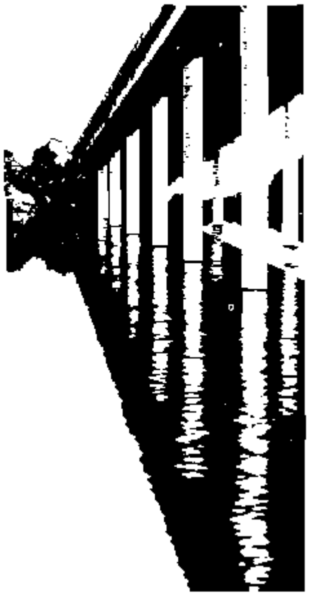

**AQUATIC PLANT CONTROL RESEARCH PROGRAM**

MISCELLANEOUS PAPER A-90-2

# PILOT STUDY: CARBOHYDRATE ALLOCATION IN HYDRILLA BIOTYPES

by

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Carbohydrate allocation in monoecious and dioecious hydrilla (*Hydrilla verticillata* (L.f.) Royle) was examined under various water temperature and photoperiod regimes. Propagule production of monoecious hydrilla was also evaluated under different shoot-cutting treatments. Both hydrilla biotypes accumulated starch (35 percent) in shoots under short photoperiods, but only monoecious plants were able to form tubers and turions. High temperature (35° C) promoted fragmentation in both biotypes after 8 weeks of growth.

Cutting to 1-cm height decreased shoot starch content and inhibited tuber production in monoecious hydrilla. Shoot removal as a possible means of decreasing propagule formation in monoecious hydrilla is suggested.

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# Preface

This study was sponsored by the US Army Corps of Engineers (USACE), Directorate of Civil Works, through the Aquatic Plant Control Research Program (APCRP). Funds were provided by the USACE under Department of the Army Appropriation No. 96X3122, Construction General. Technical Monitor for the USACE was Mr. James W. Wolcott. The APCRP is managed by the US Army Engineer Waterways Experiment Station (WES) under the Environmental Resources Research and Assistance Programs, Mr. J. Lewis Decell, Manager.

The principal investigator for this study was Dr. Howard E. Westerdahl, Aquatic Processes and Effects Group (APEG), Environmental Research and Simulation Division (ERSD), Environmental Laboratory (EL), WES. The study was conducted and the report prepared by Dr. George J. Pesacreta, APEG. Technical assistance was provided by Dr. Kien T. Luu, Ms. Cindy Waddle, Ms. Cindy Teeter, and Mr. David Stuart, APEG. Dr. Thai Van, USDA Aquatic Weed Laboratory, Fort Lauderdale, FL, provided hydrilla tubers used in the study. Reviews of the report were provided by Drs. Kurt D. Getsinger, Luu, and Westerdahl, APEG. The report was edited by Ms. Jessica S. Ruff of the WES Information Technology Laboratory.

The investigation was performed under the general supervision of Dr. John Harrison, Chief, EL; Mr. Donald L. Robey, Chief, ERSD; and Dr. Thomas L. Hart, Chief, APEG.

Commander and Director of WES was COL Larry B. Fulton, EN. Technical Director was Dr. Robert W. Whalin.

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# Contents

# PILOT STUDY: CARBOHYDRATE ALLOCATION IN HYDRILLA BIOTYPES

## Introduction

### Background

1. The submersed macrophyte hydrilla (*Hydrilla verticillata* (L.f.) Royle) is a major nuisance in waterways managed by the US Army Corps of Engineers (Environmental Laboratory 1988). Present management techniques have provided some short-term control of the plant; however, a better understanding of hydrilla's life cycle and identification of weak points in that cycle are needed to improve the effectiveness of existing control tactics.

2. The relationship between carbohydrate allocation and seasonal growth characteristics has been used to determine weak points in the life cycle of nuisance, perennial plants to improve control techniques (Linscott and McCarthy 1962; Schirman and Buchholtz 1966; Klingman, Ashton, and Noordhoff 1975; McAllister and Haderlie 1985). As with their terrestrial counterparts, perennial aquatic plants may rely on stored carbohydrate reserves for survival through winter and initiation of spring growth. Moreover, recovery from periods of stress caused by fluctuating water temperatures, drought, nutrient depletion, and turbidity may be dependent on carbohydrate reserves.

3. Linde, Janisch, and Smith (1976) identified a relationship between carbohydrate reserves and growth cycle events in cattails (*Typha latifolia* L.). When the pistillate spike was lime green in color and the staminate spike appeared dark green, carbohydrates were at their lowest level in the plant. This information allowed the timing of a control strategy to coincide with the color of the pistillate and staminate spikes.

4. A literature survey on hydrilla revealed no studies relating weak points in the plant's growth cycle to carbohydrate allocation (Pesacreta and Luu 1988). Starch levels have been determined in ungerminated hydrilla tubers (Miller, Garrard, and Haller 1976); however, starch and sugar changes in tubers following germination and other growth periods have not been studied.

5. This pilot study was designed to assess carbohydrate allocation patterns in monoecious and dioecious biotypes of hydrilla. This information will be used to design additional studies for determining potential weak points in the growth cycle of hydrilla.

### Objectives

6. The objectives of this study were to (a) determine changes in carbohydrate levels of hydrilla biotypes, during the first 8 weeks of growth, when grown under different light and temperature regimes; (b) determine diel variations of carbohydrate levels in hydrilla biotypes at constant light and temperature; and (c) test whether tuber production in monoecious hydrilla can be decreased by partial removal of the shoots.

## Materials and Methods

### General preparations

7. Plants grown in these experiments were from monoecious and dioecious hydrilla tubers. Ungerminated tubers were soaked for 20 min in 1.3-percent sodium hypochlorite solution and thoroughly rinsed to inhibit fungi and bacteria (Sutton 1986). Tubers were germinated on a 16:8 hr light-dark (L-D) photoperiod at 22° and 32° C for the carbohydrate allocation experiment; 14:10 L-D photoperiod at 27° C for the tuber production experiment; and 12:12 L-D photoperiod at 27° C for the diel experiment. Germinated tubers were placed in cold water (12° C) to stop growth until enough tubers with similar shoot length were obtained for studies. Planting containers (2-ℓ) were filled with sediment from Brown's Lake, Vicksburg, MS, and covered with washed silica sand (5-mm depth). Three germinated tubers were planted below the sediment in each container, with 1-cm shoots piercing out of the sand.

8. Studies were conducted in 1,200-ℓ fiberglass tanks with a water depth of 90 cm. A nutrient solution (Smart and Barko 1984) was added to the overlying water to supply major ions to the plants. Nitrogen and phosphorus were omitted from the nutrient solution, since previous studies have shown that hydrilla is capable of mobilizing these nutrients from the sediment (Barko and Smart 1980, 1981). Water temperatures were maintained (±1° C) with thermostatically controlled Remcor liquid circulators. Lighting was maintained at ~500 $\mu E\ m^{-2}\ sec^{-1}$ supplied by Sunbrella fixtures with 400-W multivapor and sodium iodide lamps.

9. Plants were harvested to determine dry weights, free sugars, and starch. Harvested plants were washed thoroughly to remove attached sediment and debris. Plant parts (shoot, root, tuber, newly formed tuber or newly formed turion) were dried at 55° C for 48 hr, cooled in a desiccator, weighed,

and ground to pass through a 1-mm screen in a cyclone mill (Udy Corporation, Boulder, CO). Free sugars and starch were measured in plant parts and determined in duplicate by a modification of the procedure of Swank et al. (1982). Extracts for starch were incubated for 15 min at 55° C with 1 unit of amyloglucosidase (Sigma No. A-3042) per milliliter to achieve complete starch hydrolysis (Nelson 1944). Free sugars were also determined on extracts not incubated with amyloglucosidase.

### Carbohydrate levels in hydrilla biotypes

10. Experiment 1. Plants were grown on a 16:8 L-D photoperiod for 4 weeks and then changed to an 8:16 L-D photoperiod for 4 weeks to promote propagule production. Containers of germinated tubers of each biotype were placed in separate tanks at water temperatures of 22 ± 1° and 32 ± 1° C. Three containers were removed from each temperature treatment at 2, 4, 6, and 8 weeks. All plant material was harvested from each container to determine biomass (dry weight), free sugars, and starch content in shoots, roots, and tubers. A completely randomized design was used with three replications (three containers); treatments were the four sampling dates.

11. Experiment 2. An experiment to examine diel variation in carbohydrate levels was initiated at 4 weeks postplanting for both monoecious and dioecious biotypes under a 12:12 L-D photoperiod at 27° C. Shoots were harvested at 3-hr intervals for 2 days and were analyzed for free sugars and starch. A completely randomized design was used with three replicates (three containers); treatments were different harvesting times.

### Effect of shoot cutting on tuber production in monoecious hydrilla

12. Germinated monoecious tubers were grown at 27° C on a 14:10 L-D photoperiod for 4 weeks, and then changed to a 10:14 L-D photoperiod for a 4-week period (Experiment 3). Three cutting regimes were used for plants: plants that were not cut (control); plants cut at week 4 (single cut); and plants cut at weeks 4 and 6 (multiple cut). Plants were cut at a point 1 cm above the hydrosoil. A completely randomized design was used with three replicates (three containers). Fifteen treatments were the combination of five harvesting times (0, 2, 4, 6, and 8 weeks) and three cutting regimes. Measured parameters were biomass, free sugars, and starch content for shoots roots, and tubers.

13. The Bayesian Least Significance Difference Test (BLSD) was used to separate the effects of treatment means (Smith 1978). The Student's t-test was used to compare treatment effects between biotypes.

## Results and Discussion

### Comparison of hydrilla biotypes

14. Biomass. Mean shoot weight of monoecious plants was significantly ($p \leq 0.05$) greater than dioecious plants grown at 22° C, 2 weeks following planting; yet, no difference in weight was found during subsequent weeks (Figure 1). Initial production rates for plants at 32° C were similar; however, from the 4- to 8-week period, dioecious hydrilla biomass was still increasing while biomass for the monoecious plants did not change. By week 8, shoot weight of dioecious plants was significantly higher than monoecious plants at 32° C, and shoot fragmentation occurred in both biotypes at 32° C, but not at 22° C. Root production was similar between biotypes at both temperatures. Growth studies by Stewart and Van (1985) at 22° and 30° C and by Spencer and Anderson (1986) at 24° C showed similar biomass production between these biotypes, but they did not report shoot fragmentation. Apparently, the warm water temperature (32° C) promoted shoot fragmentation in both biotypes.

15. Monoecious plants produced a mean (±1 standard error) of 10.7 (±1.3) new tubers and 6.5 (±0.5) new turions per container at 22° C, and 5.1 (±0.6) new tubers per container at 32° C by week 8. The dioecious biotype did not produce additional vegetative propagules. Lack of tuber production by the dioecious biotype and tuber production by the monoecious biotypes agrees with studies by Spencer and Anderson (1986). These researchers speculated that monoecious hydrilla would colonize new environments more rapidly than dioecious hydrilla because of increased tuber production.

16. Carbohydrates. Free sugars were lowest during the initial sampling periods. Free sugars in germinated tubers were low, but as shoots matured, the amount of free sugars increased in the tubers of both biotypes at 22° and 32° C (Figures 2 and 3). The highest amounts of free sugars in tubers were found at weeks 2 and 4. The increased trend of free sugars in tubers during the first 4 weeks indicates the active conversion of starch into free sugars

for young shoot growth. No discernible pattern for free sugars was apparent for shoots and roots.

17. The trend for starch was similar for both hydrilla biotypes. At 22° and 32° C, the initial concentrations of shoot starch were low, i.e., near 8 to 10 percent (Figures 4 and 5). Shoot starch by week 6 had increased approximately fivefold for all treatments. Starch was evidently building up in growing shoots, while in contrast it was drastically decreasing in the tubers that were supporting shoot growth. By week 8, starch decreased in shoots of dioecious hydrilla at 32° C. Starch content from recovered shoot fragments was low for both biotypes.

18. The highest starch was found in the freshly planted tubers and newly formed tubers and turions. Starch content of freshly planted tubers was approximately 55 to 60 percent for both biotypes. The greatest change in tuber starch concentration occurred in all tanks during the first 2 weeks of development, as tuber starch decreased by at least 50 percent. Following this initial depletion, the decrease in tuber starch concentration was less noticeable. In dioecious hydrilla, complete depletion of tuber starch occurred at 32° C by week 8.

19. Root starch levels increased with time in monoecious hydrilla at 32° C and in both biotypes at 22° C. Roots contained lower concentrations of starch than any other plant part. Haller (1974) also found starch concentrations low from roots of dioecious hydrilla in Florida ponds.

20. Starch levels in ungerminated monoecious and dioecious tubers were similar to levels reported for dioecious hydrilla by Miller, Garrard, and Haller (1976); however, these authors did not report starch levels in germinated tubers. The level of tuber mass and starch depletion following germination in this study demonstrated that starch reserves decrease following germination, but some starch does remain. This differs from studies on (*Elodea nuttallii* Planch.), where starch reserves in underground organs were depleted by 0.4 day following spring growth (Best and Dassen 1987). The authors speculated that proteins were serving as an alternative energy source, maintaining the plant through spring growth and development. Hydrilla apparently did not use all of its tuber carbohydrate reserves to sustain the plant past initial growth periods, since nearly 12 to 15 percent starch remained in the tubers in many of the experiments. Young plants were able to produce photoassimilates before starch levels in tubers were exhausted.

21. Both hydrilla biotypes accumulated a greater percentage of starch in shoots when exposed to a short photoperiod. Similarly, Haller (1974) found that dioecious hydrilla shoots collected in September contained three times more starch than shoots collected in January. Haller speculated that hydrilla was storing starch in the shoots as food reserves for overwintering. The present studies confirm that hydrilla does accumulate starch in shoots. The starch level in shoots (30 to 35 percent) is twice that found in shoots of Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Titus and Adams 1979, Perkins and Sytsma 1987).

22. Elevated starch concentrations in shoots suggest that hydrilla stems may serve as temporary storage sites for starch, before translocation to newly formed tubers and turions. High starch content in shoots also ensures the survival potential of hydrilla when fragmentation occurs.

23. In general, diel free sugars and starch from shoots did not differ significantly in a 2-day experiment (Figure 6). Free sugars were near 3 to 6 percent for both biotypes. There was more change in starch levels with concentrations ranging from 5 to 11 percent. No cyclic patterns of free sugars and starch could be associated with the onset of day or night conditions. Guha (1965) studied daytime patterns of carbohydrates in hydrilla stems and leaves and found that starch levels increased from 0600 to 1800 hr; however, free sugars and total sugars had no appreciable fluctuation during the day. Similarly, Titus and Adams (1979) reported there was no diel difference in total nonstructural carbohydrates (TNC) concentration for shoots from Eurasian watermilfoil.

### Cutting effects on monoecious hydrilla

24. Biomass. Prior to cutting treatments, no substantial differences in shoot biomass were noted among uncut, single, and multiple cut treatments. Plants cut at weeks 4 and 6 had significantly less shoot biomass by week 8 compared with uncut treatments (Figure 7). Increased shoot biomass was observed for the single cut treatment by the end of the experiment, but not in the multiple cut treatment. The root biomass trend was similar to shoot biomass, with more root mass produced in control plants compared with cut treatments.

25. Carbohydrates. Trends of free sugars were not apparent between cutting treatments. Slightly more free sugars (~10 percent) were produced at

6 and 8 weeks in shoots and roots for the uncut treatment compared with cut plants (~5 to 8 percent). Free sugars in tubers were greater following germination, but trends varied by weeks 6 and 8 (Figure 8).

26. Starch in shoots was similar between treatments prior to cutting. Following cutting at week 4, starch in shoots was significantly lower in the single and multiple cut plants compared with the control plants (Figure 9). Starch in the control plants continued to increase on a short photoperiod to week 8. Starch increased in the single cut treatment by week 8, but at a lower level than the control. Starch in the multiple cut treatment remained significantly lower following cutting to termination of the experiment.

27. Differences in propagule production were noteworthy among treatments. The control treatments produced a mean (±1 SE) of 18 (±2.1) tubers per container, while the single and multiple cut treatments produced 1 (±1.4) and 0 tubers, respectively (Figure 9). Therefore, even under short-day conditions (10 hr of light), few tubers were produced in the cut treatments by removing overlying shoot mass.

28. Shoot accumulation of starch by hydrilla may precede translocation of carbohydrates to storage organs (e.g., tubers and turions) for overwintering. Starch levels in the shoots were greatest following exposure to a short photoperiod. Perhaps the shorter photoperiod triggered the accumulation of starch in the shoots. In fact, this phenomenon has been reported for other submersed macrophytes. Results from two field studies showed that an accumulation of TNC in Eurasian watermilfoil roots occurred as day length shortened in autumn (Titus and Adams 1979, Kimbel and Carpenter 1981).

29. Preventing starch storage in shoots decreased the amount of carbohydrates available for translocation to tubers, thus reducing the formation of overwintering propagules. Cutting plants after the change to a short-day photoperiod dramatically restricted tuber production. This finding could be most significant for monoecious hydrilla growing in the Middle Atlantic States, where appreciable biomass does not overwinter and most reinfestation is by tuber and turion production (Environmental Laboratory 1985; Harlan, Davis, and Pesacreta 1985). It appears that if harvesting or chemical treatments were timed to the period prior to starch accumulation in shoots, propagule formation would be curtailed.

## Conclusions and Recommendations

30. The most significant accumulation of starch in both hydrilla biotypes occurs in the shoots, with the greatest accumulation of starch occurring when plants are exposed to short photoperiods. Starch levels in tubers of both biotypes decrease following tuber germination, with the greatest decrease occurring during the first 2 weeks of plant development. Under these experimental conditions, only monoecious plants were able to form tubers and turions.

31. Diel variations of carbohydrate concentrations in both hydrilla biotypes were insignificant, when plants were exposed to a 12:12 L-D photoperiod and a constant temperature of 27° C. High temperatures (32° C) enhanced fragmentation in both hydrilla biotypes after 8 weeks of growth. Cutting treatments resulted in the suppression of propagule formation for the duration of the study in monoecious hydrilla.

32. Environmental chamber studies can provide insight into carbohydrate cycling of submersed species such as hydrilla; however, the restrictions of sediment nutrients and growing space limit these studies to a relatively short duration (8 to 12 weeks). Long-term field or pond studies should be conducted before potential weak points in the life cycle of hydrilla can be determined.

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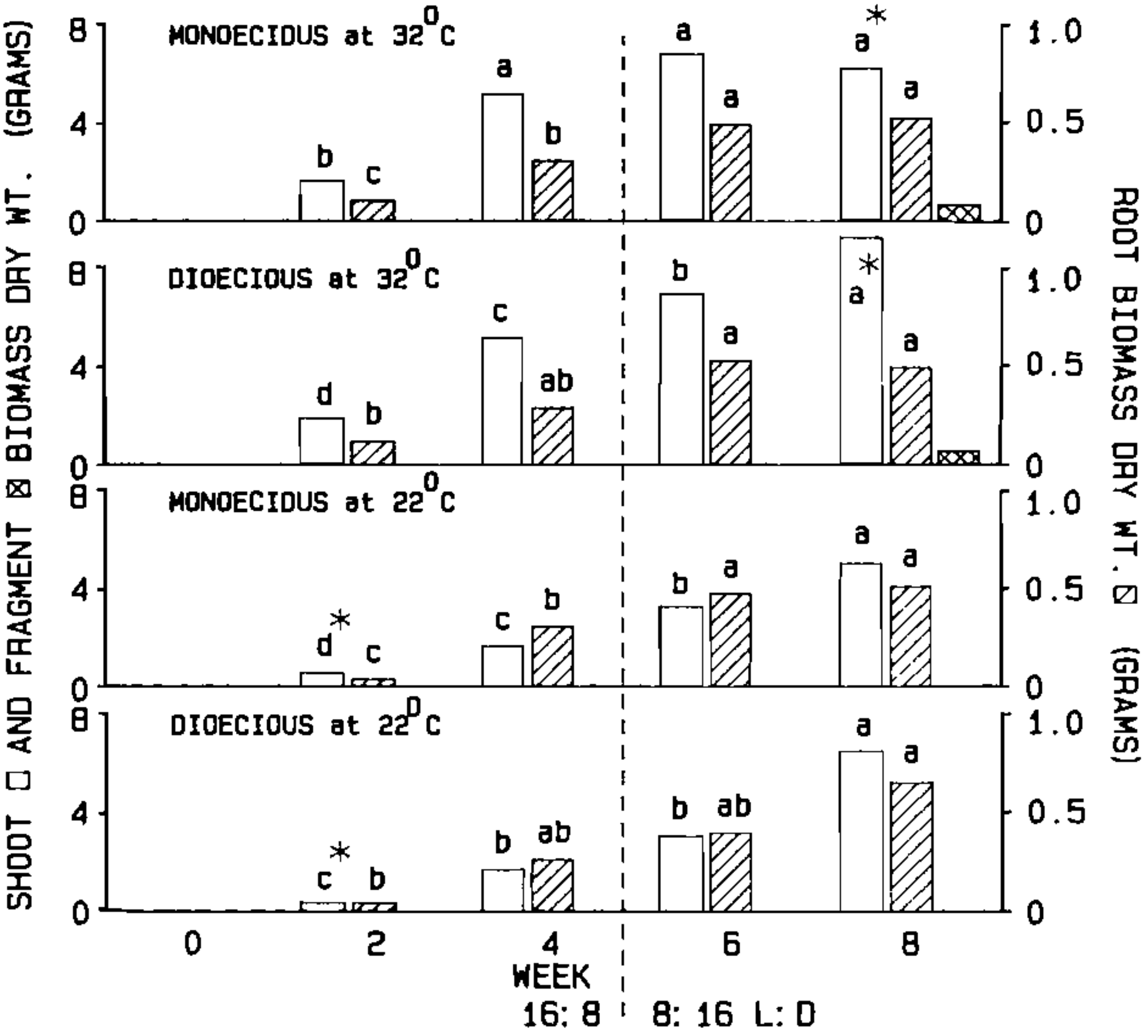

Figure 1. Mean shoot, shoot fragment, and root dry weight for hydrilla grown at 22° and 32° C. Means within a row for shoot or roots followed by the same letter were not significantly different according to BLSD test ($p \leq 0.05$). Differences between biotypes at each week and temperature noted by asterisks (Student's t-test: $p \leq 0.05$). Change in photoperiod at week 4 is indicated by dashed line

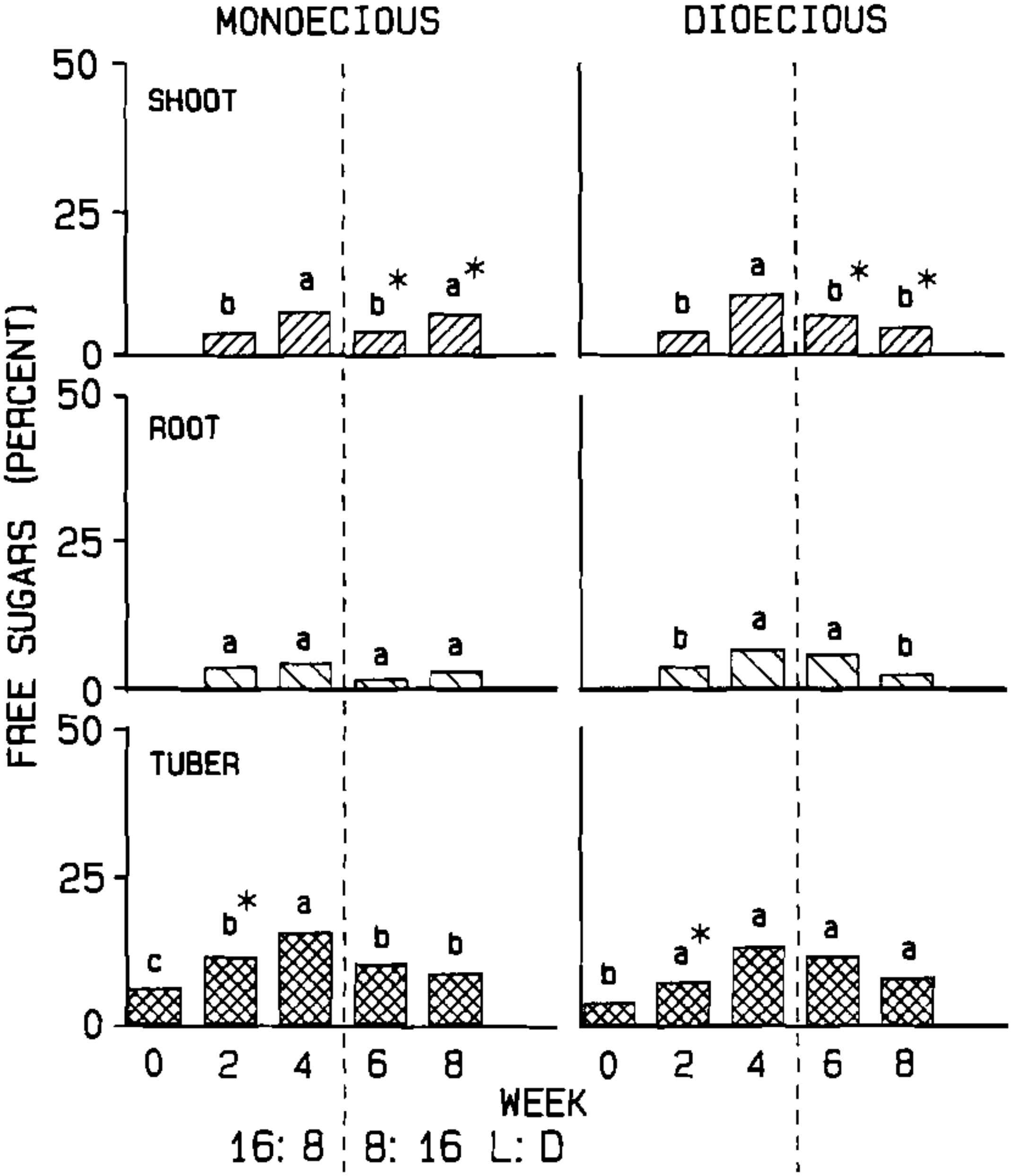

Figure 2. Free sugars in hydrilla grown at 22° C. Means within a row for each biotype followed by the same letter are not significantly different according to the BLSD test ($p \leq 0.05$). Differences between biotypes at each week noted by asterisks (Student's t-test: $p \leq 0.05$). Change in photoperiod at week 4 is indicated by dashed line

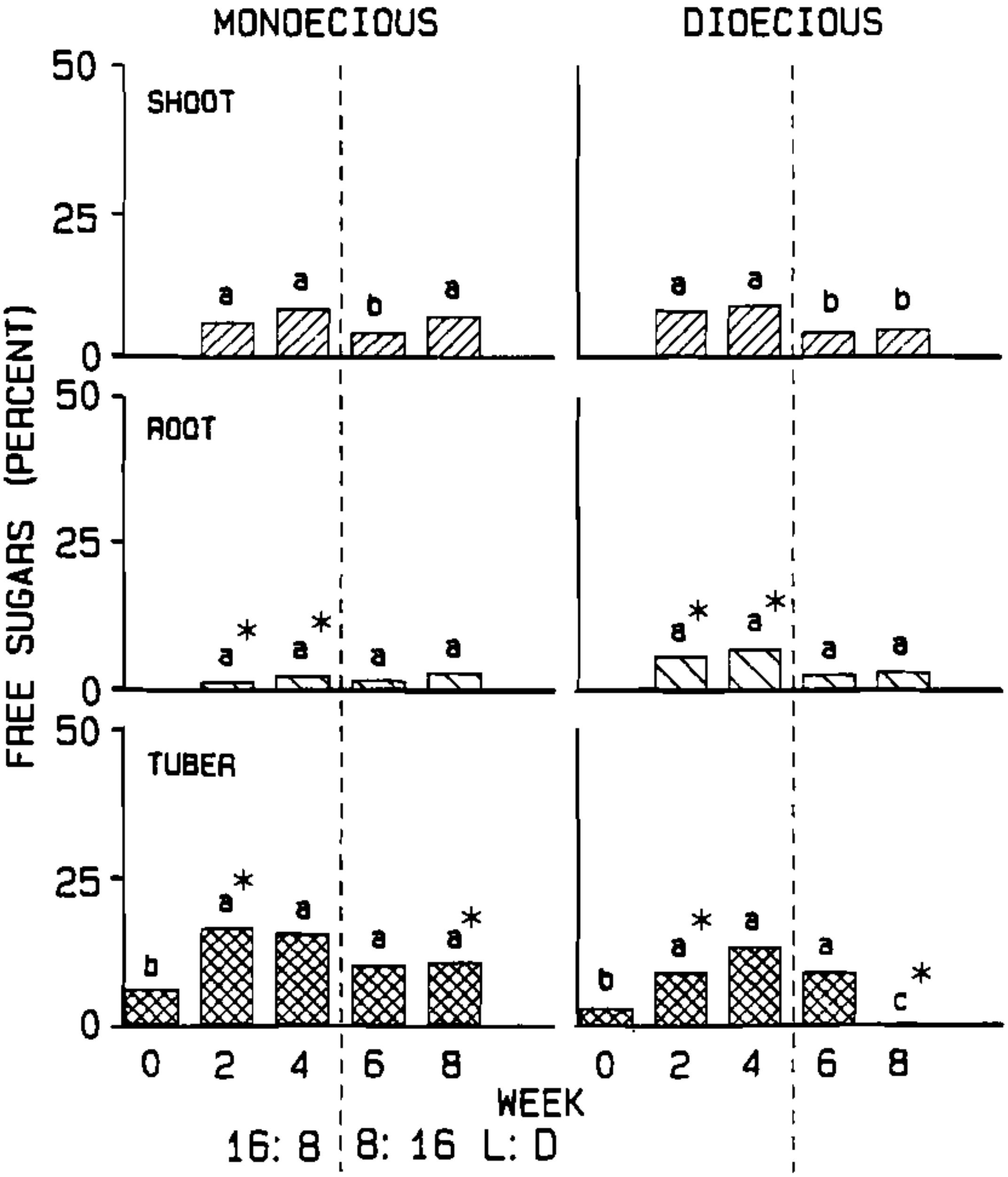

Figure 3. Free sugars in hydrilla grown at 32° C. Means within a row for each biotype followed by the same letter are not significantly different according to BLSD test ($p \leq 0.05$). Differences between biotypes at each week noted by asterisks (Student's t-test: $p \leq 0.05$). Change in photoperiod at week 4 is indicated by dashed line

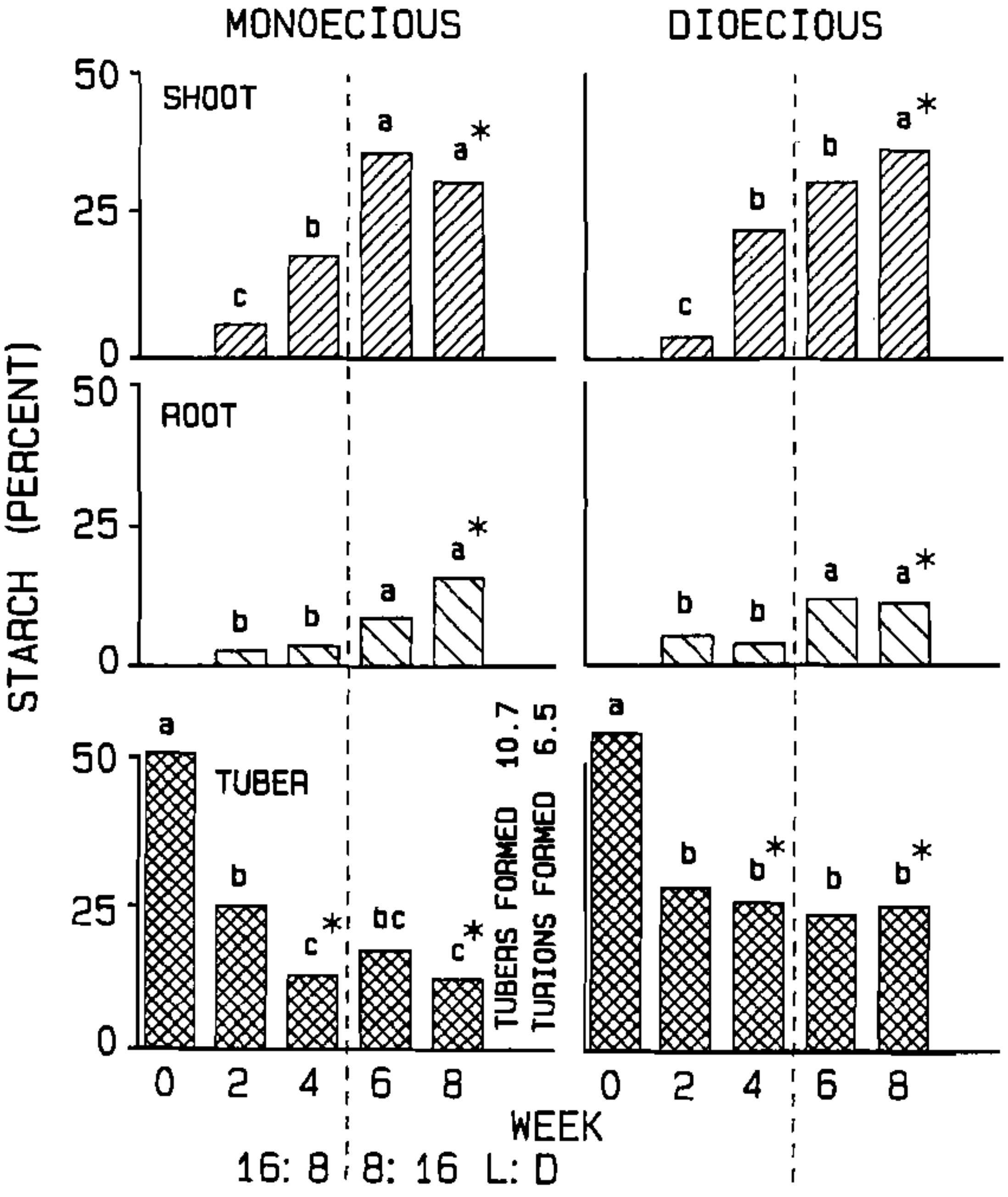

Figure 4. Starch in hydrilla grown at 22° C water temperature. Means within a row for each biotype followed by the same letter are not significantly different according to the BLSD test ($p \leq 0.05$). Differences between biotypes at each week noted by asterisks (Student's t-test: $p \leq 0.05$). Change in photoperiod at week 4 is indicated by dashed line

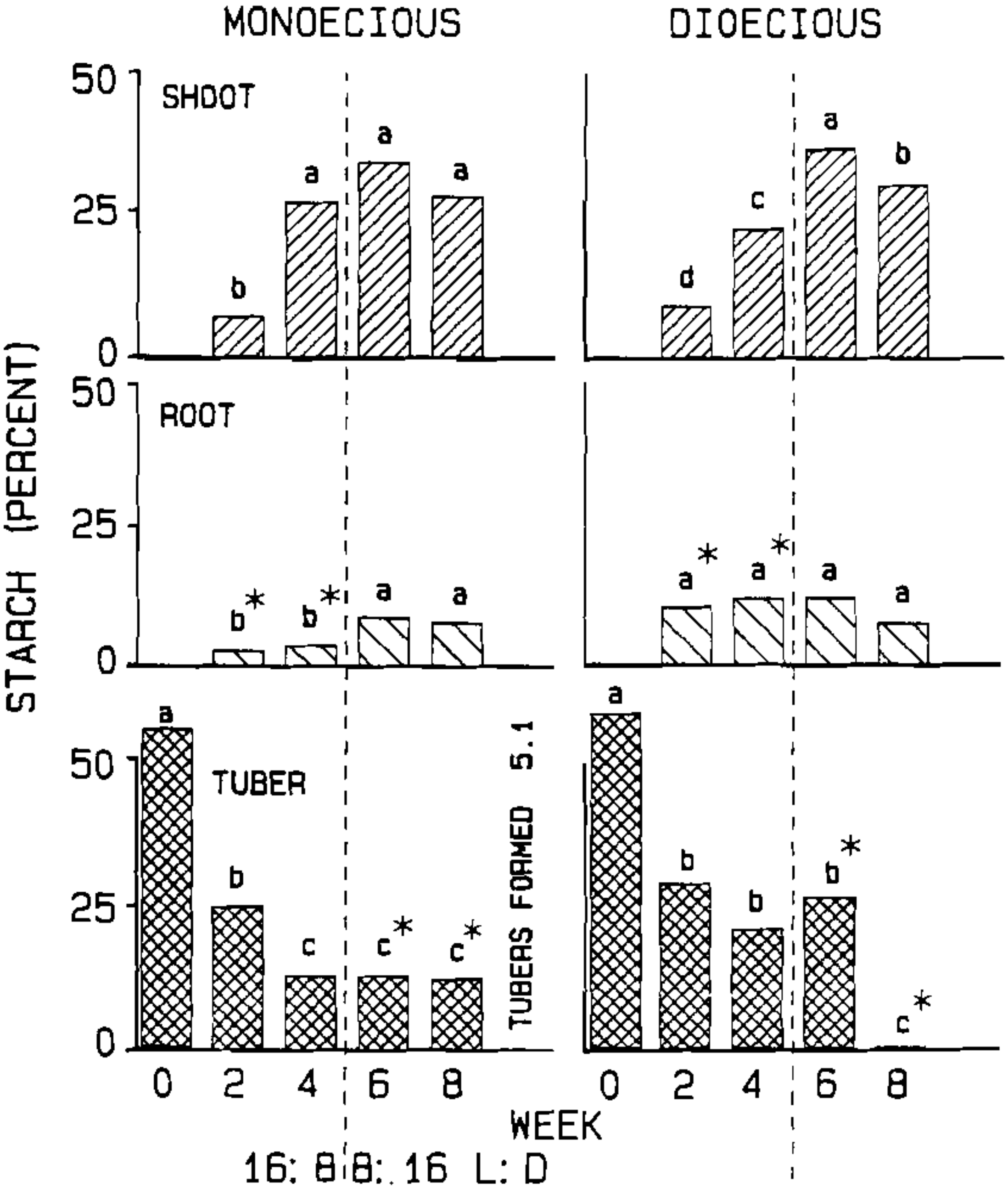

Figure 5. Starch in hydrilla grown at 32° C. Means within a row for each biotype followed by the same letter are not significantly different according to BLSD test ($p \leq 0.05$). Differences between biotypes at each week noted by asterisks (Students t-test: $p \leq 0.05$). Change in photoperiod at week 4 is indicated by dashed line

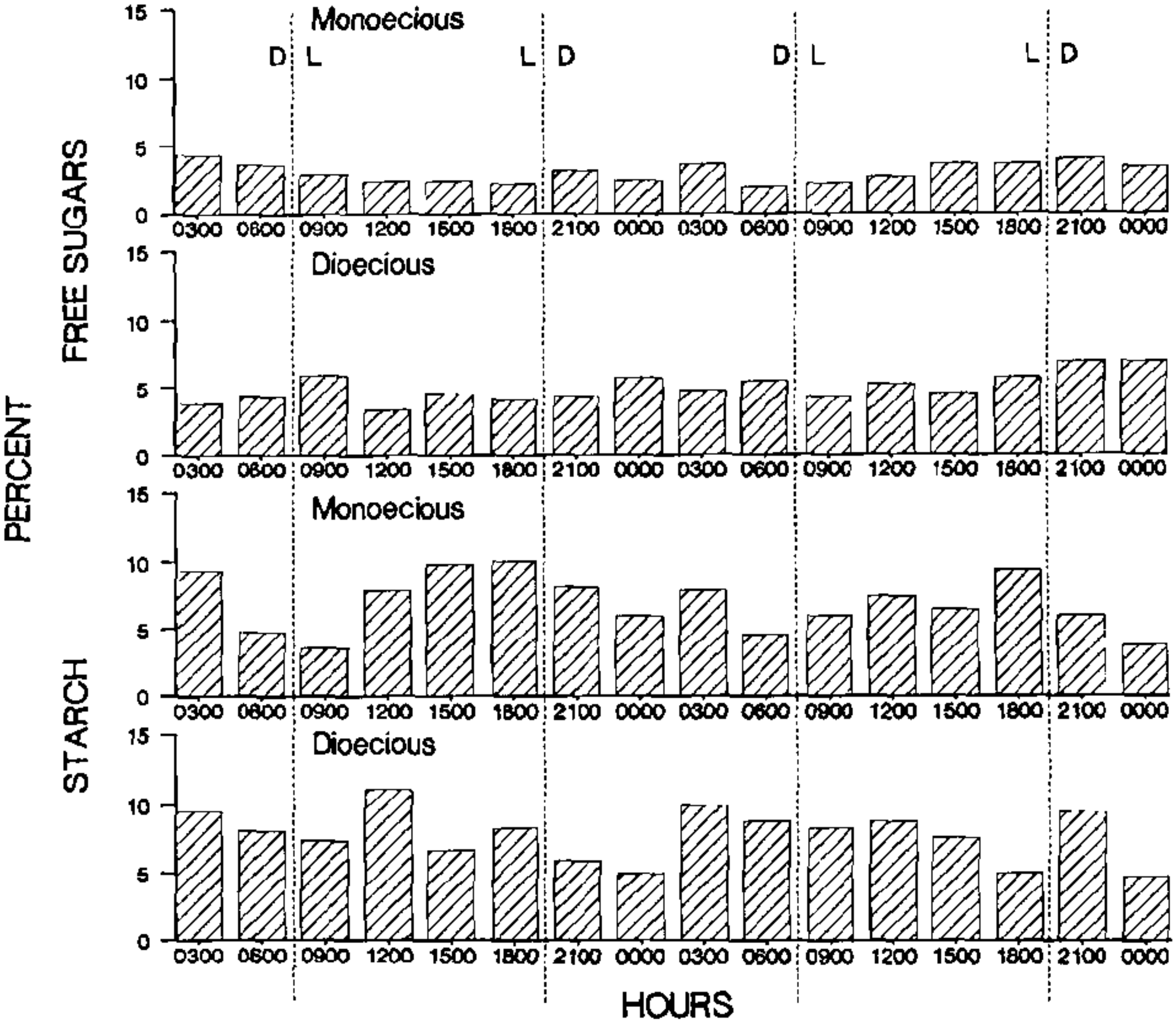

Figure 6. Diel studies of free sugars and starch in hydrilla shoots grown at 27° C with a 12:12 L-D photoperiod for 4 weeks prior to sampling. No differences were found between hours for free sugars and starch according to BLSD test ($p \leq 0.05$)

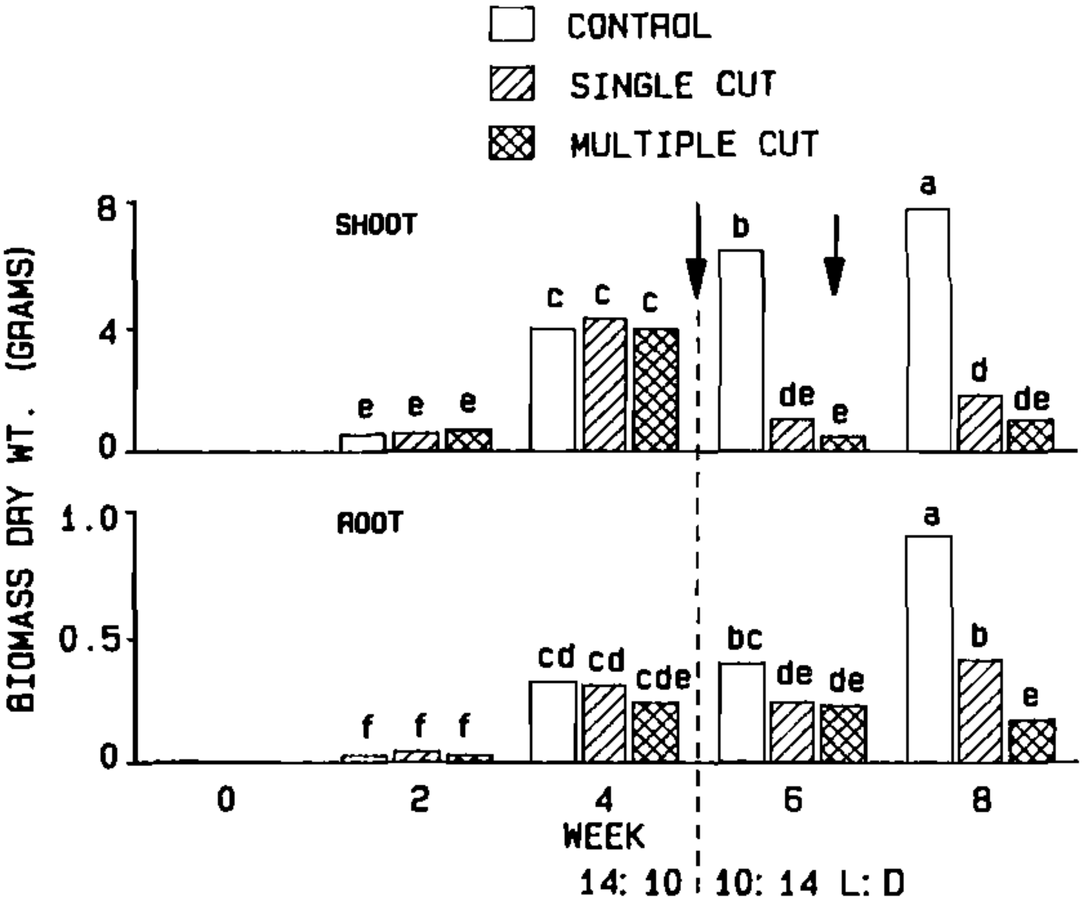

Figure 7. Mean shoot and root dry weight for monoecious hydrilla at control (no cutting), single cutting (arrow at week 4), or multiple cutting (arrows at weeks 4 and 6) grown at 27° C. Means within a row for each biotype followed by the same letter are not significantly different according to BLSD test ($p \leq 0.05$). Change in photoperiod at week 4 is indicated by dashed line

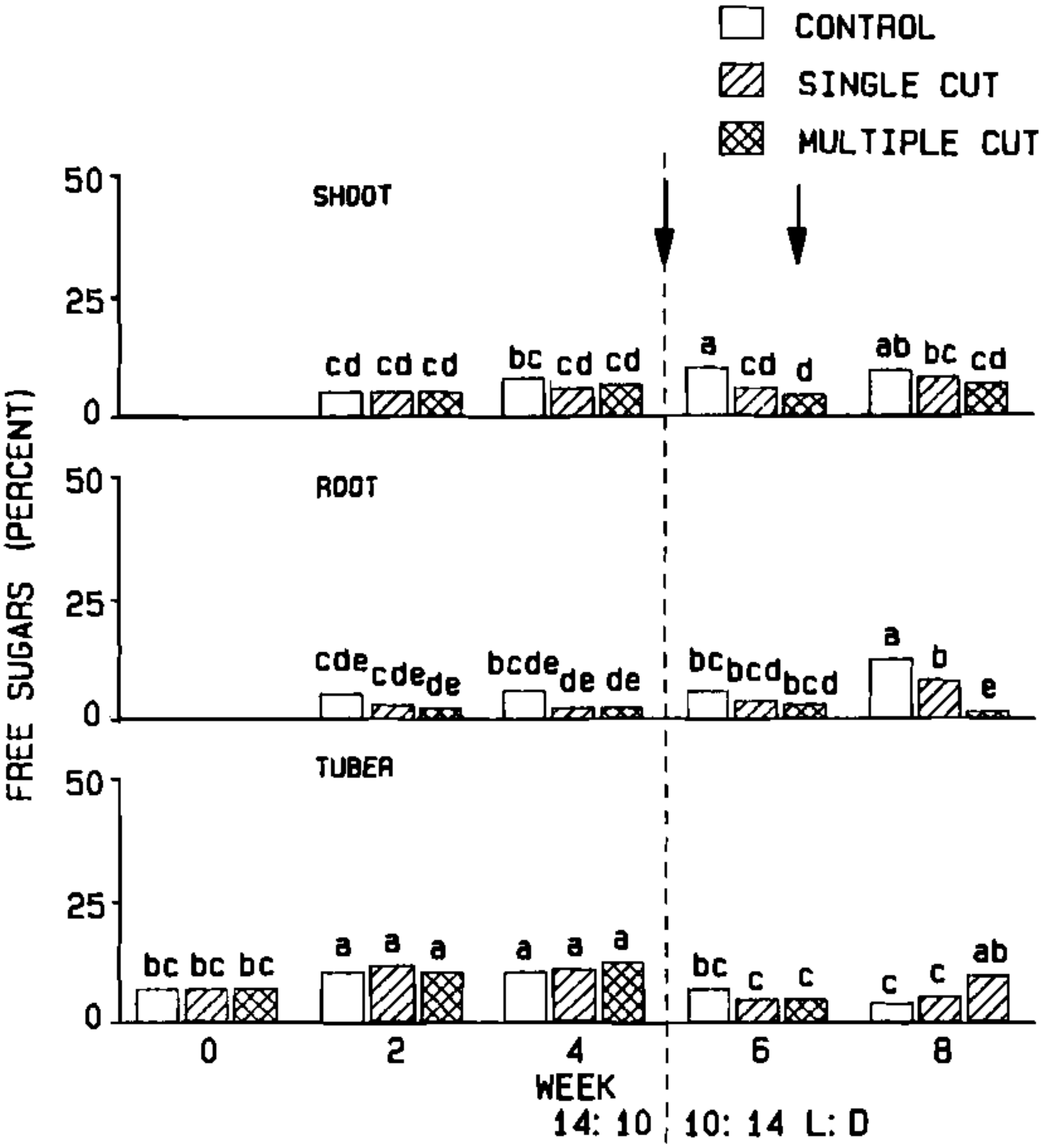

Figure 8. Free sugars in monoecious hydrilla at control (no cutting), single cutting (arrow at week 4), or multiple cutting (arrows at weeks 4 and 6) grown at 27° C. Means within a row for each biotype followed by the same letter are not significantly different according to BLSD test ($p \leq 0.05$). Change in photoperiod at week 4 is indicated by dashed line

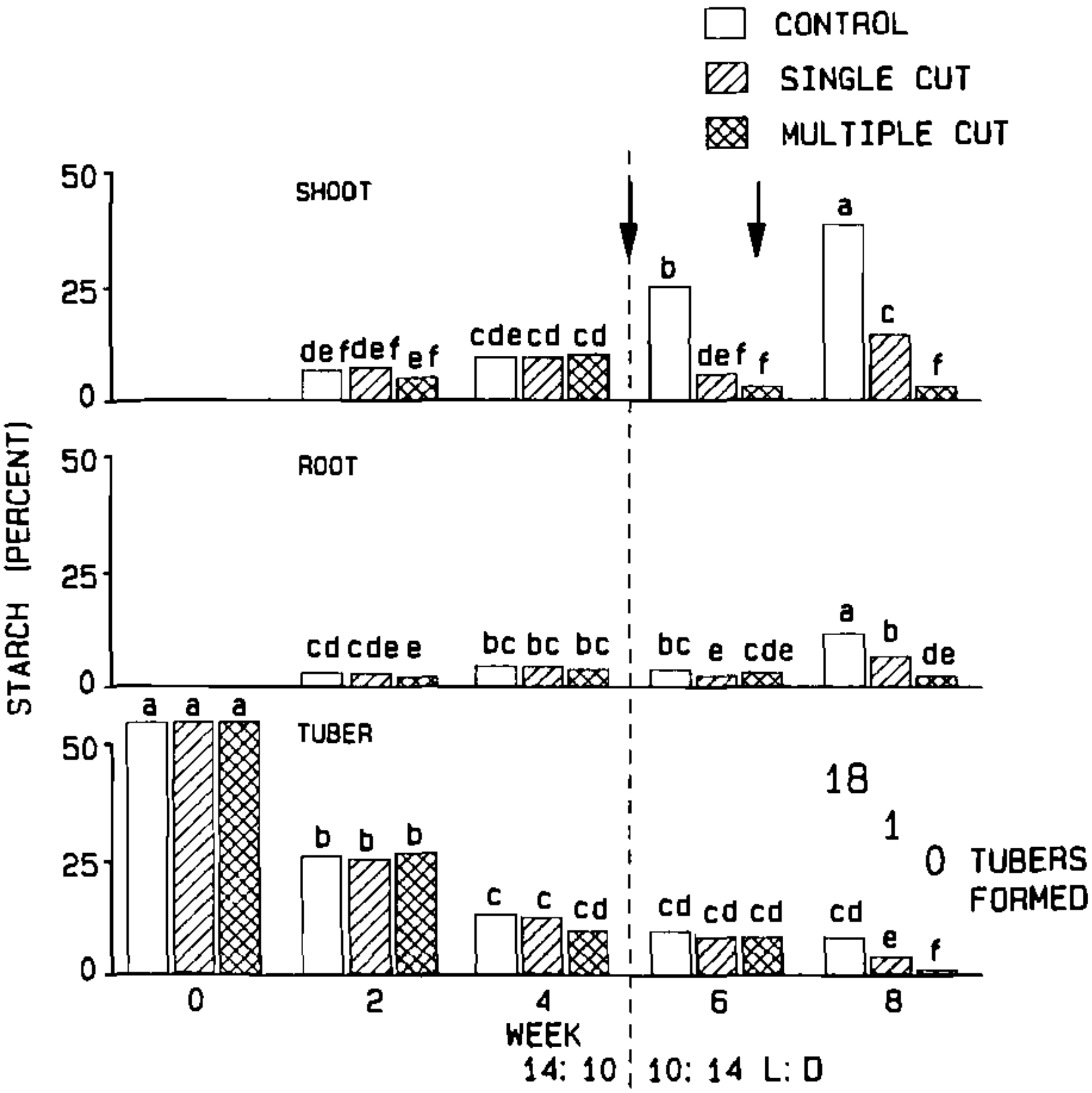

Figure 9. Starch in monoecious hydrilla at control (no cutting), single cutting (arrow at week 4), or multiple cutting (arrows at weeks 4 and 6) grown at 27° C. Means within a row for each biotype followed by the same letter are not significantly different according to BLSD test ($p \leq 0.05$). Change in photoperiod at week 4 is indicated by dashed line