

Commercial in Confidence

Client Report No.

**Improving predictions of pest mortality
prior to pest eradication operations: The
influence of host species and
provenance on the efficacy of *Bacillus
thuringiensis* var. *kurstaki***

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Date: **June 2005**
Client: **Forest Biosecurity Research Council (FBRC) and
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Contract No:

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EXECUTIVE SUMMARY

1. An apparent host plant-mediated effect on the efficacy of Btk on painted apple moth was confirmed.
2. Secondary hosts, which prolong painted apple moth generation times, promote the efficacy of Btk.
3. Primary hosts ameliorate the effect of Btk.
4. Artificial diet, which is the best known growth medium for painted apple moth, does not ameliorate the toxicity of Btk.
5. Trials suggest that the mediation of Btk efficacy is not related to plant architecture or overt secondary plant chemistry.
6. It is postulated that some component within the living tissue of the primary host plant is responsible for suppressing the toxicity of the Btk γ -endotoxin.
7. On plant hosts, Btk efficacy was inversely proportional to the larval growth rate of painted apple moth larvae on those hosts.
8. It is proposed that larval growth rate be used as a predictor for the efficacy of Btk in field operations.



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Information for Ensis abstracting:

Contract number	FBRC Milestone
Client Report No.	
Products investigated	
Wood species worked on	
Other materials used	
Location	

INTRODUCTION

Bacillus thuringiensis var. kurstaki (Btk) is the insecticide of choice for aerial operations over urban areas, as it is considered environmentally benign. Its high specificity, but limited efficacy, is seen as a judicious compromise in such situations. However, adequate application rates and frequency become paramount to the success of eradication programmes and it is important to understand and minimise parameters which may further compromise efficacy.

Host-plant mediated effects on the efficacy of Btk have been recorded for a number of pests. Factors affecting Btk efficacy have been inferred from variation in host plant secondary chemical compounds, pH and the presence of protease inhibitors, as well as a possible feeding deterrence from Btk itself.

Apparent host-plant-Btk interactions were noted in bioassays supporting the painted apple moth, *Teia anartoides* (Lepidoptera: Lymantriidae), eradication programme currently underway in Auckland. Eradication programmes are often instigated when invasive alien species of unknown calibre threaten crops and indigenous ecosystems. In many instances the establishment of alien invertebrates results in unpredictable novel pest-plant interactions. A predictor for the outcome of such interactions on the efficacy of Btk would not only enhance pest assessments, but would also enable efficiencies in application rates and frequencies and could lead to improved formulations, better Bt strains selection and the management of resistance to Btk.

The painted apple moth is an Australian polyphagous defoliator with the potential to significantly damage forest and horticultural crops. Although considered a polyphagous defoliator painted apple moth larvae are primarily found on *Acacia* species. Larvae feeding on secondary hosts typically exhibit lower growth rates and higher natural mortality than on primary hosts (Charles *et al.* 2004). The plants trialed in this study included primary and secondary hosts of the painted apple moth which had a selection of foliage types and plant architecture. Larval growth rate on the various host plants was assessed as a predictor of Btk efficacy. Trials with provenance selections of one host plant were utilised to eliminate the possible influence of plant architecture on the interception of Btk.

MATERIALS AND METHODS

Foray Btk was sourced from stocks used for the painted apple moth eradication programme. The Btk was applied to cut plant samples passing under an applicator on a moving belt 'track sprayer'. Belt speed and both Btk dilution and pump delivery rate can be regulated to deliver nominal doses by way of the CDA ULV8 applicator delivering droplets of 120 μ vmd. The equipment is designed to mimic that used in operational spray programmes

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and dose rates are given as litre/hectare equivalents. A range of dose rates were trialed for different host plants to achieve a sub-lethal dose for the instar and host plant under trial. Dose was measured by the colorimetric analysis of the Btk deposition on mylar sheets that were run consecutively with the plant samples. All plant material was sourced from **ensis** Rotorua campus, except for *Paraserianthes lophanta*, which was collected from roadside trees at Pyes Pa. Test plants included at least three trees of *Acacia mearnsii*, *A. dealbatum*, *A. decurrens*, *P. lophanta*, *Sophora microphylla* and *Pseudotsuga menziesii*. Foliage of provenances of *P. menziesii* were taken from a research plot established in the 1940's.

Larvae for the bioassays were provided from the painted apple moth colony maintained on artificial diet within the **ensis** quarantine facility. Bioassays were run at $22^{\circ}\pm 1^{\circ}\text{C}$ and 16 hour photoperiod within the same facility. Five or ten replicates were run of each host plant in bioassays which utilised either 10 first instar larvae, 5 third instar larvae, or 1 fifth instar larva per replicate. For each bioassay, control and treated plant samples were secured individually, each in a two-pot system (Matsuki *et al.* 2001), which maintained plant turgor, while providing a dry test arena around the plant for the insects. For each bioassay the control and sprayed replicates were arranged as five or ten randomised blocks within the facility.

Larvae were placed within the test arenas and mortality and/or development was recorded at one to three day intervals. Larvae from the control replicates were used to determine larval growth rates. At the end of trials larvae were killed in alcohol, then air-dried and weighed when they had reached a stable weight. Pupal fresh weights were used to determine the growth rate of fifth instar larvae.

The study was designed firstly to confirm the host-mediated differential in Btk toxicity and its relationship with larval growth rate. The trials required the determination and application of sub-lethal doses on the various hosts to provide a variability of response that would allow statistical correlation. Results were then applied in the Douglas fir provenance trial to confirm the relationship between larval growth rate and Btk toxicity, while eliminating any overt influences of plant architecture and chemistry.

1. THE EFFECT OF HOST PLANT SPECIES ON Btk EFFICACY

Bioassays of first, third and fifth instar painted apple moth larvae were run for the six host plants with Btk applications of 2 or 3.5 l/ha.

Results:

Similar differential mortality was seen across all plant hosts for all instars. Larvae feeding on the *Acacia* species consistently showed better survival and faster growth than those on other hosts (Figs 1-3). *Sophora microphylla* was

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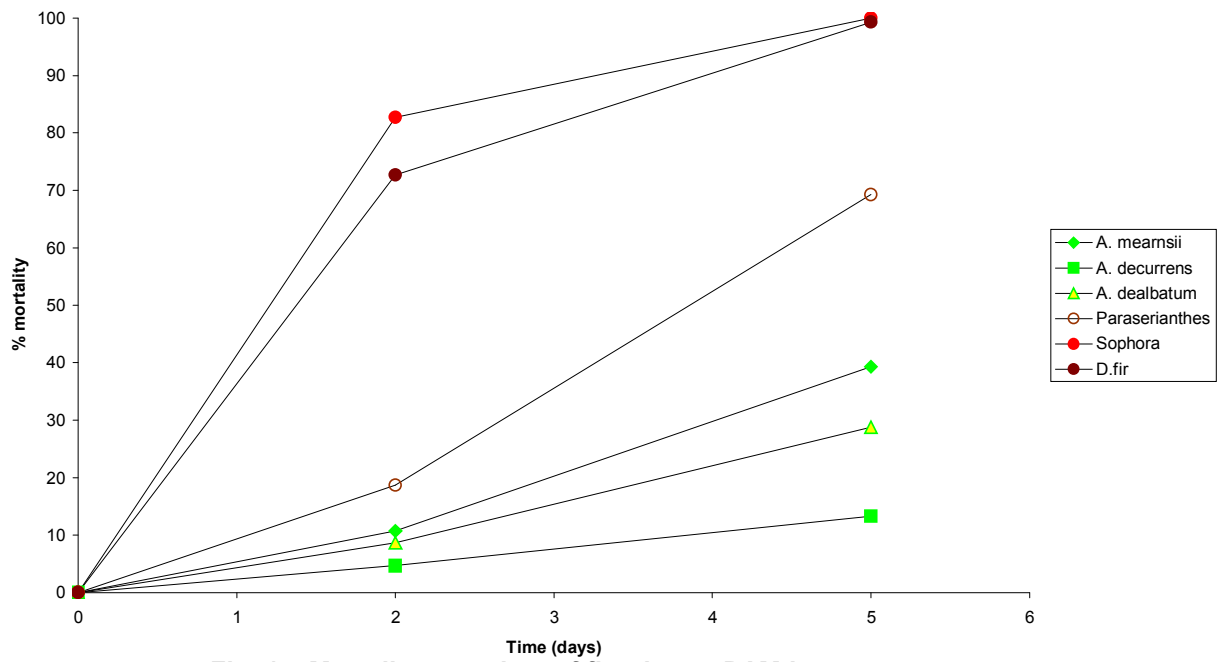


Fig. 1 – Mortality over time of first instar PAM larvae on hosts sprayed with 2L/ha Btk equivalent

% mortality 3rd instar PAM on various hosts with 2L/ha Btk

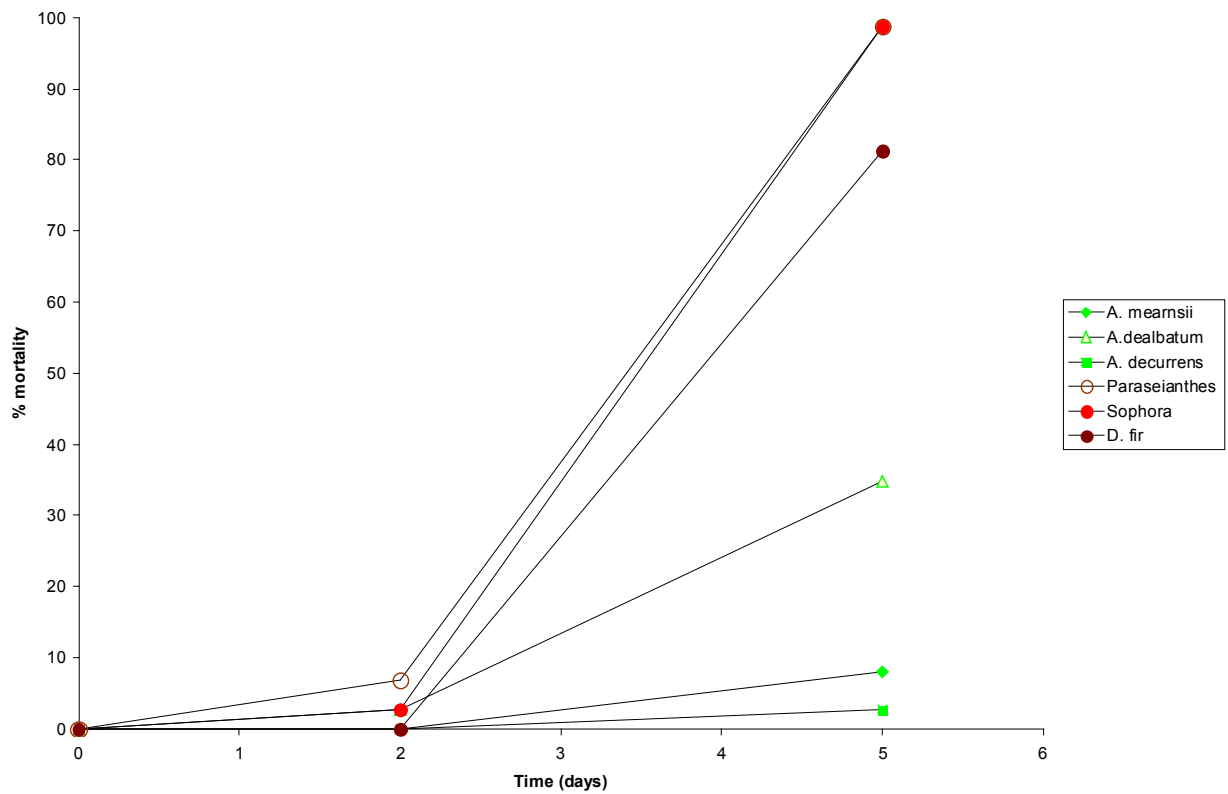


Fig. 2 – Mortality over time of third instar PAM larvae on hosts sprayed with 2L/ha Btk equivalent

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% mortality 5th instar PAM on hosts with 3.5L/ha Btk

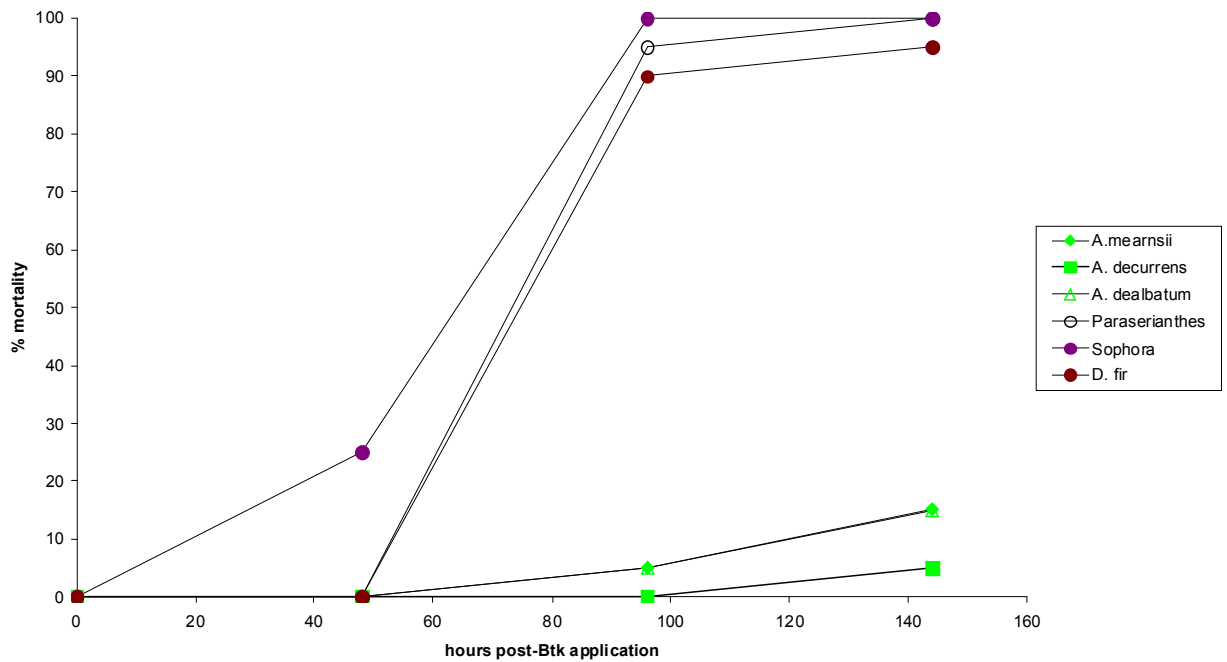


Fig. 3 – Mortality over time of fifth instar PAM larvae on hosts sprayed with 3.5L/ha Btk equivalent

consistently the poorest host showing less feeding on controls (Image 1) and no apparent feeding on sprayed foliage (Image 2). On *Acacia* species larvae continued to feed, albeit slowly, on foliage sprayed with Btk (Images 2 & 3). Interestingly, fifth instar larvae placed on artificial diet, which is demonstrably the best substrate for larval growth, died rapidly with no apparent feeding (Image 4).



Image 1: Species difference in feeding on controls after 48hrs.

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Image 2: Species difference in feeding on sprayed hosts after 48hrs



Image 3: Paired 'least feeding' control & 5L/ha treatment after 48hrs

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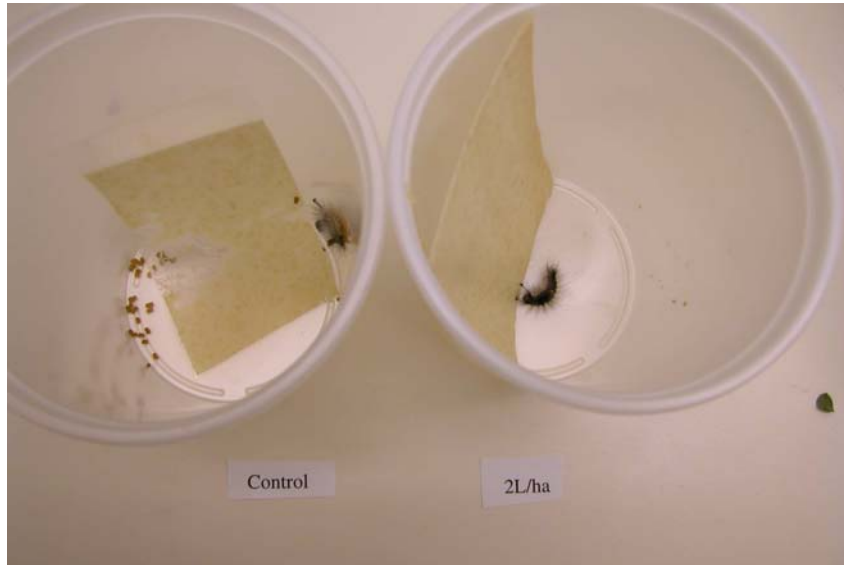


Image 4: Feeding & mortality on diet control & 2L/ha after 48hrs

Larval mortality was significantly and negatively correlated to growth of the larvae on their respective untreated host plants (Figs 4 & 5). Larvae feeding on *A. mearnsii* developed at a faster rate than larvae on other species and only those larvae feeding on *Acacia* species developed to the next instar during the trials (Fig 6).

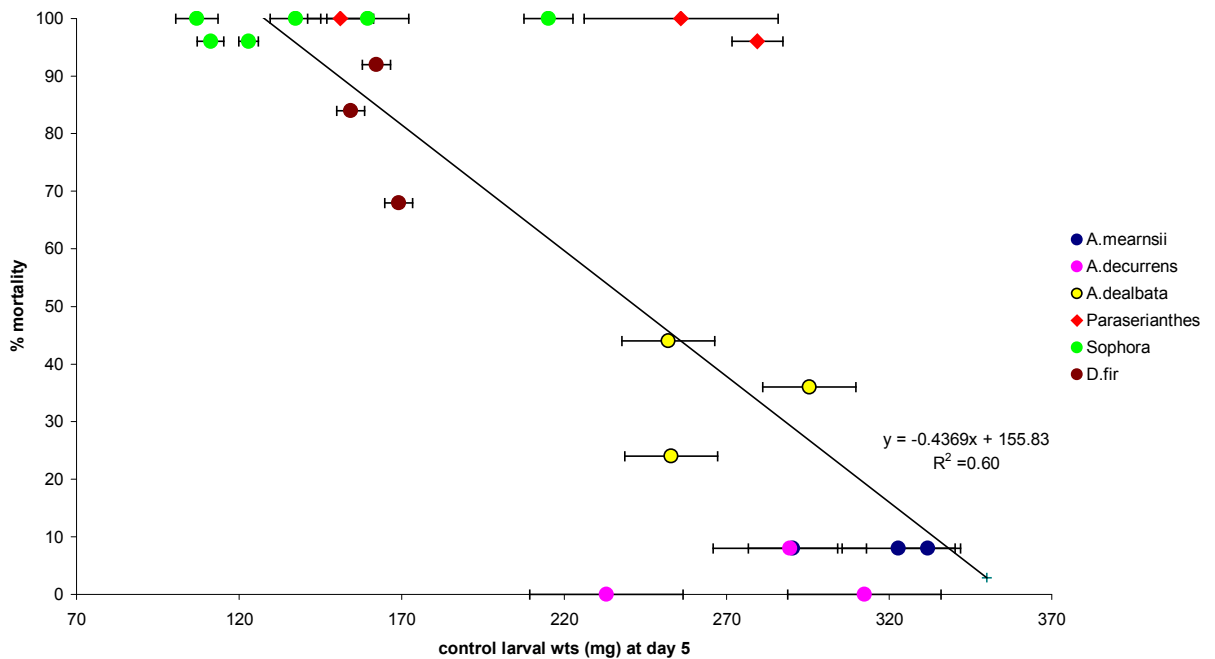


Fig. 4 – The relationship between the growth of first instar PAM larvae feeding on untreated host plants and the larval mortality on the same host species treated with 2 l/ha Btk equivalent

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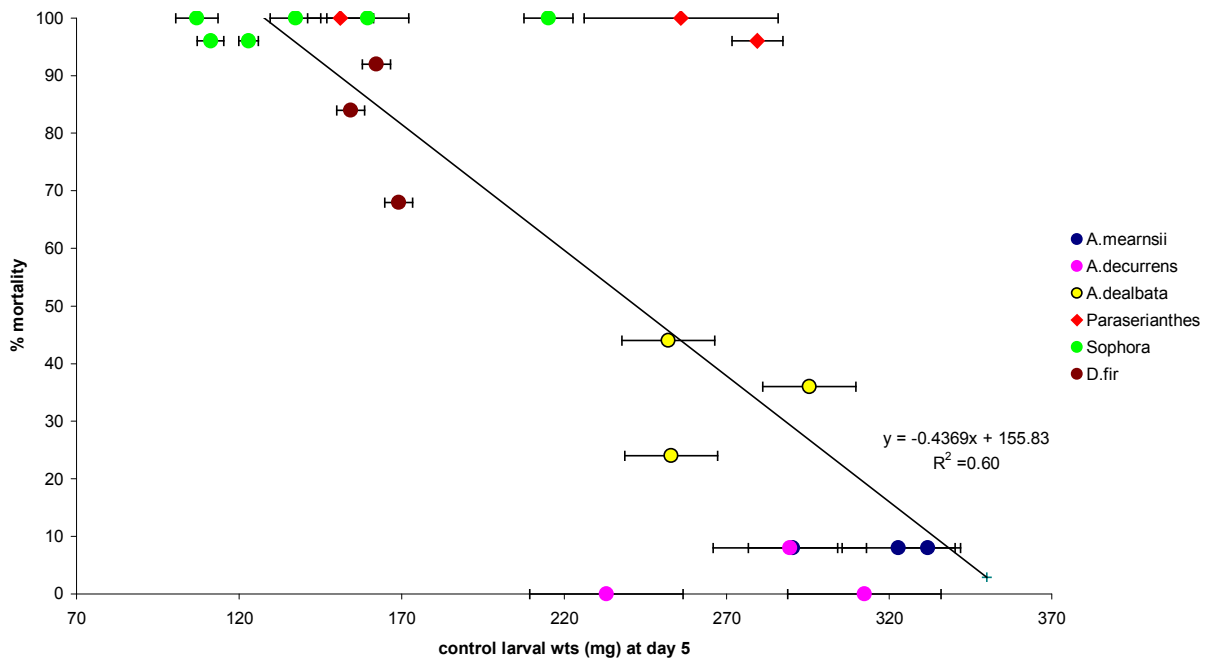


Fig. 5 – The relationship between the growth of third instar PAM larvae feeding on untreated host plants and larval mortality on the same host species treated with 2 l/ha Btk equivalent

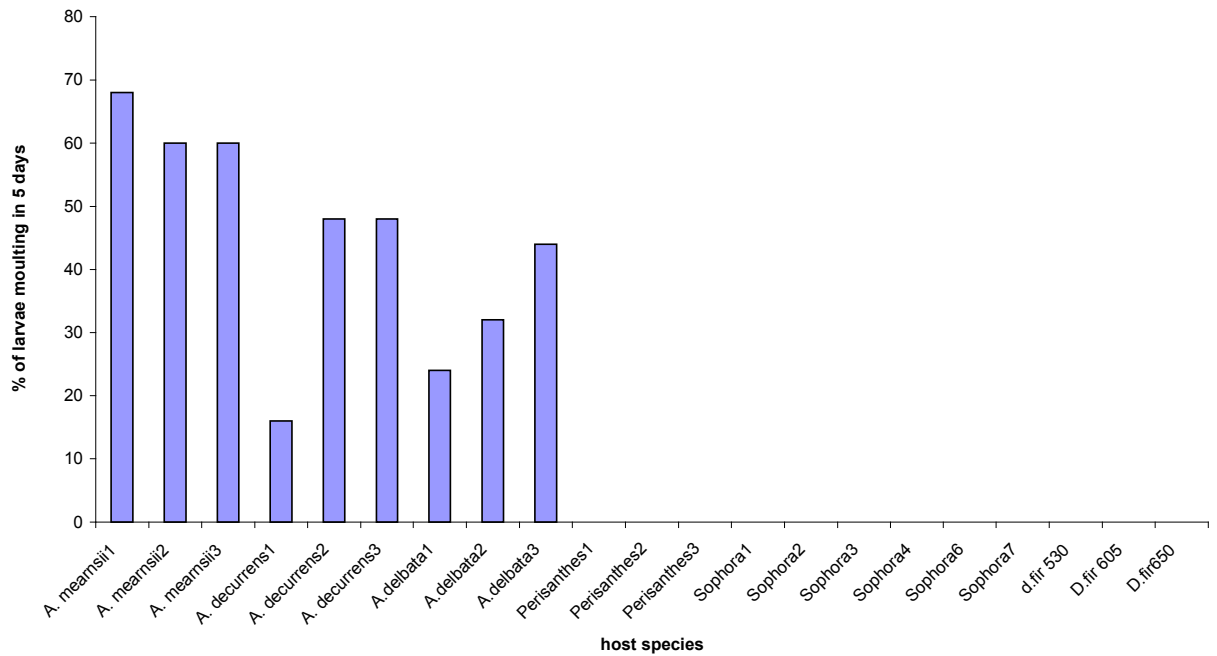


Fig. 6 – The proportion of larvae on untreated host plants entering the next instar over 5 days

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2. DOSE RATE RESPONSE ON DIFFERENT HOST PLANTS

Bioassays were run of:

- third instar larvae on 17 provenances of Douglas fir treated at a nominal 0.5 l/ha
- third instar larvae on foliage from one tree of *Sophora microphylla* treated at nominal dose rates of 0.05 to 0.45 l/ha
- fifth instar larvae on foliage of one tree of *A. mearnsii* and artificial diet, at nominal dose rates of 2, 4 and 6 l/ha. Artificial diet was spread as a 1mm film on mylar sheet with a TLC spreader and exposed to Btk at the same time and in the same manner as the mylar test sheets.

Results:

At 0.5 l/ha Btk the mortality of third instar larvae on all provenances of Douglas fir was rapid and the correlation between larval survival (measured in days) and actual dose rate (as by mylar sheets) was only weakly negative and not statistically significant (Fig 7). Similarly the correlation between larval survival and growth rate was only weakly positive, but not statistically significant (Fig 8).

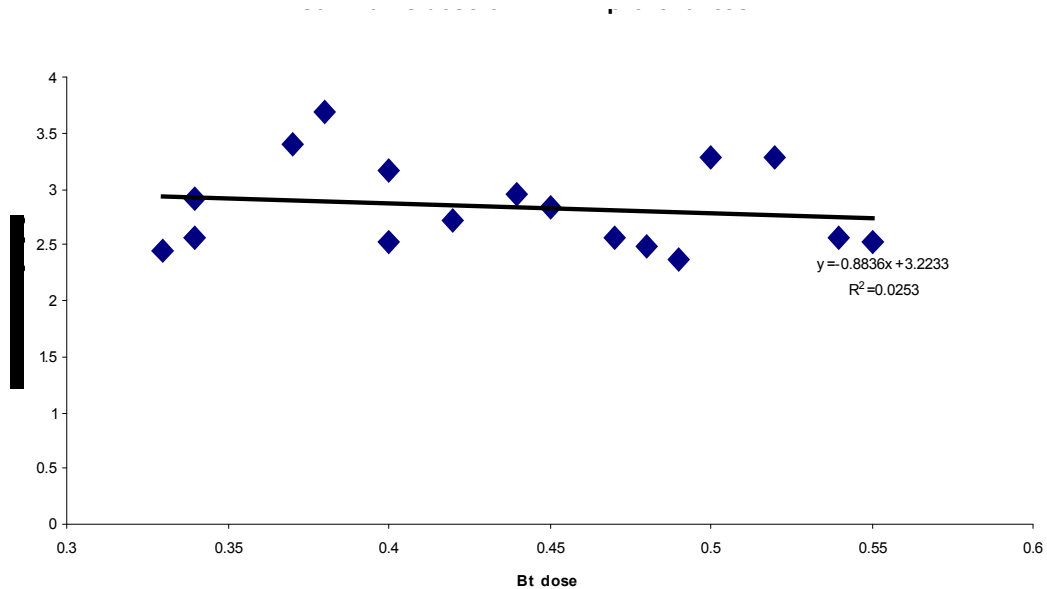


Fig. 7 – Average days of survival of third instar PAM larvae feeding on 17 provenances of Douglas fir treated with 0.5 l/ha Btk equivalent

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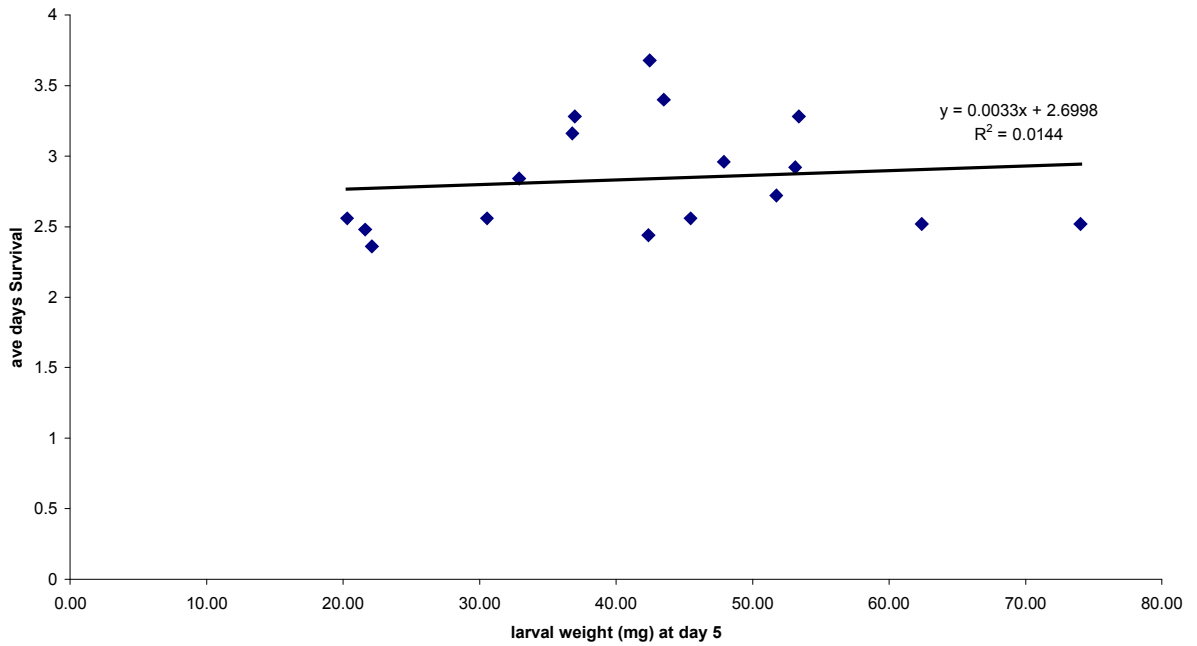


Fig. 8 – The relationship between the growth of third instar PAM larvae feeding on 17 untreated provenances of Douglas fir and the days of survival following feeding on the same provenances sprayed with 0.5 l/ha Btk equivalent

On *S. microphylla* mortality was positively correlated to actual dose rate, but was not statistically significant unless the zero rate was included (Fig 9). The variability of the actual dose rates achieved is shown in Fig. 10.

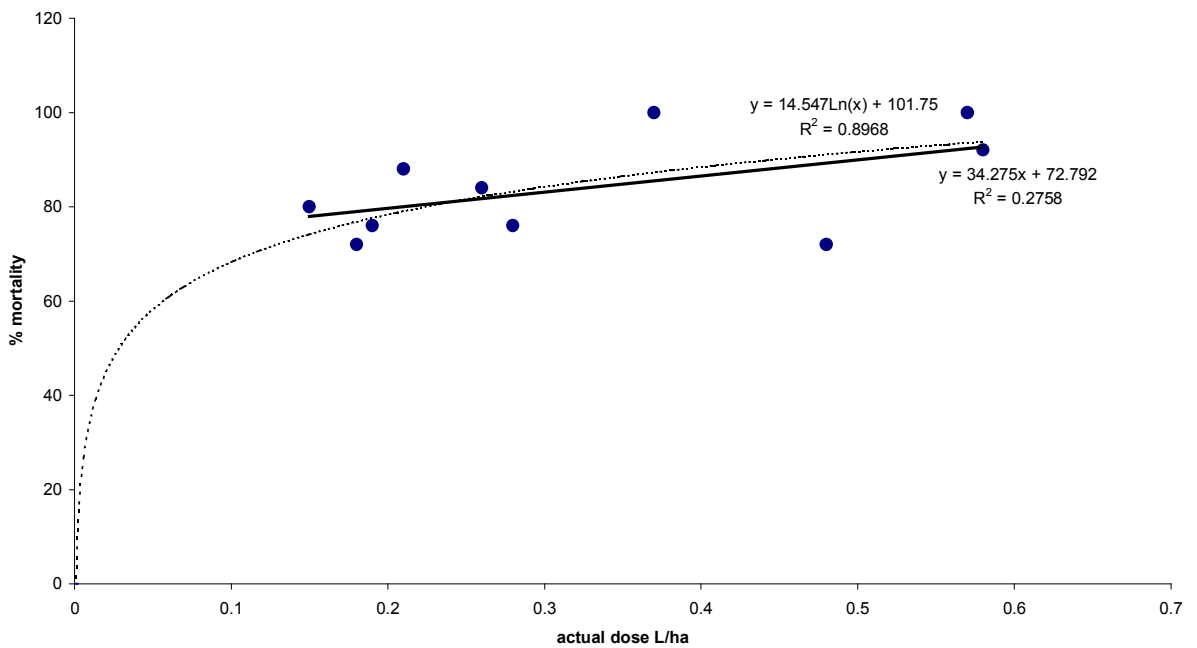


Fig. 9 – The mortality of third instar PAM larvae feeding on *Sophora microphylla* sprayed at various rates with Btk

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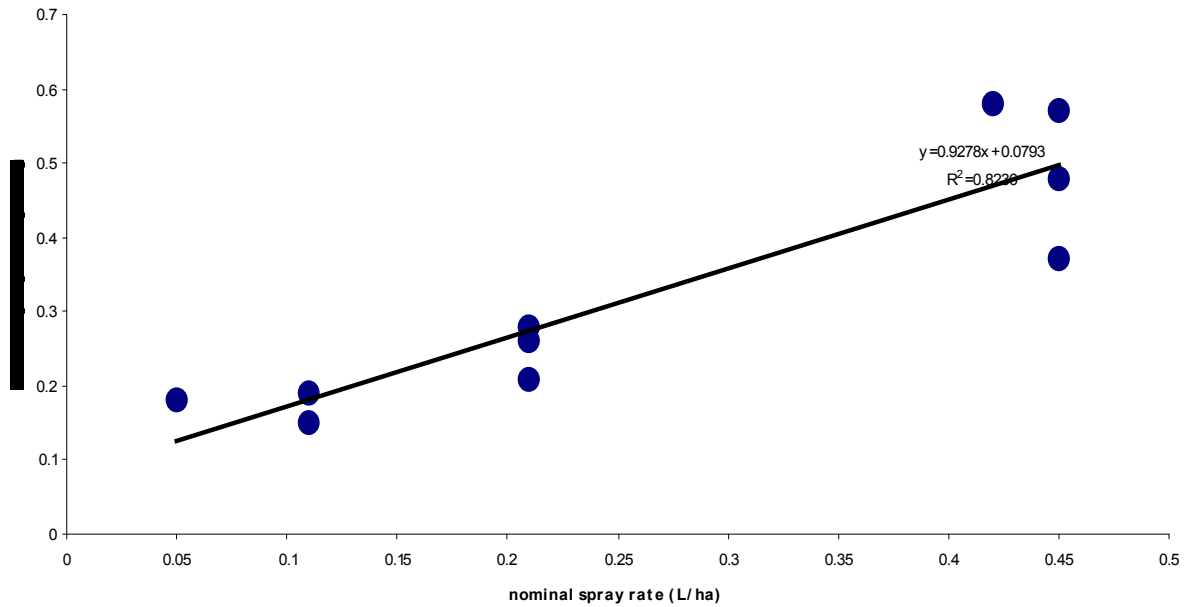


Fig. 10 – The variation in actual spray deposition recorded by varying Btk concentration and track spray belt speed

On *A. mearnsii*, fifth instar mortality was considerably lower and took longer to achieve than that on artificial diet (Fig 11) but was positively correlated to dose rate (Fig 12). Dose rate also had a significant impact on subsequent larval growth rate and the final pupal weights achieved (Fig 13).

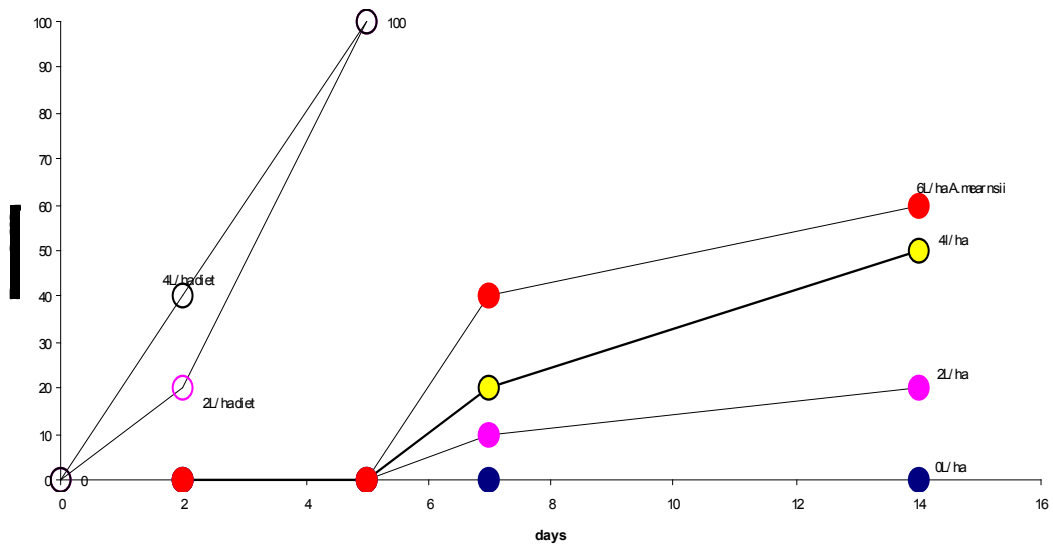


Fig. 11 – The mortality over time of fifth instar PAM larvae feeding on artificial diet or *A. mearnsii* sprayed with Btk at various nominal rates

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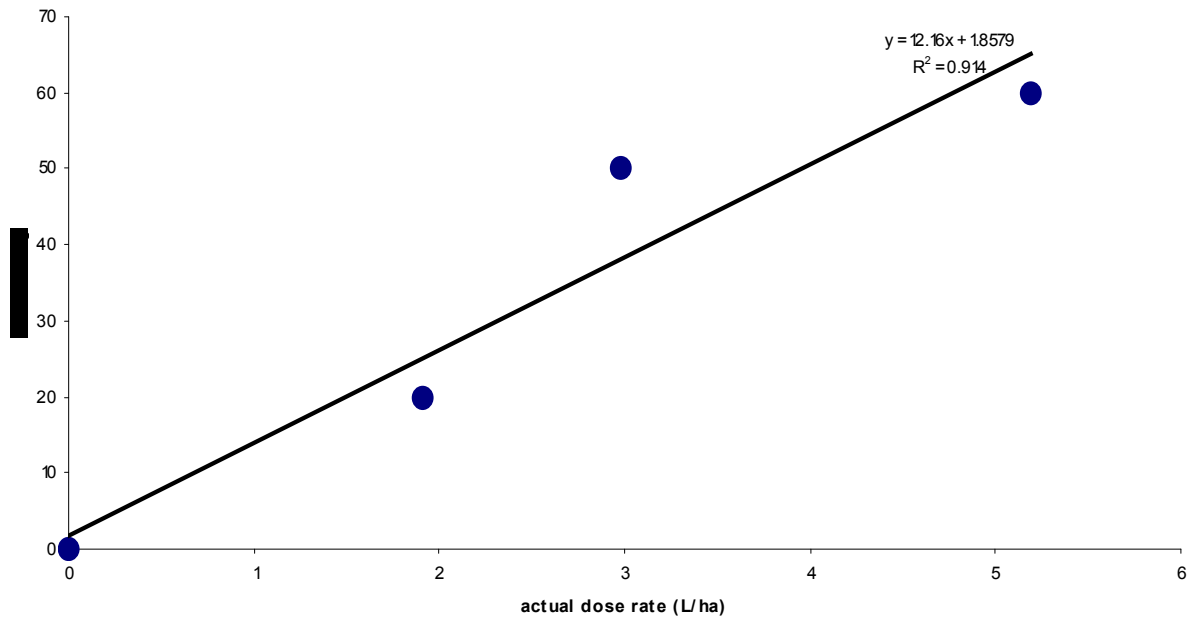


Fig. 12 – The relationship between Btk dose and the mortality of fifth instar PAM larvae feeding on treated *A. mearnsii*

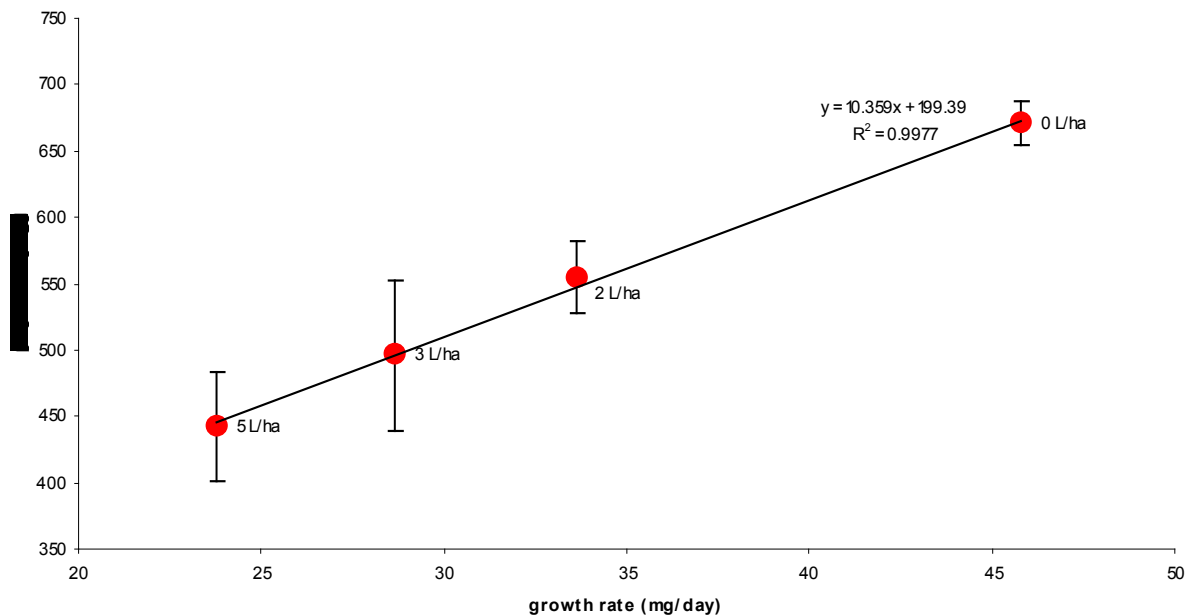


Fig. 13 – The effect of dose rate on the pupal weight and growth rate of fifth instar PAM larvae feeding on treated *A. mearnsii*

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3. **PROVENANCE TRIALS OF THE EFFECT OF GROWTH RATE ON *Btk* EFFICACY**

A nominal dose of 0.1 l/ha Btk was applied to the foliage taken from 11 provenances of Douglas fir. The mortality of third instar larvae was recorded until pupation occurred. For surviving larvae this necessitated the addition of untreated foliage from day 12. The control larvae were grown on to pupation on untreated foliage, then weighed and sexed.

Results:

Actual dose rate varied only slightly (0.125 ± 0.003 L/ha) within and between provenances. Mortality even at this low dose was high over the range of provenances and continued after the introduction of untreated foliage (Fig 14). However, the correlation between larval mortality on treated foliage and the growth rate of the larval controls on untreated foliage of the individual provenances was statistically significant (Fig 15). When treated with Btk, those provenances supporting the fastest larval growth produced the lowest larval mortality.

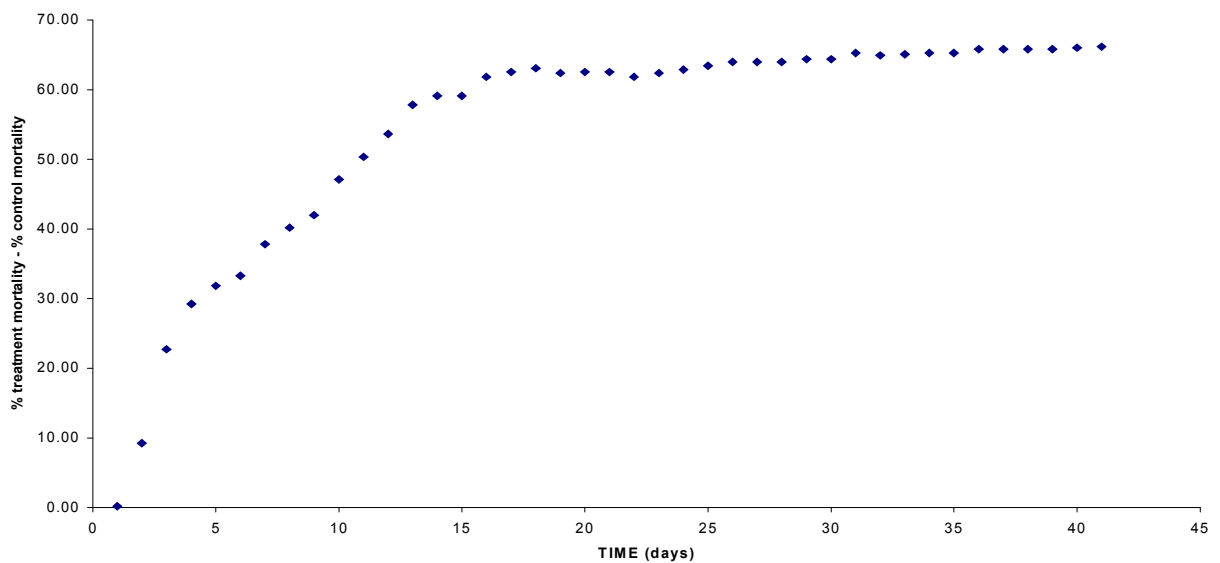


Fig. 14 – The combined mortality, over time, of third instar PAM larvae feeding on 11 Douglas fir provenances treated with 0.1 l/ha Btk equivalent

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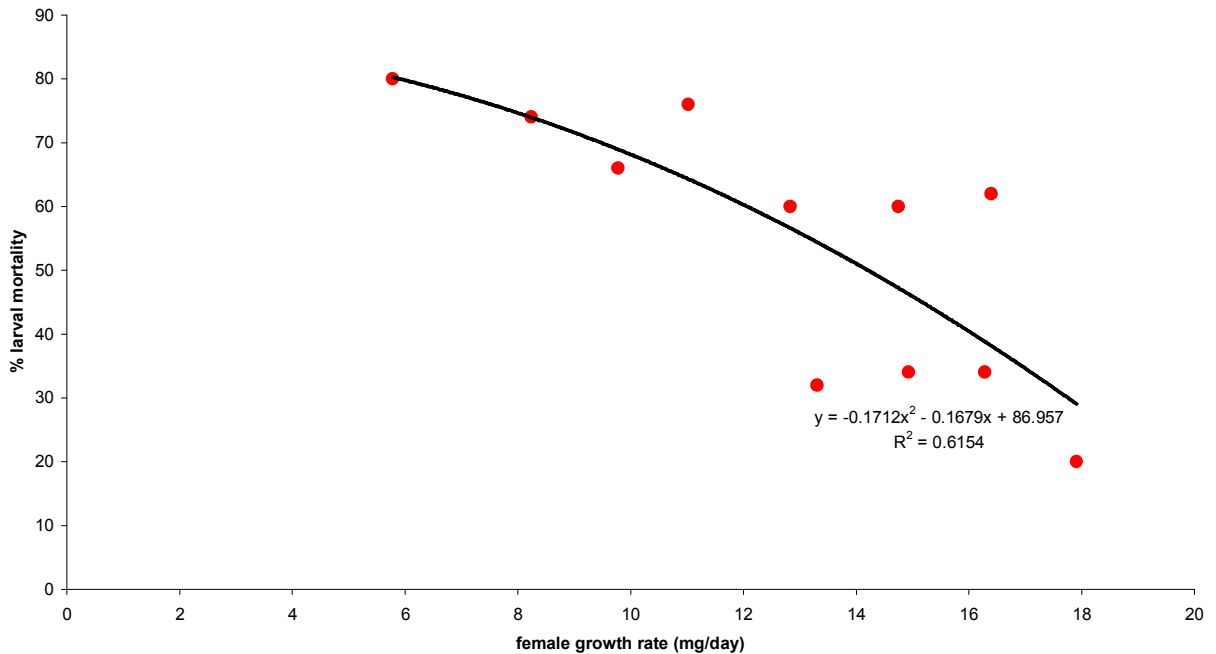


Fig. 15 – The relationship between the growth rate of PAM larvae feeding on 11 provenances of Douglas fir and the mortality of larvae feeding on the same provenances treated with 0.1 l/ha Btk equivalent

DISCUSSION

It would appear that larval growth rate is a strong predictor for the efficacy of Btk on different host plants. On poor hosts, natural mortality would be compounded, even at very low doses, by Btk. For *Sophora* and Douglas fir a response threshold could probably be measured at doses below 0.1l/ha, or a fiftyth of the 5l/ha recommended application rate, but that is beyond the capabilities of the Btk delivery system used in this study. The dose required to ensure mortality on these hosts negates the need for a phagostimulant to encourage the consumption of Btk. Often there was no evidence (in the form of frass production or plant damage) of feeding on these hosts. On *Acacia* species, which provide good larval growth, feeding is not prevented by the presence of Btk. Rather the consumption of Btk appears to affect the growth rate and the subsequent final pupal weights of surviving larvae are lowered. This may be the result of a cost to the insect in continually repairing the midgut wall damaged by Btk. The long term effects of Btk ingestion was seen in trials in which development to pupation was observed. The non-lethal effects on fifth instar larvae are not insignificant and would markedly affect population dynamic predictions that are based on mortality alone.

There has been some debate as to the role of the specific host plant attributes which may lead to either ameliorating or synergistic interaction with Btk. A

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high gut pH is needed to activate Btk and variation in plant-mediated midgut pH has been postulated as a mechanism in moderating Btk efficacy (Schultz & Lechowicz 1986). However, Kouassi *et al.* (2001) found in two-host trials, that the more acidic host produced higher mortality than the less acidic, more favoured, host. Secondary plant compounds have also been implicated in the mediation of Btk efficacy. However, the interaction, although strong *in vitro*, is weak *in vivo*, and plants appear to contain simultaneously, different allelochemical constituents that may have opposite effects on the toxicity of Btk (Hwang *et al.* 1995).

The artificial diet used in these trials is the same used in the routine rearing of the painted apple moth for over thirty consecutive generations. It probably has the least allelochemical 'contamination' of all the material tested in this study. It is also the best growth medium, producing larger pupae, faster, than any foliage. However, when sprayed with Btk it is one of the most 'toxic' substrates, eliciting rapid and total mortality, with minimal feeding, at dose rates far below those recommended for operational control. *Acacia mearnsii*, on the other hand, supports good larval growth, is a preferred natural host, but elicits far less Btk-induced toxicity than artificial diet, or the less preferred host plants.

The mode of action of a host-based, larval growth rate-mediated Btk efficacy demonstrated in this study is not known. It may be due to some effect akin to a peroxidase or anti-oxidant availability within the host plant tissue, which neutralises the Btk endotoxin. It is obviously not available in the agar/wheatgerm-based, processed diet.

There is evidence of a dose response for Btk on all substrates, even though it is severely truncated on poor hosts. It is possible that the host plant architecture, or the spray interception profile, of the plant influences the deposition of Btk and hence mediates in the dose rate ingested by the insect. The spray dosage on different plant samples could be determined by the application, and subsequent recovery, of a dye. However, the Douglas fir provenance trial, which should have eliminated any interception effect, still demonstrated a correlation between larval growth rate and Btk efficacy. The same trial should have eliminated any overt secondary chemical components that varied between the tested plant species. However, variable intra-specific host-mediated responses to Btk have been found for Gypsy moth (Hwang *et al.* 1995).

The growth rate effect demonstrated in this study has important implications for the design of eradication programmes. The determination of larval growth rate is relatively simple, less time consuming, and more predictive than chemical analyses of host plants. The only proviso being that plants to be tested should be taken from the area in which the control programme is to be implemented. For any eradication programme it is obviously necessary to determine the hosts providing the highest larval growth rates within the terrain to be sprayed. These plants will not only promote the fastest and most prolific

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population growth, but will also be home to those larvae least affected by Btk. The role of secondary hosts can largely be ignored when spraying mixed species canopies. Larvae on these trees naturally grow more slowly, produce fewer offspring and are more susceptible to both natural mortality and particularly to Btk.

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