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Abstract

Temperature inducible phenotypic plasticity is a major player in plant reponses to climate warming. Functional responses and their role in determining thermal plasticity of plants remain poorly understood. Our objective was to compare trait responses of six populations of *Ludwigia peploides* resulting from seed from Oceanic climate and from Mediterranean climate after an exposure at three temperatures (16 °C, 24 °C, and 30 °C). A comparative analysis showed that at 30°C the six populations of *L. peploides* shared different morphological responses, whereas a common pattern of morphological responses were found for the six populations at 16 °C. At 16 °C, the growth was very low suggesting a stress. At 30 °C, the three Mediterranean populations of *L. peploides* accumulated ≈7 fold more total biomass than the populations from Oceanic region. Despite drawing similar response pattern to temperature, the populations showed several different metabolic responses. The thermal plastic responses to the highest temperature differed according to the origin of the populations. The Mediterranean populations of *L. peploides* could be better adapted to rising temperature. These abilities could allow them to take advantage from climate warming if the temperature is not warming up to temperature above a critical threshold.

Keywords : morphological traits, physiological traits, metabolome approach, climate warming, *Ludwigia peploides*,

Introduction

Environmental changes, including climate change, have profound impacts on life history traits of aquatic plant species, in turn affecting population dynamics and community structure in lakes and streams (Meerhof al., 2012; Salinas et al., 2018). In addition to rising temperature, several climatic models predict that the frequency and magnitude of temperature extremes will likely increase in the future (Seneviratne et al., 2012). These worldwide changes in climatic features will modify climate envelopes, increasing the number of suitable habitats and promoting the establishment and spread of invasive species (Bellard et al., 2013; Gillard et al., 2017a). Warming freshwaters have profound effects on aquatic plant species (Madsen & Brix, 1997; Hussner et al., 2014; Hyldgaard et al., 2014), on the community composition (Netten et al., 2010; 2011) or long term effects within thermal abnormal waters (Hussner, 2014). Consistently, Da Silveira & Thiébaut (2017) reported the beneficial effects of rising temperature for the relative growth rate of the Canadian pondweed *Elodea canadensis* Michaux and the Brazilian waterweed *Egeria densa* Planch. and concluded that warming should further support a shift in the geographical range of *E canadensis*.

The ever-changing conditions of habitat characteristics of living organisms give a central role to phenotypic plasticity, i.e. the ability of a particular genotype to express a range of phenotypes across different environments (Bradshaw, 1965), which may be adaptive (van Kleunen & Fischer, 2005; Richards et al., 2006). By allowing real-time changes of the morphology and physiology of the individual, environmental modifications can be tracked in order to maintain the highest possible fitness (Richards et al., 2006; Nicotra et al., 2010). Adaptive plasticity has been reported as one of the key features of invasive populations as compared with natives (Davidson et al., 2011), with invasive macrophytes being able, for instance, to invade climatic niches quite different than those of their area of origin (Gillard et al., 2017a).

A range of physiological mechanisms such as accumulation of osmolytes or changes in the composition of sugars or on the carbohydrate contents, can filter the effects of thermal fluctuations in ectothermic animals and plant species (Colinet et al., 2015, Sulmon et al., 2015; Koussoroplis et al., 2017), enhancing the level of trait plasticity. In this context, plant functional traits, which connects growth, reproduction and survival to the fitness of the individual (Violle et al., 2007), represent straightforward measures for the evaluation of organism responses to environmental factors, including habitat temperature. For instance, Riis et al. (2012) showed increased Relative Growth Rate values in *E. canadensis* grown at 25 °C as compared with their relatives maintained at 20 °C or 30 °C. This example of *E. canadensis* illustrates, that warming as such will not generally causes faster growth, whereas Li et al. (2017) showed that the total biomass and number of flowers of *Myriophullum spicatum* (L.) increased with climate warming and heat wave events. Moreover, physiological traits, including metabolic activities, are core mechanisms allowing plants to cope with climate change (Sun et al., 2016). Consistently, the accumulation of osmolytes (i.e. low-molecular weight organic compounds) has been repeatedly demonstrated in plants exposed to challenging temperature conditions (reviewed in Vincente et al., 2016). Osmolytes stabilize proteins to thermal and chemical denaturation. Other examples include the temperature-induced changes in the composition of primary metabolites like sugars (Thouvenot et al., 2015). Notably decreased sugar levels were reported in different plant species in response to increasing temperature (Sun et al., 2016; Gillard et al., 2017b). Sucrose can be quickly mobilised and stored throughout the plant and used for respiratory needs while gross morphological and biochemical changes occur during acclimation (Sun et al., 2016). As a result, examining functional traits of invasive populations provides a useful framework for the assessment of their responses to future climate change. As local conditions are strong drivers of phenotypic plasticity (King et al., 2018), the responses of geographically separated populations collected from contrasted climatic conditions would provide valuable comparisons

of their potential differences (Sultan & Spencer, 2002; Drenovsky et al., 2012; Gillard et al., 2020), in turn improving information for making predictions of future geographic expansion.

The breadth of the thermal tolerance range of organisms often matches with the climatic conditions of the habitats and regions where it thrives, as described by the climatic variability hypothesis (CVH). Accordingly, a positive relationship between thermal tolerance ranges and latitude has been reported in the worldwide submerged plant *Stuckenia pectinata* (L.) (Pilon & Santamaria, 2002). In this species, higher-latitude populations could escape from low temperatures by growing later in the season, or by postponing their reproductive phase. Another example is provided by the comparison of southern and northern European submerged plants which may show a perennial or winter-annual life cycle in the south, while at higher latitudes populations are most frequently summer annuals (Van Wijk, 1988). This illustrates the importance of considering intraspecific variation in plasticity and trait when investigating the effects of climate change on living organisms. This knowledge will refine our understanding of the range of responses exhibited by invasive plants, and improve predictions of their potential proliferation and spread success under climate warming.

Among the most invasive aquatic plants in the world, the creeping water primrose *Ludwigia peploides* subsp. *montevidensis* (Spreng.) P.H.Raven causes many significant changes to ecological processes and structures by reducing water flow and by altering hydrological regimes (Dandelot et al., 2005). Dense infestations of emergent macrophytes can dramatically reduce the dissolved oxygen (DO) concentrations in water by reducing water circulation and increasing biological oxygen demand from high biomass production and subsequent in situ decomposition of organic matter. *L. peploides* was introduced from the South America to Montpellier in France in the 1830s, likely as a result of ornamental plantings (Dandelot et al., 2005). It has since become a widespread aquatic invasive plant in the south of France and along the Atlantic coast. In the northwestern part of France, *L. peploides* started to

106 colonize the Loire River watershed by the end of the 1970s, and has spread rapidly among
107 several water bodies (Ruaux et al., 2009). Nowadays, *L. peploides* has a wide distribution in
108 Europe (EPPO, 2011; Thouvenot et al., 2013). Recent modeling efforts using CLIMEX (EPPO
109 2011) and species distribution models (Gillard et al., 2017a) predict that *Ludwigia* spp. has the
110 potential to spread invasively into higher latitudes in Europe. At the European level, the water
111 primrose belongs to the first European Union list of 37 invasive species adopted by the
112 European Commission in July 2016. The plant demonstrates a high degree of phenotypic
113 plasticity, which allows it to adapt to a broad range of growing conditions and water regimes
114 (Ruaux et al., 2009; Billet al., 2018). It is mainly aquatic, but is also able to colonise terrestrial
115 habitats such as riverbanks and wet meadows (Thouvenot et al., 2013). Under controlled
116 conditions, Yen & Myerscough (1989) noted the absence of *L. peploides* when the temperature
117 of the water remains below 20 °C, and when present, cooler temperatures can highly delay the
118 development of water primroses (Thouvenot et al., 2013). *Ludwigia peploides* has weak growth
119 at atmospheric temperatures of 10 °C and very high growth at 40 °C, but without flower
120 production (Yen & Myerscough, 1989).

121 In the present study, we aimed at comparing the effects of warming temperature on the
122 morphological and physiological traits of the invasive water primrose *L. peploides* collected
123 from two French different geographic regions. Three independent populations were sampled
124 respectively from Mediterranean and from Oceanic regions in France, and subsequently
125 cultured under controlled conditions. To explore whether trait values varied among these
126 populations when exposed at three different temperatures, and if the source regions were
127 predictors of these differences, we measured morphological (main shoot length, number of
128 lateral branches and leaves, relative growth rate, above and below ground biomass, total
129 biomass) and physiological (flavonols, anthocyanins, chlorophyll, and nitrogen balance
130 indexes), in addition to obtaining metabolic phenotypes of the plants (GC-MS metabolomics).

We hypothesized that 1) individuals of *L. peploides* would exhibit variation in plant trait values when grown at increasing temperatures; 2) Mediterranean populations would perform better than those from Oceanic climate at high temperatures.

Materials and methods

Sampling localities

For the purpose of this work, we selected six populations of *L. peploides* from two source regions (three populations of northwestern France and three populations of southeastern France) differing in their climatic variables. For each sampled population, mature capsules were collected in October 2017 from individuals separated by at least 10 m in order to increase the likelihood of subsequently working with distinct individuals.

One set of three distinct populations was sampled from southeastern France (hereafter referred to as Mediterranean France): the sampling habitats are located in the Köppen climate zone Csa, corresponding to a hot-summer Mediterranean climate and a dry summer season. In this region, the three populations were collected from (i) a small drained pond with emergent wetland plants (Marais de Coute, MACO), (ii) Canal des Capettes (CCAP), a tributary artificial channel of Petit Rhône River, and (iii) in a marshland (population CAM) (Table 1).

A second set of three populations was collected from three sites in northwestern France (hereafter referred to as Oceanic France) which is characterized by a temperate Oceanic climate having a warm summer season, but no dry season; these sampling sites are categorized as Cfb according to the Köppen climate classification (Belda et al., 2014).. These three populations of *L. peploides* were sampled from (i) oxbows of the Loire River at Ile Joli Coeur (LRJC), (ii) at Port de Vallières (LRPV), and (iii) in a marshland (population BRB) (Table 1).

After collection, capsules were dried out at ambient temperature before being stored at 4 °C in the dark until being cultured. In March 2018, twenty capsules of each population were soaked in tap water at 4 °C for a week, and then dissected to extract seeds. For each population,

200 seeds (10 seeds from each of the 20 capsules) were placed in a climatic chamber cycling from 19 °C to 24 °C (night / day) with a 14 h day/10 h night photoperiod to produce seedlings for the subsequent setting of the experiment. Seedling emergence and seedling growth were monitored over a period of 42 days. In early April 2018, the length of the primary stem of the largest seedlings was monitored every other day, and all seedlings were grown until there were a minimum of 35 individuals having primary stem lengths of at least 4 cm in each population. In mid-April, a stratified random selection of the plant based on primary stem length was done, allowing to sample 24 individuals from each population for the measurements of morphological and physiological traits, and nine individuals from each population for the metabolomic approach.

Description of the thermal treatments

The seedlings were transplanted individually into 200 cL pots (L x W x H; 5 cm x 5 cm x 7 cm) filled with a 1:1 (v/v) mixture of fine sand and potting soil. Initial nitrogen (N), phosphorus (P) and potassium (K) primary macronutrient concentrations were at NPK 14-10-18 percentages by soil mass. Plants were then placed for a period of four weeks into three growing chambers differing by their diurnal temperature regimes: for each population, one plant group (N= 8 replicates) was maintained at 16/11 °C (day/night, 14/10 h) (mean annual temperature of Oceanic region), a second plant group at 24/19 °C (mean annual temperature of Mediterranean region) (day/night, 14/10 h), and a third group at 30/25 °C (Climate warming, worse scenario) (day/night, 14/10 h). The climate warming temperature has been chosen according to the worst ICPP climatic scenario (RCP 8.5). For the daylight period (14h), a photosynthetic photon flux density of 350 µmol m^{-2} s^{-1} was used for all experimental conditions. Relative humidity was set to 60%. The three different temperature regimes will have strongly different vapour pressure deficits (VPD), and higher VPD might result in acclimations to reduce the water loss (physiologically by e.g. increased stomatal resistance), which will affect

181 growth and biomass related traits as well as biochemical characteristics. The water level was
182 adjusted manually during the growth period.

183 *Measurement of physiological traits during the thermal treatment*

184 We used a functional trait approach to monitor the physiological responses of individual
185 *L. peploides* plants of the six populations during the four-week exposure at one of the three
186 temperature regimes. Four physiological traits were simultaneously monitored *in vivo* using a
187 non-destructive measurement device called the Dualex Scientific+™ sensor. This hand-held
188 leaf-clip sensor (Cerovic et al., 2012; Burling et al., 2013) allows obtaining indicator values of
189 the amounts of flavonols (Flav), anthocyanins (Anth.) and chlorophyll (Chl.) in the leaves. The
190 Chl index related to the chlorophyll content (between 0 and 150) is a proxy to the Photosynthetic
191 yield. The Flav. index related to the flavonol content or to phenolics accumulation is an
192 indicator to the defense mechanisms against pathogens and herbivores. The Anth. index related
193 to anthocyanin is an indicator of an exposure to stress (shading conditions, nutrient deficiencies,
194 temperature stress etc.). The sensor also calculates the nitrogen balance index (NBI), which
195 represents an indicator of changes in C/N allocation as a result of N-deficiency (from 0 to 100)
196 rather than a measure of leaf nitrogen content *per se*. The measurements of physiological traits
197 were all taken from one apical leaf of one individual per population of *L. peploides* and per
198 thermal regime every 4-5 days over the four-week period, thus ending up with six different
199 temporal measures repeated on the same individuals.

200 *Measurement of morphological traits at the end of the thermal treatment*

201 At the end of the four-week exposure, for each experimental condition and for each
202 population, the main shoot length was measured and the lateral branches and the number of
203 leaves were counted. The roots and shoots were harvested separately, placed into paper bags,
204 dried out at 65 °C for 72h, and weighed (dry mass). The Relative Growth Rate (RGR; d^{-1}) was
205 calculated, as proposed by Hunt (1990):

206 RGR stem = (ln L2 - ln L1)/(T2 - T1)

207 where L1 and L2 represent total shoot length at the beginning of the experiment (T1)
208 and at the end of the experiment (T2), respectively.

209 *Metabolomic analyses*

210 At the end of the thermal treatment, the leaves of each plant were collected, resulting in
211 the constitution of 3 to 5 replicates per population and per temperature regime. After collection,
212 leaves were snap-frozen into liquid nitrogen and stored at -80 °C. Before the analyses, leaves
213 were lyophilized, and reduced into powder. For each replicate, 5 to 10 mg of plant material
214 were weighed. We used the extraction procedure described by Serra et al. (2013). Briefly, each
215 replicate was homogenized in 1125 µL of a solution of ice-cold methanol/chloroform (2:1, v/v).
216 Then, a volume of 750 µl of ultra-pure water was added. Samples were homogenized and
217 centrifuged for 10 min at 4,000 g (4°C). Twenty µL of the upper phase containing metabolites
218 was transferred to new glass vials. Samples were vacuum dried (Speed Vac Concentrator,
219 MiVac, Genevac Ltd., Ipswich, England). The derivatization of the samples was conducted
220 with a CTC CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland), as described
221 in Khodayari et al. (2013). The GC-MS platform consisted of an Agilent 7890B gas
222 chromatograph coupled to a 5977B mass spectrometer. The injector was held at 250 °C, and
223 the temperature of the oven ranged from 70 to 170 °C at 5 °C per min, from 170 to 280 °C at 7
224 °C per min, and from 280 to 320 °C at 15 °C per min; at the end of the temperature ramps, the
225 oven remained at 320 °C for 4 min. A 30 m fused silica column (HP5 MS 30 m, I.D. 0.25 mm,
226 thickness 0.25 µm, 5% Diphenyl / 95% Dimethylpolysiloxan, Agilent Technologies) was used,
227 with helium as the gas carrier at 1 mL per min. For the transfer line and ion source, temperatures
228 were 280 and 230 °C, respectively. The split mode (split ratio: 2:1) was used for the injection
229 of 1 µL of each sample, and detection was realized by electronic impact (electron energy: 70

230 eV), in full scan mode. The detected peaks were annotated with MassHunter. Concentration of
231 each metabolite was calculated using individual quadratic calibration curves.
232 *Statistical analysis*
233 All analyses were performed with R (R Core Team, 2016). Morphological traits were
234 analysed using generalized linear models (GLMs) with « population » (6 levels) and
235 « temperature » (3 levels) as fixed and crossed variables. The effects of each variable and the
236 interaction were analysed via the analysis of deviance (ANOVA function in "car" package;
237 Fox, 2003). Post-hoc comparisons were based on least-squares means using the "lsmeans"
238 package (Russell, 2016). For physiological traits, data from the three individuals (and
239 populations) originating from Oceanic region (i.e. BRB, LRJC, LRPV; Table 1) or from
240 Mediterranean region (i.e. CAM, MACO, CCAP; Table 1) were grouped to assess the "Region"
241 effect. As physiological traits were repeatedly measured on the same individuals during
242 growing period, the identity of each plant was included in a generalized linear mixed-effects
243 model as a random effect to account for repeated measures, using lmer function in the "lme4"
244 package for R (Bates et al., 2015). The model tested the effects of time (6 time points), region
245 (OFR vs. MFR) and growing temperature regime (16, 24, 30 °C), as well as all interactions.
246 The effects of variables were assessed with ANOVA function using the "car" package and post-
247 hoc comparisons were done using the "lsmeans" package. Metabolic compositions of the plants
248 were compared using between-class PCA in the "ade4" package in R (Dray & Dufour, 2007).
249 Monte Carlo tests were performed to examine the significance of the difference among the
250 classes (based on 1,000 simulations). To identify the variables (i.e., metabolites) contributing
251 the most to the PCA structure separation, the correlations to the principal components (PCs)
252 were extracted and integrated into correlation circles; correlations values of each compound to
253 PC1 and PC2 are shown in Table S1.

Results

Changes in plant morphological traits

The population, temperature, and the interaction of these terms, significantly affected all of the measured morphological traits related to growth, biomass, leaf and shoot production (Table 2). At 16 °C, the below and above ground biomasses were very low in individuals of *L. peploides* (Fig. 1). No lateral shoots were produced, there was low production of new leaves, all of these measurements ending up in a very small RGR value for all populations. While no striking difference was observed for the production of new leaves among the six populations exposed at 24 °C, the production of lateral shoots, below and above ground biomasses, and total biomass tended to be significantly higher in the three Mediterranean populations as compared with their three Oceanic relatives. A similar pattern was reported from the plants exposed at 30 °C (Fig. 2). At this temperature, the production of lateral shoots, above ground biomass and total biomass were higher than those measured at 24 °C for the plants from the Mediterranean region. Overall, a higher number of new leaves produced in plants from both geographic regions exposed at 30 °C as compared with those that were exposed at 24°C, RGR was highly significantly increased from 16° C to 24 °C, but then remained more or less similar when temperature was further increased.

Changes in plant physiological traits

The effects of geographic origin of the plants (Mediterranean versus Oceanic), temperature, and the interaction of these terms, on the physiological traits of the plants are presented in Table 3. All physiological traits were significantly affected by the temperature of the experiment, the geographic origin of the plants, the timing of the measurement, and the interaction temperature : timing of the measure. A similar changing pattern was observed for the six populations of *L. peploides*, with Nitrogen balance and chlorophyll indexes being increased from 16 to 30 °C, while flavonol and anthocyanin indexes were decreased over this

thermal range (Fig. 2). Conversely, the indices depicting Nitrogen balance index, chlorophyll and anthocyanin amounts were higher in Mediterranean plants at 16 °C, and the difference with Oceanic plants disappeared when temperature was increased, except for chlorophyll.

Metabolic phenotypes of the six populations of L. peploides

The PCA showed that 86% of the changes in metabolite amounts were explained by the two first axes (Fig. 3). The first axis of the PCA accounted for 68% of the total inertia, and was mainly constructed by the higher amounts of galactose, glucose 6P, inositol, malate acid, ornithine, succinic acid and quinic acid, which were measured in higher amounts in individuals of *L. peploides* exposed at 16 °C. The second axis of the PCA, accounted for 18% of the total inertia, was mainly defined by the higher amounts of aspartic acid, fumaric acid, and glycine which tended towards higher concentrations at 30 °C (Fig. 3).

In addition to the similar temperature-driven metabolic pattern reported from the six *L. peploides* populations, in-depth analysis of the measured amounts of the different metabolites revealed several differences (Fig. 4). Specifically, seedlings from the LRPV population were characterized by their high levels of galactose, glucose 6P, inositol, malic acid, mannose, ornithine, saccharose, and xylose (Fig. 4). A clear cut separation appeared between MACO population at 30 °C and the others populations both at 24 °C and 30 °C in the second axis. At 30 °C, seedlings from the MACO population were characterized by their high levels of ethanolamine, glycine and fumaric acid (Fig. 4).

Discussion

Changes in morphological and physiological traits with increasing temperature

In this study, we were interested in determining if geographically separated populations of *L. peploides* from distinct climatic conditions would be characterized by different abilities to cope with warming temperature. Using populations of the invasive macrophyte *L. peploides* exposed at three different temperatures, several changes in morphological and physiological

traits were found, thus confirming the local adaption of the sampled populations, in line with the climate variability hypothesis. At the lowest temperature we tested (16 °C), most of the traits (biomass accumulation, Relative Growth Rate, production of new leaves, nitrogen uptake, photosynthetic activity) remained at very low levels in seedlings from all populations. The quasi absence of plant growth is consistent with a former study conducted in *Ludwigia grandiflora* (Michx.) Greuter & Burdet, and reporting a weak growth at 10 °C (Yen & Myerscough, 1989). Our results suggest that 16 °C may be largely suboptimal for the plants, as supported by the increased values of the anthocyanin index. These water soluble pigments enhance cold tolerance when accumulated in plant tissues, as earlier reported in a range of plant taxa (reviewed in Chalker-Scott 1999). Moreover, the inhibition of stem length and lateral shoot production are also well-characterized responses of plants exposed at low temperatures (Da Silveira & Thiébaut, 2017, Riis et al., 2012). Conversely, the growth and development of the individuals from the six tested populations exposed at 24 °C continued, with significant energy allocation to growth and nitrogen uptake, as revealed by the NBI index. Previous study showed that *L. peploides* was characterized by a rapid growth and allocation of most biomass and nitrogen into above ground plant parts when developing under Mediterranean climates (Rejmánková, 1992).

The optimum development of emerged leaved plants such as *Ludwigia* occurs between 25 °C and 35 °C and these air temperatures will likely occur along the Mediterranean coast during summer. The Oceanic region actual air temperatures are frequently lower than the above suggested thermal optima, optimal photosynthetic activities may be currently limited to short climatic windows in summer periods. Thus, the current temperature characteristics of Oceanic regions should highly restrict the development of this plant species, also because other abiotic factors (e.g. irradiance and CO_2 availabilities) that can also constrain the performance of photosynthetic reactions may be characterized by different variation patterns than those of

329 temperature. Rising air temperature increases saturated water vapor pressure. An increase in air
330 vapor pressure deficit (VPD), affects plant physiology independently of other drivers associated
331 with climate change, for example elevated carbon dioxide concentrations ([CO_2]). Stomatal
332 conductance declines under high VPD and transpiration increases in most species up until a
333 given VPD threshold, leading to a reduction of photosynthesis and growth, and higher risks of
334 carbon starvation (Day, 2013).

335 The chlorophyll index was almost doubled in Oceanic *L. peploides* plants exposed at 30
336 °C relative to those exposed at 16 °C. Yet, the lower values of chlorophyll for these populations
337 are intriguing, and this typical response of cold-stressed plants, and symptomatic of oxidative
338 stress, has not been observed in plants from the Mediterranean region. The Mediterranean
339 populations perform better than the Oceanic populations with regard to chlorophyll, at all
340 temperatures. Our second hypothesis was validated. More interesting, the Mediterranean
341 populations produced more chlorophyll at 16 °C than the Oceanic populations, even though
342 they are adapted to higher temperatures. The apparent higher sensitivity of chlorophyll
343 production, and thus photosynthetic activities of these individuals suggests that populations
344 from this region should highly benefit from future warming.

345 Interestingly, most of the differences observed for trait responses among the six plant
346 populations were largely determined by the climate regions where seeds were collected. At 30
347 °C, the three populations originating from the Mediterranean region allocated more energy to
348 biomass in general, and to the production of lateral shoots rather than to apical growth. These
349 results are congruent with available literature (Rejmankova, 1992), and with a mesocosm
350 experiment conducted in California (Gillard et al., 2020). Indeed, Gillard et al. (2020)
351 established that a higher climatic similarity in the source and invaded regions greatly assists the
352 establishment and performance of *L. peploides*. Overall, we found that Mediterranean
353 populations of *L. peploides* performed significantly better at 30 °C than those from Oceanic

populations. This finding, in accordance with the climatic variability hypothesis, suggests that the populations have adapted locally to their environmental conditions. As a result, the larger dissimilarity between the temperature characteristics of their original habitat and the thermal regimes may contribute to explaining the highly reduced performance of these populations at high temperature.

Different metabolic responses to temperature

Several studies have employed metabolic profiling to describe changing physiological dynamics in plants exposed to different temperature regimes, in addition to evidencing the set of metabolites characterizing acclimation to cold or heat temperatures (reviewed in Shulaev et al., 2008). Variations in metabolite contents is essential for maintaining optimal metabolic phenotype and overall, trait-environment matching; during changes of temperature conditions, amino acid, sugar, and Krebs cycle intermediate metabolites acting as osmolytes, antioxidants, byproducts of stress exposure, and signal transduction are expected to vary (Xu et al., 2020). In our study, higher levels of galactose, glucose 6P, and succinic acid were measured from the leaves of *L. peploides* after they were exposed at low temperature for 4 weeks. The accumulation of soluble sugars is common in plant leaves whose growth is limited by low temperatures, as for instance found in young tomato plants (Klopotek & Klaring, 2014). Variations of the amounts of soluble sugars and of some Krebs cycle intermediates, e.g. the accumulated galactose, glucose 6P, malic and succinic acids, parallel the reduced growth rates of *L. peploides* exposed at 16 °C. Reductions in carbohydrate concentrations in macrophytes typically occur when plants are relying on stored energy to initiate growth of plant tissues until photosynthesis can begin (Wersal et al, 2011). Indeed, Wersal et al., (2011) showed that stolons were the primary storage location of starch in *Myriophyllum aquaticum* (Vell.) Verdc.. Earlier study also showed that the transfer of *Arabidopsis thaliana* (L.) Heynh. (Brassicacea) to cold temperature, followed by their return at optimal temperature, increased the need for carbon

source when growth was resumed, as revealed by lowing amount of sugars and TCA intermediates (Pagter et al. 2017). It has also been suggested that fumaric acid can be highly accumulated in the leaves of *Arabidopsis thaliana* (Chia et al., 2000), acting as a carbon reservoir for sustaining development and growth of the plant. In this study, the metabolite concentrations of the six *L. peploides* populations exposed at 16 °C can be likely considered as a reliable metabolic signature of depressed growth and development of the plants.

Oxygen deficiency in plant tissues often correlates with an elevation of the amounts of alanine and, but to a lesser extent, of succinic acid (Billet et al., 2018). In *L. peploides* exposed at 16 °C, the higher concentrations of lactic acid, an end-product of anaerobic respiration, suggest that both populations may have activated fermentation (Sun et al., 2016). We suggest that low temperature-induced fermentation may serve as an additional way of energy production, in order to meet the demands of plant tissues that could not be fulfilled by respiratory pathways alone.

Amino acids serve as precursors for a large array of metabolites having multiple functions in plant growth and response to various stresses (Sun et al., 2016). These compounds are osmolytes that can accumulate in stressed plants (Shulaev et al., 2008). Yet, we did not find clear signs of amino acid accumulation in any of the six populations exposed at 16 °C. As mentioned earlier, temperatures below 20 °C progressively reduce the development of *L. peploides*, which is halted around 10 °C. Our metabolic profiles are highly consistent with this earlier suggestion, by showing that the 16 °C temperature is restricting for the six populations, but not stressful. This idea is also congruent with field observations, as the spread of *L. peploides* in the Oceanic region was particularly evident when summer temperatures reach 24 °C, a finding that may also apply for *Ludwigia grandiflora* (Gillard et al., 2017b).

In the present study, the effect of temperature on the physiological responses of *L. peploides* was also compared for both regions. Higher levels of fumaric and malic acids were

404 measured at 30 °C than at 24 °C. These changes may indicate a stimulation of the photosynthetic
405 activity in this C4 species, in turn contributing to the higher biomass accumulation we
406 measured. Photoassimilates may be used for lateral growth, which would be consistent with the
407 observed significant increase of this morphological trait at 30 °C for Mediterranean populations.
408 A similar malic acid accumulation has been shown in other C4 macrophytes (Beer & Wetzel,
409 1982; Bowes et al., 2002), including in *Ludwigia hexapetala* (Gillard et al.,2017b). At 30 °C, a
410 strong decrease in Glucose 6P and in saccharose contents in Oceanic populations of *L. peploides*
411 was measured, whereas this change was less evident in Mediterranean populations. This high
412 temperature may have stimulated the photosynthesis of the Oceanic populations of *L. peploides*,
413 which would have resulted in enhanced carbohydrate production, *in fine* contributing to the
414 greater apical growth of Oceanic populations at 30 °C. Importantly, the allocation of energy to
415 apical growth and not to the lateral growth is usually considered as an escape strategy (Puijalon
416 et al., 2008) to avoid stress due to the high temperature

417 Indeed, a range of physiological adjustments are likely to take place for maintaining
418 homeostasis within the organisms through acclimation responses. Physiological responses
419 include the accumulation of compatible solutes in the form of polyols, sugars or amino acids,
420 meaning that metabolic processes should be highly solicited during thermal stress.

421 **Conclusion**

422 In this study we showed that at 30 °C the six populations of *L. peploides* shared different
423 morphological and physiological responses, whereas at 16 °C the growth for the six populations
424 was very low suggesting a stress. At 30°C, the Mediterranean populations of *L. peploides*
425 perform better than the Oceanic populations. The Mediterranean populations of *L. peloides* can
426 cope well with high temperatures. Although they did not produced higher biomass than the
427 oceanic populations at low temperature, they were able to induce a higher photosynthetic
428 activity and nutrient uptake than the oceanic populations. The Mediterranean populations of *L.*

peloides are characterized by a higher plasticity than the oceanic ones. Indeed, our results suggest that the populations growing in the oceanic region somehow lost some of their plasticity. The present results lead to the conclusion that those exotic species whose native habitats are warmer than their introduced ranges would have an advantage in an increasingly heat climate. Research on the response of this species to increasing temperatures in parallel to other environmental factors such as increasing CO_2 is required to improve our understanding of the impact of global warming on the invasiveness of aquatic plants. For instance Riis et al. (2012) showed that rate of branching degree for three invasive aquatic plants (*Egeria densa, Elodea canadensis* and *Lagarosiphon major* (Ridl.) Moss) suggests that temperature did not influence the general developmental pattern of these plants and light and temperature effects on stem length can counteract each other.

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617

618 Figure Caption

619 Figure 1. Morphological traits of six populations of *L.peploides* exposed at three temperatures.
620 Different letters indicate significant differences for *L. peploides* with temperature and
621 population (ANOVA, $p < 0.05$). Bars indicate standard deviation.

622 Figure 2: Responses of physiological traits of *L. peploides* populations coming from two
623 different regions (Oceanic and Mediterranean regions) to three temperatures. NBI =
624 Nitrogen Balance Index, Chl = chlorophyll index, Flav = flavonol index and Anth =
625 anthocyanin index.

626 Fig 3: A) Between-PCA based on the 25 metabolites identified and quantified by GC-MS. A
627 plot of the first two principal components (PC1 vs. PC2) is shown in panel A. Lines link
628 individuals to their respective centroids (n = 3 to 5). Populations at 16, 24 and 30°C are
629 shown in blue, black, and red, respectively. B) Correlation circles from the PCA. C)
630 Histogram of the 1000 simulated values of the Monte Carlo randomization test of the
631 between-PCA. The observed value is given by the vertical line, at the right of the
632 histogram ($P < 0.001$). D) Bar chart showing the percent of inertia explained by each
633 principal component (PC) of the between PCA. Only the first two components (PC1,
634 PC2) were considered (black bars), as they accounted for 68 and 18% of intertia
635 respectively.

636 Fig.4: Changes in Malic acid, Fumaric acid, Aspartic acid, Succinic acid, Alanine and Lactic
637 acid in seedlings of *L. peploides* exposed at three temperatures (16 °C, 24 °C and 30
638 °C). The seedlings were issues from three Mediterranean populations (CAM, MACO,
639 CCAP) and from three Oceanic populations (LRPV, LRJC, BRB).

640

641 Table 1 – Location of six populations where the capsules of *Ludwigia peploides* subsp.
642 *montevidensis* were collected. M 1,2,3= Mediterranean region, O1,2,3= Oceanic region.

Seed source region code	Population code	Name of waterbody – Site name	GPS coordinates
M1	CAM	Camargue	43.535972 ; 4.753578
M2	MACO	Marais de Coute	43.584092; 4.366019
M3	CCAP	Canal des Capettes	43.606264; 4.336035
O1	BRB	Brière	47.32599; -2.267511
O2	LRJC	Loire River at Ile Joli Coeur	47.318086; 0.405323
O3	LRPV	Loire River at Port de Vallieres	47.386011; 0.608789

643

Table 2. Effects of the temperature, population and the interaction of these two terms on the morphological traits. The comparisons were made by running generalized linear models with temperature and population as fixed and crossed variables. The effects of each variable and their interaction were analyzed with ANOVA. The resulting values of the LR Chisquares, degree of freedom (df), and p values are presented in the table. Significant differences ($p < 0.05$) are in bold.

Morphological traits	Temperature effect			Population effect			Temperature x poplulation effect		
	LR Chisq	df	p	LR Chisq	df	p	LR Chisq	df	p
Total biomass	209.09	2	**<0.001**	170.68	5	**<0.001**	142.90	10	**<0.001**
Below mass	31.227	2	**<0.001**	34.408	5	**<0.001**	42.261	10	**<0.001**
Aboveground mass	218.94	2	**<0.001**	167.35	5	**<0.001**	143.65	10	**<0.001**
Number of new leaves	559.98	2	**<0.001**	36.35	5	**<0.001**	29.06	10	**<0.001**
Number of branches	550.32	2	**<0.001**	389.10	5	**<0.001**	229.72	10	**<0.001**
RGR	1282.13	2	**<0.001**	58.90	5	**<0.001**	30.26	10	**<0.001**

650 Table 3. Linear fixed model results. NBI = Nitrogen Balance Index, Chl = chlorophyll index, Flav = flavonol index and Anth = anthocyanin index.

651 Significant p-values at 5% significance level are in bold.

	NBI			Chl			Flav			Anth		
	Chisp	df	p	Chisp	df	p	Chisp	df	p	Chisp	df	p
temperature	304.92	2	**<0.001**	43.47	2	**<0.001**	158.25	2	**<0.001**	209.51	2	**<0.001**
time	512.19	5	**<0.001**	335.75	5	**<0.001**	39.70	5	**<0.001**	186.64	5	**<0.001**
region	10.86	1	**<0.001**	17.15	1	**<0.001**	6.00	1	**<0.001**	46.36	1	**<0.001**
temperature:time	783.65	10	**<0.001**	342.07	10	**<0.001**	388.72	10	**<0.001**	682.87	10	**<0.001**
temperature: region	8.63	2	**<0.001**	1.60	2	0.450	3.24	2	0.198	8.38	2	**<0.001**
time: region	9.33	5	0.100	5.81	5	0.330	0.15	5	0.999	28.70	5	**<0.001**
temperature: time: region	14.38	10	0.156	39.26	10	**<0.001**	14.53	10	0.150	63.43	10	**<0.001**

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