SCIENTIFIC NOTE

Cold requirements to facilitate mass emergence of spruce beetle (Coleoptera: Curculionidae) adults in the laboratory

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ABSTRACT— The spruce beetle, *Dendroctonus rufipennis* Kirby (Coleoptera: Curculionidae, Scolytinae), is a native disturbance agent of spruce (*Picea* spp.) forests in North America. Based on field observations, it is widely accepted that new adults must overwinter regardless of the length of the life cycle. We tested the effect of different lengths of time at 4° C on spruce beetle emergence. Our objective was to determine a protocol for rearing spruce beetle to facilitate laboratory-based research. We found that for spruce beetles from north–central Alberta and southern British Columbia, 70 d at 4° C led to rapid mass emergence of adults. Adults also emerged in the absence of a cold period, but over an extended period of time.

Key words: spruce beetle, *Dendroctonus rufipennis*, bark beetle, rearing, emergence, diapause, overwintering

The spruce beetle *Dendroctonus rufipennis* Kirby (Coleoptera: Curculionidae, Scolytinae) is a native disturbance agent of spruce (*Picea* spp.) forests in North America. Large-diameter weakened and injured trees, fresh-cut stumps, cull logs, windthrow, and drought-stressed trees are the preferred hosts (Dyer and Taylor 1971; Safranyik 2011; Hart *et al.* 2013). Populations may build up in these hosts and spill over into mature healthy standing trees once the preferred hosts have been depleted (Safranyik *et al.* 1983; Safranyik 2011). Controlled rearing of spruce beetle in the laboratory may facilitate experiments aimed at understanding factors affecting the population dynamics and control of this economically important insect.

Bark beetles are easily reared in logs in the laboratory; however, some species require a cold period to complete their life cycle (Ryan 1959). A two-year life cycle is common throughout much of spruce beetle's range; under warmer conditions, the life cycle may be completed in one year and, in areas with cool, wet summers, the life cycle may take as long as three years (Massey and Wygant 1954; Knight 1961; Berg *et al.* 2006; Werner *et al.* 2006). Adult beetles emerge from overwintering in the natal host to attack new trees in the spring, usually in late May or June, although attacks can occur throughout the summer. Females bore into the inner bark, where they are joined by a male, mate, construct egg galleries, and lay eggs. The majority of the life cycle is completed under the bark, with larvae mining the inner bark before pupating and eclosing to new adults. Cool temperatures trigger what has been described as a facultative larval diapause in the two- and three-year life cycles, although the conditions that trigger the diapause and instar sensitive to the cue might vary geographically (Dyer and Hall 1977; Hansen *et al.* 2011). Based on numerous field observations, new teneral adults always overwinter once, regardless of the length of the life cycle, and it is widely accepted that new adults must overwinter to become sexually mature (Massey and Wygant 1954). Although it has not been demonstrated experimentally, it is now widely accepted that spruce beetle has an obligatory adult diapause (e.g., Raffa *et al.* 2015). New teneral adults overwinter in windthrow or standing trees where they developed; however, a proportion of beetles in standing trees may emerge in the fall, move to the base of the same tree, and bore under the bark where they overwinter in groups insulated from cold winter temperatures and

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woodpecker predation by the snow pack (Massey and Wygant 1954; Knight 1961; Fayt et al. 2005).

In this paper, we report the effect of different lengths of time at 4° C on spruce beetle emergence. Our objective was to determine a protocol for rearing spruce beetle in the laboratory that would lead to mass emergence of new adults. We selected 4° C as our treatment temperature, because it met the adult cold diapause requirements of Douglas-fir beetle *D. pseudotsugae* Hopkins (Ryan 1959) and it could be easily achieved with a regular refrigerator. We used two distant populations in the trial: Grande Prairie (GP) in north–central Alberta (N54.860800, W118.713567; 649 m) and Placer Creek (PL) in southern British Columbia (N49.15332, W120.47453; 650 m).

Spruce bolts (35-cm-long logs) were cut from a recently infested tree at each site and transported to the laboratory at the Pacific Forestry Centre, in Victoria, British Columbia. Five bolts were cut at GP in late-May 2013 and four bolts were cut at PL in mid-June 2013. The GP tree was likely pure white spruce (*Picea glauca* (Moench) Voss) and the PL tree was likely pure Engelmann spruce (*P. engelmannii* Parry ex Engelm.), although introgression between the species beyond their reported ranges is possible (see Maroja *et al.* 2007 and references therein). Parent beetles were constructing galleries and laying eggs at the time the material was collected. The bolts were placed vertically in emergence cages and held in a room at approximately 22° C with a photoperiod of 16 light:8 dark until 29 August 2013, when the presence of fully darkened teneral adults was confirmed by removing several small pieces of bark. As developing larvae were reared at 22° C, the facultative larval diapause was not triggered. At this time, one bolt from each population was left at 22° C and the other bolts were placed in a walk-in cold room at 4° C. The cold room was dark, except when someone transferred material in or out of it. One bolt from each population was removed after 15 (GP only), 30, 50 and 70 days in the cold room and placed back at 22° C. Henceforth in this report, we use the population code followed by the number of days at 4° C to refer to the different treatments. For example, GP30 and GP0 refer to insects from Grande Prairie, which were held at 4° C for 30 d and 0 d, respectively, while PL30 and PL0 refer to insects from Placer Creek receiving those treatments.

Emerging beetles were collected at least three times per week. Average daily emergence was calculated by dividing the number of beetles collected by the number of days since the last collection. After at least 10 days of zero beetles emerging from a bolt, the bark was removed and any teneral adults remaining under the bark were counted and recorded as alive or dead.

The majority of beetles in all treatments emerged. Eighty-nine percent or more of the beetles emerged, with two exceptions: PL0, 28% of the total number of beetles failed to emerge and were found dead under the bark; and GP50, 18% of the total number of beetles failed to emerge and most of these beetles were found alive under the bark (Table 1). For rapid mass emergence, the best cold treatment was 70 d for both beetle populations (Figure 1). Beetles subjected to 70 d of cold had a notable increase in emergence within 10 d after the cold treatment was terminated, and the vast majority of beetles emerged rapidly within a 10-d period (Figure 1). Beetles in the 50-d treatment also emerged soon after being removed from the cold room. However, after approximately 65% and 80% of the beetles had emerged from PL50 and GP50, respectively, emergence plateaued for almost two weeks (Figure 1). The remaining beetles emerged from PL50 but, in the case of GP50, the bolt was peeled after 10 d of no emergence and 17% of the total number of beetles were still under the bark and were alive (Table 1); these beetles may also have emerged given more time. Our results are similar to what Ryan (1959) reported for Douglas-fir beetle, which also overwinters as a teneral adult: 90 d of cold treatment was more effective than 50 d for triggering emergence. He also tested a number of cold temperatures from 0.5 to 12.5° C, and found that 6.6° C was the optimum cold temperature, although emergence following 90 d at 6.6 and 4° C were similar.

Table 1

Percentage of spruce beetles emerging from bolts (short logs) after 0, 15, 30, 50 or 70 d at 4° C. Insects were fully darkened teneral adults when cold treatments were initiated. After at least 10 d of no emergence, the bark was removed and the number of live and dead beetles remaining under the bark was recorded. Bolts were cut at two sites: Grande Prairie (GP), in north–central Alberta, and Placer Lake (PL), in southern British Columbia.

Site	Days at 4° C	Total Beetles (n)	Emerged (%)	Unemerged Dead (%)	Unemerged Live (%)
GP	0	98	96	4	0
	15	129	94	6	0
	30	272	94	6	0
	50	328	82	1	17
	70	169	99	1	0
PL	0	117	72	28	0
	30	259	95	5	0
	50	281	98	2	0
	70	282	89	11	0

Emergence tended to be slower from bolts receiving 30 d or less of cold treatment (Figure 1). Emergence from both GP30 and PL30 did not notably increase until after 21 d at 22° C, and emergence was also delayed after the cold treatment was terminated for GP15, although once it started it was relatively rapid (Figure 1). In the absence of a cold treatment, beetles emerged over an extended period of time (over 100 d) and it took longer for beetles to start emerging from GP0 than from PL0. The new adults may have been at different levels of maturation, as the populations may have received slightly different degree day accumulations based on temperatures in the field and when the trees were infested and cut. In addition, there may be geographic variation in the developmental rates of bark beetles (Bentz *et al.* 2001) or the proportion entering diapause (McKee and Aukema 2015). We also cannot determine if beetles were emerging to overwinter at the base of the tree or to disperse to attack a new host tree. Ryan (1959) determined that Douglas-fir beetles in diapause had underdeveloped reproductive organs. We did use a number of the new adults emerging from GP70 and PL70 in another experiment. These beetles entered fresh bolts and successfully reproduced, indicating they were sexually mature upon emergence; however, we did not use beetles from the other cold treatments, so their level of sexual maturity remains unknown.

Our results indicate that a cold period promotes the rapid mass emergence of new spruce beetle adults. In the absence of a cold period, 72% of PL beetles and 96% of GP beetles still emerged, although they emerged slowly over approximately 100 d. We have demonstrated that 70 days of cold treatment at 4° C is sufficient to trigger mass emergence of adult spruce beetles. This treatment can be used to rear insects in the laboratory, thereby facilitating experimental research.

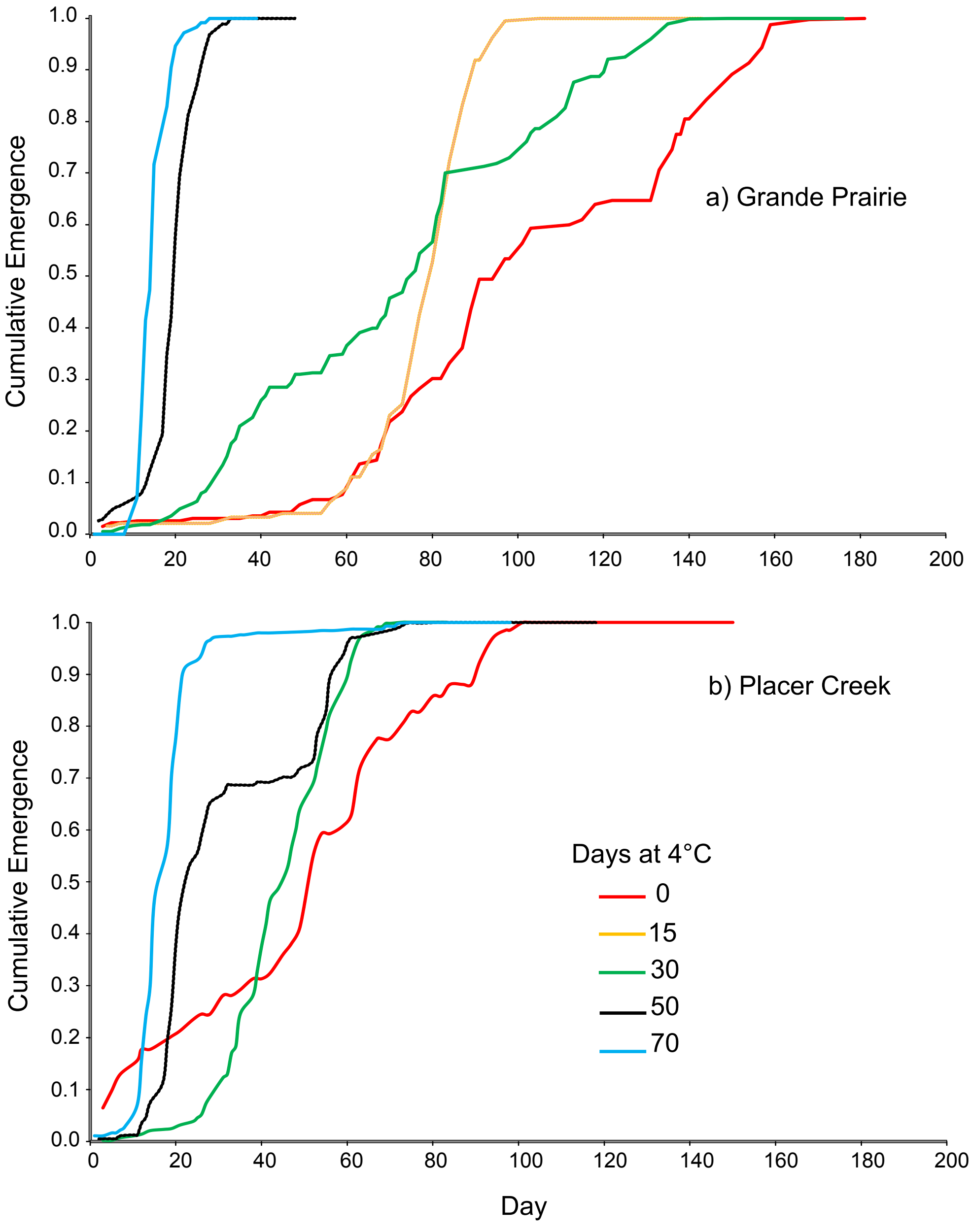

Figure 1. Cumulative emergence of all spruce beetles in bolts (short logs) after 0, 15, 30, 50 or 70 d exposure to 4° C before being held at 22° C (day 0). Bolts were cut at two sites: a) Grande Prairie (GP), in north–central Alberta, and b) Placer Lake (PL), in southern British Columbia.

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