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Assessing Benthic Barriers vs. Aggressive Cutting as Effective Yellow Flag Iris (*Iris pseudacorus*) Control Mechanisms

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An experiment was initiated to study the effects of rubber benthic barriers vs. aggressive cutting on the invasive aquatic emergent plant, yellow flag iris. Treatments were compared against a control at two locations within British Columbia, Canada (Vaseux Lake and Dutch Lake). Yellow flag iris response was significantly different between the two sites, but biologically the results were identical: the benthic barrier killed yellow flag iris rhizomes within 70 d of treatment. Over the extent of the research, at Vaseux Lake the effect of aggressive cutting was no different from the control, while aggressive cutting was statistically no different than the benthic barrier at Dutch Lake. Vegetation regrowth approximately 200 d after the benthic barriers were removed was not detected at either location. These results indicate that rubber benthic barriers may be an effective treatment for yellow flag iris and maybe suitable for other, similar species.

Nomenclature: Yellow flag iris, *Iris pseudacorus* L. IRPS.

Key words: Integrated pest management, mechanical control, yellow flag iris.

Yellow flag iris (*Iris pseudacorus* L.) is one of many invasive species in North America altering ecosystem processes. Yellow flag iris is an emergent species found specifically along calm shorelines of fresh, brackish, and saline water bodies (Pathikonda et al. 2008, Sutherland and Walton 1990). In western North America, yellow flag iris typically occurs in monocultures, or in mixed stands with common cattail (*Typha latifolia* L.) (Lakela 1939; Preece 1964; Rubtzoff 1959) and can grow in water depths ranging from 0 to 100 cm (0 to 39 in) (Preece 1964). While yellow flag iris is typically associated with sites with continuous high soil-water content, it can grow in dry, sandy soils (Dykes, 1974 in Sutherland 1990). Rhizomes placed indoors, without water, can grow for 3 mo (Sutherland 1990).

Yellow flag iris tolerates a wide range of soil pH ranging from 3.6 to 7.7 (Unit of Collaborative Plant Ecology, unpublished, in Sutherland 1990), but prefers high-nutrient sites (Ellenberg 1979 in Sutherland 1990). Once established, yellow flag iris is known to change the hydrology, and ecosystem complexity and functioning of an area, reducing habitat suitability for native plant and animal species (Clark et al. 1998; Pathikonda et al. 2008; Raven and Thomas 1970, Thomas 1980). Yellow flag iris readily invades new areas via seeds and rhizome fragments. The species has a very high carbohydrate storage capacity in the rhizomes (Taylor unpublished in Sutherland 1990) and is able to quickly colonize from rhizome fragments. During peak storage capacity, yellow flag iris rhizomes soluble carbohydrate values may be as high as 80% of the dry matter (Hanhijarvi and Fagerstedt 1994). The high carbohydrate content may allow single populations to expand rapidly. For example, in Ireland, populations 20 m (66 ft) across are thought to have originated from a single clone (Sutherland 1990). The success of this species may be due, in part, to the high buoyancy displayed by the seeds, which can float for over a year before establishing on a suitable substrate (Coops and Van Der Velde 1995; van den Broek et al. 2005).

Once established, yellow flag iris is able to dominate a site due to flooding and anoxia tolerance. This species has been documented to persist in areas that are flooded for over 6 mo of the year, due, at least in part, to a high carbohydrate storage capacity (Hanhijarvi and Fagerstedt 1994). While some plants will cease growth under unfavorable conditions, yellow flag iris continues to utilize nonreducing sugar (fructan) stores. Typically, yellow flag

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Management Implications

Rubber matting (benthic barriers) appeared to work well on the emergent aquatic invasive plant yellow flag iris. In less than 3 mo, rhizomes that were treated with the benthic barrier had very few living cells. Additionally, no regrowth from rhizomes was documented the following growing season, further indication of the successful effects of the barriers. Aggressive cutting is also used as a yellow flag iris treatment. Our research found that depending on the site, aggressive cutting may be no different than the untreated control; or it may be no different than the benthic barrier. More research is required to understand how environmental parameters may affect aggressive cutting of yellow flag iris.

The abundant exposed soil following benthic barrier treatment, the presence of cattails, and the limited number of yellow flag iris germinants indicates that treated sites have a period of time posttreatment when restoration with desirable species could be implemented.

iris uses rhizome carbohydrates to put out aboveground growth, thus ensuring that while the ecosystem is anoxic, the plant is able to access oxygen via its aboveground organs. Ostensibly, it seems that when shoot growth is prevented, yellow flag iris plants have a tendency to utilize carbohydrates, which may be detrimental to survival. Previous research found that under laboratory conditions, when kept in anoxic, dark conditions, the rhizomes of yellow flag iris were dead after 35 d (Hanhijarvi and Fagerstedt 1994). Lowered survival rates under anoxic conditions may be due to the fact that yellow flag iris continues to utilize stored carbohydrates thus triggering a suite of toxic processes (Hanhijarvi and Fagerstedt 1994).

Typically, wetland species down-regulate metabolism during prolonged anoxia (Schlüter and Crawford 2001). However, when devoid of oxygen, total nonsoluble and soluble carbohydrates contained within the rhizomes of yellow flag iris drops to about 20% of original levels within just 2 wk (Schlüter and Crawford 2001) suggesting that the plant is actively transporting carbohydrates to maintain leaf tissue. The active transport of carbohydrates out of the rhizomes to feed metabolic processes in the leaf tissue would be critical to the establishment of a connection with atmospheric oxygen to ensure survival in anoxic conditions. The end product (acetaldehyde) created during anaerobic metabolism would typically be released via diffusion out of the leaf surface (Schlüter and Crawford 2001). The toxic effects of acetaldehyde on plant development and growth are well documented (Perata and Alpi 1991). Kimmerer and Kozlowski (1982) demonstrated a linear relationship between acetaldehyde production and necrosis in birch and pine leaves. The same researchers summarize data of many species that exhibit increased acetaldehyde production under stressed conditions (Kimmerer and Kozlowski 1982). Atkinson et al. (2008) monitored acetaldehyde concentrations in the xylem sap and leaves of intact *Forsythia* plants and found that acetaldehyde concentrations in the xylem sap increased fourfold and increased 10-fold in the leaf tissue, following 3 d under flooded conditions, vs. under well-drained control conditions. Therefore, the active transport to leaves of the toxic by-product acetaldehyde is critical to ensuring survival under flooded conditions.

Based on the laboratory results by Hanhijarvi and Fagerstedt (1994) and a hypothesized understanding of the metabolic process that might be underway in riparian ecosystems, we tested three research questions: (1) Does a nonporous, rubber benthic barrier create an oxygen deprived condition? (2) Could a benthic barrier result in greater rhizome mortality than aggressive cutting? (3) How long would it take for cellular health to decline under the conditions created by the benthic barrier?

Materials and Methods

Treatments. Treatments were installed at two locations within southern British Columbia, Canada: Dutch Lake, north of Kamloops (120.0573E 51.6480N), and Vaseux Lake, south of Penticton, BC (125.5467E 49.3049N). Treatments were installed June 10 and 12, 2014, at Dutch Lake and June 17 at Vaseux Lake. During installation, at both locations, yellow flag iris plants were in the full flowering stage with no signs of senescence.

Five sites were selected at Vaseux Lake and four at Dutch Lake, using a completely randomized design. Each site represented an individual population of yellow flag iris, measured at 5 m by 2 m, and was 100% yellow flag iris by foliar cover. At each site, three treatments were installed: an untreated control, removal of aboveground vegetation only (vegetation only), and removal of aboveground vegetation combined with the addition of a benthic barrier treatment (benthic barrier). The treatments were installed parallel to the water–shoreline interface. While lake levels fluctuated over the course of the study, the rhizomes were not below water at the time of treatment installation at Vaseux Lake. At Dutch Lake, all rhizomes were 25 to 30 cm below water at the time of treatment installation, and remained submerged throughout the duration of the study.

The control and vegetation-only treatments were installed on infestations measuring a minimum of 1 m^2, and the benthic barrier treatments were 0.55 m^2. The benthic barrier treatment included a rectangular sheet metal (2-mm-thick [0.08-in-thick]) frame (90 cm long by 60 cm wide by 30 cm tall) and a 7-mm-thick sheet of rubber matting (the "benthic barrier"). The sheet metal frame was installed into the soil to a depth of 15 cm to prevent neighboring rhizomes from entering the treatment area. The metal frame was tested to determine if the frame affected rhizome viability ($n = 24$); no effect was found (P

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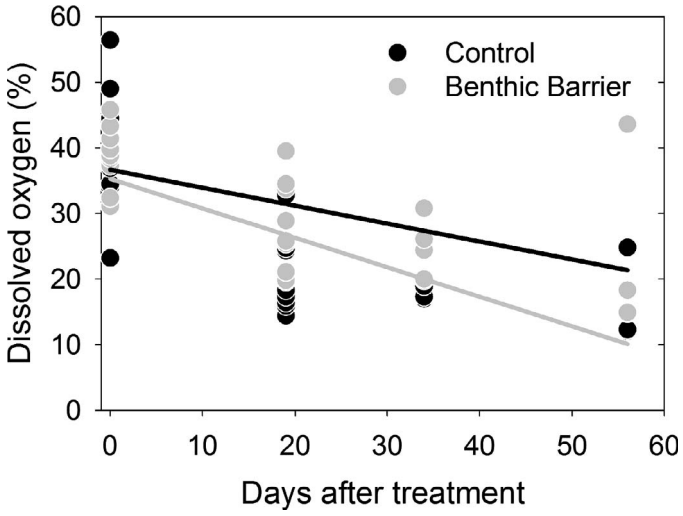

Figure 1. A comparison of percentage of dissolved oxygen within the top 5 cm of the vadose zone in the control and the benthic barrier treatments at Vaseux and Dutch lakes (combined).

= 0.9). Aboveground vegetation was clipped at the vegetation-only and benthic barrier treatment locations to a height of 0 to 4 cm.

After the vegetation was removed, a small water sampler was installed 3 cm below soil surface at all the control and benthic barrier locations. The water sampler consisted of polyvinyl chloride (PVC) pipe (2 cm), cut to a 9-cm length, with 12 0.5-cm holes drilled in the sides; mosquito netting (1.2-mm mesh size) was wrapped around the pipe three times and secured with cable ties. The water samplers were capped with a standard PVC cap and sealed with silicon. A 0.5-cm hole was drilled through one cap to allow polyethylene tubing to be inserted. The tubing was sealed with silicon. For the benthic barrier treatments, rubber matting was cut to the exact dimensions to fit inside the sheet metal frames. A 0.5-cm hole was drilled in the mat and the water sampler tubing was pulled through. Then, the rubber matting was installed on top of the rhizome–soil interface, within the sheet metal frame. The rubber matting was held firmly in place by four 13-kg (29-lb) blocks of cement measuring 0.4 m long by 0.2 m wide by 0.15 m tall. The end of the polyethylene tubing was folded over and securely fastened closed to prevent atmospheric oxygen from entering the treatment areas.

Field Measurements. Using a 2-cm-diam soil punch corer (AMS, Idaho Falls, ID), three rhizome core samples were removed from each of the control, vegetation-only, and benthic barrier treatments at each site 20, 34, 56, 70, and 150 d posttreatment (for example: 3 treatments × 5 sites at Vaseux Lake × 3 cores per treatment × 5 sampling dates). Once collected, rhizome core samples were immediately stored in a cooler with ice to prevent cellular degeneration and transported back to the laboratory. During each rhizome sampling period, water was extracted from both the control and benthic barrier treatments using the water extraction sampler, and tested for percentage of dissolved oxygen and for temperature (Extech DO600, Extech, Wilmington, NC). Soil temperature was recorded at 3 to 5 cm depth each sampling date. Average iris regrowth (cm) was also recorded where applicable. At the final sampling date (150 d after treatment), the benthic barriers were removed.

Both sites were visited approximately 1 yr after treatment (July 2015) to assess if the rhizomes undergoing the various treatments had exhibited regrowth and which plant species, if any, were colonizing the sites. At the same time, rhizome samples were collected and rhizome viability assessed following the procedures outlined below.

Laboratory Analysis. Rhizome core samples were prepared within 24 h of field collection. Each rhizome core (three cores per treatment per sampling date) was prepared for apoptosis analysis following the procedures outlined by Sigma-Aldrich (Sigma-Aldrich, no date). Once prepared, the rhizome slices were examined under a microscope at 10× magnification (Nikon Eclipse E400, Nikon, Tokyo, Japan) with a UV lamp and a fluorescein isothiocyanate, 515 to 555–nm filter (ThermoFischer Scientific, Waltham, MA). Two microscope viewing fields (2 μm) were assessed per rhizome slice. The number of living cells within each microscope viewing field was counted.

Statistical Analysis. Using JMP 11.0 statistical software (SAS, Cary, NC), ANOVA was used to compare means of live cell counts within each sampling date. The data was natural log transformed to normalize the data and then back transformed for presentation and discussion. Logistic regression was used to determine the log-likelihood of mortality. Means were separated using Tukey's HSD ($P = 0.05$). Simple linear regression and descriptive statistics were used for analysis of dissolved oxygen and vegetation regrowth, respectively.

Results

Dissolved Oxygen. Both the control and benthic barrier treatments started with approximately 35% dissolved oxygen (Figure 1). Over the course of 56 d, the amount of dissolved oxygen decreased in both the control and benthic barrier treatments (Figure 1). While dissolved

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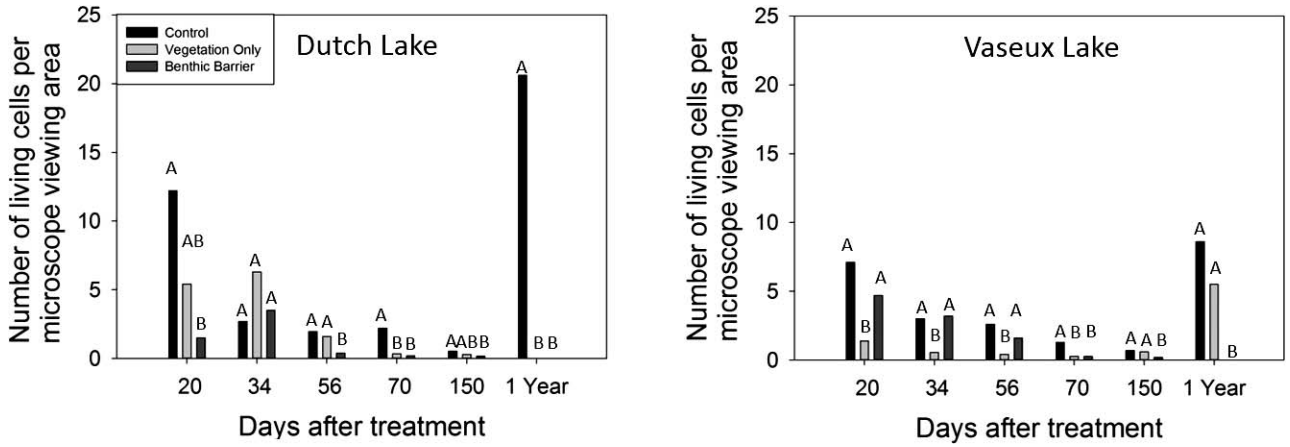

Figure 2. Number of living cells per microscope viewing area within a rhizome slice 20 d to approximately 1 yr after treatment at Dutch Lake (left) and Vaseux Lake (right). Different letters are significantly different ($P < 0.05$) within each time period.

oxygen under the benthic barrier decreased more rapidly than the control, the decline was not significant ($P > 0.05$).

Rhizome Viability. The rhizome response to the treatments was significantly different at Dutch Lake and Vaseux Lake and therefore the two locations will be discussed separately.

At Dutch Lake, there was no significant effect of treatments (measured as number of living cells per microscope viewing field) until 56 d after treatment (Figure 2a). By 150 d, the rhizomes under the control treatment had significantly higher numbers of living cells (0.54 living cells per viewing area) than the benthic barrier (0.16 living cells per viewing area), but the difference between the benthic barrier and the vegetation-only treatment (0.29 living cells per viewing area) was not significant (Figure 2a).

In terms of predicting the likelihood of mortality under each treatment, at Dutch Lake, 150 d after treatment, plants were seven times (95% confidence interval [CI] = 2 to 30 times) more likely to have no living cells present if treated with the benthic barrier than the control; and 2.5 times (95% CI = 0.7 to 11 times) more likely than the vegetation-only treatment. There was no difference in likelihood of cell mortality between the vegetation-only treatment and the control.

At Vaseux Lake, 70 d after treatment, there were significant differences in the effect of the benthic barrier and the vegetation-only treatment compared to the control (Figure 2b). By 150 d after treatment, rhizomes from the control sites had significantly higher numbers of living cells per microscope viewing section (0.70 living cells) than under the benthic barrier (0.19 living cells) ($P < 0.05$); there was no significant difference between the control and the vegetation-only treatments ($P < 0.05$; Figure 2b).

By 150 d after treatment at Vaseux Lake, plants treated with the benthic barrier were 3.6 times (95% CI = 1.2 to 11) more likely to have no living cells present in the rhizome slice when compared to the control, and 2.8 times (95% CI = 1.0 to 8.4) more likely when compared to the vegetation-only treatment. There was no difference between the vegetation-only and control treatments.

Vegetative Regrowth 150 d after Treatment. The benthic barriers were removed at 150 d after treatment. At Dutch Lake there was no stem regrowth at 150 d after treatment for either the benthic barrier or the vegetation-only treatments. All treatments were submerged for the duration of the study. The water depth at Dutch Lake ranged from 15 to 46 cm, averaging 43 ± 1.5 cm ($\alpha = 0.05$) for the majority (3 mo) of the study. At 150 d after treatment the water level dropped to 15 cm. During the length of the research, rhizomes at Vaseux Lake were not submerged at any time; the average water depth was 0.8 ± 0.4 cm ($\alpha = 0.05$). At Vaseux Lake, the vegetation only treatment had an average regrowth of 41.2 ± 30.9 cm ($\alpha = 0.05$).

Rhizome Viability 1 yr after Treatment. Both sites were assessed approximately 1 yr after treatment, or approximately 200 d after the benthic barriers were removed. At both lakes, the response 1 yr after benthic barrier treatment was very similar. All benthic barrier sites had no regrowth from preexisting yellow flag iris rhizomes. Rhizome viability could not be determined because the rhizomes were in an advanced stage of decomposition. At both lakes control rhizomes had recovered to pretreatment levels. Dutch Lake control rhizomes had 20.6 living cells per

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microscope viewing area (± 5.1) and Vaseux Lake rhizomes had 8.6 (± 2.7) (Figure 2). As expected, at Dutch Lake, the vegetation-only treatment exhibited identical results to the benthic barrier in that the rhizomes were too decomposed to sample. However, at Vaseux Lake, the vegetation-only treatment was no different from the control with 5.5 living cells per microscope viewing area (± 2.0).

Vegetation Recolonization 1 yr after Treatment. Vegetation regrowth followed a similar pattern to rhizome health. At Dutch Lake, where rhizome mortality under the benthic barrier was similar to that at the aggressive cutting sites, bare ground across both treatment conditions was approximately 95% (± 2%) while vegetation at the control location was 100% yellow flag iris. Vaseux Lake, where there was no significant difference in rhizome mortality between the aggressive cutting and the control, vegetation cover was 100% yellow flag iris. The benthic barrier sites averaged 93% (± 4%) bare ground. At both lakes sparse recolonization was comprised of cattail and yellow flag iris seedlings.

Discussion

The nature of plant species anoxia tolerance is complex and mechanisms developed to improve gas exchange and survive adverse conditions is both species-specific (Kennedy et al. 1992) and seasonally variable (Crawford 2003). Plant species tend to exhibit two responses to stress; they either initiate dormancy in an attempt to avoid the stress conditions, or they outgrow the stress conditions through adjustments in morphology (Armstrong et al. 1994; reviewed in Crawford 2003). Rapid carbohydrate consumption in yellow flag iris after initiation of anoxic conditions is well documented (Schlüter and Crawford 2001; Hanhijarvi and Fagerstedt 1994) and may be attributed to a survival mechanism that enables the species to adjust through shoot extension (Summers et al. 2000). However, when shoot extension is prevented by way of the benthic barrier, then diffusion of toxic acetaldehyde is prevented. Therefore, the innate characteristic of yellow flag iris to continue respiration under anaerobic conditions (rather than going dormant), combined with inability of the plants to move toxic acetaldehyde out of the rhizomes may have worked in tandem to generate rapid cellular death.

Yellow flag iris at the two locations, Vaseux Lake and Dutch Lake, differed in their responses to aggressive cutting (vegetation-only treatment). At Dutch Lake, where the treatments were submerged (water levels ranging from 15 to 46 cm over the course of the study), there was no difference between the benthic barrier and vegetation-only treatments. This may be due to very slow oxygen diffusion in water. Oxygen moves 1,000 times slower in water than in air (Sairam et al. 2008); thus, surface water may provide a barrier to oxygen acquisition as well as acetaldehyde diffusion out of the rhizomes. At Vaseux Lake, where the water level was often 0 cm, the rhizomes may have been able to diffuse the acetaldehyde into the atmosphere and more readily initiate shoot regrowth. The differing plant response under varying conditions is currently being studied (Tarasoff et al., unpublished data)

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