Environmentally Friendly Strategies for Controlling the European Grapevine Moth, *Lobesia botrana* (Lepidoptera: Tortricidae)

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Abstract

Terlemezyan, H., M. Sargsyan, H. Harutyunyan, S. Sargsyan and N. Zarikian. 2023. Environmentally Friendly Strategies for Controlling the European Grapevine Moth, *Lobesia botrana* (Lepidoptera: Tortricidae). Arab Journal of Plant Protection, 41(3): 272-277. https://doi.org/10.22268/AJPP-041.3.272277

The European grapevine moth, *Lobesia botrana* (Denis & Schiffermüller, 1775) (Lepidoptera: Tortricidae), a palearctic pest, has been the target of an international control campaign. Agricultural production areas in Armenia were identified as lacking effective control in 2019-2020. The results of using a mixture of bacterial and chemical insecticides (BT_{TER-55} + Coragen, BT_{TER-94} + Coragen) were effective in controlling this pest. Coragen combined with sublethal concentration of bacterial insecticides (BT_{TER-55} + Coragen, BT_{TER-94} + Coragen) showed high biological efficiency against young larvae of grapevine moth under vineyard conditions. It has been proven also that, Coragen together with the applied insecticides that fell on the soil after spraying were less dangerous ecologically since they reduced the number of soil-living ammonifiers for only a short period (1-2 months). In addition, results obtained confirmed that the bacterial insecticides BT_{TER-55} and BT_{TER-94} differed in morphological and physiological features, particularly in the size of vegetative cells and spores formed by them, as well as in the size of insecticidal crystalline bodies, colonies shape and assimilation of carbon and nitrogen sources.

Keywords: Armenia, European grapevine moth, insecticide, Invasive pests, Plant protection.

Introduction

The European grapevine moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) historically has been a pest in the Mediterranean regions of Europe, Asia, North Africa, and then introduced into the Americas with the first detection in Chile in 2008 (Ioriatti *et al.*, 2011; 2012). Grapevine flowers and berries are beloved hosts for this moth. Also, they can be found on olive flowers, blueberries, and plums. An increase in the yield of viticulture is often hindered by the European grapevine moth *Lobesia botrana* (Denis & Schiffermüller, 1775) (Lepidoptera), affecting the crop's yield and quality (Arestova & Ryabchun, 2017; Thiéry *et al.*, 2018). Thus developing and implementing control approaches that are highly effective and environmentally safe are strongly needed. Because chemical control of garden pests caused a number of undesirable consequences for the environment (Belyaev & Nozdrenko, 2004; Ivantsova, 2004), special attention was paid to the use of microbiological, especially BT-type bacterial preparations, which showed high biological effectiveness against harmful insects, and at the same time was safe for humans, warm-blooded animals, beneficial entomofauna and fish (Africyan, 1973).

It is known that the dominant part of the sprayed pesticides (60-99%) eventually drop into the soil in different ways (through spraying, precipitation, falling of leaves, etc.) (Chigarev *et al.*, 1974; Yablokov, 1987). Plant protection research often pays attention to the economic effectiveness of the insecticides used, but their impact on the soil fertility indices is neglected, which from an ecological point of view makes it impossible to predict and prevent the undesirable manifestations of the insecticides applied (Sargsyan, 2013).

Thus, this study summarized the results of the bacterial and chemical insecticides against European grapevine moth (EGM) larvae of early instars and assessed the influence of the insecticides that fell on the soil through spraying (both individual and combined with sub-lethal concentration) on the quantity of the ammonifiers (ammonifying bacteria) that defined the fertility of light-brown soil.

Materials and Methods

In Armenia, *L. botrana* typically completes three generations, and this study was conducted on the second generation, and control was based on data obtained from pheromone traps. The second generation of male adults were collected using pheromone traps ("Ararat" traps made in Armenia) in the vineyard of Arpi region, Vayots Dzor province (Republic of Armenia) during the period June 2019 to June 2020.

On one hectare, three traps were placed one meter above ground level. Every 2-3 days, the traps were inspected and data was collected. As a result, the 1st instar larvae of the second generation of the European grapevine moth (EGM) were collected. The preparations evaluated in this study were as follows: Local BTTER-55 and BTTER-94 bacterial insecticides (named after the founder and one of the authors of this paper, Hrant Terlemezyan) that were isolated from the larvae of the naturally deceased *Lobesia botrana* (Denis & Schiffermüller, 1775) and apple moth, *Hyponomeuta malinellus* (Zeller, 1838); Lepidocide

https://doi.org/10.22268/AJPP-041.3.272277

(commercial bacteriological preparation powder BA 3000 UA/mg, produced by "Sibbiopharm" (Russian Federation); Coragen (active ingredient: chlorantraniliprole, produced by Syngenta (Switzerland); and Arrivo (active ingredient cypermethrin, produced by FMC (USA). In addition, grapevine variety Areni, light-brown soil, and soil-inhab (ammonifiers) were used. Bacterial (as cultuyre fluids) and chemical (as water suspension) insecticides were tested individually and in combination against early-instars of EGM larvae. The Armbiotechnology Research and Production Center produced the BT_{TER-55} and BT_{TER-94} culture fluids, at a concentration of 600 million spores/ml. Insecticides with sub-lethal concentrations were included in the "culture fluid + chemical preparation" combinations. The culture fluid and the water suspension of the chemical preparation were diluted three and four times, respectively as compared with the lethal concentration.

The spraying was done in June, early in the morning, with an OVT-1A tractor sprayer. The working fluid consumption was 1000 litres per hectare. Each treatment was replicated three times. The control was an unsprayed vineyard patch infested with EGM larvae. The reference preparations were Lepidocide from the bacteriological group and Arrivo from the chemical group.

The biological effectiveness of the tested preparations was assessed in accordance with the methodological guidelines reported earlier (Fedorinchik, 1973; Ganiev & Nedorezkov, 2006). The size of vegetative cells, spores, and crystalline bodies were determined as describe earlier (Egorova, 1976). The Hiss's serum water medium was prepared using a previously described method (Labinskaya, 1963).

The number of ammonifiers in light-brown soil (both sprayed and unsprayed (control) with insecticides were determined during the growth period (June-September) under laboratory conditions (Netrusov, 2005). The experimental data was then statistically analysed (Ashmarin *et al.*, 1962; Berstein, 1968; Birkhofer *et al.*, 2017; Orlov, 2006).

Results and Discussion

The preparations BT_{TER-55} and BT_{TER-94} are both prokaryotic rod-shaped vegetative bacteria with approximate dimensions of 4.02 × 1.52 and 3.84 × 1.20 μm, respectively (Table 1). BT_{TER-55} and BT_{TER-94} pathogens formed intracellular oval spores (with mean sizes of 1.78 x 0.90 and 1.60 x 0.84 μm, respectively) and toxic crystalline bodies with mean dimensions of 1.62 x 1.28 and 1.48 x 1.16 μm , respectively) at a young stage (3 days after sowing). The synthesis of spores and crystalline bodies in vegetative cells occurred simultaneously in a 1: 1 ratio.

It was significant that, during the ageing phase, in addition to sporulation, the strain BT_{TER-55} synthesised morphologically different shapes (oblique, oval, square, irregular, boat-shaped, and so on) as compared to only diagonal crystals by the BT_{TER-94} strain.

There was no correlation between the insecticidal activity of BT bacteria and the appearance of crystalline bodies (Olekh, 1974), who reported that the biological effectiveness of a BT bacterial pathogen is dependent on the physicochemical properties of the crystalline body, particularly the endothelial crystal structure and the amino acids contained within it.

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The aerobic crystallising bacteria BT_{TER-55} and BT_{TER-94} develop well at temperature of 28-30°C on MPA (meat-peptone broth) nutrient medium and form colonies with diameters of 7.4 and 8.2 mm, respectively, and both strains form a fine membrane on the medium surface. BT_{TER-55} assimilates glucose, fructose, sucrose, and arabinose from carbon sources while growing in a Hiss's serum water medium, whereas BT_{TER-94} assimilates glucose, fructose, raffinose, and glycerin. BT_{TER-55} misappropriates valin and peptone from the nitrogen sources of Beyerink's nutrient mediums, liquefies meat peptone gelatin (MPS) (in the affected area by the bacteriological needle, at the 2/3 of the longitudinal test tube column). BT_{TER-94} liquefies MPS as well, but only at the one third of the longitudinal test tube. Nitrate salts (KNO_3, $NaNO_3$, $Ca(NO_3)_2$) were not absorbed by these strains. The data obtained showed that Coragen when sprayed at 0.2 l/ha had a high biological effectiveness (97.1%) against early instars of EGM larvae, 7 days after spraying as well as 4 days after spraying (94.3%) (Table 1).

Table 1. Vegetative cells and spores of BT-type bacterial insecticides present in colonies based on MPA nutrient medium and average size of endothelial crystalline bodies (μm).

	Vegetative cells		spores		crystalline bodies	
Preparation	**length**	**width**	**length**	**width**	**length**	**width**
BT_{TER-55}	4.02	1.52	1.78	0.90	1. 62	1.28
BT_{TER-94}	3.84	1.20	1.60	0.84	1.48	1.16

When compared with the chemical preparations, bacterial BT_{TER-55} and BT_{TER-94} culture fluids sprayed at the rate of 3 l/ha each showed relatively low biological effectiveness of 52.0% and 60.7%, 4 days after spraying, and 88.0 and 89.3%, 7 days after spraying, respectively.

The sub-lethal concentration combinations of BT_{TER-55} + Coragen and BT_{TER-94} + Coragen demonstrated high biological effectiveness against phytophagus larvae (7 days after spraying) that reached 90.9% and 92.3%, respectively. The observed high rates of effectiveness were most likely conditioned by the stimulating effect of Coragen sub-lethal concentration and the combined effect with the bacterial insecticide. Seven days after treatment, the reference preparations, bacterial Lepidocide and chemical Arrivo, gave 86.2% and 90.0% biological effectiveness against early instars of EGM larvae, respectively (Table 2).

Table 2. Mean biological effectiveness of the individual and combined insecticides against early instars of *Lobesia botrana* larvae in the vineyard.

Treatments	Consumption rate of the commercial preparations (l/ha, kg/ha)	Biological effectiveness (%) following treatment in days	
		4 days	7 days
$B_{TER\ -94}$ + Coragen	1.0+0.05	69.2	92.3
Coragen	0.2	94.3	97.1
$BT_{TER\ -55}$	3.0	52.0	88.0
$BT_{TER\ -94}$	3.0	60.7	89.3
$BT_{TER\ -55}$ + Coragen	1.0+0.05	66.7	90.9
Lepidocide (reference preparation)	2.0	62.1	86.2
Arrivo (reference preparation)	0.38	80.0	90.0

According to Student t standard calculation indices, the biological effectiveness of using Coragen was significantly different from the BT_{TER-55} treatment recorded 7 days after spraying. BT_{TER-94} and Lepidocide (reference preparation) treatments (at P=0.05 and n=3), had calculated indices of 4.030, 3.326 and 4.232, respectively, which exceeded the Student t standard tabular value of 3.182). In all other cases, Student t standard calculation indices, which ranged from 0.381 to 3.122, confirmed that there was no significant difference in the biological effectiveness of the individual experimental treatments, as compared to the reference preparations. The statistical figures of the average numbers of dead EGM larvae presented in Table 3 (coefficient of variation and experimental error) fluctuated in the range of 6.41-9.18 and 3.7-5.3%, respectively, confirmed that the results of the experiments were significant. The square deviation value was used as an auxiliary parameter when calculating the coefficient of variation, experimental and average errors. Based on previous work (Emcev *et al.*, 2005), ammonification, which plays an important role in soil fertility, occured when inaccessible organic nitrogen is converted into a mineral nitrogen was easily assimilated by higher plants and soil-inhabiting bacteria.

Based on the aforementioned arguments and experimental data from previously published work (Kazaryan, 2007), indicated that BT-type bacterial insecticides, which drop on the light-brown soil following spraying, remain in the mentioned soil for 4 months with the tendency to decrease the number of ammonifiers involved in the nitrogen cycle. Laboratory tests confirmed that, in response to spraying, ammonifiers quantities significantly decreased for two months (June-July) due to the effects of fallen coragen and arrive chemical insecticides on the soil, compared to the unsprayed control (Table 4). The quantities of ammonifiers were restored in the following months and did not differ significantly from the control (unsprayed soil).

Table 3. Statistical figures of the average quantity of the dead young instars of *Lobesia botrana* larvae obtained 7 days after spraying.

Treatments*	ANDL**	SDM	CV (%)	ME	SE (%)
Coragen	34	2.827	8.31	1.632	4.8
$BT_{TER\ -55}$	22	1.483	6.74	0.856	3.9
$BT_{TER\ -94}$	25	1.732	6.93	1.000	4.0
$BT_{TER\ -55}$ + Coragen	30	2.182	7.27	1.260	4.2
$BT_{TER\ -94}$ + Coragen	24	1.538	6.41	0.888	3.7
Lepidocie (reference preparation)	25	2.295	9.18	1.325	5.3
Arrivo (reference preparation)	27	1.936	7.17	1.118	4.1

* The dosage used is as described in Table 2.

** ANDL: Average number of the dead larvae on 150 grapevine leaves SDM: Square of deviation from the mean; CV: Coefficient of variation; ME: Mean error; SE: Experimental error

Bacteriological studies revealed that non-sporulating bacteria were quantitatively dominant in ammonifiers grown on MPA nutrient medium, followed by actinomycetes and microscopic fungi with a decreasing order. The quantities of actinomycetes and microscopic fungi were negligible in both the experimental and control (unsprayed) treatments. The number of non-sporulating bacteria increased during the growing season, peaking in July and gradually decreased over the following two months. From June to September, the number of soporulating bacteria and actinomycetes increased, whereas microscopic fungi decreased.

Previous work (Emcev & Mishustin, 2005), indicated that the mineralization dynamics (ammonification) of organic compounds containing soil nitrogen depend on soil temperature, humidity and air saturation during the growth period. These factors most likely also influenced the dynamics of the number of ammonifiers in this study. The species composition of ammonifiers is currently being determined. Based on the results obtained in this study, it can be concluded that the BT_{TER-55} and BT_{TER-94} bacterial insecticides differed from each other in terms of vegetative cells, spores, crystalline bodies, and colony sizes, as well as the shape of crystalline bodies produced and the peculiarity of carbon source assimilation. The biological effectiveness of coragen chemical preparation against early-instar of *L. botrana* larvae has been demonstrated. The overall biological effectiveness of the Lepidocide bacterial preparation against phytophagous early-instar larvae was 86.2-89.3% using BT_{TER-55}, BT_{TER-94} culture fluids, and insecticides water suspension. Combinations of chemical and bacterial insecticides with sub-lethal concentrations against EGM larvae demonstrated biological effectiveness comparable with that of Coragen.

It was confirmed that Coragen, Arrivo, and combined insecticides are less harmful because they suppressed the number of soil-inhabiting ammonifiers for a short period of 1-2 months, and it was revealed that the ammonification process in the soil involves sporulating and non-sporulating bacteria, actinomycetes, and microscopic fungi, the quantities of which change during the growth period period, because of the EGM population in the soil.

Table 4. Average statistical figures of the number of ammonifiers after spraying with insecticides in the laboratory experiments of 2019 and 2020.

Treatments*	Months	Average number of the ammonifiers in 1 gr of the dry soil (millions)	Square deviation	Variation coefficient (%)	Average error	Experimental error (%)	Student $t_{standard}$ calculation index
Coragen	June	5.22	0.478	9.16	0.214	4.1	10.894
	July	9.70	0.628	6.47	0.281	2.9	3.570
	August	7.88	0.617	7.83	0.276	3.5	0.603
	September	5.90	0.487	8.25	0.218	3.7	1.016
$BT_{TER\ -55}$	June	10.02	0.718	7.17	0.321	3.2	0.914
	July	13.00	1.308	10.06	0.585	4.5	0.362
	August	7.60	0.476	6.26	0.213	2.8	1.332
	September	6.62	0.740	11.18	0.331	5.0	0.663
$BT_{TER\ -94}$	June	11.24	1.205	10.72	0.539	4.8	2.431
	July	13.42	1.140	8.49	0.510	3.8	0.829
	August	8.20	0.458	5.58	0.205	2.5	0.096
	September	6.62	0.622	9.40	0.278	4.0	0.729
$BT_{TER\ -55}$ + Coragen	June	7.46	0.534	7.16	0.239	3.2	5.077
	July	11.80	1.136	9.63	0.507	4.3	0.893
	August	8.34	0.803	9.63	0.359	4.3	0.339
	September	5.72	0.498	8.71	0.223	3.9	1.46
$BT_{TER\ -94}$ + Coragen	June	6.98	0.843	12.08	0.377	5.4	4.907
	July	10.98	1.129	10.28	0.505	4.6	1.770
	August	8.70	0.740	8.51	0.331	3.8	1.064
	September	6.48	0.420	6.48	0.188	2.9	0.481
Lepidocide (reference preparation)	June	10.80	1.183	10.95	0.529	4.9	1.813
	July	12.40	0.776	6.26	0.347	2.8	0.285
	August	7.86	0.720	9.16	0.322	4.1	0.600
	September	6.20	0.416	6.71	0.186	3.0	0.268
Arrivo (reference preparation)	June	4.90	0.559	11.41	0.250	5.1	10.995
	July	8.56	0.630	7.36	0.282	3.3	9.072
	August	7.94	0.461	5.81	0.206	2.6	0.529
	September	6.11	0.546	8.94	0.244	4.0	0.460
Control soil (unsprayed)	June	9.58	0.642	6.70	0.287	3.0	-
	July	12.64	1.498	11.85	0.670	5.3	-
	August	8.16	0.693	8.49	0.310	3.8	-
	September	6.30	0.619	9.83	0.277	4.4	-

* The dosage used is as described in Table 2.

The Student $t_{standard}$ tabular index (at P=0.05 and n=5) was equal to 2.571, and the "-" control soil index was included in the Student $t_{standard}$ calculation formula.

الملخص

ترلمزيان، هرانت، ماسيس سارجيسيان، هاروتيون هاروتيونيان، سونا سارجيسيان ونوشيغ زاريكيان. 2023. طرائق صديقة للبيئة لمكافحة عثّة العنب الأوروبية (*Lobesia botrana*, Lepidoptera: Tortricidae). مجلة وقاية النبات العربية، 41(3): 272–277.
https://doi.org/10.22268/AJPP-041.3.272277

تمّ في السنوات الاخيرة إطلاق حملة دولية لمكافحة حشرة عثّة العنب الأوروبية، وقد حُدِّدت مناطق الإنتاج الزراعي في أرمينيا كمناطق تعوزها السيطرة الفعالة عليها خلال الفترة 2019–2020. شملت الدراسة استخدام المبيدات الحشرية البكتيرية والكيميائية (BT_{TER-55} + Coragen، BT_{TER-94} + Coragen) لمكافحة هذه الآفة. بيّنت النتائج أن استخدام توليفة من المبيد Coragen مع تراكيز شبه قاتلة من المبيد البكتيري قد أعطى كفاءة حيوية عالية ضدّ اليرقات الصغيرة لعثّة العنب في مزارع كروم العنب. كما ثبت أيضاً أن كمية المبيد Coragen مع المبيدات الحشرية المستخدمة التي سقطت على التربة بعد الرش كانت أقل خطورة من الناحية البيئية حيث أنها قلّلت من أعداد البكتيريا المحولة للأمونيا في التربة لمدة قصيرة فقط (شهر إلى شهرين). كما تمّ التأكيد على أن المبيدات الحشرية البكتيرية BT_{TER-55} و BT_{TER-94} تختلف في سماتها الشكلية والوظيفية/الفيزيولوجية، ولا سيما في حجم الخلايا الخضرية والأبواغ المتشكلة، فضلاً عن أحجام الأجسام البلورية للمبيدات الحشرية والمستعمرات المتشكلة، وتمثيل مصادر الكربون والآزوت.

كلمات مفتاحية: أرمينيا، عثّة العنب الأوروبية، مبيدات، آفات غازية، وقاية النبات.

عناوين الباحثين: هرانت ترلمزيان[1]، ماسيس سارجيسيان[1]، هاروتيون هاروتيونيان[1]، سونا سارجيسيان[1] ونوشيغ زاريكيان[2*]. (1) المركز العلمي لتقييم وتحليل المخاطر في منطقة سلامة الأغذية، SNCO، 2/107 طريق ماسيس السريع، يريفان، أرمينيا؛ (2) المركز العلمي لعلم الحيوان وعلم البيئة المائية التابع للأكاديمية الوطنية للعلوم لجمهورية أرمينيا، يريفان، أرمينيا. *البريد الإلكتروني للباحث المراسل: noushigz@hotmail.com

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Received: May 31, 2022; Accepted: January 16, 2023

تاريخ الاستلام: 2022/5/31؛ تاريخ الموافقة على النشر: 2023/1/16