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Original Article

Winter warming effects on overwinter survival, energy use, and spring emergence of *Cerotoma trifurcata* (Coleoptera: Chrysomelidae)

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Abstract

1 Bean leaf beetle *Cerotoma trifurcata* (Förster) (Coleoptera: Chrysomelidae) is a pest of soybean in USA and is becoming a concern in Canada. The projected increase in winter temperatures under climate change could affect overwinter survival, timing of spring emergence, and ultimately, the severity of this pest.

2 We assessed the potential effects of warmer winters in field experiments performed in three consecutive years. Three warming levels were applied: heated ~4°C above ambient, unheated with snow cover left intact, and unheated with snow cover removed. Survival and date of emergence were assessed in all years, and beetle lipid content was analyzed in one year to determine rates of energy use.

3 Overwinter survival was 6.5–14.5% among years. Winter warming inconsistently affected overwinter survival, increasing survival in one winter, decreasing survival in the warmest winter, and having no effect in one winter. Beetles that received supplemental winter warming emerged ~2 weeks earlier in spring, and lipid content did not differ among treatment groups.

4 Earlier spring emergence may allow for the production of an additional generation per year of *C. trifurcata* under future climate change. However, further experiments are required to establish the relation between overwinter survival and subsequent beetle population growth to determine potential pest status and best management practices under future climate conditions.

Keywords: bean leaf beetle, climate change, overwintering energetics, phenology, soybean pest, temperature increase

Introduction

Ectothermic animals such as insects are vulnerable to winter temperatures, and their overwinter survival, body condition, and timing of emergence could be affected by climate change-induced increases in the occurrence and frequency of winter warming events (IPCC (Intergovernmental Panel on Climate Change), 2013; Williams *et al.*, 2015). The winter conditions insects experience result from interactions between temperature, snow cover, and thermal variability (Williams *et al.*, 2015). Although low temperatures are the most obvious (and most studied) source of mortality (Bale & Hayward, 2010), overwintering insects also contend with energy drain, water loss, and pathogen pressure, among other stressors (Danks, 2000; Sinclair *et al.*, 2013; Sinclair, 2015), and herbivorous insects are under phenological pressure to emerge in synchrony with their host plants with sufficient post-winter energy reserves to maintain fitness (Williams *et al.*, 2015). In nature, most thermal regimes fluctuate, and such fluctuations can have unexpected consequences, depending on whether the fluctuations lead to cold mortality or energy savings at low temperatures, and energy drain or damage repair at higher temperatures (Colinet *et al.*, 2015). Thus, warmer winters could increase survival by reducing chilling injury (Bale & Hayward, 2010) or allowing earlier spring emergence (Hahn & Denlinger, 2007; van Asch *et al.*, 2007; Pozsgai & Littlewood, 2011), but could decrease survival by reducing insulating snow cover (Lawrence & Slater, 2010), leading to exposure to lethal air temperatures (Joshi *et al.*, 2009; Bale & Hayward, 2010) or increased rates of energy use that deplete energy reserves (Bosch & Kemp, 2003; Irwin & Lee, 2003; Williams *et al.*, 2003; Hahn & Denlinger, 2007, 2011). The relative importance of each of these scenarios remains to be determined and is likely to be both region and species specific.

Current understanding of the relationship between temperature and survival is based mainly on results from laboratory studies under relatively constant temperatures, which are poor approximations for performance outcomes under fluctuating temperatures (Colinet *et al.*, 2015). Studies using controlled fluctuating temperatures show that sufficient frequency and duration of warm periods can allow repair of accumulated chilling injuries and improvements in low-temperature survival (Renault *et al.*, 2004; Colinet *et al.*, 2006; Marshall & Sinclair, 2011). However, energy demands are greater under fluctuating temperatures because metabolic rates increase at higher temperatures (Colinet *et al.*, 2015), so cumulative warming duration may deplete energy stores. Overwintering in the field often involves additional mortality risks from desiccation, predators, and pathogens (Payah & Boethel, 1986; Lam & Pedigo, 2000), as well as exposure to unpredictably changing temperatures that require continuous physiological adjustments (Colinet *et al.*, 2015; Kingsolver *et al.*, 2015). Thus, a greater understanding of the relative importance of survival and energy use for overwintering insects under field conditions is required.

The bean leaf beetle *Cerotoma trifurcata* (Förster) (Coleoptera: Chrysomelidae) is a pest of soybean *Glycine max* (L.) Merrill and other legumes. It is native to eastern North America, but sources of anecdotal information disagree about when its range extended into southern Ontario, Canada (Chittenden, 1898; McCreary, 2013). Coincident with increases in soybean production, *C. trifurcata* has increasingly been considered a pest of potential economic importance (Witkowski & Echtenkamp, 1996; McCreary, 2013) because of its ability to cause feeding damage to soybean plant tissues and root nodules (Smelser & Pedigo, 1992; Lundgren & Riedell, 2008) and to transmit *Bean pod mottle virus* and fungi that reduce grain yield and quality (Shortt *et al.*, 1982; Giesler *et al.*, 2002).

Ceratoma trifurcata overwinters as an adult under leaf litter and is freeze intolerant, with Minnesota populations having a mean supercooling point of –8.9°C in February (Carrillo *et al.*, 2005). It currently produces 2–3 generations/year in central and southern United States (Pedigo, 1994), and 1 generation/year in southern Ontario (McCreary, 2013). An increase in the number of generations produced per year could be problematic because although insecticide treatments can suppress overwintered and first-generation populations, subsequent generations can still reach economic injury levels (Bradshaw *et al.*, 2008). Overwintering stress could be a bottleneck limiting the ability of *C. trifurcata* to attain high population numbers and high reproductive fitness in its northern range. If so, increasing temperatures might allow expansion of its distribution (Berzitis *et al.*, 2014), improved survival and fitness, and development of additional generations.

Our objective was to determine the likely effects of increasing winter temperatures on overwintering *C. trifurcata*. Using field experiments conducted in three years, we examined the effect of warmer winters on the overwinter survival, rates of energy use, and date of spring emergence of *C. trifurcata*. First, we hypothesized that increased winter temperatures with coincident decreased snow cover affect *C. trifurcata* overwinter survival and timing of emergence. We predicted that beetles would emerge earlier and have better survival with supplemental heating during winter, but that the loss of snow cover would reduce the positive effect of heating in colder winters. Second, we hypothesized that increasing mean temperatures lead to increased overwinter energy use. We predicted that beetle lipid content would decrease faster in beetles that received supplemental winter heating.

Materials and Methods

Winter warming experiment

An outdoor field experiment was repeated in 2011, 2012, and 2013, with slight differences in timing and sample sizes. Adult *C. trifurcata* were collected each fall from soybean fields near Wroxeter (43.86° N, 81.19° W) and Ennotville (43.65° N, 80.32° W), Ontario. Beetles were collected over a period of 2–4 weeks when soybean plants were physiologically mature. After collection, beetles were maintained in outdoor mesh cages (Bugdorm BD2120, MegaView Science, Taiwan) for up to 8 weeks and were provided with potted soybean and alfalfa plants. The outdoor conditions allowed natural acclimation of beetles to decreasing temperatures and photoperiod. In preparation for the experiments, beetles were brought indoors briefly for counting in December (2011) or November (2012, 2013), placed in 6-L pots (20 cm diameter) containing packed soil collected from an agricultural field (Elora, Ontario: 43.64° N, 80.41° W; Gleyed Brunisolic Grey Brown Luvisol developed on loam till), and covered by 4 cm (depth) of loose leaf litter collected from mixed-deciduous hedgerows at the Elora agricultural field. There were 25, 70, and 50 beetles/pot in 2011, 2012, and 2013, respectively, reflecting beetle availability. The pots were then buried with their tops level with the soil surface in an open area on the University of Guelph campus (43.527° N, 80.229° W) that had been prepared by light tilling. Pots were covered by mesh to prevent beetle escape, and left to overwinter.

Three levels of a warming treatment were applied to the buried pots in a randomized complete block design with six (2011) or eight (2012, 2013) replicates: heated ~4°C above ambient temperature, unheated with snow cover left intact (control), and unheated with snow cover removed. Snow was removed manually by sweeping it off of the mesh within 24 h of snowfall. Heating prevented snow accumulation, so the snow removal treatment controlled for this effect. Heat lamps (model 100011596, Zilla, Franklin, Wisconsin) were installed ~30 cm above all pots, but only those in the heated group contained 150 W ceramic bulbs (ExoTerra PT-2047, Hagen, Montreal, Quebec). Ambient temperature at the soil surface under the leaf litter was measured at 15-min intervals in each pot using a sensor connected to an Argus greenhouse control system (Argus Control Systems, White Rock, British Colombia) that turned the heat lamps on and off to maintain 4°C differential between heated and control pots. Although heat lamps tended to run continuously, realized temperature differences were often <4°C (Fig. 1). In 2013, a Hobo Pro v2 data logger (U23-001, Onset Computer Corporation, Bourne, Massachusetts) was placed under the leaf litter in one control and one heated pot in the same block to record humidity at 30-min intervals.

In spring (mid-March 2012, early April 2011, 2013), each pot and lamp were covered by an emergence tent (Bugdorm BD2400, MegaView Science) with the bottom cut open and the edges buried under the soil to prevent beetle escape. The mesh was then removed from the pots, and potted alfalfa plants were placed in each tent as a food resource. Tents and foliage were searched thoroughly for emerged beetles daily after 10:00 h, when *C. trifurcata* is reported to be most active (Krell *et al.*, 2003); beetles that had emerged were removed and counted. Tents were searched daily for 2-3 weeks after the last emerged beetle detection, after which pots were removed, and the leaf litter (and soil in 2011) were searched for live beetles.

The proportion of individuals surviving the winter was calculated based on the total number placed in the pot at the start of the experiment and the number that emerged in the spring. The dates of first and median emergence were determined for pots with at least one emerging beetle. Based on daily minimum and maximum temperatures recorded under the leaf litter, the number of degree-days accumulated at first emergence was calculated for each pot for 2011 and 2012 beginning March 1 and using a base of 10°C, estimated as the lower developmental threshold in Ontario (McCreary, 2013). Several malfunctioning temperature probes in May prevented degree-day calculations for 2013 (heat lamps were left on in affected pots during this time).

Lipid analysis

In 2013, rates of energy use by *C. trifurcata* were compared in the heated and control groups by collecting individuals for lipid analysis before the experiment began (baseline) and at three times during the winter. Fifteen baseline individuals were sampled in November prior to placing beetles into pots. For the next two sampling times, 15 individuals were enclosed in a 10 cm diameter flattened mesh bag filled with leaf litter, and one bag was placed between the soil surface and leaf litter in each heated and control pot at the beginning of the experiment. Bags were removed from one-half of the replicates on 28 January and from the other half on 13 March, taking care to minimize disturbance to the overlying leaf litter. Bags were allowed to warm at room temperature for 3 h, and then all live individuals, identified as those whose legs moved when their body was prodded, were collected. For the final sampling time, individuals were collected immediately following emergence in spring. All collected beetles were lyophilized for 48 h (Free Zone 4.5-L Freeze Dry System model 7751020, Labconco, Kansas City, Missouri) and stored at –20°C until analysis.

Lyophilized beetles were sexed, and neutral lipids and soluble protein were quantified individually in one female and one male from each pot as available. Because we expected lipids to compose the majority of the body mass and to decline during winter, we used protein as an estimate of metabolizing tissue mass to standardize body size. Beetles were homogenized in 100 μL 0.05% Tween 20 and diluted tenfold in Tween 20 solution. One aliquot was taken for lipid extraction and quantification while another was centrifuged for 10 min at 12,000 ×*g* and used for protein assay. Lipids were extracted using a Folch extraction, with the diacylglycerol glyceryl 1,3 distearate (D8269, Sigma-Aldrich Canada) added as an internal standard, and measured by thin layer chromatography coupled to a flame ionization detector (TLC-FID; Iatroscan MK-6 TLC-FID Analyzer, Iatron Laboratories, Tokyo, Japan) following the protocol of Williams et al. (2011). Total protein content was measured spectrophotometrically in triplicate using a bicinchoninic acid assay based on the methods of Gefen et al. (2006).

Statistical analysis

Proportion survival was compared among treatments using generalised linear mixed models with a binomial distribution and log-log link function. Maximum likelihood estimation was performed via the Laplace approximation in SAS 9.4 (SAS Institute, Cary, North Carolina). Warming was a fixed effect, block was a random effect, and years were analysed separately due to different numbers of blocks among years. The block effect was excluded from the model for 2011 because it did not explain any variance and caused a nonpositive definite covariance matrix. Overdispersion was evaluated using the Pearson χ^2 statistic divided by the degrees of freedom. Significant warming effects were decomposed using Tukey-Kramer tests.

Date of first emergence, median date of emergence, and degree-days accumulated at first emergence were compared among treatments using mixed-model ANOVA and restricted maximum likelihood estimation, followed by Tukey's multiple comparisons for significant effects, in JMP 12 (SAS Institute). Warming was a fixed effect, block was a random effect, and years were analysed separately. Degree-day data were log transformed. The analysed data met statistical assumptions in all years, except date of first emergence and median date of emergence in 2012; transformation did not improve these data (emergence timing was more variable in heated than other groups), and they were left untransformed for analysis.

Lipid content was compared using ANCOVA followed by Tukey's multiple comparisons in JMP 12. Treatment, time, sex, and their interactions were fixed effects, and protein was a covariate. Because the unit of replication differed, baseline data were excluded from the ANCOVA. Data were Box-Cox transformed to meet statistical assumptions. Data are archived at the University of Guelph Agri-environmental Research Data Repository (Berzitis *et al.*, 2013).

Results

Microclimate conditions

Ambient air temperatures were warmer in 2012 than in 2011 or 2013 (see Supporting Information, Appendix S1 for further details). Temperatures under the leaf litter differed substantially among treatments and years (Fig. 1a-c). On average, heated pots were warmer than control pots, and more so in March–April than January–February. In contrast, pots with snow removed were colder than control pots when snow was present (Jan–Feb 2011 and 2013), but did not differ in the absence of snow cover (Mar–Apr 2011 and 2013, and most of 2012). Temperature variability was similar among treatments within years (see Supporting Information, Appendix S1). Mean vapour pressure in 2013 was slightly lower and more variable in the heated (mean 660 Pa, range 210–1240 Pa) than the control pot (700 Pa, 350–1180 Pa; Fig. 1d).

Overwinter survival

At least one beetle survived in each pot except for one control pot in 2011, one heated pot in 2012 and 2013, and two snow removal pots in 2013. Across all treatments, survival was 13.3% in 2011, 14.5% in 2012, and 6.5% in 2013 (range of 0–44% survival among pots). Across all years and treatments, survival was 11%. Warming increased overwinter survival compared to the control in 2011 ($F_{2,15} = 4.38$, $P = 0.03$, $\chi^2/df = 1.71$), although care should be taken in interpreting this result because the *P* value is fairly large and the data are somewhat overdispersed. Warming decreased survival compared to the control and snow removal pots in 2012 ($F_{2,14} = 17.6$, $P = 0.002$, $\chi^2/df = 1.41$; Fig. 2). There was no effect of warming in 2013 ($F_{2,14} = 2.11$, $P = 0.2$, $\chi^2/df = 1.12$).

Lipid content

Triacylglycerols (TAG) were the only neutral lipids detected in *C. trifurcata* samples. TAG was significantly lower in spring-emerged (April/May) beetles compared to those sampled in January and March (Table 1 and Fig. 3). TAG content did not differ for any other effect or interaction, and the effect of the protein covariate was nonsignificant (Table 1).

Date of emergence and degree-day accumulation

Cerotoma trifurcata emerged significantly earlier in the heated than control and snow removal groups in all years (2011: $F_{2,9} = 38.2$, $P < 0.001$; 2012: $F_{2,13} = 11.3$, $P = 0.001$; 2013: $F_{2,11} = 7.81$, $P = 0.009$; Fig. 4). Beetles began to emerge approximately 12 d (2013) to 22 d (2012) earlier in the heated than the other groups. Median date of emergence was also significantly earlier in the heated than control and snow removal groups in all years (2011:

$F_{2,9} = 23.0$, $P < 0.001$; 2012: $F_{2,14} = 10.0$, $P = 0.002$; 2013: $F_{2,11} = 16.9$, $P < 0.001$; data not shown). Median emergence was approximately 8 to 15 d earlier in the heated than the other groups. There was no difference in the number of degree-days (base 10°C) accumulated at the date of first emergence among treatment groups (2011: $F_{2,9} = 0.50$, $P = 0.6$; 2012: $F_{2,14} = 2.34$, $P = 0.13$).

Discussion

Consistent with our predictions, *C. trifurcata* that experienced supplemental winter warming emerged significantly earlier than individuals in the control and snow removal groups across all years of study. Calculated degree-day accumulation at first emergence did not differ among the treatment groups, suggesting that increased late winter and early spring temperatures can advance the phenology of *C. trifurcata*. Contrary to our predictions, supplemental winter warming in the field did not consistently increase the cold survival of *C. trifurcata,* but was contingent upon the year of the experiment. The depletion of *C. trifurcata* lipid energy reserves did not differ among heated, control, and snow removal treatment groups, but was strongly affected by time, with lipid content lower upon spring emergence than at any other sampling time.

Temperature and beetle survival

Cerotoma trifurcata experienced very low winter survival in our field experiment: <15% in any year of the study and as low as 6.5% in 2013. These low rates of survival are consistent with estimates of 11–23% overwinter survival in soybean fields in Iowa, USA (Lam & Pedigo, 2000), which has a similar climate to southern Ontario, and are greater than the low of 0% survival in soybean fields in North Carolina, USA (Boiteau *et al.*, 1980), which has a more moderate climate. However, most *C. trifurcata* tend to overwinter in less exposed areas than soybean fields such as field margins and woodlands, where individuals shelter inside rolled leaves and hollow acorns (Lam & Pedigo, 2000; Lam *et al.*, 2002); survival may be somewhat higher in these habitats across the beetle's U.S. range, e.g., 18–51% in Iowa (Lam & Pedigo, 2000), 34–59% in Minnesota (Carrillo *et al.*, 2005), 33% in North Carolina (Boiteau *et al.*, 1980), and 4–67% in Louisiana (Payah & Boethel, 1985). Thus, overwinter survival of *C. trifurcata* appears to be highly variable, and potentially lower in Ontario than in other areas of its range.

Contrary to expectations, the application of supplemental winter warming did not consistently improve *C. trifurcata* overwinter survival, but differed by year, likely due to complex interactions of temperature and snow cover. In the warmest winter (2012), supplemental warming decreased beetle survival, suggesting that *C. trifurcata* overwinter survival in Ontario could remain low under climate warming. There are a number of hypotheses for decreased beetle survival at warmer winter temperatures. First, higher metabolism under warmer winter temperatures could lead to higher rates of energy use and reduced survival (Irwin & Lee, 2003; Hahn & Denlinger, 2007; Sinclair, 2015). Our measurements of *C. trifurcata* lipid content in 2013 suggest that this was likely not the case: lipid content was relatively stable until March, suggesting that beetles do not begin depleting this energy reserve until early spring. Additionally, Boiteau et al. (1979), who visually classified the size of *C. trifurcata*'s internal fat body as lean, intermediate, or fat in dissected beetles, noted little depletion of fat reserves during diapause. Although *C. trifurcata* activity has been examined during and postdiapause (Boiteau *et al.*, 1979; Schumm *et al.*, 1983), little is known about what triggers natural diapause termination, which information would be helpful in predicting *C. trifurcata* responses to changing climate.

A second hypothesis for decreased survival at warmer winter temperatures is that exposure to warmer temperatures could result in the loss of cold tolerance, increasing susceptibility to subsequent cold snaps (Williams *et al.*, 2015). Increasing temperature is a primary cue for diapause termination in other temperate chrysomelid species (Lefevere & de Kort, 1989; Watanabe & Tanaka, 1998; Nahrung & Allen, 2004). There is likely a complex relationship between survival and temperature such that low temperatures contribute to energy savings for the beetle but cause chilling injuries to accumulate when temperatures fall below a threshold (Sinclair, 2015). Experiments using fluctuating thermal regimes would be required to address this hypothesis.

Third, increased variability in winter climate could cause greater variation in soil moisture content due to snowmelt, drying, and refreezing (Williams *et al.*, 2015), leading to unpredictable physiological responses and survival outcomes for insects. In the Colorado potato beetle (*Leptinotarsa decemlineata*), a temperate, freeze-intolerant chrysomelid in which adults overwinter in soil and experience high overwinter mortality, beetles have higher freezing points and mortality in moist than in dry substrate (Costanzo *et al.*, 1997). Monitoring changes in soil moisture and *C. trifurcata* water content among winter warming treatments would allow further examination of this hypothesis.

Increased temperatures and earlier spring emergence

Supplemental winter warming resulted in consistently earlier spring emergence of *C. trifurcata* across all years of study, ranging approximately 12–22 d earlier than in the control and snow removal groups but with similar degree-day accumulation. This advance in phenology of *C. trifurcata* could be beneficial or detrimental to survival, depending on climate variability in any given year. In years when late winter and early spring temperatures are highly variable, earlier emergence could increase the risk of mortality due to early reductions in the beetle's cold tolerance (Hayward *et al.*, 2005; Sobek-Swant *et al.*, 2012), increasing its vulnerability to subsequent cold spells (Bale & Hayward, 2010; Williams *et al.*, 2015). For example, in our field experiment, some *C. trifurcata* in the heated treatment group emerged in mid-March 2012 when air temperatures surpassed 20°C; these beetles were collected as soon as they emerged, but if they had remained outdoors, it is possible that the subsequent decrease in temperature to a low of 6°C for three weeks could have caused potentially fatal accumulation of chilling injuries.

Successful earlier emergence and survival of *C. trifurcata* would effectively increase the length of its growing season and allow greater accumulation of developmental degree-days (Ziter *et al.*, 2012; Berzitis *et al.*, 2014), which could allow the development of a second reproductive generation of *C. trifurcata*. The comparable dates of emergence of beetles from the heated group in this study (27 April on average) and beetles in Iowa at present (23 April and 4 May; Smelser & Pedigo, 1991), where there are two generations, support this possibility. However, phenological synchrony of *C. trifurcata* and its food resources will be important for subsequent population growth and pest status. Although *C. trifurcata* is native to North America and, prior to soybean emergence, feeds on wild native legumes, several herbaceous plant species, and leguminous crops such as alfalfa and sweet clover (Waldbauer & Kogan, 1976; Helm *et al.*, 1983; Hammack *et al.*, 2010), it is possible that those resources are not abundant enough to support development of large populations. For example, Jeffords et al. (1983) noted large *C. trifurcata* populations and subsequent pest problems in Illinois, USA in a year when its emergence was well synchronized with soybean emergence, but small early season *C. trifurcata* populations and few subsequent pest problems in a year when soybean planting was delayed because of wet spring weather.

There have been suggestions that later soybean planting dates might be useful for management of *C. trifurcata* by causing mortality of newly emerged overwintering beetle populations prior to soybean emergence due to a lack of food resources (Hammack *et al.*, 2010). However, in field studies in central Iowa and eastern Nebraska, USA that compare effects of early and late planting (usually early vs. late May) on *C. trifurcata* abundance and feeding damage as well as prevalence of *Bean pod mottle virus* in soybean, results differ among studies and are highly dependent on early spring and early fall weather conditions (Pedigo & Zeiss, 1996; Witkowski & Echtenkamp, 1996; Krell *et al.*, 2005). In Ontario, where appropriate soybean maturity groups range from group III in a small area of southwestern Ontario to group 00 elsewhere in southern Ontario (OSACC 2015), early soybean planting (early May) is a strategy used to maximize grain yield (Andrews 2011). In addition, hay (including alfalfa and other legumes) comprises the third largest crop area in Ontario after corn and soybean (OMAFRA 2015), which would present an abundant early spring food resource for *C. trifurcata*. Field observations also suggest that *C. trifurcata* migrates from senescing soybean fields late in the growing season to soybean fields that are not yet mature (Pedigo & Zeiss, 1996; Bradshaw *et al.*, 2008), indicating that late planted soybean fields could be at risk for *C. trifurcata* colonization if growers do not synchronize their planting efforts. Therefore, field studies in Ontario are required to determine how overwinter survival affects population development, and how management practices might be best adjusted to address *C. trifurcata* in combination with other weed and pest issues.

Supporting Information

Appendix S1. Further details of microclimate conditions under the leaf litter in experimental pots in the winter warming experiment, 2011-2013.

References

Andrews, B. (2011) How early is early for soybeans? Top Crop Manager, March 2011, print. [online] URL: http://www.agannex.com/field-crops/how-early-is-early-for-soybeans

Asch, M. van, Tienderen, P.H. van, Holleman, L.J.M. & Visser, M.E. (2007) Predicting adaptation of phenology in response to climate change, an insect herbivore example. *Global Change Biology*, **13**, 1596–1604.

Bale, J.S. & Hayward, S.A.L. (2010) Insect overwintering in a changing climate. *Journal of Experimental Biology*, **213**, 980–994.

Berzitis, E.A., Hunter, K., Sinclair, B.J., Hallett, R.H. & Newman, J.A. (2013) Winter warming effects on overwinter survival, energy use, and spring emergence of the bean leaf beetle *Cerotoma trifurcata* 2011 to 2013 [South-central Ontario, Canada].

Berzitis, E.A., Minigan, J.N., Hallett, R.H. & Newman, J.A. (2014) Climate and host plant availability impact the future distribution of the bean leaf beetle (*Cerotoma trifurcata*). *Global Change Biology*, **20**, 2778–2792.

Boiteau, G., Bradley, J.R. & Vanduyn, J.W. (1979) Bean leaf beetle: some seasonal anatomical changes and dormancy. *Annals of the Entomological Society of America*, **72**, 303–307.

Boiteau, G., Bradley, J. & Vanduyn, J. (1980) Bean leaf beetle (Coleoptera, Chrysomelidae): seasonal history of the overwintering population in eastern North Carolina. *Journal of the Georgia Entomological Society*, **15**, 138–151.

Bosch, J. & Kemp, W.P. (2003) Effect of wintering duration and temperature on survival and emergence time in males of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). *Environmental Entomology*, **32**, 711–716.

Bradshaw, J.D., Rice, M.E. & Hill, J.H. (2008) Evaluation of management strategies for bean leaf beetles (Coleoptera: Chrysomelidae) and bean pod mottle virus (Comoviridae) in soybean. *Journal of Economic Entomology*, **101**, 1211–1227.

Carrillo, M.A., Koch, R.L., Burkness, E.C., Bennett, K., Ragsdale, D.W. & Hutchison, W.D. (2005) Supercooling point of bean leaf beetle (Coleoptera: Chrysomelidae) in Minnesota and a revised predictive model for survival at low temperatures. *Environmental Entomology*, **34**, 1395–1401.

Chittenden, F.H. (1898) Insects injurious to beans and peas. In *Yearbook of the United States Department of Agriculture*. Government Printing Office, Washington, D.C., pp. 233–260.

Colinet, H., Renault, D., Hance, T. & Vernon, P. (2006) The impact of fluctuating thermal regimes on the survival of a cold-exposed parasitic wasp, *Aphidius colemani*. *Physiological Entomology*, **31**, 234–240.

Colinet, H., Sinclair, B.J., Vernon, P. & Renault, D. (2015) Insects in fluctuating thermal environments. *Annual Review of Entomology*, **60**, 123–140.

Costanzo, J.P., Moore, J.B., Lee, R.E., Kaufman, P.E. & Wyman, J.A. (1997) Influence of soil hydric parameters on the winter cold hardiness of a burrowing beetle, *Leptinotarsa decemlineata* (Say). *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology*, **167**, 169–176.

Danks, H.V. (2000) Dehydration in dormant insects. *Journal of Insect Physiology*, **46**, 837–852.

Gefen, E., Marlon, A.J. & Gibbs, A.G. (2006) Selection for desiccation resistance in adult *Drosophila melanogaster* affects larval development and metabolite accumulation. *Journal of Experimental Biology*, **209**, 3293–3300.

Giesler, L.J., Ghabrial, S.A., Hunt, T.E. & Hill, J.H. (2002) *Bean pod mottle virus:* a threat to U.S. soybean production. *Plant Disease*, **86**, 1280–1289.

Hahn, D.A. & Denlinger, D.L. (2007) Meeting the energetic demands of insect diapause: nutrient storage and utilization. *Journal of Insect Physiology*, **53**, 760–773.

Hahn, D.A. & Denlinger, D.L. (2011) Energetics of insect diapause. *Annual Review of Entomology*, **56**, 103–121.

Hammack, L., Pikul, J.L. & West, M.S. (2010) Phenology and abundance of bean leaf beetle (Coleoptera: Chrysomelidae) in eastern South Dakota on alfalfa and soybean relative to tillage, fertilization, and yield. *Environmental Entomology*, **39**, 727–737.

Hayward, S.A.L., Pavlides, S.C., Tammariello, S.P., Rinehart, J.P. & Denlinger, D.L. (2005) Temporal expression patterns of diapause-associated genes in flesh fly pupae from the onset of diapause through post-diapause quiescence. *Journal of Insect Physiology*, **51**, 631–640.

Helm, C.G., Jeffords, M.R., Post, S.L. & Kogan, M. (1983) Spring feeding activity of overwintered bean leaf beetles (Coleoptera, Chrysomelidae) on nonleguminous hosts. *Environmental Entomology*, **12**, 321–322.

IPCC (Intergovernmental Panel on Climate Change). (2013) *Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge.

Irwin, J.T. & Lee, R.E. (2003) Cold winter microenvironments conserve energy and improve overwintering survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis*. *Oikos*, **100**, 71–78.

Jeffords, M.R., Helm, C.G. & Kogan, M. (1983) Overwintering behavior and spring colonization of soybean by the bean leaf beetle (Coleoptera, Chrysomelidae) in Illinois. *Environmental Entomology*, **12**, 1459–1463.

Joshi, A., Olson, D.L. & Carey, D.R. (2009) Overwintering survival of *Aphthona beetles* (Coleoptera: Chrysomelidae): a biological control agent of leafy spurge released in North Dakota. *Environmental Entomology*, **38**, 1539–1545.

Kingsolver, J.G., Higgins, J.K. & Augustine, K.E. (2015) Fluctuating temperatures and ectotherm growth: distinguishing non-linear and time-dependent effects. *Journal of Experimental Biology*, **218**, 2218–2225.

Krell, R.K., Pedigo, L.P. & Babcock, B.A. (2003) Comparison of estimated costs and benefits of site-specific versus uniform management for the bean leaf beetle in soybean. *Precision Agriculture*, **4**, 401–411.

Krell, R.K., Pedigo, L.P., Rice, M.E., Westgate, M.E. & Hill, J.H. (2005) Using planting date to manage bean pod mottle virus in soybean. *Crop Protection*, **24**, 909–914.

Lam, W.-K.F. & Pedigo, L.P. (2000) A predictive model for the survival of overwintering bean leaf beetles (Coleoptera: Chrysomelidae). *Environmental Entomology*, **29**, 800–806.

Lam, W.-K.F., Pedigo, L.P. & Hinz, P.N. (2002) Spatial distribution and sequential count plans for overwintering bean leaf beetles (Coleoptera: Chrysomelidae). *Journal of Agricultural and Urban Entomology*, **19**, 73–84.

Lawrence, D.M. & Slater, A.G. (2010) The contribution of snow condition trends to future ground climate. *Climate Dynamics*, **34**, 969–981.

Lefevere, K.S. & Kort, C.A.D. de. (1989) Adult diapause in the Colorado potato beetle, *Lepinotarsa decemlineata:* effects of external factors on maintenance, termination and post-diapause development. *Physiological Entomology*, **14**, 299–308.

Lundgren, J.G. & Riedell, W.E. (2008) Soybean nitrogen relations and root characteristics after *Cerotoma trifurcata* (Coleoptera: Chrysomelidae) larval feeding injury. *Journal of Entomological Science*, **43**, 107–116.

Marshall, K.E. & Sinclair, B.J. (2011) The sub-lethal effects of repeated freezing in the woolly bear caterpillar *Pyrrharctia isabella*. *Journal of Experimental Biology*, **214**, 1205–1212.

McCreary, C. (2013) *Development of a degree day model and economic thresholds for* Cerotoma trifurcata *(Coleoptera: Chrysomelidae).* (MSc thesis).

Nahrung, H.F. & Allen, G.R. (2004) Overwintering ecology of *Chrysophtharta agricola:* mechanisms of reproductive diapause induction and termination. *Australian Journal of Zoology*, **52**, 505–520.

OMAFRA (Ontario Ministry of Agriculture, Food, and Rural Affairs). (2015) *Estimated area, yield, production and farm value of specified field crops, Ontario, 2011-2015.* Ontario Ministry of Agriculture, Food, and Rural Affairs, Guelph, Ontario. [online] URL: http://www.omafra.gov.on.ca/english/stats/crops/estimate_new.htm#metric

OSACC (Ontario Soybean and Canola Committee). (2015) *Ontario soybean maturity conversions.* Ontario Soybean and Canola Committee, Ontario. [online] URL: http://www.gosoy.ca/mat_groups.php

Payah, W. & Boethel, D. (1985) Overwintering bean leaf beetles, *Cerotoma trifurcata* (Forster) (Coleoptera, Chrysomelidae): density of populations, temporal patterns. *Environmental Entomology*, **14**, 65–73.

Payah, W. & Boethel, D. (1986) Impact of *Beauveria bassiana* (Balsamo) Vuillemin on survival of overwintering bean leaf beetles, *Cerotoma trifurcata* (Forster), (Coleoptera, Chrysomelidae). *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie*, **102**, 295–303.

Pedigo, L.P. (1994) Bean leaf beetle. In *Handbook of soybean insect pests* (ed. by Higley, L.G. & Boethel, D.J.). Entomological Society of America, Lanham, Maryland, pp. 42–44.

Pedigo, L.P. & Zeiss, M.R. (1996) Effect of soybean planting date on bean leaf beetle (Coleoptera: Chrysomelidae) abundance and pod injury. *Journal of Economic Entomology*, **89**, 183–188.

Pozsgai, G. & Littlewood, N.A. (2011) Changes in the phenology of the ground beetle *Pterostichus madidus*. *Insect Science*, **18**, 462–472.

Renault, D., Nedved, O., Hervant, F. & Vernon, P. (2004) The importance of fluctuating thermal regimes for repairing chill injuries in the tropical beetle *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) during exposure to low temperature. *Physiological Entomology*, **29**, 139–145.

Schumm, M., Stinner, R.E. & Bradley, J.R. (1983) Characteristics of diapause in the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera, Chrysomelidae). *Environmental Entomology*, **12**, 475–477.

Shortt, B.J., Sinclair, J.B., Helm, C.G., Jeffords, M.R. & Kogan, M. (1982) Soybean seed quality losses associated with bean leaf beetles and *Alternaria tenuissima*. *Phytopathology*, **72**, 615–618.

Sinclair, B.J. (2015) Linking energetics and overwintering in temperate insects. *Journal of Thermal Biology*, **in press**.

Sinclair, B.J., Ferguson, L.V., Salehipour-shirazi, G. & MacMillan, H.A. (2013) Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. *Integrative and Comparative Biology*, **53**, 545–556.

Smelser, R.B. & Pedigo, L.P. (1992) Soybean seed yield and quality reduction by bean leaf beetle (Coleoptera, Chrysomelidae) pod injury. *Journal of Economic Entomology*, **85**, 2399–2403.

Smelser, R. & Pedigo, L. (1991) Phenology of *Cerotoma trifurcata* on soybean and alfalfa in central Iowa. *Environmental Entomology*, **20**, 514–519.

Sobek-Swant, S., Crosthwaite, J.C., Lyons, D.B. & Sinclair, B.J. (2012) Could phenotypic plasticity limit an invasive species? Incomplete reversibility of mid-winter deacclimation in emerald ash borer. *Biological Invasions*, **14**, 115–125.

Waldbauer, G.P. & Kogan, M. (1976) Bean leaf beetle Coleoptera, Chrysomelidae: phenologial relationship with soybean in Illinois. *Environmental Entomology*, **5**, 35–44.

Watanabe, M. & Tanaka, K. (1998) Adult diapause and cold hardiness in *Aulacophora nigripennis* (Coleoptera: Chrysomelidae). *Journal of Insect Physiology*, **44**, 1103–1110.

Williams, C.M., Henry, H.A.L. & Sinclair, B.J. (2015) Cold truths: how winter drives responses of terrestrial organisms to climate change. *Biological Reviews*, **90**, 214–235.

Williams, C.M., Thomas, R.H., MacMillan, H.A., Marshall, K.E. & Sinclair, B.J. (2011) Triacylglyceride measurement in small quantities of homogenised insect tissue: comparisons and caveats. *Journal of Insect Physiology*, **57**, 1602–1613.

Williams, J.B., Shorthouse, J.D. & Lee, R.E. (2003) Deleterious effects of mild simulated overwintering temperatures on survival and potential fecundity of rose-galling *Diplolepis* wasps (Hymenoptera: Cynipidae). *Journal of Experimental Zoology Part A-Ecological Genetics and Physiology*, **298**, 23–31.

Witkowski, J.F. & Echtenkamp, G.W. (1996) Influence of planting date and insecticide on the bean leaf beetle (Coleoptera: Chrysomelidae) abundance and damage in Nebraska soybean. *Journal of Economic Entomology*, **89**, 189–196.

Ziter, C., Robinson, E.A. & Newman, J.A. (2012) Climate change and voltinism in Californian insect pest species: sensitivity to location, scenario and climate model choice. *Global Change Biology*, **18**, 2771–2780.

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Table 1. ANCOVA results for effects of warming treatment, sampling date, and beetle sex on Box-Cox transformed beetle lipid (triacylglycerol) content.

Source	df[a]	F ratio	P value
Protein	1	0.79	0.40
Treatment	1	0.34	0.56
Date	2	897.6	< 0.001
Sex	1	0.19	0.67
Treatment × Date	2	1.07	0.35
Treatment × Sex	1	0.57	0.45
Date × Sex	2	0.12	0.89
Treatment × Date × Sex	2	2.55	0.09

[a] Error df = 38.

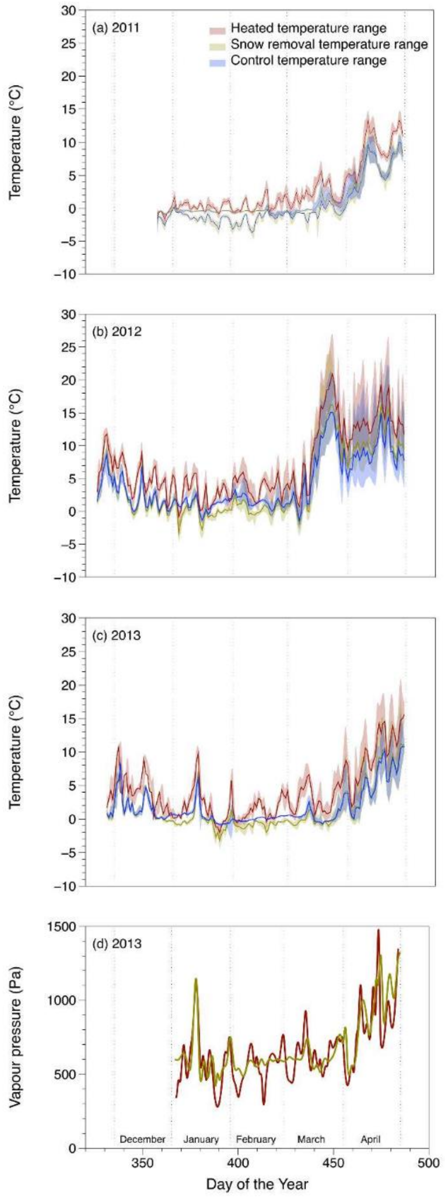
Figure captions

Figure 1. Mean and range of daily minimum and maximum temperatures (°C) recorded under the leaf litter in the heated, control, and snow removal treatment groups over the first four months of the year for 2011 (a), 2012 (b), and 2013 (c), and (d) mean daily vapour pressure (Pa) recorded under the leaf litter in one set of replicates from the heated and control treatment groups in 2013. Dates <365 indicate early winter in the previous year. Solid lines indicate means; thicker, semi-transparent shading indicates the difference between minimum and maximum temperatures in (a–c).

Figure 2. Proportion of *Cerotoma trifurcata* surviving the winter in heated, control, and snow removal treatment groups in 2011, 2012, and 2013 (mean ± SE). Means and SEs are back-transformed from generalized linear mixed models using the inverse link function and the delta method. Different lowercase letters indicate significant differences within years (Tukey-Kramer, α =0.05).

Figure 3. Triacylglycerol content (mg/mg soluble protein content) of *Cerotoma trifurcata* in November 2012 (prior to overwintering) and from heated and control groups in January, March, and at spring emergence in April or May 2013 (mean ± SE). Different lowercase letters indicate significant differences (Tukey's HSD, α = 0.05); numbers indicate sample sizes.

Figure 4. Date of first emergence of *Cerotoma trifurcata* from heated, control, and snow removal treatment groups in 2011, 2012, and 2013 (mean ± SE). Different lowercase letters indicate significant differences within years (Tukey's HSD, α = 0.05).



Mean and range of daily minimum and maximum temperatures (°C) recorded under the leaf litter in the heated, control, and snow removal treatment groups over the first four months of the year for 2011 (a), 2012 (b), and 2013 (c), and (d) mean daily vapour pressure (Pa) recorded under the leaf litter in one set of replicates from the heated and control treatment groups in 2013. Dates <365 indicate early winter in the previous year. Solid lines indicate means; thicker, semi-transparent shading indicates the difference between minimum and maximum temperatures in (a–c).
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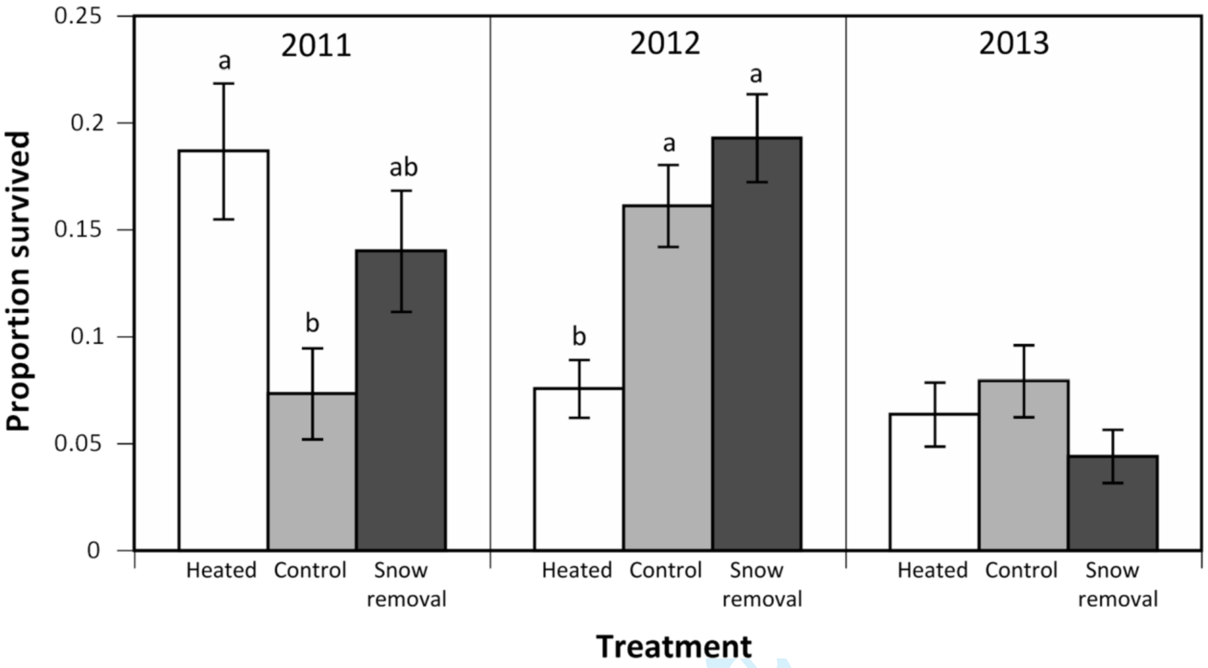

Fig.2 – Berzitis et al.

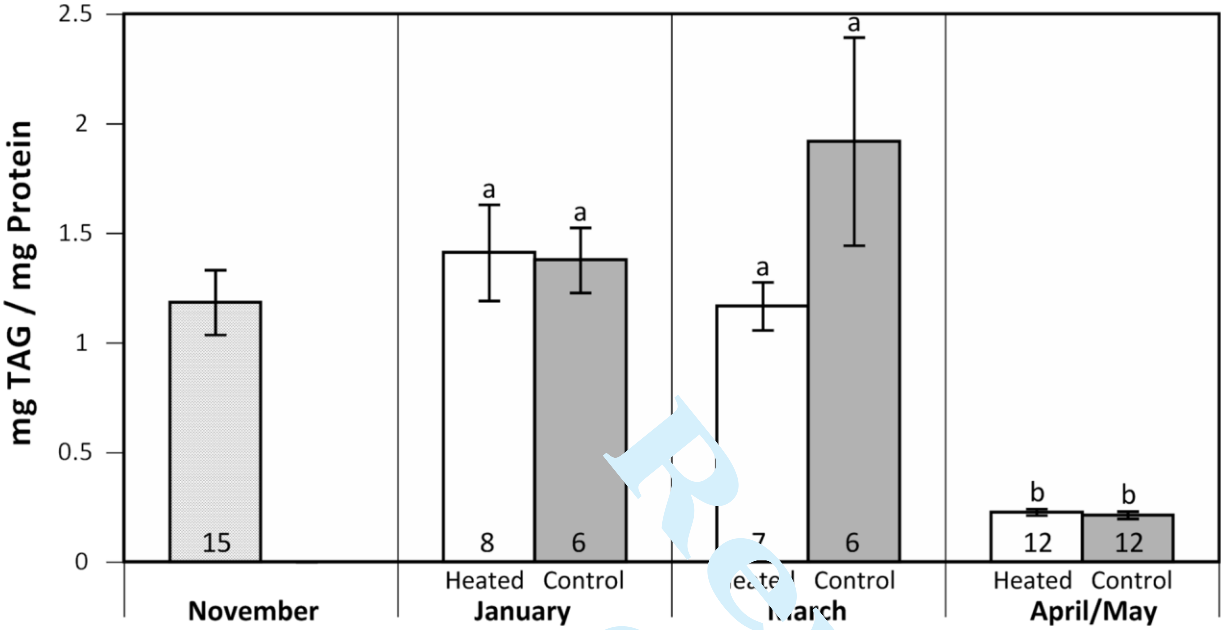

Fig.3 – Berzitis et al.

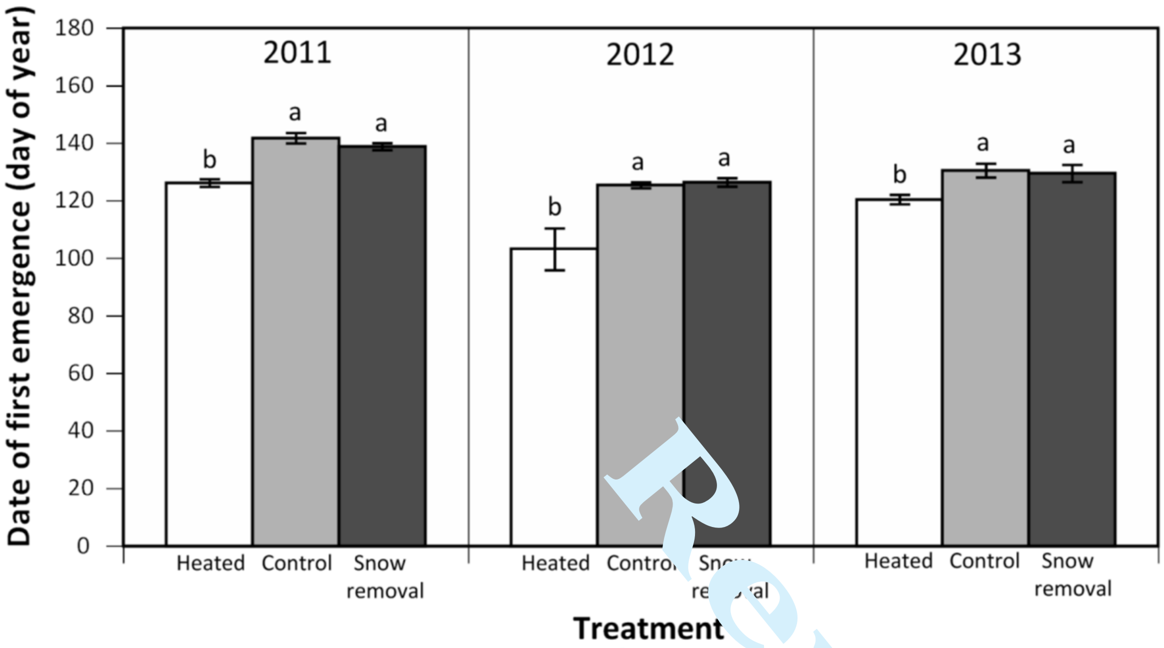

Fig.4 – Berzitis et al.