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Humidity Control of a Novel Fern Gametophyte Dessication Chamber

Jessica Moser
Florida State University

Lauren Haberstock

Stephen Davis
Pepperdine University

Helen Holmlund
Pepperdine University

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Jessica Moser, Lauren Haberstock, Stephen Davis Ph.D., Helen Holmlund Ph.D.

Introduction

Drought is typically a major threat to plants, but there are some unique ecosystems in which plants thrive under such conditions. In coastal southern California, much of the landscape is made up of drought tolerant chaparral shrublands. The diversity in the chaparral is surprising; several species of drought tolerant ferns thrive in the chaparral understory despite ferns' typical requirement for readily available water (2016, Holmlund et al.). Like all land plants, ferns experience alternation of generations, meaning their lives have two stages: haploid gametophytes and diploid sporophytes. However, fern gametophytes are independent and free-living, with fertilization taking place externally (on the substrate) rather than within the sporophyte tissue (2016, Hauffler et al.). Fern gametophytes are independently photosynthetic and typically only one cell layer thick, making them particularly vulnerable to dehydration. Thus, the long summer droughts in southern California pose a challenge not only for large diploid individuals (sporophytes) but also for the small haploid gametophytes. Research has indicated that some gametophytes are desiccation tolerant (capable of surviving near-complete desiccation, or -100 MPa), but the extent and under what circumstances is unclear (2022 Regier, 2007; Watkins et al.; 2013, Testo & Watkins).

Prior research has established drying speed and substrate is important for full gametophyte resurrection in two fern species (2022, Regier; 2020, Oliver et al.). Watkins et al. has established drying and resurrection rates in tropical fern gametophytes (2007). In that experiment, the authors dried fern gametophytes for 45 minutes. Subsequent testing partially dried the gametophytes and afterwards rehydrated them. Chaparral gametophytes may be significantly more delicate and require longer dry down periods using an appropriate substrate (2022, Regier). Other research indicates that desiccation tolerance varies wildly among ferns and factors such as temperature can impact gametophyte recovery and growth (2013, Testo & Watkins). Numerous strategies have been employed to aid in the dry down of gametophytes. Current techniques include leaving gametophytes on filter paper, using salts to control adequate humidity, and using a dew point generator (2013, Testo & Watkins; 2007, Watkins et al.; 2022, Regier). Each technique has used a chamber of variable size and substrate, which may contribute to variability in desiccation and rehydration tolerance amongst gametophytes as they hold water to a varying extent (2022, Regier; 2007 Watkins et al.).

To address the difficulty of adequate drying time for the desiccation and recovery of chaparral gametophytes, I have designed a chamber using 3D printing technology that has ports to attach the chamber to regulatory devices and a lid to regulate light and water retention. Salts and a dew point generator were used to test the efficacy of this device. Our primary hypothesis is that the chambers will effectively regulate humidity within them. Our secondary hypothesis is that the dew point generator will provide the most control over relative humidity within the chambers.

Design

The gametophyte chambers were printed using black polylactic (PLA) plastic on an Ultimaker 3 Extended printer (Dynamism, Chicago, IL, USA). Boxes were printed to contain 60mm by 15mm Fisherbrand petri dishes for easy transport of gametophytes. Each box had two holes on opposing sides for 6.30 mm tubing to fit. One tube connected to the dew point generator , and the other acted as an output for air to prevent pressure buildup. A 4.5mm x 5.0mm divot was added to the chamber for the insertion of a HOBO sensor model MX2302A (Onset Computer Corporation, Bourne, MA, USA). Initial trials used parafilm as a suitable cover, but subsequent trials used lids made from transparent PLA plastic to allow for reuse and ease of placement.

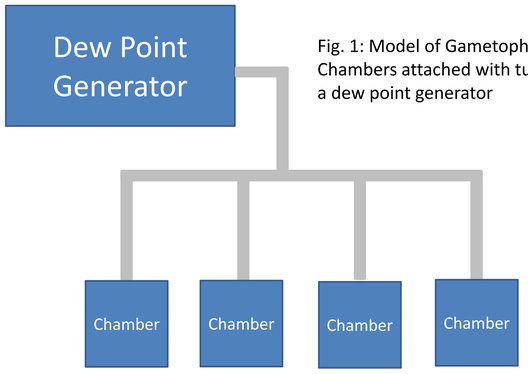

Fig. 1: Model of Gametophyte Chambers attached with tubing to a dew point generator

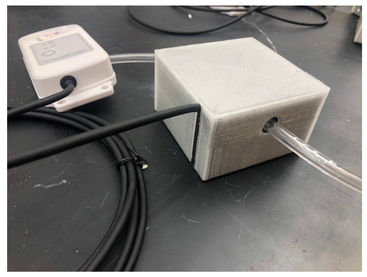

Fig. 2: Gametophyte Chamber attached to a HOBO sensor and tubing. The HOBO sensor continuously measured temperature and relative humidity inside the chamber throughout the experiments.

Results

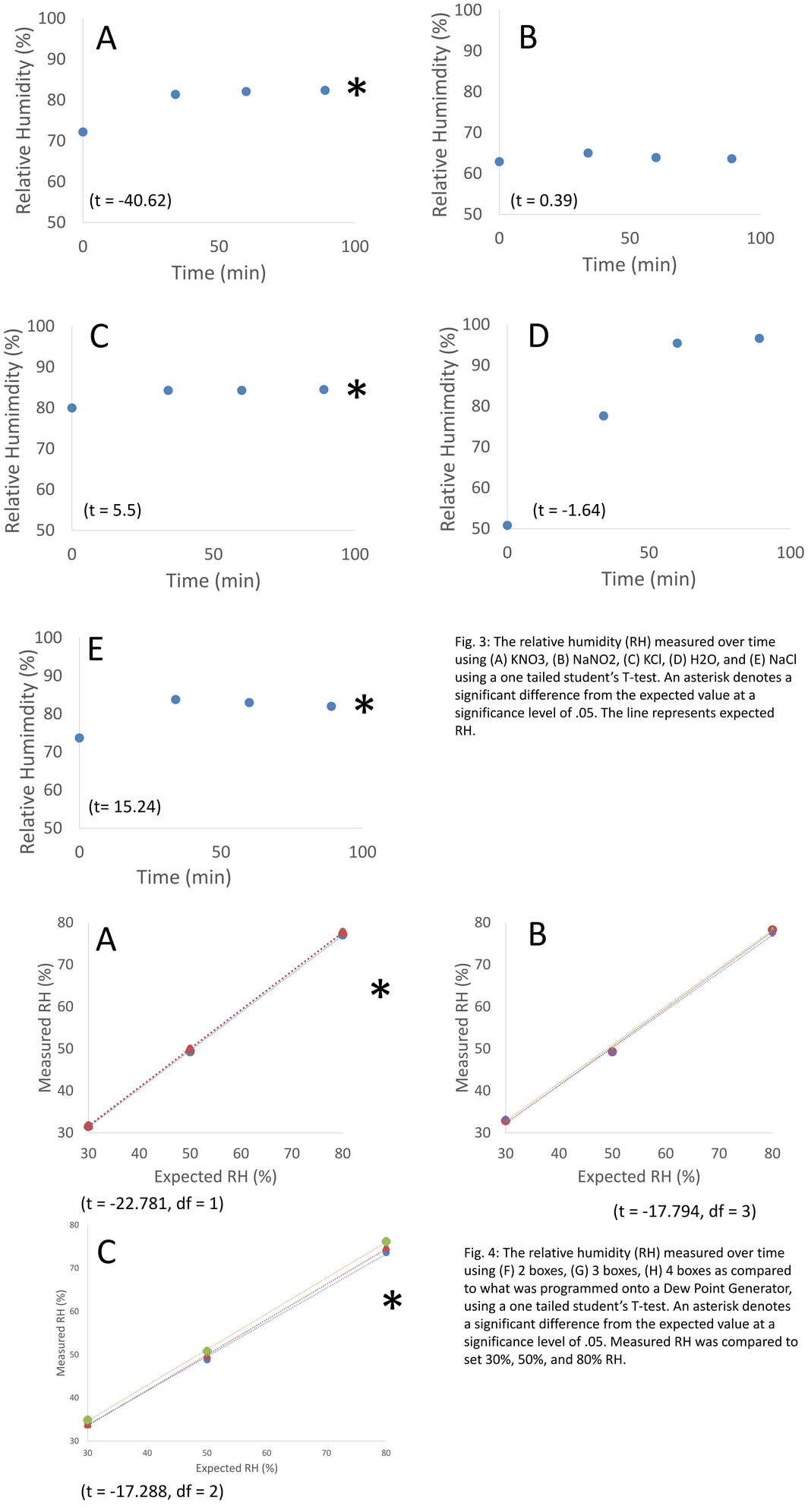

Fig. 3: The relative humidity (RH) measured over time using (A) KNO3, (B) NaNO2, (C) KCl, (D) H2O, and (E) NaCl using a one tailed student's T-test. An asterisk denotes a significant difference from the expected value at a significance level of .05. The line represents expected RH.

Fig. 4: The relative humidity (RH) measured over time using (F) 2 boxes, (G) 3 boxes, (H) 4 boxes as compared to what was programmed onto a Dew Point Generator, using a one tailed student's T-test. An asterisk denotes a significant difference from the expected value at a significance level of .05. Measured RH was compared to set 30%, 50%, and 80% RH.

Methods

Fig. 5: In the first set of experiments, relative humidity was controlled with a dew point generator… (a little bit about how it works)

Picture of salts

Fig. 6: In the second set of experiments, relative humidity was controlled using salt solutions… (include salt names and expected RH for each.)

Conclusion

1.Using a DPG, there was a significant difference found when increasing the chamber size from one to four chambers between actual and set RH.
2. Manually created salts were effective in controlling relative humidity.
3. The chambers provide adequate space for desiccating and rehydrating plants with precision.
4. This design set up can be used in the future to test drought tolerance of various plant species.

Literature Cited

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Acknowledgements

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