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The overwintering biology of the acorn weevil, *Curculio glandium* in southwestern Ontario.

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1 The overwintering biology of the acorn weevil, *Curculio glandium* in southwestern
2 Ontario

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15

16 **ABSTRACT**

17 The acorn weevil, *Curculio glandium*, is a widespread predator of acorns in eastern
18 North America that overwinters in the soil as a larva. It is possible that low
19 temperatures limit its northern geographic range, so we determined the cold tolerance
20 strategy, seasonal variation in cold tolerance, and explored the physiological plasticity
21 of overwintering larvae. Weevil larvae were collected from acorns of red and bur oak
22 from Pelee Island, southwestern Ontario in fall 2010 and 2011. *Curculio glandium*
23 larvae are freeze avoidant and larvae collected from bur oak acorns had lower
24 supercooling points (SCPs: -7.6 ± 0.36 °C, LT₅₀: -7.2 °C) than those collected from red
25 oak acorns (SCPs: -6.1 ± 0.40 °C, LT₅₀: -6.1 °C). In the winter of 2010-2011, SCPs and
26 water content decreased, however these changes did not occur in 2011-12, when winter
27 soil temperatures fluctuated greatly in the absence of the buffering effect of snow. To
28 examine whether larvae utilize cryoprotective dehydration, larvae from red oak acorns
29 were exposed to -5 °C in the presence of ice for seven days. These conditions decreased
30 the SCP without affecting water content, suggesting that SCP and water content are not
31 directly coupled. Finally, long-term acclimation at 0 °C for six weeks slightly increased
32 cold tolerance but also did not affect water content. Thus, although larval diet affects
33 cold tolerance, there is limited plasticity after other treatments. The soil temperatures
34 we observed were not close to lethal limits, although we speculate that soil temperatures
35 in northerly habitats, or in years of reduced snow cover, has the potential to cause
36 mortality in the field.

37

38 Keywords: Curculionidae, freeze avoidance, supercooling point, acclimation,
39 cryoprotective dehydration

40

41 **Introduction**

42 Because insects are ectotherms, and winter cold affects survival and
43 reproduction, it is commonly predicted that warmer winters might lead to poleward
44 expansion of temperate insects (Danks, 1991; Denlinger and Lee, 2010; Semel and
45 Andersen, 1988). Insect cold tolerance is generally divided into freeze avoidance
46 (insects that are killed by freezing) and freeze tolerance (insects that can withstand
47 internal ice formation) (Denlinger and Lee, 2010). Because freeze-avoidant species
48 cannot survive when ice forms in their bodies, their supercooling points (SCP, the
49 temperature at which freezing occurs) equates to their lower lethal temperature (LLT).
50 Freeze avoiding insects often seasonally depress the SCP to ensure they remain
51 unfrozen. For example the SCP of prepupae of the emerald ash borer, *Agrilus*
52 *planipennis*, is about -20 °C in October and decreases to -30 °C from December to
53 February (Crosthwaite et al., 2011). The SCP can be decreased by various
54 physiological means, including dehydration and accumulation of colligative
55 cryoprotectants, such as glycerol (Chown and Nicolson, 2004). By contrast,
56 freeze-tolerant species can survive the freezing of their body fluids, although most of
57 them do not tolerate intracellular ice formation (Sinclair and Renault, 2010). The
58 SCPs of freeze-tolerant insects are higher than their LLTs and SCP does not usually
59 decrease substantially in winter. Nevertheless, many cryoprotectant mechanisms may
60 be shared between freeze tolerance and avoidance, including polyols and antifreeze
61 proteins (Denlinger and Lee, 2010).

62 The temperature conditions in overwintering microhabitats affect both the
63 survival and fitness of insects. In some species, warm winter temperatures decrease
64 fitness by increasing consumption of energy reserves (e.g., Irwin and Lee, 2002;

65 Marshall and Sinclair, 2012; Williams et al., 2012). By contrast, other species are
66 killed by low winter temperatures (e.g., Roland and Matter, 2013). Soil is a common
67 overwintering site for temperate insects (Danks, 1991) and buffers the extremes of
68 overwinter temperatures, especially in the presence of snow cover (Marshall and
69 Sinclair, 2012). The high moisture content of soil has facilitated the development of a
70 cryoprotective dehydration strategy to enhance cold tolerance in permeable
71 invertebrates overwintering in the soil (Holmstrup et al., 2002). In frozen soil, these
72 organisms lose water to the lower energy-state ice, dehydrating them and increasing the
73 concentration of their body fluids such that they remain unfreezeable (Pedersen and
74 Holmstrup, 2003). For example, the Antarctic midge *Belgica antarctica* decreases the
75 melting point of its body fluid to about -3 °C when exposed to ice (Elnitsky et al., 2008).
76 In theory, cryoprotective dehydration should be confined to permeable insects
77 (Holmstrup et al. 2002). However, insects can rapidly change their cuticular
78 permeability (e.g. Bazinet et al. 2011), dehydration is associated with cold tolerance in
79 coleopteran larvae that might be expected to resist dehydration (e.g. Sformo paper), and
80 has not been well-explored in temperate holometabolous insects, so it is possible that
81 other insects that overwinter in the soil may utilize this strategy.

82 Acorn weevils of the genus *Curculio* damage mature acorns with potential
83 impacts on the fitness of oak trees in north America and Europe (Gibson, 1982; Semel
84 and Andersen, 1988). Female weevils lay eggs in young acorns and larvae feed and
85 develop within the acorns. After the ripe acorns fall to the ground, fully developed
86 larvae cease feeding and burrow into the soil to overwinter (Pélisson et al., 2012;
87 Venner et al., 2011). Some *C. glandium* larvae in southeastern France might
88 experience two winters in the soil by developing to the adult stage (Venner et al., 2011)

89 but the overwintering stage for the second year in other populations, including in North
90 America, is not clear. Although there is no apparent preference for oak species,
91 (Crawley and Long, 1995; Espelta et al., 2009; Pélişson et al., 2012), the balance of
92 tannin, protein and fat in acorns varies among oak species (Shimada and Saitoh, 2006).
93 The quality of diet influences cold tolerance in insects and spiders (Kořtál et al., 2012;
94 Tanaka, 1994; Worland and Lukeřova, 2000), but it is not clear whether there is a
95 relationship between nutrition of acorns and weevil cold hardiness.

96 We investigated the overwintering biology of *C. glandium* overwintering in the
97 soil in a temperate North American location: Pelee Island, southwestern Ontario,
98 Canada. To determine the cold tolerance strategy, we measured lower thermal limits
99 and water balance during winter and examined the contribution of dehydration and
100 exposure to survival during a long-term mild cold temperature. We also compared the
101 overwinter biology of *C. glandium* larvae collected from acorns of different *Quercus*
102 species. This is the first study of overwintering in this species, and one of only a few
103 that examines holometabolous insects that overwinter in the soil.

104

105 **Material and methods**

106 *Insect collection and laboratory maintenance*

107 To obtain *Curculio glandium* larvae, fallen acorns from bur oak, *Quercus*
108 *macrocarpa*, and red oak, *Quercus rubra*, were collected in late September and early
109 October from deciduous forests on Pelee Island, Ontario, Canada (41°77'N, 82°71'W)
110 in 2010 (both species) and 2011 (red oak only). To collect weevils, acorns (separated
111 by oak species) were placed on a plastic screen with 2 cm × 2 cm grids over c. 10 cm of
112 moist soil in a plastic bin at 4 °C. Larvae emerging from the acorns fell through the

113 screen and burrowed into the soil, which was riddled daily to collect larvae. Larvae
114 were transferred to 120 ml plastic jars containing 100 ml soil (30-50 individuals per jar)
115 and stored at approximately 4 °C until use in experiments. Immediately prior to use in
116 experiments, larvae were removed from soil and blotted on a paper towel to remove soil
117 and excess water from the surface.

118

119 *Supercooling point*

120 To measure the SCP, larvae were placed in 1.7 ml microcentrifuge tubes in
121 contact with 36-AWG type-T copper-constantan thermocouples (Omega, Laval, Quebec,
122 Canada). Thermocouples were connected to a Picotech TC-08 thermocouple interface
123 and PicoLog software (Pico Technology, Cambridge, UK). The tubes containing weevils
124 were inserted into wells in an aluminium block that was cooled by methanol circulated
125 from a Lauda Proline 3530C refrigerated bath (Lauda, Wurzburg, Germany). The
126 weevils were allowed 30 min to equilibrate at 0 °C, and then cooled from 0 °C to -30 °C
127 at 0.1 °C min⁻¹. SCP was determined as the lowest temperature before the exotherm
128 was observed. After measuring the SCP, the water content of each larva was measured
129 gravimetrically as described below.

130

131 *Water content*

132 After assessing survival or measuring SCP, the larvae were weighed (wet mass)
133 and placed in 1.5 ml tubes. To speed drying, they were pierced ventrally with a needle.
134 The larvae were dried at 70 °C for at least five days, and then reweighed (dry mass).
135 The water content was calculated as the difference between fresh and dry mass of each
136 individual.

137

138 *Acute cold tolerance*

139 To examine survival after a short cold exposure, weevils from red and bur oak
140 acorns collected in 2010 were exposed to seven temperatures (0, -3, -6, -7, -9, -12 and
141 -15 °C) and those from red oak acorns collected in 2011 were exposed to six
142 temperatures (-2, -4, -6, -8, -10 and -12 °C). Eight to 20 individuals were used at each
143 test temperature. A weevil was put in a 1.7 ml microcentrifuge tube and the tubes were
144 cooled from 0°C at 0.1°C min⁻¹ to the chosen temperature, held at that temperature for
145 30 min, and then warmed to 4 °C min at 0.1°C min⁻¹. After rewarming, larvae were
146 kept at room temperature for 15 min and survival was assessed in this experiment.
147 Preliminary studies showed that survival rates estimated 15 min and 24 h after
148 rewarming were identical. Weevils were considered alive if they crawled voluntarily.
149 The median lower lethal temperature (LT₅₀), the temperature that caused 50 % mortality
150 was estimated with logistic regression in R v 3.0.3 (R Core Team, 2013).

151

152 *Overwintering in the field site*

153 For overwintering in the field, thirty larvae were placed with 100 ml sandy
154 loam soil in a 100 ml plastic jar (5.5 cm diameter, 6.5 cm depth) pierced with small
155 holes at the top and bottom. Three jars from each of red or bur oak acorns collected in
156 2010 and seven jars containing larvae from red oak acorns collected 2011 were placed
157 in the field. Jars containing weevil larvae were buried at 5 cm depth in tilled soil in
158 London, Ontario, Canada (43°00'N, 81°15'W) on 18 December 2010, and 12 November
159 2011. This depth was chosen because Semel and Anderson (1988) showed that most
160 acorn weevil larvae burrowed up to 21 cm and about 30 % of larvae was in a depth in

161 less than 4 cm. Larvae were collected through the winter every two or four weeks and
162 SCP and water content measured.

163

164 *Microclimate temperatures*

165 Temperature data were collected at two sites: the acorn collection site (Pelee
166 Island, ON) and the tilled soil site where weevils were buried to study seasonal changes
167 in cold tolerance (London, ON). iButton Thermochron DS1922L data loggers (Maxim
168 Integrated Products, Sunnyvale, CA, USA) were buried approximately 5 cm depth at the
169 same location as the sites collected acorns on Pelee island or included in a jar containing
170 larvae (see above) in London. Temperatures on Pelee Island and the London site were
171 recorded every 60 and 30 min, respectively.

172

173 *The effect of cryoprotective dehydration on SCP and water content*

174 To assess if *Curculio glandium* larvae utilize cryoprotective dehydration to
175 enhance their cold tolerance, larvae collected from red oak acorns in 2011 were placed
176 in 1.7 ml microcentrifuge tube with holes at the top. A group of five tubes, each
177 containing a larva, was put in a 120 ml Parafilm-sealed jar containing approximately 80
178 ml of crushed ice. A total of eight jars containing larvae and ice were placed in an
179 environmental test chamber (Tenney ETCU 16-RCW2.5, Thermal Product Solutions,
180 New Columbia, PA, USA) at 0 °C for one day, and cooled at 1 °C/day to -5 °C, where
181 they were held for seven days. To measure SCPs and water contents, four jars were
182 removed from the chamber on the first day that temperature reached at -5 °C and on the
183 last day of cold exposure. Under the experimental conditions, it was expected that
184 larvae were exposed to desiccation caused by the vapour pressure difference between

185 ice and their supercooled body water (Holmstrup and Sømme, 1998; Irwin and Lee,
186 2002; Lundheim and Zachariassen, 1993).

187

188 *The effect of acclimation on cold tolerance*

189 To study the effect of long-term cold exposure on *C. glandium* larvae,
190 individuals from red oak acorns collected in 2011 were kept at 0 °C for 2, 4 and 6 weeks.
191 Larvae were placed individually in 1.7 ml microcentrifuge tubes and five tubes with
192 larvae were contained in a 50 ml plastic jar. To expose larvae to 0 °C, jars were buried
193 in a slurry of crushed ice and water, and placed in a Styrofoam box inside a
194 SanyoMR-153 incubator (Sanyo Scientific, Bensenville, IL, US) set at 5 °C. Ice was
195 changed or added at least once a week and an iButton data logger was placed in the box
196 to confirm the temperature. The SCPs and water contents of 8-21 larvae per time point
197 were measured as described above.

198

199 *Statistical analysis*

200 Supercooling points were natural-log-transformed prior to further analysis.
201 The SCPs, body mass and dry mass of larvae kept at 4 °C were compared among three
202 groups, which larvae from bur and red oaks collected in 2010 and larvae from red oak
203 collected in 2011, using one-way ANOVA followed by Tukey's *post hoc* test (SigmaPlot
204 v10; Systat Software, Inc., Chicago, IL, USA). Differences in water content were
205 investigated using analysis of covariance (ANCOVA) of water mass with wet mass as a
206 covariate followed by Tukey's *post hoc* test in SPSS (v. 20; IBM, NY, UAS).
207 Supercooling points, body mass and dry mass of larvae from field-acclimated larvae
208 were separately compared among sampling times within a winter or species of oak trees

209 using one-way ANOVA followed by Tukey's *post hoc* test (SigmaPlot) and to compare
210 water content among sampling times ANCOVA followed by Tukey's *post hoc* test
211 (SPSS) was performed. Pearson's product-moment correlation was calculated between
212 SCP and water content in field-acclimated larvae (across all years) in SigmaPlot.
213 One-way ANOVA followed by Tukey's *post hoc* test (SigmaPlot) was used to compare
214 SCPs, body mass and dry mass among control and treated groups in the cryoprotective
215 dehydration and acclimation experiments and ANCOVA followed by Tukey's *post hoc*
216 test (SPSS) was used for comparison of water contents.

217

218 **Results**

219 *Cold tolerance strategy and acute cold tolerance*

220 Larvae were exposed to six or seven test temperatures for 30 min to determine
221 LT_{50} . The LT_{50} of larvae collected in 2010 was $-6.1\text{ }^{\circ}\text{C}$ and $-7.2\text{ }^{\circ}\text{C}$ for larvae from red
222 oak and bur oak acorns, respectively. The LT_{50} of larvae collected in 2011 from red
223 oak was $-6.5\text{ }^{\circ}\text{C}$. No larvae survived internal ice formation regardless of oak tree
224 species or sampling year and all unfrozen larvae survived cold exposure ($N = 16$).

225 The SCPs of larvae from bur oak acorns were significantly lower than SCPs of
226 larvae from red oak acorns in 2010 (Fig. 1A, $F_{2, 140} = 6.810$, $P < 0.001$). Larvae
227 collected from red oak acorns in 2011 had lower SCPs than larvae collected in 2010 (P
228 < 0.001). Larvae collected from bur oak acorns had significantly higher water contents
229 than those collected from red oak acorns in 2010 (Fig. 1B, $F_{2, 139} = 11.183$, $P < 0.001$).
230 The water contents of larvae from red oak acorns in 2010 were significantly lower than
231 those of larvae collected in 2011 ($P < 0.001$). There was no significant difference in
232 wet (Fig. 1C, $F_{2, 140} = 1.62$, $P = 0.201$) or dry ($F_{2, 140} = 0.80$, $P = 0.452$) mass among

233 larvae collected in different years or from different species of acorns.

234

235 *Microclimate temperatures*

236 Microclimate temperatures did not drop below -2 °C during the winters of
237 2010-2011 or 2011-2012 at the sites where weevil larvae were buried and acorns
238 containing larvae collected (Fig. 2). At both sites, temperatures remained between 0 and
239 +2 °C from late December 2010 to early March 2011. The Pelee Island site in
240 2011-2012 was slightly warmer; the temperature remained above 1 °C all winter (Figs
241 2B, 2D).

242

243 *Seasonal changes in cold tolerance and water content*

244 Seasonal changes in cold tolerance and water content of weevils buried in the
245 field varied among species of oak and years (Fig. 3). Larvae from bur oak acorns
246 collected in 2010 had significantly lower SCPs in January and February than in March
247 (Fig. 3A, $F_{2,39} = 12.69$, $P < 0.001$). In larvae from red oak acorns collected in 2010,
248 the mean SCP in January was significantly lower than that in March ($F_{2,34} = 13.82$, $P <$
249 0.001), but the SCP increased to -7 °C in February. However, there was no significant
250 difference in SCPs of larvae from red oak acorn among sampling periods in 2011 ($F_{6,76}$
251 $= 1.831$, $P = 0.104$).

252 In larvae collected in 2010, water content showed a pattern similar to SCP (Fig.
253 3B). In larvae from bur oak acorn, water content was about 0.45 mg H₂O/mg dry mass
254 in January and February and significantly increased in March ($F_{2,36} = 25.37$, $P < 0.001$).
255 January water content of larvae from red oak acorns collected in 2010 was significantly
256 lower than that of larvae in February and March ($F_{2,33} = 14.82$, $P < 0.001$). Weevil

257 larvae collected in 2011 did not show a decrease in water content; in fact water content
258 gradually increased through the experimental period (Fig 3B). There was a significant
259 positive correlation between SCPs and water content in weevil larvae buried in the field
260 (Fig. 4, $r_s = 0.68$, $P < 0.001$).

261

262 *Cryoprotective dehydration*

263 To determine whether acorn weevil larvae utilize cryoprotective dehydration to
264 increase cold hardiness, larvae from red oak acorns in 2011 were exposed to -5 °C at
265 high vapour pressure deficit for seven days. The SCPs were significantly decreased by
266 the exposure to low temperature and high vapor presser conditions (Fig. 5B, $F_{2,81} =$
267 4.171, $P = 0.019$) and larvae after seven days exposure to -5 °C had significantly lower
268 SCPs than those maintained at 4 °C. Water contents were significantly different among
269 control and treated groups (Fig 5C, $F_{2,80} = 9.816$, $P < 0.001$). The water contents on
270 the first day the temperature reached -5 °C were significantly lower than those of control
271 larvae, but the water contents of larvae after seven days exposure to -5 °C did not differ
272 significantly from those of control larvae. Neither fresh nor dry body mass differed
273 among control and groups exposed to low temperature (wet mass: $F_{2,81} = 0.670$, $P =$
274 0.52; dry mass: $F_{2,81} = 2.76$, $P = 0.07$; Fig. 5D).

275

276 *The effect of long-term acclimation on cold hardiness*

277 Weevil larvae obtained from red oak acorns collected in 2011 were acclimated
278 at 0 °C for 6 weeks. Exposure to 0 °C for two and four weeks decreased the mean SCP
279 from -8.6 ± 0.37 °C (mean \pm S.E.) to -11.3 ± 0.87 °C and to -11.5 ± 0.78 (Fig. 6A;
280 $F_{3,106} = 4.957$, $P = 0.003$) but after additional cold exposure the SCP of [who?] did not

281 differ significantly from control larvae. Water content of larvae kept at 0 °C for six
282 weeks was significantly compared to control and larvae kept at 0 °C for two and four
283 weeks (Fig. 6B, $F_{3,88} = 9.285$, $p < 0.001$). Neither wet nor dry body mass differed
284 significantly among groups (Fig. 6C, wet mass, $F_{3,105} = 2.267$, $p = 0.085$; dry mass,
285 $F_{3,105} = 0.932$, $p = 0.428$).

286

287 **Discussion**

288 *Curculio glandium* larvae survived subzero temperature exposure for several
289 days and were killed by internal ice formation irrespective of oak species and sampling
290 years, suggesting that acorn weevil larvae are freeze-avoidant. Both freeze-tolerant
291 and -avoidant species have been reported in Curculionidae species overwintering as
292 adults (Coulson and Bale, 1996; Kandori et al., 2006; Košťál and Šimek, 1996; van der
293 Merwe et al., 1997). However, all species studied thus far that overwinter as larvae are
294 freeze avoidant, including *C. glandium* in this study (Coyle et al., 2011; Watanabe and
295 Tanaka, 1997). The larvae of *Phullobius oblongus*, *Polydrusus sericeus*, *Barypeithes*
296 *pellucidus* are also freeze avoidant, and their SCPs are between -9 and -13 °C in winter
297 (Coyle et al., 2011). Thus the SCPs of *C. glandium* larvae are not especially low
298 compared to other freeze avoidant weevil larvae. Microclimate data showed that
299 weevil larvae are not likely to be exposed to low sub-zero temperatures, because they
300 are protected from freezing in their buffered overwintering site. Larvae may be even
301 better-protected from low temperatures than our data show, because our data loggers
302 were, at 5 cm depth, probably near the upper limit of the range of overwintering depths
303 (Semel and Andersen, 1988). Thus, larvae may temperature and weevil larvae that
304 stayed closed to the bottom of containers were likely exposed to slightly higher

305 temperatures than larvae that stayed at the top of the jar.

306 Most freeze-avoidant insects decrease their SCP to survive low winter
307 temperatures in winter (Denlinger and Lee, 2010). Clear seasonal changes of SCPs
308 were observed during the winter of 2010-2011, although snow cover kept the 5 cm
309 depth soil temperature at about 0.5 °C. The SCP also decreased in larvae acclimated at
310 0 °C for four weeks, although the SCP did not appear to decrease in individuals kept at 0
311 °C for six weeks. Additionally, seven days' exposure to -5 °C induced lower SCPs
312 than 4 °C when larvae were cooled to -5 °C gradually. In many insect species, survival
313 rates are increased by acclimation to low temperatures (ref), and it appears that the cold
314 tolerance of acorn weevil larvae is enhanced by exposure to both prolonged mild and
315 short severe temperatures. However, SCPs of weevil larvae from red oak did not
316 decline during the winter in 2011 - 2012 and the temperatures in overwintering site
317 fluctuated more in 2011-2012 than in 2010 - 2011. These variations of cold tolerance
318 in larvae in the soil may result from the different microclimate conditions to which
319 larvae were exposed during winter, perhaps driven by variation in both air temperature
320 and snow cover. This variation in acclimation means that winters with reduced snow
321 cover (or extreme low temperatures) that lead to enhanced frost penetration could kill
322 weevil larvae if prior conditions have not been favourable for acclimation. Moreover,
323 the thermal conditions during development in the acorns and the moisture environment
324 of the soil probably vary among years, and could cause interannual differences in cold
325 tolerance.

326 Weevil larvae from red and bur oak acorns collected in 2010 decreased their
327 cold tolerance in February and March, respectively. An internal factor like the
328 termination of diapause may be the cause of the decline of cold tolerance, because in

329 2010-2011, winter temperatures at 5 cm depth in soil remained at about 0.5 °C until
330 March. Although there is no information about diapause in *C. glandium*, diapause is
331 intimately associated with cold tolerance in many species (Lee and Denlinger, 1991).
332 The SCPs of the beetle *Aulacophora nigripennis* increase in February and termination
333 of diapause might be related to reduction of cold tolerance (Watanabe and Tanaka,
334 1998). Therefore, understanding the dynamics of diapause in *C. glandium* and
335 clarifying the relationship between diapause and cold tolerance would be a useful next
336 step in understanding the plasticity of overwintering in this species.

337 Field-acclimated larvae showed a positive correlation between SCP and water
338 content (Fig. 4), and we therefore investigated whether larvae utilize cryoprotective
339 dehydration (Elnitsky et al., 2008; Holmstrup et al., 2002; Holmstrup and Sømme,
340 1998). Although exposure to low temperatures in the presence of ice increased cold
341 tolerance, a change of water content did not appear to effect this change directly.
342 Therefore, we conclude that *C. glandium* larvae do not utilize cryoprotective
343 dehydration to increase their cold tolerance. As shown in microclimate data, the site
344 where weevil larvae overwinter was not frozen, which means that the opportunity for
345 cryoprotective dehydration may not exist in the field. Furthermore, *C. glandium*
346 appears to be relatively dehydration-resistant (H. Udaka, unpublished observations),
347 which would further impede the development of a cryoprotective dehydration strategy,
348 which relies on a very permeable cuticle (Holmstrup et al., 2002). Nevertheless, there
349 are striking changes in water content among years, which may result from among-year
350 differences in soil moisture, which we did not measure. While these differences are
351 reflected in cold tolerance (Figure 4), they do not appear to be modified by the animal
352 specifically as part of the overwintering programme.

353 The species of oak tree on which the weevils fed affects their cold tolerance;
354 weevil larvae from bur oak acorns had lower SCPs than those fed on red oak acorns in
355 2010. Although red oak acorns have tannin levels three times higher than bur oak
356 acorns (Dixon et al., 1997), and tannin has negative effects on growth rate in some
357 insects (Bernays, 1981; Manuwoto and Scriber, 1986), there was no significant
358 difference in body mass between larvae fed on bur oak and red oak acorns. Thus, the
359 difference in nutrition may affect the ratio of body components or potential investment
360 into cryoprotectants, rather than growth rate or body size *per se*. In the absence of
361 other tradeoffs, we would expect that this indirect impact of diet on overwintering
362 biology could lead to selection for oviposition on bur oak acorns, although at present it
363 is not clear that weevils preferentially choose one species over another. Because the
364 acorns came from a number of individual trees, and the larvae were assigned randomly
365 to measures and treatments, we are unable to determine whether differences among
366 individual trees within a species also affect cold tolerance. However, the trees have
367 very similar locations and growth forms on Pelee Island, so we do not expect any
368 systematic effects of tree health or location to override the coarse-scale differences in
369 nutritive environment.

370

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379

References

- 380
381
- 382 Bernays, E., 1981. Plant tannins and insect herbivores: an appraisal. *Ecol. Entomol.* 6,
383 353-360.
- 384 Chown, L.S., Nicolson, W.S., 2004. *Insect physiological ecology : mechanisms and*
385 *patterns.* Oxford ; New York : Oxford University Press.
- 386 Coulson, S.J., Bale, J.S., 1996. Supercooling and survival of the beech leaf mining
387 weevil *Rhynchaenus fagi* L. (Coleoptera: Curculionidae). *J. Insect Physiol.*
388 42, 617-623.
- 389 Coyle, D.R., Duman, J.G., Raffa, K.F., 2011. Temporal and species variation in cold
390 hardiness among invasive rhizophagous weevils (Coleoptera:
391 Curculionidae) in a northern hardwood forest. *Ann. Entomol. Soc. Am.* 104,
392 59-67.
- 393 Crawley, M.J., Long, C.R., 1995. Alternate bearing, predator satiation and seedling
394 recruitment in *Quercus robur* L. *J. Ecol.* 83, 683-696.
- 395 Crosthwaite, J.C., Sobek, S., Lyons, D.B., Bernards, M.A., Sinclair, B.J., 2011. The
396 overwintering physiology of the emerald ash borer, *Agrilus planipennis*
397 fairmaire (coleoptera: buprestidae). *J. Insect Physiol.* 57, 166-173.
- 398 Danks, H.V., 1991. Winter habitats and ecological adaptations for winter survival, in:
399 Lee, R.E., Denlinger, D.L. (Eds.), *Insects at low temperature.* Springer, pp.
400 231-259.
- 401 Denlinger, D.L., Lee, R.E., 2010. *Low temperature biology of insects.* Cambridge
402 University Press, Cambridge.
- 403 Dixon, M.D., Johnson, W.C., Adkisson, C.S., 1997. Effects of caching on acorn tannin
404 levels and blue jay dietary performance. *The Condor* 99, 756-764.

405 Elnitsky, M.A., Hayward, S.A., Rinehart, J.P., Denlinger, D.L., Lee Jr, R.E., 2008.
406 Cryoprotective dehydration and the resistance to inoculative freezing in the
407 Antarctic midge, *Belgica antarctica*. J. Exp. Biol. 211, 524-530.

408 Espelta, J.M., Bonal, R., Sánchez-Humanes, B., 2009. Pre-dispersal acorn predation in
409 mixed oak forests: interspecific differences are driven by the interplay
410 among seed phenology, seed size and predator size. J. Ecol. 97, 1416-1423.

411 Gibson, L.P., 1982. Insects that damage northern red oak acorns. US Department of
412 Agriculture, Forest Service, Northeastern Forest Experiment Station.

413 Groffman, P.M., Driscoll, C.T., Fahey, T.J., Hardy, J.P., Fitzhugh, R.D., Tierney, G.L.,
414 2001. Colder soils in a warmer world: a snow manipulation study in a
415 northern hardwood forest ecosystem. Biogeochemistry 56, 135-150.

416 Henry, H.A., 2008. Climate change and soil freezing dynamics: historical trends and
417 projected changes. Climatic Change 87, 421-434.

418 Holmstrup, M., Bayley, M., Ramløv, H., 2002. Supercool or dehydrate? An
419 experimental analysis of overwintering strategies in small permeable arctic
420 invertebrates. Proc. Natl. Acad. Sci. U. S. A. 99, 5716-5720.

421 Holmstrup, M., Sømme, L., 1998. Dehydration and cold hardiness in the Arctic
422 Collembolan *Onychiurus arcticus* Tullberg 1876. J. Comp. Physiol. B 168,
423 197-203.

424 Irwin, J.T., Lee, R.E., Jr., 2002. Energy and water conservation in frozen vs.
425 supercooled larvae of the goldenrod gall fly, *Eurosta solidaginis* (Fitch)
426 (Diptera: Tephritidae). J. Exp. Zool. 292, 345-350.

427 Kandori, I., Kimura, T., Tsumuki, H., Sugimoto, T., 2006. Cold tolerance of the sweet
428 potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae), from

- 429 the Southwestern Islands of Japan. Jpn. J. Appl. Entomol. Zool. 41, 217-226.
- 430 Košťál, V., Šimek, P., 1996. Biochemistry and physiology of aestiv-ohibernation in the
431 adult apple blossom weevil, *Anthonomus pomorum* (Coleoptera:
432 Curculionidae). J. Insect Physiol. 42, 727-733.
- 433 Košťál, V., Šimek, P., Zahradníčková, H., Cimlová, J., Štětina, T., 2012. Conversion of
434 the chill susceptible fruit fly larva (*Drosophila melanogaster*) to a freeze
435 tolerant organism. Proc. Natl. Acad. Sci. U. S. A. 109, 3270-3274.
- 436 Kreyling, J., Henry, H.A., 2011. Vanishing winters in Germany: soil frost dynamics and
437 snow cover trends, and ecological implications. Clim. Res. 46, 269.
- 438 Lee, R.E., Denlinger, D.L., 1991. Insects at low temperatures. Chapman & Hall.
- 439 Lundheim, R., Zachariassen, K.E., 1993. Water balance of over-wintering beetles in
440 relation to strategies for cold tolerance. Journal of Comparative Physiology
441 B 163, 1-4.
- 442 Manuwoto, S., Scriber, J.M., 1986. Effects of hydrolyzable and condensed tannin on
443 growth and development of two species of polyphagous lepidoptera:
444 *Spodoptera eridania* and *Callosamia promethea*. Oecologia 69, 225-230.
- 445 Marshall, K., Sinclair, B., 2012. Threshold temperatures mediate the impact of reduced
446 snow cover on overwintering freeze-tolerant caterpillars.
447 Naturwissenschaften 99, 33-41.
- 448 Pélişson, P.-F., Bel-Venner, M.-C., Rey, B., Burgevin, L., Martineau, F., Fourel, F.,
449 Lecuyer, C., Menu, F., Venner, S., 2012. Contrasted breeding strategies in
450 four sympatric sibling insect species: when a proovigenic and capital
451 breeder copes with a stochastic environment. Funct. Ecol. 26, 198-206.
- 452 Pedersen, P.G., Holmstrup, M., 2003. Freeze or dehydrate: only two options for the

453 survival of subzero temperatures in the arctic enchytraeid *Fridericia ratzeli*.
454 J. Comp. Physiol., B 173, 601-609.

455 R Core Team, 2013. R: A Language and environment for statistical computing. R
456 Foundation for Statistical Computing, Vienna, Austria.

457 Roland, J., Matter, S.F., 2013. Variability in winter climate and winter extremes reduces
458 population growth of an alpine butterfly. Ecology 94, 190-199.

459 Semel, B., Andersen, D.C., 1988. Vulnerability of acorn weevils (Coleoptera:
460 Curculionidae) and attractiveness of weevils and infested *Quercus alba*
461 acorns to *Peromyscus leucopus* and *Blarina brevicauda*. Am. Midl. Nat. 119,
462 385-393.

463 Shimada, T., Saitoh, T., 2006. Re-evaluation of the relationship between rodent
464 populations and acorn masting: a review from the aspect of nutrients and
465 defensive chemicals in acorns. Popul. Ecol. 48, 341-352.

466 Sinclair, B.J., Renault, D., 2010. Intracellular ice formation in insects: unresolved after
467 50 years? Comp. Biochem. Physiol. A Mol. Integr. Physiol. 155, 14-18.

468 Tanaka, K., 1994. The effect of feeding and gut contents on supercooling in the house
469 spider, *Achaearanea tepidariorum*. Cryo-letters 15, 361-366.

470 van der Merwe, M., Chown, S.L., Smith, V.R., 1997. Thermal tolerance limits in six
471 weevil species (Coleoptera, Curculionidae) from sub-Antarctic Marion
472 Island. Polar Biol. 18, 331-336.

473 Venner, S., Pelisson, P.F., Bel-Venner, M.C., Debias, F., Rajon, E., Menu, F., 2011.
474 Coexistence of insect species competing for a pulsed resource: toward a
475 unified theory of biodiversity in fluctuating environments. PLo
476 S One 6, e18039.

- 477 Watanabe, M., Tanaka, K., 1997. Overwintering status and cold hardiness of *Hypera*
478 *punctata*. *Cryobiology* 35, 270-276.
- 479 Watanabe, M., Tanaka, K., 1998. Adult diapause and cold hardiness in *Aulacophora*
480 *nigripennis*(Coleoptera: Chrysomelidae). *J. Insect Physiol.* 44, 1103-1110.
- 481 Williams, C.M., Marshall, K.E., MacMillan, H.A., Dzurisin, J.D.K., Hellmann, J.J.,
482 Sinclair, B.J., 2012. Thermal variability increases the impact of autumnal
483 warming and drives metabolic depression in an overwintering butterfly.
484 *PLoS One* 7, e34470.
- 485 Worland, M.R., Lukešová, A., 2000. The effect of feeding on specific soil algae on the
486 cold-hardiness of two Antarctic micro-arthropods (*Alaskozetes antarcticus*
487 and *Cryptopygus antarcticus*). *Polar Biol.* 23, 766-774.
- 488
- 489

490 **Figure legends**

491 Fig.1. SCPs (A), water content (B), and body mass (C) of *Curculio glandium* larvae
492 from bur oak acorns collected in 2010 and red oak acorns collected in 2010 and 2011.
493 Weevil larvae were stored at 4 °C for a minimum of two weeks after collection, without
494 any cold exposure. Different letter indicate the data were significantly different (Tukey's
495 *post hoc* test, $P < 0.05$). Values are mean \pm SE. N= 25- 72.

496

497 Fig.2. The soil temperature at 5 cm depth in the site where weevil larvae buried in
498 London, Ontario in 2010 - 2011 (A) and 2011 – 2012 (B) and the acorns with weevil
499 larvae collected on Pelee island in 2010 - 2011 (C) and 2011 - 2012 (D). Dashed lines
500 indicate 0 °C.

501

502 Fig. 3. Seasonal changes in SCP (A) and water content (B) of *Curculio glandium* larvae
503 from January to March 2011 (open symbols) and from October 2011 to March 2012
504 (filled symbols). Weevil larvae were obtained from red oak acorns (circles) and bur oak
505 acorns (triangles). Weevils collected from Pelee Island in fall 2010 were buried in the
506 field on 18 December 2010 and those collected in 2011 fall were buried on 12
507 November 2011. The same letters indicate SCP and water content were not significantly
508 different (Tukey's *post hoc* test, $P < 0.05$). Mean \pm S.E. N = 8- 21.

509

510 Fig.4. The relationship between SCPs and water content in *Curculio glandium* larvae
511 buried in the fields in 2010 - 2011 and 2011 – 2012. Weevil larvae were obtained from
512 bur oak acorns collected in 2010 and red oak acorns collected in 2010 and 2011.
513 Weevils collected from Pelee Island in fall 2010 were buried in the field on 18

514 December 2010 and those collected in 2011 fall were buried on 12 November 2011.

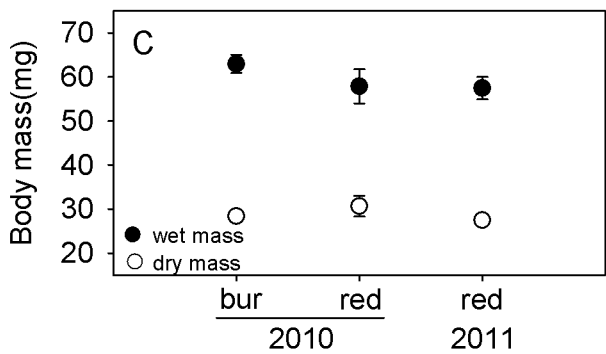
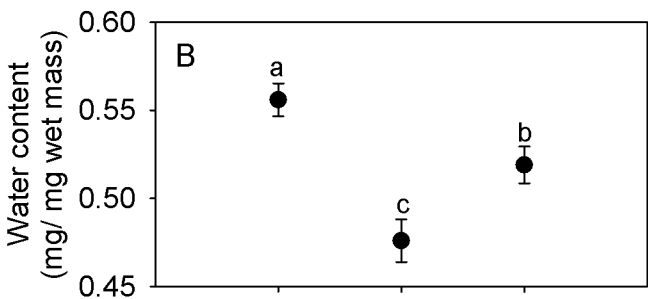
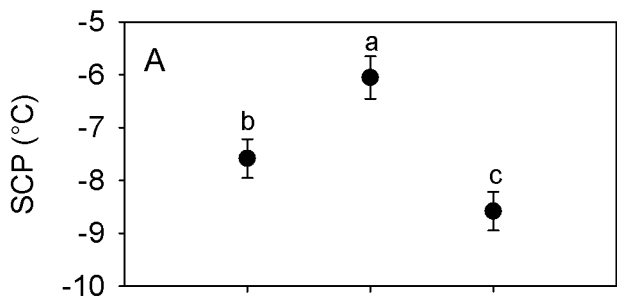
515 The data are derived from Figs 1A and 1B. N = 162.

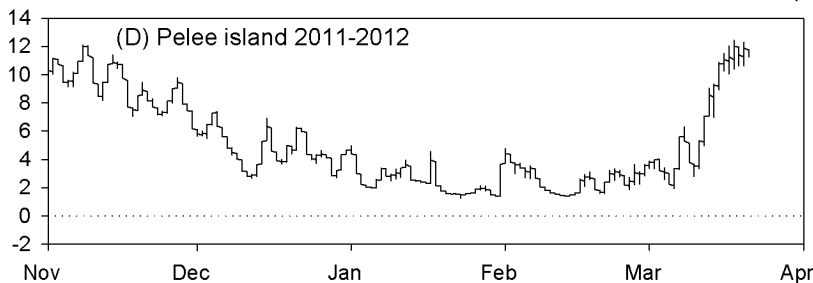
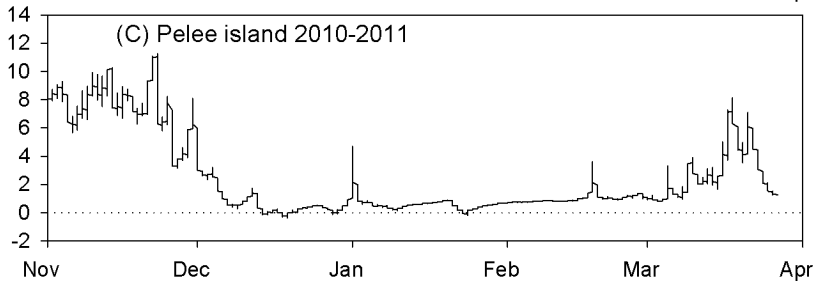
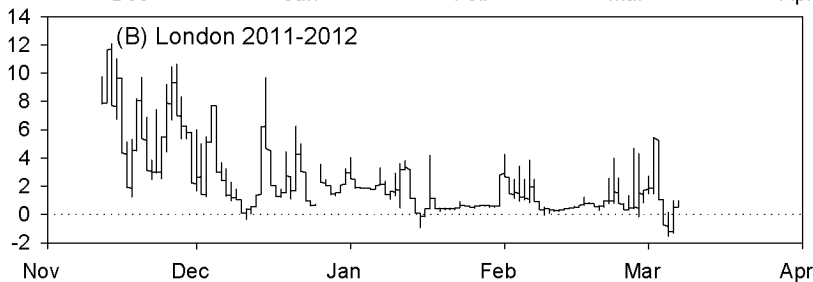
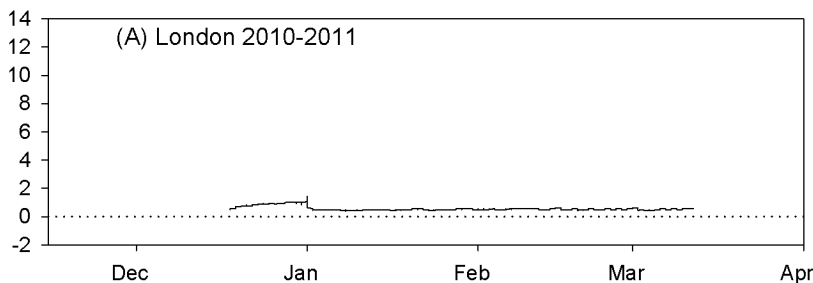
516

517 Fig.5. The experimental design (A) and the effect of cryoprotective dehydration on
518 SCPs (B), water content (C), body mass (D) in *Curculio glandium* larvae obtained from
519 red oak acorns in 2011. Larvae were kept at 0 °C for 1 day and temperature was
520 decreased 1 °C/ day and held at -5 °C for seven days. Supercooling points (SCPs) were
521 measured on (i) the first day temperature reached at -5 °C and (ii) seven days after
522 exposure to -5 °C. The same letters indicate SCP and water content data were not
523 significantly different (Tukey's *post hoc* test, $P < 0.05$). N= 18- 46.

524

525 Fig.6. The effects of long-term acclimation on SCPs (A), water content (B) and body
526 mass (C) in larvae of *Curculio glandium*. Larvae collected from red oak acorns in 2011
527 were used and SCPs were measure in weevil larvae exposed to 0 °C for 2, 4, and 6
528 weeks. The same letters indicate SCPs were not significantly different (Tukey's *post hoc*
529 test, $P < 0.05$). N= 12- 46.





Month

Fig3

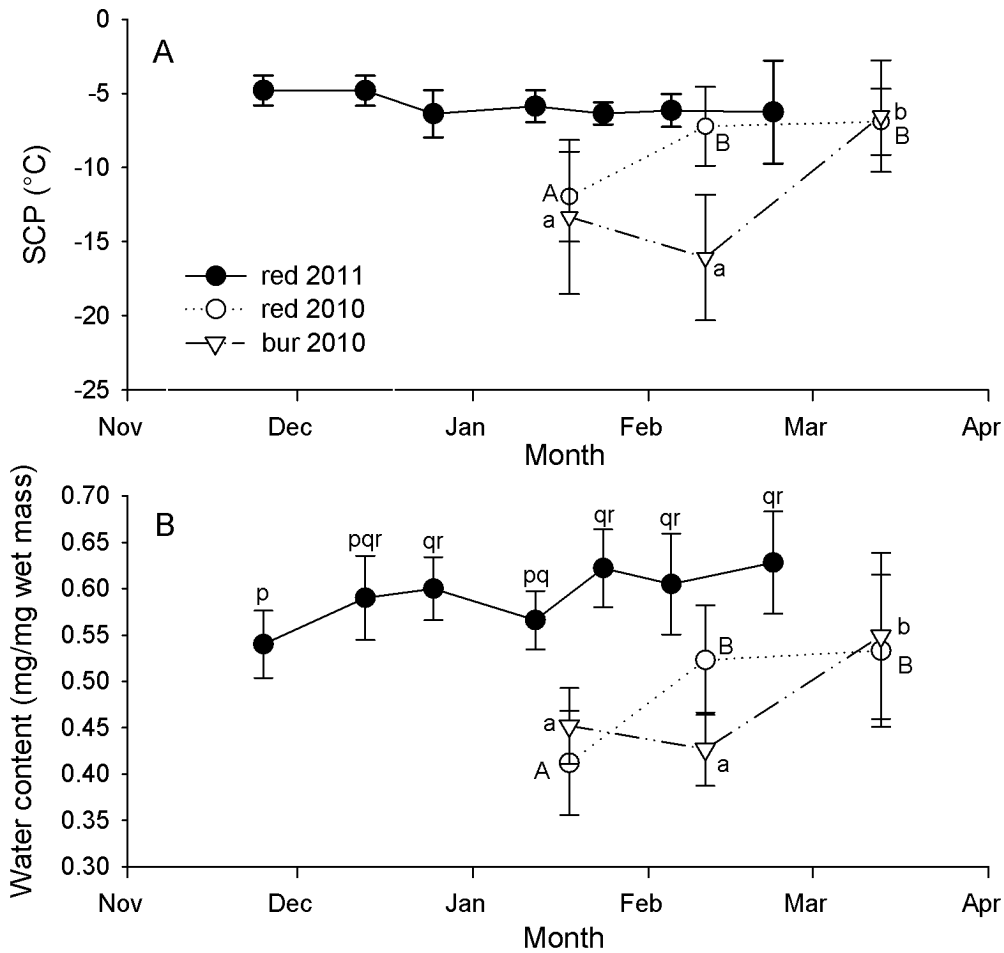


Fig 4

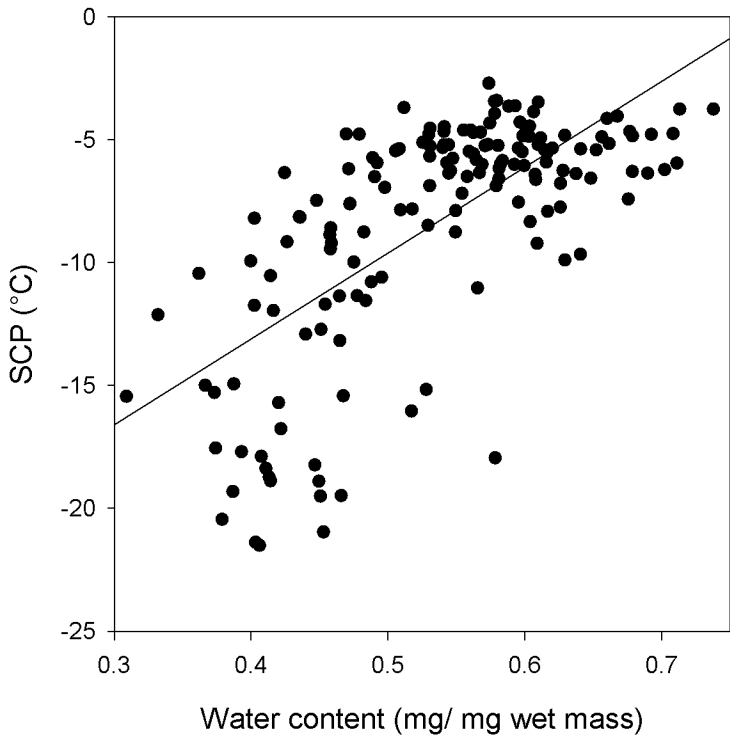


Fig 5

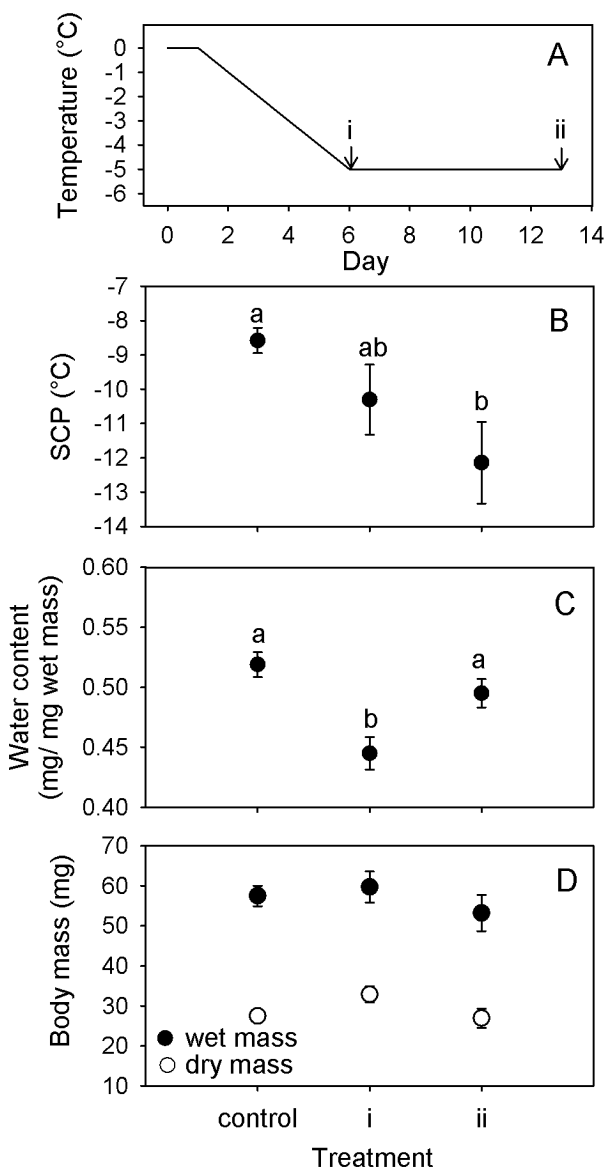


Fig 6

