SCION

New Zealand Journal of Forestry Science

42 (2012) 15-23

www.scionresearch.com/nzjfs

published on-line:
18/01/2012

Rearing and storing *Arhopalus ferus* life stages in the laboratory for experimental purposes

Kees (C. W.) van Epenhuijsen*, Ken G. Somerfield and Duncan Hedderley

The New Zealand Institute for Plant & Food Research Limited, Private Bag 11600, Palmerston North 4442, New Zealand

(Received for publication 16 December 2010; accepted in revised form 26 October 2011)

*corresponding author: *vanepenhuijsenkh@xtra.co.nz*

Abstract

It can be difficult to provide large numbers of fresh forest insects for use in fumigation experiments. This paper reports on studies aimed at providing large numbers of fresh burnt pine longhorn beetles (*Arhopalus ferus* Mulsant) (Coleoptera: Cerambycidae). Burnt pine longhorn beetle is one of the most important pests of quarantine concern associated with export of New Zealand radiata (*Pinus radiata* D.Don) logs, particularly to India, China and other Asian countries.

The timing of the collection of hundreds of adults, obtaining eggs from them and then storing the eggs for use in fumigation trials must be synchronised with the timing of the trial itself. Three separate experiments were conducted to improve rearing and storage of burnt pine longhorn beetles. In the first experiment, burnt pine longhorn beetle eggs were laid by field-collected adults. Larvae were successfully reared on an artificial (huhu) diet at 20 °C ± 1.5 °C and pupated after 216 days (males) and 227 days (females). Adults emerged after a further 2.5 weeks. These adults stayed alive in the laboratory for up to 46 days at 20 °C. In a second experiment field-collected burnt pine longhorn beetle adults of mixed age were stored at 4, 6, 8, 10, 12 and 14 °C for up to 10 weeks. The longest survival time was at 6 °C (50% still alive after 28 days). In a third study we found 12 – 14 °C was the best temperature for storing eggs. The total life times, times to pupation and adult survival times were not significantly different between males and females. Males tended to be more variable than females particularly in the time spent as larvae and as adults.

On average females were longer than males and had larger elytra. The antenna length : elytra size ratio (AL/ES) was higher for males than females.

Keywords: *Arhopalus ferus*; diet; life stages; *Pinus radiata*; rearing; storing; survival.

Introduction

Adult burnt pine longhorn (BPL) beetles (*Arhopalus ferus* Mulsant) (Coleoptera: Cerambycidae) fly at dusk and through part of the night and are attracted to security lights and light traps in areas close to the forest. Burnt pine longhorn beetles attack logs, stumps and standing dead or dying pine trees (mainly *Pinus radiata* D. Don). Early larval stages of the beetle feed in the inner phloem of the tree. Larvae at Formby, England showed the greatest increase in weight on slightly decayed wood invaded by fungi but on sound wood they lost weight (Wallace, 1954). This is contrary to findings in New Zealand (Hosking & Bain, 1977). Trees damaged by fire are particularly favoured by the feeding stages of this insect. The beetles contribute to the devaluation of logs by vectoring sap-stain causing fungi (New Zealand Farm Forestry Association, 2010). Any life stage of BPL found on logs for export can be cause for rejection by importing countries and

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

consequently such logs require fumigation treatment, either in New Zealand or at their destination. A quarantine issue of great importance is the sheltering of adults among logs and sawn timber at sawmills and ports.

The majority of individuals complete their life cycle in one year (Brockerhoff & Hosking, 2001) but in one study in New Zealand it took two years for about a third of the population. The lifecycle was estimated at one to two years depending on the temperature and the stage entering sapwood (Hosking & Bain, 1977). The nutritional value of inner bark and sapwood of *Pinus radiata* (D.Don) to *Arhopalu*s larvae was studied when reared in bark in a rearing chamber by Hosking and Hutcheson (1979).

Rearing various species of longicorn beetles (Cerambycidae) in the laboratory is considered difficult because of their very long life cycles, high incidence of cannibalism amongst adults (Larsson, 2010) and high mortality rate during the larval stage (van Epenhuijsen, unpublished data). Wang et al. (2002) stated that, in terms of time, labour and the number of resulting adults, collecting *Oemona hirta* (F) larvae in the field in autumn and then transferring them onto an artificial diet was the most effective method for maintaining a laboratory colony of this beetle. Gardiner (1970) reared 49 cerambycid species on the diet and found that most completed their life cycles under laboratory conditions in less than half the time it would take in the field. Burnt pine longhorn adults reared on an artificial diet produced viable eggs. *Anoplophora glabripennis* Motschulsky (Cerambycidae) has been found to lay eggs even without successful sperm transfer (Melody Keena personal communication, 2011). Previous studies showed BPL adults of mixed sexes and unknown age stored at 8, 12, 16 and 20 °C survived best at 8 °C, which was for an average of 21.4 days. Burnt pine longhorn adults collected from Nelson Port survived 17.1 days longer on average when stored at 8 °C than at 20 °C (van Epenhuijsen et al., 2009)[1].

Obtaining a plentiful supply of all life stages of BPL beetles was the focus of our research. We aimed to improve rearing and storage of BPL beetles to provide large numbers of fresh insects for log-disinfestation trials. We report on three experiments herein.

Materials and methods

Field collection of BPL adults

From the last week in December, burnt pine longhorn adults were collected from the ground beneath light towers and adjacent to an ultra-violet (UV) light trap present at the site's wood fired co-generating heating unit at the port in Eves Valley (Nelson) and at the premises of the timber merchant, KLC Ltd in Rotorua. The adults were collected after dusk when they were active, i.e. moving rapidly across the ground or had just flown and landed at the site. They were probably not more than two days old (Steve Pawson, personal communication, 2011).

For each of the experiments, two to three hundred adults were collected. They were placed in yoghurt containers filled with moderately compressed crumpled paper towel to reduce cannibalism and sent in polystyrene boxes with cool pads to Palmerston North by courier within 10 hours of capture.

Experiment 1 - Rearing BPL eggs from field-collected BPL adults

Egg laying by field-collected BPL beetles started upon arrival at ambient temperature. Two to three hundred eggs were harvested over a period of 2 – 3 days and stored at 12 °C. To obtain neonate larvae, eggs on paper towel were held in Petri dishes at 20 °C ± 1.5 °C for 10 – 12 days. Newly emerged larvae were assigned to each of four temperature groups (12, 15, 18 and 20 °C; all ± 1.5 °C). Small larvae can be easily collected from the Petri dishes in which the eggs have hatched as they will survive for up to 5 days without food.

Each group of 4 replicates contained 17 larvae and was placed on 8 grams of diet in a small Petri dish (35 mm diameter). The diet used was a huhu diet (developed by Rogers et al. (2002)) for *Prionoplus reticularis* White from a diet originally developed by Gardiner (1970). Because the diet was moist, no attempt was made to control humidity. Observations started on 21 December 2008.

Larvae that attained about 19 mm in length (which only occurred with the 18 °C and 20 °C groups) were each moved to separate, larger Petri dishes (85 mm diameter) containing fresh huhu diet to avoid cannibalism. Weekly weighing of these larvae continued through to the start of pupation. Petri dishes were placed on an "island" in glycerine-filled trays which were placed on boards painted with anti-mite paint (Artilin SA, France) to avoid contamination from mould mites (*Tyrophagus putrescentiae* Shrank).

Adults that emerged from pupae were left for 2 – 3 days to allow their exoskeleton to fully sclerotise. They were then placed singly in 120 mL containers with metal mesh on both ends. A piece of very slightly damp paper facial tissue was placed in each container and the containers were kept at 20 °C in the dark. Adults were checked every 2 – 3 days until they died. The sex of each of these beetles was confirmed by Landcare Research, Auckland.

[1] van Epenhuijsen, C. W., Somerfield, K. G., & Hedderley, D. (2009). *Rearing and storage conditions for burnt pine longhorn (*Arhopalus ferus*) and golden haired bark beetle (*Hylurgus liginiperda*)*. The New Zealand Institute for Plant & Food Research Ltd. Report No. 2388.

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

Data were collected on the weight of each individual male and female adult when they emerged, the time it took them to pupate, the time they spent as a pupa, their adult longevity and total lifetime. Data were also collected on differences between the male and female adults in body length, elytra and antennae size.

Experiment 2 - Effect of storage temperature on longevity of field-collected BPL adults of unknown age

The BPL adults were assigned randomly to one of the storage temperature treatments before 11 am on the day they were received. Two trials were conducted. Run 1 started on 26 February 2010 with eight BPL adults per container and Run 2 started on 2 March 2010 with five BPL adults per container. Each container was 120 mL in volume with fine stainless steel mesh at each end. Damp facial tissue was loosely crumpled and placed in each container. The containers were stored at 8 °C, 10 °C, 12 °C and 14 °C for Run 1 and at 4 °C, 6 °C, 8 °C, 10 °C, 12 °C and 14 °C in Run 2. In Run 1, there were three containers at each temperature; in Run 2 there were four containers at each temperature; so total numbers of adults per replicate per temperature were 20 – 24. The individual containers were placed in large 230 x 230 x 120 mm closed containers (darkened). All fridges and cool rooms used for storage were checked and calibrated for temperature. Run 1 was assessed for 24 days (from 2 March until 26 March 2010) and Run 2 for 67 days (from 5 March up to 11 May). Containers were removed from refrigerated storage and allowed to warm to room temperature prior to observation of the contents. The BPL adults were removed every 2 – 3 days to check how many were left alive.

Longevity was analysed by fitting log-Normal distributions to the numbers of beetles dying in each interval, using Minitab software (Minitab 15, 2006).

Experiment 3 - Effect of storage temperature on hatch rate of eggs from field-collected BPL adults

To obtain eggs, field collected mixed sex adults of unknown age which flew into the collection points were placed at 20 °C ± 1.5 °C in groups of 20 – 25 in large 4.5 L closed plastic containers filled with crumpled paper towel to prevent cannibalism. The paper received a light water spray before the lid was closed. The adults were held in the laboratory at ambient temperature. Egg batches deposited on the paper were cut out during the following 2 – 3 days. The eggs were counted, and then stored for 1 – 2 days at 12 °C until enough eggs had been collected for the start of the trial. Eggs were found by holding the paper towards a light source as the eggs can be easily confused with excreta deposits, which were the same colour.

Four separate runs were conducted, starting on different dates using 2 – 3 day old eggs (total of 6855 eggs). Eggs for Run 4 were taken from adult BPL collected last in the season. Groups of 68 – 180 eggs from at least three egg batches were stuck onto double-sided adhesive tape (Sellotape, New Zealand, 50 mm) in the centre of the bottom of Petri dishes (diameter 85 mm). Fewer eggs were used in Runs 1 & 2 because lower numbers of eggs were laid at these times. The dishes were sealed with Parafilm. The Petri dishes (five dishes per storage temperature) were placed in a large closed plastic container in cool rooms in complete darkness. Run 1 was conducted at 8 °C only. Run 2 was conducted at 10 °C only. Runs 3 & 4 were conducted at 8, 10, 12 and 14 °C. Petri dishes were taken out of the cool rooms after five weeks and were placed at 20 °C in the laboratory in January (L16 : D8) on racks placed over glycerine-filled trays. Observations on larval emergence were made without opening the Petri dishes. Egg hatch in each dish was assessed on a single occasion, after most eggs had hatched and the head capsules of larvae inside any unhatched eggs had stopped moving. The total number of larvae that had emerged from the eggs and had stuck to the double-sided tape was counted. Larvae that had not left the egg shell completely were not counted as hatched. Untreated (i.e. unstored) controls using fresh eggs were kept at ambient temperature 20 °C ± 1.5 °C in 16 h light : 8 h dark regime.

Results

Experiment 1 - Rearing BPL eggs from field-collected BPL adults

Larvae from eggs stored at either 8 °C or 10 °C did not emerge until after the 4- or 5-week storage period had ended. When stored at 14 °C, all eggs hatched before the storage period was over. In order to maximise the storage life of eggs, the storage temperature should be below 14 °C and preferably in the range of 8 – 10 °C. Rearing larvae at 20 °C ± 1.5 °C was more successful than at the other temperatures tested. At 12 °C, the larvae failed to gain weight, and none survived more than 56 days. At 15 °C, some larvae gained weight, but none survived more than 56 days. At 18 °C, only 3 of the 17 larvae survived more than 21 days (similar to 15 °C), but the ones which survived did gain weight (Figure 1) although they did not eventually pupate. At 20 °C, 12 of the 17 larvae survived more than 140 days and pupated, producing six males and six females. The mean weight of the survivors increased from 4.2 mg (12 January 2009) to 515.2 mg after 140 days (2 June 2009). Weights then fell before increasing again in some cases.

When BPL larvae were reared from egg-hatch in groups of 5, 10 or 15 insects on diet in Petri dishes (35 mm), each group was reduced by cannibalism to a single larva after 3 – 4 weeks (van Epenhuijsen, unpublished data).

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

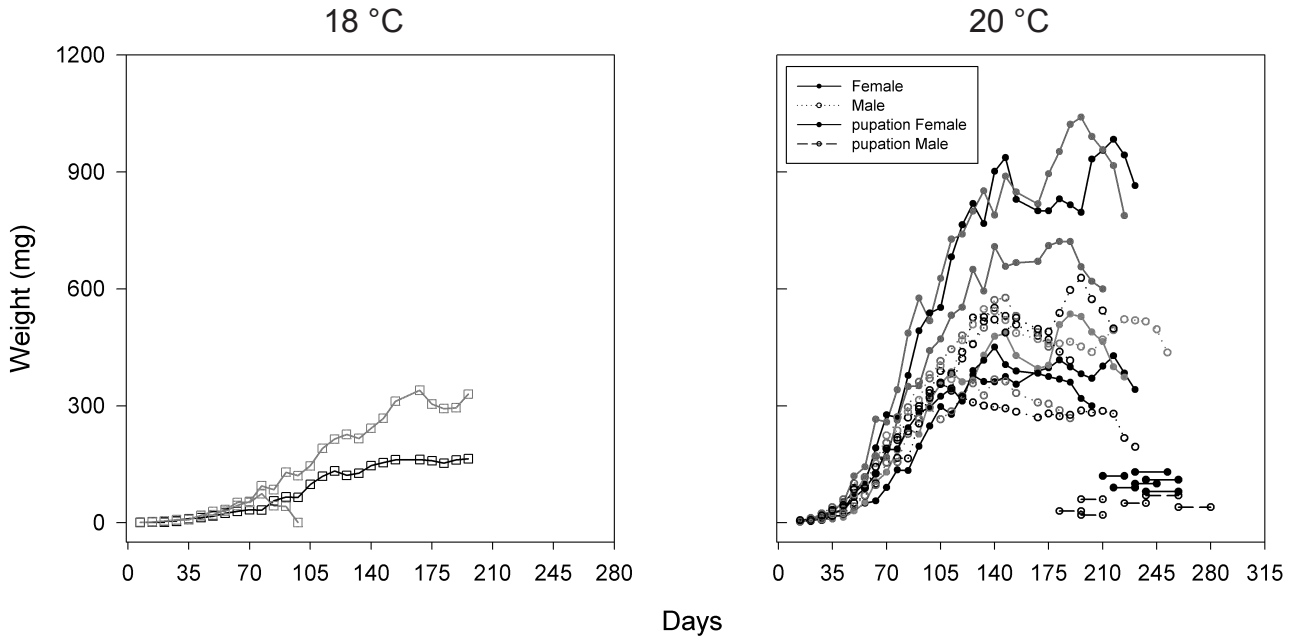

FIGURE 1: Weights (mg) of BPL larvae stored at 18 or 20 °C ± 1.5 °C through to death (18 °C) or pupation (20 °C). Each line represents an individual larva. Sex was determined after pupation, and is shown. Data from the 12 and 15 °C groups are not shown.

Results from the weekly weighings of the larvae are shown in Figure 1. Pupation occurred between 180 – 260 days and lasted about 2.5 weeks at 20 °C. No pupation occurred at 18 °C. No weighing was carried out during pupation, as the pupae were too fragile. At 20 °C, 71% of the mature larvae successfully pupated.

The survival of six male and six female adults, from egg-hatch to death, was observed for up to 40 weeks after hatching (Table 1). Time between the emergence of the adult from the pupa (eclosion) to death was recorded as adult longevity. These adults stayed alive in the laboratory for up to 46 days at 20 °C.

There was a strong negative correlation between adult survival time and time from egg to pupation ($r = -0.95$, $P < 0.001$); larvae which pupated earlier tended to live longer as adults.

Total lifetimes, times to pupate, pupation times and adult longevity did not differ significantly between males and females (t-test *P*-values are 0.161, 1.000, 0.399, 0.600 and 0.197 respectively). However, the males tended to be more variable than the females, particularly for the time spent as larvae and as adults (F test for equality of variance, *P*-values were 0.140, 0.220, 0.600, 0.060, 0.070 respectively; the t-test results were largely unchanged when adjusted for the unequal variances).

The weights of the larvae at maturity (Table 2) did not appear to be associated with the lengths of any of the life stages (Table 1).

On average, the females were longer and had larger elytra than the males (t-test *P*-values were 0.052 and 0.028; variability was similar for these measures), but the antennae lengths did not differ significantly (t-test *P*-value 0.166) (Table 2). The antenna length/elytra size ratio (AL/ES) was higher for males than for females (t-test *P*-value 0.002). Wang and Leschen (2003) report similar results in their study of BPL beetles. There were strong correlations between final

TABLE 1: Mean time for various life cycle events of six female and six male BPL adults reared from eggs at 20 °C (with standard errors in brackets).

Sex	Time to pupate (days)	Time as pupae (days)	Adult longevity (days)	Total lifetime (days)
Female	227 (4.69)	17.5 (1.54)	30.9 (3.43)	277.9 (2.94)
Male	215.6 (12.04)	16.1 (1.47)	45.5 (8.61)	277.9 (5.32)

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

TABLE 2: Mean body length, antennae length, elytral size and final weight (with standard errors) of six female and six male BPL adults reared in captivity.

Sex	Final larval weight (mg)	Body length (mm)	Antennae length (mm)	Elytra size (mm)	Ratio of antennae length : elytra size
Female	544 (99)	21.4 (1.12)	11.3 (0.80)	15.8 (0.79)	0.72 (0.04)
Male	377 (48)	18.3 (0.86)	13.5 (1.18)	13.1 (0.64)	1.02 (0.06)

larval weight, body length and elytra size (between 0.91 and 0.96, $P < 0.001$). There was no significant (overall) correlation between elytra length and the other body size measures, but that seemed to be because of a marked male-female difference – males generally have longer antennae for their size than females.

Experiment 2 - Effect of storage temperature on longevity of field-collected BPL adults of unknown age

Combined results for two runs are shown in Figure 2. Storage at 6 °C resulted in the best survival. Temperatures from 8 °C to 14 °C were increasingly unfavourable to adult survival. Several distributions for survival time were tested and the log-Normal distribution gave the best fit to all temperatures (Figure 2).

Where there were two runs of each experiment (at 8, 10, 12 or 14 °C), there was an indication that in Run 2 the adults lived longer than those in Run 1 at the same temperature ($P = 0.008$).

Survival declined up to the maximum temperature in the trial (14 °C) where 50% of adults survived an average of only 7.1 days. Data from Run 2 only suggests that survival was also reduced at low temperatures, with 50% survival occurring after 14.9 days at 4 °C. The beetles stored at 4 °C initially showed no movement after they were removed from the refrigerator. Some movements occurred shortly afterwards as they warmed up. The adults stored at 6 °C recovered quickly after they were removed from the refrigerator. Seventy-five percent of adults survived to 19.2 days at 6 °C, and 7.5 days at 4 °C. The longest surviving beetle lived for 70 days at 6 °C.

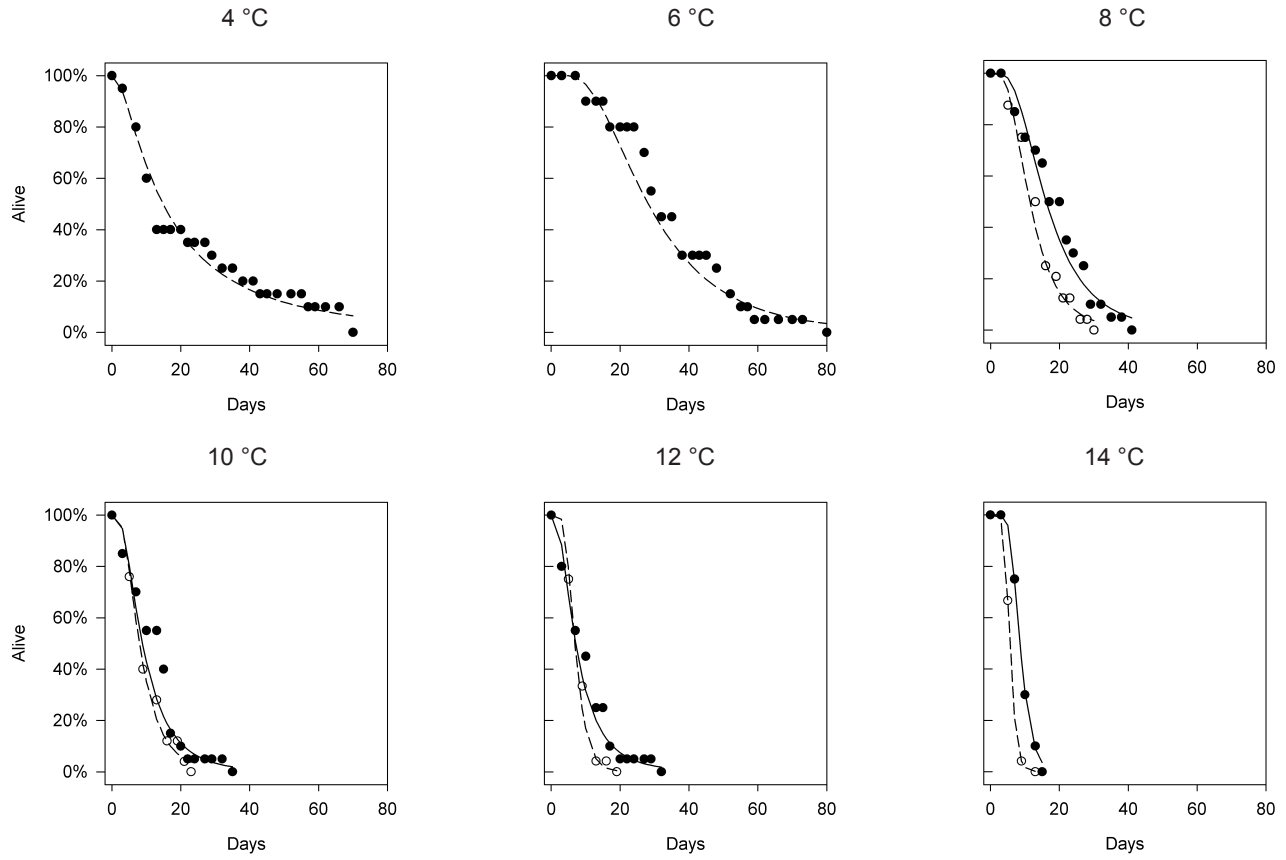

FIGURE 2: Survival of field-collected BPL adults at six temperatures (unfilled symbols and solid lines are from Run 1; filled symbols and dashed lines are from Run 2).

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

The results from Experiment 1 showed that newly emerged reared adults were kept alive with a probability of 75% survival for 15 days at 20 °C. Field-collected adults of unknown age can be kept alive with a probability of 75% survival for 35 – 45 days at 6 °C.

For trial purposes, it is recommended that BPL adults be stored at temperatures no higher than 10 °C. The present data indicate an optimum storage temperature of 6 °C.

Experiment 3 - Effect of storage temperature on hatch rate of eggs from reared BPL adults

Egg hatch after 1 – 5 weeks in storage at 8, 10, 12 or 14 °C is shown in Figure 3.

A binomial generalised linear mixed model was fitted to the data, with a separate linear trend over time (on the logit scale) for each temperature as fixed terms, and random effects for experiment/date, and experiment

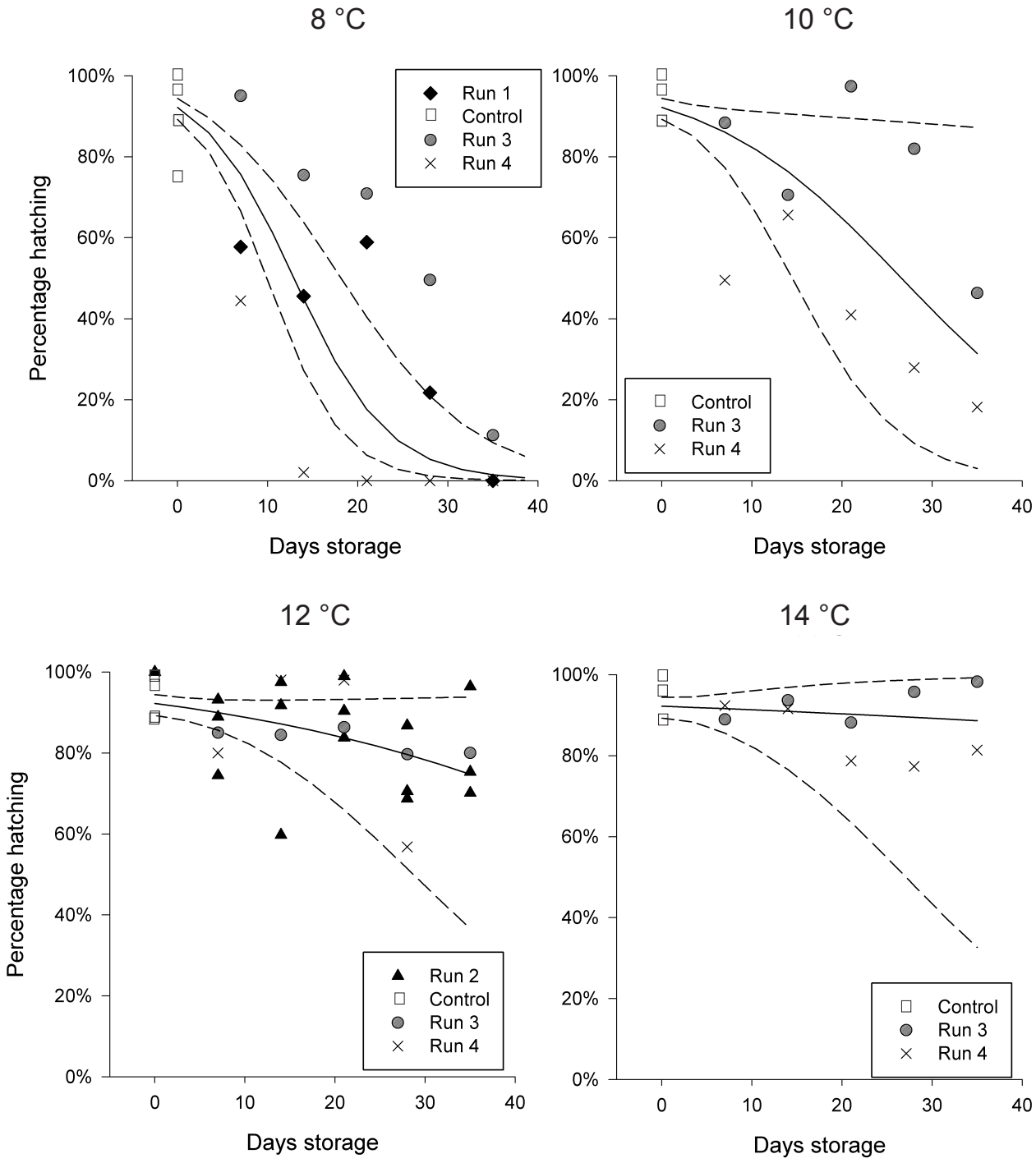

FIGURE 3: Proportion of BPL eggs hatching after storage at 8, 10, 12 or 14 °C for 1 – 5 weeks. The fitted line from the binomial generalised linear mixed model is shown (–) as are the + and - 95% confidence intervals (---).

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

x slope. The model suggests 12 – 14 °C is the best temperature range to store eggs for larval production. More than 75% of eggs hatched after 5 weeks' storage at these temperatures. The model also suggests less than 1% of eggs will hatch at 8 °C after 37 days (95% confidence interval, 29 days to 38+ days). The most consistent egg hatch occurred at 14 °C. At either 12 °C or 14 °C, developing head capsules were seen in some eggs before the 4 and 5 week treatment periods were over and, at 14 °C, some head capsules were visible and some eggs hatched fully before the storage period was over. This did not occur at either 8 °C or 10 °C.

Discussion

Rearing cerambycid larvae on synthetic diet is well documented but presents a challenge when large numbers are required. De Viedma et al. (1985) in Spain reared 26 species of cerambycid on artificial diet at 25 °C. They report *Arhopalus ferus* completed its life cycle, adults mated, eggs being laid and hatching, and larvae developing to adults. Hosking and Hutcheson (1979) in New Zealand stated that larvae of *A. ferus* showed a strong preference for inner bark of pine (*Pinus radiata)* under field conditions with a growth rate four times that of sapwood-fed individuals. They reported that nitrogen concentration and soluble carbohydrate levels were much higher in the inner bark than the sapwood. In our trials, young larvae have preferred feeding under the bark of trial logs. Rogers et al. (2002) successfully reared the native cerambycid *Prionoplus reticularis* (huhu) on artificial diet containing 5% pine (*Pinus radiata)* sawdust at 20 °C. The larval period was reduced to about 250 days compared to at least 2 years in the field. We found that burnt pine longhorn larvae would not complete development at 18 °C on artificial diet, but readily did so at 20 °C. Rogers et al. also found that rearing huhu larvae at the higher temperature of 25 °C resulted in higher mortality and reduced larval weight.

Gardiner (1970) in Canada reared 17 cerambyid species from eggs. This author says the technique has proved useful in reducing rearing time in the production of larvae and pupae. Gardiner says the addition of ground plant tissue to the diet appears to function only by speeding acceptance of the diet by young larvae. Payne et al. (1975) in the USA tested five diets for rearing larvae of *Prionus imbricornis*. They also found that initially larvae developed much faster on diets supplemented with sawdust from the natural host. Wang et al. (2002) found that adults of the lemon tree borer *Oemona hirta* from natural hosts laid more eggs than those reared on artificial diet.

Keena (2006) found the optimum temperature for median longevity was 18 °C for *Anoplophora glabripennis* (Cerambycidae) in North America. Our data indicates that adults should be stored at temperatures no higher than 10 °C, and that the optimum storage temperature is 6 °C.

Keena (2006) estimated the lower threshold for egg hatch as 10 °C. We found 10 °C to be a safe minimum temperature for storing *A. ferus* eggs. The binomial generalised linear mixed model fitted to our data suggested 12 – 14 °C is the best temperature to store eggs for larval production, with more than 75% of eggs hatching after 5 week's storage. Keena and Moore (2010) estimated the lower threshold temperature for development of instars 1 – 5 and the pupal stage as 10 °C, and as near 12 °C for the higher instars. The development rate was less temperature-sensitive for instars 5 – 9.

The reduced egg hatch at 8 °C and 10 °C is probably due to a number of factors. Dr Melody Keena (personal communication, 2011) has advised us that there may be differences among adult BPL beetles collected at different times of the season. There were probably genetic differences in the response of eggs from different females to temperature. Also, eggs laid later in the season may not have been provisioned the same as the eggs laid earlier. Dr Keena's work with Asian longhorn borer has shown the effects of genetics on hatch especially at low temperatures. She has observed that females that emerge earlier in the season tend to be the ones that develop faster and their eggs tend to need fewer heat units to hatch. She suggests this could be happening with *Arhopalus ferus.*

The *Arhopalus ferus* adults reared on artificial diet in our laboratory produced viable eggs. M. Keena (personal communication, 2011) has informed us that *Anoplophora glabripennis* has been found to lay unfertilized eggs even without successful sperm transfer.

Rearing *Arhopalus ferus* larvae individually on huhu diet until they reach the required size for trial work is one way, although time consuming and labour intensive, to obtain reasonable numbers of insects for experiments. For quarantine purposes where larger numbers are required it may be preferable to treat the insects in their natural environment by using infested pine logs, although these logs may also contain *Prionoplus reticularis* (huhu) and other insects[2].

[2] Brash, D. W., Klementz, D., Bycroft, B. L., Page, B. B. C., van Epenhuijsen, C. W., Somerfield, K. G., & Hedderley, D. (2010). *Phosphine fumigation of insect infested logs.* The New Zealand Institute for Plant & Food Research Limited Confidential Report No. 4236.

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

Conclusions

Planning for fumigation trials is facilitated by knowing the optimum storage time for BPL eggs and adults.

It was relatively easy to rear BPL beetles from eggs to adults in the laboratory. However, cannibalism prevents rearing large numbers together. Development took an average of 278 days at 20 °C ± 1.5 °C. We found that BPL adults of known age (reared in the laboratory) have an average longevity of 45.5 days (males) and 31 days (females) at 20 °C ± 1.5 °C. Adults of unknown age collected in the field survived longest at 6 °C: 45 days for males and 35 days for females.

Burnt pine longhorn beetle eggs can be stored for 4 – 5 weeks. Eggs stored at 12 – 14 °C should give maximum numbers of larvae. Eggs can also be stored at 10 °C for up to 15 days and 75% of the eggs will still hatch after being removed from storage.

The long lifecycle of BPL (about 278 days in our studies) would make larger scale rearing of adults impractical for regular trials.

Acknowledgements

We thank Brent Whibley (Koputara Timber Limited, KLC, and Rotorua); Steve Pawson and Jessica Kerr (Scion); and Terry Westbury (Carter Holt Wood Products, Eve's Valley, Nelson) for the supply of BPL adults. Ann Barrington (Plant & Food Research) made the huhu diet and Darren Ward (Landcare Research) identified the genders of the laboratory-reared adult BPL. We also wish to acknowledge the financial contribution to this project from STIMBR (Stakeholders in Methyl Bromide Reduction) and the MAF Sustainable Farming Fund.

We thank Dr Melody Keena, Research Entomologist (Director's Representative), USDA Forest Service, Northern Research Station, Hamden, USA for advice on cerambycid egg laying and hatching, and for commenting on the low level of egg hatching observed in our *Arhopalus ferus* at 8 °C and at 10 °C.

We also thank Don Brash for comments on the manuscript.

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ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)