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

	European and Mediterranean Plant Protection Organisation		
	Organisation Européenne et Méditerranéenne pour la Protection des Plantes		
	Guidelines on Pest Risk Analysis		
	Lignes directrices pour l'analyse du risque phytosanitaire		
	Decision-support scheme for quarantine pests Version N°3		
PEST RISK ANALYSIS FOR *Hydrocotyle ranunculoides*			
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: - M. Guillaume Fried, LNPV Station de Montpellier, SupAgro (fried@supagro.inra.fr), - M. Andreas Hussner, Institut für Botanik, Universitaet Duesseldorf (andreas.Hussner@uni-duesseldorf.de), - M. Jonathan Newman, CEH Wallingford (jone@ceh.ac.uk), - Ms Gritta Schrader, Julius Kühn Institut (JKI) (gritta.schrader@jki.bund.de), - M. Ludwig Triest, Algemene Plantkunde en Natuurbeheer (APNA) (ltriest@vub.ac.be) - M. Johan van Valkenburg, Plant Protection Service (J.L.C.H.van.valkenburg@minlnv.nl)
1 What is the reason for performing the PRA?			*Hydrocotyle ranunculoides* originates from the American continent and was introduced 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*, *H. leucocephala*, and *H. natans* which is a synonym of *H. ranunculoides*). 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 *H. ranunculoides* and there are concerns that it may be able to enter and establish in further countries. This PRA assesses the risks of its further introduction into other EPPO countries and its current and predicted impact. An initial EPPO 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)
2B Indicate the taxonomic position		Kingdom: *Plantae* Class: *Magnoliopsida* (Dicotyledons) Family: *Apiaceae*
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 only partly valid (out of date, applied in different circumstances, for a similar but distinct pest, for another area with similar conditions)?	Yes	The present PRA consists in an update of the earlier EPPO PRA.
Stage 2A: Pest Risk Assessment - Pest categorization		

6 Specify the host plant species (for pests directly affecting plants) or suitable habitats (for non parasitic plants) present in the PRA area.		Freshwater bodies and ecosystems: ponds, ditches, marshes, waterways etc, more particularly, in static or slow-flowing waters (Newman & Dawson, 1999). In waters of high nutrient content the species thrives extremely well (EPPO, 2009).
7. Specify the pest distribution		*Native range:* *H. ranunculoides* 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 *et al*., 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 *et al*., 1979), Ecuador, Paraguay, Peru, Uruguay (Mathias & Constance 1976). *Introduced range:* **EPPO region**: Belgium, France, Germany, Italy, the Netherlands, the United Kingdom, Ireland (Maguire *et al*., 2008; EPPO Datasheet, 2009). According to Flora Iberica (ref), the mention of *H. ranunculoides* in Spain (Tutin *et al*., 1964-1980) could have resulted from confusions with small forms of *H. vulgaris* or *H. verticillata*. **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 *Hydrocotyle* species revealed that the species can be separated from other resembling *Hydrocotyle* species (van der Wiel *et al*., 2009). Chromosome number: 2n=24, 48 (according to Constance *et al*. (1976) ; Tomei *et al*. (1989) et Pimenov *et al*. (2003), cited by Reduron (2007). There is a wide range of polyploids within the genus *Hydrocotyle*, with up to 15-ploidy (Moore, 1971, Federov, 1974). Baker *et al*. (1997) found four distinct groups of *H. ranunculoides* 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, *H. ranunculoides* is variable and was divided into 8 varieties and 3 forms which would require further investigation (Eichler, 1987): 1. var. *adoensis* 2. var. *brasiliensis* 3. var. *genuina* Urban (épithète non admise = var. *ranunculoides*) I f. *genuina* Urban (épithète non admise = f. *ranunculoides*) 4. var. *incisa* 5. var. *incisocrenata*

		6. var. *lobata* II f. *minima* Kuntze III f. *minima* Hochstetter *ex* Richard (même plante que var. *minima*) 7. var. *minima* (Hochstetter *ex* Richard) Engler (même plante que f. *minima*) 8. var. *natans* (Cirillo) Urban (même plante que f. *natans*) IV f. *natans* (Cirillo) Urban (même plante que var. *natans*) 9. var. *ranunculoides* V f. *ranunculoides* 10. var. *sibthorpioides* (= *H. sibthorpioides* 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, *H. ranunculoides* can cause major problems in nature reserves and recreation areas as well as in intensely managed waterways (Baas & Duistermaat, 1999; Newman & Dawson, 1999). *H. ranunculoides* 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 *H. ranunculoides* 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 *H. ranunculoides*. In Belgium, the species is considered invasive, and voluntary actions are being taken between the nursery industry and the Belgian Biodiversity Platform (see website http://ias.biodiversity.be/; 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 pests directly affecting plants) or one suitable habitat (for non parasitic plants) occur in the PRA area (outdoors, in protected cultivation or both)?	Yes	Suitable habitats for the plant are static or slow-flowing and occasionally flowing freshwater bodies and ecosystems: ponds, ditches, marshes, waterways (Newman & Dawson, 1999). These habitats occur in the EPPO region.
15. If a vector is the only means by which 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)		Not applicable
16. Does the known area of current 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)?	Yes	The plant is already established in part of the PRA area.

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	*H. ranunculoides* 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 *et al*., 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 *et al*., 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 *Hydrocotyle ranunculoides* 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 + uncertainty	Explanatory text of rating and 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 list them		Pathways are: - 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 *H. ranunculoides* 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 *H. leucocephala* (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 Q 1.33 on spread helped by human activities for data on trade within the EPPO 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 *H. ranunculoides* being produced and sold is considered to be very low. - Intentional import for non ornamental uses EFSA (2007) identified another pathway to be considered in the PRA which is the introduction of *H. ranunculoides* being used in phytoremediation (Bretsch, 2004) due to its ability to accumulate heavy metals and phosphorous (Poi de Neiff *et al*. 2003) and the general interest in the use of aquatic macrophytes for bioremediation (Vajpayee *et al*. 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. http://www.scientificjournals.com/sj/all/AutorenProfil/AutorenId/5118 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.), *Phytoremediation: Transformation and Control of Contaminants.* (pp. 85-119). Hoboken, NJ: Wiley-Interscience, Inc. Prof. Dr. Peter Schroeder, working for the German Research Center for Environmental Health (http://www.scientificjournals.com/sj/all/AutorenAnzeigeESS/autorenId/1136) have been contacted but no answer was received. In the EPPO region, other species are usually used for phytoremediation including *Phragmites australis*, *Typha* spp., etc (Cooper, 2001). Trials have been made in

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		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 *H. ranunculoides* 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), *H. ranunculoides* 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 free-floating plants. Some *Hydrocotyle* spp. produced within the EPPO region have been found to be contaminated with *H. ranunculoides* (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		<u>see Q. 1.1.</u> Identified pathways are: - trade for ornamental and aquarium purposes on the Internet

Question	Rating + uncertainty	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 *H. ranunculoides* 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	*H. ranunculoides* 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 *H. ranunculoides* (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 species is needed to complete the life cycle or for a critical stage of the life cycle such as transmission (e.g. vectors), growth (e.g. root symbionts), reproduction (e.g. pollinators) or spread (e.g. seed dispersers), how likely is the pest to come in contact with such species?	No Uncertainty: low	No other species is needed to complete the life cycle of the plant. The plant is able to reproduce vegetatively.
1.19. How similar are the climatic conditions that would affect pest establishment, in the PRA area and in the current area of distribution?	largely similar Uncertainty: Medium	*H. ranunculoides* is already established in several EPPO member countries (Belgium, France, Italy, the Netherlands, United Kingdom, Ireland, quite recently also Germany). The climatic conditions experienced over winter result in a smaller suitable area restricted to the margins of waterbodies (Newman, 2003). 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 *H. ranunculoides* 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). 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

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		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. Additional shortage of water during summer would also limit the success 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/completely similar Uncertainty: low	*H. ranunculoides* 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: *H. ranunculoides* 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>

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		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). <u>Water quality (see map in Appendix 1)</u> 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 *H. ranunculoides* 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/l +/- 2.1 [1.3-8.5], phosphorus: 21 µM+/- 10 [7-45], nitrogen: 116 µM +/- 77 [11-241]. In Belgium, summer field measurements found *H. ranunculoides* on sites with the following ranges of (Nijs *et al*., 2009): O2: 6-11 mg/l pH: 6.7 – 7.5 conductivity: 232-699 µSiemens/cm Total Phosphate (PT): 0.066-0.82 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. <u>Physical characteristics of waterbodies</u> Experiments show that under stable water level regimes, *H. ranunculoides* adopted different morphologies, with highest biomass occurring in fully aquatic conditions (Hussner & Meyer, accepted). Water level fluctuation limit or decrease the biomass accumulation (Hussner, pers. comm., 2009). In its native range in Argentina, Gantes & Sánchez Caro (2001) studied the distribution

Question	Rating + uncertainty	Explanatory text of rating and uncertainty
		of aquatic plant in streams and reported that emergent plants including *H. ranunculoides* 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). <u>Salinity</u> The salinity tolerance of *H. ranunculoides* 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, *H. ranunculoides* has substantial metal absorption capacities (Pinochet *et al*., 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 *et al*., 2009). *H. ranunculoides* may be able to produce allelopathic anti-algal compounds (Della Greca *et al*., 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 (*Myocastor coypus*) can eat *H. ranunculoides* (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 + uncertainty	Explanatory text of rating and uncertainty
1.24. To what extent is the managed environment in the PRA area favourable for establishment?	Highly Favourable Low uncertainty	The optimal habitat of *H. ranunculoides* are static or slow-flowing waters, and the creation of the slowing down of waters by creating dams may favour the establishment of the plant. Restoration of water bodies and the creation of new ponds would encourage the establishment of the species to new sites. Two factors contribute to the establishment of *H. ranunculoides*: - 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 management practice will fail to prevent establishment of the pest?	Very likely Low uncertain ty	Existing mechanical water management strategies often favor the spread and invasion of *H. ranunculoides* by increasing fragmentation (Newman, pers. comm., 2009), see also questions 1.33 & 1.34. According to Hussner & Lösch (2007), the high regeneration capacities from stem fragments is very likely to result in the dispersal of the species after mechanical control. Pot (2000) described this problem for the management of *H. ranunculoides* populations in the Netherlands.
1.26. Based on its biological characteristics, how likely is it that the pest could survive eradication programmes in the PRA area?	Very likely in heavy infested areas. Uncertai nty: low	Eradication is very difficult or even impossible in water bodies with heavy infestation. However, according to the Dutch experience, local eradication is possible if it is started early and the water system is reasonably accessible. In the Netherlands as a whole, eradication is not possible anymore. Dutch waterboards are currently successful in early detection by visual inspection and in local eradication of small infestations by careful manual work. In the UK, mechanical control is combined with applications of herbicides but did not 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 + uncertainty	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, *H. ranunculoides* 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 cm² 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 *H. ranunculoides* 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 + uncertainty	Explanatory text of rating and uncertainty
1.28 How likely are relatively small populations to become established?	Very likely Uncertainty: low	Founder populations may have low genetic diversity but vegetative spread ensures that 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 various introductions from a single clone available at aquatic garden centres and 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 Uncertainty: low	The species occurs in the USA, Central and South America, Australia, Africa and parts of Europe, suggesting that this species is adaptable to different climatic conditions (see Q. 7 for distribution). The plant can establish in different freshwater bodies and ecosystems: *H. ranunculoides* 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 *et al*., 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 *H. ranunculoides* (van Valkenburg, pers. comm., 2009). The species is present on all continents except Antarctica.

Question	Rating + uncertainty	Explanatory text of rating and 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 m² was completely invaded in three years (Hussner, pers. comm., 2009). Observations in the UK highlight that the species grows

Question	Rating + uncertainty	Explanatory text of rating and uncertainty
	long distance: unlikely Uncertainty: 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, *H. ranunculoides* 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 + uncertainty	Explanatory text of rating and 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, *Hydrocotyle ranunculoides* is present in Sardinia (Central-West Sardinia, channels in agricultural area, wetlands) where it is invasive (Brundu *et al*., 2003). The species is only recorded as naturalised in two other Italian regions (i.e. Toscana and Campania) (Celesti-Grapow *et al*., 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 *H. ranunculoides*. 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 *H. ranunculoides* into some EPPO countries: **Germany** In Germany no suppliers have been identified selling *H. ranunculoides* under that name (PPP-index, 2009). Other *Hydrocotyle* spp. are traded which could be mislabeled (A. Hussner, pers. comm., 2009). **France** In France, a website selling *Hydrocotyle natans* (a synonym for *H. ranunculoides*) has been identified (http://www.floraquatic.com/-50029/hydrocotyle-natans-500039.html). Furthermore, seven nurseries, throughout the whole country, are selling *H. vulgaris* and/or *H. leucocephala* which could well be *H. ranunculoides* (G Fried, pers. comm., 2009). **UK** Due to the high invasiveness of *H. ranunculoides*, the UK Royal Horticultural Society banned this plant from its shows and gardens (Shaw, 2003). *H. ranunculoides* is not cultivated or deliberately sold. There are some specialist aquatic nurseries (about 5) that supply *H. vulgaris*. The specimens of this species sent for identification to J. Newman in 2006 confirm that they were *H. vulgaris*. Other species sold are *H. umbellata*, *H. nova zealandae*, and *H. sibthorpiodes*. *H. ranunculoides* 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 + uncertainty	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 *H. ranunculoides*, and possibly other names, but some centres have been asked to withdraw the species from trade from 2009 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 *H. ranunculoides*. 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 *H. ranunculoides* 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. Within a catchment, biological characteristics of the plant make it difficult to contain.

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 *H. ranunculoides* 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 *H. ranunculoides* 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 + uncertainty	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 *H. ranunculoides* 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 *H. ranunculoides* over and above normal operating costs for this plant (van Valkenburg, pers. comm., 2009). In Flanders, the estimated cost for the management of *H. ranunculoides* 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 *H. ranunculoides* 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 *H. ranunculoides* (Newman, pers. comm.., 2009). In 6 years, the costs were multiplied 7 times. Flooding caused by the plant may also have an economic impact due to loss of crops (Newman, pers. comm., 2009).
2.2. How great a negative effect is the pest likely to have on crop yield and/or quality in the PRA area without any control measures?	Minimal Medium uncertainty	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 structures.

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, *H. ranunculoides* will not be controlled. Even with phytosanitary measures, *H. ranunculoides* is very difficult to control. Mechanical control has to be done very carefully. If it is not done properly, spread can be promoted, as *H. ranunculoides* 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 Medium uncertainty	Control costs could be similar to those already spent in infested parts of the PRA area. See 2.1. A weevil, *Listronotus elongatus*, 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 + uncertainty	Explanatory text of rating and uncertainty
2.6. How important is environmental damage caused by the pest within its current area of distribution?	Major Low uncertainty	Since 2005 (date of the previous PRA), much more information on environmental impacts was made available. 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 cover from 50% to 10 (Nijs *et al*., 2009). In Sardinia, the species is considered invasive, and although no specific impacts have been studied, the thick coverage of the species at the surface of the water is considered to outcompete other species (G Brundu, pers. comm., 2009). In the PRA area, where present, *H. ranunculoides* competes with many plant species due to its ability to establish in different habitats. Examples: different *Carex*/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 *H. ranunculoides'* capability to build floating carpets that shade out other plants. Data on impacts in dense infestation are rare because of dangerous surveillance conditions underneath dense floating 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 + uncertainty	Explanatory text of rating and uncertainty
		pers. comm., 2009). The EWG considered that *H. ranunculoides* 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 *H. ranunculoides* 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 note for question 2.6)?	Major Low uncertainty	see for question 2.6. Environmental impact is supposed to be the same wherever the species grows in suitable conditions. For instance, in Essonne (France) and in Italy, similar impacts can be expected as in the 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 + uncertainty	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 *H. ranunculoides* 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 + uncertainty	Explanatory text of rating and 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, already present in the PRA area, will not reduce populations of the pest below the economic threshold?	Very likely Uncertainty: low	In the UK, there are no natural enemies recorded. In Germany, observations showed that coypus (*Myocastor coypus*) – which is non-native can feed on *H. ranunculoides* (Hussner & Lösch, 2007) but it is unlikely that this species can reduce or contain all the populations of *H. ranunculoides* below the economic threshold, considering its rapid establishment and spread in invaded areas.
2.12. How likely are control measures to disrupt existing biological or integrated systems for control of other pests or to have negative effects on the environment?	Very likely Uncertainty: low	Both chemical and mechanical management measures will have negative effects on the environment. Mechanical control would remove considerable number of invertebrates (Dawson *et al*., 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 (J Newman, pers. comm., 2009). 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 toxicity 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).
2.13. How important would other costs resulting from introduction be?	Minor to moderate	Publicity may be provided by the horticultural industry. Some funds for research into control methods may be invested. For example, the development of some research on biological control agents in the UK would cost approximately £500.000 (Newman, pers.

Question	Rating + uncertainty	Explanatory text of rating and uncertainty
	Uncertainty: low	comm., 2009).
2.14. How likely is it that genetic traits can be carried to other species, modifying their genetic nature and making them more serious plant pests?	Unlikely Uncertainty: medium	Pollen flow could happen with the native *H. vulgaris* or with other exotic *Hydrocotyle* spp. but has never been documented, and there is no information available about hybridization of *Hydrocotyle* species. The EWG considered that it is unlikely that genetic traits could be carried to other *Hydrocotyle* spp.
2.15. How likely is the pest to cause a significant increase in the economic impact of other pests by acting as a vector or host for these pests?	Unlikely Uncertainty: medium	There are no records of *H. ranunculoides* as a vector or host of other pests. The EWG considered that it is unlikely that *H. ranunculoides* would increase the economic impact of other pests.
2.16. Referring back to the conclusion on endangered area (1.35), identify the parts of the PRA area where the pest can establish and which are economically most at risk.		Freshwater bodies and ecosystems: ponds, ditches, marshes, waterways etc, more particularly, in 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 are likely to limit the species. (See Annexe 1). The species may be limited by acidic soils (see Appendix 1).
Degree of uncertainty **Estimation of the probability of introduction of a pest and of its economic consequences involves many uncertainties. In particular, this estimation is an**	**Low**	The areas of uncertainty identified are the following: - study on the varieties and forms, and the ones considered invasive. - the amount of internet trade - the amount of production in the EPPO region - the amount of exchange between gardeners and hobbyists,

Question	Rating + uncertainty	Explanatory text of rating and uncertainty
extrapolation from the situation where the pest occurs to the hypothetical situation in the PRA area. It is important to document the areas of uncertainty (including identifying and prioritizing of additional data to be collected and research to be conducted) and the degree of uncertainty in the assessment, and to indicate where 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.**		- Information of the situation in Italy - why did the plant disappeared from a river in Corsica (non found since 1968). - effects on water quality (e.g. O_2 content) and secondary effects on biota. Research needs identified: - data on outcompeted native species and their potential for recovery. - The effect of climatic change on the distribution and impacts of the plant - Biological control
Evaluate the probability of entry and indicate the elements which make entry most likely or those that make it least likely. Identify the pathways in order of risk and compare their importance in practice.	**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 *H. ranunculoides* being produced and sold is considered to be very low.

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 *Hydrocotyle ranunculoides* 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? **If yes, go to 3.11,** **If no, go to 3.3**	Yes	
3.11.If the pest is a plant, is it the commodity itself? **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**	Yes	**--**

3.29. Are there effective measures that could be taken in the importing country (surveillance, eradication) to prevent establishment and/or economic or other impacts? If yes, possible measures: internal surveillance and/or eradication campaign, go to 3.30	yes	Prohibition of the import, selling, planting, holding, movement, causing to grow in the wild, and possession of the plant. Due to the high invasiveness of *H. ranunculoides*, the UK Royal Horticultural Society banned this plant from its shows and gardens (Shaw, 2003). In January 2001, the Dutch Ministry of nature conservation and food quality prohibited the sale and possession of this plant (Staatsblad van het Koninkrijk der Nederlanden, 2000). In Scotland, the Wildlife and Countryside act added *H. ranunculoides* 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 have been intentionally imported'.

3.30.Have any measures been identified during the present analysis that will reduce the risk of introduction of the pest? List them. **If yes, go to 3.31** **If no, go to 3.38**	Yes	Prohibition of the import, selling, planting, holding, movement, causing to grow in the wild, and possession of the plant is the most efficient measure.
3.31.Does each of the individual measures identified reduce the risk to an acceptable level? **If yes, go to 3.34** **If no, go to 3.32**	depends on situation	In countries where the species is present, control measures of infestations within countries are not efficient if the plant is frequently reintroduced. Prohibition of selling is therefore necessary. When *H. ranunculoides* is not yet present in a country, prohibition of selling may be sufficient combined with the knowledge on action plans for early intervention in case the plant occurs.
3.32.For those measures that do not reduce the risk to an acceptable level, can two or more measures be combined to reduce the risk to an acceptable level? **If yes, go to 3.34** **If no, go to 3.33**	Yes	National measures Prohibition of selling, planting, holding, movement, causing to grow in the wild, and possession of the plant in the EPPO region is necessary. Moreover, the plant has to be controlled where it occurs. If these measures are not implemented by all countries, they will not be efficient since the species would spread from one country to another. In addition, it has to be combined with international measures. International measures Accurate identification of all *Hydrocotyle* spp. traded in the EPPO region should be encouraged. Methods of DNA bar-coding are available (see van der Wiel *et al*., 2009). Prohibition of import into the EPPO region of species labelled as *H. natans* and *H. americana* which are synonyms for *H. ranunculoides* and within the countries.

3.33.If the only measures available reduce the risk but not down to an acceptable level, such measures may still be applied, as they may at least delay the introduction or spread of the pest. In this case, a combination of phytosanitary measures at or before export and internal measures (see question 3.29) should be considered. **Go to 3.34**		
3.34.Estimate to what extent the measures (or combination of measures) being considered interfere with trade. **Go to 3.35**		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.
3.35.Estimate to what extent the measures (or combination of measures) being considered are cost-effective, or have undesirable social or environmental consequences. **Go to 3.36**		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** **If no, go to 3.37**	Yes	Prohibition of import, trade, planting, holding and movement of the plant.
3.37.Envisage prohibiting the pathway **For pathway-initiated analysis, go to 3.43 (or 3.39),** **For pest-initiated analysis go to 3.38**	Yes	This is one of the options.
3.38.Have all major pathways been analyzed (for a pest-initiated analysis)? **If yes, go to 3.41,** **If no, Go to 3.1 to analyze the next major pathway**	Yes	
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 would qualify as quarantine pests, and select only those that provide phytosanitary security against all 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 **Go to 3.42**		Intentional import of the plant for ornamental purposes: high Phytoeremediation: low
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. **Go to 3.43**		Import and trade of *H. ranunculoides* to the EPPO region and its sale within it should be prohibited.
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. **Summarize the conclusions of the Pest Risk Management stage. List all potential management options and indicate their effectiveness. Uncertainties should be identified.**		**Major pathway is trade of the plant for ornamental purposes** <u>International measures</u> Prohibition of import and trade in the EPPO region and within the countries will effectively prevent further introduction into the EPPO region combined with accurate identification of species and synonyms. <u>National measures</u> 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. <u>Integrated management plan for the control of existing infestations</u> 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 surveillance in the countries at risk. - 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. <u>Monitoring and review</u> 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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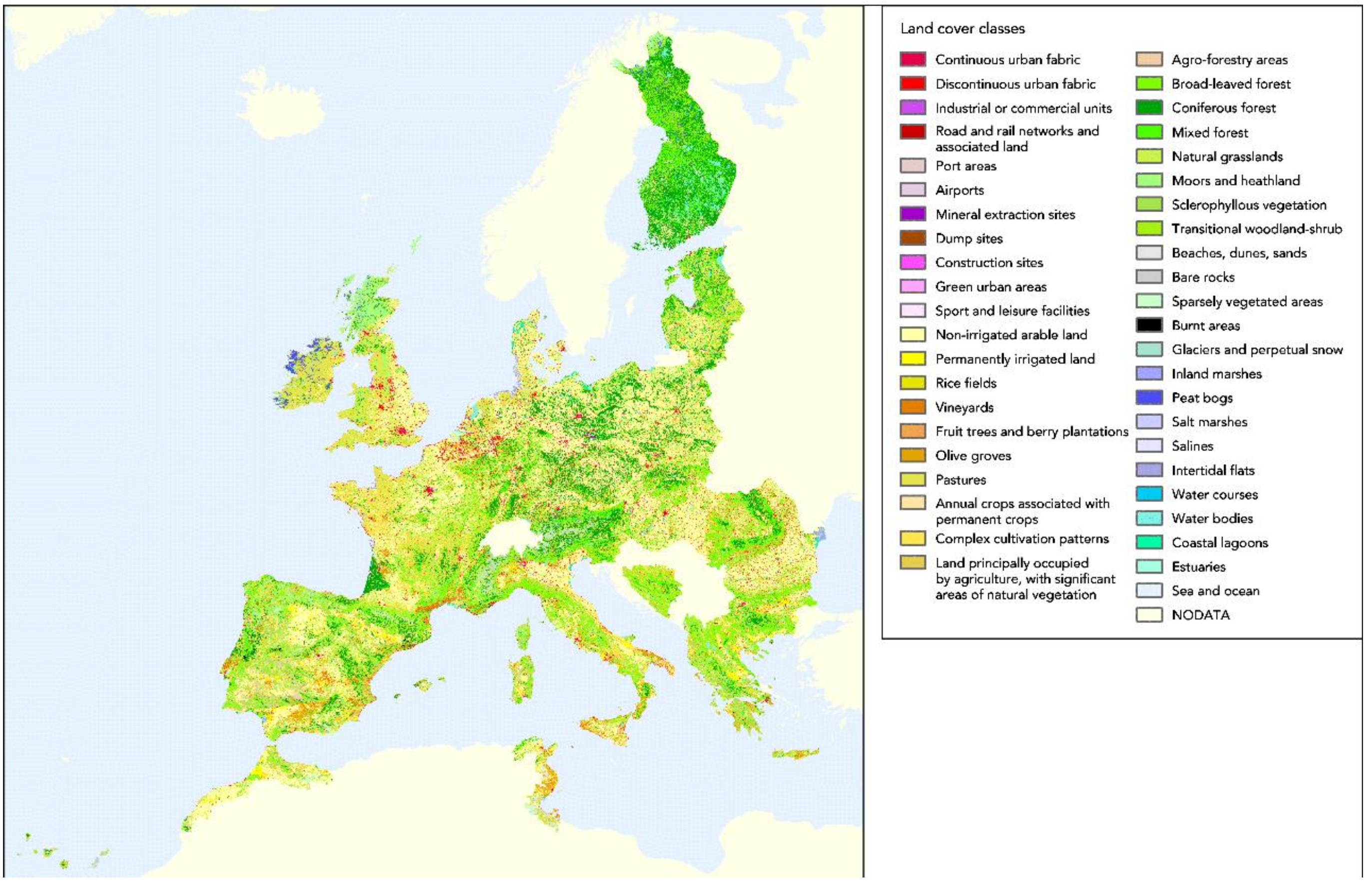
Appendix 1

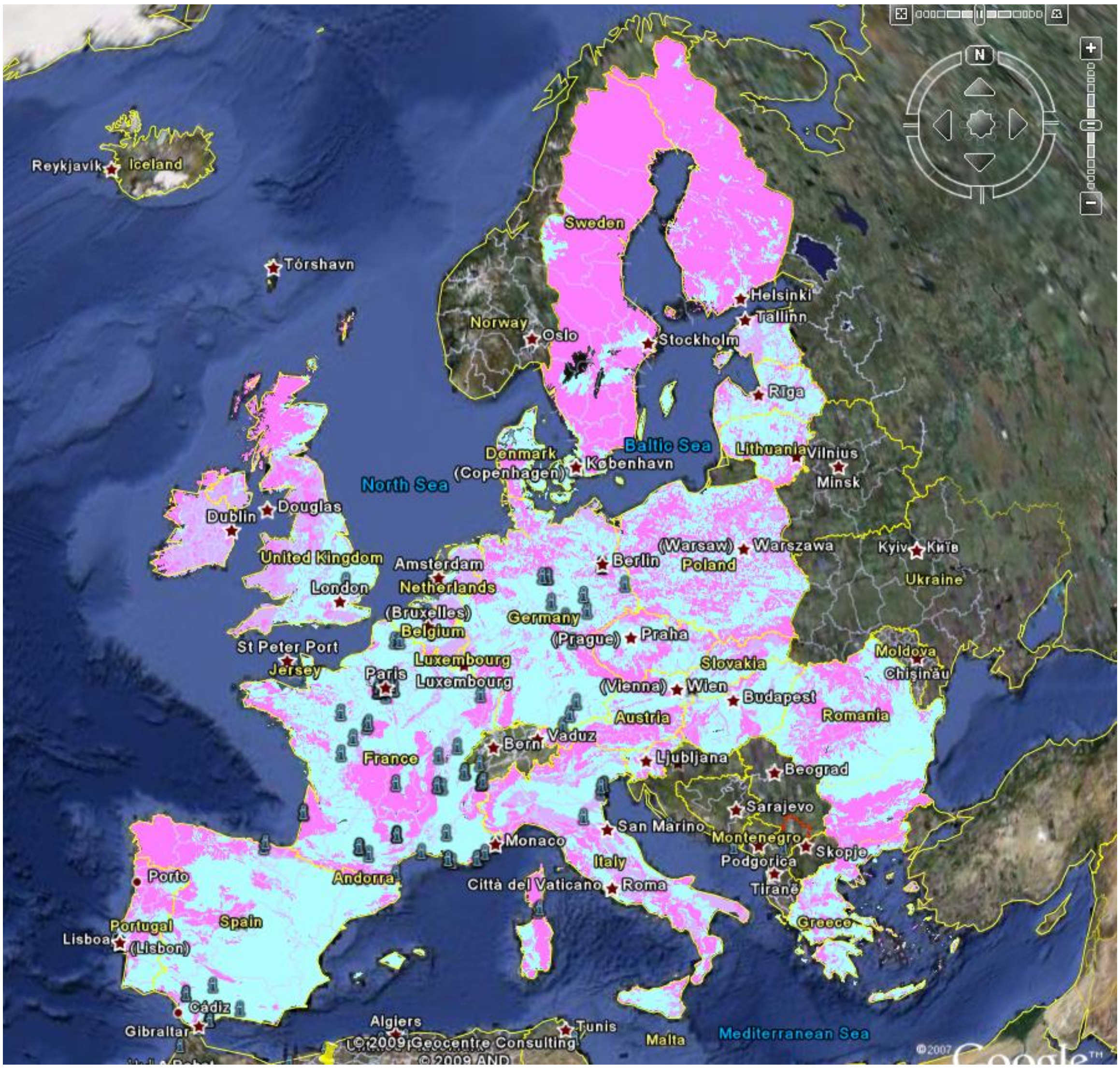
Maps relevant for the distribution of *Hydrocotyle ranunculoides*

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 (http://eusoils.jrc.ec.europa.eu/, 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.*


Reykjavík
Iceland
Tórshavn
Sweden
Helsinki
Tallinn
Norway
Oslo
Stockholm
Riga
Denmark
(Copenhagen)
København
Baltic Sea
Lithuania
Vilnius
Minsk
North Sea
Dublin
Douglas
United Kingdom
London
Amsterdam
Netherlands
(Bruxelles)
Belgium
Germany
Berlin
(Warsaw)
Warszawa
Poland
Kyiv
Київ
Ukraine
(Prague)
Praha
St Peter Port
Jersey
Paris
Luxembourg
Luxembourg
Slovakia
Moldova
Chişinău
(Vienna)
Wien
Budapest
Austria
Romania
Bern
Vaduz
France
Ljubljana
Beograd
Sarajevo
San Marino
Montenegro
Skopje
Podgorica
Monaco
Italy
Porto
Andorra
Città del Vaticano
Roma
Tiranë
Portugal
Spain
Greece
Lisboa
(Lisbon)
Cádiz
Gibraltar
Algiers
Tunis
Malta
Mediterranean Sea
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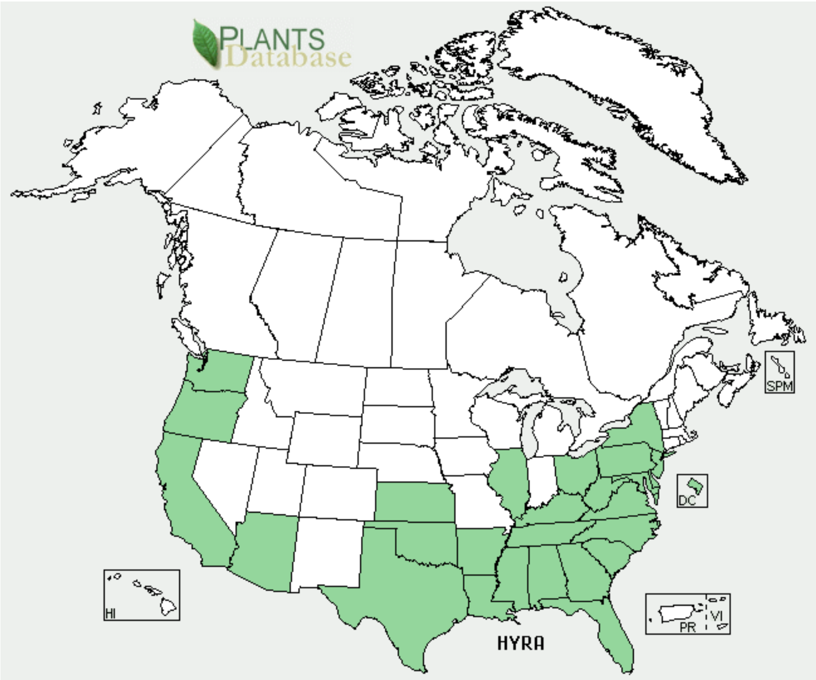
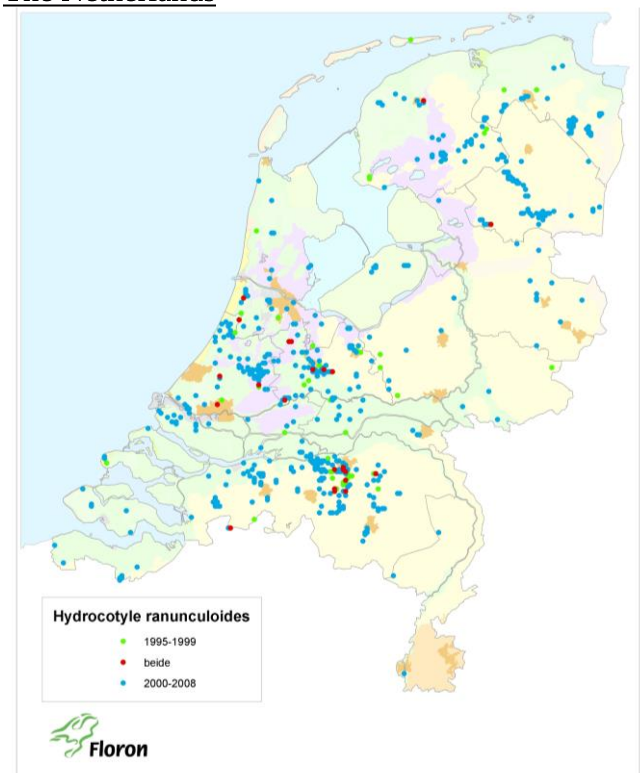
Appendix 2

Maps of occurrence and spread in countries of the EPPO region

North- America
Map available at http://plants.usda.gov/java/profile?symbol=HYRA
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

<u>Germany</u>

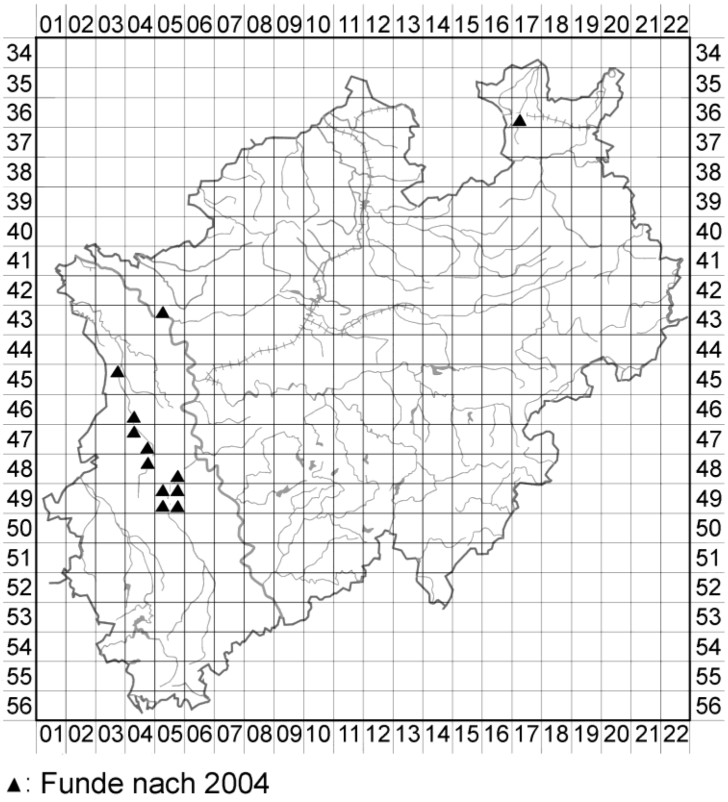
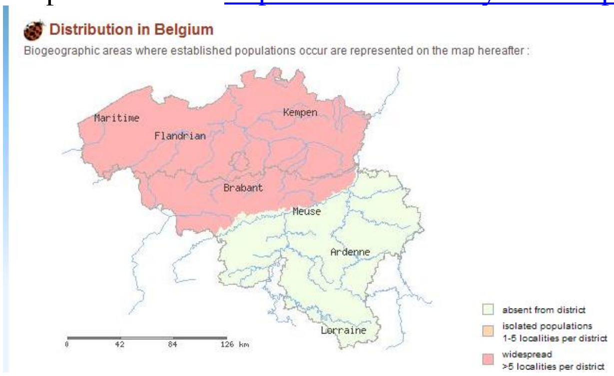


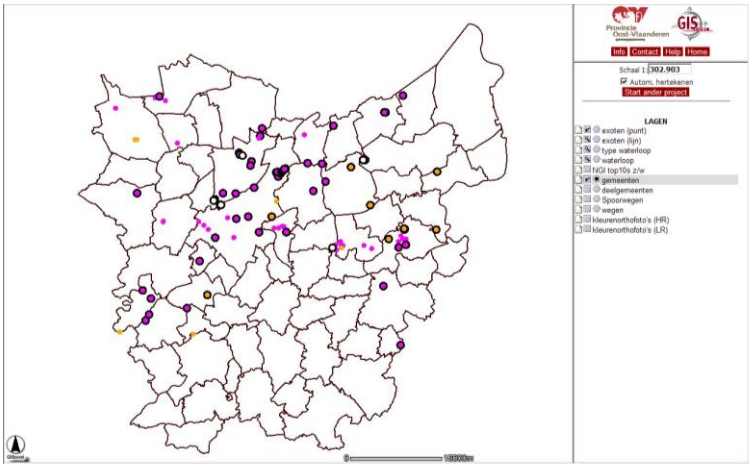
Figure: Known occurrences of *Hydrocotyle ranunculoides* in North Rhine-Westphalia (Germany) in 2008, species was present in all lower parts of the rivers Erft (a tributary of the River Rhine) and Niers (Hussner 2008).

Belgium

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

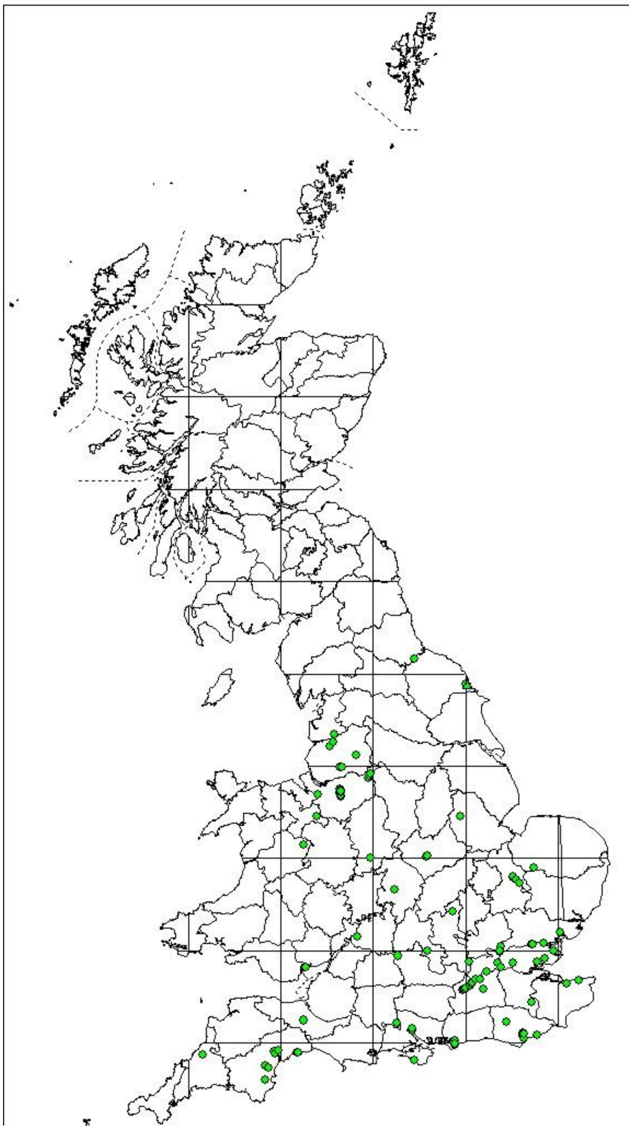


Atlas Flora of Flanders, available at http://www.gisoost.be/exoten/ (go to “volledig gebied”)



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.

<u>The UK</u>



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): http://www.gbif.org/, USDA http://plants.usda.gov, 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 ranunculoides 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).

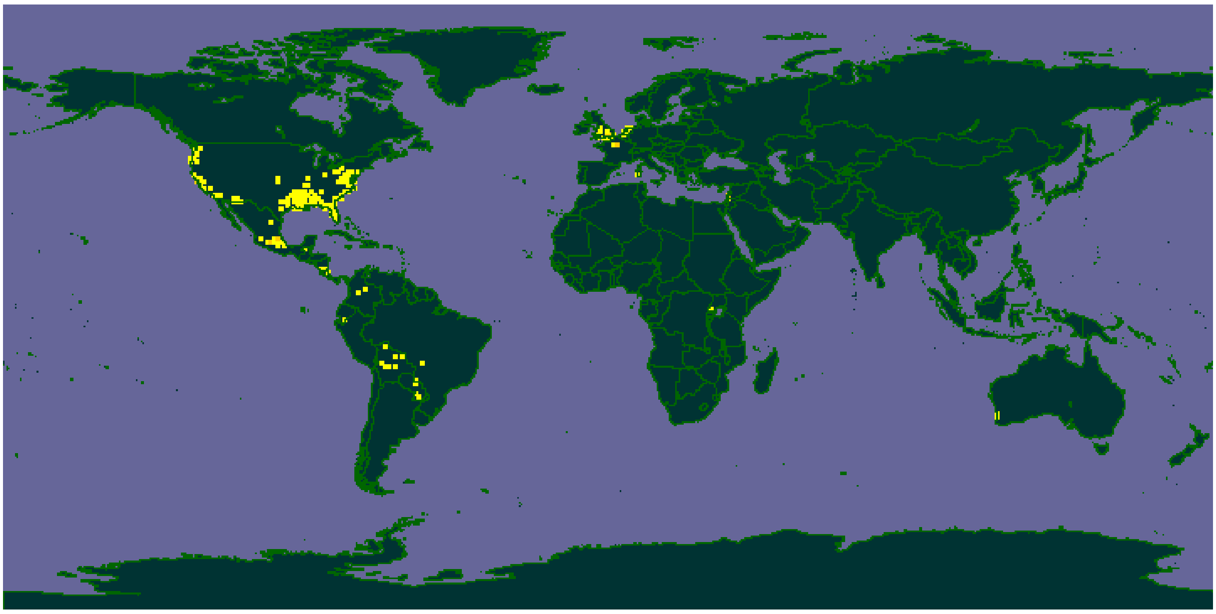


Fig. 1. World distribution of *Hydrocotyle ranunculoides* according to the GBIF.
This map is incomplete for data in Africa, in South America and in Europe.

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 (http://loarie.stanford.edu/calflora/index.php) 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					
☑ Temperature Index					
DV0	DV1	DV2	DV3		
1	24	33	35		
☐ Light Index					
☐ Diapause Index					
☑ Cold Stress					
TTCS	THCS	DTCS	DHCS	TTCSA	THCSA
1	-0.001	9	-0.001	0	0
☑ Heat Stress					
TTHS	THHS	DTHS	DHHS		
35	0.001	0	0		
☐ Dry Stress					
☐ Wet Stress					
☐ Cold-Dry Stress					
☐ Cold-Wet Stress					
☐ Hot-Dry Stress					
☐ Hot-Wet Stress					
Day-degree accumulation above DV0					
DV0	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-days per Generation					
PDD					
0					

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°C, the maximum lethal temperature is set at DV3=35°C and optimal growth are set between DV1=24 and DV2=33°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

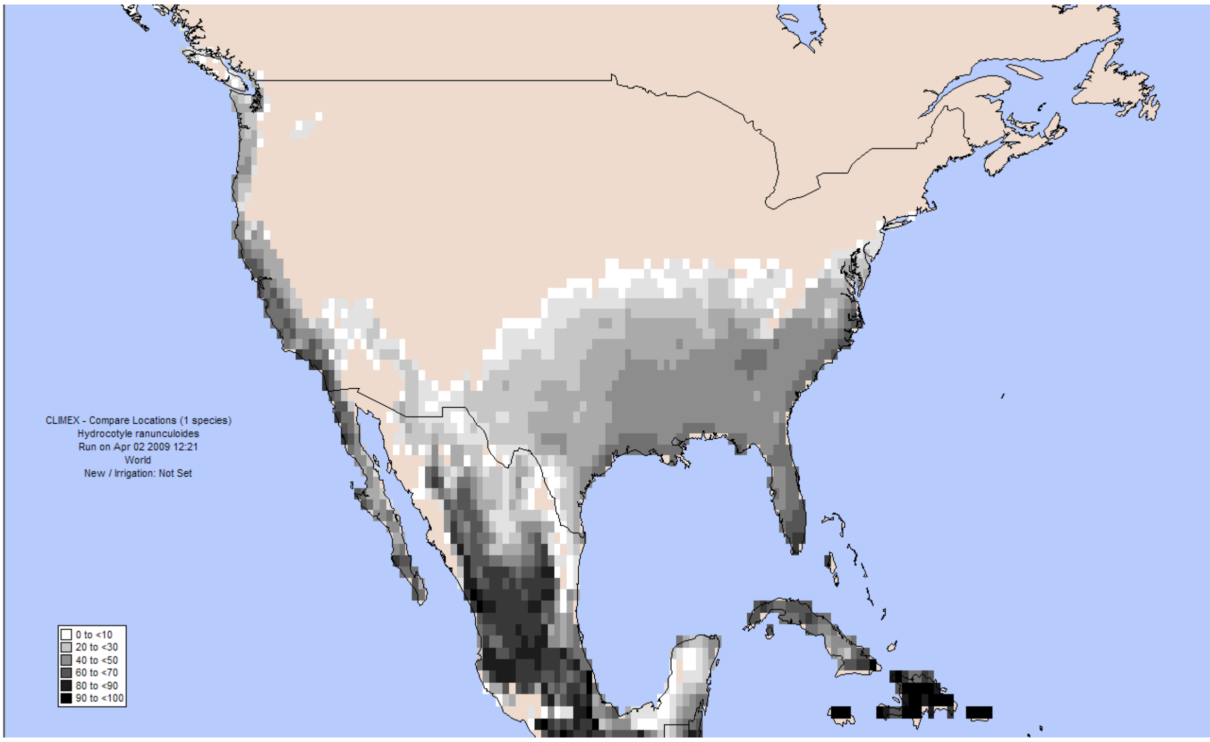


Fig. 3. Potential distribution of *Hydrocotyle ranunculoides* in North America

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

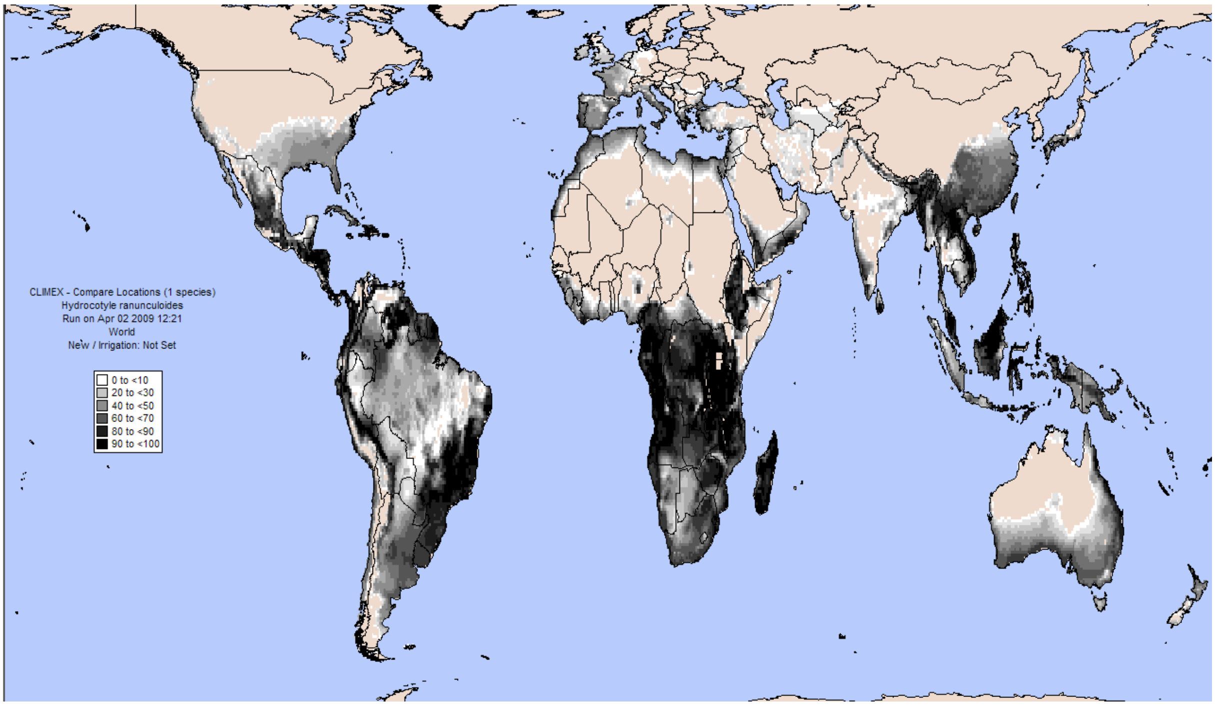


Fig. 4. Potential distribution of *Hydrocotyle ranunculoides* in the world.

The world distribution fits with known occurrences of the species.

4. Climatic prediction for the EPPO region

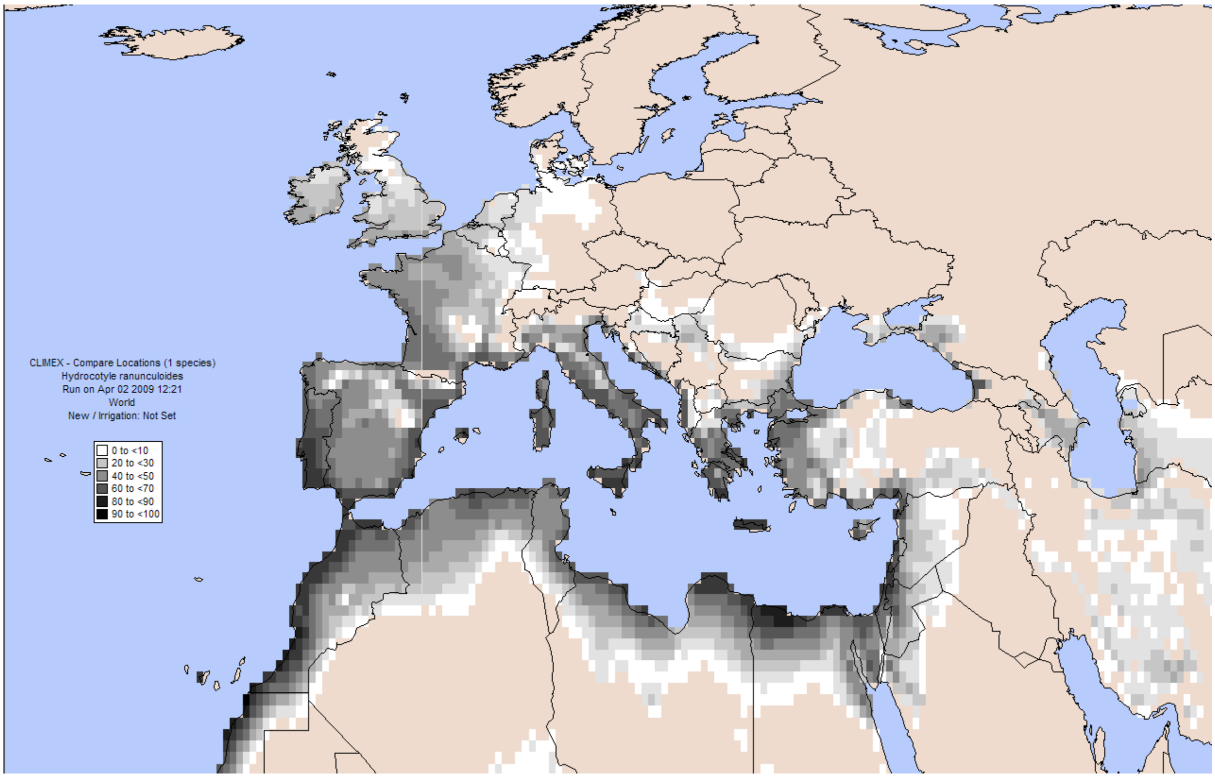


Fig. 5. Potential distribution of *Hydrocotyle ranunculoides* in Europe.

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.

References

Ackerly lab California Flora Climate Database (http://loarie.stanford.edu/calflora/index.php

Hussner A & Lösch R (2007) Growth and photosynthesis of *Hydrocotyle ranunculoides* L. fil. In *Central European Flora*, **202**: 653-660.

Kasselmann C (1995) Aquarienpflanzen. Egen Ulmer GMBH & Co., Stuttgart. 472 pp. (In German)

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