Australian Government
Grape and Wine Research and Development Corporation

Minimisation of pesticide residues in grapevines by improving the efficacy and application of the biological insecticide, *Bacillus thuringiensis*

FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **DAV 92/2**

Principal Investigator: **Dr Mark Smith**

Research Organisation: **Agriculture Victoria**
Date: **December 1995**

GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

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Funding Agency: Grape and Wine Research and Development Corporation

Other Funding Agencies:

Rural Industries Research and Development Corporation
Abbott Agricultural Products Division
Hoechst Schering AgrEvo Pty Ltd
Nufarm Chemicals Pty Ltd
Sandoz Australia Pty Ltd
Silvan Pumps and Sprayers Pty Ltd (in-kind support)

December 1995

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Non-technical summary

The major pest of grapevines in southeastern Australia is lightbrown apple moth (LBAM). Caterpillars (larvae) feed on berries and cause direct yield loss and promote bunch rot infections. Control measures have recently relied on regular application of synthetic insecticides. However, in line with the grape and wine industry's aim to maintain high quality products with low chemical residues, the biological insecticide, *Bacillus thuringiensis* (Bt), has been promoted as a replacement or supplement to chemical insecticides.

Viticulturists have used Bt products for several years now, but levels of control of LBAM have been variable and sometimes unacceptably poor. Possible reasons for poor performance included product variability, degradation through UV radiation, poor application methods and timing of sprays, and incompatibility with tank mixers. This project was commenced with the objectives of identifying the reasons for poor efficacy of Bt on grapevines, to improve coverage and adhesion of Bt formulations on the leaves and bunches using tank adjuvants (wetters and stickers), to determine the compatibility of Bt with common tank mixers (e.g. fungicides), and to inform the industry of the ideal dose for application of Bt on grapevines. It was intended that the subsequent improvement of Bt performance would encourage replacement of synthetic insecticides.

The principal research tools employed were application and bioassay techniques to measure the efficacy of Bt. Bt could be applied to leaves in precise amounts, or under simulated applications or at controlled droplet sizes using different application and bioassay procedures.

Laboratory bioassays identified that Bt efficacy is lower on grapevine leaves compared to equivalent doses on other leaf hosts. Possible reasons for lower efficacy on grapevine leaves are differences in spray retention properties and chemical composition of the leaves. Relatively high acidity of grapevine leaves (pH 3.3 to 3.9) may inhibit the action of the Bt toxin, as it requires a strong alkaline environment to be activated in the insects gut. High levels of leaf tannins can also inhibit Bt toxin activity. In laboratory experiments, there was some differences in efficacy of commercial Bt products, but it was unclear whether the differences were relevant to field use.

LBAM field populations vary in their susceptibility to Bt. Limited testing of field collections in laboratory bioassays revealed a six-fold difference in dose-mortality response. This variation in susceptibility to Bt has not been noted before and helps explain the range of control successes and failures reported by viticulturists.

Several common tank mixers, mainly fungicides, were screened for compatibility with Bt. Most of those tested were found to be compatible, with no significant loss of efficacy when mixed for up to two hours with Bt. Laboratory and field trials of Bt with certain tank adjuvants (wetters/stickers) often gave an increase in efficacy, but the benefits were not always repeatable.

Three field trials at vineyards in the Yarra Valley examined different application methods, different doses of Bt, and effects of an adjuvant on coverage and efficacy. Bioassay of leaf deposits suggests a rate of 375 gm/ha, or greater, or 3 times the current label rate was necessary for adequate control of the more resistant strain of LBAM. Applications by a high volume air-blast sprayer produced greater coverage of liquid on leaves when compared to a low volume air-shear machine. However, the amount of Bt active per unit area of leaf surface ('deposit') was less variable and a better indication of LBAM mortality.

The main recommendation from this research is to increase the rate of Bt per ha. In practice, some regular Bt users already follow a 2-4 times label rate (i.e. 250-600 gms/ha). One product, Biobit HPWP (Nufarm), has been registered at 500-700 gm/ha. The move to higher rates should lead to better control of the pest but only if they are used in conjunction with good timing and spray application. Timing can be improved through better monitoring using moth traps and inspections of vines for eggs and caterpillars The recommended wetter and sticker on the Bt label may provide increased efficacy at the correct rate. Our field trials suggest that there is more work required to define the best method for spray application, targettting and coverage of Bt. The issues are machinery type, set up (e.g. nozzle type and fan speed), water volumes and ideal targets (e.g. flower, bunch, shoot tips and back or front of leaves).

Background to project

The grape and wine industry aims to maintain high quality products with low chemical residues, particularly for export markets. A major pest of grapevines is lightbrown apple moth *Epiphyas postvittana*) because feeding by caterpillars can destroy the berries and more importantly promote infection of the bunches by fungal rots, primarily *Botrytis cinerea* (Buchanan 1977; Baker & Lang 1983; Baker & Bailey 1993). As a replacement or adjunct to chemical insecticides, the biological insecticide, *Bacillus thuringiensis* (Bt), has been used to provide residue-free control of this caterpillar pest (Childs 1993). Bt products have been used by viticulturists for several years but with variable, and at times unacceptable, levels of control of LBAM.

The original project funded by GWRDC and RIRDC between 1992-94 aimed at developing new improved formulations of Bt using Daratech's controlled release technology. This research produced more persistent formulations primarily through protection of the Bt toxin and crystal from degradation by ultraviolet light and is reported separately. However, there arose some basic concerns about the performance of Bt, which were not necessarily related to issues of formulation improvement.

An industry meeting in July 1994 between Agriculture Victoria staff, GWRDC and the major producers or sellers of Bt products confirmed that Bt performance could be variable and identified some possible causes for this variability. The most likely reasons were UV inactivation, poor application methods, poor timing, and incompatibility with tank mixers. Research at the Institute had identified more fundamental reasons that related to rate of application or grams of product per hectare. The meeting concluded that there was a need to improve growers confidence in Bt, and this could only be achieved by improving its efficacy and application in grapevines.

The following objectives were proposed for 1994/95:

(i) To minimise the use of synthetic insecticides, and their consequent residues, in grapes and wine through improved efficacy and application of formulations of Bt.

(ii) To identify reasons for the relatively poor efficacy of Bt on grapevines.

(iii) To improve coverage and adhesion of Bt formulations on the leaves and bunches using wetters and stickers, and thus increase the efficacy of Bt.

(iv) To identify compatible and incompatible chemicals applied with Bt formulations by screening common tank mixers, such as fungicides.

(v) To inform the industry of the best and worst tank adjuvants and mixers, and ideal dose for application of Bt on grapevines.

The time lines and milestones were:

Sept - Dec 94	Develop alternative spray techniques for leaf bioassays
Oct 94 - Feb 95	Screen Bt products provided by Bt companies in lab. bioassay
Oct 94 - May 95	Test effects of commercial wetters, stickers and spreaders on Bt efficacy using a sprayed leaf bioassay
Feb - June 95	Test effects of commercial adjuvants (i.e. wetters, etc.) for compatibility with Bt using the direct-dose bioassays
Jan - Feb 95	Field trial the best adjuvants using commercial sprayers in vineyards in Yarra Valley. Bioassay spray deposits.
Oct 94 - June 95	Use fluorescent marker techniques to study behaviour of spray deposits of Bt with and without adjuvants
Jan - June 95	Screen the compatibility of common tank mixers (e.g. fungicides)
June 95	Report findings to Bt companies and grape and wine industry

Introductory technical information

Bacillus thuringiensis is a gram positive, aerobic, endospore-forming bacterium. Bt is recognised by its parasporal body (known as the crystal). Bt products used in grapevines contain two active ingredients, the protein crystal toxin and the bacterial spore, but do not have any live bacterial cells. The variety of Bt in the commercial products, Biobit® HPWP (Nufarm), Delfin® WG (Sandoz) and Dipel Forte® (Abbott), is *Bacillus thuringiensis* var *kurstaki*, specific to caterpillar species. These products are all rated at 32,000 International Units (IU) of potency/mg and manufactured from *Bacillus thuringiensis* Berliner var. *kurstaki*. Biobit and Dipel Forte are HD-1 strain whereas Delfin has the SA-11 strain.

The caterpillar eats Bt-infected plant material and the protoxin is activated by the high pH in the insect's mid gut. The insect's gut membrane is disrupted and spores and crystals enter the haemocoel. The major causes of mortality are gut paralysis, breakdown of the gut wall and subsequent starvation and septicaemia (Gill et al. 1992). The specificity of Bt means that it does not interfere with natural biological control by wasp parasitoids, lacewing predators or spiders in the vineyard (Childs 1993). Some of the disadvantages of Bt are its lack of contact activity and persistence in comparison to the chemical alternative, chlorpyrifos.

Our research on the development of improved Bt formulations had shown that the recommended dose of Bt was probably too low for control of LBAM larvae. We also found that the dose was better when applied to some leaf hosts, such as French beans, but not when applied to grapevine leaves. The problem may have been one of poor spreading or sticking to the leaf surface, or antagonistic agents in leaves may have reduced the toxicity of the Bt.

The efficacy of commercial Bt formulations varied in laboratory bioassays but it was unclear whether the differences were significant or relevant for extrapolation to field use. We also discovered variability in Bt efficacy with certain tank adjuvants (wetters and stickers).

Bt is often mixed in the tank with other pesticides, such as fungicides, but their compatibilities were uncertain. For example, tank additives of high pH may have prematurely activated the Bt protein toxin in the tank and therefore reduce its effectiveness after application in the crop.

Varying susceptibility of insect populations to Bt has been reported for other insect pests, such as the budworms, *Helicoverpa punctigera* and *H. armigera* in Australia (Forrester 1995). Tabashnik et al. (1990) found that field populations of the diamondback moth (*Plutella xylostella*) differed by 7-fold even though they had minimal exposure to Bt sprays. It was considered important to test if LBAM populations differ in their susceptibility to Bt.

It was not clear as to which were the best application methods for Bt in grapevines. Viticulturists use a range of water volumes from low volume air-shear machines to high volume air-blasters. Field trials with spray machinery manufacturers were required to compare spray machinery, rates of Bt, high and low volume application and effect of adjuvants.

Research methodology

1. Basic methods of application and bioassay of Bt

The key to rapidly and efficiently obtaining relevant data on Bt efficacy was to develop and use several methods of Bt application and repeatable bioassay techniques.

a) Application techniques

i) 'Discrete dose' method. A 2 µl drop of Bt solution was delivered to a known area of leaf surface (5 mm^2) using a micropipette. Drops were applied to leaves in the microtitre tray bioassay system described below. The leaf surface was confined to ensure that the dose was consumed by an LBAM larva within 3 days of feeding. The method was designed to eliminate the type of application method as a factor that may cause efficacy differences between treatments of Bt. It was also useful for studying compatibility of Bt with common tank mixers.

ii) Atomiser. A hand held atomiser was used to simulate a simple spray application by spraying potted plants to insipient runoff with a fine mist of Bt solutions.

ii) Potter spray tower. A Potter spray tower was used to apply a more consistent dose of Bt in a finer spray (≈ 50 µm droplets) to a leaf surface. A leaf was placed at 45° angle to the direction of spray and a 2.0 ml sample of Bt applied at a pressure of 40 kPa.

iii) Commercial spray machinery. Field trials were performed using a Silvan Turbomiser air-shear sprayer for medium (200 L/ha) volume application and a Silvan air-blast orchard sprayer for high (1000 L/ha) volume application. Leaves were picked when dry and bioassayed in the laboratory.

(b) Maintenance of lightbrown apple moth colony

Six males and six females were aspirated from the diet and placed into a plastic cup ('Cafe Bar cup') with 10% honey solution in a small lid filled with cotton wool, and sealed with the lid provided for the cup. The cups were then stored at 19 °C for egg laying. Eggs were laid after 2-3 days on the corrugated sides of the cups. Egg laying also occurred on the lids. Laying occurred for about one week with fresh eggs harvested daily and moths placed into new cups. As the eggs matured their colour changed from pale green to yellow through to a dark olive/brown, which indicated that the eggs were close to hatching. This process took about one week at 19 °C. Eggs were sometimes stored for several weeks by placing in sealed plastic bags at 4 °C; however eggs that were stored for longer periods of time had reduced viability. Twenty-five to thirty large egg masses from freshly laid cups were placed at 19 °C for 6-7 days in a plastic bag until the eggs were ready to hatch.

An artificial diet (Singh et al. 1985) was prepared and poured into the bottoms of 10 plastic boxes. Once the diet had cooled and set the surface is broken up and 2-3 black egg masses are placed upright into the diet (about 100 eggs/container). The boxes were sealed with a lid that has also some tissue attached which helps provide a surface for some larvae to pupate on. Containers were stored at 19°C and adult moths began to emerge within 6 weeks of colony being set up. On emergence of adults the tissues were removed from the containers and any larvae or pupae from the tissue were placed onto the diet. Adult moths were collected daily using a small vacuum aspirator and placed into plastic cups (six males and six females) to mate and lay eggs. The colony was set up once a week to provide a constant supply of eggs and larvae.

b) Bioassay methods

i) 'Leaf sandwich' method. Whole leaves were sandwiched between two perspex sheets with a grid of 2.5 mm diameter holes. The sandwich was then held between two microtitre trays (Inter Med), with the top tray having the bases removed from the wells. When bolted together, this provided a 2.5 mm diam. leaf surface area within each cell on which individual larvae could feed. Bt doses were applied to each well, as above in the discrete dose application, or leaves could be treated by spray application prior to being placed into the sandwich. A newly-emerged (< 24 hours old) LBAM larva was placed in each cell and the cell sealed with a steel ball-bearing. There were generally 30-40 larvae set up for each replicate and 3-4 replicates per treatment. The insects were then stored at 25 ^{0}C and 12L:12D for 3 days, at which time mortality was assessed.

ii) Leaf disk method. Leaf disks of 35 mm diameter were cut from treated leaves and placed in 40 mm diameter glass petri-dish lids. Fifteen to twenty LBAM larvae were placed on the disk, which was sealed with the petri-dish base and an elastic band. Mortality of larvae was assessed after 3 days.

2. Establishing mortality-dose response curves

An initial requirement of this research was to obtain base-line data on Bt efficacy by measuring the mortality response of LBAM to Bt. This was first performed using the discrete dose bioassay method with an unregistered liquid form of Bt, Biobit XLP [Novo Nordisk; 20.1 million IU/ml] and a commercial powder formulation, Delfin® WG (Sandoz; 32 million IU/mg). Percentage mortality results were adjusted for control mortality using Abbott's formula (Finney, 1971) and analysed using probit analysis in the statistical package, POLO-PC (LeOra Software, 1987).

3. Identifying reasons for the relatively poor efficacy of Bt on grapevines

a) Comparing relative efficacy of Bt products

An initial requirement in assessing factors which influence Bt activity was to determine the relative efficacy of the common Bt products used by viticulturists, namely, Biobit HPWP®

(Nufarm), Delfin WG® (Sandoz) and Dipel Forte® (Abbott). Their efficacies were compared by screening with LBAM larvae on French bean leaves sprayed in a Potter tower at 2 ml of 2 doses, 0.94 and 1.88 mg of product/ml (30,000 and 60,000 IU/ml respectively). Although 2 ml was put into the tower, only 5.5% or 0.11 ml deposited on the target area (70.8 cm^2). If the solution was evenly spread over the leaf surface, this resulted in an estimated 1.5 and 3.0 µg of product/cm^2 of leaf surface respectively, or 0.08 and 0.16 µg of product/LBAM larva respectively (2.6 and 5.2 IU/LBAM larva respectively).

b) Effect of leaf type on Bt efficacy

In laboratory trials, French bean and grapevine plants (var. Chardonnay and Cabernet Sauvignon) were sprayed with several concentrations of Biobit XLP to insipient runoff with the atomiser, under a Potter tower and using the discrete dose assays. After drying the leaves were placed in the leaf-sandwich bioassay system and mortality assessed after 3 days.

c) Variability in dose-mortality response of LBAM populations to Bt

Several laboratory colonies of LBAM were sourced from different field collections and used for bioassays. The original colony used in 1993 was commenced from specimens collected from grapevines in the Mildura region during the summer of 1992-93. In early 1994, an increasingly larger proportion of eggs were not hatching, although they appeared fertile. The reason was thought to be inbreeding, as no fresh field specimens had been introduced for over twelve months. Therefore, we started new colonies from larval infestations from grapevines in Yarra Valley vineyards. During Oct-Nov 1994, we suspected that neonate larval susceptibility to Bt in our laboratory had changed because variations in Bt efficacy were noted. This could be explained by the use of larvae from the different colonies. Dose-response bioassays were conducted to determine any differences in susceptibility between the Mildura and Yarra Valley strains.

Note that all larvae used for screening adjuvants, tank mixers and for field trial bioassays were from the population sourced at Yarra Ridge Vineyard, Yarra Glen.

d) Screening of common tank mixers for compatibility with Bt

Several common tank mixers (Table 1), mainly fungicides, were screened for compatibility with Biobit XLP. The bioassay incorporated bean leaves with the microtitre tray method. Bt was applied at 1.0 and 4.0 µg/cm², and fungicides were mixed at the label rate. A fungicide-only treatment was included to determine any toxicity toward LBAM. Treatments were applied to the leaf surface immediately after mixing (ie. 0 hours) and after two hours agitation. The pH of each treatment was also recorded at 0 and 2 hours.

Table 1. Common tank mixers screened in Bt compatibility trials.

Product	Chemical Group
Bayfidan®	Triadimenol (demethylation inhibitor)
Blue Shield® DF	Copper hydroxide
Fulasin®	Ziram (dithiocarbamate)
Dithane® DF	Mancozeb (dithiocarbamate)
Kumulus® DF	Wettable sulphur
Oxydul®	Copper oxychloride
Ronilan®	Vinclozolin (dicarboximide)
DC-Tron®	Petroleum spray oil

4. Improving performance of Bt through increased rate, adjuvants and spray application.

a) Laboratory screening of adjuvants for effects on efficacy

Several commercial adjuvants (wetters and stickers) were screened at label rate (0.01%) to determine whether the efficacy of Biobit HPWP, Delfin WG and Dipel Forte could be enhanced. The adjuvants screened: were: Agral®, Bond®, Citowett®, Chemwett®, Latron®,

Pulse® and Tween®. Bt was applied at 1.5 µg product/cm², with and without adjuvant, using the Potter tower technique. Mortality was assessed using the leaf disk bioassay method.

b) Comparing effects of rate, adjuvants and spray machinery on Bt efficacy in vineyard trials

Three trials were conducted in Yarra Valley vineyards between January and March 1995 to test key outcomes of the laboratory research in the field. The aims were to: (i) compare the efficacy of Bt products at the registered rate of 125 g/ha and at increased rates; (ii) compare the efficacy of Bt products applied with low (200 L/ha) and high (1000 L/ha) water volumes; (iii) determine the effect of a wetter (Citowett) applied at the registered rate of 0.01% on the efficacy of Bt; (iv) determine the coverage and droplet distribution for the above application methods using water-sensitive cards, and relate spray coverage to Bt efficacy.

Details of trial sites, spray machinery and treatments. Trial #1 - Hoddles Creek Beenak Vineyard, Hoddles Creek; Pinot Noir grapevines (minimal pruned); 11 January 1995. Growers air-shear Silvan Turbomiser spraying at 200 L/ha.

Trial #2 - Yarra Ridge Winery, Yarra Glen; Cabernet Sauvignon grapevines (hanging cane); foliage of 1.7 m high, 1 m wide, 0.8 m clearance and medium to high density; 9 February 1995. The sprayers were: (1) three point linkage LD50 Silvan Turbomiser fitted with HiLo nozzles and spraying at a water rate of 200 L/ha.; (2) trailed Silvan air-blast orchard sprayer with 1 m diameter axial fan; fan blade pitch 45°; PTO 540 RPM and fan gear box was stuck in upper position; 1000 L tank; water rate was 1000 L/ha; 5 pink ceramic 1.5 hollow cone nozzles were used; swirl plates were metal 1.2, 1.8, 1.2, 1.2, 1.0 in order from the top of the machine; operating pressure 400 psi.; tractor speed 7.5 km/h.

Trial #3 - Fernhill Vineyard, Yeringberg; Chardonnay grapevines (hanging cane); foliage: 2.2 m high; 1.3 - 1.5 m wide; 0.8 m clearance and medium to high density. row spacing 3 m; trellis height 1.6 m; 16 March 1995. The sprayers were: (1) a Silvan trailed LD50 Turbomiser with a 500 mm, 30 hp centrifugal fan; 200 L/ha; (2) air-blast orchard sprayer; 1000 L/ha; same machine as trial 2; operating at 9 km/h; 6 pink ceramic 1.5 hollow cones were used.

For each treatment (Table 2), at least ten panels were sprayed down each side of the same row, leaving a buffer of three rows between each treatment.

Table 2. Bt treatments applied in vineyard trials.

TRIAL 1			TRIAL 2			TRIAL 3		
Bt product	Rate (g/ha)	Volume (L/ha)	Bt product	Rate (g/ha)	Volume (L/ha)	Bt product	Rate (g/ha)	Volume (L/ha)
Dipel	125	200	Dipel	125	200	Dipel*	125	200
Dipel*	375	200	Dipel*	375	200	Dipel*	250	200
Delfin	125	200	Dipel	125	1000	Dipel*	125	1000
Delfin	375	200	Dipel*	375	1000	Dipel*	250	1000
Control	0		Control	0		Control	0	

* These treatments applied with and without a wetter (Citowett®) at the registered rate of 0.01%.

Measurement of bioefficacy. Sprayed leaves from the lower canopy (about 80 cm above ground) were picked from the fifth and sixth panels, where tractor speed and spray application should have been consistent. Observation of leaves and water-sensitive cards just after spraying showed that the middle and lower canopy leaves received the highest spray volume. Leaves were assayed in the laboratory with neonate LBAM larvae using the microtitre tray and leaf disk bioassays for samples from Trials 1 and 2. Leaves from Trial 3 were screened using the leaf disk assay only. There were three replicates for each treatment using the microtitre tray method and six replicates for the leaf disk method. Mortality was assessed after 3 days.

Measurement of spray coverage and deposit. Coverage of spray was measured during Trials 2 & 3. The coverage of spray on the vines was measured using water sensitive cards. Thirty-six cards were sprayed during each run. The cards were placed on both the top and bottom of leaves positioned in nine sections as shown in Figure 1 at each of two sites. Due to the requirement of a leaf to fasten the card to, there was no fixed position for each card within each

section. The water sensitive cards change colour from yellow to a dark purple wherever water touches them. This change in colour provides a substantial contrast suitable for Computer Visual Analysis. Two sites of a centimetre square are measured for percentage cover on each card. Unfortunately there is no measure of the quantity of chemical retained on the wetted area, with this method. For the purposes of discussion we have treated the wetted area as proportional to the assumed chemical retained. The deposit is therefore taken as proportional to the tank mix concentration multiplied by the percentage coverage.

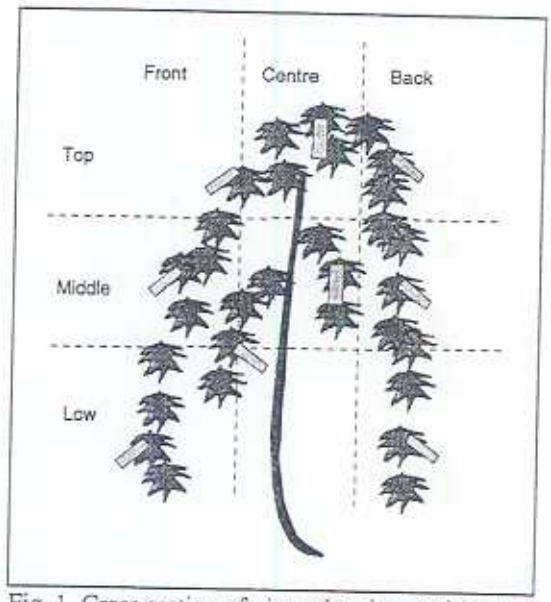


Fig. 1 Cross section of vines showing positions of water sensitive cards in 9 sections.

Results & Discussion

1. Establishing mortality-dose response curves

Accurate and repeatable bioassays that determine the dose-mortality response of insects are essential for meaningful comparisons of different preparations or formulations of Bt. An example of a dose-mortality response curve for the discrete dose bioassay method is shown in Figure 2a. Note that the % mortality figures were adjusted for natural responsiveness (i.e. control mortality) using Abbott's formula. This data was then subjected to probit analysis in

which it was transformed to log dose-probits and fitted with a line of best fit using maximum likelihood estimation (Fig. 2b). Note that doses of Delfin WG are expressed in International Units (IU) of active ingredient per ml to allow comparison with the liquid formulation Biobit XLP; Biobit XLP has 20,100 IU/ml as compared to 32,000 IU/mg in the powder formulations, Biobit HPWP, Delfin WG and Dipel Forte.

Table 3. Probit analysis of log dose-mortality response data for discrete dose assays of LBAM on Bt (Biobit XLP & Delfin WG) treated bean leaves.

Product	Dose range (IU/LBAM larva)	LC_{50} (LCL - UCL)[a] (IU/LBAM larva)	LC_{90} (IU/LBAM larva)	Slope[b]	Chi-squared (χ^2)	Heterogeneity	df[c]
Biobit XLP	0.065- 6.350	1.956 (0.897-3.994)	18.709	1.03	27.3	3.4	8
	0.201-3.216	0.835 (0.443-1.250)	4.844	1.67	61.4	4.7	13
	0.101-1.608	0.814 (0.516-1.298)	7.821	1.30	36.2	2.8	13
Delfin WG	0.101-32.0	0.979 (0.380-0.221)	39.713	0.80	55.0	3.4	16
	0.101-32.0	0.868 (0.527-1.317)	9.306	1.24	25.5	1.6	16
	0.64-10.12	0.585 (0.236-0.951)	10.401	1.03	17.7	1.4	13
	0.32-5.12	1.908(1.307-2.771)	10.520	1.73	37.7	2.9	13
	0.32-5.12	0.899 (0.618-1.215)	6.502	1.49	9.2	1.1	8

[a] Lower and upper 95% confidence limits of the LC_{50}.
[b] Slope of the line of best fit.
[c] df is the degrees of freedom.

Table 3 shows probit analysis of results from a number of discrete dose bioassays with Biobit XLP and Delfin WG. An examination of the slopes of the lines, ranged from 0.8 to 1.7, suggests there was some variation in the dose-mortality relationship from assay to assay. However, the LC_{50} values (i.e. lethal concentration producing 50% mortality) were relatively consistent, about 0.8-0.9 IU/LBAM larva., while LC_{90} values were generally about 5-11 IU/LBAM larva. Consequently we tried to perform paired-comparison bioassays (i.e. two treatments set up at the same time) and repeated assays, where possible, to account for the variation that may occur between bioassays.

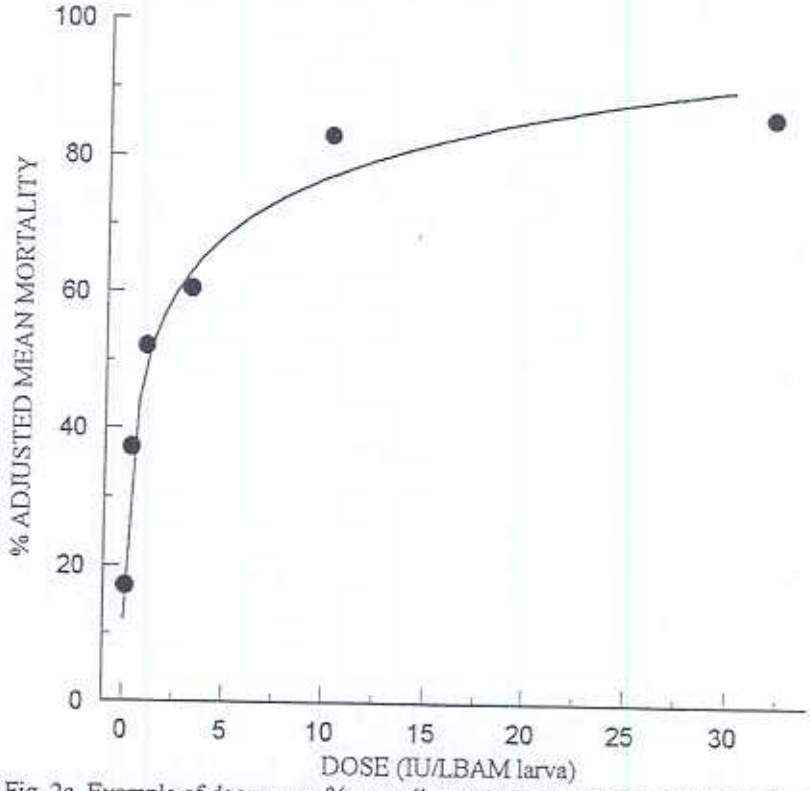


Fig. 2a Example of dose-mean % mortality response curves for LBAM on bean leaves treated with doses of Bt (Delfin WG) in a discrete dose assay (SAN-1).

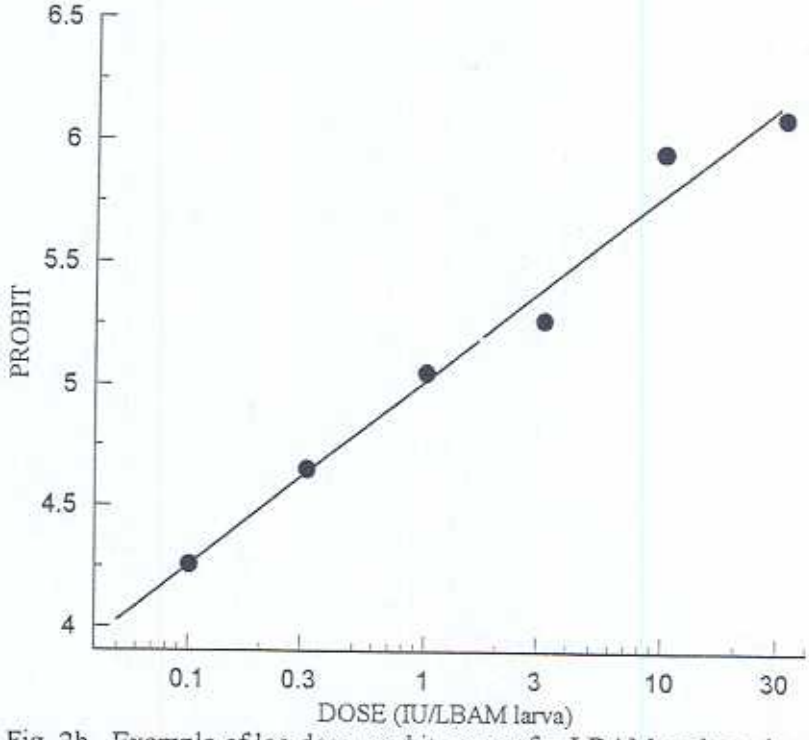


Fig. 2b Example of log dose-probit curves for LBAM on bean leaves treated with doses of Bt (Delfin WG) applied using discrete dose assay. Log and probit transformed data allows linear regression analysis.

2. Identifying reasons for the relatively poor efficacy of Bt on grapevines

a) Comparing relative efficacy of Bt products

Relative efficacies of various commercial powder products were compared by screening with LBAM larvae on French bean leaves sprayed in a Potter tower at 2 doses. After repeating the bioassays 9 times, the mean larval mortality ranged from 40 to 62% at the 1.5 µg/cm² dose and from 79 to 87% for the 3.0 µg/cm² dose (Table 4). If the solution sprayed through the Potter tower was evenly spread over the leaf surface, then 2.6 and 5.2 IU of Bt respectively would have been consumed by each LBAM larva. It was interesting to note that these values, 2.6 and 5.2 IU/LBAM, approximated the LC_{50} - LC_{60} and LC_{80} - LC_{90} ranges estimated from the discrete dose bioassays for Delfin WG (Table 3).

Table 3. Mean adjusted %mortality (± SEM) of LBAM larvae bioassayed on French bean leaves sprayed in a Potter tower with common Bt products at 1.5 and 3.0 µg/cm².

Product	Bt dose (µg/cm²)	
	1.5	3.0
Biobit HPWP	58.6 ± 4.8	87.3 ± 6.1
Delfin WG	61.7 ± 4.1	81.1 ± 10.7
Dipel Forte	40.1 ± 5.4	79.3 ± 2.1

The similarity between LC values suggested that the Potter tower was a reasonably accurate and repeatable method of spray application. The potency of Dipel Forte was significantly lower than for Biobit HPWP and Delfin WG at 1.5 µg/cm² whereas all products had similar potency at 3.0 µg/cm².

b) Effect of leaf type on Bt efficacy.

Preliminary trials, spraying trellised grapevines with a mist blower and collecting leaves for bioassay, failed to provide significant mortality of LBAM at the registered rates of Dipel Forte

(i.e. 12.5 g/100L or 125 g/ha). Increasing the rates by 2-fold similarly failed to achieve mortalities greater than about 30% (unpublished data). We therefore decided to test out the application and bioassay system by applying Bt solutions to potted grapevines and French beans using a simple hand held atomiser. Leaves were again placed in the leaf sandwich bioassay system and fed to young LBAM larvae. Larvae fed on treated French bean leaves were easier to kill than larvae fed on grapevines at the same application doses (Fig. 3).

To test this phenomenon further, leaves of the 2 host types were sprayed with a more uniform and controlled spraying technique, the Potter tower. A significantly higher mortality was achieved again on the French bean leaves (Fig. 4). We speculated that the difference in mortality may have been simply due to the behaviour of a product during spray application, such as poorer spreading or retention of active Bt on grapevine leaves in comparison to bean leaves.

To eliminate any effects of application, Bt doses were applied in known doses per unit area of leaf (i.e. in a discrete dose assay). Only a few trials were conducted but it appeared there was a difference in mortality according to host plant, though of a lower magnitude than the other trials (Fig. 5). Particular leaf chemistry of grapevines, lower pH or higher tannin content, may explain some of the host differences. Grapevine leaves are relatively acidic (pH 3.3-3.9 for grapevines compared to 6.0 for beans) and may inhibit action of Bt toxin and spores, which require an alkaline pH of 10 to be activated in the larval gut (Gringorten et al, 1992). Other factors, such as leaf tannins, may reduce Bt activity. For example, the mortality of gypsy moth (*Lymantria dispar*) larvae was much lower when fed high-tannin oak leaves treated with Bt as compared to low-tannin oak varieties (Appel & Schultz, 1994). Navon et al. (1993) showed that purified tannin from cotton fed to *Heliothis virescens* larvae reduced feeding and mortality caused by the Bt endotoxin.

We concluded that some of the poor performance of Bt on grapevines was a product of a leaf type that may not retain Bt sprayed onto its surface and have leaf chemistry antagonistic to the activation of Bt.

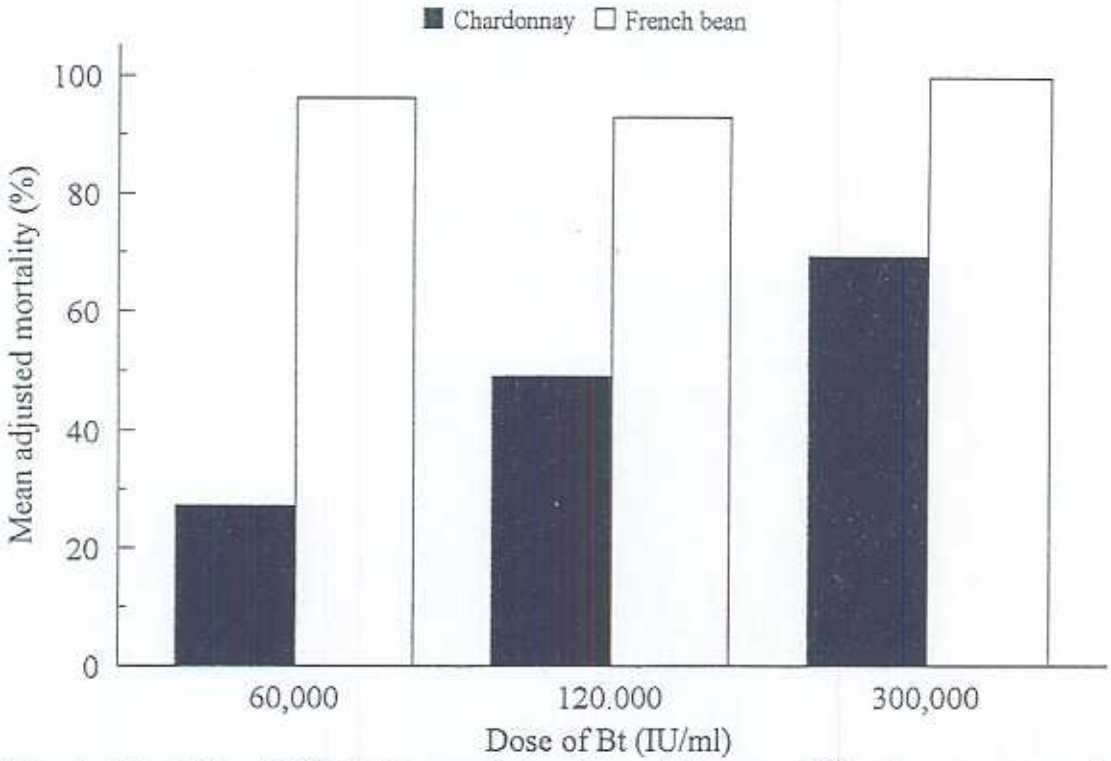


Fig. 4 Mortality of LBAM larvae fed on French bean and Chardonnay grapevine leaves sprayed with 3 doses of Biobit XLP applied using a Potter tower.

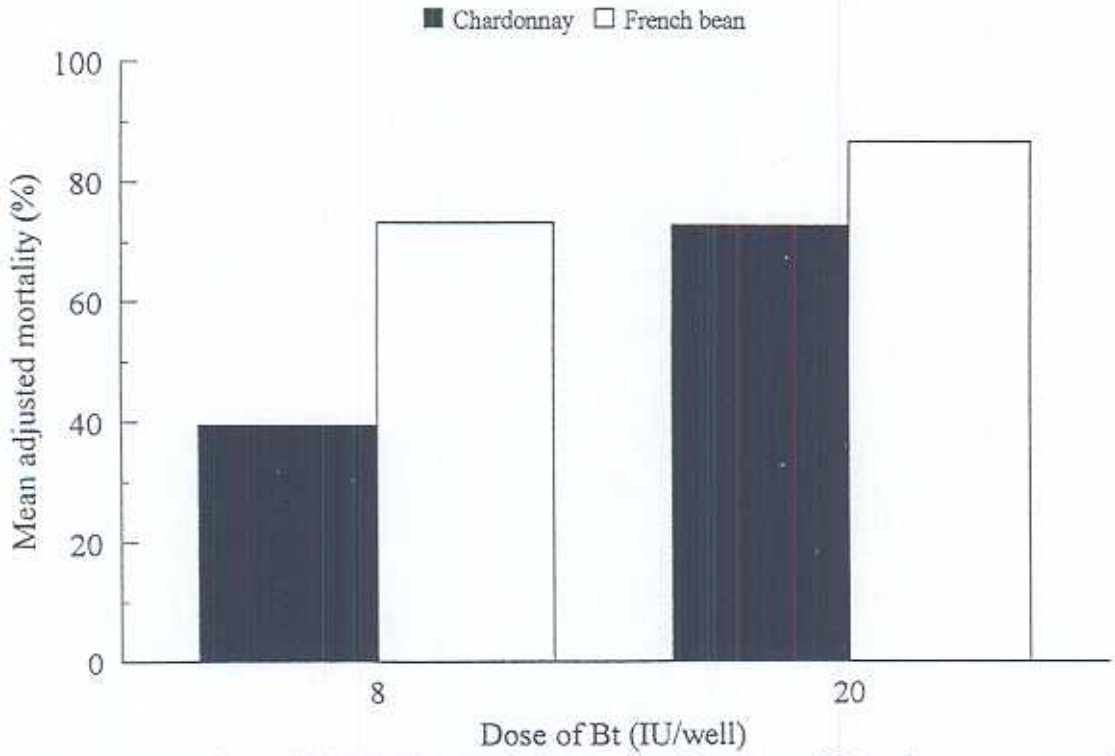


Fig. 5 Mortality of LBAM larvae fed on French bean and Chardonnay grapevine leaves treated with discrete doses of Biobit XLP applied as 2 microlitres/well.

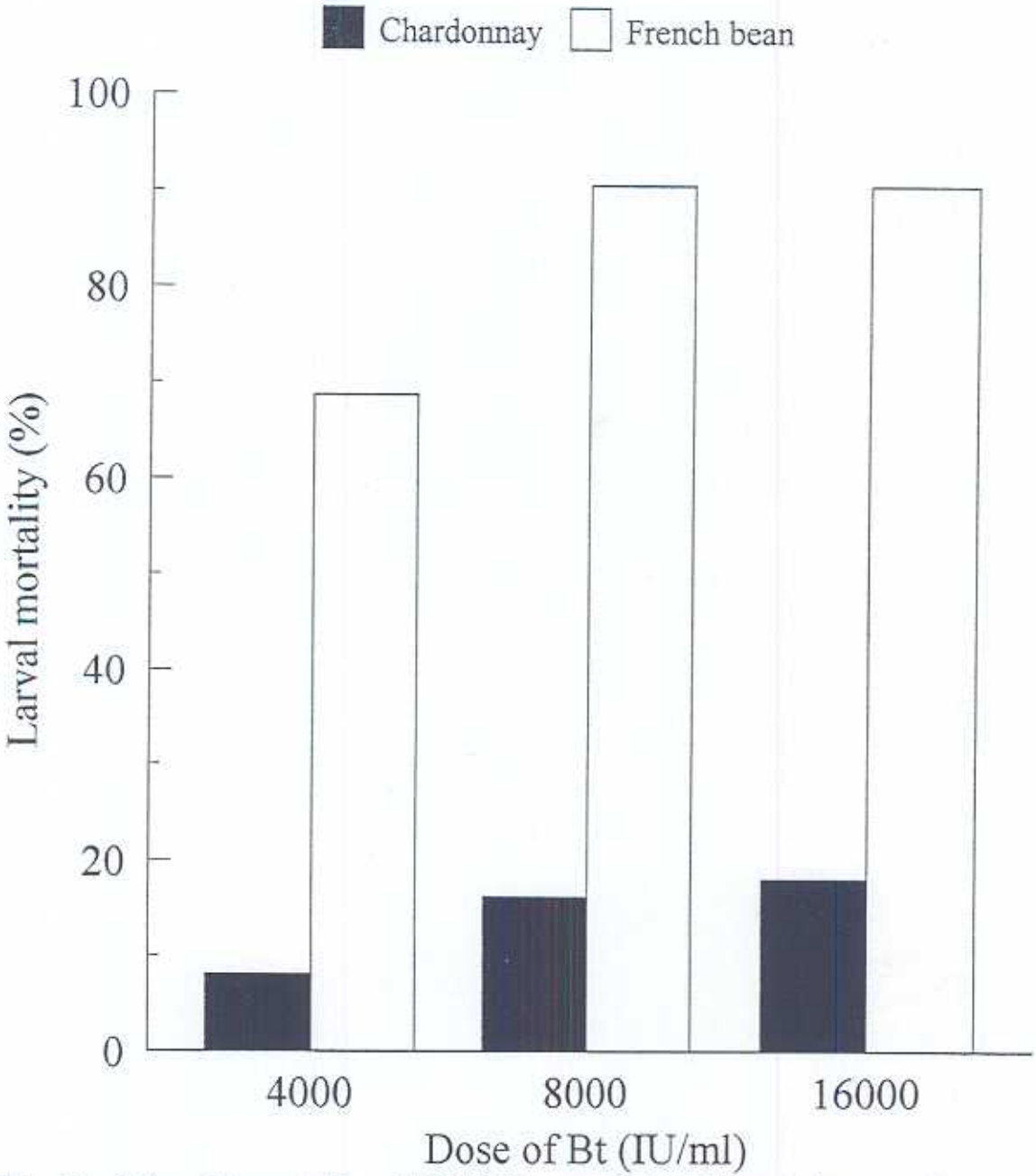


Fig. 3 Mean % mortality of LBAM larvae fed on French bean and grapevine (Chardonnay) leaves sprayed with various doses of Biobit XLP applied with a hand-held atomiser.

c) 'Resistance' of LBAM populations to Bt

Bioassay results of the Yarra Valley colony were compared with results of an early bioassay of the Mildura strain, which had a similar slope of the regression line, about 0.8 (see Table 3). Probit analysis showed that the slopes of the lines were the same (i.e. not significantly different) and hence the intercepts could be compared statistically to examine differences in dose-mortality response of the LBAM populations to Bt (Fig. 6). The estimated LC_{50} for the Yarra Valley strain was 6 IU/LBAM larva, significantly greater than the LC_{50} for the Mildura strain at 1 IU/LBAM larva. A difference of this magnitude did not occur within the experimental variability of discrete dose assays with the Mildura strain (Table 3). We concluded that larvae from the Mildura colony were 6 times more susceptible to Bt than larvae from the Yarra Valley strain.

This parallels the findings for testing of *Helicoverpa punctigera* and *H. armigera* by (Forrester 1995). Variation in susceptibility to Bt by different strains of LBAM has not been noted before, and helps to explain the reported range of successes by growers in controlling this pest with Bt. It is doubtful that the resistant strains have developed through exposure to Bt, rather that they display natural variations in tolerance levels. Further research on Bt efficacy against strains of LBAM over south eastern Australia would help to identify the extent of variability in Bt tolerance and help identify suitable field rates for control in specific regions.

d) Screening of common tank mixers for compatibility with Bt

The effect on LBAM mortality of Bt and tank mixers, and the Bt and mixers alone was assessed for seven products commonly combined with Bt in spray vats (Fig. 7-14). To some extent, all mixer products used on their own were toxic to newly emerged larvae. Some caused significant mortality: Blue Shield, Oxydul and the mineral oil insecticide D-C Tron had mortalities ranging from 42 to 87%. Therefore, it was impossible to distinguish if these products reduced the efficacy of Bt. Current advice to growers is that Bt should not be left standing in the tank for too long if mixed with copper hydroxide fungicides (Childs 1994) because their alkalinity can activate the Bt toxin in the tank. Blue Shield, a copper hydroxide based product, may have been compatible with Bt in our trial because it was only slightly alkaline (pH 7.9) when in solution with Bt.

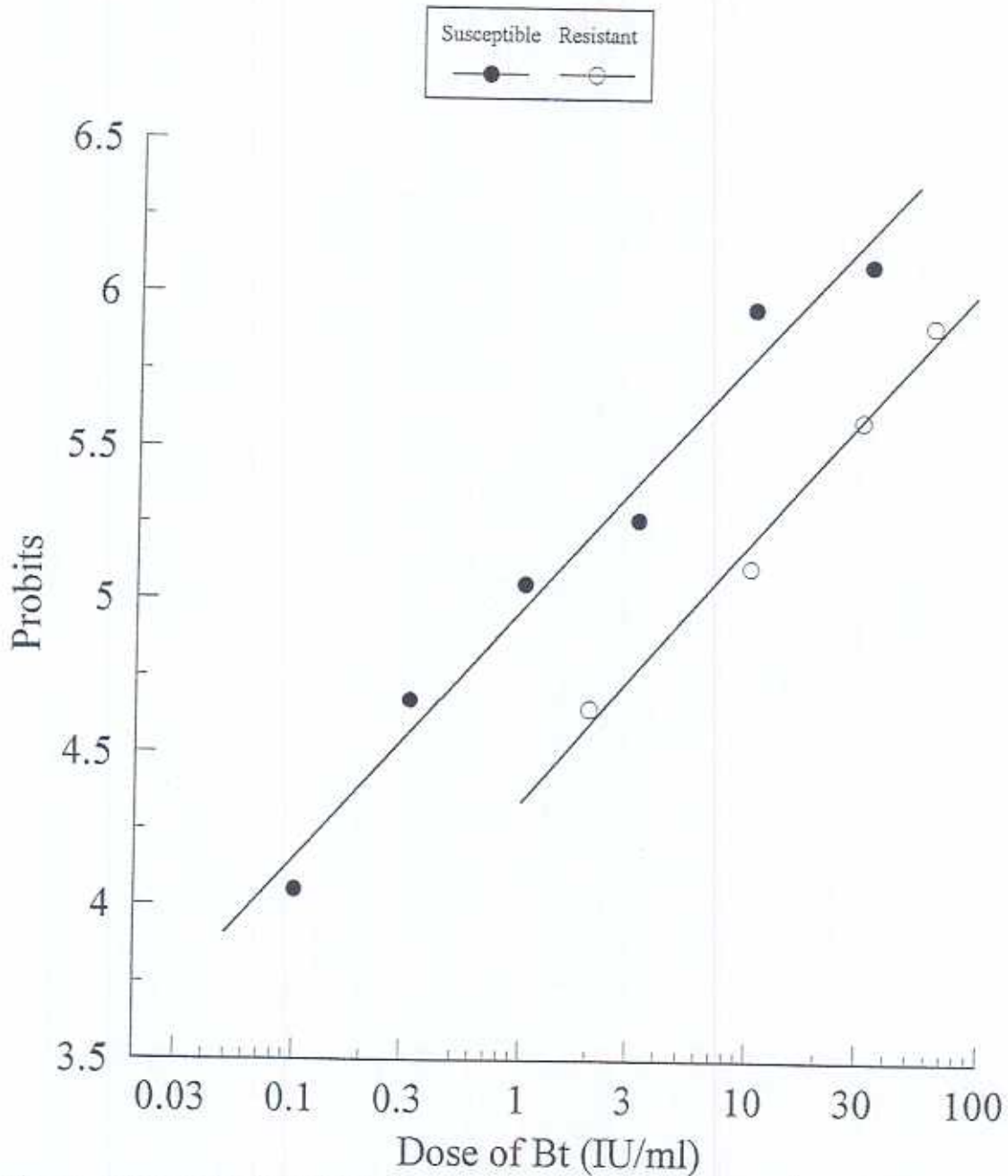


Fig. 6 Resistant (Yarra Valley) and susceptible (Mildura) strains of LBAM assayed on Bt treated leaves sprayed with Potter tower onto French bean leaves.

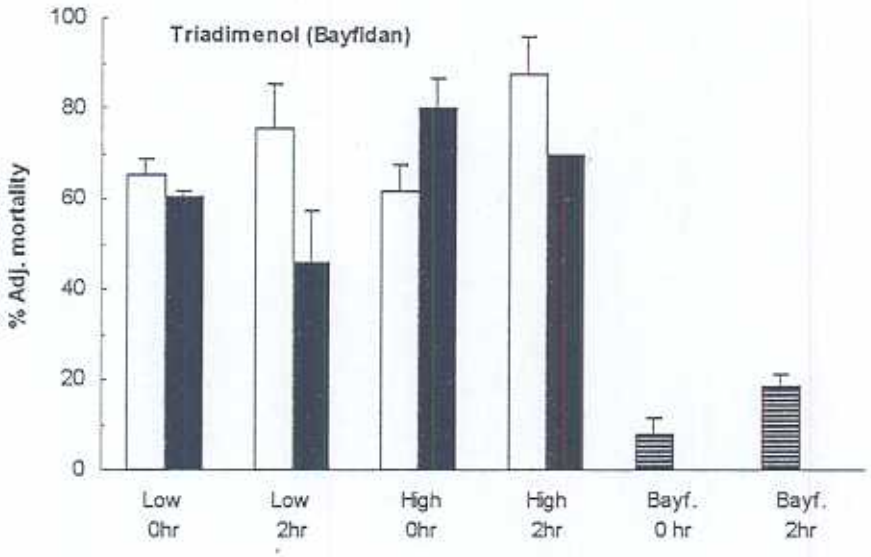


Fig 7. Mean % mortality (standard error of mean) of LBAM larvae fed on bean leaves treated with Bt (white bars) at 2 doses (low = 1000 IU/ml; high = 4000 IU/ml), Bt and Bayfidan 250EC mixtures (black bars), and Bayfidan only (grey bars) applied to bean leaves at 0 and 2 hours after mixing.

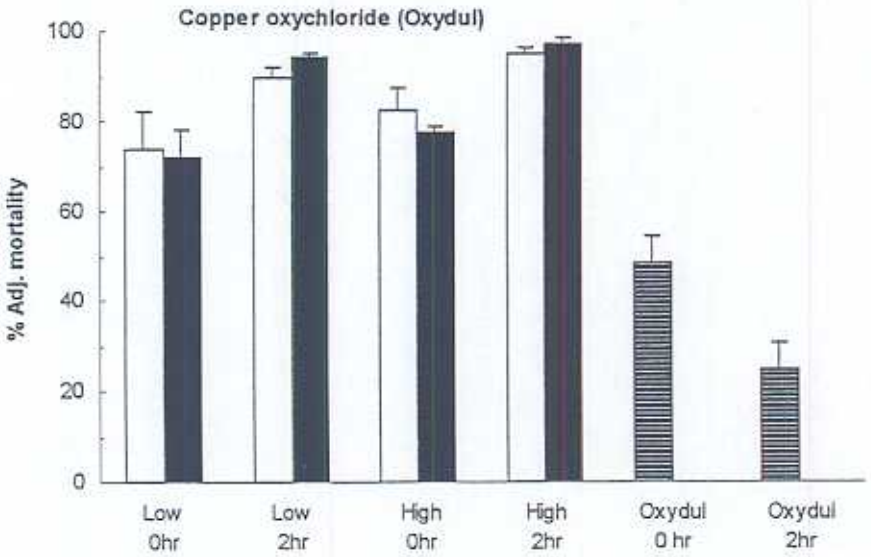


Fig 8. Mean % mortality (standard error of mean) of LBAM larvae fed on bean leaves treated with Bt (white bars) at 2 doses (low = 1000 IU/ml; high = 4000 IU/ml), Bt and Oxydul mixtures (black bars), and Oxydul only (grey bars) applied to bean leaves at 0 and 2 hours after mixing.

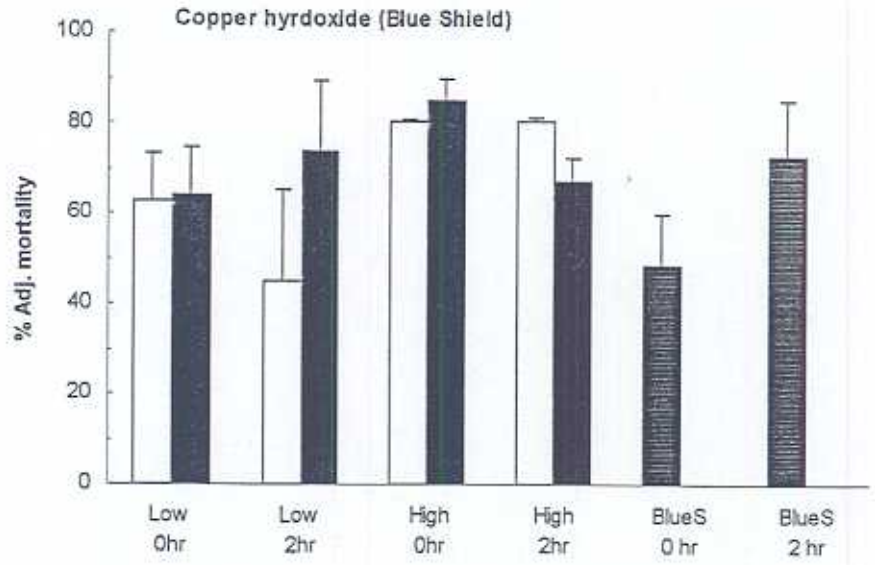


Fig 9. Mean % mortality (standard error of mean) of LBAM larvae fed on bean leaves treated with Bt (white bars) at 2 doses (low = 1000 IU/ml; high = 4000 IU/ml), Bt and Blue Shield mixtures (black bars), and Blue Shield only (grey bars) applied to bean leaves at 0 and 2 hours after mixing.

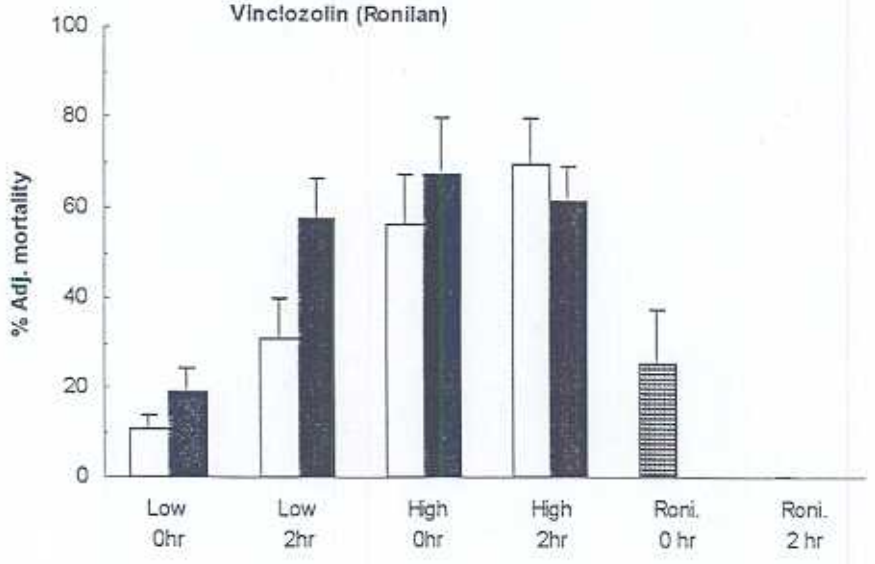


Fig 10. Mean % mortality (standard error of mean) of LBAM larvae fed on bean leaves treated with Bt (white bars) at 2 doses (low = 1000 IU/ml; high = 4000 IU/ml), Bt and Ronilan mixtures (black bars), and Ronilan only (grey bars) applied to bean leaves at 0 and 2 hours after mixing.

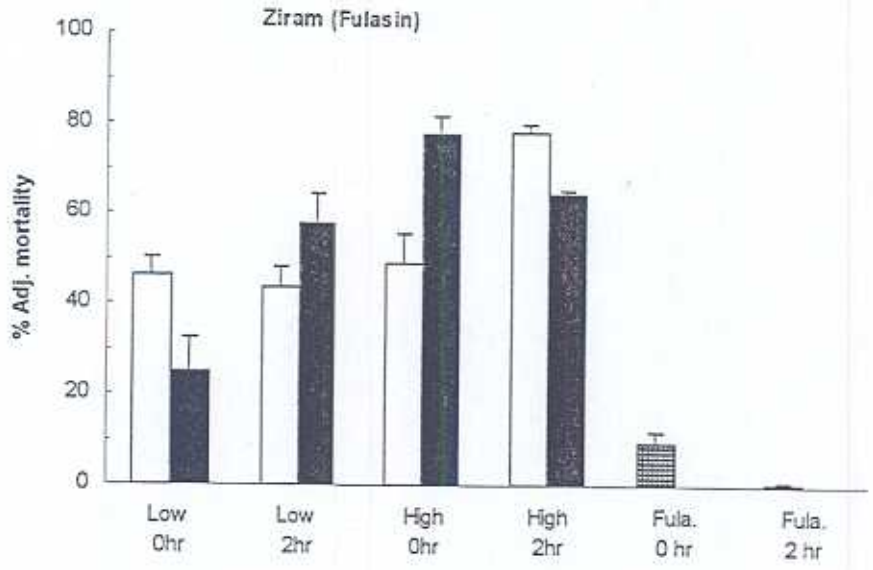


Fig 11. Mean % mortality (standard error of mean) of LBAM larvae fed on bean leaves treated with Bt (white bars) at 2 doses (low = 1000 IU/ml; high = 4000 IU/ml), Bt and Fulasin mixtures (black bars), and Fulasin only (grey bars) applied to bean leaves at 0 and 2 hours after mixing.

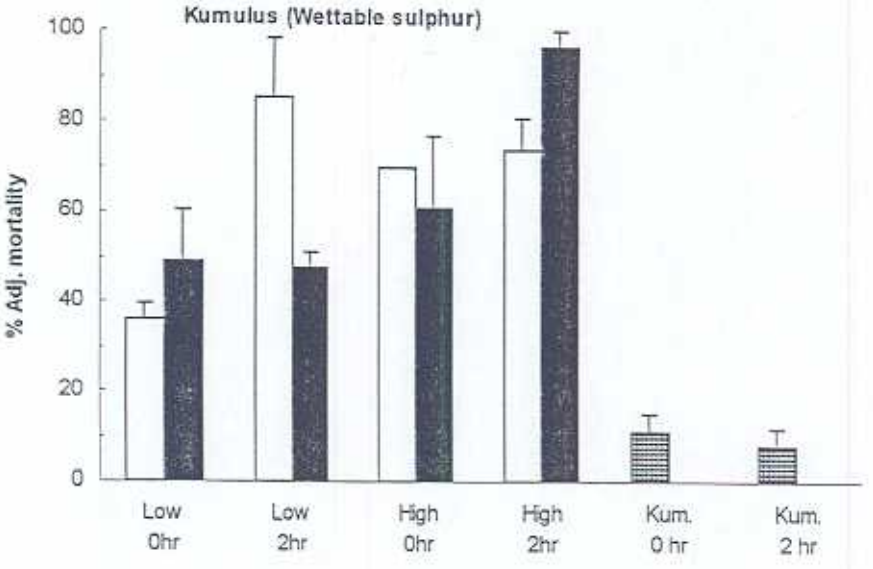


Fig 12. Mean % mortality (standard error of mean) of LBAM larvae fed on bean leaves treated with Bt (white bars) at 2 doses (low = 1000 IU/ml; high = 4000 IU/ml), Bt and Kumulus mixtures (black bars), and Kumulus only (grey bars) applied to bean leaves at 0 and 2 hours after mixing.

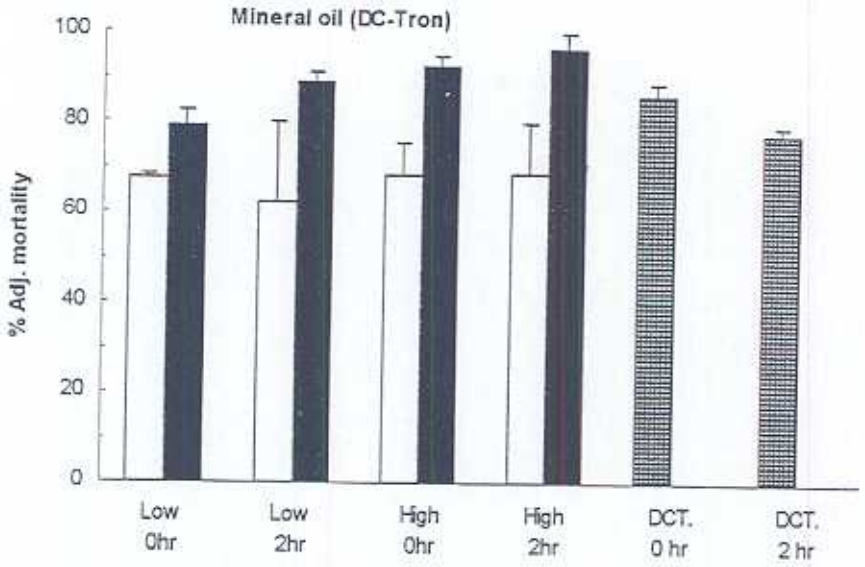


Fig 13. Mean % mortality (standard error of mean) of LBAM larvae fed on bean leaves treated with Bt (white bars) at 2 doses (low = 1000 IU/ml; high = 4000 IU/ml), Bt and DC-Tron mixtures (black bars), and DC-Tron only (grey bars) applied to bean leaves at 0 and 2 hours after mixing.

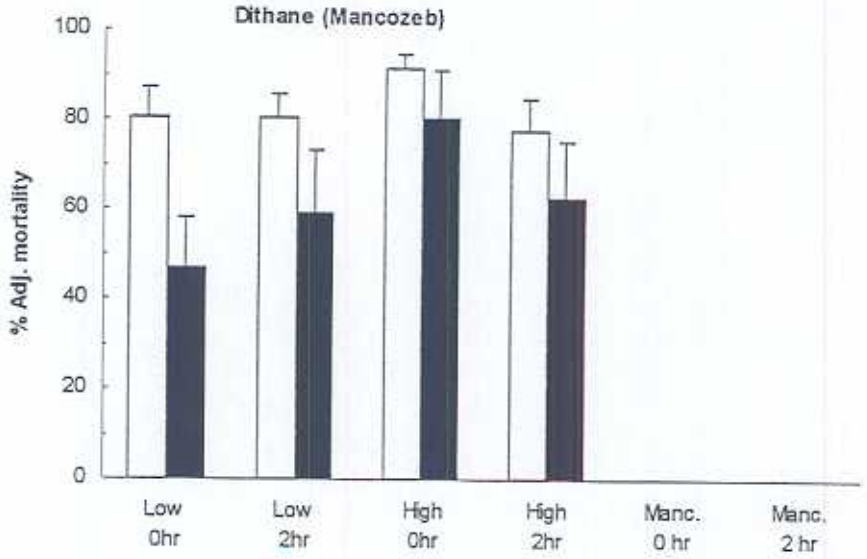


Fig 14. Mean % mortality (standard error of mean) of LBAM larvae fed on bean leaves treated with Bt (white bars) at 2 doses (low = 10000 IU/ml; high = 30000 IU/ml), Bt and Dithane mixtures (black bars), and Dithane only (grey bars) applied to bean leaves at 0 and 2 hours after mixing. Note that these treatments were applied under the Potter tower and there was no Mancozeb only treatment.

Of those mixers that were less toxic in their own right, most had no effect on efficacy of Bt at either dose. Two exceptions were triadimenol (Bayfidan), with the efficacy appearing to drop after 2 hrs of mixing, and mancozeb (Dithane) at the low dose of Bt. We concluded that all the mixers tested would not significantly reduce the efficacy of Bt in normal use. This list of test products was not exhaustive and other products or multiple combinations of these products may affect Bt in spray tanks.

4. Improving performance of Bt through increased dose, use of adjuvants and spray application.

a) Laboratory screening of adjuvants for effects on efficacy

The screening trials indicated that a concentration of 1.6 µg/cm² was close to the LC_{50} of most products to LBAM, and was a suitable concentration for screening adjuvants as it would allow any positive or negative effects to be shown. The efficacy of Bt on LBAM was not significantly enhanced by most of the adjuvants screened at label rate (Fig. 15-23). The 2 exceptions were Latron and Citowett. Latron appeared to increase the efficacy of Biobit HPWP and Dipel Forte when sprayed in the Potter Tower (Fig. 16). The addition of Citowett increased activity of Delfin WG and Dipel Forte in the Potter Tower (Fig. 17) but the same adjuvant sprayed with the hand atomiser resulted in lower efficacy (Fig. 18). The reason for this difference in behaviour is unknown.

Our laboratory trials suggested that use of wetters as per label recommendations may increase efficacy. This conclusion is supported by an increase in efficacy of Dipel Forte when applied with 0.01% Citowett in the first field trial (see below).

b) Comparing effects of rate, adjuvants and spray machinery on Bt efficacy in vineyard trials

In trial #1, the mortality of LBAM in the leaf sandwich bioassay was low for each Bt product and application rate (Fig. 24 a). Mortality ranged from 12.8% for Dipel Forte at 125g/ha to 26.4% for Dipel Forte at 375g/ha plus 0.01% Citowett. Low larval mortalities were also obtained using the leaf disk method, except for Dipel at 375 g/ha plus Citowett at 48.8% (Fig. 24 b).

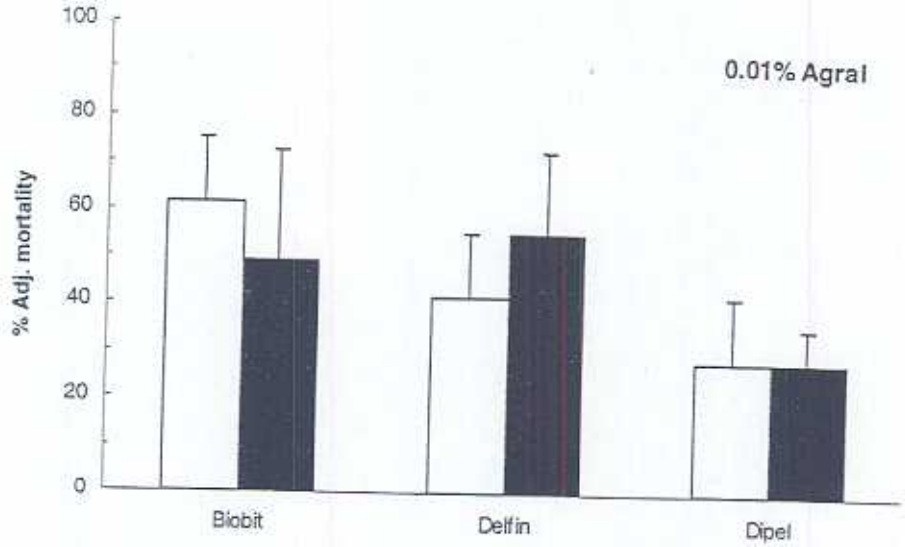


Fig. 15 Mean % mortality of LBAM larvae fed on grapevine leaves treated with Bt products, Biobit HPWP, Delfin WG and Dipel Forte, applied with and without 0.01% Agral in a Potter tower.

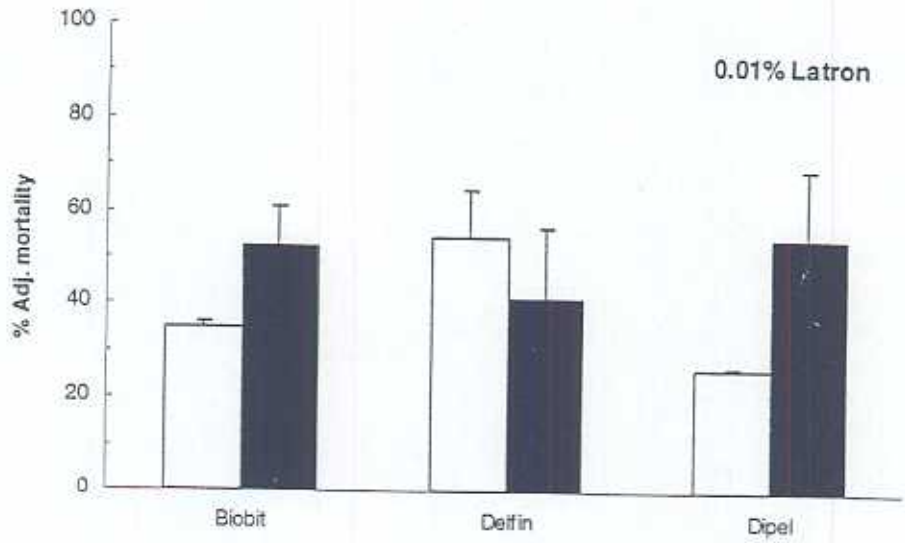


Fig. 16 Mean % mortality of LBAM larvae fed on grapevine leaves treated with Bt products, Biobit HPWP, Delfin WG and Dipel Forte, applied with and without 0.01% Latron in a Potter tower.

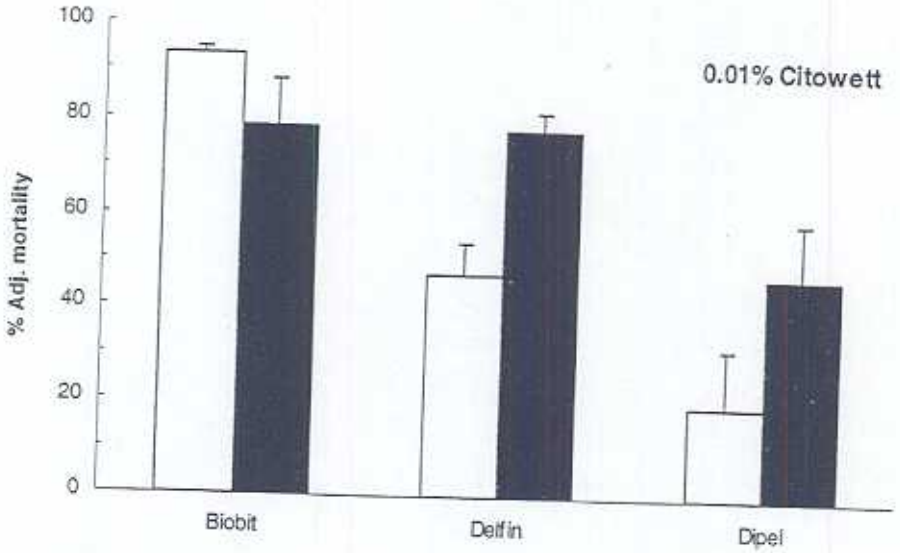


Fig. 17 Mean % mortality of LBAM larvae fed on grapevine leaves treated with Bt products, Biobit HPWP, Delfin WG and Dipel Forte, applied with and without 0.01% Citowett in a Potter tower.

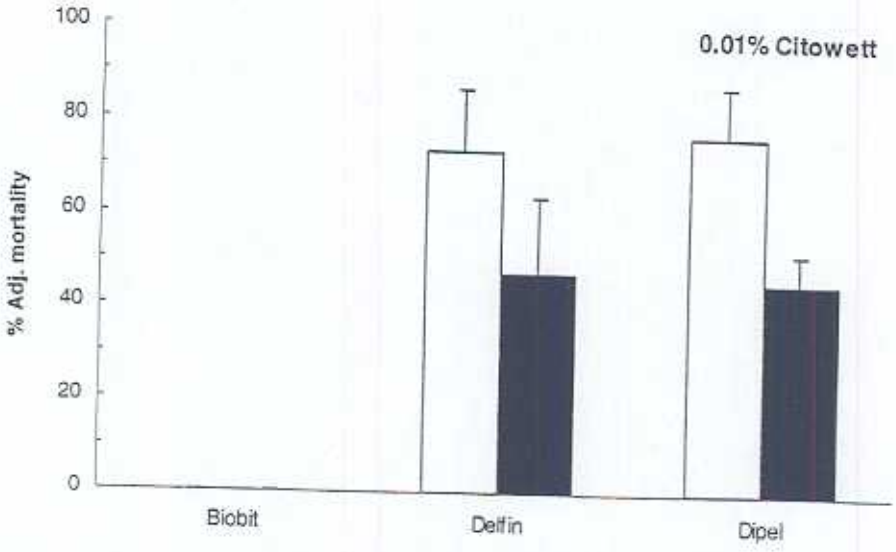


Fig. 18 Mean % mortality of LBAM larvae fed on grapevine leaves treated with Bt products, Delfin WG and Dipel Forte, applied with and without 0.01% Citowett using a hand-held atomiser. Note that there was no Biobit HPWP treatment.

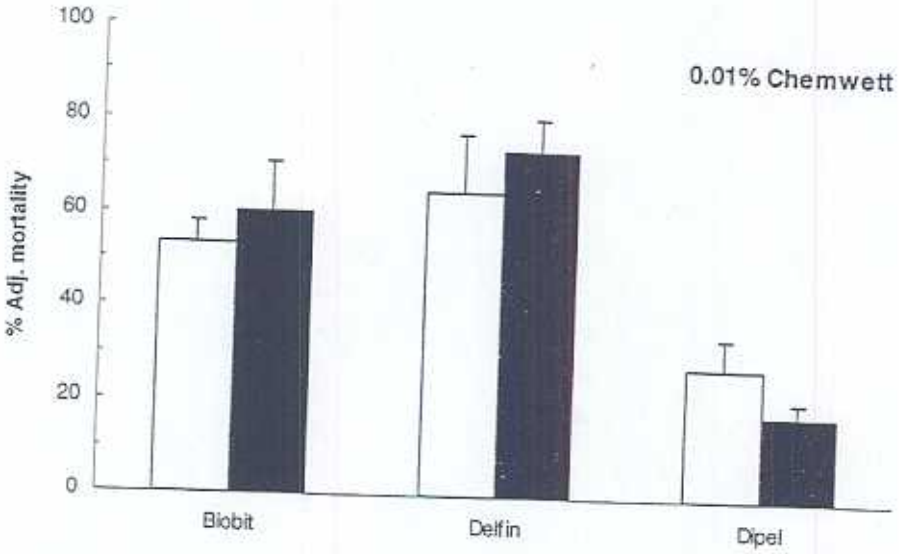


Fig. 19 Mean % mortality of LBAM larvae fed on grapevine leaves treated with Bt products, Biobit HPWP, Delfin WG and Dipel Forte, applied with and without 0.01% Chemwett in a Potter tower.

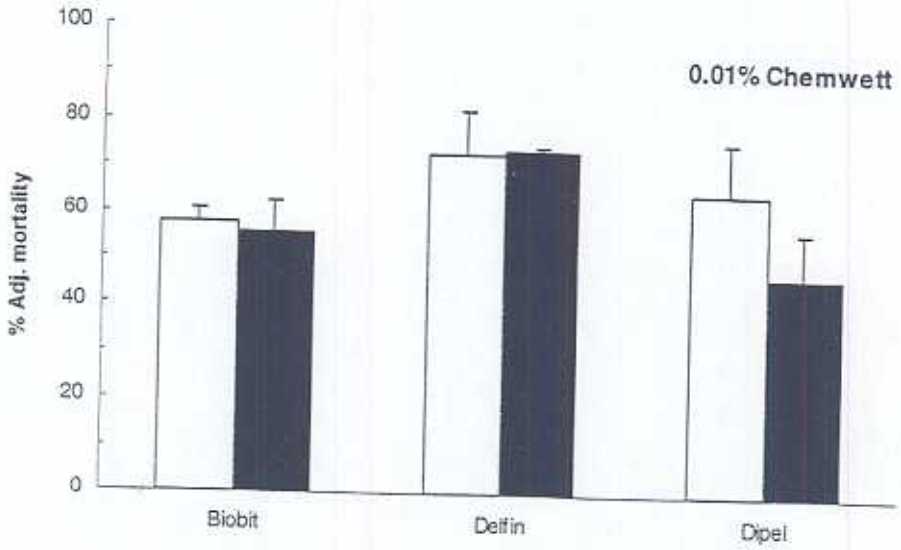


Fig. 20 Mean % mortality of LBAM larvae fed on grapevine leaves treated with Bt products, Biobit HPWP, Delfin WG and Dipel Forte, applied with and without 0.01% Chemwett using a hand-held atomiser.

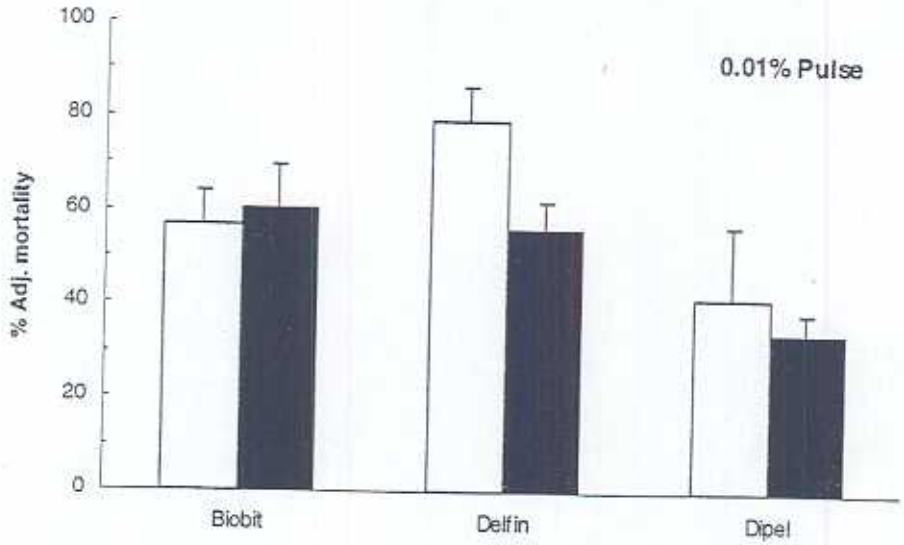


Fig. 21 Mean % mortality of LBAM larvae fed on grapevine leaves treated with Bt products, Biobit HPWP, Delfin WG and Dipel Forte, applied with and without 0.01% Pulse in a Potter tower.

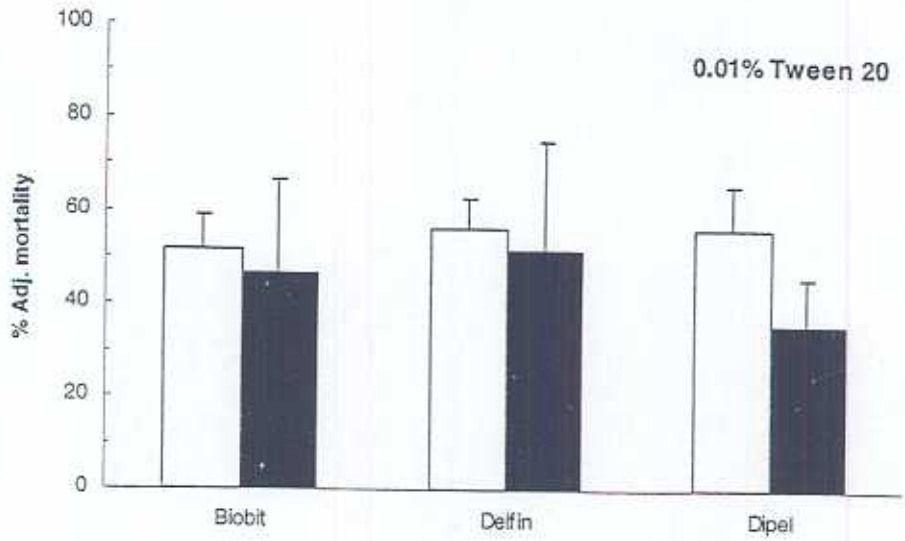


Fig. 22 Mean % mortality of LBAM larvae fed on grapevine leaves treated with Bt products, Biobit HPWP, Delfin WG and Dipel Forte, applied with and without 0.01% Tween 20 in a Potter tower.

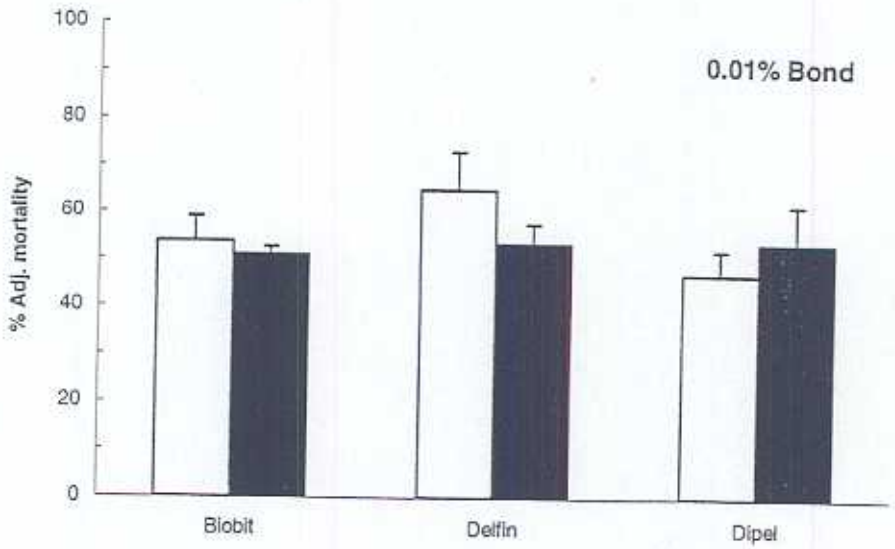


Fig. 23 Mean % mortality of LBAM larvae fed on grapevine leaves treated with Bt products, Biobit HPWP, Delfin WG and Dipel Forte, applied with and without 0.01% Bond in a Potter tower.

According to the bioassays, control of LBAM in this trial would have been inadequate even though rates of application were up to 3 times the label rate. It was difficult to determine the reasons for poor control. It may have simply been the testing against the resistant strain of LBAM, which may need still higher doses to control it. The highest LBAM mortality achieved with the addition of Citowett suggested there may have been a problem with application and adherence to the leaf surface. Citowett may have enhanced the coverage of Bt on the leaf surface by reducing surface tension of the spray droplets and therefore promoting adhesion to the leaf rather than shattering and bouncing off the surface. Another reason may have been related to the growers spray equipment, which was not specifically calibrated for the trial.

Unlike the first trial, satisfactory control of LBAM was obtained at the high rate of Bt in trial #2. Mortalities were about 20-40% at the registered field rate and up to 87% for 3 times label rate (Fig. 25). Significant differences in mortality occurred between dose, method of bioassay, and volume of application. Both assay methods showed an increase in mortality

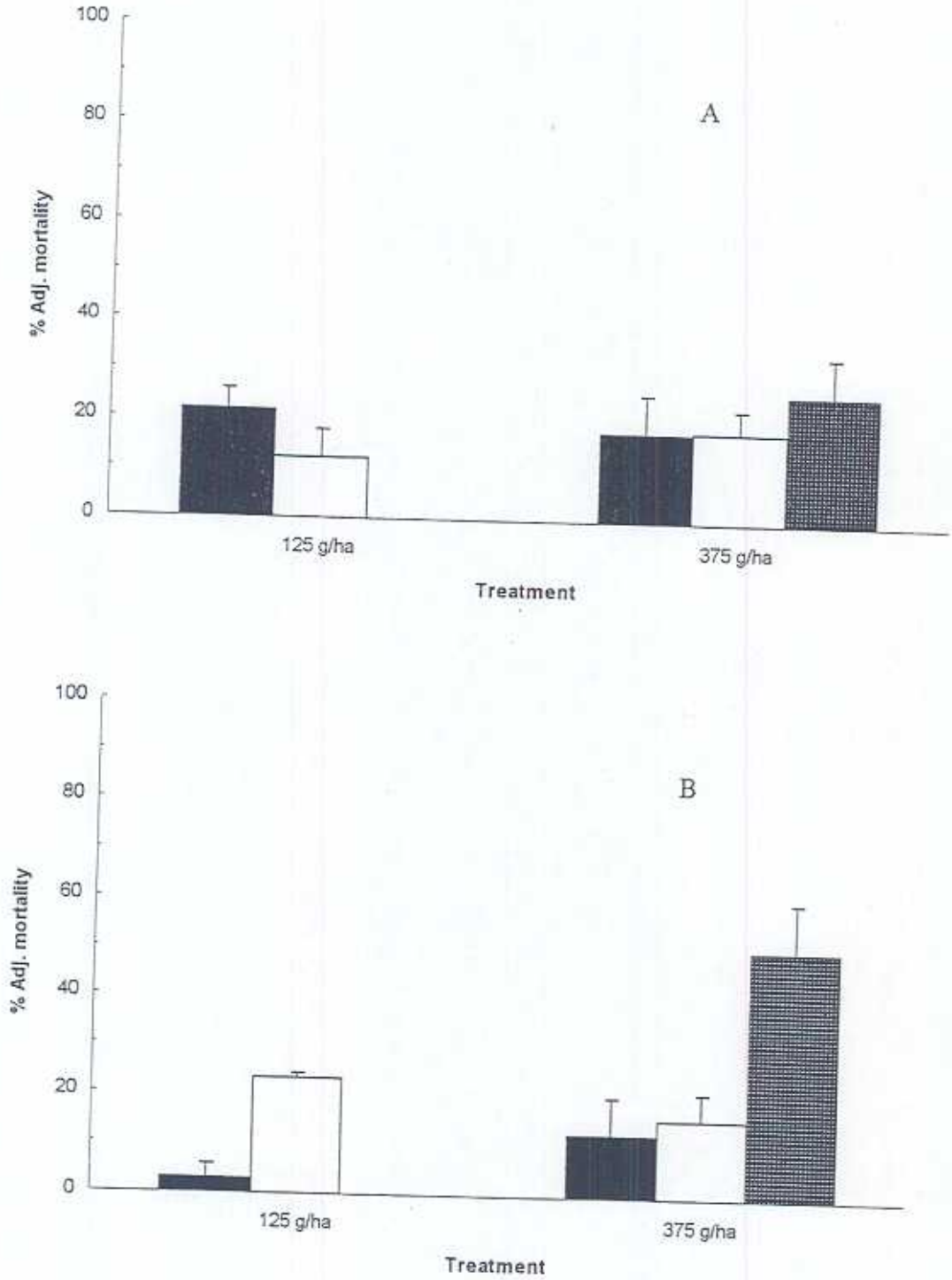


Fig. 24 Mean % mortality (standard error of mean) of LBAM larvae in a bioassay using the (A) '**leaf sandwich**' and (B) **leaf disk** methods on leaves collected from spray application trial #1. Treatments were Delfin WG (black bar) and Dipel Forte (white bar) at 125 and 375 g/ha, and Dipel Forte at 375 g/ha plus 0.01% Citowett (grey bar); all applied at 200 L/ha.

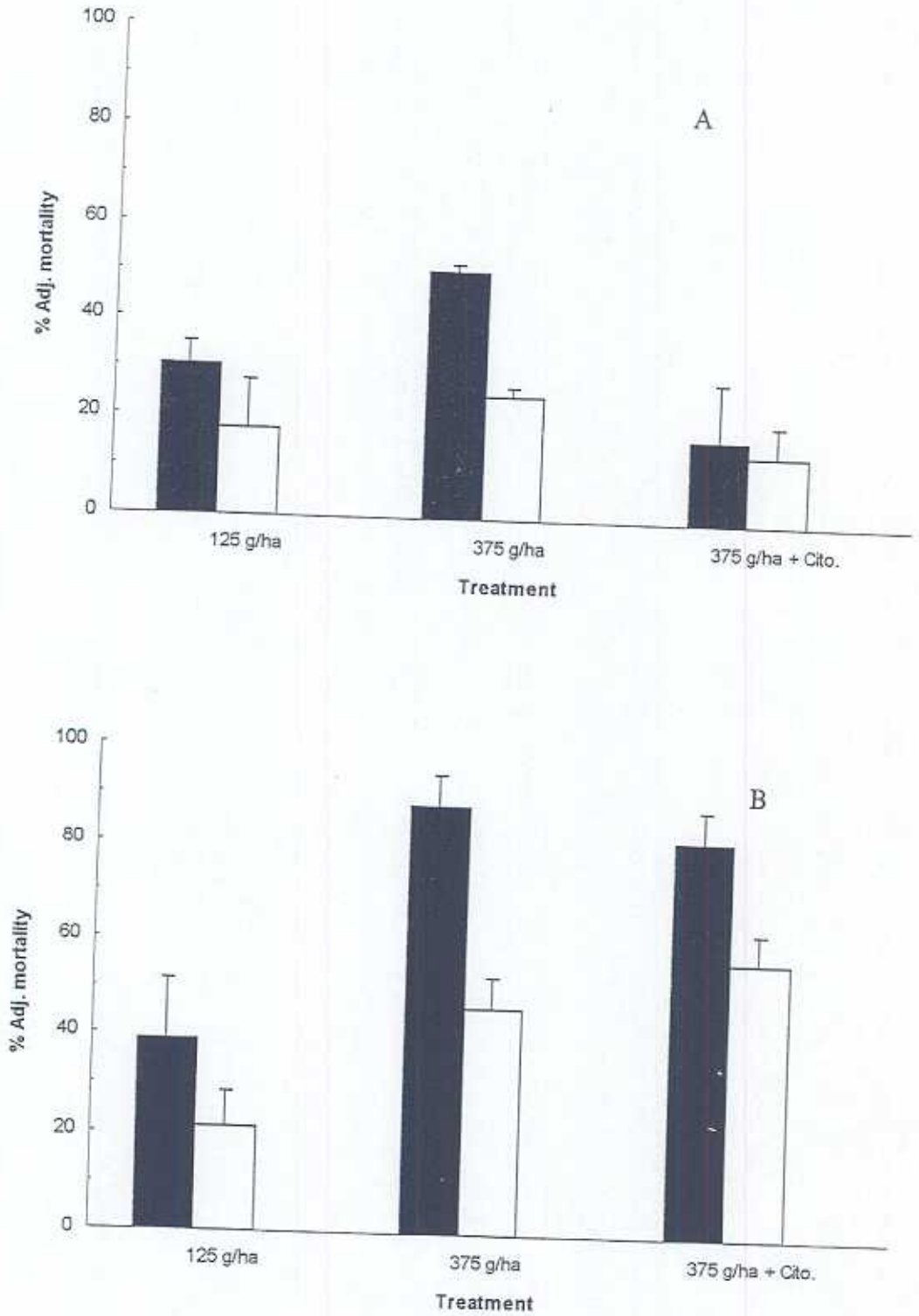


Fig. 25 Mean % mortality (standard error of mean) of LBAM larvae in a bioassay using the (A) **'leaf sandwich'** and (B) **leaf disk** methods on leaves collected from spray application trial #2. Treatments were Dipel Forte at 125 and 375 g/ha, and 375 g/ha plus 0.01% Citowett at two volumes 200 L/ha (black bar) and 1,000 L/ha (white bar).

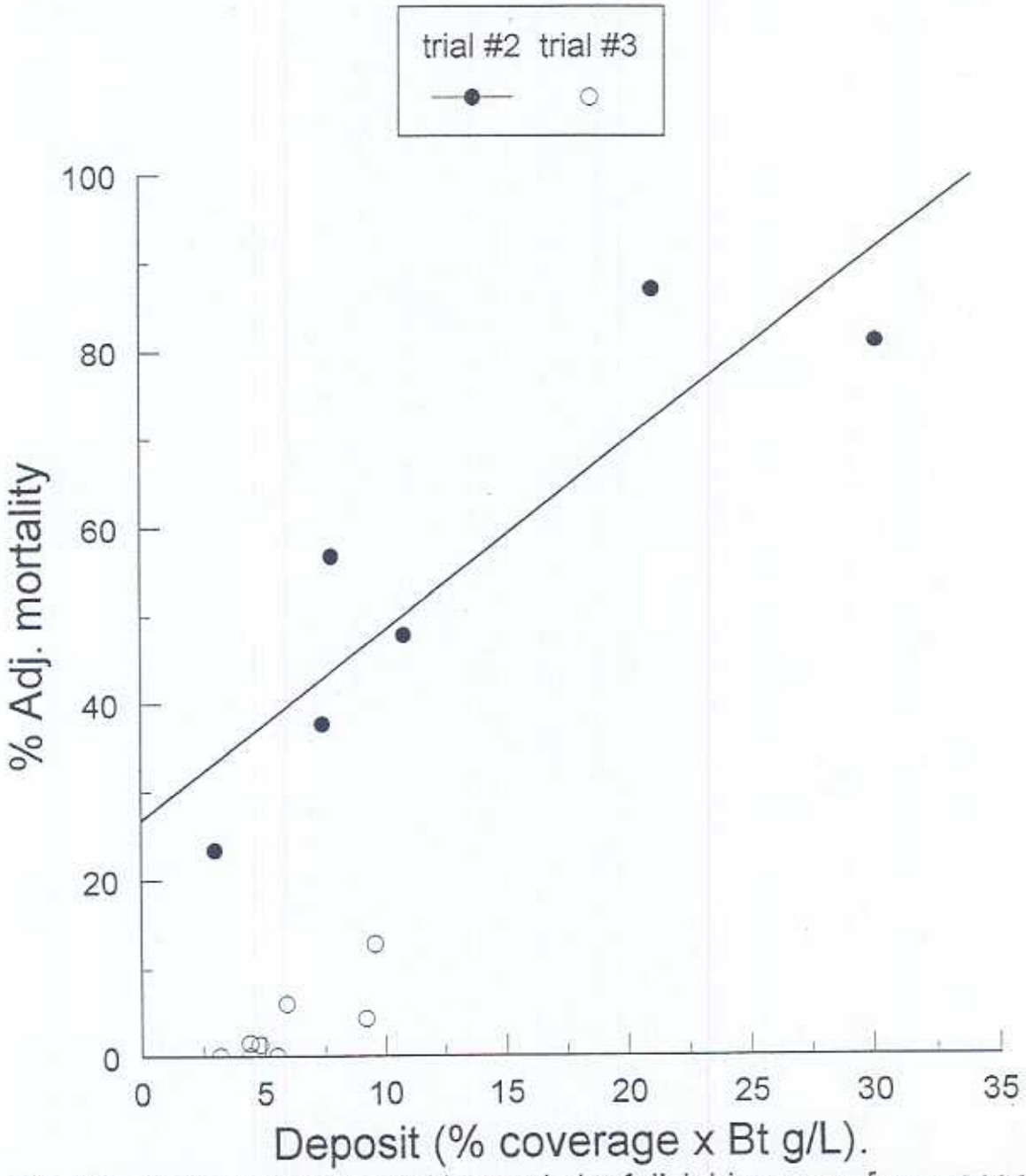


Fig. 26 % Mortality of LBAM larvae in leaf disk bioassays for a range of Bt deposits from vineyard trials #2 and #3.

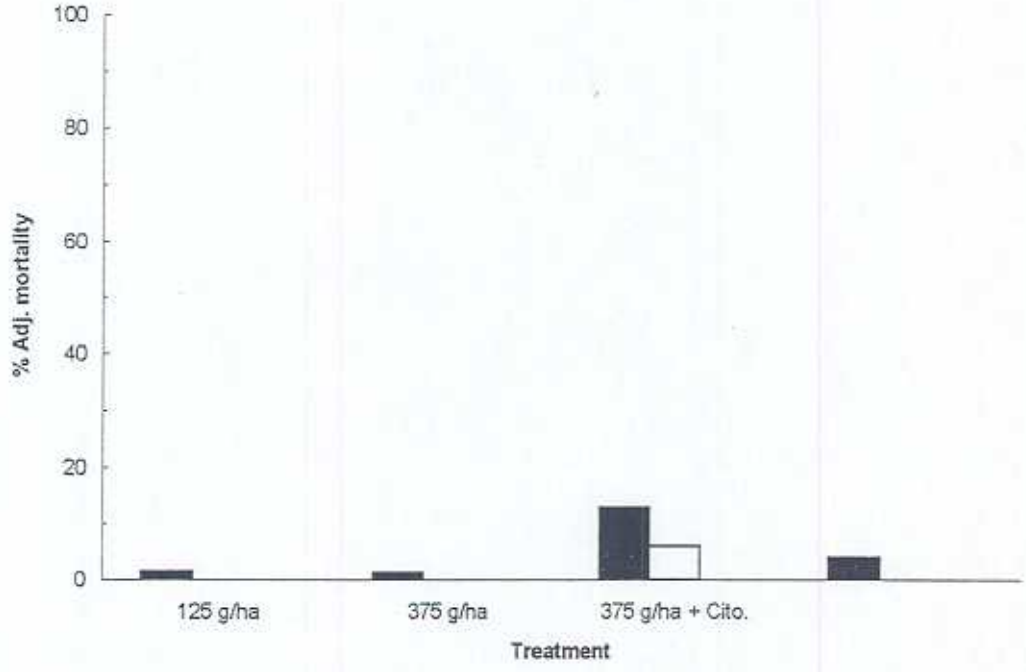


Fig. 27 Mean % mortality of LBAM larvae in a bioassay using the **leaf disk** method on leaves collected from spray application trial #3. Treatments were Dipel Forte at 125 and 250 g/ha, with and without 0.01% Citowett at two volumes 200 L/ha (black bar) and 1,000 L/ha (white bar).

with dose, but the leaf disk method produced comparatively higher rates of mortality. Higher mortalities suggested that the leaf disk assay method was probably a better (but not ideal) simulation of feeding conditions as larvae were able to consume Bt deposits as they graze over the leaf surface. In the leaf sandwich method, larvae had restricted feeding areas within the tray cells, and the method was more suited to discrete dose assays. Young first instar larvae disperse from the egg masses to the shoots and shelter within the folded terminal leaf (Baker and Bailey 1993). Investigation of feeding behaviour and targeting of Bt sprays within these shoot tips may help improve the efficacy of Bt.

Bt treatments applied using low volume yielded higher mortality than the same treatment applied using high volume application. An examination of the % coverage of Bt solutions on spray cards showed the high volume treatments had 21 to 29% coverage, which was greater

than the low volume treatments at 11 to 16% (Table 4). However, % coverage is not a measure of the amount of active ingredient (gms of Bt) per unit area (cm^2 of card). 'Deposit', or % coverage adjusted for concentration of the spray solution, was a better measure for comparing treatments. Deposit values were higher for the low volume treatments than for the high volume ones, and more closely reflected the trend in mortality observed in bioassays. Mean % mortality in the leaf disk assays was significantly correlated with mean deposit values on the spray cards ($r^2 = 0.79$; $F = 15.29$; $df = 5$; $P < 0.05$) (Fig. 26).

In trial #3, LBAM mortalities for all treatments were very low (Fig. 26). The inclusion of Citowett at both water volumes and Bt concentrations did not increase mortality. The highest adjusted mortality was only 12.8% for Dipel Forte at twice label rate and 1000 L/ha.

Like trial #1, the low mortality was an indication that the rates of 125 and 250 g/ha were too low for adequate control of young LBAM larvae. Results from trial #2 suggest that 375 g/ha may be the more appropriate dose. After a series of trials in grapevines, Nufarm had registered the Novo Nordisk product, Biobit HPWP, at 4 to 6 times the current rate of some other products (i.e. 500-700 g/ha).

In trial #2, coverage of the water sensitive cards was 5 to 9 times higher at 1000 L/ha (Table 5). Deposit values were similar at the 125 g/ha rate of Bt whereas the air blast sprayer had nearly twice the deposit at the 250 g/ha rate. Unlike trial #2, there was no obvious trend between deposit and mortality of LBAM in bioassays. The size of the deposits in trial #3 were lower than in trial #2 at comparable rates of Bt but not enough to explain low levels of mortality (Fig. 26). For example, deposit values of 3-11 in high volume spraying in trial #2 gave 20-40% mortality whereas values of 9 in trial #3 appeared to give negligible mortality, after adjustment for control mortality. Irrespective of bioassay results, there were large differences in % coverage between the trials. These differences were probably due to the combined effects of variations in weather, vine topography, canopy volume/ha, and the set up of the spray machines on the days.

Reasons for the discrepancy between trials in the deposit-mortality relationships were unclear. One explanation may be quality of the leaf. Perhaps the Chardonnay variety (trial #3) was

Table 4. Coverage (%) and deposit (% x g/L) data from spray cards for Dipel Forte applied at 2 rates (highest rates with and without 0.01% Citowett) and 2 application volumes in Trial #2.

	Air shear sprayer (200 l/Ha)			Air blast sprayer (1000 l/Ha)		
	125 g/Ha	375 g/Ha	375 g/Ha +Citowett	125 g/Ha	375 g/Ha	375 g/Ha +Citowett
Coverage (%)	11.9	11.2	16.0	24.2	28.7	20.9
Deposit (% x Bt g/l)	7.4	21.0	30.1	3.0	10.8	7.8

Table 5. Coverage (%) and deposit (% x g/L) data from spray cards for Dipel Forte applied at 2 rates, with and without 0.01% Citowett, and 2 application volumes in Trial #3.

	Air shear sprayer (200 l/Ha)				Air blast sprayer (1000 l/Ha)			
	125 g/ha	125 g/ha + Cito.	250 g/ha	250 g/ha + Cito.	125 g/ha	125 g/ha + Cito.	250 g/ha	250 g/ha + Cito.
Coverage	5.1%	7.1%	4.7%	4.4%	35.0%	38.7%	38.5%	36.9%
Deposit (% x Bt g/l)	3.2	4.4	5.9	5.5	4.4	4.8	9.6	9.2

more inhibitory of Bt activity than Cabernet Sauvignon (trial #2) or the older leaf age in trial #3 meant the leaves contained higher levels of Bt-inhibiting tannins. Another explanation was proposed after an examination of treated leaves under a Scanning Electron Microscope. This SEM view failed to reveal any of the distinctive bipyramidal Bt crystals or spores on the leaf surface. The only deposit was a far smaller particle, which also appeared on the unsprayed leaves, and we believed to be remnants of a sulphur application that occurred a few weeks before the trial. However, a tank mix sample was tested for particle content and found to contain the expected size range of particles. Although this was only a preliminary finding, it suggests that a closer study of the behaviour of Bt solutions through spray nozzles and machinery may provide clues as to the inconsistent performance of Bt in these trials.

Acknowledgments

The researchers would like to thank the Grape and Wine Research and Development Corporation, Rural Industries Research and Development Corporation, Abbott Agricultural Products Division, Hoechst Schering AgrEvo Pty Ltd, Nufarm Chemicals Pty Ltd, Sandoz Australia Pty Ltd and Silvan Pumps and Sprayers Pty Ltd for their support. We are also indebted to the managers who cooperated in the field trials: Steve Sadlier (Yarra Ridge Winery); Keith Harris (Hoddles Creek Beenak Vineyard) and Stewart McNabb (Fernhill Vineyard).

Implications and recommendations

Major findings and implications:

* Major Bt products had different potencies against first instar LBAM in laboratory bioassays but it is unclear if these differences extrapolate to efficacy in vineyards.
* Efficacy of Bt appears to be inhibited by grapevine leaf chemistry and poor retention or spreading/sticking on the leaves.
* There is natural variation in susceptibility of LBAM populations to Bt.
* Label rates for Bt in grapevines are too low at 125 g/ha, especially for the 'resistant' strains of LBAM.
* Bt is compatible with common tank mixers examined in this study
* Wetters and stickers may enhance Bt efficacy if used according to recommended rates.
* Spray volume does not appear to be important for coverage and efficacy of Bt..

Recommendations:

* Better control of LBAM with Bt may be achieved by:
    1. Using higher rates of Bt (greater than or equal to 375 gm/ha of 32,000 IU/mg products). [Nufarm had registered Biobit HPWP at 500-700 g/ha]
    2. Timing sprays to coincide with young larvae (egg hatch).
    3. Ensuring calibration of sprayers and good coverage.

* Further research is required to:
    1. Fully test the LBAM strains from separate geographical regions for resistance to Bt.
    2. Confirm the higher rates of Bt achieve satisfactory control in commercial vineyards.
    3. Define the best method for spray application, targetting and coverage of Bt, with special emphasis on machinery set up (e.g. nozzle type and fan speed), and ideal targets, like flower clusters and shoot tips.

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