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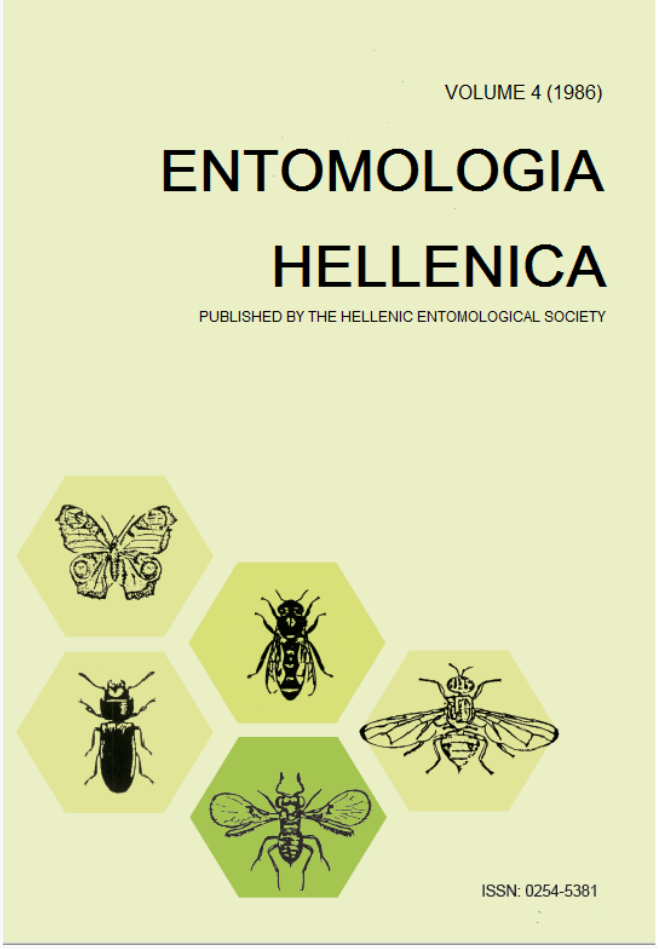


# Effectiveness of Bacillus thuringiensis Berliner var. kurstaki on the grape berry moth Lobesia botrana Den. and Shiff. (Lepidoptera, Tortricidae) under field and laboratory conditions in Crete

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# Effectiveness of *Bacillus thuringiensis* Berliner var. Kurstaki on the Grape Berry Moth *Lobesia botrana* Den. and Shiff. (Lepidoptera, Tortricidae) under Field and Laboratory Conditions in Crete[1]

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**ABSTRACT**

The effectiveness of *Bacillus thuringiensis* Berliner var. Kurstaki (Dipel Abbot Lab.) against the grape berry moth (*Lobesia botrana* Den. & Shiff.) was evaluated under laboratory and field conditions. Under laboratory conditions (24±0.5°C, 55±5% r.h., 2,000 Lux, and 16 hours light daily) *B. thuringiensis* was compared to triflumuron and methomyl on egg hatching and shallow entries per grape berry. *B. thuringiensis* had no effect on egg hatching while methomyl and triflumuron had ovicidal action. Shallow entries per grape berry were 0.9-1, 0,0 and 4.55 for *B. thuringiensis*, triflumuron, methomyl and control, respectively. Under field conditions, using as criteria for the timing of sprays: a. pheromone and food trap catches and b. visual counting of egg laying and a threshold of 20-30 eggs/100 grapes, two applications of *B. thuringiensis* at Kastelli Pediados in 1981 and four at Peza in 1982 were made both at the 2nd and 3rd flights. Its effectiveness was 96-100% and 92% at Kastelli Pediados and 73-75% at Peza.

## Introduction

The microbial insecticide *Bacillus thuringiensis* Berliner (Bt) possesses a number of important attributes that favor its use in a pest management programme. Among these are its generally nontoxic nature to both plants and vertebrates and its relatively specific action on certain insect species (Burges and Hussey 1971, Yamvrias 1962, 1964, Oatman and Legner 1964). It has shown considerable promise in the control of several important lepidopterous pests while having no adverse effect on beneficial species (Frischknecht and Muller 1976). So, an effective biological control agent as part of an integrated control programme could contribute to grape berry moth (GBM) control, limiting the use of toxic chemicals hazardous to the environment and human health. It must be noticed that growers of table grapes are among the heavier users of pesticides.

Many authors have described the susceptibility of GBM to a variety of strains of Bt (Roehrich 1970, Stoeva 1978, Akhmedov 1974, Buess and Bassand 1976, Boller and Remund 1981, Baillod et al. 1985). Bt has been also used effectively in the field mainly against lepidopterous pests e.g. the Mediterranean flour moth *Anagasta kuehniella* Zeller (Yamvrias 1962), the olive moth *Prays oleae* Bern (Yamvrias 1964, 1972), and the eye spotted bud moth *Spilonota ocellana* Denis et Shiff. (Oatman and Legner 1964). Because neonate larvae of *L. botrana* feed on berry tissue for only a short period before entering the berry, dosage and timing of applications of the control agent are quite critical and may be the main cause of the variable effectiveness observed in field tests (Buess and Bassand 1967, Boller and Remund 1981, Valli 1978, Schmid and Antonin 1977,

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Roehrich 1970, Fillip and Alexandri 1975, Colbrie 1980). Several feeding stimulants increase the effectiveness of Bt such as 1% sugar (Boller and Remund 1981, Baillod and Schmid 1978), 5% sucrose (Abdul-Sattar et al. 1982). It is also known that UV affects the viable spores of Bt (Falcon 1971, Burges and Hussey 1971, Roehrich 1970) but the crystalline toxin remains unaffected. The special type of installation (crevatina) of table grape cv Razaki permits good coverage of the berries since most grapes are suspended beneath the horizontal wire frame used to support the vine shoots while UV is greatly impeded by leaves arranged over the grapes. For the above reasons we thought that it would be very interesting to test the effectiveness of a certain Bt formulation in the field under the conditions of Crete island. Good control on the grape berry moth could reduce the use of broad spectrum insecticides hazardous to the beneficial fauna and human health.

## Materials and Methods

The experiments were carried out at Kastelli Pediados in 1981 and at Peza in 1982 which are the main areas of Crete growing table grapes cv Razaki. The timing of applications was determined by the following criteria: a. pheromone (Pherocon 1C Zöecon) and food traps (water: vinegar 6‰: sugar, 0.940 lt: 40cm$^3$: 20gr) checked and serviced three times a week, b. visual counting of egg laying on grape berries at intervals of three to four days (period shorter than the development time of eggs from oviposition to hatch in the field, Table 1), starting point of egg counts was the first burst of flights, c. the threshold for application was 20-30 eggs/100 grapes cumulatively. The application was repeated if the egg laying period was over 10 days. Counts of egg laying were on 16 grapes per plot (four grapes per plant sampled). The experiments were arranged in a randomized block design with four replications of 16 vine plants per plot (4X4) of which only the four internal ones were sampled. Spraying was applied by a Knapsack sprayer, and a volume of 10 lt of insecticide solution/block was used. In April and May sprays could not be timed properly during the 1rst flight because of inclement weather, thus we treated the 2nd and 3rd flights. There were used: a. the commercial formulation Dipel (*B. thuringiensis* var. Kurstaki from Abbot Laboratories) I.U. 16,000 in dose 20 gr WP/10 lt water, b. the feeding stimulant CoAX (cottonseed flour, disaccharide, vegetable lipil oil, ethoxylated ester Zöecon) in dose 10 gr/10 lt water in combination with a half dose of Dipel (10 gr/100 lt water).

Laboratory studies were designed to determine the efficacy of *B. thuringiensis* sprayed on grape berry moth eggs in the field in comparison with methomyl and triflumuron (chitin synthesis inhibitor). Triflumuron has ovicidal properties, although the embryo develops the larva is unable to hatch, and exhibits reduced harmful effect on the beneficial fauna (Hammann and Sirrenberg 1980, Schmidt and Dorntlein 1980). This compound has not been registered in Greece as yet. Methomyl, a conventional insecticide with ovicidal properties on grape berry moth eggs (Marcelin 1983) has a restricted use in Crete because of absence of experimental data under local field conditions, while it has been widely used against greenhouse pests.

All eggs were obtained from a six month old laboratory culture (24±0.5°C, 55±5% r.h., 2,000 Lux 16 hours daily). Strips of parafilm paper on which the eggs had been laid were immersed in the solution of *B. thuringiensis* in the dosages mentioned above with gentle agitation for 20 seconds. Methomyl (Lannate Du Pont) and triflumuron (Sir Bayer) concentrations

TABLE 1. Fluctuation of egg density (eggs/100 grapes) of grape berry moth (GBM) on table grape cv Razaki at Kastelli Pediados and Peza in 1981 and 1982, respectively.

| Kastelli Pediados 1981 | | | | Peza 1982 | | | |
|---|---|---|---|---|---|---|---|
| II gen. | | III gen. | | II gen. | | III gen. | |
| 20/6/81 | 2 | 18/8/81 | 0 | 22/6/82 | 6 | 18/8/82 | 14 |
| *23/6/81 | 8 | 20/8/81 | 2 | 26/6/82 | 15 | 20/8/82 | 26 |
| 26/6/81 | 1 | 23/8/82 | 10 | *30/6/82 | 29 | *23/8/82 | 39 |
| 30/6/81 | 0 | *26/8/81 | 7 | 5/7/82 | 10 | **27/8/82 | 52 |
| | | 30/8/81 | 0 | 10/7/82 | 12 | *1/9/82 | 10 |

* Dates of application of GBM.
** Repetition of application because of a 25 mm precipitation on 24/8/82.

TABLE 2. Effectiveness of Bt and Bt (half dose)+feeding stimulant according to mean number of injuried grape berries under field conditions in Crete. In each column, means followed by the same letter are not significantly different by Duncans multiple range test (P=0.05). Volume of spray/application was 10kg/4 vines plot. Applications were at Kastelli: 1981 June 23, Aug. 26 and Peza: 1982 June 30, Aug. 23 and 27, Sept. 1.

| | Kastelli Ped. 1981 | | Peza 1982 | |
|---|---|---|---|---|
| Treatment | II gen. | III gen. | II gen. | III gen. |
| Bt | 0.00 a | 0.3 a | 2.8 a | 6.4 a |
| Bt (1/2 dose)+CoAX | 0.06 a | 0.3 a | 2.5 a | 3.7 a |
| Control | 1.43 b | 3.4 b | 10.2 b | 17.0 b |

were 0.03% and 0.013% a.i., respectively. Unripe grape berries of cv Razaki were also immersed in the same manner and the treated eggs and berries were transferred to glass Petri dishes in a temperature cabinet (24±0.5°C, 50±5% r.h., 2,000 Lux for 16 hours daily). One hundred eggs from each treatment were examined to determine percent egg hatch. Also, 25 newly hatched larvae were transferred individually to the treated berries. Seven days later mortality and shallow entries were measured. There were four replicates.

## Results and Discussion

The effectiveness of *B. thuringiensis* was noticeable, 96-100 and 92% in the 2nd and 3rd generation respectively, at Kastelli Pediados in 1981 but at Peza it was moderate, 73-75% and 63-79% in the 2nd and 3rd generation respectively, in 1982 (Table 2). The difference was possibly due to the higher egg density (Table 1) and the longer egg laying period in 1982. There was no significant difference between the effectiveness of the normal dose of Bt (20 gr/10 lt water) and half dose combined with a feeding stimulant. In laboratory tests, Bt had no effect on egg hatching but a significant mortality on newly hatched larvae (75-85%) was measured four to five days later (Table 3). The infested larvae stopped feeding from the 2nd day but caused shallow entries on grape berries. These shallow entries were significantly fewer (0.9-1.5) than those of the control (4.55) but under favourable conditions (high humidity and low temperature) the *Botrytis cinerea* infestations throve. These conditions usually occur in mid-autumn and coincide with grape picking. Methomyl and triflumuron had en excellent ovicidal action (100%). The embrya developed normally in treated eggs but were unable to hatch.

Triflumuron was tested in the field against the grape berry moth at Peza the same period (1982) and gave good control (Roditakis unpublished). Many insect growth regulator

TABLE 3. Effectiveness of Bt and Bt+feeding stimulant on egg hatching (48h-old eggs) and survival of larvae under laboratory conditions (24±0.5° C, 55±5% r.h., 2,000 Lux 16h daily). In each column, means followed by the same letter are not significantly different by Duncan's multiple range test (P=0.05).

| Treatment | Eggs unhatched/ 100 eggs | Dead larvae | Shallow entries/ grape berry |
|---|---|---|---|
| Bt | 11 a | 75 a | 1.5 a |
| Bt+CoAX | 11 a | 85 a | 0.9 a |
| Control | 15 a | 6 b | 4.55 b |
| Triflumuron | 100 b | – | 0.0 c |
| Methomyl | 100 b | – | 0.0 c |

substances have been widely used in the field against GBM in an integrated pest management programme in the last ten years. The absence of harmful effects on many beneficial arthropods (honey bees, syrphids, chrysopids, coccinelids, predacious mites, and parasitic hymenoptera) has been observed repeatedly (Frischknecht and Muller 1976, Tranfaglia and Viggiani 1976, Voight et al. 1979). On the other hand mixtures of Bt with insecticides at sublethal doses such as phosmet, phosalone etc. have been used successfully (Fillip and Alexandri 1975). The use of ovicides could be part of an integrated pest control program on table grapes. Certainly we need a series of tests on mixtures of Bt with ovicides at sublethal doses, especially those having the least harmful effects on beneficial fauna and human health.

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KEY WORDS: Biological control, Grape berry moth, *Lobesia botrana*, *Bacillus thuringiensis*, Feeding stimulant, Methomyl, Insect growth regulator, Razaki, Table grape

# Αποτελεσματικότητα του *Bacillus thuringiensis* Berliner var. Kurstaki στην Ευδεμίδα του Αμπελιού *Lobesia botrana* Den. and Shiff. (Lepidoptera, Tortricidae) σε Συνθήκες Αγρού και Εργαστηρίου

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## ΠΕΡΙΛΗΨΗ

Αξιολογήθηκε η αποτελεσματικότητα του *Bacillus thuringiensis* (Dipel) στην ευδεμίδα του αμπελιού *Lobesia botrana* Den. και Shiff. στην επιτραπέζια ποικιλία Ραζακί στον αγρό και σε συνθήκες Εργαστηρίου. Οι ψεκασμοί διενεργήθηκαν βάσει των παρακάτω κριτηρίων: α. συλλήψεις ακμαίων σε παγίδες φερομόνης και σε τροφοπαγίδες, β. οπτική εκτίμηση των εναποθέσεων των αυγών σε ορισμένα χρονικά διαστήματα, γ. κριτήριο επεμβάσεων εφ' όσον η πυκνότητα εναποθέσεων ήταν 20-30 αυγά/100 σταφύλια αθροιστικά και επανάληψη όταν η περίοδος εναποθέσεων ήταν μεγαλύτερη των 10 ημερών από την επέμβαση. Διενεργήθηκαν 2 ψεκασμοί συνολικά το 1981 στο Καστέλι (Ηρακλείου Κρήτης) για την 2η και 3η γενεά και 4 ψεκασμοί στα Πεζά (Ηρακλείου Κρήτης) το 1982 στη 2η και 3η γενεά. Χρησιμοποιήθηκαν το Dipel IU 16.000/mg στη δόση 20gr/10 lt νερό και ο συνδυασμός Dipel 10 gr/10 lt νερό με ένα ελκυστικό τροφής CoAX στη δόση 10g/Kg νερό. Η αποτελεσματικότητα του *B. thuringiensis* σε θνησιμότητα προνυμφών ήταν 96-100% και 92% στη 2η και 3η γενεά αντίστοιχα στο Καστέλι το 1981 και 73-75% και 63-79% στα Πεζά το 1981 στη 2η και 3η γενεά αντίστοιχα, χωρίς σημαντικές διαφορές μεταξύ των επεμβάσεων.

Στο εργαστήριο (24±0.5°C, 55±5% r.h., 2.000 Lux και 16 ώρες φως ημερησίως) αξιολογήθηκε η αποτελεσματικότητα του *B. thuringiensis* στα αυγά και στις προνύμφες που εξέρχονται σε σύγκριση με το methomyl 0.03% δ.ο. και το triflumuron 0.013% δ.ο. (παρεμποδιστής σύνθεσης χιτίνης). Ο *B. thuringiensis* δεν επηρεάζει την εκκόλαψη των αυγών ενώ στις εξερχόμενες προνύμφες τρεφόμενες με ράγες που έχουν ψεκαστεί πριν 48h προκαλεί θνησιμότητα 75-85% ύστερα από 4-5 ημέρες. Τα methomyl και triflumuron έχουν αυγοκτόνο δράση (100%). Ο συνδυασμός του *B. thuringiensis* με ένα αυγοκτόνο που είναι όσο το δυνατόν χαμηλής τοξικότητας και ακίνδυνο για τα ωφέλιμα έντομα και τον άνθρωπο, είναι πολύ πιθανό να αποτελέσει καλή μέθοδο καταπολέμησης του *L. botrana* στην πράξη εφ' όσον το *B. thuringiensis* μόνο του δεν μπορεί να προστατέψει τις ράγες από τις μικρές πληγές που προξενούν οι νεαρές προνύμφες του εντόμου μέχρι να θανατωθούν από τη δράση του βακίλου. Οι μικρές αυτές πληγές μπορεί αργότερα να αποτελέσουν εστίες μόλυνσης από το μύκητα *Botrytis cinerea*.