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The effect of pH on *in vitro* growth of protonemata, asexual propagules, or gametophytes fragments of four Neotropical moss species

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Abstract: Many abiotic factors affect bryophyte survival. Ecosystem's abiotic factors have been altered by anthropogenic disturbance and this has taken a toll in bryophyte species diversity. Bryophyte *in situ* or *ex situ* culture can be used to assess how these abiotic factors affect plant growth, restoration, and conservation of endangered species. For Neotropical species, it is poorly understood how an *ex situ* technique like *in vitro* culture may affect these plants. In addition, information regarding how pH can affect these species is lacking; especially when the medium pH is a factor that can influence germination and differentiation of spore, asexual propagules, protonemata or gametophyte modules. Therefore, the effect of media pH on gametophyte modules (*Neckeropsis disticha*), spores (*Octoblepharum albidum* and *Vesicularia vesicularis* var. *vesicularis),* and asexual propagules (*Calymperes afzelii*) were studied to observe species requirements for optimal module growth. Sterile bryophyte modules were inoculated into petri dishes that contained different pH treatments (pH 4, 5, 6; multiple pH values in the range 4-5 and 5-6) and MS medium. Variation in plant modules growth (length) and survival were annotated, as well compared among pH treatments for each species. Species demonstrated various patterns of module growth, according to treatments. Among these, *N. disticha*'s fragmented gametophytes growth varied. C. *afzelii* grew best at pH 6.0, while *O. albidum* optimal growth was at pH 5.0. *Vesicularia vesicularis* var. *vesicularis* protonemata grew best when pH was decreased (4.0). The decrease/increase of pH may or not alter growth patterns; this will depend on the species requirement/tolerance for a range/niche of pH. Furthermore, this study showed that the frequently used pH 5.8 is not necessarily required for these Neotropical species and therefore culture methods should be revised when propagating bryophyte species; especially when studying threatened species.

Keywords: pH, bryophyte niche, micropropagation, *in vitro* culture, Neotropics

Introduction

Abiotic factors affect bryophyte growth, development, and reproduction. These factors (humidity, photoperiod, temperature, habitat mineral composition, water availability and pH) along with biotic factor will determine if a bryophyte population survives or not (Goffinet and Shaw 2009). To ensure their survival it is necessary to understand how these abiotic factors relate to plant growth. For example, da Silva *et al.* (2010) found that for *Bryum argenteum* Hedw., light and nutrient are necessary for spore germination phases to occur; in contrast, darkness and water cause spore swelling and protrusion of the germ tube. In *Thamniopsis incurva* (Hornsch.) W.R. Buck, protonemata growth augmented with increased irradiance, but low water availability (-0.2 to -1.0 MPa) affected spore germination. High salt (NaCl) concentration can reduce chlorophyll content for *Atrichum undulatum* (Hedw.) and *B. argenteum*; however, these bryophytes could tolerate higher salt concentration than the ferns *Asplenium viride* Britton*, Ceterach officinarum* DC, and *Phyllitis scolopendrium* (L.) Newman (Bogdanović *et al.* 2011). Nutrient enrichment stimulated growth in *Sphagnum squarrosum* Crome and *Scorpidium scorpoides* (Hedw.) Limpr., but *Sphagnum subniten* Russ. and Warnst. ex Warnst nor *Calliergonella cuspidata* (Hedw.) Loeske did not respond (Kooijman and Bakker 1995). Abiotic factors are variable in an environment and bryophytes are well adapted to these. However, if a disturbance influences their habitat to a state that is outside of their abiotic suitable ranges, then this can cause a threat to their populations (Vitt 2000, Paulissen *et al.* 2004, Porley and Hodgetts 2005), have it decreased, and even go extinct (McClean *et al.* 2011, Verhoeven *et al.* 2011). Though, the main focus of these studies on abiotic