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TOXICITY OF THE NORFLURAZON TO THE AQUATIC MACROPHYTE *VALLISNERIA AMERICANA* (MICHX.)

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Vallisneria americana *(Michx.) (common name tapegrass) is a submersed, vascular aquatic plant that reproduces vegetatively and by seed. The objective of this study was to determine the no-observable-effects concentrations (NOECs) and lowest-observable-effects concentrations (LOECs) for tapegrass exposed to the herbicide norflurazon (0–0.1 mg/L) following a 14-d exposure and a postexposure period. The primary symptom of norflurazon toxicity was bleaching of newly emerged leaf blades at concentrations of 0.04 mg/L and higher after 14 d of exposure. Leaf greenness effect levels were 0.04 mg/L (NOEC) and 0.06 mg/L (LOEC). All other endpoints measured resulted in a NOEC greater than 0.1 mg/L following the exposure period. Latent effects were observed 14 d postexposure for new leaf production and fresh weight gains, with a NOEC and LOEC of 0.08 and 0.1 mg/L, respectively. Total leaf growth was the least sensitive endpoint measured. Following the exposure/postexposure periods, significant effects on vegetative reproduction were apparent, with no effects occurring at concentrations up to 0.08 mg/L, but with significant reduction at the 0.1 mg/L treatment level. Root and stolon dry weights were significantly reduced at the 0.1 and 0.08 mg/L treatments, respectively. Total soluble sugars (TSS) and hexose content in shoots was reduced at concentrations of 0.04 mg/L and higher. TSS, hexose, and sucrose contents were higher in roots of plants exposed to 0.1 mg/L. Some recovery was apparent for all treatment concentrations following the postexposure period, indicating that the effects were at least partially reversible.*

Vallisneria americana (Michx.) (common name American tapegrass) is a submersed, vascular aquatic plant that reproduces vegetatively and by seed. It has tape-like leaves 20–50 mm wide and grows up to several meters in length (Hoyer et al., 1996). This plant is commonly found in both still and running waters, and provides valuable food resources for waterfowl and refuge for aquatic invertebrates and fish populations (Hoyer et al., 1996). One objective of the Comprehensive Everglades Restoration Project (CERP) is to manage

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water quality in the North Fork of the St. Lucie River at levels suitable for establishing/reestablishing tapegrass meadows (SFWMD, 2002). However, discharges of herbicides from surrounding land uses drained by Ten Mile Creek, Canal-23, Canal-24, and urban areas may significantly retard establishment, development, and reproduction of tapegrass.

One herbicidal active ingredient of possible concern is norflurazon [4-chloro-5-methylamino-2-(α,α,α-trifluoro-*m*-tolyl)-3(2*H*-pyridazinone)]. This fluorinated pyridazinone (phenylpyridazinone) is moderately water soluble (28 mg/L), and relatively nonvolatile (2.66×10^{-9} kPa at 20°C) (Vencill, 2002). It is labeled for pre- and postemergent control of grassy and broadleaf weeds in citrus production and other agronomic crops. Norflurazon blocks carotenoid biosynthesis by inhibiting phytoene desaturase (Vencill, 2002). Carotenoids play a protective role by dissipating excess energy from photosystem II. Without these protective molecules, triplet chlorophyll and singlet oxygen species form, resulting in lipid peroxidation and ultimately the destruction of chlorophyll and membrane lipids (Vencill, 2002). The most visible symptom of norflurazon activity is bleaching of plant leaves. Through a quarterly sampling program conducted by the South Florida Water Management District (SFWMD), norflurazon was detected in surface water samples from several local waterways, some of which drain into the St. Lucie River. Its presence was reported in 89, 100, and 93% of samples collected from the C-44 and C-25 canals, and Ten Mile Creek, respectively, at concentrations ranging from 0.04 to 1.6 μg/L (Pfeuffer, 1998, 1999; Pfeuffer & Matson, 2000, 2001, 2002, 2003). The land areas draining into these tributaries are primarily agricultural.

Since no information was available regarding the toxicity of norflurazon to *V. americana* (Michx.), this study was initiated to determine the no-observable-effects concentration (NOEC) and lowest-observable-effects concentration (LOEC) for a variety of endpoints following a 14-d exposure and a 14-d postexposure period.

MATERIALS AND METHODS

Plant Culturing

All plants utilized in these assays were offspring from stock plants originally collected from Lake Okeechobee, FL. Stock plants were collected from northern Lake Okeechobee in November 2001 by SFWMD staff. These plants were transplanted into 1135-L RubberMaid cattle watering tanks located inside of an enclosed greenhouse (UF/IFAS IRREC, Fort Pierce, FL). Fine grade play sand was added to each tank to serve as a substrate (5.1 cm depth). Stock plants were fertilized using Florikan controlled-release fertilizer (13-13-13). Stock tanks were filled with aged tap water, originating from the Fort Pierce Utilities Authority.

Subcultures of the stock plants were established under controlled laboratory conditions in 38-L glass aquaria. Offspring produced vegetatively from these

plants were used in each toxicity assay. This arrangement allowed plants to acclimate to testing conditions for several weeks before tests were initiated. These plants were grown under a 16-h light: 8-h dark photoperiod using GE Plant and Aquarium wide-spectrum fluorescent lights (~76 μmol at 30 cm height). The water temperature was maintained at 25°C using aquarium heaters. Readily available play sand was also used as the culture substrate. However, in this case the sand was acid washed prior to use. Synthetic, reconstituted, very hard water was used for culturing and testing plants (APHA, AWWA, & WEF, 1995). General characteristics of the reconstituted water included: pH 8.2 ± 0.2, electrical conductivity 826 ± 14 μS, alkalinity 207 ± 7 mg/L as $CaCO_3$, and hardness 226 ± 6 mg/L as $CaCO_3$.

Toxicity Testing Procedures

Toxicity tests were conducted on individual plants grown within individual 1-L glass cylinders. Plants of relatively uniform size (fresh weight range: 0.44–1.34 g) and appearance were chosen for the assay. Norflurazon was administered to four individual, replicate plants for each treatment concentration. Plants were carefully removed from the testing stock cultures, taking care not to damage roots. Following collection of nondestructive data (see next sections), plants were assigned an individual identification code and placed in unspiked assay water until placement in testing cylinders.

For placement in individual 1-L glass cylinders, 100 ml of acid-washed sand was first poured into each cylinder. One gram of slow-release fertilizer (Nutricote, 13-13-13) was then added, followed by approximately 100 ml of treatment solution. Fertilizer sources and rates were based on recommendations from SFWMD staff, UF/IFAS Center for Aquatic Plants, and aquatic plant nurseries. Each plant was then placed within the cylinder using a glass tube. While holding the plant roots above the previously poured sand, an additional 100 ml sand was added to secure the plant roots within the substrate. The balance of the 1-L treatment volume was then added to the cylinder. The final water level was marked on the outside of each container. Water volume within each cylinder was maintained by daily additions of deionized water to the marked level.

Testing solutions were made by dilution of a 10-mg/L stock solution. For range-finding assays, concentrations of 0.001, 0.01, 0.1, 1, and 10 mg/L were evaluated (data not shown). Based on the results, definitive tests were initiated to determine the no-observable-effects concentration (NOEC) and the lowest-observable-effects concentration (LOEC). Concentrations evaluated for NOEC/LOEC determination included 0, 0.02, 0.04, 0.06, 0.08, and 0.1 mg/L norflurazon. Herbicide concentrations were confirmed using a Hewlett Packard 5890 Series II gas chromatograph equipped with a flame ionization detector and DB-5 column. For confirmations, two 200-ml sample aliquots for each treatment concentration were extracted using activated C_{18} columns following the method of Keese et al. (1994). After air-drying the columns, norflurazon was eluted with 2 ml pesticide-grade acetone. Recoveries were greater than 90%.

Measured Endpoints

Growth Nondestructive growth measurements included: (1) fresh weight gains after 14-d exposure and 14-d postexposure periods, (2) increases in total leaf length after 14 d of exposure and 14 d postexposure, and (3) production (quantity and fresh weight) of stolons and asexually produced offspring. Using an Ohaus top-loading balance (model TS 400s), all fresh weights were taken after removing excess moisture from plants by gently blotting with paper towels. Plants were then immediately rewetted to avoid desiccation while other measurements were taken. Destructive analyses were conducted at the end of the study. All plants were divided into shoots, roots, and stolons. Plant parts were freeze-dried over a 1-wk period and dry weights of each were recorded. Subsamples of these tissues were then used for destructive chlorophyll and carbohydrate analyses.

Carbohydrates Procedures for total soluble sugars and starch determination were modified as described by Haissig and Dickson (1979) and Miller and Langhans (1989). Glass Pasteur pipettes with glass-wool plugs were loaded with 50 mg of each freeze-dried sample. Three extractions with 1.5 ml of 12 methanol:5 chloroform:3 water (by volume; MCW) were carried out for soluble sugar extraction before the residue was used for starch extraction.

Total soluble sugars (TSS; sucrose, glucose, and fructose) were analyzed using a Waters 2695 high-pressure liquid chromatograph (Waters Technological Corporation, Milford, MA) equipped with a Waters 2414 refractive index detector (Waters Technological Corporation, Milford, MA) and a BioRad Aminex HPX-87C column (BioRad Laboratories, Hercules, CA). Column and detector temperatures were maintained at 80°C and 50°C, respectively. HPLC-grade water was used as the mobile phase, at a flow rate of 0.6 ml/min.

For starch analysis, the tissue residue was dried overnight at 60°C, suspended in 4 ml Na acetate buffer (100 m*M*, pH 4.5) and placed in a boiling water bath overnight. After cooling to room temperature, 1 ml amyloglucosidase solution (from *Rhizopus* mold, Sigma-Aldrich Co., St. Louis, MO; 50 units enzyme/assay in 0.1 *M* pH 4.5 Na acetate buffer) was added to each test tube. Samples were incubated for 48 h at 55°C with occasional agitation. Glucose determinations via the glucose oxidase and peroxidase enzymatic method were completed on 100-μl samples (Haissig & Dickson, 1979). Absorbance at 450 nm was determined using a Beckman DU-64 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA), and starch content was calculated based on the regression equation of the glucose calibration curve (0.0 to 0.6 μmol).

Leaf Greenness and Chlorophyll *a* Nondestructive measurements of leaf greenness (SPAD) were taken using a Minolta chlorophyll meter (SPAD-502) before treatment and after the 14-d exposure and 14-d postexposure periods. SPAD values, defined by Minolta, provide an indicator of the relative amount of chlorophyll present in plant leaves. These measurements are based on the amount of light transmitted by the leaf in two wavelength regions in which the absorbance of chlorophyll is different (Minolta, 1989). Chlorophyll *a*

was extracted from plant tissues using *N,N*-dimethylformamide (DMF) over a 48- to 72-h period, in darkness, and at 5–7°C. This measurement was not made following the initial 14-d exposure because of its destructive nature and the limited quantity of available plants. Absorbance of extracts was then measured at 664 and 647 nm using a Cary 300 (Varian, Inc., Walnut Creek, CA) spectrophotometer. Chlorophyll *a* was calculated using the method outlined by Moran (1982).

Latent Effects/Recovery

Following collection of nondestructive data after the 14-d exposure period, individual plants were rinsed with deionized water and were planted in community 18.9-L containers (substrate and media same as already described) along with other plants from the same treatment concentration in order to observe possible latent effects or recovery. After 2 d, half of the water was replaced with newly reconstituted water to dilute pesticide concentrations that may have resulted from depuration. At the end of this 2-wk recovery/depuration period, the previously mentioned endpoints were remeasured, with the addition of total chlorophyll *a*, total soluble sugars, and starch measurements.

Statistical Analysis

All data were subjected to an analysis of variance (ANOVA). Treatment means were separated from control means using calculated least significant differences ($p = 0.05$) for each endpoint (SAS Institute software, Cary, NC).

RESULTS

Growth

No significant differences in fresh weight gains were observed following the 14-d exposure period at any of the treatment concentrations (Table 1). Plants gained an average of 0.68 to 0.99 g fresh weight during this period. However, following the 14-d postexposure period, some latent effects were observed at the highest concentration (0.1 mg/L), with fresh weight gains being 65% lower than controls.

No significant differences were observed for new leaf production following the 14-d exposure period (Table 1). New leaf production during this period ranged from 2.8 to 6. However, following the 14-d postexposure period, new leaf production was reduced 47% for the plants exposed to 0.1 mg/L, relative to controls. Likewise, no significant differences were observed for total plant leaf growth following the exposure period. Total plant leaf lengths increased on average from 15 to 30.3 cm during this period. Following 14 d of exposure, leaf length of plants exposed to 0.1 mg/L norflurazon was only 62% of that observed in the controls, yet statistically insignificant due to the large amount of variability observed within the treatment groups.

TABLE 1. Summary of *Vallisneria americana* (Michx.) (American Tapegrass) Growth Measurements Following 14-d Exposure and 14-d Postexposure Periods to Varying Concentrations of Norflurazon

	Fresh weight production (g)				New leaf production (#)				Total leaf growth (cm)			
	14-d Exposure		14-days Postexposure		14-d Exposure		14-d Postexposure		14-d Exposure		14-d Postexposure	
Norflurazon (mg/L)	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
0	0.99	0.11	1.18	0.25	4.3	0.45	10.3	0.18	21.5	0.43	36.0	0.62
0.02	0.80	0.18	0.98	0.18	4.0	0.20	8.5	0.43	23.0	0.21	35.5	0.24
0.04	0.76[a]	0.16	1.23	0.12	2.8	0.91	9.0	0.45	19.4	0.40	44.1	0.30
0.06	0.95	0.39	0.79	0.43	4.5	0.13	10.5	0.42	24.8	0.40	34.3	0.53
0.08	0.68	0.39	0.73	0.54	3.8	0.46	10.3	0.33	15.0	0.15	39.5	0.22
0.1	0.72	0.45	0.41[a]	0.29	6.0	0.61	5.5[a]	0.43	30.3	0.33	22.5	0.54

Note. CV, coefficient of variation; $n = 4$ for all treatments.

[a]Indicates mean is significantly different from control; ANOVA ($p = .05$).

At the end of the 14-d exposure + 14-d postexposure periods, plants exposed to the 0.1-mg/L norflurazon treatment vegetatively produced an average of 50% fewer offspring relative to the controls and other concentrations (Table 2). Between 7 and 9 total offspring were produced by each set of 4 plants exposed to 0.08 mg/L and less, with the controls producing a total of 8. Plants exposed to 0.1 mg/L only produced a total of 4 offspring. While not statistically different, the mean fresh weight of the offspring produced by plants exposed to 0.1 mg/L was 41% lower relative to controls (Table 2). Stolon production by the parent plants was also markedly reduced with the 0.1 mg/L treatment. Mean stolon fresh weights in this treatment were reduced 57% relative to controls. Stolon fresh weights for the 0.02, 0.04, 0.06, and 0.08 mg/L treatments were similar to the controls.

Norflurazon treatment did not affect mean shoot dry weight of plants, regardless of concentration (Table 3). However, root dry weights were reduced

TABLE 2. Summary of *Vallisneria americana* (Michx.) (American Tapegrass) Vegetative Reproduction Measurements Following 14 d of Exposure and 14 d Postexposure to Norflurazon

Norflurazon (mg/L)	Offspring quantity		Offspring fresh weight (g)		Stolon fresh weight (g)	
	Mean	CV	Mean	CV	Mean	CV
0	2.0	0.0	0.39	0.33	0.21	0.31
0.02	1.8	0.5	0.38	0.57	0.17	0.54
0.04	1.8	0.9	0.50	0.43	0.15	0.76
0.06	2.0	0.4	0.40	0.62	0.20	0.38
0.08	2.3	0.4	0.43	0.50	0.15	0.31
0.1	1.0[a]	0.0	0.23	0.27	0.09[a]	0.60

Note. CV, coefficient of variation; $n = 4$ for all treatments.
[a]Indicates mean is significantly different from control; ANOVA ($p = .05$).

TABLE 3. Summary of *Vallisneria americana* (Michx.) (American Tapegrass) Dry Weights Following 14 d of Exposure and 14 d Postexposure to Norflurazon

Norflurazon (mg/L)	Dry weight (mg)					
	Shoots		Roots		Stolons	
	Mean	CV	Mean	CV	Mean	CV
0	199.4	0.2	102.6	0.4	14.1	0.1
0.02	208.7	0.2	69.1	0.5	9.7	0.5
0.04	222.0	0.2	58.4	0.4	9.7	0.3
0.06	214.6	0.4	60.2	0.2	10.9	0.3
0.08	185.0	0.3	63.3	0.5	7.8[a]	0.4
0.1	163.8	0.2	35.3[a]	0.3	5.7[a]	0.6

Note. CV, coefficient of variation; $n = 4$ for all treatments.
[a]Indicates mean is significantly different from control; ANOVA ($p = .05$).

by 66, 45, and 60% for the 0.06-, 0.08-, and 0.1-mg/L treatments, respectively, relative to the controls. The 0.08- and 0.1-mg/L treatments reduced stolon dry weight by 45 to 60%.

Carbohydrates

Average total soluble sugars in the shoots ranged from 14.9 to 31.2 mg/g dry weight (Table 4). Relative to the controls, TSS levels in plant shoots were significantly reduced when exposed to the 0.04, 0.06, 0.08, or 0.1 mg/L treatments. Reductions ranged from 48 to 59% of control levels. Within the roots, TSS ranged from 3.2 to 9.2 mg/g dry weight. Root TSS was similar to the controls at all concentrations except 0.1 mg/L, where a two-fold increase was observed. Total TSS in the stolons generally decreased as norflurazon concentration increased.

Hexose levels in the shoots ranged from 10.0 to 25.1 mg/g dry weight, and were statistically lower than controls at the 0.04-, 0.06-, and 0.1-mg/L treatments at the end of the assay (Table 4). Hexose levels in the roots ranged from 2.9 to 7.7 mg/g dry weight. Hexose levels in the roots were not significantly different than the controls in any of the treatments. Total hexose levels in stolons generally decreased with increasing norflurazon concentrations.

Sucrose levels in the shoots ranged from 4.6 to 6.9 mg/g dry weight and were statistically similar to the controls at all treatment levels (Table 4). Sucrose levels in the roots ranged from 0.1 to 1.5 mg/g dry weight. Similar to TSS and hexose, levels in the roots were significantly higher (5.2-fold) than controls in the 0.1-mg/L treatment only. Total sucrose levels in stolons generally decreased with increasing norflurazon concentrations.

Starch levels in the shoots and roots were highly variable and nonsignificant, ranging from 1.7 to 11.1 and from 10.3 to 43.1, respectively (Table 5).

Leaf Greenness and Chlorophyll *a*

Summaries of the leaf greenness measurements after the 14-d exposure and 14-d postexposure periods are listed in Table 6. After 14 days exposure, leaf greenness was significantly reduced at the 0.04-, 0.06-, 0.08-, and 0.1-mg/L treatments relative to controls. However, following the 14-d postexposure period, leaf greenness was restored to control levels, indicating recovery was in progress. No significant differences were observed in total chlorophyll *a* levels at the end of the study (Table 6).

DISCUSSION

A summary of NOECs and LOECS is shown in Table 7. The primary symptom of norflurazon toxicity was bleaching of newly emerged leaf blades, which is consistent with the mode of action resulting in the destruction of chlorophyll. This was readily visible at concentrations of 0.04 mg/L and higher after 14 d of exposure. Following the 14-d exposure period, leaf greenness measurements were the only measurements that indicated effects, having a NOEC

TABLE 4. Summary of Total Soluble Sugar (TSS), Hexose, and Sucrose Content in *Vallisneria americana* (Michx.) (American Tapegrass) Following 14 d of Exposure and 14 d Postexposure to Norflurazon

	TSS (mg/g dry weight)					Hexose (mg/g dry weight)					Sucrose (mg/g dry weight)				
	Shoots		Roots		Stolons,	Shoots		Roots		Stolons,	Shoots		Roots		Stolons,
Norflurazon (mg/L)	Mean	*CV*	Mean	*CV*	total	Mean	*CV*	Mean	*CV*	total	Mean	*CV*	Mean	*CV*	total
0	31.2	0.24	4.7	0.43	69.8	25.1	0.32	4.4	0.39	52.7	6.1	0.19	0.3	1.24	17.1
0.02	20.8	0.31	4.1	0.13	58.0	13.9	0.40	4.0	0.11	43.6	6.9	0.24	0.1	2.00	14.4
0.04	14.9[a]	0.21	3.2	0.68	60.7	10.0[a]	0.12	2.9	0.71	49.9	4.8	0.61	0.3	1.34	10.7
0.06	18.3[a]	0.21	5.9	0.55	45.8	10.9[a]	0.33	5.3	0.68	38.9	7.3	0.05	0.6	1.11	7.0
0.08	18.0[a]	0.20	3.4	0.47	42.0	13.4[a]	0.30	3.0	0.47	36.9	4.6	0.18	0.4	0.94	5.1
0.1	17.3[a]	0.10	9.2[a]	0.34	47.7	11.3[a]	0.11	7.7	0.40	44.9	5.9	0.21	1.5[a]	0.38	2.8

Note. CV, coefficient of variation; $n = 4$ for all treatments.
[a]Indicates mean is significantly different from control; ANOVA ($p = .05$).

TABLE 5. Summary of Starch Content in *Vallisneria americana* (Michx.) (American Tapegrass) Following 14 d of Exposure + 14 d Postexposure to Norflurazon

Norflurazon (mg/L)	Starch (mg/g-dw)				
	Shoots		Roots		Stolons, total
	Mean	*CV*	Mean	*CV*	
0	8.9	1.40	21.2	0.39	74.5
0.02	1.9	0.51	13.1	0.20	40.0
0.04	1.7	0.99	29.6	0.88	27.5
0.06	4.7	0.87	21.3	0.42	41.3
0.08	4.0	0.72	10.3	0.71	12.4
0.1	11.1	1.11	43.1	0.55	39.5

Note. CV, coefficient of variation; $n = 4$ for all treatments.

TABLE 6. Summary of *Vallisneria americana* (Michx.) (American Tapegrass) Leaf Greenness and Chlorophyll *a* Measurements Following 14 d of Exposure and/or 14 d Postexposure to Norflurazon

Norflurazon (mg/L)	Leaf greenness (SPAD)					
	14 d Exposure		14 d Postexposure		Chlorophyll *a*, 14 days Postexposure	
	Mean	*CV*	Mean	*CV*	Mean	*CV*
0	11.7	0.4	10.8	0.3	4.43	0.34
0.02	9.7	0.4	14.1	0.6	5.97	0.16
0.04	7.4	0.5	11.5	0.7	5.68	0.40
0.06	3.5[a]	0.8	13.6	0.5	4.53	0.31
0.08	2.9[a]	1.1	12.0	0.7	4.35	0.42
0.1	2.9[a]	1.0	10.9	0.7	2.95	0.80

Note. CV, coefficient of variation; $n = 4$ for all treatments.
[a]Indicates mean is significantly different from control; ANOVA ($p = .05$).

of 0.04 mg/L and LOEC of 0.06 mg/L, consistent with observations. All other endpoints indicated a NOEC greater than 0.1 mg/L. However, latent effects were observed after the 14-d postexposure period for new leaf production and fresh weight gains, with NOEC and LOEC of 0.08 and 0.1 mg/L, respectively. Total leaf growth was the most insensitive endpoint measured. This is likely because of the gross nature of the measurements. The 14-d exposures were enough to destroy chlorophyll, but not enough to result in destruction of cell membranes, which would have resulted in loss of measured lengths. Consequently, when the norflurazon was no longer present, plant resources shifted to production of new chlorophyll. Following the exposure and postexposure periods, vegetative reproductive effects were apparent, with no effect occurring up to 0.08 mg/L, but with a significant reduction in offspring production and stolon fresh weights at the 0.1-mg/L treatment level. Fresh weight was the

TABLE 7. Summary of No-Observable-Effects Concentrations (NOEC) and Lowest-Observable-Effects Concentrations (LOEC) for *Vallisneria americana* (Michx.) (American Tapegrass) Exposed to Norflurazon

Endpoint	NOEC (mg/L)	LOEC (mg/L)
Growth		
Fresh weight gains—14 d exposure	>0.1	>0.1
Fresh weight gains—14 d postexposure	0.08	0.1
New leaf production—14 d exposure	>0.1	>0.1
New leaf production—14 d postexposure	0.08	0.1
Total leaf growth—14 d exposure (cm)	>0.1	>0.1
Total leaf growth—14 d postexposure (cm)	>0.1	>0.1
Vegetative reproduction		
Offspring quantity	0.08	0.1
Offspring fresh weight	>0.1	>0.1
Stolon fresh weight	0.08	0.1
Dry weights		
Shoots—14 d exposure + 14 d postexposure	>0.1	>0.1
Roots—14 d exposure + 14 d postexposure	0.08	0.1
Stolons—14 d exposure + 14 d postexposure	0.06	0.08
Leaf greenness/chlorophylla		
Leaf greenness—14 d exposure	0.04	0.06
Leaf greenness—14 d postexposure	>0.1	>0.1
Chlorophyll *a* (after 14 d of exposure + 14 d postexposure)	>0.1	>0.1
Carbohydrates, shoots		
Total soluble sugars (TSS)	0.02	0.04
Hexose	0.02	0.04
Sucrose	>0.1	>0.1
Starch	>0.1	>0.1
Carbohydrates, roots		
Total soluble sugars (TSS)	0.08	0.1
Hexose	>0.1	>0.1
Sucrose	0.08	0.1
Starch	>0.1	>0.1

least sensitive measurement taken for offspring. Shoot dry weights were not affected by norflurazon. However, root and stolon dry weights were significantly reduced at the 0.1- and 0.08-mg/L treatment levels, respectively. Plants exposed to these levels of norflurazon likely suffered the most destruction of chlorophyll and cell membranes, thus requiring more mobilization of stored energy reserves for recovery. This increased mobilization and use of stored energy reserves could manifest itself as lower root and stolon dry weights, since these organs are typically used for storage.

Sucrose and starch are two primary products of photosynthesis in plants. Sucrose is a polysaccharide comprised of D-glucose and D-fructose monomers linked by a glycosidic bond. Both monomers contain six carbon atoms and are generically referred to as hexoses. TSS includes sucrose as well as the monomeric components D-glucose and D-fructose. Starch is a common name for the insoluble form of glucose. The net outcome of the Calvin cycle is the production

of triose phosphate molecules that are used for starch synthesis within chloroplasts or for sucrose synthesis in the cytosol (Hall & Rao, 1999). The sucrose formed in the cytosol is available for transport to metabolic sinks such as roots, seeds, and tubers (Hall & Rao, 1999). The starch (glucose) formed in the chloroplasts is broken down to soluble sugars or sugar phosphates and utilized for respiration by the plant during periods of darkness or limited photosynthesis (Hall & Rao, 1999). The lower amounts of TSS and hexose in the shoots may be indicative of increased use of the D-glucose, D-fructose, and sucrose through metabolism for regeneration of photosynthetic machinery and for new growth. This is supported by the observation that the significant reductions in TSS and hexose occurred at the same treatment levels where plants showed visual signs of bleaching after the 14-d exposure period. The higher amount of sucrose in the roots at the 0.1-mg/L treatment level is likely indicative of increased transport into the roots from the shoots. These plants had significantly lower root dry weights, which are expected since the plants were visually devoid of chlorophyll pigments following the 14-d exposure. During the postexposure period, more sucrose transport into the roots was likely needed to support regeneration of roots. The increased levels of TSS and hexose observed in the roots may be indicative of increased metabolism associated with root growth and mobilization of transportable carbohydrates into the roots. The apparent numerical decrease in TSS, hexose, and sucrose within the stolons is expected at the higher concentrations since the majority of carbohydrate metabolism and transport would be directed toward metabolism, growth, and repair, but not reproduction. However, this is speculative based on the data, since there was not enough material available to provide adequate repetitions for statistical analysis.

While plant growth and reproduction were affected by some norflurazon concentrations, some recovery was apparent at all levels following the postexposure period, indicating that the effects were at least partially reversible. Based on these results, frequent 2-wk exposures of plants to 0.08 mg/L norflurazon or higher would likely result in reduced recruitment via asexual reproduction. Within the context of the St. Lucie River, these effective concentrations are at least an order of magnitude greater than the concentrations reported based on the SFWMD quarterly sampling program (Pfeuffer, 1998, 1999; Pfeuffer & Matson, 2000, 2001, 2002, 2003). However, much uncertainty exists regarding actual norflurazon concentrations throughout the year because only four samples were analyzed each year. Since plants were destructively analyzed at the end of the assay, no conclusion can be made regarding the effects on recruitment through sexual reproduction. It is also likely that differences in the other measured endpoints may become more pronounced under frequent 2-wk exposure scenarios since more metabolic resources would be devoted to plant reconstruction at any given time. Additional studies are needed to better characterize temporal concentrations of norflurazon in the St. Lucie River and the effects of this herbicide on seed germination.

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