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Conservation and fruit biology of Sichou oak (*Quercus sichourensis*, Fagaceae) – A critically endangered species in China

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ABSTRACT

Several conservation programs have been started for the critically endangered Sichou oak (*Quercus sichourensis*) since 2007. These programs include detailed field investigations, seedling cultivation and research on the fruit biology of the species. In this study, we first report on the five mature individual trees found in our 9-year field investigation. Thus far, a total of 10 mature individuals have been recorded. All *Q. sichourensis* trees are healthy and most produce healthy acorns. Acorns of *Q. sichourensis* are large with dry masses of 8.0–14.0 g. These acorns had high moisture contents at collection and died shortly after (7–28 d) when dried with silica gel. Characteristics of *Q. sichourensis* acorns varied between populations. Compared with the acorns from Funing, the acorns collected from Ceheng were bigger, more viable (germination percentage was up to 96%), less sensitive to desiccation, and germinated faster. *Q. sichourensis* occurs in regions with a distinct 5–6 month dry season. Habitat degradation is largely responsible for the rareness of *Quercus sichorensis*, but desiccation sensitivity of the acorns may also limit the regeneration of the species and potentially lead to its continued rareness. As a species with extremely small populations (PSESP), *Q. sichourensis* is facing high risk of extinction and should be defined as a Critically Endangered species in the global IUCN Red List.

1. Introduction

As a “foundation” component in many forests of the northern hemisphere, oaks (*Quercus* spp.) harbor a large numbers of species (such as insects, rodents and jays; Vander Wall, 2001) and provide valuable ecosystem goods and services to humanity, including food, timber, clean water, cultural values, and climate moderation. However, because of various natural (e.g. climate change, severe predation, disease) and human-induced disturbances (e.g. deforestation for timber and agriculture), many oaks are now in decline (Helama et al., 2016; Sallé et al., 2014), and some are even facing extinction. In the newly released IUCN Red List of Threatened Species (2016), 14 oak species are recorded as endangered or critically endangered species. However, Sichou oak (*Quercus sichourensis*), a critically endangered species from subtropical forests of China, is not on this list.

Q. sichourensis is an evergreen oak endemic to China. It belongs to the subgenus *Cyclobalanopsis*, and in Flora of China, where *Cyclobalanopsis* is classified as an independent genus in Fagaceae, it is also named *Cyclobalanopsis sichourensis* (Huang et al., 1999). *Q. sichourensis* can be easily distinguished in the forests because it is taller than most other *Quercus* species (Fig. 1A) and produces very unique acorns (Fig. 1B). Acorns of *Q. sichourensis* are big and entirely enclosed by a highly lignified cupule (Fig. 1B). To our knowledge, of 450 species in the genus *Quercus*, this type of cupule is only known to occur in *Q. sichourensis*, and occasionally in *Quercus lamellose*, whose cupule is usually fleshier and encloses 2/3–4/5 of the acorn (Huang et al., 1999).

Specimens of *Q. sichourensis* were first collected near the town of Liuhe in present Malipo County, southeast Yunnan, China in 1947 (Liuhe belonged to Xichou County at the time of collection). Based on these specimens, *Q. sichourensis* was described as a new species in 1951. In 1964, new specimens were collected in Funing, southeast Yunnan. This species was not recorded again until 2006, when

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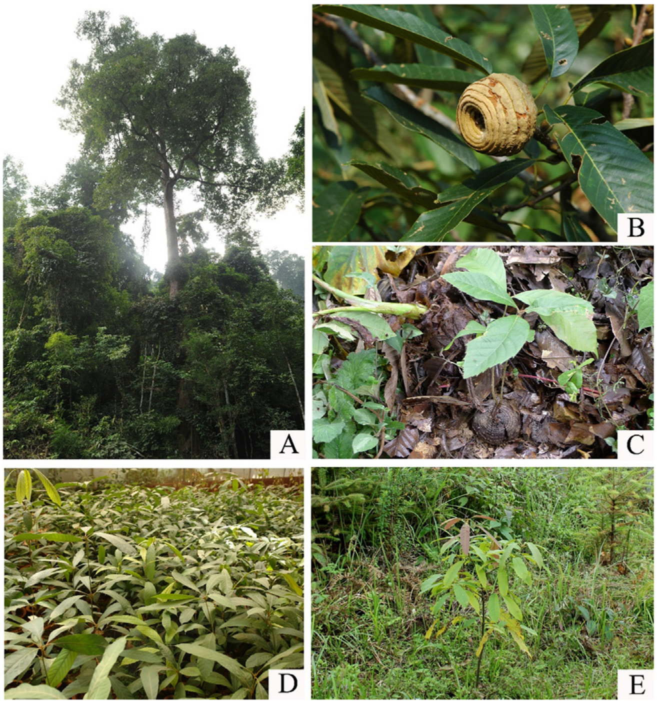

Fig. 1. Plants, acorns and seedlings of *Q. sichourensis* in the field or in the green house: (A) a natural individual in the forest; (B) the acorn; (C) naturally established seedlings which were rarely found during our investigation; (D) cultivated seedlings in the green house; (E) a young plant of *Q. sichourensis* which was planted in a reserve of Maguang, Yunnan, China.

we found one individual tree in Ceheng, Guizhou (Chen et al., 2007).

Q. sichourensis used to play an important role in the subtropical forests of karstic (limestone) areas. It was a common species in the late 1940s. Over the next half century, *Q. sichourensis* populations declined severely. By 2007, we found that it was facing an extremely high risk of extinction: there were only three known localities and a total of five living individuals in the wild. In its type locality of Liuhe (Xia et al., 2008), there were no individuals remaining.

The rareness of *Q. sichourensis* results mainly from habitat degradation and from deforestation for timber and agriculture (Xia et al., 2008). However, the desiccation sensitivity (recalcitrance) of its acorns (Xia et al., 2012) might also have limited the regeneration of the species and could potentially led to its continued rareness. The Asian monsoon system, which mainly shapes the Chinese climate, causes distinct wet (May–October) and dry (November–April) seasons across the ranges of *Q. sichourensis*. In the same regions, the disadvantages of acorn desiccation sensitivity are obvious. In the event of dry periods after dispersal, many acorns or germinated acorns die in a long dry spell. However, with limited knowledge of its fruit biology, the effects of desiccation sensitivity on the survival of *Q. sichourensis* acorns remain unclear.

The conservation of *Q. sichourensis* is crucial and urgent. In this study, we report a detailed field investigation for this species across southwest China between 2007 and 2016, which aimed to look for and protect the remaining individuals of this species. To increase the population of the species, acorns of *Q. sichourensis* were collected during the 9-year investigation, and seedlings were cultivated. We also investigated the desiccation tolerance and germination of acorns from *Q. sichourensis.* Knowledge of *Q. sichourensis* fruit biology will facilitate the understanding of its rareness and help plan appropriate conservation strategies (Corlett, 2016).

2. Materials and methods

2.1. Field investigation

Field investigations started in 2007. Eight field investigations were undertaken over the last 9 years, covering most of east and southeast Yunnan, southwest Guizhou and west Guangxi. In these areas, we visited local people and surveyed all possible forests and rural lands in search of additional populations of *Q. sichourensis*. For each individual tree, we recorded the health status, then measured the diameter at breast height (DBH), the diameter of the tree trunk at 1.3 m above the ground as well as the tree height, and the height from the ground surface to the tree crown (Fang et al., 2012).

2.2. Germination tests and seedling cultivation

Acorns of *Q. sichourensis* were collected in Funning and Ceheng at the time of their natural dispersal between 2009 and 2015. Once collection was finished, acorns were delivered by car to the lab directly. Fresh acorns with cupules were planted in the green house within a week of receipt. Acorns collected in late October 2015 were used for germination and desiccation tolerance studies. Germination tests were conducted on acorns with cupules, and without cupules. For acorns without cupules, a total of 32 (split over 4 sub-samples of 8 acorns) from Funning and 24 acorns (split over 4 sub-samples of 6 acorns) from Ceheng were incubated at a wide temperature range between 5 and 40 °C. Acorns were sown on 1 cm thick layer of 1% agar in distilled water inside closed plastic boxes (size: 175 × 105 × 65 mm). For acorns with cupules, a total of 24 acorns (split over 4 sub-samples of 6 acorns) from Funning were incubated at 15, 20 and 25 °C, and a total of 50 acorns (split over 10 sub-samples of 5 acorns) from Ceheng were incubated at 25 °C. Only 24–50 acorns were used due to the difficulty of obtaining large numbers; similarly low numbers of seeds have been used elsewhere for large seeded tree species (e.g. Daws et al., 2004). Germination was defined as radicle emergence by at least 2 mm. Germination was recorded until no further germination was observed for at least 1 month.

The mean time to germination (MTG) was calculated using the following equation:

$$\mathrm{MTG} = \sum (\mathrm{n} \times \mathrm{d}) \Big/ \mathrm{N}$$

where n is the number of seeds germinated between scoring intervals; d the incubation period in days at that time point; and N the total number of seeds germinated in the treatment (Tompsett and Pritchard, 1998).

2.3. Initial acorn moisture contents and desiccation responses

Fruit dry mass and initial moisture contents were determined by drying 10 individual acorns at 103 °C for 17 h (ISTA, 2007). Moisture contents are expressed on a fresh weight basis (% fr.wt). To desiccate acorns from Funing, seven samples of 40 acorns without cupules, and nine samples of 40 acorns with cupules were placed in press seal polythene bags with an equal weight of freshly regenerated silica gel. Acorns with cupules from Ceheng were desiccated in the same way, but only 30 acorns were used for each desiccation bag. All the bags were held at 15 °C. These acorns were dried for different periods to reach a range of target moisture contents. For acorns in each desiccation bag, the post-desiccation moisture contents were determined by using 10 and 5 acorns for the Funing and Ceheng collections, respectively. At the same time, the remaining 30 (Funing collection) and 25 (Ceheng collection) acorns in the same desiccation bags were used for post-desiccation germination tests at 25 °C.

3. Results

Five individual trees of *Q. sichourensis* were found during our field investigation in 2011 (Table 1): four trees are in declining subtropical evergreen forest in Funing and one tree is in Sichou County, which is close to the type locality of Liuhe. Thus far, a total of 10 trees have been found and recorded (Table 1). All the trees are healthy and seven of them produce healthy acorns. All the trees distributed in the subtropical evergreen forest in Funing are now protected by the local government and the habitats of these trees are also protected as a reserve.

Q. sichourensis produces acorns annually. The mature acorns disperse from October to mid-December. Unlike acorns of other congeners, acorns of *Q. sichourensis* usually don't detach from the cupules. At the time of dispersal, acorns from both Funing and Ceheng had high moisture contents in embryonic axes (53.4–59.6%), cotyledons (42.3–45.3%) and cupules (58.8–67.7%).

Compared with Ceheng acorns, Funing acorns had lower dry mass and a lower initial germination percentage (Table 2, Fig. 2). They died within 7 days of desiccation while the moisture contents for both axes (44.0%) and cotyledons (31.6%) was still high. Funing acorns covered by cupules dried more slowly, but they died at higher moisture contents of 53.5% (axes) and 40.8% (cotyledons). Acorns collected in Ceheng were dried with cupules. These acorns died after 28 days' desiccation with a lower moisture content for both axes (23.9%) and cotyledons (21.6%) (Table 2).

Germination behaviors of acorns from the two collections were different. For acorns collected in Ceheng, high germination percentages were recorded (75–95.8%) in a temperature range between 10 and 35 °C. At 5 and 40 °C, acorns had no or low germination percentages. Between 10 and 30 °C, acorns from Ceheng germinated faster as the temperature increased (Fig. 2). A high temperature (40 °C) and lower temperatures (10 and 15 °C) prolonged germination time. Acorns collected in Funing germinated in a more narrow temperature range between 20 and 35 °C. The highest germination of Funing acorns was 62.5% at 25 °C. At the same temperatures, acorns from Funing germinated more slowly than the ones from Ceheng. For acorns covered with cupules, acorns collected in Funing reached a germination percentage of 45.8% (MTG = 71 d) at 25 °C but failed to germinate at 15 and 20 °C. Acorns (with cupules) collected in Ceheng had a germination percentage of 54% at 25 °C and the MTG was 25 d.

In the green house, 2650 acorns germinated and developed into seedlings. Of these, 2500 seedlings are still growing in the green house in Kunming Institute of Botany, Chinese Academy of sciences (KIB). Another 50 seedlings were planted in the KIB botanic gardens and 100 seedlings were planted in a provincial reserve located in Maguang, Yunnan, for the purposes of conservation and research.

4. Discussion

Plant species with extremely small populations (PSESP) refers to naturally rare species and species with low numbers due to serious human disturbance in recent times (Ma et al., 2013; Volis, 2016). The concept of PSESP was first proposed in China and is intended to protect plant species with extremely small numbers that are unable to survive long-term in the wild (Ren et al., 2012). PSESP are defined by several key characteristics: small existing populations, restricted habitat, serious human disturbance and extremely high risk of extinction (Ma et al., 2013). *Q. sichourensis* meets these criteria completely and is now on the PSESP list. With such small numbers of mature individuals, *Q. sichourensis* also meets the criteria for inclusion on the IUCN Red List of "Critically Endangered" species. To strengthen public awareness of the need to conserve both this species and its habitat, here we recommend that this species be added to the IUCN Red List.

Due to habitat degradation and the small number of mature individuals, *Q. sichourensis* populations are unlikely to expand naturally. At present, the local government of Funing has set up a reserve to protect the eight trees located in subtropical evergreen forest. Located in strongly disturbed areas, the remaining two individuals face high risks in Sichou County and Ceheng.

This study presents the variances in acorn characteristics between different populations (Funing and Ceheng). Generally, acorns from Ceheng were bigger, more viable and able to survive much longer in drought conditions (Table 2; Fig. 2), whereas acorns from Funing were smaller, less viable and less desiccation tolerant. The recalcitrance/desiccation-sensitivity of *Q. sichourensis* acorns was previously reported by Xia et al. (2012). However, the previous work did not evaluate the desiccation tolerance of the acorns. This study provides this information and also presents the variance in acorn desiccation tolerance between different populations. Farrant et al. (1988) and Berjak et al. (1989) suggested there were marked differences in the responses of seeds, which led to the suggestion of highly-, moderately-, and minimally recalcitrant behavior within the recalcitrant or 'desiccation intolerant' category. Acorns from Funing died at a high moisture content level (44–53.5% for axes, and 31.6–40.8% for cotyledons), and while this could be associated with low quality acorns, it is still possible to define Funing acorns as highly recalcitrant. In contrast, acorns from Ceheng tolerated drying to lower moisture contents and were less recalcitrant.

Quercus species are usually highly viable and non-dormant, but their germination rates vary (Xia et al., 2015). Generally, species

Table 1
Records for the living individuals of *Quercus sichourensis* in the field. Trees no.1–8 grow in the same subtropical evergreen forests in Funing, Yunnan.

Trees	Habitats and locations (coordinates, altitudes)	DBH (cm)	Tree height (m)	Year of being fund	Health status	Protection status
1	Subtropical evergreen forest, Funing, Yunnan	88.2	42.4	2007	Healthy and produces acorns	Protected
2	(N 23°44′, E 104°53′, 1017–1002 m)	50.0	36.8	2007	Healthy and produces acorns	Protected
3		71.0	42.6	2011	Healthy and produces acorns	Protected
4		96.8	37.6	2007	Healthy and produces acorns	Protected
5		120.1	45.2	2007	Healthy and produces acorns	Protected
6		118.2	34.8	2011	Healthy and produces acorns	Protected
7		32.5	18.0	2011	Healthy but not produces acorns	Protected
8		84.1	34.0	2011	Healthy but not producs acorns	Protected
9	On a farm in Sichou, Yunnan (N 23°20′, E 104° 44′, 1403 m)	74.2	27.8	2011	Sprouted from a fallen tree, healthy but produces aborted acorns	Not protected
10	On a farm in Ceheng, Guizhou (N 24°54′, E 105°58′, 760 m)	119.4	28.8	2006	Healthy and produces acorns	Not protected

Table 2
Fruit characteristics and desiccation study results for *Quercus sichourensis* from Funing and Ceheng. Data are means ± standard errors.

Location (sample)	Fresh						Desiccated					
	Dry mass (g)	Initial Germ % (at 25 °C)	Moisture contents				Drying days	Germ% (at 25 °C)	Moisture content			
			Axis	Cotyledons	Coat	Cupule			Axis	Cotyledons	Coat	Cupules
Funing (acorn)	8.0 ± 0.5	62.5 ± 7.2	59.6 ± 1.8	45.3 ± 0.7	40.6 ± 0.4	–	7	0	44.0 ± 0.9	31.6 ± 0.8	19.8 ± 0.6	–
Funing (acorn + cupule)	12.0 ± 0.9	45.8 ± 12.5	59.6 ± 1.8	45.3 ± 0.7	40.6 ± 0.4	67.7 ± 1.7	9	0	53.5 ± 2.0	40.8 ± 1.4	33.3 ± 1.0	24.4 ± 1.5
Ceheng (acorn)	14.4 ± 0.7	75.0 ± 4.8	53.4 ± 1.1	42.3 ± 0.5	39.3 ± 0.5	–	–	–	–	–	–	–
Ceheng (acorn + cupule)	25.2 ± 1.6	54.0 ± 11.3	58.5 ± 4.0	42.9 ± 1.4	37.5 ± 0.9	58.8 ± 3.5	28	0	23.9 ± 1.3	21.6 ± 0.5	19.0 ± 0.4	14.6 ± 0.1

Germ%: germination percentage.

from subgenus *Cyclobalanopsis* germinate more slowly (up to 4.7 times slower) than other species from subgenus *Quercus* (Xia et al., 2015). Besides, seeds/fruits of a large size usually take a longer time to germinate because larger surface-area-to-mass ratio would reduce the water absorption capacity (Kikuzawa and Koyama, 1999; Norden et al., 2009). *Q. sichourensis* (subgenus *Cyclobalanopsis*) acorns are large (up to 25 times' bigger than acorns from other species; Xia et al., 2012) and are therefore expected to germinate slowly. In fact, they germinated faster than many *Quercus* species (Xia et al., 2015). At suitable temperatures (20–35 °C), acorns collected in Ceheng had MTGs of 15 d or less, and these acorns reached high germination percentages in a wider temperature range (10–35 °C). Acorns from Funing had prolonged MTGs of 22–70 d at temperatures of 25–35 °C and only germinated in a more narrow temperature range.

Desiccation-sensitive seeds/fruits are shed at high moisture contents and in a metabolically active state progressing to germination (Berjak et al., 1989). Species with desiccation-sensitive seeds/fuits are largely restricted to regions with comparatively high rainfall (Tweddle et al., 2003). But when occurring in dry land, many of these species may time seeds to shed coincident with the peak in annual rainfall (>60 mm) (Pritchard et al., 2004). *Q. sichourensis* is distributed in a more humid area than many of the dominant or important oak species (e.g. *Quercus schottkyana*) in southwest China. However, its acorns disperse between October and December, at the beginning of the 6-month dry season. While the total rainfall during the dry season is c. 209 mm compared with 978 mm for the wet season (Fig. 2), the monthly rainfalls in the dry season range from 16 to 85 mm. In the lab, acorns of *Q. sichourensis* lose viability quickly and at high moisture contents. In the habitats, post-dispersal drought may largely limit the survival of these acorns.

Rapid germination may have the potential advantage of reducing the risk of desiccation-induced mortality and fruit predation of desiccation-sensitive seeds/fruits (Tweddle et al., 2003; Daws et al., 2005). The rapid germination concurrent with the higher rainfall in October (c. 82 mm) might offset some of the negative effects of desiccation. Unlike most *Quercus* species, acorns of *Q. sichourensis* disperse with their cupules. Although cupules may slightly prolong the survival time in response to desiccation, overall, cupules have negative effects on germination in this species: decreasing germination percentages, prolonging germination

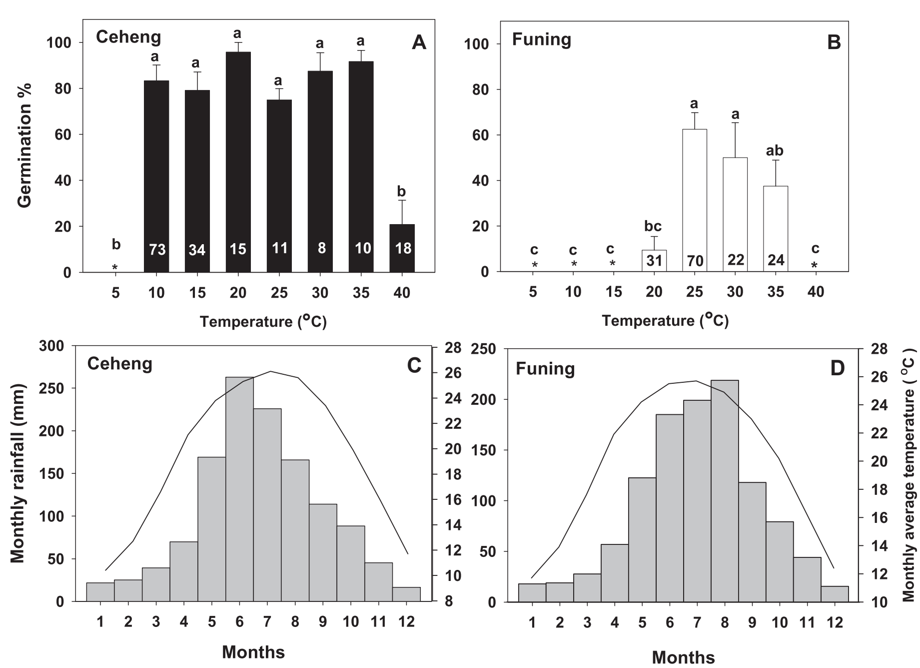

Fig. 2. Finial germination percentages and MTGs (numbers of days in the bars) of *Q. sichourensis* collected from Ceheng (A) and Funning (B); and the monthly rainfall (showed with bars) and monthly average temperatures (showed with lines) in Funing (C) and Ceheng (D). Bars (in plot A, B) with the same letters (a–c) are not significantly different from each other ($p \geq 0.05$). The analysis was accessed by ANOVA using Minitab 17. * represents treatments obtaining no survival. Climate data in plots C and D are obtained from Climatic Data Center, National Meteorological Information Center (CMA, 2015).

time and narrowing the temperature range in which acorns germinate. Seedlings from large seeds/fruits are usually larger and have a higher probability of survival in drought (Leishman and Westoby, 1994). In wetter years, some quickly developing seedlings may still have a chance to survive in the field (Fig. 1C).

5. Conclusions

Q. sichourensis is a productive species. We found that its acorns were viable and germinated rapidly but lost viability quickly in drought conditions. Habitat degradation combined with the high desiccation sensitivity of its acorns might have led to the rareness of this species, and are now factors limiting the development of its populations. *Q. sichourensis* is still facing a high risk of extinction and should be defined as a Critically Endangered species on the IUCN Red List.

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