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Two stages of dormancy in turions of *Potamogeton crispus* L.

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ABSTRACT

Vegetative buds (turions) are the major source of propagation for the aquatic invasive angiosperm, *Potamogeton crispus* L. A better understanding of the factors that regulate turion dormancy and sprouting would lead to better methods of population control. Turions were collected and divided into two age groups: new, current season turions; and overwintered turions that were at least 1 year old. Sprouting was monitored in these two groups exposed to varying light durations and temperatures. Glucose and starch content and photosynthesis were also determined. Current season turions are dormant but metabolically active over a 6 weeks period. A small percentage of these new turions sprouted in response to short day-long night light durations at a temperature of 23 °C. Turions that did not sprout progressed to a deeper stage of dormancy similar to overwintered turions. Overwintered turions were not photosynthetically active and had stable carbohydrate levels. Under laboratory conditions, a large percentage of these turions sprouted in response to warming to 23 °C and further stimulated by exposure to any light duration. These different stages of dormancy based on age can explain much of the variability in turion sprouting reported by other researchers. A small percentage of both types of turions maintain a prolonged state of dormancy that is not broken by external stimuli.

1. Introduction

Potamogeton crispus L. is a submerged aquatic plant that is native to Europe, Asia and parts of Africa. It was first reported in the USA in 1859. It is currently widespread in temperate lakes in North America where it is a major nuisance species. Dense plant growth interferes with commercial and recreational uses of lakes. In addition, *P. crispus* changes the ecological characteristics of aquatic systems by solubilizing phosphorus and fixed nitrogen from the sediments which contribute to algal growth. This release of nutrients and algal growth leads to displacement of native plants and associated animal species (see Bolduan et al., 1994 for a review).

The unique life cycle of *P. crispus* in temperate regions makes it especially difficult to manage. As in many other aquatic plants, reproduction is mainly vegetative rather than by seed (Sculthorpe, 1967). In *P. crispus*, reproduction is due to vegetative buds called turions. The turions form on mature plants in late spring (June). The turions consist of 2-7 dormant buds covered with a hard structural carbohydrate. Turions are typically 1–4 cm long and approximately 1 cm wide although morphology can vary. They are initial green in color but change to orange or brown hues after release from the parent plant (Heuschele, 2013). As the parent plant senesces, turions with a few attached leaves are released into the water and float to new locations. The leaves soon decay and the turions sink to the sediment. The turions are dormant over the summer and a percentage of them sprout as lake temperatures decline in the autumn (September) and start to grow. Growth stops as temperatures drop in the winter but the plants remain viable under ice-cover. The small *P. crispus* plants resume growth when temperatures rise in the spring before most native plants have begun to grow. The cycle then repeats. Current management of *P. crispus* involves treating the adult plants with various herbicides in spring before turions form (Netherland et al., 2000; Poovey et al., 2002). However, subsequent sprouting of turions that remain in the sediment can restore the plant population (Johnson, 2010). Under controlled conditions, Yeo (1966) has determined that one turion can give rise to a plant that will produce up to 945 new turions. Effective management of *P. crispus* requires a better understanding of how to manipulate turion dormancy and sprouting.

Several research groups have studied turion dormancy under both laboratory and field conditions. Temperature and light duration are the chief abiotic triggers to end dormancy. However, different research groups have reported varied results. For example, Kadono (1982) found that 100% of turions would sprout in the light at 25 °C. In contrast, Waisel (1971) found only 25% sprouting at 20 °C. Rogers and Breen (1980) recorded a 58% maximum

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sprouting of turions in the light at 15 °C. Sastroutomo (1981) found that green turions would sprout at 100% if subjected to an extreme temperature pretreatment. His brown turions sprouted at a lower rate. Some of these contradictory results may be due to the different ages of the turions. In this work, we specifically collected *P. crispus* turions at different times of the year and divided them into two different age groups: newly-formed turions that were taken directly from plants in late spring and overwintered turions that were taken from sediments in early spring and were at least 1 year or more older. We then determined the effects of light and temperature on sprouting. We also determined metabolic activity both before and after sprouting in these two groups. Major differences were found between these two different age groups which can explain much of the variability observed by previous researchers. The factors that determine whether a turion remains dormant or sprouts depend on the stage of dormancy.

2. Materials and methods

2.1. Turion collection

Turions were collected from Lake Sarah, Hennepin County, Minnesota, USA (T119N, R24W, Sec. 34 and 35). This 227 ha lake was chosen because it is heavily infested with *P. crispus* and has not been extensively treated with herbicides. Samples were collected from three to four different sites at the west end of the lake in May before plants produce new turions. These turions were designated the overwintered population and are a minimum of 1 year old. Turions were taken directly from mature plants at the same sites in June and were the current season population. Collections were done over four seasons from 2009 to 2012. Between 900 and 1500 current season turions were collected in a single year. Overwintered turions were less common (100–400 per season). Turions were also collected in September of 2008 to provide a field population comprised of both populations. A ponar grab (Wildco, Wills Point, Texas, USA) and dip-net were used to harvest the overwintered and mixed populations of turions from the sediment. Turions were washed and submerged in distilled water (pH 6.5). A subset of each population was frozen immediately on return to the laboratory and stored at −20 °C to provide the initial measurements for chemical analyses. Additional turions that were not used immediately in sprouting experiments were stored at 4 °C in the dark. The mixed population of turions collected in September was sorted by color. All green turions were assumed to be the current season's production. Orange and brown turions were assumed to be a mixture of current season and overwintered populations. Black turions were previously determined to be dead and were discarded.

2.2. Turion sprouting conditions

To determine the effects of light duration on turion sprouting, sets of 60 ± 15 turions were exposed to 10:14 light:dark cycles (L:D) and 16:8 L:D to simulate autumn and summer light durations, respectively. Four 40-W Philips Econ-o-watt fluorescent bulbs were used for the 16:8 L:D cycles with a photosynthetically active radiation (PAR) value of 23 μmol of photons $m^{-2}\,s^{-1}$. The autumn 10:14 L:D exposures were conducted in a separate room with similar lighting and a PAR value of 28 μmol of photons $m^{-2}\,s^{-1}$. The temperature in both rooms was maintained at a constant 23 °C. Additional sets of turions were placed in complete darkness, one set at 23 °C and a second set, at 4 °C. Turions were monitored for sprouting and the numbers were recorded. Sprouted turions were removed from each experimental set as soon as they were visible. The sprouted buds were removed from the turions and both parts were pooled by week of sprouting and frozen at −20 °C. One additional set of unsprouted current season turions (200 ± 50) was placed in dim light (PAR = 2.7 μmol of photons $m^{-2}\,s^{-1}$) at 4 °C to simulate over-wintering conditions. After 7 weeks of this treatment, these turions (approximately 50 for each condition) were placed in one of the four different experimental light and temperature conditions described above.

2.3. Glucose and starch analyses

Pooled turions samples were analyzed for glucose using a modification of the Trinder method for measuring glucose in blood samples (Trinder, 1969). In this assay, glucose oxidase catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide. The peroxide then reacts with 4-aminoantipyrine and p-hydroxybenzene sulfonate to form a colored quinoneimine product. This reaction is catalyzed by a peroxidase. The Trinder reagent was prepared fresh when needed and contained 0.5 mM 4-aminoantipyrine, 20 mM p-hydroxybenzene sulfonate, 15 U ml^{-1} glucose oxidase from *Aspergillus niger*, and 10 U ml^{-1} of horseradish peroxidase in 100 mM Tris–HCl buffer, pH 7.5. All chemicals were purchased from Sigma Diagnostics (St. Louis, Missouri, USA).

Turions (5–10 for each analysis) were lyophilized to dryness and ground to a fine powder. Aliquots were removed, weighed (20 ± 5 mg) and suspended in 0.5 ml distilled, deionized water. Tannins in the samples inhibit enzyme activity and were removed by adding a known volume of a slurry of 30% diethylaminoethyl (DEAE) cellulose (GE Healthcare, Piscataway, New Jersey, USA). This mixture was incubated for 10 min at 23 °C and then centrifuged at 8500 × *g* for 6 min. Aliquots of the supernatant were added to 0.5 ml Trinder reagent for a total volume of 0.6 ml. A sample containing a known concentration of glucose was used as an internal standard. The reaction tubes were incubated at 37 °C for 30 min and then placed on ice to stop the reaction. The absorbance at 506 nm was determined in an 8450A diode array spectrophotometer (Hewlett-Packard, California, USA). The glucose concentration in the samples was determined from a standard curve.

Starch content was determined by suspending 20 ± 5 mg of ground turion sample in 0.5 ml of acetate buffer, pH 4.8. A DEAE slurry (0.5 ml) was added and the mixture was treated as described above to remove tannins. After tannin removal, 50 μl of amyloglucosidase (10 mg ml^{-1}; from *A. niger*) were added and the mixture was further incubated at 60 °C for 20 min. The mixture was placed on ice to stop the reaction and centrifuged at 8500 × *g* for 10 min. Aliquots of the supernatant were analyzed using the Trinder protocol. The concentration of starch in the sample was calculated by subtracting the amount of free glucose from the total glucose obtained after amyloglucosidase digestion. Corn starch was used as a standard to verify the amount of digestion.

2.4. Chlorophyll and tannin analyses

Turions (5–6 per analysis) were lyophilized and ground to a fine powder. For chlorophyll analysis, 20 ± 5 mg of powder were suspended in 1 ml of 95% ethanol and incubated at 4 °C for 16 h in the dark. The suspensions were centrifuged at 8500 × *g* for 10 min and the visible absorption spectra (400–700 nm) of the supernatants were determined. The concentration of chlorophyll *a* was calculated using the extinction coefficient, ϵ = 82.04 mg ml^{-1} at 662 nm (Arnon et al., 1974).

For tannin analysis, 20 ± 10 mg of lyophilized turion powder were suspended in water and stirred for approximately 16 h at 4 °C. The suspension was centrifuged as above and the water-soluble phenolic content of the supernatant was determined with the Folin–Ciocalteau reagent (Gross et al., 1996). Tannic acid was used as a standard.