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Full-text article

DETERMINATION OF *TRICHOGRAMMA EUPROCTIDIS* EFFICACY AGAINST THE KEY PEST, EUROPEAN GRAPEVINE MOTH, *LOBESIA BOTRANA* (LEPIDOPTERA: TORTRICIDAE) IN THE AEGEAN REGION VINEYARDS, TURKEY

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European grapevine moth *Lobesia botrana* is the most important pest causing crop losses in most of Türkiye's grape growing regions, feeding mainly on inflorescence and grape berries. This study was carried out to determine the release efficiency and the most efficient dose of the native egg parasitoid, *Trichogramma euproctidis* against *L. botrana* in an organic vineyard located in the Menemen district of Izmir, in the Aegean Region, which has the largest grape production area. In this context, two different release doses at 75 000 and 100 000 parasitoids/ha were applied to find the most effective one. The effectiveness of the parasitoid was compared in terms of pest infestation rates in grape bunches from parasitoid-released and non-released plots just prior to harvest. The infestation rates in plots released 100 000 parasitoids/ha (12, 4 and 5%) and 75 000 parasitoids/ha (7.5, 11 and 1 1%) were lower than control (25, 33 and 70%) in 2017, 2018 and 2019, respectively. In 2018 and 2019, the high release dose (100 000 parasitoids/ha) applied five or six times in total was the most promising with the lowest pest infestation rates. In this study, the most effective release dose (100 000 parasitoids/ha) of the native egg parasitoid, *T. euproctidis,* was determined for the first time in Türkiye. In conclusion, the results obtained from the study will encourage the use of this parasitoid in organic and integrated grape growing programs.

Keywords: egg parasitoid, grape, biological control, pest

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Introduction

In Türkiye, 4 165 000 tons of grapes are produced from 384 537 hectares of vineyard area. The Aegean Region, on the other hand, ranks first in Türkiye with its viticulture area of 110 000 hectares. Today, "Sultani Çekirdeksiz (=Sultana)" variety (*Vitis vinifera* L.) takes the first place in terms of export-oriented table grape production in Türkiye and 95% of grape exports are seedless grapes. Manisa, Denizli and Izmir, which are among the provinces of the Aegean Region, are in the first place in production (Anonymous, 2022). European grapevine moth, *Lobesia botrana* (Denis and Schiffermuller) (Lepidoptera: Tortricidae), which is the major pest in the vineyards of Türkiye, directly damages on the vine by feeding on flowers, unripe and mature grape berries. Additionally, it causes fruit juice to flow from the punctures out of mature berries and creates a suitable environment for saprophyte fungi and afterwards mycotoxin development during unsuitable storage conditions of dried berries and thus indirectly, causing considerable losses in both quality and yield of grape (Altındişli and Özsemerci, 2013). *L. botrana* control is almost the most important source of input and if this pest is not controlled, there is a loss in yield between 45–92% (Önçağ, 1975). Due to intensive and unconscious chemical control practices, pests become resistant to insecticides and cause disruption of the natural balance by damaging beneficial insects in the ecosystem. For these reasons, it is necessary to develop environmentally friendly control methods that will be an alternative to chemical control of *L. botrana*. Integrated pest management and organic farming are controlled, certified agricultural production systems that increase the feasibility of biological control applications and give importance to the environment and human health. It is of great importance to use natural enemies against *L. botrana* in grape growing areas where these agricultural programs are implemented.

A number of studies have been carried out to describe parasitoids, specifically or occasionally associated with European grapevine moth (Lucchi et al., 2016; Scaramozzino et al., 2017; 2018). Most of the species associated with EGVM (>95%) are either parasitic Hymenoptera belonging to the families Braconidae, Chalcididae, Elasmidae, Eulophidae, Ichneumonidae, Pteromalidae and Trichogrammatidae, or belong to Tachinidae (Diptera). Egg parasitoids of the genus *Trichogramma* are one of the most used beneficial insects in biological control, especially against moth species (Pinto, 2006; Querino et al., 2010). *Trichogramma* species are the most widely exploited and used for pest management across the world. There are 230 recorded species and the highest numbers of species have been described from the USA, India, Brazil, China, Russia and Portugal (Jalali et al., 2016; Carlos et al., 2022; Di Giovanni et al., 2022) However, it has been proven that the low effectiveness of some *Trichogramma* applications in biological control is due to the selection of unsuitable species and their application in unfavourable ecological conditions (Reda, 2004; Moreau et al., 2009). Therefore, native species are preferred over exotic species because of their possibility of adapting to climate, habitat and host conditions (Hassan, 1994; Smith, 1996). It was noticed that, in releasing the indigenous *Trichogramma* *bourarchae* for the control of the grape moth *L. botrana* was more effective than *T. evanescens* in reducing grape yield particularly (Kordy et al., 2014). Indigenous species that are collected from the same region should always be prefered (Van Lenteren et al., 2003).

A limited number of studies have been conducted abroad on the use of different *Trichogramma* egg parasitoid species against the harmful insect *L. botrana* in the vineyard within the scope of biological control. In the Aegean Region, *Trichogramma euproctidis* was found to be the most common and intense species in *L. botrana* eggs (Özsemerci et al., 2016). However, a comprehensive study has not yet been conducted in Türkiye with regard to the release efficacy and effective dose of the egg parasitoid *T. euproctidis* against this pest. Polat and Özpınar (2007), released *T. evanescens* against *L. botrana* only once and neither effective release dose nor efficiency were investigated in the study. In the second study, single release of *T. evanescens* was applied with a single dose in field conditions, and the effective dose was not revealed (Ayvaz et al., 2008).

In this study, it was aimed to determine the effective release dose and release number to reveal the effectiveness of the mass-produced *T. euproctidis* parasitoid, which is the indigenous species found in the Aegean Region. The effective release dose and release number findings obtained from the study to determine the effectiveness of the parasitoid will contribute to support and using of this parasitoid in organic agriculture, integrated pest and integrated crop management programs. The possibility to suppress the population of *L. botrana* by using native parasitoid in alone or, if necessary, as a complementary treatment using together with a biological pesticide is a very important step for the sustainability of grape growing. As a result, healthy and environmentally friendly products will be obtained and a positive contribution to sustainable grape production will be made by disseminating and putting the local input into practice.

Materials and Methods

The main material of the study consisted of "Sultani Çekirdeksiz" vineyard in Menemen county of Izmir Province, the Flour moth [*Ephestia kuehniella* Zeller (Lep.: Pyralidae)], the egg parasitoid [*Trichogramma euproctidis* Girault (Hymenoptera: Trichogrammatidae)], biological stages (egg, larva, pupae and adult) of the European grapevine moth [*Lobesia botrana* (Dep. Tortricidae), pheromone traps (Pherocon 1C, Trece®) and release cards.

Production of laboratory host *Ephestia kuehniella*

Fresh eggs of the laboratory host, *E. küehniella,* were needed for the mass rearing of *T. euproctidis*. *T. euproctidis* were reared in the climate room conditioned at 25 ±1 °C temperature, 70 ±5 % relative humidity and a photoperiod of 16:8 (L:D). Larvae of *E. küehniella*, were reared in an artificial diet consisting of ¼ corn flour, 2/4 wheat flour and ¼ beaten pistachio nut mixtures in plexiglas cages. In order to facilitate pupae formation, cardboard was placed in the culture cages in rolls during the last larval stage (Tunçyürek, 1972).

Emerging *E. kuehniella* adults were transferred to the egg laying cages. One part of the eggs taken daily from these cages were used in parasitoid rearing and the other part of them were used in the rearing of host *E. kuehniella*. In order to prevent embryo development of one-day-old *E. kuehniella* eggs, they were exposed to UV irradiation for 20–25 minutes and stored in the refrigerator at +4 °C. Then, these eggs were glued homogeneously on 200 x 100 mm paper, which was moistened with water. After water dries, the papers were cut into pieces 100 x 1.5 mm) and placed in glass tubes for parasitoid rearing (Tunçyürek, 1972; Uzun, 1989; Cerutti et al., 1992; Goncalves et al., 2005).

Rearing of the egg parasitoid *Trichogramma euproctidis*

In this context, first, the native egg parasitoid species were collected directly from each generation of the host pest eggs incubated in the laboratory in order to check for parasitism. Samples of parasitized eggs were sent for species characterization to Dr. Fahriye Sumer Ercan (Ahi Evran University, Faculty of Engineering and Architecture, Department of Genetics and Bioengineering, Kırşehir–Türkiye) and to Richard Stouthammer (Department of Entomology, University of California, Riverside, USA) for validation of results. Parasitoid samples identified as *T. euproctidis* were reared on *E. kuehniella* eggs in incubator adjusted to 28±1 °C temperature, 65 ± 10 % relative humidity and a photoperiod of 16:8 (L:D) conditions. Rearing was conducted in glass tubes by using eggs glued on the paper cards. Diluted honey solution was smeared on these cards as food for adult parasitoids. Paper cards carrying daily eggs of *E. kuehniella* were taken into glass tubes and offered to newly emerged *T. euproctidis* adults transferred into these tubes for parasitization. Parasitoid adults that completed their development and emerged were transferred to other tubes with fresh *E. kuehniella* egg cards. In this way, parasitoids were continuously reared throughout the year until the end of the study.

Determining efficiency of *Trichogramma euproctidis* against *Lobesia botrana*

The study was conducted in a Sultani Çekirdeksiz vineyard of three-ha trained as "T" trellising system (3 x 2 m) in Research and Application Farm of Ege University in Menemen County in Izmir. Plots are comprised of two different release doses (75 000 and 100 000 parasitoids/ha) and unreleased (Control). Each parasitoid releasing plot includes four parallel rows (100 m in length), considering each row as one replication (Reda, 2004). Control plot consisted of 10 rows. Seventy rows were left between control plot and parasitoid released plots, whereas 10 rows were reserved as buffer area between two parasitoid-released plots.

The egg parasitoid *T. euproctidis* has been released against first, second, third and fourth generation of *L. botrana*. Parasitoid release dates have been chosen in each generation of the pest considering egg-laying period. For the determination of the beginning of egg laying, the criteria of Forecasting System have been followed. One each pheromone trap (Pherocon 1 C, Trece®) was installed in the control and parasitoid-released plots when the accumulation of daily maximum temperatures reached to 1000 °C. The monitoring traps have been checked two or three times until first adult has been captured, then once a week. Parasitoid release dates were timed according to:

a) When the capture of adults began in traps,

b) The accumulation of daily effective temperatures reached to 95–100, 450–460 and 970–980 degree days,

c) Phenological growth stages of grapevine reached to Inflorescence separated, Berries pea-sized and Veraison in 1st, 2nd and 3rd generations, respectively, and

d) Monitoring population fluctuation and new egg laying in 4th generation (Altindisli, 2014).

When the infestation rate of *L. botrana* exceeded 5–6% in grape flowers or bunches, bioinsecticide based on *Bacillus thuringiensis* var. *kurstaki* was applied against larvae (Altindisli et al., 2016). Tractor-mounted orchard sprayer (Model: ATA 400 L-capacity, equipped with 12 ceramic hollow cone nozzles, Agrotek Spraying Machinery & Agricultural Technology Import Export Agricultural Products Industry Trade. Ltd.-Manisa-Türkiye) has been used in the vineyard for pesticide applications. High working pressure (25 bar) and different application rates (700–1100 l / ha) according to grape phenological stages have been used during sprayings.

By estimating the egg laying date of the pest in each generation, release bags containing parasitoid carrier cards were placed in each row at 5 m intervals at the height of the clusters (approximately 1.2 m) (Reda, 2004). Two different doses (75 000 and 100 000 parasitoids/ha) have been applied in each releasing time to determine the most effective dose. *E. kuehniella* eggs of different ages including pre-adult (larval, prepupal and pupal) stages of the parasitoid have been used during releases to cover egg laying period of *L. botrana* as long as possible (Uzun et al., 1996). All application procedures have been similar in two parasitoid-released plots. Biological features of the parasitoid during releases have been explained in Table 1.

Assessment

Grape bunches were checked in parasitoid releasing and control plots to decide the time of parasitoid release and to determine infestation rates. The infestation rates in each plot just before harvest have been analysed statistically to evaluate the effectiveness of the parasitoid against the pest. In order to determine the effect of the parasitoid, 25 grape bunches from each replication, (totally 100 bunches per plot) were checked before harvest. Infection rates in the parasitoid-released and control plots were determined and compared by X^2 test ($P<0.05$) by using the SPSS Statistics Software 21.

Table 1. Some biological features of *Trichogramma euproctidis* in release cards

Таблица 1. Некоторые биологические особенности *Trichogramma euproctidis* в карточках выпуска

Biological Stage	Ratio (%)	Developmental period (Day)
Larva	20	3
Prepupa	20	4
Pupa	30	6
Pupa	30	7

Results

Population fluctuation in pheromone traps

Pheromone traps were hanged into trial vineyard on 8 March 2017, 9 March and 9 March 2019. First adults were captured on 24 March 2017, 15 March 2018 and 29 March 2019, respectively. Weekly trap catches and climatic data regarding average temperature and relative humidity in 2017–2019 are presented in Figure 1, 2 and 3 respectively.

As seen in Figure 1, three generations of *L. botrana* have been clearly observed in the trial vineyard in 2017. Generally, adult population of each generation in parasitoid released plots was lower than control. The highest number of adults captured in the release plots were 33, 40 and 38 observed on 14 April, 07 June and 27 July, respectively. Adults number observed in the control plots, on 14 April, 07 June and 27 July were 40,

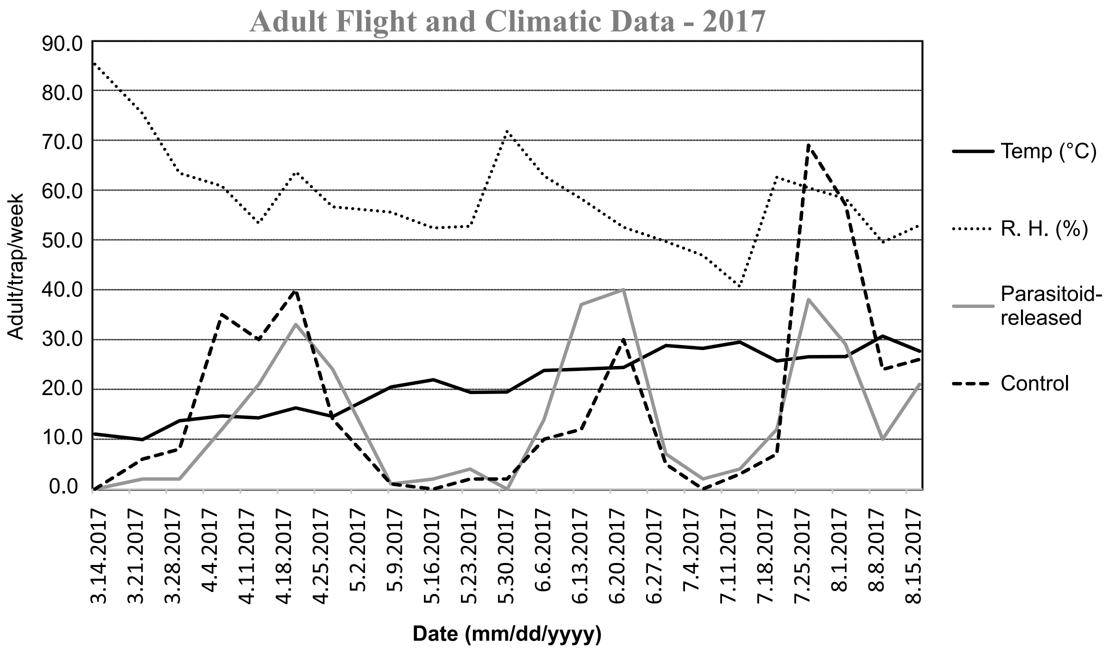

Figure 1. Weekly trap catches of *Lobesia botrana* in parasitoid-released and control plots and climatic data regarding average temperature and relative humidity in Menemen-Izmir in 2017

Рисунок 1. Еженедельные уловы *Lobesia botrana* на участках, где были выпущены паразитоиды, и на контрольных участках, а также климатические данные о средней температуре и относительной влажности в Менемен-Измире в 2017 году

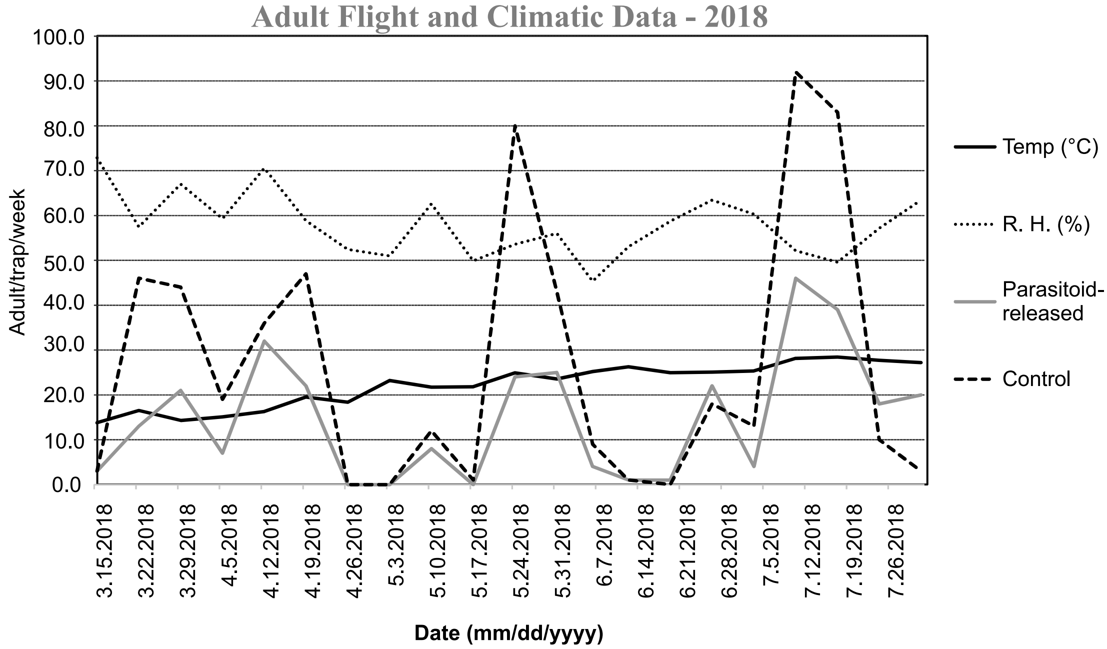

Figure 2. Weekly trap catches of *Lobesia botrana* in parasitoid-released and control plots and climatic data regarding average temperature and relative humidity in Menemen-Izmir in 2018

Рисунок 2. Еженедельные уловы *Lobesia botrana* на участках, где были выпущены паразитоиды, и на контрольных участках, а также климатические данные о средней температуре и относительной влажности в Менемен-Измире в 2018 году

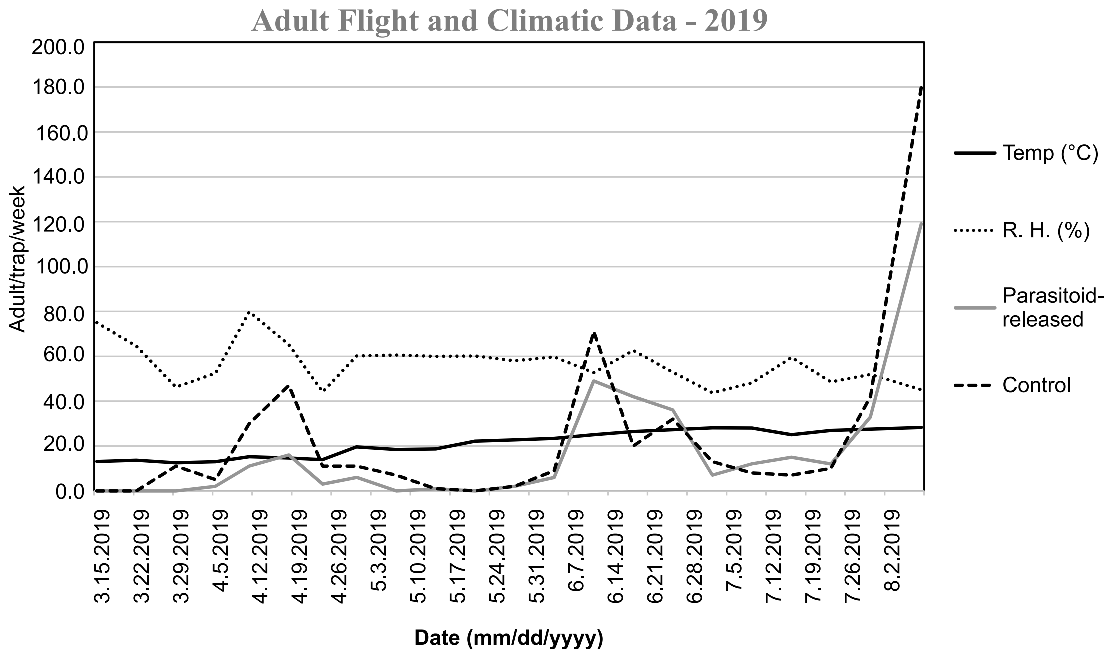

Figure 3. Weekly trap catches of *Lobesia botrana* in parasitoid-released and control plots and climatic data regarding average temperature and relative humidity in Menemen-Izmir in 2019

Рисунок 3. Еженедельные уловы *Lobesia botrana* на участках, где были выпущены паразитоиды, и на контрольных участках, а также климатические данные относительно средней температуры и относительной влажности в Менемен-Измире в 2019 году

30 and 69 respectively, Similarly in Figure 2, adult population of each generation in parasitoid released plots was lower than control in 2018. In the release plots, number of adults observed on 12 April, 31 May and 10 July, were 32, 25 and 46 respectively. In the control plot, on 19 April, 25 May and 10 July, the highest number were detected as 46, 80 and 92 adults, respectively. However, earlier adult emergence and two peaks were observed in the first generation probably due to warmer winter and extreme climatic conditions such as heavy rain between 24 and 26 March and low temperature (min. 4 °C) on 30 March 2018. Rainy period has been repeated in the first three weeks of June 2018, inhibiting adult flight and decreasing trap catches. It caused overlapping of second and third generations in June and July by occurring egg-laying period with one-week interval on 19 and 26 June 2018 and longer egg laying period in July 2018 (Table 2). Grapevine phenology and *L. botrana*

biology developed earlier in 2018. Grape berries matured and were harvested two weeks earlier than 2017 (Table 3).

As shown in Figure 3, adults of *L. botrana* have been observed in the monitoring traps throughout the season in the control and release plots since March 29, 2019. In the release plots, three peaks were observed on 18 April, 12 June and 8 August 2019, capturing 16, 55 and 119 adults. In the control plot, on the same dates, the highest number were detected as 47, 80 and 180 adults, respectively. Studies have shown that the first adults of *L. botrana* emerge in the second half of March in the vineyards of Izmir (Özsemerci et al., 2016). Similarly, it has been determined that the first adult flight of *L. botrana* in the vineyards of Hatay Province begins in March and the pest generally produces three generations a year, once in April, June and July in the southern Türkiye (Çağlar, 2009).

Determining efficiency of *Trichogramma euproctidis* against *Lobesia botrana*

Dates when the Forecasting criteria including egg detection were met and used for the timing of *Trichogramma* releases, were given in Table 2, whereas dates of counting, complementary B.T. applications against *L. botrana* and infestation rates in each generation of the pest were presented in Table 3.

Table 2. Dates of Forecasting criteria for the timing of *Trichogramma* releases against *Lobesia botrana* in Menemen-Izmir in 2017, 2018 and 2019

Таблица 2. Даты прогнозирования критерии определения сроков выпуска *Trichogramma* против *Lobesia botrana* в Менемен-Измире в 2017, 2018 и 2019 годах

2017			
Peak (Gen.)*	d-d*	Egg det.*	Phenological stage
04/14 (1.)	92	n.d.*	Inflorescence sep.
-	400	05/31	Pea-sized berry
06/07 (2.)	465	06/06	Pea-sized berry
-	979	07/11	Before veraison
07/27 (3.)	1216	07/27	Before veraison
-	1430	08/09	Maturation
08/17 (4.)	1558		Maturation
2018			
Peak (Gen.)*	d-d*	Egg det.*	Phenological stage
04/19 (1.)	95	n.d.*	Inflorescence vis.
-	435	05/18	Pea-sized berry
05/25 (1.)	476	05/22	Berries small
-	850	06/19	Bunch closure
06/26 (2.)	970	06/26	Berries still green
-	1085	07/03	Before veraison
07/10 (3.)	1284	07/10	Veraison
2019			
Peak (Gen.)*	d-d*	Egg det.*	Phenological stage
04/18 (1.)	134	n.d.	Inflorescence vis.
06/11 (2.)	438	05/31	Pea-sized berry
-	589	06/11	Pea-sized berry
	707	06/20	Bunch closure
-	1008	07/09	Before veraison
-	1204	07/23	Veraison
08/08 (4.)	1329	07/30	Maturation

*: Peak (Gen.): Peak (Generation); d.d.: degree-day; Egg det.: Egg detection; n.d.: not detected.

As shown in Table 2 and 3, the first release of the parasitoid has been made on 19 April 2017 in the trial plots even before egg laying since other necessary criteria such as flight peak, phenology and degree-day not to get risk in the beginning. When the damage of the first generation larvae became noticeable in the inflorescences, very high infestation rates were detected in two parasitoid release plots (21.6 and 22.9 %) and in the untreated control plot (30.7 %) in the assessment on 18 May 2017. It was observed that a single release is not sufficient to be effective during the long egg-laying period in the first generation. Trandeva (1993) reported that *L. botrana* lays a large number of eggs during the first generation, but the egg parasitoids cannot be effective in this period due to precipitation and low average temperatures, and the effectiveness of the releases begins to increase from the second generation.

Table 3. Dates of counting, parasitoid release, BT applications and infestation rates of *Lobesia botrana* in Menemen-Izmir in 2017, 2018 and 2019

Таблица 3. Даты учета, выброса паразитоидов, применения BT и уровня заражения *Lobesia botrana* в Менемен-Измире в 2017, 2018 и 2019 годах

2017					
Count	Release	B.T. Appl.*	Infestation rate (%)		
			75000	100000	Control
04/19	04/19	-	-	-	-
05/18	05/31	-	21.6	22.9	30.7
06/06	06/07	05/06	10.0	22.0	24.0
07/06	07/11	-	7.0	10.0	39.0
07/11	07/27	-	6.7	1.7	39.4
07/27	08/01	-	7.3	7.4	33.0
08/09	08/09	-	14.0	10.0	43.1
08/17 (harvest)			7.5	12.5	25.0
2018					
Count	Release	B.T. Appl.*	Infestation rate (%)		
			75000	100000	Control
04/19	-	04/20	14.0	10.0	38.0
05/18	05/18	-	8.0	5.0	16.0
05/22	05/25	-	5.0	4.0	25.0
05/31	-	-	12.0	6.0	20.0
06/26	06/27	-	11.0	3.0	26.0
07/23	07/23	07/10	13.0	4.0	30.0
07/31 (harvest)			11.0	4.0	33.0
2019					
Count	Release	B.T. Appl.*	Infestation rate (%)		
			75000	100000	Control
05/31	05/31	04/26	7.0	3.0	14.0
06/11	06/12	-	9.0	4.0	12.0
06/20	06/20	-	8.0	4.0	27.0
06/25	-	-	20.0	5.0	41.0
07/02	07/09	-	14.0	6.0	38.0
07/09	07/23	-	20.0	4.0	41.0
07/23		-	12.0	5.0	48.0
07/30	07/31	-	-	-	-
08/08 (harvest)			11.0	5.0	70.0

*: B.T. Appl.: Bioinsecticide application based on *Bacillus thuringiensis* var. *kurstaki.*

Since spraying could not be done against the first generation of *L. botrana*, infestation rates of second generation were determined as 10 and 22% in the release plots on 6 June 2017. One day before parasitoid release, a complementary application with *Bacillus thuringiensis* var. *kurstaki* was made against the second-generation larvae to help suppressing high population density (Table 3). Although more parasitoids were released, the infestation rate in clusters was found to be higher in 100 000 parasitoids/ha plot compared to 75 000 parasitoids/ha plot in 2017.

It was considered that higher infestation rate in the 100 000 parasitoids/ha application occurred because the 10 rows of safety strips left between the 100 000 parasitoids/ha application plot and control plot were not sprayed. For this reason, in the experiment conducted in 2018, the number of rows between 100 000 parasitoids/ha releasing plot and control plot as safety increased from 10 to 70 and were applied regularly with B.T. In the plots that had a total of 7 times parasitoid releases during the season in 2017, the infestation rate in the clusters was found to be lower (7.5 and 12.5%) than in the control (25%), but it is seen that it is not at an acceptable level.

In 2018, when the injury of first generation became clear, a very high infestation level by larvae was detected in the release and control plots (14%, 10% and 38%) in the assessment on April 19. Therefore, an application with B.T. was made on 20 April 2018 against the first generation before the start of parasitoid releases. This application has been very useful in reducing the population level of the pest. Between 18 May and 10 July, from the second generation to the end of the third generation for 8 weeks, the pest could be controlled through four releases, especially in 100 000 parasitoids/ha-released plot. However, due to an unexpected problem on the rearing of *T. euproctidis*, no release could be made on 10 July 2018, and it was obligatory to spray with B.T. once again (Table 3). After this date, the problem in production was solved and a release against the fourth generation was made on July 23, 2018. After this last release, the density of the pest did not increase until harvest. In the final assessment made on July 31, 2018, the infestation rates were 33% in the control, 4% in 100 000 parasitoids/ha, and 11% in 75 000 parasitoids/ha-released plots. Harvest in the trial area started on 31 July 2018. With the release of 100 000 parasitoids/ha for 5 times in total, the infestation rate of *L. botrana* in the clusters was the lowest compared to the control and at an acceptable level in terms of the success (<5–6%) (Altindisli et al., 2016).

Based on grape phenology, pest biology and accumulation of degree-day, an application was made against the first generation of *L. botrana* using B.T. on April 26, 2019, in order to reduce the population density. This application has been very useful in controlling the population of the pest, and the infestation level did not increase after the releases of parasitoids until harvest, especially in the application of 100 000 parasitoids/ha. In the control plot, the infestation rate of the pest reached a very high level. Because of the counting made on May 31, 2019, at the beginning of the second generation damage, larval infestation rates of 7%, 3% and 14% were determined in the parasitoid release and control plots, respectively (Table 3). The first parasitoid release was made same day just after the assessment count.

Afterwards, as newly laid *L. botrana* eggs were found on 11 June 2019, the second parasitoid release was made on 12 June 2019. The pest was monitored until the harvest and when new eggs were found in the vineyard, parasitoid release was repeated if more than one week had passed since the previous release. Although adults were regularly captured in the traps in July, a significant peak did not occur in the third generation (Table 2). Harvest in the experimental area started on August 8, 2019, when adult numbers began to increase in the traps.

In the last assessment before harvest on August 8, the infestation rate was recorded as 70% in the control, 5% in the application of 100 000 parasitoids/ha, and 11% in the application of 75 000 parasitoids/ha (Table 3). With the release of 100 000 parasitoids/ha 6 times in the season, it is seen that the infestation rate in the clusters is the lowest compared to the control and is at an acceptable level in terms of the success of the efficacy. In summary, no other insecticide was used in 2017, 2018 and 2019, except for biological pesticide applications of B.T. recommended by us in order to reduce the initial population density of *L. botrana*, which was very high in all the plots. As known, B.T. used to control this pest in vineyards has been found to be harmless against many Trichogramma species (Smith, 1996; Reda, 2004).

In this study, the parasitoid carrier cards in the release bags were brought to the laboratory and examined under a microscope one week after each release to determine the hatching rates of parasitoid eggs in the nature. The hatching rates of parasitoid eggs released in the field were found to be 86%, 83% and 90% in 2017, 2018 and 2019, respectively. Reda (2004) obtained similar results with 77.7–97.4% parasitoid emergence rates in the released labels in the efficacy study of many Trichogramma species against *L. botrana* in the vineyard.

As stated in biological control studies using Trichogramma species, evaluating parasitism rates alone is insufficient to determine the effectiveness of the parasitoid. It has been reported that the efficacy of egg parasitoids from *Trichogramma* and other genera can only be interpreted in relation to an indicator showing host population level per unit area (for example, decrease in infestation rate) (Smith, 1996; Reda, 2004). For this reason, this study was designed in such a way as to make an evaluation on the infested bunch number only. In order to determine the effectiveness of the released parasitoid, the infestation rates before harvest were compared and evaluated (Table 4).

The two applications experiment were compared in pairs with the Chi-square method and grouped statistically. There was no difference between the two release doses in 2017 in terms of infestation rates in the clusters (X^2= 1.510; *S.d*= 2; *p*= 0.219); A difference was found in 2018 (X^2=33.859; *S.d*= 2; p= 0.00) and 2019 (X*2* =126.20; *S.d=2*, p=0.00). As a result of the pairwise comparisons, the control plot formed a different group from *Trichogramma*-released plots in three years: in 2017 – [(X^2= 13.469; p.d= 1 p= 0.001); in 2018 – (X^2=4.348; p.d= 1 p= 0.037) and in 2019 – X^2 =2.496; p.d=1; p=0.118)] (Table 4).

In 2018 and 2019, the lowest infestation rates were determined as 4% and 5% for two consecutive years, respectively. Accordingly, it is thought that the release of 100 000 parasitoids/ha provides the lowest cluster infestation rate compared to the control and remains below 5–6%, which is an acceptable level for the success of environmentally friendly control (Anonymous, 2014; Altindisli et al., 2016).

Table 4. Infestation rates of *Lobesia botrana* in Menemen-Izmir just before harvest in 2017, 2018 and 2019

Таблица 4. Уровень заражения *Lobesia botrana* в Менемен-Измире непосредственно перед сбором урожая в 2017, 2018 и 2019 гг.

Treatment	2017				
	Total # of bunches	Infested bunch		Uninfested bunch	
		#	Ratio (%)	#	Ratio (%)
75 000 parasitoids/ha	106	8	7.5 *a*	98	92.5
100 000 parasitoids/ha	120	15	12.0 *a*	105	87.0
Control	104	26	25.0 *b*	78	75.0
	2018				
	Total # of bunches	Infested bunch		Uninfested bunch	
		#	Ratio (%)	#	Ratio (%)
75 000 parasitoids/ha	100	11	11.0 *b*	89	89.0
100 000 parasitoids/ha	100	4	4.0 *c*	96	96.0
Control	109	36	33.0 *a*	73	76.0
	2019				
	Total # of bunches	Infested bunch		Uninfested bunch	
		#	Ratio (%)	#	Ratio (%)
75 000 parasitoids/ha	100	11	11.0 *b*	89	89.0
100 000 parasitoids/ha	100	5	5.0 *c*	95	95.0
Control	100	70	70.0 *a*	30	30.0

Discussion

In the research carried out within the scope of integrated control in the vineyards in Bulgaria, *Trichogramma dendrolimi*, *T. embryophagum* and *T. pintoi* have been released three times against the first and second generations of *L. botrana* (400 000, 600 000 and 400 000 parasitoids/ha) as well as sprayings of insecticides and fungicides that are necessary. *T. embryophagum* and *T. dendrolimi* suppressed *L. botrana* population below the targeted level, keeping infestation rates at 2 % and 5.8 %, respectively in 1993 (Trandeva, 1993). In Germany, Reda (2004) released different strains of 11 *Trichogramma* species (220 000 parasitoids/ha) once against the second and third generations of *L. botrana* in vineyard areas where pesticides are also used. The highest reduction in cluster infestation rate was recorded in the plots where *T. cacoeciae* (Cac-94) was released with an average of 83.2 %. Unlike this study, the lowest infestation rates were obtained with higher parasitoid number and less release number.

El Wakeil et al. (2009,) obtained the lowest cluster infestation rates of 9.2 % and 4.4 %, respectively, applying two release doses (50 000 parasitoids/ha and 75 000 parasitoids/ha) of the egg parasitoid *Trichogramma evanescens* four times against *L. botrana* in vineyard areas in Egypt. In this study, a similar lowest infestation rate of 4 % was obtained by using higher parasitoids (100 000 parasitoids/ha) and release numbers at shorter release interval.

As can be seen above, successful results were obtained below the targeted levels in studies conducted with different species, doses, release numbers and intervals regarding the release efficiency of *Trichogramma* species against different population densities of *L. botrana* in different countries and grape varieties. To date, the effectiveness of *Trichogramma* egg parasitoids released against *L. botrana* in vineyards has varied according to the selected parasitoid species and quality (lifetime, egg laying, search capacity and hatching rates), release rate and frequency, pest density, variable climatic and growing conditions, release and evaluation criteria (Smith, 1996; Moreau et al., 2009). For example, in the studies conducted by Trandeva (1993), Reda (2004) and El Wakeil et al. (2009), other parameters such as day-degree and plant phenology in the Forecasting System were not used to determine the release time of the egg parasitoid, apart from adult flight monitoring of the pest with pheromone traps. In addition, higher doses and longer release intervals have been used (Trandeva, 1993; Reda, 2004). Except for the study conducted by El Wakeil et al., (2009) in Egypt, the lowest number of parasitoids and the lowest cluster infestation rates were obtained in this study.

In the Aegean Region, where viticulture is the most common and economically important, *Lobesia botrana* is the key pest of grapevines as it directly damages the fruit. When any wrong application is made regarding the control of the pest in the first generation, the population of the pest is usually high in the following generations and the damage increases. In order for the biological control of the pest to be successful, the management strategy is to reduce the population density with an appropriate insecticide against the first generation, preferably with B. T.. Then, when the eggs of the second, third and, if necessary, fourth generations begin to appear or when they are expected to be laid, it should be suppressed by releasing 100 000 *T. euproctidis* parasitoids/ha up to six times with 1–2 week intervals. Depending on the continuation of the oviposition period, the number of releases should be reduced or increased.

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Полнотекстовая статья

ОПРЕДЕЛЕНИЕ ЭФФЕКТИВНОСТИ *TRICHOGRAMMA EUPROCTIDIS* ПРОТИВ ОСНОВНОГО ВРЕДИТЕЛЯ, ГРОЗДЕВОЙ ЛИСТОВЕРТКИ *LOBESIA BOTRANA* (LEPIDOPTERA: TORTRICIDAE), НА ВИНОГРАДНИКАХ ЭГЕЙСКОГО РЕГИОНА, ТУРЦИЯ

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Европейская гроздевая листовертка *Lobesia botrana* – это наиболее опасный вредитель, вызывающий потери урожая в большинстве регионов выращивания винограда в Турции, питаясь в основном соцветиями и гроздьями винограда. Это исследование было проведено для определения эффективности выпуска и наиболее эффективной дозы местного паразитоида яиц *Trichogramma euproctidis* Girault (Hymenoptera: Trichogrammatidae) против *L. botrana* в органическом винограднике, расположенном в районе Менемен-Измир Эгейского региона, который имеет самую большую площадь производства винограда. Были применены две разные дозы выпуска, 75 000 и 100 000 паразитоидов/га, чтобы выбрать более эффективную. Эффективность паразитоида сравнивали по степени заражения вредителями гроздей винограда с участков, где производился и не производился выпуск, непосредственно перед сбором урожая. Зараженность участков, на которых было выпущено 100 000 паразитоидов/га (12, 4 и 5%) и 75 000 паразитоидов/га (7.5, 11 и 11%), была ниже, чем на контрольных участках (25, 33 и 70%), в 2017, 2018 и 2019 годах, соответственно. В 2018 и 2019 годах наиболее перспективной, показавшей самую низкую зараженность вредителем, оказалась более высокая доза выпуска (100 000 паразитоидов/га) с кратностью применения пять или шесть раз. В этом исследовании впервые в Турции была определена наиболее эффективная доза выпуска (100 000 паразитоидов/га) *T. euproctidis*. Результаты, полученные в этом исследовании, будут способствовать использованию этого паразитоида в программах органической и интегрированной борьбы с вредителями на винограде.

Ключевые слова: яйцевой паразитоид, виноград, биологический контроль, вредитель

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