SUPPLY OF BURNT PINE LONGHORN LARVAE FOR DISINFESTATION TRIALS

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A regular supply of insects of known age and standard quality is necessary for disinfestation work. Burnt pine longhorn (BPL), *Arhopalus ferus* (Mulsant) (Cerambycidae: Coleoptera), is a xylophagous beetle species accidentally introduced to New Zealand from Europe (Hosking & Bain 1977). Eggs are laid on the bark of dead and dying standing trees and larvae burrow into the wood. Adults, eggs and larvae can occur in association with export logs and sawn timber, which may require phytosanitary treatment. Here we describe a method using artificial diet as a reliable means of supplying six-week-old larvae for disinfestation trials.

Comparisons of artificial diets

We compared an artificial diet developed for a similar beetle species (Rogers et al. 2002) and five variations of the diet for their effects on larval survival and weight at five weeks after inoculation. Preparation of diet followed the method described by Rogers et al. (2002). Eggs of BPL laid on wax paper sections were collected from containers holding adult beetles (n=30-40). Newly hatched larvae were handled using a fine camel-hair brush. Experiments with larvae were all carried out in controlled temperature rooms at 20^{o}C and a photoperiod of 16:8 (L:D).

The establishment of newly hatched larvae on diet can be improved by adding some dried and powdered host material (Gardiner 1970). The diet of Rogers et al. (2002) ('standard diet') contains pine wood sawdust. This was replaced by pine bark sawdust, based on field observations of superior larval feeding performance on bark (Hosking & Hutcheson 1979). Both the standard and bark sawdust diets were further modified by reducing cellulose and glucose and by reducing the amount of mould inhibitor. The former modification was to assess how relative changes to carbohydrate and protein affect larval growth. The latter modification was test whether reduced mould inhibitor was associated with high mortality for young larvae (Wang et al. 2014).

Survival after five weeks was superior (average 75.6%) for larvae reared on diets with pine bark sawdust compared with survival on the diets with pine wood sawdust (average 22.8%) (Table 1). There was no effect of reducing cellulose or mould inhibitor. Average weight for larvae on diets with pine bark sawdust was greater (average 20.8 mg) than for larvae on diets with pine wood sawdust (average 9.3 g).

Table 1 Survival and mean weight of burnt pine longhorn larvae reared on artificial diets for five weeks at 20^{o}C and a 16:8 L:D photoperiod. Mean weights with the same letter do not differ at $P<0.05$. SE, one standard error of the mean.

Diet	Key ingredients and changes	%Survival	Mean weight ± SE (mg)
Standard	Pine wood sawdust	37.9	10.5±1.7 a
1	+ Reduced cellulose	15.8	10.2±2.6 a
2	+ Reduced mould inhibitor	14.6	7.1±2.8 a
3	Pine bark sawdust	72.5	22.8±1.3 b
4	+ Reduced cellulose	77.1	19.5±1.2 b
5	+ Reduced mould inhibitor	77.1	20.2±1.3 b

Supply of larvae for trials

Further modification was made to improve the rearing method. Reduced cellulose powder lowered the viscosity of the diet, enabling it to be dispensed into individual tubes following the method of Singh et al. (1983). Test tubes with a reduced cellulose, bark sawdust diet were then inoculated with a single larva, and plugged tightly with cotton wool. Test tubes were superior to other methods, as they proved less labour-intensive to prepare with diet, and contamination from mould was confined to individual tubes rather than entire trays. The success of the rearing method was assessed by recording the number of larvae surviving after six weeks at 20^{o}C for 15 batches of 512-650 larvae inoculated for disinfestation research. Of a total of 8740 larvae inoculated individually into test tubes with diet 5, 2.4% failed to establish or died before six weeks and a further 0.6% were discarded as being too small for convenient handling (average weight 8 mg). This gave an average yield of suitable larvae for disinfestation trials of 97% (range 94.7-99.3%) six weeks after inoculation of diet with neonates.

DISCUSSION

Our aim was to develop a reliable method for rearing large numbers of BPL on artificial diet for disinfestation research. After initial experiments, one of us (AMB) was able to rear >8000 larvae to six weeks of age and a size suitable for disinfestation trials with a mortality rate of less than 3%. Replacing pine wood sawdust with pine bark sawdust was associated with an improved survival and growth for BPL and it is possible that components of pine bark are phagostimulants for newly hatched larvae. In conclusion, our experiments indicated that a diet with pine bark sawdust was consistently superior to a diet with pine wood sawdust in terms of establishment of newly hatched larvae and early larval growth. A method derived from experiments had negligible loss of larvae, minimal labour requirements, and met the need of a reliable supply of larvae for disinfestation trials.

ACKNOWLEDGEMENTS

Funding for this study was provided by the Stakeholders in Methyl Bromide Reduction (STIMBR) project and by The New Zealand Institute for Plant & Food Research Ltd. We thank reviewers of the poster for valuable comments. This poster is based on work reported in the New Zealand Plant Protection journal (http://www.nzpps.org/journal.php).

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