Impact of respiration and CO_2 on the survival of *Epichloë festucae* var. *lolii* strain AR37 in a cultivar of perennial ryegrass during short-term seed storage

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During storage, ryegrass seeds undergo cellular respiration to stay alive. When seeds respire, they take in oxygen (O_2) while releasing an equal amount of carbon dioxide (CO_2). Oxygen reduces seed longevity due to the oxidation of cells and membranes, even at ambient temperatures, but the effect of increased CO_2 concentration on seed and *Epichloë* endophyte viability is unknown. Two experiments were conducted to identify (i) whether seed respiration rate was affected by storage temperature and time, and whether this affected the viability and survival of the endophyte in seed and (ii) to determine whether a reduction in atmospheric O_2 concentration, and therefore increased CO_2 concentration, would affect seed and endophyte survival during storage. The first experiment was set up using a split-plot design with seeds in airtight containers. There were two endophyte treatments, with endophyte AR37 (E+), and endophyte-free (E-) three storage temperatures (5°C, 20°C, and 30°C), and six storage sampling periods (1, 2, 4, 8, 16, and 32 days). The hypotheses were that high CO_2 concentration resulting from the respiration of seeds would (i) have a negative host-endophyte effect, decreasing the survival of AR37 in an airtight container, (ii) E+ seeds would have a higher respiration rate than E- seeds, and that (iii) increasing storage temperature and storage time would decrease endophyte viability. The second experiment, designed as a completely randomised block, used ascorbic acid dust (ascorbic acid -AA) to create anoxic conditions, elevating relative carbon dioxide concentrations. The experiment was conducted in airtight glass vials with E+ and E- seeds at 20°C only, for the same storage periods as experiment one. Unsealed vials were used as control treatments. The hypotheses were that (i) high concentrations of CO_2 (greater than 400ppm) and increasing storage time would have a negative host-endophyte effect, (ii) anoxic conditions would not affect seed survival, and (iii) germination would differ between E+ and E- seeds. The endophyte viability and seed germination were assessed using an established tissue-print immunoblot procedure, and top of paper method respectively. Endophyte survival after storage was significantly reduced at all three storage temperatures, dropping from 86% at the beginning of the experiment to a grand mean of 77%; the loss in viability was lower at 5°C (-2.6%) than the other two temperatures (-11%). Seed respiration rate (mean=1.83 $LogCO_2mg\ g^{-1}\ h^{-1}$) increased significantly with storage time at all three temperatures. E+ seeds had a significantly higher respiration rate (1.923 $LogCO_2mg\ g^{-1}\ h^{-1}$) than E- seeds (1.748 $LogCO_2mg\ g^{-1}\ h^{-1}$). Within a storage temperature, the respiration rate did not differ between E+ or E- seeds. When stored under anoxic and high CO_2 concentration conditions, AR37 survival fell significantly to 72% after 32 days at 20°C. However, germination was not affected for either E+ or E- seeds. While seed germination was not affected by the modified storage conditions used a suitable explanation for the loss of AR37 viability remains to be determined. Further studies on the biochemical changes in the seed resulting from exposure to high concentrations of CO_2 are required.

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