the flights of both GBM. Thereby, parasitism potential of *Trichogramma* at this site was significantly lower than that those in Sand and Berg Rottland sites. In addition, there was no a third activity peak for *Trichogramma*.

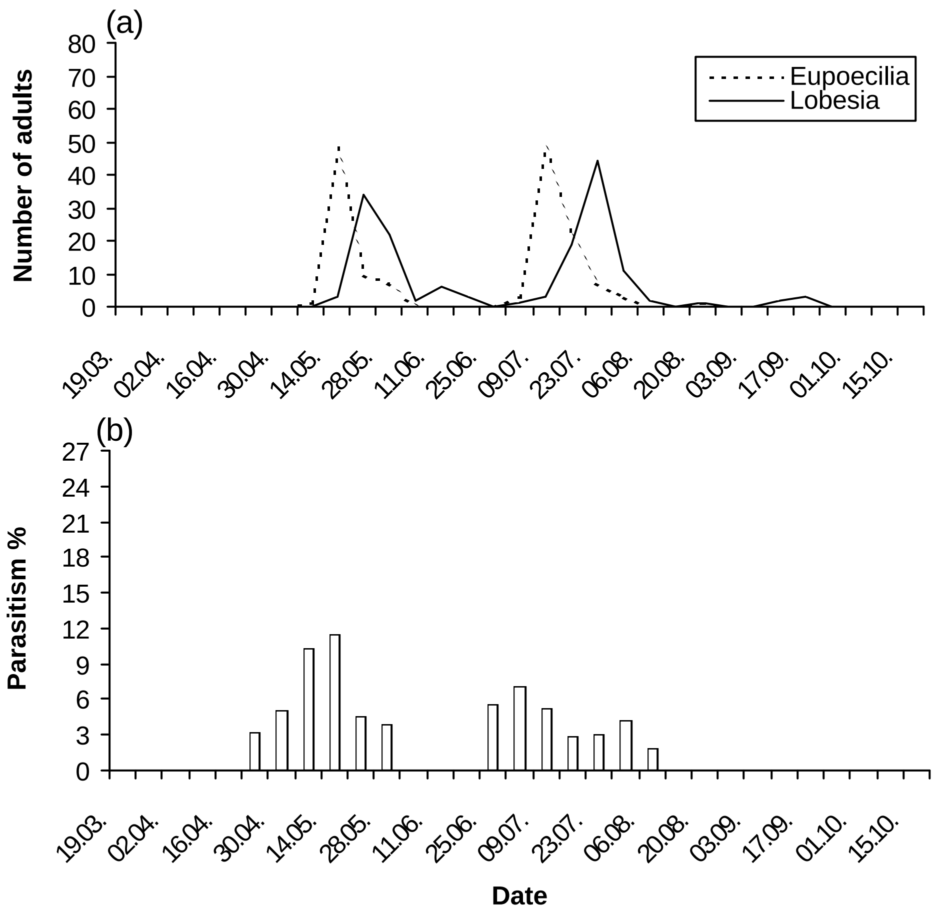


Fig. 12: Flights of grape berry moths (a), parasitism activity of *Trichogramma* in the vineyard (b) (Fuchsberg, 2001).

Mäuerchen site and Glass houses

The flight patterns of both GBM in 2001 (Fig. 13 a) showed peaks during the first generation, from 10 to 31 May, from 17 May to 14 June for *Eupoecilia* and *Lobesia*, respectively. The flights of second generation began at 5 July to 16 August and at 12 July to 16 August, for *Eupoecilia* and *Lobesia*, respectively. A third generation of *Lobesia* was detected during September. Whereas in 2002, the flights of both GBM were started one week earlier than in 2001 and without a third generation for *Lobesia* (Fig.13 b). On the other hand, the flight densities of the GBM were lower in 2002 than in 2001. The site Mäuerchen was ecologically managed and in the midst of a large pure vineyards area. Also, there were no hedge plants around it. During the 2001 and 2002 survey, there were no *Trichogramma* detected. Also, there were no *Trichogramma* in the glasshouses.

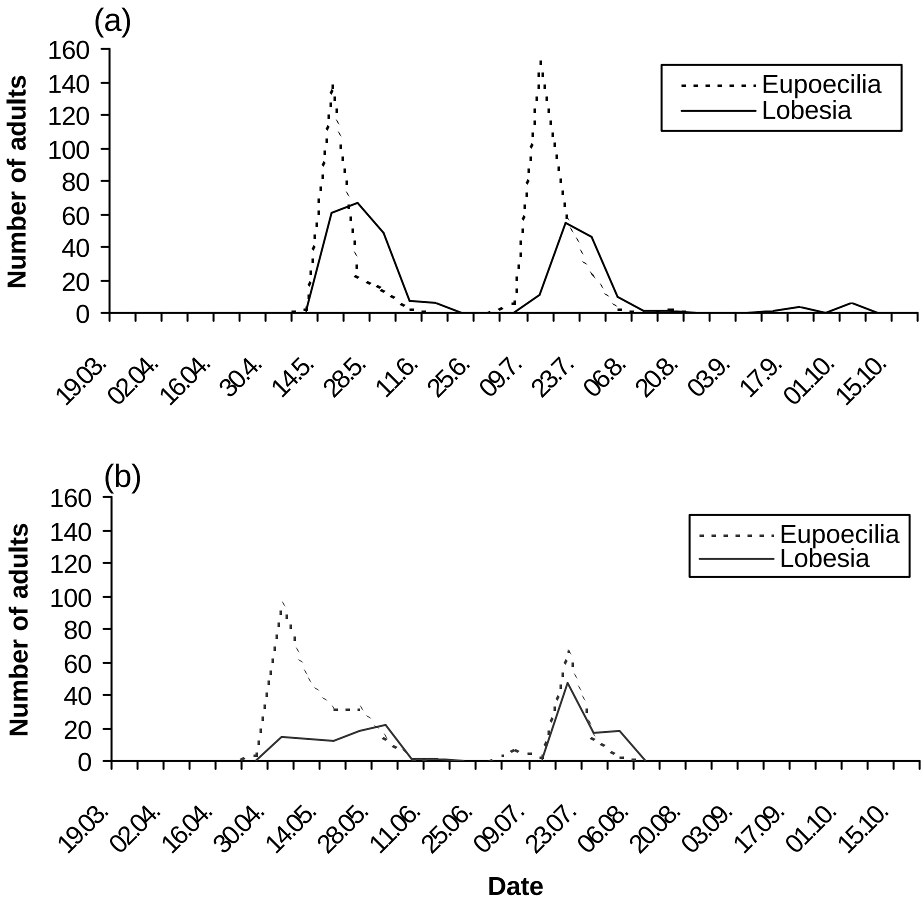


Fig. 13: Flight activities of grape berry moths in Mäuerchen; (a) in 2001, (b) in 2002.

b) Distribution of *Trichogramma* within vineyard rows

Distribution patterns of *Trichogramma* within rows of the vineyards which were surrounded by hedge strips (Sand & Berg Rottland) were similar and are shown in Figure 14 a & b. In both vineyards, parasitism activity in the edge rows was significantly higher than that in middle rows, and decreased gradually in the center vine rows (Fig. 14 a & b). Whereas, in Fuchsberg the distribution pattern of *Trichogramma* was different from those of both Sand and Berg Rottland

(Fig.14 c). Thereby, the results pointed clearly to the influence of hedge strips on the population dynamics and parasitism activities of *Trichogramma* in the neighbouring vineyards.

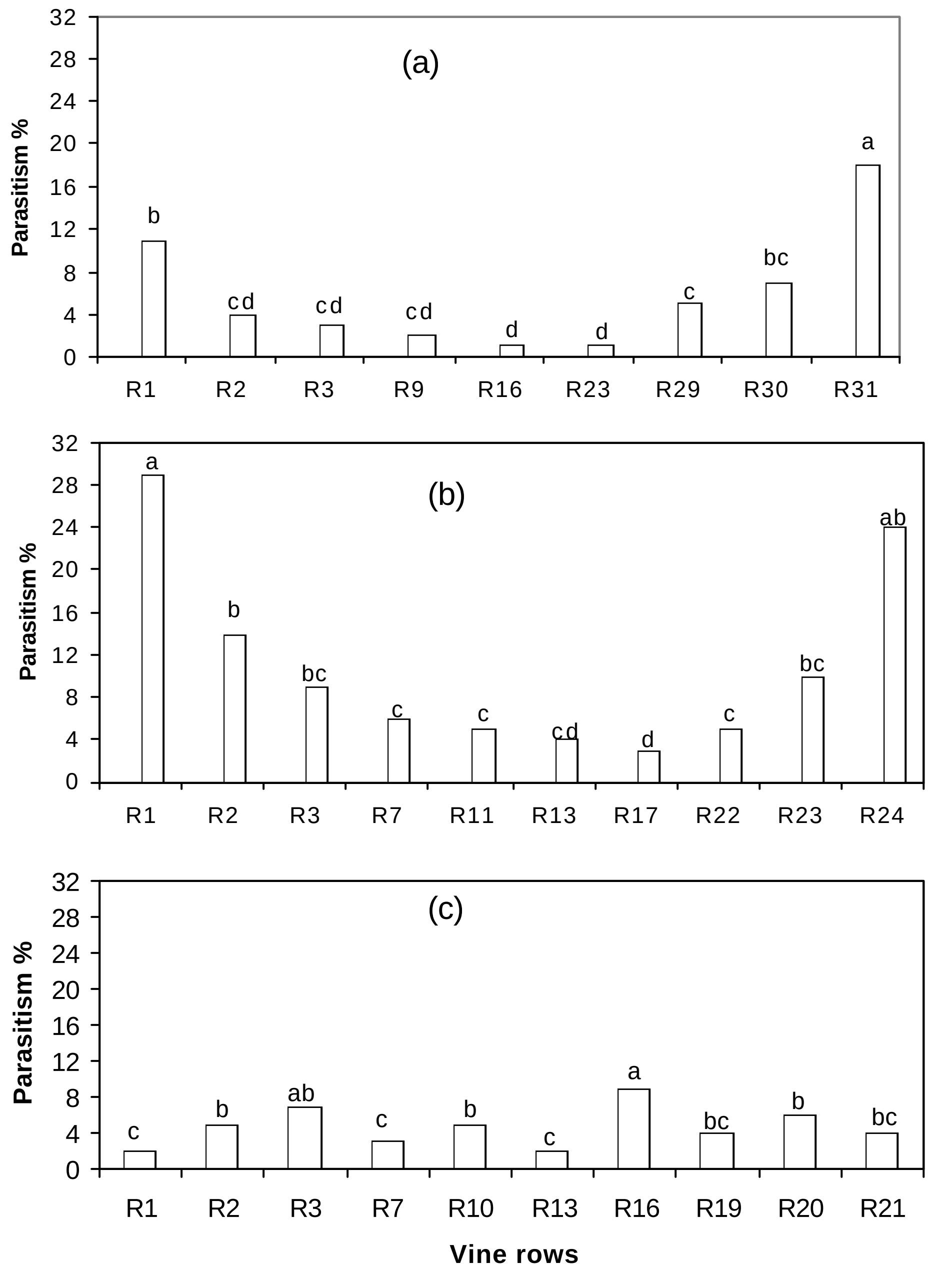


Fig.14: Distribution of *Trichogramma* among rows inside the vineyard (R= row), (a) Sand; (b) Berg Rottland and (c) Fuchsberg. Different letters indicate significant differences (P < 0.05, Tukey, HSD-test).

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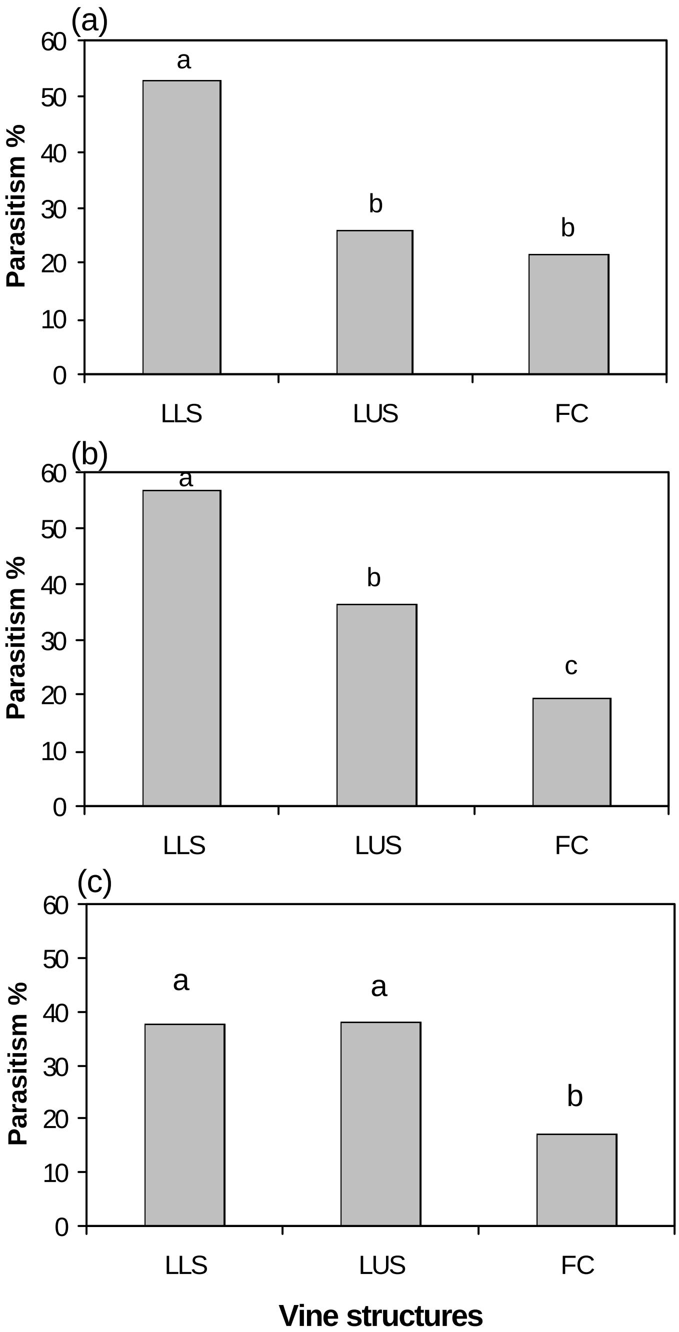
c) Distribution of *Trichogramma* on various vine structures and on canopy height levels

At all localities, *Trichogramma* showed a significantly higher activity on leaf lower side (LLS) of the vine than on both leaf upper side (LUS) and flower cluster (FC) (Fig.15 a, b and c). The lowest parasitization activity (16.9 %, at Fuchsberg) was recorded on the flower clusters. The dynamics of parasitism in various canopy height levels for the different vineyards are presented in Figure 16 a, b and c. Parasitism activity of *Trichogramma* was highest at 80 – 120 cm for all localities and significantly varied between 72.5 %, 64.2 % and 63.9 % for Sand, Berg Rottland and Fuchsberg, respectively. Only a few cards with parasitized eggs were detected above 150 cm for all localities. Thereby, the lowest activity zone of *Trichogramma* was recorded above 150 cm and reached 0 %, 2.9% and 6.4% for Fuchsberg, Sand and Berg Rottland respectively. Whereas, at 120 –150 cm parasitism activity varied from 23.5% to 36.1 %.



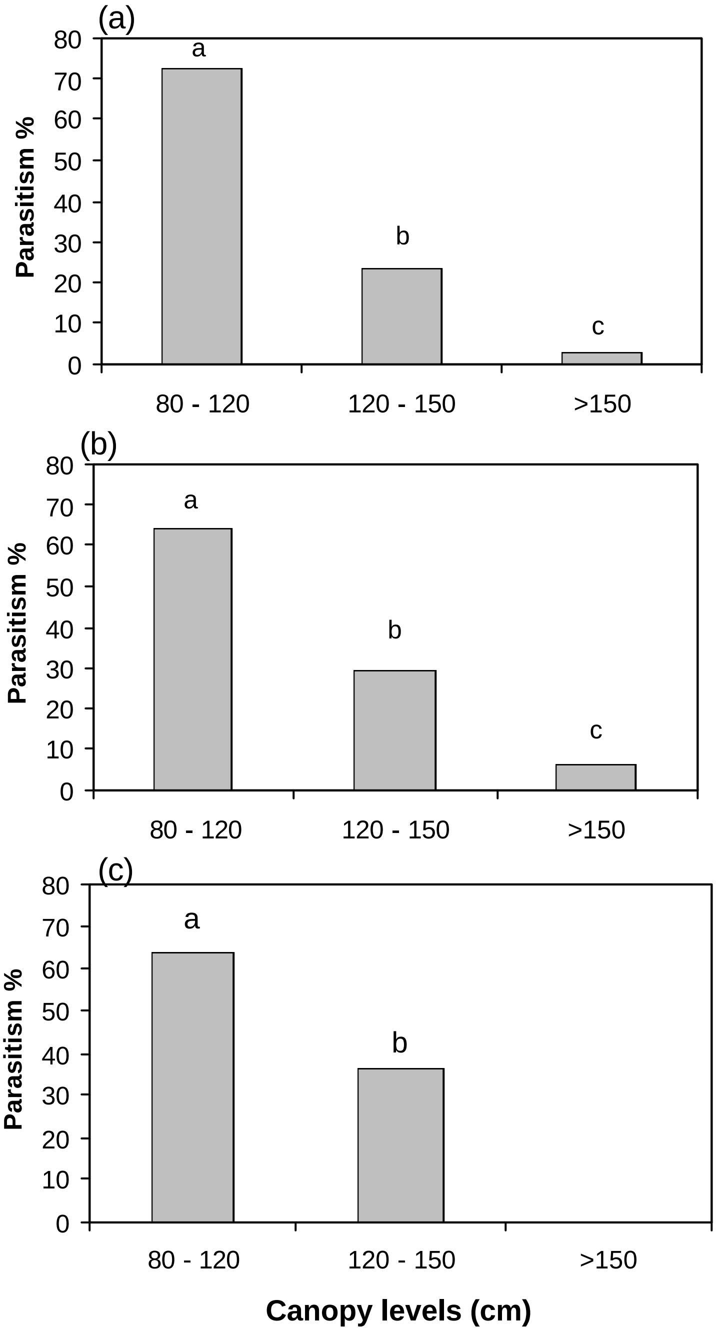


Fig. 15: Distribution of *Trichogramma* on various vine structures (a) Sand, (b) Berg Rottland and (c) Fuchsberg. Different letters indicate significant differences. LLS: leaf lower side, LUS: leaf upper side and FC: flower cluster.

Fig.16: Vertical distribution of *Trichogramma* on various canopy height levels; (a) Sand, (b) Berg Rottland and (c) Fuchsberg. Different letters indicate significant differences ($P < 0.05$, Tukey, HSD-test).

d) Spectrum and fluctuation of natural *Trichogramma* spp.

Only two *Trichogramma* species were detected in all vineyard localities and the surrounded hedges. The species which were found were *T. cacoeciae** and *T. evanescens**. However, the population size of *T. cacoeciae was* significantly higher in all localities than of *T. evanescens* (Fig. 17 a, b and c). The highest population size of *Trichogramma* was detected in Berg Rottland and Sand (Fig.17 a & b) then followed by Fuchsberg (Fig.17 c). Three activity periods of *Trichogramma* were recorded in both Sand and Berg Rottland, whereas only two were observed at Fuchsberg. *T. evanescens* was recorded earlier at both Sand and Berg Rottland than *T. cacoeciae* (Fig.17 a & b). Whereas, at Fuchsberg both *Trichogramma* species were recorded in the same time (Fig.17 c). On the other hand, *T. cacoeciae* occurred for a longer time and more often than *T. evanescens* in all localities.

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* The identification of *Trichogramma* was conducted by: both Dr. J. Monje, Institute of Phytomedicine 360,University of Hohenheim, Stuttgart, Germany; and Dr. A. Herz, BBA Darmstadt, Germany.

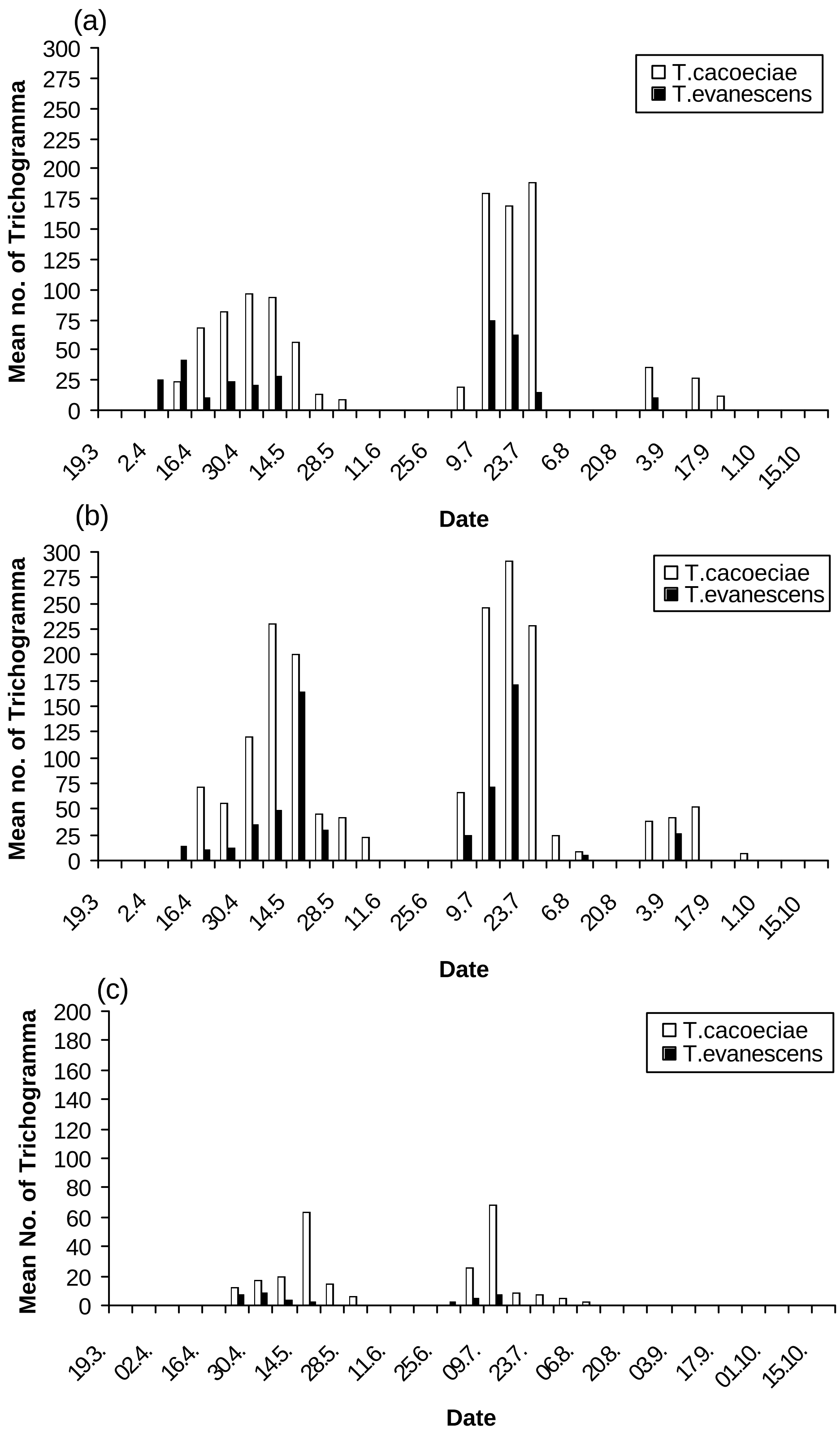

Fig.17: Population fluctuation of various *Trichogramma* species in vineyards, (a) Sand, (b) Berg Rottland and (c) Fuchsberg.

e) Analysis of hedge plants

Hedge plants were divided into three groups according to its location around the vineyard and the predominant plant species (see table 4). *Trichogramma* dynamics varied significantly among the various hedge plant combinations (Fig.18 a &b). The best results in baiting parasitoids have been obtained in hedge combinations group B (62.1 %) and group A (53.2 %) in Sand and Berg Rottland, respectively (Fig.18 a & b). Details of results showed that activities of *Trichogramma* were highest in both Sand and Berg Rottland where hedge plants were combinations of *Prunus* spp., *Rubus* spp. and *Ligustrum* vulgare. Parasitism activity of *Trichogramma* was lowest at group A (11.7%) and Group B (16.1%) for Sand and Berg Rottland, respectively.

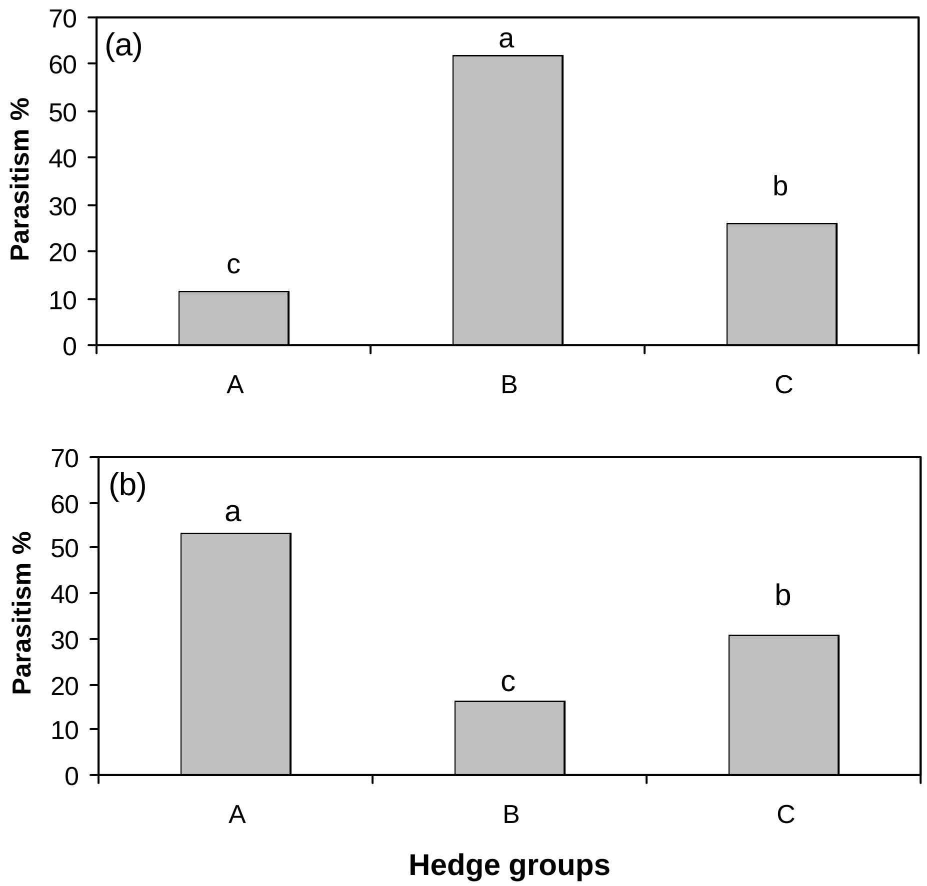

Fig.18: Parasitism activity of *Trichogramma* at various hedge plant combinations (a) Sand and (b) Berg Rottland. Different letters indicate significant differences (P < 0.05, Tukey, HSD-test).

4.2.2 Dispersal behavior of *Trichogramma* in the vineyards

a) Horizontal dispersal

Figure 19 a and b, shows the search capacity of both *T. cacoeciae* (Cac-01) and *T. evanescens (*Eva-01) during the first three days after release. Parasitism was significantly higher on the releasing row (RR) than on both direct neighbouring rows west (DNRw) and east (DNRe) for *T.cacoeciae* (Cac-01) and *T. evanescens* (Eva-01). Moreover, the longitudinal searching capacity of *T. cacoeciae* (Cac-01) and *T. evanescens* (Eva-01) was also significantly higher in the releasing row (reached 7 and 6 meters, respectively) than in neighbouring rows (reached 2 meter and one meter for *T. cacoeciae* and *T. evanescens*, respectively). For both *Trichogramma* species, parasitism activity declined gradually in the releasing row from the releasing point. There were no

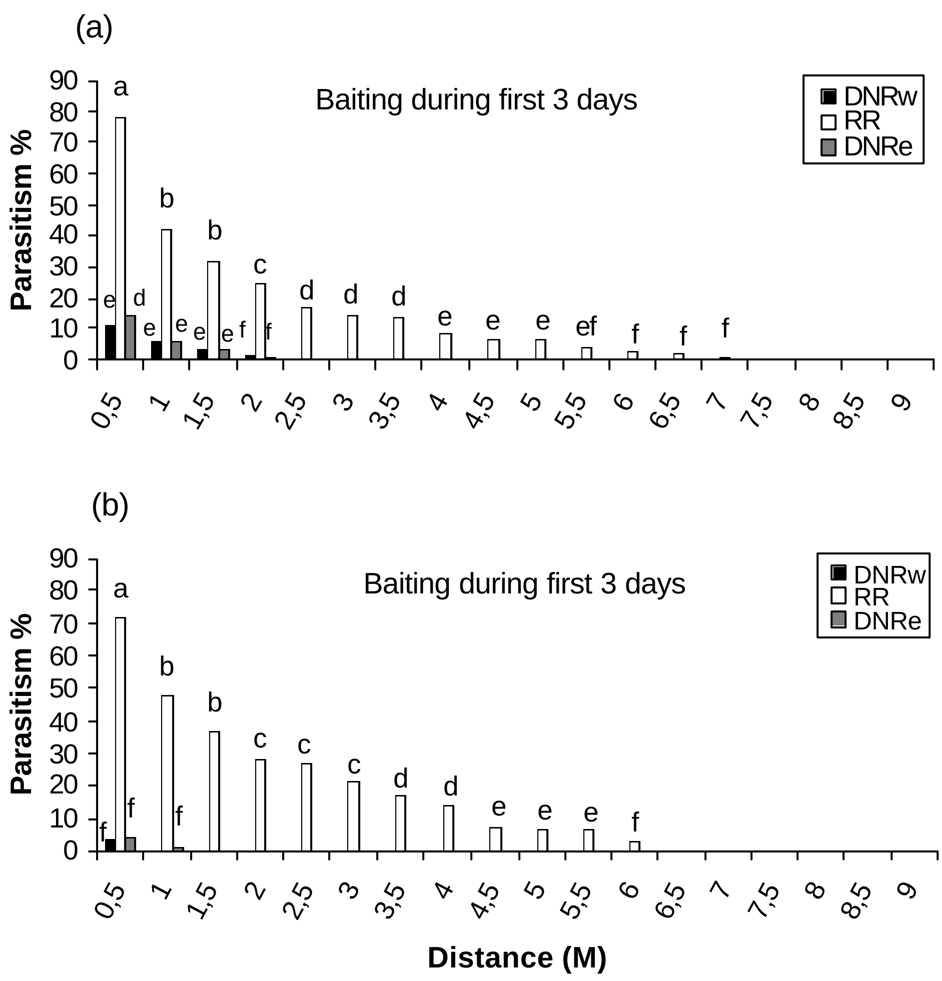

Fig. 19: Dispersal of *T.cacoeciae*-01 (a) and *T. evanescens*-01 (b) from the releasing point during the first 3 days after releasing. Different letters indicate significant differences ($P < 0.05$, Tukey, HSD-test).

significant differences of the parasitism between the two neighbouring vine rows (west and east). On the other hand, baiting activity during the second three days after release was significantly lower than that of the first baiting period. The longitudinal search capacity reached 6 and 5.5 m for *T. cacoeciae* (Cac-01) and *T. evanescens* (Eva-01), respectively (Fig. 20 a & b).

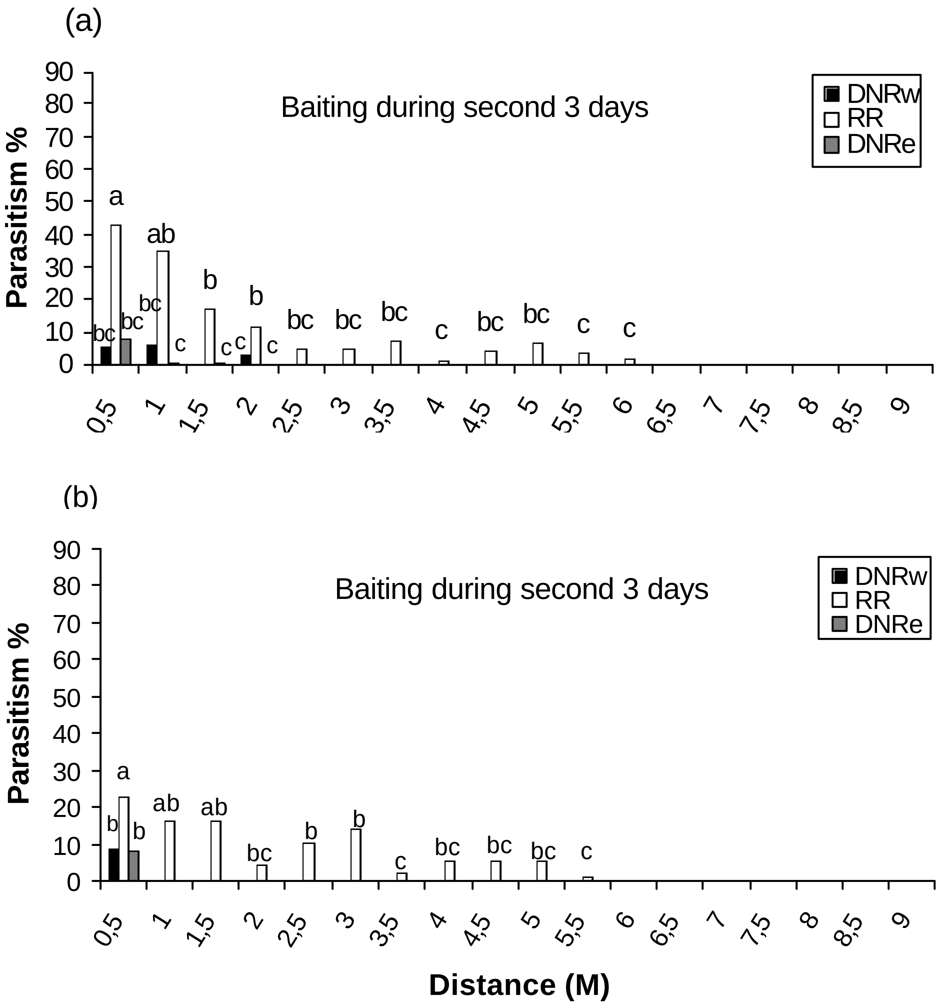

Fig. 20: Dispersal of *T. cacoeciae* (Cac-01) (a) and *T. evanescens* (Eva-01) (b) from the releasing point during the second 3 days after releasing. Different letters indicate significant differences ($P < 0.05$, Tukey, HSD-test).

b) Vertical dispersal

The lowest height level (80 - 102 cm) of the canopy was the most active zone for both *Trichogramma* species (Fig. 21 a & b). During the first baiting period, parasitism dynamics of *T. cacoeciae* differed significantly among canopy levels and reached 71%, 22.3 % and 6.7% for height levels 80 -120, 120 -150 and >150 cm, respectively. For the second baiting period it reached 84.1%, 12.6 % and 3.2% for the heights 80-120, 120-150 and >150 cm, respectively (Fig. 21 a). For *T. evanescens* there was no significance between the lowest (51.3%) and middle (43.2%) canopy levels during the first baiting period. However, baiting during the second period showed significant differences among height levels and reached 87.1%, 8.7% and 4.3% for 80 -120, 120 -150 and >150 cm respectively (Fig. 21 b). In general, the rate of parasitism in the lowest canopy level during the first baiting period was lower than that of the same level during second baiting period for *T. cacoeciae* and *T. evanescens.*

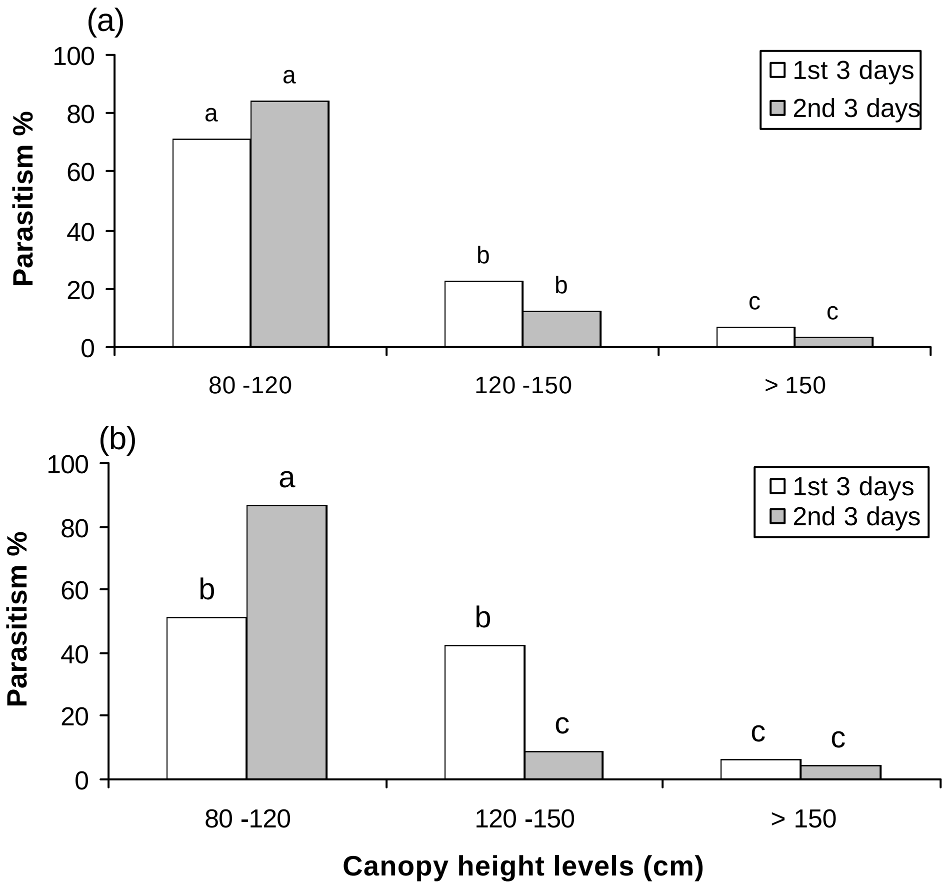

Fig. 21: Vertical dispersal of *T. cacoeciae* (Cac-01) (a) and *T. evanescens* (Eva-01) (b) in the vine canopy. Different letters indicate significant differences ($P < 0.05$, Tukey, HSD-test).

4.2.3 Biological control of grape berry moths by releasing *Trichogramma* spp. in the vineyards

In Fuchsberg site, *Trichogramma* releases were conducted to control the second generation of *E. ambiguella* and *L. botrana* in 2002 as well as the second and third generation in 2003 (Fig. 22 a & b). Infestation level by GBM was significantly higher in 2002 (16.2%) than that in 2003 (6.9%). Although, a third generation was recorded for *Lobesia* in 2003. In 2002 among the three treatments, the release of *T. cacoeciae* (Cac-94) showed the lowest damage level (2.7%) followed by *T. cacoeciae* (Cac-com) (3.6%) and *T. evanescens* (Eva-01) (4.1%) (Fig. 23 a). Thereby, *T. cacoeciae* (Cac-94) was the most efficient (infestation reduction: 83.3%) followed by *T. cacoeciae* (Cac-com) (77.6%) and *T. evanescens* (Eva-01) (74.5%) (Fig. 24 a). In 2003, infestation levels by GBM were recorded 1.8%, 2.7% and 1.3% for releases of *T. cacoeciae* (Cac-com), *T. evanescens* (Eva-com) and *T. dendrolimi* (Den-com), respectively (Fig. 23 b). *T. dendrolimi* (Den-com) showed the highest efficacy (infestation reduction: 81.9%). For *T. cacoeciae* (Cac-com) and *T. evanescens* (Eva-com), the pest infestation reductions were 73.3% and 62.8%, respectively (Fig. 24 b).

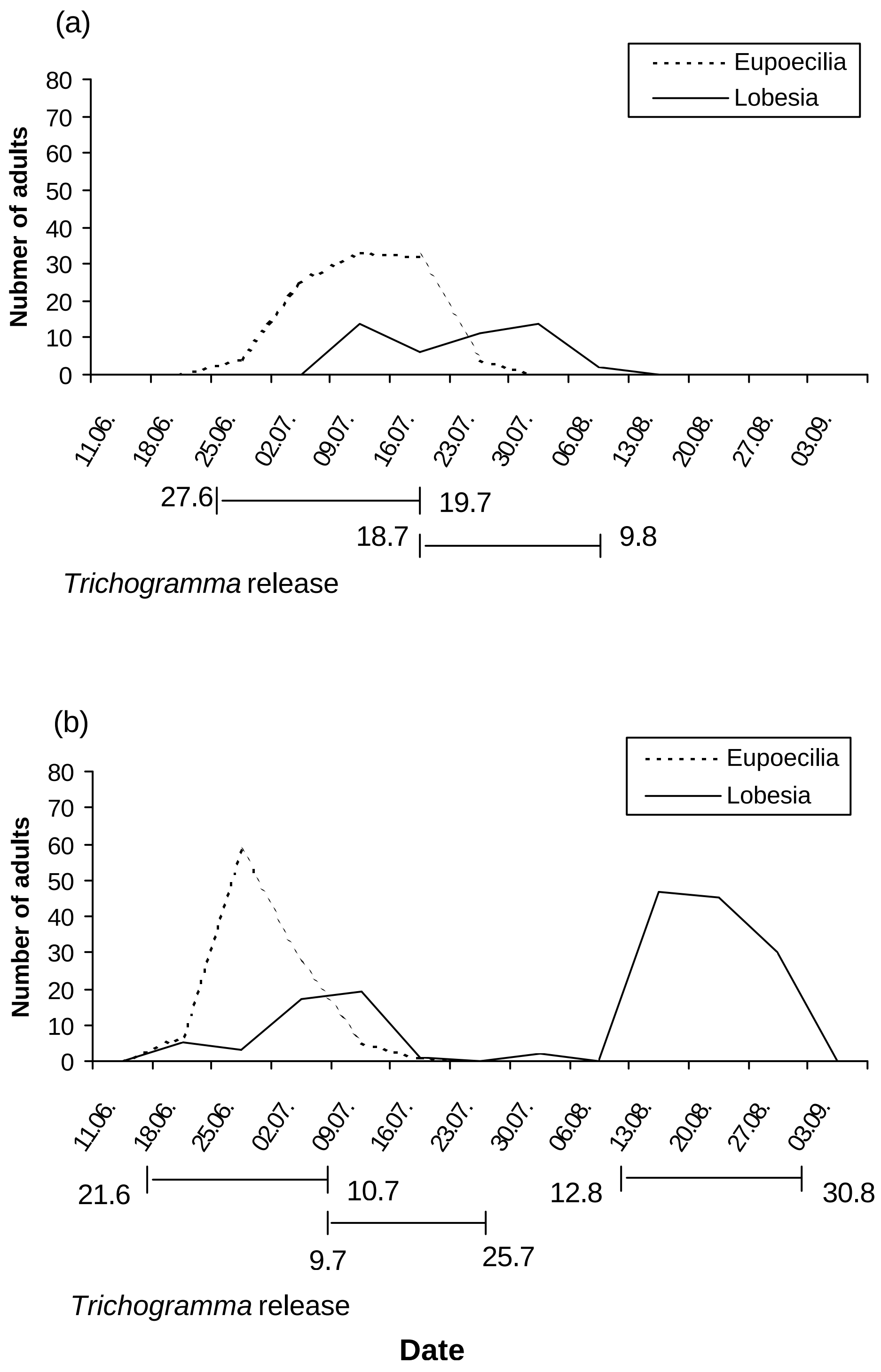

Fig. 22: Numbers of *E. ambiguella* and *L. botrana* captured in pheromone traps and releasing dates of *Trichogramma* in 2002 (a) and 2003 (b).

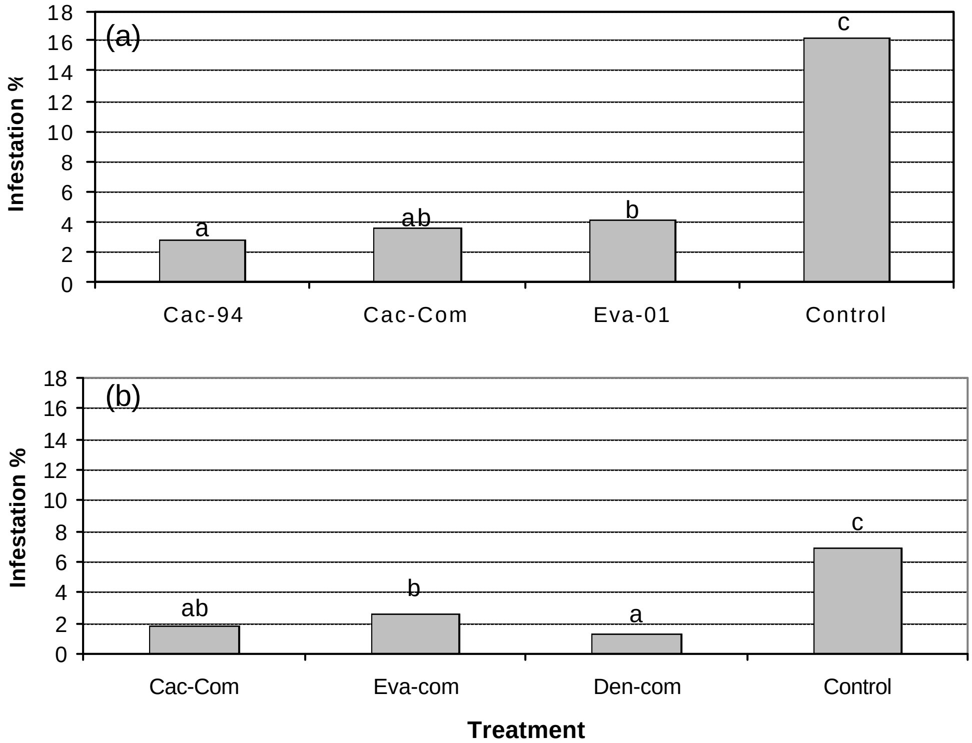

Fig.23: Infestation rates by grape berry moths, *E. ambiguella* and *L. botrana* and the released *Trichogramma* strains (a) in 2002 (b) in 2003. Different letters indicate significant differences (P < 0.05, Tukey, HSD-test).

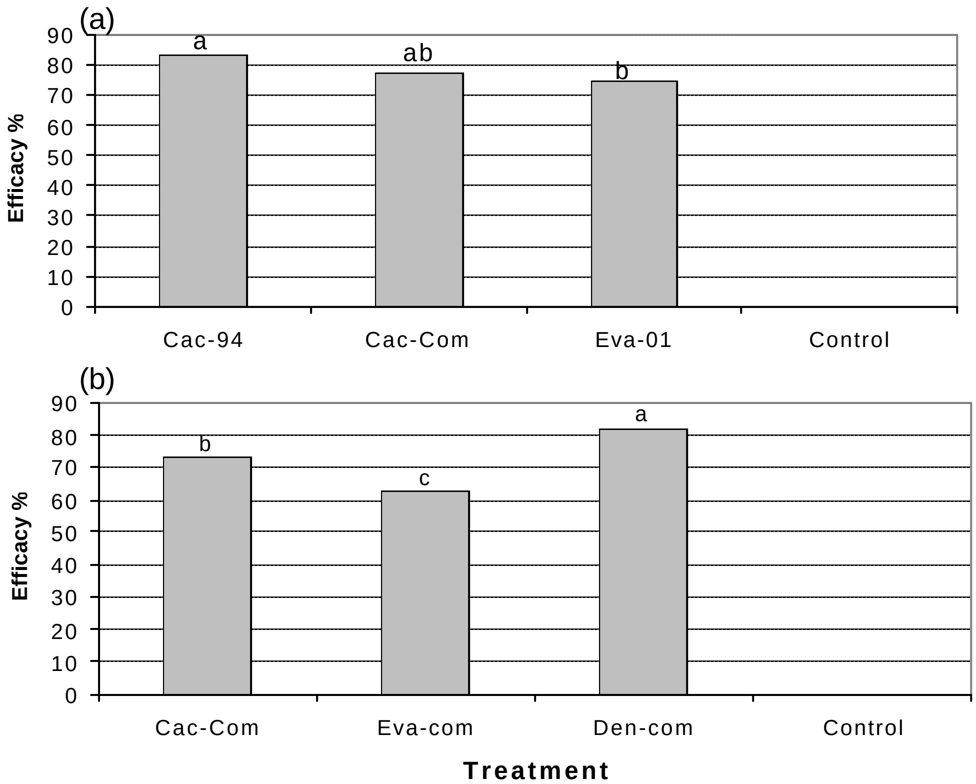

Fig.24: Efficacy of various releases of *Trichogramma* strains (a) in 2002 (b) in 2003. Different letters indicate significant differences (P < 0.05, Tukey, HSD-test).

4.2.4 Conclusions of the field studies

It could be shown that two *Trichogramma* species (*T. cacoeciae* and *T. evanescens*) do occur in the field in the Rheingau area. Their occurrence depends on the presence of hedges, especially this with *Prunus* spp., *Rubus* spp. and *Ligustrum vulgare*. There were no *Trichogramma* detected at an ecologically managed vineyard for two years survey.

So, a basic natural protection of grape against GBM is given. But the migratory potential of the very tiny *Trichogramma* spp. has been shown to be limited. So, additional measures are necessary. The searching capacity of *T. cacoeciae* (Cac-01) and *T. evanescens* (Eva-01) in the vineyard was determined. It was significantly higher in the releasing row (reached 7 and 6 meters, respectively) than in neighbouring rows (reached 2 meter and one meter for *T. cacoeciae* and *T. evanescens*, respectively). The inundative field release of commercial *Trichogramma*, timed according to the GBM flight, proved to be very efficient.

5 DISCUSSION

5.1 General

Biological control by using beneficial arthropods includes import (classical), augmentation, and conservation of beneficial organisms such as parasitoids and predators for the regulation of population densities of noxious organisms (WAAGE and GREATHEAD 1986, van DRIESCHE and BELLOWS 1996). Grape berry moths (GBM) *Eupoecilia ambiguella* Hb. and *Lobesia botrana* Schiff. are the most important pests of vineyards. They cause considerable losses in both quality and yield of the grapes (KAST 1990). As stated in the introduction already, the economic importance of GBM depends strongly on the developmental stage of the grapevine. Before and during flowering the larvae at first penetrate single flower buds and later on start to tie together several flower buds, building glomerules in which they stay and continue their feeding activities. In this stage (during the 1st generation) the tolerance level for grape berry moths infestation is relatively high and depends on the ability of the grape variety to compensate the damage (ROEHRICH and SCHMID 1979). Whereas, the second generation of the GBM is the most important whereby, considerable economic damage for grapevine can be recorded (CASTANEDA 1990, WÜHRER *et al.* 1995). Parasitic wasps are already well known since a long time as a limiting factor for the grape berry moths. However, little information exists about the efficiency grade of various *Trichogramma* spp. against the GBM. The successful use of egg parasitoids of the genus *Trichogramma* in biological control is greatly dependent on the suitability of the chosen *Trichogramma* species. Therefore, by means of useful promotion and selection of effective *Trichogramma spp.* there are promising species for controlling the GBM.

5.2 Laboratory Experiments

5.2.1 Host acceptance

The results demonstrated that the eggs of grape berry moths (GBM) *Eupoecilia ambiguella* and *Lobesia botrana* were accepted as hosts by females of all tested *Trichogramma* strains. However, they greatly varied in their acceptance grades for the two pests eggs. *Trichogramma evanescens* (Eva-

01), *T. cacoeciae* (Cac-01) and *T. exiguum* (Exi) showed a strong acceptance for both the GBM eggs over other *Trichogramma* strains. Eggs of *L. botrana* were higher accepted as host by almost all of *Trichogramma* strains tested than eggs of *E. ambiguella.* CASTANEDA *et al.* (1993) tested 4 *Trichogramma* strains against the GBM eggs and found that all of them had accepted *Lobesia* eggs (80.6%) at a higher rate than *Eupoecilia* eggs (67.6%). This is in agreement with our results. These differences in acceptance degrees of GBM eggs by *Trichogramma* might be related to differential quality and quantity of the nutritional components of the eggs of the two pests. For example, BIEVER (1972) found differences in the parasitism rates between individuals of *T. minutum* reared on two different host species, and rearing of *T. evanescens* on different hosts resulted in differences in acceptance rates as well (BOLDT 1974). SENGONCA *et al.* (1990) reported that only 14 out of 18 insect species offered were accepted and parasitized by *T. semblidis*. PAK (1988) found that acceptance of *Pieris brassicae* eggs varied among *Trichogramma* strains, whereas *Mamestra brassicae* eggs were readily accepted by all the strains tested. HASSAN and GUO (1991) reported that only 3 out of 20 strains tested against European corn borer accepted the corn borer eggs. However, host acceptance is not the only parameter, in order to determine the effectiveness of various *Trichogramma* strains. The actual mortality rate of the target host eggs is more important yet (THOMPSON 1928). WÜHRER and HASSAN (1993) reported that all of 47 *Trichogramma* strains tested had accepted the eggs of diamondback moth *Plutella xylostella* as host. SAKR (2003) tested 15 strains against *Cydia pomonella* and found all tested strains accepted *C. pomonella* eggs as host. EL-WAKEIL (2003) found that both *T. minutum* and *T. pretiosum* had accepted the eggs of *Helicoverpa armigera* as host.

Acceptance or rejection of host eggs by *Trichogramma* is dependent on the composition of the accessory gland secretion coating the chorion (PAK 1988, PAK *et al*. 1990). Ovarian eggs of *Pieris brassicae* dissected from gravid females lack the accessory gland coating. Such eggs were more attractive to female *T. buesi* Voegelé than normally laid eggs coated with the accessory gland secretion (PAK 1988). Although the accessory gland secretion of *P. brassicae* was a deterrent for *T. buesi*, this effect was not consistent. The same accessory gland secretion promoted the acceptance of *P. brassicae* by *T.*

maidis Voegelé (PAK 1988). Ovarian eggs of *P. brassicae* were rejected more frequently by *T. maidis* than normally laid eggs. The secretion of the accessory gland of *M. brassicae* was a positive recognition for both *T. buesi* and *T. maidis*. Ovarian eggs of *M. brassicae* were rejected by more than 50% of the females tested, whereas over 90% of normally laid eggs induced oviposition (PAK 1988). Thus, *Trichogramma* could distinguish among the secretions of different host species, and this sensitivity differed between various *Trichogramma* strains.

According to the results of the present experiment, it is evident that, among the 11 species/strains tested, field species *T. cacoeciae* (Cac-01) and *T. evanescens* (Eva-01) were most effective and highly accepted the GBM eggs. These species were therefore selected for further experiments and included in field experiments.

5.2.2 Host preference

Host preference is an important criterion for selecting the best *Trichogramma* species for use in biological control. A suitable *Trichogramma* strain should be chosen before field release. In general, most *Trichogramma* species exhibit a strong preference for certain hosts, crops and climatic conditions. The effectiveness of *Trichogramma* in the field largely depends on its searching behavior (habitat location, host location), host preference (recognition, acceptance, suitability), and tolerance to environmental conditions. Different methods were tried by several authors to test some of these factors (KOCHETOVA 1969, GONZALEZ *et al.* 1970, TAYLOR and STERN 1971, DOLPHIN *et al*. 1978, NEED and BURBUTIS 1979, van LENTEREN *et al*. 1982, HASSAN *et al*. 1988, HASSAN 1989, HASSAN and WÜHRER 1997, ALMATNI *et al*. 2001, EL-WAKEIL 2003, SAKR 2003).

Preference behavior of a single *Trichogramma* female released on different types of host eggs could be assessed by either direct or indirect method (HASSAN 1994). The direct method is based on the direct observation of the parasitoid and the continuous recording of all its activities (DIJKEN *et al.* 1986). Whereas, the indirect method observations and preference behavior of the parasitoid were assessed after five days by counting the number of parasitized eggs of each host (HASSAN 1989, HASSAN and GUO 1991, WÜHRER and

HASSAN 1993). However, all the activities of tested parasitoids can be recorded by the direct method but it is time costly and does not admit to test more adults at the same time. By the indirect method many parasitoids can be simultaneously tested at the same time. Both of the two methods were compared for measuring host preference of several *Trichogramma* species (WÄCKERS *et al.* 1987).

In the present study, the majority of *Trichogramma* species/strains tested strongly preferred *L. botrana* eggs over both *S. cerealella* and *E. ambiguella* eggs. By contrast, only *T. cacoeciae* (Cac-01), *T. cacoeciae* (Cac-sit) and *T. bourarachae* (Bou) preferred *E. ambiguella* eggs compared to *S. cerealella* eggs. These results correspond to those of SENGONCA *et al.* (1990). These authors, however, found that *T. semblidis* used to chose primarily eggs with biggest volumes and spherical form. Furthermore, in choice experiments, *T. semblidis* preferred eggs of both *Hypaena proboscidalis* L. (Lep.: Noctuidae) and *Aglais urticae* L. (Lep.: Nymphalidae) more than *E. ambiguella* eggs. In contrast to these results, CASTANEDA *et al*. (1993) tested the host preference of four *Trichogramma* species against *E. ambiguella* and *L. botrana* eggs and found no significant differences of the host preference between eggs of the two pests. ALMATNI *et al*. (2001) investigated the host preference of four strains of *T. cacoeciae* against the eggs of *C. pomonella* and *L. botrana.* He found that three strains had slightly preferred *C. pomonella* over *L. botrana* eggs and the fourth strain had equally chosen both of them. SAKR (2003) found that only six out of 15 *Trichogramma* strains tested showed a strong preference for *C. pomonella*.

Host finding by *Trichogramma* is primarily influenced by ecological factors, whereas host acceptance and preference is influenced by chemical factors and host suitability by mechanical, chemical and physiological factors (QUEDNAU 1955). HASSAN (1989) found that *T. dendrolimi* had nearly equal preferences among the target tortricids *C. pomonella, Adoxophyes orana* and the factitious host *S. cerealella*. WÜHRER and HASSAN (1993) found that only four out of 47 strains tested showed stronger preference for *P. xylostella* over *S. cerealella* eggs. QUEDNAU (1956) studied the host preference of three *Trichogramma* species towards four different lepidopterous pests by estimating the parasitization capacity by offering host eggs to single parasitoid females. Host preference tests of two *Trichogramma* species toward 70 lepidopterous species were conducted by placing samples of host eggs in parasitoid emerging units included *S. cerealella* eggs (SCHIEFERDECKER 1969).

WÜHRER and HASSAN (1993) mentioned that *T. pretiosum* had a high parasitization capacity of 53.7 eggs per female, but it showed no preference in parasitism between *Plutella* and *Sitotroga* eggs. In contrary, *T. cacoeciae* had a low parasitization capacity but it exhibited a clear preference for *Plutella* eggs. WÜHRER (1996) recommended the selection of *T. ostrinia* and *T. pretiosum* as a result of their preference behaviour and parasitism capacities to control *P. xylostella* and *Heliothis armigera*, respectively.

The present study indicated that**, the emergence rates** of all *Trichogramma* strains were clearly higher from *S. cerealella* parasitized eggs than those of *E. ambiguella* and *L. botrana*. Moreover, emergence rates of all *Trichogramma* tested were higher from the parasitized eggs of *L. botrana* than *E. ambiguella* eggs. The low emergence rate of *Trichogramma* strains from eggs of *E. ambiguella* and *L. botrana* may be due to the low host suitability. SALT (1938) defined host suitability as the ability of a parasitoid to develop completely within the host egg to a fertile individual. SAJAP and LEWIS (1988) detected a low emergence rate (36%) of *T. nubilale* from eggs of *Ostrinia nubilalis* which were infected by the microsporidian, *Nosema pyraustae*. Contrary, SCHULD (1994) found no influence of microsporidia on the emergence rate of *T. chilonis*. Moreover, SAKR (2003) reported that the emergence rates of nearly all *Trichogramma* strains tested were higher from *S. cerealella* eggs than from *C. pomonella* eggs. WÜHRER (1996) found that emergence rates of all *Trichogramma* strains from parasitized eggs of *S. cerealella* were higher than those from *P. xylostella* eggs. These results indicate that emergence potential of *Trichogramma* may be influenced by the host egg size.

Host egg size can have an influence on host choice because simply larger hosts are perceived earlier than smaller ones (PAK *et al.* 1991, BRUINS *et al*. (1994). By contrast, MONJE *et al*. (1999) observed that the majority of *T. pretiosum* females attacked the smaller eggs of *S. cerealella* first. Even females that were reared on *Diatraea rufescens* and encountered eggs of this host first abandoned them after having parasitized on average only two eggs. The rearing host has only a weak influence on preference compared with the effect of the host offered. MONJE *et al*. (1999) found that 67.0 and 63.3% of females of *T. galloi* and *T. pretiosum*, respectively, attacked the eggs of a certain host first regardless on which host they were reared.

HASSAN (1989) screened 17 *Trichogramma* strains for their suitability against the codling moth *C. pomonella* as well as the tortricids *Adoxophyes orana* and *Pandemis heparana*. He reported that strains of *T. dendrolimi* had the highest fecundity with all the three hosts tested. However, these strains were shown to have nearly equal preference between the target tortricids *C. pomonella, A. orana* and *S. cerealella*.

Sex ratio (female: male) of the tested *Trichogramma* strains was higher than 1:1. There were two strains tested thelytokous. Average female portions of all tested *Trichogramma* strains were 76.5%, 73.2% and 70.2% from the parasitized eggs of *Sitotroga*, *Lobesia* and *Eupoecilia*, respectively. BIGLER (1994) reported that a high portion of females is an advantage and serves as a quality criterion not only for mass rearing of *Trichogramma* but also for *Trichogramma* release. It was reported that the sex ratio of *T. galloi* and *T. cordubensis* was not affected by exposure to higher temperature (32ºC) with *T. cordubensis,* and 34°C with *T. dendrolimi* (CABELLO and VARGAS 1988, CONSOLI and PARRA 1995).

Generally, it was recommended that in choice experiments, the distance between host eggs needs to be similar adjusted to avoid inaccurate results caused by earlier perception of larger hosts (BRUINS *et al*. 1994). In the comparison carried out in the present study between *L. botrana*, *E. ambiguella* and *S. cerealella,* the eggs were distributed regularly (Fig. 1) around a honey/agar drop to reduce the effects of egg size and overlap of olfactory cues as much as possible.

According to the results of this study, as mentioned, almost all of tested *Trichogramma* strains preferred eggs of *L. botrana* stronger than *E. ambiguella*. Moreover, both of female portions and emergence rates from parasitized eggs of *L. botrana* were higher than those in *E. ambiguella*. The natural occurrence of *L. botrana* and *E. ambiguella* populations in the German viticulture is not equal, depending on the predominant climatic factors in the vineyards. It was reported that, *L. botrana* populations clearly predominated in warm and dry sites, whereas *E. ambiguella* predominates in the damp and cold ones (HOLST, personal comm.). Therefore, in areas in which only one of them occur, a release of a *Trichogramma* strain with pronounced preference and efficiency is required.

5.2.3 Analysis of various life history parameters of *Trichogramma* spp.

The 14 *Trichogramma* species/strains tested showed variations in **generation time**, **longevity**, **parasitism potential** and **reproduction potential** depending on the rearing host and/or the tested strain. In the present experiment, no significant differences occurred between *E. ambiguella* or *L. botrana* as rearing hosts on the generation time of various *Trichogramma* strains tested. By contrast, generation time varied significantly among various *Trichogramma* strains tested in the same host. PRATISSOLI and PARRA (2000) found a non significant differences in the generation time of *T. pretiosum* when reared in either *Phthorimaea operculella* (Zeller) or *Tuta absoluta* (Meyrick). There is a reciprocally proportional relation between developmental time and temperature (HARRISON *et al*. 1985, HAILE *et al*. 2002). SAKR (2003) found that the generation time of *T. cacoeciae* at 25°C was 10.2 days and 52.2 days at 10°C. However, temperature seems to be not the only key factor which affect the developmental time. WÜHRER (1996) found that generation time of *T. ostriniae* was 8.75 in *P. xylostella* compared to 9.13 days in *S. cerealella*. He stated also, generation time is dependent not only on the host type but also on *Trichogramma* strain.

Longevity is influenced by host species. Longevity of *Trichogramma* species/strains reared on *Lobesia* eggs was significantly longer compared to *Eupoecilia* eggs. The same trend has been reported also by BUTLER and LOPEZ (1980). PRATISSOLI and PARRA (2000) reported that longevity of *T. pretiosum* was significantly affected when reared in eggs of *P. operculella* compared to *T. absoluta*. For example, longevity of *T. minutum* and *T. pretiosum* reared on *H. armigera* eggs was longer than on *Sitotroga* eggs (EL-WAKEIL 2003). In the present study, *T. cacoeciae* strains showed the longest life span among the 14 tested strains, corroborating the results of SAKR (2003). By comparing the longevity of *T. chilonis* and *T. ostriniae* HIRASHIMA *et al*. (1990) selected *T. chilonis* to control the diamondback moth *P. xylostella*. ZHANG *et al*. (2001) demonstrated that *T. chilonis* and *T. nerudai* were more efficient against *P. xylostella* than *T. pretiosum* and *T. ostriniae* according to their longevity and fertility. This could be considered in the selection of *Trichogramma* species/ strains for biological control.

In the present study, parasitism potential of *Trichogramma* species/strains was strongly dependent on the host species. These results correspond to that of CASTANEDA (1990). Parasitism potential of *T. cacoeciae* strains are clearly higher than nearly all other *Trichogramma* strains. HASSAN and GUO (1991) selected *T. evanescens* and *T. ostriniae* to control the European corn borer based on their fertility. WÜHRER (1996) recommended *T. pretiosum* to control *H. armigera* as a result of its high parasitism and searching capacity.

Total progeny production of various *Trichogramma* species/strains tested varied significantly depending on host species. *T. cacoeciae* strains exhibit thelytokous parthenogenesis (producing females without fertilization). All other species were arrhenotokous. The reproduction potential of *T. cacoeciae* strains was the highest among other strains in either *Eupoecilia* or *Lobesia* eggs. However, *Lobesia* eggs were more suitable for the majority of strains tested than *Eupoecilia* eggs. SENGONCA *et al*. (1990) found that the reproduction potential of *T. semblidis* was highly affected by host species offered. He added that progeny production of *T. semblidis* were 28.9, 28.5 and 13.5 descendants per female when reared on *A. urticae*, *E. ambiguella* and *M. brassicae* eggs, respectively.

OCHIEL (1989) detected significant effects of temperature on progeny production of *T*. *exiguum* when tested at 18 and 30°C. He also found no significant difference in the proportion of female offspring at all temperatures tested. In the contrary, the female : male ratio of *T*. *principium*, was affected by temperature, females being slightly less abundant (1:1.3) at 18°C (SAKR 2003). The same trend was reported in *T*. *pretiosum* and *T*. *exiguum* when retained at 15 and 35°C (HARRISON *et al*. 1985).

In this study, the relative high proportion of females detected in *T*. *principium* is in agreement with both observations of SCOTT *et al*. (1997) and SAKR (2003). It is noticeable that a *Trichogramma* strain could gain good life history parameters by rearing on a suitable host.

Therefore, to be an effective biocontrol agent, *Trichogramma* species/strain should be characterized with: high parasitism rate, long life span, short generation time, high rates of emergence and reproduction. However, it is difficult to collect all of these advantages in one *Trichogramma* species. For example, *T. exiguum* recorded the shortest generation time i.e. more generations per year than all other strains, but with low emergence and reproduction potential. *T. minutum* recorded high emergence rates but its other life history parameters were not on the same level. *T. cacoeciae* strains recorded the best life history parameters (specially field strains) among all other strains, but its generation time was the longest. As mentioned above, there were variations among various *Trichogramma* strains in their life history parameters. Therefore, various life history parameters and origin (native or exotic) of the chosen strain should be considered in release programs against the GBM. It is known that the native species are preferred as biological control agents when both of native and exotic species have the equal potentiality advantages (HASSAN 1994). The local species are already adapted and tolerate the climatic conditions and have less non-target effects when released in a given environment.

It may guarantee a successful biological control program, considering to release two or three species at the same time. For example, *T. evanescens* and *T. ostriniae* were simultaneously used to control the European corn borer (HASSAN and GUO 1991). Moreover, *T. chilonis* and *T. nerudai* were also released against *P. xylostella* at the same time (ZHANG *et al*. 2001)

The study revealed also that, *T. cacoeciae* females evenly laid their eggs in their whole lifetime and promised to have a persistent effect in biological control.

In the present study, *T. cacoeciae* (Cac-01), *T. evanescens* (Eva-01), *T. minutum*, *T. principium* and *T. semblidis* were the best candidates for further experiments on the basis of their longevity, parasitism potential, emergence and reproductive rate. In general, if a mixture of the selected strains from *T. cacoeciae* (Cac-01), *T. evanescens* (Eva-01), *T. semblidis*, and *T. minutum* are used at the same time, the control effect may be supplemented with each other and guarantee an optimum biological control. This study also indicated that *T. cacoeciae* strains live longer and parasitize more host eggs. It was also found that *T. cacoeciae* (Cac-01) *and T. evanescens* (Eva-*01)* which were collected only one year earlier from the field performed better than those which were earlier collected. Newly collected *Trichogramma* strains from the field were more effective against *E. ambiguella* and *L. botrana* in host acceptance and preference and other life history parameters experiments than strains reared in the laboratory for a long time.

Results of laboratory experiments suggest a potential for the best *Trichogramma* species/strains to be used in vineyard augmentation efforts. However, additional studies are needed to evaluate the potential impact of *Trichogramma* in vineyards. It is useful to incorporate *Trichogramma* in biocontrol strategies of grape berry moths, since this will minimize insecticide costs and problems**.**

5.3 Field Experiments

5.3.1 Survey of *Trichogramma* in the vineyards and surrounding biotopes

a) Population fluctuation of *Trichogramma*

In vineyards managed according to IPM, *Trichogramma* were regularly proven, being far more widespread in those surrounded by hedge strips. Whereas, there were no *Trichogramma* recorded in the ecological managed vineyards.

Natural parasitism of grape berry moths (GBM) eggs by *Trichogramma* was observed in several countries worldwide. Eggs of *L. botrana* were naturally parasitized by both *T. evanescens* in the USSR (TELENGA 1934) and *T. pintoi* in Ukraine (FURSOV 1994). In France, DUGAST and VOEGELE (1984) recorded seven *Trichogramma* species which parasitized eggs of *E. ambiguella* and *L. botrana*. In Alsace vineyards, BARNAY *et al.* (2001) recorded four native *Trichogramma* species, *T. cacoeciae*, *T. daumalae*, *T. evanescens* and *T. principium*, the first two species being more widespread. In Germany, eggs of *E. ambiguella* and *L. botrana* were naturally parasitized by *T. semblidis* in Ahr valley vineyards (SENGONCA and LEISSE 1987, SCHADE and SENGONCA 1998) and by *T. evanescens* in vineyards of the Rheingau (CASTANEDA 1990), natural parasitism reaching up 40 to 50%. In an apple orchard, SAKR *et al.* (2002) found eggs of *C. pomonella* naturally parasitized by *T. cacoeciae* and *T. evanescens* in south Hessia (Germany).

In the present study, two native species of *Trichogramma*, *T. cacoeciae* and *T. evanescens* were discovered in vineyards and bordering area, the first species being more widespread. At all localities, *T. evanescens* was detected few days earlier than *T. cacoeciae*. It could be explained by the short developmental time needed for this species. These results correspond to BARNAY *et al.* (2001), SAKR *et al*. (2002) and IBRAHIM *et al*. (2003).

Three distinct activity periods of *Trichogramma* were observed in vineyards which were surrounded by hedges, whereas, only two periods in the non-surrounded. It is noticeable that *Trichogramma* was detected one week earlier at hedge strips than in vineyards. *Trichogramma* showed the greatest activity from April to the beginning of June, in July and in September. First and second activity periods corresponded with the first and second generation of GBM. However, the first activity period of *Trichogramma* was the greatest. This trend had already been reported for *T. evanescens* (TELENGA 1934), *T. semblidis* (SENGONCA and LEISSE 1987, SCHADE 1990) and for four *Trichogramma* species (BARNAY *et al*. 2001). KOT (1964) reported that *Trichogramma* was more abundant during July, August and at the beginning of September than in spring. During the second generation, parasitism activity was less compared to the first one. It may be due to the massive increase of vegetation in summer and this increase could constitute an obstacle for the parasitoids to find the host eggs. Moreover, *Trichogramma* have to explore a larger surface to find the eggs. Several authors have observed the same trend (LUKIANCHUK and SMITH 1997, WANG and HOSMER 1997).

CASTANEDA (1990) observed three activity periods for *T. evanescens,* in May June and July in vineyards of the Rheingau . SAKR *et al.* (2002) detected also three activity periods for *T. cacoeciae* and *T. evanescens.*

During the third activity period, *Trichogramma* was almost only in hedges. Details of results showed that *Trichogramma* was widespread at the lowest canopy level (80-120 cm) and preferably on the underside of the leaves. HASSAN (personal comm.) reported that *Trichogramma* preferred the lowest level of the canopy because of the high relative humidity. In this study, interruptions in parasitism activity were detected for some weeks, it could be due to that *Trichogramma* were reproducing in other biotopes in the vicinity of vineyards.

Although natural *Trichogramma* could be detected in some vineyards, they do not succeed to control the GBM. STELLWAAG (1928) and BALACHOWSKY (1966) reported that *Eupoecilia* and *Lobesia* are polyphageous species, they could exploit numerous host plants. This led to the suggestion, that females could lay their eggs on other host plants than grapes (in its vicinity) and these eggs could be used by *Trichogramma*. BARNAY *et al.* (2001) found that the number of *Lobesia* caught with pheromone traps in hedges was higher than that in the vineyard.

b) Effect of sulfur

As mentioned above, there were no *Trichogramma* detected in an ecologically managed vineyard (Mäuerchen) for two successive years of survey, although these vineyards were characterized by a very species-rich vegetative cover with a multiplicity of flower plants. The missing of *Trichogramma* could be explained as a result of the permanent and intensive application of sulfur in that vineyard. The effect of sulfur on *Trichogramma* and other beneficials was studied by many authors. For example, HASSAN *et al.* (1994) reported that sulfur caused 80-99% mortality for *Trichogramma* adult stages and was harmless for the immature stages, in addition to its high persistence in the treated environment (> 30 days). SENGONCA and LEISSE (1989) mentioned that *Trichogramma* might suffer from pesticides applications. SENGONCA (2002) pointed out that, pesticides are not only killing natural enemies but also eliminate their hosts or preys and starve them.

c) The role of Hedge plants

Hedges are a key component of agroecosystem biodiversity, that can be functional for pest management. In many cases natural vegetation around crop fields harbors alternate hosts or preys for natural enemies, thus providing seasonal resources to bridge gaps in the life cycles of entomophageous insects (ALTIERI 1991). The present study demonstrated clearly differences in the dynamics of native *Trichogramma* between edge and middle vine rows in the same vineyard. *Trichogramma* was more abundant in the edge vine rows (near hedges) than in middle ones. *Trichogramma* species showed periodical movements between vineyards and hedges. This movement behavior enabled *Trichogramma* to avoid the seasonal host scarcity in the vineyard, to exploit the complementary insect host availability on hedge plants and grape vine during the season.

The role of the different hedge plants on the presence of *Trichogramma* was analyzed. *Trichogramma* was clearly more active at locations where combinations of *Rubus fruticosus, Prunus spinosa* and *Ligustrum vulgare* than at the other hedge plant combinations. For example, SCHADE (1990) found that the close proximity of brambles (*Rubus fruticosus*) or of nettles (*Urtica dioica*) which were inhabited by several butterfly host-species, favored parasitization of *Trichogramma* in vineyards. Moreover, SCHADE and SENGONCA (1998) surveyed 29 lepidopterous species on brambles near vineyards in the Ahr valley, 14 of them being Tortricids; additionally, 23 out of 29 Lepidopteran species could be shown to be parasitized by *T. semblidis*.

PONTI *et al*. (2003) investigated the population dynamics of the parasitoid *Anagrus atomus* (Hym.: Mymaridae), both inside vineyards and in surrounding hedges in central Italy. They observed, a complementary distribution of *A. atomus* between grape and surrounding hedges, especially bramble hedges. Therefore, natural hedges play an important ecological role for egg parasitoids, they support alternate hosts and avoid local extinction of *Anagrus* wasps. The investigations show that, hedge cultivations have a great importance as hide shelters for egg parasitoids. Moreover, *Trichogramma* could move and survive more easily in hedges, which offer more host eggs than the almost bare grapevine. The study confirms the positive influence of existing diversity on stable agroecosystems, therefore it suggests conservation and keeping of hedges around fields/vineyards.

So negative effects, like those of sulfur and other pesticides application on beneficial arthropods, could possibly be minimized and/or avoided by keeping hedge strips. SENGONCA (2002) reported that unfavorable environmental conditions and lack of overwintering shelters during hibernation could cause high mortality among beneficial insects.

5.3.2 Dispersal behavior of *Trichogramma* in the vineyards

One of the most important factors in a *Trichogramma* inundation programme is the distance which the emerging parasitoids can disperse from a release point (ANDOW *et al*. 1993, McDOUGALL and MILLS 1997). The presence of *Trichogramma* adults at one point was measured by many authors using various techniques (HENDRICKS 1967, KOLMAKOVA and

MOLCHANOVA 1981, van den BERG *et al.* 1987). However, two (direct and indirect) methods were used for measuring the dispersal of tiny wasps like *Trichogramma* in the field. The direct methods included, yellow colored water traps, sticky card traps, and suction traps or sweep nets (STERN *et al*. 1965). Whereas, the indirect measurement of dispersal, based on distributing baiting devices and estimating parasitism by using either eggs of the target pest as bait (KANOUR and BURBUTIS 1984, van HEININGEN *et al*. 1985, HAWLITZKY *et al.* 1994) or by using alternative host eggs (HASE 1925, KOT 1964, NEUFFER 1987, CHERNYSHEV *et al*. 1988, BIGLER *et al*. 1990, MAINI *et al*. 1991). The results of this study showed that for both *Trichogramma* species released, parasitism activities were higher in the release row than neighboring vine rows. Moreover, parasitism activities declined gradually in the same vine row from the release point, also declined by time i.e. it was higher in the first three days of monitoring after release than in the second three days. Similar results were obtained in comparative studies with four *Trichogramma* species in vineyards of the Rheingau by CASTANEDA (1990). McDOUGALL and MILLS (1997) found that parasitism activity of *T. platneri* declined with increasing distance from the release point. Parasitism of sentinel eggs declined from a mean of 62% at the release point to < 10% at 14 meters away during the first three days after release. Parasitism also decreased by 50% during the second three days compared to the first three days. The same trend was also reported for a forest by SMITH (1988) and for an apple orchard (SAKR 2003). In the present study, parasitism dynamics were slightly higher in the eastern neighbour vine row than the western one. Parasitism efficiency can be limited by several factors. For example, parasitism was higher at the cooler side of citrus trees than at the warmer side (NEWTON 1988). SCHREAD (1932), ALLEN and GONZALEZ (1974) detected that parasitism activity was higher on plants or trees at the same distance from the release point within rows than across rows. Both NEWTON (1988) and SMITH (1988) have found no effect of wind on parasitism of baiting eggs. This study indicated also that the dispersal potential of *T. cacoeciae* was higher than that of *T. evanescens,* reaching up to seven and six m, respectively, from the release point. Parasitism efficiency at the release vine stock during the first three days were 78.6% and 71.9% for *T. cacoeciae*-01 and *T. evanescens*-01, respectively. McDOUGALL and MILLS (1997) recorded

81.6% parasitism in the first three days after releasing *T. platneri*, SAKR (2003) achieved 68.0% parasitism in the first three days after releasing *T. dendrolimi*.

The present study also indicated that at the lowest height level of the canopy (80-120 cm) was the zone of the highest parasitism activity for both *Trichogramma* species. It is also noticeable that, parasitism activity in that height level was higher during the second three days after release than that during first three days. CASTANEDA (1990) tested the vertical distribution of *T. cacoeciae, T. evanescens, T. embroyphagum* and *T. dendrolimi* at the vine canopy. He found that parasitism rate was the highest in the lowest level of the canopy for the first three species, while the fourth species preferred the zone >150 cm height of canopy. YU *et al*. (1984) found that the vertical distribution of *T. minutum* within apple trees was skewed toward the lower pail of the tree canopy.

In general releases, it is important to know how far from a release point *Trichogramma* would reach so that an acceptable level of parasitism can be achieved. The results of the present study suggest that significant differences in dispersal capacity can exist between different species or strains of *Trichogramma*. Therefore, dispersal capacity can be seen as a suitable trait for the selection of effective strains for use in biological control as mentioned by HASSAN (1994).

5.3.3 Biological control of grape berry moths (GBM) by releasing *Trichogramma* spp. in the vineyards

Currently, *Trichogramma* species/strains are the most widely augmented insect biological agents in the world (LOSEY *et al*. 1995). It was reported also that *Trichogramma* was employed as biological agents against Lepidoptera pests in more than 20 crops throughout the world (BURGIO and MAINI 1995). However, the widespread application of these parasitoids can be explained by their relative facility and low cost of rearing, making them competitive with chemicals and reduced environmental impact.

The present study was carried out to compare the effectiveness of various (laboratory and commercial) *Trichogramma* species for controlling the grape berry moths in the vineyards. In the present study, the efficacy (damage reduction) of *Trichogramma* strains used varied between 62.8% and 83.2% in two years, depending clearly on both the species used and infestation rate by GBM. It was also noticeable that newly collected *Trichogramma* species/strains performed better than older ones. A similar trend was observed by SAKR (2003). In a comparative study, CASTANEDA (1990) released *T. cacoeciae*, *T. dendrolimi* and *T. embryophagum* to control the second generation of the GBM in the Rheingau vineyards. He found that efficacy varied among the strains reaching up to 83.3%, 71.2% and 53.9% for *T. cacoeciae*, *T. dendrolimi* and *T. embryophagum,* respectively.

In the present study, *Trichogramma cacoeciae* strains were more efficient, leading at the average to reduction in infestation levels reaching 83.2%. These results correspond to results reported by CASTANEDA (1990) and SAKR (2003). In contrast, *T. dendrolimi* achieved a higher reduction of infestation compared to that reported by CASTANEDA (1990). This can be the result of differences in infestation rates by the GBM. ZIMMERMANN *et al.* (1997) released *T. cacoeciae* to control the GBM in the Rheingau vineyards and recorded damage reduction reaching up to 60%. In laboratory experiments, *T. cacoeciae* showed higher performance against eggs of *E. ambiguella* and *L. botrana* than other strains tested. Moreover, *T. cacoeciae* lived longer than both *T. evanescens* and *T. dendrolimi* and laid its eggs evenly in the whole lifetime.

According to the results of the present study, laboratory experiments, e.g. various life history parameters of a particular *Trichogramma* strain, show obviously indicators for its performance in the field. HASSAN (1989) released *T. dendrolimi* to control *C. pomonella* and recorded an efficacy of 60%. In the last few years, releasing combinations of various *Trichogramma* species against Lepidopteran pests are recommended. HASSAN (1993) recorded 11% enhancement of efficacy when released a combination of *T. cacoeciae* and *T. dendrolimi* compared to separate releases against *C. pomonella*. On the contrary, SAKR (2003) released a combination of *T. dendrolimi* and *T. cacoeciae* resulted a lower reduction of damage than using *T. cacoeciae* alone.

The results of this study clearly show that, by using the suitable *Trichogramma* species, the efficacy of inundative release can be significantly improved. In both laboratory and field experiments, *T. cacoeciae* showed a higher survival and a higher preference for grape berry moths eggs than other tested species. Moreover, *T. cacoeciae* showed a higher dispersal potential and searching capacity in the vineyards than *T. evanescens*. Therefore, *T. cacoeciae* is recommended to control the grape berry moths due to its high efficiency compared to other strains.

6 SUMMARY

The present work was carried out during the period from November 2000 to September 2003 in order to survey naturally occurring populations of *Trichogramma* in the Rheingau area, and to evaluate the efficiency of 17 *Trichogramma* species/strains against the grape berry moths (GBM) *Eupoecilia ambiguella* Hb. and *Lobesia botrana* Schiff. Two species *Trichogramma cacoeciae* (Cac-01) and *T. evanescens* (Eva-01) were recently collected from the vineyards of Rheingau during the study. Three species *T. cacoeciae* (Cac-com), *T. dendrolimi* (Den-com) and *T. evanescens* (Eva-com) were commercially produced species. The remaining species were successively reared either on GBM eggs (*T. cacoeciae* Cac-Ea and *T. cacoeciae* Cac-Lb) or on *Sitotroga cerealella* eggs.

Host acceptance experiments with 11 *Trichogramma* species/strains revealed that all *Trichogramma* tested accepted the GBM eggs as host, however varied in its grade of acceptance. The field species *T. cacoeciae* (Cac-01) and *T. evanescens* (Eva-01) were the most effective ones and highly accepted the GBM eggs.

Host preference experiments showed that the majority of *Trichogramma* species/strains tested strongly preferred *L. botrana* eggs over both *E. ambiguella* and *S. cerealella* eggs. *T. cacoeciae* (Cac-01), *T. cacoeciae* (Cac-sit) and *T. bourarachae* (Bou) preferred *E. ambiguella* eggs compared to *S. cerealella* eggs.

Emergence rates of all *Trichogramma* strains were clearly higher from *S. cerealella* parasitized eggs than those from *L. botrana* and *E. ambiguella* eggs. Moreover, emergence rates of all *Trichogramma* tested were higher from the parasitized eggs of *L. botrana* than from *E. ambiguella* eggs. The highest emergence rate was achieved by the field species *T. cacoeciae* (Cac-01), with 97.4%, 89.5% and 86.5% from the parasitized eggs of *S. cerealella*, *L. botrana* and *E. ambiguella*, respectively. The lowest emergence rates were 77.7% (in *Sitotroga* eggs), 67% (in *Lobesia* eggs) and 52.4% (in *Eupoecilia*) for *T. evanescens* (Eva-01), *T. cacoeciae* (Cac-sit) and *T. bourarachae*, respectively**.**

Sex ratio (female : male) of the tested *Trichogramma* strains was higher than 1. The lowest female ratio was (41.8%) detected for *T. minutum* in *Eupoecilia* eggs. Average female offspring ratios of all tested *Trichogramma* strains were 76.5%, 73.2% and 70.2% from the parasitized eggs of *Sitotroga*, *Lobesia* and *Eupoecilia*, respectively.

In order to evaluate the suitability of 14 *Trichogramma* species/strains for controlling the grape berry moths, their various **life history parameters** during their life span were determined.

There were no significant differences between *E. ambiguella* or *L. botrana* as rearing hosts on the **generation time** of various *Trichogramma* strains tested. By contrast, generation time varied significantly among various *Trichogramma* strains tested in the same host. The longest developing time (12.7 days) was recorded for *T. cacoeciae*. Whereas, the shortest developing time was 7.6 days for *T. exiguum*.

Longevity of *Trichogramma* species/strains reared on *Lobesia* eggs was significantly longer compared to *Eupoecilia* eggs. *T. cacoeciae* strains lived significantly longer than all other strains. In *Lobesia* eggs, the life span of various *Trichogramma* species/strains ranged from 7.6 to 27 days for *T. piceum* and *T. cacoeciae* (Cac-Ea), respectively. Whereas, in *Eupoecilia* eggs, the life span of various *Trichogramma* species/strains ranged between 5.3 to 20.4 days for *T. piceum* and *T. cacoeciae* (Cac-sit), respectively.

Parasitism potential of *Trichogramma* species/strains was strongly dependent on the host species. Parasitism potential of *T. cacoeciae* strains was clearly higher than in nearly all of other *Trichogramma* strains. Parasitism potential of *Trichogramma* strains varied from 81.4 (*T. evanescens* Eva-01) to 104.2 (*T. cacoeciae* Cac-01) parasitized eggs in *Lobesia* eggs. By contrast, it ranged from 34.4 (*T. evanescens* Eva-01) to 64.1 (*T. cacoeciae* Cac-01) parasitized *Eupoecilia* eggs.

The **reproduction potential** of *T. cacoeciae* strains was the highest among all of other *Trichogramma* strains in either *Eupoecilia* or *Lobesia* eggs. However, *Lobesia* eggs were more suitable for the majority of strains tested than *Eupoecilia* eggs. The reproductive potential of various *Trichogramma* strains reared on *Lobesia* eggs varied significantly between 13.3 and 74.6 individuals per female for *T. bourarachae* and *T. cacoeciae* (Cac-01), respectively. In *Eupoecilia* eggs, reproduction rate of females of *T. cacoeciae*

(Cac-01) was the highest (43.3), whereas it was the lowest for *T. exiguum* (13.5).

Reproduction of **daughter offspring** of various *Trichogramma* females reared in *Eupoecilia* eggs ranged from 4.6 to 43.3 daughters per female for *T. exiguum* (Exi) and *T. cacoeciae* (Cac-01), respectively. However, in *Lobesia* eggs it ranged from 9.1 to 74.6 daughters per female for both *T. bourarachae* (Bou) and *T. cacoeciae* (Cac-01), respectively.

In the present study, *T. cacoeciae* (Cac-01), *T. evanescens* (Eva-01), *T. minutum*, *T. principium* and *T. semblidis* were the best candidates on the basis of their longevity, parasitism potential, emergence and reproductive rate.

In general, the newly collected *Trichogramma* species (*T. cacoeciae* Cac-01 and *T. evanescens* Eva-01) had a better performance compared to other laboratory strains. The results of this study can be used to select the suitable *Trichogramma* species/strains for biological control programs.

Natural occurrence and the population fluctuation of native *Trichogramma* species in both the vineyards of Rheingau and surrounding hedges were studied. There were no *Trichogramma* recorded in the ecologically managed vineyards. In vineyards managed according to IPM, *Trichogramma* were regularly proven, being more widespread in those surrounded by hedge strips. Three distinct activity periods of *Trichogramma* were observed in vineyards which were surrounded by hedges, whereas, only two periods in the non-surrounded. *Trichogramma* showed the greatest activity from April to the beginning of June, in July and in September. *Trichogramma* was detected one week earlier at hedge strips than in vineyards. Two native species of *Trichogramma*, *T. cacoeciae* and *T. evanescens* were discovered in vineyards and bordering areas, the first species being more frequent. *T. evanescens* was recorded few days earlier than *T. cacoeciae*. Both *Trichogramma* spp. were widespread at the lowest canopy level (80-120 cm) and preferably on the underside of the leaves. Moreover, *Trichogramma* was more abundant in the edge vine rows (near hedges) than in middle ones.

Trichogramma species showed periodical movements between vineyards and hedges. *Trichogramma* was clearly more active at locations with combinations of *Rubus fruticosus, Prunus spinosa* and *Ligustrum vulgare* than at the other hedge plant combinations.

Therefore, negative effects like those of sulfur and other pesticides application on beneficial arthropods, could be minimized and/or avoided by keeping hedge strips which consider as hiding and reservoir shelters for natural enemies.

The dispersal potential of *Trichogramma cacoeciae* (Cac-01) and *T. evanescens* (Eva-01) was tested in the vineyards. For both *Trichogramma* species released, parasitism activities were higher in the release row than in neighbouring vine rows. Moreover, parasitism activities declined gradually in the same vine row from the release point, also declined by time i.e. it was higher in the first three days of monitoring after release than in the second three days. The dispersal potential of *T. cacoeciae* was higher than in *T. evanescens,* and reached seven and six m from the release point, respectively. Parasitism efficiency at the release vine stock during the first three days were 78.6% and 71.9% for *T. cacoeciae* (Cac-01) and *T. evanescens* (Eva-01), respectively. The present study also emphasized that, the lowest height level of the canopy (80-120 cm) was the highest activity zone for both *Trichogramma* species.

Field experiments were conducted to evaluate the efficacy of *Trichogramma* species, to control *L. botrana and E. ambiguella* in two successive years. The efficacy (damage reduction) of *Trichogramma* strains used varied between 62.8% and 83.2%. In the present study, *Trichogramma cacoeciae* strains were more efficient in controlling the grape berry moths, with 77.6% and 83.2% reduction in grape damage for *T. cacoeciae* (Cac-com) (commercial strain) and *T. cacoeciae* (Cac-94) (vineyard strain), respectively. *T. evanescens* (Eva-01) (vineyard strain) achieved 74.5% reduction in grape damage, whereas the commercial strain recorded only 62.8%. It was also found that newly collected *Trichogramma* species/strains performed better than commercial ones.

The results of this study showed that, *T. cacoeciae* and *T. evanescens* and their strains could be potential candidates for future mass rearing and field release programs for biocontrol of grape berry moths in the vineyards.

7 Zusammenfassung

Die vorliegende Arbeit wurde in der Zeit von November 2000 bis September 2003 durchgeführt, um einmal das natürliche Vorkommen von *Trichogramma*-Arten im Rheingau zu untersuchen und um die Wirksamkeit von 17 *Trichogramma*- Arten/Stämmen gegen die Traubenwickler *Eupoecilia ambiguella* Hb. und *Lobesia botrana* Schiff zu ermitteln. Zwei *Trichogramma*-Arten, *Trichogramma cacoeciae* (Cac-01) und *T. evanescens* (Eva-01) wurden während der Untersuchungen neu in den Weinbergen geködert. Drei Arten, *T. cacoeciae* (Cac-com), *T. dendrolimi* (Den-com) und *T. evanescens* (Eva-com) waren kommerziell genutzte Stämme. Die Arten *T. cacoeciae* (Cac-Ea) und *T. cacoeciae* (Cac-Lb) wurden ausschließlich auf Traubenwicklereiern gezüchtet. Alle andren Arten wurden ständig in kleinen Laborzuchten auf *Sitotroga cerealella* Eiern vermehrt.

Wirtsakzeptanz: Experimente zum Wirtswahlverhalten wurden mit 11 *Trichogramma*-Arten/Stämmen durchgeführt. Die Ergebnisse zeigten, dass alle geprüften *Trichogramma* Traubenwicklereier als Wirt annahmen, aber in unterschiedlich starkem Umfang. Unter den 11 getesteten Arten, waren die Freilandstämme *T. cacoeciae* (Cac-01) und *T. evanescens* (Eva-01) am effektivsten und nahmen die Traubenwicklereier zu einem hohen Prozentsatz an.

Wirtspräferenz: Die meisten der geprüften *Trichogramma*-Arten/Stämme zeigten eine stärkere Präferenz für Eier von *L. botrana* als für Eier von *E. ambiguella* und *S. cerealella*. Nur *T. cacoeciae* (Cac-01), *T. cacoeciae* (Cac-sit) und *T. bourarachae* (Bou) bevorzugten Eier von *E. ambiguella.*

Schlupfrate: Die Schlupfrate aller *Trichogramma*-Stämme war aus den parasitierten Eiern von *S. cerealella* am höchsten, gefolgt von der aus Eiern von *L. botrana* und *E. ambiguella*. Die beste Schlupfrate wurde bei dem Freilandstamm *T. cacoeciae* (Cac-01) aus parasitierten Eiern von *S. cerealella* mit 97,4% erzielt. Während die Schlupfrate aus *L. botrana*-Eiern 89,5% betrug und mit *E. ambiguella*-Eiern nur ein Wert von 86,5% erreicht wurde. Die niedrigsten Schlupfraten ergaben sich mit 77,7% in *Sitotroga*-Eiern bei *T. evanescens* (Eva-01), in *Lobesia*-Eiern mit 67% bei *T. cacoeciae* (Cac-sit) und in *Eupoecilia*-Eiern mit 52,4% für *T. bourarachae*.

Geschlechterverhältnis: Das Verhältnis von Weibchen : Männchen der getesteten *Trichogramma*-Stämme war höher als 1. Der niedrigste Weibchen-anteil mit 41,8% wurde für *T. minutum* in *Eupoecilia*-Eiern ermittelt. Der durchschnittliche Anteil an weiblichen *Trichogramma* aller geprüften Stämme war 76,5% aus *Sitotroga*-, 73,2% aus *Lobesia*- und 70,2% aus *Eupoecilia*-Eiern.

Um die Eignung von 14 *Trichogramma*-Arten/Stämmen für die Bekämpfung von Traubenwicklern zu ermitteln, wurden verschiedene Lebensparameter untersucht.

Generationsdauer: Alle *Trichogramma*-Arten/Stämme zeigten hinsichtlich der Generationsdauer keine signifikanten Unterschiede zwischen den Wirten *E. ambiguella* und *L. botrana*. Die Entwicklung der Arten/Stämme im gleichen Wirt weißt dagegen deutliche Unterschiede auf. Die längste Entwicklungsdauer von 12,7 Tagen wurde für *T. cacoeciae* notiert, während die kürzeste 7,6 Tage bei *T. exiguum* festgestellt wurde.

Lebensdauer: Bei ständiger Verfügbarkeit von frischen Wirtseiern war die Lebensdauer von adulten *Trichogramma*-Arten/Stämme aus *Lobesia*-Eiern signifikant länger als aus *Eupoecilia*-Eiern. Die *T. cacoeciae*-Stämme lebten erheblich länger als die Stämme aller anderen Arten. In *Lobesia*-Eiern lag die Lebensdauer zwischen 7,6 Tagen für *T. piceum* und 27 Tagen für *T. cacoeciae* (Cac-Ea). Während in *Eupoecilia*-Eiern die Lebensdauer 5,3 Tage für *T. piceum* und 20,4 Tage für *T. cacoeciae* (Cac-sit) betrug.

Parasitierungspotential: Dieses wurde bei den untersuchten *Trichogramma* -Arten durch die Wirtseier deutlich verändert. Das Parasitierungspotential der *T. cacoeciae*-Stämme war höher als das fast aller anderen *Trichogramma Arten/Stämme.* So variierte es in *Lobesia*-Eiern von 81,4 parasitierten Eiern/Weibchen bei *T. evanescens* (Eva-01) bis zu 104,2 bei *T. cacoeciae* (Cac-01). Im Vergleich dazu betrug das Parasitierungspotential in *Eupoecilia*-Eiern nur 34,4 bei *T. evanescens* (Eva-01) und 64,1 bei *T. cacoeciae* (Cac-01).

Reproduktivität: Die Reproduktivität der *T. cacoeciae*-Stämme war die höchste von allen Stämmen. Es zeigte sich, dass *Lobesia*-Eier für die Mehrheit der geprüften Stämme geeigneter als *Eupoecilia*-Eier waren. Das reproduktive Potential der verschiedenen *Trichogramma*-Stämme schwankt zwischen 13,3 Nachkommen pro Weibchen für *T. bourarachae* (Bou) und 74,6 für *T. cacoeciae*

(Cac-01) in *Lobesia*-Eiern. In *Eupoecilia*-Eiern wurde bei *T. cacoeciae* (Cac-01) mit 43,3 Nachkommen pro Weibchen der höchste Wert festgestellt, während der niedrigste für *T. exiguum* bei 13,5 Nachkommen pro Weibchen lag.

Die Anzahl weiblicher Nachkommen der verschiedenen *Trichogramma*-Arten aus *Eupoecilia*-Eiern, lag bei *T. exiguum* bei 4,6 pro Weibchen und mit 43,3 bei *T. cacoeciae* (Cac-01). In *Lobesia*-Eiern wurden für *T. bourarachae* 9,1 Nachkommen pro Weibchen und bei *T. cacoeciae* (Cac-01) sogar 74,6 weibliche Nachkommen gefunden.

Nach den Untersuchungsergebnissen waren *T. cacoeciae* (Cac-01), *T. evanescens* (Eva-01), *T. minutum, T. principium* und *T. semblidis* die besten Arten in Bezug auf Lebensdauer, Parasitierungspotential, Schlupfrate und Reproduktionsrate. Im allgemeinen erzielten die frisch geköderten *Trichogramma*-Arten, *T. cacoeciae* (Cac-01) und *T. evanescens* (Eva-01), bessere Ergebnisse verglichen mit anderen Laborstämmen. Die Ergebnisse dieser Studie sollen helfen, eine Auswahl von geeigneten *Trichogramma*-Arten oder Stämmen für die biologische Bekämpfung zu erleichtern.

Natürliches Auftreten: Weiterhin wurde das natürliche Auftreten und die Populationsfluktuation von einheimischen *Trichogramma*-Arten in Weinbergslagen und angrenzenden Hecken des Rheingaus untersucht. In ökologisch bewirtschafteten Rebflächen, inmitten eines großen reinen Rebareals, wurden keine *Trichogramma* gefunden, obwohl sich diese Flächen durch eine sehr artenreiche Begrünung mit einer Vielzahl von annuellen Blütenpflanzen auszeichneten. Dagegen wurden regelmäßig in den integriert bewirtschafteten Weinbergen *Trichogramma* nachgewiesen. Sie waren auch in den umgebenden Heckenstreifen weit verbreitet. Drei eindeutige Aktivitätsperioden von *Trichogramma* wurden in den Weinbergen beobachtet, die durch Hecken umgeben waren, während nur zwei Perioden in nicht von Hecken umgebenen Weinbergsanlagen zu beobachten waren. *Trichogramma* zeigte im Freiland Parasitierungsaktivitäten von April bis Anfang Juni, im Juli und im September. Aktive *Trichogramma* wurden eine Woche früher in den Heckenstreifen ermittelt als in den Weinbergen. Die einheimischen Arten, *Trichogramma cacoeciae* und *T. evanescens,* wurden in den Weinbergen und angrenzenden Hecken nachgewiesen. *T. cacoeciae* trat immer stärker als *T. evanescens* auf. *T.*

evanescens wurde dafür ca. 7 Tage früher als *T. cacoeciae* nachgewiesen. Im unteren Laubwandbereich (80-120 cm) war *Trichogramma* am weitesten verbreitet und hielt sich vorzugsweise auf der Blattunterseite auf. *Trichogramma* konnte häufiger in den Randzeilen nahe den Hecken, als in Rebzeilen des mittleren Weinbergsbereichs nachgewiesen werden. *Trichogramma* zeigte periodische Bewegungen zwischen Weinbergen und Hecken. Die Schlupfwespen wurden häufiger in den Hecken mit *Rubus fruticosus*, *Prunus spinosa* und *Ligustrum vulgare* nachgewiesen als in Hecken ohne diese Pflanzen. Daraus folgt, dass dem Anpflanzen von Hecken als Refugien für Eiparasitoide eine große Bedeutung zukommt. So könnten durch Heckenstreifen negative Auswirkungen, wie die des Schwefeleinsatzes auf Nutzarthropoden verringert werden.

Verbreitungspotential: Auch Untersuchungen zum Verbreitungspotential von *Trichogramma cacoeciae* (Cac-01) und *T. evanescens* (Eva-01) wurden in den Weinbergen durchgeführt. Für beide *Trichogramma*-Arten, war die Ausbreitung in der Freilassungszeile höher als in benachbarten Rebzeilen. Die Verbreitung vom Freilassungspunkt war bei *T. cacoeciae* war in der Rebzeile höher, bis zu 7 Metern, als dies von *T. evanescens* (6 m).

Die Parasitierung in den ersten drei Tagen nach Freilassung war größer als in den folgenden drei Tagen. Die Parasitierungsleistung am Freilassungsrebstock während der ersten drei Tage betrug 78,6% für *T. cacoeciae* (Cac-01) und 71,9% für *T. evanescens* (Eva-01). Die vorliegenden Ergebnisse zeigen, dass im unteren Laubwandbereich (80-120 cm) sich die höchste Aktivitätszone für beide *Trichogramma*-Arten befindet.

Feldversuch: In einem Feldversuch über zwei aufeinander folgende Jahre wurde die Wirksamkeit von drei *Trichogramma*-Arten zur Bekämpfung der Traubenwickler *L. botrana* und *E. ambiguella* überprüft. Der Wirkungsgrad der verwendeten *Trichogramma* lag zwischen 62,8% und 83,2%. Die *Trichogramma cacoeciae*-Stämme waren zur Bekämpfung von Traubenwicklern am effektivsten. *T. cacoeciae* (Cac-94), ein Weinbergstamm, war mit einem Wirkungsgrad von 83,2% leistungsfähiger als *T. cacoeciae* (Cac-com) ein kommerzieller Stamm, mit 77,6%. Mit *T. evanescens* (Eva-01), einem Weinbergstamm, wurde ein Wirkungsgrad von 74,5% erzielt, während mit *T. evanescens* (Eva-com) nur 62,8% erreicht wurden. Die neu geköderten

Trichogramma-Arten waren effektiver als die Laborstämme. Die vorliegenden Untersuchungsergebnisse zeigen, dass die Arten *T. cacoeciae* und *T. evanescens* und deren Stämme als potentielle Kandidaten für zukünftige Feldfreilassungen und Massenzuchten im Rahmen von Pflanzenschutz-programmen zur biologische Bekämpfung von Traubenwicklern sehr gut geeignet sind.

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