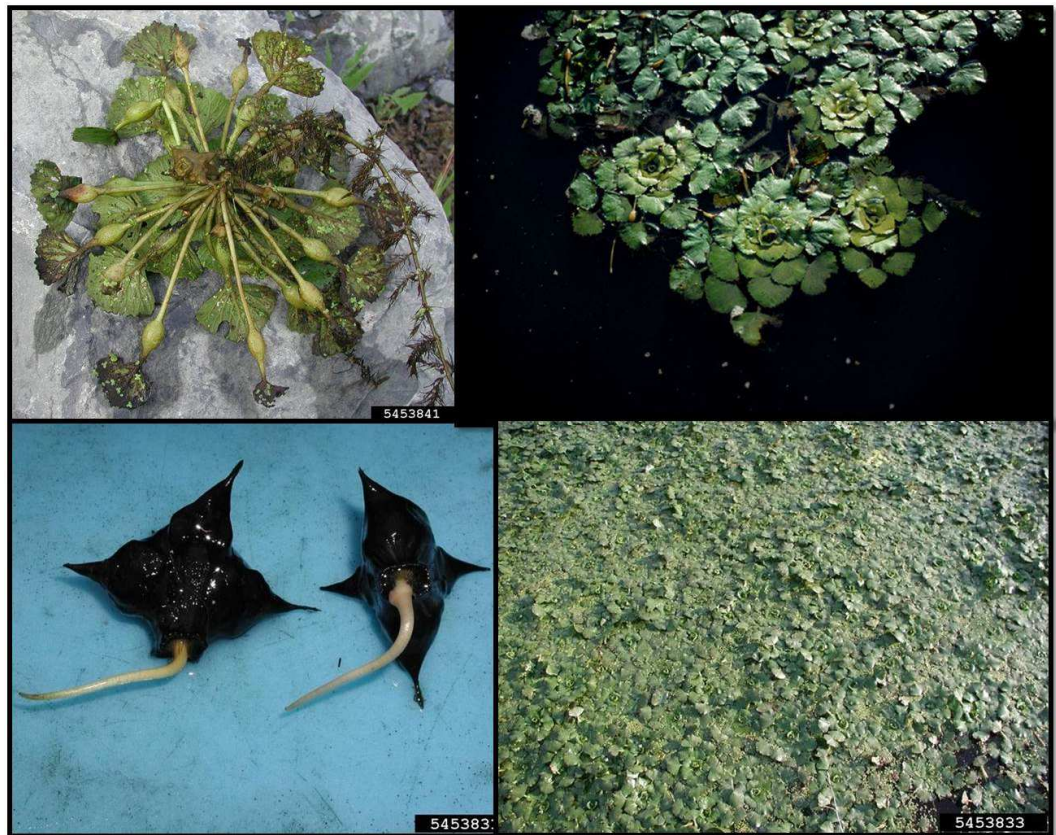


Weed Risk Assessment for *Trapa natans* L. (Lythraceae) – Water chestnut

Michigan
Department of
Agriculture
and Rural
Development

September 14,
2015

Version 1



Top left: An upsidedown plant showing the inflated petioles that keep individual leaves bouyant. Top right: growth form in water. Bottom left: Barbed nuts of *Trapa natans* Bottom right: *Trapa natans* infestation. (All images Leslie J. Mehrhoff, University of Connecticut, Bugwood.org).

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Introduction The Michigan Department of Agriculture and Rural Development (MDARD) regulates aquatic species through a Prohibited and Restricted species list, under the authority of Michigan’s Natural Resources and Environmental Protection Act (NREPA), Act 451 of 1994, Part 413 (MCL 324.41301-41305). Prohibited species are defined as species which “(i) are not native or are genetically engineered, (ii) are not naturalized in this state or, if naturalized, are not widely distributed, and further, fulfill at least one of two requirements: (A) The organism has the potential to harm human health or to severely harm natural, agricultural, or silvicultural resources and (B) Effective management or control techniques for the organism are not available.” Restricted species are defined as species which “(i) are not native, and (ii) are naturalized in this state, and one or more of the following apply: (A) The organism has the potential to harm human health or to harm natural, agricultural, or silvicultural resources. (B) Effective management or control techniques for the organism are available.” Per a recently signed amendment to NREPA (MCL 324.41302), MDARD will be conducting reviews of all species on the lists to ensure that the lists are as accurate as possible.

We use the United States Department of Agriculture’s, Plant Protection and Quarantine (PPQ) Weed Risk Assessment (WRA) process (PPQ, 2015) to evaluate the risk potential of plants. The PPQ WRA process includes three analytical components that together describe the risk profile of a plant species (risk potential, uncertainty, and geographic potential; PPQ, 2015). At the core of the process is the predictive risk model that evaluates the baseline invasive/weed potential of a plant species using information related to its ability to establish, spread, and cause harm in natural, anthropogenic, and production systems (Koop et al., 2012). Because the predictive model is geographically and climatically neutral, it can be used to evaluate the risk of any plant species for the entire United States or for any area within it. We then use a stochastic simulation to evaluate how much the uncertainty associated with the risk analysis affects the outcomes from the predictive model. The simulation essentially evaluates what other risk scores might result if any answers in the predictive model might change. Finally, we use Geographic Information System (GIS) overlays to evaluate those areas of the United States that may be suitable for the establishment of the species. For a detailed description of the PPQ WRA process, please refer to the *PPQ Weed Risk Assessment Guidelines* (PPQ, 2015), which is available upon request.

We emphasize that our WRA process is designed to estimate the baseline—or unmitigated—risk associated with a plant species. We use evidence from anywhere in the world and in any type of system (production, anthropogenic, or natural) for the assessment, which makes our process a very broad evaluation. This is appropriate for the types of actions considered by our agency (e.g., State regulation). Furthermore, risk assessment and risk management are distinctly different phases of pest risk analysis (e.g., IPPC, 2015). Although we may use evidence about existing or proposed control programs in the assessment, the

ease or difficulty of control has no bearing on the risk potential for a species. That information could be considered during the risk management (decision making) process, which is not addressed in this document.

***Trapa natans* L. – Water chestnut**

Species Family: Lythraceae (NGRP, 2015; Hummel & Kiviat, 2004)

Information Synonyms: *Trapa natans* has, at times, been split into numerous, narrowly-defined species (Weakley, 2015; Mabberley, 2008). However, currently the genus *Trapa* is recognized to include just one polymorphic species (Weakley, 2015). Several of these species are used as synonyms for *Trapa natans* and are still in use today by some researchers, primarily *Trapa bispinosa* (Agrawal & Mohan Ram, 1995) and *Trapa bicornis* (Hummel & Kiviat, 2004). For this review, *Trapa natans* was treated as a single species, and the above synonyms were included in the search for material.

Common names: Water chestnut, water caltrop, water nut, singhara nut, bull nut (Hummel & Kiviat, 2004).

Botanical description: *Trapa natans* is a rooted aquatic herb (Agrawal & Mohan Ram, 1995; Shalabh et al., 2012) which grows in water at a depth of 1.2-1.6 m, with a maximum growth depth of about 2 m (Dementeva & Petushkova, 2010). Floating leaves are arranged in a rosette, with serrated upper leaves up to “5 cm wide and broadly rhomboid, triangular, deltoid or broadly ovate” (Mikulyuk & Nault, 2009). For a full botanical description, see eFloras (Haynes, 2015).

Initiation: In accordance with the Natural Resources and Environmental Protection Act Part 413, the Michigan Department of Agriculture and Rural Development was tasked with evaluating the aquatic species currently on Michigan’s Prohibited and Restricted Species List (MCL 324.41302). The USDA Plant Epidemiology and Risk Analysis Laboratory’s (PERAL) Weed Team worked with MDARD to evaluate and review this species.

Foreign distribution: *Trapa natans* has a very broad native distribution that includes many countries in Africa, Europe, and Asia (NGRP, 2015; GBIF, 2015). This species has become naturalized in India (Bhatt et al., 2012), Japan (Kadono, 2004), and Singapore (Keng, 1990), and was first detected in Canada in southern Quebec in 1998 (Darbyshire, 2003), where it is currently considered invasive (OIP, 2015). *Trapa natans* is extensively cultivated in Asia for consumption (Raju, 1999; von Mueller, 1888; Mabberley, 2008) and medicinal purposes (Shalabh et al., 2012), but it is not known to be cultivated elsewhere.

U.S. distribution and status: *Trapa natans* is present and has naturalized in several states: California, Connecticut, Delaware, Massachusetts, Maryland, New Jersey, New Hampshire, New York, Pennsylvania, Vermont, and Virginia (Kartesz, 2015). This species is regulated in Alabama, Arizona, Connecticut, Illinois, Indiana, Michigan, Oregon, South Carolina, Vermont, Washington, and Wisconsin (National Plant Board, 2015). *Trapa natans*

does not appear to be cultivated in the United States to any extent, including in botanical gardens. Eradication programs include Lake Champlain, where the state of New York and Vermont, as well as the U.S. Fish and Wildlife Service, the Army Corps of Engineers, and the Lake Champlain Basin Program have collaborated on a management program from the 1960s until the early 2000s (Naylor, 2003). Maryland's Department of Natural Resources has also established a management and control program, which focuses on preventing establishment in areas where *T. natans* has not yet established, as well as mechanical control methods in areas where it has (Naylor, 2003).

WRA area¹: Entire United States, including territories.

1. *Trapa natans* analysis

Establishment/Spread Potential *Trapa natans* has already demonstrated to be invasive in the United States (Hummel & Kiviat, 2004) where it exhibits “explosive growth” (Ding & Blossey, 2005). Within its introduced range, it grows very quickly within waterways, and *T. natans* naturalizes and spreads in areas where it has been introduced. *Trapa natans* has a very dense growth habit (Tall et al., 2011; Swearingen et al., 2002; ISSG, 2005; Strayer et al., 2003). It is prone to both natural (Swearingen et al., 2002; Hummel & Kiviat, 2004; Pemberton, 2002) and human-mediated (Dementeva & Petushkova, 2010; Hummel & Kiviat, 2004) dispersal; this species may spread via fishing nets (Dementeva & Petushkova, 2010) and boats (Hummel & Kiviat, 2004), as well as water currents (van der Pijl, 1982; Pemberton, 2002), birds (Swearingen et al., 2002; Hummel & Kiviat, 2004), and animals (Swearingen et al., 2002; Hummel & Kiviat, 2004). The seeds have a high germination rate of up to 87% (field studies conducted by Kurihara & Ikusima, 1990). We had a low amount of uncertainty for this risk element.
Risk score = 18 Uncertainty index = 0.08

Impact Potential *Trapa natans* poses the biggest impact within natural systems. It alters nutrient regimes (Tall, Caraco, & Maranger, 2011; Caraco & Cole, 2002) and prevents up to 95% of light from permeating through the water column (Tall et al., 2011; Groth et al., 1996), which inhibits photosynthesis at lower levels and prevents oxygenation of deeper waters. Further, *T. natans* displaces native macrophytes (Strayer et al., 2003; Hummel & Kiviat, 2004) and reduces species diversity (Pemberton, 2002; Countryman, 1977; Hummel & Kiviat, 2004). This species also poses a danger to the public, including injury from stepping on the barbed fruits (Kaufman & Kaufman, 2007; Hummel & Kiviat, 2004) and drowning in its thick growth (Hummel & Kiviat, 2004). This species also reduces the recreational usage of an area that it has invaded (Pemberton, 2002; Swearingen

¹ “WRA area” is the area in relation to which the weed risk assessment is conducted [definition modified from that for “PRA area”] (IPPC, 2012).

et al., 2002; Ding et al., 2006; Hummel & Kiviat, 2004). We found no evidence of impacts in agricultural systems. We had a low amount of uncertainty for this risk element.

Risk score = 3 Uncertainty index = 0.07

Geographic Potential Based on three climatic variables, we estimate that about 82.3 percent of the United States is suitable for the establishment of *Trapa natans* (Fig. 1). This predicted distribution is based on the species' known distribution elsewhere in the world and includes point-referenced localities and areas of occurrence. The map for *Trapa natans* represents the joint distribution of Plant Hardiness Zones 3-13, areas with 0-100+ inches of annual precipitation, and the following Köppen-Geiger climate classes: tropical rainforest, tropical savanna, steppe, Mediterranean, humid subtropical, marine west coast, humid continental warm summers, humid continental cool summers, subarctic, and tundra.

The area of the United States shown to be climatically suitable (Fig. 1) is likely overestimated since our analysis considered only three climatic variables. Other environmental variables, such as pH, water turbidity, and wave turbulence, may further limit the areas in which this species is likely to establish. *Trapa natans* inhabits temperate to tropical water bodies in sluggish areas with slower water flow (Hummel & Kiviat, 2004).

Entry Potential We did not assess the entry potential of *Trapa natans* because it is already present in the United States (Ding et al., 2006; Countryman, 1977).

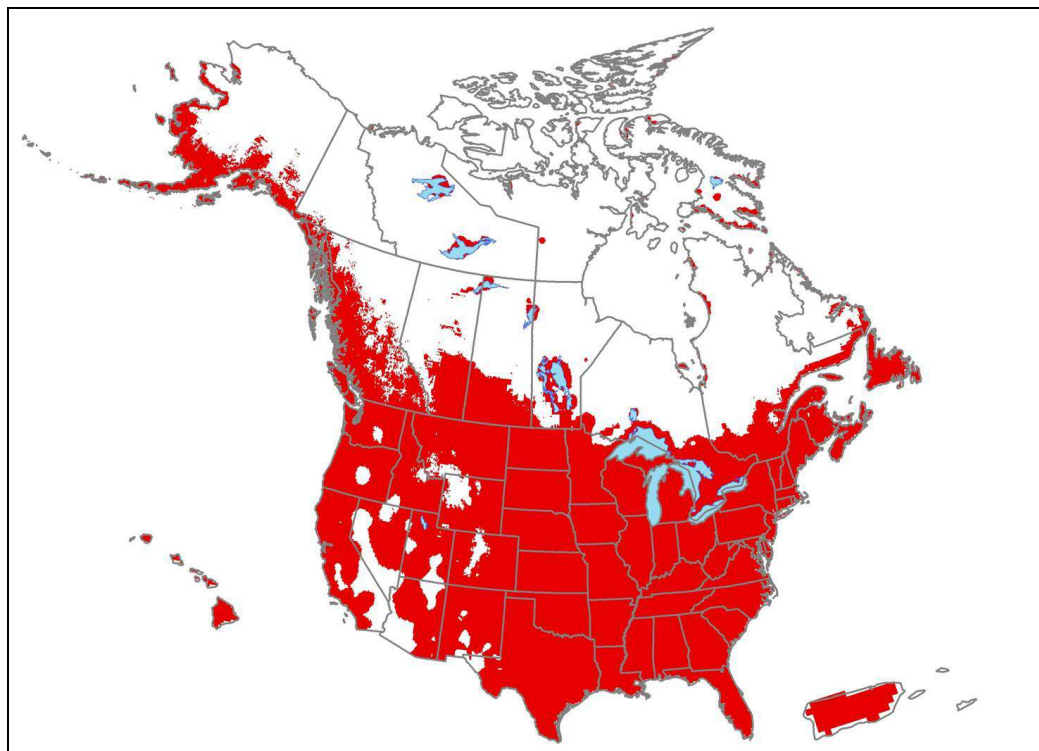


Figure 1. Predicted distribution of *Trapa natans* in the United States. Map insets for Alaska, Hawaii, and Puerto Rico are not to scale.

2. Results

Model Probabilities: P(Major Invader) = 87.1%
P(Minor Invader) = 12.4%
P(Non-Invader) = 0.4%

Risk Result = High Risk

Secondary Screening = Not Applicable

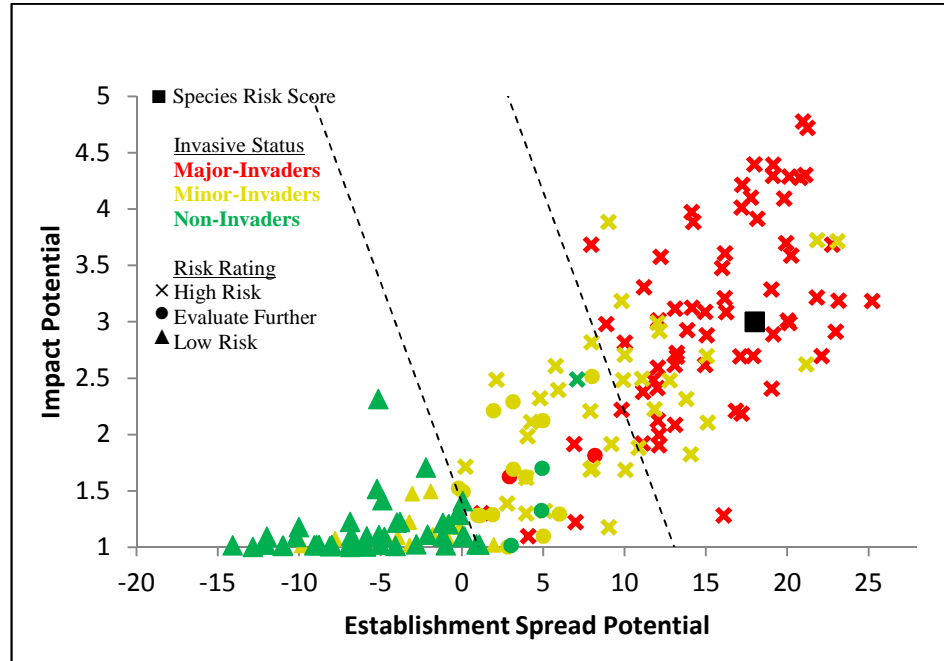


Figure 2. *Trapa natans* risk score (black box) relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols). See Appendix A for the complete assessment.

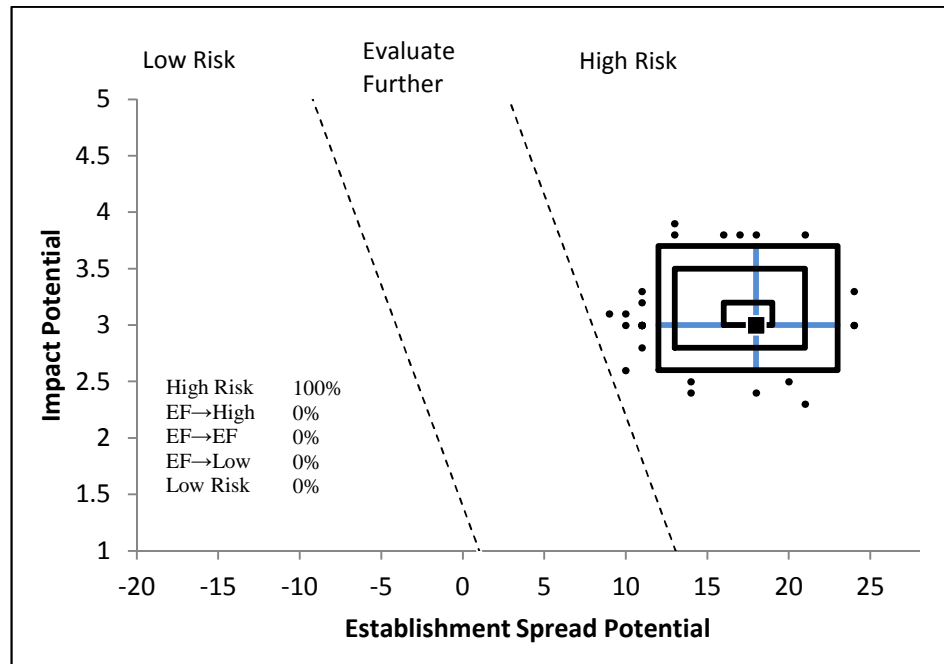


Figure 3. Model simulation results (N=5,000) for uncertainty around the risk score for *Trapa natans*. The blue “+” symbol represents the medians of the simulated outcomes. The smallest box contains 50 percent of the outcomes, the second 95 percent, and the largest 99 percent.

3. Discussion

The result of the weed risk assessment for *Trapa natans* is High Risk (Fig. 2). When compared with the species of known weeds used to validate the WRA model, this species ranked amongst other High Risk weeds. Our categorization of “High Risk” is well supported by the uncertainty analysis (Fig. 3). *Trapa natans* has been the focus of several management and eradication programs, most notably within Lake Champlain in the northeastern United States and within Maryland, near the Chesapeake Bay (Naylor, 2003). Control measures that have been most effective are mechanical hand pulling (Groth et al., 1996; Countryman, 1977) as the concentration of herbicide necessary to control growth is harmful to both native flora and fauna (Hummel & Kiviat, 2004). In Lake Champlain, more than \$5 million was spent on control between 1982 and 2003 (Kaufman & Kaufman, 2007), and the state of New York and Vermont, as well as the U.S. Fish and Wildlife Service, the Army Corps of Engineers, and the Lake Champlain Basin Program have collaborated on this management program for decades. The Maryland Department of Natural Resources’ 2003 management plan outlined a \$27,000 plan for control and management, with additional funds allocated for prevention of introduction and communication efforts (Naylor, 2003). In the Chesapeake Bay region alone, \$2.8 million has been spent in the past 20 years for control and monitoring programs (Eyres, 2009). This species also poses a unique human health hazard for an aquatic macrophyte; this plant produces barbed nuts (Swearingen et al., 2002; Ohwi, 1984; Pemberton, 2002) that pose a significant hazard to swimmers, boaters, and fishermen (Kaufman & Kaufman, 2007; Hummel & Kiviat, 2004; Swearingen et al., 2002), as well as those involved with the hand removal of the species. This species has been used in phytoremediation experiments (Sweta et al., 2015) and it is capable of removing large amounts of nitrogen from an aquatic system (Tall et al., 2011).

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Appendix A. Weed risk assessment for *Trapa natans* L. (Lythraceae). Below is all of the evidence and associated references used to evaluate the risk potential of this taxon. We also include the answer, uncertainty rating, and score for each question. The Excel file, where this assessment was conducted, is available upon request.

Question ID	Answer - Uncertainty	Score	Notes (and references)
ESTABLISHMENT/SPREAD POTENTIAL			
ES-1 [What is the taxon's establishment and spread status outside its native range? (a) Introduced elsewhere =>75 years ago but not escaped; (b) Introduced <75 years ago but not escaped; (c) Never moved beyond its native range; (d) Escaped/Casual; (e) Naturalized; (f) Invasive; (?) Unknown]	f - negl	5	<i>Trapa natans</i> has a very broad native distribution that includes many countries in Africa, Europe, and parts of Asia (NGRP, 2015; GBIF, 2015). This species has been introduced and become naturalized elsewhere (NGRP, 2015), including India (Bhatt et al., 2012), Japan (Kadono, 2004), and Singapore (Keng, 1990). This species was first reported for the U.S. in 1886 (Wibbe, 1886) and since then it has spread to several northeastern states (Kartesz, 2015; Pemberton, 2002). It was first detected in Canada in southern Quebec in 1998 (Darbyshire, 2003) and is expected to spread down the St. Lawrence River system (de Lafontaine & Costa, 2002). <i>Trapa natans</i> is considered one of the worst invasive aquatic species in India (Bhatt et al., 2012) where the species is categorized as invasive (i.e., spreading) (Khuroo et al., 2007; Jaryan et al., 2012). <i>Trapa natans</i> exhibits "vigorous spread" in Japan (Kurihara & Ikusima, 1991). After its initial introduction in Massachusetts, <i>T. natans</i> ' "explosive" spread (Ding & Blossey, 2005) extended the species' introduced range throughout the Northeastern United States and as far south as Chesapeake Bay (Ding et al., 2006). Within Lake Champlain, (located within the borders of New York, Vermont, and Quebec) total <i>T. natans</i> biomass increased tenfold within two years following the abandonment of the control program; 8 "bushels" (286 lbs.) were hand pulled in 1967, while control ceased in 1968, and 80 "bushels" (1.5 tons) were then pulled in 1969 (Groth et al., 1996; Countryman, 1977). Alternate answers for the Monte Carlo simulation are both e.
ES-2 (Is the species highly domesticated)	n - low	0	This species is sometimes used in food or used as a source of starch (Raju, 1999; von Mueller, 1888; Mabblerley, 2008). <i>Trapa bicornis</i> and <i>Trapa bispinosa</i> , which are cultivated as a food item in Asia, have seeds with two stout horns (Keng, 1990) and are considered to be agricultural selections of <i>T. natans</i> (Pemberton, 2002). Researchers are evaluating the potential use of <i>Trapa natans</i> in a variety of areas including phytoremediation (Sweta et al., 2015) and human nutrition (Stoicescu et al., 2012). However, we found no evidence that the species overall is highly domesticated or has been bred to reduce traits associated with weed potential.
ES-3 (Weedy congeners)	n - low	0	The genus <i>Trapa</i> includes just this one polymorphic species, which at times has been split into numerous, narrowly-defined species (Weakley, 2015; Mabblerley, 2008). None of the narrowly-defined species that the genus has been split into is considered a significant weed (Randall, 2012).
ES-4 (Shade tolerant at some stage of its life cycle)	n - negl	0	<i>Trapa natans</i> grows in full sun environments (Wisconsin Sea Grant, 2015; Hummel & Kiviat, 2004) and does not tolerate any shade (Golden, 2015).

Question ID	Answer - Uncertainty	Score	Notes (and references)
ES-5 (Plant a vine or scrambling plant, or forms tightly appressed basal rosettes)	n - negl	0	<i>Trapa natans</i> is neither a vine nor does it form tightly appressed basal rosettes; it is a rooted aquatic herb (Agrawal & Mohan Ram, 1995; Shalabh et al., 2012).
ES-6 (Forms dense thickets, patches, or populations)	y - negl	2	In parts of the Hudson River during the summer months, <i>Trapa natans</i> forms dense populations (Tall et al., 2011). Plants can form dense mats (Swearingen et al., 2002) sometimes covering several miles (ISSG, 2005). <i>Trapa natans</i> often occurs at densities between 100-1000 g dry weight/m ² (Strayer et al., 2003) and may grow to densities of up to 50 plants per square meter (Tsuchiya & Iwaki, 1984).
ES-7 (Aquatic)	y - negl	1	<i>Trapa natans</i> is an aquatic species (Mabberley, 2008) with a floating rosette of leaves and a central stem that is rooted (Ohwi, 1984; Pemberton, 2002; Groth et al., 1996).
ES-8 (Grass)	n - negl	0	This species is not a grass; rather it is a member of the Lythraceae family (NGRP, 2015; Hummel & Kiviat, 2004).
ES-9 (Nitrogen-fixing woody plant)	n - negl	0	We found no evidence that this species fixes nitrogen, nor is it in a plant family known to have N-fixing capabilities (Martin and Dowd, 1990). Furthermore, this is not a woody plant, but rather a rooted aquatic herb (Agrawal & Mohan Ram, 1995; Shalabh et al., 2012).
ES-10 (Does it produce viable seeds or spores)	y - negl	1	<i>Trapa natans</i> produces viable seeds (Cozza et al., 1994). Populations are persistent through spontaneous dissemination of seeds (von Mueller, 1888). Kurihara & Ikusima (1990) found an 87% germination rate in the field.
ES-11 (Self-compatible or apomictic)	y - negl	1	Floral biology of <i>Trapa natans</i> favors self-pollination (Kadono & Schneider, 1986) and self-pollination is possible before the flower opens (Hummel & Kiviat, 2004). Insect movement within the flower results in the anther sacs being 'pushed' against the stigma, facilitating self-pollination (Kadono & Schneider, 1986). Caging experiments conducted by Kadono and Schneider (1986) indicate that <i>Trapa natans</i> is "both self- and cross-compatible as well as apomictic".
ES-12 (Requires specialist pollinators)	n - negl	0	Flowers are insect pollinated (Swearingen et al., 2002; Mikulyuk & Nault, 2009; National Park Service, 2015). No further information is provided about the types of insects, indicating that these are generalist pollinators. However, field experiments and observations conducted by Kadono and Schneider (1986) state that "insects captured and examined for pollen revealed minimum amounts. These observations suggest that insects play a minimum role as cross-pollinators". We are answering no, due to the majority of literature pointing to insect pollination, but with low uncertainty given the observations by Kadono and Schneider (1986).
ES-13 [What is the taxon's minimum generation time? (a) less than a year with multiple generations per year; (b) 1 year, usually annuals; (c) 2 or 3 years; (d) more than 3 years; or (?) unknown]	b - low	1	<i>Trapa natans</i> plants are annuals (Swearingen et al., 2002; ISSG, 2005; Pemberton, 2002) and reproduce naturally only by seed (Countryman, 1977). Parent plants produce seeds by late June and die by fall, killed by the first frost (Countryman, 1977; Hummel & Kiviat, 2004). Seeds generally germinate the next year (Cozza et al., 1994), but seeds may remain dormant in the seed bank and remain viable for 3-12 years (Mabberley, 2008; Pemberton, 2002; Kurihara & Ikusima, 1990; Hummel & Kiviat, 2004). Most seeds germinate within two years (Mabberley, 2008). While this species is able to regenerate

Question ID	Answer - Uncertainty	Score	Notes (and references)
			from vegetative fragments (Kaufman & Kaufman, 2007; ISSG, 2005), <i>T. natans</i> lacks a form of natural vegetative fragmentation (Agrawal & Mohan Ram, 1995). Therefore, we are answering b, and alternate answers for the Monte Carlo simulation are both c.
ES-14 (Prolific reproduction)	n - low	-1	<i>Trapa natans</i> often grows to densities of up to 50 plants per square meter (Tsuchiya & Iwaki, 1984), and very high density beds can produce about 100 rosettes/m ² (Hummel & Kiviat, 2004). Seeds stored under natural conditions (in lakes) had a germination rate of about 80 percent (Cozza et al., 1994). Single seeded fruit germinate early in the spring and can produce 10 to 15 plant rosettes, each of which can produce 15 to 20 seeds (ISSG, 2005). Very high density beds tend to be less sexually productive than low density beds (Hummel & Kiviat, 2004; Groth et al., 1996), yet calculating that each rosette can produce 15 to 20 seeds, these very high density beds can produce 1500 to 2000 seeds, which falls below our threshold of 5000. Therefore, we answered no.
ES-15 (Propagules likely to be dispersed unintentionally by people)	y - low	1	<i>Trapa natans</i> may be introduced to new sites via fish nets (Dementeva & Petushkova, 2010). Barbs can cling to nets, wooden boats, clothing, construction equipment, and other vehicles (Hummel & Kiviat, 2004).
ES-16 (Propagules likely to disperse in trade as contaminants or hitchhikers)	n - high	-1	We found no evidence that this species is dispersed as a contaminant of agricultural, forestry, or horticultural products. It does not seem likely that seeds or vegetation would be dispersed in this manner, due to seed and fruit morphology (see ES-17).
ES-17 (Number of natural dispersal vectors)	3	2	Fruit and seed description for questions ES-17a through ES-17e: Fruit are woody with 2-4 sharp barbs that are derived from the calyx and bear a single seed (Swearingen et al., 2002; Ohwi, 1984; Pemberton, 2002). Fruits are buoyant (Swearingen et al., 2002) and weigh six grams (Mikulyuk & Nault, 2009).
ES-17a (Wind dispersal)	n - negl		We found no evidence that propagules are wind dispersed, and given the size and weight of the fruits, it would be nearly impossible for them to disperse in this manner.
ES-17b (Water dispersal)	y - negl		While the flowers are borne above water, as the plant meristem develops the fruit end up developing in the water (Pemberton, 2002). When mature, the fruit detach from the plants and float for some time, eventually falling to the sediment layer where the barbs help anchor the seeds in the hydrosol (van der Pijl, 1982; Pemberton, 2002). Nuts and rosettes that are broken off can float to other areas on currents (Swearingen et al., 2002).
ES-17c (Bird dispersal)	y - high		Fruit cling to birds (Swearingen et al., 2002). Barbs cling to the plumage of Canadian geese (Hummel & Kiviat, 2004). We are answering yes, but with high uncertainty given that the size and weight of the fruit will most likely limit this kind of dispersal over long distances.
ES-17d (Animal external dispersal)	y - high		Fruit cling to animals (Swearingen et al., 2002). Barbs cling to mammal fur (Hummel & Kiviat, 2004). We are answering yes, but with high uncertainty given that the size and weight of the fruit will most likely limit this kind of dispersal over long distances.
ES-17e (Animal internal dispersal)	n - low		We found no evidence that this species is dispersed internally;

Question ID	Answer - Uncertainty	Score	Notes (and references)
dispersal)			moreover the woody barbs and husk of the fruit will most likely deter animals from eating it.
ES-18 (Evidence that a persistent (>1yr) propagule bank (seed bank) is formed)	y - low	1	Seeds are viable for up to 12 years (Mabberley, 2008), although most will germinate within the first two years (Swearingen et al., 2002). In one experimental study, some seeds remained dormant until the second year, at which time they germinated at the same rates as seeds that were dormant for only one winter season; this study suggests that plants are producing seeds that are physiologically heteromorphic (i.e. seeds of the same generation have different growth functionality) (Cozza et al., 1994). Seed longevity is three years under natural conditions. Seeds that do not germinate the spring after they are released become part of the seed bank and may germinate at a later date (Kurihara & Ikusima, 1990). Seed banks may persist 10-12 years in sediment (Hummel & Kiviat, 2004).
ES-19 (Tolerates/benefits from mutilation, cultivation or fire)	y - mod	1	<i>Trapa natans</i> fragments will reestablish a plant (Kaufman & Kaufman, 2007; ISSG, 2005). When raking or pulling plants, floating, uplifted plants and plant parts can spread the plant to new locations (Swearingen et al., 2002; Groth et al., 1996). Detached ramets are capable of producing further ramets and seed, which may develop at any point downstream of the parent plant (Groth et al., 1996). The plant is commonly fragmented by mechanical removal and control methods (Kaufman & Kaufman, 2007) and cutting from boats, ropes, etc. (Hummel & Kiviat, 2004). We found no information regarding vegetative regeneration rates, so we are answering yes with moderate uncertainty.
ES-20 (Is resistant to some herbicides or has the potential to become resistant)	n - low	0	We found no evidence this species is resistant to herbicides. Furthermore, it is not listed by Heap (2013) as a weed that is resistant to herbicides. The herbicide 2,4-dichlorophenoxy acetic acid (2,4-D) has been used successfully to treat <i>T. natans</i> infestations, however the high concentrations used are detrimental to both native plants and other wildlife (Hummel & Kiviat, 2004).
ES-21 (Number of cold hardiness zones suitable for its survival)	11	1	
ES-22 (Number of climate types suitable for its survival)	10	2	
ES-23 (Number of precipitation bands suitable for its survival)	11	1	
IMPACT POTENTIAL			
General Impacts			
Imp-G1 (Allelopathic)	n - low	0	We found no evidence that this species is allelopathic. This species is a freshwater aquatic plant, and allelopathy is not normally associated with freshwater aquatic environments (Gross, 2003).
Imp-G2 (Parasitic)	n - negl	0	We found no evidence that this species is parasitic. Furthermore, <i>T. natans</i> does not belong to a family known to contain parasitic plants (Heide-Jorgensen, 2008; Hummel & Kiviat, 2004).
Impacts to Natural Systems			
Imp-N1 (Changes ecosystem)	y - negl	0.4	Dense mats of <i>T. natans</i> block 95 percent of light from entering

Question ID	Answer - Uncertainty	Score	Notes (and references)
processes and parameters that affect other species)			the water column, thereby inhibiting photosynthesis and oxygenation at lower levels (Tall et al., 2011; Groth et al., 1996). Plants vent oxygen directly into the atmosphere, depleting oxygen from the surrounding water (Tall, Caraco, & Maranger, 2011) and causing hypoxia and anoxia. In a study conducted in Hudson River tidal areas, Caraco and Cole (2002) measured dissolved oxygen (DO) levels of native plants beds and <i>Trapa natans</i> beds, and that from July-August, DO in native macrophyte beds (<i>Vallisneria americana</i>) never declined below 5 mg/L, and varied between 6.3 and 11.8 mg/L, while beds of <i>Trapa natans</i> had DO levels lower than 2.5 mg/L, with measurements that varied between 0 and 6 mg/L. Furthermore, decaying plants reduce oxygen levels in the water which increases the chance for fish kills (Kaufman & Kaufman, 2007; Swearingen et al., 2002). Because aquatic species with floating leaves deliver oxygen directly into the atmosphere, fixed carbon is retained in the aquatic system (Pierobon et al., 2010; Strayer et al., 2003; Goodwin et al., 2008). In the tidal portion of the Hudson River, beds of <i>Trapa</i> remove significant amounts of nitrogen each year because the low oxygen levels they create when the tide runs out promotes microbial activity which denitrify the system through the production of nitrous oxide and nitrogen gas (Tall et al., 2011). In fact, although the large <i>Trapa</i> beds in this system represent only 2.7% of the total area of the tidal Hudson, they remove between 70% and 100% of the total N in this river (Tall et al., 2011).
Imp-N2 (Changes habitat structure)	y - low	0.2	<i>Trapa natans</i> displaces submerged native vegetation in the Hudson River (Strayer et al., 2003). <i>Trapa natans</i> can cover 100% of the water's surface and block 95% of sunlight, shading out all submerged vegetation (Hummel & Kiviat, 2004). Only tall, emergent species are able to grow in water chestnut beds, and are unaffected by its interspecies competition (Hummel & Kiviat, 2004).
Imp-N3 (Changes species diversity)	y - low	0.2	<i>Trapa natans</i> can dominate ponds, shallow lakes, and river margins, displacing native vegetation due to heavy shading of submersed and other floating plants (Pemberton, 2002) and outcompeting native plants for sunlight (ISSG, 2005; Countryman, 1977; Swearingen et al., 2002). In the Hudson River, <i>Trapa natans</i> has replaced the native submerged species water celery (<i>Vallisneria americana</i> Michx.) and clasping pondweed (<i>Potamogeton perfoliatus</i> L.), as well as the introduced species Eurasian watermilfoil (<i>Myriophyllum spicatum</i> L.) (Hummel & Kiviat, 2004). <i>Trapa natans</i> is of little use to wildlife (Swearingen et al., 2002; Countryman, 1977) and crowds out desirable aquatic plants which provide food and shelter to fish and waterfowl (Countryman, 1977) Displacement of submersed plants by <i>T. natans</i> is believed to cause the loss of many animal species and their replacement by more tolerant, more common, and in some cases non-native species (Hummel & Kiviat, 2004). Pemberton, 2002; Countryman, 1977; Hummel & Kiviat, 2004).
Imp-N4 (Is it likely to affect federal Threatened and Endangered species?)	y - low	0.1	There is concern that <i>T. natans</i> populations in the Connecticut River will spread into the tidal marshes of that area, which have exceptional significance for rare plants and animals (Hummel

Question ID	Answer - Uncertainty	Score	Notes (and references)
			& Kiviat, 2004). This species greatly reduces sunlight (Tall et al., 2011; Groth et al., 1996) and depletes oxygen in the water column it occupies, which may lead to deaths of native wildlife (Kaufman & Kaufman, 2007; Swearingen et al., 2002). Further, <i>T. natans</i> outcompetes and crowds out native species (ISSG, 2005; Countryman, 1977; Swearingen et al., 2002). The displacement of native species is believed to have replaced native wildlife populations as well (Hummel & Kiviat, 2004). These effects on natural ecosystems and native populations indicate that this species is likely to have a very serious impact on T&E species in areas which it invades.
Imp-N5 (Is it likely to affect any globally outstanding ecoregions?)	y - negl	0.1	<i>Trapa natans</i> ' predicted distribution in the United States includes globally outstanding ecoregions as defined by Ricketts et al. (1999). <i>Trapa natans</i> is already present as a noxious weed in areas of Pennsylvania and Maryland (Pennsylvania Sea Grant, 2008) which occur in a globally outstanding ecoregion (Ricketts et al., 1999). <i>Trapa natans</i> may move to nearby counties in globally outstanding ecoregions via the dispersal methods discussed in ES-17. This species alters nutrient regimes within areas it becomes established in, creating hypoxic and anoxic zones (Pierobon et al., 2010; Strayer et al., 2003; Goodwin et al., 2008), encouraging microbial communities to further denitrify the water column under <i>T. natans</i> beds (Tall, Caraco, & Maranger, 2011). <i>Trapa natans</i> outcompetes native species to replace the submerged vegetation layers, shading them out (Strayer et al., 2003; Pemberton, 2002) and provides little use to native wildlife and waterfowl (Countryman, 1977; Swearingen et al., 2002). Dense mats of <i>T. natans</i> block 95% light attenuation in the water column beneath them, altering the oxygenation of the natural system and increasing the chance for fish kills (Tall, Caraco, & Maranger, 2011; Swearingen et al., 2002; Kaufman & Kaufman, 2002).
Imp-N6 [What is the taxon's weed status in natural systems? (a) Taxon not a weed; (b) taxon a weed but no evidence of control; (c) taxon a weed and evidence of control efforts]	c - negl	0.6	<i>Trapa natans</i> is a natural areas weed in Australia (Randall, 2007). Control methods are described in several sources (Swearingen et al., 2002). The Nature Conservancy has organized teams of volunteers to pull rosettes from the environment (Pemberton, 2002). Hand removal of small populations is best because it uproots easily and helps prevent additional spread (ISSG, 2005). Chemical and machine removal is more effective for large populations (ISSG, 2005). The US Department of Agriculture's Agricultural Research Service has sponsored research to identify suitable biological control agents (Pemberton, 1999). Field experiments by Ding et al. (2006) showed promise for biocontrol of <i>T. natans</i> in natural areas by <i>Galerucella birmanica</i> , a leaf beetle. In Lake Champlain, more than \$5 million was spent on control between 1982 and 2003 (Kaufman & Kaufman, 2007). The Maryland Department of Natural Resources' 2003 management plan outlined a \$27,000 plan for control and management, with additional funds allocated for prevention of introduction and communication efforts (Naylor, 2003). Alternate answers for the Monte Carlo simulation are b.
Impact to Anthropogenic Systems (cities, suburbs, roadways)			

Question ID	Answer - Uncertainty	Score	Notes (and references)
Imp-A1 (Negatively impacts personal property, human safety, or public infrastructure)	y - negl	0.1	<i>Trapa natans</i> may have played a role in the drowning deaths of a woman and two children in the Hudson River in July 2001 due to entanglement (Hummel & Kiviat, 2004). Nuts that wash up on the shoreline are hazardous to walkers and bathers due to the sharp spines (Kaufman & Kaufman, 2007). Specialized methods for control are needed to prevent injury to people (Swearingen et al., 2002). Nuts float to shores where the sharp spines are a nuisance to bare feet (ISSG, 2005). Barbed spine-tips may break off in the skin and have caused infection (Hummel & Kiviat, 2004). The Asian custom of eating raw water chestnut contributes to the ingestion of the giant intestinal fluke (<i>Fasciolopsis buski</i>) larvae that cause fasciolopsiasis (Hummel & Kiviat, 2004).
Imp-A2 (Changes or limits recreational use of an area)	y - negl	0.1	<i>Trapa natans</i> limits recreation and navigation (Pemberton, 2002). Dense growth of <i>T. natans</i> eliminates or severely impedes most recreational activities such as swimming, fishing from the shoreline, the use of small boats, and even duck hunting (Pemberton, 2002; Swearingen et al., 2002; Ding et al., 2006; Hummel & Kiviat, 2004). These large mats make areas inaccessible to fishermen (ISSG, 2005; Ding et al., 2006). Swimming and other beach-related activities are also hindered by the sharp nut hulls that accumulate on shores (Hummel & Kiviat, 2004; Swearingen et al., 2002)
Imp-A3 (Affects desirable and ornamental plants, and vegetation)	n - low	0	We found no evidence that this species affects ornamental plants and vegetation.
Imp-A4 [What is the taxon's weed status in anthropogenic systems? (a) Taxon not a weed; (b) Taxon a weed but no evidence of control; (c) Taxon a weed and evidence of control efforts]	c - low	0	Classified by the Weed Science Society of America as a weed (WSSA, 2010) and considered a weed in China (Zhang, 2000). Eradication efforts are currently in place for a population discovered in the Erie Canal (Clay, 2011) including hand pulling and monitoring for any re-establishment of the species in the area. Volunteers in New York utilize hand pulling as a control effort for the population in the Oswego River, near Battle Island, both popular tourist and recreation sites. Volunteers conduct these control efforts annually in an attempt to suppress and eradicate the local population (Yablonski, 2015). Therefore, we answered c, with alternate answers of b.
Impact to Production Systems (agriculture, nurseries, forest plantations, orchards, etc.)			
Imp-P1 (Reduces crop/product yield)	n - mod	0	Although this species is a weed of rice in India (Moody, 1989; Raju, 1999) we found no evidence that it reduces yield.
Imp-P2 (Lowers commodity value)	n - mod	0	We found no evidence that this species lowers commodity value.
Imp-P3 (Is it likely to impact trade?)	n - mod	0	<i>Trapa bicornis</i> is regulated in New Zealand where it is prohibited from sale, propagation, and distribution (BOP Environment, 2004; APHIS, 2015). <i>Trapa</i> spp. is regulated in Australia and Nauru (APHIS, 2015). Within the United States, <i>T. natans</i> is regulated in Alabama, Arizona, Connecticut, Illinois, Indiana, Michigan, Oregon, South Carolina, Vermont, Washington, and Wisconsin (National Plant Board, 2015). While this species is regulated in trade, we found no evidence that <i>T. natans</i> is likely to follow a pathway of trade as a contaminant, due to the size and morphology of its seeds.

Question ID	Answer - Uncertainty	Score	Notes (and references)
			<i>Trapa natans</i> is cultivated as a food product within Asia (von Mueller, 1888; Mabberley, 2008; Keng, 1990), but is unlikely to move as a contaminant.
Imp-P4 (Reduces the quality or availability of irrigation, or strongly competes with plants for water)	n - mod	0	We found no evidence that this species affects the quality or availability of water.
Imp-P5 (Toxic to animals, including livestock/range animals and poultry)	n - low	0	We found no evidence that this species is toxic to animals.
Imp-P6 [What is the taxon's weed status in production systems? (a) Taxon not a weed; (b) Taxon a weed but no evidence of control; (c) Taxon a weed and evidence of control efforts]	b - low	0.2	<i>Trapa natans</i> has been identified as a weed of rice in India (Raju, 1999; Moody, 1989). However, we found no evidence that this species is being controlled in this system. Therefore, we answered b. Alternate answers for the Monte Carlo simulation were both a.
GEOGRAPHIC POTENTIAL			Unless otherwise indicated, the following evidence represents geographically referenced points obtained from the Global Biodiversity Information Facility (GBIF, 2015).
Plant hardiness zones			
Geo-Z1 (Zone 1)	n - negl	N/A	We found no evidence that it occurs in this hardiness zone.
Geo-Z2 (Zone 2)	n - mod	N/A	We found no evidence that it occurs in this hardiness zone.
Geo-Z3 (Zone 3)	y - low	N/A	A few points in Russia. It was noted in one source (i.e. Cozza et al., 1994) that this species undergoes a "chilling period" necessary for germination which may adapt the species for growth in cold areas, but we were unable to verify this. However, this study also found that seeds stored at 4°C had a higher germination rate than seeds not stored at such low temperatures. Consequently, we are answering yes, but with moderate uncertainty.
Geo-Z4 (Zone 4)	y - negl	N/A	A few points each in Canada, Russia, India, and China.
Geo-Z5 (Zone 5)	y - negl	N/A	A few points in the United States and Russia.
Geo-Z6 (Zone 6)	y - negl	N/A	The United States, Japan, Russia, Poland, and Austria.
Geo-Z7 (Zone 7)	y - negl	N/A	The United States, Japan, and France.
Geo-Z8 (Zone 8)	y - negl	N/A	China, Japan, France, Spain, Belgium, Germany, and Italy.
Geo-Z9 (Zone 9)	y - negl	N/A	China, Japan, France, and Greece.
Geo-Z10 (Zone 10)	y - negl	N/A	The United States, South Africa, Namibia, Botswana, Zambia, China, and Japan.
Geo-Z11 (Zone 11)	y - negl	N/A	South Africa, Zambia, and China.
Geo-Z12 (Zone 12)	y - negl	N/A	Sudan, Uganda, Burkina Faso, China, and Thailand.
Geo-Z13 (Zone 13)	y - low	N/A	A few points in Thailand.
Köppen -Geiger climate classes			
Geo-C1 (Tropical rainforest)	y - mod	N/A	One point in Thailand
Geo-C2 (Tropical savanna)	y - negl	N/A	Zambia, Sudan, Uganda, Burkina Faso, and Thailand.
Geo-C3 (Steppe)	y - negl	N/A	South Africa, Namibia, Botswana, Spain, and China.
Geo-C4 (Desert)	n - low	N/A	We found no evidence that it occurs in this climate class.
Geo-C5 (Mediterranean)	y - negl	N/A	The United States, Turkey, Greece, and Algeria.
Geo-C6 (Humid subtropical)	y - negl	N/A	The United States, South Africa, Zambia, China, and Japan.
Geo-C7 (Marine west coast)	y - negl	N/A	France, Spain, Germany, Belgium, and the Netherlands.

Question ID	Answer - Uncertainty	Score	Notes (and references)
Geo-C8 (Humid cont. warm sum.)	y - negl	N/A	The United States, Japan, and South Korea.
Geo-C9 (Humid cont. cool sum.)	y - negl	N/A	The United States, Canada, China, Japan, and Russia
Geo-C10 (Subarctic)	y - mod	N/A	A few points in mountainous areas of France and Greece. It was noted in one source (i.e. Cozza et al., 1994) that this species undergoes a “chilling period” necessary for germination which may adapt the species for growth in cold areas, but we were unable to verify this. However, this study also found that seeds stored at 4°C had a higher germination rate than seeds not stored at such low temperatures. Consequently, we are answering yes, but with moderate uncertainty.
Geo-C11 (Tundra)	y - mod	N/A	Two points in mountainous regions in France. It was noted in one source (i.e. Cozza et al., 1994) that this species undergoes a “chilling period” necessary for germination which may adapt the species for growth in cold areas, but we were unable to verify this. However, this study also found that seeds stored at 4°C had a higher germination rate than seeds not stored at such low temperatures. Consequently, we are answering yes, but with moderate uncertainty.
Geo-C12 (Icecap)	n - low	N/A	We found no evidence that it occurs in this climate class.
10-inch precipitation bands			
Geo-R1 (0-10 inches; 0-25 cm)	y - mod	N/A	A few points in Uganda. There is no reason that this species couldn't survive in this precipitation band, as long as there is a permanent body of water.
Geo-R2 (10-20 inches; 25-51 cm)	y - negl	N/A	The United States, Botswana, Namibia, Burkina Faso, and Spain.
Geo-R3 (20-30 inches; 51-76 cm)	y - negl	N/A	South Africa, Zambia, Sudan, Burkina Faso, France, and Spain.
Geo-R4 (30-40 inches; 76-102 cm)	y - negl	N/A	South Africa, France, Belgium, Germany, and Russia.
Geo-R5 (40-50 inches; 102-127 cm)	y - negl	N/A	The United States, Canada, Zambia, China, and Japan.
Geo-R6 (50-60 inches; 127-152 cm)	y - negl	N/A	The United States, Japan, and France.
Geo-R7 (60-70 inches; 152-178 cm)	y - negl	N/A	China, Japan, and France.
Geo-R8 (70-80 inches; 178-203 cm)	y - negl	N/A	China, Japan, Thailand, and France.
Geo-R9 (80-90 inches; 203-229 cm)	y - negl	N/A	China, Japan, and France.
Geo-R10 (90-100 inches; 229-254 cm)	y - negl	N/A	China and Japan.
Geo-R11 (100+ inches; 254+ cm)	y - negl	N/A	China, Japan, Thailand, and Myanmar.
ENTRY POTENTIAL			
Ent-1 (Plant already here)	y - negl	1	We did not evaluate the entry potential of this species because it is already present and invasive in the United States (Ding et al., 2006; Countryman, 1977). It was cultivated in Asa Gray's botanical garden at Harvard University, in 1874 (Countryman, 1977). First observed to have escaped in North America in Concord, MA, in 1886 (Ding et al., 2006; Countryman, 1977).

Question ID	Answer - Uncertainty	Score	Notes (and references)
Ent-2 (Plant proposed for entry, or entry is imminent)	-	N/A	
Ent-3 (Human value & cultivation/trade status)	-	N/A	
Ent-4 (Entry as a contaminant)			
Ent-4a (Plant present in Canada, Mexico, Central America, the Caribbean or China)	-	N/A	
Ent-4b (Contaminant of plant propagative material (except seeds))	-	N/A	
Ent-4c (Contaminant of seeds for planting)	-	N/A	
Ent-4d (Contaminant of ballast water)	-	N/A	
Ent-4e (Contaminant of aquarium plants or other aquarium products)	-	N/A	
Ent-4f (Contaminant of landscape products)	-	N/A	
Ent-4g (Contaminant of containers, packing materials, trade goods, equipment or conveyances)	-	N/A	
Ent-4h (Contaminants of fruit, vegetables, or other products for consumption or processing)	-	N/A	
Ent-4i (Contaminant of some other pathway)	-	N/A	
Ent-5 (Likely to enter through natural dispersal)	-	N/A	