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Effect of Temperature on Rhizome Regrowth and Biomass in *Muhlenbergia sobolifera*, a Shade-Tolerant C_4 Grass

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ABSTRACT

Muhlenbergia sobolifera, a rhizomatous, perennial C_4 grass, is restricted to understory areas which have cooler temperatures than are common in the habitats of the majority of C_4 species. The purpose of the present study was to examine the responses of rhizome regrowth and biomass production to temperatures lower than optimal for C_4 species (10°C, 15°C and 20°C) and compare them to responses at 30°C, more often favorable for plants utilizing the C_4 photosynthetic pathway.

Timing of emergence from rhizomes, stem number and height, biomass, and shoot/root ratios were measured in plants of *Muhlenbergia sobolifera* grown at each temperature regime. Shoots emerged more quickly from rhizomes as growth temperature increased. Plants produced more stems, greater total stem height, and more biomass at 20°C than at any other temperature regime. Proportional biomass allocation to shoots increased with temperature up to 20°C and then remained unchanged. These responses to growth temperature may be important adaptations which contribute to the survival of *Muhlenbergia sobolifera* in relatively cool, deeply shaded understory habitats in the hardwood forests of the United States. Maximum growth at 20°C, 10-15°C lower than for many high-light C_4 grasses, may result in maximization of photosynthetic surface area at temperatures similar to those in early spring before the forest canopy closes. Early production of large stem and leaf structures would enhance photosynthetic activity throughout the summer in which low levels of diffuse light are interrupted only by intermittent sunflecks. Maturation and inflorescence production in plants grown at 10°C, 15° and 20°C were slower than at 30°C, which may result in an extended growing season, delaying seed production until October when temperatures are cooler and the forest canopy more open.

INTRODUCTION

Geographical distribution of grasses is known to be correlated with photosynthetic pathways (Chazdon 1978; Hattersley 1983; Rundel 1980; Teeri and Stowe 1976; Tieszen et al. 1979; Vogel et al. 1986), but there is little consensus concerning which environmental factor is most important in establishing this relationship (Batanouny et al. 1988; Teeri and Stowe 1976; Tiezsen et al. 1979; Barnes and Harrison 1982).

Based on the physiological characteristics of the C_4 pathway (high temperature optimum for photosynthesis, lack of photosynthetic saturation with respect to light, absence of apparent photorespiration, and high water-use efficiency; Black 1971), it has been inferred that C_4 plants are optimally adapted to climates which are hot, dry, and sunny. However, an increasing number of C_4 species have been reported from cool (Caldwell et al. 1977; Rundel 1980; Potvin et al. 1985), shaded (Winter et al. 1980; Pearcy 1983; Ward and Woolhouse 1986; Smith and Martin 1987), and moist habitats (Rundel 1980; Pearcy 1983; Pendergrast 1989).

Muhlenbergia sobolifera is a rhizomatous, perennial grass which grows in the understory of hardwood temperate forests in North America (Pohl 1969). It possesses Kranz anatomy, low interveinal distances, low lateral cell counts, late season flowering, and carbon isotope ratios typical of C_4 plants (Smith and Martin 1987a). It is the most shade-tolerant C_4 species reported, occuring in deeply shaded understory habitats (<10% full sun), and yet, when grown in the laboratory, this species demonstrated higher photosynthetic rates and produced more biomass at light levels 10 times greater (Smith and Martin 1987 a,b). The temperate forest habitats in which *M. sobolifera* occurs experience lower maximum temperatures during summer than the surrounding high-insolation prairies. *Muhlenbergia sobolifera* begins above-ground growth in March before the forest canopy closes, when temperatures are near 0°C, and completes seed production in October when the canopy is again more open (M. Smith, personal observation).

Generally, plants native to cooler habitats exhibit optimum photosynthesis and growth at lower temperatures than plants native to warmer environments (Berry and Bjorkman 1980). Due to the early spring emergence of *M. sobolifera*, and its occurrence in relatively cool, low-light habitats, it was hypothesized that this species would exhibit a lower optimum temperature for growth than the characteristic 30-45°C reported for most C_4 grass species (Ludlow 1976; Gifford 1974; Ehleringer 1978; Kemp and Williams 1980).

The purpose of this investigation was to determine the effect of temperature on rhizome regrowth of *M. sobolifera*. Time of shoot emergence from rhizomes, total height and biomass were measured and inflorescences counted.

MATERIALS AND METHODS

Rhizomes of *M. sobolifera* were collected from the understory of an oak-hickory forest 14 km S of Lawrence, KS (Douglas Co.). The population is on a steep bank above a stream; soil is clayey and is covered with a thick layer of leaf litter, closely underlain by a limestone substrate. Several large trees (*Quercus rubra, Q. alba*, and *Ulmus rubra*) shade the population. During the summer, photon flux density (PFD) in this understory area is approximately 10-25 umol $m^{-2}s^{-1}$, but transitory sunflecks occur throughout the day (Smith and Martin 1987a). *Muhlenbergia sobolifera* is the only grass in the area, growing among more typical understory species: *Adiantum pedatum*, *Sanicula canadensis*, and *Thuidium delicatulum*.

For the present study, plants were collected on January 7, 1988, placed in plastic bags with adequate moisture, and kept cool while being transported. Upon arrival at Southern Illinois University, rhizomes were washed clean of the original soil, shoots removed, and rhizomes placed in flats lined with moist Promix potting soil (Premier Brands, Inc., New Rochelle, N.Y.). Rhizomes for all growth temperature regimes were stored at 4°C in a cold-room until needed. A series of growth experiments conducted from 1983-1985 showed no evidence of changes in biomass production or photosynthetic rates in plants grown at different intervals after collection when rhizomes had been stored in the dormant state (Smith and Martin 1987 a,b).

Rhizomes were removed from the cold-room, individually weighed, and planted in 168 cm^3 pots with Promix (pH 7.1, closely resembling the 7.3 measured in soil from the collection site). Rhizomes were placed just below the soil surface (simulating their position in nature) and placed in a Sherer growth chamber (Model CEL-7HL). Four separate groups of rhizomes were potted, each approximately five weeks apart. Each group was exposed to a different temperature regime (variation in temperature across the growth area for day/night stated parenthetically for the following temperature settings): 10°C (10.5 ± 1.1 / 9.1 ± 0.5), 15°C (15.4 ± 1.1 / 12.8 ± 0.7), 20°C (22.5 ± 1.3 / 19.1 ± 0.6), or 30°C (32.5 ± 1.3 /29.9 ± 0.6). Due to the death of 3 plants in two of the treatment groups, sample sizes at the termination of the experiment were as follows: 25 at 10°C and 20°C; and 22 at 15°C and 30°C.

Plants were placed in the growth chamber immediately after potting, kept well-watered with deionized water, given 100 ml of 1/4-strength Hoagland's solution (Hoagland and Arnon 1938) weekly. Pots were moved every day in a clockwise pattern to reduce the position-effect caused by variability in environmental conditions in different areas of the growth chamber. Photon flux density was maintained at 500±15 umol m^{-2} s^{-1} at the tops of the plants by adjusting growth shelves. Photoperiod was 12 h light/12 h dark and relative humidity 40% day/50% night. Temperature, PFD, and humidity were continuously recorded by a Weathertronics hygrothermograph (Model 5020) and a LI-1000 datalogger (LI-COR, Lincoln, Neb) and quantum sensor (LI-COR 190SA). Time of emergence was the number of days from planting until the shoots emerged.

Plants from all growth regimes except 10°C were harvested 30 days after emergence when shoots were mature and exhibited evidence of developing inflorescences. Shoots grown at 10°C developed slowly and were allowed to grow for 38 days until the first signs of inflorescence production were noted. Plants were removed from the growth chamber, number and height of stems recorded and inflorescences counted. Rhizomes (including attached roots) and shoots were separated, placed in an oven at 80°C, and weighed after 8 days.

Because rhizomes varied in size, all biomass data were statistically analyzed using analysis of covariance (MANOVA $SPSS^x$) with the initial rhizome weight as the covariate (Smith and Martin 1987a; Sokal and Rohlf 1981). The temperature at which plants were grown was the independent variable. Day of emergence, number of stems per rhizome, mean total dry weight, and total height (calculated as the mean of the total length of all shoots arising from each rhizome), were the dependent variables. The resulting adjusted means were compared for significant difference using Hochberg's GT2 statistic. This method uses studentized maximum modulus distribution and is recommended when sample sizes are different (Sokal and Rohlf 1981). In case of significant differences in mean values, $P<0.05$ unless otherwise stated. Shoot/root ratios were calculated using adjusted means and included all below-ground biomass as "roots". An arcsine transformation was performed before shoot/root data were statistically analyzed to normalize the data.

RESULTS

The mean number of days required for shoot emergence decreased with increase in growth temperature (Fig. 1). The differences were statistically significant, except between 20°C and 30°C.

Plants grown at 20°C produced significantly more stems (Fig. 2) than plants at any other growth temperature. There were no differences in the mean number of stems produced in plants grown at 15°C versus 30°C, and plants grown at 10°C produced significantly fewer stems than plants at 20°C or 30°C.

Mean plant height was significantly greater at 20°C (Fig. 3) than at any other growth temperature. Height of plants at 30°C was significantly greater than height of plants grown at 10°C or 15°C, but only 62% of the the mean plant height at 20°C. Results for dry weight (Fig. 4) were consistent with those for height. Shoot/root ratios (Fig. 5) increased significantly with increase in growth temperature from 10°C to 20°C, but remained unchanged from 20°C to 30°C.

Only plants grown at 30°C (8 out of 22) produced mature inflorescences by the end of the growth period.

DISCUSSION

The successful emergence of *M. sobolifera* from rhizomes at 10°C is consistent with its performance in understory areas in Kansas, Missouri, and Illinois, where emergence in the field usually occurs in March (personal observation). The heavy, sprawling rhizomes containing stores of carbon necessary for re-emergence are located close to the soil surface, and are likely to respond more quickly to changes in air temperature than roots (Antos 1988). The initiation of shoot growth at cool spring temperatures, before the forest canopy has closed, may contribute significantly to the success of this species in establishing substantial aboveground biomass before light levels decrease. Successful emergence at relatively cool temperatures effectively extends the portion of the growing season when insolation is unimpeded by the forest canopy. During this period *M. sobolifera* produces a large aboveground structure, maximizing interception of sunlight throughout a summer of low levels of diffuse light and occasional sunflecks.

Production of structural material was significantly greater at 20°C than at any other growth temperature. This results in greater biomass, as well as more and taller stems produced per rhizome. Due to the biochemical properties of the C_4 metabolic pathway (Black 1971), one would expect higher biomass production in C_4 species at 30°C compared to 20°C. This has been demonstrated for several C_4 grasses (Glasziou et al. 1965; Kawanabe 1968; Dunkin and Hesketh 1968; Kemp and Williams 1980), but Hardacre and Turnbull (1986) reported greater leaf area and dry weight production at 20°C than at 28°C in *Zea mays*.

In the present study, plants of *M. sobolifera* grown at 30°C exhibited visible signs of senescence (maturation of inflorescences and yellowed leaves). All plants from this temperature regime possessed noticeable red pigmentation in the leaves which we presumed were anthocyanins, but identification was not verified. The production of anthocyanins has been associated with increased temperatures and senescence in marigolds (Armitage and Carlson 1981). Apparently, *M. sobolifera* develops and senesces more quickly at higher temperatures, completing its vegetative and reproductive growth in a short time (30 days in the present study). This developmental pattern has been observed in prairie grasses, and is the basis for niche differentiation in *Agropyron smithii* (C_3) and *Bouteloua gracilis* (C_4), species which co-occur in the western plains (Kemp and Williams 1980). *Bouteloua gracilis* remains dormant in the cool, early spring days, completing its vegetative growth during the hottest time of the summer when C_3 grasses are no longer competing for water and nutrients. For *M. sobolifera*, however, which is not exposed to high levels of insolation during the hottest days of the summer, early emergence and prolonged, slow development under cool conditions are more advantageous than a rapid growth response at higher temperatures. Maintenance of metabolic activity for an extended period of time at moderate temperatures, and delay of inflorescence production until fall when the forest canopy is again relatively open, appear to be successful physiological and phenological adaptations to cool understory habitats.

Several studies have reported increased carbon allocation to aboveground biomass, instead of roots and rhizomes, in shade-adapted plants in response to decreasing PFD (Boardman 1977; Bjorkman 1981). Such development results in proportionally greater leaf production, maximizing light-harvesting surfaces in the plants. Little has been reported concerning the effects of temperature on shoot versus root development in C_4 species. However, Hardacre and Turnbull (1986), in their study of *Zea mays*, reported that the relative allocation of carbon to shoots increased as growth temperature increased from 16°C to 28°C. In a high-light C_4 species such as *Zea mays,* the significance of this change in carbon allocation is not clear, as maximum leaf area and biomass were produced at an intermediate temperature, but maximum growth rate occurred at 28°C. In *M. sobolifera*, shoot/root ratios increase and then stabilize at 20°C, at which temperature biomass production is greatest. This pattern of carbon allocation may be important to the survival of *M. sobolifera* in understory habitats. A large shoot/root ratio at 20°C is consistent with the exploitation of the high-light period for maximum above-ground growth in spring before canopy closure.

SUMMARY

Muhlenbergia sobolifera possesses morphological, phenological, and physiological characteristics which may contribute to its survival in cool, shaded understory habitats. Its large, sprawling rhizomes, containing stored carbon necessary for re-emergence, lie close to the soil surface and respond quickly to warming air temperatures in early spring. Optimal stem, leaf, and biomass production occur at 20°C, resulting in maximal photosynthetic potential developing before the forest canopy closes. Spring carbon allocation to above-ground biomass and sustained growth and development over an extended period allow the plants to remain metabolically active until flower production is initiated and seed development completed in the relatively open fall canopy. This suite of characteristics may be important in the survival of *M. sobolifera* in its forest understory habitat.

LITERATURE CITED

Antos, J.A. 1988. Underground morphology and habitat relationships of three pairs of forest herbs. Amer. J. Bot. 75(1):106-113.

Armitage, A.M., W.H. Carlson. 1981. The effect of quantum flux density, day and night temperature and phosphorus and potassium status on anthocyanin and chlorophyll content in marigold leaves. J. Amer. Soc. Hort. Sci. 106(5):639-642.

Barnes, P.W., A.T. Harrison. 1982. Species distribution and community organization in a Nebraska sandhill mixed prairie as influenced by plant/soil-water relationships. Oecologia 52:192-201.

Batanouny, K.H., W. Stichler, H. Ziegler. 1988. Photosynthetic pathways, distribution, and ecological characteristics of grass species in Egypt. Oecologia 75:539-548.

Berry, J., O. Bjorkman. 1980. Photosynthetic response and adaptation to temperature in higher plants. Ann. Rev. Plant Physiol. 31:491-543.

Bjorkman, O. 1981. Responses to different quantum flux densities. Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (eds.). Physiological Plant Ecology. I. Responses to the physical environment. Springer-Verlag, Berlin, pp. 57-107.

Black, C.C. 1971. Ecological implications of dividing plants into groups with distinct photosynthetic production capacities. Adv. Ecol. Res. 7:87-114.

Boardman, N.K. 1977. Comparative photosynthesis of sun and shade plants. Ann. Rev. Plant Physiol. 28:355-377.

Caldwell, M.M., Osmond, C.B., Nott, D.L. 1977. C_4 pathway photosynthesis at low temperature in cold-tolerant *Atriplex* species. Plant Physiol. 60:157-164.

Chazdon, R.L. 1978. Ecological aspects of the distribution of C_4 grasses in selected habitats of Costa Rica. Biotrop. 10:265-269.

Dunkin, W.G., Hesketh, J.D. 1968. Net photosynthetic rates, relative leaf growth rates, and leaf numbers of 22 races of maize grown at eight temperatures. Crop Sci. 8:670-674.

Ehleringer, J.R. 1978. Implications of quantum yield differences on the distributions of C_3 and C_4 grasses. Oecologia 31:255-267.

Gifford, R.M. 1974. A comparison of potential photosynthesis, productivity and yield of plant species with differing photosynthetic metabolism. Aust. J. Plant Physiol. 1:107-117.

Glasziou, K.T., Bull, T.A., Hatch, M.D., Whiteman, P.C. 1965. Physiology of sugarcane. VII. Effects of temperature, photo-period duration, and diurnal and seasonal temperature changes on growth and ripening. Aust. J. Biol. Sci. 18:53-66.

Hardacre, A.K., Turnbull, H.L. 1986. Growth development of maize (*Zea mays* L.) at five temperatures. Ann. Bot. 58:779-787. Hattersley, P.W. 1983. The distribution of C_3 and C_4 grasses in Australia in relation to climate. Oecologia 57:113-128.

Hoagland D.R., Arnon, D.I. 1938. The water-culture method for growing plants without soil. Calif. Agric. Exp. Sta. Circ. 347.

Kawanabe, S. 1968. Temperature responses and systematics of the Gramineae. Proc. Jap. Soc. Plant Taxon. 2:17-20. Kemp P.R., Williams, G.E. III. 1980. A physiological basis for niche separation between *Agropyron smithii* (C_3) and *Bouteloua gracilis* (C_4). Ecology 61:846-858.

Ludlow, M.M. 1976. Ecophysiology of C_4 grasses. Lange, O.L., Kappen, L., Schulze, E.D. (eds.) Water and Plant Life; Problems and Modern Approaches. Springer-Verlag, Berlin, pp. 365-386.

Pearcy, R.W. 1983. The light environment and growth of C_3 and C_4 tree species in the understory of a Hawaiian forest. Oecologia 58:19-25.

Pendergrast, H.D.V. 1989. Geographical distribution of C_4 decarboxylation types and associated structural variants in native Australian C_4 grasses (Poaceae). Aust. J. Bot. 37: 253-273.

Pohl, R.W. 1969. *Muhlenbergia*, subgenus *Muhlenbergia* (Gramineae) in North America. Am. Mid. Nat. 82(2):512-542.

Potvin, C., Strain, B.R., Goeschl, J.D. 1985. Low night temperature effect on photosynthate translocation of two C_4 grasses. Oecologia 67:305-309.

Rundel, P.W. 1980. The ecological distribution of C_4 and C_3 grasses in the Hawaiian Islands. Oecologia 45:354-359.

Smith, M., Martin, C.E. 1987a. Growth and morphological responses to irradiance in three forest understory species of the C_4 grass genus *Muhlenbergia*. Bot. Gaz. 148:141-148.

Smith, M., Martin, C.E. 1987b. Photosynthetic responses to irradiance in three forest understory species of the C_4 grass genus *Muhlenbergia*. Bot. Gaz. 148:275-282.

Sokal, R.R., Rohlf, F.J. 1981. Biometry. 2nd ed. W.H. Freeman and Co. San Francisco, 859 pp.

Teeri, J.A., Stowe, L.G. 1976. Climatic patterns and the distribution of C_4 grasses in North America. Oecologia 23:1-12.

Tieszen, L., Senyimba, M., Imbamba, S., Troughton, J. 1979. The distribution of C_3 and C_4 grasses and carbon isotope discrimination along an altitudinal and moisture gradient in Kenya. Oecologia 37:337-350.

Vogel, J.C., Fuls, A., Danin, A. 1986. Geographical and environmental distribution of C_3 and C_4 grasses in the Sinai, Negev, and Judean deserts. Oecologia 70:258-265.

Ward, D.A., Woolhouse, H.W. 1986. Comparative effects of light during growth on the photosynthetic properties of NADP-ME type C_4 grasses from open and shaded habitats. I. Gas exchange, leaf anatomy and ultrastructure. Plant Cell Environ. 9:261-270.

Winter, K., Schmitt, M.R., Edwards, G.E. 1982. *Microstegium vimineum*, a shade-adapted C_4 grass. Plant Sci. Lett. 24:311-318.

Fig. 1. Number of days from day of planting to shoot emergence from rhizomes grown under four temperature regimes. Bars with different letters are statistically different (P<0.05).

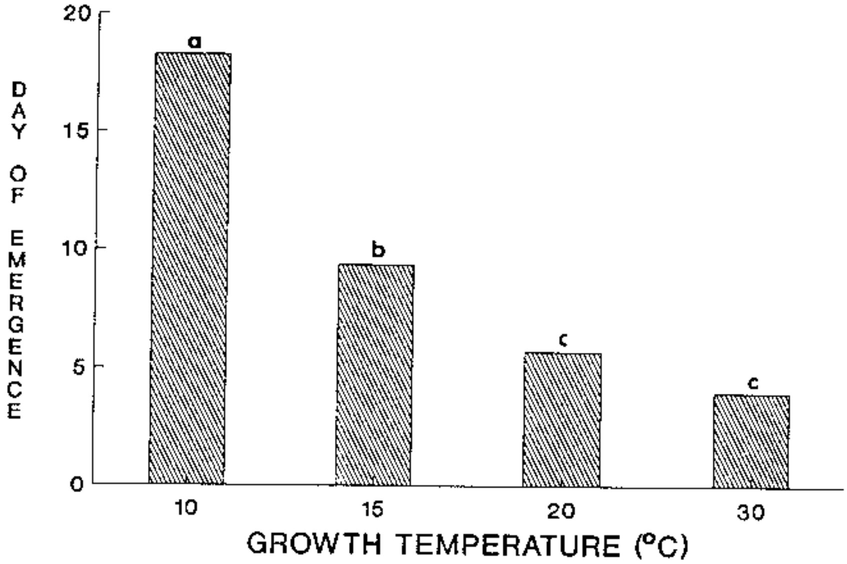

Fig. 2. Number of stems produced by each rhizome grown under four temperature regimes. Bars with different letters are statistically different (P<0.05).

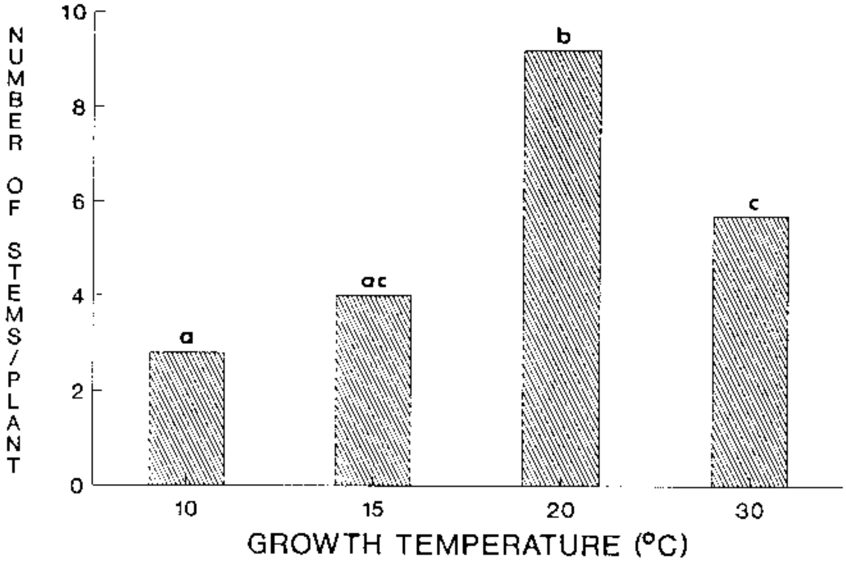

Fig. 3. Mean total height produced by each rhizome grown under four temperature regimes. Bars with different letters are statistically different ($P<0.05$).

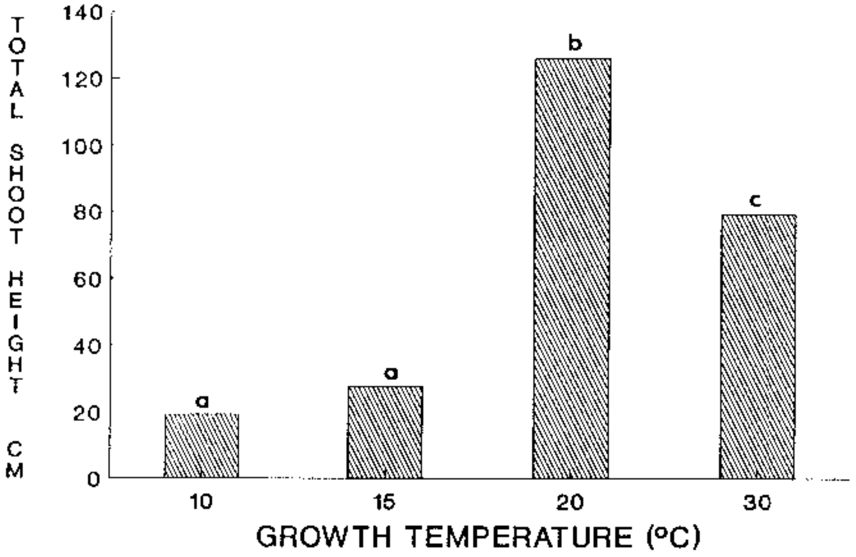

Fig. 4. Mean total dry weight produced by each rhizome grown under four temperature regimes. Bars with different letters are statistically different ($P<0.05$).

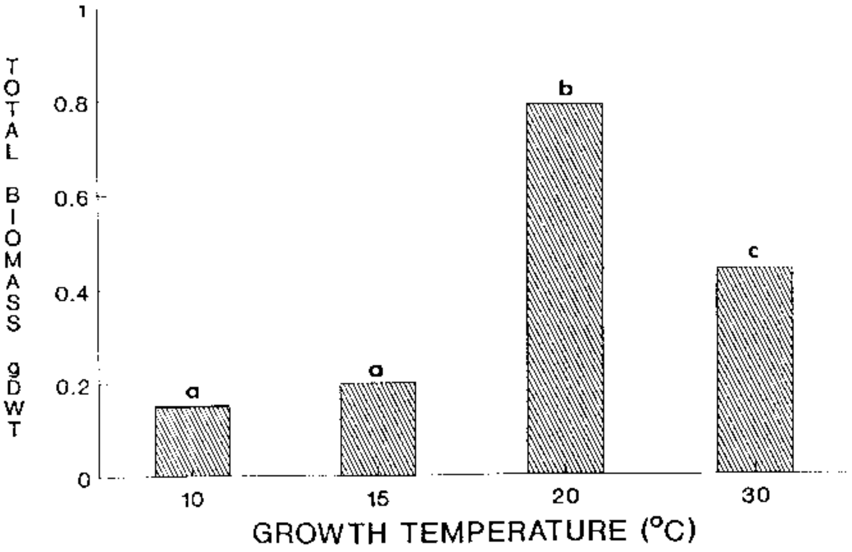

Fig. 5. Shoot/root ratios for plants grown under four temperature regimes. Bars with different letters are statistically different ($P<0.05$).