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Acorn weight as determinant of germination in red and white oaks: evidences from a common-garden greenhouse experiment

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6 **26 1 Introduction**

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8 **27** Seed weight is a fundamental life history trait of plants and ecologists have been largely interested in
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10 **28** determining how it influences tree recruitment (Silvertown 1989; Leishman et al. 2000). In highly
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12 **29** competitive habitats, such as forest understories, it was proposed that natural selection favors tree
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14 **30** species with heavier seeds because this accelerates seedling development and enhances their
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16 **31** competitive ability for light and soil resources (Foster 1986; Silvertown 1989; Westoby et al. 1992;
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18 **32** Leishman and Westoby 1994; Bruun and Ten Brink 2008; Yi and Wang 2016). Conversely, lighter-
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20 **33** seeded species are adapted to stressful habitats, such as forest clearings, where small seed sizes would
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22 **34** confer them resistance to desiccation until favorable conditions for germination occur (Foster 1986;
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24 **35** Silvertown 1989; Leishman et al. 2000). Within tree species, on the other hand, it was reported that
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26 **36** heavier seeds give rise to seedlings that are more resistant to drought and herbivory, which increases
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28 **37** their establishment chances (Bonfil 1998; Chacón et al. 1998; Chacón and Bustamante 2001; Gómez
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30 **38** 2004; Huerta-Paniagua and Rodríguez-Trejo 2011). These interspecific and intraspecific effects of
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32 **39** seed weight on the development and survival of early life-cycle stages of trees are well documented
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34 **40** (Fenner and Thompson 2005), but the effects this trait on seed germination have received less
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36 **41** attention. This could be due to the greater interest that plant ecologists have put in analyzing how seed
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38 **42** weight relates with the fate of tree seedlings, as they are critical for forest regeneration, while their
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40 **43** consequences on germination remained as experimental anecdotes (e.g., Bonfil 1998; Gómez 2004).

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42 **44** Germination starts with the development of the embryo after seed imbibition and this results in the
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44 **45** emergence of the radicle (Fenner and Thompson 2005). Germination is proposed to increase with seed
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46 **46** weight because heavier seeds are assumed to have larger cotyledonary reserves (Gómez 2004), but no
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48 **47** previous study has analyzed whether this trait constitutes an adaptation that evolved from different
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50 **48** environmental pressures. Tree species belonging to the same phylogenetic lineage (e.g., the same
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52 **49** genus) could be useful to elucidate whether seed weight, besides affecting the fate of their seedlings,
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54 **50** also influences germination. This is because closely related species usually produce seeds with similar
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56 **51** morphoanatomical features but, if they differ in seed weight and variability in this trait affect their
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7 52 germination, this may be reflecting adaptation of species to germinate under different environmental
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9 53 conditions. Further, if seed weight varies within each tree species and this also relates to germination,
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11 54 these intraspecific relationships should be more similar among species that are phylogenetically closer
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13 55 to each other (e.g., species that diversified more recently from a common ancestor within the genus)
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15 56 than among phylogenetically distant species (e.g., species groups whose ancestors diverged much
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17 57 earlier within the genus). This would occur if seeds of the ancestors that gave rise to different species
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19 58 groups within the genus were subjected to different selective pressures.

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21 59 To assess these interspecific and intraspecific effects of seed weight on germination, we focused on
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23 60 oaks (*Quercus* spp., Fagaceae) because these trees produce acorns (nut-type fruits that contain a single
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25 61 seed) that largely differ in their weight, both across and within species (Long and Jones 1996; Bonfil
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27 62 1998; Khan and Shankar 2001; Zavala-Chávez 2001; Landergott et al. 2012). Because the elevated
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29 63 phylogenetic diversity of this plant group, oaks have been divided in several infrageneric categories.
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31 64 Nixon (1993) used morphological traits and DNA sequences to split this genus in two broad
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33 65 subgenera, including *Cyclobalanopsis* (only present in eastern Asia and northern Oceania) and
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35 66 *Quercus* (distributed across all continental masses of the northern hemisphere). Additionally, Nixon
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37 67 (1993) split the subgenus *Quercus* in three phylogenetic sections: (1) *Lobatae*, or red oaks, which are
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39 68 in America only (from USA to Colombia), (2) *Protobalanus*, or golden oaks, which are restricted to
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41 69 northwest Mexico and southwest USA (California Peninsula), and (3) *Quercus*, or white oaks, which
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43 70 spread across the entire northern hemisphere (North and Central America, Europe, Asia and Northern
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45 71 Africa). Mexico is the most important diversification center of the subgenus *Quercus*, harboring more
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47 72 than 160 species (Valencia 2004). Further, red and white oaks widely overlap their distribution ranges
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49 73 across this country (Romero-Rangel et al. 2014), which provides exceptional opportunities to assess
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51 74 whether acorn biomass influences germination across and within species.

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53 75 Acorns of white and red oaks are highly recalcitrant and germinate in short time after primary
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55 76 dispersion, which occurs during the rainy season (Zavala-Chávez and García-Moya 1996). On this
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57 77 issue, it was reported that acorns of Mexican oaks lose viability when their water contents decrease
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7 78 below 25-30%, but they need to be close to their maximum hydration capacity to germinate (Zavala-
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9 79 Chávez and García-Moya 1996; Zavala-Chávez 2008). Further, viable acorns of white oaks have been
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11 80 reported to be heavier than those of most red oaks (Zavala-Chávez and García-Moya 1996; Zavala-
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13 81 Chávez 2004; Rubio-Licona et al. 2011). Therefore, if acorn weight influences germination, white oaks
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15 82 can be expected to have higher germination rates (i.e., fraction of acorns that germinate across time
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17 83 within a cohort) and higher germination percentages (i.e., final fraction of germinated acorns of a
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19 84 cohort) than red oaks when their acorns are sowed under the same environmental conditions (e.g., a
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21 85 common-garden experiment). Nevertheless, as the fresh weight of viable acorns includes both water
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23 86 and nutrients, it would also be necessary to determine how variability in this trait relates with their dry
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25 87 biomass, which would be indicative of the amount of nutrients accumulated within acorns, and percent
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27 88 moisture content, which would be indicative of the amount of water that viable acorns require to
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29 89 germinate. These relationships would provide insights about what of these variables (dry biomass or
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31 90 water content) is the main responsible of intraspecific differences in germination. Further, as maternal
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33 91 effects can also cause intraspecific differences in dry biomass and water content, germination
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35 92 probability (i.e., the chance of germinating) and germination speed (i.e., the time they require to
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37 93 germinate) are also feasible to increase with acorn fresh weight.

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39 94 In this study, we analyzed the interspecific and intraspecific germination patterns of red and white
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41 95 oaks from Sierra Madre Oriental, México. For this, we firstly assessed the fresh weight of viable
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43 96 acorns produced by different species of both phylogenetic sections and performed common-garden
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45 97 greenhouse experiment addressing the following questions: (i) does the fresh weight of viable acorns
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47 98 differ between red and white oaks, as well as among species of these two groups?; (ii) do differences in
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49 99 this trait lead to different germination rates and germination percentages in red and white oaks?; (iii)
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51 100 does intraspecific variability in acorn fresh weight affect germination probability and germination
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53 101 speed within oak species?; and (iv) are these relationships more similar among oak species from the
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55 102 same phylogenetic section that across species from different sections? After performing that
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57 103 experiment, we focused in determining whether the fresh weight of acorns related with their dry
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7 104 biomass and water content in order to determine what of these traits may be behind of the relationships
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9 105 between acorn weight and germination.

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13 107 **2 Materials and Methods**

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15 108 **2.1 Oak species and acorn collection**

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17 109 This study was performed with oak species from Sierra de Álvarez, a mountain range (2000-2500 m
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19 110 elevation) that comprises the westernmost section of Sierra Madre Oriental. Sierra de Álvarez is
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21 111 located 25 km to the east of the city of San Luis Potosí, central Mexico, and it harbors eight red oak
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23 112 species (section *Lobatae*) and nine white oak species (section *Quercus*) (García-Sánchez and Aguirre-
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25 113 Rivera 2011). Most these oaks have mast reproduction and produce acorns every 2-5 years, but these
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27 114 events are not always synchronized among species. For this reason, we focused on those species that
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29 115 produced acorns in 2015 and 2017, which included seven red oaks (*Quercus affinis* Scheidw., *Quercus*
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31 116 *castanea* Née, *Quercus crassifolia* Humb. & Bonpl., *Quercus eduardii* Trel., *Quercus jonesii* Trel.,
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33 117 *Quercus mexicana* Humb. & Bonpl. and *Quercus viminea* Trel.) and three white oaks (*Quercus laeta*
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35 118 Lieb., *Quercus polymorpha* Schldl. & Cham. and *Quercus potosina* Trel.).

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37 119 Acorns collected in 2015 were used to assess whether fresh weight influences their germination,
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39 120 while acorns collected in 2017 we used to assess whether fresh weight relates to their dry biomass and
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41 121 water content. In both years, collections were conducted immediately after acorns were released from
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43 122 parental trees. In 2015, this occurred between early August and middle September, while in 2017 this
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45 123 occurred between middle September and late October. Acorn collections included ten trees of each
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47 124 species and, beneath the canopy of each individual, we collected 100 mature acorns to capture as much
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49 125 variability in acorn weight as possible. Acorns belonging to each species were pooled in a single
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51 126 sample and carried to the laboratory to assess their viability with the float test (Gribko and Jones
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53 127 1995). We did this because acorns collected in the field can quickly lose viability due desiccation or
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55 128 parasitism by insects or fungi (Ramos-Palacios et al. 2014). For this, acorn cupules were removed and
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57 129 nuts were placed in 20 L containers filled with water. After 2 h in water, sunken acorns were assumed

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7 130 as viable, while floating acorns were assumed as unviable (Gribko and Jones 1995; González-
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9 131 Salvatierra et al. 2013; Ramos-Palacios et al. 2014). With this noninvasive test we retain more than
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11 132 500 viable acorns of each species at each year. These viable acorns were stored in refrigerated
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13 133 chambers (5° C) to prevent desiccation and retard their germination. As germination trials described
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15 134 below started in middle September 2015, acorns of those species collected in August of that year (*Q.*
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17 135 *affinis*, *Q. crassifolia*, *Q. eduardii*, *Q. jonesii*, *Q. laeta*, *Q. viminea* and *Q. potosina*) were stored by a
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19 136 month, while acorns of species collected in September (*Q. castanea*, *Q. mexicana* and *Q. polymorpha*)
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21 137 were stored by a few days. The relationships of the fresh weight of acorns vs. their dry biomass and
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23 138 water content were assessed in late October 2017 and, thus, these acorns were stored by less than a
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25 139 month.

141 2.2 Common-garden germination experiment

142 To assess effects of acorn fresh weight on germination, we performed a common garden experiment
143 with the acorns collected in 2015. This experiment was conducted in a ventilated greenhouse located at
144 the city of San Luis Potosí, México. The roof of the greenhouse was covered with Raschel mesh (60%
145 shade) to approximate temperature and light conditions to those of forest understories, were
146 germination of most oak species occur (Zavala-Chávez 2001). During the experiment, we used
147 automatized sensors to measure these two environmental variables in the greenhouse and the
148 understory of an oak forest near to the city. The average temperature of the air in the greenhouse was
149 19.7 °C, while temperature in the forest was 18.3 °C (see Online Resource 1). Photon flux density
150 during daytime (07:00 to 19:00 h) averaged 153 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the greenhouse and 134 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in
151 the forest (see Online Resource 1). These values indicate that temperature and light conditions were
152 similar between the greenhouse and the forest.

153 For the common-garden germination experiment, we randomly selected 100 viable acorns of each
154 oak species during September 14th 2015 and placed them in water during 48 h. We soaked these acorns
155 to approximate them to their maximum hydration capacity, as this is necessary to promote their

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7 156 germination (Zavala-Chávez and García-Moya 1996; Zavala-Chávez 2008). Soaking was also applied
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9 157 to standardize the germination capability across acorns because, otherwise, comparisons of
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11 158 germination responses across and within oak species could be influenced by differences in the
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13 159 moisture content that acorns had at the moment they were collected in the field. The fresh weight of
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15 160 these fully hydrated acorns was used as predictive variable in the analyses of their germination
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17 161 responses. For this, acorns were dried with paper towels after they were soaked and all of them were
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19 162 weighed in an analytical balance (resolution 0.1 mg, Sartorius, Germany) to determine their fresh
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21 163 weight. These acorns were sowed in individual plastic cups (50 mL) filled with a mixture of sterilized
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23 164 forest soil (80%) and perlite (20%). Because previous observations in the field indicate that recently
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25 165 released acorns can germinate on the soil surface, they were half-buried in the germination substrate
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27 166 (see Online Resource 1). We did not remove the pericarps of acorns because these structures prevent
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29 167 seed desiccation (Zavala-Chávez and García-Moya 1996). On all cases, acorns were assumed to have
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31 168 germinated when the radicle emerged from the pericarp (see Online Resource 1).

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33 169 Germination cups were randomly distributed on an experimental table in the greenhouse (see
34
35 170 Online Resource 1) and they were watered until saturating the substrate. We perforated the bottom of
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37 171 the cups to allow drainage of excess water and applied the same watering procedure every two days to
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39 172 maintain acorn moisture. Acorn germination was recorded daily between September 16th and
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41 173 November 15th (60 days). After finishing the experiment, we assessed whether ungerminated acorns
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43 174 were viable. For this, a small incision was performed on the pericarp of ungerminated acorns and they
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45 175 were incubated in solution 1% (mass/volume) of 2, 3, 5-triphenyltetrazolium chloride in dark, at 20-25
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47 176 °C, during 24 h. If seed embryo is alive, the colorless tetrazolium salt reacts with the hydrogen
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49 177 released by active dehydrogenase enzymes and it is reduced to formazan, a red non-diffusible dye
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51 178 (Black et al. 2006). This test indicated that all ungerminated acorns lost their viability during the
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53 179 experiment.

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57 181 **2.3 Fresh weight, dry biomass and moisture content of acorns**
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7 182 Acorns collected in 2017 were used to assess whether their fresh weight after soaking, dry biomass and
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9 183 moisture content differed between phylogenetic sections and among oak species, as well as to assess
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11 184 whether these variables were related between them. For this, we randomly selected 100 viable acorns
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13 185 of each oak species and soaked them as described above. After soaking, each acorn was weighed to
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15 186 determine their fresh weight (*FW*). They were later placed in individual paper envelopes and dried in a
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17 187 ventilated stove at 60 °C until their dry biomass (*DB*) remained constant. These data were later used to
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19 188 estimate the percent water content (*PWC*) of each acorn as $PWC = (FW - DB)/FW \times 100$.

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22 23 190 **2.4 Statistical analyses**

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25 191 If acorn fresh weight positively influences germination rates, the proportion of germinated acorns
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27 192 across time should be higher in oak species that produce heavier acorns, as compared to those that
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29 193 produce lighter ones. These effects were firstly tested at the level of oak phylogenetic section using the
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31 194 data of the greenhouse experiment conducted in 2015. For this, we assessed whether the fresh weight
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33 195 of soaked acorns differed between white and red oaks (section *Quercus* vs. section *Lobatae*) using a
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35 196 Student's *t*-test, where each acorn was included as a replicate of the respective phylogenetic section.
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37 197 After that, we performed a failure time analysis to assess differences in germination rates between
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39 198 white and red oaks. In this test, acorns of red and white oaks had a value of 0 (zero) at the beginning of
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41 199 the experiment, which was turned into 1 (one) on the date in which they germinated. Ungerminated
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43 200 acorns retained the zero-value until the end of the experiment. These binary data (0-1) was used to
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45 201 calculate standardized germination rates with the Kaplan-Meier's method (Kaplan and Meier 1958),
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47 202 which were compared between phylogenetic sections with Gehan-Wilcoxon tests (Kleinbaum and
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49 203 Klein 2012). We later assessed these effects at the species level. For this, one-way ANOVA and *post*
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51 204 *hoc* Tukey tests were used to compare the fresh weight of acorns (after soaking) among oak species,
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53 205 and failure-time-analyses similar to those described above were used to assess differences in
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55 206 germination rates. Additionally, the influence of acorn fresh weight on germination was assessed
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57 207 across oak species with a simple linear regression, where the final germination percentage of each

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208 species (fraction acorns germinated by the end of the experiment) was used as response variable and
209 their average acorn fresh weight was used as predictive variable. If acorn fresh weight positively
210 influences germination, this interspecific analysis should indicate a positive relationship between
211 germination percentage and acorn weigh across oak species.

212 To assess intraspecific effects of acorn fresh weight on germination we also used the data of the
213 experiment conducted in 2015. We determined whether variability in this trait influences germination
214 probability within each oak species using logistic regressions based on models of type
215 $Y = e^{(\alpha+\beta X)} / [1 + e^{(\alpha+\beta X)}]$. In this model, Y is a binary response variable (0-1; ungerminated and
216 germinated acorns, respectively), X is the continuous predictive variable (acorn fresh weight), e is the
217 Napier constant (2.718...), and the letters α and β are the parameters that define the shape of
218 regression function (Zar 2010). These regressions calculate the occurrence probability of an event as
219 the values of the predictive variable increases, and their accuracy is provided by the number of cases
220 (0-1) correctly classified by the model (Zar 2010). If acorn fresh weight influences germination, the
221 logistic curve would indicate the critical weight that viable acorns must reach to germinate and, after
222 this value, it would show how germination probability increases with acorn fresh weight. After these
223 analyses, we focused on germinated acorns only and determined whether fresh weight influences
224 germination speed (time elapsed between acorn sowing and germination) within each oak species
225 using simple linear regressions. In these analyses, the response variable was the germination speed of
226 each acorn (days after sowing) and the predictive variable was its fresh weight after soaking. If acorn
227 fresh weight positively influences germination speed, the number of days elapsed between sowing and
228 germination must decrease as fresh weight increases.

229 Fresh weight after soaking, dry biomass and water of acorns collected in 2017 were compared
230 between phylogenetic sections with Student's t -test, and among oak species with one-way ANOVA
231 and *post hoc* Tukey tests. To assess whether fresh weights of soaked acorns related with dry biomass
232 (DB) or water content (PWC) within each oak species, we used simple linear regressions. All statistical
233 analyses described in this section were conducted in R 3.3 (R Foundation 2016) and the datasets

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7 234 generated and analyzed during the current study are available in the Zenodo repository

8 235 [<https://doi.org/10.5281/zenodo.1100945>].

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11 237 **3 Results**

12 238 **3.1 Interspecific effects of acorn fresh weight on germination**

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16 239 In 2015, fully hydrated acorns of white oaks were heavier than those of red oaks ($2.16 \text{ g} \pm 0.039 \text{ S.E.}$

17 240 vs. $0.961 \text{ g} \pm 0.024 \text{ S.E.}$, $t = 26.725$, d.f. = 998, $p < 0.001$). Differences in acorn fresh weight after

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19 241 soaking were also found among oak species ($F_{(9,990)} = 391.327$, $p < 0.001$). Pairwise comparisons

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21 242 between species indicated that the fresh weight of acorns did not differ between the white oaks *Q.*

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23 243 *laeta* and *Q. potosina*, also indicating that these species had heavier acorns than all other ones (Table

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25 244 1). They were followed by the acorns of the red oak *Q. affinis* and the white oak *Q. polymorpha*, which

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27 245 were heavier than those of the remaining species (Table 1). The red oaks *Q. eduardii*, *Q. jonesii*, *Q.*

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29 246 *mexicana* and *Q. viminea* had lighter acorns than the other species, while *Q. castanea* and *Q.*

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31 247 *crassifolia* had intermediate acorn fresh weights (Table 1).

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33 248 Acorns of white oaks had higher germination rates than those of red oaks (Gehan-Wilcoxon

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35 249 statistic = 4.676, d.f. = 1, $p < 0.001$; Fig. 1). Differences in germination rates were also found among

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37 250 species (Gehan-Wilcoxon statistic = 31.743, d.f. = 9, $p < 0.001$). Pairwise comparisons of germination

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39 251 rates indicated that the acorns of the three white oaks included in the experiment (*Q. laeta*, *Q.*

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41 252 *polymorpha* and *Q. potosina*) germinated faster than those of most red oaks (Fig 1). The only

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43 253 exception was the red oak *Q. affinis*, whose germination rates were similar to those of white oaks. (Fig.

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45 254 1). No difference in germination rates was found among the other red oak species (Fig. 1).

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47 255 Germination percentages across oak species were positively related with increases in the average

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49 256 weight of fully hydrated acorns ($F_{(1,8)} = 539.288$, $p < 0.001$, $r^2 = 0.850$). In this relationship, those oaks

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51 257 with acorn fresh weights above 1.7 g (*Q. laeta*, *Q. polymorpha*, *Q. potosina*, and *Q. affinis*) had the

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53 258 highest germination percentage, while oaks with acorn fresh weight below 0.7 g (*Q. eduardii*, *Q.*

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55 259 *jonesii*, *Q. mexicana* and *Q. viminea*) had the lowest germination percentage (Fig. 2). Oak species with

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7 260 intermediate acorn fresh weights (*Q. crassifolia* and *Q. castanea*) were in the middle section of this
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9 261 relationship (Fig. 2).

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12 263 **3.2 Intraspecific effects of acorn fresh weight on germination**

14 264 In all oak species, logistic regressions indicated that germination probability increased with the fresh
16 265 weight of fully hydrated acorns (Table 1). However, these relationships fitted better in red oaks than in
18 266 white ones (Fig. 3). These results are supported by the proportion of cases correctly classified by
20 267 logistic models, which surpassed 85% for red oaks and were below 70 % for white oaks (Table 1). On
22 268 the other hand, germination speed decreased with acorn fresh weight in all red oaks, but these variables
24 269 were not related in any white oak (Table 1, Fig. 4).

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28 271 **3.3 Fresh weight, dry biomass and moisture content of acorns**

30 272 Fresh weights (after soaking) of acorns collected in 2017 concurred with those obtained in 2015, as
32 273 these values were higher in white oaks than in red oaks ($2.02 \text{ g} \pm 0.036 \text{ S.E.}$ vs. $0.964 \text{ g} \pm 0.021 \text{ S.E.}$, t
34 274 $= 26.763$, $\text{d.f.} = 998$, $p < 0.001$). Further, acorns of white oaks collected in 2017 had higher dry
36 275 biomasses and higher water contents than those of red oaks (dry biomass: $0.821 \text{ g} \pm 0.015 \text{ S.E.}$ vs.
38 276 $0.584 \text{ g} \pm 0.014 \text{ S.E.}$, $t = 9.545$, $\text{d.f.} = 998$, $p < 0.001$; water contents: $59.3\% \pm 0.402 \text{ S.E.}$ vs. $40.8\% \pm$
40 277 0.264 S.E. , $t = 28.999$, $\text{d.f.} = 998$, $p < 0.001$). All these acorn variables also differed among oak species
42 278 (fresh weight: $F_{(9,990)} = 312.552$, $p < 0.001$; dry biomass: $F_{(9,990)} = 320.772$, $p < 0.001$; water content:
44 279 $F_{(9,990)} = 965.273$, $p < 0.001$). The white oak *Q. potosina* had the highest acorn fresh weight after
46 280 soaking (Table 2), followed by the white oaks *Q. polymorpha* and *Q. laeta* and the red oak *Q. affinis*
48 281 (Table 2). Acorn fresh weights of the other red oaks (*Q. castanea*, *Q. crassifolia*, *Q. eduardii*, *Q.*
50 282 *mexicana* and *Q. viminea*) were lower than those recorded for the former species (Table 2). The
52 283 highest acorn dry biomass, however, was recorded for *Q. affinis*, followed by the three white oaks and,
54 284 later, by the remaining red oak species (Table 2). The acorns of the three white oak species had higher
56 285 water contents than those of red oaks (Table 2). Further, within all oak species, the fresh weight of

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286 acorns after soaking increased with their dry biomass (Table 2, Fig 5), but no relationship was found
287 between fresh weight and water content of acorns (see Online Resource 1).

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289 **4 Discussion**

290 Our results indicate that increasing acorn fresh weight improves germination in the ten oak species
291 included in the common garden experiment and, as fresh weights of acorns after soaking were
292 positively related with their dry biomasses, it is feasible to propose that these effects are due to
293 differences in the amount of nutritional reserves accumulated in the acorns. The germination patterns
294 assessed with different response variables (i.e., germination rates and germination percentage for
295 interspecific effects, and germination probability and germination speed for intraspecific effects) were
296 consistent among and within species, suggesting that acorn weight is an important life history trait that
297 influences germination in oaks.

298 White oaks (phylogenetic section *Quercus*) had heavier acorns than red oaks (phylogenetic section
299 *Lobatae*) and our results indicate that this may be linked with the higher germination rates observed for
300 white oaks. Variability in propagule weight across plant groups has been associated with the output of
301 differential selective pressures that adapted them to germinate in different environments (Foster 1986;
302 Silvertown 1989; Westoby et al. 1992; Leishman and Westoby 1994; Geritz et al. 1999; Burslem and
303 Miller 2001; Bruun and Ten Brink 2008; Venable and Rees 2009). The differential germination
304 responses that white and red oaks exhibited under the same environmental conditions may be
305 reflecting this kind of adaptation. Otherwise, if acorn weight is irrelevant for germination, all species
306 should have displayed similar germination rates irrespectively of the phylogenetic section they belong
307 to. As the common garden experiment attempted to emulate the shaded conditions of the forest
308 understory, it can be proposed that white oaks are better adapted than red oaks to germinate in this kind
309 of habitats. This earlier germination of white oaks could allow them to quickly occupy sites available
310 for seedling establishment beneath the forest canopy, which in turn may confer them some advantages

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311 over red oaks at the recruitment stage. Nevertheless, it is important to highlight that detailed field
312 experiments are still required to test the validity of this proposal.

313 Besides these differences in germination rates between red and white oaks, germination
314 percentages increased with acorn fresh weight across oak species. This suggests that variability in this
315 trait, which seems to be related with the dry biomass of acorns, also influences germination across red
316 and white oak species. Several authors have proposed that heavier-seeded tree species belong to late-
317 successional forest stages and, because they germinate beneath the forest canopy, their seeds would
318 require large reserves to support the development of seedlings (Foster 1986; Silvertown 1989; Venable
319 and Brown 1988; Leishman et al. 2000; Yi and Wang 2016). Conversely, lighter-seeded species have
320 been associated with disturbed sites where germination mainly depends on stochastic environmental
321 factors and, thus, producing large and heavy seeds would not represent any advantage because all
322 seeds have the same chance of germinating (Gross 1984; Jurado and Westoby 1992; Long and Jones
323 1996; Khurana and Singh 2001). As our experiment emulated understory conditions and germination
324 percentages increased with acorn fresh weight, it can be proposed that oak species with heavier acorns
325 would belong to late forest stages, while oaks with lighter acorns would belong to early forest stages.
326 However, comprehensive field experiments are required to elucidate whether oak species that produce
327 differentially weighed acorns are actually correspond with different successional stages of forests.

328 At the intraspecific level, increasing acorn fresh weight led to higher germination probabilities in
329 all oak species. These effects may be due to differential maternal effects among parental trees, or even
330 among different branches of the same tree, where the amount of reserves that propagules receive
331 depends on the physiological status of these individuals and/or branches (Roach and Wulff 1987;
332 Gómez 2004). In this way, heavier acorns may have larger reserves of proteins and carbohydrates in
333 their cotyledons, which could provide readily available energy to stimulate the elongation of the
334 embryonic axis after imbibition (Tripathi and Khan 1990; Khan and Shankar 2001; Purohit et al. 2003;
335 Gómez 2004; Quero et al. 2007; Lopes-Souza and Fagundes 2014). Alternatively, these effects could

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7 336 be due to differential loads of germination phytohormones among acorns of different weight, such as
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9 337 gibberellins and cytokinins (Farnsworth 2000).

10 338 When the intraspecific analyses focused on germinated acorns only, all red oak species displayed
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12 339 higher germination speeds as acorn fresh weight increased, but these relationships were not found in
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14 340 any white oak. Several studies have previously reported higher germination speeds with increasing
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16 341 seed weight, and these relationships have been mainly attributed to maternal effects as those described
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18 342 in the former paragraph (Khan and Shankar 2001; Purohit et al. 2003; Gómez 2004; Lopes-Souza and
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20 343 Fagundes 2014). However, as far as we are aware, this is the first study reporting these relationships
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22 344 between germination speed and acorn weight in red oaks, which complement the strong positive
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24 345 effects of acorn weight on germination probability that we found for these species. These results then
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26 346 suggest that acorn weight is a critical trait involved in the germination of oaks belonging to the
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28 347 phylogenetic section *Lobatae*. On the other hand, the weaker relationships between germination
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30 348 probability and acorn weight in species of the phylogenetic section *Quercus*, as well as the lack of
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32 349 effect of this trait on germination speed, suggest that germination of white oaks do not fully depend on
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34 350 acorn weight. These differential germination responses between red and white oaks could be related to
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36 351 the marked discrepancies in acorn weight that we observed among species of these two phylogenetic
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38 352 sections, where even the smaller acorns of white oaks were heavier than the largest acorns of most red
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40 353 oak. Thus, while acorn weight seems to be a strong determinant of germination in red oak species, the
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42 354 influence of this trait on germination of white oak species would be negligible.
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46 356 **5 Conclusions**

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49 357 The fresh weight of viable acorns is positively related to their dry biomass, while this trait is not
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51 358 affected by the water content of acorns. These results suggest that interspecific variability in fresh and
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53 359 dry weight of acorns influences germination rates and germination percentages across the oak species
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55 360 included in this study, where increasing acorn weight has positive effects on these two germination
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57 361 variables. The intraspecific variability in these traits, however, seems to have more influence on the
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362 germination of red oaks (phylogenetic section *Lobatae*) than on germination of white oaks
363 (phylogenetic section *Quercus*). Therefore, it is feasible to propose that germination across white oak
364 species is not as strongly determined by acorn weight as it is in red oaks.

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464 **Figure captions**

465 **Fig. 1** Acorn germination rates estimated with the Kaplan-Meier's method. The upper panel shows the
466 germination rates of red oaks (phylogenetic section *Lobatae*) and white oaks (phylogenetic section
467 *Quercus*) irrespectively of the species. The lower panel shows the germination rates of each oak
468 species included in the common-garden experiment. Different letters on the side of each oak name
469 indicate significant differences in germination rates between species (Gehan-Wilcoxon critical α for
470 pairwise comparisons = 0.05)

471
472 **Fig. 2** Linear relationship between germination percentage and average acorn fresh weight ($g \pm S.E.$)
473 across the ten oak species included in the common-garden experiment

474
475 **Fig. 3** Results of logistic regressions addressed to assess relationships between germination probability
476 and acorn fresh weight within each oak species included in the common-garden experiment. Note that
477 the range of values of the predictive variable (acorn fresh weight) varies across oak species. Red oaks
478 are *Q. affinis*, *Q. castanea*, *Q. crassifolia*, *Q. eduardii*, *Q. jonesii*, *Q. mexicana* and *Q. viminea*, and
479 white oaks are *Q. laeta*, *Q. polymorpha* and *Q. potosina*

480
481 **Fig. 4** Results of linear regressions addressed to assess relationships between germination speed and
482 acorn fresh weight within each oak species included in the common-garden experiment. These
483 analyses included germinated acorns only. Note that the range of values of the predictive variable
484 (acorn fresh weight) varies across oak species. Red oaks are *Q. affinis*, *Q. castanea*, *Q. crassifolia*, *Q.*
485 *eduardii*, *Q. jonesii*, *Q. mexicana* and *Q. viminea*, and white oaks are *Q. laeta*, *Q. polymorpha* and *Q.*
486 *potosina*. No regression lines are provided for white oaks because no significant effects of acorn
487 weight on germination speed were found

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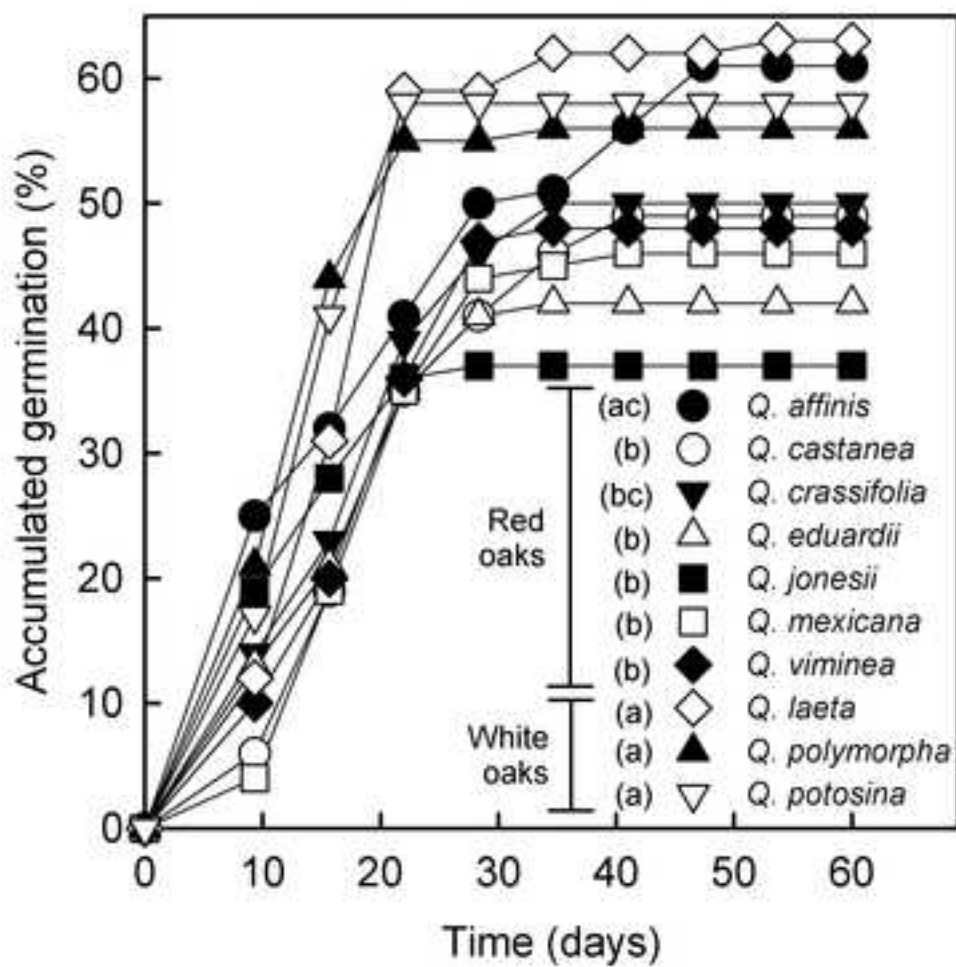
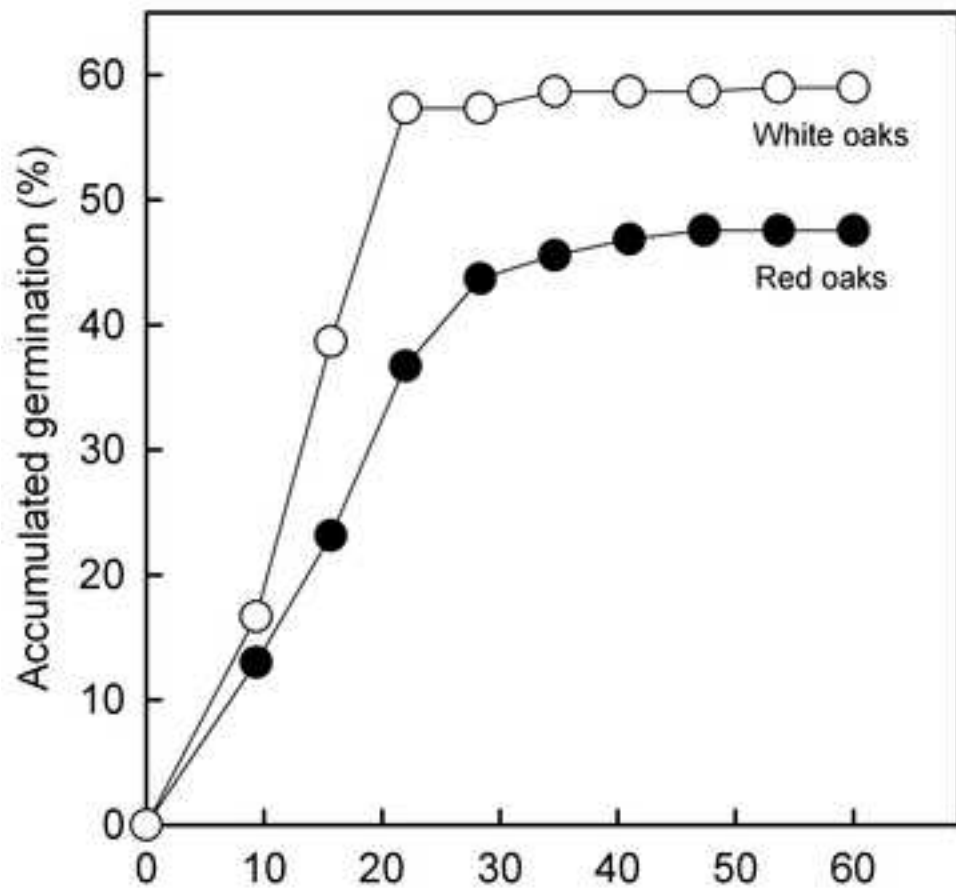
489 **Fig. 5** Results of linear regressions addressed to assess relationships between the fresh weight of
490 acorns and their dry biomass for each oak species included in the common-garden experiment. Note
491 that the range of values of both, the response variable (acorn fresh weight) and the predictive variable
492 (acorn dry biomass), vary across oak species. Red oaks are *Q. affinis*, *Q. castanea*, *Q. crassifolia*, *Q.*
493 *eduardii*, *Q. jonesii*, *Q. mexicana* and *Q. viminea*, and white oaks are *Q. laeta*, *Q. polymorpha* and *Q.*
494 *potosina*.

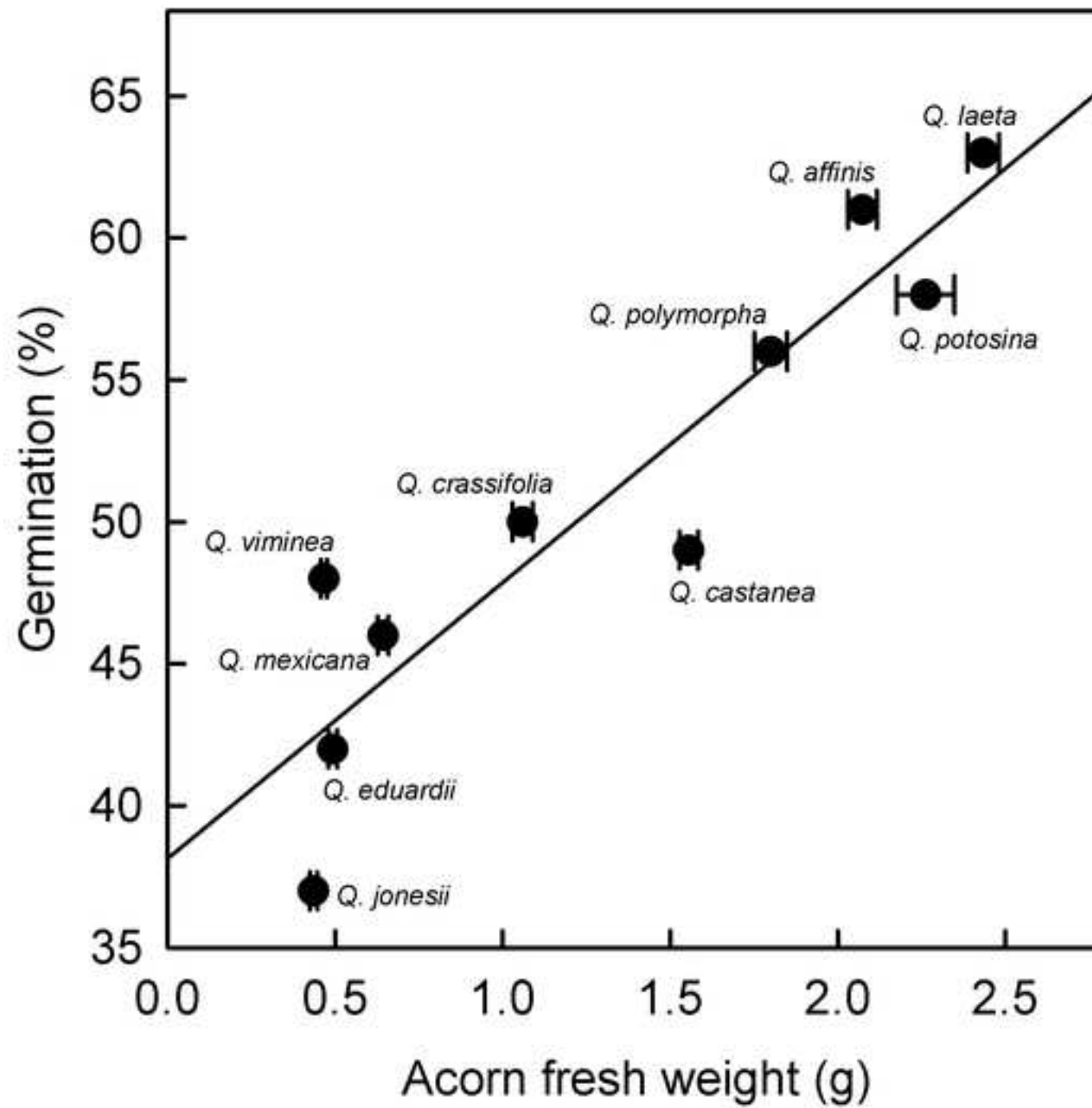
Table 1 Average fresh weight (\pm S.E.) of acorn of red and white oaks included in the common garden experiment conducted in 2015. Different letters on the side of averages indicate significant differences between oak species (Tukey test critical $\alpha = 0.05$). The table also shows the results of the logistic regressions (χ^2 -value and p -value; the percent of cases correctly classified by the model is shown in brackets) addressed to determine how germination probability relates with fresh acorn weight within each species (critical α for significant effects = 0.05), as well as the results of the simple linear regressions (F -value with degrees of freedom and p -value; the r^2 -value is shown in brackets) conducted to assess the relationships between fresh acorn weight and germination speed (critical α for significant effects = 0.05)

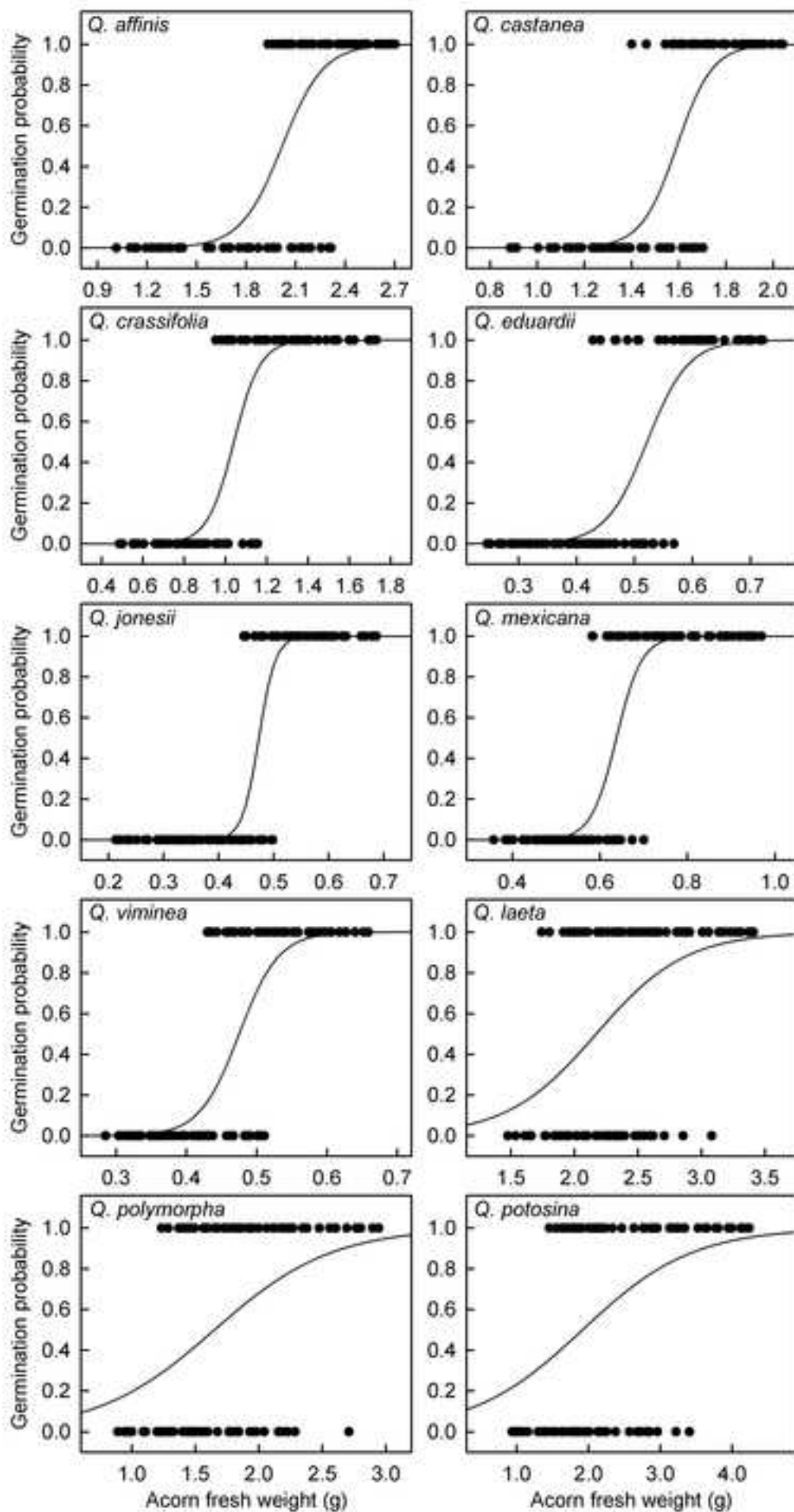
Phylogenetic section	Oak species	Fresh acorn weight after soaking (g)	Logistic regression results	Linear regression results
Red oaks (<i>Lobatae</i>)	<i>Q. affinis</i>	2.07 (± 0.043 S.E.) ^a	$\chi^2 = 71.070, p < 0.001$ (89%)	$F_{(1,59)} = 100.171, p < 0.001$ (0.629)
	<i>Q. castanea</i>	1.55 (± 0.027 S.E.) ^b	$\chi^2 = 78.357, p < 0.001$ (88%)	$F_{(1,47)} = 88.935, p < 0.001$ (0.654)
	<i>Q. crassifolia</i>	1.06 (± 0.030 S.E.) ^c	$\chi^2 = 93.850, p < 0.001$ (91%)	$F_{(1,48)} = 131.102, p < 0.001$ (0.732)
	<i>Q. eduardii</i>	0.493 (± 0.013 S.E.) ^d	$\chi^2 = 85.117, p < 0.001$ (88%)	$F_{(1,40)} = 144.077, p < 0.001$ (0.783)
	<i>Q. jonesii</i>	0.436 (± 0.011 S.E.) ^d	$\chi^2 = 100.310, p < 0.001$ (92%)	$F_{(1,35)} = 72.239, p < 0.001$ (0.674)
	<i>Q. mexicana</i>	0.643 (± 0.016 S.E.) ^e	$\chi^2 = 96.728, p < 0.001$ (91%)	$F_{(1,44)} = 42.907, p < 0.001$ (0.494)
	<i>Q. viminea</i>	0.467 (± 0.009 S.E.) ^d	$\chi^2 = 73.605, p < 0.001$ (85%)	$F_{(1,46)} = 69.558, p < 0.001$ (0.602)
White oaks (<i>Quercus</i>)	<i>Q. laeta</i>	2.43 (± 0.047 S.E.) ^f	$\chi^2 = 27.651, p < 0.001$ (66%)	$F_{(1,61)} = 0.245, p = 0.622$ (0.004)
	<i>Q. polymorpha</i>	1.78 (± 0.049 S.E.) ^g	$\chi^2 = 20.047, p < 0.001$ (67%)	$F_{(1,54)} = 1.780, p = 0.187$ (0.031)
	<i>Q. potosina</i>	2.26 (± 0.086 S.E.) ^f	$\chi^2 = 21.135, p < 0.001$ (64%)	$F_{(1,56)} = 0.263, p = 0.609$ (0.005)

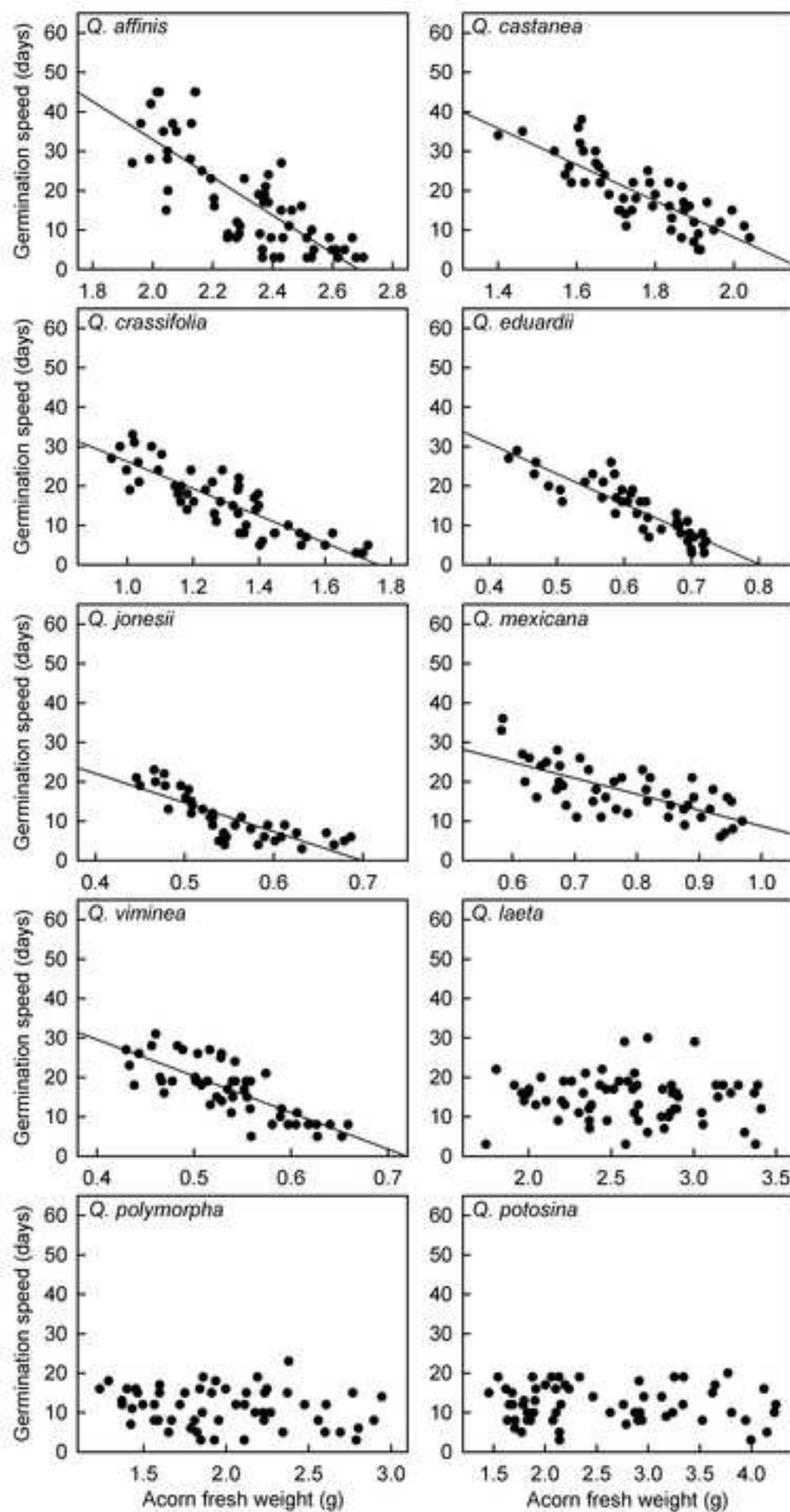
Table 2 Average fresh weight, dry biomass and percent water content (\pm S.E.) of acorns of the red and white oaks collected in 2017. Different letters on the side of averages indicate significant differences between oak species (Tukey test critical $\alpha = 0.05$). The table also shows the results of the simple linear regressions (F -value with degrees of freedom and p -value; the r^2 -value is shown in brackets) conducted to assess whether the fresh weight of acorns was related with their dry biomass (critical α for significant effects = 0.05)

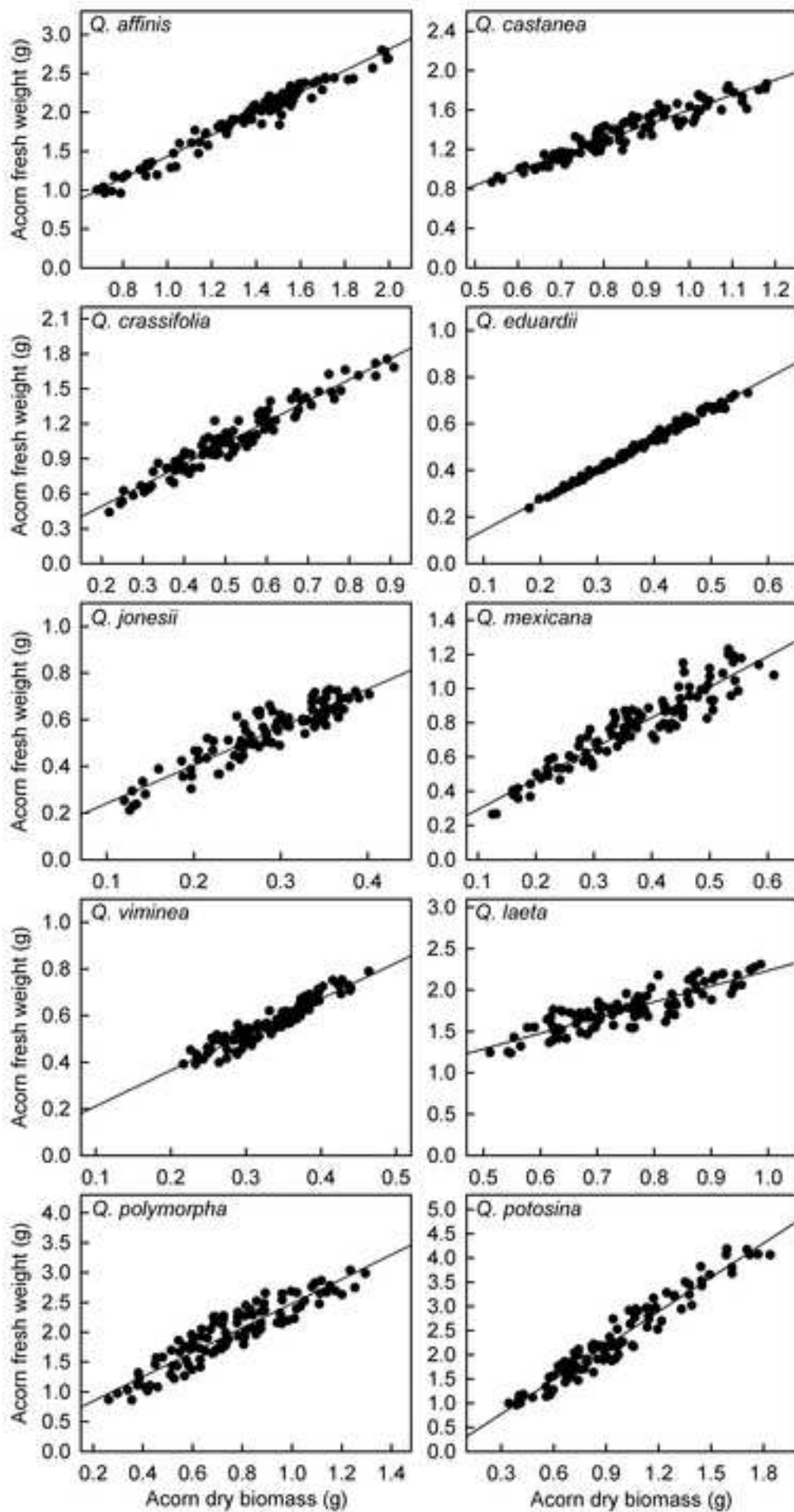
Phylogenetic section	Oak species	Fresh acorn weight after soaking (g)	Acorn dry biomass (g)	Acorn percent water content (%)	Linear regression results
Red oaks (<i>Lobatae</i>)	<i>Q. affinis</i>	1.92 (± 0.044 S.E.) ^a	1.36 (± 0.031 S.E.) ^a	29.4 (± 0.365 S.E.) ^a	$F_{(1,98)} = 2323.738, p < 0.001 (0.959)$
	<i>Q. castanea</i>	1.36 (± 0.024 S.E.) ^b	0.842 (± 0.015 S.E.) ^b	37.9 (± 0.341 S.E.) ^b	$F_{(1,98)} = 901.790, p < 0.001 (0.902)$
	<i>Q. crassifolia</i>	1.06 (± 0.029 S.E.) ^c	0.514 (± 0.015 S.E.) ^c	51.7 (± 0.379 S.E.) ^c	$F_{(1,98)} = 1349.742, p < 0.001 (0.932)$
	<i>Q. eduardii</i>	0.516 (± 0.012 S.E.) ^d	0.386 (± 0.009 S.E.) ^d	25.3 (± 0.152 S.E.) ^d	$F_{(1,98)} = 12068.524, p < 0.001 (0.992)$
	<i>Q. jonesii</i>	0.546 (± 0.012 S.E.) ^d	0.287 (± 0.007 S.E.) ^e	47.4 (± 0.547 S.E.) ^e	$F_{(1,98)} = 504.373, p < 0.001 (0.837)$
	<i>Q. mexicana</i>	0.779 (± 0.022 S.E.) ^e	0.371 (± 0.011 S.E.) ^d	52.6 (± 0.513 S.E.) ^c	$F_{(1,98)} = 695.915, p < 0.001 (0.877)$
	<i>Q. viminea</i>	0.563 (± 0.009 S.E.) ^d	0.329 (± 0.006 S.E.) ^{de}	41.6 (± 0.368 S.E.) ^f	$F_{(1,98)} = 726.034, p < 0.001 (0.881)$
White oaks (<i>Quercus</i>)	<i>Q. laeta</i>	1.77 (± 0.025 S.E.) ^a	0.755 (± 0.011 S.E.) ^f	57.3 (± 0.329 S.E.) ^g	$F_{(1,98)} = 291.639, p < 0.001 (0.748)$
	<i>Q. polymorpha</i>	1.98 (± 0.053 S.E.) ^a	0.755 (± 0.024 S.E.) ^f	62.1 (± 0.476 S.E.) ^h	$F_{(1,98)} = 558.691, p < 0.001 (0.851)$
	<i>Q. potosina</i>	2.31 (± 0.084 S.E.) ^f	0.951 (± 0.035 S.E.) ^g	58.6 (± 0.419 S.E.) ^g	$F_{(1,98)} = 1460.344, p < 0.001 (0.937)$





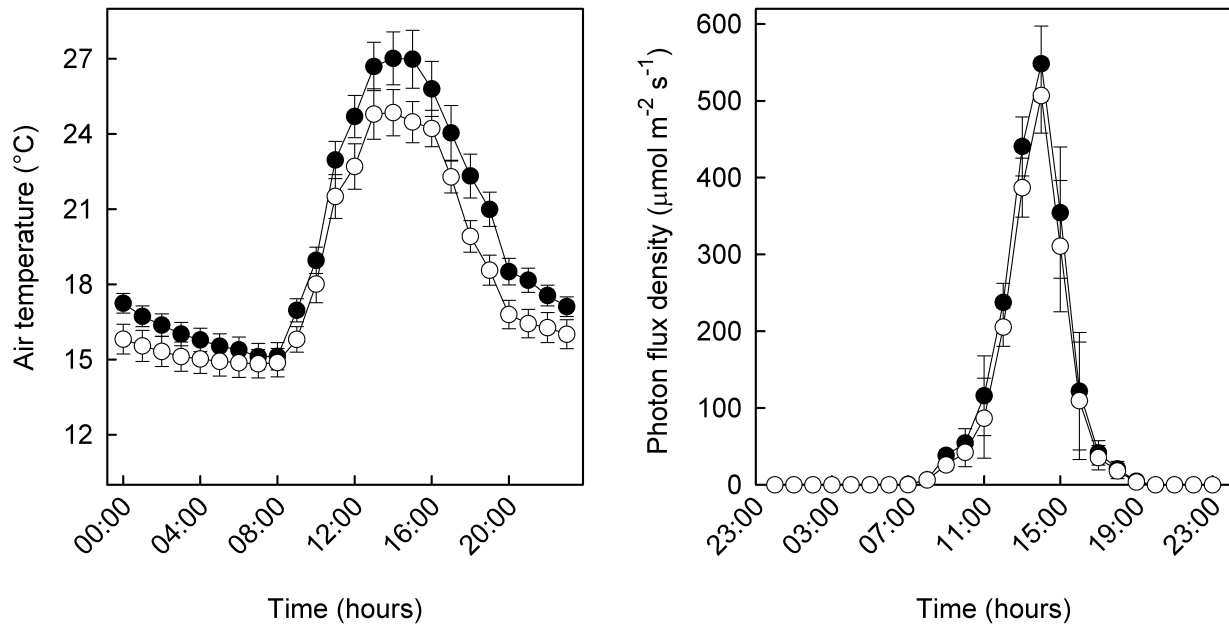




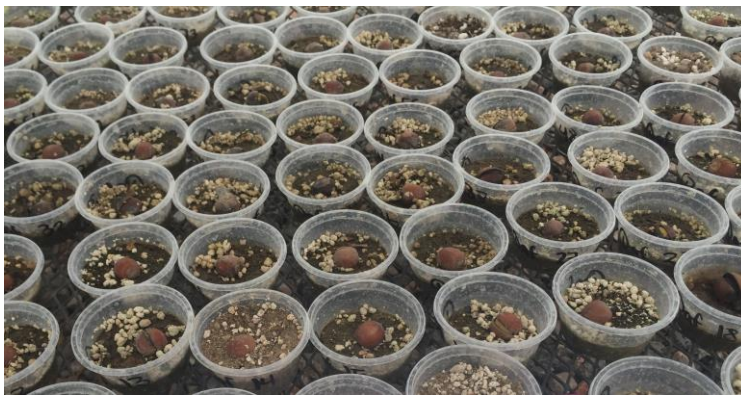


ONLINE RESOURCE 1

Air temperature and light conditions in the greenhouse and beneath the forest canopy. Automatized sensors (Hobo Pendant, Onset Computer Corporation, USA) were placed in the greenhouse where the experiment was conducted and beneath the canopy of an oak forest of Sierra de Álvarez (state of San Luis Potosí, México). These sensors were programed to measure temperature and relative humidity of the air at each hour during the 60 days of the common-garden experiment (September 16th to November 15th 2015). At the end of the experiment, these values were averaged per hour. The figures below show the daily curves (averages \pm 95% I.C.) obtained for these two variables in the greenhouse (black symbols) and beneath the forest canopy (white symbols).



Common garden experiment. The left plate shows acorns sowed in germination cups on the experimental table of the greenhouse. The right plate shows a germinated acorn of *Quercus affinis*.



Relationships between fresh weight and water content of acorns. Fresh weight of acorns (after soaking) was not related with water content in any oak species. The figure below shows the scatterplots that resulted from these regression analyses. The statistical results obtained for each oak species (F -value, degrees of freedom, p -value and r^2 -value) are shown within the respective panel (critical α for significant effects = 0.05)

