Determining the instar of mountain pine beetle (Coleoptera: Curculionidae) larvae by the width of their head capsules

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Abstract—Cut-points to distinguish larval instars of the mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae), from the measurement of head-capsule width were determined using a maximum likelihood approach. The cut-points that distinguish the four instars are suitable to classify individual larvae from field populations collected from lodgepole pine *Pinus contorta* var. *latifolia* Engelmann (Pinaceae) or jack pine (*P. banksiana* Lambert) × lodgepole pine hybrids throughout most of the beetle's current range in British Columbia and Alberta, Canada. The ability to designate the instar of field-collected larvae will facilitate the calibration of phenology and populations models that assess the climatic suitability of habitats and the potential for MPB to continue expanding its range.

Identifying the instar of insect larvae allows the description of age distributions in populations. Assessing the climatic suitability of new habitats, predicting spread potential, quantifying stage-dependent mortality, and calibrating phenology and population models all require the ability to assign instar to field-collected larvae (*e.g.*, Régnière *et al.* 2012). The efficacy of many control methods depends on their application coinciding with certain life stages and knowledge of the distribution of instars within a population over time is required to develop and apply phenology and population models.

The mountain pine beetle (MPB) *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae) is a native, eruptive bark beetle that attacks most species of pine (*Pinus* Linnaeus (Pinaceae)) in western North America. A recent epidemic in British Columbia (BC), Canada killed ~53% of the mature pine in the province and affected over 17 million ha of forested land between 1999 and 2011 (Walton 2012). During this recent outbreak, the beetle significantly expanded its range when it crossed the Rocky Mountains in northern BC; the beetle now appears to be established in northeastern BC and northcentral Alberta (AB), Canada where populations had not been reported previously (Safranyik *et al.* 2010; Bleiker *et al.* 2011). Mountain pine beetle now threatens to spread eastward across Canada's boreal forest.

A common, useful metric to determine the instar of immature insects is the size of their sclerotised head capsules, which remain relatively constant between moults, but increase in size with each instar (Daly 1985). Mountain pine beetle has four instars (Reid 1962; Amman and Cole 1983; Safranyik and Carroll 2006). Cut-points for each instar may be determined by visually identifying the lowest frequency class between the peaks on a histogram or by least-squares nonlinear regression (*e.g.*, Logan *et al.* 1998). However, these methods require large numbers of head-capsule width measurements to construct a frequency distribution and depend on the user to select appropriate frequency classes.

In this paper, we assess larval size using head-capsule widths for MPB from two regions in the historic range in BC and from the recently expanded range in northern AB. We use a maximum likelihood approach (Flaherty *et al.* 2012)

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Table 1. Location of field sites and dates when mountain pine beetle life stages were sampled in Canada.

Site name	Longitude	Latitude	Dates sampled (date/month) 2011 (trees attacked in 2010 or 2011)	Dates sampled (date/month) 2012 (trees attacked in 2011)
Southern BC				
Lower Red	−120.310	49.505	25/08, 27/09	27/06
Finnegan	−120.285	49.625	07/07	
Upper Red	−120.149	49.564	06/07, 28/09	28/06
Northwestern BC				
Big Oliver Creek	−128.287	54.813	24/06, 17/08, 06/10	20/06
Copper South	−127.694	54.783	21/06	
Northcentral AB				
Grande Prairie 1	−118.696	55.035	06/06, 16/07, 11/08, 24/09	02/06
Musreau	−118.661	54.560	04/06, 14/07, 12/08, 23/09	31/05
Hwy 40-4	−118.660	54.471	05/06, 15/07, 13/08, 25/09	01/06
Hwy 947	−116.592	54.230	08/06, 17/07	
Eagle Tower	−116.441	54.459	07/06, 18/07, 09/08, 20/09	04/06

Sites in northwestern and southern British Columbia (BC) were within the beetle's historic range and sites in northcentral Alberta (AB) were in the recently expanded range of the beetle east of the Rocky Mountains.

to determine MPB larval instar and provide cut-points that can be used to classify field-collected larvae throughout most the of the range of the beetle in Canada.

Mountain pine beetle head capsules from larvae collected in three regions were measured: southern BC, northwestern BC, and from the beetle's recently expanded range in northern AB east of the Rocky Mountains (Table 1). Larvae were sampled from 6–12 trees at each site at each sample time. Trees attacked in 2010 were sampled in the spring of 2011 and trees attacked in the summer of 2011 were sampled in the summer and fall of 2011 and again in the spring of 2012 (Table 1). Sample trees were lodgepole pine *Pinus contorta* var. *latifolia* Engelmann at field sites in BC and jack pine (*P. banksiana* Lambert) × lodgepole pine hybrids or backcrosses in AB with lodgepole pine being the recurrent parent in the region (Critchfield 1985).

A gas-powered hole saw was used to cut 10 cm diameter bark pucks between 0.5 and 2.0 m above ground on the bole of sample trees. Most sample trees were mass-attacked by beetles with successful attacks occurring around the entire circumference of the tree, but at sites with low beetle populations, a few samples trees were strip-attacked, with successful attacks around only a portion of the bole (Bleiker *et al.* 2014). Pucks were cut from the north (NW to NE) and south (SW to SE) aspects of mass-attacked trees and from the mid-point (±22°) of the successfully attacked portion of the bole on strip-attacked trees. A minimum of two pucks were sampled from each aspect if they contained <10 live individuals (eggs, larvae, pupae, and/or adults) and additional pucks were sampled until 10 or more individuals were found or a total of seven pucks had been sampled. Larvae were extracted immediately from the pucks and stored in 70% ethanol until processed in the laboratory. Head capsules were measured at the widest point in the dorsal–ventral view at 40 times magnification using an ocular micrometer on dissecting microscopes. Precision of the microscopes was 0.023 and 0.025 mm.

We estimated the means, μ_{ij} and variances, σ_{ij}^2 of head-capsule widths for each instar ($i = 1,2,3,4$) and region ($j = 1,2,3$) assuming a normal distribution. This represents 24 unknowns (12 means, 12 variances). A total of 4375 measurements were made, of which $n_1 = 1324$ came from southern BC, $n_2 = 275$ from northwestern BC, and $n_3 = 2776$ from northern AB. The number in each instar in each regional sample is not known, but the total number from each region is known. Once the number in each of the first three instars is determined, the number of fourth instars in each region is set by $n_{4j} = n_j - \sum_{i=1}^{3} n_{ij}$. Thus, three instar numbers from each region are

Table 2. Parameter estimates describing the head-capsule widths of the four instars of mountain pine beetle in southern British Columbia (BC), northwestern BC, and northcentral Alberta (AB).

Instar	Mean [μ_i (mm)]	Standard deviation [σ_i (mm)]	Number of observations (n_i)	Upper cut-point (mm)	Probability of classifying i as $i+1$	Probability of classifying $i+1$ as i
Southern BC						
I	0.476	0.028	428	0.541	0.009	0.010
II	0.612	0.031	523	0.694	0.004	0.008
III	0.881	0.078	250	1.035	0.030	0.024
IV	1.175	0.079	123			
Northwestern BC						
I	0.469	0.033	55	0.542	0.013	0.012
II	0.612	0.031	44	0.703	0.002	0.004
III	0.862*	0.059*	54	1.010	0.006	0.007
IV	1.175	0.067	122			
Northern AB						
I	0.501*	0.026	262	0.570	0.004	0.005
II	0.648*	0.030	528	0.740	0.001	0.001
III	0.891	0.049*	572	1.030	0.002	0.003
IV	1.222*	0.071	1414			

*Means significantly different from same instar in southern BC ($P < 0.001$).

free to vary, and become nine unknowns to be estimated.

The probability of observing a width w in the sample is calculated from the weighted average probability distribution of the head-capsule width within a random sample of 4375 larvae with the same regional composition as ours:

$$p(w) = \frac{\sum_i \sum_j n_{ij} \mathrm{N}\left(\mu_{ij}, \sigma_{ij}^2\right)}{4375} \quad [1]$$

where

$$N\left(\mu_{ij}, \sigma_{ij}^2\right) = \frac{1}{\sqrt{2\pi\sigma_{ij}^2}} e^{-\frac{\left(w-\mu_{ij}\right)^2}{2\sigma_{ij}^2}} \quad [2]$$

is the normal distribution with mean μ_{ij} and variance σ_{ij}^2.

The objective of the analysis is to estimate the means μ_{ij} and variances σ_{ij}^2 of head capsules (by instar and region). This was done with Procedure NLMIXED of SAS 9.2 minimising:

$$\mathrm{NLL} = -\sum_{j=1}^{4375} \mathrm{Ln}\left[p(w_j, \mathbf{A})\right] \quad [3]$$

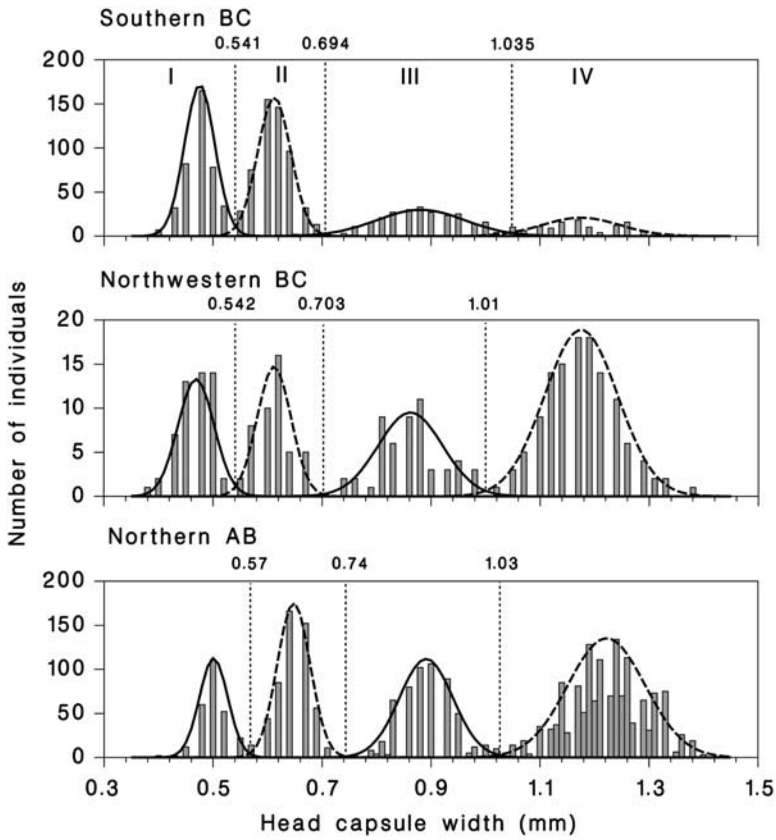
where $p(w_j, \mathbf{A})$ is the likelihood of each observation j and $\mathbf{A}$ the set of parameters to estimate (9 n's, 12 μ's, and 12σ^2's).

To test for differences in means and variances within instar, larvae from southern BC were used as a reference (*i.e.*, $\mu_{2j} = \mu_{1j} + \Delta\mu_{2j}$, $\mu_{3j} = \mu_{1j} + \Delta\mu_{3j}$, $\sigma_{2j} = \sigma_{1j} + \Delta\sigma_{2j}$, and $\sigma_{3j} = \sigma_{1j} + \Delta\sigma_{3j}$); a significant difference is found whenever $\Delta\mu_{ij} \neq 0$ or $\Delta\sigma_{ij} \neq 0$ (Table 2). Figure 1 shows histograms of the head-capsule width observations, overlaid on the estimated head-capsule distribution of the four instars (lines), for each region. From the means and variances obtained with this method, head-capsule width cut-points between successive instars and the probabilities of misclassification were determined (Flaherty *et al.* 2012).

The mean head-capsule widths obtained from this algorithm obey the Brooks-Dyar's rule, which states that the log of head-capsule width increases linearly with instar ($R^2 = 0.995$; Fig. 2; Hutchinson and Tongring 1984). There were no significant differences among regions in either intercept ($F(2,6) = 1.1$; $P = 0.393$) or slope ($F(2,6) = 0.13$; $P = 0.878$) in this linear relationship.

Larvae from northern AB had significantly wider head capsules than larvae from southern BC in the first, second, and fourth instars. Third instars collected from northern BC were significantly smaller than those from southern BC, but no differences were found in the other instars (Table 2). Factors such as competition, fungal

Fig. 1. Frequency distribution histogram of observed mountain pine beetle head-capsule widths sampled at sites in southern British Columbia (BC), northwestern BC, and northern Alberta (AB), Canada. The estimated head-capsule distribution of the four instars is overlaid (lines). Sites in BC are within the historic range of the beetle and sites in AB are within the recently expanded range.



associates, host species and quality, and growing season temperatures and length have been shown to affect MPB size (Amman 1982; Amman and Cole 1983; Safranyik and Linton 1983; Langor *et al.* 1990; Cerezke 1995; Bentz *et al.* 2001, 2011; Bleiker and Six 2007). However, the size differences in this study were very small and probably of little consequence for the purpose of instar determination in field samples. A final set of parameter values was obtained by pooling all head-capsule measurements without regional distinction. The probability of misclassifying instars was low (Table 3).

The cut-points reported here (Table 3) are suitable for classifying individual larvae from field populations of MPB from lodgepole or lodgepole × jack pine hosts throughout most of the beetle's range in western Canada.

Fig. 2. A regression showing the geometric progression in the size ($\log_e$[head-capsule width]) of mountain pine beetle larvae consistent with the Brooks-Dyar rule (see text) for populations sampled in southern British Columbia (BC), northwestern BC, and northcentral Alberta, Canada.

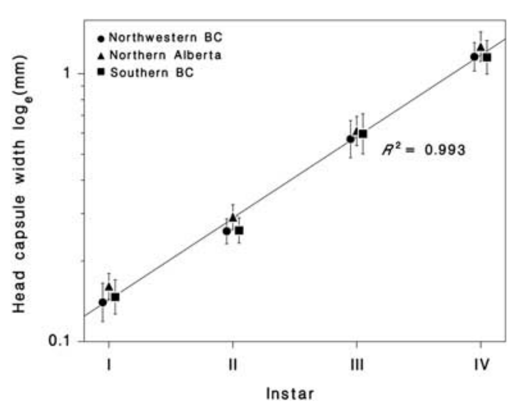


Table 3. Parameter estimates describing the head-capsule widths of the four instars of mountain pine beetle in Canada.

Instar	Mean [μ_i (mm)]	Standard deviation [σ_i (mm)]	Number of observations [n_i]	Upper cut-point (mm)	Probability of classifying i as $i+1$	Probability of classifying $i+1$ as i
I	0.485	0.030	745	0.552	0.013	0.016
II	0.629	0.036	1102	0.732	0.002	0.004
III	0.888	0.058	930	1.037	0.005	0.006
IV	1.216	0.072	1598			

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