



Title	Growth and nutrition of <i>Agelastica coerulea</i> (Coleoptera: Chrysomelidae) larvae changed when fed with leaves obtained from an O <sub>2</sub> -enriched atmosphere
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1           **Growth and nutrition of *Agelastica coerulea* (Coleoptera: Chrysomelidae) larvae**  
2           **changed when fed with leaves obtained from an O<sub>3</sub>-enriched atmosphere**

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21

## 22 **Abstract**

23 A series of laboratory no-choice assays were performed to test changes in the feeding,  
24 growth and nutrition of leaf beetle (*Agelastica coerulea*) larval instars on O<sub>3</sub>-treated  
25 leaves of Japanese white birch (*Betula platyphylla* var. *japonica*). Larvae fed with  
26 O<sub>3</sub>-treated leaves grew and developed significantly faster throughout their developmental  
27 cycle than the corresponding controls. The growth rate (GR) and consumption index (CI)  
28 were mostly decreased with age for both control and O<sub>3</sub>-treated leaves. Efficiency of

29 conversion of both ingested and digested food (ECI, ECD) showed an increase from the  
30 2<sup>nd</sup> to the 4<sup>th</sup> instar, after which they decreased significantly and reached the lowest value  
31 in the last larval instars (7<sup>th</sup>). GR, CI, ECI and ECD were greater and approximate  
32 digestibility (AD) lower in larvae fed with O<sub>3</sub>-treated leaves than those fed with control  
33 leaves. This indicated that the greater rate of growth on fumigated leaves was due  
34 primarily to a greater rate of consumption (*i.e.*, O<sub>3</sub> increased the "acceptability" of the  
35 host more than "suitability") and efficiency in converting food into body mass. Overall,  
36 larval performance seemed to have improved when fed with O<sub>3</sub>-treated leaves in these  
37 assays. This study suggests insects may be more injurious to O<sub>3</sub>-treated plants and  
38 warrants further investigations on birch-beetle interactions under field conditions.

39

40 **Keywords:** air pollution; herbivore; leaf beetle; nutrition; plant-insect; trophic interaction

41

## 42 **1. INTRODUCTION**

43 Ground-level ozone (O<sub>3</sub>) concentrations in the lower troposphere have notably increased  
44 since the preindustrial age, and remain at potentially phytotoxic levels, although control  
45 measures on precursor emissions have reduced O<sub>3</sub> peaks in some regions of Europe  
46 (Vingarzan 2004; Derwent et al. 2007; Cape 2008; The Royal Society 2008; Sicard et al.

47 2016, 2017; Solomou et al. 2018). Northeast Asia is a hot spot of O<sub>3</sub> pollution  
48 (Nagashima et al. 2017; Trieu et al. 2017; Wang et al. 2017). Domestically-produced O<sub>3</sub>  
49 and transboundary transport from outside, mainly from China, result in significant  
50 increases in O<sub>3</sub> levels in Japan in recent years (Nagashima et al. 2010, 2017; Tanimoto  
51 2009; Tanimoto et al. 2009). Furthermore, an increase in background O<sub>3</sub> levels in regions  
52 of the northern hemisphere may occur in the near future (The Royal Society 2008). O<sub>3</sub> is  
53 a gaseous pollutant which causes toxicity to plants at macro- and microscopic levels,  
54 when its concentrations exceed species-specific thresholds, through reactions in the  
55 leaves, with a subsequent production of highly oxidative intermediates (Robinson and  
56 Rowland 1996; Oksanen et al. 2013; Vaultier and Jolivet 2015). Such exceedances may  
57 result in reduction in plant vigor, suppression of yields and productivity, with further  
58 implications to ecological processes and trophic cascades (Feng et al. 2008; Lindroth  
59 2010; Ainsworth et al. 2012; Koike et al. 2013; Blande et al. 2014; Agathokleous et al.  
60 2016a; Chappelka and Grulke 2016).

61 There has been a notable progress in research on O<sub>3</sub> effects on vegetation and plant  
62 ecosystems over the last decades (Feng et al. 2008; Lindroth 2010; Ainsworth et al. 2012;  
63 Koike et al. 2013; Blande et al. 2014; Agathokleous et al. 2015a,b, 2016); however, the  
64 knowledge about O<sub>3</sub> effects on trophic interactions remains limited. The relationship

65 between plant stress and susceptibility to insects is of particular interest (White 1974,  
66 1976, 1984; Alstad et al. 1982; Hain 1987; Mattson and Haack 1987). Plant-herbivore  
67 relationships represent a dynamic equilibrium between plant defense against herbivory  
68 and insect feeding adaptation on host plants. However, environmental stresses like air  
69 pollution can shift the balance of these relationships (Hughes 1988; Hillstrom and  
70 Lindroth 2008; Lindroth 2010; Chappelka and Grulke 2016; Agathokleous 2018).  
71 Stressed plants could become more vulnerable to herbivory when biochemical changes  
72 lead to an increase in the nutritional value or to a decrease in plant chemical defenses  
73 (White 1974, 1984; Valkama et al. 2007; Ali and Agrawal 2012; Chappelka and Grulke  
74 2016). Stressed trees may be a more suitable food source for invertebrate herbivores than  
75 unstressed trees due to an increase in the tissue content of soluble nitrogenous compounds  
76 (Fred 1987; Koike et al. 2006). Although O<sub>3</sub> is known to change the palatability of leaves,  
77 how this change influences plant-insect interactions remains underexplored.

78 The insect performance depends on the level of toxins produced by plants and the quality  
79 of the insect, e.g. sequestering or stealthy (Ali and Agrawal 2012; Agathokleous 2018). In  
80 the case of O<sub>3</sub>, the level of defense chemicals produced by plants, and thereby the insect  
81 performance, depend on the exposure level or on the O<sub>3</sub> dose uptake by plants, within the  
82 framework of hormesis (Agathokleous 2018). For instance, insects may display a

83 preference towards leaves which experience short exposure to elevated O<sub>3</sub> levels and  
84 have increased palatability (Jones and Coleman 1988a; Bolsinger et al. 1991, 1992).  
85 Recently, results from laboratory assays showed that O<sub>3</sub> altered the feeding behavior of  
86 the leaf beetle *Agelastica coerulea* (Baly 1874) (hereafter leaf beetle) into leaves of  
87 Japanese white birch (*Betula platyphylla* var. *japonica*) (Agathokleous et al. 2017a). In  
88 these assays, it was found that overwintered adults preferred elevated O<sub>3</sub>-treated leaves  
89 than ambient O<sub>3</sub>-treated ones and that the feeding behavior of 2<sup>nd</sup> instar larvae was not  
90 changed when larvae could select between ambient O<sub>3</sub>-treated and elevated O<sub>3</sub>-treated  
91 leaves (Agathokleous et al. 2017a). These laboratory observations are in agreement with  
92 observations in assays with the common leaf weevil *Phyllobius pyri* L. (Coleoptera:  
93 Curculionidae) (Freiwald et al. 2008) and oppose earlier field observations where the leaf  
94 beetle deterred from grazing leaves of Japanese white birch in elevated O<sub>3</sub> sites of a Free  
95 Air Controlled Exposure (FACE) system (Sakikawa et al. 2014, 2016; Vanderstock et al.  
96 2016), a phenomenon which may be upon O<sub>3</sub>-induced degradation of biogenic volatile  
97 organic compounds (BVOCs) that repels insects (Fuentes et al. 2013; Dötterl et al. 2016;  
98 Farré-Armengol et al. 2016; Li et al. 2016). However, in O<sub>3</sub>-polluted regions, herbivore  
99 insects have no privilege to choose between O<sub>3</sub> sites, and, thus, feed on plants under  
100 stress. The implications of this feeding to the insect nutrition remain unknown.

101 In the present study, we explored the possibility that O<sub>3</sub> does affect insect nutrition  
102 indirectly, when insects consume leaves which underwent elevated O<sub>3</sub> exposure. In order  
103 to identify and quantify O<sub>3</sub> effects on insect nutrition, we conducted laboratory assays  
104 with a collection of larvae instars of the leaf beetle fed with Japanese white birch leaves  
105 obtained from sites with either background or elevated O<sub>3</sub> levels in a FACE system. We  
106 hypothesized that O<sub>3</sub> would have indirect effects on the nutrition of larvae feeding with  
107 O<sub>3</sub>-treated leaves, and the effects could vary among larval instars which differ in their  
108 anatomical and physiological characteristics.

109 We assessed larvae consumption, mass growth, efficiency of conversion of ingested food  
110 (growth efficiency, ECI), efficiency of conversion of digested food (ECD), and  
111 assimilation efficiency (approximate digestibility, AD) as effective indicators of food  
112 utilization by insects (Slansky 1985). ECI relates the total consumption (food ingested) of  
113 insect to the amount of body mass, whereas ECD ignores undigested food (Slansky 1985;  
114 Farrar et al. 1989). AD indicates the food digestibility whereas ECI and ECD indicate the  
115 insect efficiency to convert food into body mass.

116



## 117 2. MATERIALS AND METHODS

### 118 2.1. Insect eggs and leaf samples

119 Samples were collected in 2016 from Japanese white birch trees grown in the FACE  
120 system located at Sapporo Experimental Forest of Hokkaido University, Japan (43°04' N,  
121 141°20' E, 15 m a.s.l.). This birch is classified as heterophyllous shoot development type  
122 with two types of leaves, namely early leaves vs. late leaves which appear after complete  
123 expansion of the early leaves (Koike 1995; Matsuki et al. 2004). These trees were planted  
124 in the experimental plots in mid-May 2014, when they were two-year old. The plants  
125 were periodically irrigated and treated with 100 times diluted wood vinegar early after  
126 plantation in 2014 for pest management until their establishment to the experimental sites.  
127 Fertilization was never carried out. After the establishment to the sites, plants were grown  
128 naturally with no intentional irrigation or other pest management. The snow cover period  
129 in Sapporo lasted from mid-December to early-May. Meteorological data in 2016 were  
130 recorded at a station located in Sapporo (WMO, ID: 47412), at 43°03.6'N 141°19.7'E  
131 (Japan Meteorological Agency 2017). For the period May-August, the main  
132 meteorological conditions (mean  $\pm$  s.e.) were: mean monthly average of air temperature =  
133 18.95 ( $\pm$ 2.06) °C; daily maximum temperature = 23.48 ( $\pm$ 1.95) °C; daily minimum  
134 temperature = 15.50 ( $\pm$ 2.18) °C; wind speed = 4.00 ( $\pm$ 0.23) m s<sup>-1</sup>; relative humidity =

135 69.75 ( $\pm 3.94$ ) %; mean monthly total sunshine duration = 205.90 ( $\pm 16.54$ ) h; and mean  
136 monthly precipitation 137.63 ( $\pm 52.36$ ) mm, respectively. Meteorological data for the  
137 years 2014 and 2015 along with details of the study site are available in Agathokleous et  
138 al. (2017b).

139 The O<sub>3</sub>-FACE system consisted of six rings; three with ambient air and three with  
140 ambient air enriched with O<sub>3</sub> (target = 70 nmol mol<sup>-1</sup>) during daylight hours with  
141 photosynthetic photon flux density (PPFD) > 70  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (07:00–17:00). In 2014, the  
142 plants were exposed to elevated O<sub>3</sub> from August 15 to October 26, and, in 2015, from  
143 April 24 to October 26. The mean O<sub>3</sub> levels (07:00–17:00) for elevated O<sub>3</sub> plots were 60  
144 nmol mol<sup>-1</sup> in 2014 and 72 nmol mol<sup>-1</sup> in 2015, whereas for ambient O<sub>3</sub> plots were 20  
145 nmol mol<sup>-1</sup> in 2014 and 34 nmol mol<sup>-1</sup> in 2015. In 2016, the O<sub>3</sub> enrichment started on  
146 May 18, and the last leaf samples were collected on July 15. The mean O<sub>3</sub> levels  
147 (07:00–17:00) in elevated O<sub>3</sub> plots were 63.52 nmol mol<sup>-1</sup> during the period May 10 to  
148 July 15 and 60.92 nmol mol<sup>-1</sup> during the period June 21 to July 15 (duration of insect  
149 assays). Ambient O<sub>3</sub> levels were recorded from June 1 onwards. Ambient O<sub>3</sub> levels  
150 (07:00–17:00) were 5.21 and 5.84 nmol mol<sup>-1</sup> during the periods June 1 to July 15 and  
151 June 21 to July 15. Details on the FACE system and O<sub>3</sub> exposures can be found in  
152 Agathokleous et al. (2017b).

153 **2.2. Laboratory insect culture and assays**

154 The colony of the leaf beetle was started with egg patches obtained from ambient air  
155 conditions of Sapporo Experimental Forest of Hokkaido University, on June 16, 2016.  
156 Deposited eggs were selected based on the intensity of yellow color (eggs close to  
157 hatching avoided) and transferred to laboratory. Thereafter, all the processes were  
158 conducted under laboratory conditions. Eggs hatching occurred within four to five days  
159 from the collection. Newly hatched larvae were placed in plastic boxes (12 × 6 × 18 cm).  
160 Ten larvae were placed in a box replicated four times per O<sub>3</sub> treatment per instar stage,  
161 giving thus a total of 40 individual larvae per instar per O<sub>3</sub> treatment. The assays were  
162 conducted based on a completely randomized design, and the position of the boxes was  
163 rotated on a daily base. The initial weight of the larvae was measured and the culture was  
164 kept at a temperature of 25 ± 2°C, 65 ± 5 % relative humidity and 14:10 hours (D: L)  
165 photoperiod (Abu ElEla and ElSayed 2015; Adham et al 2005 a,b; Abu ElEla et al 2016).  
166 Larvae of each preceding instar molted and transferred to next stage by shedding the skin.  
167 Larvae were fed on weighed quantities of treated and untreated fresh mature leaves of  
168 Japanese white birch leaves until pupation. Fresh late leaves were collected from ambient  
169 and elevated O<sub>3</sub> plots and provided to the larvae on a daily base, until the end of larval  
170 stage. Late leaves were selected over early leaves because females of this beetle

171 commonly oviposit on late leaves and from June larvae start hatching and grazing these  
172 leaves (Agathokleous et al. 2017a). The leaves were randomly selected; however leaves  
173 with injury were excluded to minimize potential bias. To overcome limitations which  
174 may occur when using leaf disks, intact leaves were used (Jones and Coleman 1988b;  
175 Smith et al. 1994; Lacey 1997). Plant materials of each O<sub>3</sub> treatment were daily pooled,  
176 and leaf samples were randomly drawn and provided to the larvae. Feeding period lasted  
177 from June 21 to July 15.

178 In the middle of the assays, additional leaf samples, similar to those used for the assays,  
179 were collected from each plot and kept at room conditions with dry air. The weight of  
180 each leaf was measured at 0 and 22 hours, and then the leaves were dried to a constant  
181 weight. Water content in fresh leaves was 1.1 % greater in elevated O<sub>3</sub> than in ambient O<sub>3</sub>,  
182 but the difference was non-significant (data not shown). Furthermore, at 22 hours, leaves  
183 obtained from elevated O<sub>3</sub> lost 6.9 % more water than those obtained from ambient O<sub>3</sub>,  
184 however the difference was non-significant (data not shown). Therefore larval feeding  
185 was not affected by O<sub>3</sub>-mediated alteration in the dehydration of leaves.

186 The average fresh weight of larvae, faeces, and consumed leaf, and the gained weight  
187 were determined by an analytical balance (Mettler<sup>®</sup> M22). To assess the effects on larval  
188 feeding and conversion of food to biomass, consumption and utilization of ozonated and

189 control tissues were compared by means of nutritional indices (Kogan 1986; Waldbaure  
190 1968) for the 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup>, and 7<sup>th</sup> instars using standard gravimetric procedures.  
191 Consumption index (CI), mass growth rate (GR), ECD; ECI, and AD were calculated  
192 using standard gravimetric procedures described by Waldbauer (1968):

193 a)  $CI = C / [T \times A]$ , where C is the fresh weight of leaf consumed, T is the duration of  
194 feeding period and A is the mean fresh weight of the larvae during the feeding  
195 period. CI measures the amount of food eaten per unit time relative to mean  
196 weight of larvae during the feeding period.

197 b)  $GR = G / [T \times A]$ , where G is the fresh weight gain of the larvae. GR measures the  
198 amount of weight gained per unit time relative to the mean weight of the larvae  
199 during the feeding period.

200 c)  $ECI = (G/C) \times (100)$ . ECI is an overall measure of the larvae's ability to utilize  
201 ingested food for growth.

202 d)  $ECD = [G / (C - F)] \times (100)$ , where F is the weight of faeces during the feeding  
203 period. ECD is an overall measure of the larvae ability to utilize digested food for  
204 growth.

205 e)  $AD = [(C - F) / C] \times (100)$ . AD measures the larvae's ability to digest the introduced  
206 food.

207 Exuviae were measured with the faeces since they are not a part of the insect at the end of  
208 the experiment (Reese and Beck 1976; Abu ElEla and ElSayed 2015, Abu ElEla et al  
209 2016).

### 210 **2.3. Data Analysis**

211 Four values were used per O<sub>3</sub> treatment per instar stage, each of which was a robust  
212 estimate of one independent experimental unit. The alpha level was predefined at  $\alpha=0.05$ .  
213 The data of CI, GR, ECI, ECD and AD were not satisfactorily fit to Gaussian distribution  
214 and therefore were subjected to a Box-Cox power transformation (Box and Cox 1964), as  
215 described by Agathokleous et al. (2016b). Data of each response variable were subjected  
216 to repeated measures Analysis of Variances (rANOVAs) where Instar was the  
217 within-subjects factor with 6 levels and O<sub>3</sub> the categorical predictor with 2 levels.  
218 rANOVA was based on *effective hypothesis* Sums of Squares (*Type 6 SS*) straightforward  
219 computation method (Hocking 2013) with  $\sigma$ -restricted coding of effects (Hill and Lewicki  
220 2006). Type 6 SS for each effect is the difference of the model SS for all the other effects  
221 from the whole model SS, thus, providing an explicit estimate of predicted values  
222 variability for the outcome that is attributed to each effect (Hill and Lewicki 2006). When  
223 rANOVAs returned overall significances at a level of significance  $\alpha=0.05$  ( $H_0$  rejected),  
224 the data were further subjected to Bonferroni *a posteriori* test.

225 Data were processed and statistically analyzed in the software MS EXCEL 2010 (©  
226 Microsoft) and STATISTICA v.10 (© StatSoft Inc.).

227

## 228 **2. RESULTS AND DISCUSSION**

229 Based on observations, larvae from ambient treatment needed 24 days from 1<sup>st</sup> to 7<sup>th</sup>  
230 instar, whereas larvae from elevated O<sub>3</sub> needed 21 days from 1<sup>st</sup> to 7<sup>th</sup> instar.

231 **GR** (Fig 1A) significantly varied among instar stages ( $F=20.0$   $P<0.001$ ). Second and 4<sup>th</sup>  
232 instars showed similar GR which was on average 4.3 times greater than that of 3<sup>rd</sup>, 5<sup>th</sup>, 6<sup>th</sup>  
233 and 7<sup>th</sup> instars ( $P<0.05$ ). Third, 5<sup>th</sup> and 6<sup>th</sup> instars shared a similar GR, which was on  
234 average 3.5 times lower than that of 2<sup>nd</sup> and 4<sup>th</sup> instars and 4 times greater than that of 7<sup>th</sup>  
235 instar. These differences were significant except for GR between 6<sup>th</sup> and 7<sup>th</sup> instars due to  
236 large relative standard deviation (RSD) in 6<sup>th</sup> instar ( $P<0.05$ ). Seventh instars had a  
237 multi-fold lower GR than 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> instars. Larvae displayed a 137 % greater  
238 GR in elevated O<sub>3</sub> than in ambient O<sub>3</sub> ( $F=9.2$ ,  $P<0.05$ ). O<sub>3</sub> effects did not vary  
239 significantly among instar stages ( $F=1.8$ ,  $P=0.152$ ).

240 **CI** (Fig 1B) also varied among instar stages ( $F=13.8$ ,  $P<0.001$ ). Second instars had on  
241 average 2.6 times greater CI than 3<sup>rd</sup>, 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> instars ( $P<0.05$ ); they also had 1.7  
242 times greater CI than 4<sup>th</sup> instars, however the difference was non-significant. Seventh

243 instars had significantly lower CI than 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instars. Larvae showed a 115 %  
244 greater CI in elevated O<sub>3</sub> than in ambient O<sub>3</sub> ( $F=9.0$ ,  $P<0.05$ ), while O<sub>3</sub> effects did not  
245 vary significantly among instar stages ( $F=0.3$ ,  $P=0.919$ ). When food contains less  
246 nitrogen, the consumption by insects increases to compensate for nitrogen acquisition.  
247 Thus, elevated O<sub>3</sub>-treated leaves, might have lower nitrogen content than elevated  
248 O<sub>3</sub>-treated leaves, however this could only be a speculation as we have no supportive data.  
249 In the experiment of Agathokleous et al. (2017a), leaves of white birch trees were  
250 collected in July, after a similar O<sub>3</sub> exposure. By that time, leaves exposed to O<sub>3</sub> had  
251 lower content of total phenolics and condensed tannin than leaves, but no different leaf  
252 mass per area (LMA), compared to leaves exposed to ambient O<sub>3</sub>. In that no-choice  
253 laboratory assay, 2<sup>nd</sup> instar larvae and adults of the leaf beetle did not significantly  
254 increase the leaf consumption to compensate for degraded leaf palatability caused by O<sub>3</sub>.  
255 Several other investigations reported that insects showed preference towards O<sub>3</sub>-treated  
256 leaf material. For example, the monarch butterfly, *Danaus plexippus* (Lepidoptera:  
257 Nymphalidae), preferred O<sub>3</sub>-treated leaves of *Asclepias curassavica* and *A. syriaca*  
258 (Bolsinger et al. 1992), and the Mexican bean beetle, *Epilachna varivestis* (Coleoptera:  
259 Coccinellidae), preferred O<sub>3</sub>-treated leaves of soybean, *Glycine max* (L.) Merr. (Endress  
260 and Post 1985; Chappelka et al. 1988). Jones and Coleman (1988a) found that the willow



261 leaf beetle, *Plagioder a versicolor a* (Coleoptera: Chrysomelidae), not only preferred  
262 O<sub>3</sub>-treated plants but also consumed more foliage. This phenomenon might be upon  
263 decreased palatability and/or reduced defense of the leaves. Consumption of leaf area  
264 alone is not an efficient indicator of O<sub>3</sub>-induced alterations because of the several factors  
265 which interplay. This assumption relies on the fact that consumption alone cannot inform  
266 if changes are upon O<sub>3</sub>-induced changes in the palatability or defense of leaves, or if  
267 changes in consumption have any effects on insect physiological performance (Whittaker  
268 et al. 1989).

269 The extent to which leaf palatability is improved depends on the O<sub>3</sub> exposure and the  
270 subsequent plant response. Moderate increases in the levels of chemicals produced by  
271 plants may translate to stimulation of insect performance, i.e. hormesis (Ali and Agrawal  
272 2012; Agathokleous 2018). The increase in CI, due to treatment of leaves with O<sub>3</sub>,  
273 observed in this experiment is in agreement with the GR results, thus verifying earlier  
274 findings where the amount of growth reduction was generally proportional to reduced  
275 food consumption (Woodring et al. 1978; Adham et al. 2005a; Abu ElEla et al. 2016).  
276 The present results may hint to O<sub>3</sub>-induced increase in the palatability and decrease in the  
277 defense of the leaves, something that requires further investigations.

278 **ECI** (Fig 2A) showed differences among instar stages ( $F=32.0$ ,  $P<0.001$ ). Independently  
279 from O<sub>3</sub>, 3<sup>rd</sup> and 4<sup>th</sup> instars had no significantly different ECI from 2<sup>nd</sup> instars, whereas 5<sup>th</sup>  
280 and 6<sup>th</sup> instars had lower ECI compared to 2<sup>nd</sup> instars. Seventh instars had a multi-fold  
281 lower ECI than all the previous instars. Instars showed a greater ECI in elevated O<sub>3</sub> than  
282 in ambient O<sub>3</sub> ( $F=11.8$ ,  $P<0.05$ ), and O<sub>3</sub> effects did vary significantly among instar stages  
283 ( $F=7.6$ ,  $P<0.001$ ). Within each instar stage, the only significant difference was observed  
284 in the 3<sup>rd</sup> instars. Instars of ambient O<sub>3</sub> had much lower ECI at 3<sup>rd</sup> than at 2<sup>nd</sup> stage,  
285 whereas instars of elevated O<sub>3</sub> had no significantly different ECI between 2<sup>nd</sup> and 3<sup>rd</sup>  
286 stages. Thus, 3<sup>rd</sup> instars of elevated O<sub>3</sub> had greater ECI than 3<sup>rd</sup> instars of ambient O<sub>3</sub>.

287 **ECD** (Fig 2B) also showed significant differences among instar stages ( $F=105.0$ ,  
288  $P<0.001$ ). Larvae of ambient or elevated O<sub>3</sub> showed lower ECD in 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> instar  
289 stages than in 4<sup>th</sup> one. Seventh instars had a multi-fold lower ECD than all the previous  
290 instars. Fourth instars larvae were more selective feeders and choose more digestive  
291 foliage from the inter-vein regions of the leaf. Also, their metabolic rate was greater than  
292 other instar stages, and, hence, more digested food was available for conversion to body  
293 substance (i.e. ECD) (Abu ElEla and ElSayed 2015). It was noticed that 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup>  
294 instar larvae were more generalized in feeding, and they ingested different parts of  
295 foliage such as leaf veins, which contain large quantities of indigestible crude fiber.

296 Therefore, it is likely that the 7<sup>th</sup> instar larvae had lower metabolic rate than younger ones  
297 (2<sup>nd</sup> instars). The decrease in ECD indicates a less precise correspondence between larval  
298 requirements and the level of nutrients balance in the diet. Larvae showed a greater ECD  
299 in elevated O<sub>3</sub> than in ambient O<sub>3</sub> ( $F=43.5$ ,  $P<0.001$ ). ECD may decrease as a result of a  
300 compensation to increase nutrient intake in leaves with reduced nutrients, along with  
301 parallel intake of toxins. Our results are reverse, suggesting that there was no issue of  
302 O<sub>3</sub>-induced production of toxins in leaves. The O<sub>3</sub>-induced increased efficiency of larvae  
303 to convert ingested and digested food (ECI and ECD, Fig. 2A,B) observed in this study  
304 can explain the enhanced GR (Fig 1A). O<sub>3</sub> effects did vary significantly among instar  
305 stages ( $F=28.1$ ,  $P<0.001$ ). Within instars, the only difference was a lower ECD in larvae  
306 of ambient than in larvae of elevated O<sub>3</sub> at 3<sup>rd</sup> and 7<sup>th</sup> stages. Larvae of elevated O<sub>3</sub>  
307 displayed a significantly greater ECD in 3<sup>rd</sup> and 4<sup>th</sup> instar than in 2<sup>nd</sup> instar, whereas  
308 larvae of ambient O<sub>3</sub> displayed lower ECD in 3<sup>rd</sup> stage and statistically non-different  
309 ECD in 4<sup>th</sup> stage compared to 2<sup>nd</sup> stage. Greatest ECD was recorded in 3<sup>rd</sup> and 4<sup>th</sup> instar  
310 stages, when larvae were fed with elevated O<sub>3</sub>-treated leaves, in agreement with findings  
311 in *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) (Hemati et al. 2012).

312 **AD** measures the digestive availability to the larvae, which is an important aspect in a  
313 diet (Cohen 2005). AD (Fig 2B) displayed a variation within instars ( $F=104.9$ ,  $P<0.001$ ).

314 Seventh instars had greater AD than all the previous instars. Third, 4<sup>th</sup> and 5<sup>th</sup> instars had  
315 lower AD than 2<sup>nd</sup> instars. Sixth instar had significantly non-different AD than 2<sup>nd</sup> instar.  
316 Larvae displayed a lower AD in elevated O<sub>3</sub> than in ambient O<sub>3</sub> ( $F=47.1$ ,  $P<0.001$ ).  
317 Lower AD values may indicate lower suitability of leaf tissue for the larvae (Rahmathulla  
318 and Suresh 2012). However, the present results verify that the absorptive capacity of  
319 larvae (i.e. AD) is inversely proportional to ECD and ECI, as previously suggested  
320 (Waldbauer 1968; Xue et al. 2010; Teimouri et al. 2015). Increased consumption would  
321 accelerate passage of food through the gut and thereby reduce AD. In our research with  
322 the leaf beetle, we found that larvae reared on leaves treated with O<sub>3</sub> showed an increase  
323 in the consumption rates and thereby a decreased AD. Lower AD of the leaf beetle larvae  
324 indicates an adaptation to compensate for an increase in ECD and ECI which may result  
325 from a nutritional imbalance. For example, Adham et al. (2005a,b) showed that the 6<sup>th</sup>  
326 instar of *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae compensated for lower  
327 ECD by showing higher AD. O<sub>3</sub> effects did vary significantly among instar stages  
328 ( $F=41.0$ ,  $P<0.001$ ). Larvae of ambient O<sub>3</sub> had non-different AD in 3<sup>rd</sup> instar compared to  
329 2<sup>nd</sup> instar but they had lower AD in 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> instars and greater AD in 7<sup>th</sup> instar than  
330 in 2<sup>nd</sup> instar; AD was similar among 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> instars. Larvae of elevated O<sub>3</sub> had

331 lower AD in 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> instars and greater in 7<sup>th</sup> instar than in 2<sup>nd</sup> instar, but they had  
332 non-different AD between 6<sup>th</sup> and 2<sup>nd</sup> instars and among 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> instars.

333 Larvae of elevated O<sub>3</sub> had lower AD in 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> instar stages and greater AD in 6<sup>th</sup>  
334 stage than larvae of ambient O<sub>3</sub>. Among all instar stages, larvae at 3<sup>rd</sup> instar were the most  
335 nutritionally responsive to O<sub>3</sub>, as indicated by ECI, ECD and AD. In an earlier  
336 experiment, ECI, ECD and AD of 3<sup>rd</sup> and 4<sup>th</sup> instar larvae of the monarch butterfly  
337 (*Danaus plexippus* L.) fed with leaf tissues of *Asclepias curassavica* L. and *A. syriaca* L.  
338 were not different between O<sub>3</sub>-treated and O<sub>3</sub>-untreated tissues (Bolsinger et al., 1992).

339 However, small leaf disks were used in that assay, and the indices were calculated only  
340 after a short exposure (maximum 24 h) of insects to the tissues. In our assay, larvae were  
341 provided with fresh leaves on a daily base and followed over their larval life cycle. AD  
342 may not be a sensitive index to changes in leaf secondary compounds as previously found  
343 in larvae of *A. alni* L. (Firidin and Mutlu 2009).

344 Rapid growth of larvae may be associated with increased gross feed efficiency (Medrano  
345 and Gall, 1975). Larvae may display a rapid growth (growth = CI × ECD × AD) as a  
346 result of a nutritional overcompensation if O<sub>3</sub>-treated leaves have any costly effects  
347 (Manuwoto and Scriber 1985; Rahmathulla and Suresh 2012). In our assays, larvae fed  
348 with elevated O<sub>3</sub>-treated leaved (M=287.7 ±50.7) had greater growth ( $F=25.9, P<0.01$ )

349 than larvae fed with ambient O<sub>3</sub>-treated leaves (M=228.1 ±57.3), independently from  
350 larval stage. Therefore, larvae may have displayed a nutritional overcompensation. When  
351 insects consume leaf tissues which lack materials for the development of their body, ECI  
352 and ECD are expected to decrease whereas AD to increase (Teimouri et al. 2015). Our  
353 observations were reverse, suggesting that the leaf tissue quality was not degraded. When  
354 nutrients are less abundant in leaves, insects increase their consumption rate, accelerate  
355 food passage through their guts, and decrease AD. More research is required to address  
356 these effects across generations of insects fed with leaves grown under elevated O<sub>3</sub>.

357 It should be noted that the results may differ at field because O<sub>3</sub> may transform the scent  
358 or degrade leaf-emitting volatile organic compounds (VOCs), becoming thus a repellent  
359 to herbivores and imbedding the plant-insect communication (Fuhrer and Booker 2003;  
360 Lindroth 2010; Fuentes et al. 2013; Blande et al. 2014; Cui et al. 2014; Farré-Armengol  
361 et al. 2016; Li et al. 2016). Nonetheless, the results of the present study are the first of  
362 their kind and are still valuable because in an O<sub>3</sub>-polluted environment insects will not  
363 have the choice to select among O<sub>3</sub> conditions and thus will have to graze leaves at the  
364 relevant area.

365

366 **3. CONCLUSIONS**

- 367 • O<sub>3</sub> does indirectly change the growth and nutrition of the leaf beetle larvae.
- 368 • O<sub>3</sub> treatment of leaves may enhance the insect performance which may be proved
- 369 costly for the plants under field conditions.
- 370 • ECD and AD can be utilized as efficient biomarkers of O<sub>3</sub> injury.
- 371 • Third instars can serve as the most effective O<sub>3</sub> bioindicator among all the larval
- 372 instars of the leaf beetle.
- 373 • When needed, control of the leaf beetle at an O<sub>3</sub>-enriched environment should be
- 374 conducted before the 4<sup>th</sup> instar stage where larvae can cause greater injuries to
- 375 plants (greater ECI and ECD).
- 376 • Indirect O<sub>3</sub>-induced alterations of insect physiology through consumption of
- 377 ozonated leaf tissues require further experimentations to reveal potential
- 378 consequences over insect generations.

379 **References**

- 380 Abu ElEla SA, ElSayed WM (2015) The influence of cadmium on the food consumption
- 381 and utilization of the cotton leaf worm *Spodoptera littoralis* (Boisd.)
- 382 (Lepidoptera: Noctuidae). Ecol Balkan 7:81–85.
- 383 Abu ElEla SA, Nassar MM, Eesa NN (2016) Impact of lead acetate on quantitative

384 consumption and utilization of the cotton leaf worm, *Spodoptera littoralis*  
385 (Boisduval, 1833) (Lepidoptera: Noctuidae). Ecol Balkan 8: 101–106.

386 Adham FK, Gabre RM, Abu El-Ela SA, Hassan MM (2005a) Growth and feeding  
387 efficiency of cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera:  
388 Noctuidae) on cotton plant *Gossypium barbadens* (Malvaceae) grown in  
389 enriched CO<sub>2</sub> atmosphere. Bul Entom Soc Egy 82:187–196.

390 Adham FK, Gabre RM, Abu El-Ela SA (2005b) The performance parameters of  
391 *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) fed on cotton leaves  
392 grown in enriched CO<sub>2</sub> atmosphere. Bull Entom Soc Egy 82:197–205.

393 Agathokleous E (2018) Environmental hormesis, a fundamental non-monotonic  
394 biological phenomenon with implications in ecotoxicology and environmental  
395 safety. Ecotox Environ Safe 148:1042–1053. DOI:  
396 10.1016/j.ecoenv.2017.12.003 [In Press]

397 Agathokleous E, Koike T, Watanabe M, Hoshika Y, Saitanis CJ (2015a) Ethylene-di-urea  
398 (EDU), an effective phytoprotectant against O<sub>3</sub> deleterious effects and a valuable  
399 research tool. J Agric Meteor 71:185–195.

400 Agathokleous E, Saitanis CJ, Koike T (2015b) Tropospheric O<sub>3</sub>, the nightmare of wild  
401 plants: A review study. J Agric Meteor 71:142–152.



402 Agathokleous E, Saitanis CJ, Wang X, Watanabe M, Koike T (2016a) A review study on  
403 past 40 years of research on effects of tropospheric O<sub>3</sub> on belowground structure,  
404 functioning and processes of trees: a linkage with potential ecological  
405 implications. *Wat Air Soil Poll* 227:33.

406 Agathokleous E, Saitanis CJ, Stamatelopoulos D, Mouzaki-Paxinou A-C, Paoletti E,  
407 Manning WJ (2016b) Olive oil for dressing plant leaves so as to avoid O<sub>3</sub> injury.  
408 *Wat Air Soil Poll* 227:282.

409 Agathokleous E, Sakikawa T, Abu ElEla SA, Mochizuki T, Nakamura M, Watanabe M,  
410 Kawamura K, Koike T (2017a) Ozone alters the feeding behavior of the leaf  
411 beetle *Agelastica coerulea* (Coleoptera: Chrysomelidae) into leaves of Japanese  
412 white birch (*Betula platyphylla* var. *japonica*). *Environ Sci Poll Res* 24:  
413 17577–17583.

414 Agathokleous E, Vanderstock A, Kita K, Koike T (2017b) Stem and crown growth of  
415 Japanese larch and its hybrid F<sub>1</sub> grown in two soils and exposed to two free-air  
416 O<sub>3</sub> regimes. *Environ Sci Poll Res* 24:6634–6647.

417 Ainsworth EA, Yendrek CR, Sitch S, Collins WJ, Emberson LD (2012) The effects of  
418 tropospheric ozone on net primary productivity and implications for climate  
419 change. *Annu Rev Plant Biol* 63:637–661.

- 420 Ali JG, Agrawal AA (2012) Specialist versus generalist insect herbivores and plant  
421 defense. *Trends Plant Sci* 17:293–302.
- 422 Alstad DN, Edmunds GFJr, Weinstein LH (1982) Effects of air pollutants on insect  
423 populations. *Ann Rev Entomol* 27:369–384.
- 424 Blande JD, Holopainen JK, Niinements Ü (2014) Plant volatiles in polluted atmospheres:  
425 stress responses and signal degradation. *Plant Cell Environ* 37:1892–1904.
- 426 Bolsinger M, Lier ME, Lansky DM, Hughes PR (1991) Influence of ozone air pollution  
427 on plant-herbivore interactions. Part 1: biochemical changes in ornamental  
428 milkweed (*Asclepias curassavica* L.; Asclepiadaceae) induced by ozone. *Environ*  
429 *Pollut* 72:69–83.
- 430 Bolsinger M, Lier ME, Hughes PR (1992) Influence of ozone air pollution on  
431 plant-herbivore interactions. Part 2: Effects of ozone on feeding preference,  
432 growth and consumption rates of monarch butterflies (*Danaus plexippus*).  
433 *Environ Pollut* 77:31–37.
- 434 Box GEP, Cox DR (1964) An analysis of transformations. *J Roy Stat Soc B* 26:211–252.
- 435 Cape JN (2008) Surface ozone concentrations and ecosystem health: Past trends and a  
436 guide to future projections. *Sci Tot Environ* 400:257–269.
- 437 Chappelka A H, Grulke NE (2016) Disruption of the ‘disease triangle’ by chemical and

438 physical environmental change. *Plant Biol* 18:5–12.

439 Chappelka AH, Kraemer ME, Mebrahtu T, Rangappa M, Benepal PS (1988) Effects of  
440 ozone on soybean resistance to the Mexican bean beetle (*Epilachna varivestis*  
441 mulsant). *Environ Exp Bot* 28:53–66.

442 Cohen A (2015) *Insect diets: science and technology*, 2<sup>nd</sup> edition. CRC Press, Florida. 473  
443 pp.

444 Cui H, Su J, Wei J, Hu Y, Ge F (2014) Elevated O<sub>3</sub> enhances the attraction of  
445 whitefly-infested tomato plants to *Encarsia formosa*. *Sci Rep* 4:5350.

446 Derwent RG, Simmonds PG, Manning AJ, Spain TG (2007) Trends over a 20-year period  
447 from 1987 to 2007 in surface ozone at the atmospheric research station, Mace  
448 Head, Ireland. *Atmos Environ* 41:9091–9098.

449 Dötterl S, Vater M, Rupp T, Held A (2016) Ozone differentially affects perception of  
450 plant volatiles in western honey bees. *J Chem Ecol* 42:486–489.

451 Endress AG, Post SL (1985) Altered feeding preference of Mexican bean beetle  
452 *Epilachna varivestris* for ozonated soybean foliage. *Environ Pollut* 39:9–16.

453 Farrar RR, Barbour JD, Kennedy GG (1989) Quantifying food consumption and growth  
454 in insects. *Ann Entomol Soc Am* 82:593–598.

455 Farré-Armengol G, Peñuelas J, Li T, Yli-Pirilä P, Filella I, Llusia J, Blande JD (2016)

456 Ozone degrades floral scent and reduces pollinator attraction to flowers. *New*  
457 *Phytol* 209:152–160.

458 Feng Z, Kobayashi K, Ainsworth E (2008) Impact of elevated ozone concentration on  
459 growth, physiology, and yield of wheat (*Triticum aestivum* L.): a meta-analysis.  
460 *Global Chang Biol* 14:2696–2708.

461 Firidin B, Mutlu C (2009) Nitrogen utilization pattern and degradation capability of some  
462 plant secondary metabolites by *Agelastica alni* L. (Coleoptera: Chrysomelidae).  
463 *J Entomol Res Soc* 11:2.

464 Fred PH (1987) Interactions of insects, trees and air pollutants. *Tree Physiol* 3:93–102.

465 Freiwald V, Häikiö E, Julkunen-Tiitto R, Holopainen JK, Oksanen E (2008) Elevated  
466 ozone modifies the feeding behaviour of the common leaf weevil on hybrid  
467 aspen through shifts in developmental, chemical, and structural properties of  
468 leaves. *Entomol Exp Applic* 128:66–72.

469 Fuentes JD, Roulston TH, Zenker J (2013) Ozone impedes the ability of a herbivore to  
470 find its host. *Environ Res Let* 8:014048.

471 Fuhrer J, Booker F. (2003) Ecological issues related to ozone: agricultural issues. *Environ*  
472 *Int* 29:141–154.

473 Fuhrer J, Val Martin M, Mills G, Heald CL, Harmens H, Hayes F, Sharps K, Bender J,

474 Ashmore MR (2016). Current and future ozone risks to global terrestrial  
475 biodiversity and ecosystem processes. *Ecol Evol* 6:8785–8799.

476 Hain FP (1987) Interactions of insects, trees and air pollutants. *Tree Physiol* 3:93–102.

477 Hedges LV, Olkin I (1985) *Statistical methods for meta-analysis*, 1<sup>st</sup> edn. Academic Press,  
478 Orlando, FL. 369pp.

479 Hemati SA, Naseri B, Ganbalani GN, Dastjerdi HR, Golizadeh A (2012). Effect of  
480 different host plants on nutritional indices of the pod borer, *Helicoverpa*  
481 *armigera*. *J Insect Sci* 12:55

482 Hill T, Lewicki P (2006) *Statistics : methods and applications : a comprehensive reference*  
483 for science, industry, and data mining. StatSoft.

484 Hillstrom ML, Lindroth RL (2008) Elevated atmospheric carbon dioxide and ozone alter  
485 forest insect abundance and community composition. *Insect Cons Diver*  
486 1:233–241.

487 Hocking RR (1996) *Methods and Applications of Linear Models: Regression and the*  
488 *Analysis of Variance*, 3<sup>rd</sup> edition. New York, Wiley, 720p.

489 Hughes PR (1988) Insect populations on host plants subjected to air pollution. In:  
490 Heinrichs EA (Ed), *Plant Stress-Insect Interactions*. Chichester, John Wiley, pp.  
491 249-319.

492 Japan Meteorological Agency (2017) <http://www.jma.go.jp/jma/indexe.html> Website  
493 accessed on 27th May 2017

494 Jones CG, Coleman JS (1988a) Plant stress and insect behavior: cottonwood, ozone and  
495 the feeding and oviposition preference of a beetle. *Oecologia* 76:51–56.

496 Jones CG, Coleman JS (1988b) Leaf disc size and insect feeding preference: implications  
497 for assays and studies on induction of plant defense. *Entom. Exp. Appl.*  
498 47:167–172.

499 Kogan M (1986) Bioassays for measuring quality of insect food. In: Miller JR, Miller TA  
500 (Eds.), *Insect-Plant Interactions.*, New York, Springer-Verlag, pp. 155-189.

501 Koike T (1995) Vegetation Science in Forestry: Global Perspective based on Forest  
502 Ecosystems of East & Southeast Asia. In: Box E.O. et al. (Eds.), *Physiological*  
503 *ecology of the growth characteristics of Japanese mountain birch in northern*  
504 *Japan: a comparison with Japanese mountain white birch*, Kluwer Academic  
505 Publishers, The Netherlands, pp.409–422.

506 Koike T, Tobita H, Shibata T, Mastuki S, Konno K, Kitao M, Yamashita N, Maruyama, Y.  
507 (2006) Defense characteristics of seral deciduous broad-leaved tree seedlings  
508 grown under differing levels of CO<sub>2</sub> and nitrogen. *Popul Ecol* 48:23–29.

509 Koike T, Watanabe M, Hoshika Y, Kitao M, Matsumura H, Funada R, Izuta T (2013)

510 Effects of ozone on forest ecosystems in East and Southeast Asia. In: Matyssek  
511 R, Clarke N, Cudlin P, Mikkelsen TN, Tuovinen J-P, Wieser G, Paoletti E (Eds.),  
512 Climate Change, Air Pollution and Global Challenges. Elsevier Pub. pp.  
513 371–390.

514 Lacey LA (1997) Manual of techniques in insect pathology. Bath, Academic Press, 409p.

515 Li T, Blande JD, Holopainen JK (2016) Atmospheric transformation of plant volatiles  
516 disrupts host plant finding. *Sci Rep* 6:33851.

517 Lindroth RL (2010) Impacts of elevated atmospheric CO<sub>2</sub> and O<sub>3</sub> on forests:  
518 phytochemistry, trophic interactions, and ecosystem dynamics. *J Chem Ecol*  
519 36:2–21.

520 Manuwoto S, Scriber JM (1985) Consumption and utilization of experimentally altered  
521 corn by southern armyworm: Iron, nitrogen, and cyclic hydroxamates. *J Chem*  
522 *Ecol* 11:1469–1483.

523 Matsuki S, Sano Y, Koike T (2004) Chemical and physical defense in the early and late  
524 leaves in three heterophyllous birch species native to northern Japan. *Annals Bot*  
525 93:141–147.

526 Mattson WJ, Haack RA (1987) Role of drought in outbreaks of plant-eating insects.  
527 *Bioscience* 37:110–118.

- 528 Medrano JF, Gall GAE (1975) Food consumption, feed efficiency, metabolic rate and  
529 utilization of glucose in lines of *Tribolium castaneum* selected for 21-day pupa  
530 weight. *Genetics* 83:393–407.
- 531 Nagashima T, Ohara T, Sudo K, Akimoto H (2010) The relative importance of various  
532 source regions on East Asian surface ozone. *Atmos Chem Phys* 10:11305–11322,
- 533 Nagashima T, Sudo K, Akimoto H, Kurokawa, J, Ohara T (2017) Long-term change in  
534 the source contribution to surface ozone over Japan. *Atmos Chem Phys*  
535 17:8231–8246.
- 536 Oksanen E, Pandey V, Keski-Saari S, Kontunen-Soppela S, Sharma C (2013) Impacts of  
537 increasing ozone on Indian plants. *Environ Pollut* 177:189–200.
- 538 Rahmathulla VK, Suresh HM (2012) Seasonal variation in food consumption,  
539 assimilation, and conversion efficiency of Indian bivoltine hybrid silkworm,  
540 *Bombyx mori*. *J Insect Sci* 12:82.
- 541 Reese JC, Beck SD (1976) Effects of allelochemicals on the black cut worm, *Agrotis*  
542 *ipsilon*; effects of pbenzoquinone, hydroquinone, and duroquinone on larval  
543 growth, development, and utilization of food. *Ann Entomol Soc Am* 69:59–67.
- 544 Robinson JM, Rowland RA (1996) Carbohydrate and carbon metabolite accumulation  
545 responses in leaves of ozone tolerant and ozone susceptible spinach plants after



546 acute ozone exposure. *Photos Res* 50:103–115.

547 Royal Society (2008) Ground-level Ozone in the 21st Century: 1 Future Trends, Impacts  
548 and Policy Implications. Science Policy Report 15/08.

549 Sakikawa T, Oikawa M, Watanabe M, Mao Q, Koike T (2014) The effect of ozone on leaf  
550 phenology of white birch (*Betula platyphylla* var. *japonica*) grown under free-air  
551 ozone exposure. *Boreal For Res* 62:59–60. (in Japanese)

552 Sakikawa T, Nakamura M, Watanabe M, Oikawa M, Satoh F, Koike T (2016) Leaf  
553 phenology and insect grazing of Japanese white birch saplings grown under  
554 free-air ozone exposure. *J Agr Meteorol* 72: 80–84.

555 Sas Institute. 2002. SAS/STAT User's Guide. Version 9.1. SAS Institute, Cary, NC.

556 Sicard P, Serra R, Rossello P (2016) Spatiotemporal trends in ground-level ozone  
557 concentrations and metrics in France over the time period 1999–2012. *Environ*  
558 *Res* 149:122–144.

559 Sicard P, Anav A, De Marco A, Paoletti E (2017) Projected global tropospheric ozone  
560 impacts on vegetation under different emission and climate scenarios. *Atmos*  
561 *Chem Phys Discuss*. doi:10.5194/acp-2017-74

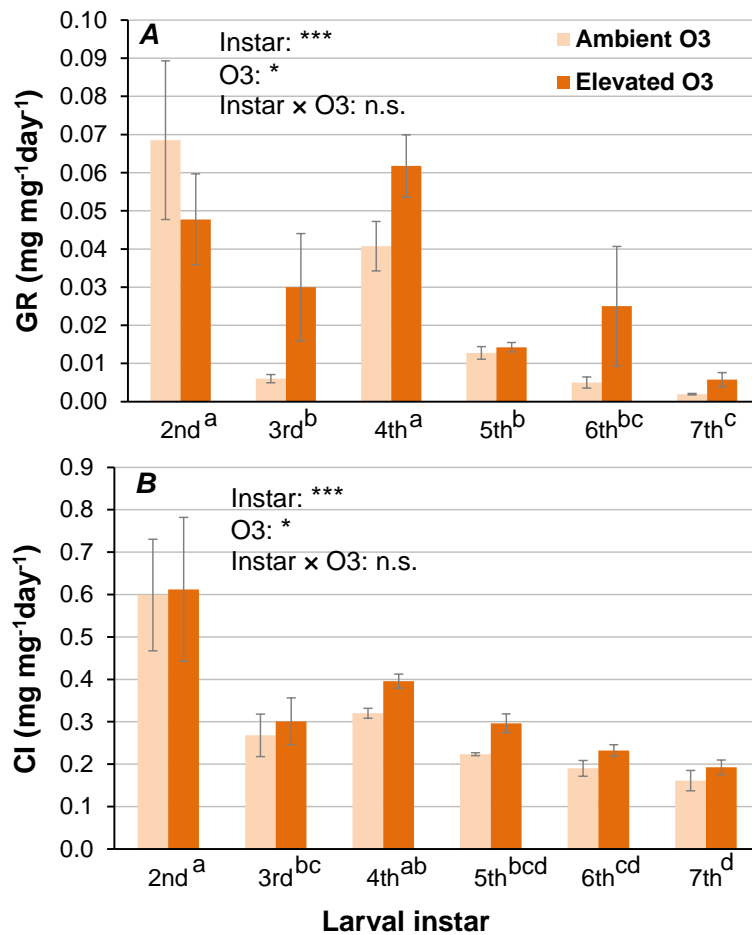
562 Slansky JrF (1985) Food utilization by insects: Interpretation of observed differences  
563 between dry weight and energy efficiencies. *Entom Exp Applic* 39:47–60.

- 564 Smith CM, Khan ZR, Dutta Pathak M (1994) Techniques for Evaluating Insect  
565 Resistance in Crop Plants. CRC Press LLC, 336p.
- 566 Solomou E, Poupkou A, Bolis S, Zanis P, Lazaridis M, Melas D (2018) Evaluating  
567 near-surface ozone levels simulated from MACC global and regional modeling  
568 systems in Eastern Mediterranean under the influence of Etesian winds. Atmos  
569 Res. doi: 10.1016/j.atmosres.2017.09.010
- 570 Tanimoto H (2009) Increase in springtime tropospheric ozone at a mountainous site in  
571 Japan for the period 1998–2006. Atmos Environ 43:1358–1363.
- 572 Tanimoto H, Ohara T, Uno I (2009) Asian anthropogenic emissions and decadal trends in  
573 springtime tropospheric ozone over Japan: 1998–2007. Geophys Res Lett 36:  
574 L23802.
- 575 Teimouri N, Sendi JJ, Zibae A, Khosravi R (2015) Feeding indices and enzymatic  
576 activities of carob moth *Ectomyelois ceratoniae* (Zeller) (Lepidoptera:  
577 Pyralidae) on two commercial pistachio cultivars and an artificial diet. J Saudi  
578 Soc Agric Sci 14:76–82.
- 579 Trieu TT, Goto D, Yashiro H, Murata R, Sudo K, Tomita H, Satoh M, Nakajima T (2017)  
580 Evaluation of summertime surface ozone in Kanto area of Japan using a  
581 semi-regional model and observation. Atmos Environ 153:163–181.

- 582 Vaultier M-N, Jolivet Y (2015) Ozone sensing and early signaling in plants: An outline  
583 from the cloud. *Environ Exp Bot* 114:144–152.
- 584 Valkama E, Koricheva J, Oksanen E (2007) Effects of elevated O<sub>3</sub>, alone and in  
585 combination with elevated CO<sub>2</sub>, on tree leaf chemistry and insect herbivore  
586 performance: a meta-analysis. *Glob Change Biol* 13:184–201.
- 587 Vanderstock A, Agathokleous E, Inoue W, Eguchi N, Nakamura M, Satoh F, Kanie S,  
588 Koike T (2016) Preliminary survey on insect grazing in white birch stands under  
589 free-air O<sub>3</sub> fumigation. *Bor For Res* 64:27–29.
- 590 Vingarzan R (2004) A review of surface ozone background levels and trends. *Atmos*  
591 *Environ* 38:3431–3442.
- 592 Waldbauer GP (1968) The consumption and utilization of food by insects. *Advanc Ins*  
593 *Physiol* 5:229–288.
- 594 Wang T, Xue L, Brimblecombe P, Lam YF, Li L, Zhang L (2017) Ozone pollution in  
595 China: A review of concentrations, meteorological influences, chemical  
596 precursors, and effects. *Sci Tot Environ* 575:1582–1596.
- 597 White TCR (1974) A hypothesis to explain outbreaks of looper caterpillars, with special  
598 reference to populations of *Selidosema suavis* in a plantation of *Pinus radiata* in  
599 New Zealand. *Oecologia* 16:279–301.

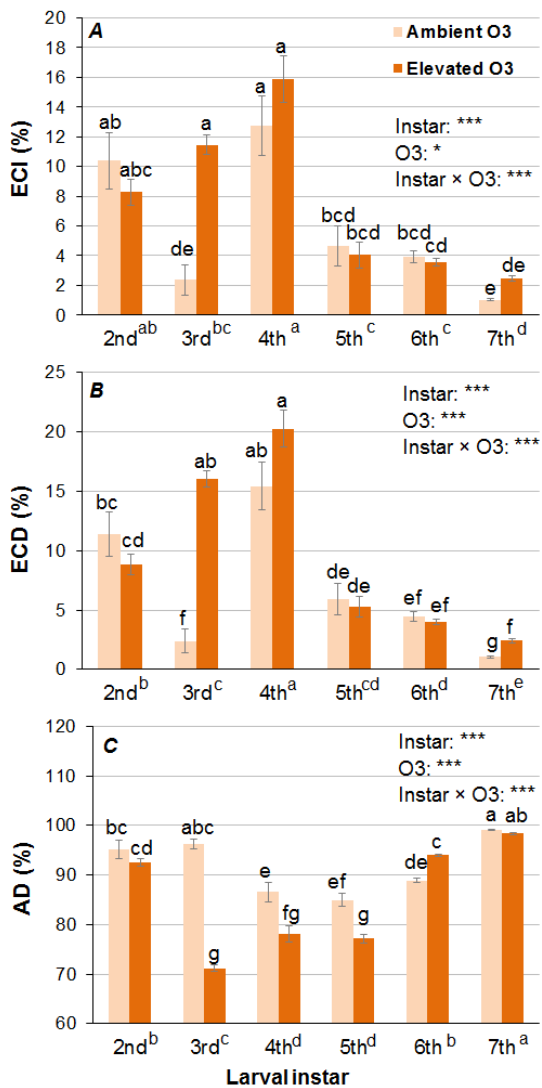
- 600 White TCR (1976) Weather, food and plagues of locusts. *Oecologia* 22:119–134.
- 601 White TCR (1984) The abundance of invertebrate herbivores in relation to the availability  
602 of nitrogen in stressed plants. *Oecologia* 63:9C~105.
- 603 Whittaker JB, Kristiansen LW, Mikkelsen TN Moore R (1989) Responses to ozone of  
604 insects feeding on a crop and a weed species. *Environ Pollut* 62:89–101.
- 605 Wolf FM (1986) *Meta-analysis: Quantitative Methods for Research Synthesis*. Beverly  
606 Hills, CA: Sage.
- 607 Woodring JP, Clifford CW, Roe RM, Beckman BR (1978) Effects of CO<sub>2</sub> and anoxia on  
608 feeding, growth, metabolism, water balance, and blood composition in larval  
609 house crickets, *Acheta domesticus*. *J Ins Physiol* 24:499–509.
- 610 Xue M, Pang Y-H, Wang HT, Li Q-L, Liu TX (2010) Effects of four host plants on  
611 biology and food utilization of the cutworm, *Spodoptera litura*. *J Insect Sci*  
612 10:22.
- 613 Yli-Pirilä P, Copolovici L, Kännaste A, Noe S, Blande JD, Mikkonen S, Klemola T,  
614 Pulkkinen J, Virtanen A, Laaksonen A, Joutsensaari J, Niinemets Ü, Holopainen  
615 JK (2016) Herbivory by an outbreaking moth increases emissions of biogenic  
616 volatiles and leads to enhanced secondary organic aerosol formation capacity.  
617 *Environ Sci Tech* 50:11501–11510.

618 **Fig 1.** Means  $\pm$  SE ( $n = 4$ ) of growth rate (GR) (A) and consumption index (CI) (B) of 2nd, 3rd, 4th, 5th,  
619 6th, and 7th larvae instars of the leaf beetle *Agelastica coerulea* (Baly, 1874) (hereafter leaf beetle) fed with  
620 leaves of Japanese white birch (*Betula platyphylla* var. *japonica*) obtained from ambient or elevated O<sub>3</sub>  
621 atmospheres, under laboratory conditions. Asterisks indicate rANOVA significant effects at  $P < 0.05$  (\*),  
622  $P < 0.01$  (\*\*) and  $P < 0.001$  (\*\*\*), whereas “n.s.” indicates non-significant effects ( $P > 0.05$ ). Different  
623 lowercase letters above instar stages show statistically significant differences among the instar stages (O<sub>3</sub>  
624 pooled). Differences are marked according to Bonferroni test ( $\alpha = 0.05$ ).



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630 **Fig 2.** Means  $\pm$  SE ( $n = 4$ ) of efficiency conversion of ingested food (ECI) (A), efficiency conversion of  
 631 digested food (ECD) (B), and approximate digestibility (AD) (C) of 2nd, 3rd, 4th, 5th, 6th, and 7th larvae  
 632 instars of the leaf beetle *Agelastica coerulea* (Baly, 1874) (hereafter leaf beetle) fed with leaves of Japanese  
 633 white birch (*Betula platyphylla* var. japonica) obtained from ambient or elevated O<sub>3</sub> atmospheres, under  
 634 laboratory conditions. Asterisks indicate rANOVA significant effects at  $P < 0.05$  (\*),  $P < 0.01$  (\*\*) and  
 635  $P < 0.001$  (\*\*\*), whereas “n.s.” indicates non-significant effects ( $P > 0.05$ ). Different lowercase letters above  
 636 instar stages show statistically significant differences among the instar stages (O<sub>3</sub> pooled). Different  
 637 lowercase letters above the means show statistically significant differences within the interaction Instar  $\times$   
 638 O<sub>3</sub>. Differences are marked according to Bonferroni test ( $\alpha = 0.05$ ).



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