Too Much of a Good Thing – Using Water to Control the Aquatic Invasive Yellow Flag Iris (Iris Pseudacorus L.)

Catherine Tarasoff (Catherine@agrowest.ca)
Agrowest Consulting Scientist https://orcid.org/0000-0003-3242-5292
Sharon Gillies
University of the Fraser Valley

Research Article

Keywords: mechanical control, yellow iris, anoxia, hypoxia, plant physiology, carbohydrate starvation

Posted Date: February 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-224348/v1

Too much of a good thing – using water to control the aquatic invasive yellow flag iris (*Iris Pseudacorus* L.)

Authors: CS Tarasoff[1]*and S Gillies[2]

[1]Agrowest Consulting Scientists Ltd., Kamloops, BC, Canada.

[2] Department of Biological Sciences, University of the Fraser Valley, 33844 King Road, Abbotsford, BC, Canada

[*] Corresponding author: Catherine Tarasoff. Agrowest Consulting Scientists Ltd., Kamloops, BC Canada

Catherine@Agrowest.ca

Acknowledgements

Funding for this research was provided by the National Wetland Conservation Fund - Environment and Climate Change Canada (Reference #: GCXE18C067). Gratitude is extended to the University of the Fraser Valley Ecology Class for field assistance installing the research plots, to Thompson Rivers University for the use of their greenhouse, and to Dustin Melan, Kristi Gordon and Doug Terpsma for additional field assistance. Thanks to Tom Pypker for project review and manuscript edits.

Abstract

Invasions of *Iris pseudacorus* L. (yellow flag iris) into wetland environments can result in changes to the functioning of the ecosystem. Field-based and greenhouse studies were initiated to study the effect of water depth on regrowth rates of cut stems of yellow flag iris. The field-based experiment occurred at 41 independent populations around the perimeter of a single wetland. The greenhouse experiment was conducted to further study the effect of water depth and duration of submersion on rhizome mortality. In both studies, treatments were compared against controls. In the field-study, yellow flag iris regrowth was significantly affected by water, though there was no relationship between water depth and percent regrowth. In the greenhouse study, there was a significant positive relationship between duration of submersion and percent mortality of rhizomes. And, there was no relationship between water depth and percent mortality; indicating that as little as 5 cm of water is sufficient to kill yellow flag iris rhizomes, if the stems are cut to the base of the plant. Our results indicate a simple technique can control yellow flag iris within aquatic ecosystems without the need for chemicals or laborious hand removal.

Nomenclature: yellow flag iris, *Iris pseudacorus* (L.) IRPS

Key words: mechanical control, yellow iris, anoxia, hypoxia, plant physiology, carbohydrate starvation

Introduction

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; Gerwing et al. 2020). In western North America, yellow flag iris typically occurs in monocultures, or in mixed stands with broadleaf cattail (*Typha latifolia* L.) (Lakela 1939; Preece 1964; Rubtzoff 1959) and can grow in water depths ranging from 0-100 cm (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 continue to grow for 3 months (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). 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 a single population to expand rapidly. For example, in Ireland, populations 20 m across are thought to have originated from a single clone (Sutherland 1990). While yellow flag iris can invade new areas via rhizome fragments, seeds are the main mode of dispersal (Gaskin et al. 2016). 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).

Yellow flag iris is able to dominate a site due to flooding and anoxia tolerance. Typically, wetland species down-regulate metabolism during prolonged anoxia (Schlüter and Crawford 2001). However, when devoid of oxygen, total non-soluble and soluble carbohydrates contained within the rhizomes of yellow flag iris drops to about 20% of their original levels within just 2 weeks (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* sp. plants and found that acetaldehyde concentrations in the xylem sap increased 4-fold and increased 10-fold in the leaf tissue, following 3 days under flooded conditions, versus 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.

Research by Tarasoff et al. (2016) found that yellow flag iris rhizomes died quickly (within 3 months) when plants were cut to the base and covered with a non-porous rubber matting. They also observed that at one site after the plants were cut, the water levels rose such that the cut bases of the plants were under at least 10 cm of water for the duration of the study. There was no significant difference between cutting the plants to the base versus cutting the plants to the base and putting the rubber matting on top. The results of this study suggest that there may be an inhibitory effect of water on the ability of yellow flag iris to regrow.

It is known that gases diffuse through water 1000 times more slowly than through the atmosphere (Sairam et al., 2008). Therefore, we hypothesize that water may act as a barrier to gas (acetaldehyde) diffusion and thus water alone may result in yellow flag iris mortality. We expect a relationship between water depth and duration of submersion and rhizome mortality; therefore, we hypothesis that a constant layer of water overtop cut yellow flag iris stems. We pose the questions: what depth of water is required and what is the optimal duration of treatment?

Materials and Methods

Field-Based Experiment

The field-based portion of the study occurred at Cheam Lake Wetlands Regional Park (49.1981, -121.7503) (Cheam), near Vancouver, British Columbia, Canada. Cheam is a 107 hectare wetland with a history of marl dredging. In 1990, the area was designated a Regional park and Ducks Unlimited Canada installed a water-flow box

100 to control lake levels. Because the lake has been dredged, it is unsafe to venture into deeper water. Therefore, we
101 chose to drop the lake levels by 40 cm to expose the bases of the plants prior to initiating the experiment.
102 September 1, 2017, the controls of the water-flow box were lowered approximately 3-4 cm per day for 14 days.
103 After 14 days, the water levels had dropped 40 cm; allowing safe access to yellow flag iris populations along the
104 deeper regions of the shoreline. On September 18, 2017, 41 unique populations of yellow flag iris were randomly
105 selected and treated; in addition to 5 untreated Control sites (n=46). Prior to treating the populations, number of
106 stems were counted and marked with 'pin-flags', number of flowering stems were counted, the population size was
107 measured, and a permanent water staff was installed.
108 September 19, 2017, the plants were treated by cutting the stem to the rhizome. If the population had a
109 terrestrial portion then the terrestrial portion was treated with rubber matting following the methods outlined by
110 Tarasoff et al. (2016) to prevent gas exchange between the treated and untreated portions. September 19, 2017
111 immediately after treatments, the water-flow control box was raised to the original height and the wetland was
112 allowed to recharge. Water levels were monitored daily; after 10 days (September 29th), the wetland water levels
113 had returned to pre-treatment levels. November 6, 2017 all 46 locations were revisited and monitored for both water
114 height and plant regrowth. May 15, 2018 all 46 locations were again revisited and monitored for both water height
115 and plant regrowth. Two of the 41 treated sites were vandalized (n=39).
116 We suspect that uneven ground surface and distance from rising water may have resulted in an uneven recharge rate
117 at the 39 sites. This uneven recharge rate would allow the treated yellow flag iris at some sites to remain exposed for
118 up to 10 days (the recharge time for the system) longer than other sites. During this time, the plant could recover
119 and form a leaf. In order to control water depth treatment, we conducted a greenhouse study.
120 *Greenhouse Experiment*
121 A greenhouse experiment was initiated to create instant water depth treatments of 0, 5, 10, 15, 20 and 25 cm. June
122 10th, 2018, prior to initiating the study, 36 yellow flag iris plants were removed and transported to the greenhouse at
123 Thompson Rivers University (Kamloops, BC, Canada). The yellow flag iris plants were divided into rhizome
124 sections of 10 cm lengths and transplanted into 1-gallon pots filled with potting soil. The rhizomes were planted just
125 at soil level to mimic natural field growth of yellow flag iris. The transplanted iris' were allowed to recover and
126 grow for three months. September 14th, 2018 the transplanted iris' were cut to their bases. To save space, 2 pots
127 were placed in each 40 liter Rubbermaid tote. The totes were filled with water such that when the 1-gallon pots

were submerged the water level was 5, 10, 15, 20, or 25 cm above the soil level. The control treatment was sub-irrigated. At 21, 68, 105, and 230 days of treatment, 3 rhizomes from each water depth were removed from the treatment and washed clean (n=36). The rhizome was sliced into 5 sections and each section was assessed for percent mortality as a function of percent necrotic tissue.

Statistical Analysis

Using JMP 14.0 statistical software (SAS, Cary, NY), descriptive statistics and regressions were used to treatment effects and the relationship between regrowth and water depth, in the field based study; and mortality versus water depth and duration of treatment in the greenhouse study ($\alpha = 0.05$).

Results

Field-Based Experiment

Results presented are for the May 15, 2018 site visit to ensure all populations had sufficient time to regrow. Of the original 41 sites, 2 were vandalized during the study and were omitted from analysis (n=39). There was no relationship between the number of stems at each population at the start of the experiment and regrowth in May 2018 (r^2= 0.01, P=0.47). Additionally, there was no relationship between water height and regrowth in May 2018 (r^2= 0.0008, P=0.86) (Figure 1).

For example, 59% of the populations treated had zero percent regrowth, 25% had more than 0% regrowth but less than 30%, 10% had more than 30% regrowth but less than 60%, and 6% had greater than 60% regrowth (Figure 2). The Control sites experienced an average increase in stems of 34% ($\pm$ 20%).

Greenhouse Experiment

Results from the greenhouse study provided some excellent insight into water treatment depth and duration. Again, there was no effect of water depth on mortality (P=0.23) nor was there an interaction between water depth and days of treatment (P=0.49); therefore, all depth treatments were combined and analyzed by days of treatment (P<0.0001). We found that while significant mortality started to occur at 105 days of treatment (69% $\pm$ 24%), 230 days of treatment were required to attain 100% mortality (Figure 3). At 230 days of treatment the Control plants exhibited 0% mortality.

Discussion

155 The nature of anoxia/hypoxia tolerance in aquatic plants is complex and species specific (Loreti and Perata 2020).
156 Regardless of the mechanism(s) of survival; for a species to be considered anoxia/hypoxia-tolerant it is not
157 necessary that every organ or tissue survive the oxygen deprivation. All that is required is the survival of the
158 essential organs that support regrowth for survival once plants are returned to favourable conditions. Roots are
159 relatively sensitive to oxygen deprivation, and the survival capacity of most tolerant species resides in the shoots or
160 rhizomes. Schlüter and Crawford (1982) studied *I. pseudacorus* anoxia tolerance extensively and found that
161 rhizomes could withstand approximately 65 days of total anoxia and regrow upon return to oxygenated conditions.
162 This ability to withstand long durations of anoxia is likely linked to the very high carbohydrate reserves found
163 within *I. pseudacorus* rhizomes (approximately 40 mg/g FW total soluble carbohydrates and 140 mg/g FW total
164 non-soluble carbohydrates) (Schlüter and Crawford 1982). Schlüter and Crawford (1982) documented a sharp
165 decline in carbohydrates and a steady increase in ethanol content in the rhizomes till about 20 days after treatment;
166 after which, ethanol content reached a steady state.

167 Interestingly, plants have two growth responses to anoxia: some species experience shoot elongation and, if
168 successful, are able to 'reconnect' with the atmosphere (Voesenek et al. 2004); while others initiate a quasi-
169 dormancy to survive the anoxic condition and will not grow until oxygen is available (Barclay and Crawford 1982).
170 The advantage in the latter adaptation is that plants seem to outlast anoxia by reducing metabolic activity. However,
171 reduced metabolic activity cannot be sustained indefinitely; and thus, carbohydrate starvation has long been
172 regarded as one of the main causes of cell death under anoxia (Schlüter and Crawford 1982). The results from the
173 current study indicate that *I. pseudacorus* utilizes the second, quasi-dormancy, mechanism to survive anoxic
174 conditions. Ironically, the rhizomes of yellow flag iris hold more than enough carbohydrate reserves to produce a
175 leaf and reconnect with the atmosphere. Only 5 cm of surface water is required to prevent shoot growth, however
176 upwards of 240 days is required to completely kill the rhizomes through carbohydrate starvation.

177 For land managers using water as a control mechanism for yellow flag iris some key protocols must be
178 observed to ensure success. Often, yellow flag iris populations are a mixed condition where part of the population
179 is terrestrial and part is in deep water. In a mixed condition population, it is imperative that the terrestrial portion be
180 treated with a benthic barrier following the guidelines outlined by Tarasoff et al. (2016) to the point where the water
181 depth is at least 5 cm deep year round. Treating the terrestrial portion will ensure that the deep-water portion does
182 not receive oxygen via the interconnected rhizome network. If the deep-water portion does not remain under at least

5 cm of water year round, then cutting alone will not result in mortality. If the water drops below the cut rhizome surface, yellow flag iris will quickly send up a leaf and reconnect to the atmosphere. Next, when cutting the deep water populations, success hinges on cutting the leaves to the base of the plant. Lastly, monitoring treated areas and removing any rogue leaf formation may further improve success rates. Future research should involve testing this technique at multiple sites and across a variety of ecological conditions. The authors also encourage testing this technique on other, biologically similar, species.

Declarations

Funding - Funding for this research was provided by the National Wetland Conservation Fund -Environment and Climate Change Canada.

Conflicts of interest – Not Applicable

Availability of data and material – The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Code availability – Not Applicable

Author's contributions – All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Catherine Tarasoff and Sharon Gillies. The first draft of the manuscript was written by Catherine Tarasoff and edited by Sharon Gillies. All authors read and approved the final manuscript.

Ethics approval – Not Applicable

Consent to participate – Not Applicable

Consent for publication – Not Applicable

Figure Captions

Fig. 1 The relationship between percent plant regrowth recorded May 2018 and water height above cut stems of treated yellow flag iris populations at Cheam Wetlands (Vancouver, BC)

Fig. 2 Levels of control, measured by percent regrowth, at 39 treated yellow flag iris sites at Cheam Wetlands (Vancouver, BC)

Fig. 3 The relationship between days of water treatment (5,10, 15, 20 or 25 cm water depth) and percent rhizome mortality of cut yellow flag iris plants grown under greenhouse conditions ($\pm$ 95% CI)

221 **Works Cited**

222

223 Atkinson CJ, Harrison-Murray RS, Taylor JM (2008) Rapid flood induced stomatal closure accompanies xylem sap
224 transportation of root-derived acetaldehyde and ethanol in *Forsythia*. Environ. Exp. Bot. 64:196-205
225 Barclay AM, Crawford, RMM (1982) Plant growth and survival under strict anaerobiosis. J. Exp. Bot. 33:541-549
226 Clark FH, Mittrick C, Shonbrun S (1998) Rogues gallery: New England's notable invasives. Conservation Notes of
227 the New England Wild Flower Society 2:19-26
228 Coops H, Van Der Velde G (1995) Seed dispersal, germination and seedling growth of six helophyte species in
229 relation to water-level zonation. Freshw. Biol. 34:13-20
230 Crawford RMM (2003) Seasonal differences in plant responses to flooding and anoxia. Can. J. Bot. 81:1224-1246
231 Gaskin JF, Pokomy ML, Mangold JM (2016) An unusual case of seed dispersal in an invasive aquatic; yellow flag
232 iris (*Iris pseudacorus*). Biol. Invasions 18:2067-2075
233 Gerwing, TG, Thomson, HM, Brouard-John, EK et al. (2021) Observed dispersal of invasive yellow flag iris (*Iris*
234 *pseudacorus*) through a saline marine environment and growth in a novel substrate, shell hash. Wetlands 41:
235 https://doi.org/10.1007/s13157-021-01421-w
236 Hanhijarvi AM, Fagerstedt KV (1994) Comparison of the effect of natural and experimental anoxia on carbohydrate
237 and energy metabolism in *Iris pseudacorus* rhizomes. Physiol. Plant. 90:437-444
238 Kimmerer TW, Kozlowski TT (1982) Ethylene, ethlane, acetaldehyde, and ethanol production by plants under
239 stress. Plant Physiol. 69:840-847
240 Lakela O (1939) A floristic study of a developing plant community on Minnesota Point, Minnesota. Ecology
241 20:544-552
242 Loreti E, P Perata (2020) The many facets of hypoxia in plants. Plants (Basel) 9:745
243 Pathikonda S, Ackleh AS, Hasenstein KH, Mopper S (2008) Invasion, disturbance, and competition: modeling the
244 fate of coastal plant populations. Conserv. Biol. 1:164-173
245 Perata P, Alpi A (1991) Ethanol –induced injuries to carrot cells. The role of acetaldehyde. Plant Physiol. 95:748-
246 752
247 Preece SJ (1964) *Iris pseudacorus* in Montana. Proceedings of the Montana Academy of Sciences 24:1-4
248 Raven PH, Thomas JH (1970) *Iris pseudacorus* in western North America. Madrono 20:390-391

249 Rubtzoff P (1959) *Iris pseudacorus* and *Caltha palustris* in California. Leaflets of Western Botany 9: 31-32
250 Sairam R, Kumutha D, Ezhilmathi K, Deshmukh PS, Srivastava GC (2008) Physiology and biochemistry of
251 waterlogging tolerance in plants. Biol. Plant. 52:401–412
252 Schlüter U, Crawford RMM (2001) Long-term anoxia tolerance in leaves of *Acorus calamus* L. and *Iris*
253 *pseudacorus* L. J. Exp. Bot. 52: 2214-2225
254 Sutherland WJ, Walton D (1990) The changes in morphology and demography of *Iris pseudacorus* L. at different
255 heights on a saltmarsh. Funct. Ecol. 4: 655-659
256 Sutherland WJ (1990) Biological flora of the British Isles: *Iris pseudacorus* L. J. Ecol. 78: 833-848
257 Tarasoff CS, Streichert K, Gardner W, Heise B, Church J, Pypker TG (2016) Assessing benthic barriers vs.
258 aggressive cutting as effective yellow flag Iris (*Iris pseudacorus*) control mechanisms. Invasive Plant Sci Manag
259 9:229–234
260 Thomas LK Jr (1980) The impact of three exotic plant species on a Potomac island. National Park Service Scientific
261 Monograph Series No. 13. Washington, DC: U.S. Department of the Interior, National Park Service
262 Van Den Broek T, Van Diggelen R, Bobbink R (2005) Variation in seed buoyancy of species in wetland ecosystems
263 with different flooding dynamics. J. Veg. Sci. 16: 579-586
264 Voesenek LACJ, Riijnders J, Peeters A, Van de Steeg HM, De Kroon H (2004) Plant hormones regulate fast shoot
265 elongation under water: from genes to communities. Ecology 85: 16-27

Figures

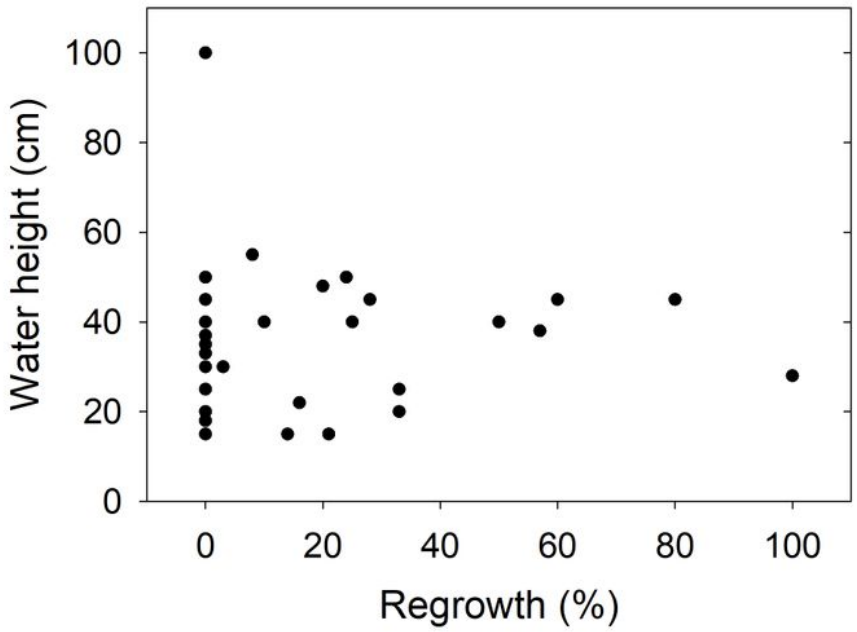

Figure 1

The relationship between percent plant regrowth recorded May 2018 and water height above cut stems of treated yellow flag iris populations at Cheam Wetlands (Vancouver, BC)

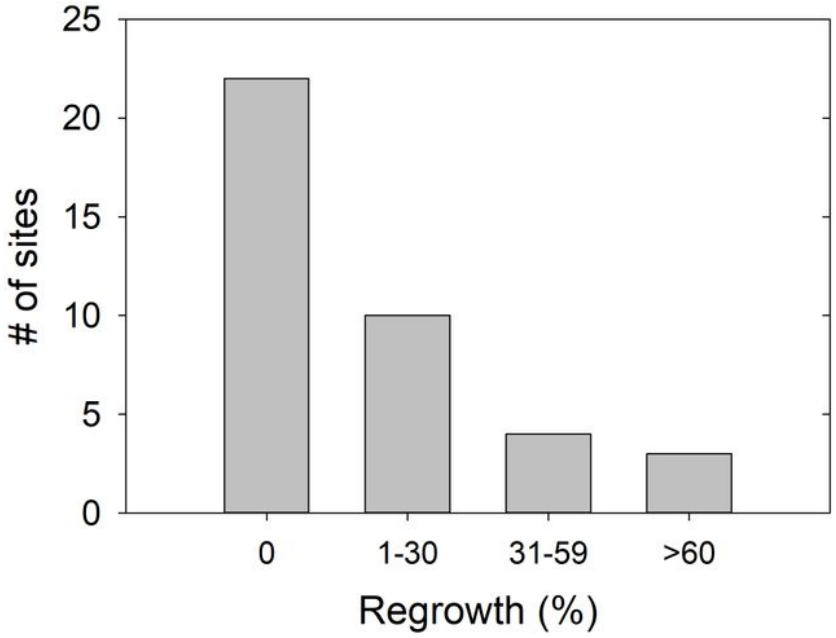

Figure 2

Levels of control, measured by percent regrowth, at 39 treated yellow flag iris sites at Cheam Wetlands (Vancouver, BC)

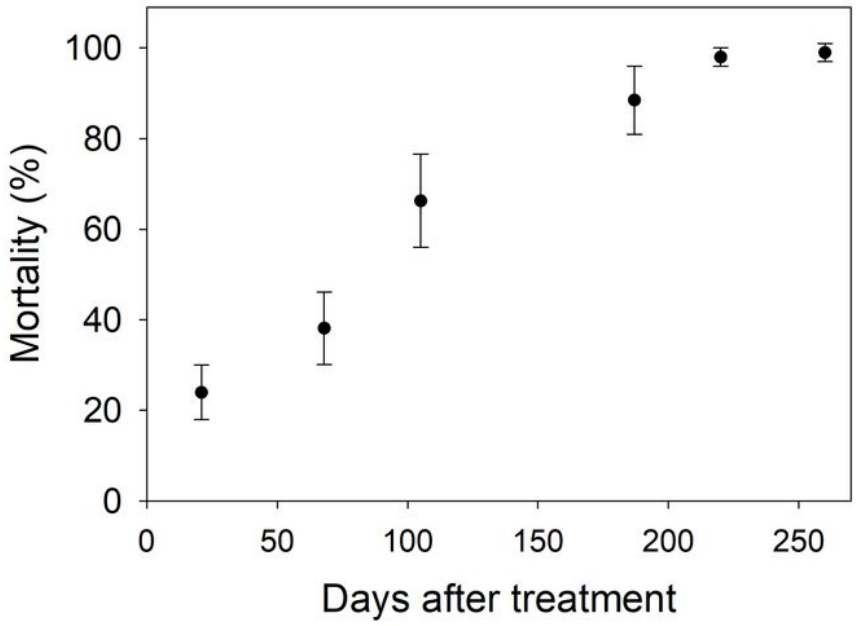

Figure 3

The relationship between days of water treatment (5,10, 15, 20 or 25 cm water depth) and percent rhizome mortality of cut yellow flag iris plants grown under greenhouse conditions (± 95% CI)