MORTALITY OF HUHU (*PRIONOPLUS RETICULARIS*) SUBJECTED TO HEAT AND CONTROLLED ATMOSPHERE TREATMENTS

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ABSTRACT

Eggs and larvae of huhu (*Prionoplus reticularis* White) were treated at elevated temperatures and varying controlled atmosphere (CA) conditions. At 35°C in air, more than 9.4 days were needed to achieve 99% mortality (LT_{99}) of huhu larvae. Treatment time decreased with increasing temperature to 3 h at 45°C in air. Eggs and neonate larvae (mean weight: 1.25 mg) were more susceptible to the heat treatments than were larger larvae (≥ 100 mg). When treating larger huhu with controlled atmosphere conditions of 100% N_2, 100% CO_2, or a 50% N_2 / 50% CO_2 mixture at 20°C, less than 36% mortality was observed after 11 days' exposure. However, increasing the treatment temperature to 40°C during 100% N_2, 100% CO_2, and 50% N_2 / 50% CO_2 treatments resulted in LT_{99} of 8.3, 6.9, and 7.6 h respectively. The 100% CO_2 controlled atmosphere treatment was most effective. There was no statistical difference in mortality responses to the 100% N_2 or 50% N_2 / 50% CO_2 treatment. All controlled atmosphere treatments at 40°C were significantly better at controlling larger larvae than was the 40°C air treatment.

Keywords: temperature; controlled atmospheres; quarantine; *Prionoplus reticularis*; *Pinus radiata*.

INTRODUCTION

Exports of New Zealand logs and poles in 1997 exceeded 5.5 million m³, and contributed about $639 million to total export forestry income (Anon. 1997). The native New Zealand huhu beetle was identified in a pest risk assessment study (USDA 1992) as one of five major quarantine insect pests which would pose a risk if present on New Zealand *Pinus radiata* D. Don logs imported into the United States. Huhu infest dead parts of living trees as well as stumps, logs, and untreated sawn timber. The eggs are laid in cracks in timber or under the bark. Larvae feed in sapwood and heartwood and in the worst case may completely destroy the wood, leaving only a thin outer shell (Hosking 1978).

Fumigation with methyl bromide (Cross 1992) was identified as "…probably effective but needs research…" (USDA 1992) and is currently the only accepted quarantine treatment

against huhu before export to markets such as the United States. While North Asian markets do not require mandatory methyl bromide fumigation, substantial volumes of logs are fumigated at the port of arrival (Maud 1995). An estimated 4.8 million m^3 of New Zealand logs were fumigated with methyl bromide worldwide in 1996 to kill forestry quarantine pests (J. Maud, Ministry of Agriculture and Forestry, pers. comm.). However, methyl bromide has been identified as an environmentally hazardous toxic gas and as a major contributor to ozone depletion (WMO 1994). Implementation of environmentally sustainable postharvest disinfestation methods against forestry pests will allow continued access for New Zealand logs to overseas markets and may provide the New Zealand forestry industry with a competitive advantage over those countries which have not developed such quarantine treatments.

Some chemical treatment alternatives, such as phosphine or sulphuryl fluoride, have been investigated for their activity against pests but they require long treatment times (3–5 days), do not control all insect life stages, and are registered in only a few countries (Banks 1994; UNEP 1995; USDA 1991). .

Overseas research indicates that non-chemical treatments such as dry heat are effective against insects and nematodes in lumber (Dwinell 1990; USDA 1991). Low oxygen conditions combined with high levels of carbon dioxide control the immature and adult stages of some bark beetle species and other wood-attacking insects (Schroeder & Eidmann 1986; Paton & Creffield 1987). Low oxygen conditions may also enhance the activity (Paster *et al.* 1990) of natural products which have shown potential against huhu (B.H.Rohitha pers. comm.). Low oxygen / moderate carbon dioxide combination treatments can control horticultural pests such as leafrollers (Whiting *et al.* 1991, 1992; Dentener *et al.* 1992), mealy bugs (Dentener *et al.* 1992), and mites (Whiting & van den Heuvel 1995). Raising the treatment temperature to above ambient can further enhance the efficacy of controlled atmosphere treatments and reduce the exposure time needed for quarantine security (Whiting *et al.* 1995). Although elevated temperatures, reduced oxygen atmospheres, or combination treatments are well researched for horticultural pests, the response of huhu to these treatments has been unknown.

Successful development of insect quarantine treatments requires that a standardised protocol is followed to determine treatment efficacy without loss of product quality (Paull & Armstrong 1994; Shannon 1994). Here we present the first part of an ongoing study to determine the tolerance of eggs and larval stages of huhu off logs to a range of heat and controlled atmosphere treatments, either alone or in combination. Studies carried out to determine heat transfer into *P. radiata* logs, and subsequent mortality of huhu larvae, will be presented in future papers.

METHODS

Insect numbers or treatment conditions presented are described as mean ± standard error of the mean, with the number of observations used for analysis in parentheses (n). Two main sets of experiments were conducted. In the first, the effect on huhu mortality of heat treatments in air at 35°, 40°, and 45°C was tested. In the second, temperatures were fixed at 20° or 40°C and the effect on insect mortality of combining heat with controlled atmosphere was investigated with 100% N_2, 100% CO_2, and a 50% N_2 / 50% CO_2 mixture.

Treatment Procedures

Heat treatments

Experiments were carried out in a controlled-temperature room using air flowing through perspex rectangular chambers organised in two horizontal banks of four to five chambers. Each chamber had an internal dimension of 250 (L) × 250 (W) × 300 (H) mm, with a volume of 18.75 litres. A chamber airflow rate of ≈313 ml/min (approximately one chamber air change every hour) was used.

The relative humidity was maintained at 80.3 ± 1.6% (n=78) by bubbling air through 1-litre Agee humidifying jars containing water or a water/glycerol mixture (Forney & Brandl 1992). Access to each chamber for insect removal was through a marine style "porthole" with screw-in-lid (165 mm diam.). Huhu were exposed to temperatures of 35°, 40°, and 45°C, which were sensor controlled. Heat treatment experiments were carried out at "static" temperatures, i.e., the temperature was established before the insects were exposed. Temperatures were maintained on average at 35.3° ± 0.06°C (n=51), 40.4° ± 0.05°C (n=131), and 45.4° ± 0.05°C (n=132). Temperature recordings were taken during the treatments with a Grant® Squirrel data logger (Model 1200 series; Grant Instruments, Barrington, England). One temperature probe was placed inside each of the treatment chambers, and relative humidity was measured with Vaisala (UK) Ltd probes (type Grant VH-L; Grant Instruments) in at least one chamber during each experiment. All temperature probes were calibrated against a RT200 resistance reference thermometer (Industrial Research Ltd, Lower Hutt, New Zealand). Temperatures recorded in the experiments were corrected using calibration errors associated with the individual probes before readings were analysed. All heat treatments were carried out at a photoperiod of 16:8 (light:dark) hours.

Based on the results obtained in the heat treatment research, further experiments combining heat with controlled atmosphere were carried out at 20°C, and a maximum temperature of 40°C, to allow expression of mortality due to both treatment components.

Controlled atmospheres

Experiments with 100% N_2, 100% CO_2, or a 50% N_2 / 50% CO_2 mixture were done in a controlled-temperature room using atmospheres flowing through perspex chambers as described above. The atmospheres were generated from carbon dioxide (food grade), compressed air, and nitrogen. Nitrogen was either supplied by bottle (oxygen-free grade), or generated using an Isolcell nitrogen separator (Isolcell Italia, Laives, Italy), capable of varying levels of nitrogen purity with a maximum supply of 11 m^3 N_2/hour with a nominal purity of 99.9% (based on an air feed of 90 m^3/hour). All gases were mixed in a manifold and supplied to the experimental chambers. A chamber flow rate of ≈313 ml/min (approximately one atmosphere change every hour) was used. Relative humidity in each treatment chamber was obtained as described above. The atmosphere inside each chamber and the manifold was sampled regularly throughout the experiment. Samples (1 ml) were analysed using an ADC (The Analytical Development Company Ltd - Hoddesdon, England) analyser with a 50 ml/min nitrogen carrier gas, equipped with a paramagnetic (oxygen) and an infrared (carbon dioxide) transducer. The concentrations of oxygen and carbon dioxide were calculated by comparison with certified standard reference gases containing a range of oxygen and carbon dioxide mixtures in nitrogen. The following controlled atmospheres were observed after a 5-hour establishment period at 20°C:

(i)   100% N_2 atmosphere (0.49 ± 0.01% O_2, balance N_2; n=67);

(ii)   100% CO_2 atmosphere (0.01 ± 0.00% O_2, balance CO_2; n=68); or

(iii) a 50% N_2 / 50% CO_2 mixture (0.27 ± 0.01% O_2, 48.50 ± 0.32% CO_2, balance N_2; n=58).

During the 40°C treatments, the controlled atmosphere compositions were:

(i)   0.34 ± 0.03% O_2, balance N_2 (n=16);

(ii)   0.27 ± 0.04% O_2, balance CO_2 (n=15); and

(iii) 0.37 ± 0.03% O_2, 49.9 ± 0.26% CO_2, balance N_2 (n=37).

Larvae were exposed to temperatures of 20°C (20.7° ± 0.02°C; n=28) or 40°C (40.2° ± 0.05°C; n=87). All controlled atmosphere experiments were "ramped" from ambient conditions to target temperature and controlled atmosphere with a photoperiod of 16:8 (L:D) hours. During 100% CO_2 and 100% N_2 experiments at 40°C, all temperatures were within 0.5°C of target in <180 min. During the 50% CO_2 / 50% N_2 experiments, this time was extended to ≈210 min.

Insects

Eggs

Field-collected egg batches of varying age found on felled *P. radiata* logs were used to determine egg tolerance at elevated temperatures. Huhu eggs are very susceptible to drying out (Edwards 1961). Experiments were therefore carried out only at temperatures of 40° and 45°C which were associated with short (≤24 h) treatment durations for larvae ≥100 mg.

Five different egg batches were used for each experiment, and each egg batch was divided into six smaller lots which were evenly allocated to the five treated and one non-treated (control) sample points. Therefore each time sample point (or treatment unit) contained eggs from the five batches (mean: 34 ± 1.5 eggs; n=174). All experiments were replicated at least twice. Eggs were placed on filter paper inside a plastic specimen jar (53 mm high × 44 mm diam.) and stored closed with a screw-top lid overnight at 20°C, 70% RH, and a photoperiod of 16:8 (L:D) hours until treated. Before treatment the lid was removed, and eggs in containers were exposed to elevated temperatures for five different time periods (40°C 3–24 h, n=2; 45°C 1.5–3.5 h, n= 6) to obtain a range of mortalities. After treatment, the screw-top lid was replaced, and eggs in jars were placed at 20°C, 70% RH, and a photoperiod of 16:8 (L:D) hours until assessment. Control eggs were treated similarly but not exposed to heat treatment. Filter paper was attached to the inside of the lid with double-sided tape, and moisture inside the jar was maintained on a weekly basis by wetting the filter paper with 10 μl water. Eggs were progressively assessed three times per week for larval hatch during a 6-week period following treatment. Treated egg lots were used for analysis only when egg hatch occurred from the same egg batch in the associated control lot during the 6-week period.

Neonate larvae

Larvae ≤96 hours old (weight: 1.25 ± 0.04 mg; n=25) and hatched from field-collected egg batches were used for heat treatment experiments at 35°, 40°, and 45°C. On average, 19.9 ± 0.14 (n=33) larvae representing one sample point were placed inside a specimen jar (as

described above) on top of a folded strip ($\approx$30 cm^2) of 1-ply natural cellulose paper towel and exposed for 0.25 to 126.5 h, depending on the temperature. Control larvae were treated similarly but not exposed to heat treatment. Experiments at each temperature were replicated 8–13 times. After treatment, larvae in jars were stored as described above until assessment 3–4 days later.

Larvae ≥ 100 mg

No instar determination has been described for this species, and head capsule measurements are not indicative of instar (P.J.Lester pers. comm.). Therefore, huhu larvae ≥ 100 mg were collected from *P. radiata* logs and stumps in Riverhead Forest north of Auckland, transported to the laboratory in insulated containers, and held overnight at 20°C, 70% RH, and a photoperiod of 16:8 (L:D) hours before treatment the next day.

Larger huhu larvae were exposed in glass tubes to a range of treatment conditions for five different time periods (from 6 to 264 h depending on heat and controlled atmosphere conditions) to provide a range of mortalities. Since our heat experiments had shown that eggs and neonate larvae were more susceptible to heat treatments than were larvae ≥ 100 mg, controlled atmosphere experiments were carried out only with larger larvae. After treatment, all insects were held for 3–4 days at 20°C, 70% RH, and a photoperiod of 16:8 (L:D) hours until assessment. Control larvae were treated similarly but not exposed to the treatment. All experiments were replicated at least three times.

Average larval numbers per sample point during heat and controlled atmosphere treatments of larger larvae were 45.1 ± 2.2 (n=41; 3–5 replicates) and 51.9 ± 1.5 (n=44; 3 replicates) respectively. All controls were stored at 20°C, 70% RH, and a photoperiod of 16:8 (L:D) hours until the last sample point in the treatments was assessed.

Determination of Mortality

Neonate and larger larvae were recorded as "live" (movement) or "dead" (no movement) when gently prodded with blunt forceps and observed by binocular microscope (20× magnification) or naked eye. Egg viability was determined by counting the number of hatched larvae in lots with known total egg numbers.

Statistical Analysis

Time-mortality data for each experiment were analysed with a complementary log-log model $\log(-\log(1-p)) = a + bt$, where p = expected mortality and t = time (Preisler & Robertson 1989). This gave approximate linearity and determined the estimated times for 50% (LT$_{50}$) and 99% mortality (LT$_{99}$). These estimates were calculated after allowance for the control mortality as the time to achieve a mortality of $c + (1-c) \times d$, where c = control mortality and d = required level of mortality (0.50 or 0.99, for LT$_{50}$ and LT$_{99}$ respectively).

The model was fitted using a robust version of the generalised linear model procedure in S-PLUS (Statistical Sciences 1995). Mortality was the dependent variable, with time and life stage as the explanatory variables. This model assumes that variance is proportional to that of a binomial distribution. The robust version reduces the weight given to points lying away from the body of the data (Waddell *et al.* 1997).

For each treatment and life stage combination a mean, and corresponding standard error of the mean (SEM), and 95% confidence interval (CI) were determined (Finney 1971) to make comparisons where appropriate.

RESULTS

Heat Treatment

An estimated 139 hours at 35°C was needed to achieve 99% mortality of neonate huhu larvae (Table 1). This exposure time reduced with increasing temperatures to 1 hour at 45°C.

For larger huhu larvae, an estimated 226 hours' treatment time at 35°C was required to achieve 99% mortality. This exposure time was reduced to 3 hours by increasing the temperature to 45°C (Table 1). Overall, larvae $\geq$100 mg were more tolerant to heat treatment than were neonate larvae (p = 0.01).

TABLE 1–Time in hours (plus 95% confidence intervals — CI) required for 50% (LT_{50}) and 99% (LT_{99}) mortality of huhu larvae exposed to different heat treatment.

Temperature (°)	LT_{50}	95% CI	LT_{99}	95% CI	No. replications
Neonate larvae ($\approx$1.25 mg; control mortality: 5.9 $\pm$ 0.02% [n $\approx$ 16])					
35	88	82–93	139	128–154	13
40	5.8	5.5–6.0	7.8	7.4–8.5	8
45	0.61	0.5–0.7	1.0	0.9–1.6	8
Larvae ($\geq$ 100 mg; control mortality: 5.6 $\pm$ 0.02% [n=11])					
35	135	111–164	226	187–273	3
40	11.5	9.5–13.9	24	20.0–28.6	3
45	2.2	1.9–2.4	3.0	2.5–3.6	5

Mortality in the control groups of field-collected egg batches varied greatly, with an average mortality of 49.7 $\pm$ 0.06% (n=34). Egg survival after heat treatment was observed at one sample point at 45°C (8.7% hatched after 2.5 h) and at three sample points at 40°C (12.5–38.5% hatched after 3 and 5 hours' exposure), but two of these lots had no hatched larvae in the associated control batch, and were therefore excluded from the analysis. As a consequence, no LT values could be calculated for eggs treated at 40–45°C. However, it is clear from the high mortality rate (survival in only four out of 134 samples tested) that eggs were more susceptible than huhu larvae to heat treatment at elevated temperatures.

Controlled Atmospheres

Exposure at 20°C for up to 264 h to a 100% CO_2 atmosphere caused 0–17.4% (average: 5.3 $\pm$ 0.01%) mortality of huhu larvae. Similarly, exposure to a 100% N_2 or a 50% N_2 / 50% CO_2 atmosphere at 20°C caused 0–16.7% (4.8 $\pm$ 0.01%) and 0–36.4% (6.6 $\pm$ 0.02%) mortality respectively. Owing to low insect mortality associated with the controlled atmosphere treatments at 20°C, no LT values could be calculated. When treating larger larvae with 100% N_2 at 40°C, an estimated 8.3 hours were required to achieve 99% mortality (Table 2). This reduced to an LT_{99} of 6.9 hours under a 100% CO_2 CA, and to an LT_{99} of 7.6

TABLE 2–Time in hours (plus 95% confidence intervals — CI) required for 50% (LT_{50}) and 99% (LT_{99}) mortality of huhu larvae $\geq$ 100 mg exposed to controlled atmospheres at 40°C of 100% N_2, 100% CO_2, and a 50% N_2 / 50% CO_2 mixture.

Treatment configuration	LT_{50}	95% CI	LT_{99}	95% CI	No. replications
Larval control mortality: 4.56 ± 0.02% [n=9]					
100% N_2	6.8	6.1–7.6	8.3	7.2–9.6	3
100% CO_2	6.0	5.7–6.2	6.9	6.4–7.3	3
50% N_2 / 50% CO_2	6.9	6.7–7.1	7.6	7.3–7.8	3

hours when treated with a 50% N_2 / 50% CO_2 mixture. When LT_{50} and LT_{99} values for the three controlled atmosphere configurations were compared, the 100% CO_2 treatment was significantly (p = 0.01) more efficient in killing huhu larvae than the 100% N_2 or 50% N_2 / 50% CO_2 treatments. However, there was no statistical difference in larval response to the 100% N_2 and 50% N_2 / 50% CO_2 treatments. All controlled atmosphere conditions at 40°C (Table 2) were significantly better at killing larger huhu larvae than was the 40°C and air treatment (Table 1).

DISCUSSION

Huhu eggs and neonate larvae were more susceptible than larger larvae to heat treatment. Exposure to 35°C in air required more than 226 hours to achieve 99% mortality of all life stages tested. However, increasing temperatures to 40°C significantly reduced exposure time for 99% kill to 24 hours, with a further reduction to 3 hours at 45°C. Heat treatments have also been used successfully to treat pinewood nematodes in lumber and wood chips (Dwinell 1990; Dwinell, Magnusson & Tomminen 1994). One of the conclusions of a USDA efficacy review of control measures for potential pests of imported Soviet timber was that different forms of heat "...appear most promising..." (USDA 1991). A study by Dwinell, Avramidis & Clark (1994) confirmed the efficacy of applying heat $\geq$ 56°C to lumber to control nematodes, using a radio-frequency/vacuum dryer.

At 20°C, larger huhu larvae ($\geq$100 mg) were very tolerant to low oxygen conditions. This is similar to results described for wood-boring insects exposed to a range of low oxygen conditions (with moderate to high carbon dioxide levels) at 30°C (Paton & Creffield 1987). When the temperature of the controlled atmosphere treatments in our study was raised to 40°C, 99% of larger larvae could be killed in an estimated 8.3 hours. Similar reductions in LT values by increasing treatment temperatures during controlled atmosphere treatments have been observed for various coleopteran species in stored products (Donahaye *et al.* 1994), *Cydia pomonella* L. in walnuts (Soderstrom *et al.* 1996), and *Epiphyas postvittana* (Walker) (Whiting *et al.* 1991) and *Tetranychus urticae* Koch (Whiting & van den Heuvel 1995) on fresh produce. All treatments against huhu which combined 40°C with controlled atmosphere resulted in LT_{99}s significantly lower than those for 40°C air treatment.

A 100% CO_2 treatment was more effective than a 100% N_2 treatment or a 50% N_2 / 50% CO_2 treatment. Schroeder & Eidmann (1986) also found that controlled atmospheres containing high concentrations of carbon dioxide were more effective against bark beetles than those containing mainly nitrogen. Similar results were observed by Soderstrom *et al.*

(1996) when treating diapausing *Cydia pomonella* larvae. However, the opposite trend applied to treatment of *C. pomonella* adults and eggs (Soderstrom *et al.* 1991), and mortality responses of other insect species may be more rapid to mixtures of carbon dioxide in air than to "pure" carbon dioxide (Nicolas & Sillans 1989).

Research has shown that larvae of woodborers are tolerant to atmospheres of pure carbon dioxide and carbon dioxide in air (Paton & Creffield 1987). These authors discussed the possibility of high physiological adaptation to these altered atmosphere conditions since low oxygen concentrations may occur in waterlogged logs or logs with high moisture content. Carbon dioxide concentrations as high as 12% have been reported in decaying beech logs in wet locations (Paim & Beckel 1964). Mortality may be due to immediate or latent effects of carbon dioxide such as anaesthesia (Nicolas & Sillans 1989). In some insect species (e.g., *Drosophila*) the anaesthetic effect of carbon dioxide decreased as temperature increased.

Heat and controlled atmosphere treatments have been tested successfully in our research against huhu eggs and larvae. Treating huhu in logs with controlled atmosphere may also assist with the control of wood-decaying and sapwood-staining fungi (Scheffer 1985). In addition, low oxygen tension enhanced the efficacy of natural products such as essential oils (Paster *et al.* 1990) which could also be active against forestry pests.

Applying heat (kiln drying) is already an established process in the timber industry and combination treatments for the preservation of wood are common (Burton *et al.* 1991; Wenlong He *et al.* 1997). Using elevated temperatures to eradicate mesophilic organisms in wood shows most promise (Dwinell 1996), and has a high probability of successful mitigation (Morrell 1995). Under New Zealand conditions, heat could be applied using conventional means or by utilising low-grade thermal energy resources currently available (Ian Thain, pers. comm.).

Successful implementation of a heat disinfestation treatment for New Zealand logs requires acceptance of efficacy data by Government agencies of the importing country as well as a treatment that will maintain product quality and does not have a negative effect on any associated processing treatments. Jones (1973) found that 12-hour hot-water treatment at 49°C sterilised oak wilt fungus without deleterious effect on log quality. Tejada *et al.* (1997) treated fresh logs of fir, Japanese cedar, oak, and ash for 70 hours with high-temperature smoke and found that the treatment reduced the residual stresses in the wood and as a consequence improved the dimensional wood stability. Steam and dry heat can penetrate logs and raise internal temperatures to levels that effectively control pests without causing wood damage (USDA 1991). Depending on the species, heating wood above the boiling point of water may reduce wood strength, and consideration should therefore be given to the ultimate end product when developing heating regimes (Morrell 1995).

In collaboration with the New Zealand forestry industry, further research will be required to apply successful treatment conditions to insects in logs using larger-scale treatment facilities similar to those described by Dentener *et al.* (1996) for fresh produce, and to determine the effect of potential huhu disinfestation treatments on product quality.

ACKNOWLEDGMENTS

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