EVALUATING THE BENEFITS OF DOSE-RESPONSE BIOASSAYS DURING AERIAL PEST ERADICATION OPERATIONS

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ABSTRACT

Between January 2002 and May 2004 a series of aerially applied sprays of Btk (*Bacillus thuringiensis* var. *kurstaki*) were made to areas of Auckland and Hamilton, New Zealand. The purpose of the sprays was to eradicate the painted apple moth (PAM; *Teia anartoides*) from Auckland and the Asian gypsy moth (AGM; *Lymantria dispar*) from Hamilton. In both operations, bioassays were used to test the effectiveness and consistency of spray applications. Bioassays were undertaken by placing foliage samples (*Acacia mearnsii*) at a selection of locations in the spray zone next to cards on which spray deposition was measured. After spraying, PAM larvae were placed on each plant and mortality recorded. Results indicated that there was a strong relationship between droplet density on cards and PAM mortality on plants. Once this relationship was determined, further bioassays were of limited additional value to simply measuring deposition on cards.

Keywords: bioassay, aerial spraying, *Bacillus thuringiensis*, *Teia anartoides*, *Lymantria dispar*.

INTRODUCTION

Painted apple moth (PAM; *Teia anartoides* Walker, Lymantriidae), a native of Australia, was first found in the west Auckland suburb of Glendene in May 1999 (Richardson 2002). In October 2001, the Ministry of Agriculture and Forestry (MAF) decided to supplement ground-based attempts at eradication of PAM by using aerial application of *Bacillus thuringiensis* var. *kurstaki* (Btk) formulated as Foray 48B. Between January 2002 and May 2004, 40 aerial sprays were applied, plus a number of repeat aerial applications onto "hotspot" areas where PAM activity was most active and proved difficult to control. Although some ground-based applications were also made, the sampling described here is only in relation to aerial spraying.

In March 2003 a single Asian gypsy moth (AGM; *Lymantria dispar* L., Lymantriidae) was caught in a pheromone trap in Hamilton, placed as part of the MAF national gypsy moth surveillance programme. A decision was made to implement an eradication plan based around aerial spraying with Foray 48B. A total of eight aerial sprays were applied at approximately weekly intervals between 8 October 2003 and 29 November 2003.

Bioassays were used in both Auckland and Hamilton to provide an index of the biological effectiveness of spray applications during the course of the eradication programme. The purpose of this paper is to determine whether using bioassays is a useful monitoring technique during pest eradication operations.

METHODS

Spray target areas and application methods

Over the course of the PAM eradication programme the spray zone varied from about 600 ha to over 8,000 ha. Spray applications were undertaken using two fixed-wing aircraft (Friendship (F-27) and an Air Tractor (AT-602)) and a BK-117C twin-engine helicopter. The BK-117 was generally used for smaller areas with steep or uneven topography, or that were too close to physical hazards for fixed wing aircraft to operate. Table 1 summarises the spray operating parameters for the three aircraft. Although the Civil Aviation Authority required a minimum release height of 45 m above the ground for spraying in an urban environment, the helicopter could fly lower when spraying over uninhabited parks and reserves. The spray equipment was set up to produce a droplet spectrum with a volume median diameter of about 120 μm at the point of release. All aircraft were fitted with global positioning systems and spray controllers (to adjust flow rate for changes in ground speed).

For AGM, the spray target zone included an area of about 1.6 km radius (1250 ha) centred on the AGM find. All applications were made using a fixed wing AT-602 (Table 1) with the same set up as for PAM.

TABLE 1: Operational details for the helicopter and fixed wing applications.

Parameter	BK-117C	AT-602	F27
<i>Aircraft spraying system</i>			
Atomiser	AU6539	AU5000	CP-03
Number of atomisers	6	12	42
RPM for rotary nozzles	7000	6500 (blade size Ex 1772/2 and angle 55°)	N/a
Nozzle orientation, orifice size, and pressure	N/a	N/a	55°; 0.125 orifice; 20 psi)
Flow rate (litres/min)	10.4	99 ¹ / 131 ²	236
<i>Operational conditions</i>			
Lane separation (distance between flight lines) (m)	25	45	90
Spraying speed (km/h)	50	263 ¹ / 250 ²	315
Minimum release height (m)	45 ³	45	45
Maximum wind speed (km/h)	18	18	18
Foray 48B application rate (litres/ha)	5	5 or 7 ⁴	5

¹For application rate of 5 litres/ha.

²For application rate of 7 litres/ha.

³Unless spraying over non-residential areas.

⁴5 litres/ha for PAM and AGM spays 1 and 2 and 7 litres/ha for AGM for sprays 3 to 8.

Spray deposition monitoring

During each spray operation, spray deposition cards were placed on the ground to monitor droplet density (number/cm²). Methods have been presented elsewhere (Richardson et al. 2003) and are only summarised briefly here. The relevance of the deposition cards to the bioassay data is that the droplet density measured on cards was used as an index of the dose received by bioassay plants that were placed next to a selection of cards.

A card consisted of a strip of paper (approximately 150 mm long and 105 mm wide) stapled to a rigid cardboard backing. Cards were processed in the laboratory (Richardson et al. 2003) where droplet density and the card area covered by droplet stains were measured using image analysis. This method of droplet sampling is simple, low cost, and easily implemented from an operational point of view. However, although the

technique is effective for measuring droplet density (counting the individual droplets) it is not ideal for measuring droplet spread areas (on the card surface) as there may be inconsistencies in droplet spread due to different batches of paper and the effects of dew or high humidity.

In both the PAM and AGM operations, cards were placed in linear transects distributed through the spray zones. Each transect consisted of 10 to 15 cards placed at approximately 10 m intervals. Total numbers of transects typically ranged from 18 to 30 for the PAM sprays with most AGM sprays having 12 transects.

For the PAM sprays each foliage sample was adjacent to a single card. However, for most AGM sprays an additional three cards were placed around bioassay plants (i.e. a total of four). The purpose of these extra cards was to test whether using four cards provided a better overall estimate of the dose reaching the bioassay plants than a single card.

The plant canopy (or other object) cover around each spray card was estimated to qualitatively describe the degree to which cards were shaded from depositing droplets. A five-point cover class was used, from 1=open to 5=dense cover, where cover included both vegetation and any man-made structures (Richardson et al. 2003).

Bioassays

Bioassays were also carried out during most PAM sprays and all AGM sprays to confirm efficacy of the applications. The general procedure was to place potted seedlings of *Acacia mearnsii* (Charles et al. 2005) at a selection of deposit card locations. An additional set of unsprayed control plants were also set aside (or covered). Typically, bioassays were undertaken at between five and eight transects. At each selected transect, bioassay plants were placed next to usually four or five of the ten cards. In most sprays this gave a total of between 30 to 40 bioassay plants.

After spraying, plants were taken to a quarantine facility. In quarantine, four or five foliage samples (replicates) were taken from each plant and each was placed in a 395 ml plastic container. Ten first instar PAM larvae were placed on each replicate (Charles et al. 2005) and mortality was recorded after 96 and 168 hours. PAM was also used for the AGM operation because there is no AGM colony in New Zealand.

Analysis

The purpose of the analysis was to determine the level of PAM mortality for different sprays and to try and attribute observed variation in mortality to differences between operations in either droplet density or cover class.

For ease of interpretation, spray deposition rate was organised, on the basis of number of droplets per cm², into five classes: 0 (controls), 1–10, 11–20, 21–30 and >30. A logistic regression was used to relate mortality to droplet density, cover class and spray operation number. The regression included terms for deposition rate (fitted either as linear or class terms), cover class and spray number. By including both deposition rate and cover class in the model, least squares mean mortalities adjusted to the mean deposition rate and cover class were obtained for each spray operation. The analysis was performed using the SAS procedure GENMOD.

RESULTS AND DISCUSSION

There was very little difference in Foray 48B droplet density on one card versus the mean of four cards (Fig. 1). There was a linear relationship between the two variables, with an intercept of 0 and a slope close to 1 ($R^2=0.97$). In other words, using one card to estimate droplet density on bioassay plants should be adequate in future operations and all analyses presented below use deposition measured on one card only.

FIGURE 1: Foray 48B droplet density measured on one card relative to the mean the density from four cards.

A logistic regression of the form:
 $\%mortality = 100/(\exp(a+bx)-1)$ (1)
 where x is droplet density and a and b are regression coefficients, indicated that PAM and AGM mortality was, as expected, strongly influenced by droplet deposition (Fig. 2). The difference in mortality between the control plants and all sprayed plants was highly significant ($P < 0.0001$), while for the sprayed plants, the effect of droplet density (log transformed) on mortality was also highly significant ($P < 0.0001$). As long as deposition was greater than 10 drops/cm², mortality was over 80%.

FIGURE 2: Mean mortality versus droplet density from all bioassays in both the PAM and AGM operations. The error bars are 95% confidence intervals.

A similar logistic regression model fitted using cover class rather than droplet density showed that there was also a significant reduction in mortality with increasing cover ($F_{4,698}=21.29$, $P < 0.0001$) (Fig. 3). Given that many bioassay plants and cards were not placed in the open, the mean deposition rate across all sprays of 23 drops/cm² indicates the overall effectiveness of the applications.

FIGURE 3: Mean mortality versus vegetation cover class after adjusting to a common Foray 48B droplet density of 23 droplets/cm² in both the PAM and AGM operations. The error bars are 95% confidence intervals.

When both deposition rate and cover were included together in a multiple logistic regression model of the form:

$$\%mortality = 100/(\exp(a+bx_1+cx_2)-1) \tag{2}$$

where x_1 is droplet density, x_2 is cover and a , b and c are regression coefficients, both variables remained statistically significant (droplet density: $F_{1,697}=50.94$, $P<0.0001$; cover class: $F_{4,697}=7.67$, $P<0.0001$). This model indicated that mortality was slightly lower for sample plants situated under denser cover, even after taking account of the lower droplet density on these plants (Fig. 3). The F-statistic for cover class in this model was reduced by two thirds compared to the logistic regression presented above, implying that most of the reduction in mortality under increased cover can be directly related to the lower deposit rate. However, the multiple regression model (equation 2) does suggest that the effect of cover on mortality cannot be fully explained by deposit density alone. One possibility is that the average size of droplets under cover may be smaller because large droplets are more effectively removed at the top of the plant canopy. An analysis of data from the AGM operation suggests strongly that this is so. Droplet density and the average droplet spread area decreased sharply with increasing cover class (Table 2).

TABLE 2: Average Foray 48B droplet density and average droplet spread area in each cover class for the AGM operation.

Cover class	Droplet density (N/mm ²)	Average spread area of each droplet (mm ²)
1	0.30 a ¹	0.49 a
2	0.30 a	0.48 a
3	0.18 b	0.37 b
5	7.20 c	0.18 c

¹Values in a column followed by the same letter do not differ significantly ($P>=0.05$).

A logistic regression (equation 2) adjusted to the average droplet density of 23 droplets/cm² showed that mean mortality remained fairly consistent at about 80% over most of the sprays (Fig. 4). However, there were still some significant fluctuations between sprays even after accounting for differences in droplet density and cover ($P<0.0001$). In particular, there was a marked reduction in mortality in several bioassays conducted for both the PAM and AGM operations in October and November 2003 (Fig. 4).

FIGURE 4: Adjusted mean mortality for each spray operation. Values are adjusted to a common Foray 48B droplet density of 23 droplets/cm² using a logistic regression.

Notes taken during the bioassay assessments suggest that the lower mortality was probably related to quality of the bioassay plants used at this time, which had particularly low leaf areas. In the worst cases, larvae were placed on stems with very little foliage meaning that some larvae would not be able to ingest much foliage or, consequently, Btk. In other words, the reduction in mortality was more likely to have been caused by some variation in the bioassay procedures rather than any factors related to the spray operations themselves. This result highlights a deficiency with the bioassay technique as a means of assessing spray operation effectiveness. Introducing new variables, e.g. foliage sample size, leaf area, and foliage quality, leads to difficult to control variation in the bioassay procedure.

Unpublished data (B. Richardson) have shown that season of application also had an effect on PAM mortality, presumably due to factors relating to foliage age. However, effects such as this are, arguably, more effectively determined by specific experimental approaches rather than from extrapolation during the routine bioassays as described here.

In conclusion, bioassays to assess pest mortality as part of an aerial spray monitoring programme were initially valuable to determine a field relationship between droplet density on deposit cards and pest mortality. However, once this relationship was determined, further bioassays were of limited additional value to simply measuring consistency of spray deposition on cards (Richardson et al. 2003, 2004). If bioassays are desired to verify product potency at the time of spraying, it may be preferable to directly measure potency in a laboratory assay (e.g. Frankenhuysen et al. 1997). Alternatively, if a field assessment is required, variability due to foliage quality or plant size/architecture effects on deposition could be minimised by replacing foliage samples with standard Petri dishes containing artificial diet.

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