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Risk analysis of the Water Pennywort, *Hydrocotyle ranunculoides* (L.F., 1781)

Risk analysis report of non-native organisms in Belgium

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Adopted in date of : 11 March 2013

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Etienne Branquart (Cellule Espèces Invasives, Service Public de Wallonie) developed the risk analysis template that was used for this exercise.

The general process of drafting, reviewing and approval of the risk analysis for selected invasive alien species in Belgium was attended by a steering committee, chaired by the Federal Public Service Health, Food chain safety and Environment. RBINS/KBIN was contracted by the Federal Public Service Health, Food chain safety and Environment to perform PRA's for a batch of species. ULg was contracted by Service Public de Wallonie to perform PRA's for a selection of species. INBO and DEMNA performed risk analysis for a number of species as in-kind contribution.

Steering committee members were:

Tim Adriaens	Research Institute for Nature and Forest (INBO)
Olivier Beck	Brussels Environment (BIM)
Roseline Beudels-Jamar	Royal Belgian Institute of Natural Sciences (RBINS/KBIN)
Etienne Branquart	Département de l'Etude du Milieu Naturel et Agricole (DEMNA)
Jim Casaer	Research Institute for Nature and Forest (INBO)
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Hans Van Gossum	Agency for Nature and Forest (ANB)
Hugo Verreycken	Research Institute for Nature and Forest (INBO)

Rationale and scope of the Belgian risk analysis scheme

The Convention on Biological Diversity (CBD) emphasises the need for a precautionary approach towards non-native species. It strongly promotes the use of robust and good quality risk assessment to help underpin this approach (COP 6 Decision VI/23). More specifically, when considering trade restrictions for reducing the risk of introduction and spread of a non-native organisms, full and comprehensive risk assessment is required to demonstrate that the proposed measures are adequate and efficient to reduce the risk and that they do not create any disguised barriers to trade. This should be seen in the context of WTO and free trade as a principle in the EU (Baker et al. 2008, Shine et al. 2010, Shrader et al. 2010).

This risk analysis has the specific aim of evaluating whether or not to install trade restrictions for a selection of absent or emerging invasive alien species that may threaten biodiversity in Belgium as a preventive risk management option. It is conducted at the scale of Belgium but results and conclusions could also be relevant for neighbouring areas with similar eco-climatic conditions (e.g. areas included within the Atlantic and the continental biogeographic regions in Europe).

The risk analysis tool that was used here follows a simplified scheme elaborated on the basis of the recommendations provided by the international standard for pest risk analysis for organisms of quarantine concern[1] *produced by the secretariat of the International Plant Protection Convention (FAO 2004). This logical scheme adopted in the plant health domain separates the assessment of entry, establishment, spread and impacts. As proposed in the GB non-native species risk assessment scheme, this IPPC standard can be adapted to assess the risk of intentional introductions of non-native species regardless the taxon that may or not be considered as detrimental (Andersen 2004, Baker et al. 2005, Baker et al. 2008, Schrader et al. 2010).*

The risk analysis follows a process defined by three stages : (1) the <u>initiation process</u> which involves identifying the organism and its introduction pathways that should be considered for risk analysis in relation to Belgium, (2) the <u>risk assessment stage</u> which includes the categorization of emerging non-native species to determine whether the criteria for a quarantine organism are satisfied and an evaluation of the probability of organism entry, establishment, spread, and of their potential environmental, economic and social consequences and (3) the <u>risk management stage</u> which involves identifying management options for reducing the risks identified at stage 2 to an acceptable level. These are evaluated for efficacy, feasibility and impact in order to select the most appropriate. The risk management section in the current risk analysis should however not been regarded as a full-option management plan, which would require an extra feasibility study including legal, technical and financial considerations. Such thorough study is out of the scope of the produced documents, in which the management is largely limited to identifying needed actions separate from trade restrictions and, where possible, to comment on cost-benefit information if easily available in the literature.

This risk analysis is an advisory document and should be used to help support Belgian decision making. It does not in itself determine government policy, nor does it have any legal status. Neither should it reflect stakeholder consensus. Although the document at hand is of public nature, it is important to realise that this risk assessments exercise is carried out by (an) independent expert(s)

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A weed or a pest organism not yet present in the area under assessment, or present but not widely distributed, that is likely to cause economic damages and is proposed for official regulation and control (FAO 2010).

who produces knowledge-based risk assignments sensu Aven (2011). It was completed using a uniform template to ensure that the full range of issues recognised in international standards was addressed.

To address a number of common misconceptions about non-native species risk assessments, the following points should be noted (after Baker et al. 2008):

- *Risk assessments are advisory and therefore part of the suite of information on which policy decisions are based;*
- *The risk assessment deals with potential negative (ecological, economic, social) impacts. It is not meant to consider positive impacts associated with the introduction or presence of a species, nor is the purpose of this assessment to perform a cost-benefit analysis in that respect. The latter elements though would be elements of consideration for any policy decision;*
- *Completed risk assessments are not final and absolute. New scientific evidence may prompt a re-evaluation of the risks and/or a change of policy.*

Figure 1 : *Hydrocotyle ranunculoides* (Photo : Jo Packet ; INBO).

Executive summary

PROBABILITY OF ESTABLISHMENT AND SPREAD (EXPOSURE)

- Entry in Belgium

H. ranunculoides was first observed in the Belgian wild in 1999. It is produced and sold in Belgium as an ornamental plant for aquariums and ponds (sometimes under erroneous and misleading names). Most introductions into the wild occur when plant fragments are released after aquarium/pond cleaning or incautious disposal of plant. Secondary invasion may occur through natural dispersion from populations in the Netherlands or France.

- Establishment capacity

H. ranunculoides grows in stagnant and slowly running freshwater. It colonizes the shallow parts and banks of rivers, streams, ditches, mill weirs, ponds, lakes, pits, canals and freshwater marshes. It supports tidal conditions or strong irregular water-level variations and grows on all types of soil, including peat. It even grows on drained soils. Most freshwater bodies and slow flowing streams of the Belgian territory, including endangered, sensitive areas and Natura2000 habitats are suitable for *H. ranunculoides* establishment. Future climate change may enhance further invasion in the Ardennes and Lorraine.

- Dispersion capacity

Natural dispersion readily occurs through water flow. Dispersal may be enhanced by human activities such as transport with boats or machines used to clear watercourses. Intentional introduction in ponds and accidental introduction such as release after aquarium or pond cleaning or incautious plant residues disposal also enhance propagation of *H. ranunculoides*.

EFFECT OF ESTABLISHMENT

- Environmental impacts

With a high capacity to cover large surface and an extremely fast growth rate, *H. ranunculoides* is able to outcompete most native aquatic and many riparian species. The floating mats affect the penetration of light available for photosynthesis and reduce oxygen levels in the water column which can result in fish mortality and influence invertebrate life. Water flow may be impeded causing accelerated silting-up and altered succession, loss of open water at the margins for wildlife and increasing flood risk.

RISK MANAGEMENT

The main current pathway of introduction of *Hydrocotyle ranunculoides* in Belgium remains its sale as an ornamental plant for aquariums and ponds, and its subsequent release in the wild. This pathway is however decreasing thanks to education actions carried out in the country (e.g. in the framework of the AlterIAS LIFE project). Enhancing awareness-raising on risks associated with dumping of garden waste in natural areas could be appropriate for existing populations. Once established, natural spread may occur, especially in flowing waters. As a result, secondary invasions within Belgium, but also from French or Dutch populations, are very likely.

Prohibition of importation, ban of trade and holding of *H. ranunculoides* in Belgium, though necessary as a preventive measure could not be enough to prevent further entry and establishment because (1) the species is already spreading in the country, and (2) natural spread from neighbouring countries is expected since dense populations occurs close to the Belgian border. Nevertheless, such measures might slow down its current spread.

Hydrocotyle ranunculoides is difficult to detect at early stage of invasion, and therefore control or eradication actions often start when the plant is already well-established.

Since chemical weed control in an aquatic environment is extremely restricted in Belgium and because the results should be of practical use, control should focus on prevention and non-chemical methods (in this case mechanical removal).

Résumé

PROBABILITE D'ETABLISSEMENT ET DE DISSEMINATION (EXPOSITION)

- Introduction en Belgique

H. ranunculoides a été observée pour la première fois en Belgique dans la nature en 1999. Cette espèce est produite et vendue en Belgique comme plante ornementale pour les aquariums et les étangs (parfois sous des noms erronés ou trompeurs). La majorité des introductions dans la nature se font par le biais de fragments de plantes rejetés après le nettoyage des aquariums/étangs ou l'élimination négligée/non contrôlée de la plante. Une seconde voie d'introduction est la dispersion naturelle à partir des populations présentes aux Pays-Bas ou en France.

- Capacité d'établissement

H. ranunculoides pousse dans les eaux stagnantes et les cours d'eau à débit lent. Elle colonise les parties peu profondes et les berges des cours d'eau ainsi que des fossés, des barrages de moulin, les étangs, les lacs, les puits, les canaux et les marais d'eau douce. Elle supporte les marées, les variations fortes et irrégulières du niveau de l'eau et pousse sur tous les types de sols, y compris la tourbe. Elle pousse même sur et les sols drainés. La plupart des plans d'eau douce et des courants à débit lent du territoire, y compris les zones menacées et sensibles ainsi que les sites Natura 2000, offrent un habitat qui convient à *H. ranunculoides.* L'évolution du changement climatique pourrait favoriser son envahissement dans les Ardennes et en Lorraine.

- Capacité de dispersion

La dispersion naturelle de *H. ranunculoides* s'effectue rapidement par les courants d'eau. Dans beaucoup de cas, elle peut être accélérée par les activités humaines, notamment le transport par bateau ou les machines utilisées pour nettoyer les cours d'eau. L'introduction délibérée dans les étangs et les introductions accidentelles (par rejet négligé/non contrôlé de résidus de la plante après nettoyage d'aquariums ou des étangs) favorisent aussi la propagation de *H. ranunculoides* dans le milieu naturel.

EFFET DE L'ETABLISSEMENT

- Impacts environnementaux

Un taux de croissance extrêmement élevé et la capacité de couvrir de grandes surfaces permettent à *H. ranunculoides* d'exclure par compétition la majorité des espèces indigènes aquatiques et riveraines. Des tapis flottants se forment rapidement et empêchent la pénétration de la lumière nécessaire pour la photosynthèse des autres plantes aquatiques. Ceci diminue la concentration en oxygène dans la colonne d'eau et peut provoquer la mort de nombreux invertébrés et poissons. Le débit du cours d'eau peut être entravé ce qui peut accélérer l'envasement et perturber les successions. Les larges populations d'*H. ranunculoides* réduisent considérablement les portions d'eau libre disponibles sur les berges pour la faune et augmente le risque d'inondation.

GESTION DES RISQUES

La principale voie d'introduction actuelle de *H. ranunculoides* en Belgique est la vente comme plante ornementale pour les aquariums et les étangs et son rejet subséquent dans la nature. Cette voie d'introduction diminue toutefois grâce aux actions éducatives et de sensibilisation menées dans le pays (p. ex. dans le cadre du projet AlterIAS LIFE). Pour les populations existantes, il est utile d'accroître la conscientisation des propriétaires d'étangs et du grand public sur les risques liés aux abondons sauvages de déchets de jardin dans la nature. Une fois l'espèce établie dans le milieu naturel, la dissémination peut avoir lieu, et de manière particulièrement rapide dans les eaux vives. Par conséquent, des envahissements secondaires sont très probables, à partir des populations déjà établies en Belgique mais aussi à partir des populations françaises ou néerlandaises.

L'interdiction d'importation, de commerce et de détention de *H. ranunculoides* en Belgique ne suffira probablement pas pour empêcher les nouvelles introductions et établissements du fait que (1) l'espèce se dissémine déjà largement sur le territoire et que (2) on s'attend à sa dispersion naturelle à partir des pays voisins en raison de la présence de populations denses à proximité des frontières belges. Quoi qu'il en soit, ces mesures peuvent ralentir sa dissémination actuelle.

Hydrocotyle ranunculoides est difficile à détecter à un stade précoce de développement. De ce fait, les actions de contrôle ou d'éradication sont souvent mises en place seulement à partir du moment où la plante est déjà bien établie rendant le travail extrêmement difficile et coûteux.

Etant donné que la lutte chimique contre les plantes envahissantes est très réglementée dans les milieux aquatiques en Belgique, les efforts entrepris pour contrôler les populations de *H. ranunculoides* porteront essentiellement sur la prévention et l'application de méthodes non chimiques (dans ce cas, l'arrachage mécanique).

Samenvatting

WAARSCHIJNLIJKHEID VAN VESTIGING EN VERSPREIDING (BLOOTSTELLING)

- Introductie in België

Hydrocotyle ranunculoides werd voor het eerst in 1999 in het wild in België waargenomen. Deze soort wordt in België geproduceerd en verkocht als sierplant voor aquaria en vijvers (vaak onder een foutieve soortnaam). De introductie in het wild gebeurt heel vaak door plantfragmenten die na het schoonmaken van aquaria/vijvers afgevoerd worden of door het onvoorzichtig wegwerpen van de plant. Secundaire invasie wordt veroorzaakt door natuurlijke verspreiding vanuit populaties in Nederland of Frankrijk.

- Vestigingsvermogen

Hydrocotyle ranunculoides groeit in stilstaand of langzaam stromend zoet water. De plant koloniseert de ondiepe delen en de oevers van rivieren, stromen, sloten, dammen, vijvers, meren, groeven, kanalen en zoetwatermoerassen. De plant verdraagt getijden en heel onregelmatige waterpeilschommelingen en groeit op alle grondsoorten, ook op veengrond. Ze groeit ook op goed gedraineerde bodem. De meeste zoetwaterpartijen en traag stromende waterlopen op het Belgische grondgebied, waaronder bedreigde, kwetsbare gebieden en Natura2000 habitats zijn geschikt voor vestiging van de soort. De klimaatverandering kan in de toekomst de verdere invasie in de Ardennen en in Lotharingen in de hand werken.

- Verspreidingsvermogen

Deze plant kan zich op een natuurlijke manier snel verbreiden via de stroming van het water. De verbreiding kan in de hand worden gewerkt door menselijke activiteiten zoals transport met boten of via machines die voor het schoonmaken van de waterlopen worden ingezet. De opzettelijke introductie in vijvers en onopzettelijke introductie na het schoonmaken van aquaria of vijvers of het onachtzaam dumpen van plantenresten werken de verspreiding van *H. ranunculoides* ook in de hand.

EFFECTEN VAN DE VESTIGING

- Milieu-impact

Doordat grote waternavel zich aan een hoog tempo ontwikkelt en grote oppervlakken kan innemen, verdringt de soort de meeste inheemse waterplanten en tal van oeversoorten. De drijvende, monospecifieke matten tasten de doordringbaarheid van de waterkolom voor licht aan (belangrijk voor de fotosynthese) en doen het zuurstofgehalte in de waterkolom dalen, wat op zijn beurt kan leiden tot vissterfte en het leven van ongewervelden beïnvloedt. De plantenmatten kunnen de stroming van het water belemmeren waardoor er verzilting en wijziging van de stroomsnelheid optreedt. Bovendien treedt verlies van open water aan de rand op voor dieren en brengt dit een verhoogd risico op overstroming met zich.

RISICOBEHEER

De voornaamste actuele introductiewegen van *Hydrocotyle ranunculoides* in België blijven de verkoop als sierplant voor aquaria en vijvers en het aansluitend dumpen van plantenmateriaal in het wild. Dankzij nationale educatieve acties (bv. in het kader van het AlterIAS LIFE project) lijkt de verkoop stilaan over zijn hoogtepunt heen. Voor bestaande populaties kan bewustzijnverhoging bij vijvereigenaars en het grote publiek rond de gevaren van het dumpen van tuinafval in de natuur soelaas bieden. Eens de plant zich heeft gevestigd, verspreidt ze zich via natuurlijke weg, met name via stromend water. Bijgevolg is het erg waarschijnlijk dat er binnen België secundaire invasies ontstaan, maar ook dat Franse of Nederlandse populaties hun weg naar België zullen vinden.

Hoewel als preventieve maatregel noodzakelijk, volstaat een verbod op de invoer en het bezit van en de handel in *H. ranunculoides* in België niet om de verdere introductie en vestiging ervan te voorkomen omdat (1) de soort zich over het land verspreidde, en (2) er een natuurlijke verspreiding vanuit de buurlanden wordt verwacht, doordat belangrijke populaties zich dichtbij de Belgische grenzen ophouden. Toch kunnen dergelijke maatregelen de huidige verspreiding temperen.

Hydrocotyle ranunculoides is in een vroeg invasiestadium moeilijk op te sporen. Daarom starten de bestrijdings- of uitroeiingsacties vaak pas als de plant al goed gevestigd is.

Omdat chemische onkruidbestrijding in een aquatisch milieu in België aan verregaande beperkingen is onderworpen, moeten de bestrijdingsacties zich toespitsen op preventie en niet-chemische methodes (in dit geval mechanisch verwijderen met manuele nazorg).

STAGE 1: INITIATION

Precise the identity of the invasive organism (scientific name, synonyms and common names in Dutch, English, French and German), its taxonomic position and a short morphological description. Present its distribution and pathways of quarantine concern that should be considered for risk analysis in Belgium. A short morphological description can be added if relevant. Specify also the reason(s) why a risk analysis is needed (the emergency of a new invasive organism in Belgium and neighboring areas, the reporting of higher damages caused by a non native organism in Belgium than in its area of origin, or request made to import a new non-native organism in the Belgium).

1.1 ORGANISM IDENTITY

Scientific name : *Hydrocotyle ranunculoides* (L.F., 1781)

Synonyms:
Hydrocotyle adoënsis Hochst. 1841
Hydrocotyle americana Walt. 1788
Hydrocotyle batrachioides DC 1830
Hydrocotyle cymbalarifolia Muhl. 1813
Hydrocotyle natans Cirillo 1788
Hydrocotyle nutans G. 1830
Hydrocotyle ranunculoides f. minima Kuntze 1898
Hydrocotyle ranunculoides var. *genuina* Urban 1879
Hydrocotyle ranunculoides var. *natans* (Cirillo) Urban 1879

Common names : Water-Pennywort, Floating Pennywort, Floating Marsh-Pennywort, Irish Marsh Pennywort, Greater Water Pennywort (GB), Hydrocotyle flottante, Hydrocotyle fausse-renoncule, Hydrocotyle à feuilles de renoncule (F), Grote Waternavel, Amerikaanse Waternavel (NL), Großer Wassernabel (DE), Sombrerito de agua, Redondita de agua (Sp).

Taxonomic position: Domain: Eukaryota / Kingdom: Plantae / Phylum: Spermatophyta / Subphylum: Angiospermae / Class: Dicotyledonae / Order: Apiales / Family: Apiaceae / Genus: *Hydrocotyle* / Species: *Hydrocotyle ranunculoides*

1.2 SHORT DESCRIPTION

Hussner et al. (2012) provide a clear description of *Hydrocotyle ranunculoides*: « *H. ranunculoides* is an entirely glabrous, stoloniferous, perennial aquatic plant species. Stems float in the water or creep onto the shore and the plants root freely from nodes at about 3-10 cm intervals, roots are profuse and hairlike. The alternate leaves are emergent or floating and sit above the horizontal stem on fleshly petioles of up to 40 cm long. The leaves are non-peltate, suborbicular to reniform with a cordate base, (25) 40 – 100 (180) mm in diameter, and usually broader than long. They are shallowly or deeply incised into 3-7 rounded, crenate or lobulate subequal lobes [Figure 1]. Leaf matter extends up to 40 cm above the water surface and the interwoven mat of roots and stems can sink up to 50 cm into the water. The flowers are hermaphrodite, white and grouped by 5 to 10 in a small umbel. The inflorescence is borne on a leafless stalk, 1-6 cm in length and remaining shorter than the petioles. The flowers lack sepals, and have 5 unconnected petals and 5 stamens. The ovary is inferior, two-lobed and has 2 styles. The schizocarp fruits are brownish, nearly round and flat, 2 – 2.5 mm long and 3 – 3.5 mm wide, with faint ribs and divided into two halves, each with a small persistent stalk (Tutin et al., 1968; Martin & Hutchins, 1981; Casper & Krausch, 1981; Huckle, 2002; Hussner & van de Weyer, 2004). Van de Wiel et al. (2009) developed a DNA barcode that discriminates against closely related species ». The chromosome number of the species is reported as 2n=24 (Federov, 1974).

1.3 ORGANISM DISTRIBUTION

Native range

The origin of *Hydrocotyle ranunculoides* is most often considered to be North America (e.g. Newman & Duenas, 2010), but the presence of co-evolved insect herbivores suggests a South American origin with spread through Central America to North America at some time in the recent past (Newman et al., 2009). The native range of *H. ranunculoides* is, in fact, poorly defined and literature abounds in confusing and contradictory information on this issue. For instance, the species is sometimes considered to be native to the Americas (e.g. EPPO, 2009c), although some authorities believe that the plant is also native from parts of Africa (e.g. Hussner et al., 2012).

Introduced range: the case of Europe

Hydrocotyle ranunculoides is reported from Europe (Italy) since the beginning of the 17th century (Columna, 1616; Figure 2). Dortel et al. (2011) explain:

« *H. ranunculoides* est décrit dans un ouvrage italien du début du 17ème siècle (Columna, 1616) dans la région de Naples, où la plante est nommée « *Ranunculus Aquaticus umbilicato folio* » et citée " *in palustris stagnantibus* " : la planche illustrée qui accompagne la description en latin ne laisse pas de doute sur l'identité de la plante [Figure 2]. En 1833, Antonii Bertelonii indique toujours la présence d'*H. natans* (synonyme [of *H. ranunculoides*]), qu'il a récolté en Campanie à Naples où elle était abondante, mais aussi en Sicile (près de Syracuse) et en Sardaigne, et même à Melazzo, commune du Piémont situé au nord du pays. De nos jours, la plante est toujours signalée en Sicile, Sardaigne et Campagnie, ainsi qu'en Toscane, Latium et Calabre ; elle n'est plus indiquée dans le Piémont. Tous ces éléments portent à penser qu' *H. ranunculoides* est présent sans interruption depuis au moins le début du 17ème siècle. L'arrivée initiale en Italie d'une espèce du nouveau monde pose question : les jardins botaniques ont importé des plantes en Italie depuis le 16ème siècle et une acclimatation ancienne n'est donc pas à exclure. Malgré tout, *H. ranunculoides* est considérée comme une plante non indigène localement invasive en Sardaigne (où elle était déjà présente au moins depuis 1833). »

Minus cogn. ſtirp. & rar. 315

Ranunculus Aquaticus vmbilicato folio. Cap. CXLIII.

MAGIS affinitatem ſequentes, quàm effigiem, huc inſerendam cenſuimus hanc: ne ſimilitudine foliorum deceptus aliquis pro Cotyledone accipiat, atque vt vulgò dicimus, *feruentem aquā vſtulato addat*: Acris enim & exulcerans natura cum ſit, contrario Cotyledoni verò refrigeranti efficacia magno detrimento experiretur. In paluſtribus ſtagnantibusq. aquis oritur hæc innatās tenuiſſimis fibris limo nixa, è ſingulis geniculis repentis caulis rubeſcentis carnoſi exeuntibus; ex quibus folia prodeunt ſemipedalibus rotundis læuibus carnoſiſq. petiolis in ſummo tenuioribus inſidentia, orbiculata læuia, per ambitum rotundis crenis diuiſa Cotyledonis paria & admodum æmula prima facie, vt quiſque fallatur: ſed huic Maluæ modo circa petiolum diuiſa ſunt & aperta folia, [non vt Lobellius & Pena integra & vmbilicata depinxerũt,] & quinq. maioribus inciſuris partita, vt in Cotyledone. Flores in nudis petiolis carnoſis læuibus ſemipedalibus Ranunculo ſimiles, atque etiam capitula & ſi rotundiora, & ſemina. Acri ſapore eſt atque exulcerante: Quare non Cotyledon ſed Ranunculus appellanda effigie Cotyledonis, rotundifolius. Miramurque Penam & Lobellium pro Plinij Callitriche folijs lenticulæ ſupponere hanc Cotyledone maiora quandoque folia habentem nedum paria. Pro qua nos aliam aquaticam depinximus Neapoli in Sebeti paluſtribus obſeruatam aquaticæ lenticulæ folijs binis in tenuiſſimis caulibus fructu iuxta illa tetragono: Sed nobis Plinius de Trichomane loqui videtur: ideo nec noſtra Callitriche habenda.

Nominis cauſa. Vires. Locus. Deſcriptio. Sapor. Lib. 25. c. 11.

Ranunculus trichophyllon aquaticus medioluteus. Cap. CXLIV.

IISDEM locis innatat & hæc, eidem familiæ connectenda: flore & fructu ſimilis, atque etiam acrimonia; Folijs capillaribus Abrotoni maritimi, vel Artimiſiæ leptohyllæ tenuioribus breuibus petiolis, repenti cauliculo carnoſo geniculato densè hærentibus, ex quorum cauis veluti ex folliculis, vt in Vmbelliferis thyrſuli nudi breues digitales producuntur elati, floribus quinquepartitis ſtellatis albis, ſed circa centrum luteis Iunio, vt medio luteo flore appellerur. Fructus deinde capitatus rotundus ſquamoſus ſemine

Rr 2 ſuccedit,

Nominis cauſa. Sapor. Deſcriptio.

316 Fabii Columnæ

Figure 2 : Description and illustration of *Hydrocotyle ranunculoides*, under the name *Ranunculus Aquaticus umbilicato folio* in Columna (1616) (Book available online at: http://www.animalbase.uni-goettingen.de/zooweb/servlet/AnimalBase/home/reference?id=2308).

However, the first note of concern over the potential of *H. ranunculoides* to become a weed was only published in 1936 (Mathias, 1936). Moreover, so far, the species has mainly expressed invasiveness in North-Western European countries (namely, Belgium, France, Germany, the Netherlands, and the United Kingdom), while the areas which seem to have the most suitable climatic conditions (see § 2.1.4.B) are the Mediterranean and the Atlantic areas. The invasiveness of *H. ranunculoides* may therefore be due to other elements such as the use of the plant, the eutrophication of waters (EPPO, 2009c), or the dispersion of particular horticultural variety (or varieties). The plants reported as invasive in northern Europe are believed to originate from North America (Hussner *et al.*, 2012).

Detailed presence (both native and introduced ranges) of *H. ranuculoides* by continent (Figure 3; EPPO, 2009c; www.cabi.org; www.q-bank.eu; and references there-in):

- **North America**: Canada (British Columbia, Quebec), 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), Mexico.
  [Further details on records in the USA can be found on the USDA-NCRS website (http://plants.usda.gov/java/profile?symbol=HYRA)].

- **Central America & Caribbean**: Costa Rica, Cuba, Guatemala, Nicaragua, Panama.

- **South America**: Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, Uruguay.
- **Europe**: Belgium, France, Germany, Ireland, Italy, The Netherlands, Spain [however, presence in this country is uncertain as it is possible that records referred to misidentified *H. vulgaris* or *H. verticillata* specimens (EPPO, 2009c)], United Kingdom.
  [According to GB Non-Native Species Secretariat, the species is also present in Austria, Denmark and Portugal (Lansdown, 2011), however EPPO (2009c) indicates that these mentions are inaccurate].

- **Africa**: Angola, Congo Democratic Republic, Ethiopia, Kenya, Madagascar, Malawi, Rwanda, Sudan [only "possible" according to EPPO (2009c)], Zimbabwe, Zambia, Uganda, Tanzania.

- **Asia**: Iran [this record is, however, dubious since we did not find any mention about *Hydrocotyle ranunculoides* in Naqinezhad et al. (2007), the single publication regularly cited when documenting the presence of the species in Iran], Israel, Lebanon, Japon [National Institute for Environmental Studies-NIES: http://www.nies.go.jp/biodiversity/invasive/DB/detail/81150e.html), Syria, Yemen.

- **Oceania**: Australia (Queensland, Western Australia) [since 1983 according to Ruiz-Avila & Klemm (1996)].

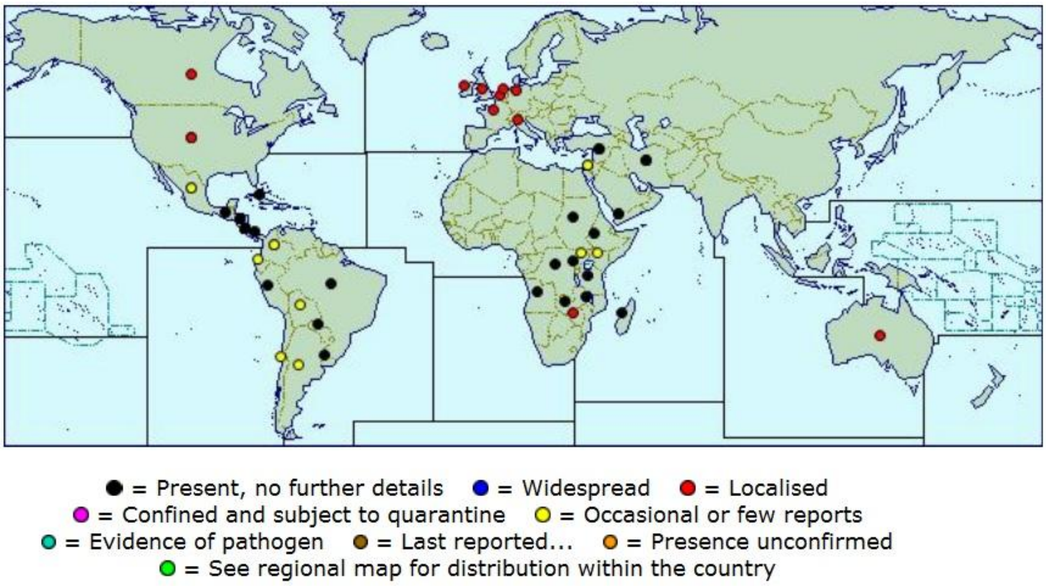


Figure 3: Geographic distribution of *Hydrocotyle ranunculoides.* Source: http://www.cabi.org

1.4 REASONS FOR PERFORMING RISK ANALYSIS

In its introduced range (e.g. Europe), *H. ranunculoides* competes with many native plant species. These include littoral marsh plants as well as submerged aquatic plants. These are overgrown and shaded out by the extensive beds or floating carpets. Species richness of native aquatic plants may be reduced by more than 50% and submerged species may even disappear entirely (Nijs et al., 2009).

Hydrocotyle ranunculoides can cause major problems in nature reserves, recreation areas and intensely managed waterways (EPPO, 2009a; Williams et al., 2011). The floating mats not only affect the penetration of light available for photosynthesis, but also reduce oxygen levels in the water column which can result in fish mortality and influence invertebrate life (EPPO, 2009a; Stiers et al., 2009). If leading to sediment anoxia, the release of nutrients and potentially toxic substances can be enhanced. Rapid biomass accumulation fuels decomposition processes, alters the composition of the bottom substrate and expedites the infilling of shallow standing waters. In flowing waters, drainage is impeded and siltation increases with heavy infestation (Hussner et al., 2012).

The characteristics that indicate its invasiveness are typical of many aquatic weeds (McChesney, 1994; Newman & Dawson, 1999): high growth rates, adaptability to prevailing nutrient conditions, very effective vegetative propagation, plasticity in growth response, overwintering to avoid low temperature stress, resistance to herbivory, resistance to chemical control, and absence of specific pests and diseases in introduced environments.

H. ranunculoides has already proved to be difficult to control because of rapid growth rates combined with an ability to regrow from a single node (Newman & Dawson, 1999; Hussner et al., 2012). It is very likely to spread around watercourses and become a major nuisance for water resource managers in the future.

In Belgium, the Netherlands, and the UK in particular, it is considered a serious invader having escaped into the wild following its introduction to Europe in the 1980s through the aquatic nursery trade (often wrongly labeled, as the native *H. vulgaris* for instance [EPPO, 2009c]). The serious threat it poses to habitats has led to it being black listed in Belgium (Branquart, 2012), added to Schedule 9 of the Wildlife and Countryside Act in the UK (www.cabi.org), and banned in the Netherlands (Pot, 2002). It has spread into water bodies in a number of other European countries including France, Germany and Italy (see § 1.3).

It is expected that *H. ranunculoides* will profit from increasing temperatures in Europe and become more invasive in Central and North Europe (Hallstan, 2005; Hussner & Lösch, 2007; Hussner, 2008; Fried et al., 2009).

2.1 PROBABILITY OF ESTABLISHMENT AND SPREAD (EXPOSURE)

Evidence should be available to support the conclusion that the non-native organism could enter, become established in the wild and spread in Belgium and neighbouring areas. An analysis of each associated pathways from its origin to its establishment in Belgium is required. Organisms intentionally imported maybe maintained in a number of intended sites for an indeterminate period. In this specific case, the risk may arise because of the probability to spread and establish in unintended habitats nearby intended introduction sites.

2.1.1 Present status in Belgium

Specify if the species already occurs in Belgium and if it makes self-sustaining populations in the wild (establishment). Give detail about species abundance and distribution within Belgium when establishment is confirmed together with the size of area suitable for further spread within Belgium.

The species was first noticed in 1998 in different water bodies around Ghent (Verloove & Heyneman, 1999). In 2004, the species was already widespread and well established in Flanders, especially in the East-Flanders and Antwerp provinces (Denys et al., 2004; Figure 4). Nowadays, the species continues its spread, mainly in Flanders (Figure 5) but it is also present in the Brussels region and, since 2000, in part of the Walloon region (Verloove, 2006, Adriaens et al., 2009; Figure 6).

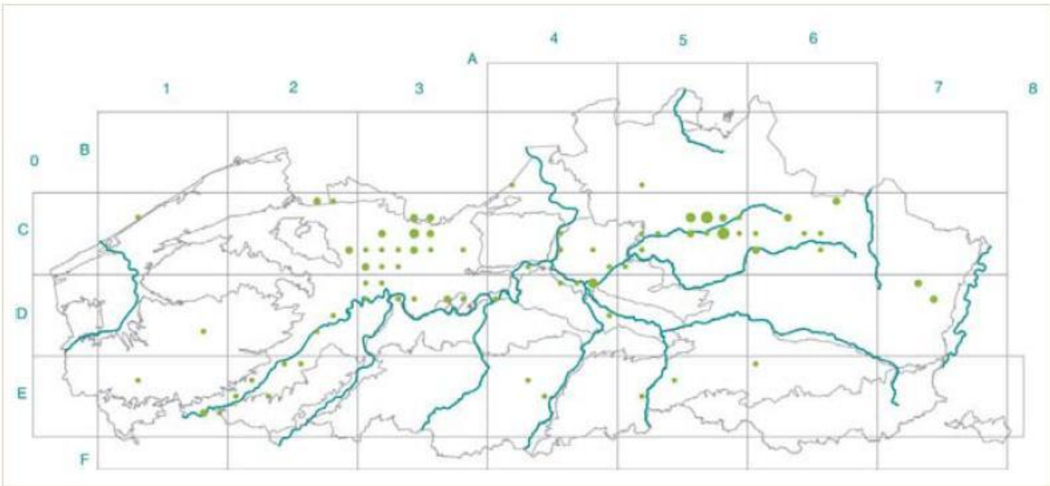
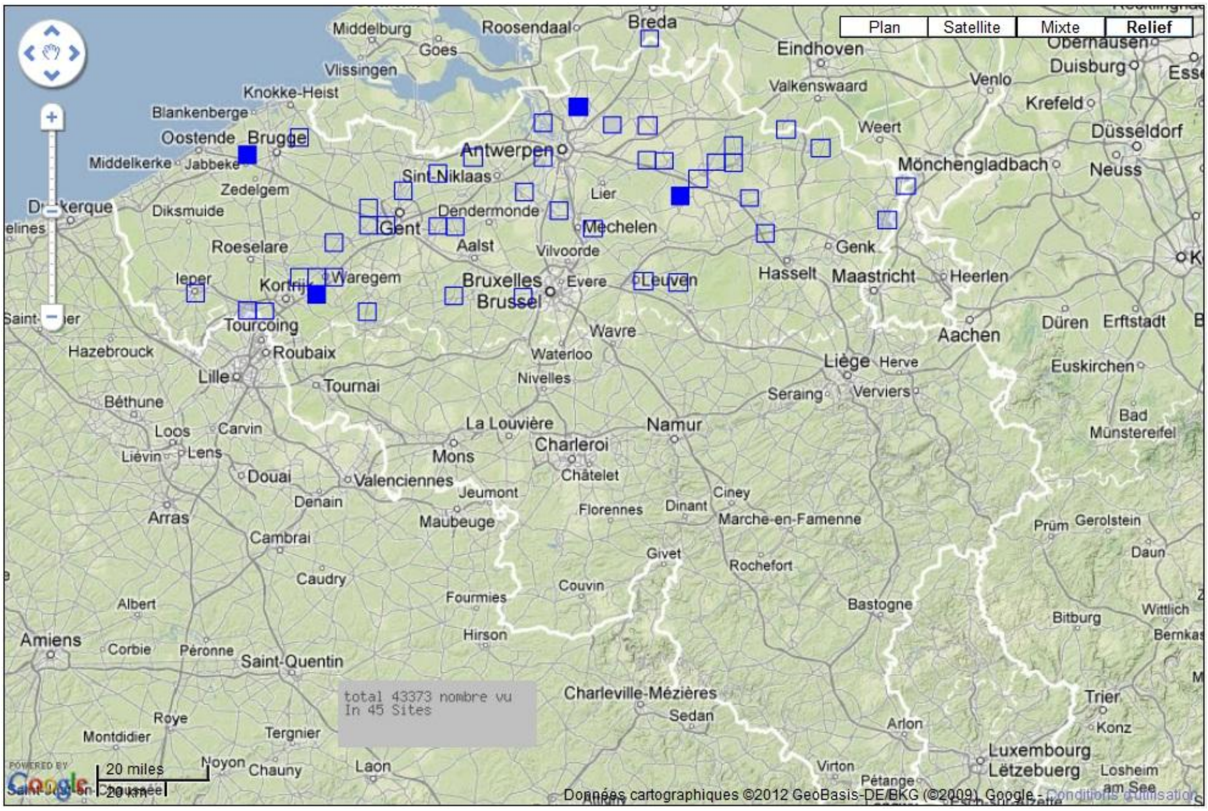
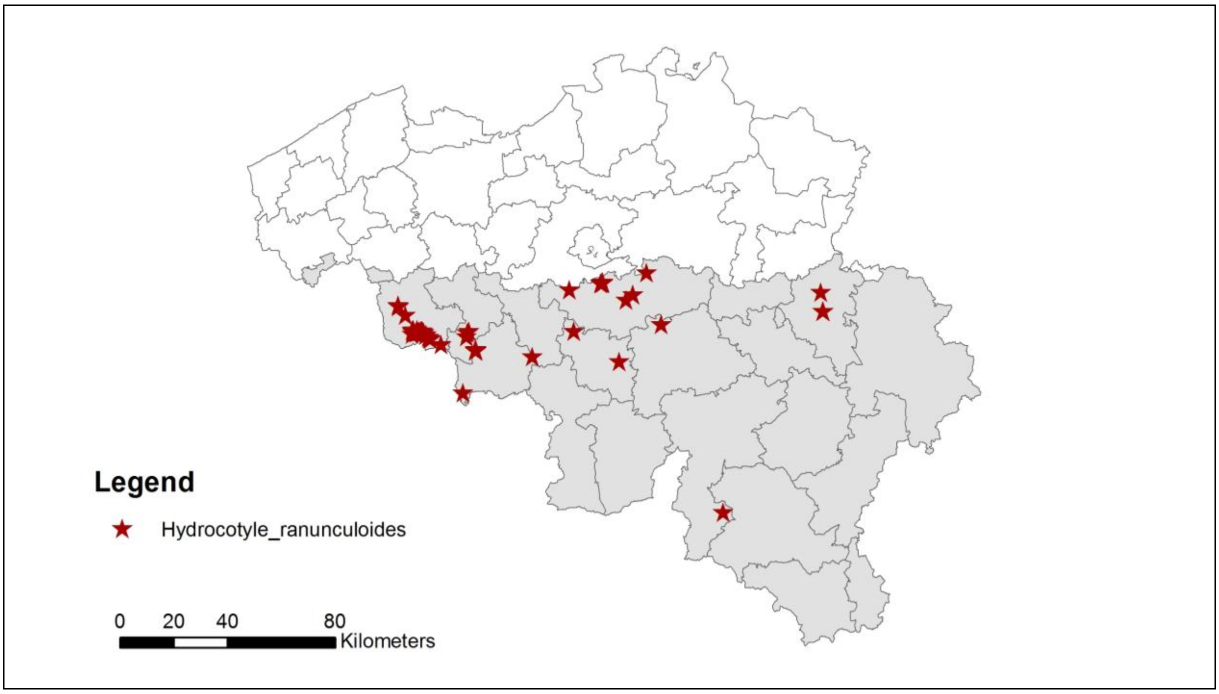


Figure 4: Distribution of *H. ranunculoides* (green dots) in the Flemish region in 2004 (from Denys et al., 2004).



<u>Figure 5:</u> Geographic localizations of *Hydrocotyle ranunculoides* sightings in the Flemish region from Jan. 2000 to Dec. 2012. The squares on the map are based on the local utm (gmap_local); the transparancy represents the maximum number of individuals; blue squares means only observations with the tag escape are known (Source: http://observations.be).



<u>Figure 6:</u> Geographic localizations of *Hydrocotyle ranunculoides* sightings in Wallonia (Data from DEMNA [Département de l'Etude du Milieu Naturel et Agricole], 2000-2012; Map by Yaëlle Bouyer, RBINS).

2.1.2 Present status in neighbouring countries

Mention here the status of the non-native organism in the neighbouring countries.

- **England:**

In the 1980's, *Hydrocotyle ranunculoides* was brought to Great Britain by the aquatic nursery trade to sell as a plant for tropical aquaria and garden ponds (Newman & Duenas, 2010). In September 1990, *H. ranunculoides* was first recorded as a naturalized alien when discovered in the River Chelmer at Chelmsford (although the specimen remained unidentified until September 1991) (Payne, 1994). By 1992, it was present in the Chelmer-Blackwater canal, up to 12 km downstream of Chelmsford (Payne, 1994). The following reported occurrence was made in *ca.* 1994 on the River Wey (Newman & Dawson, 1999). Two years later, the growth of *H. ranunculoides* along this channel necessitated cutting to maintain navigation and access to the water for anglers (Newman & Dawson, 1999). Further reports indicate that the species spread rapidly over the country (Burton, 1996, 1998). It is considered that the presence of *H. ranunculoides* in the wild is a likely result of release from aquaria and garden ponds (Newman & Dawson, 1999; Newman & Duenas, 2010).

Nowadays, *H. ranunculoides* is reported from about 150 sites in the south of England and south Wales (Newman & Duenas, 2010). More precisely the species is concentrated in an area around and to the north of London into coastal Norfolk, with a few records along the south coast, the Gwent Levels and the West Midlands, from Cheshire northwards (National Biodiversity Network's Gateway, 2013; Figure 7).

H. ranunculoides is one of five aquatic plants which are to be banned from sale in the UK from April 2014 (Kinver, 2013).

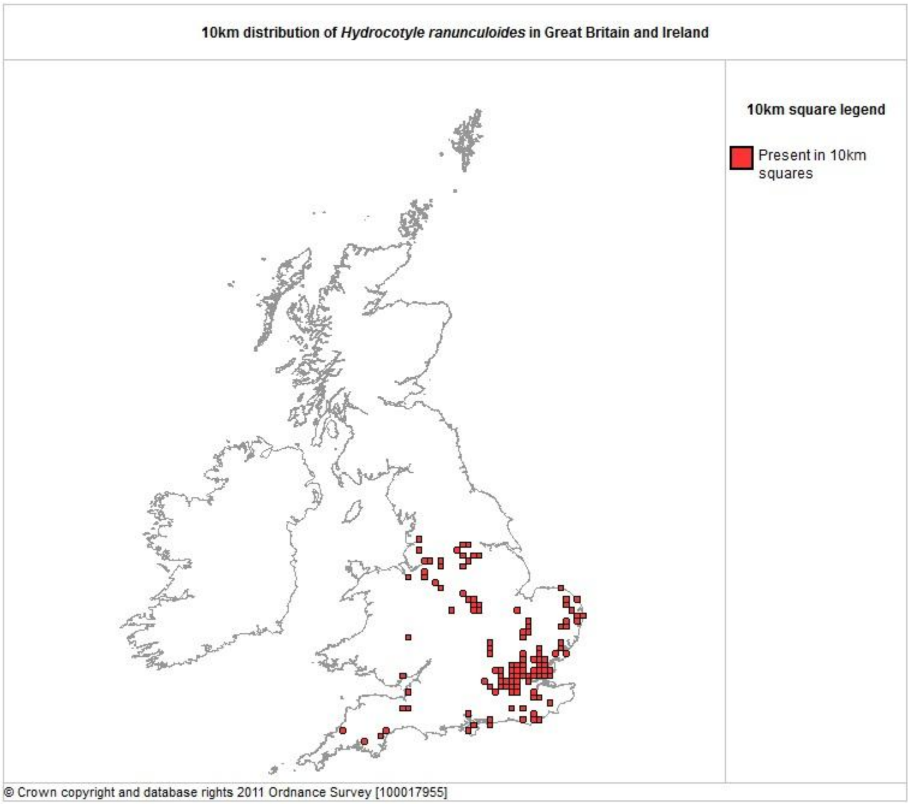


Figure 7: *Hydrocotyle ranunculoides* geographic distribution in the UK (Source: National Biodiversity Network's Gateway, 2013: http://data.nbn.org.uk/gridMap/gridMap.jsp?allDs=1&srchSpKey=NHMSYS0000459812).

- **Ireland:**

The earliest record of *H. ranunculoides* in Ireland was made on 31 August 2002 at the flooded clay pits at Glastry in County Down (Day, 2004). The species was subsequently recorded at Dunadry, County Antrim on 14 February 2004 (Day, 2004). The number of sites in Ireland appears to have remained limited so far (Hackney, 2006) but the species is considered locally established (Minchin, 2007) and continues to be recorded in sites where it was previously unknown (e.g. Comber, county Down [National Biodiversity Data Centre, 2012]; Figure 8).

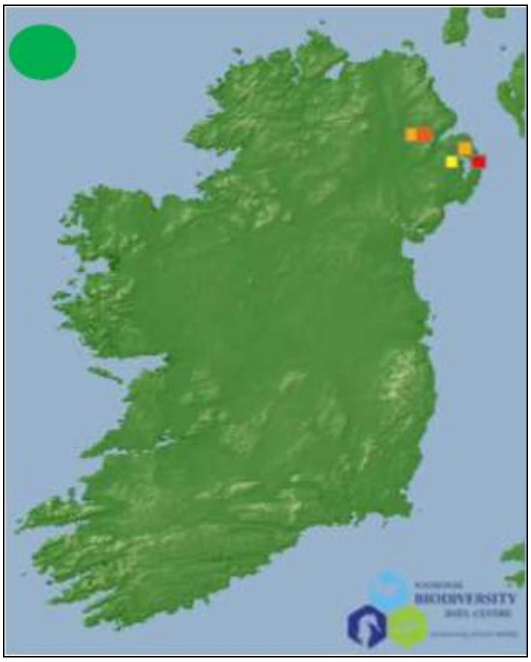


Figure 8: Geographic distribution of *H. ranunculoides* in Ireland. The green dot on the upper left corner means that all known records are displayed. The color intensity of the record square from yellow to red reflects the increase in the number of records for each 10 km² (from National Biodiversity Data Centre, 2012).

- **France**:

H. ranunculoides was first discovered in France in the 40's in Essonne, near Paris (Dortel et al., 2011), although Thiébaut (2007) indicates that the species was already found in this country in 1820. Today the species can be found in Aquitaine (4 stations), Nord-Pas de Calais (around 10 stations, where the plant is locally invasive), Normandie (4 stations), Picardie (one observation in 2005, at Mont-l'Évêque, where the few plants were immediately killed), Bretagne (1 station), Pays de Loire (4 stations), Ile-de-France (at least 10 stations) and Rhône-Alpes (Dortel et al., 2011; Levy et al., 2011; Figure 9). A single station was known from Corsica but was not found since 1968 (Dortel et al., 2011).

In the Nord-Pas de Calais region, the species was first found in 2004 in the Scarpe canal, close to Saint-Amand-Les-Eaux (Toussaint & Hendoux, 2005). In 2005, the species was found in the Vieille Lys

River, near Haverskerque (Toussaint & Hendoux, 2005). In 2006, a mechanical removal of the Vieille Lys River's population was attempted under scientific supervision (Toussaint, 2007). The population was, however, still reported in the following years (Toussaint et al., 2008; Levy et al., 2011). In 2008, the species was well established in the ponds of Lac du Héron (Villeneuve-d'Ascq) and at Prés du Hem (Armentières) (Toussaint et al., 2008). In 2011, the species was observed in nearly 10 localities, where it was often spreading (Levy et al., 2011; Figure 9).

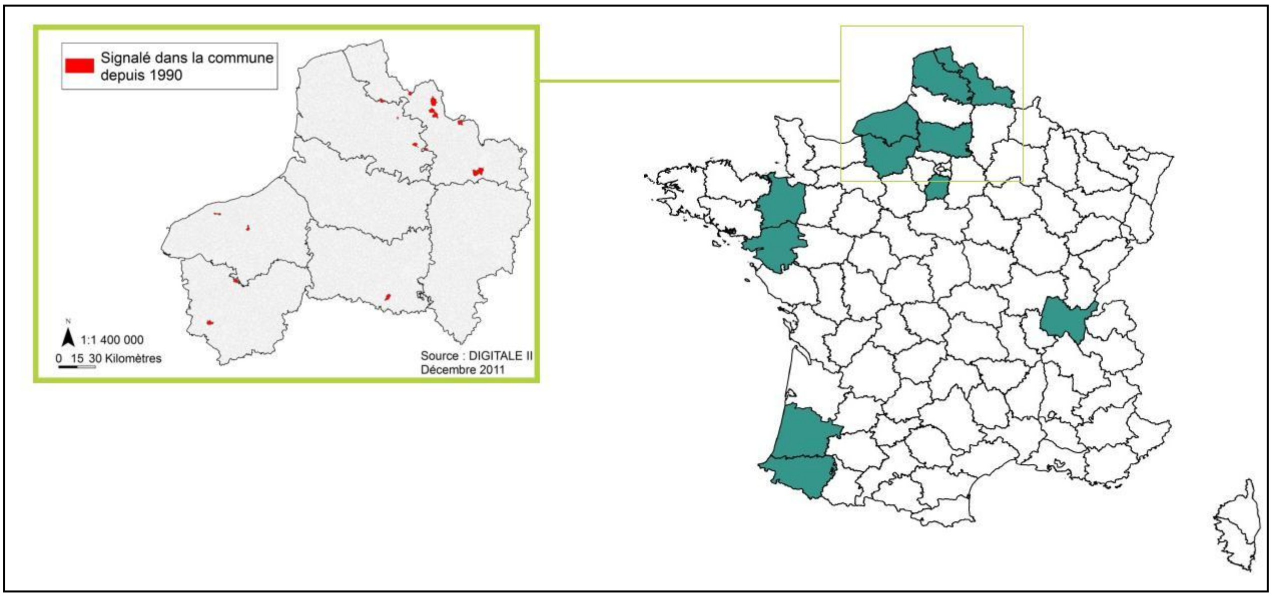


Figure 9: French departments where *Hydrocotyle ranunculoides* is reported, with detailed distribution (data from 1990 to 2011) for the North-West region (box on the left). Modified from Levy et al. (2011) and from « Réseau des Conservatoires botaniques nationaux », http://www.centrederessources-loirenature.com/mediatheque/especes_inva/fiches_FCBN/Fiche%20-%20Hydrocotyle%20ranunculoides_sr.pdf.

- **Germany**:

Floating Pennywort was recorded for the first time in Germany in 2003 (Hussner, 2008; Hussner et al., 2010) from several water bodies in North Rhine-Westphalia (Hussner & van de Weyer, 2004; Hussner et al., 2005). Since then the species is considered as rare but has invaded numerous small lakes in North Rhine-Westphalia (Hussner et al., 2010). It is also present in Nieder-Sachsen (Figure 10) and is expected to potentially spread rapidly to most of the western parts of Germany (Hussner, 2007, 2008; Hussner & Van de Weyer, 2004; Hussner et al., 2005; Hussner & Lösch, 2007; Hussner et al., 2010).

- **The Netherlands:**

In the Netherlands, *H. ranunculoides* was first detected in 1994 in a watercourse near Utrecht (Baas & Holverda, 1996a, 1996b; Baas & Duistermaat, 1999; Pot, 2002; Nienhuis, 2008), although initial introduction possibly occurred as early as the late nineteen seventies (Jansen, 2010). In 1995, the watercourse was fully overgrown over a distance of 2 km. In 1998, the species has become a prominent nuisance in North Brabant province (e.g. in the Dommel River; Nienhuis, 2008). In 2000, a leaflet was distributed to warm water managers and the public about this plant (Pot, 2002). However, in 2004, the plant had spread all over the country in ditches, canals, brooks and smaller rivers, except in brackish localities (Nienhuis, 2008). The plant, commercially available in the Netherlands as an ornamental plant until 2001 (Pot, 2002; EPPO, 2006), has probably escaped from garden ponds (De Mars & Bouman, 2002; Pot, 2002; Nienhuis, 2008).

H. ranunculoides is now considered naturalized and well established. It still can be found throughout the country (Figure 11), and it causes serious problems in some localities (Baas & Duistermaat, 1999; Pot, 2002).

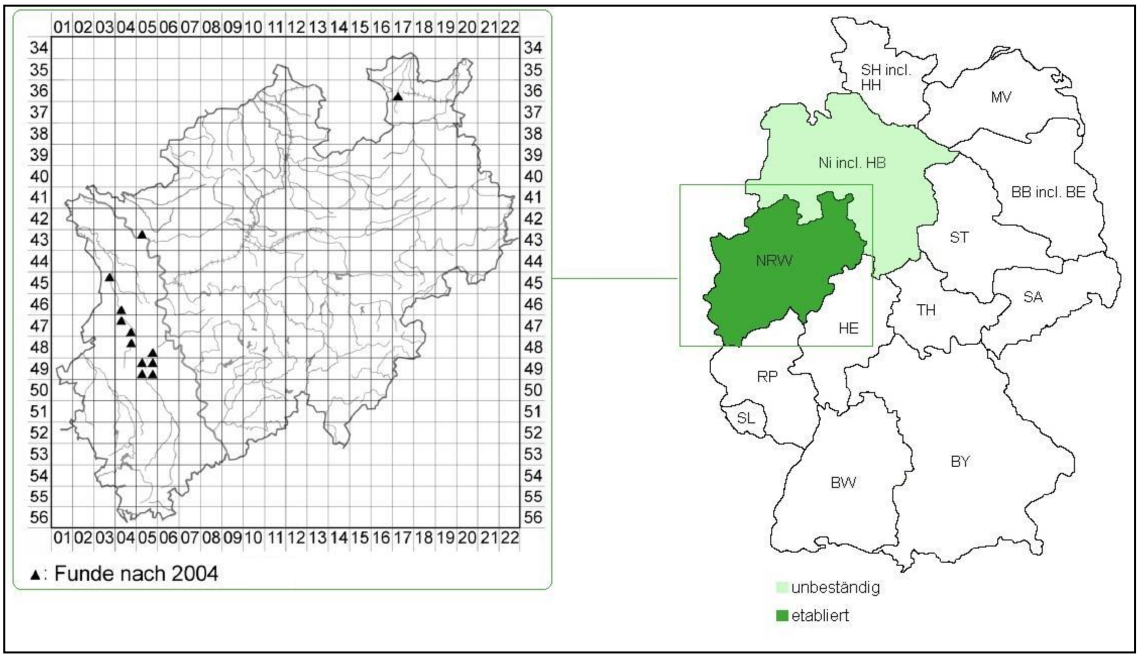


Figure 10: Geographic distribution of *Hydrocotyle ranunculoides* in Germany, with detailed distribution (2004-2008) for the North Rhine-Westphalia state (box on the left). Modified from Hussner (2008, 2010).

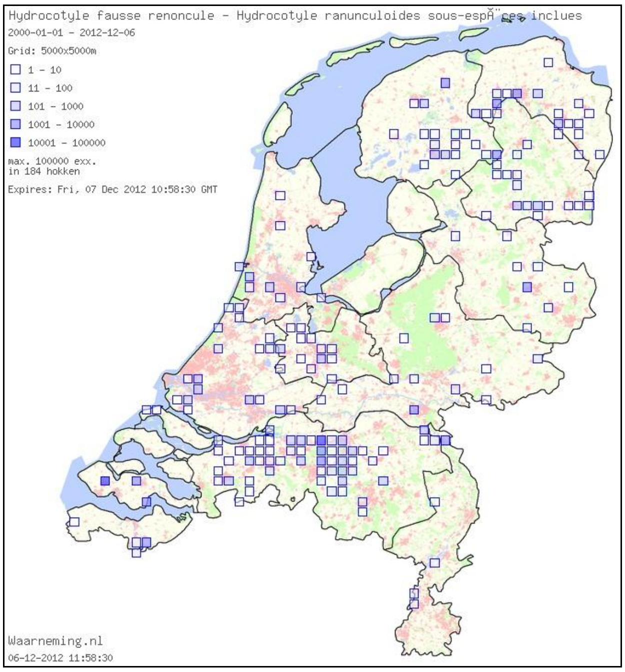


Figure 11: Geographic localizations of *Hydrocotyle ranunculoides* sightings in the Netherlands from Jan. 2000 to Dec. 2012 (Source: http://waarneming.nl).

2.1.3 Introduction in Belgium

Specify what are the potential international introduction pathways mediated by human, the frequency of introduction and the number of individuals that are likely to be released in Europe and in Belgium. Consider potential for natural colonisation from neighbouring areas where the species is established and compare with the risk of introduction by the human-mediated pathways. In case of plant or animal species kept in captivity, assess risk for organism escape to the wild (unintended habitats).

In Belgium, the species has been intentionally introduced for horticultural purposes and for experimental water-treatment (Verloove & Heyneman, 1999). It subsequently escaped and colonized several water courses by hydrochory (Verloove & Heyneman, 1999; Hussner et al., 2012). Colonization of isolated water bodies mainly results from discarded plants or intentional planting (Hussner et al., 2012). Increased flooding was suggested to facilitate dispersal (Hussner et al., 2012).

The species is still sold in Belgium (sometimes under an erroneous scientific name [Branquart, pers. comm. in Brunel 2009; EPPO, 2009c], as a tropical aquarium plant or for garden ponds (Haldford et al., 2011; Figure 12). A recent study suggested that Belgian horticulture professionals had, until recently, a poor understanding of ecological issues caused by invasive plants, resulting from a lack of information and awareness (Vanderhoeven et al., 2011). Fortunately, progress has been made and it becomes well known that *H. ranunculoides,* and other plants as well, may become invasive in Belgium. The species is now black listed (Branquart, 2012) and several professional and non-professional horticulturists and gardeners have agreed with a code of conduct on invasive plants in Belgium (Halford et al., 2011), developed by the AlterIAS LIFE project (Alternatives for invasive plants, http://www.alterias.be/). As a result, the species progressively disappears from catalogues of aquatic nurseries (e.g. Figure 13).

It is a notable and important advancement as *Hydrocotyle ranunculoides* is no longer imported (EPPO, 2009c). Indeed, the species has not been captured as imported during a recent study on imported plants in 10 European and Mediterranean countries (namely, Austria, Czech Republic, Estonia, France, Germany, Hungary, the Netherlands, Latvia, Switzerland and Turkey), indicating that the main pathway of dispersion for the species is production and trade within the studied region (Brunel, 2009).

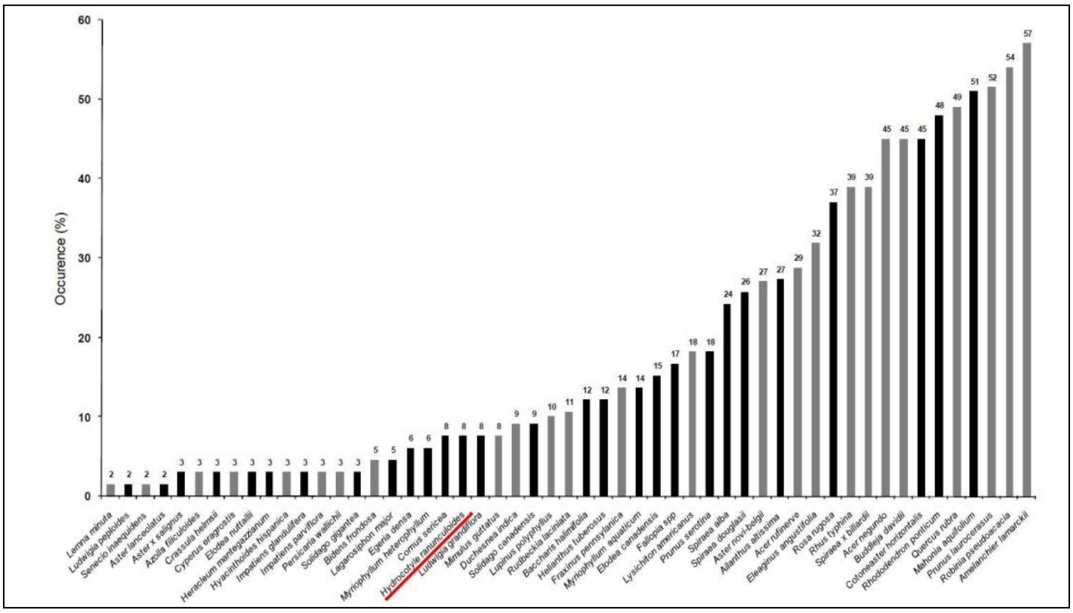


Figure 12: Occurrence of invasive plants (terrestrial and aquatic) sold in nurseries in Belgium (n = 67). Black bars: black listed species. Grey bars: watch listed species. *Hydrocotyle ranunculoides* (underlined in red) is present in 8% of the Belgian nurseries studied (modified from Halford et al., 2011).

HOUTTUYNIA cordata Chameleon (Feuillage panaché/gestreept blad)	9x9 cm	A	rose/roos	30	+10/0	VI à VIII
HOUTTUYNIA cordata Chameleon (Feuillage panaché/gestreept blad)	2,5 L	E	brun/bruin	30	+10/0	VI à VIII
HOUTTUYNIA cordata Plena (blanc/wit)	9x9 cm	A	rose/roos	30	+10/0	VI à VIII
HOUTTUYNIA cordata Plena (blanc/wit)	2,5 L	E	brun/bruin	30	+10/0	VI à VIII
HYDROCLEYS nymphoïdes(jaune/geel)	9x9 cm	B	vert/groen	5	-15/-20	VI à IX
~~HYDROCOTYLE leucocephala~~ PLANTE INVASIVE - INVASIEVE PLANT	~~9x9 cm~~	~~A~~	~~rose/roos~~	~~10~~	~~0/-30~~	--

3

PLANTES AQUATIQUES & DE MARAIS
VIJVER - EN MOERASPLANTEN

	FORMAAT POT TAILLE	PRIJS CODE PRIX	KLEUREN CODE COULEUR	cm	cm	
HYDROCOTYLE vulgaris	9x9 cm	A	rose/roos	10	0/-10	--
HYPERICUM elodes (jaune/geel)	9x9 cm	A	rose/roos	5	+10/-5	VI à VII
IRIS kaempfery (ensata) (mélange/mixt)	9x9 cm	A	rose/roos	70	+10/0	VI à VII

Figure 13: Extract from the 2013 catalogue of Aqua Production, one of the most important nurseries of aquatic plants in Belgium (http://www.aquaproduction.be/fr/plantes-aquatiques-lagunage.html). The species *Hydrocotyle leucocephala* (a possible miss-identification of *H. ranunculoides* [Branquart, pers. comm. in Brunel 2009]) was deleted from the list of sold plants. The remaining *Hydrocotyle* species (*H. vulgaris*) is native in Europe.

The plant is established and spreading in North of France and in the Netherlands (see § 2.1.2), sometimes at very close distance from the Belgian border. Because *H. ranunculoides* is easily dispersed by water and can rapidly regenerate from single small fragment (Newman & Dawson, 1999; Hussner & Lösch, 2007; Dortel et al., 2011), invasion from these neighboring populations is very probable.

ENTRY IN BELGIUM

***H. ranunculoides* is produced and sold in Belgium as an ornamental plant for aquariums and ponds (sometimes under erroneous and misleading names). Most introductions into the wild occur when fragments of plant are released after aquarium/pond cleaning or incautious disposal of plant remains. This is also valid for neighboring countries, enhancing the probability of a secondary invasion through natural dispersion of existing populations in the Netherlands or France.**

2.1.4 Establishment capacity and endangered area

Provide a short description of life-history and reproduction traits of the organism that should be compared with those of their closest native relatives (A). Specify which are the optimal and limiting climatic (B), habitat (C) and food (D) requirements for organism survival, growth and reproduction both in its native and introduced ranges. When present in Belgium, specify agents (predators, parasites, diseases, etc.) that are likely to control population development (E). For species absent from Belgium, identify the probability for future establishment (F) and the area most suitable for species establishment (endangered area) (G) depending if climatic, habitat and food conditions found in Belgium are considered as optimal, suboptimal or inadequate for the establishment of a reproductively viable population. The endangered area may be the whole country or part of it where ecological factors favour the establishment of the organism (consider the spatial distribution of preferred habitats). For non-native species already established, mention if they are well adapted to the eco-climatic conditions found in Belgium (F), where they easily form self-sustaining populations, and which areas in Belgium are still available for future colonisation (G).

A/ Life-cycle and reproduction

Hydrocotyle ranunculoides is capable of both sexual and asexual reproduction, although production of viable seeds was apparently never observed so far in Western Palearctic (Dortel et al., 2011; Hussner et al., 2012; but Newman & Duenas (2010) suggested that introduction by seed may have occurred in at least two sites in the UK through sewage treatment works]. Vegetative reproduction is thus the most common way of dispersion (Hussner & Lösch, 2007). It takes the form of ramets detaching from parent mats to spread downstream and colonize further sites (Hussner & Lösch, 2007). Regeneration capacity of *H. ranunculoides* is high since a new plant can be formed from a single node fragment (Newman & Dawson, 1999; Hussner & Lösch, 2007; Dortel et al., 2011).

Hussner (2008) and Hussner & Lösch (2007) described the life cycle of *Hydrocotyle ranunculoides* in Germany (Erf River, North Rhine-Westphalia):
Starting from small plants or fragments, plants start growing slowly in spring as soon as the ice melts. Small leaves (up to 10 cm^2) are formed that float on the water surface for the most part (Figure 14a). With increasing temperature, photoperiod and light intensity, the leaves become larger and reach a height of up to 40 cm above the water (Figure 14b). Growth rate is highest in the summer months, June and July. The plants flower and fruit between May and October as the stands get more and more dense [In the Netherlands, *H. ranunculoides* has also been found to flower and fruit as early as May (Meijden et al., 2001)]. With temperature and light availability decreasing in autumn, plants develop smaller fresh leaves (Figure 14c). At this time, plants have both floating and submerged leaves. Most of the leaves die off as night frosts set in (floating leaves die when enclosed in ice, but submerged stems and leaves survive the winter [Figure 14d]). Plants grow out again in spring from the persisting small submerged plants and leafless stolons (Hussner et al., 2012).

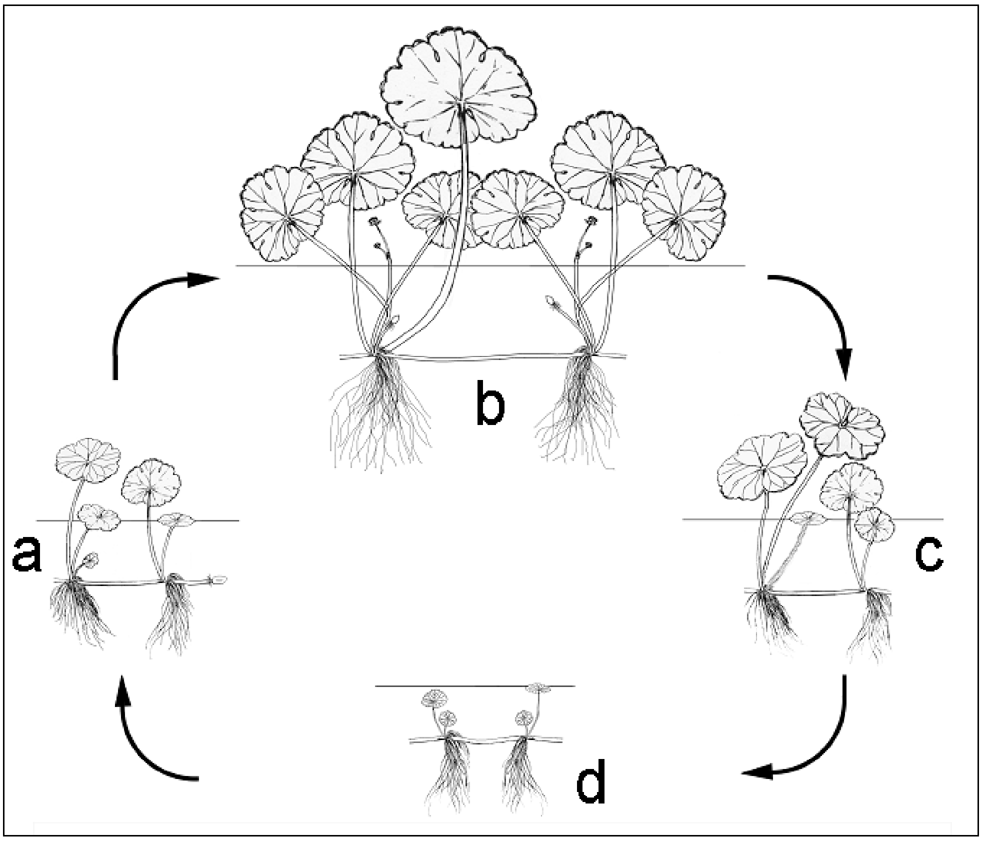


Figure 14: Life cycle of *Hydrocotyle ranunculoides* in Germany (Erf River, North Rhine-Westphalia; Source: Hussner & Lösch, 2007).

Growing rate and regeneration capacity of *H. ranunculoides* are impressive. Indeed, this species grows and regenerates rapidly forming floating mats that may extend up to 20 cm per day, for instance in the UK (Newman et al., 1999). 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). Growth in waste water treatment systems has reached 19.7 tons per hectare (Boyd & Bayne, 1988). Studies in Germany showed increased growth under high nutrient conditions up 0.132 g g^{-1} dry weight d^{-1} (Hussner & Lösch, 2007). In Australia, *H. ranunculoides* doubles its biomass in 3 days, and in the UK doubling times vary between 4 and 7 days in summer, depending on the availability of nitrate (Newman & Duenas, 2010).

B/ Climatic requirements[2]

The species is endangered in parts of its native range (USDA; http://plants.usda.gov/java/profile?symbol=HYRA), where it is vulnerable to low temperatures (EPPO, 2009c). 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 remaining

[2] Organism's capacity to establish a self-sustaining population under Atlantic temperate conditions (Cfb Köppen-Geiger climate type) should be considered, with a focus on its potential to survive cold periods during the wintertime (e.g. plant hardiness) and to reproduce taking into account the limited amount of heat available during the summertime.

dormant to avoid low temperatures and new plants can grow up in spring from these overwintering parts (Hussner & Lösch, 2007; EPPO, 2009c; Hussner et al., 2012).

H. ranunculoides is an helophyte and thus prefers sunny conditions (Hussner & Lösch, 2007). It reaches maximum photosynthetic rates at temperatures between 25 and 35°C, 18 µmol CO_2 $m^{-2}s^{-1}$ and high photon flux densities (~ 800 µmol photons $m^{-2}s^{-1}$; Hussner & Lösch, 2007).

Potential distribution of *H. ranunculoides* in Europe was mapped using the CLIMEX software and its current distribution in Central and North America (Figure 15). Results indicate that the species could widely established, mainly in regions with Atlantic or Mediterranean climatic conditions characterized by mild winters (Fried et al., 2009). Moreover, Hussner & Lösch (2007) believe that global warming, including longer growing seasons and higher temperatures in summer, will support the further spread of the species. In this direction, Hallstan (2005) describes the risk of future establishment in Sweden (i.e. in a country much higher in latitude than Belgium!) of *Hydrocotyle ranunculoides* according to different climate change scenarios (Figure 16), and concludes that this will become more likely as temperatures increase.

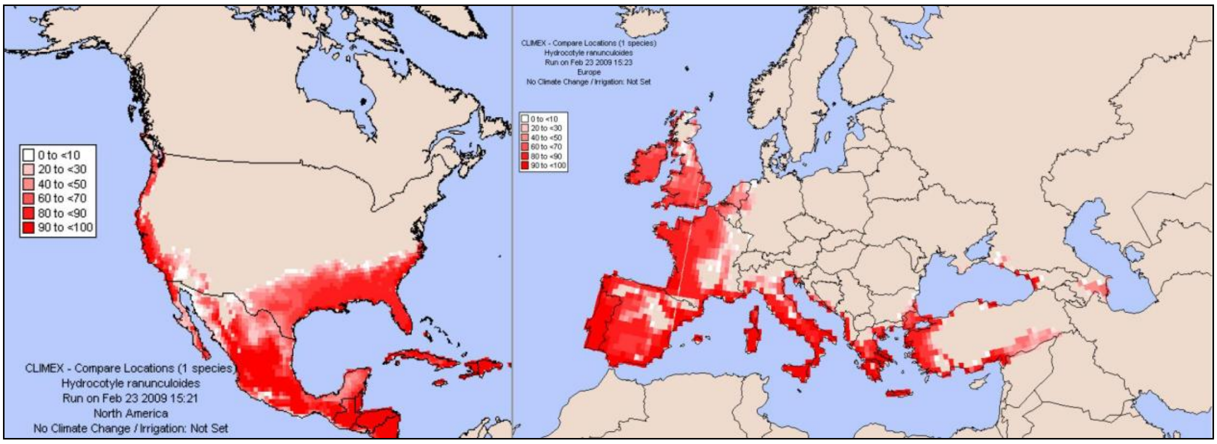


Figure 15: Potential distribution map of *H. ranunculoides* in Europe (right) according to climate occurring in its current distribution range in North and Central America (left) (from Fried et al., 2009).

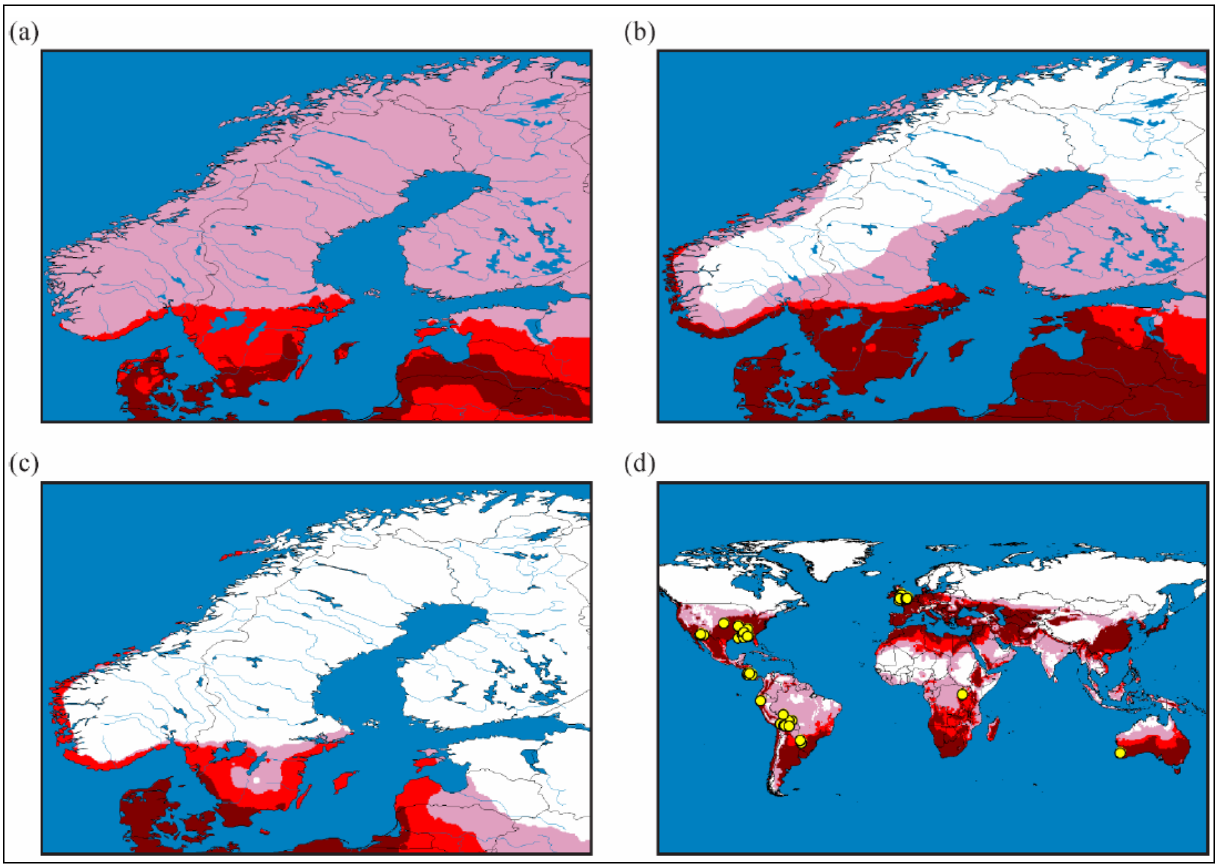


Figure 16: Modeled establishment possibility for *Hydrocotyle ranunculoides* for (a) 2071-2100 climate, A2 emission scenario; (b) 2071-2100 climate, B2 emission scenario; (c) 1961-1990 climate (SweClim) and (d) 1961-1990 climate (IPPC). Yellow dots: actual occurrences; white area: no probability, pink, red and dark red areas: low, intermediate and high probability, respectively. Scenarios A2 and B2 correspond to those produced by the Intergovernmental Panel on Climate Change (IPCC), see references and details in Hallstan (2005) (Source: Hallstan, 2005).

C/ Habitat preferences[3]

Hydrocotyle ranunculoides grows in stagnant and slowly running freshwaters (Newman & Duenas, 2010), such as the shallow parts and banks of rivers, streams, ditches, mill weirs, ponds, lakes, pits, canals and freshwater marshes (Hussner & Meyer, 2009; Hussner et al., 2012). McChesney (1994) identifies two distinct habitats where *H. ranunculoides* occurs: high altitude tropical lakes of East Africa and South America; and low altitude coastal regions of the temperate zone of the USA, South America, Australia and Europe. The species supports tidal conditions or strong irregular water-level variations and grows on all types of soil, including peat (Hussner et al., 2012). It even grows on drained soils but performs best under waterlogged conditions (Hussner & Meyer, 2009). Once established, it is able to spread into deeper water by forming extensive floating mats. This growth form allows it to cope with frequent water-level changes (Hussner et al., 2012). It grows best at high-nutrient sites, tolerating turbid water and organic pollution (Newman & Dawson, 1999; Baas, 2001; Pot, 2002; Hussner & Lösch, 2007; Hussner & Meyer, 2009). Establishment also occurs on banks which remain barren of any other vegetation (Hussner et al., 2012). Although highly eutrophic, base-rich sites may be especially susceptible; invasive behavior may also occur in more nutrient-poor and

[3] Including host plant, soil conditions and other abiotic factors where appropriate.

even acid conditions (de Mars & Bouman, 2002), although the species is considered to be limited by acidic waters (EPPO, 2009c).

Toussaint et al. (2008) offer a simplified summary of the needs of *H. ranunculoides* along a scale from 1 to 5 (Table below; for clarity the scale meaning is namely explained within the cells).

Factor	**1**	**2**	**3**	**4**	**5**
Water					**Aquatic plant**
pH			**Neutrophilous**		
Nutrients				**Prefers eutrophic to hypertrophic conditions**	
Organic Matter			**Prefers soil with medium organic matter quantity**		
Granulometry					**Prefers silt soils; supports anoxic conditions**
Light				**Helophyte**	
Salt	**Not halophile**				

D/ Food habits[4]

Not applicable

E/ Control agents

In Germany, observations showed that the introduced 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 (EPPO, 2009c).

During summer, cattle may eat the plant when it grows at the water margins, but this again has not prevented the establishment of the species, and may even encourage the spread of the plant due to fragmentation (Newman, pers. comm., in EPPO 2009a).

In native range, herbivory has been observed on *Hydrocotyle* species by the co-evolved, leaf-feeding weevil *Lixellus elongatus* (Coleoptera: Curculionidae) in Argentina (Cordo *et al.*, 1982). A large suite of other arthropods have been recorded as natural enemies from Argentina and neighboring countries, including stolon mining Diptera, Lepidoptera (Arctiidae, Noctuidae), and Hemiptera (Newman & Duenas, 2010; Walsh & Maestro, 2011). *L. elongatus* is, however, the most common and widespread herbivore on *H. ranunculoides*, and is currently the main organism being evaluated for quarantine studies in England (Walsh & Maestro, 2011). According to these authors, results are promising (see also § 3.3).

[4] For animal species only.

The US National Fungus Collections Database (Farr & Rossman, 2011) lists the pathogens found associated with *H. ranunculoides*, mostly from California and southeastern states. These include *Cercospora hydrocotyles*, *Entyloma fimbriatum*, *Entyloma hydrocotyles*, *Physoderma hydrocotylidis* and *Puccinia hydrocotyles* (from the USA and Chile).

F/ Establishment capacity in Belgium

Due to the high regeneration capacity of its shoots and fragments (Newman & Dawson, 1999; Hussner & Lösch, 2007; Dortel et al., 2011), *H. ranunculoides* can reach new regions very easily carried by waterfowl (Huckle, 2002), via water courses and by human intervention (Hussner et al., 2012). Its dispersion capacity is therefore high. Hussner et al. (2012) explain:
"Both intentional (e.g. through the aquatic nursery trade) and unintentional distribution (e.g. by boating) commonly occur. Flooding allows it to become widely established in river valleys. Management activities or water sport activities that result in the fragmentation of plants facilitate dispersal. New shoots are formed even from small stem fragments. Up to 90% of stem fragments 1 cm in length and with only one node, with or without leaves, regenerate within one week; single leaves and internodes fragments do not regenerate. Although suggested, dispersal by means of seeds is not yet documented".

Moreover, climatic conditions in Belgium, as well as abiotic characteristics of a number of Belgian streams and waterbodies, are within the tolerance range of the species (as its recent and rapid spread over the country clearly demonstrates; see § 2.1.1.). Consequently, establishment capacity of *Hydrocotyle ranunculoides* in Belgium is high. Nevertheless, it is likely that winter conditions present in the southern part of the country (Ardennes, Lorraine) are severe enough to limit its establishment there.

G/ Endangered areas in Belgium

Most freshwater bodies and slow flowing streams of the territory (including endangered and sensitive areas) are to be considered as suitable for *H. ranunculoides* establishment (Figure 17). For instance, in 2009, the species was recorded in the Natura 2000 site BE32012 "Bord nord du bassin de la Haine [data from DEMNA]). Similarly, the species is found in several Flemish natural reserves (Stiers et al., 2011).

Nowadays climatic conditions (linked with altitude) may play a role in keeping the species from invading harsher climatic parts of Wallonia (south of the Meuse) but, with predicted increasing temperatures due to climate change, the species could spread further south through the Ardennes and Lorraine.

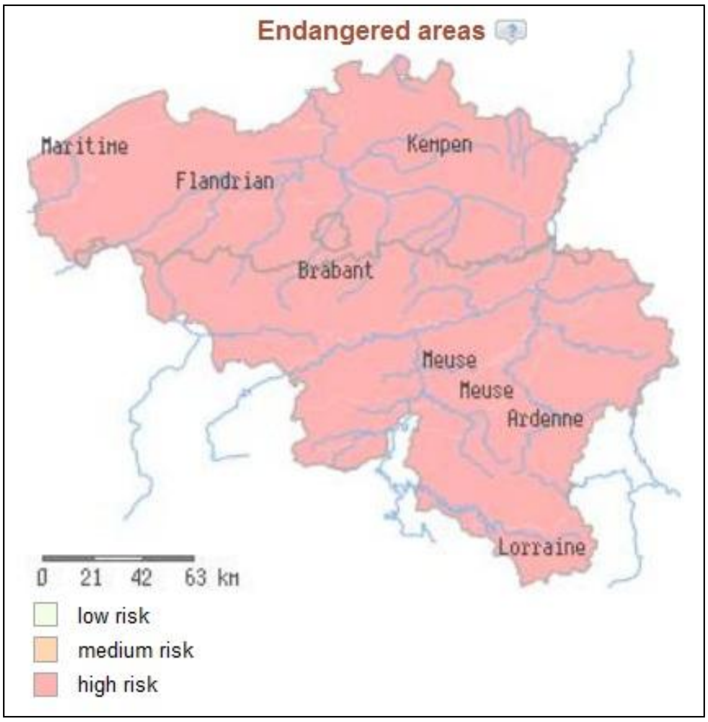


Figure 17: Potential dispersion of *H. ranunculoides* in endangered areas in Belgium (Source: http://ias.biodiversity.be).

Establishment capacity in the Belgian geographic districts:

Districts in Belgium	**Environmental conditions for species establishment**[5]	**Environmental conditions for species establishment under increasing temperature due to climate change**
Maritime	Optimal	Optimal
Flandrian	Optimal	Optimal
Brabant	Optimal	Optimal
Kempen	Optimal	Optimal
Meuse	Optimal	Optimal
Ardenne	Suboptimal	Optimal
Lorraine	Suboptimal	Optimal

ESTABLISHMENT CAPACITY AND ENDANGERED AREAS IN BELGIUM

Most freshwater bodies and slow flowing streams of the territory (including endangered and sensitive areas) are suited for *H. ranunculoides* establishment. Nowadays climatic rigors of southern Wallonia may play a role in keeping the species from establishment south of the Meuse. Future climate change may enhance further invasion in the Ardennes and Lorraine.

[5] For each district, choose one of the following options : optimal, suboptimal or inadequate.

2.1.5 Dispersion capacity

Specify what is the rate of dispersal once the species is released or disperses into a new area. When available, data on mean expansion rate in introduced territories can be specified. For natural dispersion, provide information about frequency and range of long-distance movements (i.e. species capacity to colonise remote areas) and potential barriers for spread, both in native and in introduced areas, and specify if the species is considered as rather sedentary or mobile. For human-assisted dispersion, specify the likelihood and the frequency of intentional and accidental movements, considering especially the transport to areas from which the species may easily colonise unintended habitats with a high conservation value.

A/ Natural spread

- **Natural Dispersal (non-biotic)**

Natural dispersal through water current occurs within contiguous systems once the plant has become established by other means (Hussner & Lösch, 2007; Hussner 2008; Hussner et al., 2012). In flowing water of normalized brooks (e.g. De Dommel River in the Netherlands), the species may distribute downstream tens of kilometers per year (Nienhuis, 2008). Strong winds may also fragment the plant and facilitate its dispersion (Levy et al., 2011). It is, however, unlikely that colonization of new isolated ponds results from natural dispersion.

- **Vector Transmission (biotic)**

Biotic dispersion of *Hydrocotyle ranunculoides* is not well-documented, although Huckle (2002) indicates that the plant can reach new regions very easily carried by waterfowl. We acknowledge that it seems indeed possible that birds roosting on mats may transport fragments to other locations. In addition, animal (e.g. birds, mammals) foraging may result in the fragmentation of the plant, which may facilitate its dispersion, and eventually results in the production of new populations (Levy et al., 2011).

B/ Human assistance

It is likely that the majority of sites where the species now occurs outside its natural range are the result of deliberate or involuntary human introductions through cultivation, planting for water treatment or for aquarium and pond ornament (Newman & Dawson, 1999; De Mars & Bouman, 2002; Pot, 2002; Nienhuis, 2008; Newman & Duenas, 2010). In this direction, *H. ranunculoides* has been intentionally introduced and sold as an ornamental plant for tropical aquarium and garden ponds in the UK, the Netherlands and Belgium (Verloove & Heyneman, 1999; EPPO, 2006; Newman & Duenas, 2010; Hussner et al., 2012).

Once escaped in the wild, natural (see §2.1.5.A) and/or human-facilitated dispersion may occur. For instance, transport on machinery used in agriculture to clear watercourses may be a factor in local spread. Similarly, management activities, fish-stocking, water sports and other recreational activities may result in the fragmentation of plants and facilitate the dispersion of the species (Levy et al., 2011; Hussner et al., 2012 [see also § 2.1.4.F]).

Although the trade of *H. ranunculoides* in Europe is decreasing, the species is still sold in several countries, including Belgium. As a result, it may still be introduced locally into water systems, following cleaning of aquaria and ponds. It is therefore likely that, without the introduction of restrictive legislation and enforcement, further establishment will occur. Similar patterns of introduction are noted for other aquatic plants in most countries with aquatic horticultural trade (www.cabi.org).

DISPERSAL CAPACITY

Natural dispersion readily occurs through water flow. Dispersion may be enhanced by transport on boats or machines used to clear watercourses. Intentional introduction (for water treatment) or accidental introduction (e.g. released after aquarium or pond cleaning or incautious plant residues disposal) also enhance dispersal capacity.

2.2 EFFECTS OF ESTABLISHMENT

Consider the potential of the non-native organism to cause direct and indirect environmental, economic and social damages as a result of establishment. Information should be obtained from areas where the pest occurs naturally or has been introduced, preferably within Belgium and neighbouring areas or in other areas with similar eco-climatic conditions. Compare this information with the situation in the risk analysis area. Invasion histories concerning comparable organisms can usefully be considered. The magnitude of those effects should be also compared with those caused by their closest native relatives.

2.2.1 Environmental impacts

Specify if competition, predation (or herbivory), pathogen pollution and genetic effects is likely to cause a strong, widespread and persistent decline of the populations of native species and if those mechanisms are likely to affect common or threatened species. Document also the effects (intensity, frequency and persistency) the non-native species may have on habitat peculiarities and ecosystem functions, including physical modification of the habitat, change to nutrient cycling and availability, alteration of natural successions and disruption of trophic and mutualistic interactions. Specify what kind of ecosystems are especially at risk.

A/ Competition

The high “Leaf Area Index” of *Hydrocotyle ranunculoides* (up to 5.47 ± 0.2) indicates that the species is able to outcompete submerged vegetation, especially the native *H. vulgaris* (Hussner & Lösch, 2007). In this direction, *H. ranunculoides* is a superior competitor of many native plant species in its introduced European range. These include littoral marsh plants, such as species of *Carex*, *Juncus*, *Myosotis* and *Rorippa*, as well as submerged aquatic plants (Hussner et al., 2012). Because of its rapid growth, *H. ranunculoides* can form dense canopies which occupy a large amount of space (Stiers et al., 2011). Native plants are overgrown and shaded out by these extensive beds or floating carpets (Hussner, 2008; Stiers *et al.* 2009, 2011). Moreover, *H. ranunculoides* may be able to produce allelopathic anti-algal compounds (Della Greca et al., 1994a, 1994b). In invaded ponds in Belgium, species richness of native aquatic plants and of submerged species may be reduced by more than 50% and up to 100%, respectively (Nijs et al., 2009; Figure 18).

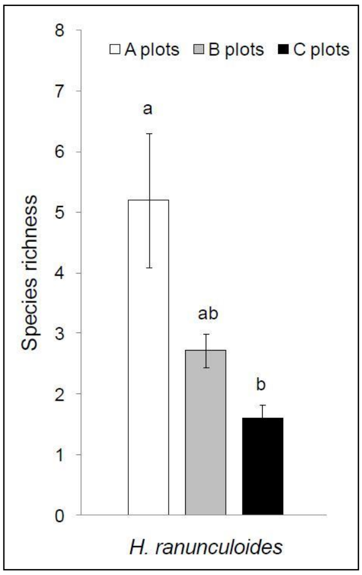


Figure 18: Impact of colonization of *H. ranunculoides* (n = 38) on native plant species richness in (A) uninvaded, (B) semi-invaded, and (C) and heavily invaded (i.e. the plant cover is > 75%) 4 m^2-plots in Belgium ponds. Given are means ± SE; different letters indicate significant differences amongst invasion categories ($p < 0.05$) (Modified from Nijs et al., 2009).

B/ Predation/herbivory

Hussner (2008) describes grazing of non-native Coypu, *Myocastor coypus,* on *H. ranunculoides* stands in North Rhine Westphalia. The animals only eat the leaves, clearing small patches in the dense canopy. Hussner (2008) also reports infestation by greenflies (Hemiptera: Aleyrodidae) on some plants grown in greenhouses. When the plant forms a dense mat over the surface of the water, cattle will eat the leaves on the water margins, if accessible (EPPO, 2009c). To our knowledge, the palatability for fish is not documented.

C/ Genetic effects and hybridization

In a short genetic study of *H. ranunculoides* in the UK (Bakner et al., 1997 in EPPO, 2009d), four groups of the species were distinguished in the UK population by AFLP analysis, suggesting four potential independent introductions. One clone was found to be relatively similar to the native *H. vulgaris*, perhaps indicating hybridization. In the same study, the chromosome number was found to be 96, indicating tetraploidy (based on the chromosome number reported by Federov (1974) over the same species: 2n=24). This may indicate that the horticulturally derived weed of Europe is different to the native species. This could explain the only recent invasiveness of the species despite its presence in Europe since at least the beginning of the 17^{th} century (Columna, 1616; Figure 2). Nevertheless, further work is needed to confirm this hypothesis (www.cabi.org).

D/ Pathogen pollution

No pathogen pollution reported.

E/ Effects on ecosystem functions

In Belgium, Nijs et al. (2009) investigated whether *H. ranunculoides* modifies the invertebrate, phytoplankton and zooplankton abundance and diversity. They found no clear support for impacts of this species on overall species diversity, but it negatively affected invertebrate and zooplankton abundance. They explained these results by reduced space, sunlight and oxygen exchange in invaded ponds. Nevertheless, phytoplankton density increased in highly invaded ponds, which may be caused by the entrapment capacity of the invasive species. Presenting complementary results obtained in the same ponds as Nijs et al. (2009), Stiers et al. (2009, 2011) showed that taxonomic compositions of aquatic invertebrate assemblages in invaded ponds differed from uninvaded ones. Moreover, sensitive benthos, such as mayflies, were completely absent from invaded ponds. The authors therefore concluded that the introduction of *H. ranunculoides* has caused significant ecological alterations in the aquatic vegetation and the detritus community of Belgian ponds.

More generally, dense floating mats of *H. ranunculoides* on the water surface cause many significant changes of ecological processes and structures (EPPO, 2009c; Dortel et al., 2011; Hussner et al., 2012) by :

- reduction in water flow, increasing water temperature by sunlight absorbing and allowing insect proliferation (e.g. mosquitoes);
- increased eutrophication and sedimentation resulting in acceleration of ecological succession (rapid biomass accumulation fuels decomposition processes, alters the composition of the bottom substrate and expedites the infilling of shallow standing waters);
- decrease in O_2 concentration, reducing oxygen availability for fish and invertebrates (Hussner & Lösch, 2007; Hussner & Meyer, 2009), especially during *H. ranunculoides'* decay (EPPO, 2009b). If leading to sediment anoxia, the release of nutrients and potentially toxic substances can be enhanced (Hussner et al., 2012);
- loss of accessible open water at the margins for wildlife (e.g. birds, fish), restricting access to feeding and resting spaces (Williams et al., 2011);
- loss of light for photosynthesis of native plants (Hussner & Lösch, 2007);
- increased flood risk.

ENVIRONMENTAL IMPACTS

With a high surface cover capacity and an extremely fast growth rate, *H. ranunculoides* is able to outcompete most native aquatic and water banks species. The floating mats affect the penetration of light available for photosynthesis and reduce oxygen levels in the water column which can result in fish mortality and influence invertebrate life. Water flow may be impeded causing acceleration of ecological succession, loss of accessible open water at the margins for wildlife and increasing flood risk.

2.2.2 Other impacts

A/ Economic impacts

Describe the expected or observed direct costs of the introduced species on sectorial activities (e.g. damages to crops, forests, livestock, aquaculture, tourism or infrastructures).

- **Positive impact**:

Due to its vigorous growth and its propensity for nutrient bioaccumulation (De Maeseneer, 2000; Bretsch, 2004) and other substances or enhancing sedimentation, the species has found some uses in water treatment (Verloove & Heyneman, 1999). For instance, it has been tested as a treatment for the removal of wastewater nutrients with some success (Boyd & Bayne, 1988). However, accumulation of heavy metals can make disposal of harvested plant material problematic.

Similarly, the plant has been proposed, in reason of its attractive rapid growth rate, and of its high crude protein and high digestibility indices, as a suitable livestock food source (www.cabi.org). Nevertheless, its use as fodder for livestock (Leeflang, 2008) or for energy production does not appear to be worthwhile (Wolverton & McDonald, 1981; Pot, 2008). Moreover, as explained above, its capacity to accumulate heavy metals can be problematic and may prohibit its use as food for cattle.

At last, Della Greca et al. (1994a, 1994b) isolated oleanane glycosides and polyoxygenated oleanane triterpenes from dried whole plant material with potential antialgal properties.

- **Negative impact**:

The economic impacts of this species mainly concern the causation of flooding events where channels and water control structures are blocked (e.g. Odé, 2009; CBS et al., 2011 ; Figure 19), with potential economic impacts, on crops for instance (Kelly, 2006). In addition, strongly invaded waters may lose their attractiveness and safety for leisure and recreation (EPPO, 2009b; Williams et al., 2011; Hussner et al., 2012). Prevention of navigation is also a problem in some watercourses (Williams et al., 2011). At last, Nienhuis (2008) indicates that water from ponds with large mats of *H. ranunculoides* becomes not drinkable for cattle without health risk due to the accumulation of toxic compounds like H_2S, NH_3 and NO_2.

Costs of control are another economic factor. An outbreak on the Canning River in Western Australia in 1991 reportedly cost AUS$ 2 million and the species is still present in Australia (EPPO, 2009c).

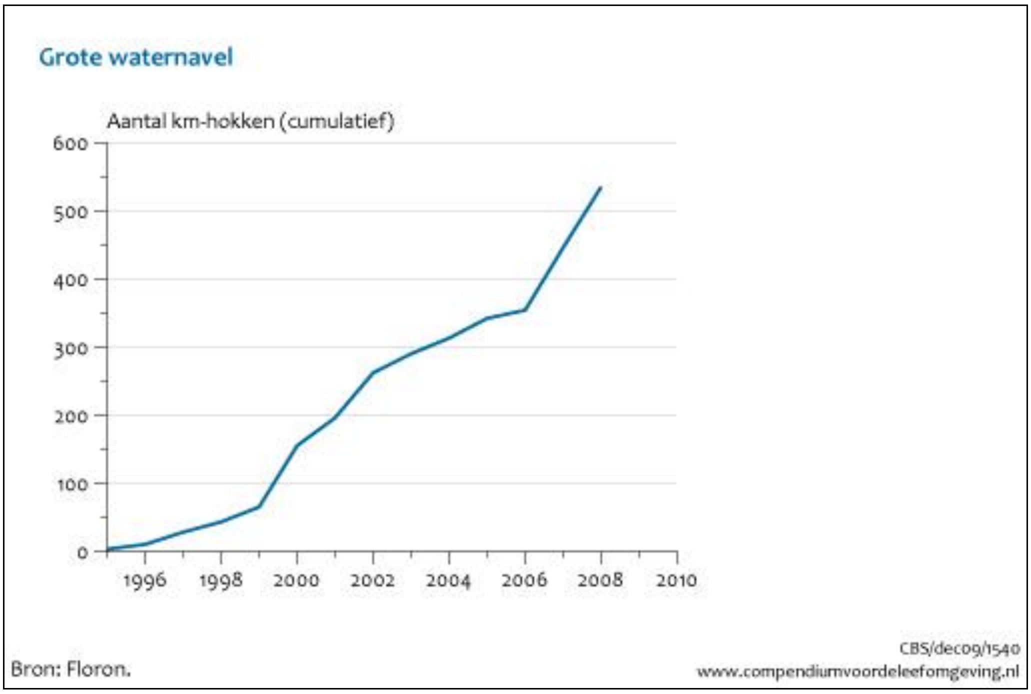


Figure 19: Cumulative number (km) of water structures blocked by *Hydrocotyle ranunculoides* in the Netherlands since its discovery in 1994 (Odé, 2009; CBS et al., 2011).

Control of floating pennywort has already cost millions of Euros in Europe (EPPO, 2009c), and increasingly frequent flooding is transporting the plant to new systems. The invasion of this invasive weed will severely limit the chance water bodies reaching good ecological status as defined by the Water Framework Directive (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32000L0060:EN:NOT).

Annual costs to the UK economy in terms of management, disposal and its effect on tourism have been estimated in a recent review of non-native species impacts in the UK to a total of £25,467,000 for *H. ranunculoides* (Williams et al., 2011). Similarly, water boards in the Netherlands have spent well over 2 million € in 2007 to control *H. ranunculoides* (EPPO, 2009c). In Flanders, the estimated cost for the management of *H. ranunculoides* was estimated in 2009 at 1.5 million euros per year (needed during 3 years from 2009) (EPPO, 2009c).

B/ Social impacts

Describe the expected or observed effects of the introduced species on human health and well-being, recreation activities and aesthetic values.

To our knowledge, there are no known effects on human health. However, *H. ranunculoides* forms dense interwoven mats of vegetation which can quickly cover the water surface interfering with the ecology and amenity uses of the water body. Strongly affected waters lose their attractiveness, safety for recreation (angling, swimming, and boating) and aesthetic value (Dortel et al., 2011; Williams et al., 2011).

In addition, vegetation mats may mislead on the firmness of the substratum and may thus increase drowning of both persons and animals (Dortel et al., 2011). At last, toxic compounds like H_2S, NH_3 and NO_2 accumulate under the vegetation and the water may become malodorous (Nienhuis, 2008).

STAGE 3 : RISK MANAGEMENT

The decision to be made in the risk management process will be based on the information collected during the two preceding stages, e.g. reason for initiating the process, estimation of probability of introduction and evaluation of potential consequences of introduction in Belgium. If the risk is found to be unacceptable, then possible preventive and control actions should be identified to mitigate the impact of the non-native organism and reduce the risk below an acceptable level. Specify the efficiency of potential measures for risk reduction.

3.1 RELATIVE IMPORTANCE OF PATHWAYS FOR INVASIVE SPECIES ENTRY IN BELGIUM

The relative importance of intentional and unintentional introduction pathways mediated by human activities should be compared with the natural spread of the organism. Make use e.g. of information used to answer to question 2.1.3.

- **Intentional import as an ornamental aquatic plant for use outdoors and in aquariums (see also § 2.1.3):**

H. ranunculoides has been imported in Europe but is not considered to be imported anymore because local production is far more cost effective than importation (van Valkenburg, pers. comm., in EPPO 2009a; Brunel, 2009). The species is known to be produced and traded within Europe and the plant is more likely to be introduced by aquarium/pond trade through the Internet rather than direct retail (Newman, pers. comm., in EPPO, 2009a). The volume of *H. ranunculoides* being produced and sold is however considered to be very low (EPPO, 2009a; Halford et al., 2011; Figure 12).

The actual trade volume is, in fact, difficult to ascertain because of the misapplied names regularly given to *H. ranunculoides* in plant nurseries (EPPO, 2009c). For instance, *H. ranunculoides* could be traded under the misapplied name *H. vulgaris* or the synonym *H. natans.* In Belgium, the species has also been sold as *H. leucocephala* (Branquart, pers. comm. in Brunel, 2009). Moreover, other *Hydrocotyle* species are in trade, which although being different species could be mislabelled (*H. umbellata, H. novae-zeelandiae, H. verticillata, H. moschata, H. sibthorpioides*). At last, *H. ranunculoides* is cited as *H. americana* in various catalogues (Brickell, 1996).

Human activities are considered as the major cause of introduction into the wild with subsequent invasion (Verloove & Heyneman, 1999; Hussner et al., 2012). Fortunately, progress has been made and it becomes well known that *H. ranunculoides,* and other plants as well, may become invasive in Belgium. As a result, the species progressively disappears from catalogues of aquatic nurseries (see § 2.1.3; Figure 13).

- **Intentional introduction for non-ornamental uses:**

EFSA (2007) identified another pathway to be considered: introduction of *H. ranunculoides* 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).

In Europe, other species are usually used for phytoremediation including *Phragmites australis, Typha* spp., etc. (Cooper, 2001). As these plants are native in Belgium, they should be preferentially used in place of *H. ranunculoides*. Nevertheless, trials have been made in Belgium, and the species was planted along watercourses in the Ghent area, from where it spread towards the border of the

Netherlands (EPPO, 2009c). The species has also been tested for phytoremediation in Germany under controlled situation (Hussner, pers. comm., in EPPO 2009a).

- **Unintentional introduction: hitch-hiking with other aquatic ornamental plants:**

Some *Hydrocotyle* spp. produced in Europe have been found to be contaminated with *H. ranunculoides* (J van Valkenburg, pers. comm., in EPPO 2009c; Figure 20). Such contamination is considered as a secondary spread pathway.

Figure 20: *Hydrocotyle ranunculoides* (Grote waternavel) as a contamination on ornamental plants of the native *H. vulgaris* (Gewone waternavel) produced in the Netherlands (Photo: J. van Valkenburg in EPPO, 2009c). These species can, however, be easily differentiated by their leaves (see Figure 21).

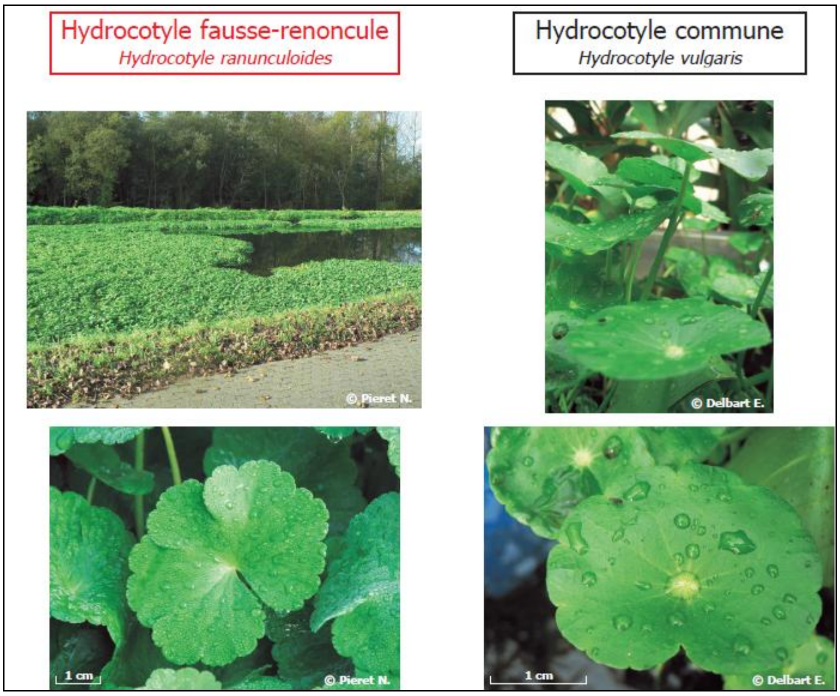


Figure 21: Differences between *Hydrocotyle ranunculoides* (left) and the native *H. vulgaris* (right). *H. vulgaris* has peltate leaves (i.e. having the stalk attached to the centre of the lower surface) whereas *H. ranunculoides* has cordate ones (i.e. heart-shaped leaves, with the petiole attached to the cleft) (Source: Pieret et al., 2007).

- **Natural and human assisted spread** (see also §2.1.5A and 2.1.5B)**:**

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 whereby plant fragments enter the water system (J. Newman, pers. comm., in EPPO 2009a). Subsequently, natural dissemination through water system and accidental transport of plant parts or debris (e.g. on boats, machines or epizoochory) are highly probable and are to be considered as playing a role in *H. ranuculoides* dissemination as important as intentional plantation.

3.2 PREVENTIVE ACTIONS

Which preventive measures have been identified to reduce the risk of introduction of the organism? Do they reduce the risk to an acceptable level and are they considered as cost-effective? Specify if the proposed measures have undesirable social or environmental consequences. Consider especially (i) the restrictions on importation and trade and (ii) the use of specific holding conditions and effect of prohibition of organism introduction into the wild.

In Belgium (and other non-native countries of the species) several actions can be undertaken in order to limit introduction of *Hydrocotyle ranunculoides*:

- **Action 1: Amend existing legislation.**

Legislation should be strengthened to ensure a total ban on import and possession of potential invasive plants such as *H. ranunculoides* and closely related species*.*

- **Action 2: Highlight, support and promote Invasive Species Codes of Practice.**

A priority action to prevent the spread and release of invasive species such as *H. ranunculoides* is to promote wide use and implementation of the Invasive Species Codes of Practice (ISCP; Table below), recently developed in Belgium through the AlterIAS LIFE project (http://www.alterias.be), and to support these with literature and information leaflets for both the horticultural sector and the general public.

Table: Invasive Species Codes of Practice for the industry and the general public.
Source: http://www.alterias.be/fr/que-pouvons-nous-fairen/les-codes-de-conduite-sur-les-plantes-invasives

ISCP for horticultural professionals	**ISCP for the general public**
1.Be informed about the Belgian alien species list	1.Be informed about the Belgian alien species list
2.Stop selling and/or planting invasive alien species	2.Avoid buying and planting alien species
3.Spread information about invasive alien species to customers and the general public	3.Choose non-invasive native plants as an alternative to alien species
4. Promote the use of alternative, non-invasive	4.Do not dump vegetal residues in nature

plants	
5. Take part in early invasive alien species detection actions	5. Share your knowledge and awareness about invasive plants and issues related to their introduction

Indeed, *H. ranunculoides* is valued as an ornamental plant, therefore educational programs must be directed to educate the public about the dangers this plant poses outside its native range. Teaching water managers how to clean equipment in a way that decreases the chance of transmission is one way to lessen the prevalence of human-mediated transport. Additionally, information should be disseminated regarding responsible propagation and cultivation of this species if it remains to be sold (which is an undesired scenario).

In Belgium, a large information campaign was already promoted by AlterIAS. Such initiatives enhance awareness of the risks caused by invasive species such as *H. ranunculoides,* facilitate early warning and correct identification and provide valuable measures for careful culture and manipulation, as well as trade reduction, by proposing alternative garden plants (Figure 22) through detailed Invasive Species Codes of Practice, targeting the public at large as well as retailers. As the species is still easily available in Belgium (Figure 12), there is an opportunity for education at various points along the horticultural trade pathway from distributor to introduction. Fortunately, as explained in §2.1.3, several nurseries of aquatic plants have already agreed with the Invasive Species Codes of Practice (Figure 13).

Figure 22: Extract from a leaflet produced by AlterIAS. Its aim is to encourage both horticultural professionals and amateur gardeners to plant native species rather than invasive ones. Here are presented the plants proposed as alternatives to *Hydrocotyle ranunculoides* [in addition to the three species presented on this leaflet, Dortel et al (2011) propose *Ranunculus peltatus, Apium nodiflorum, Nasturtium officinale*, and *Menyanthes trifoliata* as alternatives]. The entire document is available at: http://www.alterias.be/images/stories/downloads/folder_brochures/folder_aquatic_final_fr.pdf.

- **Action 3: Public sector bodies adopt Invasive Species Codes of Practice**

All public sector organizations should lead by example and adopt the Invasive Species Codes of Practice in their relevant work areas. This is key to the success of both existing codes (for professionals in horticulture and for general public). Government agencies should also incorporate the philosophy of the codes into tenders and procurement procedures and ensure that suppliers and contractors for public works are abiding the codes.

(i) Prohibition of organism importation, trade and holding

Being sold as a tropical aquarium plant in Europe, and also as an outdoor pond plant, it is likely, without the introduction of restrictive legislation and enforcement, that further spread of *H. ranunculoides* will occur. Similar patterns of introduction are noted for other aquatic plants in most countries with an aquatic horticultural trade (www.cabi.org).

- **Prevention methods :**

Hussner et al. (2010) consider that the increase in species number and abundance of aquatic plants is probably caused by enhanced trading and increased invasibility by water eutrophication / re-oligotrophication and climate change. They made proposal of a trading ban for highly invasive non-indigenous aquatic plants with which we agree. This will not stop their natural spread, but should reduce the risk of further unintended entry and thus can be a major control factor.

Such a trading ban was already adopted by several European countries. In the Netherlands, sale and possession of *H. ranunculoides* is prohibited since 2001 (Pot, 2002; EPPO, 2006). In Great Britain, the Royal Horticultural Society prohibited this plant due to its high invasiveness, and the plant has to be banned from sale from April 2014 (Kinver, 2013). Distribution is also prohibited in Switzerland (AFCS, 2008). Hussner et al. (2010) recommend including *H. ranunculoides* on the German black list and prohibition of its trade. Moreover, in recognition of the problems caused by *H. ranunculoides* in Great Britain and the Netherlands, the European Plant Protection Organization, placed the species on the A2 Action list in 2005 and considers it a quarantine pest (EPPO, 2006).

While recognizing the potentially invasive behavior of *H. ranunculoides*, the EFSA Scientific Panel on Plant Health (EFSA, 2007) points out that such behavior does not occur throughout the region and concludes that the key factors determining invasiveness are insufficiently identified in the Pest Risk Analysis submitted by EPPO (2006) to justify actions at the European Community level. Verbrugge et al. (2012) conclude, however, that the risk classification of *H. ranunculoides* is quite similar among European countries.

In Belgium, the species figures on the national black list of invasive species (Branquart, 2012; http://ias.biodiversity.be/ias/species/show/63) and legislation to ban its commercial use and distribution is being prepared (Adriaens et al., 2009). Meanwhile, as explained above, representatives of the sector approved a code of conduct for invasive plants and most wholesalers voluntarily removed *H. ranunculoides* from their catalogue, whilst the ban in the Netherlands also seems to affect commercial availability.

- **Information and awareness :**

The Belgian Federal services for Public health, Food safety and Environment and the National Biodiversity Platform launched an information campaign on alien species which also focuses on *Hydrocotyle*. The Walloon Ministry of Environment has undertaken similar action. In Flanders, provincial and local authorities raised awareness by distributing free brochures and information sheets among stakeholders, adding warnings to their internet sites, organizing information and training sessions and contributing to meetings of professional organizations and interest groups. Several overviews were published in popular and semi-professional journals, and press releases were issued. Overall, response was especially good among nature conservationists, farmers and water managers. As explained in § 3.2. above, the AlterIAS LIFE project has already made a lot to disseminate information about the problematic of invasive plant species, including *H. ranunculoides* (e.g. Figure 21).

(ii) Use of specific holding conditions and effect of prohibition of organism introduction into the wild

A large campaign of information with proposed alternatives for limiting invasive species introduction has been promoted by AlterIAS (Figure 22). Such initiatives enhance awareness of the risks caused by invasive species such as *H. ranunculoides* and provide valuable measures for careful holding/manipulations, reduce trade, accurately identify specimens and propose alternative plants for gardens through detailed Invasive Species Codes of Practice targeting the public at large as well as garden plants retailers. In Belgium, Garden Centres 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 Species (Branquart, 2008; Figure 13).

3.3 CONTROL AND ERADICATION ACTIONS

Which management measures have been identified to reduce the risk of introduction of the organism? Do they reduce the risk to an acceptable level and are they considered as cost-effective? Specify if the proposed measures have undesirable social or environmental consequences. Consider especially the following questions.

(i) Can the species be easily detected at early stages of invasion (early detection)?

H. ranunculoides may spread and disseminate by way of small, but viable, fragment of the plant. Detection at an early stage of invasion is very unlikely, however due to its extremely high growth rate it is often detected when already well established.

(ii) Are they some best practices available for organism local eradication?

The side effect of chemicals and even biological control means can often be as detrimental or even worse for the environment at large, native species and human health.
The precautionary principle should be applied as a general rule.

According to the "EPPO Pest Risk Analysis" (EPPO, 2009a, 2009b, 2009c), without phytosanitary measures, *H. ranunculoides* will not be controlled. Even with phytosanitary measures, *H. ranunculoides* is very difficult to control.

- **Mechanical control**

Mechanical control has to be done very carefully. If it is not done properly, spread can be enhanced, as *H. ranunculoides* spreads very effectively by fragmentation and water movements (Newman & Dawson, 1999; Pot, 2000; Hussner, 2008; Dortel et al., 2011). *H. ranunculoides* can be cut with weed cutting buckets or boats (Newman & Duenas, 2010). These techniques will only offer a very short-term reduction in the local extent of the plant, as it is capable of growing back rapidly from single nodes. Re-cutting will be necessary throughout the growth season. However, without thorough removal of all cut material the inevitable spread of the plant downstream will be exacerbated (Newman & Duenas, 2010). Where cutting is deemed appropriate, the affected areas should be carefully fenced or, netted off, to reduce the risk of downstream infestation and to insure that all cut plant material can be removed from the water body (Haury et al., 2010). This is especially important in flowing situations. Mechanical removal can be practiced to reduce the biomass for subsequent chemical treatment and to ease access for herbicide application, especially in dense masses. A better option is to remove as much of the plant biomass as possible and then to go over the area handpicking the remaining fragments. Although this technique has eradicated the plant for the upper reaches of the Chelmer River and the Lee River (both located in the UK), success rate is generally low (e.g. Haury et al., 2010). It is, however, the current best option for control (Newman & Duenas, 2010; Dortel et al., 2011; Delbart et al., *in press*).

When mechanical control has to be done? According to Newman (pers. comm. in Dortel et al., 2011), to prevent the spread of *Hydrocotyle ranunculoides*, the best way is to remove as much of plants before winter dormancy. This prevents the spread of fragments in the hydrosystem and provides effective control of spring populations. It is necessary to repeat manual or mechanical harvesting in early spring (i.e. before the development of the emergent phase). Removal should continue during the summer period. It is especially important to do not wait July to intervene, because the growth rate is maximum and very large volumes are then to extract.

In the Netherlands, mechanical control methods are used with a globally high success to remove *H. ranunculoides* from infested waters (Invexo, 2012). For good results, repeated manual removal of remaining plants afterwards appears necessary. In Belgian Flanders, provincial and local authorities collaborate with the Flemish Environment Agency in a monitoring and control program that addresses both public and private water bodies. The size of reported populations is assessed by trained personnel, followed by control aimed at eradication. This consists of mechanical removal according to a strict protocol, avoiding techniques producing plant fragments, and manual harvesting of all plants remaining after three weeks by a private contractor (Invexo; Figure 23). Chemical treatment is not allowed. Manual intervention needs to be continued until the site is cleared and efficiency strongly depends on the quality of the work. Consequently, good project management is essential. On private premises, initial removal can be carried out at no expense to the proprietor if prospects for success and follow-up by the land owner are good. Results so far seem promising (Veraart & Soens, 2010).

In Wallonia too, mechanical control allowed water managers to obtain good results. For instance, at Plancenoit (near Lasnes), a population of *Hydrocotyle ranunculoides* covering 170 m^2 was almost entirely eradicated (a 99% decrease in cover and biomass was obtained) in one year thanks to four manual removals carried out between August and November 2011. The total operation cost reached 1000 Euro (Delbart et al., 2012). These authors indicate that manual eradication of *H. ranunculoides* populations can be achieved when the plant cover is inferior to 500 m^2.

In another direction, the effects of hydrogen peroxide treatment and torching were tested on potted plants (van der Burg, 2010; van der Burg & Michielsen, 2010; Figure 24). In contrast to the former, repeated short burning with a butane torch in early spring or after rigorous removal may be effective to remove stands growing on certain types of embankments. Field testing remains necessary.

Besmettingsgraad van Vlaamse waterlopen 2009-2011.

	Vlaamse Milieu-maatschappij		Provincie Oost-Vlaanderen		Provincie Antwerpen		Totaal	
Waterlopen in beheer	448,6 km		3775,2 km		2779,7 km			
2009 Besmetting bij start	146,1 km	100 %	117,7 km	100 %	69,5 km	100 %	333,4 km	100 %
2011 Besmettingsgraad 0	95,9 km	65,6 %	76,8 km	65,3 %	50,5 km	72,7 %	223,2 km	67,0 %
2011 Besmettingsgraad 1	39,8 km	27,3%	30,9 km	26,2 %	4,1 km	5,9 %	74,9 km	22,5 %
2011 Besmettingsgraad 2	10,2 km	7,0 %	5,4 km	4,6 %	6,9 km	9,9 %	22,5 km	6,7 %
2011 Besmettingsgraad 3	0,2 km	0,1 %	4,6 km	3,9 %	8,0 km	11,5 %	12,8 km	3,8 %

Figure 23: Infestation of Flemish waters by invasive plant species (mainly *Hydrocotyle ranunculoides*) from 2009 to 2011. Mechanical control methods were used to remove the invasive plants, with relatively high success (Source: Invexo, 2012).

Figure 24: Experimental treatment of *Hydrocotyle ranunculoides* by flaming (Source: van der Burg & Michielsen 2010).

- **Chemical control**

In the UK, applications of glyphosate to dense infestations of *H. ranunculoides* appear to have little effect on the plant (Newman & Dawson, 1999). The waxy leaf surface may restrict glyphosate uptake such that the amount available to reach new leaves growing from the submerged, interconnected mat of root material is too low to kill the plant. 2,4-D amine applied at 4.23 kg ha^{-1} active ingredient (a.i.) was considerably more effective, giving complete control within 6 weeks of treatment (Newman & Dawson, 1999). The treatment should be done at the end of the growing season when submerged apical stem tips are no longer present, as these are unaffected by the herbicide (Newman & Dawson, 1999; Hussner et al., 2012).

Nevertheless, according to some authors, herbicides containing glyphosate can work well on *Hydrocotyle ranunculoides* (Newman & Duenas, 2010) but a very low volume apparatus should be used to apply a low volume of concentrated herbicide to the leaf surface in order for any control to be achieved. Normal applications of 6 L/ha in 200 L water do not work. Applications should be made in water volumes of between 50 and 80 L / hectare, using 6 L per hectare of product. This is equivalent of an application rate of between 15 and 24 L per hectare of product at a normal dilution rate in 200 L water per hectare. Specialist contractors may be required to undertake this work. Decomposition of the remaining plant material is often slow, as *H. ranunculoides* typically forms extensive beds, and may take as long as six weeks in slow flowing water bodies (Newman & Duenas, 2010). As *H. ranunculoides* forms such thick beds of vegetation conventional spray applications may not reach all the leaves at the first attempt. Small leaves under the main canopy may be shaded from the herbicide by those above leading to incomplete control and a source from which the plant will regrow. It is therefore essential to plan a follow-up treatment in any chemical control programme which allows spot treatment or removal by hand, of any remaining stands of *H. ranunculoides* about 2 to 4 weeks after the first herbicide application (Newman & Duenas, 2010; Hussner et al. 2012). Although the growth of *H. ranunculoides* is noticeable throughout the season (it may completely cover small slow flowing channels or ditches in the late summer [Figure 1]) it does not usually reach nuisance proportions on larger water bodies until later in the summer or early autumn, with the peak growth starting in early July. However, treatment earlier in the season will reduce man-hours, equipment and chemicals needed to control the weed at a later date (Newman & Duenas, 2010). Ease of control also depends on pesticide legislation. Mechanical control is combined with the application of herbicides in the UK.

In a recent review of techniques used to control invasive amphibious plants, Delbart et al. (*in press*) noted that chemical methods have never allowed water managers to eradicate *Hydrocotyle ranunculoides in situ* so far.

- **Environmental control**

There are several methods that may be used, none of which give a complete solution. **Shade** may be an effective method of control as the plant does not establish well in shaded conditions, and is best achieved by planting trees on the south side of the water body (Newman & Duenas, 2010). This is unlikely to be practical to implement on larger water bodies. **Increasing flow** will restrict the growth of *H. ranunculoides in situ* but may increase the spread of the plant downstream. **Increasing rooting depth** to below 1 meter may reduce the ability of *H. ranunculoides* to root at the margins. This, however, is unlikely to be a feasible option in most small ponds or ditches. Reducing the amount of suitable rooting substrate by **piling** or preventing access to suitable areas by **netting** off sections may prove effective (Newman & Duenas, 2010). At last, **dredging** does not seem to be an effective method to eradicate *H. ranunculoides* (Haury et al., 2010).

All these environmental options are likely to be expensive to implement, may modify the native habitats, and are often untested (Newman & Duenas, 2010).

- **Biological control**

There are no known methods of biological control appropriate for use in non-native populations of Europe. Research is being conducted on biological control by weevils (especially the non-native ***Listronotus elongatus***) and other potential natural enemies (Gassman et al., 2006; Sheppard et al., 2006; Shaw & Tanner, 2008; Newman & Duenas, 2010; Walsh & Maestro, 2011). *Listronotus elongatus* has indeed been demonstrated to feed exclusively on *H. ranunculoides* in Argentina, and extensive damage caused by relatively small numbers of this weevil to the plant in Argentina was observed (Newman & Duenas, 2010). This agent is currently under study in the United Kingdom (Walsh & Maestro, 2011). The adult weevil feeds on the leaves by scraping away the leaf surface and forming discrete holes, some of which become infected by unidentified pathogens. The adult females lay eggs in the base of the petiole and the larvae develop and burrow down into the stolon (Newman & Duenas, 2010). Preliminary observations indicate that larval damage is restricted to the stolon around the base of each petiole, possibly allowing other larvae to occupy neighboring petiole / stolon sections.

Other insects, e.g. Lepidopteran larvae (moth), also appear to feed well on this plant (Newman & Duenas, 2010; Walsh & Maestro, 2011). Non-native Coypus, *Myocastor coypus,* feeds on *H. ranunculoides* stands in North Rhine-Westphalia but the herbivory pressure is considered to be not enough to control the plant (EPPO, 2009c). Hussner (2008) also reports infestation by greenflies (Hemiptera: Aleyrodidae) under greenhouse conditions, but these insects are also an unlikely suitable control agent in the field. The palatability for fish is not documented.

According to Walsh & Maestro (2011), there are at least four other natural enemies that deserve some attention: notably the petiole and stolon mining *Hydrellia* and the leaf mining *Monochaetoscinella*, both still unidentified at the species level, and probably undescribed. The moth *Paracles quadrata* (Arctiidae) may also be interesting, although its specificity is questionable.

(iii) Do eradication and control actions cause undesirable consequences on non-target species and on ecosystem services ?

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), and could also negatively impact native plants. Experiments in the UK concluded that the impact of mechanical control is severe on non-target organisms, but limited in the short term as recover occurs by recolonization in a relatively short time (J Newman, pers. comm., in EPPO 2009a).

- Chemical control of large stands can lead to the de-oxygenation 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 de-oxygenation of the waterbody due to decomposition of treated plant material, not to direct toxicity of the herbicide (EPPO, 2009d). 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., in EPPO 2009a).

(iv) Could the species be effectively eradicated at early stage of invasion?

Eradication of the species is possible at small spatial scale and at very early stage of invasion (EPPO, 2006). Mechanical control appears to be the most environmental friendly mean of action and must be achieved immediately after detection (Dortel et al., 2011). Due to the high growth rate of the species, the time frame before eradication becomes really problematic (or impossible) is very short.

(v) If widely widespread, can the species be easily contained in a given area or limited under an acceptable population level?

Eradication is very difficult or even impossible in water bodies with heavy infestation (EPPO, 2009a, 2009b, 2009c, 2009d). However, according to the Dutch experience, local eradication is possible if it is started early and the water system is reasonably accessible (2009d; Invexo, 2012; Figure 23). 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 (Newman & Duenas, 2010). Successful chemical control has been achieved on an experimental basis using glyphosate combined with either the adjuvant TopFilm at 850 mL / ha up to the end of June, or with Codacide Oil from July onwards (EPPO, 2009d). This technique has been used on several small infestations with good success, although more than one year's treatment is required. In several EU member states, herbicide application in aquatic environments/biotopes is prohibited or highly restricted.

In Belgium, it is probably not anymore possible to eradicate the plant from the country. Actions are only possible in small water-bodies and require early detection and repeated action (L Triest, pers. comm., in EPPO 2009a; Invexo, 2012).

RISK MANAGEMENT

The main current pathway of introductions of *Hydrocotyle ranunculoides* in Belgium remains its sale as an ornamental plant for aquariums and ponds, and its subsequent release in the wild. This pathway is however decreasing thanks to education actions carried out in the country (e.g. in the framework of the AlterIAS LIFE project). Once established, natural spread may occur, especially in flowing waters. As a result, secondary invasions within Belgium, but also from French or Dutch populations, are very likely.

A unified, strengthened legislation should be established <u>in Europe</u> to ensure a total ban on import, trade and holding of *Hydrocotyle ranunculoides* and other (potentially) invasive aquatic plants. Unfortunately, for *H. ranunculoides*, prohibition of importation, trade and holding in

Belgium could not be enough to prevent its entry and establishment because (1) the species is already spreading in the country (especially in the Flemish Region, but also more recently in the two other regions), and (2) natural spreading from neighbouring countries are expected since dense populations are now close to the Belgian border. Nevertheless, such measures would certainly slow down its current spread.

Hydrocotyle ranunculoides **is difficult to detect at early stages of invasion, and therefore control or eradication actions often start when the plant is already well-established.**

Since chemical weed control in an aquatic environment is extremely restricted in Belgium and its different regions and because the results should be of practical use, the practical control options should focus on prevention and non-chemical methods (mechanical removal in the case of *H. ranunculoides*).

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