

EFFECTS OF TEMPERATURE ON SETA ELONGATION IN *ATRICHUM UNDULATUM*^{1, 2, 3}

DENNIS WM. STEVENSON⁴, JAMES R. RASTORFER, AND RAY E. SHOWMAN

Department of Botany, The Ohio State University, Columbus, Ohio 43210

ABSTRACT

Field-collected gametophytes of *Atrichum undulatum* were placed in two growth chambers which were maintained at the same light intensity and light period, but at two different temperature regimes. After sporophyte development, differences in seta lengths were observed. Measurements of cell lengths revealed that attached setae grown in the high-temperature regime (12°-22°C) were longer than those grown in a low-temperature regime (3°-12°C), as a result of both more cell divisions and a larger average cell length. Thus, temperature appeared to influence both cell division and cell elongation in the setae of *Atrichum undulatum*.

INTRODUCTION

Sporophytes of most liverworts and mosses (Bryophyta) consist of a basal foot attached to the gametophyte and a seta or stalk, which supports a spore-bearing capsule at its apex. Lengths of setae at sporophyte maturity differ among species, varying from a minute structure to a conspicuous organ which may exceed 5 cm (Watson, 1964). Setae of the Common Hair-Cap Moss (*Polytrichum commune*) are reported to vary from 6 to 12 cm in length (Welch, 1957), but unfortunately it is not clearly understood whether these differences are caused by genetic factors or environmental factors or both. A few studies have suggested that seta elongation can apparently be influenced by environmental factors (e.g. temperature; light intensity; day length), and that there may be interactions of internal plant factors (e.g. age of sporophyte; hormone balance) with environmental factors which affect seta elongation in bryophytes (Asprey, Benson-Evans, and Lyon, 1958; Crombie and Paton, 1958; Benson-Evans, 1961; Hughes, 1962; Slade, 1965).

Hughes' (1962) work was particularly important in illustrating an apparent influence of different day lengths on sporangial development and seta length of *Polytrichum aloides*. Plants with young sporophytes were collected in the field by him and subsequently exposed to different light periods in the laboratory. Short days of six hours of light per day appeared to stimulate sporangial development and also resulted in shorter setae (averaging 26.5 mm). In contrast, long days of eighteen hours of light per day appeared to delay sporangial development and also resulted in longer setae (averaging 45.7 mm). He examined the central strand of macerated setae of sporophytes exposed to short days and long days and concluded that an increase in cell length alone accounted for the differences in seta lengths. Our study was undertaken to determine the effects of different temperatures on length of setae, with respect to cell size and cell number, of *Atrichum undulatum*.

MATERIALS AND METHODS

Gametophyte sods of *Atrichum undulatum* (Hedw.) Beav. were collected on 6 June, 1968, south of Bonham Road above Harkers Run, Section 13, Oxford Township, Butler County, Ohio. The collected specimens were divided into two equal portions. Each portion was then placed in a plastic crisper having six drainage holes (1/4 inch in diameter) and containing about one inch of vermiculite

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⁴Present Address: Department of Botany, University of California, Davis, California 95616.

on the bottom, on which the *Atrichum* gametophytes were placed. Then each crisper was covered with a clear plastic lid and placed in one of two different growth chambers.

Both growth chambers had the same length and timing of light and dark periods and the same light intensities, but each had different temperature regimes (fig. 1).

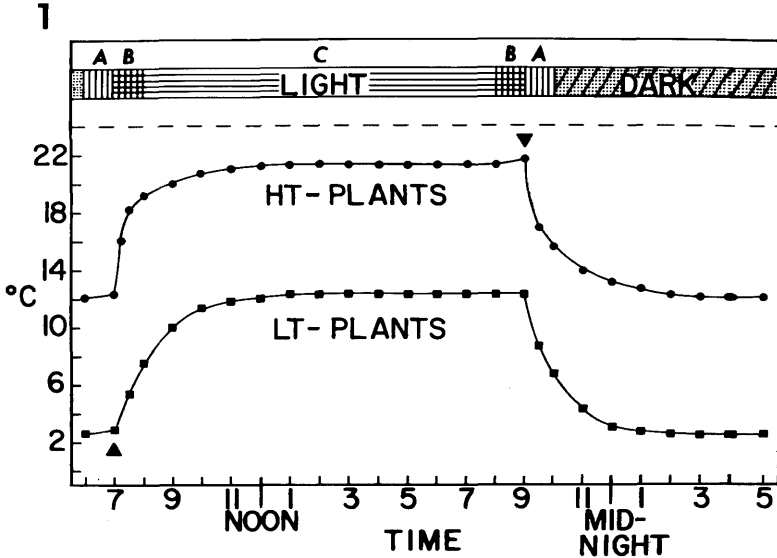


FIGURE 1. Light and temperature conditions imposed on gametophytes and sporophytes of *Atrichum undulatum*. A—incandescent light at 0.117 mW cm^{-2} ; B—incandescent + fluorescent light at 2.64 mW cm^{-2} ; C—fluorescent light at 2.50 mW cm^{-2} . Arrows indicate clock times for temperature cycle switching.

Spectral light intensities were measured at plant level (beneath the plastic lid) with an ISCO spectroradiometer at 25 nm increments from 400–750 nm. Corrected spectral intensities were integrated and are reported in milliwatts per centimeter squared (mW cm^{-2}) (fig. 1). The growth chambers were programmed for high- and low-temperature cycles. Temperatures were measured with a YSI Thermistemp Tele-Thermometer and a recorder, employing a thermistor probe placed among the gametophytic shoots (inside closed crisper). Although the diurnal temperature patterns were similar in both situations, temperatures at plant level in the growth chamber having the low-temperature regime (LT) reached a minimum of 3°C during the dark period and a maximum of 12°C during the light period. Temperatures at plant level in the growth chamber having the high-temperature regime (HT) ranged from 12° to 22°C (fig. 1). Once a week the plants were supplied with a one-tenth concentration of Hoagland solution.

Developing sporophytes were first noticed six weeks after the gametophytes were placed in the high- and low-temperature growth chambers. Whether fertilization occurred in the field or in the growth chambers is unknown. After further sporophyte development, a substantial difference in seta lengths was quite apparent. Sporophytes were harvested on 28 and 30 October 1968, before capsule dehiscence. Some sporophytes were used for measurements of total seta lengths and others were killed and fixed in FPA (1 part formalin, 1 part propionic acid, and 18 parts 50% ethanol). Several fixed setae were cleared and macerated in a 4–5% sodium hypochlorite solution (full strength Clorox) and subsequently mounted in a Hoyer medium (Anderson, 1954). Portions of other fixed setae were

parafin-embedded and sectioned transversely and longitudinally on a rotary microtome. These sections were then stained with safranin O and aniline blue.

OBSERVATIONS

Setae of attached sporophytes cultured at the higher temperatures (HT setae) were longer than were those cultured at the lower temperatures (LT setae). The mean length for HT setae was 41.4 mm, whereas the mean length for LT setae was 13.3 mm (Table 1).

TABLE 1

Ranges, means (\bar{X}), and standard deviations (SD) for seta lengths and seta-cell lengths of *Atrichum undulatum*

	Seta Lengths (mm)		Cell Lengths (μ)			
			Macerated Setae		Sectioned Setae	
	HT	LT	HT	LT	HT	LT
Range {	31.1	10.6	182.0	113.0	90.0	40.0
to			to	to	to	to
\bar{X}	45.3	17.3	518.0	378.0	270.0	230.0
SD	41.4	13.3	335.0	239.0	164.0	108.0
N	2.7	1.8	74.0	49.2	35.9	30.4
	20.0	20.0	500.0	400.0	450.0	450.0

HT refers to plants cultured in the high temperature regime (12° to 22°C) and LT to those cultured in the low temperature regime (3° to 12°C).

Cell measurements were made from macerated HT and LT setae (Table 1). In addition, lengths of cells in longitudinal sections of HT and LT setae were measured randomly as a control. Ranges in cell lengths in these sectioned setae were much lower than were ranges found in macerated setae. Furthermore, cells tended to be shorter in the central strand and inner cortex than in the outer cortex and epidermis (fig. 2).

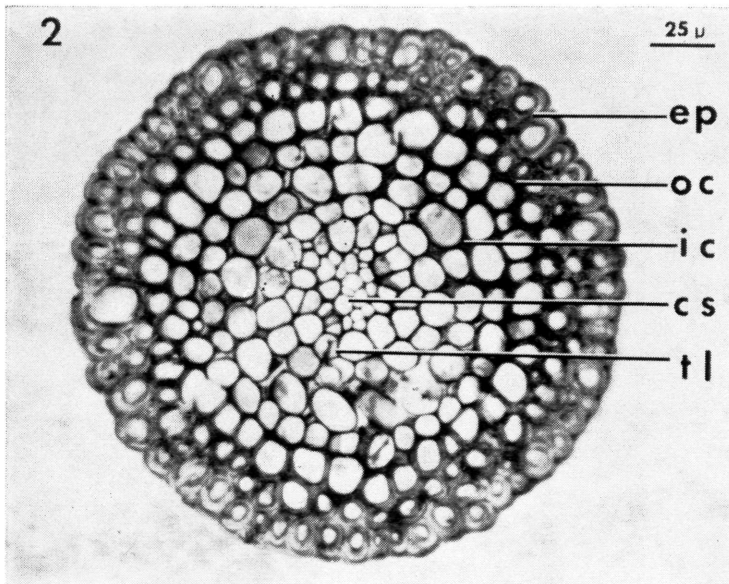


FIGURE 2. Transverse section through a seta of *Atrichum undulatum* from a plant cultured in the low temperature regime (3°–12°C); ep—epidermis; oc—outer cortex; ic—inner cortex; tl—layer of cells with slightly thickened walls and; cs—central strand.

To resolve the difference in cell-length ranges between macerated and sectioned setae, transverse and longitudinal sections were placed in the depressions of micro-culture slides. Paraffin was removed from the sections, slide depressions were filled with a 4-5% sodium hypochlorite solution, and cover slips were applied. After a few hours, all cells of the inner cortex and inward were dissolved, while cells of the outer cortex and epidermis were still intact, but were separated from each other. Based on these observations, cell-length measurements obtained from macerated setae were considered to represent cells of the epidermis and outer cortex. On the other hand, cell-length measurements of sectioned setae were made only on cells of the inner cortex, the layer of cells with slightly thickened walls, and the of central strand (fig. 2). Because of this difference, data on cell-length measurements of macerated setae were handled separately from those of sectioned setae.

Cell lengths of macerated HT setae ranged from 182 to 518 μ , with a mode of 267-280 μ (fig. 3) and a mean of 335 μ (Table 1). In contrast, the range of cell

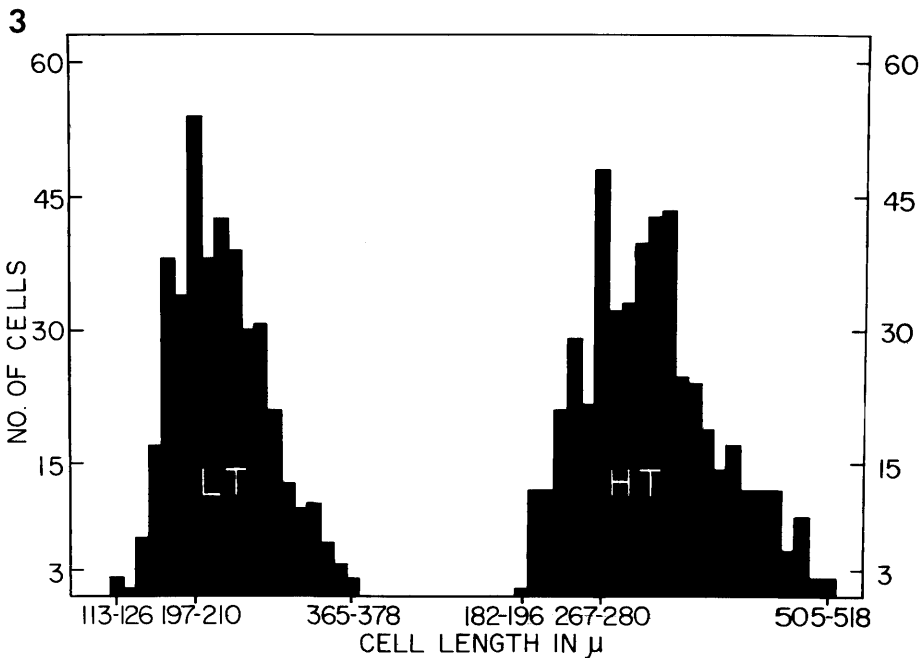


FIGURE 3. The distribution of cell lengths measured in macerated setae of *Atrichum undulatum*.

lengths in macerated LT setae was much lower, from 113 to 378 μ , with a mode of 197-210 μ (fig. 3) and a mean of 239 μ (Table 1). Sectioned setae had lower ranges of cell lengths in both HT and LT setae than did those in macerated HT and LT setae. Cell lengths of sectioned HT setae ranged from 90 to 270 μ , with a mode of 150-160 μ (fig. 4) and a mean of 164 μ (Table 1). In sectioned LT setae, the range of cell lengths was lower, from 40 to 230 μ , with a mode of 100-120 μ (fig. 4) and a mean of 108 μ (Table 1).

Ratios of mean cell lengths for HT setae to LT setae were 1.4:1 and 1.5:1 for macerated and for sectioned setae, respectively (Table 2). The ratio of combined data (cell lengths) for macerated and sectioned HT setae to combined data for macerated and sectioned LT setae was 1.4:1, as would be expected (Table 2).

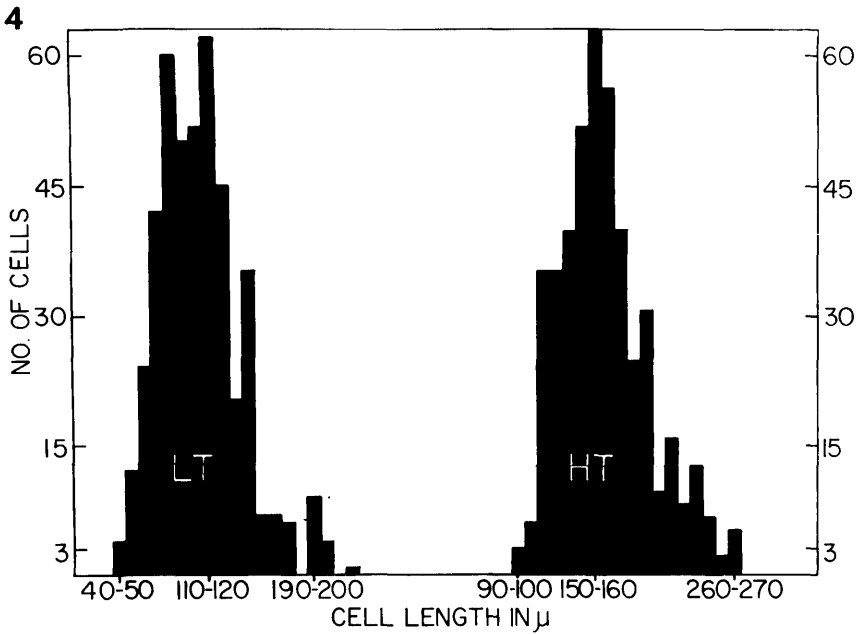


FIGURE 4. The distribution of cell lengths measured in sectioned setae of *Atrichum undulatum*.

On the other hand, the ratio of mean cell lengths for macerated to sectioned HT setae was 2.1:1 and was essentially the same as the comparable ratio for LT setae, which was 2.2:1 (Table 2).

Besides the observations mentioned above, other anatomical differences between HT and LT setae were observed. These included differences in setae

TABLE 2
Ratios of mean seta lengths and mean seta-cell lengths of *Atrichum undulatum*

Seta Lengths	Cell Lengths		
	Macerated Setae	Sectioned Setae	Macerated + Sectioned Setae
HT	HT	HT	HT
LT	LT	LT	LT
$\frac{HT}{LT} = 3.1:1$	$\frac{HT}{LT} = 1.4:1$	$\frac{HT}{LT} = 1.5:1$	$\frac{HT}{LT} = 1.4:1$
	$\frac{\text{Macerated HT Setae}}{\text{Sectioned HT Setae}} = 2.1:1$	$\frac{\text{Macerated LT Setae}}{\text{Sectioned LT Setae}} = 2.2:1$	

diameters and in the amount of secondary thickening of cortical-cell walls. Diameters of HT setae averaged 230 μ, whereas those of LT setae were somewhat larger and averaged 262 μ (Table 3). The ratio of these diameters, however, was quite small, amounting to only a factor of approximately 1.2 (LT:HT). The cortex of HT setae had more cell layers with secondary-wall thickening than did the cortex of LT setae (fig. 5, 6). Phloroglucinol staining indicated that the thick-walled cells of the outer cortex were not lignified. However, these cells did stain with Sudan IV, which indicated that a lipid compound was present in the secondary

TABLE 3
*Mean setae diameters (μ) of *Atrichum undulatum**

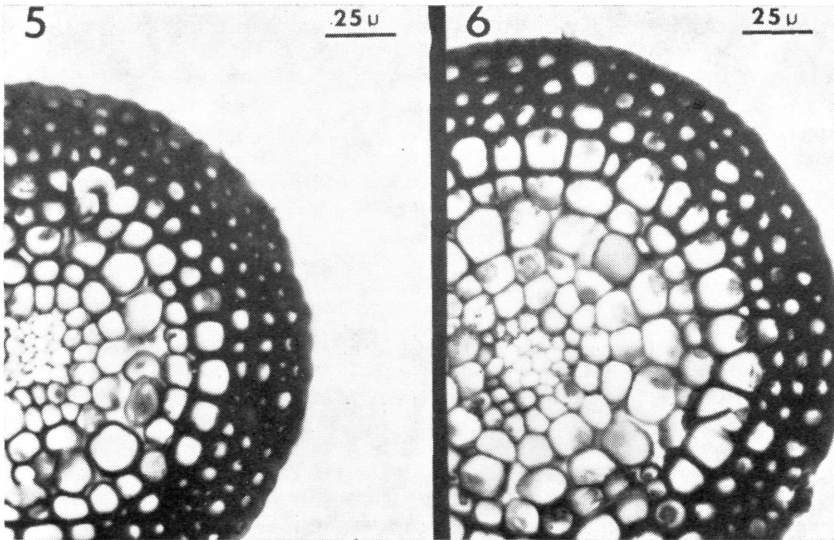
Seta No.	HT			LT		
	\bar{X}	SD	N	\bar{X}	SD	N
1	238	6.26	5	258	1.78	5
2	220	2.28	5	265	7.09	5
3	234	1.41	5	266	2.79	5
4	229	2.17	5	260	2.74	5

thickening of the cell walls. Unfortunately, there appear to be no histochemical tests to distinguish between different lipid compounds such as cutin, suberin, or lectin.

DISCUSSION

Setae of plants cultured in the high-temperature regime (HT) (12° – 22° C) were longer, by a factor of 3 (Table 2), than setae of plants cultured in the low-temperature regime (LT) (3° – 12° C). This difference in seta lengths may have resulted from the cells of HT setae being longer, or occurring in greater number, or from a combination of these, as compared with cells of LT setae.

Mean cell lengths for macerated and sectioned HT setae were longer than were those for LT setae (Table 1; fig. 3, 4). Although the ratio of seta lengths (HT:LT) was 3.1:1, the ratio of mean cell lengths (HT:LT) was only 1.4:1 (Table 2). The difference in these ratios indicates that there were both longer cells and more transverse cell divisions in the longer HT setae than in the shorter LT setae, apparently a result of the different temperature regimes in which the plants grew.



FIGURES 5-6. Transverse sections through setae of *Atrichum undulatum*. Both sections at the same magnification. 5—Seta from a plant cultured in the high temperature regime (12° – 22° C). 6—Seta from a plant cultured in the low temperature regime (3° – 12° C).

Both HT and LT setae had longer cells in macerated material than in sectioned material (Table 1; fig. 3, 4). Also, the ratio of mean cell lengths (macerated: sectioned) for both HT and LT setae was approximately 2:1 (Table 2). These observations indicate that cells of both types of setae were about twice as long, on the average, in the epidermis and outer cortex as were cells in the inner cortex, in the layer of cells with slightly thickened walls, and in the central strand.

Diameters of LT setae were somewhat larger than were those of HT setae (Table 3; fig. 5, 6). Cell diameters appeared to be larger in LT setae than in HT setae (fig. 5, 6); however, transverse sections were not adequately examined, with respect to cell diameters and cell numbers, to confirm this impression. Therefore, the larger diameters of LT setae may have resulted from greater radial cell expansion, or more longitudinal cell divisions, or both of these.

Although not proven, it seems likely that genetic variability was probably of minor importance in affecting the anatomical differences observed between HT and LT setae, because the plants were collected from one large colony and the sporophytes were harvested at random. Because the plants were cultured under similar conditions, except for temperature, it is apparently temperature that has influenced the differential growth of the cells in *Atrichum undulatum* sporophytes.

Slade (1965) found that temperature had apparent influences on seta elongation of the liverwort, *Pellia epiphylla*. He showed that the rates of seta elongation in this plant increased with increasing temperatures up to an optimal temperature of 20°C. At higher temperatures, the rates of elongation decreased to a maximal temperature of about 30°C. In addition, final seta lengths of the same liverwort were apparently affected by temperature. Plants exposed to 5°C had longer setae after three weeks than did those grown at higher temperatures (10°, 15°, 20°, and 25°C), even though setae of plants grown at higher temperatures had higher rates of elongation (Slade, 1965).

In contrast to Slade's (1965) observations, we found longer setae on plants cultured at the higher temperatures (12°–22°C) than on plants cultured at the lower temperatures (3°–12°C), which indicates that the optimal temperature for maximal seta elongation may be higher for *Atrichum undulatum* than for *Pellia epiphylla*. Unfortunately, this difference in apparent temperature optima for seta elongation of these taxa can not be readily explained at this time, because there seems to be no clear understanding of the mechanism (or mechanisms) by which temperature influences internal factors of bryophytes (such as rates of enzymatic reactions or hormone production or both), that in turn might affect the growth and differentiation of sporophytes. Likewise, the mechanism (or mechanisms) by which temperature affected the differences observed in our study between setae of plants cultured in the high- and low-temperature regimes requires exploration.

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