Short-day Exposure Period For Subterranean Turion Formation in Dioecious Hydrilla

J. N. THAKORE, W. T. HALLER AND D. G. SHILLING

ABSTRACT
Two experiments conducted in the greenhouse determined that a minimum of 20 short-days (SD) were required for induction of hydrilla [Hydrilla verticillata (L.f.) Royle] subterranean turions. Fertilizer was applied to the plants in one study but not the other. There were 15 treatments ranging from 0 to 60 sequential days under SD conditions. Measured parameters included shoot weight, subterranean turion number and weight. The unfertilized plants began producing subterranean turions only after exposure to 20 SD, while in the other study, the fertilized plants produced subterranean turions only after being exposed to 38 SD. The shoot weight of the unfertilized plants decreased with increasing SD exposure, whereas that of the fertilized plants increased. The weight of subterranean turions produced did not significantly differ between the two studies. The unfertilized plants produced 0.17 subterranean turions per day after induction while the fertilized plants produced 0.99 per day. Key words: Photoperiod, subterranean turions, fertilizer, plant nutrients, tubers.

INTRODUCTION
Hydrilla is an exotic, submersed aquatic macrophyte that has grown aggressively and occurs throughout the Southeastern United States (Langeland 1990). As many as 10 times more subterranean turions (also called tubers) than axillary turions may be produced in a hydrilla infested area (Mitra 1995). Subterranean turions contain one apical meristem capable of regenerating a plant and may remain dormant in the hydrosoil for 1 to 5 years (Van and Steward 1990, Yeo et al. 1984). Subterranean turions are the primary reason that hydrilla rapidly regrows after management procedures since hydrated subterranean turions are capable of regenerating a plant and may remain dormant in the hydrosoil for 1 to 5 years (Van and Steward 1990, Yeo et al. 1984). The critical day length for optimum subterranean turion induction while the fertilized plants produced 0.89 per day. Studies on the effects of temperature, nutrient levels, and plant growth regulators on subterranean turion production have been carried out, but the number of SD necessary to induce subterranean turion production in dioecious hydrilla has not been determined (Netherland 1997). This study was conducted to determine the number of SD required for subterranean turion formation in dioecious hydrilla under two nutrient levels.

MATERIALS AND METHODS
The study was conducted in two greenhouses, a short-day (LD) greenhouse, in Gainesville, Florida in the winter of 1994 and repeated in the winter of 1995. The temperatures were maintained at 25 to 30°C in the LD greenhouse and between 18 to 22°C in the SD greenhouse. The LD photoperiod was 16 hours extended with fluorescent and incandescent lights, with a quantum flux density between 500 and 800 µE m⁻² s⁻¹ (LI 185 Lamda Quantum Meter). The SD greenhouse received 8 to 10 hours ambient light conditions of 800 to 1000 µE m⁻² s⁻¹. Subterranean turions were collected from established hydrilla populations grown in outdoor 900 L concrete tanks were washed, and sprouted in distilled water at 22°C under LD conditions. Sixteen sprouted turions were planted in 30 by 24 by 12 cm plastic containers filled to the depth of 5 cm with potting soil and sand. Fourteen grams of Osmocote were added to each container in the second study. Sprouted subterranean turions were planted in 60 containers for each study. The plants were placed in 540 L fiberglass tanks (well water) in the LD greenhouse. After a month of growth under LD conditions, plants were transported to the SD greenhouse for exposure to the SD treatments. The treatments ranged from 4 to 60 days under LD conditions and progressed sequentially in increments of 4 days. Every 4 days, 4 containers of hydrilla were transferred from the SD greenhouse back to the LD greenhouse to continue growth. A control group was maintained under LD conditions throughout the study.

A completely randomized design was used for the experiment. Each SD exposure treatment was replicated four times using four containers. After the plants were transferred back to the LD greenhouse, they were allowed to grow for 60 days before harvesting. After 60 LD, shoots were severed at the hydrosoil, washed vigorously to remove all soil particles, and...
dried for 2 weeks at 60°C. The soil was also washed thoroughly, recognizable subterranean turions attached to rhizome tips and detached from rhizomes were collected, counted, and dried for two weeks at 60°C. Shoot and turion dry weights were determined.

Analysis of variance (ANOVA) was carried out to measure the difference in treatment effects between the two studies. Since the treatment effects of study 1 (unfertilized) were significantly (p < 0.05) different from the treatment effects of study 2 (fertilized), the studies were not pooled and statistical analysis was carried out separately for each study. For each study, one-way analysis of variance was used to measure the effects of SD conditions on the dependant variables—shoot weight, subterranean turion number, and subterranean turion weight per container—and orthogonal contrasts were performed for each study by grouping the treatment means. Treatments that caused the formation of subterranean turions were designated as inductive and those that did not cause formation were designated as non-inductive treatments. Orthogonal contrasts were carried out to measure the difference between the two groups. Simple linear regression was conducted for analysis of shoot biomass and non-linear regression of subterranean turion number.

RESULTS AND DISCUSSION

There was a significant year by treatment interaction (p < 0.05) for the shoot weight and subterranean turion number; therefore, the studies will be discussed separately.

STUDY 1 (UNFERTILIZED)

There were significant differences in shoot biomass, subterranean turion number, and subterranean turion biomass resulting from the length of exposure to SD conditions (p < 0.05). The treatment means were pooled into two groups and analyzed to determine at what point the SD conditions cause significantly different outcomes in the shoot biomass, and subterranean number and biomass. The first 20 days of SD were grouped together because there was no subterranean turion production during this interval. This grouping was contrasted with the grouping of all means after 20 SD. The contrast indicated that after 20 SD, the shoot weight, turion number, and turion biomass of hydrilla were significantly (p < 0.05) different from the first part of the study. The estimated extent of the differences between the first 20 SD and the rest are presented in Table 1.

The shoot biomass (per container) was greater during the first 20 days than during the rest of the study. This observed decrease in shoot biomass has been found to be characteristic of hydrilla in nature during the winter months (MacDonald 1994). This decline in shoot biomass may be due to the onset of subterranean turion production at day 20 when the plants began allocating carbohydrates to the subterranean turions rather than above-ground growth (Figure 1).

Regression analysis was carried out to further examine the change in shoot weight with increasing SD conditions. The regression line describes the decrease in shoot biomass due to increasing SD conditions.

Table 1: Comparison of pooled non-inductive and inductive means for subterranean turion number, shoot and subterranean turion biomass per container in study 1 (unfertilized) and study 2 (fertilized).

<table>
<thead>
<tr>
<th>Plant component</th>
<th>H0: ( \mu_{\text{Preinduction}} = \mu_{\text{Postinduction}} )</th>
<th>Standard Error of differences</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot biomass</td>
<td>7.2 vs. 4.65</td>
<td>0.569</td>
<td>0.0001**</td>
</tr>
<tr>
<td>Turion number</td>
<td>0 vs. 3.25</td>
<td>0.65</td>
<td>0.0001**</td>
</tr>
<tr>
<td>Turion biomass</td>
<td>0 vs. 3.12</td>
<td>0.12</td>
<td>0.0034*</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot biomass</td>
<td>11.2 vs. 18.7</td>
<td>1.08</td>
<td>0.0001**</td>
</tr>
<tr>
<td>Turion number</td>
<td>0.3 vs. 11.8</td>
<td>0.72</td>
<td>0.0001**</td>
</tr>
<tr>
<td>Turion biomass</td>
<td>0.03 vs. 1.35</td>
<td>0.36</td>
<td>0.0047</td>
</tr>
</tbody>
</table>

Figure 1. The influence of SD exposure period on the shoot biomass (dry wt; container) of unfertilized and fertilized hydrilla.
that there is a linear increase in subterranean turion produc-
tion once it has been induced by a given number of SD. 
Whereas the step function model assumes that there is a
threshold effect due to SDs and after induction the number
of subterranean turions produced remains the same for all
inductive treatments. Statistical analyses using both models
demonstrated that the linear plateau model better explains
the observed data. Also, the biological effects due to SD did
not resemble a threshold type response modeled by the step
function equation. Therefore, the linear plateau model was
employed to determine the pattern of subterranean turion
production in increasing SD.
The plants began forming rhizomes after 20 SD (data not
shown) but subterranean turions were not produced until
hydrilla was exposed to 24 SD. The linear plateau regression
model calculated that after 20 days in SD conditions, hydrilla
increased subterranean turion production at the rate 0.17
subterranean turions per day (Figure 2). There is no $R^2$
value to determine the fit of the equation in non-linear regression,
but the slope of the linear plateau model had the lower and
upper limits of the confidence interval (CI) above zero $[0.12
\leq b \leq 0.22]$ indicating that the slope is significantly greater
than zero at the 95% level.

**STUDY 2 (FERTILIZED)**
Analysis of variance indicated there was a significant ($p <
0.05$) difference in shoot biomass, subterranean turion num-
ber, and subterranean turion biomass among treatments
(Table 1).
The first 40 means were grouped as non-inductive and
contrasted with the inductive means (SD > 40). The shoot
biomass of the second part of the study was significantly
higher ($p < 0.05$) and the estimated difference between the
two groups was calculated (Table 1).
Regression was carried out to predict the change in shoot
biomass due to the change in SD conditions (Figure 1). In
contrast to the first year study, the shoot biomass of hydrilla
increased with increasing SD in the second study which is
most likely due to the addition of fertilizer in the second
study.
After 20 SD days there was a decrease in the shoot biomass
of unfertilized plants (study 1) which coincided with the
onset of subterranean turion production. The fertilized
plants, on the other hand, began increasing shoot biomass.
Since we assume there were ample nutrients in study 2, the
vegetative growth may have been prolonged due to less nutri-
tion stress. Due to the delay in reproduction, plants may have
had a longer period to increase top growth, causing an
increase in shoot biomass.
Analysis of variance indicated that subterranean turion
production for the second study was significantly influenced
by the duration of SD ($p < 0.05$). Orthogonal contrasts were
carried out to determine how the inductive means differed
from non-inductive treatment means in their effect on sub-
terranean turion number and weight. The number and bio-
mass of the subterranean turions were significantly greater in
the second part of the study than in the first part. The esti-
mated differences between the two treatment groups are
listed (Table 1). For further analysis, non-linear regression
was carried out to predict the change in subterranean turion
production with increasing SD. The linear plateau model cal-
culated that 38 days of exposure to SD conditions were criti-
cal for subterranean turion induction in the fertilized plants.
After 38 SDs, hydrilla increased subterranean turion produc-
tion at the rate of 0.89 subterranean turions per day (Figure
2). The slope was significant and positive for the linear pla-
teau model with a CI of $[0.63 \leq b \leq 1.16]$.
A possible mechanism that may explain the difference in
shoot biomass and subterranean turion production in Study
2 is possible differences in endogenous hormonal regula-
tion. Several researchers have demonstrated that interaction
among phytotremes are involved in asexual reproduction
in plants (Mohr and Schopfer 1995). Turion formation in
*Spirodela polyrrhiza* was found to be controlled by ABA and
cytokinins (Smart and Trewavas 1983a, Smart and Trewavas
1983b). Although ABA is not directly responsible for subter-
rande turion production in hydrilla, it seems to play an indi-
trect role (MacDonald, 1994). Rhizome formation in
dioecious hydrilla depends on a the SD stimulus, and ABA
may be produced after rhizome differentiation to maintain
dormancy and induce swelling of the rhizome tip (Mac-
donald 1994).
The experiments reported here showed that 20 to 24 SD
stimulate subterranean turion production in unfertilized

Figure 2. The influence of SD exposure period on the subterranean turion
production (number per container) in unfertilized and fertilized hydrilla.
plants, and fertilized plants will begin producing subterranean turions after 38 to 44 days of SD conditions. The fertilized plants were more vigorous throughout the study and increased in shoot biomass throughout the study in contrast to the unfertilized plants. The number of subterranean turions produced and the rate of production were significantly higher in fertilized plants, but the onset of turion production was delayed. We also found that once subterranean turions were induced there was no statistically significant difference in turion biomass between the fertilized and non-fertilized plants (data not shown).

The results from these experiments may help in planning more effective and economically feasible herbicide treatment strategies which could be accomplished by applying herbicides or other management strategies at a time that would prevent subterranean turion formation.

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LITERATURE CITED


