FORMAT FOR A PRA RECORD (version 3 of the Decision support scheme for PRA for quarantine pests)

European and Mediterranean Pl	European and Mediterranean Plant Protection Organisation	
Organisation Européenne et Mé	diterranéenne pour la Protection des Plantes	
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Guidelines on Pest Risk Analy	/SIS	
Lignes directrices pour i anar	yse du risque phytosanitaire	
Decision-support scheme for a	quarantine pests Version N°3	
PEST RISK ANALYSIS FOR Hydrocotyle ranunc	culoides	
Stage 1: Initiation	Note: the EPPO datasheet should be considered conjointly with this PRA.	
	The EWG was held on 2009-03-23-25, and was composed of the following experts:	
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1 What is the reason for performing the	Hydrocotyle ranunculoides originates from the American continent and was introduced	
PRA?	into the EPPO region as an ornamental plant for tropical aquaria and garden ponds,	
	where it is still sold under its correct name, sometimes under other names (H. vulgaris,	
	<i>H. leucocephala</i> , and <i>H. natans</i> which is a synonym of <i>H. ranunculoides</i>). The plant was	
	first recorded as naturalised in the south-east of the UK in the 1980s (Newman, 2003).	
	Naturalisation in the Netherlands and in Belgium was recorded in the last decade of the	
	twentieth century (Baas & Duistermaat, 1999; Baas & Holverda, 1996; Krabben &	
	Rotteveel, 2003; Verloove 2006, Invasive Species in Belgium Website). Deleterious	
	impacts have been reported in these three countries. The species is also recorded in	

		France, Ireland, Italy, Germany (see EPPO, 2009) but several EPPO countries are still
		free from <i>H. ranunculoides</i> and there are concerns that it may be able to enter and
		establish in further countries. This PRA assesses the fisks of its further introduction into other EPPO countries and its current and predicted impact
		An initial FPPO PRA was performed and approved in 2005. After the proposal of listing
		this species in the Directive 2000/29 the European Food Safety Authority reviewed the
		initial PRA and made some comments. The initial PRA is therefore revised in the view
		of the EFSA comments and of information having become available after the initial PRA
		(EFSA, 2007).
2 Enter the name of the pest		Hydrocotyle ranunculoides L. f.
2A Indicate the type of the pest		Aquatic freshwater plant (macrophyte)
		King Lange Directory
2B Indicate the taxonomic position		Kingdom: Planiae
		Family: Aniaceae
		Tanny. Aplaceae
3 Clearly define the PRA area		EPPO region
4 Does a relevant earlier PRA exist?	Yes	Schrader G, Rotteveel T & Bacher R (2005) Pest Risk Analysis: Hydrocotyle
		ranunculoides, 38pp
5 Is the earlier PRA still entirely valid, or	Yes	The present PRA consists in an update of the earlier EPPO PRA.
only partly valid (out of date, applied in		
different circumstances, for a similar but		
distinct pest, for another area with similar conditions)?		
Stage 2A: Pest Risk Assessment - Pest categor	ization	

6 Specify the host plant species (for pests	Freshwater bodies and ecosystems: ponds, ditches, marshes, waterways etc, more
directly affecting plants) or suitable	particularly, in static or slow-flowing waters (Newman & Dawson, 1999).
habitats (for non parasitic plants) present	In waters of high nutrient content the species thrives extremely well (EPPO, 2009).
in the PRA area.	
7. Specify the pest distribution	Native range:
	<i>H. ranunculoides</i> is considered to be native to North and South America (Everett 1981). Nevertheless, natural enemies are only reported from South America, but not from North America (Cordo <i>et al.</i> , 1982). Some studies are in progress to determine with accuracy the native area of the plant (Newman, pers. comm., 2009).
	North America: Canada (British Columbia, Quebec), Mexico, the USA (Alabama, Arizona, Arkansas, California, Delaware, Florida, Georgia, Illinois, Kansas, Louisiana, Maryland, Mississippi, New Jersey, New York, North Carolina, Ohio, Oklahoma, Oregon, Pennsylvania, South Carolina, Tennessee, Texas, Virginia, Washington, West Virginia). In some States (Illinois, New Jersey, New York) it is considered as an endangered species. Further details on American records can be found in USDA (2004).
	Central America and Caribbean : Costa Rica, Cuba, Guatemala, Nicaragua, Panama. Martin & Hutchins (1981) indicate presence in Tropical America generally.
	South America: Argentina, Bolivia, Brazil, Chile, Columbia (Holm <i>et al.</i> , 1979), Ecuador, Paraguay, Peru, Uruguay (Mathias & Constance 1976).
	Introduced range:
	EPPO region : Belgium, France, Germany, Italy, the Netherlands, the United Kingdom, Ireland (Maguire <i>et al.</i> , 2008; EPPO Datasheet, 2009). According to Flora Iberica (ref), the mention of <i>H. ranunculoides</i> in Spain (Tutin <i>et al.</i> , 1964-1980) could have resulted from confusions with small forms of <i>H. vulgaris</i> or <i>H. verticillata</i> .
	Asia: Lebanon (Conroy, 2006), Iran (Naqinezhad et al., 2007), Israel (old record), Syria

		(Mouterde, 1966), Yemen (Wood, 1997).
		 Africa: Angola, Ethiopia, Kenya, Malawi, Tanzania, Uganda, Democratic Republic of Congo (Gonçalves, 1978), Madagascar, Rwanda (Troupin, 1978), Zimbabwe (Chikwenhere, 2001). Possibly also Sudan. Oceania: Australia (Queensland, Western Australia) (Ruiz Avila & Klemm, 1996). Note: the fact that it is endangered in its northern range of distribution in North America is considered to be due to sub-optimal climatic conditions. Although mentioned as present in Austria in the previous PRA, the species does not occur in this country (F Essl, pers. comm., 2009). It is as well not recorded in Denmark (H E Svart, pers. comm., 2009) and Portugal (H Marchante, pers. comm., 2009).
8. Is the organism clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	Yes	Recent pilot study on barcoding <i>Hydrocotyle</i> species revealed that the species can be separated from other resembling <i>Hydrocotyle</i> species (van der Wiel <i>et al.</i> , 2009).Chromosome number: 2n=24, 48 (according to Constance <i>et al.</i> (1976) ; Tomei <i>et al.</i> (1989) et Pimenov <i>et al.</i> (2003), cited by Reduron (2007). There is a wide range of polyploids within the genus <i>Hydrocotyle</i> , with up to 15-ploidy (Moore, 1971, Federov, 1974). Baker <i>et al.</i> (1997) found four distinct groups of <i>H. ranunculoides</i> in the UK population which can be separated by AFLP analysis, meaning that there are different genotypes represented into the UK. Additionally, this study identified that the populations introduced within the UK were very similar from the ones originating from the Netherlands.According to the literature, <i>H. ranunculoides</i> is variable and was divided into 8 varieties and 3 forms which would require further investigation (Eichler, 1987):1. var. <i>adoensis</i> 2. var. <i>brasiliensis</i> 3. var. genuina Urban (épithète non admise = var. <i>ranunculoides</i>)I f. genuina Urban (épithète non admise = f. <i>ranunculoides</i>)4. var. <i>incisa</i> 5. var. <i>incisocrenata</i>

		 6. var. <i>lobata</i> II f. <i>minima</i> Kuntze III f. <i>minima</i> Hochstetter <i>ex</i> Richard (même plante que var. <i>minima</i>) 7. var. <i>minima</i> (Hochstetter <i>ex</i> Richard) Engler (même plante que f. <i>minima</i>) 8. var. <i>natans</i> (Cirillo) Urban (même plante que f. <i>natans</i>) IV f. <i>natans</i> (Cirillo) Urban (même plante que var. <i>natans</i>) 9. var. <i>ranunculoides</i>
		V f. <i>ranunculoides</i> 10. var. <i>sibthornioides</i> (= H. <i>sibthornioides</i> espèce différente)
		VI f. terrestris
		There is uncertainty about the extent to which different levels of ploidy between
		populations influences invasiveness.
9. Even if the causal agent of particular symptoms has not yet been fully identified, has it been shown to produce consistent symptoms and to be transmissible?	Not applicable	
10. Is the organism in its area of current distribution a known pest (or vector of a pest) of plants or plant products?	Yes	In its introduced range, <i>H. ranunculoides</i> can cause major problems in nature reserves and recreation areas as well as in intensely managed waterways (Baas & Duistermaat, 1999; Newman & Dawson, 1999). <i>H. ranunculoides</i> can displace native flora through competition, and fauna by habitat modification (Krabben & Rotteveel, 2003). In the Netherlands, this is the only plant species which transport, possession and trade is prohibited because of its huge impacts and costs of management (Netherland Act on Flora and Fauna, J van Valkenburg, pers. comm., 2009). In the UK, the review of the Wildlife Act instigated a ban species list for 32 plant species of which <i>H. ranunculoides</i> was one (see http://www.defra.gov.uk/wildlife-countryside/pdf/wildlife-manage/non- native/consultation.pdf). The Royal Horticulture Society banned this plant from their shows. The Ornamental Aquatic Trades Association in the UK adopted a voluntary ban on the trade of <i>H. ranunculoides</i> . In Belgium, the species is considered invasive, and voluntary actions are being taken between the nursery industry and the Belgian Biodiversity Platform (see website <u>http://ias.biodiversity.be/</u> ; Branquart 2008).

12 Does the pest occur in the PRA area?	Yes	The species occurs into the wildin Belgium, France, Germany, Ireland, Italy, the Netherlands and the United Kingdom (Hussner, pers. comm., Hegi, 1975, Pignatti, 1982).
13. Is the pest widely distributed in the PRA area?	No	This species is widespread and spreading rapidly in almost all the Netherlands (Krabben & Rotteveel, 2003) in the United Kingdom (Newman, 2003), and in Belgium (Branquart, 2008), while its presence is more localized in France, Germany, and Italy (EPPO, 2009) where invasion is at an early stage.
14. Does at least one host-plant species (for	Yes	Suitable habitats for the plant are static or slow-flowing and occasionally flowing
pests directly affecting plants) or one		freshwater bodies and ecosystems: ponds, ditches, marshes, waterways (Newman &
suitable habitat (for non parasitic plants)		Dawson, 1999). These habitats occur in the EPPO region.
occur in the PRA area (outdoors, in		
protected cultivation or both)?		
15. If a vector is the only means by which		Not applicable
the pest can spread, is a vector present in		
the PRA area? (if a vector is not needed or		
is not the only means by which the pest can		
spread go to 16)	X 7	
16. Does the known area of current	Yes	The plant is already established in part of the PRA area.
distribution of the pest include ecoclimatic		
conditions comparable with those of the		
PRA area or sufficiently similar for the		
pest to survive and thrive (consider also		
protected conditions)?		

17. With specific reference to the plant(s) or habitats which occur(s) in the PRA area, and the damage or loss caused by the pest in its area of current distribution, could the pest by itself, or acting as a vector, cause significant damage or loss to plants or other negative economic impacts (on the environment, on society, on export markets) through the effect on plant health in the PRA area?	Yes	 <i>H. ranunculoides</i> causes significant problems in areas where it has been introduced. The species is reported to be invasive in Australia (Ruiz Avila & Klemm 1996), the UK, the Netherlands, Belgium and Africa (CABI, 2005). The functioning of water ecosystems can be dramatically changed: In Belgium, it has been observed to reduce by more than 50% the number of native aquatic plant species and up to 100% of the submerged species, and to reduce the native cover from 50% to 10% (Nijs <i>et al.</i>, 2009); It increases flood risk (Newman & Dawson, 1999) which may result in blockage of agricultural drainage networks, raising the ground water level which causes impacts on plant communities and grazing pastures, as well as potential economic impacts on crops (Kelly, 2006); Strongly invaded waters lose their attractiveness and safety for recreation (boating, fishing); Loss in water quantity; Plants may accumulate heavy metals where available (Pinochet <i>et al.</i>, 2002), making disposal of plant material problematic.
18. This pest could present a risk to the PRA area.	Yes	Dense mats of vegetation can seriously affect species, habitats and ecosystems and their use. There is a high risk of spread of <i>Hydrocotyle ranunculoides</i> in still and slow flowing waterbodies in countries where it is already established, and there is a high risk of introduction where it is not already present and conditions (habitats, climate) are suitable.
19. The pest does not qualify as a quarantine pest for the PRA area and the assessment for this pest can stop.		

Section 2B: Pest Risk Assessment - Probability of introduction/spread and of potential economic consequences

Question	Rating +	Explanatory text of rating and uncertainty
	uncertainty	
		Note: If the most important pathway is intentional import, do not consider entry,
		but go directly to establishment. Spread from the intended habitat to the
		unintended habitat, which is an important judgement for intentionally imported
		organisms, is covered by questions 1.33 and 1.35.
1.1. Consider all relevant pathways and		Pathways are:
list them		- Intentional import as an ornamental aquatic plant for use outdoors and in aquariums
		From the isolated nature of the sites in which the plant has been observed, it can be
		suggested that they are almost all derived from human activity, whether by direct
		planting, by throwing away unwanted plants, or through cleaning of tropical aquaria or
		garden ponds where the plant fragments enter the water system (J. Newman, pers.
		comm., 2009). The plant is more likely to be introduced by aquarium trade through the
		Internet rather than direct retail (Newman, pers. comm., 2009).
		The species has been imported into the EPPO region but is not considered to be
		imported anymore because local production is far more cost effective than
		importation (van Valkenburg, pers. comm., 2009). The species is known to be
		produced and traded within the EPPO region.
		The actual sale of <i>H. ranunculoides</i> is difficult to ascertain because of the
		misapplied names. H. ranunculoides could be traded under the misapplied name
		Hydrocotyle vulgaris or the synonym H. natans. In Belgium, the species has also
		been sold as <i>H. leucocephala</i> (E. Branquart, pers. comm. 2009).
		Other Hydrocotyle species are in trade, which although being different species
		could be mislaballed (H. umbellata, H. novae zeelandiae, H. verticillata, H.
		moschata, H. sibthorpioides).
		H. ranunculoides is cited as H. americana L. in various catalogues (Brickell (ed),
		1996).
		See $O(1,33)$ on spread helped by human activities for data on trade within the EDPO
		countries
		countries.

Question	Rating + uncertainty	Explanatory text of rating and uncertainty
		As the plant is no longer imported, but is produced and traded, the entry pathway is not further considered. The volume of <i>H. ranunculoides</i> being produced and sold is considered to be very low.
		- <u>Intentional import for non ornamental uses</u> EFSA (2007) identified another pathway to be considered in the PRA which is the introduction of <i>H. ranunculoides</i> being used in phytoremediation (Bretsch, 2004) due to its ability to accumulate heavy metals and phosphorous (Poi de Neiff <i>et al.</i> 2003) and the general interest in the use of aquatic macrophytes for bioremediation (Vajpayee <i>et al.</i> 1995). Experts on phytoremediation were contacted to gather additional information.
		Dr McCutheon, Hydrologist and Environmental Engineer for the University of Georgia was contacted, and reported that the community working on phytoremediation is concerned about the use of alien species and typically limit itself to screening and selecting suboptimal plant species from indigenous communities. <u>http://www.scientificjournals.com/sj/all/AutorenProfil/AutorenId/5118</u>
		Mr Marmiroli from the University of Parma was contacted, but no answer was received. Marmiroli, N., & McCutcheon, S.C. (2003). Making phytoremediation a successful technology. In McCutcheon, S.C., & Schnoor, J.L. (Eds.), <i>Phytoremediation:</i> <i>Transformation and Control of Contaminants</i> . (pp. 85-119). Hoboken, NJ: Wiley- Interscience, Inc.
		Prof. Dr. Peter Schroeder, working for the German Research Center for Environmental Health (<u>http://www.scientificjournals.com/sj/all/AutorenAnzeigeESS/autorenId/1136</u>) have been contacted but no answer was received.
		In the EPPO region, other species are usually used for phytoremediation including <i>Phragmites australis, Typha</i> spp., etc (Cooper, 2001). Trials have been made in

Question	Rating +	Explanatory text of rating and uncertainty
	uncertainty	 Belgium, and the species was planted along watercourses in the Ghent area, from where it spread towards the border of the Netherlands (See Appendix 2). The species has also been tested for phytoremediation in Germany under controlled situation (Hussner, pers. comm., 2009). If an EPPO country was willing to use <i>H. ranunculoides</i> for phytoremediation, the species is already available in the region. <u>Unintentional introduction: hitch-hiking with other aquatic ornamental plants</u>. According to Maki & Galatowitsch (2004), <i>H. ranunculoides</i> has not been found as a contaminant of other traded aquarium plants in Minnesota (USA). In their study, a total of 681 individual plants (corresponding to 123 species) were ordered from vendors across the USA between May and September 2001, and were composed of the following types: 66 emergent plants, 16 submersed plants, 34 floating leaved plants and 6 freefloating plants. Some <i>Hydrocotyle</i> spp. produced within the EPPO region have been found to be contaminated with <i>H. ranunculoides</i> (J van Valkenburg, pers. comm., 2009). Such contamination is considered as a spread pathway (see Q. 1.33 and picture in Appendix 4) <u>Natural and human assisted spread</u> are considered in the dedicated section (Q 1.32 and 1.33). EFSA (2007) suggested the exchange of plant material between hobby gardeners and aquarium holders, and this is considered as local human activities as well.
1.2. Estimate the number of relevant pathways, of different commodities, from different origins, to different end uses.		
1.3. Select from the relevant pathways, using expert judgement, those which appear most important. If these pathways involve different origins and end uses, it is		<pre>see Q. 1.1. Identified pathways are: - trade for ornamental and aquarium purposes on the Internet</pre>

Question	Rating +	Explanatory text of rating and uncertainty
sufficient to consider only the realistic worst-case pathways. The following group of questions on pathways is then considered for each relevant pathway in turn, as appropriate, starting with the most important.		 trade for ornamental and aquarium purposes in direct retail use for phytoremediation. The plant is more likely to be traded for ornamental and aquarium purposes through the Internet rather than direct retail. Entry is not considered because the most important pathway is intentional import.
1.15. Do other pathways need to be considered?	no	
Conclusion on the probability of entry. Risks presented by different pathways.	High Low uncertainty	The plant has already entered the EPPO region. The plant is no longer imported, but is produced and traded. The volume of <i>H. ranunculoides</i> being produced and sold is considered to be very low.
1.16. Estimate the number of host plant species or suitable habitats in the PRA area (see question 6).	Few Uncertai nty: low	 <i>H. ranunculoides</i> grows in static, slow-flowing, and occasionally flowing water bodies. Habitats include managed and unmanaged lakes, ponds, ditches, canals rivers and streams. It grows best in water bodies with high contents of nitrate and phosphate, and/or organic matter, but is not restricted to these habitats. Extension to areas of saline and brackish waters is unlikely because it has been shown that salinity inhibits growth of <i>H. ranunculoides</i> (Stockley, 2001). According to the CORINE Land Cover nomenclature, the suitable habitats are: Continental waters (water courses, water bodies) Banks of continental water, riverbanks/canal sides (dry river beds)
1.17. How widespread are the host plants or suitable habitats in the PRA area? (specify)	Very widespread Uncertainty: low	Freshwater bodies and ecosystems abound in the EPPO region, particularly slow-flowing water bodies, ditches, canals, lakes and ponds. see CORINE LAND COVER (2009) in Appendix 1. CORINE Land Cover reports in Europe (http://dataservice.eea.europa.eu/dataservice/viewdata/viewpvt.asp): - 1.082.068 ha of inland marshes

Question	Rating + uncertainty	Explanatory text of rating and uncertainty
	· · · · ·	- 807.977 ha of water courses
		- 3.073.442 ha of water bodies.
1.18. If an alternate host or another	No	No other species is needed to complete the life cycle of the plant. The plant is able to
species is needed to complete the life cycle		reproduce vegetatively.
or for a critical stage of the life cycle such	Uncertainty:	
as transmission (e.g. vectors), growth (e.g.	low	
root symbionts), reproduction (e.g.		
pollinators) or spread (e.g. seed		
dispersers), how likely is the pest to come		
in contact with such species?		
1.19. How similar are the climatic	largely	<i>H. ranunculoides</i> is already established in several EPPO member countries (Belgium,
conditions that would affect pest	similar	France, Italy, the Netherlands, United Kingdom, Ireland, quite recently also Germany).
establishment, in the PRA area and in the		The climatic conditions experienced over winter result in a smaller suitable area
current area of distribution?	Uncertai	restricted to the margins of waterbodies (Newman, 2003).
	nty:	
	Medium	The species is endangered in parts (U.S. federal states of Illinois, New Jersey and New
		York (New York Environmental Regulations, 2000; USDA, 2004)) of its native range
		where it is vulnerable to low temperatures. However, in its introduced range, even if
		emergent leaves die at the first night frosts and floating leaves die when enclosed in ice,
		leaves of <i>H. ranunculoides</i> submerged below ice cover are reported to survive the winter
		months, and new plants can grow up in spring from these overwintering parts (Hussner & $1 - 2007$)
		Losch, 2007).
		In Western Europe nonulations may be strongly reduced during cold winters, but
		in western Europe populations may be strongly reduced during cold winters, but
		recovery occurs quickly in the following season.
		Optimum temperatures for gas exchange (linked with photosynthesis) at the leaves
		surface have been recorded to be comprised between 25°C and 32°C (Hussner &
		Lösch, 2007). At 35°C, the gas exchanges dropped
		The species being aquatic, it is not considered to be susceptible to air drought or
		humidity as long at it rooted in water. The species prefers growing in full sun, and

Question	Rating +	Explanatory text of rating and uncertainty
		 is limited by shade. According to the Climex simulation, the Atlantic and Mediterranean areas of the EPPO region that are characterized by mild winters are the most at risk. (see Appendix 3). The countries at risk are: Albania, Algeria, Austria, Azerbaijan, Belgium, Bosnia & Herzegovina, Bulgaria, Croatia, Cyprus, Denmark, France (including Corsica), Greece, Ireland, Israel, Italy (including Sardinia and Sicilia), Jordan, Germany (mostly western part), Hungary, Moldavia, Morocco, Portugal, Romania, Russia (Black Sea), Serbia, Slovenia, Spain, Switzerland, Tunisia, Ukraine Black Sea), the United Kingdom, Republic of Macedonia, Romania, Turkey. Nevertheless, so far, the species has expressed invasiveness in North-Western EPPO countries (Belgium, the Netherlands, United Kingdom,), while the areas which seem to be the most suitable are the Mediterranean and the Atlantic areas. This may be due to other elements such as the use of the plant and the eutrophication of waters. There is some uncertainty, how the plant would perform in Northern and Eastern Europe. However, severe continental winters and hot and dry summers (e.g. in continental conditions) are likely to limit distribution of the species.
1.20. How similar are other abiotic factors that would affect pest establishment, in the PRA area and in the current area of distribution?	largely/c ompletel y similar Uncertai nty: low	 <i>H. ranunculoides</i> is found in static, slow-flowing and occasionally flowing water bodies, especially ditches, canals, lakes and ponds. In the Netherlands, the species is found over a broad range of water quality conditions: from mesotrophic pools to the eutrophic lake Ijsselmeer margins. The environmental conditions in such habitats are present in most if not all EPPO countries. It is also important to note that eutrophic conditions are preferred: <i>H. ranunculoides</i> shows a much higher growth rate in high nutrient conditions, while maintaining similar rates of growth to native species in low nutrient conditions (Newman, 2002). <u>Sediments nutrients</u>

Question	Rating +	Explanatory text of rating and uncertainty
	uncertainty	
		In controlled conditions, optimal growth was observed in water with 20 mg N l ⁻¹ with a N uptake rate of 41 mg N g-1 (dw) of plant tissue (Reddy & Tucker, 1985). In Germany, fields' measurements showed that monospecific stands could occur in water with 6.2 to 11.5 mg of NO_{3-N} / kg of sediment and 2.9 to 61.9 mg of P_2O_5 / 100 g sediment (Hussner & Lösch, 2007).
		Water quality (see map in Appendix 1) In an area in France, the species remained confined to a restricted pond, most probably
		due to acidic waters which limit the vigour of the species (E Tabacchi, pers. comm., 2009).
		In the EPPO region, there are no macronutrients limitations.
		Arocena & Mazzeo (1994) showed the importance of alkalinity, total phosphorus and total inorganic nitrogen in the development of several macrophytes. Optimal
		development of <i>H. ranunculoides</i> was recorded in waters with the following mean values
		(extrema between brackets): total suspended solids: 63 mg +/- 52 [21-213] pH=7.1 +/-
		0.4 [6.5-7.9], alkalinity: 5.0 meq/1 +7-2.1 [1.3-8.5], phosphorus: 21 μ M+7-10 [7-45], nitrogen: 116 μ M +7.7 [11-241] In Belgium summer field measurements found H
		<i>ranunculoides</i> on sites with the following ranges of (Nijs <i>et al.</i> , 2009):
		O2: 6-11 mg/l
		pH: $6.7 - 7.5$
		Total Phosphate (PT): $0.066-0.82 \text{ mg/l}$
		Soluble reactive phosphorus: 0.005-0.21 mg/l
		Dissolved inorganic nitrogen: 0.018-4.14 mg/l
		These data show no particular preference for specific water quality parameters.
		Physical characteristics of waterbodies
		Experiments show that under stable water level regimes, <i>H. ranunculoides</i> adopted
		different morphologies, with highest biomass occurring in fully aquatic conditions
		(Hussner & Meyer, accepted). Water level fluctuation limit or decrease the biomass
		In its native range in Argentina, Gantes & Sánchez Caro (2001) studied the distribution

Question	Rating +	Explanatory text of rating and uncertainty
	uncertainty	
		of aquatic plant in streams and reported that emergent plants including <i>H. ranunculoides</i> were relatively ubiquitous with the independence of their distribution in relation to the hydrological variables: current velocity (from ~ 0 to 35cm/s), stream width (from 100 to 700cm), stream depths (from 7cm to 50cm). The EWG concluded that in the EPPO region, the species grows in waterbodies with velocities up to 1 m/s and depth up to several metres.
		<u>Water flow velocity</u> In the UK, significant infestations were found in 4 locations (River Chelmer, River Wey, Pevensey Levels, Gwent Levels), all of which are slow-flowing rivers or wide channels, which could be an abiotic factor favouring infestations (Newman & Dawson, 1999). Static and very slow flowing waters are considered to be optimal habitats (Newman, pers. comm., 2009).
		Salinity The salinity tolerance of <i>H. ranunculoides</i> has been tested in a study by the Centre for Aquatic Plant Management, UK. The results of the study show a decrease in leaf number and an increase in leaf death rate above 6.5 ppt salinity. The effect is sharply marked, with a 0.5 ppt increase causing a dramatic effect (Rothamsted Research, 2000). As a comparison for salinity levels, undiluted seawater has a salinity of 35 ppt, and eutrophic fresh water of 4 ppt. The salinity tolerance is possibly physiologically linked with a capacity to take up metals from water, <i>H. ranunculoides</i> has substantial metal absorption capacities (Pinochet <i>et al.</i> , 2002).
		These abiotic factors are very common and largely similar to the ones in the native range.
		The EFSA opinion suggested that the levels of eutrophication in water bodies as monitored by the Water Information System for Europe (WISE) of the European Environment Agency should be taken into account. The species is not borne to euthrophic waters, and the level of eutrophication does therefore not influence the distribution of the species. These maps have been checked by the EWG but are not considered to provide any accurate additional information.

Question	Rating + uncertainty	Explanatory text of rating and uncertainty
1.21. If protected cultivation is important in the PRA area, how often has the pest been recorded on crops in protected cultivation elsewhere?	Not relevant	
1.22. How likely is it that establishment will occur despite competition from existing species in the PRA area?	Very likely Uncertainty: low	 The high Leaf Area Index of up to 5.47 +-0.2, is an indication that the species is able to outcompete submerged vegetation (Hussner & Lösch, 2007). In Belgium, it has been observed to reduce by more than 50% the number of native aquatic plant species (up to 100% of the submerged species (Nijs <i>et al.</i>, 2009). <i>H. ranunculoides</i> may be able to produce allelopathic anti-algal compounds (Della Greca <i>et al.</i>, 1994).
1.23. How likely is it that establishment will occur despite natural enemies already present in the PRA area?	Very likely Low Uncertainty	In Germany, observations showed that coypus (<i>Myocastor coypus</i>) can eat <i>H.</i> <i>ranunculoides</i> (Hussner & Lösch, 2007). Some populations were partially grazed by this mammal, which exclusively eats the leaf lamina of these plants. However, grazing does not prevent the establishment of the species. During summer, cattle will eat the plant when it grows at the water margins, but this again has not prevented the establishment of the species, and even encourages the spread of the plant due to fragmentation (Newman, pers. comm., 2009).

Question	Rating +	Explanatory text of rating and uncertainty
	uncertainty	
1.24. To what extent is the managed	Highly	The optimal habitat of <i>H. ranunculoides</i> are static or slow-flowing waters, and the
environment in the PRA area favourable	Favourable	creation of the slowing down of waters by creating dams may favour the establishment of
for establishment?		the plant.
	Low	Restoration of water bodies and the creation of new ponds would encourage the
	uncertainty	establishment of the species to new sites.
		Two factors contribute to the establishment of <i>H. ranunculoides</i> :
		- high nutrient levels through agricultural, urban and industrial run-offs favour the rapid
		growth
		- and impoundment of waters by creating dams, altering hydrological regimes.
1.25. How likely is it that existing pest	Very	Existing mechanical water management strategies often favor the spread and invasion of
management practice will fail to prevent	likely	H. ranunculoides by increasing fragmentation (Newman, pers. comm., 2009), see also
establishment of the pest?		questions 1.33 & 1.34.
	Low	
	uncertain	According to Hussner & Lösch (2007), the high regeneration capacities from stem
	ty	fragments is very likely to result in the dispersal of the species after mechanical control.
		Pot (2000) described this problem for the management of <i>H. ranunculoides</i> populations
		in the Netherlands.
1.26. Based on its biological	Very	Eradication is very difficult or even impossible in water bodies with heavy infestation.
characteristics, how likely is it that the	likely in	However, according to the Dutch experience, local eradication is possible if it is started
pest could survive eradication	heavy	early and the water system is reasonably accessible. In the Netherlands as a whole,
programmes in the PRA area?	infested	eradication is not possible anymore. Dutch waterboards are currently successful in early
	areas.	detection by visual inspection and in local eradication of small infestations by careful
		manual work.
	Uncertai	In the UK, mechanical control is combined with applications of herbicides but did not
	nty: low	eradicate or contain the plant. Successful chemical control has been achieved on an
		experimental basis using glyphosate as Roundup Pro Biactive combined with either the
		adjuvant TopFilm at 850 mL / ha up to the end of June, or with Codacide Oil from July
		onwards. This technique has been used on several small infestations with good success,

Question	Rating +	Explanatory text of rating and uncertainty
		 although more than one year's treatment is required (Newman, pers. comm., 2009). In some other EU member states, herbicide application in aquatic environments/biotopes is prohibited. Additionally, restrictions in the use of some herbicides due to new EU environmental regulation is an important factor to take into account when assessing the likelihood of control/containment/eradication. In Belgium, it is not anymore possible to eradicate the plant from the country, and actions are only possible in small waterbodies and require early detection and repeated action (L Triest, pers. comm., 2009).
1.27. How likely is the reproductive strategy of the pest and the duration of its life cycle to aid establishment?	Very likely Uncertainty: low	In Germany, <i>H. ranunculoides</i> shows a rapid growth with a maximal growth rate in the summer months June and July (Hussner & Lösch, 2007). Starting from small plants or fragments, plants grew slowly in spring and formed small, up to 10 cm ² large leaves, which mostly floated on the water surface and reached a height of up to 40 cm above water level. The plants flowered and fruited between May and October and the stands got more and more dense. With a decrease in temperatures and light availability in autumn, plants developed smaller new leaves and most of the leaves died at the first night frosts. However, in its introduced range, even if emergent leaves die at the first night frosts and floating leaves die when enclosed in ice, leaves of <i>H. ranunculoides</i> submerged below ice cover are reported to survive the winter months, and new plants can grow up in spring from these overwintering parts (Hussner & Lösch, 2007). From these small submerged plants and leafless overwintering stolons plants again grew out in spring. The same strategy is observed in the UK, in the Netherlands and in Belgium.
		Its regeneration capacity is high as it can form new shoots even from small stem fragments (1 cm in length with one node and with or without leaves). The development of new shoots takes a maximum of 1 week when regenerating from cuttings that were made up by a node with one leaf, and a maximum of 2 weeks if regeneration occurred from a node without attached leaves (Hussner & Lösch, 2007). Vegetative growth occurs without any contact with soil.

Question	Rating + uncertainty	Explanatory text of rating and uncertainty
		In the UK, the seeds never reached maturity to be able to germinate (J Newman, pers. comm., 2009) and seed germination has never been observed in other EPPO countries, but the plant reproduces very efficiently vegetatively.
		In Italy (Toscana and Campania), the species is considered to be at an early stage of invasion, but is currently in isolated systems.
		In most places where it has been observed, the species showed an invasive behaviour. The EWG concluded that the species may have similar population dynamics when introduced in suitable conditions.

Question	Rating +	Explanatory text of rating and uncertainty
	uncertainty	
1.28 How likely are relatively small	Very	Founder populations may have low genetic diversity but vegetative spread ensures that
populations to become established?	likely	this will not be a problem. In the UK, the initial populations in 29 sites when assessed in
		1999 in the south east of England and South Wales, is likely to have originated from
	Uncertai	various introductions from a single clone available at aquatic garden centres and
	nty: low	nurseries in the UK (Newman & Dawson, 1999). In 2008, the plant is present in at least
		156 sites in the whole UK (see distribution map in Appendix 2).
		The EWG concluded that populations can originate from one single individual vegetative propagule.
1 29 How adaptable is the pest?	Moderate	The species occurs in the USA Central and South America Australia Africa and parts of
	Wiodelate	Europe, suggesting that this species is adaptable to different climatic conditions (see Q. 7
	Uncertainty:	for distribution).
	low	
		The plant can establish in different freshwater bodies and ecosystems: <i>H. ranunculoides</i>
		grows either in water, often by forming floating mats, or as a helophyte in riparian
		vegetation.
		See question 1.20 which gives range of values for several parameters of water
		composition.
		The species adapts its morphology depending on growth conditions and time of year.
		showing high inherent adaptability (Ruiz-Avila and Klemm, 1996; Newman & Dawson
		(1999); Eichler, 1987). Depending on nutrient availability, the species adapts biomass
		allocation to various plant components (e.g. in low nutrient conditions, root biomass
		dominates whereas in higher nutrient conditions, leaves and above water biomass
		dominate). This also happens in different conditions of water availability (Newman &
		Duenas, submitted, 2009). The growth form imparts resistance to glyphosates and to 2-4
		D amine because of submerged apical meristems (Newman <i>et al.</i> , 2001).
		The species is not adapted to salinity, change of water level, and drought (when not
		rooted in water).
		Based on this information, it is assumed that adaptability of the species is moderate.

Question	Rating + uncertainty	Explanatory text of rating and uncertainty
1.30. How often has the pest been introduced into new areas outside its original area of distribution? (specify the instances, if possible)	Often Uncertainty: low	The plant has been introduced in Australia (Ruiz-Avila and Klemm, 1996), in Europe, in Asia (see Q 7 for distribution). The herbarium specimen from 1838 for Ethiopia (Database for national herbarium in the Netherlands) has been checked and is truly <i>H. ranunculoides</i> (van Valkenburg, pers. comm., 2009). The species is present on all continents except Antarctica.

Question	Rating +	Explanatory text of rating and uncertainty
	uncertainty	
1.31. If establishment of the pest is very unlikely, how likely are transient populations to occur in the PRA area through natural migration or entry through man's activities (including intentional release into the environment)?	Not relevant	Establishment of the pest has already occurred in some countries of the EPPO region.
Conclusion on the probability of establishment	High Low uncertainty	The pest has already established in at least 6 countries of the EPPO region, the probability of establishment is therefore very high. According to the climatic prediction, additional countries are at risk (e.g.: Mediterranean countries, Black Sea area). The countries at risk are: Albania, Algeria, Austria, Azerbaijan, Belgium, Bosnia & Herzegovina, Bulgaria, Croatia, Cyprus, Denmark, France (including Corsica), Greece, Ireland, Israel, Italy (including Sardinia), Jordan, Germany (mostly western part), Hungary, Moldavia, Morocco, Portugal, Romania, Russia (Black Sea), Serbia, Slovenia, Spain, Switzerland, Tunisia, Ukraine, the United Kingdom, Republic of Macedonia, Romania, Turkey. Nevertheless, so far, the species has expressed invasiveness in North-Western EPPO countries (Belgium, the Netherlands, United Kingdom,), while the areas which seem to be the most suitable are the Mediterranean and the Atlantic areas. This may be due to other elements such as the use of the plant and the eutrophication of waters. There is some uncertainty, how the plant would perform in Northern and Eastern Europe. However, severe continental winters and hot and dry summers (e.g. in continental conditions) are likely to limit distribution of the species. (See Appendix 3).
1.32. How likely is the pest to spread rapidly in the PRA area by natural means?	short distance: very likely	The species has not yet been observed to reproduce by seeds in the EPPO region (EWG, pers. comm., 2009). Vegetative reproduction has lead to rapid spread in the UK, the Netherlands and Australia. In Germany, a surface of ca. 2000 m ² was completely invaded in three years (Hussner, pers. comm. 2009). Observations in the UK highlight that the species grows

Question	Rating +	Explanatory text of rating and uncertainty
	uncertainty	
	long distance: unlikely Uncertai nty: low	rapidly throughout river systems once established, displacing native vegetation and becoming dominant in less than 2 years (Newman & Dawson, 1999). There is no evidence of natural spread between different water bodies, as natural spread seems to be local. Spread from garden ponds is more likely to occur through human activities. Waterfowl can spread viable fragments of the plant (Huckle, 2002), but the EWG considered it unlikely.
		Once in a watercourse that is favourable to its growth, <i>H. ranunculoides</i> spreads very effectively by fragmentation and water movement. The most important time for fragment movement is winter due to the disruption of the mats by higher water velocities, lower water and air temperatures, reduced growth rates and increasing senescence. In contrast, in summer conditions with slow flow of water, high growth rates and stronger plant tissue, the plants resist fragmentation better. J Newman (pers. comm., 2009) has shown that, of 100 apparently dead brown stems (subjected to January frosts), 9% were capable of regrowth in culture medium in a glasshouse at 20° C. In contrast, all green stem nodes regrew when potted in these conditions at the same time. Flooding and summer storm events are important for transporting fragments within the same system and between different parts of the same catchement (Newman, pers. comm., 2009).
		The natural spread is very likely to occur within connected water systems, but is unlikely to occur between isolated water bodies.
		Since it is difficult to determine if spread is due to natural or human assisted spread, general information is provided below: In the UK, the species has spread from 3 sites in 1989 to 156 in 2008 despite intensive management activities (see Appendix 2).
		In the Netherlands, since 1995 when it was first recorded as invasive for the Netherlands, it is now present in all Provinces, and only absent from the Wadden Islands, separated by salt water from the mainland (see map in Appendix 2).

Question	Rating +	Explanatory text of rating and uncertainty
	uncertainty	
		In Belgium, it was recorded from 3 localities in 1 Province (Oost-Vlaanderen) in 1998
		and was recorded in up to 50 localities in 2008 (see Appendix 2).
		In Germany, in 2004, the species was reported from 8 grid cells (5.81 km in length, 5.56 km in height; equivalent to a quarter of a topographical map 1:25000), and the number of grid cells more than doubled to 21 in 2008 (see map in Appendix 2).
		In France (Essonne) the species was first found in 1987 in one site, and since then, the species have been recorded in 7 new sites in the same water system. (Information provided by G. Arnal, Conservatoire Botanique).
		In Italy, <i>Hydrocotyle ranunculoides</i> is present in Sardinia (Central-West Sardinia, channels in agricultural area, wetlands) where it is invasive (Brundu <i>et al.</i> , 2003). The species is only recorded as naturalised in two other Italian regions (i.e. Toscana and Campania) (Celesti-Grapow <i>et al.</i> , in press), but might be overlooked in other regions (G Brundu, pers. comm., 2009). Pignati (1982) reported the species as present in Calabria, Campania, Lazio, Sardinia, Sicilia, Toscana, as very rare and decreasing. It is considered that many habitats where the species was recorded might have been destroyed for urbanization, or the species might even have been misidentified in the past (G Brundu, pers. comm., 2009).
1.33. How likely is the pest to spread rapidly in the PRA area by human assistance?	very likely Uncertainty: low	Spread can readily occur through the action of gardeners (gardening practice, cleaning of ponds etc), aquarists (cleaning of aquaria, exchange of plants between hobbyists), and the sewage treatment system (Newman, pers. comm., 2009). The plant is unlikely to move to new watersheds without human assistance. Maintenance work will produce copious amounts of viable plant parts which can be spread by the waterflow. Trying to remove the plant mechanically is the most important cause of spread in the Netherlands (Pot,
		2000).Modifications of chemical (eutrophication) and physical (reduction of current velocity) properties of waterbodies can also enhance the spread of <i>H. ranunculoides</i>.The following factors favour the spread of the species:

Question	Rating + uncertainty	Explanatory text of rating and uncertainty
		 linear connectivity within infested sites is a factors contributing to spread and improper management practices of the species in these systems trade pressure
		- contamination of other traded aquatic plants within the EU also favors its spread (see Q. 1.1).
		Trade of <i>H. ranunculoides</i> into some EPPO countries:
		Germany In Germany no suppliers have been identified selling <i>H. ranunculoides</i> under that name (PPP-index, 2009). Other <i>Hydrocotyle</i> spp. are traded which could be mislabeled (A. Hussner, pers. comm., 2009).
		France In France, a website selling <i>Hydrocotyle natans</i> (a synonym for <i>H. ranunculoides</i>) has been identified (<u>http://www.floraquatic.com/-50029/hydrocotyle-natans-500039.html</u>). Furthermore, seven nurseries, throughout the whole country, are selling <i>H. vulgaris</i> and/or <i>H. leucocephala</i> which could well be <i>H. ranunculoides</i> (G Fried, pers. comm., 2009).
		UK Due to the high invasiveness of <i>H. ranunculoides</i> , the UK Royal Horticultural Society banned this plant from its shows and gardens (Shaw, 2003). <i>H. ranunculoides</i> is not cultivated or deliberately sold. There are some specialist aquatic nurseries (about 5) that supply <i>H. vulgaris</i> . The specimens of this species sent for identification to J. Newman in 2006 confirm that they were <i>H. vulgaris</i> . Other species sold are <i>H. umbellata</i> , <i>H. nova zealandae</i> , and <i>H. sibthorpiodes</i> . <i>H. ranunculoides</i> was not available from any source in 2008. It is likely that new material of all of these species is created by vegetative propagation, rather than new import, although new imports cannot be ruled out (Newman, pers. comm., 2009).
		The Netherlands

Question	Rating +	Explanatory text of rating and uncertainty
		 In January 2001, the Dutch Ministry, van Landbouw, Natuurbeheer en Voedselkwaliteit, prohibited the sale and possession of this plant (Staatsblad van het Koninkrijk der Nederlanden, 2000). Belgium In Belgium, Garden Centers are still selling this plant under the name <i>H. ranunculoides</i>, and possibly other names, but some centres have been asked to withdraw the species from trade from 2000 onwards by the Belgian Forum on Invasive Alien Speceis
		(Branquart, 2008).
1.34. Based on biological characteristics, how likely is it that the pest will not be contained within the PRA area?	unlikely Uncertainty: low	 Spread via waterways makes containment difficult. For initial infestations some Dutch water districts have shown that containment is possible in fully controlled systems where water levels are artificially regulated. However, this requires considerable central organization, funding and perseverance. For completely infested water systems, containment areas must be based on watersheds, in order to take into account the likelihood of downstream spread. In the UK, initial observations suggest that the species is resistant to the herbicide glyphosate applied at 2.16 kg a.i./ha. This resistance is supposed to be due to insufficient uptake of the herbicide through the leaf cuticule. 2,4-D amine was considerably more effective, giving complete control within 6 weeks of treatment. Even so, because of the dense leaf canopy, repeated application of herbicide after 2 months was necessary to eradicate <i>H. ranunculoides</i>. See Q. 1.26 for details on a treatment programme on the Pevensey. Mechanical control is ineffective, creating fragments which disperse to recolonize downstream habitats. Additionally, it may not be possible to effectively control <i>H. ranunculoides</i> in the UK with the herbicides available and approved for use in water (Newman & Dawson, 1999). These habitats are less actively managed than in the Netherlands.

Question	Rating + uncertainty	Explanatory text of rating and uncertainty
		However between unconnected catchments, the possibility of containment is high.
Conclusion on the probability of spread	High Low uncertainty	The overall probability of spread is high, uncertainty is low. The species has expressed a high spread in the UK, the Netherlands, Belgium and Germany.
Conclusion on the probability of introduction and spread The overall probability of introduction and spread should be described. The probability of introduction and spread may be expressed by comparison with PRAs on other pests.	High Low uncertainty	Since <i>H. ranunculoides</i> is introduced intentionally as an ornamental plant and is still for sale in garden centres in some parts of Europe (e.g. France, Belgium) (see Q. 1.1) and that exchanges between gardeners and aquarists occur, the probability of introduction to areas of the EPPO region where it is currently not present is high. As far as is known, in the EPPO region, there is only a prohibition to sell it in the Netherlands, a prohibition to cause to grow the plant into the wild in Scotland, and a recommendation not to sell it in the UK. Where present, the probability of short distance spread is very high as vegetative spread is very effective for local colonization. Human activity is principally responsible for long distance spread. The presence of <i>H. ranunculoides</i> in the Netherlands, Belgium, France, Germany and its capacity for vigorous growth favours its spread to neighbouring countries. The Netherlands and the UK (apart from Northern Ireland to Ireland) are countries from where water flows into the sea without passing through other countries. This, combined with a prohibition or a code of conduct advising against sale, significantly decreases the risk of spread to neighbouring countries. Direct sale and internet sale within and from other countries clearly provides the greatest risk.

Question	Rating +	Explanatory text of rating and uncertainty
Conclusion regarding endangered areas 1.35. Based on the answers to questions 1.16 to 1.34 identify the part of the PRA area where presence of host plants or suitable habitats and ecological factors favour the establishment and spread of the pest to define the endangered area.	Medium uncertainty	Freshwater bodies and ecosystems: ponds, ditches, marshes, waterways etc, more particularly, in and static or slow-flowing waters (Newman & Dawson, 1999). According to the Climex simulation, the atlantic and mediterranean areas of the EPPO region that are characterized by mild winter are the most at risk. The countries at risk are: Albania, Algeria, Austria, Azerbaijan, Belgium, Bosnia & Herzegovina, Bulgaria, Croatia, Cyprus, Denmark, France (including Corsica), Greece, Ireland, Israel, Italy (including Sardinia), Jordan, Germany (mostly western part), Hungary, Moldavia, Morocco, Portugal, Romania, Russia (Black Sea), Serbia, Slovenia, Spain, Switzerland, Tunisia, Ukraine, the United Kingdom, Republic of Macedonia, Romania, Turkey. There is some uncertainty, how the plant would perform in Northern and Eastern Europe. However, severe continental winters and hot and dry summers (e.g. in continental conditions) are likely to limit distribution of the species. (See Appendix 3). Additional shortage of water during summer would also limit the success of the species. The species is considered to be limited by acidic waters, as shown by the map of acidity of soils in Appendix 1. Acidic soils are found in the Centre of France, in Toscana, in Corsica which may explain why the species is not as invasive yet as in other localities where it is present in the EPPO region. Acidity of soils (and therefore waters) may in future limit the species in some places like the northern Atlantic coast of Spain, West of Sardinia, Scandinavia, Western France, etc.
2. In any case, providing replies for all hosts (or all habitats) and all situations may be laborious, and it is desirable to focus the assessment as much as possible. The study of a single worst-case may be sufficient. Alternatively, it may be appropriate to consider all hosts/habitats together in answering the questions once. Only in certain circumstances will it be necessary to answer the questions		

Question	Rating + uncertainty	Explanatory text of rating and uncertainty
separately for specific hosts/habitats.		
2.1. How great a negative effect does the pest have on crop yield and/or quality to cultivated plants or on control costs within its current area of distribution?	Major Uncertainty: low	 In the Canning River in Western Australia <i>H. ranunculoides</i> became a serious problem in 1992. A program costing over AU\$ 200,000 in the first year was implemented (Atkins, 1994, Ruiz Avila, Klemm, 1996; Newman & Dawson 1999), and the species is still present in Australia. Control costs: In the Netherlands, some water boards faced a doubling of costs each year during the 1990s, and, in 2000, the total annual control costs were around 1 Million Euro (van der Krabben & Rotteveel, 2003). In 2007, in the Netherlands, 11 water boards out of 26 responded to an inquiry stating that they spent an additional 1.8 millions euros for the management of <i>H. ranunculoides</i> over and above normal operating costs for this plant (van Valkenburg, pers. comm., 2009). In Flanders, the estimated cost for the management of <i>H. ranunculoides</i> is 1.5 million euros per year (needed during 3 years from 2009) (Triest, pers. comm., 2009). In the UK, the estimate for control of the total area infested by <i>H. ranunculoides</i> by herbicides was between £250,000 and £300,000 per year (Harper, 2002). In 2008, £1.93 million were spent for the management and disposal of <i>H. ranunculoides</i> (Newman, pers. comm., 2009). In 6 years, the costs were multiplied 7 times.
2.2. How great a negative effect is the pest likely to have on crop yield and/or quality in the PPA area without any control	Minimal	There are currently no impacts recorded in crops, but the EWG considered that flooding of low lying agricultural areas is possible due to blockage of water level control
measures?	uncertainty	

Question	Rating + uncertainty	Explanatory text of rating and uncertainty
2.3. How easily can the pest be controlled in the PRA area without phytosanitary measures?	with much difficulty Uncertainty: low	 Without phytosanitary measures, <i>H. ranunculoides</i> will not be controlled. Even with phytosanitary measures, <i>H. ranunculoides</i> is very difficult to control. Mechanical control has to be done very carefully. If it is not done properly, spread can be promoted, as <i>H. ranunculoides</i> spreads very effectively by fragmentation and water movement (Pot, 2000). Ease of control also depends on pesticide legislation. Mechanical control is combined
		with the application of herbicides in the UK. In Germany, herbicide application in aquatic environments/biotopes is prohibited.
2.4. How great an increase in production costs (including control costs) is likely to be caused by the pest in the PRA area?	Moderate to major	Control costs could be similar to those already spent in infested parts of the PRA area. See 2.1.
	Medium uncertainty	A weevil, <i>Listronotus elongatus</i> , has been demonstrated to feed exclusively on pennywort species in Argentina, and further work on this potential bio-control agent is planned in the UK (Newman, 2003). The cost of a preliminary study was £30.000, but the cost of a full biological control project would be £500.000 (Newman, pers. comm., 2009).
2.5. How great a reduction in consumer demand is the pest likely to cause in the PRA area?		Not relevant.

Question	Rating +	Explanatory text of rating and uncertainty
	uncertainty	
2.6. How important is environmental	Major	Since 2005 (date of the previous PRA), much more information on environmental
damage caused by the pest within its		impacts was made available.
current area of distribution?	Low	
	uncertainty	Direct effects
		The EWG concluded that in most sites, 100% cover is often observed over large
		distances (25 km), which is detrimental for the ecosystem (see pictures in Appendix 4).
		The plant is perennial and present all year long in the UK.
		In Belgium, it has been observed to reduce by more than 50% the number of native
		aquatic plant species up to 100% of the submerged species, and to reduce the native $500(4 \pm 10)$ (N ¹¹) (100)
		cover from 50% to 10 (Nijs <i>et al.</i> , 2009).
		In Sardinia, the species is considered invasive, and although no specific impacts have
		outcompete other species (G Brundu pers comm 2009)
		outcompete other species (O Brundu, pers. comm., 2007).
		In the PRA area, where present, <i>H. ranunculoides</i> competes with many plant species due
		to its ability to establish in different habitats. Examples: different <i>Carex</i> /sedge and
		Juncus species, Rorippa amphibia, Myosotis palustris (syn. M. scorpioides), Nasturtium
		officinale (A. Hussner, pers. comm., 2009). In Germany, the native Myriophyllum
		spicatum, Callitriche spec. and Potamogeton crispus were displaced (Hussner, 2008).
		Nevertheless, these species are not endangered.
		Due to the high LAI of up to $5.57 + 0.2$ it seems obvious, that the species is able to
		outcompete submerged vegetation (Hussner & Lösch, 2007). Many more species can be
		outcompeted due to <i>H. ranunculoides'</i> capability to build floating carpets that shade out
		other plants.
		Data on impacts in dense infectation are rare because of dangerous surveillance
		conditions underneath dense floating mats
		conditions underneuth dense noating mats.
		Indirect effects
		Indirect effects on other biota and food web (phytoplankton, zooplancton, fishes) is
		caused by its summer biomass and by moments of decay (lowering of oxygen) and
		alteration of detritus (impact on macroinvertebrates) (Alien impact report, 2009; L Triest,

Question	Rating +	Explanatory text of rating and uncertainty
	uncertainty	
		 pers. comm., 2009). The EWG considered that <i>H. ranunculoides</i> causes many significant changes of ecological processes and structures by : reduction in flow; increased sedimentation resulting in acceleration of ecological succession; changes in O2 concentration; loss of accessible open water at the margins for wildlife (e.g. birds); loss of light; increased flood risk. Presence of <i>H. ranunculoides</i> prevents attainment of good ecological quality status under the Water framework Directive (http://ec.europa.eu/environment/water/water-framework/index_en.html).
2.7. How important is the environmental damage likely to be in the PRA area (see	Major	see for question 2.6.
note for question 2.6)?	Low uncertainty	Environmental impact is supposed to be the same wherever the species grows in suitable conditions.
		Netherlands, UK and Belgium. In France, the species is currently only present in 7 sites, but already exhibits up to 100% cover of water surface in some of them.

Question	Rating +	Explanatory text of rating and uncertainty
2.8. How important is social damage caused by the pest within its current area of distribution?	Minor to moderate Low uncertainty	 Effects on tourism (swimming, water sports, fishing, navigation, leisure etc.) can locally be expected to be large. As waterways covered with <i>H. ranunculoides</i> are not attractive for recreation and may hinder traffic, even the movements of boats, some profit losses have been observed in the Netherlands (van Valkenburg, pers. comm., 2009). Dense vegetation mats can present a direct safety risk to the public and livestock. Cattle have drowned in the UK (Newman, pers. comm., 2009).
		Loss of aesthetic value in nature reserves has been reported in Belgium (Triest, pers. comm., 2009). Increased costs for drainage and/or flood prevention will be borne by the users (agriculture and general society). The water boards tax the inhabitants and enterprises of their management area.
2.9. How important is the social damage likely to be in the PRA area?	Minor- moderate Low uncertainty	see for question 2.8. Social impact is supposed to be the same wherever the species grows in suitable conditions.
2.10. How likely is the presence of the pest in the PRA area to cause losses in export markets?		Not relevant
As noted in the introduction to section 2, the evaluation of the following questions may not be necessary if the responses to question 2.2 is "major" or "massive" and the answer to 2.3 is "with much difficulty" or "impossible" or any of the responses to questions 2.4, 2.5, 2.7, 2.9 and 2.10 is		

Question	Rating +	Explanatory text of rating and uncertainty
	uncertainty	
"major" or "massive" or "very likely" or		
"certain". You may go directly to point		
2.16 unless a detailed study of impacts is		
required or the answers given to these		
questions have a high level of uncertainty.		
2.11. How likely is it that natural enemies,	Very likely	In the UK, there are no natural enemies recorded.
already present in the PRA area, will not		In Germany, observations showed that coypus (<i>Myocastor coypus</i>) – which is non-native
reduce populations of the pest below the	Uncertainty:	can feed on <i>H. ranunculoides</i> (Hussner & Lösch, 2007) but it is unlikely that this species
economic threshold?	low	can reduce or contain all the populations of <i>H. ranunculoides</i> below the economic
		threshold, considering its rapid establishment and spread in invaded areas.
2.12. How likely are control measures to	Verv likelv	Both chemical and mechanical management measures will have negative effects on the
disrupt existing biological or integrated		environment.
systems for control of other pests or to	Uncertainty:	
have negative effects on the environment?	low	Mechanical control would remove considerable number of invertebrates (Dawson <i>et al.</i> ,
		1991). Experiments in the UK concluded that the impact of mechanical control on non-
		target organisms is severe, but limited in the short term as recover occurs by
		recolonisation in a relatively short time (I Newman pers comm 2009)
		recording and in a relativery short time (5 reconnal, pers. commi, 2007).
		Chemical control of large stands can lead to the deoxygenation of water due to
		decomposition of dead material (Barrett 1978)
		Experiments in the UK concluded that the effects of chemical control on large volumes
		of plant biomass are restricted to deoxygenation of the waterbody due to decomposition
		of treated plant material not to direct to vicity of the herbicide Mitigation of this effect
		can be achieved by removing the majority of the biomass prior to manual removal or
		targeted herbicide application to remaining inaccessible fragments (Newman, pers
		comm 2009)
		comm., 2007).
2 13 How important would other costs	Minor to	Publicity may be provided by the horticultural industry. Some funds for research into
resulting from introduction he?	moderate	control methods may be invested. For example, the development of some research on
	mouerate	biological control agents in the UK would cost approximately f500 000 (Nowman pars
		biological control agents in the OK would cost approximately 200.000 (Newman, pers.

Question	Rating +	Explanatory text of rating and uncertainty
	uncertainty	
	Uncertainty:	comm., 2009).
	low	
2.14. How likely is it that genetic traits can	Unlikely	Pollen flow could happen with the native <i>H. vulgaris</i> or with other exotic <i>Hydrocotyle</i>
be carried to other species, modifying	-	spp. but has never been documented, and there is no information available about
their genetic nature and making them	Uncertainty:	hybridization of Hydrocotyle species. The EWG considered that it is unlikely that genetic
more serious plant pests?	medium	traits could be carried to other Hydrocotyle spp.
2.15. How likely is the pest to cause a	Unlikely	There are no records of <i>H. ranunculoides</i> as a vector or host of other pests. The EWG
significant increase in the economic		considered that it is unlikely that H. ranunculoides would increase the economic impact
impact of other pests by acting as a vector	Uncertainty:	of other pests.
or host for these pests?	medium	
2.16. Referring back to the conclusion on		Freshwater bodies and ecosystems: ponds, ditches, marshes, waterways etc, more
endangered area (1.35), identify the parts		particularly, in static or slow-flowing waters (Newman & Dawson, 1999).
of the PRA area where the pest can		
establish and which are economically most		According to the Climex simulation, the Atlantic and Mediterranean areas of the EPPO
at risk.		region that are characterized by mild winter are the most at risk. The countries at risk are:
		Albania, Algeria, Austria, Azerbaijan, Belgium, Bosnia & Herzegovina, Bulgaria,
		Croatia, Cyprus, Denmark, France (including Corsica), Greece, Ireland, Israel, Italy
		(including Sardinia), Jordan, Germany (mostly western part), Hungary, Moldavia,
		Morocco, Portugal, Romania, Russia (Black Sea), Serbia, Slovenia, Spain, Switzerland,
		Tunisia, Ukraine, the United Kingdom, Republic of Macedonia, Romania, Turkey.
		There is some uncertainty, how the plant would perform in Northern and Eastern Europe.
		However, severe continental winters are likely to limit the species. (See Annexe 1).
		The species may be limited by acidic soils (see Appendix 1).
Degree of uncertainty	Low	The areas of uncertainty identified are the following:
Estimation of the probability of		- study on the varieties and forms, and the ones considered invasive.
introduction of a pest and of its economic		- the amount of internet trade
consequences involves many uncertainties.		- the amount of production in the EPPO region
In particular, this estimation is an		- the amount of exchange between gardeners and hobbyists.

Question	Rating +	Explanatory text of rating and uncertainty
	uncertainty	
extrapolation from the situation where the		- Information of the situation in Italy
pest occurs to the hypothetical situation in		- why did the plant disappeared from a river in Corsica (non found since 1968).
the PRA area. It is important to document		- effects on water quality (e.g. O ₂ content) and secondary effects on biota.
the areas of uncertainty (including		
identifying and prioritizing of additional		Research needs identified:
data to be collected and research to be		- data on outcompeted native species and their potential for recovery.
conducted) and the degree of uncertainty		- The effect of climatic change on the distribution and impacts of the plant
in the assessment, and to indicate where		- Biological control
expert judgement has been used. This is		
necessary for transparency and may also		
be useful for identifying and prioritizing		
research needs.		
It should be noted that the assessment of		
the probability and consequences of		
environmental hazards of pests of		
uncultivated plants often involves greater		
uncertainty than for pests of cultivated		
plants. This is due to the lack of		
information, additional complexity		
associated with ecosystems, and variability		
associated with pests, hosts or habitats.		
Evaluate the probability of entry and	High	The plant has already entered the EPPO region.
indicate the elements which make entry		The plant is no longer imported, but is produced and traded. The volume of <i>H</i> .
most likely or those that make it least	Low	<i>ranunculoides</i> being produced and sold is considered to be very low.
likely. Identify the pathways in order of	uncertainty	
risk and compare their importance in		
practice.		

Question	Rating + uncertainty	Explanatory text of rating and uncertainty
Evaluate the probability of establishment and spread, and indicate the elements which make establishment most likely or those that make it least likely. Specify which part of the PRA area presents the greatest risk of establishment.	High Low uncertainty	The pest has already established in at least 6 countries of the EPPO region, the probability of establishment is therefore very high. According to the climatic prediction, additional countries are at risk (e.g.: Mediterranean countries, Black Sea area). Spread by human activities is very effective.
List the most important potential economic impacts, and estimate how likely they are to arise in the PRA area. Specify which part of the PRA area is economically most at risk.		 Economic impacts: medium to high risk. Economic impacts include management costs of the species and flooding of areas. Any economic benefit of the introduction of this plant as an ornamental aquatic plant is heavily outweighed by management costs. Flooding may also occur. It is very likely that these impacts would occur when the plant is introduced. Environmental impacts: medium to high risk. Invasion of slow flowing waters, degradation of aquatic ecosystem, loss of biodiversity. Social impact: low-medium risk. Where it occurs, it has an impact on navigation, recreation and fishing. The part of he EPPO region which seem the most economically at risk are the Atlantic and Mediterranean areas, as well as the Black sea area.
The risk assessor should give an overall conclusion on the pest risk assessment and an opinion as to whether the pest or pathway assessed is an appropriate candidate for stage 3 of the PRA: the selection of risk management options, and an estimation of the associated pest risk.		The risk of establishment of <i>Hydrocotyle ranunculoides</i> in waterways, and negative impacts on their vegetation and use, justifies measures to prevent its further spread in the EPPO region. The pest qualifies as a quarantine pest.

This is the end of the Pest risk assessment

Stage 3: Pest risk Management

Question	Y/N	Explanatory text
3.1. Is the risk identified in the Pest Risk Assessment stage for all pest/pathway combinations an acceptable risk?	No	Medium to high economic and environmental risks and low-medium social risks have been identified.
Pathway 1		Intentional import as an ornamental aquatic plant for use outdoors and in aquariums
3.2. Is the pathway that is being considered a commodity of plants and plant products?	Yes	
If yes, go to 3.11, If no, go to 3.3		
3.11.If the pest is a plant, is it the commodity itself?	Yes	
If yes, go to 3.29, If no (the pest is not a plant or the pest is a plant but is not the commodity itself), go to 3.12		

3.29. Are there effective measures that could be taken in the	yes	Prohibition of the import, selling, planting, holding, movement, causing to grow		
importing country (surveillance, eradication) to prevent		in the wild, and possession of the plant.		
establishment and/or economic or other impacts?		Due to the high invasiveness of <i>H. ranunculoides</i> , the UK Royal Horticultural		
-		Society banned this plant from its shows and gardens (Shaw, 2003).		
If yes, possible measures: internal surveillance and/or eradication		In January 2001, the Dutch Ministry of nature conservation and food quality		
campaign, go to 3.30		prohibited the sale and possession of this plant (Staatsblad van het Koninkrijk der		
		Nederlanden, 2000). In Scotland, the Wildlife and Countryside act added H.		
		<i>ranunculoides</i> to schedule 9, which make it an offense to plant it to cause to grow		
		in the wild (Scottish Statutory Instrument 2005 No. 308). The review of the		
		Wildlife and Countryside act undertaken in 2008 in the UK proposed that H.		
		ranunculoides was added to a ban species list (see		
		http://www.defra.gov.uk/wildlife-countryside/pdf/wildlife-manage/non-		
		native/consultation.pdf).		
		Effective management methods have been developed in the Netherlands and in		
		the UK for eradication at early stages of infestation, these should be adopted by		
		countries where infestation is at an early stage, and countries where the species is		
		not present should be aware of these. The following management measures are		
		recommended:		
		- Integrated management plan for the control of existing infestations		
		The main control options are: mechanical control and herbicide application.		
		These 2 options can be integrated together as well as with a reduction in nutrient		
		input. Nevertheless, herbicides are usually prohibited in aquatic ecosystems.		
		Temporary dry out of waterbodies could also be implemented where appropriate.		
		- Monitoring/surveillance: Early detection in the countries at risk		
		- Emergency plan: rapid response to new infestations		
		- Obligations to report findings, in the whole EPPO region, especially in Western		
		Europe.		
		- Proposal of alternative non invasive aquatic species for use		
		- Legal obligation to remove invasive plants from private properties.		
		- Publicity: public awareness campaigns about the impacts of the plant with the		
		information not to use it as an ornamental, or for phytoremediation.		
		See the EPPO Standard PM 3/67 'Guidelines for the management of invasive		
		alien plants or potentially invasive alien plants which are intended for import or		
		havðbeen intentionally imported'.		

pest? List them.	
If yes, go to 3.31 If no. go to 3.38	
3.31.Does each of the individual measures identified reduce depend In countries where the species is present, control measures of infestations	within
the risk to an acceptable level? ds on countries are not efficient if the plant is frequently reintroduced. Prohibit	on of
situat selling is therefore necessary.	
If yes, go to 3.34	
If no, go to 3.32 When <i>H. ranunculoides</i> is not yet present in a country, prohibition of sell he sufficient combined with the brownledge on action plane for contry inter-	ing may
be sufficient combined with the knowledge on action plans for early inter in case the plant occurs	vention
in case the plant occurs.	
3.32.For those measures that do not reduce the risk to an Yes National measures	
acceptable level, can two or more measures be combined to Prohibition of selling, planting, holding, movement, causing to grow in the	e wild,
reduce the risk to an acceptable level? and possession of the plant in the EPPO region is necessary. Moreover, the	ne plant
has to be controlled where it occurs.	CC: . :
If yes, go to 3.34 If these measures are not implemented by all countries, they will not be e	micient
In no, go to 5.55	
in addition, it has to be combined with international measures.	
International measures	
Accurate identification of all <i>Hydrocotyle</i> spp. traded in the EPPO region	should
be encouraged. Methods of DNA bar-coding are available (see van der W	iel et
<i>al.</i> , 2009).	
Prohibition of import into the EPPO region of species labelled as <i>H. nata</i> <i>H. americana</i> which are synonyms for <i>H. ranunculoides</i> and within the c	<i>ns</i> and ountries.

The estimated value of the species to the trade is low and interference is probably quite low. UK recommends not to sell the plant - no suppliers have been identified since 2002, but there is some evidence, that the plant is sold as H. vulgaris or other names (Newman, pers. comm., 2009). In the Netherlands, sale and movement are prohibited. In Germany, no suppliers have been identified. In Belgium, a major producer of aquatic plants agreed to cease trading H. ranunculoides (E. Branquart, pers. comm., 2009). Nevertheless it is still possible that the plant is traded or will be traded in the future in the EU.
Considering the high cost of the control of the plant, compared to the benefit its trade generates, the measures are very cost-effective. Furthermore, H. ranunculoides is not an important commodity.Aquarium enthusiasts and sellers of aquatic plants are not familiar with such legislation, nor is the public, but this case could raise awareness. Non invasive substitution plants could be proposed. For instance, in Belgium, it is recommended to use Sagittaria sagittifolia, Ranunculus aquatilis and Caltha palustris in substitution (Branquart, 2008).

 3.36.Have measures (or combination of measures) been identified that reduce the risk for this pathway, and do not unduly interfere with international trade, are cost-effective and have no undesirable social or environmental consequences? If yes, For pathway-initiated analysis, go to 3.39 For pest-initiated analysis, go to 3.38 	Yes	Prohibition of import, trade, planting, holding and movement of the plant.
11 110, go to 5.57 3 37 Envisage prohibiting the nathway	Ves	This is one of the options
5.57.Envisage promoting the pathway	105	
For pathway-initiated analysis, go to 3.43 (or 3.39),		
For pest-initiated analysis go to 3.38		
3.38.Have all major pathways been analyzed (for a pest-	Yes	
initiated analysis)?		
If yos go to 2.41		
If no. Go to 3.1 to analyze the next major nathway		
3.39. Have all the pests been analyzed (for a pathway-initiated		
analysis)?		
If yes, go to 3.40,		
If no, go to 3.1 (to analyze next pest)		
3.40.For a pathway-initiated analysis, compare the measures		
appropriate for all the pests identified for the pathway that		
nrovide nhytosanitary security against all the pests		
provide phytosanitary security against an the pests.		
Go to 3.41		

3.41.Consider the relative importance of the pathways identified in the conclusion to the entry section of the pest risk assessment	Intentional import of the plant for ornamental purposes: high Phytoeremediation: low
Go to 3.42	
3.42.All the measures or combination of measures identified as being appropriate for each pathway or for the commodity can be considered for inclusion in phytosanitary regulations in order to offer a choice of different measures to trading partners.	Import and trade of <i>H. ranunculoides</i> to the EPPO region and its sale within it should be prohibited.
Go to 3.43	
3.43.In addition to the measure(s) selected to be applied by the exporting country, a phytosanitary certificate (PC) may be required for certain commodities. The PC is an attestation by the exporting country that the requirements of the importing country have been fulfilled. In certain circumstances, an additional declaration on the PC may be needed (see EPPO Standard PM 1/1(2): Use of phytosanitary certificates) Go to 3.44	
3.44. If there are no measures that reduce the risk for a pathway, or if the only effective measures unduly interfere with international trade (e.g. prohibition), are not cost-effective or have undesirable social or environmental consequences, the conclusion of the pest risk management stage may be that introduction cannot be prevented. In the case of pest with a high natural spread capacity, regional communication and collaboration is important.	

Conclusion of Pest Risk Management.	Major pathway is trade of the plant for ornamental purposes
Summarize the conclusions of the Pest Risk Management	
stage. List all potential management options and indicate	International measures Prohibition of import and trade in the EPPO region and within the countries will
their effectiveness. Uncertainties should be identified.	effectively prevent further introduction into the EPPO region combined with
	accurate identification of species and synonyms.
	National measures
	Prohibition of the import, selling, planting, holding, movement, causing to grow
	in the wild, and possession of the plant may effectively prevent further
	establishment and spread within the EPPO region.
	Integrated management plan for the control of existing infestations
	It is potentially highly effective if coupled with prohibition measures. Uncertainty
	concerns commitment to long-term implementation.
	This would require:
	- Accurate identification of the species
	- Monitoring/surveillance in the countries where it is invasive or present
	(Belgium, France, Germany, the Netherlands, the United Kingdom, Italy), and
	- Early warning consisting of exchanging information with other countries and
	rapid response
	- Control of existing populations.
	- Publicity: aquatic plants producers and sellers and aquarium enthusiasts shall be
	informed of the problem and work should be undertaken with them to explain the
	prohibition of the species, and inform consumers. Administration should also be
	warned that the plant shall not be used as a phytoremediation species.
	Monitoring and review
	Performance of measure(s) should be monitored to ensure that the aim is being
	achieved. This is often carried out by inspection of the commodity on arrival,
	noting any detection in consignments or any entries of the pest to the PRA area.
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Appendix 1

Maps relevant for the distribution of Hydrocotyle ranunculoides

CORINE land cover classification

http://dataservice.eea.eu.int/download.asp?id=5859&type=gif.



pH maps

The following map can be found on the European Soil Portal maintained by the European Commission (<u>http://eusoils.jrc.ec.europa.eu/</u>, European soil data center > Data > European soil data base > Raster version or Google earth version> chemical properties > base saturation top soil (BS TOP)

The areas in pink (darker) represent acidic soils which are not suitable for *Hydrocotyle ranunculoides*.



Appendix 2

Maps of occurrence and spread in countries of the EPPO region

<u>North-America</u> Map available at <u>http://plants.usda.gov/java/profile?symbol=HYRA</u> More detail data at the state scale are available on the website.



The Netherlands



Distribution of *Hydrocotyle ranunculoides* for the Netherlands for the period from 1995 till 2008.

In the Netherlands, since 1995 when it was first recorded as invasive for the Netherlands, it is now present in all Provinces, and only absent from the Wadden Islands, separated by salt water from the mainland

Germany



▲: Funde nach 2004

Figure: Known occurrences of *Hydrocotyle ranunculoides* in North Rhine-Westphalia (Germany) in 2008, species was present in all lower parts of the rivers Erft (a tributary of the River Rhine) and Niers (Hussner 2008).

Belgium

For the whole Belgium Map available at http://ias.biodiversity.be/ias/species/show/63



 Image: Sector Secto

Atlas Flora of Flanders, available at http://www.gisoost.be/exoten/ (go to "volledig gebied")

Pink points represent localities where *Hydrocotyle ranunculoides* is present. Orange points represent localities where *Myriophyllum aquaticum* is present. Points circled in black represent unmanaged localities.

The UK



Dots correspond to sites where *H. ranunculoides* is present.

Appendix 3

Climatic prediction on Hydrocotyle ranunculoides

The CLIMEX model is a computer programme aiming at predicting the potential geographical distribution of an organism considering its climatic requirements. It is based on the hypothesis that climate is an essential factor for the establishment of a species in a country.

For *Hydrocotyle ranunculoides*, a compare location analysis has been undertaken.

1. Geographical distribution of the species and parameters

The distribution of *Hydrocotyle ranunculoides* was assembled from several sources: Global Biodiversity Information Facility (GBIF): <u>http://www.gbif.org/</u>, USDA <u>http://plants.usda.gov</u>, ForaWeb (ttp://www.floraweb.de/), etc. Distribution data in the EPPO region have been taken from question 7 and from distribution maps provided by individual countries (see Appendix 2).

Hydrocotyle ranuculoides is native from the American continent. Its northern boundary is reached in the USA and Canada (British Columbia, Quebec) where it becomes very rare. In the USA, the plant is only present in a belt including the southern states (except New Mexico), and north, the plant is mainly found along the east and west coasts. Its southern range is more obscure but it seems present in the whole tropical America (Martin & Hutchins, 1981), in almost all south American countries (Argentina, Bolivia, Brazil, Chile, Columbia, Ecuador, Paraguay, Peru, Uruguay). In the south, the species was recorded at latitude 35.34.030 and longitude 058.03.512 in the province of Buenos Aires (Newman, unpublished) but is known to go 200 km further south (J Newman, pers. comm., 2009).



Fig. 1. World distribution of *Hydrocotyle ranunculoides* according to the GBIF. This map is incomplete for data in Africa, in South America and in Europe. *Phenology*

In Illinois (USA), the blooming period of *H. ranunculoides* occurs during the summer or early fall. In Australia, *H. ranunculoides* flowers in spring (September, October, November).

In Europe, plants grow slowly in spring and form small, up to 10 cm² large leaves. The plants flower and produce fruits between May and October. The maximal growth rate is reached during June and July (Hussner & Lösch, 2007)

Influence of climatic factors on distribution

Temperature

The species is reported to tolerate a wide range of temperatures, from 0°C up to 30°C of water temperatures (Kasselmann, 1995).

According to the climate calculations of Ackerly lab California Flora Climate Database (<u>http://loarie.stanford.edu/calflora/index.php</u>) which are based on mean climatic data where the species is recorded, the following information are available for temperatures:

- mean daily air temperature (Annual based on 18-year mean) = 14.31 °C

- minimum daily air temperature (Annual based on 18-year mean) = 1.58 °C

- maximum daily air temperature (Annual based on 18-year mean) = 30.82 °C

According to Hussner & Lösch (2007), optimal CO_2 exchange is between 25 and 32°C, meaning that optimal growth would occur at these temperatures; at 35°C, the gas exchanges dropped. Its presence in tropical America, in Africa and western Asia (Lebanon, Syria) shows however that *H. ranunculoides* could be present at higher temperatures.

Rainfall

According to the same Ackerly lab California Flora Climate Database, *H. ranunculoides* occurs in sites with 779.85 mm precipitation per year.

Fitting parameters

The parameters used in the CLIMEX model for *H. ranunculoides* are summarized in Fig 2. The role and meaning of these parameters are fully described in Sutherst *et al.* (2004), and their values are discussed below. It should be noted that the meteorological data used in this model represent long-term monthly averages, not daily values. This means that it is not possible to compare directly values derived using the model with instantaneous values derived through direct observations. This applies mostly to parameters relating to maximum and minimum temperatures.

The climatic requirements of *H. ranunculoides* were derived by fitting the predicted distribution to the known native distribution in America

		Edit Comments		Copy to Clipboard			
□ Moisture	Index						
🔽 Tempera	ture Index						
DVO	DV1	DV2	DV3				
1	24	33	35				
Light Ind	ex						
🗖 Diapause	e Index						
Cold Stre	255						
TTCS	THCS	DTCS	DHCS	TTCSA	THCSA		
1	-0.001	9	-0.001	0	0		
I Heat Stre	ess						
TTHS	THHS	DTHS	DHHS				
35	0.001	0	0				
Dry Stres	55						
□ Wet Stre	55						
Cold-Dry	Stress						
Cold-We	t Stress						
Hot-Dry	Stress						
Hot-Wet	Stress						
Day-degree	accumulat	tion above [070				
DVO	DV3	MTS					
1	35	7					
Day-degree accumulation above DVCS							
DVCS	*DV4	MTS					
1	100	7					
Day-degree accumulation above DVHS							
DVHS	*DV4	MTS					
35	100	7					
Degree-day	/s per Gene	ration					
PDD							

Fig. 2. Parameters used for Hydrocotyle ranunculoides

Moisture index

Moisture index is not considered since the plant is aquatic.

Temperature index

Based on the data described above, the minimum lethal temperature is set at $DV0=1^{\circ}C$, the maximum lethal temperature is set at $DV3=35^{\circ}C$ and optimal growth are set between DV1=24 and $DV2=33^{\circ}C$. We then modify the parameters to better fit the potential distribution to the known distribution in America.

Stresses

Wet stress is not considered since the species is aquatic. The main stresses may be the *cold stress* which seems to limit the species in its northern range and to a lesser extent the *dry stress* which might limit the presence of its preferred habitats (for example in New Mexico).

Cold stress TTCS

As the plant is known to survive to 51 consecutive days of frost (Ackerly lab California Flora Climate Database), and to tolerate temperatures from 0 to 35° C, we set TTCS at 1° C and we supposed that the cold stress accumulates moderately slowly so the rate (THCS) was set at - 0.001 (compared to *Eichhornia crassipes* for which it has been set at -0.01).

Cold stress DTCS

Additionally to be sensitive to a cold stress, the species might be sensitive to the fact that temperatures are not high enough to allow it to photosynthesise enough to offset minimum respiration demands. The parameters are therefore set (separately from the cold stress index) to 9 for DTCS. This parameter is set upon with an accumulation rate of -0.001 (DHCS) since the species is supposed to accumulate this stress slowly.

Heat stress

The plant is tolerant to temperatures of at least 30°C (Kasselmann, 1995). The plant is present in Lebanon, Syria or Yemen where temperatures are very high, the heat stress threshold was therefore set to 35°C. It is assumed that the stress accumulates quite moderately and the rate was set to 0.001 (THHS).

Dry stress

Dry stress is not considered as the species is aquatic.

2. Climatic prediction in the native range

Fig. 3. Potential distribution of Hydrocotyle ranunculoides in North America

The fitting parameters provide a distribution into North-America very close to the current distribution of the species (see appendix 2 for the distribution of the species in North America). The West and east coasts are suitable for the species, as well as the southern part of the State.

3. Climatic prediction for the world



Fig. 4. Potential distribution of *Hydrocotyle ranunculoides* in the world.

The world distribution fits with known occurrences of the species.

- CE: crest catcle (1 stores) market

 Markt

 Market
- 4. Climatic prediction for the EPPO region

Fig. 5. Potential distribution of *Hydrocotyle ranunculoides* in Europe.

According to the Climex simulation, the Atlantic and Mediterranean areas of the EPPO region

that are characterized by mild winters are the most at risk.

The countries at risk are: Albania, Algeria, Austria, Azerbaijan, Belgium, Bosnia & Herzegovina, Bulgaria, Croatia, Cyprus, Denmark, France (including Corsica), Greece, Ireland, Israel, Italy (including Sardinia), Jordan, Germany (mostly western part), Hungary, Moldavia, Morocco, Portugal, Romania, Russia (Black Sea), Serbia, Slovenia, Spain, Switzerland, Tunisia, Ukraine, the United Kingdom, Republic of Macedonia, Romania, Turkey.

Nevertheless, so far, the species has expressed invasiveness in North-Western EPPO countries (Belgium, the Netherlands, United Kingdom,), while the areas which seem to be the most suitable are the Mediterranean and the Atlantic areas. This may be due to other elements such as the use of the plant and the eutrophication of waters.

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Appendix 4

Pictures of invasion



Hydrocotyle ranunculoides as a contaminant on ornamental plants of *H. vulgaris* produced the Netherlands. Picture: J van Valkenburg



Invasion of a stream by *H. ranunculoides* in the UK. Picture: J Newman



Removal of *H. ranunculoides* in the UK. Picture: J Newman



Mechanical removal of *H. ranunculoides* in the U. Picture: J. ewman