



# Tolerance and acclimation of photosynthesis of nine urban tree species to warmer growing conditions

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**(Citation)**

Trees, 35(6):1793-1806

**(Issue Date)**

2021-12

**(Resource Type)**

journal article

**(Version)**

Accepted Manuscript

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1 Tolerance and acclimation of photosynthesis of nine urban tree species to  
2 warmer growing conditions

3

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18

19 **Abstract (250)**

20 To prepare and manage urban greenspace for a warmer future, we must select trees that can  
21 tolerate or acclimate to warmer growing conditions. Here, we compared tolerance and acclimation  
22 of photosynthesis to warmer growing conditions among urban trees species in Japan. Two paired  
23 experiments were installed where saplings of nine species were grown outside at two locations  
24 (warm- and cool-temperate climates) and in a greenhouse with and without passive warming. We  
25 compared the temperature where quantum yield declined to 50% of maximum value ( $T_{50}$ ) due to  
26 excess heat (50-60°C) and the thermal optimum for photosynthesis ( $T_{opt}$ ) among species. For two  
27 deciduous and three evergreen species,  $T_{50}$  was higher for saplings grown in warm- than cool-  
28 temperate climate.  $T_{50}$  was also higher under passive warming for three species, of which two  
29 evergreen species (*Morella rubra*, *Quercus myrsinifolia*) showed marked increases (5-7°C).  $T_{opt}$   
30 was higher under passive warming in three of six species examined, but the acclimation response  
31 of photosynthesis and stomatal conductance to warming was highly species-specific. We inferred  
32 that *M. rubra* and *Q. myrsinifolia*, which acclimated consistently to warmer growing conditions  
33 in both experiments, are heat-hardy species. Our results also suggest warm-temperate evergreen  
34 species are not necessarily more tolerant of warmer growing conditions than cool-temperate  
35 deciduous species. For example, despite its warm-temperate origin, *Machilus thumbergii* showed  
36 no acclimation response and photosynthetic rates were lower under passive warming. Our results  
37 indicate species need to be screened individually to assess their physiological tolerance and  
38 acclimation potential to warmer climate.

39

40 **Keywords:** climate change, global warming, photosynthetic acclimation, urban trees

41 **Declarations**

42

43 Funding: This research was funded by a grant from Sumitomo Forestry Inc. to Kobe University.

44

45 Conflicts of interest/Competing interests (include appropriate disclosures): none declared.

46

47 Availability of data and material (data transparency): Data will be made available in a database  
48 at Kobe University.

49

50 Code availability (software application or custom code): NA

51

52 Authors' contributions:

53 HI and SI conceived and designed the study. SI prepared the plant material and CH, SI, and HI

54 made the measurements with assistance from MN and HK. MO helped with statistical analyses.

55 HI wrote the paper and all co-authors have approved of the draft.

56

57 Ethics approval (include appropriate approvals or waivers): NA

58

59 Consent to participate (include appropriate statements): NA

60

61 Consent for publication (include appropriate statements): NA

62

63 Availability of data and material (data transparency):

64

65 **Key Message**

66 Tolerance and acclimation of photosynthesis to warmer growing conditions among nine urban  
67 tree species was species-specific indicating that individual screening is needed to prepare urban  
68 greenspace for a warmer future.

69

70 **Introduction**

71 Climate change is already affecting the global environment (Pachauri et al. 2014). Impacts on  
72 trees include, reductions in growth and increasing mortality due to drought (Carnicer et al. 2011;  
73 McDowell and Allen 2015; Williams et al.), changes in leafing and flowering phenology due to  
74 warming (Cleland et al. 2007; Menzel et al. 2006), latitudinal and altitudinal range shifts and local  
75 decline/extinction of species (Jump et al. 2009; Lenoir et al. 2008; Thomas et al. 2004). Global  
76 climate change is progressing at rates faster than any previous climate shift in Earth's history  
77 (Visser 2008; Zachos et al. 2001). Because of their slow regeneration rates, most tree species will  
78 not be able to migrate or adapt quickly enough in response to climate change (Jump and Penuelas  
79 2005). Extant trees, therefore, must acclimate in situ in order to survive.

80 Social adaptation to climate change includes redesigning and managing urban  
81 greenspace for a warmer future (Demuzere et al. 2014; Gill et al. 2007). Urban greenspace plays  
82 an important role in mitigating negative effects of climate change on our society, including  
83 buffering heat island effects and controlling stormwater runoff. (Caplan et al. 2019; Emmanuel  
84 and Loconsole 2015). These functions could be lost if tree health is not maintained and urban  
85 greenspace managed sustainably (Chen et al. 2017). For example, to adapt urban greenspace to  
86 future climate, the city of Melbourne is moving away from planting European species, which are  
87 susceptible to heat and drought, to using more tolerant native species (Nitschke et al. 2017; Norton  
88 et al. 2013). Selecting trees for adaptation of urban greenspace to climate change, however, is not  
89 a straightforward task (Roy et al. 2017). Planting species originating in warmer climates may not  
90 necessarily be the solution, because tropical and sub-tropical species are not adapted to  
91 temperature fluctuation and have low acclimation potential to changes in temperature  
92 (Cunningham and Read 2002; Perez et al. 2016). Therefore, to prepare for a warmer future, we  
93 must develop methods to assess species' tolerance and acclimation potential to warmer climate.

94 In urban areas of the warm-temperate zone, summer surface temperatures near the

95 pavement may exceed 50°C (Chen et al. 2017; Emmanuel and Loconsole 2015). When exposed  
96 to high temperatures, plants maintain leaf temperature by increasing transpiration (Pallardy 2008).  
97 This could lead to water deficit for urban trees, which have limited access to ground water (Pataki  
98 et al. 2011). Stomatal closure to maintain leaf water balance, on the other hand, could lead to  
99 overheating and ultimately to carbon starvation (McDowell et al. 2008; Sevanto et al. 2014).  
100 Physiological acclimation to high temperature can be achieved by either up-regulating  
101 photosynthesis or down-regulating respiration to maintain positive carbon balance (Dusenge et al.  
102 2019; Lombardozzi et al. 2015; Slot and Kitajima 2015). At the leaf level, photosynthetic rate  
103 generally shows a unimodal response to temperature, defining a thermal optimum for  
104 photosynthesis ( $T_{opt}$ ). Photosynthetic rate decreases at higher temperatures not only due to  
105 stomatal closure, but also slower rates of enzymatic reactions, and extreme temperatures can cause  
106 biochemical damage to photosynthetic proteins and membranes (Hikosaka et al. 2006;  
107 Kumarathunge et al. 2019; Sage and Kubien 2007; Yamori et al. 2014). Leaves may acclimate to  
108 high temperature through an upward shift in  $T_{opt}$  and/or increasing photosynthetic rates at higher  
109 temperature (Berry and Bjorkman 1980; Kumarathunge et al. 2019; Way and Yamori 2014). The  
110 thermal optimum for photosynthesis varies among species as a result of adaptation to climatic  
111 conditions in their native habitat, as well as among individuals due to photosynthetic acclimation  
112 to specific growing conditions (Cheesman and Winte 2013; O'Sullivan et al. 2017; Sendall et al.  
113 2015). Previous studies, which examined photosynthetic thermal acclimation of leaves, present  
114 mixed results. In tropical trees, experimental warming of leaves produced no or negative (down-  
115 regulation) photosynthetic acclimation responses (Doughty 2011; Slot et al. 2014). In cool-  
116 temperate trees, photosynthetic rates did not acclimate (up-regulate) in response to experimental  
117 warming, but rather decreased (Carter and Cavaleri 2018). These results suggest, photosynthetic  
118 response to warming can be highly variable among species and regions.

119 Here, we compared tolerance and acclimation of leaf photosynthetic properties to

120 warmer growing conditions among nine urban tree species commonly planted across Japan. Two  
121 paired experiments were installed. The same set of saplings were grown under ambient conditions  
122 in warm- and cool-temperate climates at two different locations. Another set of saplings were  
123 grown under controlled conditions in a greenhouse with and without passive warming. To  
124 compare thermal tolerance and thermal optimum among species, we measured threshold  
125 temperatures for loss of leaf photosynthetic function and the response of light-saturated  
126 photosynthetic rate to temperature. We inferred that species with greater thermal tolerance are  
127 those which maintain leaf photosynthetic function at higher temperatures. Species with greater  
128 thermal acclimation are those which exhibit significant responses to differences in climate and to  
129 the warming treatment.

130

### 131 **Materials and Methods**

132 Four-year-old, potted saplings of three deciduous: *Cornus florida* L., *Liquidambar styraciflua* L.,  
133 and *Zelkova serrata* (Thunb.) Makino, and six evergreen trees: *Fraxinus griffithii* C.B. Clarke,  
134 *Ligustrum lucidum* Aiton, *Ligustrum japonicum* Thunb., *Machilus thunbergii* Sieb. et Zucc.,  
135 *Morella rubra* Lour., *Quercus myrsinifolia* Blume, were used in the common garden experiment  
136 (Table 1). We installed two paired experiments in April 2019. In the “regional experiment”, four  
137 replicate pots of each species were grown outside at two locations in Japan. One set was grown  
138 in cool-temperate climate at the Monbetsu Station, Sumitomo Forestry Co. Ltd. in Monbetsu City,  
139 Hokkaido (44.36°N, 143.30°E) and another in warm-temperate climate at the Tsukuba Research  
140 Institute, Sumitomo Forestry Co. Ltd. in Tsukuba City, Ibaraki Prefecture (36.11°N, 140.02°E).  
141 We chose these two locations because they have contrasting temperatures (5.2-8.8°C difference  
142 in mean monthly temperature during the study period), while relative humidity and solar radiation  
143 were similar (Table S1). To control for differences in precipitation, the pots were watered regularly.  
144 All saplings were planted in pots (height x diameter = 186 x 205 mm) filled with 5:3:2 mixtures

145 of red ball earth, Kanuma pumice, and manure. Plant sizes are shown in Table 1. All saplings  
146 were watered for 15 min at 5:00 am and 7:00 pm each day. According to the Japan Meteorological  
147 Agency database, mean monthly temperature and total precipitation at the two locations during  
148 Jan-Nov 2019 were 8.7°C, 708.5 mm and 16.2°C, 1265.0 mm, respectively.

149 In the “greenhouse experiment”, the saplings were grown under controlled conditions  
150 in a greenhouse in Tsukuba. We placed one set (four saplings of each species) inside a 3x3 m  
151 mini-greenhouse constructed using transparent plastic sheeting to passively increase the  
152 temperature (hereafter: “passive warming”, Fig. 1). A sheet of transparent plastic was placed over  
153 the saplings outside of the mini-greenhouse (ambient/control) to maintain similar irradiance levels.  
154 We confirmed that maximum irradiance levels were similar and sufficient ( $> 1400 \mu\text{mol m}^{-2}$  on  
155 sunny days) for both treatment and control plants.

156 Temperatures varied with season (Fig. 2). The mean temperature difference between  
157 passive warming and ambient condition was 1.7°C and the mean difference in daily maximum  
158 temperature was 4.5°C. Although we did not measure air humidity in the two treatments, we  
159 acknowledge that it may have been higher inside the mini-greenhouse. We presumed, however,  
160 that higher humidity would allow the saplings to maintain higher stomatal conductance and better  
161 control of leaf temperature. Thus, we inferred that any differences found between passive  
162 warming and ambient conditions would be a conservative result.

163

#### 164 *Thermal tolerance*

165 To assess thermal tolerance, we measured quantum yield (QY) of dark-adapted leaves at various  
166 temperatures (20-60°C) using a FluoroPen FP110 (Photo System Instruments, Drásov, Czech  
167 Republic). Before measurement, we watered the pots and allowed the saplings to rehydrate in  
168 darkness in the lab overnight. Under complete darkness, we cut out leaf discs (ca. 1.5 cm diameter)  
169 using a hole-puncher from intact current-year leaves of each sapling. For each species, four leaf



170 discs (one from each sapling) were sealed in plastic bags with moist cloth to keep humidity within  
171 the bag at equilibrium with leaf moisture. The bags were then sealed and immersed for 30 min in  
172 water heated to a set temperature ( $T$ ) using a make-shift water bath consisting of a sous vide  
173 cooker (Felio F9575, Fujisho Co., Tokyo, Japan) and a 3L molybdenum pot. Then, the bags were  
174 removed from the water and allowed to cool to room temperature for 15 min before measuring  
175 QY with the FluorPen. Here, QY is a fluorometric measurement of maximum quantum yield of  
176 photosystem II, also referred to as  $F_v/F_m$  (maximum – minimum chlorophyll fluorescence  
177 relative to maximum fluorescence). The amount of time for heating and cooling were determined  
178 in a pilot study as necessary for leaves to equilibrate to heat and to room temperature so that we  
179 could determine if irreversible change had occurred. This procedure was repeated for each  
180 temperature setting. Temperature settings were varied and customized for each species to obtain  
181 good regression estimates (see below for statistical analyses). To observe seasonal change in  
182 thermal tolerance, measurements were made twice during the growing season, during and after  
183 the hottest season: Aug 6 and Sept 19 in cool-temperate climate (Monbetsu), Jul 29-Aug 1 and  
184 Sept 24-26 in warm-temperate climate (Tsukuba).

185

#### 186 *Temperature-photosynthesis relationship*

187 We measured the temperature response of light-saturated photosynthetic rate ( $A_{net}$ ) on one leaf  
188 from one representative sapling of six of the nine species (*C. florida*, *Z. serrata*, *L. lucidum*, *L.*  
189 *japonicum*, *M. thumbergii*, and *M. rubra*) in the greenhouse experiment from Sep 1-6 using a  
190 portable photosynthesis measurement system (LI-6400-XT, LI-COR Biosciences, Lincoln, NE,  
191 USA) with LED light source (LI-6400-02B). The CO<sub>2</sub> concentration in the chamber was set to  
192 400 ppm and light intensity was initially set at 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and then increased until  
193 photosynthetic rate reached light-saturation (1200 – 1400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , depending on species).  
194 Initially, room temperature was set to 20°C and the block temperature of the LI-6400 was

195 increased from 15 to 20°C. Then, room temperature was increased to 25°C and block temperature  
196 was increased from 25 to 40°C. As we increased the temperature, relative humidity was adjusted  
197 by running a commercially available humidifier (SH-OR30 WT, Topland Co., Tokyo, Japan) near  
198 the inlet of the LI-6400 to prevent vapor pressure deficit (VPD) from increasing too rapidly. At  
199 each temperature setting, leaf gas exchange was allowed to acclimate to cuvette conditions before  
200 logging ten measurements of  $A_{\text{net}}$ . Temperature settings were varied and customized for each  
201 species to obtain good regression estimates (see below for statistical analyses). From these  
202 measurements, we obtained the relationship between leaf temperature ( $T_{\text{leaf}}$ ) and  $A_{\text{net}}$ , as well as  
203 between VPD and stomatal conductance ( $G_s$ ).

204

#### 205 *Statistical analyses*

206 To assess leaf thermal tolerance of each species, measurements of QY from the four leaf disks  
207 were plotted in relation to  $T$ . We fit a logistic curve to the relationship using non-linear least-  
208 squares regression to obtain the heat tolerance curve for each species:

$$209 \quad \text{QY} = \frac{a}{1 + \exp\{b(T - T_{50})\}} \quad (\text{eq. 1})$$

210 Where  $T_{50}$  is the temperature and  $b$  is the slope at the inflection point where QY is 50% of the  
211 initial, maximum value,  $a$ . The 95% confidence intervals for the parameter estimates were  
212 obtained using the drc package in R (ver. 3.5.3, R Development Core Team).

213 The relationship between  $T_{\text{leaf}}$  and  $A_{\text{net}}$  was analyzed using quadratic regression:

$$214 \quad A_{\text{net}} = c(T_{\text{leaf}} - T_{\text{opt}})^2 + d \quad (\text{eq. 2})$$

215 Where,  $T_{\text{opt}}$  is the optimum temperature when  $A_{\text{net}}$  is highest and  $c$  is a coefficient determining the  
216 concavity of the unimodal curve. Regression analyses were conducted using JMP ver. 15J (SAS  
217 Institute Japan, Tokyo) to calculate 95% confidence intervals for each parameter estimate. The  
218 relationship between VPD and  $G_s$  was analyzed using log-linear regression:

219 
$$G_s = \alpha + \beta \ln \text{VPD} \quad (\text{eq. 3})$$

220 For each species the regression estimates were compared between treatments using analysis of  
221 covariance (ANCOVA), where treatment (ambient/warming) is the main effect and VPD is the  
222 covariate. A significant interaction term would indicate difference between treatments in the  
223 response of  $G_s$  to VPD.

224 Parameter estimates for equations 1 and 2 were considered significantly different  
225 between species and treatments if confidence intervals did not overlap. We chose this test because  
226 our sample sizes are limited and it is a more conservative test than comparing non-linear  
227 regressions between pooled vs. individual regressions using dummy variables. For a given species,  
228 significant difference in parameter estimates between climates/treatments was interpreted as  
229 photosynthetic acclimation. We also compared  $T_{50}$  of the same species between seasons to infer  
230 whether the acclimation response was reversible.

231

## 232 **Results**

### 233 *Thermal tolerance*

234 In the Jul-Aug measurement of the regional experiment (ca. four months after the experiment was  
235 initiated),  $T_{50}$  of current-year leaves was 2.7-3.9°C higher in warm-temperate climate for three  
236 evergreen (*M. rubra*, *F. griffithii*, *Q. myrsinifolia*) and two deciduous (*C. florida*, *L. styraciflua*)  
237 species (Table 2). The  $T_{50}$  of other species did not vary with climate, while for *Z. serrata*, the slope  
238 of the logistic curve was less steep in warm-temperate climate, reflecting slower decrease rate of  
239 QY in response to increasing temperature (Fig. 3). Seasonal change in  $T_{50}$  was observed only in  
240 warm-temperate climate where  $T_{50}$  was 1.7-3.5°C lower in Sep for *M. thumbergii*, *C. florida*, and  
241 *F. griffithii*.

242 In the Jul-Aug measurement of the greenhouse experiment,  $T_{50}$  of current-year leaves  
243 was 1.6-7.6°C higher under passive warming for *M. rubra*, *Q. myrsinifolia* and *C. florida* (Table

244 3). The effect of warming on  $T_{50}$  was especially marked for *M. rubra* and *Q. myrsinifolia* (7.6 and  
245 5.3°C difference, respectively). Seasonal change in  $T_{50}$  was observed mostly under passive  
246 warming. Under ambient condition,  $T_{50}$  was 1.7°C lower in Sep for *C. florida*. Under passive  
247 warming,  $T_{50}$  was 1.8-5.5°C lower in Sep for *M. rubra*, *M. thumbergii* and *C. florida*. The seasonal  
248 response of *M. rubra* was especially marked (5.5°C difference).

249 As a result of acclimation, species rank in  $T_{50}$  varied between climates and treatments  
250 (Fig. 5). In cool-temperate climate, *M. thumbergii* and *Z. serrata* had the highest  $T_{50}$ . In warm-  
251 temperate climate, there was no significant difference among the top five species, *Z. serrata*  
252 retreated from second to seventh in rank, and *L. japonicum* and *L. lucidum* had the lowest  $T_{50}$   
253 among the nine species. Of the five species whose  $T_{50}$  was higher in warm- than cool-temperate  
254 climate, *M. rubra*, *F. griffithii* and *Q. myrsinifolia* advanced markedly in rank. In the greenhouse  
255 experiment, there was very little difference in  $T_{50}$  among the nine species under ambient condition.  
256 Under passive warming, however, the  $T_{50}$  values of *M. rubra* and *Q. myrsinifolia* were  
257 significantly higher than the other species. Here again, *L. japonicum* and *L. lucidum* had the lowest  
258  $T_{50}$  among the nine species.

259

#### 260 *Temperature-photosynthesis relationship*

261 In the greenhouse experiment, patterns of photosynthetic acclimation to passive  
262 warming varied among species (Fig. 6). For *L. lucidum*, *L. japonicum* and *Z. serrata*,  $T_{opt}$  was  
263 higher under passive warming (Table 4), although for *Z. serrata*, the  $T_{opt}$  estimate under ambient  
264 condition was lower than the measured temperature range. *L. lucidum* and *L. japonicum* had the  
265 highest  $T_{opt}$  under passive warming. For *L. japonicum*,  $A_{net}$  did not change and only  $T_{opt}$  shifted  
266 upward. For *L. lucidum* and *M. rubra*, the concavity of the  $T_{leaf}$ - $A_{net}$  relationship ( $c$ ) was less  
267 negative under passive warming, i.e.,  $A_{net}$  decreased less with increasing temperature. For *Z.*  
268 *serrata*, an overall increase in  $A_{net}$  was observed under passive warming, whereas for *M.*

269 *thumbergii*  $A_{\text{net}}$  decreased. For *C. florida*,  $A_{\text{net}}$  decreased with increasing  $T_{\text{leaf}}$  under ambient  
270 condition ( $P < 0.01$ ), while it remained constant under passive warming.

271           During our measurement of  $A_{\text{net}}$ , VPD increased gradually with increasing temperature  
272 for all six species (Fig. S1). The pattern of VPD increase in relation to air temperature was similar  
273 among the four evergreen species. For the two deciduous species, *Z. serrata* and *C. florida*, VPD  
274 increased more rapidly for saplings grown under passive warming. The response of  $G_s$  to VPD  
275 varied among species. For *L. lucidum*, *L. japonicum* and *M. rubra*,  $G_s$  increased with increasing  
276 VPD and the response was more marked under passive warming than ambient condition (Fig. 7).  
277 This indicated that higher  $A_{\text{net}}$  of *L. lucidum* and *M. rubra* at high temperatures under passive  
278 warming was coupled with higher  $G_s$ . In contrast, for *Z. serrata*, *M. thumbergii* and *C. florida*  $G_s$   
279 decreased with increasing VPD, reflecting stomatal control of transpiration rate. For *Z. serrata*,  
280 and overall increase in  $G_s$  was observed under passive warming, indicating that higher overall  
281  $A_{\text{net}}$  under passive warming was coupled with higher  $G_s$ . For *M. thumbergii* and *C. florida*,  $G_s$  was  
282 less responsive to VPD under passive warming, suggesting reduced stomatal control of  
283 transpiration rate.

284

## 285 **Discussion**

### 286 *Thermal tolerance*

287 Our results indicated that species' thermal tolerance can vary widely in response to differences in  
288 regional climate, as well as to experimental warming. We expected that the evergreen species,  
289 which originate in warm-temperate to sub-tropical climates, would be more tolerant of warmer  
290 climate than the cool-temperate deciduous species. Indeed, *M. rubra* and *M. thumbergii*, both  
291 evergreen species of warm-temperate origin, had the highest  $T_{50}$  among the nine species when  
292 grown in warm-temperate climate. In addition, three of the six evergreen species showed  
293 acclimation response of  $T_{50}$  to differences in climate, although *M. thumbergii* did not. However,

294 in both experiments, *L. japonicum* and *L. lucidum*, both warm-temperate evergreen species,  
295 consistently ranked lowest in  $T_{50}$  and showed no acclimation responses. On the other hand, *C.*  
296 *florida*, a deciduous species, whose  $T_{50}$  was 3.4°C higher in warm-temperate than cool-temperate  
297 climate, ranked third in  $T_{50}$  under warm-temperate climate. This species also showed acclimation  
298 response of  $T_{50}$  in both experiments.

299 In a reciprocal transplanting experiment, Cunningham and Reed (2002) found that when  
300 grown in temperate climate, tropical species showed greater reductions in maximum net  
301 photosynthesis than temperate species grown under tropical climate. Tropical species also had  
302 narrower temperature ranges for maximum photosynthesis, suggesting lower thermal acclimation  
303 potential. These results suggest that temperate species, which are more adapted to temperature  
304 fluctuation, may have high thermal tolerance and acclimation potential than tropical and sub-  
305 tropical species. Our results indicate that species originating from warm regions may not  
306 necessarily be more tolerant or show greater acclimation potential to warmer growing conditions  
307 than those originating from cool regions.

308 Although some plants can maintain photosynthetic activity at temperatures as high as  
309 40°C (Colombo and Timmer 1992), irreversible biochemical damage occurs in many plants above  
310 55°C (Sharkey 2005). The  $T_{50}$  values of the nine street-tree species in this study were mostly  
311 around 50°C. However, for two warm-temperate evergreen species in our study, *M. rubra* and *Q.*  
312 *myrsinifolia*,  $T_{50}$  was higher than 55°C under passive warming suggesting these two species may  
313 be the most tolerant of extreme temperature among the nine species examined. *M. rubra* and *Q.*  
314 *myrsinifolia* also showed the greatest acclimation response, where  $T_{50}$  of current-year leaves was  
315 5-7°C higher under passive warming. In our experiment, warming increased mean temperature  
316 difference by 1.7°C. Maximum temperatures under warming, however, were 4-5°C higher than  
317 ambient condition, exceeding 45°C in late July (Fig. 2). Our results indicate *M. rubra* and *Q.*  
318 *myrsinifolia* are able to acclimate to such high temperatures. Several other species also showed

319 acclimation of thermal tolerance in response to different climates as well as to warming, but for  
320 many species these responses were dampened when temperatures decreased in Sep, suggesting  
321 that acclimation of thermal tolerance may be a short-term response. This may be because  
322 increasing thermal tolerance involves metabolic costs, such as production of isoprenes, heat-stress  
323 proteins, etc., strengthening of thylakoid membranes, and regeneration of rubisco (Law and S.J.  
324 1999; Sharkey 2005).

325

### 326 *Temperature-photosynthesis relationship*

327         Each of the six species examined showed unique acclimation response of the  
328 temperature-photosynthesis relationship to warming, suggesting that photosynthetic acclimation  
329 is species-specific. This could explain why previous studies of leaf warming produced such mixed  
330 results (Carter and Cavaleri 2018; Doughty 2011; Slot et al. 2014). We found that upward shifts  
331 in  $T_{opt}$  may or may not involve changes in overall photosynthetic rate. Up-regulation of  
332 photosynthetic rates, observed along with higher stomatal conductance for *Z. serrata* in this study,  
333 may reflect higher overall metabolism in response to warming (Dusenge et al. 2019). On the other  
334 hand, species showing down-regulation, such as *M. thumbergii*, which also showed lower  
335 stomatal control of transpiration under warming, could still maintain positive carbon balance if  
336 leaf respiration is also down-regulated (Araki et al. 2017; Atkin and Tjoelker 2003; Way and  
337 Yamori 2014). For *L. lucidum* and *M. rubra*, high photosynthetic rates in response to increasing  
338 temperature under passive warming were coupled with high stomatal conductance, i.e., increasing  
339 transpiration. Such species may not be able to maintain photosynthetic rate if high temperatures  
340 co-occur with drought conditions. Variable responses to higher temperature has also been found  
341 among tropical tree species (Cheesman and Winte 2013), suggesting species-specific  
342 physiological responses, including measurements of leaf photosynthesis and respiration, as well  
343 as stomatal control in response to increasing temperature and water stress, need to be examined

344 in order to predict acclimation potential of each species to warmer climate.

345

### 346 **Conclusion**

347 In the face of global climate change, we must manage forests and trees to prepare for  
348 uncertain future climate conditions (Millar et al. 2007). Urban trees and greenspace must also be  
349 managed and sustained to adapt to climate change (Roy et al. 2017). This is no easy task, and  
350 involves long-term planning at various spatial scales from replanting individual trees to  
351 redesigning cities. Our results provide a scientific basis for assessing physiological tolerance and  
352 acclimation potential of urban tree species to warmer growing conditions. Further investigation  
353 is needed to test specific responses to increasing temperature, because in our regional experiment,  
354 unknown factors other than temperature may have been confounded and in our greenhouse  
355 experiment we could not control for air humidity. Similar tests comparing urban tree species'  
356 tolerance and acclimation to changes in temperature, as well as co-varying effects of  
357 precipitation/humidity, are needed to prepare urban greenspace for a warmer future.

358

### 359 **Acknowledgements**

360 We thank Ms. H. Ishii, Ms. M. Hioki, Dr. K. Ohshiman and the staff of Sumitomo Forestry Co.  
361 Ltd. for assistance with the experiments, and K. Kuroda, W. Azuma and members of the  
362 Laboratory of Forest Resources, Kobe Univ. for guidance and advice during research.



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482  
483  
484

485 Table 1. Sizes of the potted saplings of each species used in the study. Mean  $\pm$  one s.d. of all  
 486 saplings are shown (n = 16 for each species). These were then equally distributed: three each  
 487 among the two sites and two treatments.

Species	Basal diameter (mm)	Height (cm)
<i>C. florida</i>	8.1 $\pm$ 1.0	80.9 $\pm$ 14.4
<i>L. styraciflua</i>	9.8 $\pm$ 0.8	98.1 $\pm$ 5.0
<i>Z. serrata</i>	7.7 $\pm$ 0.8	78.1 $\pm$ 15.5
<u><i>F. griffithii</i></u>	6.8 $\pm$ 0.9	94.4 $\pm$ 18.6
<u><i>L. lucidum</i></u>	8.0 $\pm$ 0.6	83.3 $\pm$ 11.1
<u><i>L. japonicum</i></u>	8.9 $\pm$ 1.2	65.2 $\pm$ 8.3
<u><i>M. thumbergii</i></u>	7.5 $\pm$ 1.0	70.2 $\pm$ 12.9
<u><i>M. rubra</i></u>	13.8 $\pm$ 2.0	81.9 $\pm$ 13.5
<u><i>Q. myrsinifolia</i></u>	8.2 $\pm$ 0.7	70.9 $\pm$ 9.5

488 Species names with underline indicate evergreen trees.

489 Table 2.  $T_{50}$  (temperature at 50% of maximum quantum yield, °C, mean  $\pm$  one s.e.) for current-  
 490 year-old leaves of the nine tree species grown in cool- and warm-temperate climates. Species are  
 491 listed in order of decreasing  $T_{50}$  in warm-temperate climate during Jul-Aug. Asterisk (\*) indicates  
 492 significantly higher value for warm- than cool-temperate climate and bold indicates significantly  
 493 lower value for Sep than Jul-Aug ( $P < 0.05$ ).

Rank	Species	Season	0-yr		
			cool	warm	
1	<u><i>M. rubra</i></u>	Jul-Aug	47.24 $\pm$ 1.0	50.62 $\pm$ 1.2	*
		Sep	48.87 $\pm$ 0.8	48.87 $\pm$ 1.4	
2	<u><i>M. thumbergii</i></u>	Jul-Aug	49.17 $\pm$ 1.1	50.52 $\pm$ 1.5	
		Sep	48.85 $\pm$ 1.0	<b>48.85<math>\pm</math>0.5</b>	
3	<i>C. florida</i>	Jul-Aug	47.03 $\pm$ 1.1	50.43 $\pm$ 0.4	*
		Sep	47.53 $\pm$ 0.6	<b>48.74<math>\pm</math>0.3</b>	*
4	<u><i>F. griffithii</i></u>	Jul-Aug	46.38 $\pm$ 1.0	50.28 $\pm$ 0.4	*
		Sep	46.74 $\pm$ 0.9	<b>46.74<math>\pm</math>0.5</b>	*
5	<u><i>Q. myrsinifolia</i></u>	Jul-Aug	46.09 $\pm$ 1.4	50.00 $\pm$ 0.4	*
		Sep	47.84 $\pm$ 0.7	47.84 $\pm$ 0.7	*
6	<i>L. styraciflua</i>	Jul-Aug	46.83 $\pm$ 0.8	49.55 $\pm$ 0.5	*
		Sep	47.59 $\pm$ 0.6	47.59 $\pm$ 1.2	*
7	<i>Z. serrata</i>	Jul-Aug	48.74 $\pm$ 0.3	48.98 $\pm$ 1.5	
		Sep	48.12 $\pm$ 0.6	48.12 $\pm$ 1.3	
8	<u><i>L. japonicum</i></u>	Jul-Aug	47.86 $\pm$ 0.5	48.68 $\pm$ 0.8	
		Sep	48.45 $\pm$ 1.0	46.97 $\pm$ 0.4	
9	<u><i>L. lucidum</i></u>	Jul-Aug	47.42 $\pm$ 0.5	48.36 $\pm$ 0.5	
		Sep	46.97 $\pm$ 0.7	46.97 $\pm$ 0.4	

494 Species names with underline indicate evergreen trees.

495 Table 3.  $T_{50}$  (temperature at 50% of maximum quantum yield, °C, mean  $\pm$  one s.e.) for current-  
 496 and one-year-old leaves of the nine tree species grown in ambient and passive warming conditions  
 497 in a greenhouse. Species are listed in order of decreasing  $T_{50}$  under passive warming during Jul-  
 498 Aug. Asterisk (\*) indicates significantly higher value for warming than ambient condition and  
 499 bold indicates significantly lower value for Sep than Jul-Aug ( $P < 0.05$ ).

Rank	Species	Season	0-yr		
			Ambient	warming	
1	<u><i>M. rubra</i></u>	Jul-Aug	50.02 $\pm$ 1.5	57.63 $\pm$ 1.3	*
		Sep	50.25 $\pm$ 0.7	<b>52.10</b> $\pm$ 1.0	*
2	<u><i>Q. myrsinifolia</i></u>	Jul-Aug	50.34 $\pm$ 0.9	55.68 $\pm$ 0.3	*
		Sep	50.35 $\pm$ 0.2	53.78 $\pm$ 0.5	*
3	<u><i>M. thumbergii</i></u>	Jul-Aug	50.70 $\pm$ 1.4	52.95 $\pm$ 1.4	
		Sep	49.00 $\pm$ 0.6	<b>49.58</b> $\pm$ 1.2	
4	<i>L. styraciflua</i>	Jul-Aug	51.04 $\pm$ 1.0	52.41 $\pm$ 0.8	
		Sep	49.62 $\pm$ 0.4	50.95 $\pm$ 1.1	
5	<u><i>F. griffithii</i></u>	Jul-Aug	50.03 $\pm$ 1.2	52.00 $\pm$ 1.2	
		Sep	50.75 $\pm$ 0.4	53.01 $\pm$ 0.4	*
6	<i>C. florida</i>	Jul-Aug	50.33 $\pm$ 0.3	51.95 $\pm$ 1.0	*
		Sep	<b>48.59</b> $\pm$ 1.3	<b>50.13</b> $\pm$ 0.3	
7	<i>Z. serrata</i>	Jul-Aug	50.11 $\pm$ 1.9	50.53 $\pm$ 1.3	
		Sep	49.20 $\pm$ 0.7	50.03 $\pm$ 1.5	
8	<u><i>L. lucidum</i></u>	Jul-Aug	50.01 $\pm$ 1.2	50.09 $\pm$ 1.6	
		Sep	48.95 $\pm$ 0.8	50.92 $\pm$ 1.2	
9	<u><i>L. japonicum</i></u>	Jul-Aug	48.33 $\pm$ 0.4	48.76 $\pm$ 1.2	
		Sep	48.37 $\pm$ 0.9	47.71 $\pm$ 0.5	

500 Species names with underline indicate evergreen trees.

501 Table 4. Regression coefficients (estimate  $\pm$  one s.e.) of the relationship between leaf temperature  
 502 and maximum photosynthetic rate ( $A_{\text{net}}$ ) for current-year leaves of the six tree species grown in  
 503 ambient and passive warming conditions in a greenhouse shown in Fig. 6. Species are listed in  
 504 order of decreasing thermal optimum for photosynthesis ( $T_{\text{opt}}$ ) under passive warming. Quadratic  
 505 regression of the form  $A_{\text{net}} = c (T + T_{\text{opt}})^2 + d$  was used. Values in bold indicate significantly higher  
 506  $T_{\text{opt}}$  and smaller  $c$  for warming than ambient condition ( $P < 0.05$ ).  
 507

	Species	Treatment	$T_{\text{opt}}$ ( $^{\circ}\text{C}$ )	$c$	$d$	$r^2$	$P$
1	<u><i>L. lucidum</i></u>	Ambient	25.86 $\pm$ 0.13	-0.034 $\pm$ 0.0011	5.63 $\pm$ 0.03	0.936	<0.01
		Warming	<b>28.63</b> $\pm$ 0.12	<b>-0.017</b> $\pm$ 0.0009	5.65 $\pm$ 0.03	0.937	<0.01
2	<u><i>L. japonicum</i></u>	Ambient	25.23 $\pm$ 0.76	-0.001 $\pm$ 0.0006	5.46 $\pm$ 0.02	0.813	<0.05
		Warming	<b>27.63</b> $\pm$ 0.10	-0.010 $\pm$ 0.0004	5.50 $\pm$ 0.01	0.884	<0.01
3	<u><i>M. rubra</i></u>	Ambient	25.35 $\pm$ 0.11	-0.015 $\pm$ 0.0007	5.75 $\pm$ 0.01	0.877	<0.01
		Warming	26.25 $\pm$ 0.21	<b>-0.005</b> $\pm$ 0.0004	6.11 $\pm$ 0.01	0.861	<0.01
4	<i>Z. serrata</i>	Ambient	17.70 $\pm$ 0.67	-0.009 $\pm$ 0.0006	6.90 $\pm$ 0.04	0.860	<0.01
		Warming	<b>22.40</b> $\pm$ 0.20	-0.009 $\pm$ 0.0003	8.33 $\pm$ 0.01	0.845	<0.01
5	<u><i>M. thumbergii</i></u>	Ambient	22.35 $\pm$ 0.36	-0.008 $\pm$ 0.0007	8.87 $\pm$ 0.04	0.920	<0.01
		Warming	21.37 $\pm$ 0.52	-0.008 $\pm$ 0.0006	5.90 $\pm$ 0.02	0.902	<0.01
6	<i>C. florida</i>	Ambient	ns	-0.055 $\pm$ 0.002*	6.73 $\pm$ 0.06	0.889	<0.01
		Warming	ns	ns	ns	ns	ns

508 Species names with underline indicate evergreen trees.

509 \*For *C. florida*, linear regression ( $A_{\text{net}} = c T + d$ ) was used.



510 **Figure captions**

511 Fig. 1. Potted saplings in the greenhouse (ambient) and double-green house (passive warming).

512 The dimensions of the double greenhouse are 3 x 3 m.

513 Fig. 2. Mean hourly temperatures during the experiment for Monbetsu City, Hokkaido (cool-

514 temperate climate) and Tsukuba City, Ibaraki (warm-temperate climate), and for ambient

515 and passive warming conditions in a greenhouse in Tsukuba. All data were taken by

516 thermometers set up beside the potted trees. Some data are missing for the greenhouse

517 during July due to mechanical failure.

518 Fig. 3. Quantum yield (QY) of current-year leaves in relation to leaf temperature for the nine tree

519 species grown in cool- and warm-temperate climates. Temperature and slope at the

520 inflection point of the logistic regression curve is  $T_{50}$  and  $b$ , respectively, in Table 2.

521 Asterisks next to species names indicate significant difference in  $T_{50}$  between climates and

522 thick lines indicate significant difference in  $b$  ( $P < 0.05$ ). Species are listed in the same order

523 as Table 2. Species names with underline indicate evergreen trees.

524 Fig. 4. Quantum yield (QY) of current-year leaves in relation to leaf temperature for the nine tree

525 species grown in ambient and passive warming conditions in a greenhouse. Temperature

526 and slope at the inflection point of the logistic regression curve is  $T_{50}$  and  $b$ , respectively, in

527 Table 3. Asterisks next to species names indicate significant difference in  $T_{50}$  between

528 treatments and thick lines indicate significant difference in  $b$  ( $P < 0.05$ ). Species are listed

529 in the same order as Table 3. Species names with underline indicate evergreen trees.

530 Fig 5. Nine urban tree species in Japan ranked in order of decreasing  $T_{50}$  (regression estimate  $\pm$

531 one standard error) in the two experiments, where saplings were grown in cool- and warm-

532 temperate climates, and under ambient and passive warming conditions in a greenhouse.

533 Species names with underline indicate evergreen trees.

534 Fig. 6. Maximum photosynthetic rate of current-year leaves ( $A_{\text{net}}$ ) in relation to leaf temperature

535 for six tree species grown in ambient and passive warming conditions in a greenhouse. Each

536 point is the mean of ten measurements taken at each temperature setting on one leaf from a

537 representative sapling for each species (standard error = 0.009~0.095  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Lines

538 indicate significant quadratic regressions (drawn using all ten observations for each

539 temperature setting) whose peak is  $T_{\text{opt}}$  (values are shown in each graph). Values in bold are

540 significantly higher for warming than ambient condition ( $P < 0.05$ ). Thick lines indicate

541 significant difference in concavity of the quadratic regression curve ( $P < 0.05$ , Table 4).

542 Species are listed in the same order as Table 4. Species names with underline indicate  
543 evergreen trees.

544 Fig. 7. Stomatal conductance of current-year leaves ( $G_s$ ) in relation to vapor pressure deficit  
545 (VPD) for six tree species grown in ambient and passive warming conditions in a  
546 greenhouse. Data are from the same measurement as Fig. 5. Lines indicate significant log-  
547 linear regressions of the form  $G_s = \alpha + \beta \ln \text{VPD}$  ( $P < 0.05$ ). For all six species, the slope  
548 and intercept were significantly different between warming and ambient conditions ( $P <$   
549  $0.05$ ). Species names with underline indicate evergreen trees.

550

551 Fig. S1. Vapor pressure deficit (VPD) in relation to air temperature ( $T_{\text{air}}$ ) for six tree species grown  
552 in ambient and passive warming conditions in a greenhouse. Data are from the same  
553 measurement as Fig. 5. Lines indicate significant linear regressions ( $P < 0.05$ ). This  
554 relationship was similar for the four evergreen species whose names are underlined ( $P <$   
555  $0.05$ ). For *Z. serrata* and *C. florida*, the relationship was significantly different between  
556 warming than ambient conditions ( $P < 0.05$ ).

557 Table S1. Monthly mean temperature, total precipitation, relative humidity and solar radiation during the study period for the two sites in the regional  
 558 experiment (Apr-Sep, 2019). Data are from the Japan Meteorological Agency database.

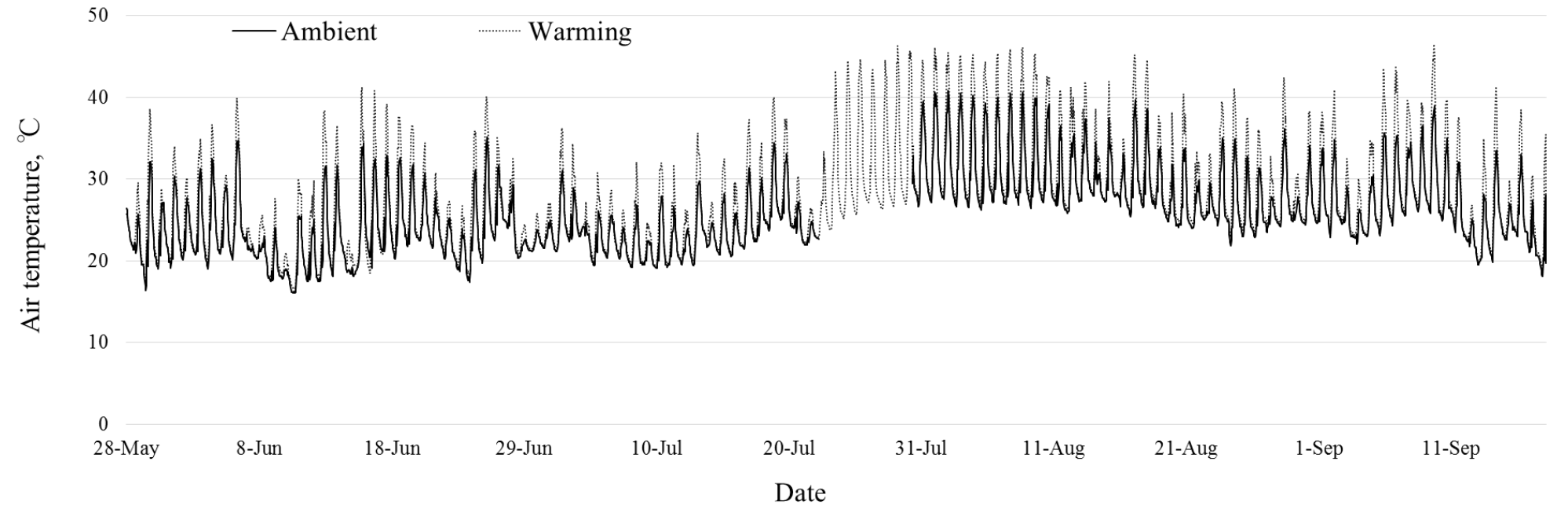
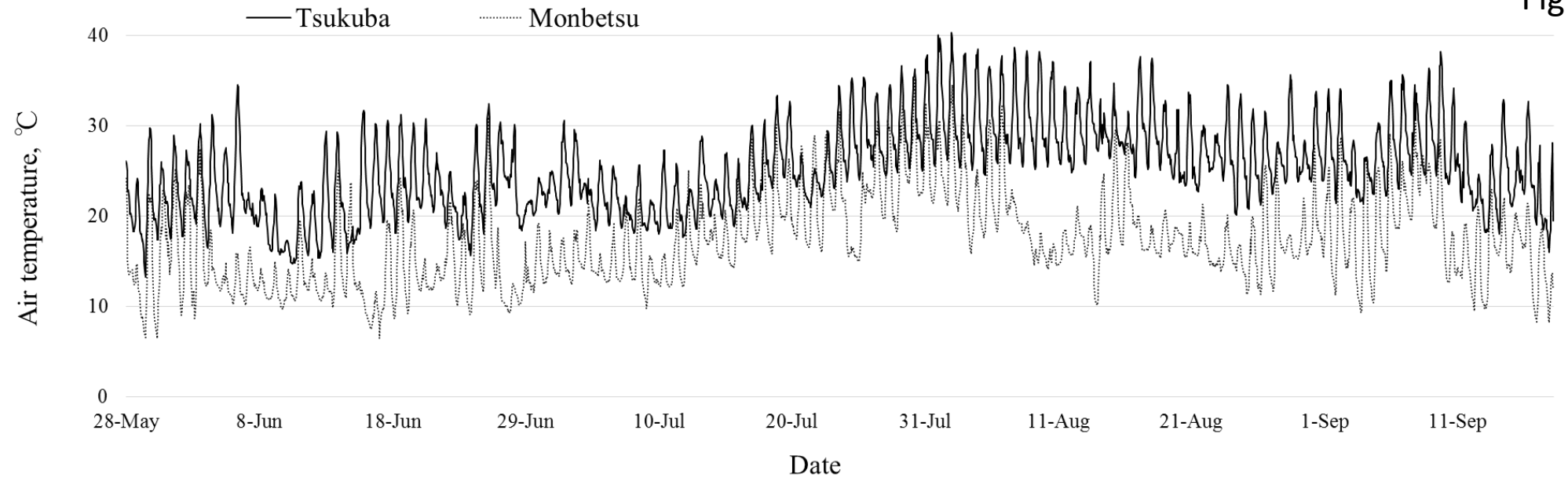
Month	Temperature(°C)		Precipitation (mm)		Humidity (%)		Solar radiation (MJ m <sup>-2</sup> )	
	Monbetsu	Tsukuba	Monbetsu	Tsukuba	Monbetsu	Tsukuba	Monbetsu	Tsukuba
Apr	5.4	12.3	15.0	82.0	63	67	18.8	18.3
May	13.5	18.7	21.5	95.5	66	70	20.1	21.2
Jun	13.5	20.9	58.5	141.5	85	82	19.2	15.9
Jul	18.1	23.5	59.0	160.0	90	88	18.6	13.5
Aug	18.6	27.4	239.5	57.5	91	83	13.8	17.3
Sep	17.4	23.8	98.5	172.5	77	82	14.1	14.6
Total			492.0	709.0			104.6	100.8

559



Fig 1

Fig 2



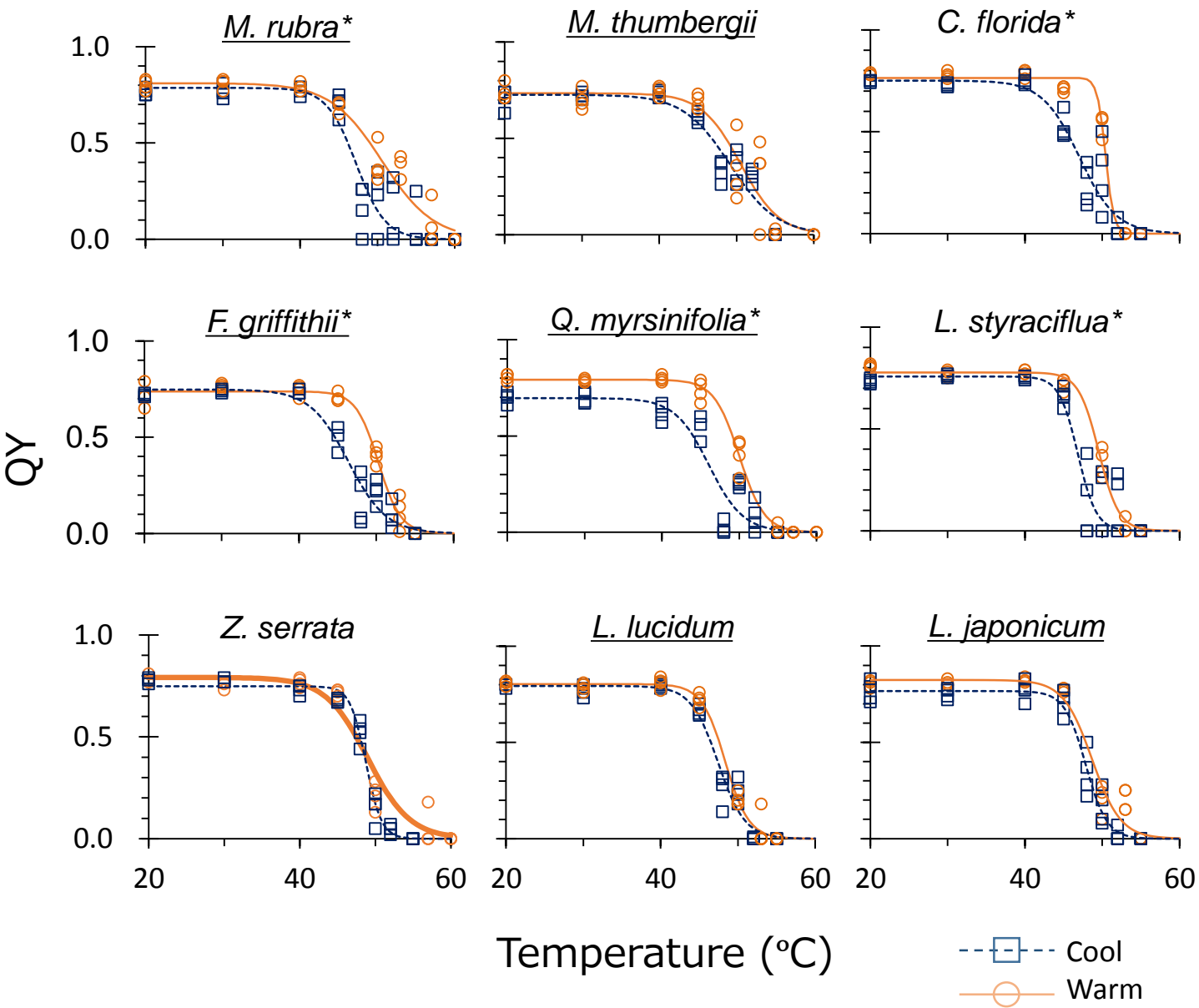


Fig 3

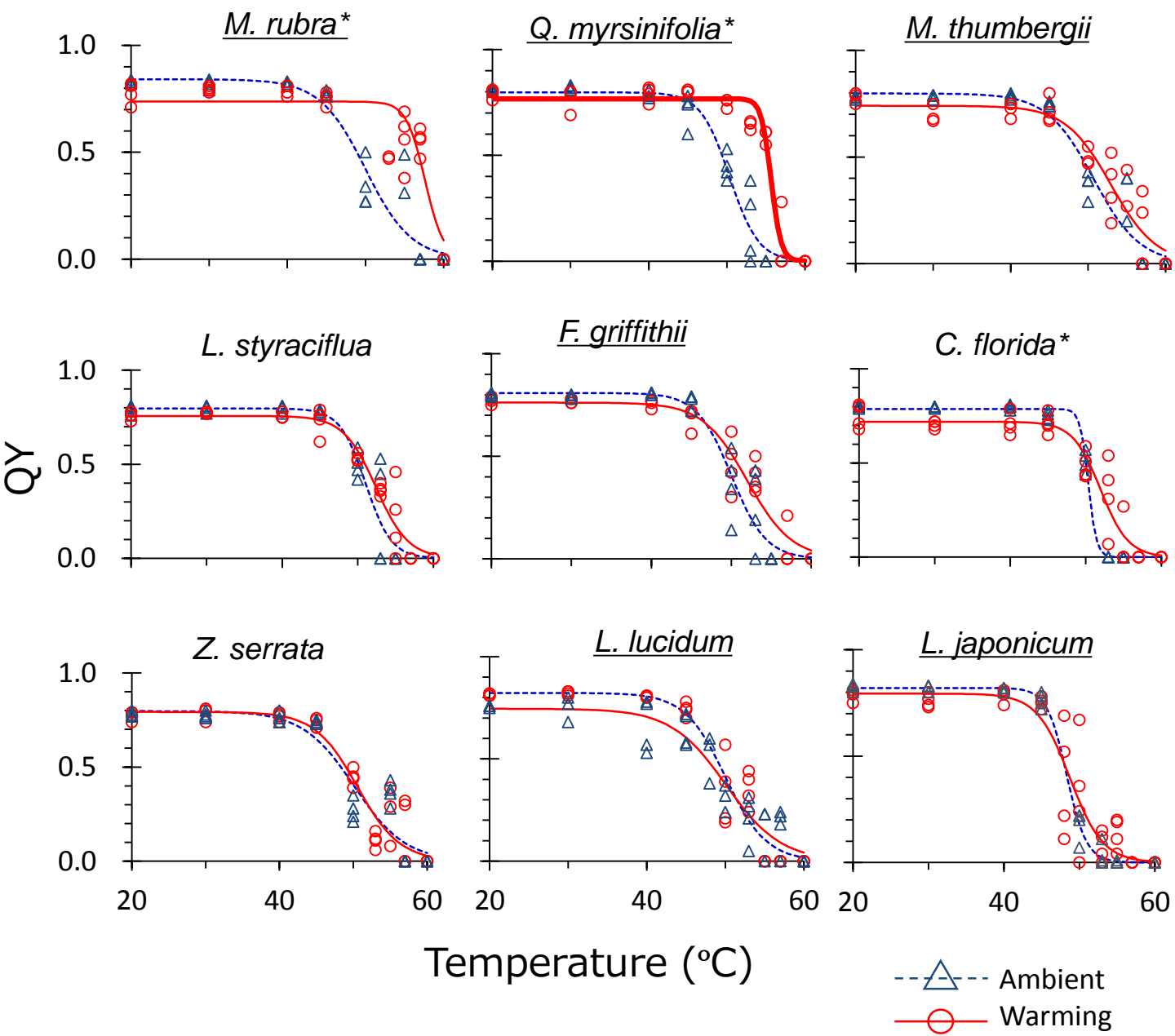


Fig 4

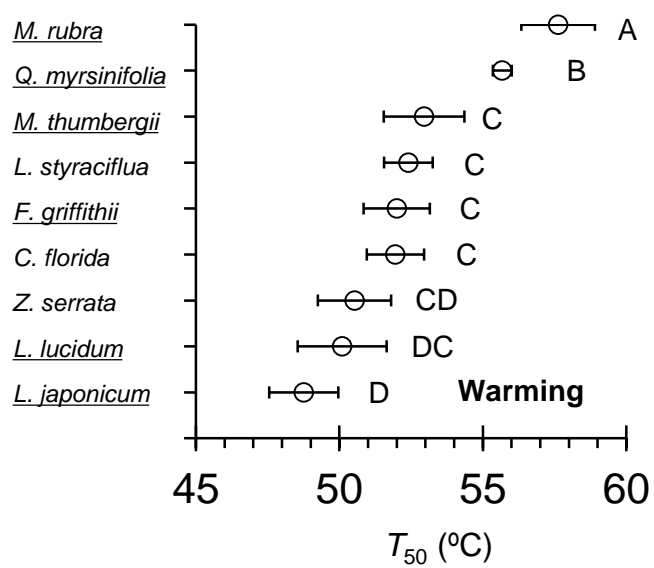
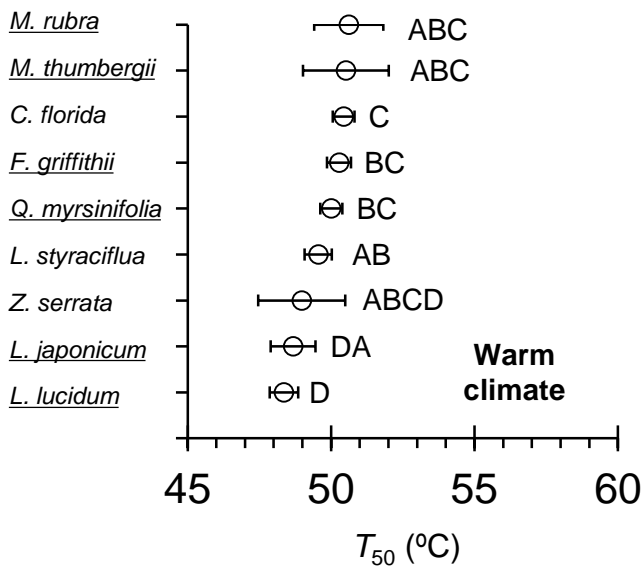
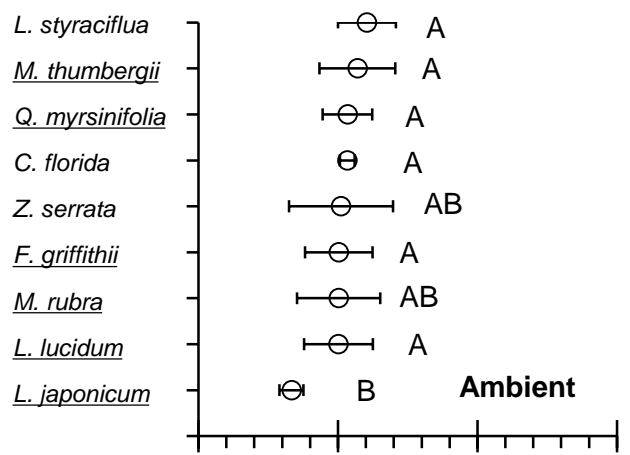
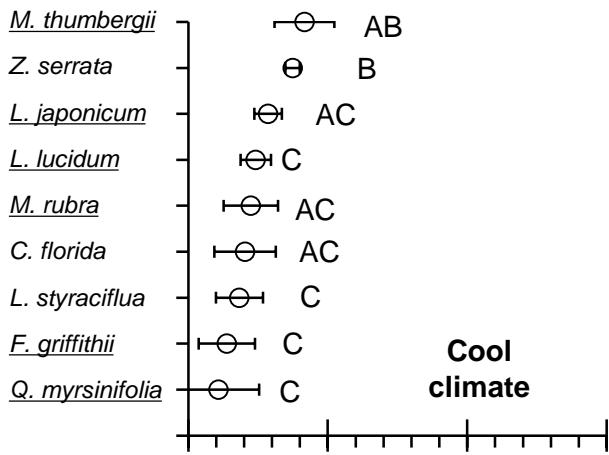


Fig 5



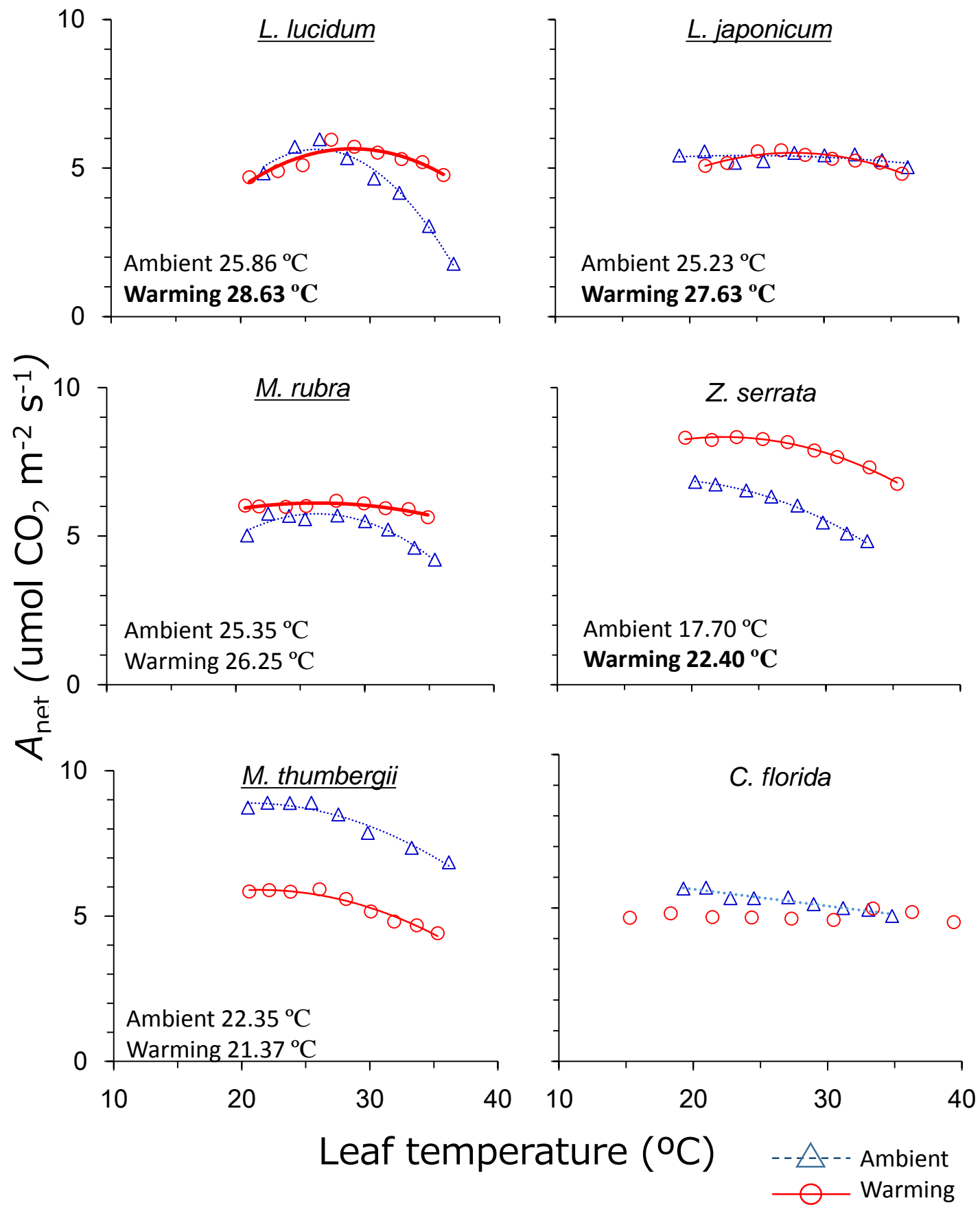


Fig 6

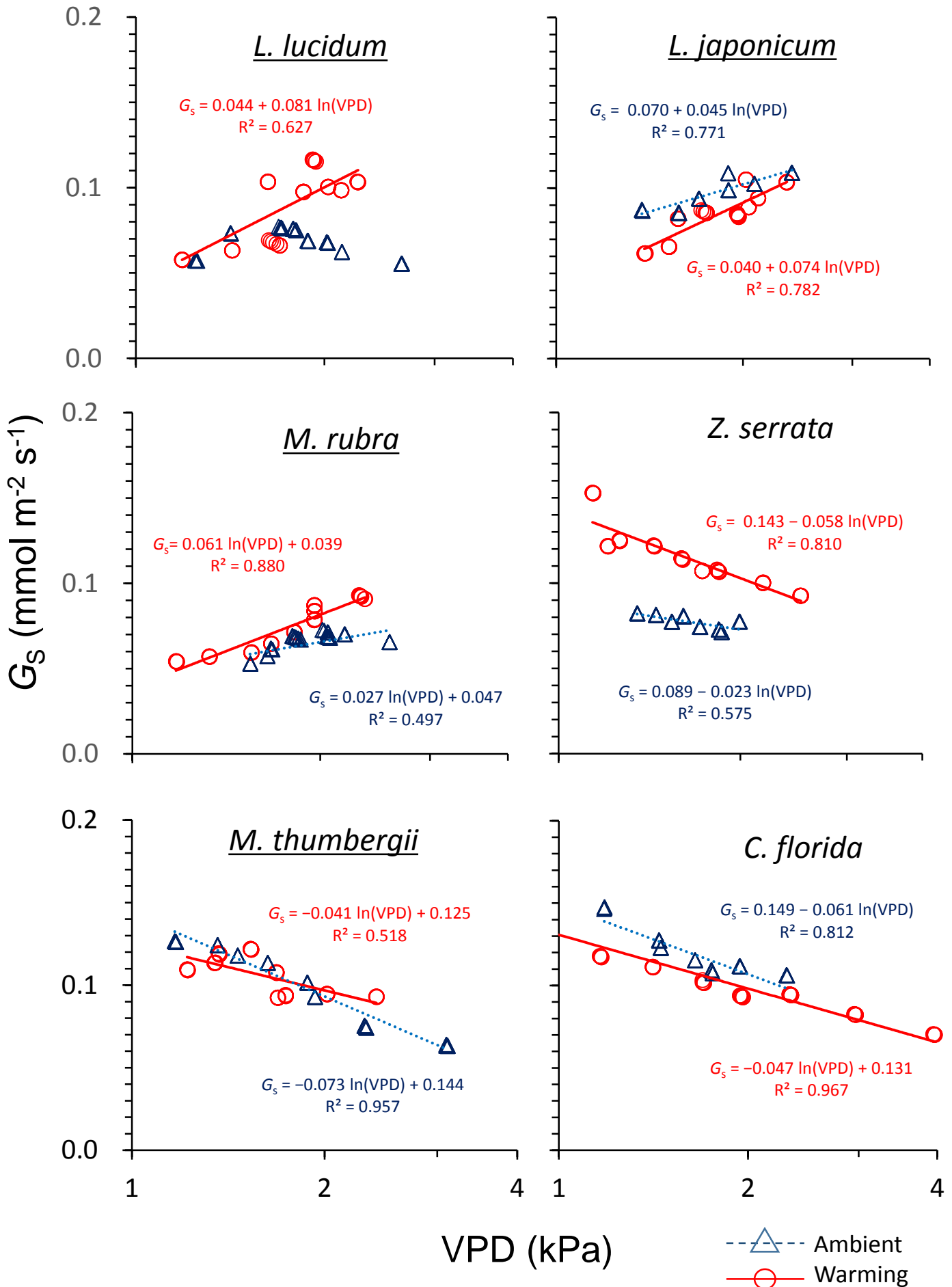


Fig 7

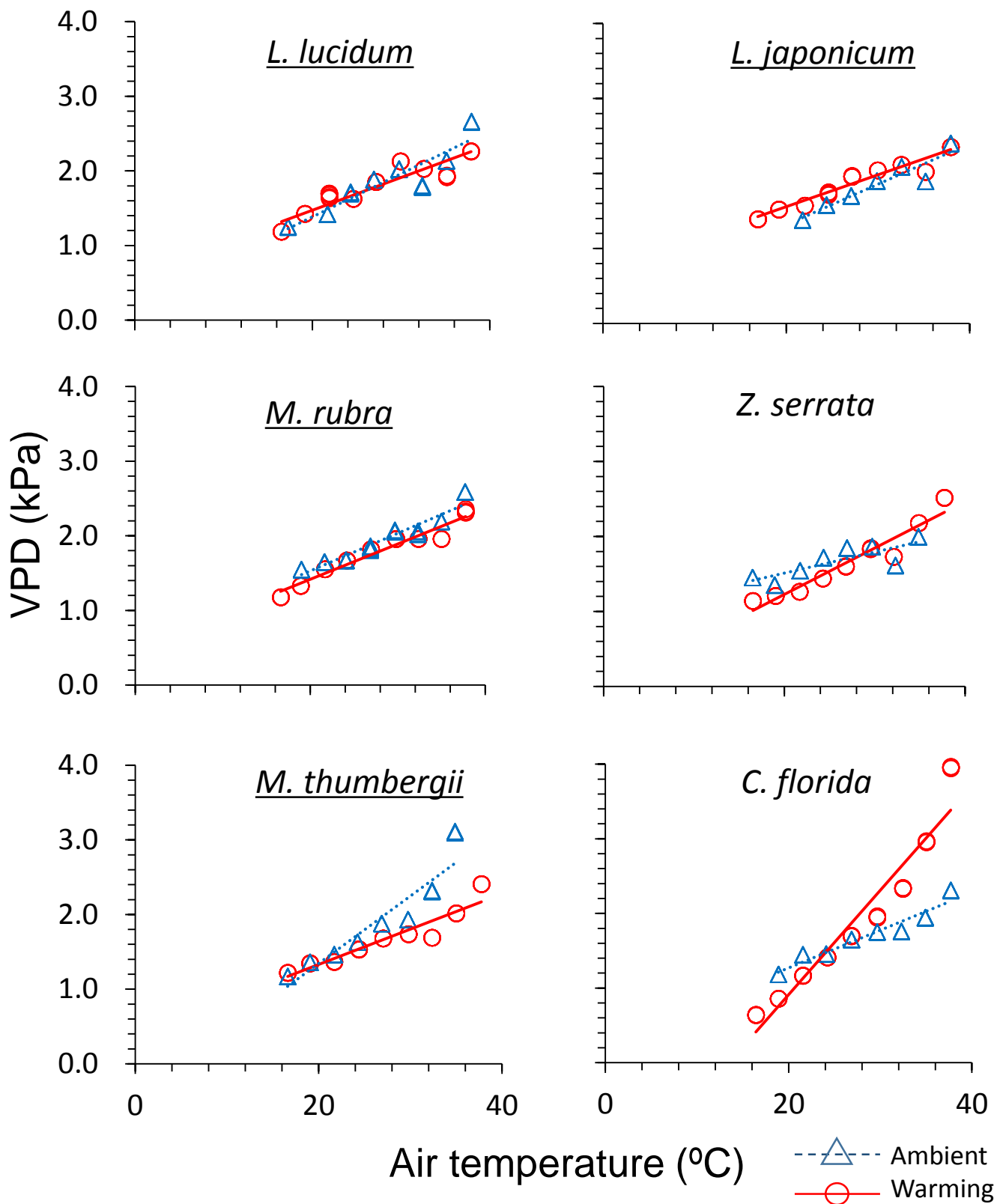


Fig S1