

# Mortality of mountain pine beetle larvae, *Dendroctonus ponderosae* (Coleoptera: Scolytidae) in logs of lodgepole pine (*Pinus contorta* var. *latifolia*) at constant low temperatures

L. SAFRANYIK and D.A. LINTON

NATURAL RESOURCES CANADA CANADIAN FOREST SERVICE  
PACIFIC FORESTRY CENTRE 506 WEST BURNSIDE ROAD VICTORIA, BC V8Z 1M5

## ABSTRACT

Mortality of mountain pine beetle larvae (*Dendroctonus ponderosae* Hopkins) in naturally infested logs of lodgepole pine (*Pinus contorta* var. *latifolia* Douglas) held at constant low temperatures was investigated. The logs were brought to the laboratory in mid-fall and stored at  $-6.6\text{ }^{\circ}\text{C}$  for 10 weeks, then at  $-12.2\text{ }^{\circ}\text{C}$  for 2 weeks prior to the start of the experiments to allow the larvae to acquire maximum cold hardiness. The logs were exposed to constant temperatures of  $-17.8$ ,  $-23.3$ ,  $-28.8$ , and  $-34.4\text{ }^{\circ}\text{C}$  for 1, 2, 4, 8, 16, and 32 days. Mortality of larvae was recorded for two size classes: 1<sup>st</sup>- and 2<sup>nd</sup>-instar larvae and 3<sup>rd</sup>- and 4<sup>th</sup>-instar larvae. Mortality in logs stored at  $-12.2\text{ }^{\circ}\text{C}$  served as control. Mortality was negatively correlated with log diameter, bark thickness, brood density, and gallery length per  $\text{m}^2$ , but only the correlation with log diameter was statistically significant. Mortality in both small and large larvae was inversely related to temperature and directly related to duration of treatment. Mortality of the small larvae was positively correlated to mortality of large larvae but mortality of the latter was significantly lower. Multiple regression was used to describe the relationship between temperature, duration of treatment, and log diameter for both larval groups. Results are discussed in relation to published information.

**Key words:** *Dendroctonus monticolae*, Scolytidae, *Pinus contorta*, mortality, low temperature, cold hardiness

## INTRODUCTION

Over winter mortality from low temperatures is one of the main factors which regulates mountain pine beetle (mpb) populations (Safranyik 1978). To improve survival, a number of arthropods rely on supercooling to avoid freezing (Gehrken 1989). Mountain pine beetle larvae acquire and increase cold-hardiness through gradual accumulation of glycerol in their blood in response to gradually decreasing temperature, and lose it in the opposite manner (Somme 1964); thus, cold-hardiness is usually greatest in the period from December to February (Wygant 1940). Unseasonably low temperatures early in the fall, or late in the spring, reduce survival rates in all developmental stages. Because mountain pine beetles generally overwinter as larvae in their host trees in British Columbia, the effects of low temperatures on larval survival are of particular interest.

The lethal low temperature threshold for exposed larvae in laboratory experiments ranges between  $-34\text{ }^{\circ}\text{C}$  and  $-37\text{ }^{\circ}\text{C}$  (Wygant 1940; Somme 1964), with more mature larvae having the lowest threshold (Amman 1973). Sub-cortical temperatures are modified by such host characteristics as bark thickness, tree diameter, and wood moisture content (Wygant 1940) and may cause significant variation in larval mortality within and among trees. The object of this experiment was to determine the effects of prolonged exposure to constant low temperatures on the survival of cold-hardened larvae within the host in relation to some host characteristics and stage of larval development.

## MATERIALS AND METHODS

The infested lodgepole pine logs used in the experiments were cut at Elk Creek, 40 km east of Canal Flats, British Columbia, during late October 1970. The logs, 40-45 cm long, were moved to the laboratory in Calgary, Alberta, and put into cold storage at  $-6.6 (\pm 2.2)$  °C until 10 January, 1970, when they were moved to the Northern Forestry Centre, Alberta, and stored at  $-12.2 (\pm 2.2)$  °C until used in the experiments.

At the start of the experiments, the logs were cut to 30 cm lengths, waxed at the ends to retard moisture loss, and randomly assigned to treatments. The temperature treatments were  $-17.8$ ,  $-23.3$ ,  $-28.8$  and  $-34.4$  °C (0,  $-10$ ,  $-20$ ,  $-30$  °F) and control. Temperature treatments were applied in a freezer unit with a minimum temperature limit of  $-36.1$  °C ( $-33$  °F). The control treatment consisted of logs stored at  $-12.2$  °C. The original design called for two bolts to be used at each temperature and duration combination, but lack of suitable material forced us to cut back to single bolts in some treatments (Table 1).

**Table 1**

Number of logs used in the mountain pine beetle low temperature mortality experiments.

Duration (Days)	Temperature (°C)				
	-12.2	-17.8	-23.3	-28.8	-34.4
1	2	2	2	2*	2
2	2	2	2	2	2
4	2	2*	2	2	2
8	1	1	1	1	1
16	1	1*	1	1	2
32	1	1*	1	1	1

\* Denotes a single log containing fewer than five larvae which was dropped from the data analysis.

The treatments were carried out during February and March, 1971. Following treatment, the logs were stored at  $1.7$  °C ( $+35$  °F) for 1 to 2 days and then at  $21.1$  °C ( $+70$  °F) for 3 to 7 days prior to inspection for brood survival. Prior to removal of the bark, log diameter (to the nearest 2.5 mm) and bark thickness were measured; the latter on four sides of the log to the nearest 0.8 mm. Moisture content of the outer sapwood to a depth of about 5 years growth was measured on  $10.2$  cm x  $2.5$  cm blocks of wood from each of four sides and expressing moisture content as percent oven dry weight (Reid 1961). The numbers of attacks and the total length of the successful egg galleries (those having produced larvae) were determined for each log and converted to a per square meter basis prior to analysis.

The bark was carefully removed and all larvae were placed in Petri dishes on moist filter paper. Obviously living larvae, as shown by movement upon gentle prodding with a probe, were separated, their head capsule widths measured to determine larval instar (Reid 1962; Amman and Cole 1985) using an ocular micrometer on a dissecting microscope, and separated into two groups: large larvae (3<sup>rd</sup>- and 4<sup>th</sup>-instar) and small larvae (1<sup>st</sup>- and 2<sup>nd</sup>-instar). Non-moving, but non-discoloured and firm larvae were kept at room temperature for a further 24 hours prior to re-assessment for survival and determination of larval size group as described above.

Only logs containing at least five larvae were used in analyses. For this reason, one log treated at  $-17.8$  °C for each of 4, 16 and 32 days and one log treated at  $-28.8$  °C for one day were not used in subsequent analyses. The data were analyzed using correlation and regression analyses, and analysis of variance. The correlation analyses focused on percentage survival by larval size group and log and attack characteristics. The analysis of

variance determined the effects and interactions of temperature and duration of exposure on larval survival. Owing to the lack of replication in some combinations of temperature x treatment duration, we used a general linear model ANOVA (Proc GLM, SAS Institute, 1985) and means were compared by averaging over all levels of the other treatments. Multiple regression analysis was used to predict larval survival in terms of treatment, log, and attack variables.

## RESULTS

The means ( $\pm$  SD), minima and maxima of the log measurements and attacks, egg gallery lengths and brood densities are given in Table 2. Among the log measurements, percent sapwood moisture content was the most variable. Sapwood moisture content ranged from 30% to 118% in individual logs. Among the attack variables, brood density was the most variable and ranged from about 60 to over 2000 per m<sup>2</sup>. On average, 56.8 % of the brood were in the 3<sup>rd</sup>- and 4<sup>th</sup>-instars; no pupae or brood adults were present in the logs.

**Table 2**

Means ( $\pm$  SD), minima and maxima of mountain pine beetle attack variables and lodgepole pine log characteristics (N=42).

Variable	Mean	Minimum	Maximum
Log diameter (cm)	24.8 (2.46)	15.2	27.4
% Sapwood moisture	61.5 (22.9)	30	118
Bark thickness (mm)	4.9 (1.04)	3.2	8.3
Attacks per m <sup>2</sup>	141.6 (60.68)	9.7	261.4
Brood per m <sup>2</sup>	896.9 (508.9)	62.4	2005.7
Gallery length (m/m <sup>2</sup> )	18.1 (7.70)	2.6	2.7

**Table 3**

Pearson correlation matrix of mountain pine beetle attack variables and characteristics of lodgepole pine logs used in the study (N=42). Correlation coefficients marked by \* and \*\* are significant at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively.

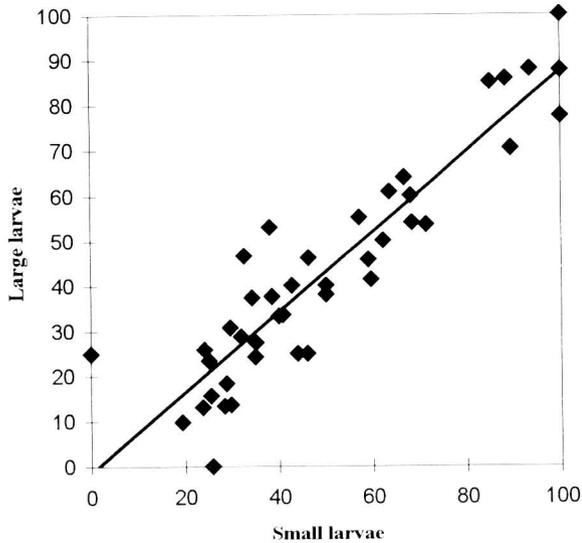
	Diameter	% Moisture	Bark Thickness (mm)	Attack per m <sup>2</sup>	Gallery per m <sup>2</sup>	Brood per m <sup>2</sup>	% Mortality Larvae	% Mortality Larvae
Diameter	1	0.41**	0.37*	0.47**	0.69**	0.53**	-0.37**	-0.45**
% Moisture <sup>a</sup>		1	-0.1	-0.06	0.43**	0.14	-0.14	-0.29
Bark Thickness <sup>a</sup>			1	0.85**	0.60**	0.42**	0.02	-0.07
Attack				1	0.74**	0.56**	-0.01	0.03
Gallery					1	0.75**	-0.18	-0.1
Brood						1	-0.21	-0.16
% Small							1	0.916**
% Large								1

<sup>a</sup> Four samples per log.

The simple correlation coefficients of all combinations of the log and brood variables are given in Table 3. Log diameter was positively and significantly correlated with each of bark thickness, sapwood moisture content, egg gallery length per m<sup>2</sup>, attack density and brood density. Bark thickness was positively and significantly correlated with each of the attack variables but sapwood moisture content was significantly correlated with egg

gallery length density only. Brood density was positively and significantly correlated with both attack density and egg gallery length density; the latter two variables were also significantly correlated.

Mortality of the small and large larvae was highly correlated ( $r=0.916$ ,  $p<0.01$ ,  $n=42$ ). The relationship between percent mortality in large larvae (Y) and that of small larvae (X) was linear at all combinations of temperature and exposure duration (Fig. 1). Both the regression coefficient and the intercept were significantly different from zero ( $p<0.01$ ,  $n=42$ ).



**Figure 1.** The relationship between percent mortality of large (3<sup>rd</sup>- and 4<sup>th</sup>-instar)(Y) and small (1<sup>st</sup>- and 2<sup>nd</sup>-instar)(X) larvae. The solid line represents the least squares equation:  $Y = -1.362 + 0.886X$ .

Analysis of variance of the combined data for small and large larvae indicated that the temperature treatment, the stage of larval development, and the interactions of stage with temperature and duration of treatment significantly affected mortality. Stage ( $F_{1,15} = 28.91$ ,  $p<0.0001$ ) and temperature ( $F_{4,13} = 13.16$ ,  $p<0.001$ ) had the greatest effect followed by the interaction of larval stage with temperature ( $F_{4,15} = 3.36$ ,  $p<0.05$ ) and duration of exposure ( $F_{6,15} = 2.98$ ,  $p<0.05$ ).

Percent mortality due to the temperature treatments, averaged over duration of exposure, is given in Table 4. Average percent mortality of the combined larval instars at the coldest temperature was significantly greater than mortality at the other temperature treatments. Average percent mortality at each of  $-28.8$  and  $-23.3$  °C was significantly different from mortality at each of  $-12.2$  and  $-17.8$  °C but not within either of these two treatment groups. The patterns of average percent mortality of small and large larvae with respect to temperature treatments were similar except that small larvae suffered greater mortality at all levels of temperature treatment (Table 4).

Percent mortality by duration of temperature exposure, averaged over temperature treatment, is given in Table 5. Larvae in the control logs suffered 27.4% average mortality (21.8% and 32.9% for small and large larvae, respectively), which was significantly lower

than any other treatment. Average percent mortality at each of 1 to 8 days of exposure differed significantly from those at 16 and 32 days of exposure but not within the respective groups. There were no differences in mortality in the controls over the duration of the experiments (Chi-square, 2df = 4.34,  $p > 0.05$ ). In general, the pattern of mortality for small larvae over duration of exposure was the same as overall larval mortality. For large larvae, mortality at each of 4 to 32 day exposures was significantly different from the control and the 1 day exposure (Table 5). There were no significant differences in mortality among exposures of 4 to 32 days.

**Table 4**

Percent mortality of small (1<sup>st</sup>- and 2<sup>nd</sup>-instar) and large (3<sup>rd</sup>- and 4<sup>th</sup>-instar) mountain pine beetle larvae maintained at different low temperatures, averaged over duration of exposure. The sample size (*N*) is the number of logs per temperature treatment.

Temperature (°C)	N	% Mortality		
		Small larvae	Large larvae	Combined
-12.2	9	32.90 ab	21.80 a	27.40 a
-17.8	6	29.65 a	24.57 a	27.11 a
-23.3	9	48.34 b	39.06 b	43.70 b
-28.8	8	43.67 ab	43.55 b	43.61 b
-34.4	10	83.93 c	75.86 c	79.89 c

\*Means followed by the same letter within columns are not significantly different ( $p > 0.05$ , Duncan's Test).

**Table 5**

Percent mortality of small (1<sup>st</sup>- and 2<sup>nd</sup>-instar) and large (3<sup>rd</sup>- and 4<sup>th</sup>-instar) mountain pine beetle larvae, at different duration of low temperature exposure, averaged over temperature treatments. The sample size (*N*) is the number of logs per temperature treatment.

Duration (days)	N	% Mortality		
		Small larvae	Large larva	Combined
0	9	32.90 a	21.80 a	27.35 a
1	7	49.79 ab	44.16 a	46.97 b
2	8	45.83 a	43.39 ab	44.61 b
4	7	50.09 ab	46.21 b	48.15 b
8	4	50.95 ab	43.85 ab	47.40 b
16	4	80.13 c	66.28 b	73.20 c
32	3	70.57 c	61.90 b	66.23 c

\*Means followed by the same letter within columns are not significantly different ( $p > 0.05$ , Duncan's Test). Zero duration of exposure indicates the control treatment.

The average percent mortality, taken over temperature treatment and duration of temperature exposure, was 50.0% for small larvae, and 42.9% for large larvae, which were significantly different ( $p < 0.001$ ).

A multiple regression of percent larval mortality of small ( $Y_1$ ) and large ( $Y_2$ ) larvae on log diameter ( $X_1$ ), duration of temperature exposure ( $X_2$ ), temperature treatment ( $X_3$ ) had the following form:

$$Y_1 = 91.55 - 5.71X_1 + 0.87X_2 - 10.88X_3 + 4.90X_3^2; R^2 = 0.68, N = 42$$

$$Y_2 = 88.40 - 6.76X_1 + 0.55X_2 + 2.68X_3^2; R^2 = 0.76, N = 42$$

All coefficients of both regressions were significantly different from 0 ( $p \leq 0.05$ ). The variable  $X_3$  was coded as 0, 1, 2, 3, and 4 in order of decreasing temperature treatment.

## DISCUSSION

The pre-treatment cold storage of the logs was sufficient to induce maximum cold hardiness in the larvae, as they required only about 2 weeks of exposure at  $-5\text{ }^{\circ}\text{C}$  constant to attain maximum accumulation of glycerol in the blood (Somme 1964). The relatively large variation in percent mortality in the control logs (C.V.=43.2 %) indicates that log characteristics had a large effect.

The strong negative correlation of log diameter with percent brood mortality is explained in part by the high negative correlation of log diameter with temperature treatment ( $r=-0.28$ ), in spite of the random allocation of logs to treatments. Temperature was the single most important variable relating to larval mortality. Log diameter directly affects the rate of cooling, due to the increase with mass in the amount of heat stored. Log diameter was significantly and positively correlated with bark thickness, percent sapwood moisture and attack, brood, and egg gallery length per  $\text{m}^2$ . As bark is a good insulator, its thickness likely reduced heat loss and the rate of cooling of the log. By creating air pockets in the inner bark, attack, brood and egg gallery length densities had similar effects. It is more difficult to assess the effect of sapwood moisture because the thermal conductivity and the specific heat of wood both increase directly with moisture content, hence, though heat may be gained or lost more readily, at a given ambient temperature more heat is stored in wood having a high moisture content.

On average, duration of temperature treatment had only a moderate effect on brood mortality. For both small and large larvae, the greatest daily change in mortality occurred following one day of exposure (Table 5). This is consistent with results by Somme (1964) and Wygant (1940) who showed that the effects of low temperature mortality usually occur within the first few hours of direct exposure. The inconsistent change in mean percent mortality with the duration of exposure in this study is likely due to the unequal replication and large variability among logs discussed above.

Mean percent mortality was the same for the control ( $-12.2\text{ }^{\circ}\text{C}$ ) and the  $-17.8\text{ }^{\circ}\text{C}$  temperature treatments because cold-hardened larvae could withstand sustained temperatures of these magnitudes (Table 4). For both small and large larvae, the largest change in mortality occurred in the treatment range from  $-23.3\text{ }^{\circ}\text{C}$  to  $-34.4\text{ }^{\circ}\text{C}$ . The maximum super cooling point of mountain pine beetle larvae is close to  $-34\text{ }^{\circ}\text{C}$  (Somme 1964). Some beetles survived even the 32 day exposure at the coldest temperature treatment, probably due to a combination of the moderating effects of log size and bark thickness, and much lower than average individual super cooling points. Since underbark temperatures were not monitored, no information is available on average temperatures and their variability in the inner bark region.

The multiple regressions predicting average percent larval mortality as a function of log diameter, larval size, temperature, and exposure, show that a large proportion of the variation in mortality can be explained by a combination of these variables. Such predictive equations have considerable utility in modeling population dynamics as well as in bark beetle management. Development of such equations should be based on well-replicated experiments that take into account the possible regional variation in cold hardiness.

These results indicate that host characteristics (such as size, bark thickness, and moisture content) and beetle variables (such as attack, egg gallery and brood densities) moderate the effects of low temperatures on mountain pine beetle survival. Mortality from low temperatures is greater for small larvae than for large larvae and the relationship between small and large larval mortality is linear within the temperature range investigated.

## REFERENCES

- Amman, G. D. 1973. Population changes of the mountain pine beetle in relation to elevation. *Environmental Entomology*. 2: 541-547.
- Amman, G. D., Cole, W. E. 1983. Mountain pine beetle dynamics in lodgepole pine forests. Part II: Population dynamics. General Technical Report, Intermountain Forest and Range Experiment Station, USDA Forest Service. No. INT-145. 59pp.
- Gehrken, U. 1989. Supercooling and thermal hysteresis in the adult bark beetle *Ips acuminatus* Gyll. *Journal of Insect Physiology*. 35:347-352.
- Reid, R. W. 1962. Biology of the mountain pine beetle, *Dendroctonus monticolae* Hopkins, in the East Kootenay region of British Columbia. I. Life cycle, brood development, and flight periods. *The Canadian Entomologist*. 94: 531-8.
- Reid, R. W. 1961. Moisture changes in Lodgepole Pine before and after attack by the mountain Pine beetle [*Dendroctonus monticolae*]. *Forestry Chronicle*: 37: 368-75, 403.
- Safranyik, L. 1978. Effects of climate and weather on mountain pine beetle populations. In: D. L. Kibbee, A. A. Berryman, G. D. Amman and R. W. Stark. (Eds.) Theory and practice of mountain pine beetle management in lodgepole pine forests. Conference held at Washington State University, Pullman, WA in 1978 (April 25-27). Forest, Wildlife and Range Experiment Station, University of Idaho, Moscow, Idaho. 224 pp.
- SAS Institute, Inc., 1985. SAS User's Guide: Statistics, Version 5 Edition. SAS Institute Inc. Cary, NC. 956 pp.
- Somme, L. 1964. Effects of glycerol on cold hardiness in insects. *Canadian Journal of Zoology*. 42: 87-101.
- Wygant, N.D. 1940. Effects of low temperature on the Black Hills beetle (*Dendroctonus ponderosae*) Hopkins. Summary, PhD. dissertation State University of New York, College of Environmental Science and Forestry. Syracuse. 57 pp.

