Ecological and Evolutionary Aspects of *Sphagnum* Biology

By

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A dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

(Botany)

at the

UNIVERSITY OF WISCONSIN-MADISON

2013

Date of final oral examination: 05/13/2013

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Dedication:

This work is gratefully dedicated to my niece (Karolina Isabelle Cruz-Cardona). Time and education taught me how to be a scientist and a researcher, but my niece taught how to be an explorer and how to take notice of the wonders of this world.

In memory of:

Santia Durán-Estevez (1942-2011) my grandmother and friend with whom I shared a passion for plant diversity and from whom I learned folk-taxonomy, traditional medicine (ethnobotany) and horticulture. My interest in cryptogamic botany would have not arisen without influence from my grandmother, for this reason this work is dedicated to her.

Juana Cabán (1916-2010) my great-grandmother from whom I learned stories relating to agriculture and plant use, stories and knowledge that will accompany me during the rest of my life.

Acknowledgements

This dissertation could have not been written without the help and guidance of Dr. Linda Graham, who not only acted as an advisor but also as a friend who never failed to encourage new research ideas and approaches to science. The experience of working with Dr. Linda Graham has enriched my academic and professional life in countless ways, from training me in laboratory, writing and presenting techniques. For this and many more things, I thank her.

I would also like to thank my wonderful graduate committee members, who guided me through different moments of my academic program. I would like to make special mention of Dr. Cécile Ané for the many hours spent providing statistical advice and guidance. I thank Dr. Joy Zedler for providing support and providing me with office space where I could work in peace and quiet, and Dr. Donna Fernandez for insightful comments about plant physiology and development. Finally I would like to thank Dr. Dana Geary for great conversations and insight into paleobiology and geology; her guidance helped me explore new ideas and take parts of this dissertation to the "next level."

Life as a graduate student can be challenging at times, but it always helps to have a strong support system from fellow students. I would like to thank all of those who, during the length of my academic program, became part of my graduate family. We laugh, we cried, and we drank (beer), but we never gave up.

Last but not least I would like to thank my biological family, my mother (Myriam Correa-Durán), my father (Concepción Cardona-Hernández), and my two sisters (Christie Cardona-Correa and Kimberly Cardona-Correa), for unconditional support during my whole life and during the length of my graduate program.

There are so many people with whom I have interacted during my graduate career that mentioning them all would be impossible, so I ask forgiveness of those who have not been mentioned but still played an important role in my success. Below you can find the names of some (but not all) the people I would like to thank.

Shana Ederer, Reese Zulkifly, Anchittha Satjarak, James Graham, Michael Piotrowski, Izak Smith, Elizabeth Georgian, Marie Trest, Andrea Herr-Turoff, Sarah Friedrich, Abigail Mazie, Lauren Moscoe, Deniz Aygoren, Isabel Rojas, Brian Walsh, James Doherty, Deborah Rook, Lalita Patel, Adam Bayless, Alyssa Deleon, Emily Sessa, Richard Hilleary, Erika Mudrack, Ava Jindra, Meredith Albrecht…

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ABSTRACT

Sphagnum peatmosses currently play a global environmental role in maintaining atmospheric chemistry and climate homeostasis and likely have done so for hundreds of millions of years in Earth's past. Modern *Sphagnum*-dominated peatlands store a large proportion of global soil carbon as peat, such long-term sequestration fostering global climate homeostasis. *Sphagnum* peat mosses also harbor microbial methanotrophs and nitrogen fixers that likewise play globally important roles in Earth's biogeochemical cycles. *Sphagnum* has also been utilized in many ways by ancient and modern cultures. Although fossil fragments of *Sphagnum*-like peatmosses are known from the Permian (Neuberg, 1956, 1960), recent molecular clock analyses suggest that peat mosses may be considerably older, and certain Ordovician microfossils were interpreted by Kroken et al. (1996) as the resistant remains of sporophytic capsule epidermis of *Sphagnum*-like early mosses. This thesis aimed to increase understanding of the ecophysiology, paleobiology and evolutionary traits of *Sphagnum,* focusing on environmental impacts occurring from Ordovician through modern times. The effects of combined temperature and UV treatments on *Sphagnum compactum* structure were determined in laboratory cultures, revealing dramatic impacts of elevated temperature, as well as temperature-UV interactions, Laboratory analyses of the environmental influences most important in controlling development and maintenance of normal dorsiventral or axial gametophytic body orientation in a suite of modern seedless plants, including *S. compactum,* revealed the strong influence of illumination direction. Finally, well-understood shallow marine mid-Ordovician carbonates (Sinnipee Group), located in Dane Co., WI, and dated at 455-460 Mya, were investigated for evidence of peatmoss fossils. The discovery and description of fragmentary Ordovician *Sphagnum*-like

leaf and stem remains from this deposit indicates that peatlands were likely present on Earth at least 150 million years earlier than previously thought. Given the global biogeochemical importance of modern *Sphagnum*, the discoveries documented in this thesis improve knowledge of Ordovician terrestrial biota and illuminate ancient to modern plant-environment interactions.

Prologue

Ever since the beginning of my graduate career, I have encountered one recurring question: "Why *Sphagnum*?" The answer to this question is not simple and could be approached from a number of angles. Since it has been stated that botanists fail to recognize the broader relevance of their own research (Hershey, 1996), I here undertake the task of explaining *Sphagnum*'s importance. This introduction is divided into four parts, each presenting different aspects. The first subsection (*Sphagnum: Ecology, Carbon Cycle and Peatland Conservation*) aims to present the reader with background regarding *Sphagnum*'s ecological importance, primarily focusing on its role in the global carbon cycle. The second sub-section (*Past and Present uses of Sphagnum*) provides the reader with information about the current and past uses of *Sphagnum* by different cultures including Native Americans and Scandinavians. The third subsection (*Preserving History: Bog Bodies and Cultural Artifacts*) presents an archeologic topic that has long interested me and that is also useful in attracting student attention to bryophytes. The fourth and final sub-section (*Research: Moving Sphagnum Research Forward*) provides an overview of my research and explains how this research adds new perspectives and approaches to the field of *Sphagnum* research.

***Sphagnum*: Ecology, Carbon Cycle and Preservation**

Sphagnum-mosses are important sinks in the global carbon cycle because they enhance organic carbon sequestration in globally extensive peatlands. *Sphagnum*-generated peat is estimated to contain ~400 gigatons of organic carbon, representing around 30% of the total soil carbon budget (Gorham, 1991). Estimates suggest that approximately 33 gigatons (~33Pg) of this stored carbon

occurs in Northern Hemisphere peatlands, especially those of Alaska and Canada (Wisser et al., 2011).

Sphagnum is noted for its ability to engineer its environment by acidifying the surrounding nutrient-poor waters. Such acidification influences peatland community composition (Crum and Anderson, 1981) and fosters peat accumulation in the ecosystem by slowing the decomposition process (Clymo, 1965; Coulson and Butterfield, 1978; Johnson and Damman, 1983; Aerts et al., 2001; Rydin and Jeglum, 2006; Pankratov et al., 2011). Peat mosses produce degradation-resistant carbon (Tsuneda, 2001 in the form of cell wall polysaccharides (Hájek et al., 2011) such as sphagnan (Painter, 1991), and/or lignin-like phenolic compounds (Kroken et al., 1996; Delwiche et al., 1989; Rozema et al., 2001). Together with favorable climatic and hydrological conditions, these materials promote peat accumulation.

Past and present uses of *Sphagnum*

Sphagnum-moss experienced a boom in biodiversity during the Miocene, when North America moved to its current position associated with a major cooling event (Shaw et al., 2010). Since then, *Sphagnum* has predominated in higher latitudes of the Northern and Southern hemispheres, and thus has been used by many indigenous cultures in their geographic locales. This subsection will describe features of *Sphagnum* moss that lend this organism to various past and present uses.

Sphagnum leaves are composed of green photosynthetic cells known as chlorophyllous cells and large, dead, porous cells known as hyaline cells. Hyaline cells store water, allowing *Sphagnum* to absorb about twenty times its dry weight in water (although the amount varies depending on the species) (Nörr, 1974; Vitt et al., 1975; Clymo and Hayward, 1982; Andrus,

1986). Because of *Sphagnum*'s water-holding ability, this moss was used by Michigan Chippewa Indians to make diapers that would keep children dry, clean and warm (Crum, 1973). Some modern companies still use *Sphagnum* to produce both adult and children's diapers and sanitary napkins (Bland, 1971; Gottesfield and Vitt, 1996). In Germany hiking boots are sometimes lined with *Sphagnum* to absorb moisture and odors (Hedenäs, 1991). *Sphagnum* is widely used in horticulture as a soil conditioner.

Northern Hemisphere *Sphagnum* has been used for heating and insulating buildings and refrigerators (Sukhanov, 1972; Ruel et al., 1977; Lewis 1981). The admirable complexions of Irish and Swedish women have been attributed to the use of *Sphagnum* as a fuel source (Drlika, 1982). *Sphagnum* is used to insulate roofs from temperature changes (Posth, 1993), playing a similar role as grass roofs commonly found on Scandinavian houses. When *Sphagnum* is used to line walls, it can diminish the propagation of sound into and within the house (Thieret, 1954).

The practice of burying foods in *Sphagnum* for preservation purposes dates back at least a couple of thousand years, as supported by Scandinavian-archeological findings of 1800 years-old wooden kegs containing foods such as butter and cheese (Ritchie 1940-1941; Raftery, 1992). The burial of root crops such as carrots in *Sphagnum* bogs as a preservation method is still practiced in Norway and Sweden (Børheim et al., 2001). Modern and traditional anglers bury catches in *Sphagnum* to preserve the freshness of the fish during long trips (Riddervold, 1990; Røsjø 1996). *Sphagnum*'s preservation capabilities are attributed to high levels of sphagnan (Børheim et al., 2001), a pectin-like polymer that lowers pH, thereby preventing growth of acid-sensitive micro-organisms (Stalheim et al., 2009). During an interview conducted by Doug Mellgren for dirtdoctor.com, T.J. Painter said that the Vikings used *Sphagnum* bog water as a

drinking source because it would stay fresh during long travels. In recent decades patents have been developed for the use of *Sphagnum*'s antimicrobial capabilities in the treatment of swimming pools (Smith, 1977).

Humans have taken advantage of *Sphagnum*'s ability to absorb liquids and deter microbial growth since as far back as the Bronze Age (Painter, 2003). *Sphagnum* has long been used as a wound covering and surgical dressing (Porter, 1917), which increased in popularity during World War I, when cotton became very expensive and scarce (Williams, 1982; Varley and Barnett, 1987a). It is reported that towards the end of War World I, the British Army used approximately one million pounds of *Sphagnum* dressings a month (Nichols 1918; 1920). The use of *Sphagnum* dressings diminished in Western countries after War World I, but the practice continues in China (Ting, 1982).

The use of *Sphagnum* dressings can be controversial, with some authors pointing out that *Sphagnum* dressings may present medical risks. For example, *Sphagnum*-moss was linked to sporotrichosis outbreaks in various states in the United States of America (Coles et al., 1982; D'Alessio et al., 1965). Sporotrichosis is a fungal infection caused when *Sporothrix schenkii* invades the human body through skin lesions, and can infect skin, lungs, central nervous system, bones and joints (Sharkey-Mathis et al., 1993). However, studies conducted on pigs showed that sterilized *Sphagnum* dressings promoted surgical wound healing (Varley and Barnett, 1987b). Research also suggests that sterilized *Sphagnum* dressings can help prevent infections and inactivate exotoxins, enzymes and lysins commonly found in pathogenic bacteria via the Maillard reaction, which inactivates ammonia, amino acids and peptides (Painter, 1998; 2003).

The Northern Hemisphere has a long history of peat harvest for desired application. Presently however, peat harvesting has become a source of major concern because harvesting is often not followed by the promotion of a new crop. Therefore, sustainable methods for peat harvesting should be developed (Lindstrom, 1980). Although over-harvesting can have negative effects on *Sphagnum* diversity, research has shown that with limited harvesting *Sphagnum* can regenerate and even experience an increase in biodiversity (Chapman et al., 2003). Further research in this area should be conducted to promote the development of new methods that would protect *Sphagnum* biodiversity, thus guaranteeing its continuous use and availability. Development of sustainable harvesting practices would not only guarantee continuous availability of *Sphagnum* as a resource, but would also protect its role as a carbon sink. Recent research suggests that restoration of peatlands can have adverse effects on the rate of carbon sequestration. Newly restored peatbogs have lower carbon sequestration rates than natural occurring peatbogs, thus limiting the roles of peatbogs as carbon sinks (Tuittila et al., 2004; Belyea and Malmer, 2008). *Sphagnum* depletion raises concern about ecological stability and potential for increase in degradation and respiration, which could turn peatbogs from a net carbon sink into a net carbon source. Promotion of sustainable harvest can mitigate the impacts that peatland destruction would cause to the global carbon cycle, as well as potentially offset effects of increasing atmospheric levels of CO_2.

Preserving History: Bog Bodies and Cultural Artifacts

As a consequence of its natural preservational qualities *Sphagnum* has also preserved pre-historic humans in the form of “bog bodies”. To this day, bog bodies are often used to introduce students to bryophytes, but are also important in archeological contexts. The majority of bog

bodies have been described from Denmark, with specimens also found in Norway, Sweden, Germany and Ireland (Glob, 1971; Connolly, 1985; Pearson, 1986; Purdy, 2000), primarily by peat-harvesters.

The exquisite preservation of bog bodies has been attributed to cold, acidic and anoxic environment as well as the preservative attributes of sphagnan (Painter, 1991). Bog bodies are so well preserved that people sometimes believed that they originated from people who had recently been murdered. Christians are known to have collected the bodies and buried them in the local church (Glob, 1971). For the most part the finding of bog bodies would go unreported by the media, and it was not until Glob published the popular book *The Bog People* (1965) that the media and researchers started to pay more attention to uncovered bog bodies (Purdy, 2000).

Controversies have surrounded the finding of bog bodies. The unique methods of bog burials led to the development of many hypothesis of cultural practice. Bodies found staked to the bottom of bogs were hypothesized to be witches that had been staked to prevent them from rising from the grave (Glob, 1971). A female body found in a bog near a royal family estate was believed to be Queen Gunhild, a beautiful and cruel Norwegian queen, who according to legend was drowned in deep Danish bog (Glob, 1956). Further historical and archeological research has suggested that the body was that of an Iron Age woman and not Queen Gunhild. Even so, modern day romantics have been inspired to write poetry, melodies and plays about this particular bog body (Glob, 1971).

A bog body known as Grauballe Man led a local elder to state that the body belonged to Red-Christian a local peat-cutter who had mysteriously disappeared (Glob, 1951). However, ^{14}C dating revealed that Grauballe Man had lived during the Late Iron Age to Roman Times (2nd

Century B.C. -4th Century A.D.) (Van der Plincht et al., 2004). Tollund Man, Lindow Man and ten other bodies have been ^{14}C dated and reported to have lived between the Late Bronze Age to the Early Roman Iron Age (840 B.C.-95 AD) (Tauber, 1979; Sellevold, et al., 1984; Pearson, 1986).

The large time interval and diverse locations at which bog bodies are found suggest that there may be many explanations. The sum of research suggests that the geographic areas where bog bodies are found were mostly dominated by a Pre-Germanic meta-culture displaying differences in religion, language and social structure, although some similarities existed (Iping-Petterson, 2011). The exquisite preservation of bog bodies has allowed researchers to ascertain mode of death and to address questions regarding the existence of human sacrifice among Pre-Germanic cultures. Researchers have noted that wounds preserved in bog bodies do not necessarily indicate that these people were victims of ritual sacrifice (Green, 1998). Only by careful analysis can bog bodies can be interpreted as human sacrifices (Green, 1998; Iping-Petterson, 2011). Some bog bodies can be classified as victims of "blood rites" or "drowning, strangulation or burial alive" (Green, 1998). Blood rites are rituals in which blood is drained from the body. An example is Grauballe Man, who suffered a cut to his throat that extended almost all the way to the gullet. Grauballe Man died as a result of the cut; even though his bones were broken, such injury is believed to result from the weight of the peat (Glob, 1971; Green, 1998). Only the Tollund man, Juthe Fen Woman, and Windeby Girl are thought to have been drowned, strangled, or buried alive (Green, 1998). Tollund Man was found with a rope around his neck suggesting he was hanged or strangled before deposition in a bog (Glob, 1971; Green, 1998; Iping-Petterson, 2011). Juthe Fen Woman and Windeby Girl were most likely either drowned or buried alive

(Glob 1971; Green, 1998). Analysis of available data suggests that sacrifices must have happened very rarely (Green, 1998).

Bog bodies were so well preserved that researchers can study more than just the cause of death, but also details such as the last meal and general health. Before death, Tollund Man and Grabauballe Man likely consumed edible weeds that included *Chenopodium*, *Camelina*, *Linum*, and *Spergula* (Glob, 1971; Rapoport et al., 1995; Green, 1998; Iping-Petterson, 2011; Karg, 2012). The eating of such weeds suggests that it was done as part of a sacrificial ritual since these plants grew hundreds of miles away from the burial site, suggesting that the food had a special significance (Green, 1998). Reconstructions of the last meal by modern researchers suggest that this meal probably had a very bad or acquired taste (Glob, 1971). The differing geographical ranges of these ritual foods provide evidence of trade. Strontium isotope measurements conducted on Denmark-bog textiles indicate that these were made of wool that was locally produced and that textiles were locally grown or imported, the latter supporting a hypothesis that regular trade occurred among groups (Frei et al., 2009; Vander Bergue et al., 2009).

Paleo-medicinal studies have been conducted on bog bodies in an attempt to study health and body development. Studies conducted on intestinal residues of bog people have indicated the widespread occurrence of parasites, e.g. whipworm eggs (*Trichuris trichuria*) found in Lindow Man (Araúgo and Ferreira, 2000; Bouchet et al., 2003). Medical-imaging research is being conducted with the hope of understanding bone structure and density, gender, age and many other characteristics that could help researchers understand the Iron Age world (Turner-Walker

and Peacock, 2004; Lynnerup, 2010). One thing is clear: without *Sphagnum*'s preservation abilities very little would be known about the life and rituals of the Nordic Iron Age Humans!

Research: Moving *Sphagnum* Research Forward

As noted, peat moss genus *Sphagnum* plays an important role in modern ecological systems by influencing carbon cycling. Microfossil evidence suggests that *Sphagnum* has been influencing biogeochemical cycling and atmospheric chemistry for hundreds of millions of years, even before the rise of vascular plants (Graham et al., 2004). In the next chapter of this thesis I describe aspects of the biology of modern *Sphagnum* that influence its roles in carbon cycling. In the following chapter I consider environmental factors that are important now and were likely important in the ancient past in determining the axial orientation of *Sphagnum* and the dorsiventral orientation of related, but earlier-diverging lineages. In the final chapter I focus on the fossil history of *Sphagnum*, important because it suggests the extent to which early land plants have influenced global biogeochemical cycles. The major goals of each of these three research projects are summarized below:

Chapter 1. The impacts of combined UV and temperature treatments on *Sphagnum compactum* biomass, cell wall components, and morphology.

Sphagnum peat mosses play a global environmental role in maintaining atmospheric chemistry and climate homeostasis. Previous research suggested that increased UV-A and UV-B resulting from ozone depletion at high latitudes play a role in growth and development of the peat moss *Sphagnum*. In view of increasing concern about the effects of global climate change on plants,

we investigated the impacts of combined temperature and UV treatments on biomass, cell wall components, and morphology of a cultivated species of *Sphagnum* moss.

Chapter 2. Experimental evidence that the direction of illumination controls gametophytic body orientation in modern bryophytes and that this process was inherited from ancestral algae.

The invasion and early colonization of land by early streptophytes was one of the most important events in Earth history. Early streptophytes, much like modern dorsiventral liverworts and axially-oriented mosses, likely contributed to soil formation, provided nutritional resources for terrestrial heterotrophs, and influenced atmospheric chemistry prior to the rise of vascular plants (Graham et al., 2004). However, the early ecophysiological characteristics of early land plants are still unknown. Early land plants likely grew on unconsolidated substrates such as mud or sand, large expanses of which occurred in Cambrian-Ordovician times. Early land plants would thus be susceptible to disturbance in dorsiventral/axial orientation as the result of aeolian processes. Physiological responses likely allowed early plants to sense and maintain optimal dorsiventral/axial orientation. In this chapter, I describe an experimental system used to distinguish the effects of light direction and gravity on the reorientation by growth of an evolutionary series of modern plant gametophytes: the charophycean alga *Coleochaete orbicularis*, the early diverging liverworts *Blasia pusilla* and *Marchantia polymorpha*, the early-diverging moss species *Sphagnum compactum*, and the model fern *Ceratopteris richardii*.

Chapter 3. *Sphagnum*-like fossils from the Ordovician

Microfossils that have been interpreted as the resistant remains of sporophytic capsule epidermis of *Sphagnum*-like early mosses (Kroken et al., 1996) have been reported from the

middle Ordovician (Caradocian, 448-468 mya) (Gray et al., 1982). This evidence has been used to model the carbon sequestration impacts of early-diverging moss lineages such as *Sphagnum*, *Polytrichum*, and *Andreaea* (Graham et al., 2004). *Sphagnum* is the earliest diverging lineage of modern mosses. However, the lack of macrofossils allied to the *Sphagnum* lineage prior to the occurrence of Lower Permian *Protosphagnum* (Neuberg, 1956) is an issue. We have found what appear to be portions of *Sphagnum*-like stem and leaves in Wisconsin Ordovician rocks. These remains suggest that the modern genus *Sphagnum* has a much longer history than previously realized, and allow inference of biogeochemical impacts of peat mosses through a long geological time period.

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Chapter 1. The impacts of combined UV and temperature treatments on *Sphagnum compactum* biomass, cell wall components, and morphology.

ABSTRACT

- *Premise of study: Sphagnum* peatmosses currently play a global environmental role in maintaining atmospheric chemistry and climate homeostasis and likely have done so for hundreds of millions of years in Earth's past. Previous research has suggested that increased temperature and UV resulting from ozone depletion at high latitudes can affect *Sphagnum* growth and development. In view of increasing concern about modern global environmental changes and evidence that ancient peatmosses likely experienced geological episodes of extreme environmental perturbation, we investigated the effects of combined temperature and UV treatments on laboratory cultures of *Sphagnum*.
- *Methods: Sphagnum compactum* was chosen as a tractable physiological model whose phylogenetic relationships indicate relatively early divergence. Three environmentally relevant temperature levels (10˚C, 20˚C, and 30˚C) and two levels of UV-A + UV-B (enhanced and reduced) were applied in a factorial experimental design. Total and acetolysis-resistant biomass, percent cellulosic biomass, and numerous morphological characteristics commonly used in peatmoss taxonomic keys were quantified and analyzed using ANOVA.
- *Key Results:* Elevated temperature was significantly associated with failure of leaf hyaline cells to differentiate and reduction in stem cortical cell layers from three to one. Enhanced UV was the major factor explaining increase in the proportion of resistant cell

wall carbon. At 30˚C and under enhanced UV *S. compactum* died within two weeks and normally rigid stem pith cells had buckled, strongly impacting morphology.

- *Conclusions*: Temperature and UV affect morphology in ways that can reduce peat moss roles in global climate stabilization, present and past.

INTRODUCTION

Sphagnum peat mosses are credited with being important sinks in the global carbon cycle by enhancing organic carbon sequestration in globally extensive peatlands. *Sphagnum*-generated peat is estimated to contain ~400 gigatons of organic carbon, representing around 30% of the total soil carbon budget (Gorham, 1991). Approximately 33 gigatons (~33Pg) of this stored carbon occurs in Northern Hemisphere peatlands, especially those of Alaska and Canada (Wisser et al., 2011).

Sphagnum is noted for its ability to engineer its environment by acidifying the surrounding nutrient-poor waters by ion exchange, releasing H^+ in the uptake of Ca^{2+} and Mg^{2+}. Such acidification influences peatland community composition (Crum and Anderson, 1981) and fosters peat accumulation in the ecosystem by slowing the decomposition process (Clymo, 1965; Coulson and Butterfield, 1978; Johnson and Damman, 1983; Aerts et al., 2001; Rydin and Jeglum, 2006; Pankratov et al., 2011). Peat mosses produce resistant carbon (Tsuneda, 2001), which may include cell wall polysaccharides (Hájek et al., 2011) and/or lignin-like phenolic compounds (Kroken et al., 1996; Delwiche et al., 1989; Rozema et al., 2001). Lignin-like phenolics are thought to confer microbial resistance, desiccation resistance and protection from harmful radiation, including ultra-violet (UV) originating from solar emissions (Graham et al., 2004).

During the past decade or so ozone (O_3) layer depletion, coincident with higher levels of UV, has become an ecological concern, particularly in high latitudes (Mandronich, 1998). Studies conducted by The World Health Organization (WHO) indicate that the rate of O_3 depletion has leveled off as the result of controls on emission of chlorofluorocarbons (CFCs), but the past damage caused by CFCs is so great that it is expected that a time period of ~50-60 years (2050-2060) will be needed for recovery to 1980's levels of surface UV (Aphalo, 2003; McKenzie et al., 2011). Models suggest that it could take longer for UV levels to fall at higher latitudes, particularly in locales affected by the Antarctic (McKenzie et al., 2011) and Arctic (World Meteorological Organization, 2007) ozone holes. For this reason, it remains important to evaluate the impact of UV on key high-latitude organisms, such as peat mosses.

Recent studies of near-Antarctic mosses, mostly located in Tierra del Fuego, Argentina, have shown that increased UV affects shoot height but not total biomass (Searles et al., 1999; Aphalo, 2003; Robson et al., 2003). A study conducted on *Sphagnum fuscum*, a Northern Hemisphere representative, showed the same pattern of reduced shoot dimensions in higher UV conditions, though no effect on total biomass was observed (Geherke, 1998). UV exposure increases the concentration of UV-absorbing flavonoids in cells of *Sphagnum* and some other mosses (Searles et al., 1999). *Sphagnum* growth rate, size, ability to store water, and number of capitula also seem to be affected by increased UV (Robson et al., 2003; 2004). In addition to increased UV exposure resulting from ozone depletion, peat mosses are also potentially vulnerable to the impacts of increasing global temperature, but the effects of increased temperature or increased temperature in combination with enhanced UV have not been adequately evaluated for peat mosses. To fill this gap we conducted experimental studies of combined temperature and UV effects on laboratory cultures of a species of *Sphagnum*.

Sphagnum compactum, whose compact structure is convenient for laboratory studies (Graham et al., 2010), and is a relatively early-diverging species within the genus *Sphagnum* (Shaw et al., 2000), was chosen as an experimental model for peat moss responses. Temperature treatments represent environments experienced by high latitude peat mosses in normal (10˚C and 20˚C) and unusually warm (30˚C) conditions. Two environmentally relevant levels of UV-A and UV-B represented reduced and enhanced conditions. In a multi-factorial experimental design, we assessed the impacts of these factors on total biomass, acetolysis-resistant biomass, percent cellulosic biomass, and numerous light microscope-level morphological characteristics commonly used in taxonomic keys.

MATERIALS AND METHODS

***Cultures and growth media*—**The relatively short gametophores of *Sphagnum compactum* cultures (originally obtained from M. Sargent, University of Illinois, Urbana-Champaign) were grown in tall petri dishes (100 X 25 mm) (Fisher Scientific, Fair Lawn, New Jersey, USA) containing 1/3 Gamborg's B5 medium (US Biological, Swampscott, Massachusetts, USA) solidified with 2% Bacto™ Agar (Becton, Dickinson and Company, Sparks, Maryland, USA). Replicate cultures for experimental treatments were maintained at 20˚C with a 16 h photoperiod for two days before treatments began. To avoid microbial contamination but also allow gas exchange to occur, experimental dishes containing *S. compactum* were sealed with a single layer of Parafilm M, which is permeable to carbon dioxide at 1200 $cc \cdot m^{-2} \cdot d^{-1}$ and oxygen at 150 $cc \cdot m^{-2} d^{-1}$ at 23˚C and 0% RH, and water vapor at 1 $g \cdot m^{-2}$ at 38˚C and 90% RH (SPI Supplies, 2010).

Experimental design—Replicate *S. compactum* cultures were treated in a multi-factorial design with three temperature conditions (10˚C, 20˚C, or 30˚C) and two UV levels, reduced

(approximately 50 μW/cm^2) and enhanced (approximately 130 μW/cm^2). In a previous pilot study it had been determined that these contrasting UV treatments were sufficient to generate different responses in *S. compactum*. In nature *Sphagnum* is known to commonly experience temperatures at 10-20˚C and sometimes as high as 30-40˚C (Proctor, 1982; Longton, 1988; Sveinbjornsson and Oechel, 1992). Summer temperatures measured at the canopy of peatmosses of northern Wisconsin bogs can reach 35-40˚C (Hanson et al., 1999). Environmental UV-A and UV-B levels were measured using a General Tools UV Light Meter (General Tools and Instruments Co., 80 White St., NY, NY). Summer (September 3, 2012) UV-A + UV-B levels on Bascom Hill at the University of Wisconsin-Madison (+43˚ 4' 27.92", -89˚ 24' 9.81") measured with this instrument were ~36,676 μW/cm^2, and spring (April 2, 2013) UV-A + UV-B levels were ~15,819 μW/cm^2. Hence, the experimental lab treatments did not exceed natural UV levels. Experimental UV treatments were continuous (no day length treatments imposed) because day length is nearly continuous during the growing season in the natural high-latitude environments in which UV effects are most likely to occur.

In each treatment, nine replicate plants received photosynthetically active radiation (PAR) provided by cool white fluorescent lamps. PAR was determined using a Quantum Scalar Laboratory Radiometer QSL-100 (Biospherical Instruments Inc., San Diego, California, USA). Fluorescent lights also emit UV radiation. For this reason, glass sheets were used to filter UV emitted by fluorescent lamps, generating reduced UV conditions. In each treatment, nine replicates received enhanced UV-A and UV-B provided by two types of 26W lamps (Repti Glo 10.0 and Repti Glo 5.0) that are designed to add essential natural UV to terraria used to house captive desert or tropical reptiles (Exo-terra, PetSmart, Phoenix, Arizona, USA). Repti Glo lamps were used in this experiment because they emit UV-A and UV-B radiation, but not UV-C.

Many UV lamps emit UV-C, radiation that is unnatural on Earth's ecosystems and is biologically more deleterious than UV-A and UV-B. Personnel entering UV treatment areas routinely wore industrial protective eyewear (Fisher Scientific, Pittsburgh, Pennsylvania, USA), which blocks 99% of UV-A and UV-B.

Replicates were limited to nine for each treatment in order to maintain uniform irradiance environment; had greater numbers of replicates been used, some would necessarily have been located outside an area of uniform irradiance conditions. To further homogenize samples within treatments, culture dish positions were regularly redistributed. All experimental treatments occurred on a 16 hr light cycle, consistent with long days that occur during the growth period of mid-high latitude peat mosses. Plants were exposed to experimental conditions for a 2-month period, consistent with the growth period of mid-high latitude peat mosses, after which some samples were harvested, dried and weighed, and aliquots used for cellulose extraction and acetolysis. Living samples were used for morphometric analyses.

Experiments conducted at 10˚C—A shelf in a 10˚C cold room was divided into two sections by a vertical reflective surface. On one side, nine culture dishes were arranged at a distance of 35 cm from glass-filtered cool-white fluorescent lamps supplemented with white light-emitting diodes (LEDs), thereby receiving an average of ~1.8×10^{16} μmol photons·m^{-2} ·s^{-1} PAR, and UV level of 47 μW/cm^2. LED supplementation was necessary to achieve PAR illumination levels equal to those present in the facilities used for the higher temperature treatments. The other side received the same PAR illumination, but from unfiltered fluorescent lamps, enhanced by UV lamps mounted at a distance of 33 cm from nine culture plates, which received a total UV dosage of 129 μW/cm^2.

Experiments conducted at 20˚C—Experiments at 20˚C were conducted on shelving located in a walk-in growth room. PAR was provided by cool-white fluorescent lamps mounted at an average distance of 40 cm from nine culture dishes, which received an average irradiance of ~$1.75x10^{16}$ µmol photons·m^{-2}·s^{-1}. One shelf received only illumination from glass-filtered fluorescent lamps (UV irradiance 55 µW/cm^2). Another shelf received enhanced UV-A and UV-B radiation from UV lamps mounted at an average distance of 23 cm from nine culture dishes, which received an average UV irradiance of 137 µW/cm^2.

Experiments conducted at 30˚C— Elevated temperature experiments were conducted in a portable growth chamber (Dual Program Illuminated Incubator 818, Precision Scientific) that was vertically divided into two sections by a black light-absorbing surface. In the reduced UV (52 µW/cm^2) section, illumination was provided only by glass-filtered cool-white fluorescent lamps oriented perpendicular to nine culture dishes, which received an average PAR irradiance of ~$1.67X10^{16}$ µmol photons·m^{-2} ·s^{-1}. The UV-enhanced received supplementary UV-A and UV-B radiation provided by Repti Glo lamps. Lamps providing UV illumination were at an average distance of 30 cm from nine culture dishes, which received an average UV dose of 126 µW/cm^2.

Harvesting and biomass assessment—After a two-month growth period, plant material was removed from three culture dishes per treatment and dried for one week at 37˚C in a Dry Type Biological Incubator (Blue M Electric Company, Blue Island, Illinois, USA). Biomass was determined using a Sartorius Analytical Weight Type 1872 (Brinkman Instruments Com. Div. of Sybron, Westbury, New York, USA). Each dried biomass sample was divided into five subsamples, used for cellulose extractions.

***Acetolysis*—**Acetolysis is an extremely hydrolytic process that involves boiling biological material for 30 minutes in a 9:1 v:v mixture of acetic anhydride: concentrated sulfuric acid. Acetolysis resistance has been used as an index of resistance to chemical or microbial hydrolysis (Kroken et al., 1996). We performed quantitative acetolysis (Graham et al., 2004; 2010) to infer the dry mass percentage of hydrolysis-resistant lignin-like phenolic polymers, which are known to be present in *Sphagnum* cell walls (Kroken et al., 1996). Acetolysis remains were dried for one week at 37˚C before weighing. In determining percent resistant carbon, the biomass of remains was divided by initial dry biomass.

Cellulose extraction--Approximately 0.3 g of dry, un-acetolyzed biomass was used for cellulose extraction. The protocol for cellulose extraction involves treatment with sodium chlorite at 60˚C overnight, in a procedure adapted from Mihranyan et al. (2004), except that a final acid hydrolysis was not performed. Extracted cellulose was air dried for two weeks before determining percent cellulose. To determine percent cellulose, the extracted cellulosic biomass is divided by initial dry biomass.

***Morphometric measurements*—**Morphological characters chosen for comparison are commonly used in keys for indentifying *Sphagnum* species: stem cross section, size of chlorophyllous cells and hyaline cells, number of pores and rings on hyaline cells, and branch leaf cross section. A 0.3% solution of crystal violet was employed to enhance contrast. For consistency, measurements were taken from between the third and fourth branches from the shoot tip.

Sphagnum grown at 30˚C and under enhanced UV was too fragile to hand section readily, so samples were embedded in plastic for thick sectioning. Ethanol dehydrated specimens were

gradually infiltrated with LR White Embedding Medium Hard Grade (Ted Pella Inc, Redding, California, USA). The plastic was polymerized for 2 days at 37˚C, and specimens were mounted and trimmed for sectioning with glass knives made with a Leica EM KMR3 (Leica Microsystems, Vienna, Austria). Sections were transferred to water drops, and after the water had evaporated, stained with 0.05% toluidine blue. Measurements were made using an Axioplan light microscope (Carl Zeiss Microscopy, Germany), and images were made using a Nikon D300s Digital Camera and Camera Control Pro software (Nikon Inc., Melville, New York, USA).

***Statistical analysis*—**Data were analyzed by means of multiple ANOVA (Minitab Statistical Software 16). Five measurements were collected and averaged per petri dish, providing three values per treatment (two UV conditions in each of three temperature conditions) for a total of 18 values. Measurements collected for *S. compactum* grown at 30˚C under enhanced UV, were not assumed to be for new growth, since the exact time of death within the two-week treatment period was undetermined. In order to focus on results most important to plant structure we established a criterion that treatments must explain at least 50% of the variation.

RESULTS

Temperature and UV treatments used in this study influenced several of the morphological characters assessed in *Sphagnum compactum*: stem total width; whole plant morphology; whole plant biomass; biomass of cellulose and acetolysis-resistant cell wall components; stem cortex structure; stem leaf dimensions; stem leaf hyaline cell dimensions; stem leaf cell hyaline cell ring number; steam leaf hyaline cell pore number and pore width; stem leaf chlorophyllous cell length; branch leaf dimensions; branch leaf hyaline cell dimensions and rings; and branch leaf

chlorophyllous cell dimensions. Characters that were not significantly influenced by the experimental treatments included cortical cell width, stem leaf chlorophyllous cell width, branch leaf hyaline cell pore number, and branch leaf hyaline cell pore width. The results will be discussed in order of statistical significance, starting with whole plant morphology.

***Whole plant morphology*—**Elevated temperature (30˚C) had a dramatic effect on whole plant morphology and survival, by comparison to lower temperatures (10-20˚C), under both reduced and enhanced UV levels. Plants grown under the lower temperature conditions displayed normal body morphology with robust stems and well-defined apical capitulum and fascicles of branches (Fig. 1A,B). When grown at 30˚C and under reduced UV *S. compactum* plants were elongated, lacked defined capitula, and displayed few to no fascicles (Fig. 1C). *Sphagnum compactum* grown at 10˚C-20˚C and under enhanced UV had normal body morphology and remained alive (Fig. 2A,B), but when grown at 30˚C and under enhanced UV, *S. compactum* died after 2 weeks (Fig. 2C).

***Stem cortex*—**Elevated temperature and UV also affected micromorphological characters, including features of the stem cortex. Stem cross-sections showed a decrease in the number of cortical cell layers (Fig. 3). We observed 2-3 layers of stem cortical cells in cross-sections of *S. compactum* grown at 10-20˚C under both enhanced and reduced UV (Fig. 3, 10-20˚C under enhanced and reduced UV), but when *S. compactum* was grown at 30˚C and under reduced UV the stem cortex was composed of a single layer of cortical cells (Fig. 3, 30˚C and under reduced UV). In contrast, cross-sections of stems of *S. compactum* grown at 30˚C under enhanced UV were not circular and cell shapes were distorted (Fig. 3, 30˚C and under enhanced UV). Under growth conditions of 10-20˚C and enhanced or reduced UV, retort cell pores were of normal

shape and position. For plants grown at 30˚C and under enhanced UV, stem morphology was so distorted that we could not determine whether retort cells had pores or not (Fig. 3, 30˚C and under enhanced UV).

***Stem leaf cell dimorphism*—**A mature *S. compactum* stem leaf, like those of other *Sphagnum* species, features a regular pattern of specialized hyaline cells and chlorophyllous cells and is thus described as dimorphic. Hyaline cells are large dead cells that serve as water storage units; chlorophyllous cells are green photosynthetic cells, which are the living component of the plant. When grown at 10-20˚C and under enhanced or reduced UV, leaf development seems normal with typical arrangement of cells (Fig. 4A). At 30˚C and under reduced UV, hyaline cells did not develop normally; some leaves were primarily composed of chlorophyllous cells (Fig. 4B).

***Plant biomass and cell wall polymer mass*—**Statistical analysis suggests that there was a difference in whole plant biomass of *S. compactum* under different treatment conditions. The temperature and UV interaction explained a considerably higher proportion of the variation in whole plant biomass than did temperature and UV (Table 1).

Higher levels of acetolysis-resistant material were observed when the percent cellulose was lower and vice versa. Percent resistant carbon was higher in plants grown in enhanced UV vs reduced UV at all temperatures tested. The biggest increase in percent resistant carbon was observed for plants grown at 10˚C in enhanced UV, by comparison to plants grown at the same temperature in reduced UV. The p-value for the interactions effect was significant (Table 1), allowing us to reject our null hypothesis that there is no temperature and UV interaction effect on resistant carbon biomass. Even so, UV explained a considerably higher proportion of the variation in resistant carbon biomass than did temperature and UV interaction (Table 1).

No difference in percent cellulose was observed between plants grown at 20-30˚C at the two UV levels tested. However, percent cellulose was significantly higher in plants grown at 10˚C and under reduced UV, by comparison to plants grown at 10˚C and under enhanced UV. The interaction p-value was significant (Table 1), rejecting the null hypothesis that there is no temperature and UV interaction effect on percent cellulose. Even so, UV explained about twice as much result of the variation in cellulose biomass than did temperature and UV interaction (Table 1).

***Stem cross-sectional dimension*—**The temperature and UV interaction effect on cortex cell width (Table 1) was not significant, but temperature explained more of the variation. The temperature and UV interaction effect on stem total width (Table 1) was significant, but temperature explained most of the variation. Pith cells located in the center of the stem provide support for *Sphagnum*. In plants exposed to enhanced UV, a dramatic decrease in stem pith width was observed in response to elevated temperatures (30˚C). For *S. compactum* grown at reduced UV, no difference in pith width was observed for plants grown at 10˚C versus 20˚C at either UV level. A decrease in pith width was observed in plants grown at 30˚C and under reduced UV, by comparison to plants grown at 10-20˚C and under reduced UV.

***Stem leaf dimensions*—**Because a large portion of the mature peat moss leaf consists of water-storing hyaline cells, changes in leaf dimensions can influence water retention. *Sphagnum compactum* grown at 10˚C and under enhanced UV had higher mean stem leaf length than plants grown at 20-30˚C and under enhanced UV. No difference in leaf length was observed for plants grown at 10˚C and under enhanced UV versus 10˚C and under reduced UV. Leaf length was significantly greater for plants grown at 10˚C or 20˚C and under reduced UV than plants grown

at 30˚C and under reduced UV. *Sphagnum compactum* grown at 20˚C and under enhanced UV had shorter leaves than plants grown at 20˚C and under reduced UV.

The results for stem leaf width were not as straight forward. No difference was observed for plants grown at 10˚C and under enhanced or reduced UV, at 20˚C and under enhanced and reduced UV, or at 20-30˚C and under enhanced UV. One clear difference occurred in stem leaf width of plants grown at 30˚C (under enhanced or reduced UV). *Sphagnum compactum* grown at 30˚C and under enhanced UV had wider stem leaves than plants grown at 30˚C and under reduced UV. P-values for temperature and UV interaction effects on both stem leaf length (Table 1) and stem leaf width (Table 1) were significant, so the null hypothesis that there is no temperature and UV interaction effect on stem leaf length and stem leaf width is rejected. For stem leaf width, temperature explained more of the variation than did temperature and UV interaction (Table 1); but for stem leaf length, temperature explained about twice as much of the variation than did UV or temperature and UV interaction (Table 1).

***Stem leaf hyaline cell dimensions*—**Hyaline cells serve as water storage units, and changes in their dimensions could potentially affect *Sphagnum*'s water absorbing capabilities. No significant difference was observed in stem leaf hyaline cell dimensions for plants grown under enhanced UV at the three temperatures tested. No significant difference in dimensions was observed for stem leaf hyaline cells for plants grown at 10-20˚C and under reduced UV. However, plants grown at 30˚C and under reduced UV had significantly shorter hyaline cells than plants grown at 10˚C-20˚C and under reduced UV. There was no difference in the length of stem leaf hyaline cells of plants grown at 10˚C and under enhanced UV versus 10˚C and under reduced UV. There was no difference in length of hyaline cells of stem leaves of plants grown at

20˚C and under reduced UV and those of plants grown at 20˚C and under enhanced UV. On the other hand, plants grown at 30˚C and under enhanced UV had longer stem leaf hyaline cells than plants grown at 30˚C and under reduced UV. The p-value for interactive temperature and UV effects on stem leaf hyaline cell length (Table 1) and stem leaf hyaline cell width (Table 1) was significant, allowing us to reject our null hypothesis that there is no temperature and UV interaction effect on stem leaf hyaline cell width and stem leaf hyaline cell length. For stem leaf hyaline cell length, temperature and UV interaction explained a higher proportion of the variation than did temperature or UV considered separately (Table 1), but for stem leaf hyaline cell width, temperature explained more of the variation (Table 1).

***Stem leaf hyaline cell rings and pores*—**Normal, mature stem leaf hyaline cells are characterized by distinctive supporting rings, as well as pores that allow contact between the external environment and the cell lumen. Stem leaf hyaline cell ring number was the same for *S. compactum* grown at 10˚C, 20˚C and 30˚C and under enhanced UV. The lowest number of stem leaf hyaline cell rings was observed in plants grown at 30˚C and under reduced UV. No difference in stem leaf hyaline cell rings was observed among plants grown at 10˚C and 20˚C in enhanced or reduced UV levels. Temperature explained more of the variation than did UV alone, or temperature and UV interaction (Table 1). No significant difference was observed in stem leaf hyaline cell pore number or stem leaf hyaline cell pore width among treatments.

***Stem leaf chlorophyllous cell dimensions*—**Chlorophyllous cells are metabolically active photosynthetic cells, which constitute the live portion of *Sphagnum*, so changes in chlorophyllous cell dimensions could affect growth and survival of the plant. There was no significant difference in stem leaf chlorophyllous cell width under conditions tested, but length

was significantly shorter for plants grown at 30˚C under reduced UV. Plants grown at 30˚C in enhanced UV had significantly longer stem leaf chlorophyllous cells than those grown at 30˚C in reduced UV, but it is important to note that plants grown at 30˚C in enhanced UV died early in the experiment. The p-value for temperature and UV interaction effects on stem leaf chlorophyllous cell length was significant, so the null hypothesis that there is no temperature and UV interaction effect on stem leaf chlorophyllous cell length can be rejected (Table 1). In fact, temperature and UV interaction explained as much as the variation as temperature alone (Table 1).

***Branch leaf dimensions*—**Branch leaf morphology differs from that of stem leaves and is a common character used in the identification of *Sphagnum* species. There was no significant difference in branch leaf length values for *S. compactum* grown under enhanced UV at 10˚C, 20˚C and 30˚C. There was also no significant difference in branch leaf length between plants grown at 10˚C and under enhanced UV versus those grown at 10˚C and under reduced UV. Plants grown at 20˚C and under enhanced UV, and plants grown at 20˚C and under reduced UV were not significantly different in branch leaf length. *Sphagnum compactum* plants grown at 30˚C and under reduced UV had shorter branch leaves than *S. compactum* plants grown at 10˚C or 20˚C and under reduced UV. *Sphagnum compactum* grown at 30˚C and under enhanced UV had longer branch leaves than plants grown at 30˚C and under reduced UV, but it is important to note that plants grown at 30˚C and enhanced UV died early in the experiment. The p-value for temperature and UV interaction was significant, and in fact, temperature and UV interaction explained more of the variation in branch leaf length than did temperature or UV alone (Table 1).

There was no significant difference in branch leaf width of plants grown at 10˚C and under enhanced UV versus the 30˚C enhanced UV treatment. Branch leaf width of *S. compactum* plants grown at 10˚C and under reduced UV is not significantly different from that of plants grown at 20˚C and under reduced UV. There was no difference in branch leaf width of *S. compactum* grown at 20˚C and under enhanced versus those grown at 20˚C and under reduced UV. The branch leaves of plants grown at 30˚C and under reduced UV are significantly narrower than those of *S. compactum* grown at 10˚C under enhanced and reduced UV. Plants grown at 30˚C and under enhanced UV had significantly wider leaves than *S. compactum* grown at 30˚C and under reduced UV. The interaction p-value for branch leaf width was significant (Table 1), so the null hypothesis that there was no temperature and UV interaction effect on branch leaf width is rejected. Interaction of temperature and UV explained more of the variation than either temperature or UV considered alone (Table 1).

***Branch leaf hyaline cell dimensions, rings and pores*—**Hyaline cells in branch leaves play a vital role in water storage, so changes in their dimensions, structural support provided by rings, or porosity could affect plant function. Plants grown at 10˚C and under enhanced UV, and at 30˚C and under enhanced UV, were not significantly different in branch leaf hyaline cell length. There was no difference in branch leaf hyaline cell length values of plants grown at 10˚C and under enhanced UV versus reduced UV. Plants grown at 20˚C and under reduced UV and at 20˚C and under enhanced UV were not significantly different in branch leaf hyaline cell length. *Sphagnum compactum* grown at 20˚C and under enhanced UV had shorter branch leaf hyaline cell length than plants grown at 10˚C and under enhanced UV, and at 30˚C and under enhanced UV. Plants grown at 10˚C and under reduced UV had shorter branch leaf hyaline cells than *S. compactum* grown at 20˚C and under reduced UV. Plants grown at 30˚C and under reduced UV

had shorter branch leaf hyaline cells than plants grown at 10˚C and under reduced UV, and at 20˚C and under reduced UV, as well as all other treatments. *Sphagnum compactum* grown at 30˚C and under enhanced UV had longer branch leaf hyaline cells than plants grown at 30˚C and under reduced UV. The interaction p-value for branch leaf hyaline cell length was significant, rejecting the null hypothesis that there was no temperature and UV interaction effect on branch leaf hyaline cell length, and temperature and UV interactions explained more of the variability than did temperature or UV alone.

Plants grown at 10˚C and under enhanced UV did not significantly differ from those grown at 20˚C and under enhanced UV, or at 30˚C and under enhanced UV, in branch leaf hyaline cell width. There was no significant difference in branch leaf hyaline cell width values for plants grown at 10˚C and under reduced UV versus at 20˚C and under reduced UV. Plants grown at 10˚C and under reduced UV did not significantly differ in branch leaf hyaline cell length from plants grown at 10˚C and under enhanced UV. There was no significant difference in branch leaf hyaline cell length between plants grown at 20˚C and under reduced UV versus 20˚C and under enhanced UV. *Sphagnum compactum* grown at 30˚C and under reduced UV had the narrowest branch leaf hyaline cells observed. Plants grown at 30˚C in enhanced UV had wider branch leaf hyaline cells than plants grown at 30˚C and under reduced UV, but it should be noted that the former died before the end of the experiment. Temperature and UV interaction effects on branch leaf hyaline cell width were significant, and explained slightly more of the variation than temperature or UV alone (Table 1).

There was no difference in the number of rings in branch leaf hyaline cells in plants grown at 10˚C and under enhanced UV versus 20˚C and under enhanced UV, and at 30˚C and under

enhanced UV. There was no difference in number of branch leaf hyaline cell rings for plants grown at 10˚C and under reduced UV versus 20˚C and under reduced UV. *Sphagnum compactum* grown at 10˚C and 20˚C and under reduced UV had more branch leaf hyaline cell rings than *S. compactum* grown at 30˚C and under reduced UV. The number of branch leaf hyaline cell rings of plants grown at 10˚C and under enhanced UV was not significantly different from those of plants grown at 10˚C and under reduced UV. There was no difference in the number of branch leaf hyaline cell rings in plants grown at 20˚C and under enhanced UV versus those grown at 20˚C and under reduced UV. The interaction p-value for the number of rings on branch leaf hyaline cells was significant (Table 1), so the null hypothesis that there was no temperature and UV interaction effect in the number of rings on branch hyaline cells was rejected, and temperature and UV interaction explains more of the variation than temperature or UV alone.

Branch leaf chlorophyllous cell dimension—As in stem leaves, branch leaves have chlorophyllous cells that constitute the living portion of leaves, so changes in dimensions have potential importance for plant function. There was no significant difference in branch leaf chlorophyllous cell length among *S. compactum* plants grown at 30˚C versus 10˚C and under enhanced UV. There was no significant difference in branch leaf chlorophyllous cell length values of plants grown at 10˚C and under reduced UV, versus at 10˚C and under enhanced UV. Plants grown at 20˚C and under enhanced UV had significantly shorter branch leaf chlorophyllous cell length than plants grown at 10˚C and under enhanced UV, and at 30˚C and under enhanced UV. *Sphagnum compactum* grown at 10˚C and under reduced UV had significantly longer branch leaf chlorophyllous cells than plants grown at 20˚C or 30˚C under reduced UV. Plants grown at 20˚C and under reduced UV had significantly shorter branch leaf

chlorophyllous cells than those grown at 10˚C and under reduced UV, and significantly longer branch leaf chlorophyllous cells than for plants grown at 30˚C and under reduced UV. There was no significant difference in branch leaf chlorophyllous cell length of plants grown at 20˚C and under enhanced UV versus 20˚C and under reduced UV. Branch leaf chlorophyllous cells of plants grown at 20˚C and under reduced and enhanced UV are significantly shorter than branch leaf chlorophyllous cells of plants grown at 10˚C and under enhanced and reduced UV, and at 30˚C and under enhanced UV (though as we have noted, the latter had died prior to end of the experiment). Branch leaf chlorophyllous cells are significantly longer for plants grown at 30˚C and under enhanced UV than plants grown at 30˚C and under reduced UV. S*phagnum compactum* grown at 30˚C and under reduced UV had significantly shorter branch leaf chlorophyllous cells than plants grown under all other treatments. The interaction p-value for branch leaf chlorophyllous cell width was significant (Table 1), so the null hypothesis that there was no temperature and UV interaction effect on branch leaf chlorophyllous cell width could be rejected, and temperature and UV interactions explained more variation than did temperature or UV alone (Table 1).

In summary, important experimental results include striking developmental and structural effects of temperature or UV or both on *Sphagnum compactum* (Table 1). In particular, the failure of hyaline cells to fully differentiate in *S. compactum* grown at 30˚C, even under reduced UV, indicates important developmental changes that could affect overall biology. The single most dramatic observed impact on *Sphagnum* was that high temperature (30˚C) coupled with enhanced UV was fatal to *S. compactum*. In plants grown at high temperature (30˚C) and under enhanced UV the stem pith was composed of collapsed cells. Even under reduced UV, plants

grown at 30˚C were unusually elongated, had few to no fascicles, and lacked a well-defined capitulum.

DISCUSSION

To our knowledge this is the first full factorial laboratory study of temperature and UV effects on taxonomically important characters and cell wall polymers of *Sphagnum*. Such full factorial studies are essential to reveal interactive effects, such as those revealed by this experiment (see Table 1). For example, the most dramatic finding of our investigation was strong interaction of temperature (30˚C) and enhanced UV (131 $\mu W/cm^2$), causing *Sphagnum compactum* to die within two weeks of treatment initiation. In addition, with the exception of biomass, all other characters we investigated had not previously been explored by prior published studies related to temperature or UV effects on *Sphagnum*. Here, we discuss the significance of our results in the contexts of 1) previous studies of temperature and UV effects on *Sphagnum*, and 2) paleoecology, paleoclimates, and recent climate change.

Temperature—The most important impacts of temperature by itself were on characters related to stem anatomy. For example, stem total width became significantly narrower as temperature increased from 20˚C to 30˚C. Stem width decrease was a function of decreases in cell width, number of cortex cells, and pith width. *Sphagnum compactum* grown at 30˚C and under enhanced UV displayed a particularly narrow stem whose pith was composed of contorted cells (see Fig. 3). These effects on stem anatomy dramatically influenced morphology by decreasing stem rigidity and stability. Because the number of stem cortical cell layers and overall body morphology are characters commonly used in species

identification (Crum and Anderson, 1981), morphological differences we observed in plants grown at 30˚C, if present in field-collections, could potentially affect taxonomic and ecological research.

In plants grown at 30˚C and under reduced UV, leaf development was dramatically affected, evidenced by reduced differentiation of leaf hyaline from chlorophyllous cells (see Fig. 4B). Since hyaline cells are involved in water storage, failure of hyaline cells to differentiate normally could affect *Sphagnum* ecosystem function at multiple levels. Our findings also suggest the possibility that reduced leaf dimorphism might have occurred in ancient periods of greenhouse warming, discussed later in more detail. Furthermore, temperature played a role in explaining the variation of stem leaf length, which was particularly short when *S. compactum* was grown at 30˚C and under reduced UV. Changes in the number of hyaline cell rings could potentially affect hyaline cell stability and support.

Other investigators have utilized growth chambers to evaluate temperature effects as we did, though few others have explored the impacts of elevated temperature. Harley et al. (1989) used growth chambers to examine the effects of temperatures in the range of 10-30˚C and under a photoperiod of 16h photoperiod, observing no significant difference in growth of an unidentified species of *Sphagnum* at 10˚C and 20˚C. These investigators also found that the irradiance at which light saturation was reached increased with temperature from 10˚C to 30˚C. Decrease in net photosynthesis was observed when *S. squarrosum*, *S. angustifolium* and *S. warnstorfii* were grown at 30˚C in a growth chamber. Correlative field measurements collected for *S. angustifolium* showed that light-saturation rates were only slightly higher at 20˚C in comparison to 10˚C, which suggests a flat

temperature response until above 20˚C (Harley et al., 1989). Although Hanson et al.'s study (1999) did not report changes in photosynthesis in field populations of *S. capillifolium* as temperature increased from 30˚C to 40˚C, increases in isoprene emissions were found and interpreted as a protective physiological response.

Other research studies have focused on temperature effects occurring in the 10-20˚C range. *Sphagnum balticum* showed a decrease in whole plant biomass and capitulum weight in response to temperatures of 14˚±3.9˚C by contrast to 11˚±4.3˚C (Gunnarsson et al., 2004). Breeuwer et al. (2009) showed that increasing temperature from 17.5˚C to 22.6˚C can negatively affect growth of *S. balticum*, *S. cuspidatum*, *S. fuscum* and *S. magellanicum* and suggested that some species might be able to deal with climate change more effectively than others. Weltzin et al. (2001) inferred that unspecified levels of environmental change can increase biomass production of *Sphagnum* Sect. *Acutifolia* and *S. megallanicum*. Gerdol et al. (2007) observed that for *S. fuscum*, *S. russowii*, *S. fallax* and *S. magellanicum*, increased temperature in the form of a seasonal heat wave (average maximum temperature of 18.2˚C in August 2003), decreased production by 30% in hummocks and 50% in lawns.

Some studies suggest that temperature increases up to 20˚C can foster *Sphagnum* growth and biomass, depending on species. Robroek et al. (2007) reported that biomass of the widely distributed *S. magellanicum* and *S. rubellum* seemed to increase as temperature increased to 20˚C, but biomass of the more northern species *S. imbricatum* and *S. fuscum* seemed unchanged as temperature increased from 15˚C to 20˚C. The apparent lack of temperature response in *S. imbricatum* was attributed to height increase that compensated

for decrease in bulk. All of these *Sphagnum* species showed an increase in capitulum water content with increased temperature that was attributed to increased evaporation and concomitant increase in capillary action (Robroek et al., 2007). Breeuwer et al. (2008) observed that for greenhouse grown *S. cuspidatum*, *S. magellanicum*, *S. balticum* and *S. fuscum*, height and biomass increased as temperature increased from 11.2˚C to 21.4˚C.

Taken together, these studies and ours indicate that although temperature responses may vary among species, *Sphagnum* biomass is generally unaffected or increases as temperature increases up to about 20˚C, but that temperatures of 30˚C and higher do occur in nature and are associated with negative impacts on development, photosynthesis, and biomass production.

UV—The major effect of enhanced UV that we observed in laboratory studies of cultured *S. compactum*—enhanced production of hydrolysis-resistant cell wall polymers—might be explained as a UV protection response, a function previously suggested by Graham et al. (2004). The concomitant reduction in cell wall cellulose found in this experiment might be explained as a redirection of limited wall biosynthesis resources.

Previous studies of UV effects on *Sphagnum* have primarily been accomplished by comparing field populations of plants that had been covered by UV-filters to uncovered plants that experienced ambient UV exposure. Previously reported effects on northern Lapland (Sweden) field populations of *S. fuscum* that were attributed to enhanced UV (ambient UV: 4.6 kJ m^{-2} h^{-1} UV-B_{BE} vs enhanced UV: 5.8 kJ m^{-2} h^{-1} UV-B_{BE}) included decreases in height, dark respiration, and shoot density (measured as number of capitula

per unit area) and increased capitulum and stem dry biomass (Gehrke, 1998), effects that we did not observe. The disparity likely results from differences in UV levels, since enhanced levels of UA-A + UV-B used in our experiments (131 $\mu W/cm^2$) were much lower than ambient levels in most modern terrestrial environments, and species differences may also have played a role.

As the result of research conducted on field populations of *Sphagnum magellanicum* in Tierra del Fuego, Argentina, Searles, et al. (1999) reported that UV-B radiation (~5.5 kJ m^{-2} d^{-1}, extrapolated from Searles, et al. figure 1, page 227) had no effect on height or amount of unspecified intracellular UV-B absorbing compounds. Differences between the observations of Gehrke (1998) and Searles et al. (1999) could have resulted from differences in research location, which imply differences in ambient UV levels and/or length of plant exposure.

In contrast to the Searles et al. (1999) report, a six-year research study conducted by Robson et al. (2003) on *Sphagnum* field populations located in Tierra del Fuego, Argentina showed that, by comparison to nearby shielded populations, unspecified levels of near-ambient UV-B decreased height and increased capitulum density and mass. These changes combined to produce increased volumetric density of *S. fimbriatum* and *S. magellanicum* (Robson et al., 2003). Morphological changes reported by Robson et al. (2003) and Gehrke (1998) in response to UV were similar, but differed from studies by Nieme et al. (2002a,b) that found no changes in *Sphagnum balticum*, *Sphagnum angustifolium* and *Sphagnum papillosum* in response to UV-B levels of 0.29 kJ m^{-2} d^{-1} to 2.33 kJ m^{-2} d^{-1}.

Temperature and UV interaction—To the best of our knowledge, our work is the first to explore potential interactive effects of temperature and UV. Our results show that the interaction of temperature and UV explain most of observed variation in biomass, stem leaf hyaline cell pore number, and branch leaf width. Furthermore, *S. compactum* grown at 30˚C and under enhanced UV, died two weeks after treatment initiation, indicating that the interaction of high temperatures (30˚C) and enhanced UV can be fatal.

***Relevance of results to paleoecology and paleoclimates*—**Thanks to a fossil record for *Sphagnum* mosses that extends back to the Permian (Neuberg 1956, 1960), and perhaps as far as the mid-Ordovician (Kroken et al., 1996), the results of our study also shed light on plant-environmental interactions in the geological past. Limitations to such extrapolations of our results include difficulties in interpreting the plant microfossil record that preceded the rise of vascular plants.

The oldest accepted plant macrofossil is that of the vascular plant *Cooksonia* described in the 1930s from Late Silurian (424-414 mya) deposits (Edwards et al., 1983; Lang, 1937). Pre-Wenlock (Late Silurian) plant fossils consist primarily of microfossils—small scraps of tissue or cryptospores (walled dispersed reproductive cells of uncertain taxonomic affinity). Cryptospores occur as monads, dyads, and tetrads that are either naked or enclosed within an envelope that is absent in all modern plant lineages (Wellman, et al. 2003; Wellman 2010). Late Ordovician and Early Silurian deposits contain ornamented hilate cryptospores and trilete cryptospores that are interpreted as products of vascular plants, since ornamented hilate spores and trilete-marked spores are produced by modern vascular plants but only a few modern bryophytes (Steemans et al., 2009).

Gray et al. (1982) described Caradocian (475-460 mya) (Late Ordovician) cryptospores that occurred in adherent tetrads (a feature of certain modern bryophytes), and so interpreted these spores as possible remains of bryophytes. In addition, Gray et al. (1982) described a scrappy sheet of cells from the Caradoc (475-460 mya) that was later interpreted as *s*porangial epidermis of a *Sphagnum*-like moss, based on similarity of distinctive features to fragmented remains of modern *Sphagnum* sporangia that survived acetolysis (Kroken et al., 1996). Later, Wellman et al. (2003) described microscopic crytospores from Mid-Ordovician (475 mya) deposits that seemed to be produced in large numbers within a sporangial covering, indicating adaptation to life on land. Wellman et al. (2003) suggested that the remains had liverwort affinities, but noted the absence from the fossil of elaters that are characteristic of modern liverwort and hornwort sporangia and which aid in spore dispersal by hygroscopic movement. Rubinstein et al. (2010) reported assemblages of monads and tetrads from early Middle Ordovician deposits (473-471 mya). Together, these tissue scrap and spore-like microfossils suggest that Ordovician landscapes featured early land plants that resembled modern early-diverging liverworts and mosses (Graham et al., 2013).

Molecular clock analyses also indicate that bryophyte-like land plants had originated by the Ordovician or earlier. Recent studies indicate that the divergence of embryophytes from streptophyte (charophycean) algae occurred during the Cryogenian Period (850-635 mya), predating fossil evidence by more than 160 million years (Zimmer et al., 2007; Clarke et al., 2011). A recent molecular clock by Magallón et al. (2013) proposes that embryophytes diverged from streptophyte algae ~916 mya. Consequently, the time at

which earliest bryophyte-like land plants appeared remains controversial (Graham et al., 2013). Even so, molecular clock and microfossil evidence is consistent with the concept that peat mosses, which diverged early from other moss lineages, were present by the mid-Ordovician and thus have had a long geological history.

Molecular clock data suggest the divergence of the recent common ancestor for modern *Sphagnum* between 7 mya to 10 mya, and divergence of Sphagnopsida from an ancestor shared with Takakiopsida between 128 mya and 319 mya (Shaw et al., 2010). *Sphagnophyllites triassicus* (Sphagnales) was described from Triassic deposits in India (Pant and Basu, 1976) and *Protosphagnum nervatum* (Sphagnopsida) was described from Permian sediments (Neuberg, 1960). In contrast to previous clock studies and fossil evidence, a recent molecular clock study by Magallón et al. (2013) suggests that the modern *Sphagnum* lineage diverged from other mosses much earlier, ~351.7 mya, during the Carboniferous. The interpretation of enigmatic microfossil cell sheets extracted from Ordovician-Devonian deposits as the remains of highly resistant *Sphagnum* sporangial epidermis (Kroken et al. 1996) suggests even earlier origin of the modern Sphagnum lineage. The recent finding of microfossils interpreted as the remains of *Sphagnum* leaves and stem pieces from well-understood mid-Ordovician dolomites (Graham et al., 2013) likewise indicates that *Sphagnum* is much older than previously realized. Given the evidence that *Sphagnum* has existed since the mid-Ordovician, this lineage has likely passed through several dramatic episodes of environmental change that are related to the experimental results reported in this article, as described next.

Ordovician paleoclimates—The end of the Ordovician period (Hirnantian) was characterized by global glaciation lasting ~1-2 my, which is associated with a mass extinction event. The relatively short period of global glaciation suggests that the cause was a decrease in atmospheric CO_2, since tectonic effects typically produce glaciations spanning more than 1 million years (Gibbs et al., 1997; Hambrey, 1985). Recent isotope-based work suggests that though the end-Ordovician glaciations reached a maximum during the Hirnantian, they were preceded by short periods of glaciation and deglaciation during the Katian (Finnegan et al., 2011). However, prior to this time, the Ordovician was considerably more moderate and congenial to the spread of early bryophyte-like terrestrial plant life.

For example, the Boda event was a short period of warming that immediately preceded the Hirnantian glaciations (Fortey and Cocks, 2005), and earlier conditions in the Ordovician were clement. Mathematical models (Berner, 1990) coupled with isotopic data collected from paleosol goethite, indicate that the pre-glaciation Ordovician atmosphere contained 14-16 times the modern levels of atmospheric CO_2, consistent with other evidence for a warm and ice-free climate (Gibbs et al., 1997). $\partial^{18}O$ apatite results by Trotter et al. (2008) indicate that Mid to Late Ordovician sea surface temperature was ~28˚C, similar to modern equatorial sea surface temperatures. Such mild climatic conditions would have fostered the growth of *Sphagnum* and other early mosses that produce lignin-like cell wall polymers postulated to sequester organic carbon (Graham et al., 2004), potentially contributing to the onset of glaciation.

Paleoclimate models estimate that a reduction of Ordovician atmospheric CO_2 from 14-16 times to 8-10 times modern levels would cause glaciations similar in magnitudes and at the

locations observed in the geologic record (Gibbs et al., 1997). It has been suggested that an increase in tectonic activity gave rise to mountains that increased weathering, as did the expansion of terrestrial floras (Lenton et al., 2012). Although increased weathering is a recognized mechanism for atmospheric carbon drawdown (Royer et al., 2007), the sequestration of carbon by early mosses during the Ordovician might also have played a role (Graham et al., 2004). In a modeling study of the early-diverging mosses *Sphagnum*, *Andreaea,* and *Polytrichum* conducted by Graham et al. (2004), it was determined that even if only 1% of resistant carbon produced by such plants had been sequestered, a decrease in 0.6%-6% of atmospheric CO_2 levels might have occurred. Such reduction could account for 1-11% of the pre-vascular plant atmospheric CO_2 levels modeled by Berner (1990). It is widely thought that continental landmasses were largely submerged by shallow seas during the Ordovician. However, periods of reduced sea levels did occur, and at least some emergent terrestrial locales are thought to have persistent throughout the Ordovician (Dott and Attig, 2004). The relatively low temperature optima displayed by many modern *Sphagnum* species that inhabit temperate-boreal and high altitude peatlands suggest the potential for ancient relatives to persist through end-Ordovician glaciations to milder Silurian-Carboniferous conditions.

Early Triassic paleoclimates—Early Triassic climate was characterized by frequently oscillating temperature that has been linked to delay in recovery from the effects of the end-Permian mass extinction event (~254 mya) (Sun et al., 2012). Rises in temperature during the Early Triassic have been attributed to episodic Siberian volcanic eruptions, which would have released mixtures of compounds that can affect global climates (Sun et

al., 2012). Aerosols produced by volcanic eruptions can accumulate in equatorial regions, creating temperature increases that taper off toward the poles. Volcanic aerosols destroy ozone, which increases surface levels of UV radiation (Robdock, 2000).

Isotope data suggest that temperatures in Early Triassic equatorial seas could have been as high as 40˚C, indicating that land temperature could have been even higher (Sun et al., 2012). Rising temperature effects at this time were indicated in biological systems by the prevalence of small taxa, changes in faunal distribution during and after the global warming event, and the relative lack of peat formation (Payne et al., 2004; Sun et al., 2012). At such high temperatures (40˚C) photosynthesis rates in *Sphagnum* would have declined since the optimum photosynthetic temperature for most *Sphagnum* species is ~20˚C (Harley, 1989). Temperatures above 35˚C are associated with predominance of photorespiration over photosynthesis in most C_3 plants, which include the peatmosses (Berry and Björkman, 1980). Increased temperature would also reduce peat accumulation by increasing microbial degradation (Broker and Peacock, 1999). Our observations that *S. compactum* died when it was grown at 30˚C and under enhanced UV, conditions similar to those present during the Early Triassic, is consistent with an environmental explanation for reduced peat formation. If *Sphagnum* were an important peat producer during the Triassic as it is in modern times, the death and decrease in *Sphagnum* biomass could have contributed to the observed decrease in peat formation in the Early Triassic.

Paleocene-Eocene thermal maximum—The Paleocene-Eocene thermal maximum was a period of rapid warming having a calibrated boundary of 55-55.5 mya that is indicated by an increase of ^{12}C-isotopes (Crouch et al., 2001; Gingerich, 2006; Moore and Kurtz, 2008;

Zachos et al., 2008). The sources for increased ^{12}C-isotopes are unknown, although some authorities suggest volcanism and accompanying injection of biogenic methane (Crouch et al., 2001; Zachos et al., 2008). A previous hypothesis that the increase in light carbon at this time was due to biomass burning has been rejected because energy requirements and amount of biomass needed would have been unrealistically high (Moore and Kurtz, 2008).

The Paleocene-Eocene thermal maximum was an increase of 10˚C in high latitude sea surface temperature that is associated with the extinction of many foraminifera and changes in distribution of dinoflagellates (Crouch et al., 2001), as well as the appearance of several modern mammalian orders (e.g. Artiodactyla, Perissodactyla and Primates) (Gingerich, 2006). The Paleocene-Eocene thermal maximum global warming event is regarded as the ancient environmental transition most proximal to current climate change. Since the order Sphagnales is known to have occurred during the Paleocene-Eocene thermal maximum, our observations on temperature effects on modern *Sphagnum* may be useful in interpreting the fossil record.

Modern Environmental change- Anthropogenic climate change is linked to the burning of fossil fuels and other human practices. Models indicate that future changes in temperature and precipitation are likely to vary by region and season (Smith et al., 2007), with temperatures in high latitudes expected to be higher than the global average (Christensen et al., 2007). The majority of models suggest increases between 2-8˚C (IPCC, 2007). A recent short-term climatic model estimates that the year 2014 will be 0.30˚±0.21˚C warmer than temperatures observed in 2004 (Smith et al., 2007). Further, Arctic ozone loss

occurred at the highest observed rates during 2011 (Manney et al., 2011), indicating potential current and future impacts of UV on high latitude vegetation.

Our observations of temperature, UV and interactive effects on features of *Sphagnum* morphology that impact its biogeochemical functions, indicate that future investigations should likewise incorporate factorial analyses allowing detection of interaction effects. Our most troubling result is that *Sphagnum* died when treated at 30˚C under UV-A and UV-B levels considerably lower than ambient. Our results indicate that temperatures above 20˚C are may be lethal to at least some species of *Sphagnum*, particularly those exposed to high levels of UV. Climate change-induced *Sphagnum* depletion will exacerbate present concerns over loss of peatlands (Freeman et al., 2001) and their ecological services resulting from land use changes. Potential losses in ecological services include peat accumulation (Gerdol et al., 2007), nitrogen fixation and methanotrophy performed by *Sphagnum*-associated microbial communities (Jassey et al., 2011) and other ecophysiological properties (Graham et al., 2010).

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FIGURES

Figure 1. *Sphagnum compactum* whole plant body morphology. (A) 10˚C, reduced UV; (B) 20˚C, reduced UV; (C) 30˚C, reduced UV.

Figure 2. *Sphagnum compactum* differential survival. (A) 10˚C, enhanced UV; (B) 20˚C, under enhanced UV; (C) 30˚C, enhanced UV.

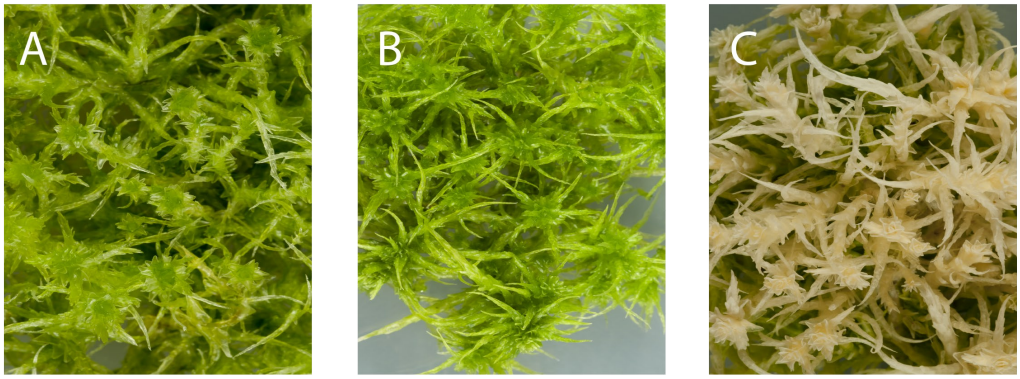

Figure 3. *Sphagnum compactum* stem cortex, shown in cross section, toluidine blue staining.

Scale bars= 100 µm

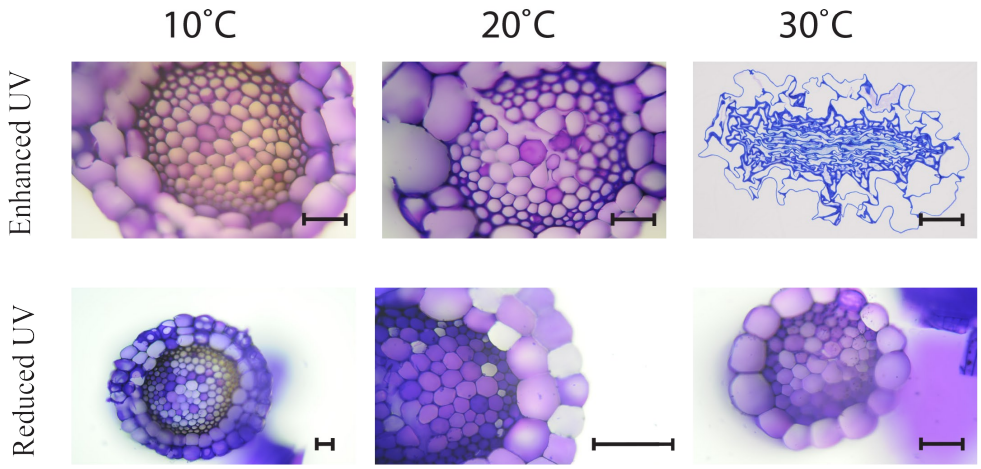

Figure 4. *Sphagnum compactum*. Effects on stem leaf cell dimensions and dimorphic development. (A) Normal leaf development (B) Abnormal leaf development. Scale bars= 100 µm

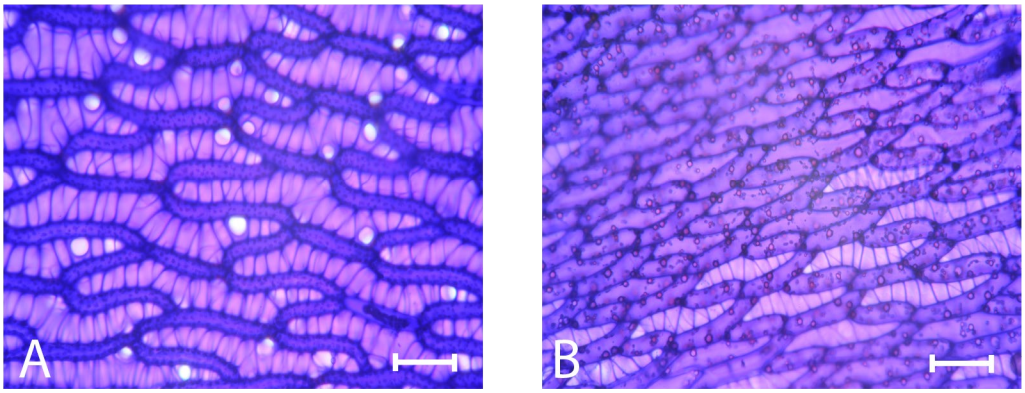

Table 1. Percent variation explained by temperature, UV and the interaction of temperature and UV (Temp&UV). P-values of <0.001 are indicated by ***, ≥0.001 to ≤0.01 are indicated by **, and p-values >0.01 but <0.05 are indicated by *.

		Percent Variation Explained		
		Temp	UV	Temp&UV
	Biomass	15.56*	5.47	63.39***
	% Resistant Carbon	3.73***	67.89***	28.33***
	% Cellulose	1.430	62.12***	33.19***
STEM	Total Width	97.19***	0.74**	1.42***
	Cortical Cell Width	9.32***	0.01	0.02
	Pith Width	66.52***	0.01	17.74**
STEM LEAF	Leaf Length	59.97***	5.39**	30.72***
	Leaf Width	43.70***	16.86***	38.96***
	Hyaline cell length	32.91***	20.48***	43.51***
	Hyaline cell width	43.17***	21.13***	33.51***
	Hyaline cell ring number	55.86***	6.96**	29.92***
	Hyaline cell pore number	26.66**	5.13*	58.51***
	Hyaline cell pore width	16.30	0.14	47.86*
	Chlorophyllous cell length	33.74***	23.53***	34.97***
	Chlorophyllous cell width	11.24	52.95**	3.46
BRANCH LEAF	Leaf Length	28.81***	19.17***	49.73***
	Leaf Width	15.25**	2.420	68.27***
	Hyaline cell length	35.89***	21.47***	42.26***
	Hyaline cell width	35.59***	19.72***	35.92***
	Hyaline cell ring number	49.99***	13.61***	35.27***
	Hyaline cell pores number	17.96*	4.83	41.82
	Hyaline cell pore width	38.80*	3.06	14.72
	Chlorophyllous cell length	36.46***	16.95***	45.65***
	Chlorophyllous cell width	17.88**	45.66***	22.55*

Chapter 2. Direction of illumination controls gametophytic body orientation in modern seedless plants and related algae.

- *Premise of study:* The environmental influences that control development and maintenance of normal dorsiventral or axial gametophytic body orientation in modern seedless plants are largely unknown. To fill this gap, an experimental laboratory system was employed to evaluate the relative effects of light direction and gravity on gametophytic body orientation in cultures of representative early-diverging streptophytes.

- *Methods:* The green alga *Coleochaete orbicularis* and green gametophytes of the liverworts *Blasia pusilla* and *Marchantia polymorpha*, the moss *Sphagnum compactum*, and the fern *Ceratopteris richardii* represented modern seedless streptophyte lineages in this study. Replicate clones were exposed to cool white illumination only from above, only from below, or from multiple directions. The spatial orientation of new growth produced during the treatment period was microscopically assessed.

- *Key Results:* For all taxa examined, light direction played a more important role in gametophytic body orientation than gravity. When lit from below, *Coleochaete* lost normal orientation, new growth of dorsiventral plant gametophytes twisted so that ventral surfaces bearing rhizoids faced the overlying air space, and axial gametophytes of *Sphagnum* moss burrowed into the substratum.

- *Conclusions:* These results indicate that: 1) directional light control of land plant gametophytic body orientation is a fundamental response, 2) early land plants inherited light-directed gametophytic body orientation from ancestral algae, 3) directional light allowed early gametophyte-dominant land plants growing on unstable substrata to

reorient after physical disturbance, and 4) directional light is key to the process by which dorsiventral or axial gametophytic plant bodies develop from spores.

INTRODUCTION

The colonization of land by early streptophytes (land-adapted plants and/or green algal ancestors) was one of the most important events in Earth history. Early gametophyte-dominated land plants that were much like modern dorsiventral liverworts and axially-oriented mosses likely contributed to soil formation, provided nutritional resources for terrestrial heterotrophs, and influenced atmospheric chemistry for tens of millions of years prior to the rise of vascular plants (Graham et al., 2004a,b). To better understand these early biogeochemical impacts, it would seem important to comprehend as much as possible about the ecophysiological properties of early land plants. Because modern land plants form a monophyletic group (Qiu et al., 2006, 2007) often referred to as the embryophytes, it is widely assumed that at least some of the features of early land plants can be inferred by interrogating modern seedless plants, particularly early-diverging liverworts and mosses. However, a number of fundamental ecophysiological properties of modern seedless plants, including the environmental factors that control body orientation, are unknown.

The bodies of early diverging thalloid liverworts are dorsiventral, with anchoring rhizoids typically produced on the ventral surface and reproductive structures on the dorsal surface, but the environmental factors that are primarily responsible for the development of single celled spores into correctly oriented dorsiventral gametophytes are not well understood. Also unknown are the environmental factors most important in orienting the axial growth typical of gametophores produced by acrocarpous mosses, which are early-diverging within the phylum

Bryophyta. The environmental factors responsible for orienting free-living, green, dorsiventral gametophytes produced by many ferns are likewise mysterious. Further, the surface-dwelling gametophytes of seedless plants can become disoriented by wind or water disturbance, and it is not known how environmental factors might relate to the re-orientation process. Based on the widespread occurrence of unconsolidated sandy substrata during the Cambrian-Ordovician time period (Dott, 2003; Avigad et al., 2005) that is widely associated with origin of earliest land plants (Graham et al. 2013), it can be inferred that early bryophyte-like land plants were probably highly susceptible to positional disturbance. Such early land plants may have adapted by sensing changes in their environment and responding by means of growth changes that restored normal dorsiventral or axial orientation. The direction of incident light and gravity present themselves as likely controlling factors. Though phototropic behavior of liverwort (Dassek, 1939) and fern (Tsuboi et al., 2006) rhizoids, phototropic and gravitropic responses of liverwort sporophytes (Thomas et al., 1987; Ellis and Thomas, 1985; Thomas et al., 2002) and phototropic aspects of moss protonemata (Mittman et al., 2009; Jaedicke et al., 2012) have been studied, the effects of these environmental factors on larger-scale gametophyte body orientation are not well understood for seedless plants.

Experiments described here tested the hypothesis that light direction is not more important than gravity in controlling gametophyte body orientation in an evolutionary spectrum of modern seedless streptophytes. The following taxa represented modern groups and stood as proxies for earliest land plants or ancestral algae: the streptophyte alga *Coleochaete orbicularis*, the early diverging liverworts *Blasia pusilla* and *Marchantia polymorpha*, the early-diverging moss species *Sphagnum compactum*, and the aquatic fern *Ceratopteris richardii*. In aquatic environments, *Coleochaete orbicularis* normally grows as attached monostromatic disks with

dorsiventral orientation indicated by dorsal hairs and position of antheridia in the closely related species *C. scutata* (Graham, 1984). *Blasia* and *Marchantia* represent thalloid liverworts that produce ventral rhizoids and dorsal asexual gemmae and gametangia (Schofield, 1985). The gametophores of *Sphagnum* and other peat mosses display axial orientation typical of the acrocarpic mosses, though very young (protonemal-stage) *Sphagnum* sporelings are monostromatic and dorsiventral (illustrated in Graham et al., 2004b), as are ferns such as the genetic model species (Cooke et al., 1995) *Ceratopteris richardii*.

MATERIAL AND METHODS

***Cultures and growth media*—**Sterilized spores of *Ceratopteris richardii* obtained from Carolina Scientific (Burlington, North Carolina, USA) were germinated and subsequent gametophyte growth conducted in 15 mm tall transparent petri plates on Basic C-Fern Medium (Carolina Scientific) solidified with 2% Bacto™ Agar (Becton, Dickinson and Company, Sparks, Maryland, USA). In preparation for experiments, 60 dishes were inoculated with sterilized spores. *Ceratopteris richardii* is a tropical fern having an optimal temperature for growth of 30˚C. For this reason, fern cultures were initiated and maintained in a culture chamber at 30˚C, 16 hr day length.

Cultures identified as the peat moss *Sphagnum compactum* having no detectable microbial contamination originated from M. Sargent (University of Illinois, Urbana-Champaign). Cultures were grown on 1/3 Gamborg's B5 medium (US Biological, Swampscott, Massachusetts, USA) solidified with 2% Bacto™ Agar (Becton, Dickingson and Company, Sparks, Maryland, USA), in 25 mm tall transparent petri plates. The relatively short stature of this species facilitates

laboratory plate cultivation. Cultures of *S. compactum* were initiated and maintained at 20˚C in a culture chamber with a 16 hr day length in a walk-in growth room.

An axenic culture of *Marchantia polymorpha* acquired by previously published procedures (Graham, et al. 2010) was maintained in standard transparent petri dishes on 1/3 Gamborg's B5 medium (US Biological, Swampscott, Massachusetts, USA) with 1% glucose (Fisher Scientific, Fair Lawn, New Jersey, USA), solidified with 2% Bacto™ Agar (Becton, Dickingson and Company, Sparks, Maryland, USA). The addition of glucose speeded the growth of replicate clonal cultures for experimental use. Cultures were maintained at 20˚C with a 16 hr day length in a walk-in growth room.

Non-axenic *Blasia pusilla* cultures were established by Alice E. Ecker from spores harvested from fresh sporangia in a collection made by Norton S. Miller in fall of 2004 from a roadside bank near Paradox, New York. *Blasia* was cultivated in standard transparent petri dishes on 1/3 Gamborg's B5 medium (US Biological, Swampscott, Massachusetts, USA) solidified with 2% Bacto™ Agar (Becton, Dickingson and Company, Sparks, Maryland, USA). Cultures were maintained at 20˚C and 16 hr day length in a walk-in growth room.

Coleochaete orbicularis originally isolated from a field collection (Graham et al., 1994) was grown in 15 mm tall transparent petri plates containing liquid DY-III medium (Lehman, 1976). Experiments were conducted in liquid media rather than on agar-solidified media because this species has been shown to display a distinctive morphology when grown on solid media or other subaerial conditions (Graham et al., 2012). Non-axenic unialgal cultures were maintained at 20˚C with a 16 hr day length in a walk-in growth room.

To avoid contamination, in all cases dishes were sealed with a single layer of Parafilm M, which is permeable to carbon dioxide at 1200 $cc \cdot m^{-2} \cdot d^{-1}$ and oxygen at 150 $cc \cdot m^{-2} \cdot d^{-1}$ at 23˚C and 0% RH, and water vapor at 1 $g \cdot m^{-2}$ at 38˚C and 90% RH (SPI Supplies, 2010). To facilitate careful evaluation of results, investigations of the five taxa were conducted serially, with care taken to ensure that temperature and irradiance levels were the same during each experiment.

Experimental design–Replicate cultures were placed on a 6 mm thick transparent plexiglass shelf, below and above which were mounted cool-white fluorescent lamps, so that dishes received an average irradiance of ~1.56×10^{16} μmol photons $\cdot m^{-2} \cdot s^{-1}$, determined with a cosine-corrected photosynthetically-active radiation meter. Cultures that were illuminated only from above were placed onto a black-painted cardboard surface through which light was unable to penetrate from below, determined by use of a light meter. Cultures that were illuminated only from below were placed nearby, but under a black-painted cardboard box through which light was unable to penetrate from above, as determined by use of a light meter. In addition, replicate cultures were placed directly and uncovered on the plexiglass shelf and thus received equal illumination from all directions.

Ninety replicate dishes, each containing multiple *C. richardii* gametophytes, were randomly divided into 3 groups of 30, one group illuminated only from above, and one group illuminated only from below, and one group illuminated from multiple directions. Of dishes receiving light only from above, 15 were placed right side up and 15 upside down. Of dishes illuminated from below and from multiple directions, 15 were placed right side up and 15 upside down.

Ninety replicate dishes, each containing multiple *S. compactum* gametophores, were maintained right side up and illuminated only from above for 2 days to allow basal portions to

attach normally to the agar substratum. Subsequently, 30 of the culture dishes were illuminated only from above; of these, 15 were placed right side up and 15 placed upside down. 30 culture dishes were illuminated only from below; 15 maintained in a right side up position and 15 upside down. In addition, 30 culture dishes were illuminated from multiple directions; 15 maintained in a right side up position and 15 upside down.

Eighty replicate dishes, each containing multiple gametophytes of *M. polymorpha*, were divided randomly into three groups, 27 plates illuminated only from above, 27 plates illuminated only from below, and 26 plates illuminated from multiple directions. In each group of dishes, approximately half were placed right side up, and the rest upside down. 70 replicate dishes, each containing multiple gametophytes of *B. pusilla*, were randomly divided into three groups, 23 plates illuminated only from above, 23 plates only from below and 24 plates illuminated from multiple directions. Each group included equal numbers of dishes oriented right side up and upside down.

Forty-five replicate dishes, each containing thalli of *C. orbicularis* large enough to produce zoospores, were randomly divided into three groups of 15 each: one illuminated only from above, one only from below, and the third from all directions.

As a consequence of differences in growth rates, the five taxa were exposed to directional light treatments for differing amounts of time. *Ceratopteris richardii* cultures were exposed to light treatments for 3 weeks, photographed after 1 week of treatment, and imaged again at the end of the growth period. *Sphagnum compactum* cultures were exposed to light treatments for 2 months and images recorded 3 weeks into the treatment and again at the end of the 2-month period. *Marchantia polymorpha* and *Blasia pusilla* were exposed to light treatments for 4 weeks and

images recorded at the end of that time. *Coleochaete orbicularis* was exposed to light treatment for 4 weeks, and was imaged 2 weeks into the treatment and again after the 1-month period had concluded.

New thallus growth—namely branch tips of gametophytes of the three bryophytes and edges of fern gametophytes—that occurred during the treatment period was imaged immediately after the end of the treatment period using a camera-enabled Leica EZ4D stereoscope (Leica, Buffalo Grove, Illinois, USA). At least 10 random images were collected of new growth for each species, in each light direction treatment, and in upside down versus right side up dishes. *Coleochaete orbicularis* was imaged with an Axioplan light microscope (Carl Zeiss Microscopy, Germany), and images made using a Nikon D300s Digital Camera and Camera Control Pro software (Nikon Inc., Melville, New York, USA). Images are archived at the University of Wisconsin-Madison, Department of Botany.

Consistent with a hypothesis that light direction is not more important than gravity in controlling gametophyte orientation, under all experimental treatments the rhizoids of dorsiventral gametophyte tissues produced during the treatment period were expected to extend into the underlying agar substratum, hair cells of dorsiventral *Coleochaete* produced during the treatment period were expected to extend into the overlying water-filled space, and capitula of axial *Sphagnum* growing during the treatment period were expected to be oriented toward the top of the overlying air-filled space. Results consistent with these expectations would indicate that gravity was a stronger control on gametophyte orientation than light direction. Exceptions to these expectations would be evidence against the null hypothesis, indicating that light direction exerts a stronger control on gametophyte orientation than does gravity.

Consequently, in the cases of dorsiventral gametophytes of *Blasia*, *Marchantia*, and *Ceratopteris*, assessment consisted of estimating the percentage of new-growth rhizoids extending into the air-filled container space versus agar substratum. In the case of axial gametophytes of *Sphagnum*, assessment consisted of making an estimated percentage of capitula that, during the treatment period, grew into the agar substratum versus extending into the air-filled container space. For *Coleochaete*, assessment consisted of making estimates of percentages of germling hair cells that extended into the liquid.

RESULTS

Fig. 1 depicts the experimental system used in this study to evaluate the relative effects of gravity and direction of illumination on the orientation of new gametophytic growth during the experimental period. For all taxa examined with the use of this experimental system, direction of illumination played a more important role in gametophytic body orientation than gravity. When illuminated only from below *Coleochaete* lost normal orientation, new growth of dorsiventral gametophytes of *Blasia*, *Marchantia*, and *Ceratopteris* twisted so that ventral surfaces bearing rhizoids faced the overlying air space, and axial gametophytes of *Sphagnum* moss burrowed into the substratum (Fig. 2a-o). None of these results would be expected unless light direction had a larger impact on gametophyte orientation than did gravity (Table 1). A hypothesis that gravity exerts more control than does direction of illumination over gametophyte orientation in seedless plants and *Coleochaete* is thus rejected.

Growth reorientation of all species having dorsivental gametophytes in response to illumination only from below, so that the ventral surfaces and rhizoid tips faced upward, was particularly dramatic evidence against a hypothesis that gravity was more important than light

direction in controlling body orientation. Comparison of expected with actual growth responses of the dorsiventral gametophytes *Blasia*, *Marchantia*, and *Ceratopteris* (see Table 1), indicates that providing illumination only from below (see Fig. 1) was critical to identifying the overriding environmental control factor—direction of illumination. This simple light-direction manipulation generated clear-cut results in all taxa investigated.

Table 1. Expected and Actual Responses of Dorsiventral Plant Gametophytes

	Initial Body Orientation	Expected Growth Response to Gravity	Expected Growth Response to Light Direction	Actual Growth Response
Direction of Illumination Treatments				
I. Illumination only from above	A. Dishes upside down; rhizoid tips extending upward	180° inversion of new growth with rhizoid tips extending downward	180° inversion of new growth with rhizoid tips extending downward	180° inversion of new growth with rhizoid tips extending downward
	B. Dishes right side up; rhizoid tips extending	No change in body orientation	No change in body orientation	No change in body orientation

	downward			
II. Illumination only from below	A. Dishes upside down; rhizoid tips extending upward	180° inversion of new growth with rhizoid tips extending downward	No change in body orientation	No change in body orientation
	B. Dishes right side up; rhizoid tips extending downward	No change in body orientation	180° inversion of new growth with rhizoid tips extending upward into the overlying air space	180° inversion of new growth with rhizoid tips extending upward into the overlying air space
III. Illumination from multiple directions	A. Dishes upside down; rhizoid tips extending upward	180° inversion of new growth with rhizoid tips extending downward	Growth in multiple orientations; rhizoid tips extending in multiple directions	Growth in multiple orientations; rhizoid tips extending in multiple directions
	B. Dishes right	No change in	Growth in	Growth in

	side up; rhizoid tips extending downward	body orientation	multiple orientations; rhizoid tips extending in multiple directions	multiple orientations; rhizoid tips extending in multiple directions

Coleochaete orbicularis- *Coleochaete orbicularis* displayed different morphology depending on the direction of illumination. In the 15 culture dishes illuminated only from above, 100% of thalli displayed the morphology typical of specimens collected from natural aquatic environments, namely monostromatic disks attached to surfaces, presence of hair cells extending upward into the overlying liquid medium (Fig. 2a). In the 15 culture dishes illuminated only from below and in the 15 culture dishes illuminated from all directions, 100% of thalli occurred as floating non-discoid aggregations of hairless cells (Fig. 2b-c).

***Blasia pusilla, Marchantia polymorpha*—**In all of the 12 culture dishes placed right side up and illuminated from above, 100% of the new growth of all gametophytes displayed normal dorsiventral orientation, with dorsal surfaces facing an air-filled space and rhizoid tips extending into the agarized medium (Fig. 2d). In all of the 14 plates of *Marchantia* placed right side up and illuminated from above 100% of new growth of all gametophytes displayed normal dorsiventral orientation, with dorsal surfaces facing an air-filled space and rhizoid tips extending into the agarized medium (Fig. 2g). New growth did not undergo change in body orientation. New growth of both liverworts displayed gemmae on the dorsal side, facing the source of

illumination. In all of the 11 culture dishes of *Blasia* and 12 *Marchantia* dishes that had initially been placed upside down, in 100% of gametophytes, new growth responded to change in illumination direction by twisting 180° to achieve normal orientation, with ventral surfaces facing downward and rhizoid tips extending downward into an air-filled space. When such dishes were inspected right side up, the gametophyte surfaces appeared fuzzy because all new growth had reoriented so that the rhizoid-rich ventral surface was conspicuous. However, such responses would be expected were either light orientation or gravity the major control on body orientation and thus did not discriminate between them (Table 1).

In contrast, when illuminated only from below, in all 12 culture dishes of *Blasia* and all 14 dishes of *Marchantia*, in 100% of gametophytes, new growth in right side up dishes had twisted 180°, so that the ventral surfaces faced upward and rhizoid tips extended into the air-filled space, away from the direction of illumination (see Table 1). In all 11 *Blasia* and 13 *Marchantia* dishes initially placed upside down and illuminated only from below, no growth reorientation was observed, but ventral surfaces of new growth of 100% of gametophytes were oriented toward the culture dish lid and rhizoid tips extended into the culture dish air space rather than into the agar substratum.

When illuminated from multiple directions, in all 12 dishes of *Blasia* and all 13 dishes of *Marchantia* placed right side up and in all 12 dishes of *Blasia* and all 13 dishes of *Marchantia* placed upside down, in 100% of gametophytes, new growth displayed abnormal growth orientation (Fig. 2c,f). Such responses were not predicted from a hypothesis that body orientation was controlled primarily by gravity, but were predicted from a hypothesis that illumination

direction was the major environmental factor controlling dorsiventral gametophyte orientation in *M. polymorpha* and *B. pusilla* (see Table 1, shaded cells).

Sphagnum compactum—In all 15 culture dishes placed right side up, and illuminated only from above, 100% of new growth was oriented with basal portions embedded in the agar substratum and terminal capitula facing the air-filled space (Fig. 2j). Branch fascicles were oriented downward, away from the source of illumination, as normally occurs in nature. However, in all 15 culture dishes placed right side up and illuminated only from below, at the end of the treatment period, 100% of terminal capitula had grown into the agar and fascicles were oriented upward, away from the source of illumination (Fig. 2k). In all 15 culture dishes placed right side up and in all 15 culture dishes placed upside down, and illuminated from multiple directions, at the end of the treatment period, 48.76% of terminal capitula had grown into the agar substratum and 51.24% into the air-filled space (Fig. 2l). These responses were consistent with a hypothesis that direction of illumination was more important than gravity in determining normal *S. compactum* axial body orientation.

In all 15 culture dishes placed upside down, and illuminated only from above, at the end of treatment period 100% of the new growth was oriented into the agar and toward the direction of light, while production of fascicles seemed to have been directed into the air-filled spaced away from the direction of light. However, in all 15 culture dishes that were oriented upside down and illuminated only from below, at the end of experimental treatment 100% of *S. compactum* displayed what would seem like normal morphology, just in inverse orientation. In the 15 cultures dishes oriented upside down and illuminated only from below, 100% of capitula grew

into the air filled spaced and in the direction of illumination, while branch fascicles occurred in contact with and in the direction as the agar medium surface.

***Ceratopteris richardii*—**In all of the 15 culture dishes placed right side up and illuminated only from above, at the end of the treatment period archegonia and antheridia had developed normally on 100% dorsal surfaces of new growth of *C. richardii* gametophytes and no rhizoids were observed to extend into the overlying air-filled space (Fig. 2m). In contrast, in all of the 15 dishes of *C. richardii* culture dishes placed right side up and illuminated only from below, at the end of the treatment period, in 100% of gametophytes the ventral surfaces of new growth faced upward and of rhizoids on those surfaces extended into air-filled space (Table 1) (Fig. 2n). In all 15 dishes placed right side up and all 15 dishes placed upside down that were illuminated from multiple directions, dorsal surfaces of 100% of new growth of *C. richardii* gametophytes were oriented in multiple directions (Fig. 2o). In the 15 petri dishes placed upside down, and illuminated only from below, at the end of treatment 100% of the new growth of *C. richardii* gametophytes occurred with dorsal sides towards the air-filled space, no rhizoids were seen to extend into the air-filled space; instead, rhizoid growth was directed toward the agar surface. However, in the 15 petri plates of *C. richardii* gametophytes were placed upside down, and illuminated only from above, 100% of the new growth occurred with the dorsal side towards the agar and rhizoids occurring into the air-filled space. These responses were consistent with a hypothesis that direction of illumination was more important than gravity in determining the normal spatial orientation of dorsivental *C. richardii* gametophytes (see Table 1).

DISCUSSION

The results reported here—that gametophytic body orientation of representative early-diverging seedless plant species is largely controlled by the direction of illumination—provides insight into the traits of earliest land plants and algal ancestors. While previous work had revealed negative phototropic growth of rhizoidal cells of *Marchantia polymorpha* and *Lunularia cruciata* (Dassek, 1939) and fern (Tsuboi et al., 2006) rhizoids, positive phototropic and gravitropic response of sporophytes of the liverwort *Pellia* (Thomas et al., 1987; Ellis and Thomas, 1985), and phototropic aspects of moss protonemata (Mittman et al., 2009; Jaedicke et al., 2012), this is the first report that larger-scale seedless plant gametophytic bodies display growth responses to changes in the direction of illumination. Identification of illumination direction as an important environmental factor in gametophyte development is important because correct spatial orientation is critical to the survival and reproduction of most seedless plants. The results of this study allow us to infer responses to changes in direction of illumination likely displayed by early liverwort- and moss-like land plants, as well as the evolutionary origin of those responses in ancestral green algae. The results of this study also suggest directions for future research into the early evolution of streptophyte light sensing and signal transduction systems, and foster teaching activities that utilize early-diverging plants.

Traits of Earliest Land Plants—Earliest land plants are inferred to have had a prostrate and relatively undifferentiated dorsiventral body, with uppermost photosynthetic and reproductive tissues and underlying ventral epidermis, many cells of which generate elongate rhizoids by tip growth, much like that of the modern thalloid liverwort *Blasia* (Crandall-Stotler et al., 2005). Photosynthetic and reproductive efficiency of earliest land plants thus depended, as do those of modern thalloid liverworts, on correct spatial body orientation. For the same reasons, the

dorsiventral gametophytes produced by many ferns, and the axial gametophytes of acrocarpous mosses must also maintain normal spatial orientation.

The uniformity of growth responses observed in an evolutionary spectrum of representative early-diverging plant species (liverworts *Blasia pusilla* and *Marchantia polymorpha*, moss *Sphagnum compactum*, and fern *Ceratopteris richardii*), namely that direction of illumination is more important than gravity in controlling gametophytic body orientation, indicates a fundamental, ancient plant trait likely characteristic of earliest land plants. Similar responses of the streptophyte alga *Coleochaete orbicularis* indicate that control of early plant gametophyte orientation by direction of incident light originated in ancestral green algae and was inherited by the earliest land plants. This hypothesis is supported by observations that branching patterns of some *Chara* species (though not other species) seem to be controlled by directional light (Schneider et al., 2006).

Control of gametophyte orientation by light direction would have allowed earliest plants to produce multicellular dorsiventral gametophytes with A) ventral rhizoids that function in attachment to the substratum and B) dorsal reproductive structures, from unicellular spores whether the substrate was horizontal, angled, or vertical. If gravity had been the controlling factor in determining gametophytic orientation, gametophytes occurring on vertical surfaces might be expected to develop different from those occurring on horizontal surfaces. Modern free-living dorsiventral plant gametophytes are not known to display structural variations correlating with position with respect to the gravity vector, as would be expected were gravity the main controlling factor. For example, diverse modern liverworts produce dorsiventral gametophytes that display the same morphology whether they grow on vertical or horizontal surfaces.

Control of gametophyte orientation by illumination direction also allows gametophytes that become spatially disoriented by wind or water to reorient by means of new growth. Such responses would have been particularly useful during the Cambrian-Ordovician time period when climatic conditions were favorable for plant growth (Graham et al., 2013), but unconsolidated, disturbance-prone sandy substrata were common (Dott, 2003; Avigad et al., 2005). This Cambrian-Ordovician time period is associated with the occurrence of microfossils resembling the terrestrial growth form of the modern streptophyte alga *Coleochaete* (Graham et al., 2012), as well as the origin and early diversification of land plants (reviewed by Graham et al., 2013). The results presented in this report indicate that early terrestrial streptophytes probably possessed the ability to respond to physical disorientation by reorienting new growth to re-establish substratum association of ventral rhizoids and production of dorsal reproductive structures in a position most favorable for wind dispersal of spores and gemmae. Mechanisms for reorientation would seem essential to the survival of early streptophytes, as well as modern plants that produce free-living, surface-growing, photosynthetic gametophytes. Similar growth responses to direction of illumination by axial gametophytes of *Sphagnum compactum* suggest that the earliest land plants having axial gametophytes, illustrated by modern acrocarpous mosses, inherited systems for detecting and responding to light direction from ancestral liverwort-like land plants.

Evolutionary Origin of Plant Light-sensing and Response Systems—The results observed in this study indicate that the species investigated possess light-sensing systems and signal transduction processes allowing the translation of variations in the direction of illumination into growth responses, and that responses are initiated by activity at the apical meristem during the generation of new tissues. Although some experts might regard the responses observed here to

represent photomorphogenesis, Matsuoka et al.'s (2007) definition of phototropism as "a mechanism to orient a whole plant to light," suggests instead that responses observed in the present study could also be interpreted as phototropism.

Phototropism in higher plants is widely regarded to be stimulated by blue light detected by the sensor phototropin, whereas phototropic responses of bryophytes and ferns have been thought to be mediated by red/far red signals detected by phytochrome. However, interactions between phototropin and the red/far red sensor phytochrome have recently been demonstrated to occur in higher plants (Lariguet et al., 2006). Consistent with this latter observation, Jaedicke et al. (2012) found that phytochrome and at least some molecules of phytochrome associate at the plasma membrane in cells of *Physcomitrella* protonemata and *Arabidopsis* (though Sakamoto and Briggs [2002] demonstrated that *Arabidopsis* phototropin is highly hydrophilic and thus not itself an integral membrane protein). Such light sensor associations may explain the intriguing evolution, on at least two independent occasions, of the chimeric red and blue light sensor neochrome, which mediates phototropic responses of fern gametophytes and the streptophyte alga *Mougeotia scalaris* (Swetsugu et al. 2005; reviewed by Wada, 2007). Evidence for growth direction responses to changes in red and far red light treatments has been observed in *Marchantia polymorpha*, suggesting the presence of phytochrome in this species (Fredericq and De Greef, 1968). However, substantial genetic evidence for presence of phototropin, phytochrome, or neochrome in the earliest-diverging streptophyte species evaluated in the present study (*Coleochaete orbicularis*, *Blasia pusilla*, *Marchantia polymorpha*, *Sphagnum compactum*) has yet to be established, but could be forthcoming as whole genomes become more available.

In *Arabidopsis*, the perception of blue light by phototropin is thought to cause changes in the location of the auxin efflux carrier PIN3 within hypocotyl endodermal cells, which results in changes in the distribution of auxin and consequent phototropic growth (Ding et al., 2011). Evidence for the occurrence of auxin regulation in four liverworts and the streptophyte alga *Nitella* (Sztein et al. 1999) suggests that part or all of a similar response pathway might have originated in streptophyte algae and have been inherited by earliest land plants, and these possibilities can be examined as genome sequences become available.

Teaching and Outreach Utility—The results reported here show that structurally normal teaching materials of *Coleochaete orbicularis* and gametophytes of *Blasia pusilla*, *Marchantia polymorpha*, and *Ceratopteris richardii* can be obtained by growing materials with illumination only from above, consistent with the natural occurrence of these organisms on opaque soil or other substrata. Growing such biological materials in transparent dishes that receive light from multiple directions is likely to produce structurally atypical results. On the other hand, applications that require unattached, suspended populations of *Coleochaete*, for example, could be achieved by providing the algae with light from multiple directions.

Finally, the simplicity and clear-cut results demonstrated by this investigation suggest that it could form the basis for early experiences in experimentation for people lacking much previous experience with plants or statistical analysis. Plexiglass supports, transparent culture dishes, and commercially available spores of *C. richardii* that can be used to easily generate cultures of fern gametophytes are all readily available to biologists interested in demonstrating the dramatic effects of variation in light direction on plant structure, which might be presented as plant acrobatics.

ACKNOWLEDGEMENTS

Sarah Friedrich assisted in production of figures.

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Figure 1. Experimental system allowing treatment with light only from above (left), only from below (center), and from multiple directions (right).

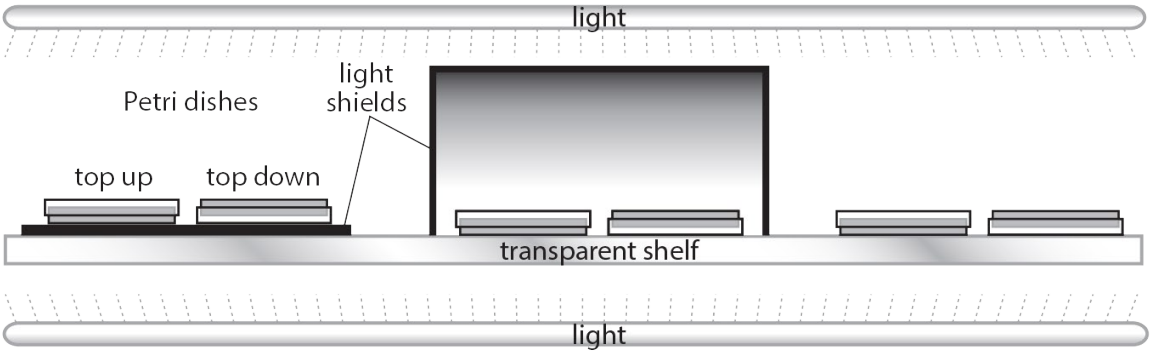
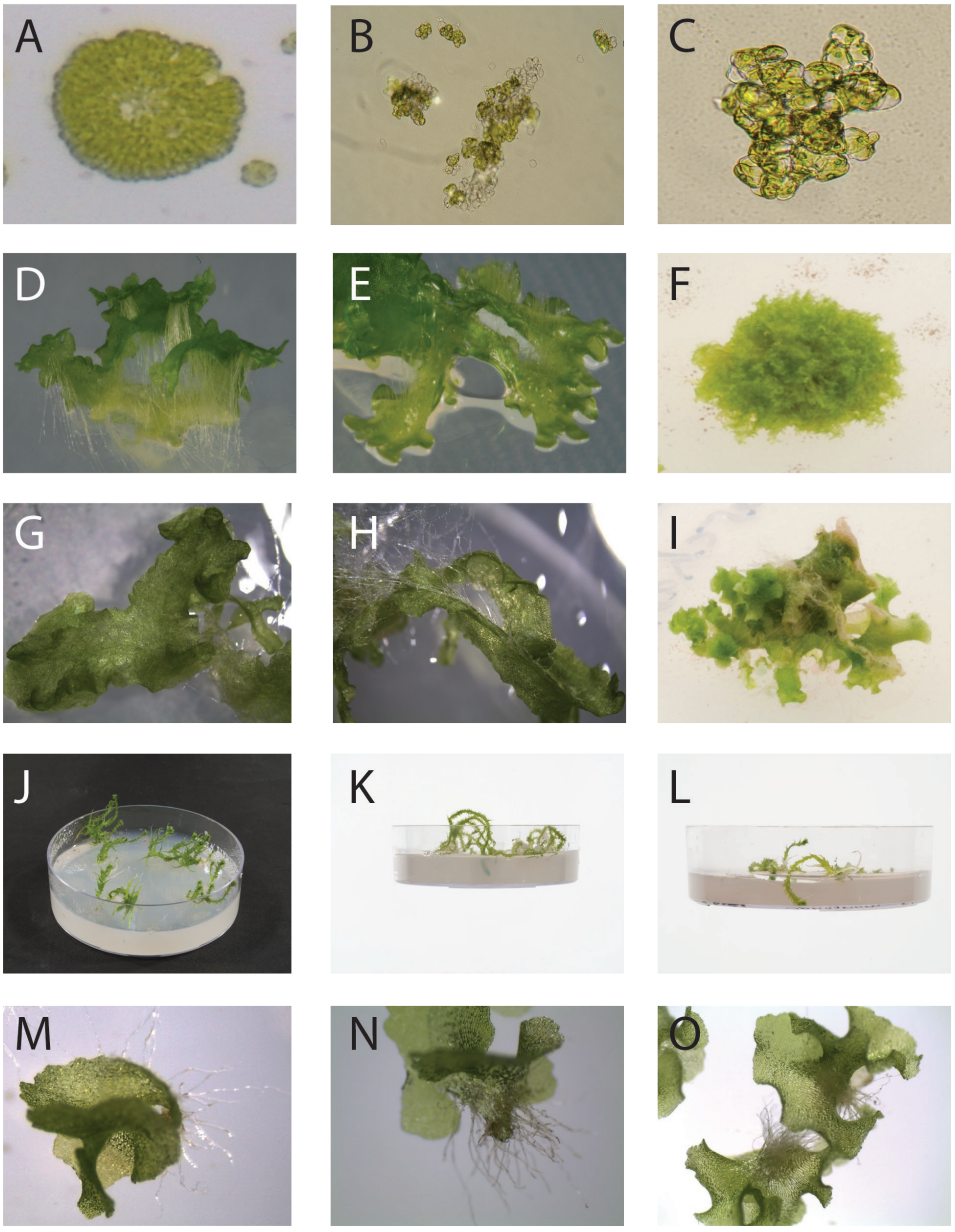


Figure 2. Representative images showing growth responses of gametophytes of five seedless streptophyte species to experimental variation in direction of illumination. A-C *Coleochaete orbicularis*, D-F *Blasia pusilla*, G-I *Marchantia polymorpha*, J-L *Sphagnum compactum*, M-O *Ceratopteri richardii*. Illumination only from above: A, D, G, J, M. Illumination only from below: B, E, H, K, N. Illumination from multiple directions: C, F, I, L, O.

Fig. 2



Chapter 3. *Sphagnum*-like Fossils from the Ordovician

ABSTRACT

- *Premise of study:* Modern *Sphagnum*-dominated peatlands store a large proportion of global soil carbon as peat, such long-term sequestration fostering global climate homeostasis. *Sphagnum* peat mosses also harbor microbial methanotrophs and nitrogen fixers that likewise play globally important roles in Earth's biogeochemical cycles. Although fossil fragments of *Sphagnum*-like peatmosses are known from the Permian (Neuberg, 1956, 1960), recent molecular clock analyses suggest that peat mosses may be considerably older. Because certain Caradocian microfossils were interpreted by Kroken et al. (1996) as the resistant remains of sporophytic capsule epidermis of *Sphagnum*-like early mosses, we examined well-understood shallow marine mid-Ordovician dolomites (Sinnipee Group), located in Dane Co., WI, and dated at 455-460 Mya, for evidence of peatmosses.
- *Methods:* From two rock collections made and processed 12 years apart, using methods designed to avoid contamination by modern materials, we isolated organic microfossils and used fluorescence and other microscopic methods to perform comparative studies.
- *Key Results:* We found numerous *Sphagnum*-like leaf and stem fragments having autofluorescence properties indicating high resistance to degradation, explaining persistence of organic remains under suboptimal preservational conditions. Morphometric analysis revealed no significant differences between microfossils obtained 12 years apart.
- *Conclusions:* Discovery of fragmentary Ordovician *Sphagnum*-like leaf and stem remains

indicates that peatlands were likely present on Earth at least 150 million years earlier than previously thought, consistent with molecular clock and other fossil evidence. Given the global biogeochemical importance of modern *Sphagnum* and associated microbes, this discovery improves knowledge of Ordovician terrestrial biota, and illuminates ancient plant-atmosphere-climate interactions.

INTRODUCTION

Molecular clock and microfossil evidence suggest that crown embryophytes diverged from algal ancestors between 568-815 mya (Zimmer et al., 2007; Clarke et al., 2011), and perhaps as long ago as ~916 mya (Magallón et al., 2013). A diversity of microfossil evidence demonstrates that early bryophyte-like plants were well established on land by the early mid-Ordovician (Rubenstein et al., 2010, Wellman et al., 2003, Strother et al., 2011), as originally suggested by Jane Grey in a now-classic 1985 article. Phylogenomic evidence indicates that modern bryophyte groups—liverworts, mosses, and hornworts—are the earliest-diverging modern land plants (Qiu et al., 2006). Molecular phylogenetic evidence indicates that *Sphagnum* peat mosses are the earliest-diverging lineage of modern mosses and molecular clock data suggest that the peat moss lineage is probably hundreds of millions of years old (Shaw et al., 2010, Magallón et al., 2013).

Determining the time when peat mosses first appeared and became abundant is important because modern *Sphagnum*-dominated peatlands play globally significant biogeochemical roles. Peatlands sequester large amounts of carbon for long periods (Gorham, 1991; Crum and Anderson, 1981), and peatmosses harbor microbial nitrogen-fixers and methanotrophs that also play global biogeochemical roles (Dedysh et al., 2001). Evidence for the existence of *Sphagnum* mosses in ancient deposits would indicate similar biogeochemical impacts. Certain middle

Ordovician (Caradocian, 448-468 mya) tissue-like microfossils (Gray et al., 1982) were interpreted as the resistant remains of sporophytic capsule epidermis of *Sphagnum*-like early mosses (Kroken et al., 1996). This evidence, coupled with carbon sequestration models based on presence of relatively high levels of hydrolysis-resistant carbon in early-diverging mosses, suggests that mosses began to play important roles in Earth's biogeochemical cycles well before the rise of vascular land plants (Graham, et al., 2004; Graham et al. 2010). Even so, fossil remains identifiable as *Sphagnum*-like leaves on the basis of distinctive cellular dimorphism, or stems having distinctive pith and cortical cells with spiral ornamentation, have not previously been recognized from deposits as old as the Ordovician.

On the basis of leaf remains, representatives of the extant order Sphagnales (*Sphagnophyllites triassicus*) were described from Triassic deposits of India (Pant and Basu, 1976), and modern *Sphagnum* spore remains were reported from the Jurassic (Oostendorp, 1987). Older fossils linked with the ancestry of modern Sphagnum include *Jungagia* (Early Permian) (Oostendorp, 1987), *Protosphagnum* described from costate leaf remains extracted from Lower Permian deposits (Neuberg, 1956, 1960), and *Vorcutannularia* (Early and Late Permian). These Permian remains have heretofore been regarded as the earliest fossil evidence for peat mosses, suggesting that modern peatland biogeochemical functions were not present during earlier times.

On the other hand, previous studies of well-understood Ordovician carbonate deposits in southern Wisconsin (dated at 460 mya) had revealed evidence of earliest known fossil glomalean fungi (Redecker et al., 2000). Because modern glomalean fungi are obligate associates of plants, including a variety of bryophytes, the Ordovician glomalean fossils suggest that early bryophyte-like land plants likely occurred in the same habitat. The description of *Sphagnum*-like fossils

from Ordovician deposits near those in which glomalean fungi occurred would support a hypothesis that peat mosses were present and exerted global biogeochemical impacts much earlier than previously thought. In this chapter we describe the discovery of *Sphagnum*-like fossils from Wisconsin Ordovician deposits and discuss their paleoecophysiological significance.

MATERIALS AND METHODS

***Sample Collection*—**In 2000 and 2012, rock samples were collected by different teams from outcrops near Country Highway P, Dane County, Wisconsin, USA, +43° 2' 44.46", -89° 39' 25.89", a dolomite deposit belonging to the Sinnipee Group composed of the Platteville, Decorah (including the Guttenberg Limestone) (Ludvigson et al., 1996, Stueber and Walter 1994, Watso and deV Klein 1989, Kolata et al. 1987, Kolata 1986, Kolata and Jollie 1982), and Galena Formations (Deininger 1964, Byers and Stasko 1978).

Stratigraphic Information—The sampled units are Ordovician in age (Mohawkian to Cincinnatian Series). All three formations are largely carbonate with interbedded shale seams. Typically shale composes less than 10% of the stratigraphic thickness. The carbonates have been variably dolomitized. Bands of nodular chert are present at some horizons. The chert is a replacement of the original carbonate lithology. Thin (mm to cm) beds of bentonite (altered volcanic ash) are present in the Sinnipee Group and have been traced laterally for large distances, demonstrating that the carbonate formations were deposited slowly, over millions of years, within an extensive epicontinental sea.

The rock is commonly blue-gray on a fresh surface and tan on a weathered surface. Bedding is wavy to nodular in outcrop, with beds ranging from centimeters to decimeters in thickness. Shale seams weather back to form distinct re-entrants. The dominant carbonate lithology is a

fossiliferous wackestone; burrows are abundant and the rock fabric is thoroughly bioturbated. The trace fossil genera *Chondrites* and *Thalassinoides* are abundant. Thin layers of fossil grainstone (centimeter-scale) punctuate the wackestones. The grainstones show scoured bases and typically are graded and/or cross-laminated, and the fossils are disarticulated and broken. Brachiopods, bryozoans, and crinoid debris dominate the macrofauna, but corals, cephalopods, bivalves, gastropods, ostracodes, and trilobites are present as well. Hardground surfaces produced by submarine cementation are common in the Sinnipee Group. They tend to be scoured to a planar surface, bored by marine organisms, and mineralized by pyrite. Locally the hardgrounds preserve remnants of an encrusting community. UW-Madison Professor Emeritus Charles Byers generated the stratigraphic description provided above, at our request.

Sample Processing—Thirty-six replicate rock samples of various sizes were obtained with the use of a geological hammer and transported to the lab in new gallon-size zip-lock plastic bags. Laboratory processing methods were developed in collaboration with the late paleontologist Jane Gray, an authority on Ordovician microfossil evidence for early land plants and microorganisms (e.g. Graham and Gray, 2001). The outer weathered rock layer was removed by chemical dissolution, exposing inner lighter colored material.

Rock samples were processed similarly during 2000 and 2012. Rocks were first rinsed with distilled water, and then within a chemical hood rocks were submerged in concentrated HCl to solubilize the outermost layer of carbonate, which was discarded to eliminate surface contamination. The remaining rock material was solubilized in new concentrated HCl, within a chemical hood, and later neutralized using distilled water until it reached a pH of 5-6. In 2000, sedimentary remains were surveyed using a stereomicroscope, and pipettes were used to isolate

organic remains. In 2012, sedimentary remains were first partitioned using a 90µm brass sieve before discarding flow through and isolating organic microfossils with the use of a stereomicroscope and pipettes. Six rocks of about one kilogram were dissolved and of these, two yielded nine microfossils similar to modern *Sphagnum* leaves.

Water mounts of organic remains were imaged with a Zeiss Axioplan fluorescence microscope (Carl Zeiss Microscopy, Germany). Digital photographs were made with the use of Nikon D300s Digital Camera and Camera Control Pro software (Nikon Inc., Melville, New York, USA). Five measurements each of hyaline cell length and width, pore number and width, and ring number were obtained for three leaf microfossil fragments collected in 2000 and three leaf microfossil fragments collected in 2012. The open source image analysis program GIMP (GNU Image Manipulator Program; www.gimp.org) was used to make these measurements from digital images of fossils. Permanent slides of microfossils were prepared using Permount Mounting Medium (Fisher Scientific, Fair Lawn, New Jersey, USA) for archiving in the UW-Madison Geology Museum.

To test the possibility that sedimentary extracts might have become contaminated with modern materials, replicate glass slides bearing drops of immersion oil were placed within the chemical hood and left for periods ranging up to 3 months, then examined microscopically for presence of cellular scraps similar to microfossils.

RESULTS

Organic microfossils isolated from dolomitic rocks confidently dated to 455-460 Mya resembled fragments of modern *Sphagnum* leaves and stems. Fossil fragments resembled

modern peat moss leaves in that cells of two structural types occurred in characteristic polygonal arrays, and the larger cell type, corresponding to hyaline cells of modern species, possessed distinctive wall pores and rings. Fossil fragments resembled modern peat moss stems in having distinctive pith with surrounding cortical cells. Morphometric features of hyaline cell features in leaf fragments found in collections made 12 years apart were not significantly different (Table 1). Microfossils displayed distinctive fluorescence features, as well as intracellular sedimentary intrusions expected to be present in fossil remains, but not modern materials.

Leaf structure—Fossil fragments interpreted as leaves were composed of two types of cells having sizes, shapes, and patterning similar to hyaline and chlorophyllose cells of modern *Sphagnum*. When describing the fossil leaf fragments, we refer to larger cells that resemble hyaline cells of modern *Sphagnum* as hyaline cells, and refer to adjacent narrower cells as chlorophyllose cells even though the fossils lack any evidence of chlorophyll. Hyaline cells of fossil leaf fragments displayed cell wall pores and rings similar to those of modern *Sphagnum*. Examples of hyaline cell rings and pores of fossil leaf fragments are shown in Figs. 1B-E.

Stem structure— Fossils found in 2000 include a portion of a stem with attached leaf bases (Fig. 1A). The stem appears to have a darkened center composed of smaller cells, interpreted as pith, surrounded by larger translucent cells, interpreted as cortical cells, whose walls display conspicuous spiral markings (Fig 1A-1C). A leaf segment attached to the stem was described, with a visible dark center mostly composed of what appear to be smaller cells and surrounded by larger translucent cells (Fig. 2D). A stem segment was

described as having a darkened center composed of small cells, surrounded by bigger translucent cells with spiral markings (Fig. 2E). The darkened small cells that are interpreted as being part of the pith are golden brown in color (Fig. 2D-2E). The surrounding translucent cells could be interpreted as part of the cortex, while the conspicuous spiral markings could be part of supportive fibrils.

Morphometric comparisons of leaf-like fragments made 12 years apart—The average length of hyaline cells for fossils collected in the year 2000 was 212±5.36 μm and the average width was 45.5±2.43 μm (Table 1), while the average length of hyaline cells for leaf fragments collected in 2012 was 220±8.50 μm and the average width was 52.5±2.33 μm (Table 1). The number of pores present in hyaline cells was variable: for the year 2000 this number averaged 4.50±0.53, and average pore width was 12.8±2.47 μm (Table 1) and for 2012 the average pore number was 3.27±0.64 and average pore width was 11.40±1.70 μm (Table 1). The average number of cell wall rings per hyaline cell for the 2000 sample was 9.59±1.54 (Table 1), while the 2012 sample had an average number of characteristic hyaline cell rings was 11.9±0.90 (Table 1).

Statistical analysis determined that there is no significant difference in hyaline cell length, hyaline cell width, hyaline cell ring number, hyaline cell pore number and hyaline cell pore width of fossils collected in the year 2000 and those collected in 2012 (Table 1). However, it is important to note that relatively small numbers of measurements were analyzed, and that this small number might impact results of the statistical analysis.

Autofluorescence— Fluorescence microscopy shows cell wall autofluorescence, blue-

white (Fig. 3A-3E) under UV excitation and yellow-green (Fig. 3F-3J) when irradiated with violet excitation (Fig. 3A-3J). Red chlorophyll autofluorescence was not observed in any of the remains.

Evidence for ancient origin—The similarity in structure of the Ordovician microfossils to portions of leaves and stems of modern peat mosses raises the issue of possible contamination of sedimentary extracts by modern materials. In both the 2000 samples and the 2012 samples, sediments belonging to the rock matrix can be found in the lumen of chlorophyllous and hyaline cells (Fig. 2A-2C), indicating intrusions to the internal lumen by external sediments (Fig. 2A-2C). Furthermore, *Sphagnum* fragments were not found in water used for neutralizing acid, eliminating lab water as a potential source of contamination. Oil traps located in the fume hood in which rocks were dissolved yielded no *Sphagnum* remains, which eliminates the fume hood as a potential source of contamination.

DISCUSSION

This study provides evidence for the presence of *Sphagnum*-like mosses in the Ordovician, more than 150 million years earlier than previously known. Qualitative and quantitative data indicate that fossil leaf and stem fragments isolated in 2000 and 2012, from rocks collected from the same location, originated from the same or a closely related species, though the remains were too fragmentary to allow comparison to modern *Sphagnum* species. Here, we: 1) compare the newly discovered Ordovician fossil fragments to later remains that have been linked with the ancestry of modern *Sphagnum*; 2) summarize the evidence that the remains represent ancient, rather than modern materials; 3) explain how

the new fossils improve understanding of Ordovician landscapes and biogeochemistry, and 4) discuss the utility of the new fossils in calibrating molecular clocks.

Comparison of the newly discovered Ordovician peat moss fossils with later *Sphagnum*-like materials—Triassic *Sphagnophyllites* a *Sphagnum*-like fossil from India, has been attributed to the Order Sphagnales, which contains the modern genus *Sphagnum*. *Sphagnophyllites* fossils consist of acostate oblong leaves, with brown cells forming meshes around hyaline regions. Brown mesh-like cell groups contain intracellular dark brown oval bodies that have been interpreted as chloroplast remains. Pant and Basu (1976) interpreted the brown mesh cells as chlorophyllous cells based on the presence of putative chloroplasts in the brown mesh cells, their location adjacent to hyaline regions, and absence of cross walls and occasional oblique partitioning, these features being reminiscent of chlorophyllous cells of modern *Sphagnum*. These authors also interpreted hyaline areas bound by brown mesh cells as hyaline cells because of their location and the presence of simple septa and thickening of cell walls, features reminiscent of hyaline cells of modern *Sphagnum*. Pant and Basu (1976) also noted that *Sphagnophyllites triassicus* leaves are similar to modern *Sphagnum* in that leaves have raised and depressed areas, and have features interpreted as remains of chlorophyllous and hyaline cells. Pant and Basu (1976) determined that *Sphagnophyllites* was different from other acostate Bryales (e.g. *Schistostega*, *Ephemerum* and *Hedwigia*) in that *Sphagnophyllites* shows chlorophyllous and hyaline cell dimorphism absent from leaves of other Bryales. These authors also stated the *Sphagnophyllites* was different from Permian fossils described by Neuberg (1960), which feature chlorophyllous and hyaline cells in addition to a central leaf costa that is missing in

Sphagnophyllites.

Neuberg (1960) described the order Protosphagnales from Permian deposits in Russia; this order contains the genera *Protosphagnum*, *Intia*, *Kosjunia*, *Vorcutannularia* and *Jungagia*. The exact features that were used to distinguish these Permian genera and species are in Russian with no accurate translation having been published to date. Based on Maslova et al. (2012), it was possible to determine that all fossil genera attributed to the order Protosphagnales contain a central leaf costae as well as chlorophyllous and hyaline cell dimorphism, even though the morphology of hyaline cells were different than those found in modern *Sphagnum*. Maslova et al. (2012) did not attempt holotype description, but instead attempted to infer stages of protosphagnalean leaf development, reaching the conclusion that leaf development differed among genera. Results reported by Maslova et al. (2012) suggested that small cells indicating early stages of development were located at the (1) top of the leaf in *Intia*, *Jungagia* and *Protosphagnum*, the (2) middle of the leaf in *Intia*, *Jungagia*, *Kosjunia* and *Vorcutannularia* and in the (3) basal part of the leaf in *Intia*, *Jungagia*, *Kosjunia* and *Vorcutannularia*.

The presence of cell dimorphism allows us to classify the Ordovician specimens described here into the order Sphagnales. If the interpretation that fossils described in 2000 (Fig. 1A, 1C) and 2012 (Fig. 2D) represent basal portions of leaves where they attach to the stem, then it is possible to infer absence of central leaf costae similar to those previously described for the Permian fossil *Protosphagnum nervatum* (Neuberg, 1960). Absence of leaf costae indicates that our fossils are not members of the Protosphagnales.

The *Sphagnum*-like fossil specimens described here lack characteristic netted brown cells that surround hyaline cells on previously described for the Triassic fossil *Sphagnophyllites triassicus* (Pant and Basu, 1976), suggesting the *Sphagnum*-like fossils described here cannot be classified with the genus *Sphagnophyllites*, but are likewise members of the Sphagnales. The new fossils indicate that the Sphagnales is older than previously recognized. These findings are consistent with the occurrence of Caradocian cell sheet microfossil remains originally described by Gray et al. (1982) that were later interpreted as remains of highly resistant *Sphagnum* sporangial epidermis (Kroken et al. 1996).

Although remains attributed to *Funaria* like moss (*Parafunaria*) have been described in Early-Middle Cambrian sediments of China (Yang et al., 2004), the low-resolution images provided in that publication do not allow detailed analysis that would be necessary for unequivocal classification. The Ordovician specimens described here represent the best and oldest known evidence for the existence of mosses in early Paleozoic sediments, and the earliest known evidence of peat mosses.

Evidence that the remains represent ancient, rather than modern materials—The collection protocol used in this study was the same as used and described in Redecker et al. (2000), which featured Ordovician *Glomales*-like fungal remains. The careful collection and extraction protocols were designed under the advice of Jane Gray (who had previously worked on Ordovician deposits), to lower the risk of modern and external contamination. The process of washing rocks with distilled water and use of HCl to solubilize outermost carbonate rock layers eliminates all modern materials. Furthermore, the lack of

contaminants in the distilled water used for neutralizing acids and in the fume hood where rocks were dissolved indicate that the Ordovician *Sphagnum*-like fossils described here did not originate from modern sources. The Ordovician *Sphagnum*-like fossils lacked red chlorophyll autofluorescence, which is indicative of dead plant material, since all living photosynthetic organisms possess autofluorescent chlorophyll. The presence of sediment intrusions in the cell lumens (see Fig. 2A-2C), indicate that the material had suffered cell wall tears that allow contact with the external environment. The sediment intrusions in the cellular lumens also indicate that the material was trapped or stored in a sediment matrix, which is indicative of fossil material.

How the new fossils improve understanding of Ordovician landscapes and biogeochemistry—The *Sphagnum*-like fossils here described add a new depth to Pre-Wenlock (Late Silurian) plant history that until now consisted mostly of cryptospores in the form of monads, dyads, and tetrads that occur either naked or enclosed within an envelope that is absent in modern plant lineages (Wellman 2010). Early Silurian and Late Ordovician deposits contain ornamented hilate and trilete cryptospores of uncertain taxonomic affinity, since ornamented hilate spores and trilete-marked spores are produced by modern vascular plants but also a few modern bryophytes (Steemans et al., 2009). Wellman et al. (2003) described microscopic cryptospores from Mid-Ordovician (475 mya) deposits that seemed to be produced in large numbers within a sporangial covering, of putative liverwort affinity. Rubinstein et al. (2010) reported assemblages of monad and tetrad cryptospores from early Middle Ordovician deposits (473-471 mya). However, Caradocian (475-460 mya) (Late Ordovician) cryptospores in adherent tetrads have been

interpreted as possible remains of modern bryophytes (Gray et al., 1982) and the same authors described a sheet of cells from the same deposits that was later interpreted as *s*porangial epidermis of a *Sphagnum*-like moss, based on similarity of distinctive features to remains of acetolyzed modern *Sphagnum* sporangia (Kroken et al., 1996).

Despite the cryptospore record, the time at which earliest mosses first appeared has been controversial (Graham et al., 2013). Some molecular clock data suggest that modern *Sphagnum* diverged from the most recent common ancestor between 7 mya to 10 mya, and divergence of Sphagnopsida from an ancestor shared with Takakiopsida between 128 mya and 319 mya (Shaw et al., 2010), but a recent molecular clock study by Magallón et al. (2013) suggests that the modern *Sphagnum* lineage diverged from other mosses much earlier, between 251 mya and 444 mya (as extrapolated from Fig. 1 in Magallón et al (2013), the value reported for the divergence of the *Sphagnum* lineage is 351.7 mya). The *Sphagnum*-like microfossil evidence described in the present report indicates that peat mosses were present by the mid-Ordovician and thus have a longer geological history than has previous been realized. This inference suggests that peat mosses may have played important global biogeochemical roles for much of the past 460 million years, particularly in times when climatic conditions were favorable to their growth.

The Ordovician (490-443 mya), for example, was a tectonically and climatic stable period. During Ordovician times, the site from which our *Sphagnum*-like fossil was described was located in the paleocontinent of Laurentia (named after the Laurentian shield that occupies most of modern day Canada) (Cocks and Torsvik, 2011). At that time Laurentia was covered by shallow epicontinental seas and surrounded by the Iapetus Ocean and the

Panthalassic Ocean (Cocks and Torsvick, 2011; Jin et al., 2013). Cocks and Torsvik (2011) opine that during the Ordovician period Laurentia remained in an equatorial position and that no glacial intervals occurred until the late-Ordovician (Hirnantian) glaciations. Figure 1 of Jin et al. (2013) and Figure 10 of Cocks and Torsvick (2011) suggest that the site from which the newly discovered *Sphagnum*-like remains were obtained was located approximately 10˚ south of the equator. It has been suggested that in the pre-Hirnantian Late Ordovician, the presence of permanent polar icecaps generated a temperature gradient between polar and tropical regions similar to that of modern times (Jin et al., 2013). $\partial^{18}O$ apatite results published by Trotter et al. (2008) indicate that Mid to Late Ordovician sea surface temperature was ~28˚C, similar to modern equatorial sea surface temperatures. These climatic conditions seem optimal for the growth and spread of early land plants, including *Sphagnum*-like mosses. It is possible, given strong carbon sequestration potential (Graham et al., 2004), that if Mid-Late Ordovician mosses were sufficiently widespread and abundant, they might have played a role in the onset of the Hirnantian glaciations.

If ancient relatives displayed the wide physiological tolerances displayed by the modern genus *Sphagnum*, they are likely to have persisted through end-Ordovician glaciated periods and other times of extreme environmental conditions into milder conditions, such as those prevailing during the Silurian-Carboniferous. Such a scenario would help to explain the occurrences of *Sphagnum*-like fossils from Permian-present times and predict that additional deposits bearing peat moss remains will be discovered.

The Ordovician *Sphagnum*-like fossils here described are also potentially important in

calibrating molecular clocks and determining fossil constraints. If the classification of the Ordovician *Sphagnum*-like fossils into Sphagnales is correct, the calibration date for first mosses moves from the Triassic to the Ordovician. Furthermore, the classification of Ordovician *Sphagnum*-like fossils as Sphagnales is a more relevant calibration tool because the commonly-used Permian-aged *Protosphagnum* belongs to the order Protosphagnales.

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FIGURES

Figure 1. *Sphagnum*-like leaf and stem fragment fossils collected in 2000. (A) Stem portion with two darkened regions interpreted as leaf bases; (B) Stem portion with leaf fragment attached; (C) Stem portion with darkened center, interpreted as pith, and larger, translucent cells in the margin, interpreted as cortex, with leaf base attached; (D) Leaf base attached to stem, with conspicuous hyaline cells, hyaline cell rings and pores; (E) Stem portion with leaf fragments, hyaline cells and chlorophyllous cells discernible. Two leaf bases are apparent. Scale bars = 100μm

Figure 2. *Sphagnum*-like leaf and stem fragment fossils collected in 2012. (A) Leaf fragment with chlorophyllous cells and hyaline cells with conspicuous rings and pores and sediment intrusions; (B) Leaf fragment showing hyaline cells, hyaline cell rings and pores; (C) Leaf portion with hyaline and chlorophyllous cells with sediment intrusions; (D) Leaf portion attached to a stem, composed of a darkened center interpreted as pith surrounded by larger translucent cells with spirals interpreted as cortex with fibrils; (E) Stem portion with darkened center interpreted as pith covered by translucent cells interpreted as cortex containing spiral markings interpreted as fribrils. Scale bars = 100 μm

Figure 3. *Sphagnum*-like fossil leaf and stem fragment epifluorescence. (A-E) Leaf and stem blue-white epifluorescence in UV excitation. (F-J) Leaf and stem yellow-green epifluorescence in violet excitation. Scale bars = 100 μm

Table 1. Comparative measurements of hyaline cell features of *Sphagnum*-like leaf fragment microfossils collected in 2000 versus 2012.

	Year (Mean±SE)				
	2000	2012	*t*	df	P-value
Hyaline cell length (µm)	212±5.36	220±8.50	-1.09	5	0.391
Hyaline cell width (µm)	45.5±2.43	52.5±2.33	-1.49	5	0.275
Number of hyaline cell rings	9.59±1.54	11.9±0.90	-1.46	5	0.281
Number of hyaline cell pores	4.50±0.53	3.27±0.64	1.82	5	0.210
Hyaline cell pore width (µm)	12.8±2.47	11.40±1.70	0.40	5	0.727

Figure 1. *Sphagnum*-like leaf and stem fragment fossils collected in 2000.

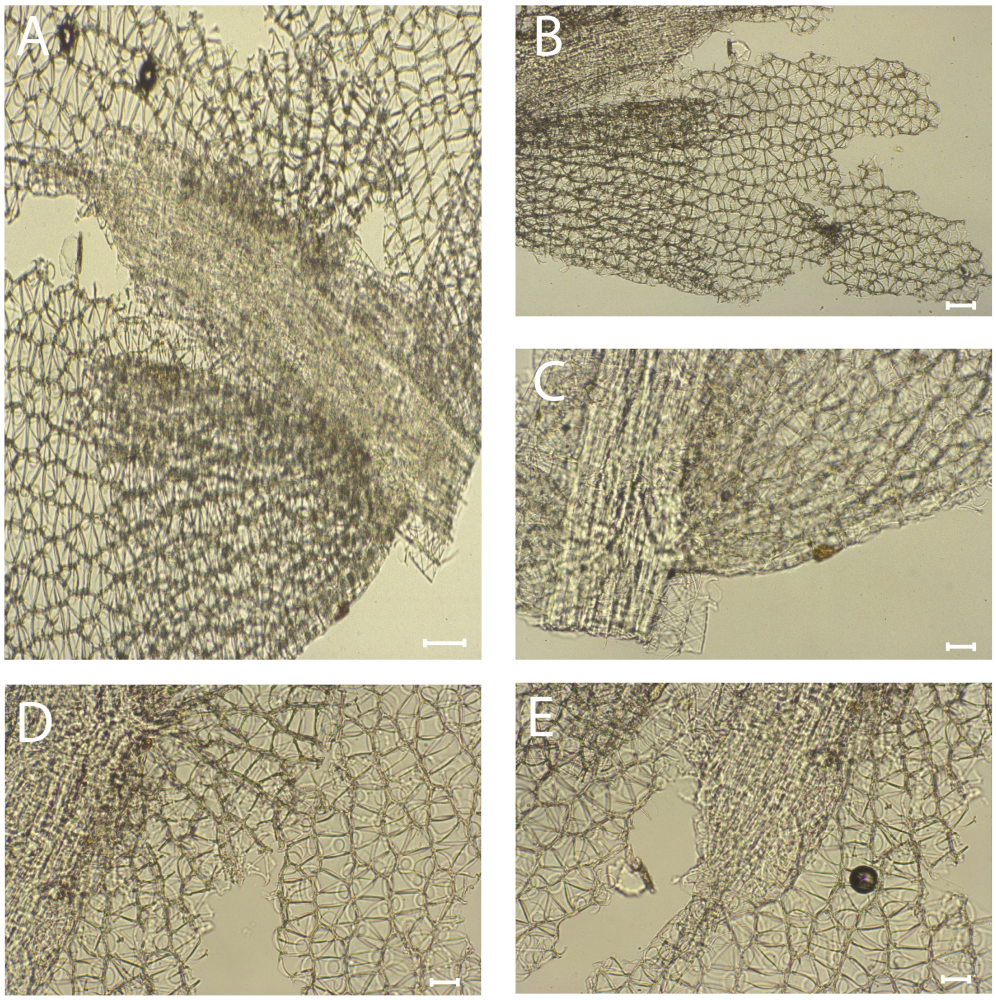


Figure 2. *Sphagnum*-like leaf and stem fragment fossils collected in 2012.

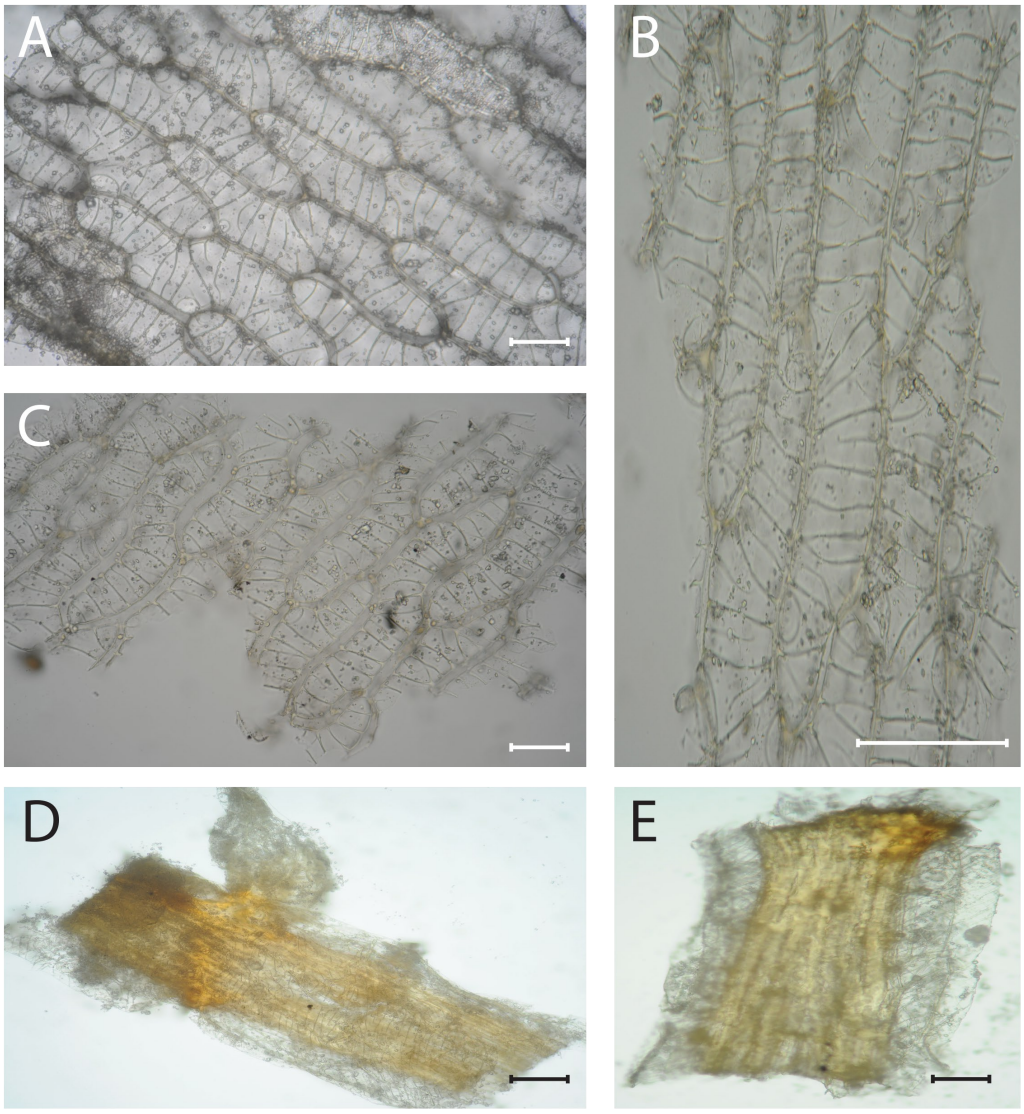
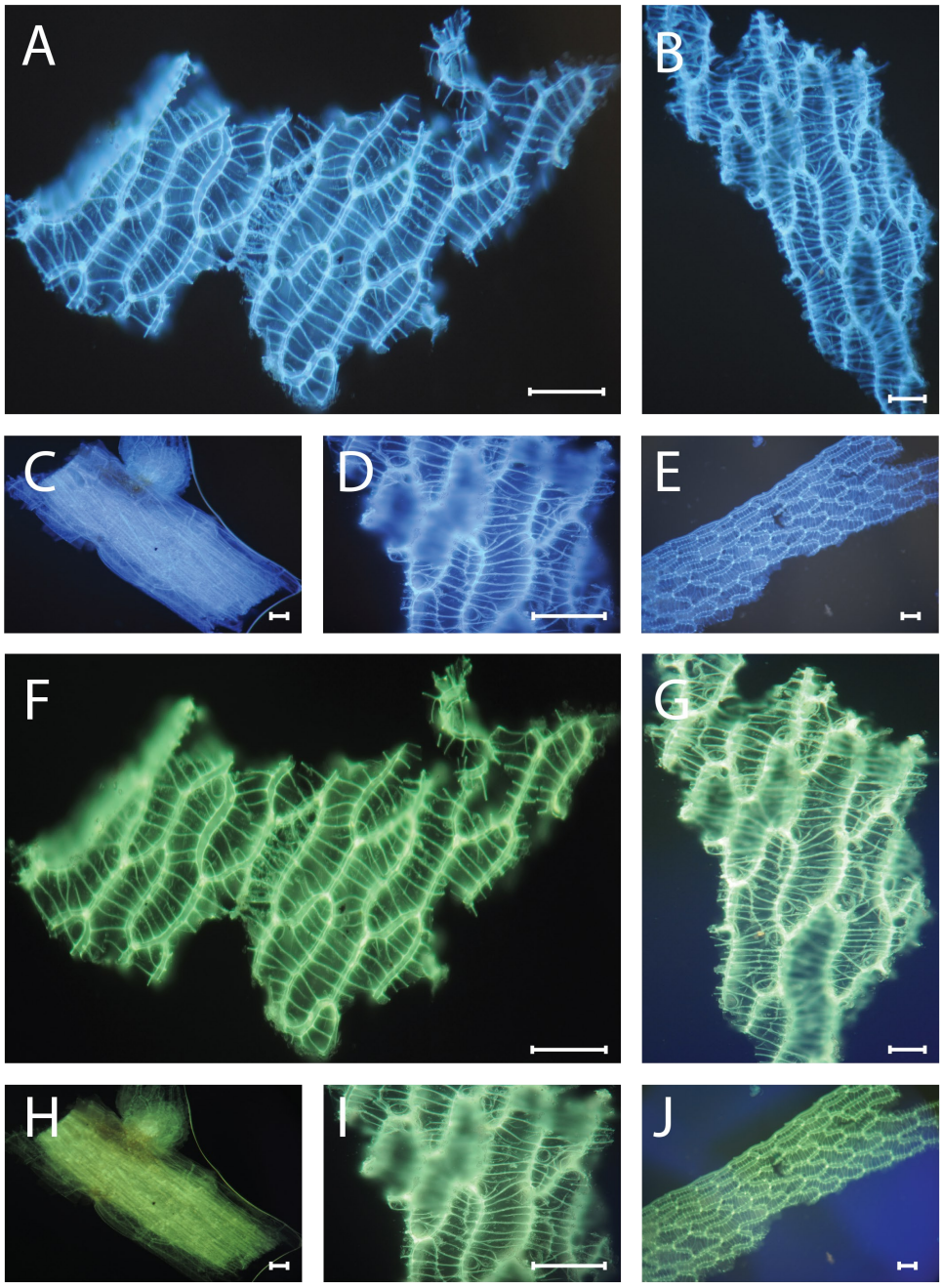


Figure 3. *Sphagnum*-like fossil leaf and stem fragment epifluorescence.



Appendix 1. Supplementary materials for chapter 1: The impacts of combined UV and temperature treatments on *Sphagnum compactum* biomass, cell wall compounds, and morphology

Table 1: P-values reported by MANOVA

		P-value		
		Temp	UV	Temp&UV
	Biomass	0.015	0.057	<0.001
	% Resistant Carbon	<0.001	<0.001	<0.001
	% Cellulose	0.160	<0.001	<0.001
STEM	Total Width	<0.001	0.001	<0.001
	Cortical Cell Width	<0.001	0.718	0.780
	Pith Width	<0.001	0.915	0.004
STEM LEAF	Leaf Length	<0.001	0.002	<0.001
	Leaf Width	<0.001	<0.001	<0.001
	Hyaline cell length	<0.001	<0.001	<0.001
	Hyaline cell width	<0.001	<0.001	<0.001
	Hyaline cell ring number	<0.001	0.001	<0.001
	Hyaline cell pore number	0.001	0.044	<0.001
	Hyaline cell pore width	0.145	0.843	0.013
	Chlorophyllous cell length	<0.001	<0.001	<0.001
	Chlorophyllous cell width	0.210	0.002	0.585
BRANCH LEAF	Leaf Length	<0.001	<0.001	<0.001
	Leaf Width	0.002	0.082	<0.001
	Hyaline cell length	<0.001	<0.001	<0.001
	Hyaline cell width	<0.001	<0.001	<0.001
	Hyaline cell ring number	<0.001	<0.001	<0.001
	Hyaline cell pores number	0.043	0.155	0.004
	Hyaline cell pore width	0.030	0.374	0.175
	Chlorophyllous cell length	<0.001	<0.001	<0.001
	Chlorophyllous cell width	0.002	<0.001	0.001

Table 2. Summary of response variables whose impact by temperature, UV, or temperature/UV interaction was statistically- ~~by~~ and for which 50% of the variation was explained.

- Temperature has statistically-significant effect
  - Stem total width
  - Stem pith width
  - Stem leaf length
  - Stem leaf hyaline cell ring number
  - Branch leaf hyaline cell ring number
- UV has statistically-significant effect
  - Percent resistant carbon
  - Percent cellulose
  - Stem leaf chlorophyllous cell width
- Temperature/UV interaction is statistically significant
  - Biomass
  - Stem leaf hyaline cell pore number
  - Branch leaf width

Table 3. Biomass

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	0.0070439	0.0070439	0.0035220	6.55	0.015	15.56
UV	1	0.0024757	0.0024757	0.0024757	4.61	0.057	5.47
Temp*UV	2	0.0286973	0.0286973	0.0143486	26.71	<0.001	63.39
Alternate Number	2	0.0016803	0.0016803	0.0008402	1.56	0.257	3.71
Error	10	0.0053730	0.0053730	0.0005373			
Total	17	0.0452702	0.0452702				
S=0.0231797	R-Sq=88.13%		R-Sq (adj)=79.82%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	0.16	0.04	0.06	0.01
20	0.08	0.02	0.17	0.04
30	0.11*	0.02	0.05	0.03

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 4. Percent resistant carbon

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	215.63	215.63	107.82	611.12	<0.001	3.73
UV	1	3919.67	3919.67	3919.67	22217.53	<0.001	67.89
Temp*UV	2	1635.40	1635.40	817.70	4634.91	<0.001	28.33
Alternate Number	2	1.00	1.00	0.50	2.82	0.107	0.02
Error	10	1.76	1.76	0.18			
Total	17	5773.46					
S=0.420026	R-Sq=99.97%		R-Sq (adj)=99.95%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	75.2	0.35	19.1	0.64
20	45.3	0.10	32.8	0.53
30	55.3*	0.70	35.4	0.25

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 5. Percent cellulose

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	60.90	60.90	30.45	2.21	0.160	1.43
UV	1	2648.22	2648.22	2648.22	192.47	<0.001	62.12
Temp*UV	2	1414.80	1414.80	707.40	51.41	<0.001	33.19
Alternate Number	2	1.32	1.32	0.66	0.05	0.953	0.03
Error	10	137.59	137.59	13.76			
Total	17	4262.84					
S=3.70931	R-Sq=96.77%		R-Sq (adj)=94.51%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	24.2	2.15	72.8	3.98
20	46.8	3.95	53.8	3.55
30	44.4*	3.33	61.5	3.11

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 6. Stem total width

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	226214	226214	113107	1528.50	<0.001	97.19
UV	1	1723	1723	1723	23.28	0.001	0.74
Temp*UV	2	3313	3313	1657	22.39	<0.001	1.42
Alternate Number	2	768	768	384	5.19	0.028	0.33
Error	10	740	740	74			
Total	17	232759					
S=8.60226	R-Sq=99.68%		R-Sq (adj)=99.46%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	530	7.78	522	17.2
20	467	2.51	523.0	14.3
30	269*	8.78	279.0	10.4

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 7. Stem cortical cell width

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	13855.2	13855.2	6927.6	108.52	<0.001	9.32
UV	1	8.8	8.8	8.8	0.14	0.718	0.01
Temp*UV	2	32.6	32.6	16.3	0.26	0.780	0.02
Alternate Number	2	325.7	325.7	162.9	2.55	0.127	0.22
Error	10	638.4	638.4	63.8			
Total	17	148600.7					
S=7.98989	R-Sq=95.70%		R-Sq (adj)=92.70%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	102	4.73	103	3.46
20	115	9.64	115	13.4
30	53.7*	3.33	48.5	12.8

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 8. Stem pith width

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	25757.1	25757.1	12878.5	36.73	<0.001	66.52
UV	1	4.2	4.2	4.2	0.01	0.915	0.01
Temp*UV	2	6867.4	6867.4	3433.7	9.79	0.004	17.74
Alternate Number	2	2586.8	2586.8	1293.4	3.69	0.063	6.68
Error	10	3506.7	3506.7	350.7			
Total	17	38722.2					
S=18.7261	R-Sq=90.94%		R-Sq (adj)=84.60%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	319	34.4	290	32.9
20	262	10.7	318	14.5
30	230*	1.76	206	21.3

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 9. Stem leaf length

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	3.53370	3.53370	1.76685	89.82	<0.001	59.97
UV	1	0.31734	0.31734	0.31734	16.13	0.002	5.39
Temp*UV	2	1.81041	1.81041	0.90521	46.02	<0.001	30.72
Alternate Number	2	0.03430	0.03430	0.01715	0.87	0.448	0.58
Error	10	0.19670	0.19670	0.01967			
Total	17	5.89245					
S=0.140250	R-Sq=96.66%		R-Sq (adj)=94.33%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	3.90	0.03	3.92	0.11
20	3.23	0.02	3.56	0.09
30	3.40*	0.23	2.26	0.21

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 10. Stem leaf width

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	2.49213	2.49213	1.24607	925.30	<0.001	43.70
UV	1	0.96142	0.96142	0.96142	713.93	<0.001	16.86
Temp*UV	2	2.22164	2.22164	1.11082	824.87	<0.001	38.96
Alternate Number	2	0.01373	0.01373	0.00687	5.10	0.030	0.24
Error	10	0.01347	0.01347	0.00135			
Total	17	5.70240					
S=0.0366970	R-Sq=99.76%		R-Sq (adj)=99.60%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	2.57	0.03	2.44	0.05
20	2.07	0.04	2.25	0.04
30	2.32*	0.04	0.88	0.07

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 11. Stem leaf hyaline cell length

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	40352	40352	20176	76.80	<0.001	32.91
UV	1	25118	25118	25118	95.61	<0.001	20.48
Temp*UV	2	53351	53351	26676	101.54	<0.001	43.51
Alternate Number	2	1170	1170	585	2.23	0.159	0.95
Error	10	2627	2627	263			
Total	17	122618					
S=16.2084	R-Sq=97.86%		R-Sq (adj)=96.36%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	352	4.42	312	25.3
20	299	12.5	337	26.4
30	336*	7.64	114	18.1

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 12. Stem leaf hyaline cell width

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	2151.84	2151.84	1075.92	108.92	<0.001	43.17
UV	1	1053.40	1053.40	1053.41	106.64	<0.001	21.13
Temp*UV	2	1670.41	1670.41	835.20	84.55	<0.001	33.51
Alternate Number	2	0.20	0.20	0.10	0.01	0.990	0.00
Error	10	98.78	98.78	9.88			
Total	17	4984.64					
S=3.14298	R-Sq=98.01%		R-Sq (adj)=96.62%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	79.0	2.60	60.1	3.06
20	45.8	0.29	55.5	4.27
30	62.7*	2.52	22.1	4.05

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 13. Stem leaf hyaline cell ring number

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	189.258	189.258	94.629	77.93	<0.001	55.86
UV	1	23.576	23.576	23.576	19.42	0.001	6.96
Temp*UV	2	101.364	101.364	50.682	41.74	<0.001	29.92
Alternate Number	2	12.498	12.498	6.249	5.15	0.029	3.69
Error	10	12.152	12.142	1.214			
Total	17	338.838					
S=1.10192	R-Sq=96.42%		R-Sq (adj)=93.91%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	20.2	2.31	23.9	1.10
20	19.1	1.03	16.4	1.40
30	18.1*	1.62	10.2	0.40

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 14. Stem leaf hyaline cell pore number

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	14.1511	14.1511	7.0756	13.83	0.001	26.66
UV	1	2.7222	2.7222	2.7222	5.32	0.044	5.13
Temp*UV	2	31.0578	31.0578	15.5289	30.32	<0.001	58.51
Alternate Number	2	0.0311	0.0311	0.0156	0.03	0.970	0.06
Error	10	5.1156	5.1156	0.5116			
Total	17	53.0778					
S=0.715231	R-Sq=90.36%		R-Sq (adj)=83.62%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	3.27	0.76	7.60	0.72
20	3.47	0.64	3.40	0.60
30	4.67*	0.81	2.73	0.23

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 15. Stem leaf hyaline cell pore width

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	5.867	5.867	2.934	2.36	0.145	16.30
UV	1	0.051	0.051	0.051	0.04	0.843	0.14
Temp*UV	2	17.224	17.224	8.612	6.91	0.013	47.86
Alternate Number	2	0.386	0.386	0.193	0.15	0.858	1.07
Error	10	12.457	12.457	1.246			
Total	17	35.985					
S=1.11609	R-Sq=65.38%		R-Sq (adj)=41.15%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	11.3	0.29	13.8	1.04
20	12.6	0.88	10.4	0.63
30	11.6*	1.50	11.0	1.35

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 16. Stem leaf chlorophyllous cell length

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	12523.5	12523.5	6261.7	27.68	<0.001	33.74
UV	1	8734.9	8734.9	8734.9	38.61	<0.001	23.53
Temp*UV	2	12981.4	12981.4	6490.7	28.69	<0.001	34.97
Alternate Number	2	618.4	618.4	309.2	1.37	0.299	1.67
Error	10	2262.5	2262.5	226.2			
Total	17	37120.6					
S=15.0416	R-Sq=93.91%		R-Sq (adj)=89.64%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	179	14.1	182	6.50
20	175.0	8.22	158	24.2
30	178*	20.9	59.3	8.75

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 17. Stem leaf chlorophyllous cell width

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	2.6886	2.6882	1.3443	1.83	0.210	11.24
UV	1	12.6672	12.6672	12.6672	17.29	0.002	52.95
Temp*UV	2	0.8286	0.8286	0.4143	0.57	0.585	3.46
Alternate Number	2	0.4119	0.4119	0.2060	0.28	0.761	1.72
Error	10	7.3281	7.3281	0.7328			
Total	17	23.9244					
S=0.856041	R-Sq=69.37%		R-Sq (adj)=47.93%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	8.67	0.76	9.83	0.58
20	9.00	0.50	11.2	0.30
30	9.20*	1.08	10.9	1.20

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 18. Branch leaf length

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	2.2065	2.2065	1.1033	69.59	<0.001	28.81
UV	1	1.4678	1.4678	1.4678	92.58	<0.001	19.17
Temp*UV	2	3.8086	3.8086	1.9043	120.12	<0.001	49.73
Alternate Number	2	0.0172	0.0172	0.0086	0.54	0.597	0.22
Error	10	0.1585	0.1585	0.0159			
Total	17	7.6586					
S=0.125910	R-Sq=97.93%		R-Sq (adj)=96.48%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	3.51	0.100	3.69	0.151
20	3.49	0.120	3.46	0.052
30	3.73*	0.041	1.87	0.019

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 19. Branch leaf width

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	0.22453	0.22453	0.11227	11.78	0.002	15.25
UV	1	0.03556	0.03556	0.03556	3.73	0.082	2.42
Temp*UV	2	1.00498	1.00498	0.50249	52.71	<0.001	68.27
Alternate Number	2	0.11160	0.11160	0.05580	5.85	0.021	7.58
Error	10	0.09533	0.09533	0.00953			
Total	17	1.47200					
S=0.0976388	R-Sq=93.52%		R-Sq (adj)=88.99%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	1.71	0.130	1.79	0.012
20	1.43	0.081	1.82	0.192
30	1.85*	0.174	1.11	0.110

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 20. Branch leaf hyaline cell length

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	27048	27048	13524	506.55	<0.001	35.89
UV	1	16182	16182	16182	606.12	<0.001	21.47
Temp*UV	2	31847	31847	15923	596.42	<0.001	42.26
Alternate Number	2	16	16	8	0.3	0.750	0.02
Error	10	267	267	27			
Total	17	75359					
S=5.16700	R-Sq=99.65%		R-Sq (adj)=99.40%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	332	2.86	333	4.01
20	307	4.51	305	4.04
30	330*	6.03	151	6.67

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 21. Branch leaf hyaline cell width

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	546.22	546.22	273.83	35.83	<0.001	35.59
UV	1	302.58	302.58	302.70	39.70	<0.001	19.72
Temp*UV	2	551.24	551.24	275.62	36.16	<0.001	35.92
Alternate Number	2	58.43	58.43	29.22	3.83	0.058	3.81
Error	10	76.22	76.22	7.62			
Total	17	1534.70					
S=2.76084	R-Sq=95.03%		R-Sq (adj)=91.56%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	53.7	3.88	54.7	3.33
20	58.3	3.82	56.5	3.04
30	56.3*	2.36	32.6	3.43

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 22. Branch leaf hyaline cell ring number

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	137.973	137.973	68.987	601.63	<0.001	49.99
UV	1	37.556	37.556	37.556	327.52	<0.001	13.61
Temp*UV	2	97.351	97.351	48.676	424.50	<0.001	35.27
Alternate Number	2	1.973	1.973	0.987	8.60	0.007	0.71
Error	10	1.147	1.147	0.115			
Total	17	276.000					
S=0.338625	R-Sq=99.58%		R-Sq (adj)=99.29%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	20.8	0.34	21.2	0.35
20	18.1	0.81	18.5	0.12
30	19.0*	0.53	9.53	0.61

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 23. Branch leaf hyaline cell pore number

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	1.1911	1.1911	0.5956	4.39	0.043	17.96
UV	1	0.3200	0.3200	0.3200	2.36	0.155	4.83
Temp*UV	2	2.7733	2.7733	1.3867	10.23	0.004	41.82
Alternate Number	2	0.9911	0.9911	0.4956	3.66	0.064	14.95
Error	10	1.3556	1.3556	0.1356			
Total	17	6.6311					
S=0.368179	R-Sq=79.56%		R-Sq (adj)=65.25%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	3.53	0.23	4.33	0.70
20	3.73	0.28	3.20	0.69
30	3.87*	0.24	2.80	0.20

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 24. Branch leaf hyaline cell pore width

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	16.651	16.651	8.325	5.06	0.030	35.80
UV	1	1.422	1.422	1.422	0.87	0.374	3.06
Temp*UV	2	6.848	6.848	3.424	2.08	0.175	14.72
Alternate Number	2	5.152	5.152	2.576	1.57	0.256	11.08
Error	10	16.442	16.442	1.644			
Total	17	46.516					
S=1.28227	R-Sq=64.65%		R-Sq (adj)=39.91%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	12.3	1.62	12.7	0.72
20	11.3	0.61	13.5	0.68
30	10.8*	0.76	10.0	2.50

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 25. Branch leaf chlorophyllous cell length

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	9941.9	9941.9	4971.0	214.67	<0.001	36.46
UV	1	4622.1	4622.1	4622.1	199.60	<0.001	16.95
Temp*UV	2	12446.5	12446.5	6223.3	268.75	<0.001	45.65
Alternate Number	2	23.2	23.2	11.6	0.50	0.620	0.09
Error	10	231.6	231.6	23.2			
Total	17	27265.3					
S=4.81214	R-Sq=99.15%		R-Sq (adj)=98.56%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	161	3.78	173	7.02
20	131	4.54	129	4.00
30	163*	4.77	57.0	2.10

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Table 26. Branch leaf chlorophyllous cell width

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	P	% Explained
Temp	2	7.8469	7.8469	3.9234	12.65	0.002	17.88
UV	1	20.0345	20.0345	20.0345	64.62	<0.001	45.66
Temp*UV	2	9.8959	9.8959	4.9479	15.96	0.001	22.55
Alternate Number	2	2.9908	2.9908	1.9479	4.84	0.034	6.82
Error	10	3.1005	3.1005	0.3101			
Total	17	43.8776					
S=0.556823	R-Sq=92.93%		R-Sq (adj)=87.99%				

Temp (˚C)	UV			
	Enhanced		Reduced	
	Mean	SE	Mean	SE
10	9.17	1.04	9.50	0.50
20	9.63	0.71	11.7	0.58
30	8.82*	0.56	12.8	0.75

* *Sphagnum* compactum when exposed to 30˚C and enhanced UV died in a 2 week period

Figure 1. Interaction plot for Biomass (g)

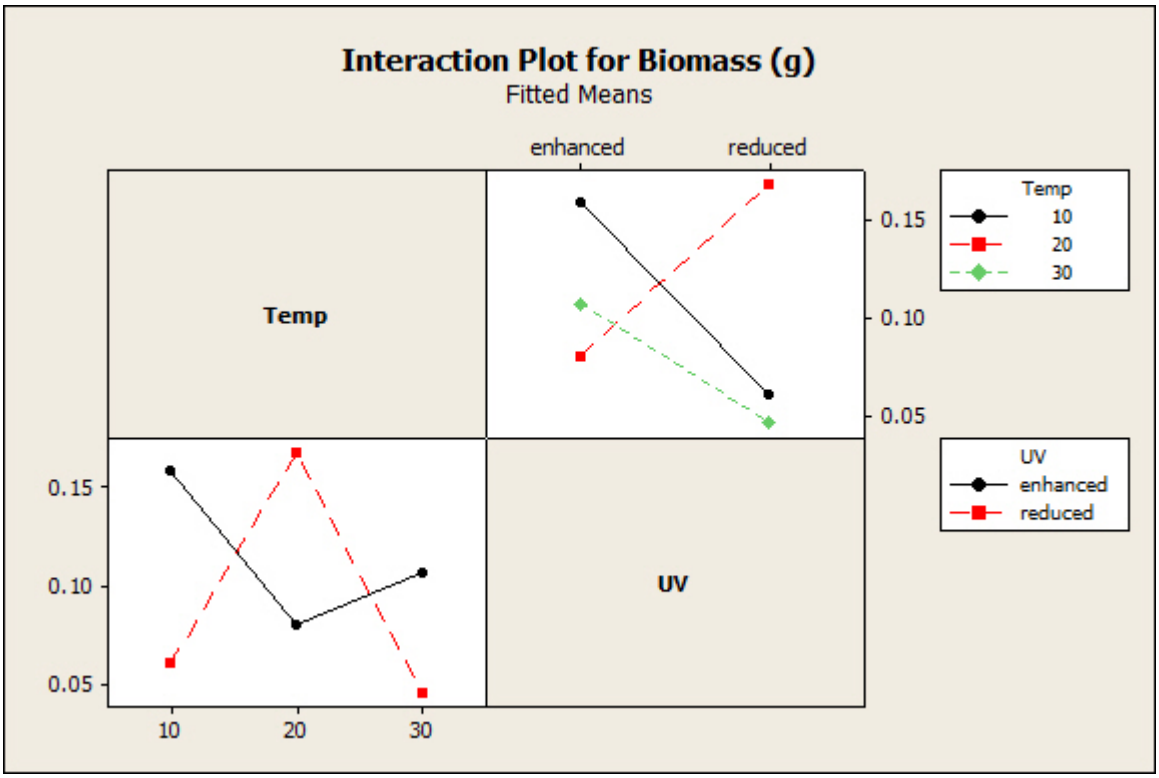


Figure 2. Interaction plot for percent cellulose

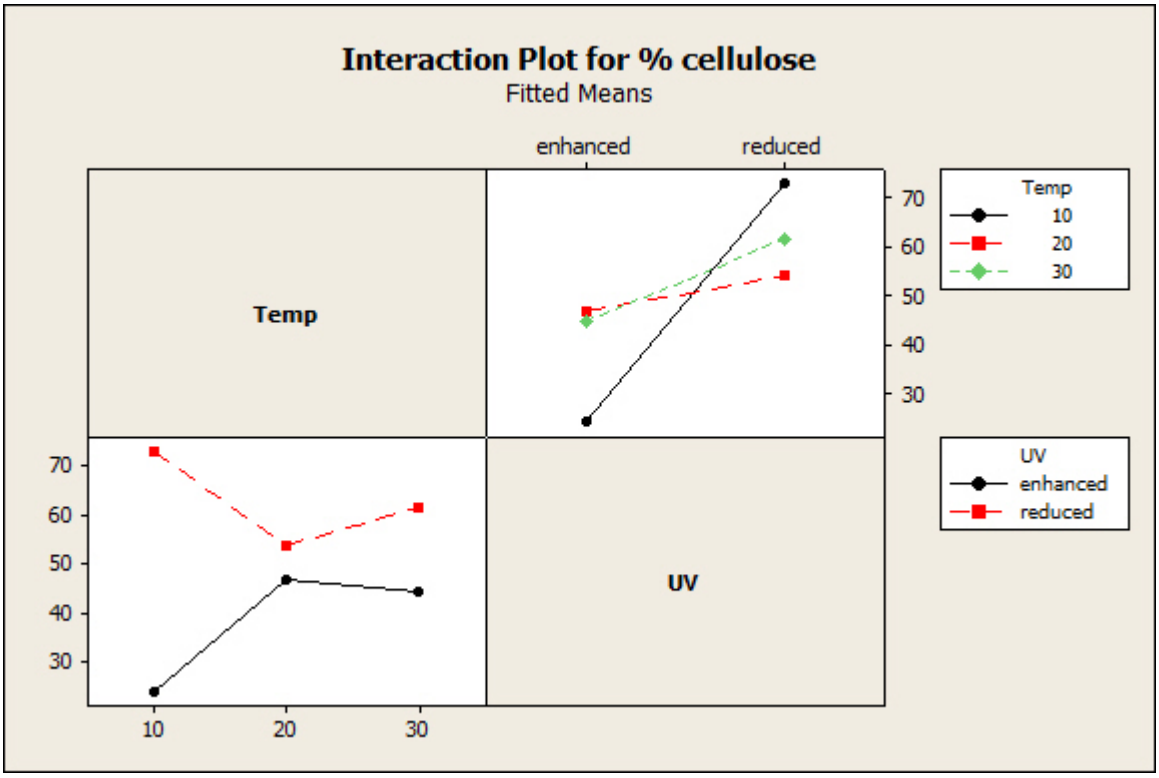


Figure 3. Interaction plot for percent resistant carbon

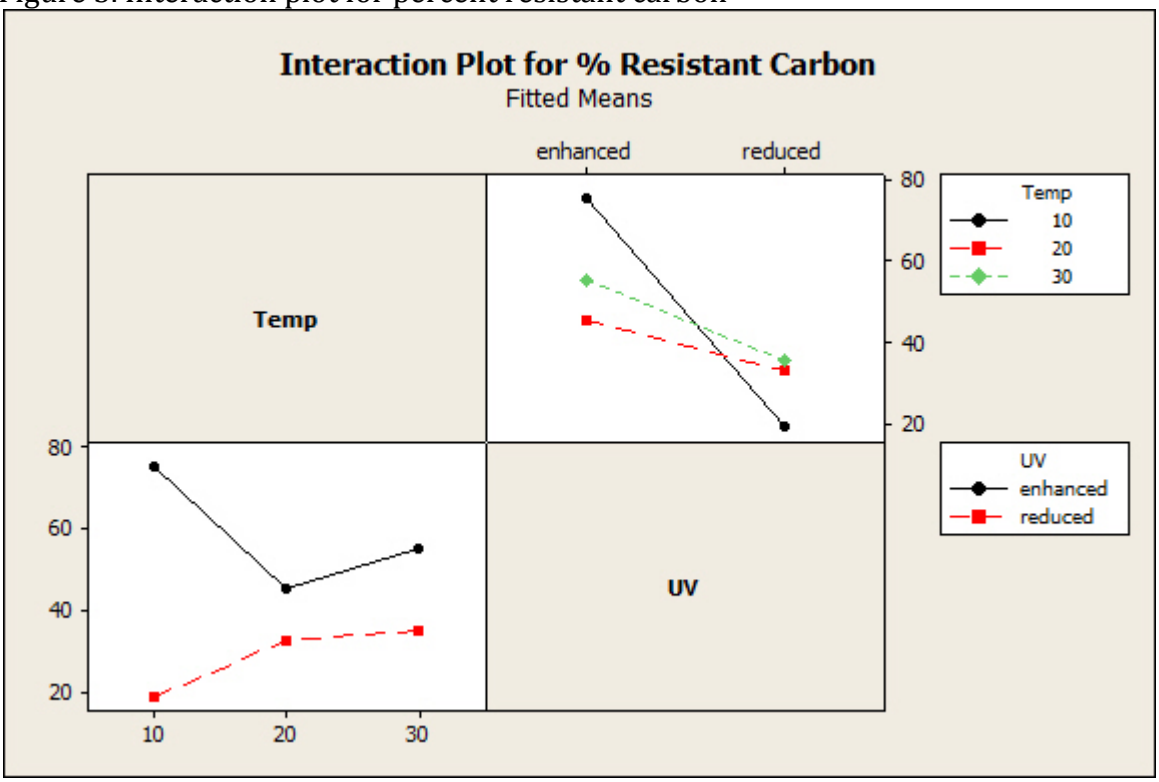


Figure 4. Interaction plot for stem total width

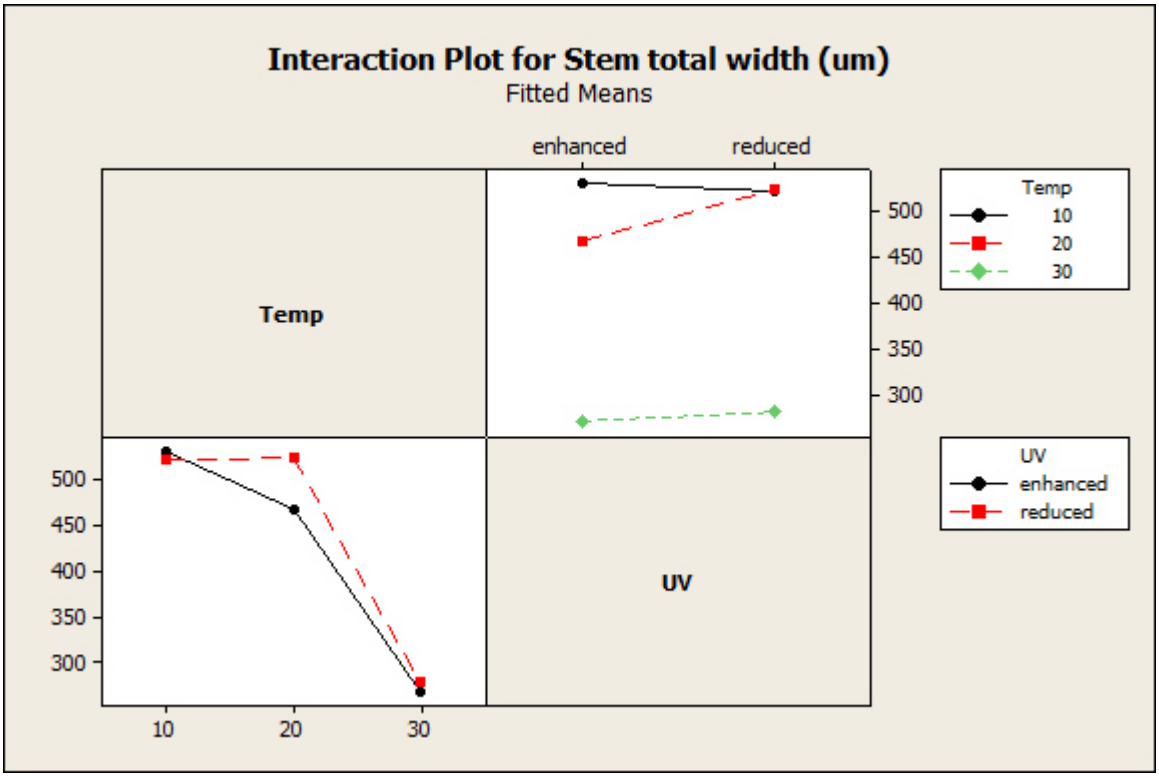


Figure 5. Interaction plot for stem cortical cell width

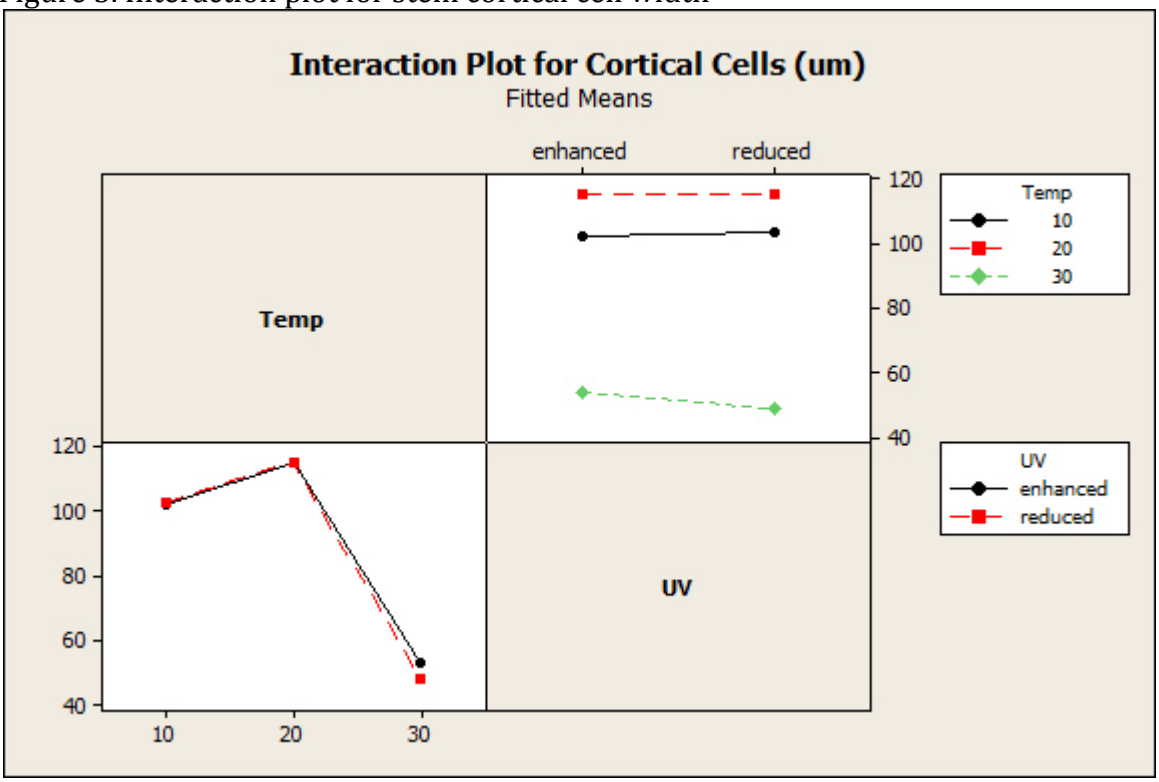


Figure 6. Interaction plot for stem pith width

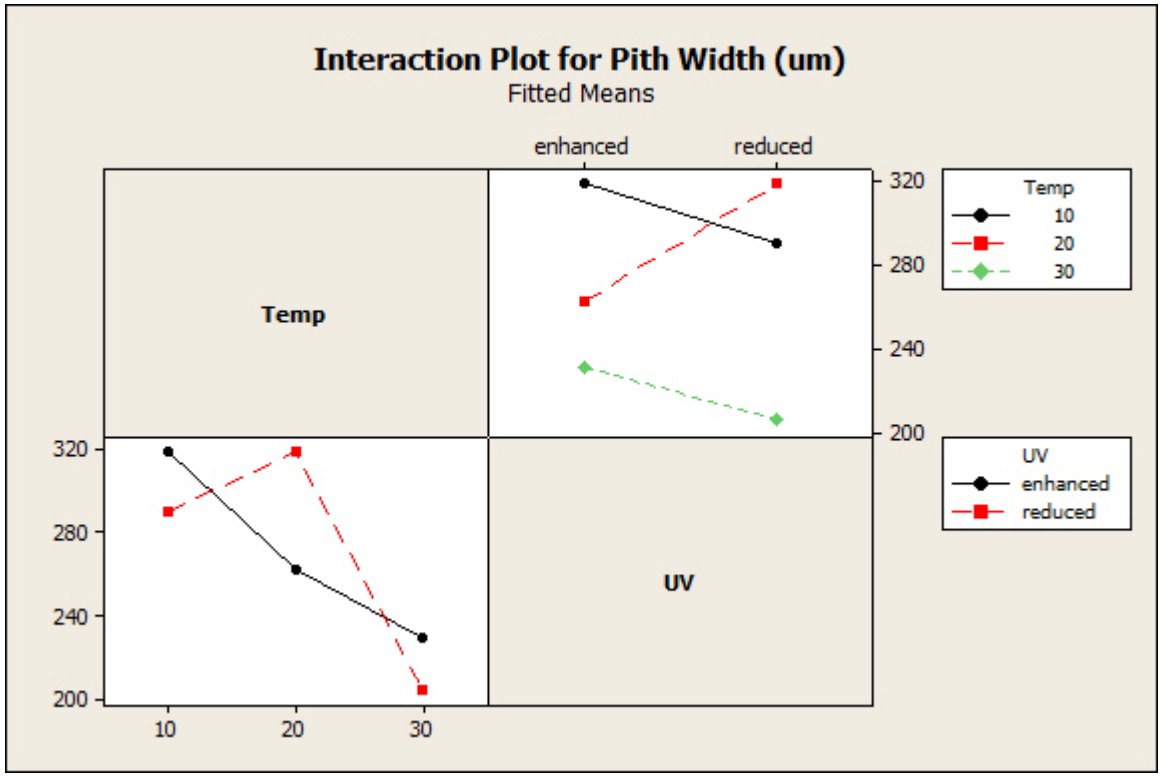


Figure 7. Interaction plot for stem leaf length

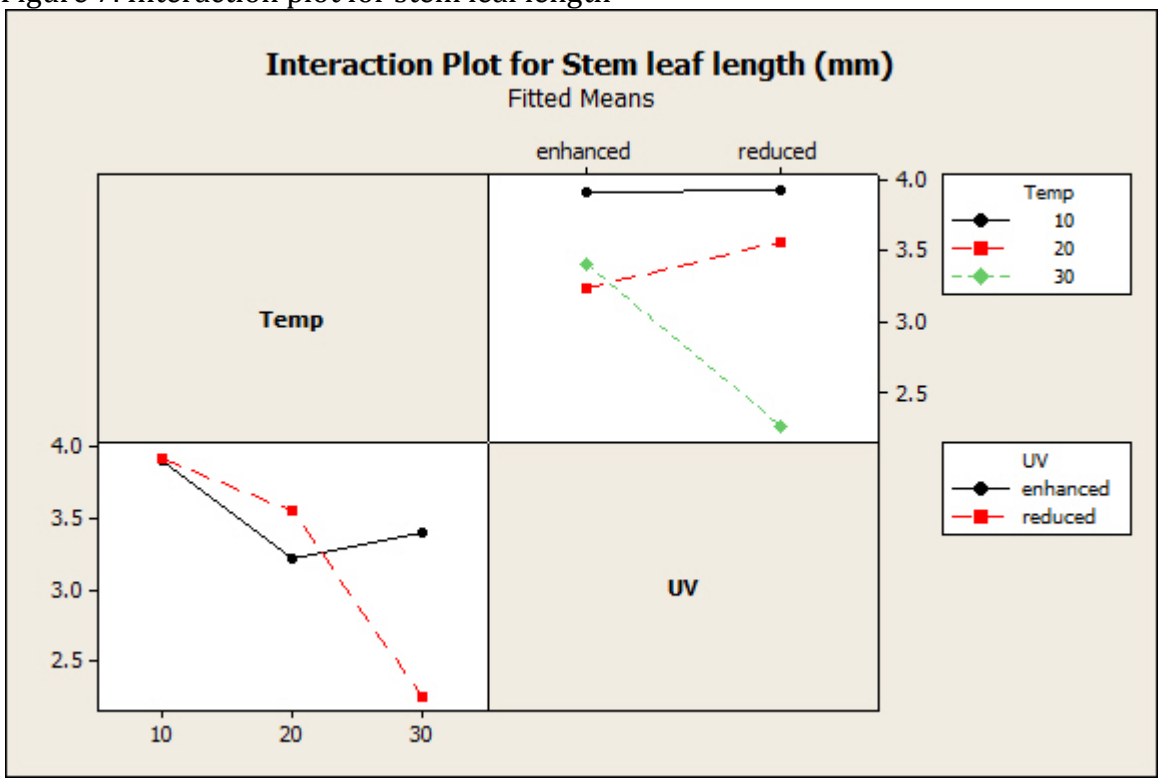


Figure 8. Interaction plot for stem leaf width

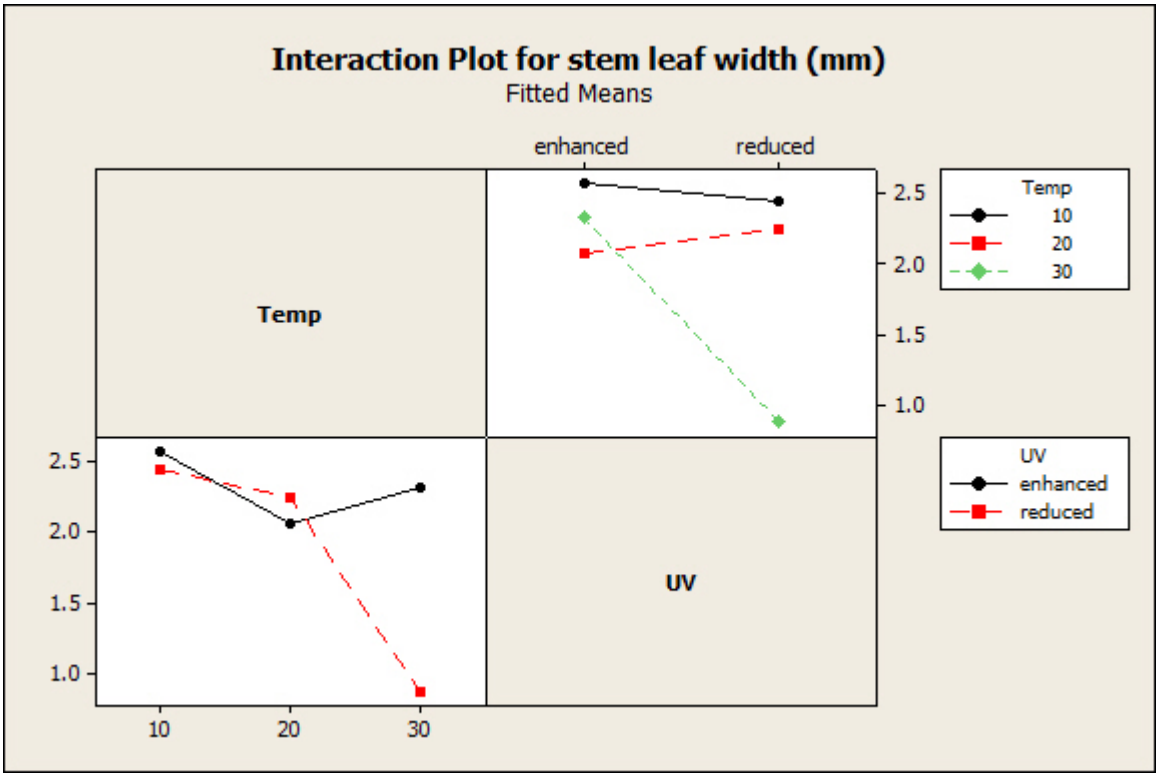


Figure 9. Interaction plot for stem leaf hyaline cell length

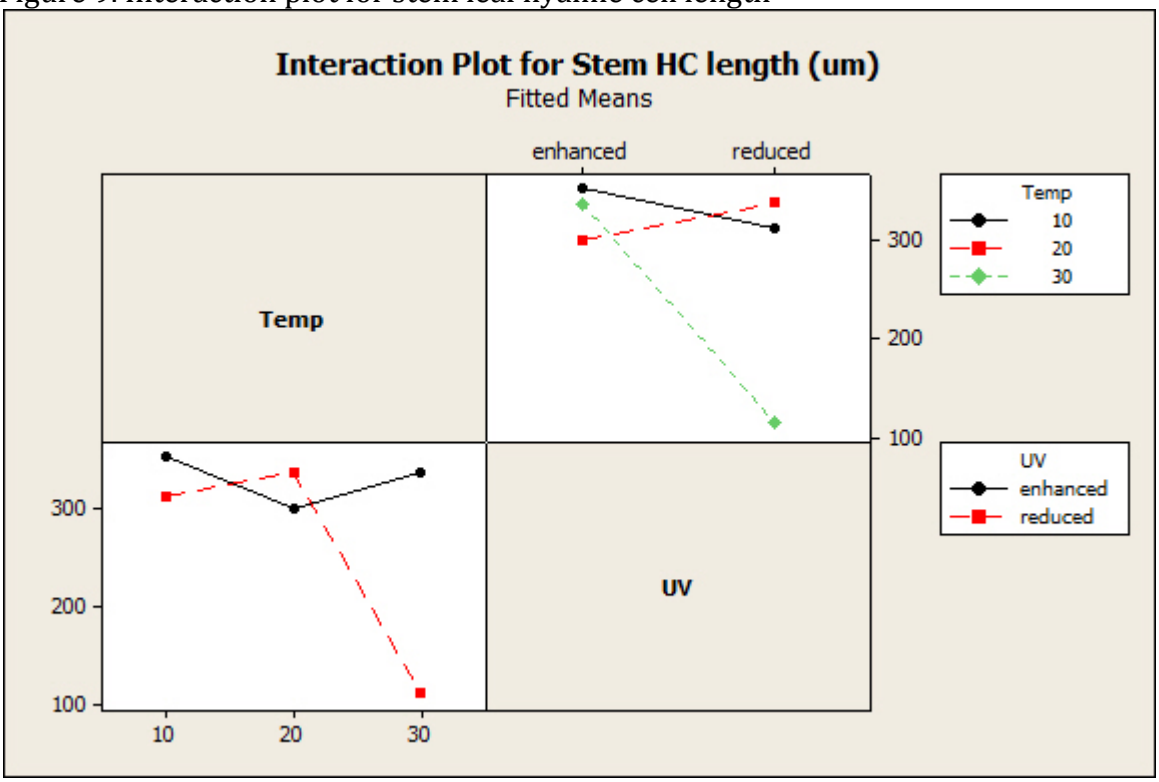


Figure 10. Interaction plot for stem leaf hyaline cell width

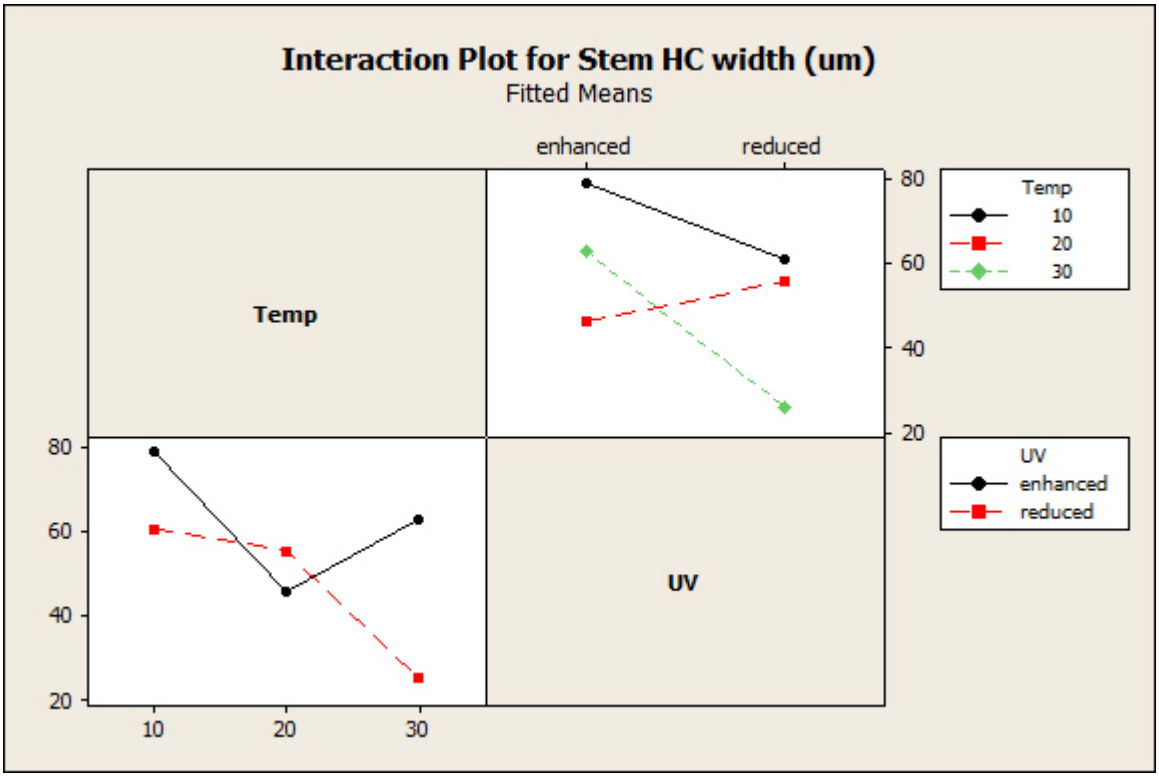


Figure 11. Interaction plot for stem leaf hyaline cell ring number

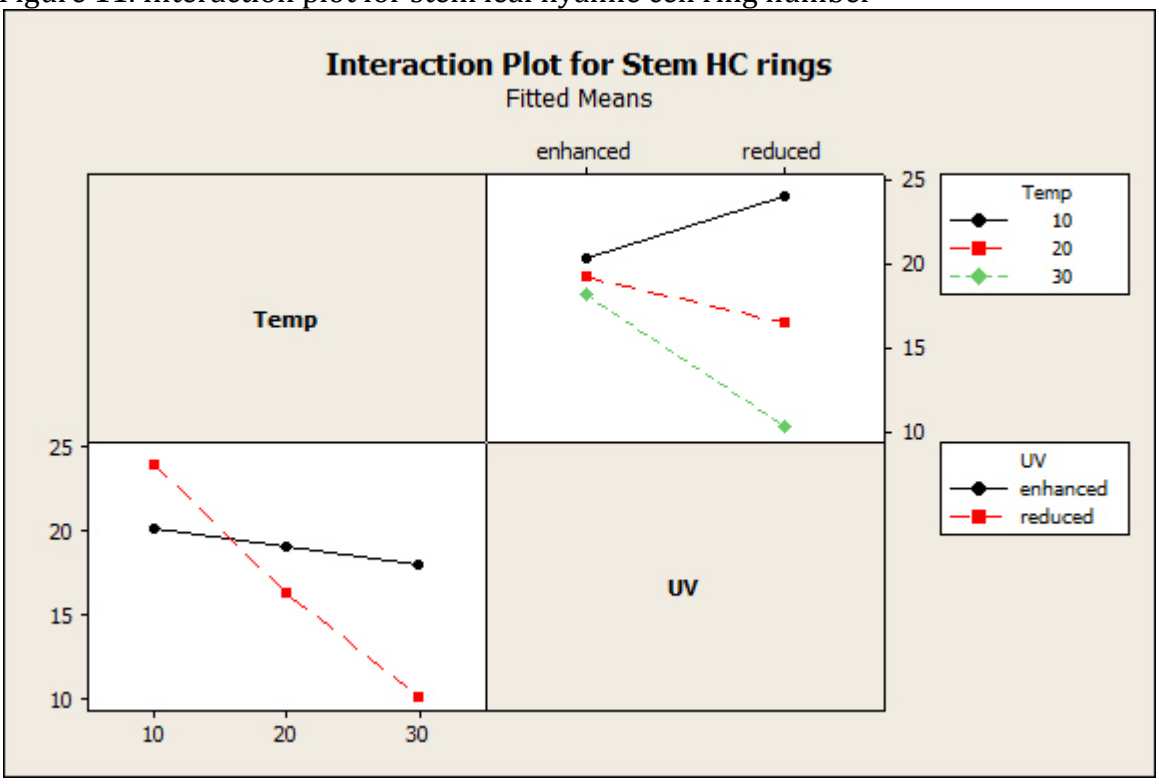


Figure 12. Interaction plot for hyaline cell pore number

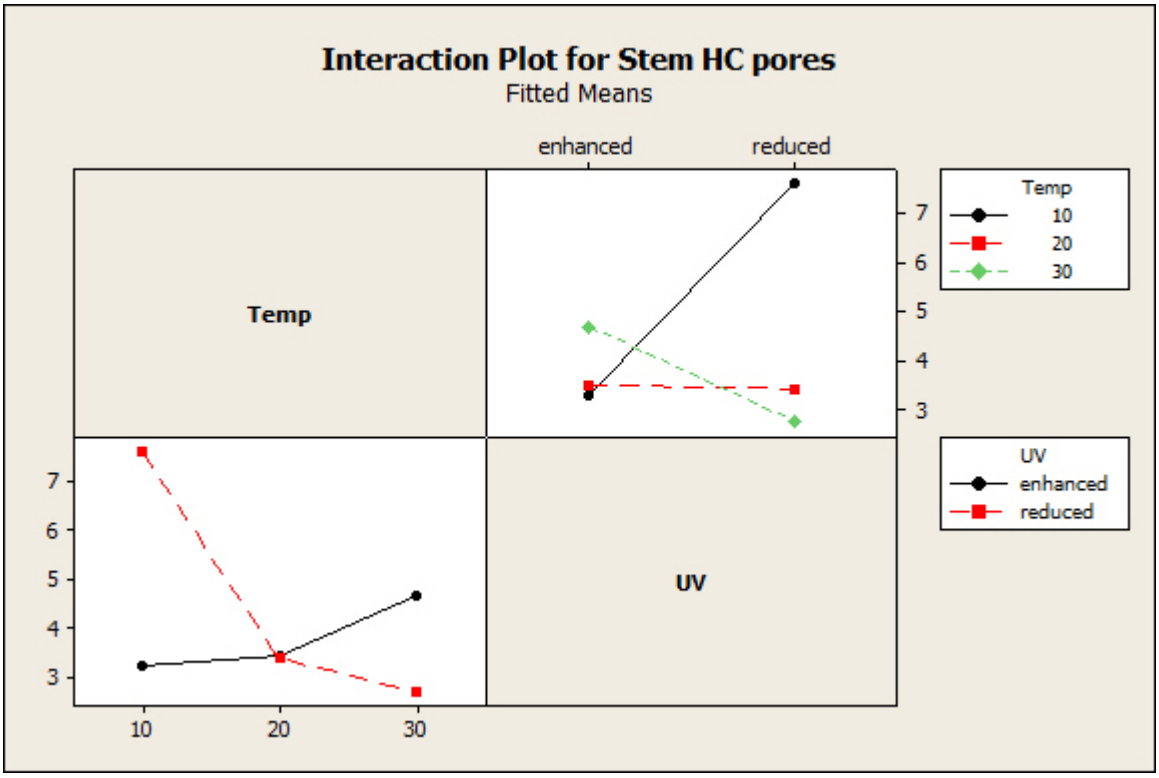


Figure 13. Interaction plot for stem leaf hyaline cell pore width

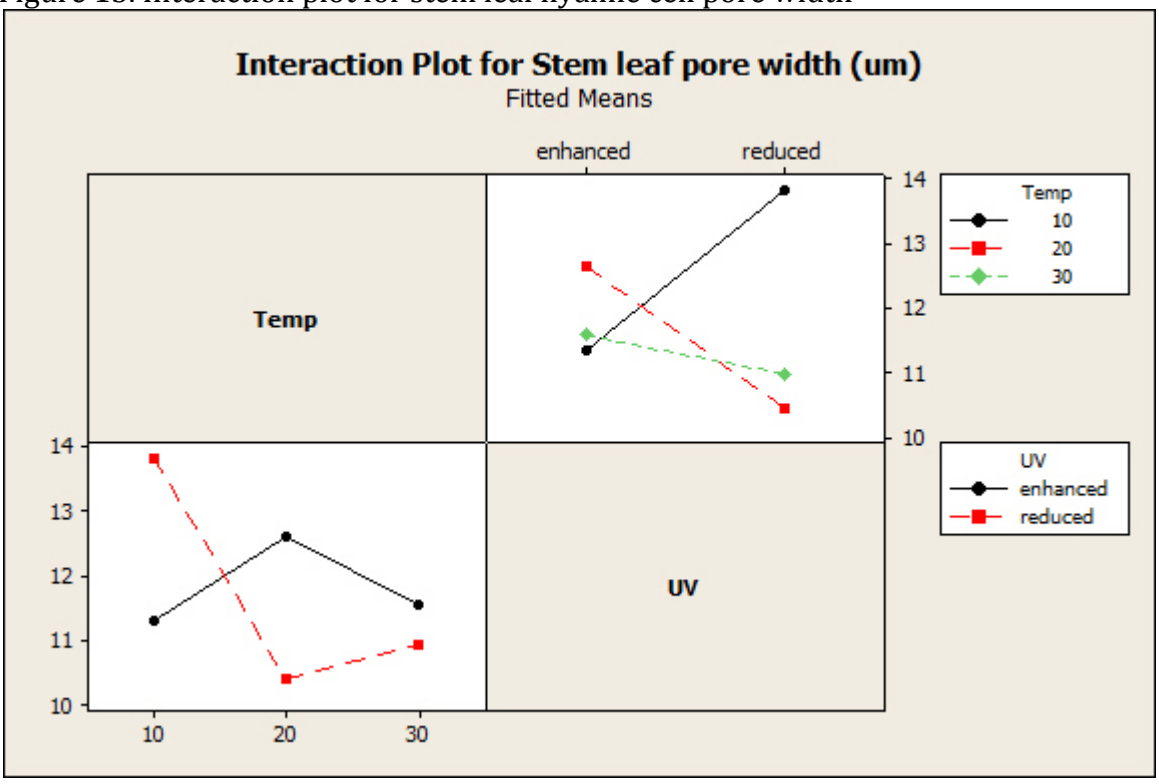


Figure 14. Interaction plot for stem leaf chlorophyllous cell length

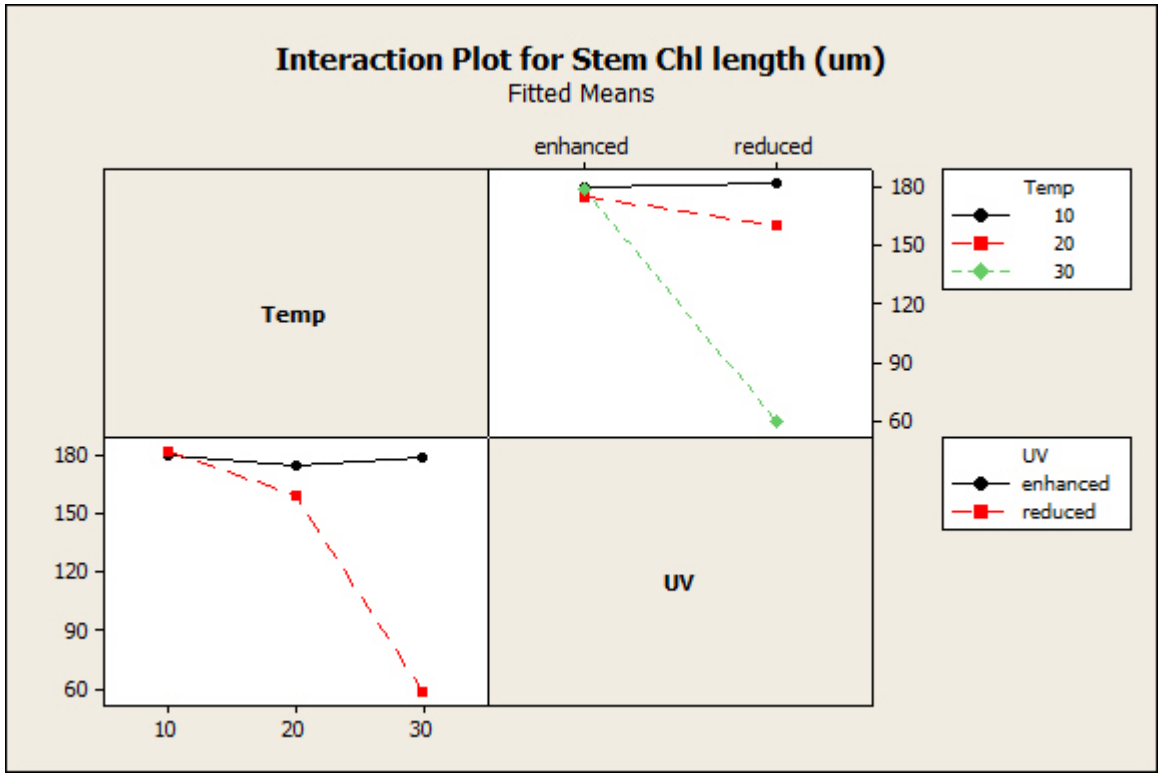


Figure 15. Interaction plot for stem leaf chlorophyllous cell width

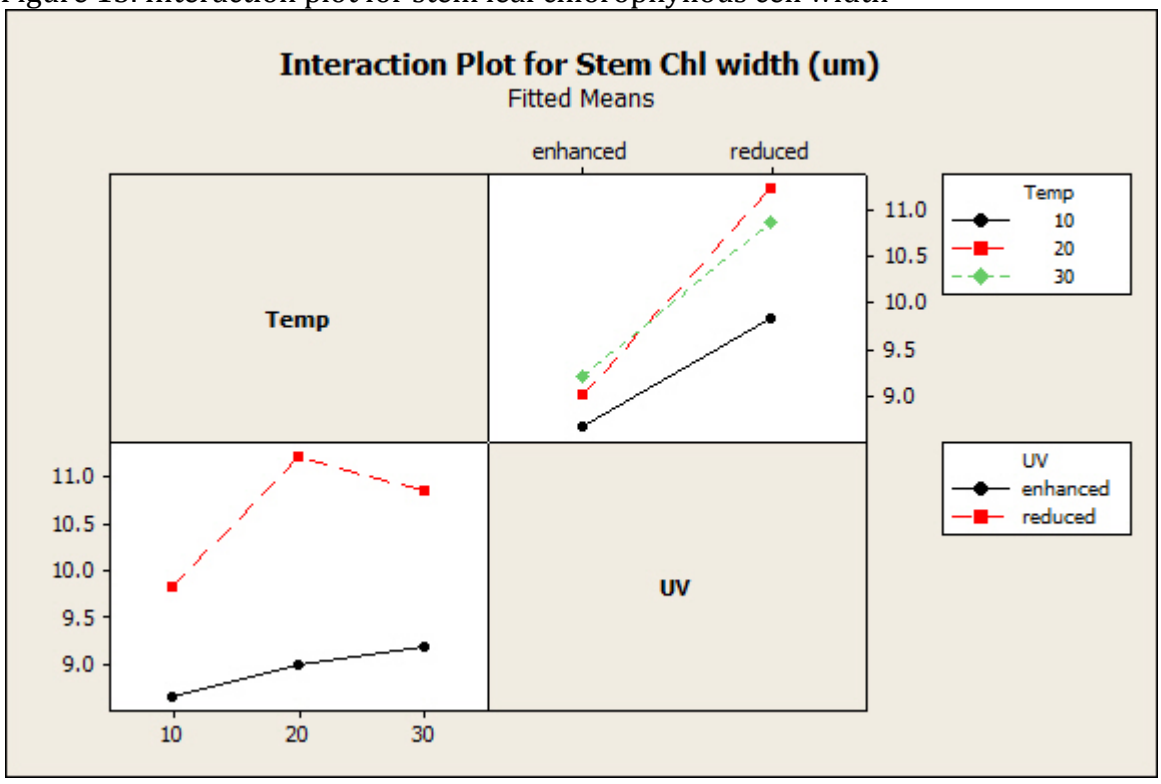


Figure 16. Interaction plot for branch leaf length

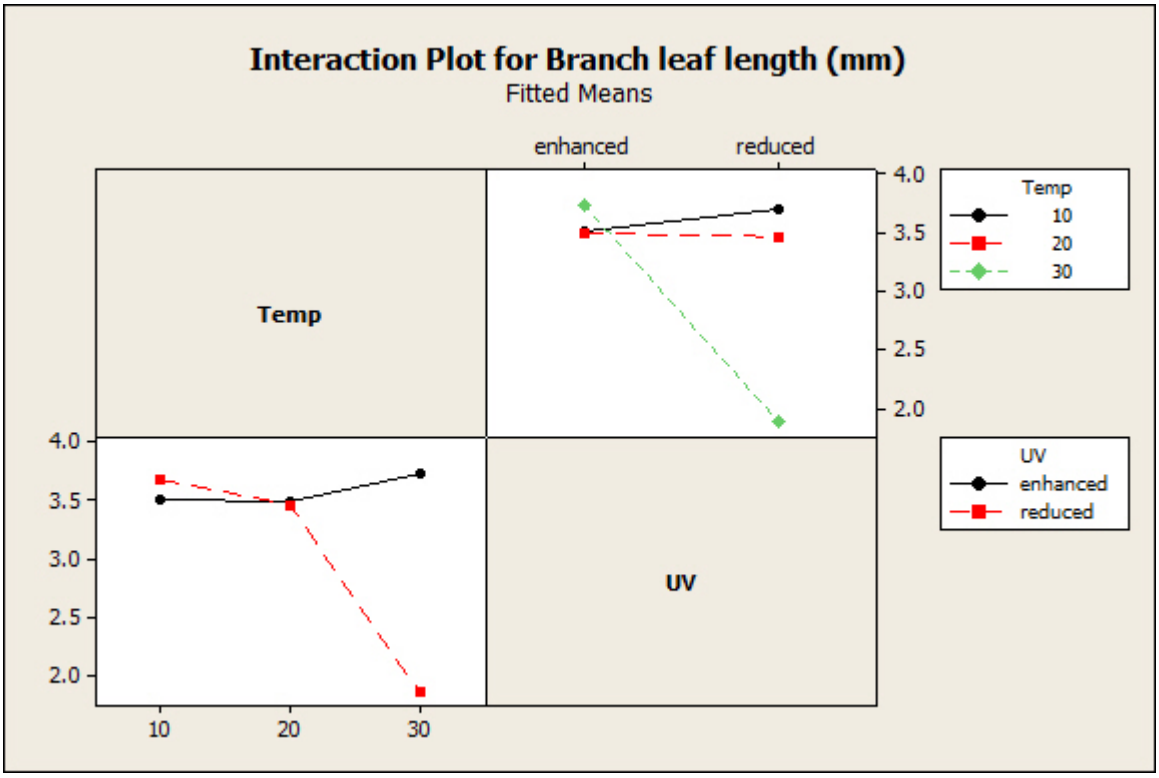


Figure 17. Interaction plot for branch leaf width

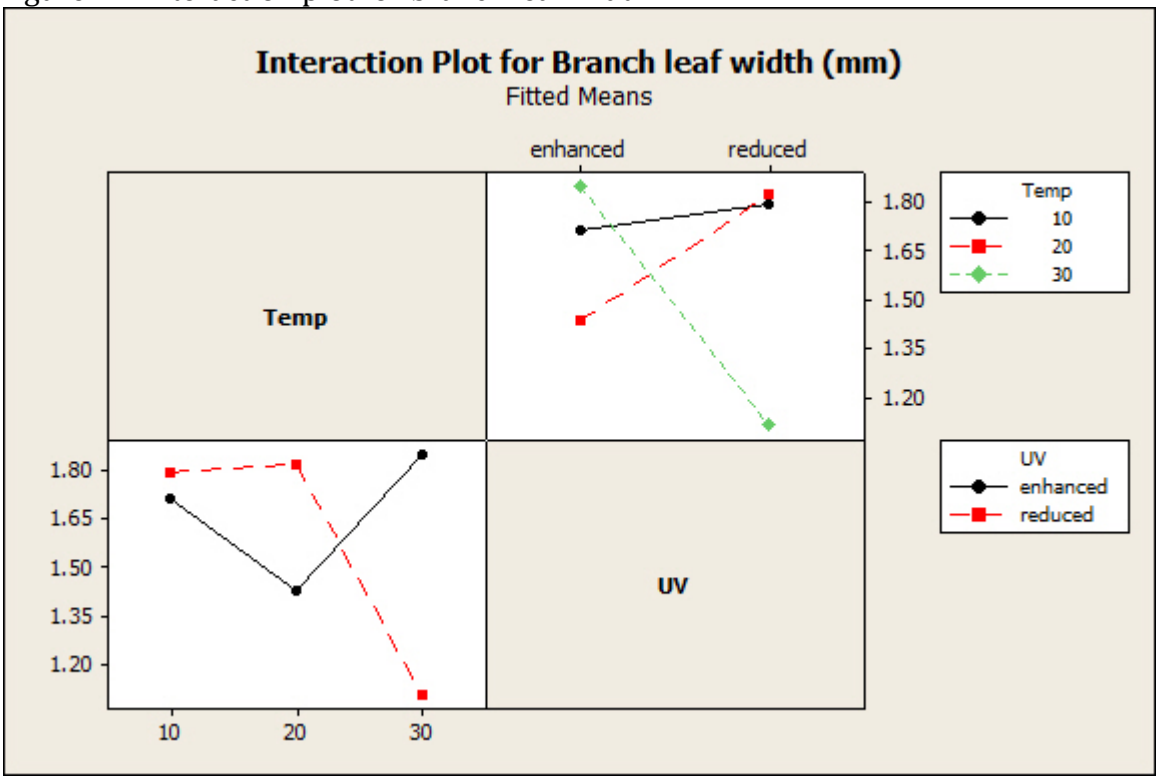


Figure 18. Interaction plot for branch leaf hyaline cell length

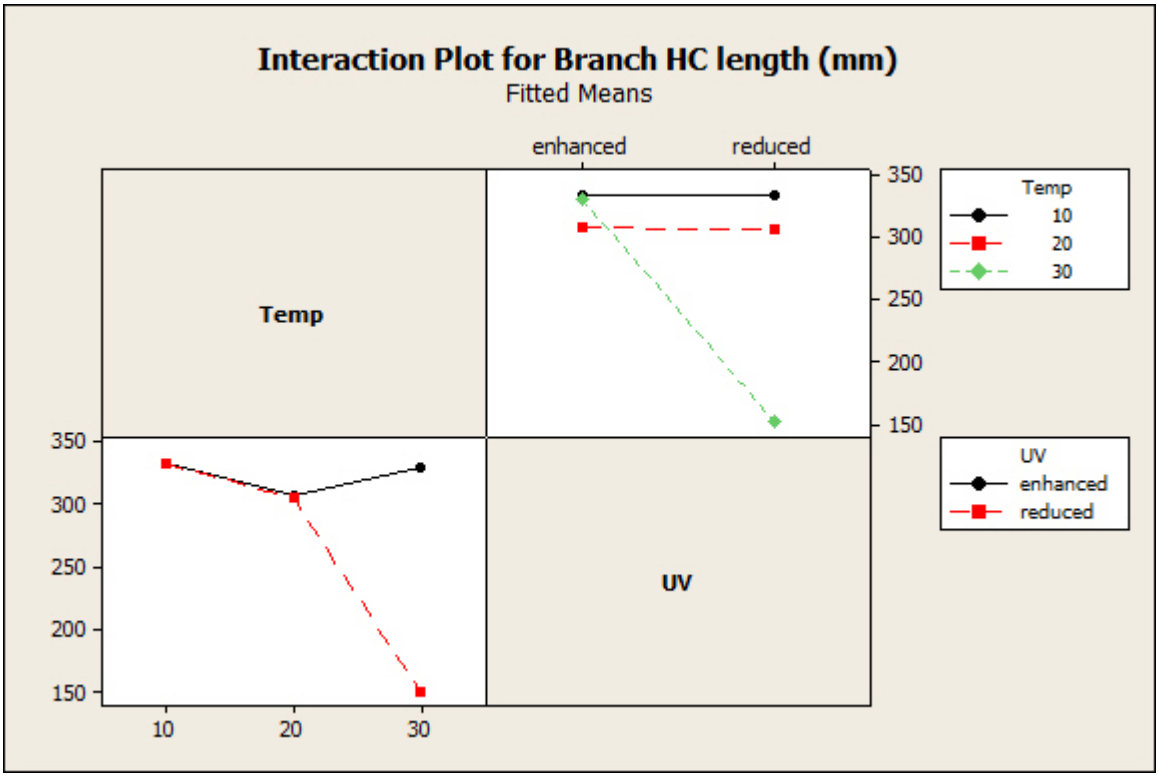


Figure 19. Interaction plot for branch leaf hyaline cell width

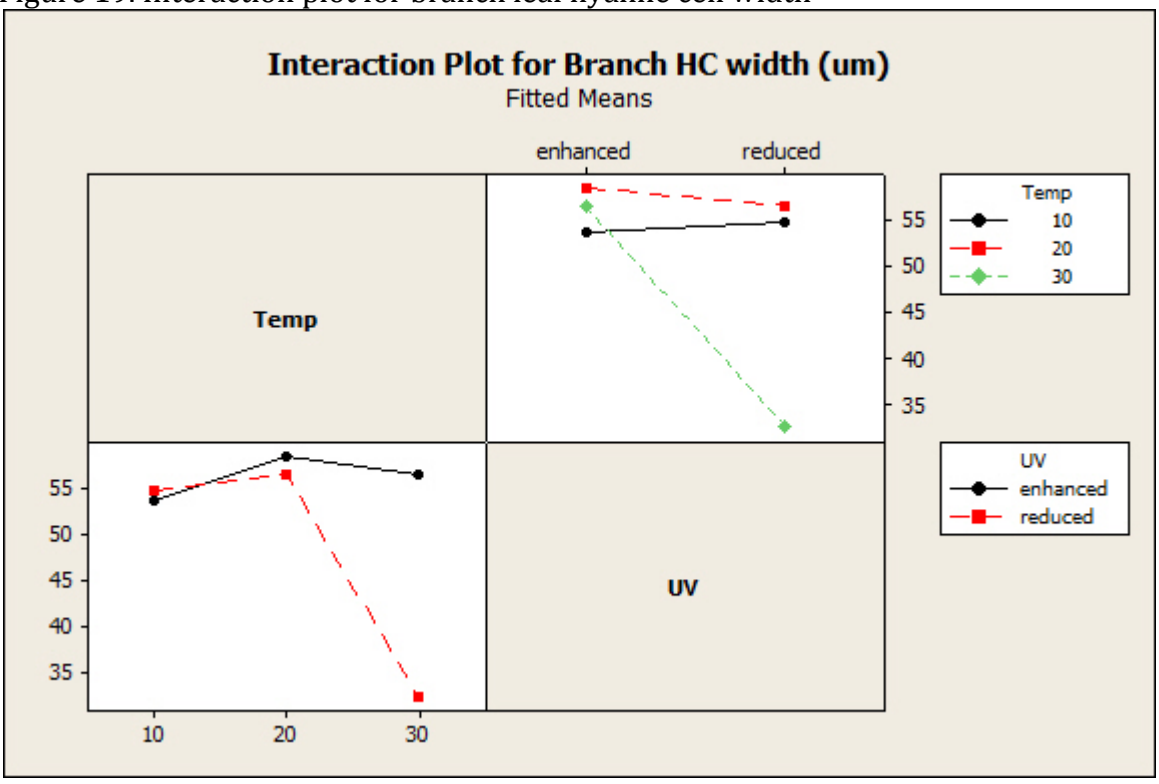


Figure 20. Interaction plot for leaf hyaline cell ring number

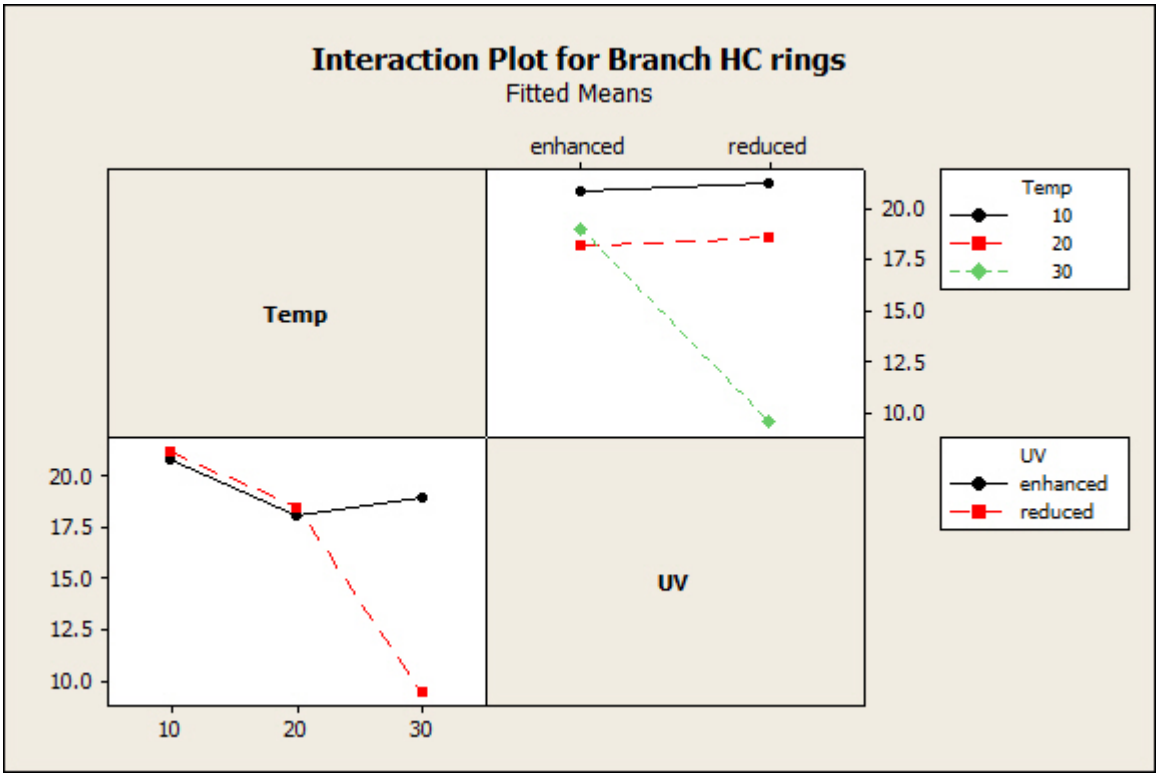


Figure 21. Interaction plot for branch hyaline cell pore number

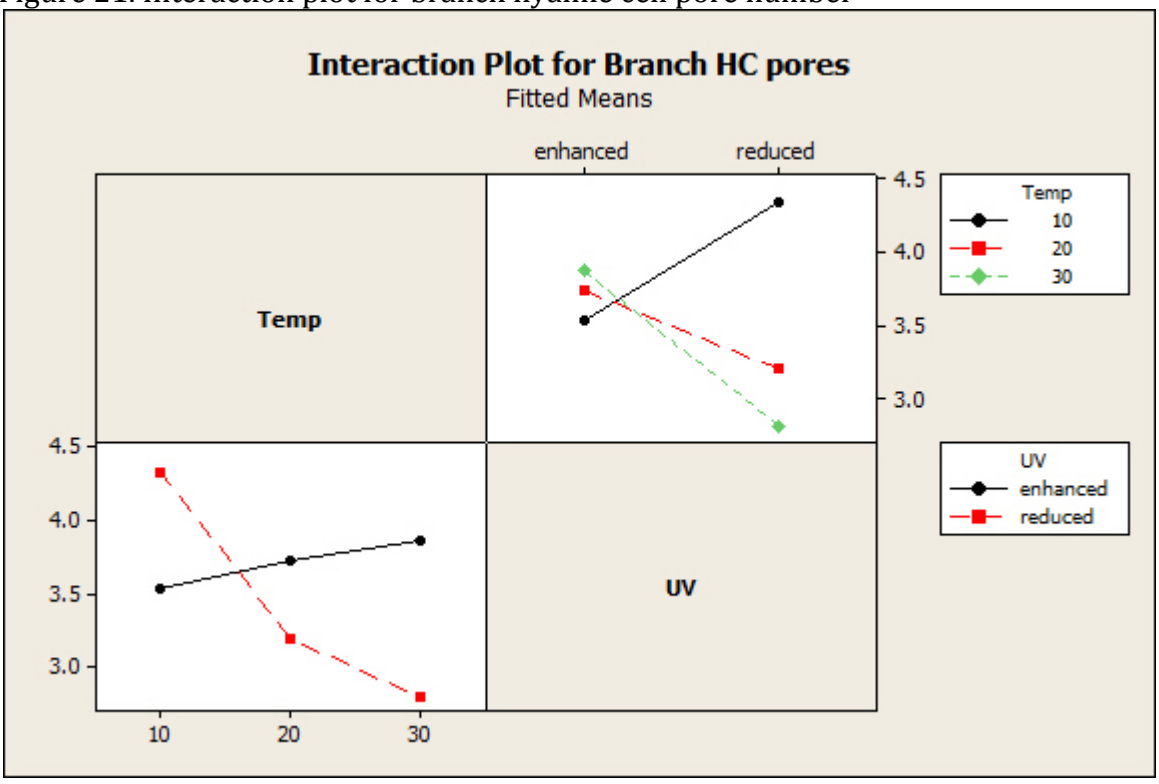


Figure 22. Interaction plot for branch leaf hyaline cell pore width

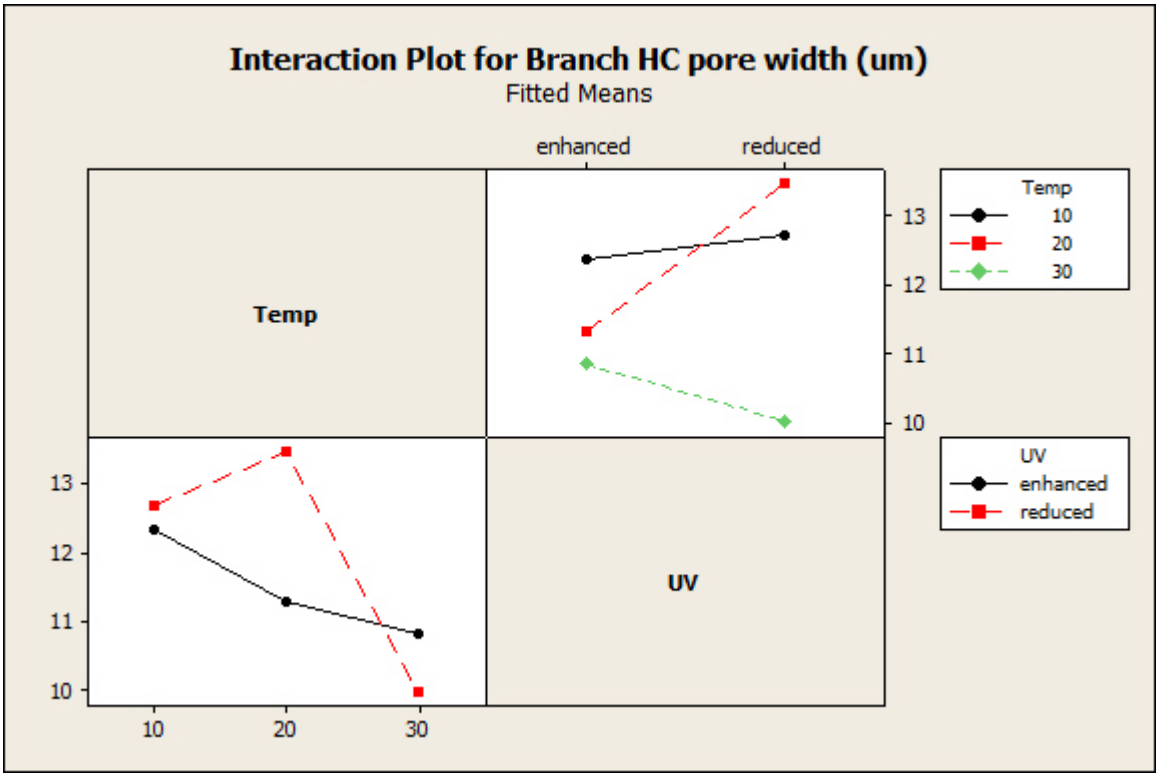


Figure 23. Interaction plot for branch leaf chlorophyllous cell length

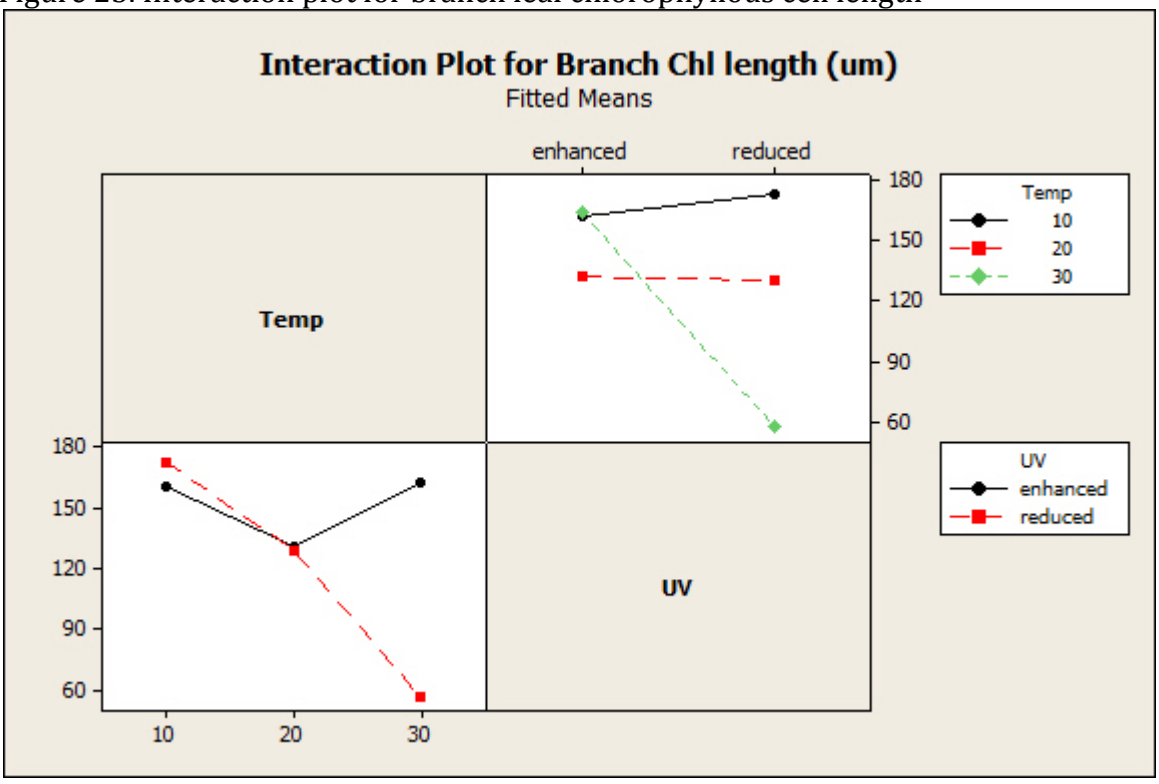


Figure 24. Interaction plot for branch leaf chlorophyllous cell width

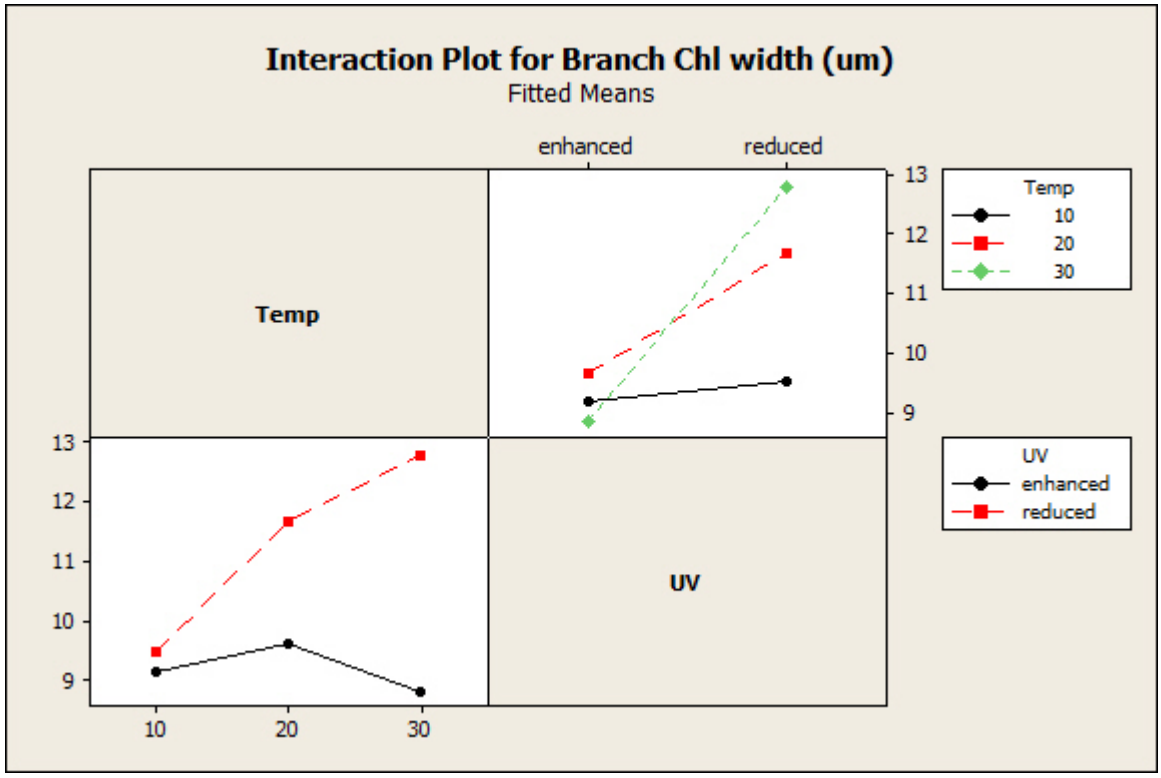


Figure 25. Interval plot for biomass

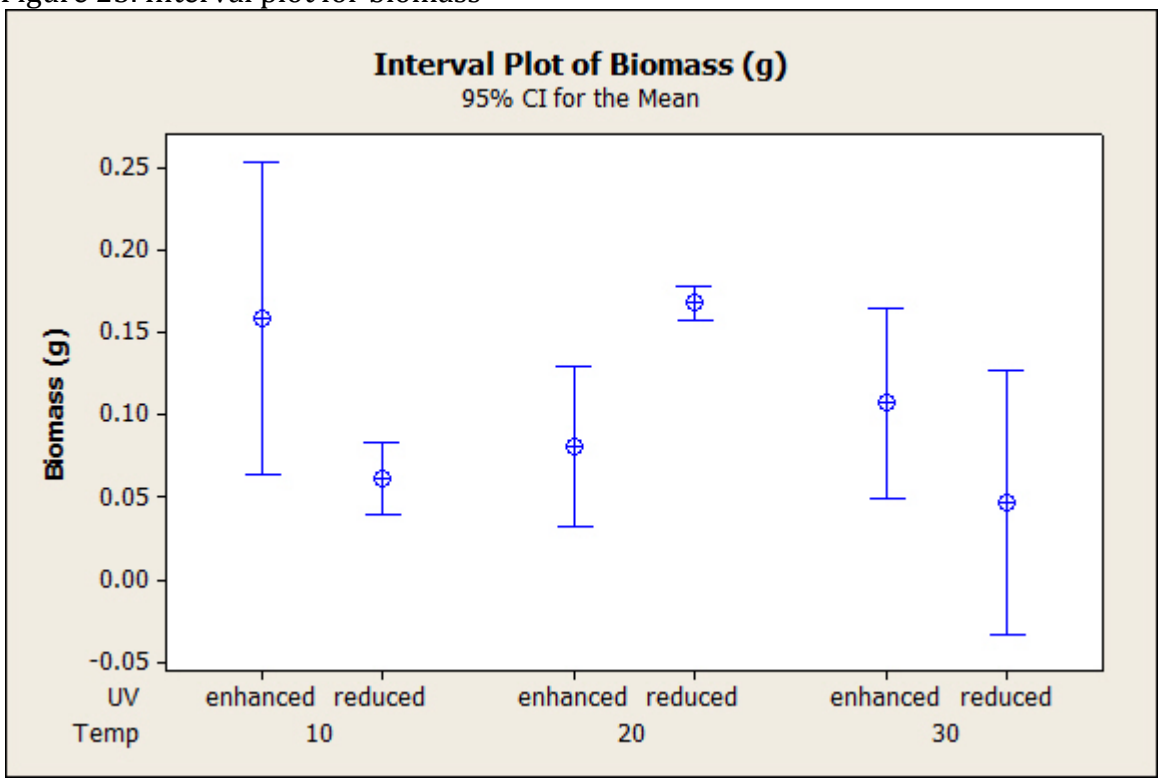


Figure 26. Interval plot for percent resistant carbon

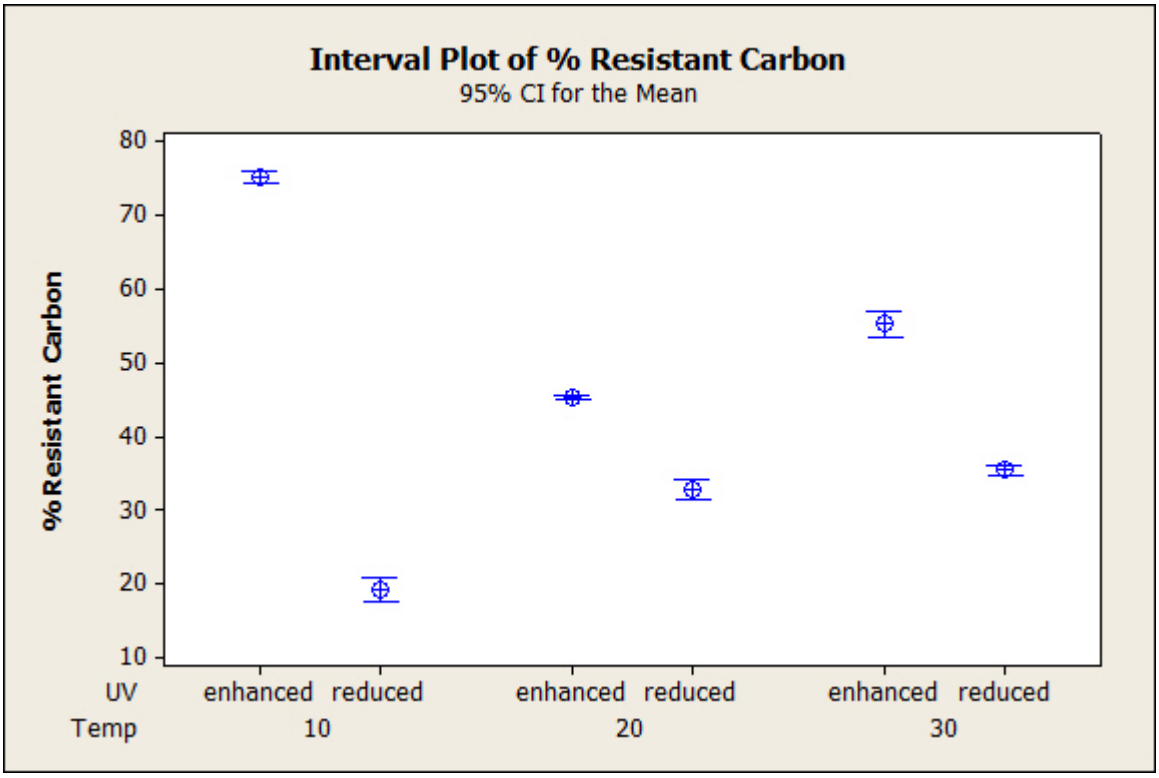


Figure 27. Interval plot for percent cellulose

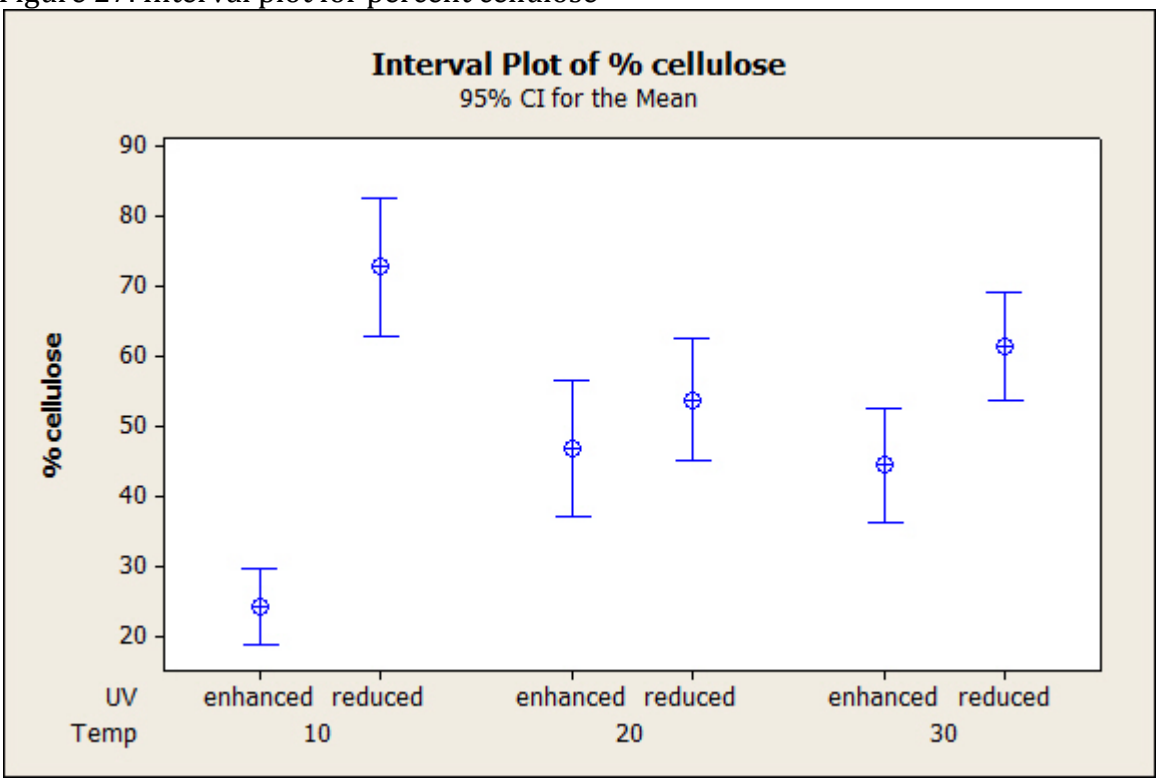


Figure 28. Interval plot for stem total width

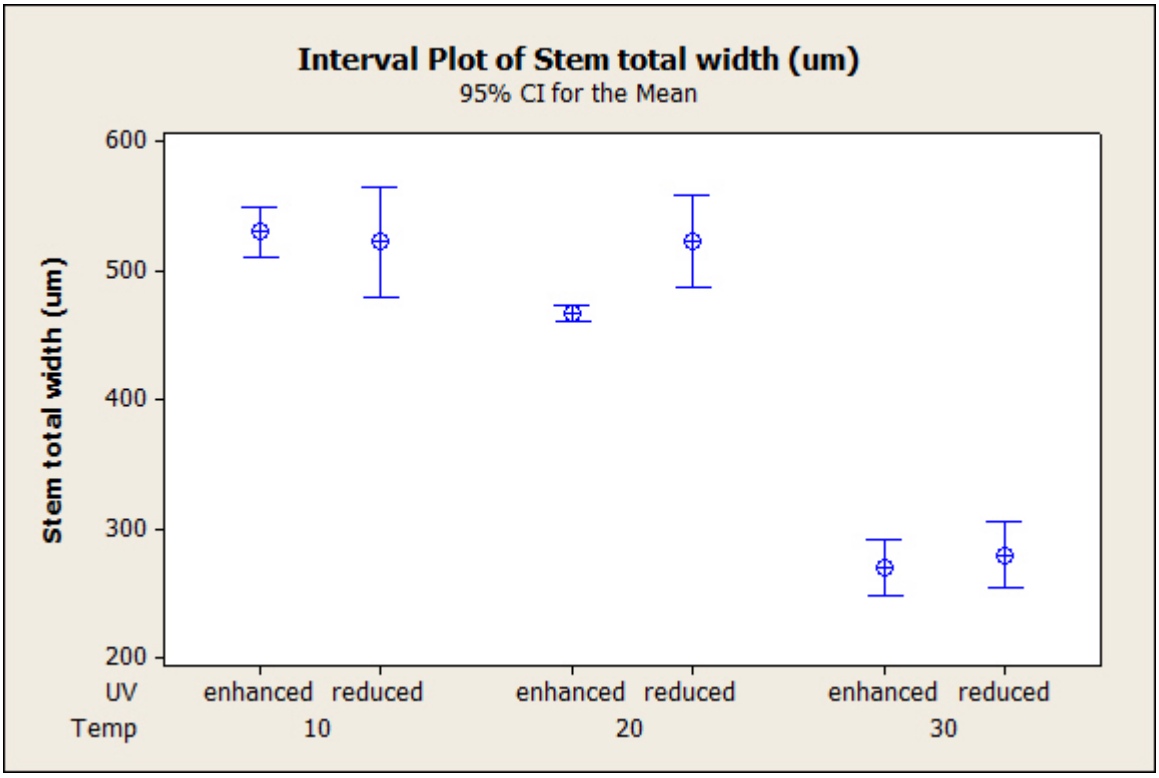


Figure 29. Interval plot for stem cortical cell width

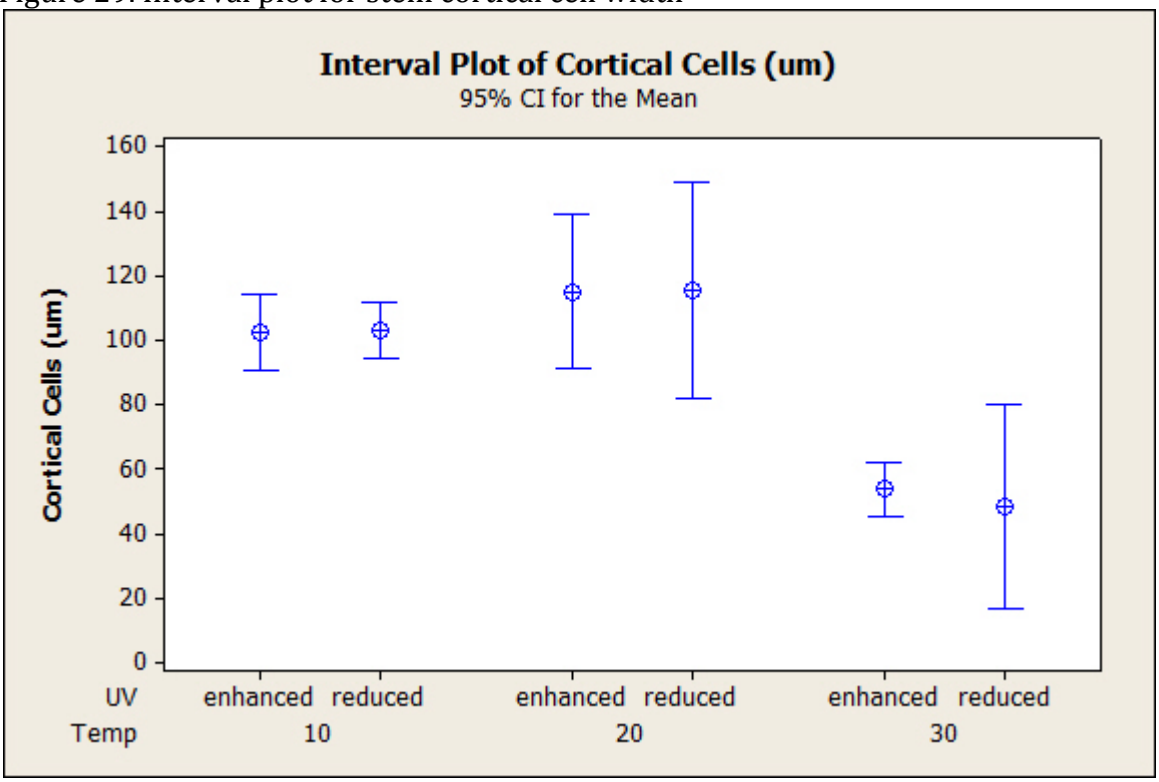


Figure 30. Interval plot for stem pith width

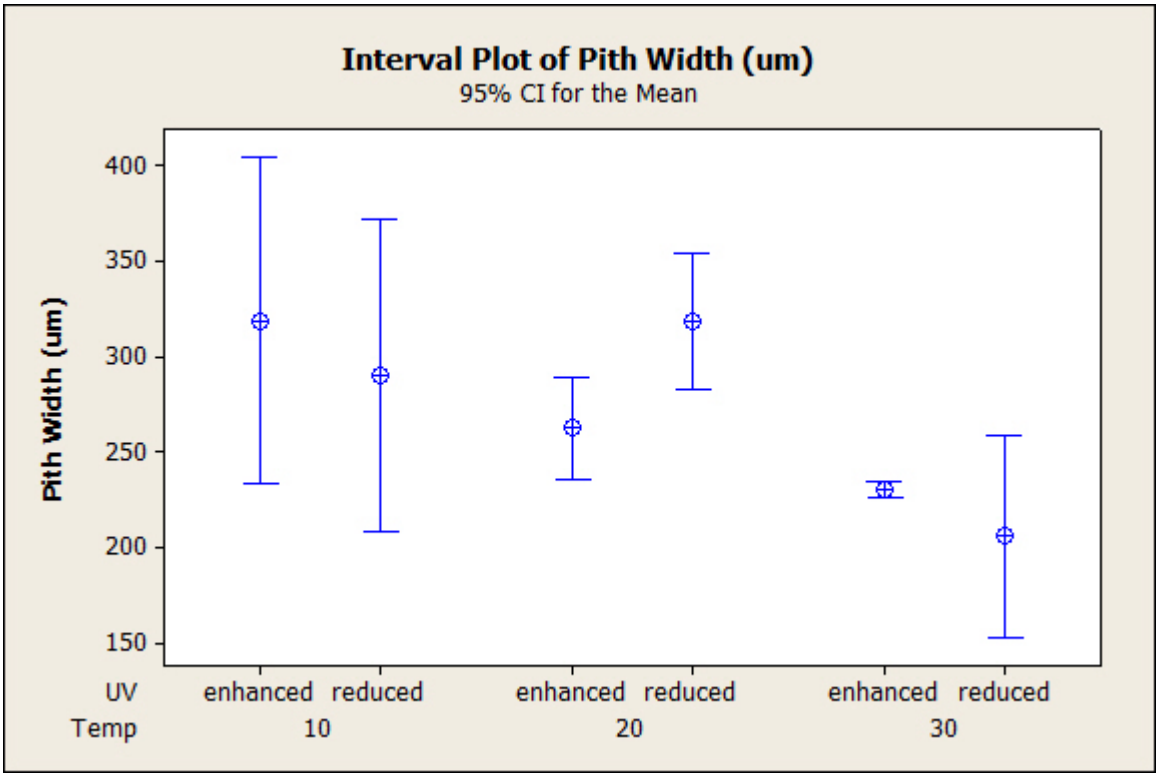


Figure 31. Interval plot for stem leaf length

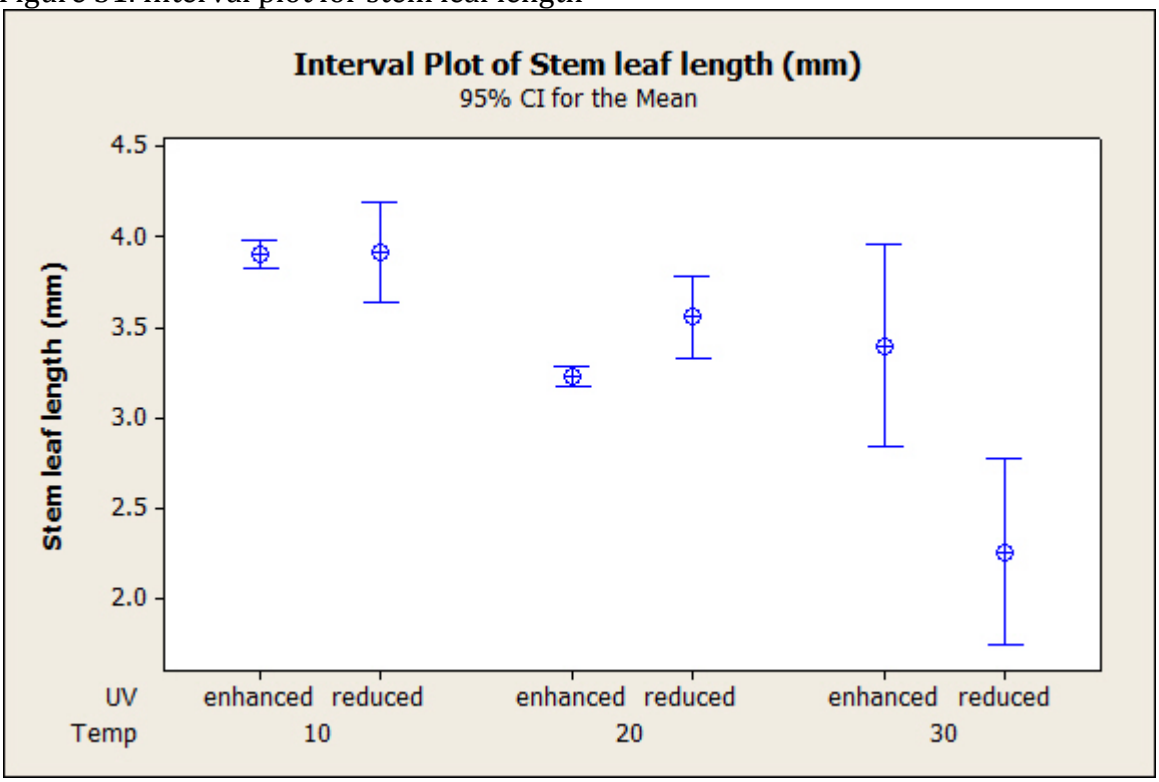


Figure 32. Interval plot for stem leaf width

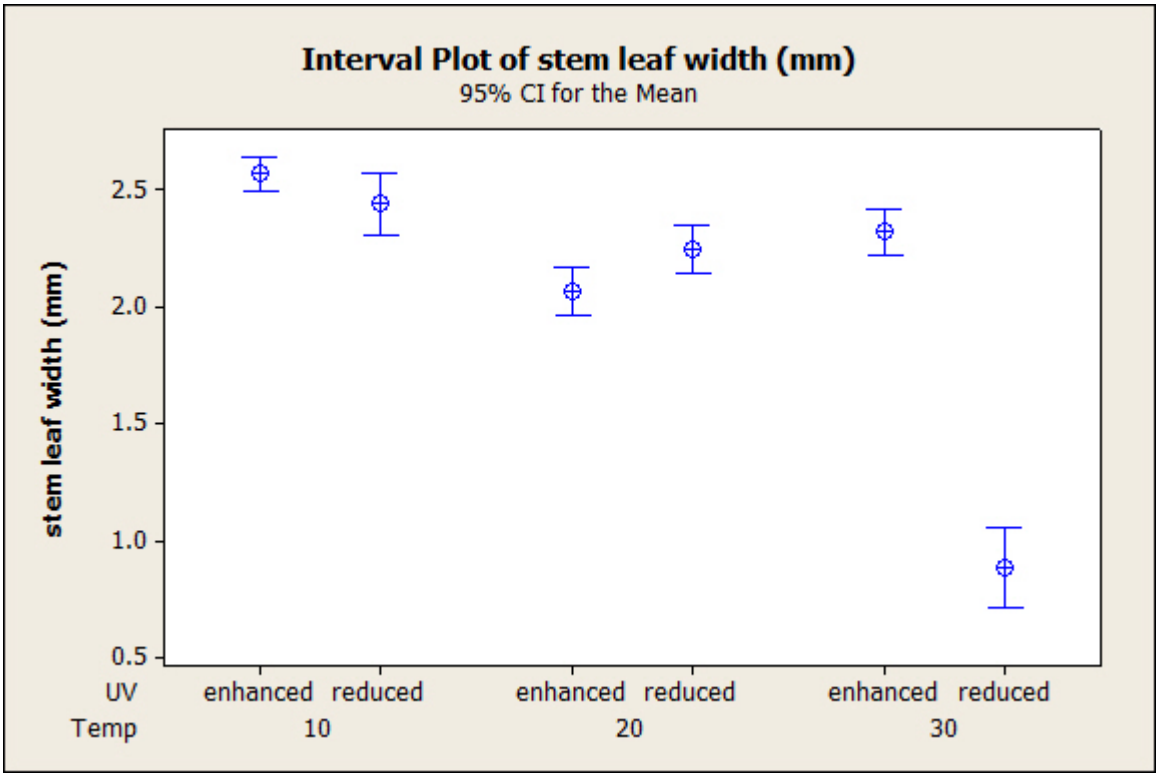


Figure 33. Interval plot for stem leaf hyaline cell length

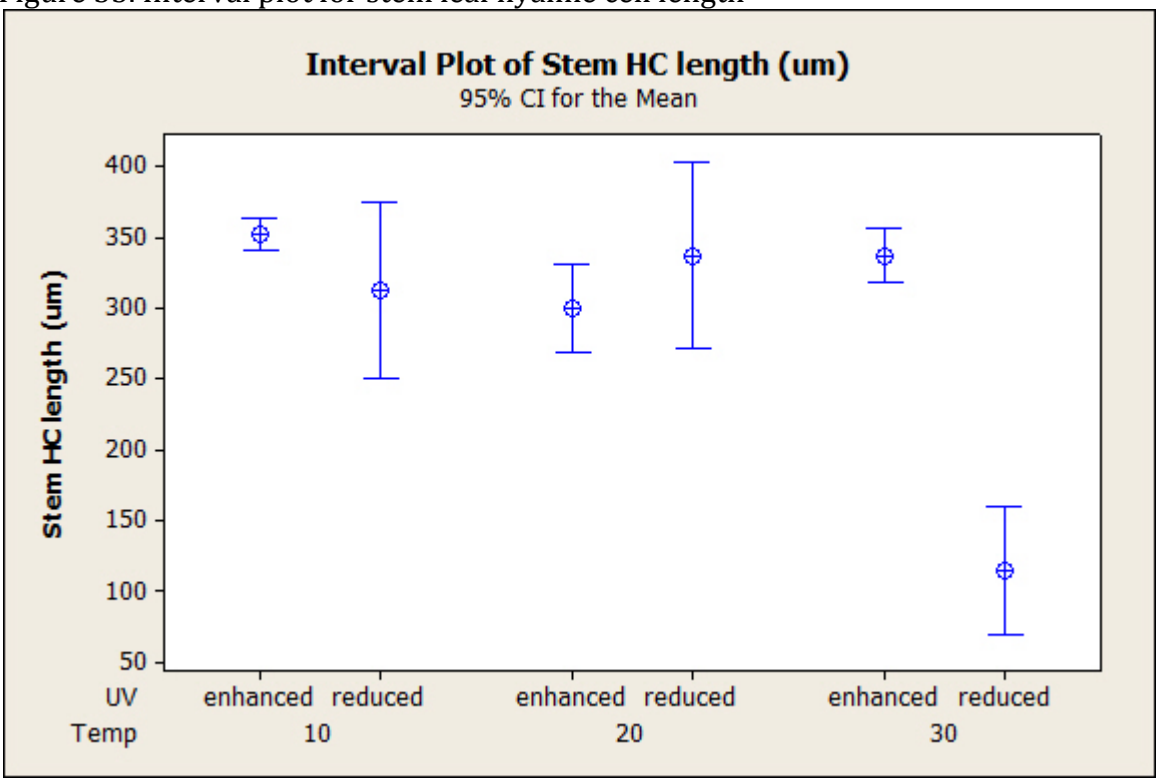


Figure 34. Interval plot for stem leaf hyaline cell width

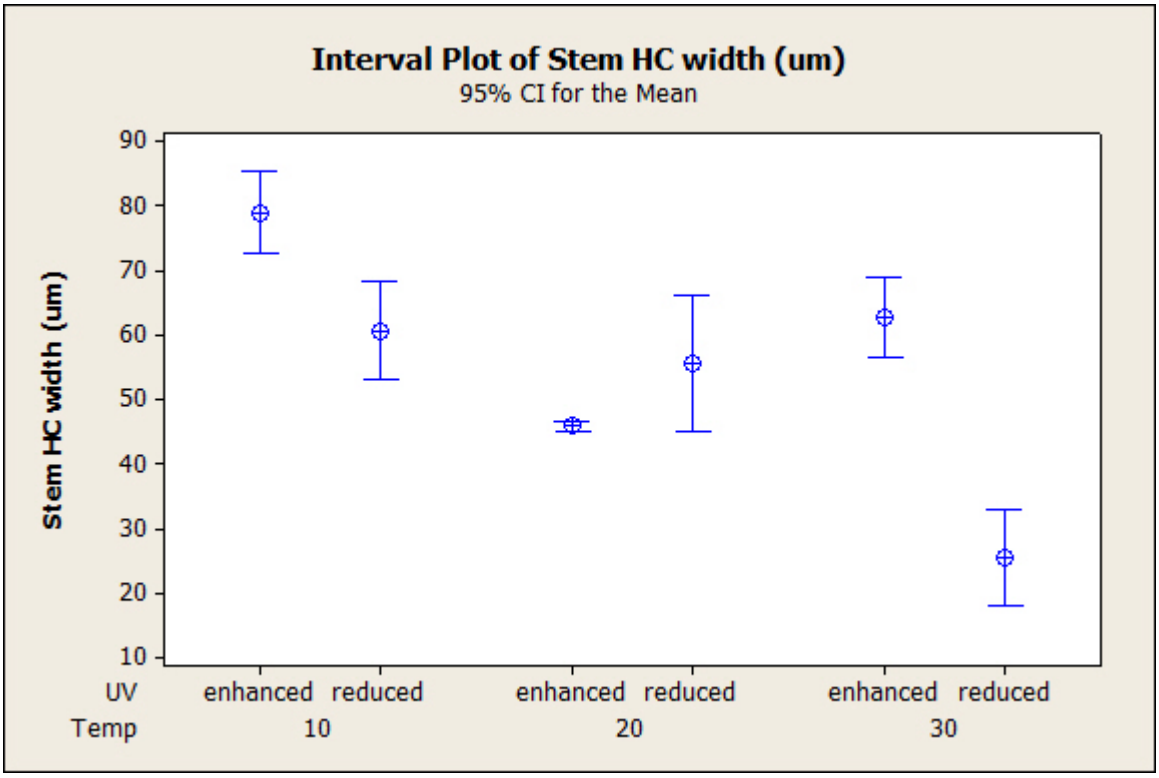


Figure 35. Interval plot for stem leaf hyaline cell ring number

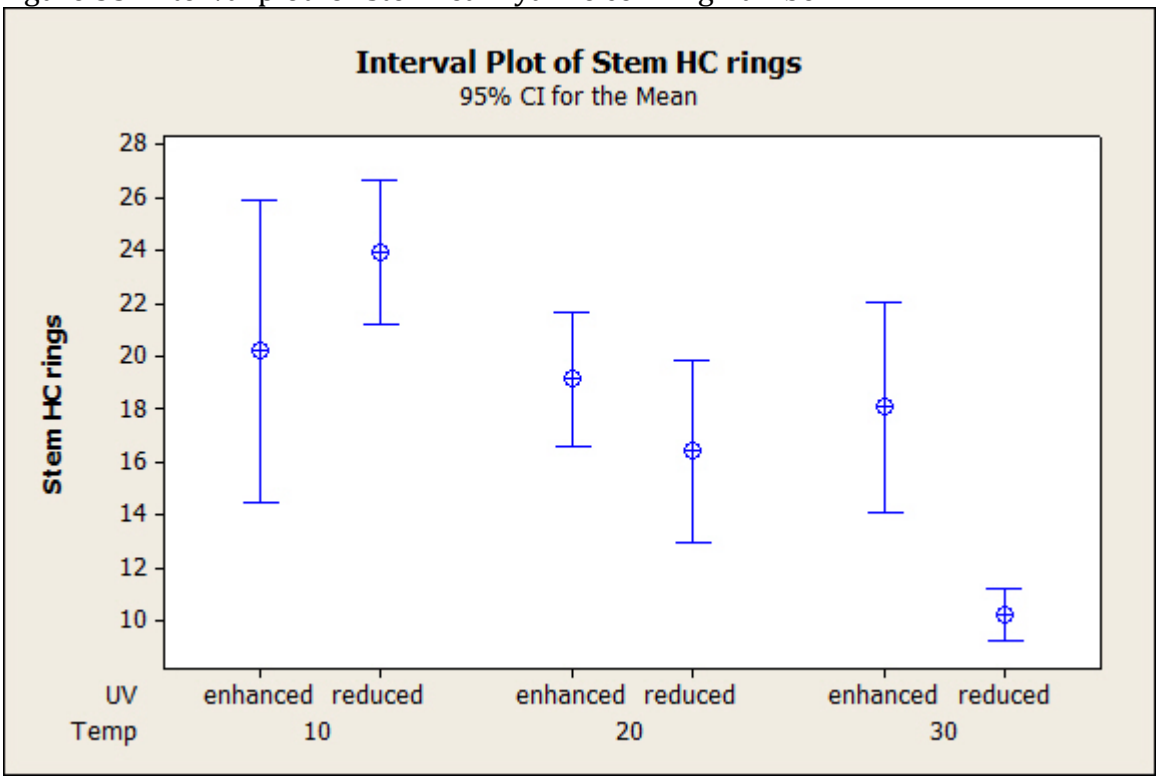


Figure 36. Interval plot for stem leaf hyaline cell pore number

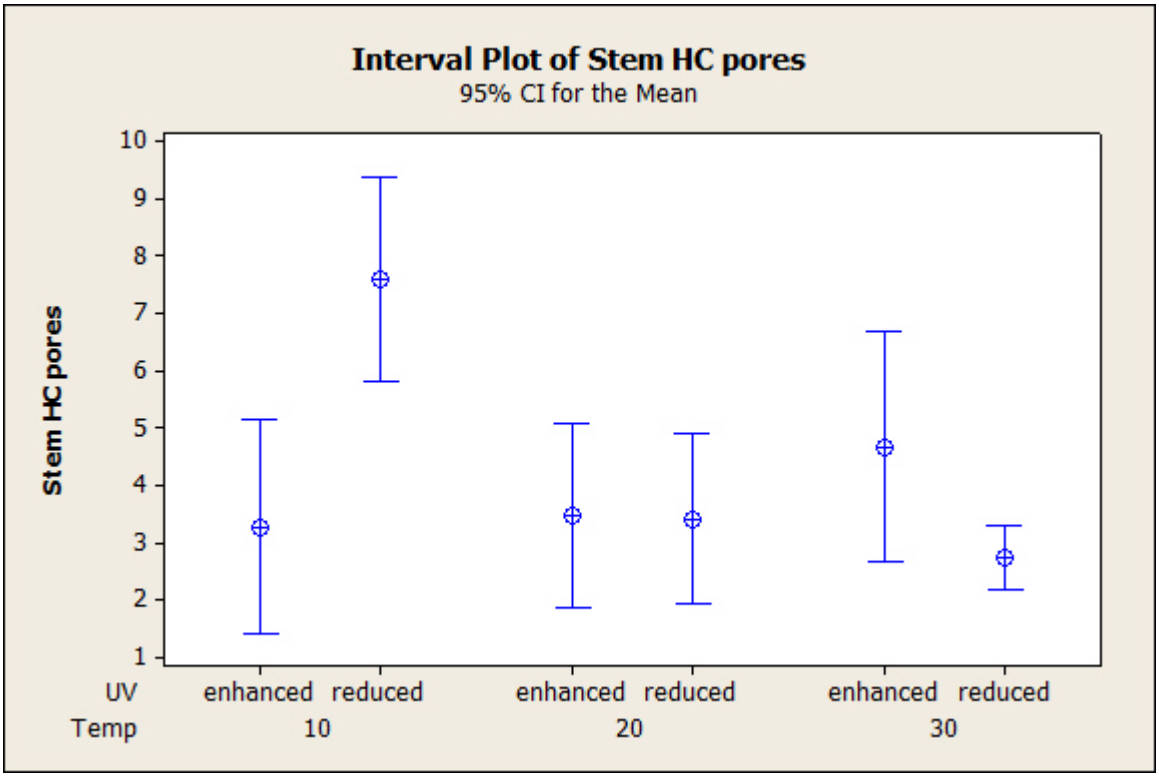


Figure 37. Interval plot for stem leaf hyaline cell pore width

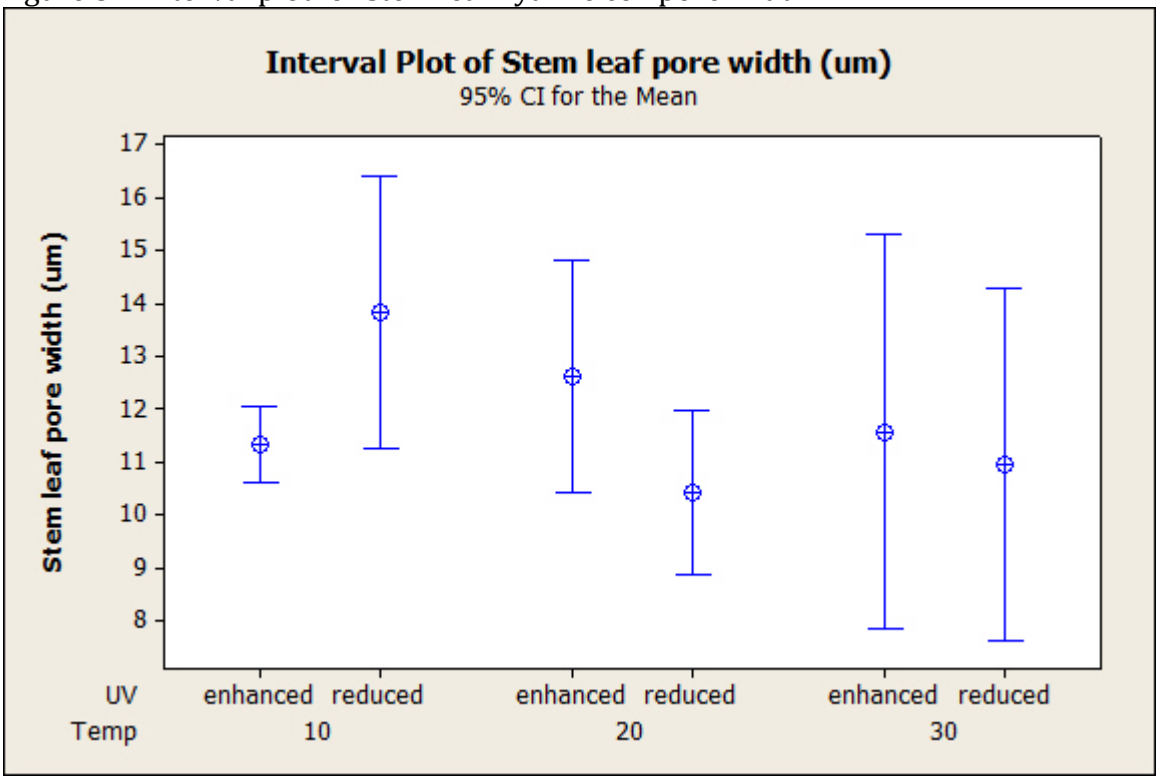


Figure 38. Interval plot for stem leaf chlorophyllous cell length

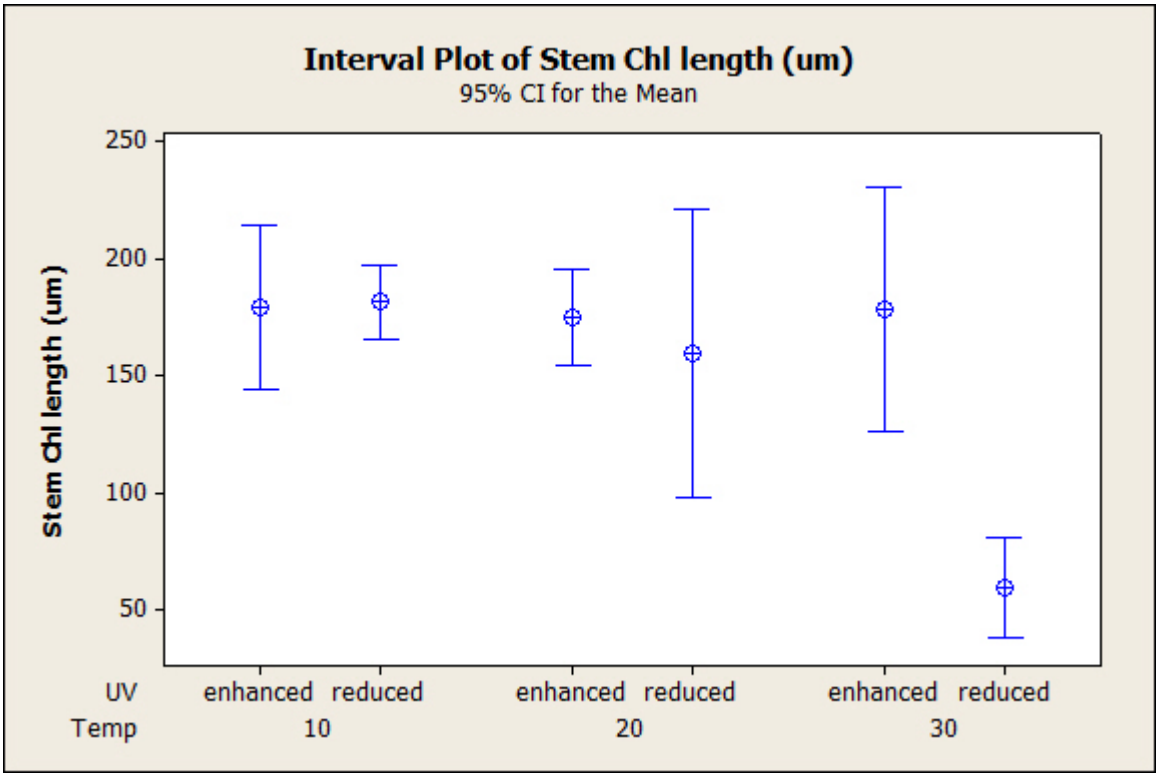


Figure 39. Interval plot for stem leaf chlorophyllous cell width

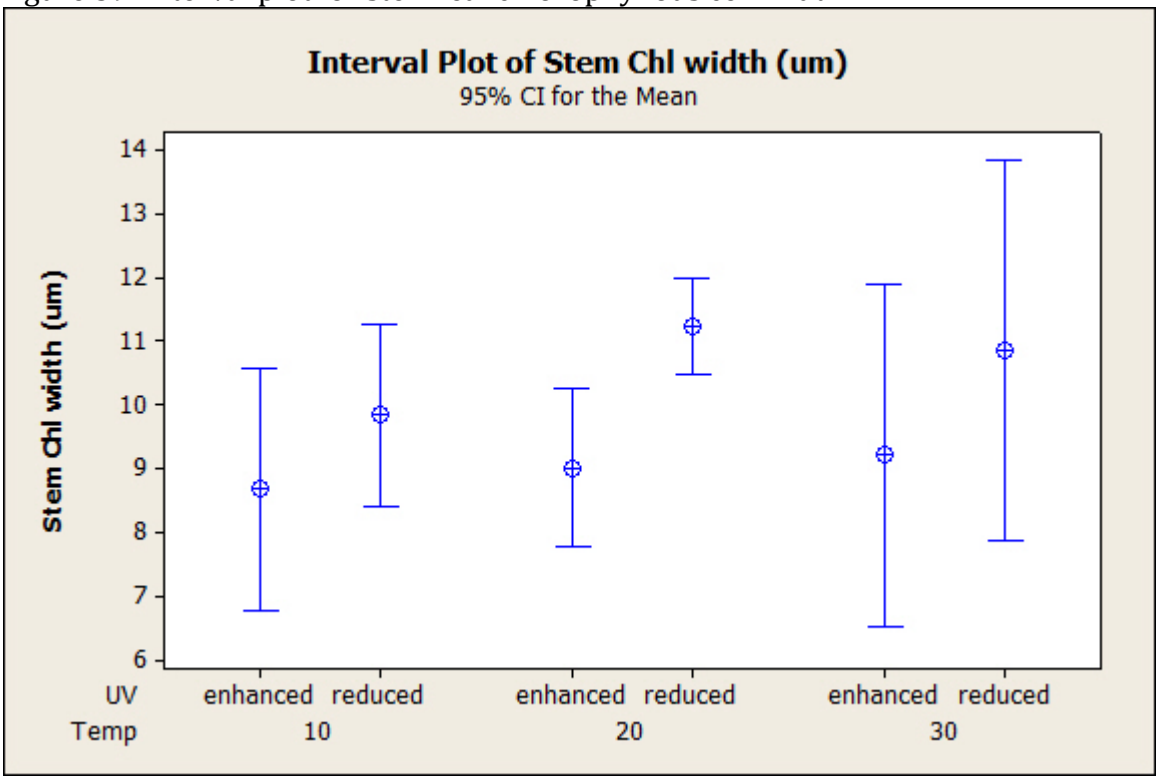


Figure 40. Interval plot for branch leaf length

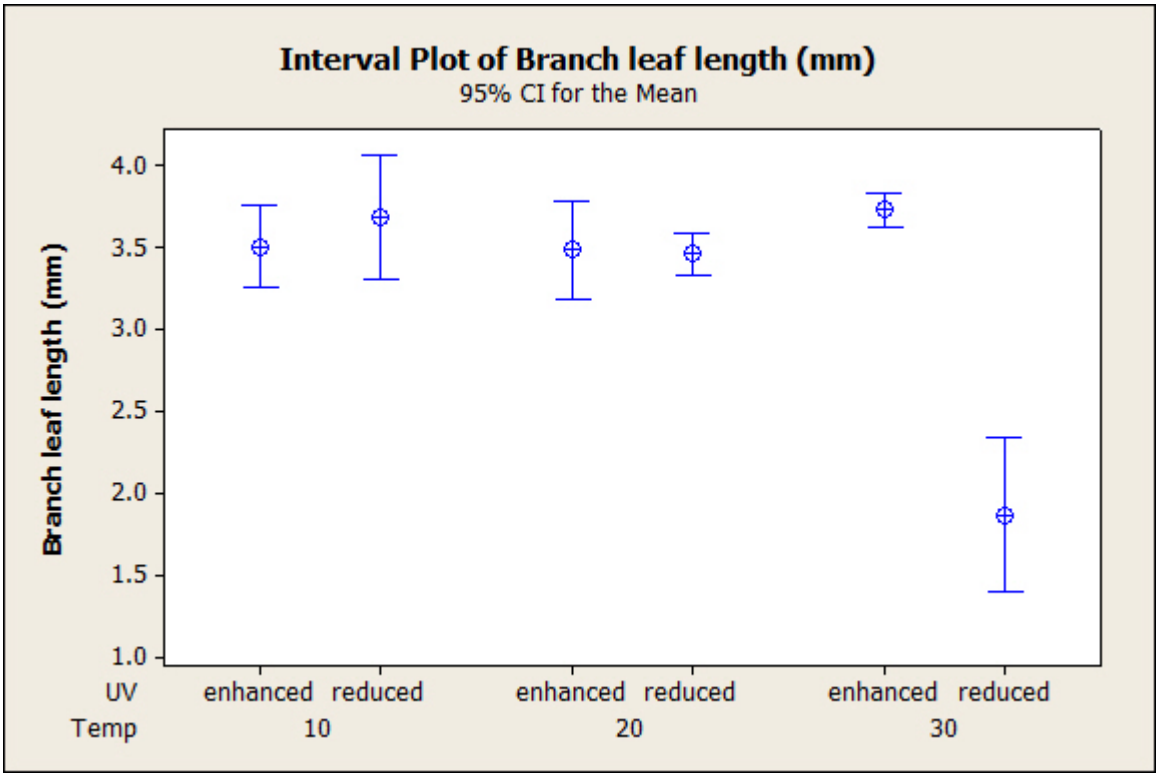


Figure 41. Interval plot for branch leaf width

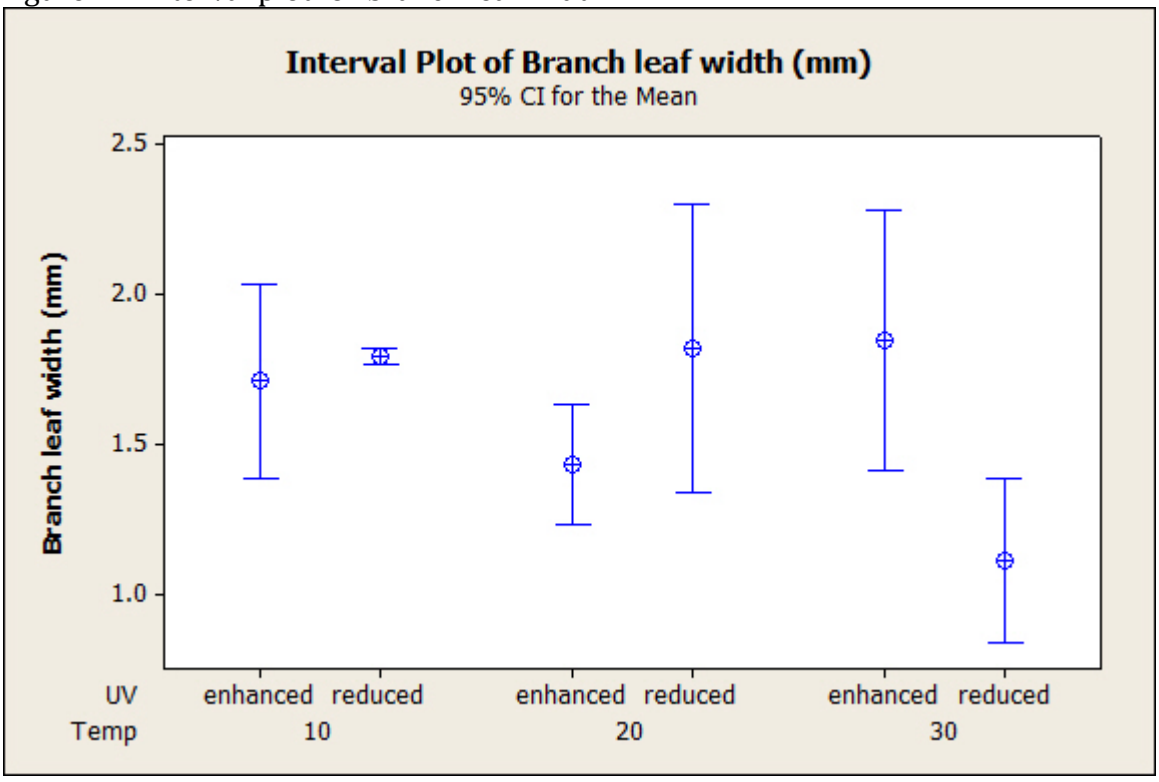


Figure 42. Interval plot for branch leaf hyaline cell length

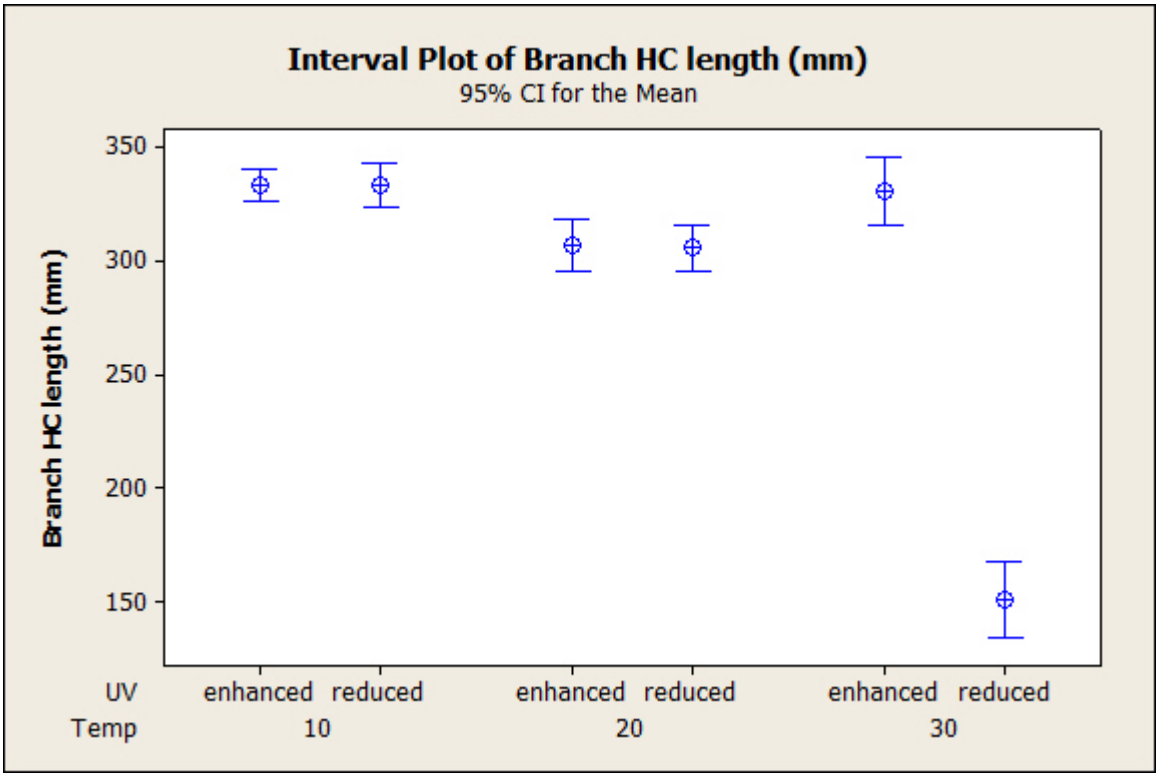


Figure 43. Branch leaf hyaline cell width

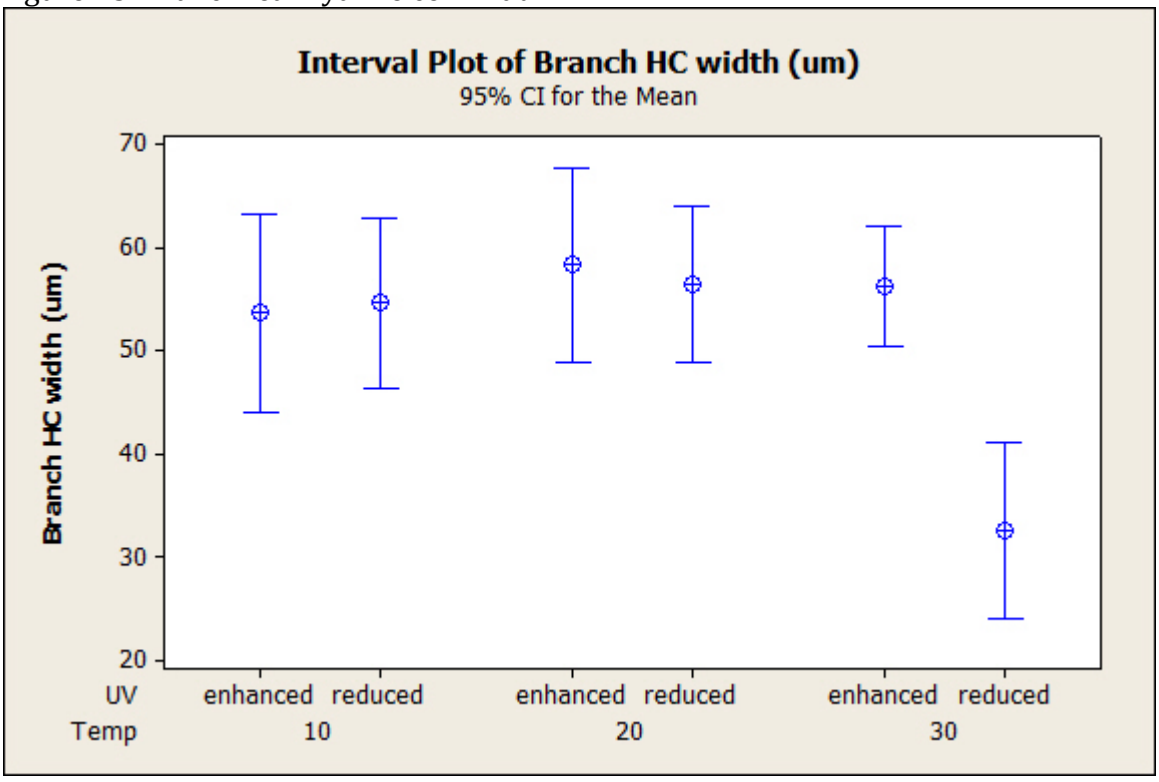


Figure 44. Interval plot for branch hyaline cell ring number

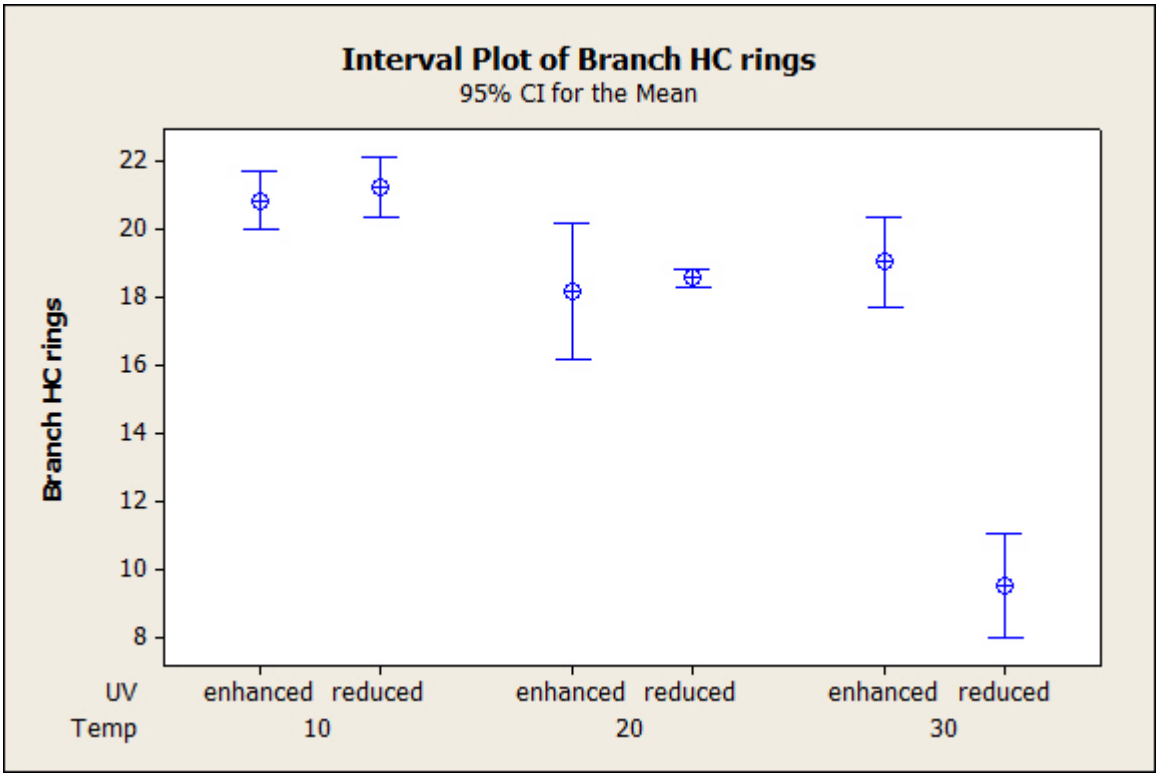


Figure 45. Interval plot for branch leaf hyaline cell pore number

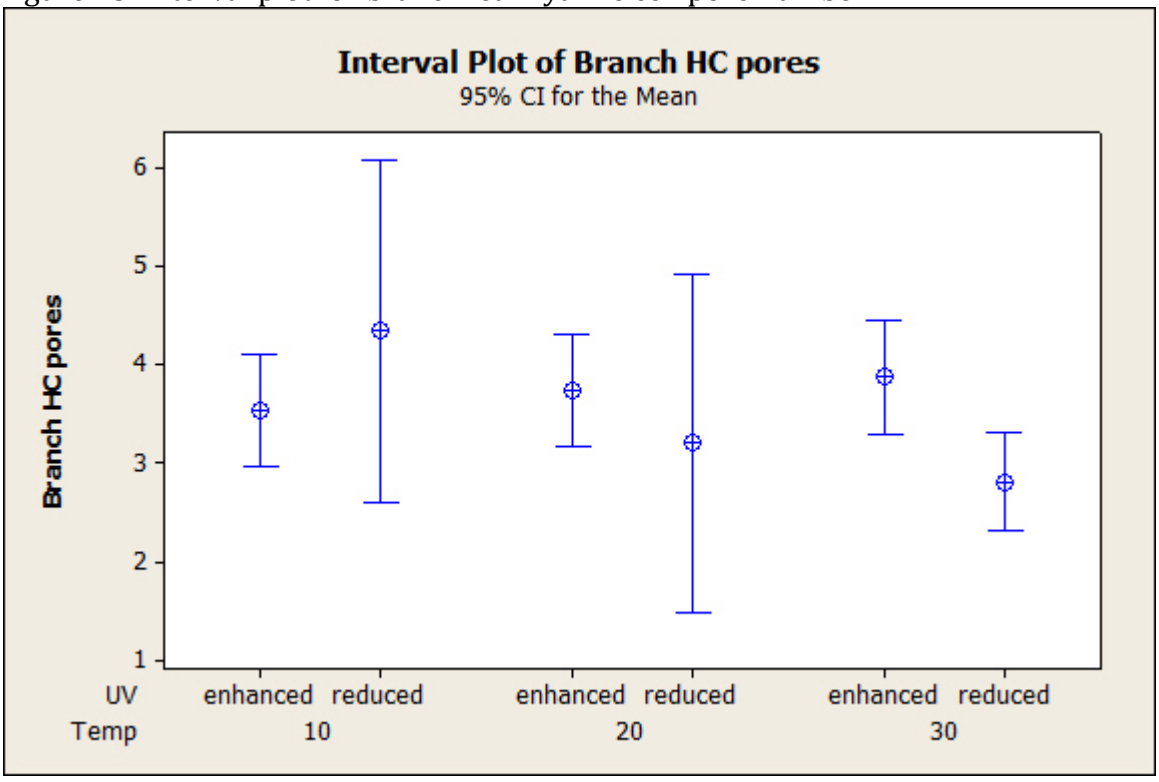


Figure 46. Interval plot for branch leaf hyaline cell pore width

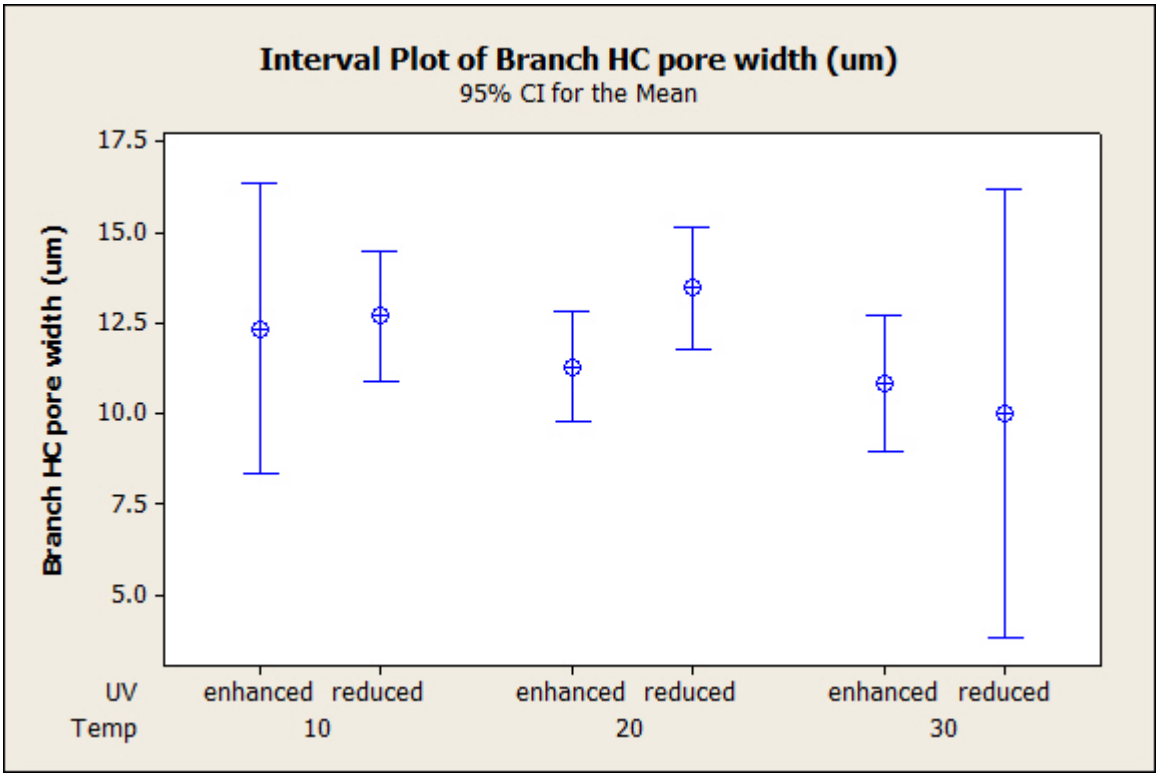


Figure 47. Interval plot for branch leaf chlorophyllous cell length

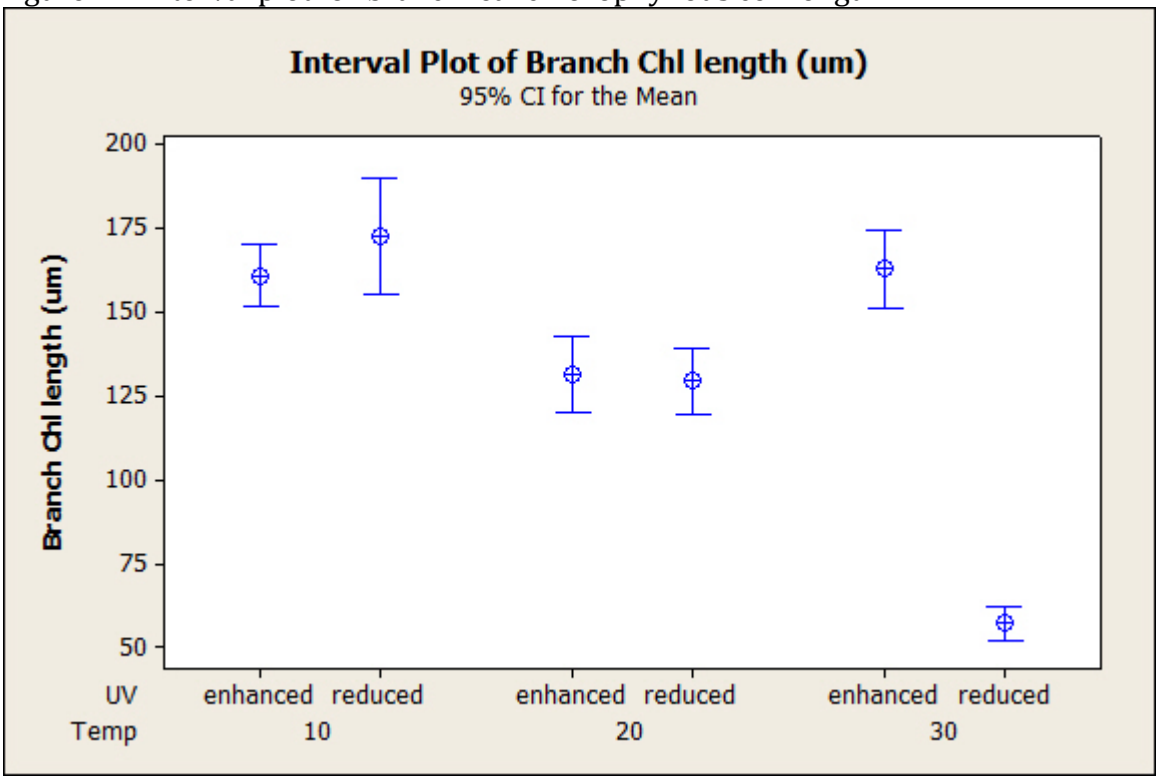


Figure 48. Interval plot for branch leaf chlorophyllous cell width