Acknowledgments. We are very grateful to Yves Carton, Minh-Ha Pham-Delègue and Louise Vet for discussion and comments, Yves Carton for providing the insect strains and Françoise Frey for her help with the insect rearing.

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0014-4754/92/090902-03$1.50 + 0.20/0

The induction of secondary seed dormancy by oxygen deficiency in a barnyard grass *Echinochloa crus-galli*

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Received 3 February 1992; accepted 28 April 1992

Abstract. At 25 °C, secondary dormancy was induced in seeds of *E. crus-galli* exposed for 100 days to oxygen deficiency. By contrast, hypoxia did not induce dormancy at 15 °C or prevent dormancy termination at 7 °C. Secondary dormancy was terminated after 2 months stratification at 7 °C. Oxygen deficiency may increase the proportion of dormant seeds in the soil, and affect the dynamics of the barnyard-grass soil seed bank.
Key words. Barnyard grass; *Echinochloa crus-galli*; oxygen deficiency; seed dormancy; soil seed bank; stratification; temperature.

In some species, secondary seed dormancy may be induced after the primary one has been terminated by afterripening (exposure of dry seed to high temperature) and/or stratification (exposure of moist seed to cold). The induction of secondary dormancy usually requires a prolonged period at conditions adverse to germination, e. g. high or low temperatures, darkness, or water stress [2], and may be viewed as an adaptation for survival. Induction of secondary dormancy by oxygen deficiency has been observed rather rarely [3].

We investigated the induction of secondary seed dormancy in a barnyard grass, *Echinochloa crus-galli* (L.) P. Beauv. In this species, a fraction of seeds (typically 1–20% in different populations) terminates dormancy after 3 months of afterripening at 25 °C [4], and this proportion may be increased by high temperature [5]. In the rest of the seed population, dormancy may be terminated by stratification at 1–10 °C [6]. Experimental induction and termination of secondary dormancy has not been studied as yet.

Material and methods

The seed material was collected at 2 localities in western Czechoslovakia, 20 km north of Prague. The seeds (spikelets consisting of a caryopsis enclosed in lemma, palea and glumae) were swept with an entomology net from plants growing within maize or sugar beet crops. The material collected at Odolena Voda (OV) and Kozomín (K) in 1990 was afterripened at 25 °C and 30% air relative humidity, for 8 months. Material FS was collected in 1989 at Odolena Voda (germination rate after 3 months of afterripening 5.0%). A part of this material was buried in the field from October 1989 to April 1990. This treatment terminated dormancy in about 95% of the seeds. The post-dormant material (F) was stored at room conditions (20 °C, 40% relative humidity) until the beginning of the experiments. The proportion of non-dormant seeds at the start of the experiment was 91.8, 21.6, 5.0 and 1.2% in F, OV, FS and K material, respectively.

The oxygen deficiency treatment consisted of submerging a packet of the seeds in water and carefully expelling the remaining air. The samples of F, OV and K seeds were then placed at a constant 7, 15, and 25 °C, in darkness. The stratification treatment for termination of secondary dormancy consisted of burying the packets of seeds in moist sand, and incubating them at 7 °C in darkness. In this experiment we used the samples of F and OV seeds

subjected to 90-day oxygen deficiency prior to the treatment (germination 9.0 and 1.1%, respectively), and an afterripened FS control with 5.0% germination. To investigate the changes in dormancy incidence, 3 × 50 seeds were taken from experimental conditions at regular intervals and left to germinate at 25 °C in darkness. Groups of 50 seeds were put into a petri dish (10 cm diam.) on a filter paper, and 5 ml of water added. The number of germinating seeds was counted after 8 days.

Results

The effect of oxygen deficiency varied with temperature. At 25 °C, 112 days oxygen deficiency caused a decrease in the proportion of germinating seeds to 10.7, 0.7 and 0.0% in F, OV and K, resp. (fig. 1). The effect was most conspicuous in F material, with a high proportion of germinating seeds at the start of the experiment. In K the germination rate first slightly increased but later fell to its original value. At 7 °C the oxygen deficiency treatment did not decrease the percentage germination. In fact, the dormancy was terminated as with the stratification treatment. In OV and K seeds germination increased from 21.6 and 1.2% to 76.7 and 74.0% respectively. The exposure to 15 °C had an intermediary effect. The germination rate was either not affected (F), slightly decreased (OV), or for a short period slightly increased (K).

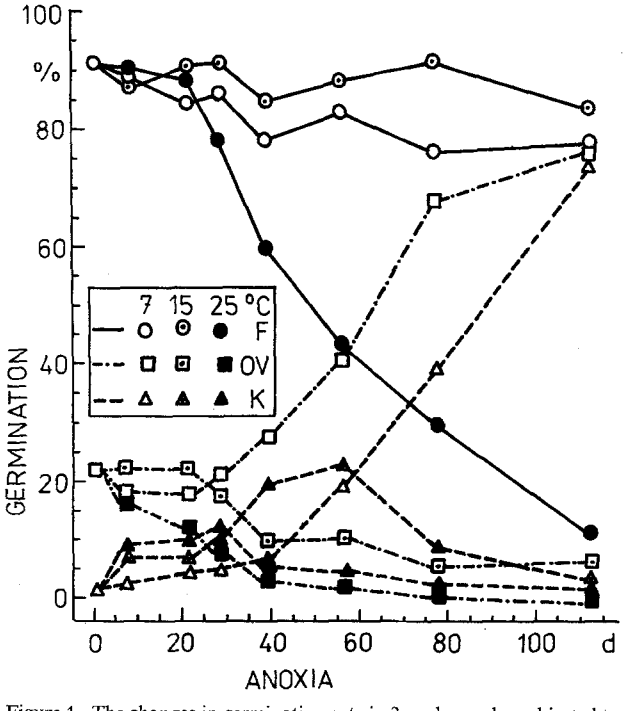

Figure 1. The changes in germination rate in 3 seed samples subjected to oxygen deficiency at temperatures of 7, 15, and 25 °C for 112 days. The symbols which distinguish between the seed materials and treatment temperatures are explained in the insert. Seed materials: F – dormancy terminated by stratification, germination rate at the start of the experiment 91.8%; OV, K – dormancy terminated by afterripening, germination rates at the start of the experiment 21.6 and 1.2%, respectively.

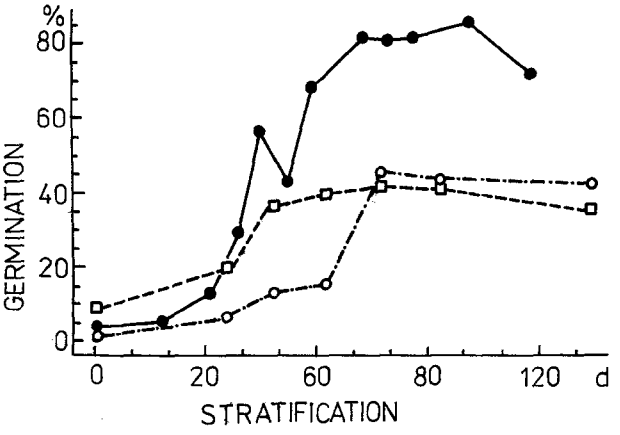

Figure 2. The increase in germination rate in 3 seed samples subjected to stratification in moist sand, at 7 °C. ●—: material FS (control), dormancy terminated by afterripening, no prior stratification or oxygen deficiency treatment, germination rate at the start of the experiment 5.0%. □– – –: material F, dormancy terminated by stratification, then subjected to 90 d oxygen deficiency treatment, germination rate at the start of the experiment 9.0%. o–·–·–: material OV, dormancy terminated by afterripening, then subjected to 90 d oxygen deficiency treatment, germination rate at the start of the experiment 1.1%.

The decrease in percentage germination after oxygen deficiency treatment was apparently due both to induction of secondary dormancy and to mortality. Stratification terminated the dormancy and enabled us to distinguish between dead and dormant seeds. The secondary dormancy in OV and K seeds was terminated by a stratification at 7 °C, similar to primary dormancy in control FS seeds subjected only to afterripening (fig. 2).

Maximum germination was attained after ca 70 days. In OV seeds, germination after stratification was even greater than before oxygen deficiency treatment. In F seeds, maximum germination (43%) was only a fraction of the percentage germination before oxygen deficiency. Some of the non-dormant seeds (ca 48%) may have died during the submersion in water.

This physiological aspect of seed dormancy may have a bearing on the field biology of *E. crus-galli*[7]. Only a small fraction, typically 5–50% of non-dormant seeds (i.e. germinating in the laboratory at 25 °C and in darkness) germinates under field conditions[8]. The rest remains in the soil and enters the soil seeds bank. The induction of a secondary dormancy may increase the survival chance of seeds which have not germinated. Secondary seed dormancy may or may not be induced in seeds buried in the field, depending on the site and depth of where they were placed[9]. This is perhaps affected by variation in soil temperature and oxygen availability. Inducing secondary dormancy may be of crucial importance for survival to the next season.

Another interesting fact was that no germination was observed in water-submerged seeds at 25 °C, in contrast to earlier studies[10]. The ability to germinate when submerged is perhaps a strain-specific character, typical for populations which are often flooded. It was absent in our

seed samples which originate from a dry temperate area (mean annual temperature 8.4 °C, and precipitation 580 mm).

1 We thank Miss Jitka Králová for her excellent technical assistance.

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0014-4754/92/090904-03$1.50 + 0.20/0

Adhesive grass spikelet with mammalian hair in Dominican amber: First fossil evidence of epizoochory

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Received 31 January 1992; accepted 19 May 1992

Abstract. Discovery of a female spikelet of the grass genus *Pharus* (Gramineae: Bambusoideae: Phareae) in association with mammalian hair in Dominican Republic amber provides the first fossil evidence of epizoochory. Hooked macrohairs on the lemma of the spikelet show that morphological modifications in grasses for dispersal by attachment to the surface of animals were present in the Late Eocene. The fossil also represents 1) the second-oldest undoubted macrofossil record of the Gramineae, 2) the earliest record of a fossil grass that can be assigned to an extant genus, 3) the earliest undoubted record of a member of the bamboo subfamily and 4) the only known fossil of *Pharus*.
Key words. Fossil grass; *Pharus*; bamboo; Dominican amber.

Studies on the highly fossiliferous amber from the Dominican Republic have led to the discovery of many new taxa[1]. The nature of the fossilization process can also result in the preservation of symbiotic associations that are rarely preserved otherwise[2, 3]. This study presents the first fossil evidence of epizoochory (dispersal by attachment to the surface of animals), by demonstrating a female spikelet of a representative of the monoecious grass genus *Pharus* R. Br. (Gramineae: Bambusoideae: Phareae) associated with mammalian hair (figs 1–3).

The fossil specimen originated from the La Toca mine, located between Santiago and Puerto Plata in the Cordillera Septentrional of the northern portion of the Dominican Republic. This mine is in the Altimira facies of the El Mamey Formation (Late Eocene), which is shale-sandstone interspersed with a conglomerate of well-rounded pebbles[4]. Differences in the magnitudes of absorption peaks in nuclear magnetic resonance spectra of the exo-methylene group of amber[5] from different mines in the Dominican Republic were used to calibrate the ages of the various mines, with the age of the Palo Alto mine (20 million to 23 million years, based on foraminifera counts) used as a standard[6]. The ages of various specimens of Dominican amber ranged from 15 million to 40 million years, with that from the La Toca mine being the oldest, some 35 million to 40 million years old (Early Oligocene to Late Eocene). This age was corroborated by recently reported dating of the La Toca mine based on coccolith diversity which produced an age of 30 million to 45 million years[7].

The amber containing the fossil has all the visual characteristics of natural Dominican amber. A series of chemical and physical tests[8] performed on a small portion of the amber piece verified that it is authentic. The piece of orange amber containing the fossil weighs 7 g and is triangular in shape, measuring 37 mm longest length, 25 mm longest width and 15 mm thick. It is deposited in the Poinar collection of Dominican amber maintained at the University of California at Berkeley.

Results

Determination of the spikelet as belonging to *Pharus* was based on the distinctive combination of characters present in extant species[9]; these characters are 1) spikelet length, 2) spikelet with a single floret, 3) glumes shorter than lemma, 4) lemma 7-nerved, 5) lemma margins strongly inrolled, 6) lemma apically tapered to a beak, 7) lemma with uncinate (hooked) macrohairs and 8) lemma curved (in some) (figs 1–3). The fossil *Pharus* represents an extinct species that will be described elsewhere.