Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention" Contract No 07.0202/2018/788519/ETU/ENV.D.2[1]

Name of organism: *Wasmannia auropunctata* (Roger, 1863)

Author(s) of the assessment:
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Risk Assessment Area: The risk assessment area is the territory of the European Union (excluding the outermost regions) and the United Kingdom.

Peer review 1: Richard Shaw
Peer review 2: Marc Kenis
Peer review 3: Alan Stewart

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[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968).

Contents

SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response:
Class: Insecta
Order: Hymenoptera
Family: Formicidae
Genus: *Wasmannia*

Scientific name: *Wasmannia auropunctata* (Roger 1863)
Original name: *Tetramorium auropunctatum* (Roger 1863) (not valid)

Synonym names: *Wasmannia atomum*, *Wasmannia australis*, *Wasmannia glabra*, *Wasmannia laevifrons*, *Wasmannia nigricans*, *Wasmannia obscura*, *Wasmannia panamana*, *Wasmannia pulla*, *Wasmannia rugosa*, *Wasmannia sulcaticeps weiseri* (Longino and Fernández 2007). A comprehensive and regularly updated list can be found at https://www.antcat.org/catalog/451265, (Bolton 2020).

Common name: Little Fire ant (Wetterer and Porter 2003).

Also known as little red fire ant, little introduced fire ant, small fire ant, West Indian stinging ant, cocoa-tree ant (English); fourmi rouge, petite fourmi de feu, fourmi électrique (French, French-New Caledonia); fourmi Sangundagenta, tsanagonawenda (Gabon). A comprehensive list of local names is provided by Wetterer and Porter (2003).

Although *W. auropunctata* is variable, there is no evidence that it is composed of multiple cryptic species (Longino and Fernández 2007). *Wasmannia auropunctata* is less than 2 millimetres in length, orange/brown in colour, and very slow-moving and sluggish. It has long, pointy spines on the propodeum, two nodes (petiole and postpetiole), and two grooves on the front of the head where the antennae can lay at rest (antennal scapes) (Cuezzo et al 2015). There is a marked negative relationship between queen size and worker size in *W. auropunctata*.

A key for separation of the taxa in the *genus Wasmannia* was provided by Longino and Fernández (2007) and by Cuezzo et al. (2015).

Because other species of *Wasmannia* are rare and inconspicuous, this assessment covers only one species, *Wasmannia auropunctata* (Roger 1863).

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response:

In practice, species from the genus *Wasmannia* may be confused with some species of the genus *Ochetomyrmex*. However, *Ochetomyrmex* have less developed antennal scrobes, the clypeal apron is lacking, and there is a slightly impressed mesonotal suture which is never present in *Wasmannia*. Species of *Ochetomyrmex* are all native to South America and are not known to be invasive nor are they recorded from Europe.

In Europe, *W. auropunctata* may be confused with species of *Solenopsis* (e.g. *Solenopsis fugax*) that are similar in size, colour and belong to the same sub-family (*Myrmicinae*). However, *Solenopsis* species do not have propodeal spines, a key trait to distinguish them. Moreover, they have a completely different ecology and behaviour. Species of the genus *Solenopsis* native to Europe are cryptic, form small colonies that live under rocks or in the litter and are almost never detectable on the soil surface. In contrast, *W. auropunctata* is not cryptic in its habits, it harbours several invasive traits, among which are behavioural and numerical dominance.
Invasive *Solenopsis* species, such as *Solenopsis invicta*, *S. richteri* and *S. geminata* are much bigger and cannot be confused with *W. auropunctata*.

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response:

A risk assessment has been carried out for New Zealand, which predicts that *W. auropunctata* would be unlikely (low risk) to establish outside but may achieve limited distribution in heated buildings (Harris et al 2005). It is predicted to have a low risk of spread from a site of establishment but the negative consequences of its presence are considered to be medium/high. The overall risk for New Zealand was considered to be low-medium. However due to the limited overlap in climatic and ecological conditions between New Zealand and the risk assessment area, this assessment has limited relevance.

No other risk assessments have been carried out for *W. auropunctata*. Several reports deal with the management of the species and are considered in the management annex (see for example Raymundo and Miller 2012; Vanderwoude et al. 2016).

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response:
W. auropunctata is native to Central and South America. It is often very common in Neotropical lowland forests (Wetterer 2013; www.antmaps.org; Guénard et al. 2017). *Wasmannia auropunctata* has been described as a true generalist in its choice of nest sites and habitats, allowing it to thrive in a wide range of conditions (Chifflet et al. 2018). The species is remarkably generalist in its habitat preference, it has invaded both open disturbed habitat and closed preserved forest in New-Caledonia (Berman et al. 2013a). It is common in habitats ranging from wet to dry and from early successional to mature.
The common ancestor of the two main clades of *W. auropunctata* occurred in central Brazil during the Pliocene (Chifflet et al. 2016). Clade A is present north and clade B south of Brazil. There are differences in the most suitable climate among clades, clade A being a tropical lineage and clade B a subtropical and temperate lineage. Only clade B reached more southern latitudes, with a colder climate than in northern South America. This differentiation in climate suitability allowed this originally tropical ant to invade temperate climates.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response:

Wasmannia auropunctata has been extraordinarily successful in spreading into several continents (Africa, part of North America and South America, Europe, Australia) and has colonized many tropical islands.

In the New world *Wasmannia auropunctata* has spread throughout the West Indies and peninsular Florida. Because its known distribution from South America through the Lesser and Greater Antilles to Florida has no large gaps, it is not possible to determine where in the West Indies *W. auropunctata* is native and where it is non-native, and it seems possible that many islands have a mix of native and exotic populations (Wetterer 2013). Indoor records of *W. auropunctata* from temperate North America are certainly introduced. It has invaded many Caribbean islands and the Galapagos islands. It is occasionally detected in heated localities in Canada (Wetterer and Porter 2003).

In the Old world, populations of *W. auropunctata* have been documented in Gabon and neighbouring countries of Cameroon and The Central African Republic. *Wasmannia auropunctata* was intentionally introduced and released in cacao plantations in Cameroon to biologically control pest insects, particularly Hemiptera (Wetterer et al 1999).

In the Indo-Pacific, the earliest records date from 1972 in New Caledonia and 1974 on the Solomon Islands. These later populations appear to be actively spreading, with recent records from Papua New Guinea and Guam. It is now spreading in many Pacific islands such as Hawaii and French Polynesia (see Wetterer (2013) for a detailed distribution and https://antmaps.org/?mode=species&species=Wasmannia.auropunctata).

In the Mediterranean basin, *W. auropunctata* has been recorded outside of the risk assessment in Israel (first detection in 2005).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded and established occurrences.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 (see also Annex V).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf (see also Annex V).

Response (6a):
Terrestrial biogeographic regions: Atlantic and Mediterranean.

Response (6b):
Terrestrial biogeographic regions: Mediterranean.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a):

- Mediterranean, Atlantic, according to Bertelsmeier et al. (2015a) (climatic suitability index[2] above 0.5, see annex I)

- Mediterranean, Atlantic, Continental, according to Federman et al. (2013) (climatic suitability index above 1, see annex II)

- Mediterranean, Atlantic, Continental, according to Coulin et al. (2019) (climatic suitability index above 0.5, see annex III). This model was built for the Mediterranean basin, and therefore does not cover the whole risk assessment area.

- Mediterranean and Atlantic according to Beckmann et al. (2019) (climatic suitability index above 0.5, see annex IV).

Response (7b):

- Mediterranean, Atlantic in 2080, according to Bertelsmeier et al. (2015a) (climatic suitability index above 0.5, see annex I)

- Mediterranean, Atlantic, Black Sea in 2070, according to Beckmann et al. (2019) (climatic suitability index above 0.5, see annex IV).

Bertelsmeier et al. (2015a), using a climate matching model (Maxent) based on present distributions, mapped suitable areas globally for *W. auropunctata*. To consider a range of possible future climates, they used downscaled climate data from three GCMs: the CCCMA-GCM2 model; the CSIRO MK2 model; and the HCCPR-HADCM3 model (GIEC 2007). They used the two extreme SRES: the optimistic B2a; and the pessimistic A2a scenario.

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[2] A threshold rule was applied whereby all pixels with a probability of presence exceeding 0.5 were classified as "suitable" area. By convention, this threshold is frequently used for binary classification for species distribution modelling (See Bertelseier et al. 2015a).

To estimate the effect of climate change on the potential distribution, Beckmann et al. (2019) computed equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip55m). Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats, the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, night time lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers) was included in the model. The index ranges between 0 and 1 and was ln+1 transformed for the modelling to improve normality. Other variables potentially affecting the distribution of the species, such as land cover were not included in the model.

Federman et al. (2013) used the Maxent model to predict potential invasion and establishment of *W. auropunctata*. Bioclimatic variables were obtained from the WorldClim dataset. These variables were derived from the monthly temperature and rainfall values, in order to generate biologically meaningful variables. The bioclimatic variables represent annual trends, seasonality, and extreme or limiting environmental factors. Yearly reference evapo-transpiration was obtained from the database of the Food and Agriculture Organization of the United Nations (FAO).

Coulin et al. (2019) analysed 19 bioclimatic variables related to temperature and precipitation at 30 arc-seconds resolution available from WorldClim. After variables selection, the remaining variables were analysed with *W. auropunctata* clade B native range presence data to fit a SDM using the Maxent procedure. To explore the link between the thermo-physiological constraints and the SDM, the lower CTmin measured in their study was evaluated by analysing the latitudinal change of the minimum temperature of the coldest month (Bio6) and its effect on the probability of presence.

A number of underlying assumptions and inherent uncertainties are generally associated with the niche modelling approach and the actual distribution is contingent on many factors. For example, this species distribution models are only based on climate data available at a coarse scale. The models do not include information on biotic interactions or other abiotic factors having an influence at a regional or global scale.

The choice of the 0.5 threshold for the climatic suitability index is arbitrary. There is uncertainty about the potential current and future geographic distribution of the species.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.
A8a. Recorded: List Member States
A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom
The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a):
W. auropunctata has been recently recorded in southern Spain, in the region of Malaga (Marbella) (Espadaler et al. 2018). Before that, it was first recorded in greenhouses in 1927 in United Kingdom, in 1952 in Germany (Geiter et al. 2002), in 1988 in the Netherlands during import inspection at the Plant Protection Service (Boer and Vierbergen 2008) and in 2006 in Italy (Wetterer and Porter 2003),

Response (8b):
To date *W. auropunctata* is known to have established populations only in southern Spain, in the region of Malaga (Marbella) (Espadaler et al. 2018). The ants were first detected by local residents around 2016 but were probably introduced more than five years previously (Espadaler et al. 2018). The origin of this invasion is unknown and requires investigation.

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a):

- France, Spain, Ireland, United Kingdom, Italy, Greece and Croatia, according to Bertelsmeier et al. (2015a) (climatic suitability index above 0.5, see annex I).

- Austria, Germany, Hungary, France, Spain, Ireland, United Kingdom, Croatia, Greece, Sweden, Slovakia, Slovenia, Czech Republic, according to Federman et al. (2013) (climatic suitability index above 1, see annex II)

- France, Spain, Portugal, Italy, Slovenia and Croatia, according to Coulin et al. (2019) (climatic suitability index above 0.5, see annex 3). This model was built for the Mediterranean basin, and therefore does not cover the whole risk assessment area.

- Cyprus, France, Greece, Italy, Portugal, Spain, according to Beckmann et al. (2019) (climatic suitability index above 0.5, see annex IV).

Response (9b):

- France, Spain, Ireland, United Kingdom, Italy and Greece in 2080, according to Bertelsmeier et al. (2015a) (climatic suitability index above 0.5, see annex I).

- Croatia, Cyprus, France, Greece, Italy, Portugal and Spain in 2070, according to Beckmann et al. (2019) (climatic suitability index above 0.5, see annex IV).

The applied methods and limitations of the mentioned models have been described in Question A7.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response:
Yes. It is considered to be amongst the most widely distributed invasive species on earth. It has colonized almost all continents and has ecological, economic, and health impacts (Holway et al. 2002). It is considered as one of the worst invasive ant species. It is present on the list of the 100 the world's worst invasive species of the IUCN (Lowe et al. 2000).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response:
Terrestrial biogeographic region: Mediterranean (Espadaler et al. 2018).

See reply to A12.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

Response: Spain

Espadaler et al. (2018) have described an infested area in Spain that has a perimeter of 1.2 km and 5.8 ha of surface. Although a number of other, native ant species are found around this infested area, none of native ant species are detected within the infested area.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the Union and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the Union or third countries shall be used, if available.

Response:
There are no known socio-economic benefits of the species.

SECTION B – Detailed assessment

Important instructions:
• In the case of lack of information the assessors are requested to use a standardized answer: "No information has been found."   • With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.   • With regard to the confidence levels, see Annex III.   • Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION

Important instructions:
• **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).   • **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild and is treated in the next section (N.B. introduction and entry may coincide for species entering through pathways such as "corridor" or "unaided)".   • The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[3] and the provided key to pathways[4].   • For organisms which are already present in the risk assessment area, only complete this section for current active pathways and, if relevant, potential future pathways.

Qu. 1.1. List relevant pathways through which the organism could be introduced. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.
For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.   In this context a pathway is the route or mechanism of introduction of the species.   The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

[3] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[4] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9

Pathway name:

a) Transport-Stowaway (Hitchhikers in or on airplane). We also considered arrivals by any vehicular means from invaded areas outside the PRA area, whose journey time would not exceed a few days.
b) Transport-Contaminant (nursery material and other matters from horticultural trade)
c) Transport-Stowaway (nests transported in container/bulk, including sea freight, airfreight, train, etc.)
d) Transportation of habitat material (soil, vegetation, wood, …)
e) Food contaminant (including live food)

Wasmannia auropunctata is considered one of the classic tramp ant species, due to its reliance on human-mediated dispersal and close association with humans (Hölldobler and Wilson 1990). It can hitchhike with many commodities through many pathways. However, only the entry of queen ants and nests present a risk of establishment.

Pathways for the introduction of *W. auropunctata* to new locations include both natural (e.g., floating on vegetation and debris), and human-mediated routes (e.g., the nursery trade, transportation in soil, packaging materials moved by road sea or air). *Wasmannia auropunctata* has been intercepted from a variety of commodities (ornamental plants and fruits) and origins (South America, Pacific islands) at US ports and airports since 1910 (Blight et al. unpublished data). One hypothesis for the introduction of *W. auropunctata* in Hawaii is transportation along with fish-tail palms. Causton et al. (2005) suggested that it is easily transported on fruits and vegetables and that the growing trade between countries has facilitated the spread of *W. auropunctata*.

Harris et al (2005) provided a very detailed analysis of potential pathways of introduction of *W. auropunctata* into New Zealand, which is also relevant for Europe.

Queens may enter the risk assessment area through the ant market on the internet. This pathway should be considered in the future if the online market of ants is not sufficiently regulated.

a) Transport-Stowaway (Hitchhikers in or on passenger aircraft). We also considered arrivals by any vehicular means from invaded areas outside the RA area whose journey time would not exceed a few days.

Qu. 1.2a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This concerns only newly mated queens. Indeed, it is very unlikely that established nests will travel in or on a passenger aircraft without being transported in containers or nursery materials. In contrast, queens during the nuptial flight periods can accidently enter a passenger aircraft.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year? including the following elements: • discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway. • an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication • if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response:

Wasmannia auropunctata has a casual association as a hitchhiker/stowaway with freight and in particular air passengers. For example, of 11 interceptions in Australia, most were from air passengers (mostly carrying plants, cut-flowers, or woven baskets or matting) (data from January 1986 to 30 June 2003; Department of Agriculture, Fisheries and Forestry, Canberra cited in Harris et al 2005).

Air passengers from South and central American countries with known infestations represent one of the most likely pathways to Europe (Foucaud et al. 2010a). There is a high travel frequency between some South American countries and Spain and Portugal. Although little data is available on ant interceptions at ports and airports, the proportion of queens recorded in these interception databases is very low. This suggests a relatively low number of newly-mated queens travelling along this pathway.

Newly emerged queens and males have wings and Torres et al. (2001) collected large numbers of *W. auropunctata* in light traps on Puerto Rico. However, such flights of males and females seem to occur only in the native range of *W. auropunctata*.

No data is available to estimate the role of propagule pressure on the likelihood of introduction, but because *W. auropunctata* mainly reproduces through budding, propagule pressure may be low. Indeed, under this scenario that only considers the transport of new queens in vehicles, propagules might concern only the species native range. Dependent colony foundation involves a queen and several workers that are unlikely to reach an aircraft without being transported in containers or nursery materials.

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
In their native range where queens have independent colony foundation, the queens are likely to be able to survive several tens of days using their own reserves before the first workers emerge. However this means of colony formation in the invaded range seems to be rare in *W. auropunctata* (Causton et al. 2005). Reserves decrease in queens that need workers to start new a colony (Hölldobler and Wilson 1990). The likelihood of survival will thus decrease with increasing travel duration, but survival is possible. Multiplication and the establishment of a small nest during such an intercontinental flight however is highly unlikely.

Sexuals are produced throughout most of the year (Passera 1994) and reproduction of ant queens can occur over several months and commodities along with which ants can be imported into Europe throughout the year.

Qu. 1.5a. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	likely	CONFIDENCE	high

Response:
N/A. There are no management practices in place against hitchhiking ants or ant queens in or on airplanes.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	likely	CONFIDENCE	high

Response:
Importation via this pathway is not likely to be detected by current surveillance. Detection rates for solitary queens or even several queens are low; in general, ants are not easy to detect in cargo airplanes and detection rate thus will be low. This is particularly true for tiny ants such as *W. auropunctata* whose workers measure around 1.5 mm and queens less than 3 mm.

Qu. 1.7a. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	moderately likely	CONFIDENCE	medium

Response:
The likelihood is scored moderately likely because the number of queen ants travelling through this pathway is expected to be relatively low and the duration of the transportation would not favour the survival of the queen.

b) Transport-Contaminant (nursery material and other material from the horticultural trade)

Qu. 1.2b. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This concerns both small groups of workers and a queen dispersing through budding, and fully developed nests (with active workers) transported in nursery material by the horticultural trade.

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:

There are very limited data on ant nests arriving through the horticultural trade in Europe. At least some nests have reached Europe (Spain) and Israel in the Mediterranean basin also. In the Netherlands, *W. auropunctata* was intercepted between two to five times (first detection in 1988) during import inspections at the Plant Protection Service (Boer & Vierbergen 2008).

Ants are not listed as quarantine pests in the EU and, therefore, records rarely appear in the national and international lists of intercepted pests. However, millions of plants arrive in Europe with soil or in pots (with substrates) from infested areas (South America, Central America, Southern US, Caribbean islands, Pacific islands and south Asia) every year and, although the soil/substrate is supposed to be sterile, infestation by ants can occur just before or during transport. The European Union (EU) imports a large volume and diversity of plants for planting every year, and the value of imported plants for planting has increased 60% over the past fifteen years (Eschen et al. 2015). For example, in the period 2013-2017, the annual volume of EU imports from the US of live plants (CN code 0602) varied between 3,000 and 5,200 tonnes with value between 11 and 16 million euro. The US was the fifth largest exporter to the EU of these products in volume and number eight in value. The US share of the total EU imports of live plants was 1% in volume and 4% in value.

Flower pots are one of the preferred habitats for invasive ants in invaded regions, in particular because of their humidity and because they are usually in contact with the ground. Other horticultural material such as mulch, hay and other plant material can also harbour ant nests.

Both multiple queened (polygyne) and single queened (monogyne) colonies occur (Wetterer and Porter 2003). *Wasmannia auropunctata* reproduces through clonal or sexual reproduction (Foucaud et al. 2009) and forms polygynous colonies only in its introduced areas or in native urban areas (Chifflet et al. 2018). The number of workers in a polygynous nest can reach around 5000 workers/m^2 in areas where it is abundant (Clark et al. 2006). It is a polydomous species, that forms supercolonies over hundreds of kilometres (Chifflet et al. 2018). Single nests of *W. auropunctata* may contain several mated queens, numerous workers, pupae, larvae and eggs. Nest densities are higher in areas where this species has become a pest in its introduced range (0.75-2.7 aggregations/ m^2 on the Galápagos) (Lubin 1984). Ant nests might travel on the pathway in large numbers as a contaminant of horticultural materials containing soil.

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
In ants, groups of active workers with queens are able to survive a few weeks with no food. In the case they do not find food resources they can eat their eggs and larvae (Modlmeieir et al. 2013). Some ant species (e.g. *Temnothorax rugatulus*) can survive for several months without food resources (Rueppell and Kirkman 2005).
Because *W. auropunctata* has a generalist diet, they are likely to find food during the transport. Tropical ants like *W. auropunctata* require moisture for their survival. However, it is unlikely to be a limiting factor along this pathway.
Sexuals are produced throughout most of the year (Passera 1994) and reproduction of ant queens can occur over several months and commodities along with which ants can be imported into Europe throughout the year.

Qu. 1.5b. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:
Horticulture plants and soils/substrates are often chemically treated before shipment but there are no known existing management practices during transport and storage under current regulations. Horticultural plants and soils/substrates can be infested after treatment either before departure or during transport. There is little information available on management during transport or its efficacy.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Fully developed nests can be detected despite the workers being tiny and similar in colour to many soils. However, a newly-founded colony of a queen(s) and workers in the soil/substrate can easily arrive undetected.

Qu. 1.7b. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Given the high numbers of horticulture items imported into Europe each year from infested areas, the probability of introduction along this pathway is likely. Since 1920 more than 60% (45 out of 76 interceptions) of the interceptions at ports and airports in US were from nursery material and other matters from horticultural trade (mostly *Vitis* plants and orchids) (Blight et al unpublished data). *Wasmannia auropunctata* is most likely to have been transported between the large islands in the Galapagos archipelago on plants and in soil (Roque-Albelo and Causton, 1999).

c) Transport-Stowaway (nests transported in container/bulk, including sea freight, airfreight, train, etc.)

Qu. 1.2c. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This section includes travelling nests that are not directly associated with the horticultural trade. Virtually any article of commerce can host hitchhiking nests of all sizes and ages, including newly-founded colonies and fully developed colonies. A free volume of 10ml should be sufficient for an incipient colony composed by a queen and a dozen of workers. There are many articles of commerce and container types that are grouped together here. This includes, e.g. sea containers but also vehicles (incl. used car parts), machinery, building material, packaging materials, bark, aquaculture material and used electrical equipment.

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.

- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:

There are very limited data on ant nests arriving in Europe. Sea containers and all articles of commerce cited above were considered by Harris et al. (2005) as the main source of transport for *W. auropunctata*. Ant nests might travel along the pathway in large numbers as stowaways in containers or other bulk freight, including soil, fruits and vegetable. However, as presented above (Q1.3b), polygynous nests can reach high densities (5000 workers/m^2 and several queens) which increases the chances of a large number of nests (group composed of workers and one or several queens) to be transported from one invaded area.

The movement of large numbers of workers increases colony survival. However, it is of less concern compared to mated queens as workers do not reproduce.

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
The chances of queens surviving transport along this pathway is very likely as workers will feed them. The likelihood of nest survival along this pathway is high. In the case that they do not find food resources they can eat their eggs and larvae. Some ant species (e.g. *Temnothorax rugatulus*) can survive for several months without food resources (Rueppell and Kirkman 2005).
However, though the likelihood of survival is high, this will decrease with increasing travel duration. Multiplication of a small nest during intercontinental translocation however is probably unlikely and will depend on the availability of resources.
Sexuals are produced throughout most of the year (Passera 1994) and reproduction of ant queens can occur over several months and commodities along with which ants can be imported into Europe throughout the year.

Qu. 1.5c. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
For most of the commodities in this pathway, there are no management practices in place.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Many of these commodities are not carefully inspected. While established nests are usually obvious, small nests are often inconspicuous. Newly-founded nests with a queen and workers could easily arrive undetected. The tiny size of both queens and workers makes the detection of this species difficult. A free volume of 10ml should be sufficient for an incipient colony composed of a queen and a dozen of workers, making their detection almost impossible.

Qu. 1.7c. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Given the high numbers and multiple types of containers, commodities and items that can be associated with *W. auropunctata*, this pathway can be considered as having a high likelihood of introduction, as determined by Harris et al. (2005). Since 1920 around 40% of the interceptions at ports and airports in US were from Yam tubers, ginger, corn but also wood pallet or crate (Blight et al unpublished data).
It is likely that *W. auropunctata* was transported between small Galapagos islands on camping provisions and equipment (Roque-Albelo and Causton 1999).

d) Transportation of habitat material (soil, vegetation, wood, …)

Qu. 1.2d. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This concerns both small groups of workers and a queen dispersing through budding, and fully developed nests (with active workers) transported in soil or vegetation.

Qu. 1.3d. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.

- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:

There are very limited data on ant nests arriving in Europe. At least some nests have reached Europe (Spain) and Israel in the Mediterranean basin also. In the Netherlands, *W. auropunctata* was intercepted between two to five times (first detection in 1988) during import inspections at the Plant Protection Service (Boer & Vierbergen 2008).

Nests are likely to be transported if the soil or vegetation of an infested sites are moved into Europe. However, the volume of such trade remains unknown, and the likelihood of the introduction of infested habitat from overseas is probably very low.

Both multiple queened (polygyne) and single queened (monogyne) colonies occur (Wetterer & Porter 2003). *Wasmannia auropunctata* reproduces through clonal or sexual reproduction (Foucaud et al. 2009) and forms only polygynous colonies in its introduced areas or in native urban areas (Chifflet et al. 2018). The number of workers in a polygynous nest can reach around 5000 workers/m^2 in areas where it is abundant (Clark et al. 1982). Single nests of *W. auropunctata* may contain several mated queens, numerous workers, pupae, larvae and eggs. Nest densities are higher in areas where this species has become a pest in its introduced range (0.75-2.7 aggregations/ m^2 on the Galápagos) (Lubin 1984). Ant nests might travel on the pathway in large numbers as a contaminant of horticultural materials containing soil.

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 1.4d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
Groups of active workers with queens are able to survive a few weeks with no food. If they do not find food resources they can eat their eggs and larvae. Some ant species (e.g. *Temnothorax rugatulus*) can survive for several months without food resources (Rueppell and Kirkman 2005).
Because *W. auropunctata* has a generalist diet, they are likely to find food during the transport. Tropical ants like *W. auropunctata* require moisture for their survival. However, it is unlikely to be a limiting factor along this pathway.

Qu. 1.5d. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	likely	CONFIDENCE	low

Response:
There is no information available on management during transport or its efficacy along this pathway.

Qu. 1.6d. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	Very likely	CONFIDENCE	medium

Response:
The probability of detection will be negatively correlated to the volume of soil of vegetation transported. Fully developed nests might be detected despite the workers being tiny and similar in colour to many soils. A newly-founded colony of a queen(s) and workers in the soil/substrate could easily arrive undetected.

Qu. 1.7d. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	likely	CONFIDENCE	low

Response:
Wasmannia auropunctata could be transported effectively along this pathway as the transfer of soil of vegetation are suitable habitat for the species survival. However, the propagule pressure is unknown, the probability of habitat material transfer from both the native and introduced ranges into Europe might be low outside of the plants for planting pathway that includes habitat.

e) Food contaminant (including of live food)

Qu. 1.2e. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	unintentional	CONFIDENCE	high

Response:
This concerns both small groups of workers and a queen dispersing through budding, and newly-mated queens transported with fruits or vegetables.

Qu. 1.3e. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	likely	CONFIDENCE	medium

Response:
There are very limited data on ant nests and queens arriving in Europe. At least some nests have reached Europe (Spain) and Israel in the Mediterranean basin also. In the Netherlands, *W. auropunctata* was intercepted between two to five times (first detection 1988) during import inspections at the Plant Protection Service (Boer & Vierbergen 2008).

Europe has a large and mature market for fresh fruits and vegetables with stable demand overall. The need for year-round availability and the interest in new exotic products maintain Europe's continuous dependence on external suppliers. With a population of more than 500 million consumers, Europe is responsible for 45% of the global trade value of fresh fruits and vegetables. Five of the global 10 importing countries are in Europe. The total import value from developing countries increased 38% in five years to 18.2 billion euros in 2018, which is significantly larger than the 3.1 billion euros in imports from developed, non-European countries, which grew by 20% in the same period (source www.cbi.eu). Some of the main countries that export fruits and vegetables to Europe are either in the native or introduced range of the species (e.g. Mexico, Peru, Brazil, Argentina, Costa Rica and Guatemala) (source www.cbi.eu).

Ants are not listed as quarantine pests in the EU and, therefore, records rarely appear in the national and international lists of intercepted pests. However, millions of tons of fruits and vegetables arrive in Europe from infested areas (South America, Central America, Southern US, Caribbean islands, Pacific islands and South Asia) every year and, although they are supposed to be washed, infestation by ants can occur just before or during transport. *Wasmannia auropunctata* has been intercepted at ports and airports in US on Yam tubers, *Zea mays* and *Zingiber officinale*. Ant interceptions on food represent 34% of the total records in US.

Both multiple queened (polygyne) and single queened (monogyne) colonies occur (Wetterer & Porter 2003). *Wasmannia auropunctata* reproduces through clonal or sexual reproduction (Foucaud et al. 2009) and forms only polygynous colonies in its introduced areas or in native urban areas (Chifflet et al. 2018). Small nests or newly-mated queens may travel on the pathway in large numbers as a contaminant of fruits and vegetables.

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 1.4e. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
The probability of nest or solitary queens' survival is very likely along this pathway as ants are likely to find food resources. Tropical ants like *W. auropunctata* require moisture for their survival. However, this is unlikely to be a limiting factor along this pathway.

Qu. 1.5e. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:
Fruits and vegetables are often washed before shipment but there are no known existing management practices under current regulations during transport and storage. Fruits and vegetables can be infested after treatment either before departure or during transport. There is little information available on management during transport or its efficacy.

Qu. 1.6e. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
A newly-founded colony of a queen(s) and workers or a solitary queen can easily arrive undetected.

Qu. 1.7e. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Given the high numbers of food (fruits and vegetable) items imported into Europe each year from infested areas, the probability of introduction along this pathway is likely. Since 1920 more than 34% of the ant interceptions at ports and airports in US were from food (mostly *Zea mays* and *Zingiber officinale*) (Blight et al unpublished data).

Qu. 1.8. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions.
Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the Union.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
The species has been already recorded/intercepted in the risk assessment area and it is likely that this will happen again, specifically with contaminated soil in the horticultural trade and/or as stowaway with container/bulk imports in sea or air freight.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Climate change is not changing the risk of introduction.

2 PROBABILITY OF ENTRY

Important instructions:

- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Entry is not to be confused with spread, the movement of an organism within the risk assessment area.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[5] and the provided key to pathways[6].
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of entry.

Qu. 2.1. List relevant pathways through which the organism could enter into the environment.
For each pathway answer questions 2.2 to 2.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.2a, 2.3a, etc. and then 2.2b, 2.3b etc. for the next pathway.
In this context a pathway is the route or mechanism of entry of the species into the environment.

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 2.2-2.8

Pathway name:

a) Transport-Stowaway (Hitchhikers in or on passenger airplane) We also considered arrivals by any vehicular means from invaded areas outside the PRA area whose journey time would not exceed a few days.
b) Transport-Contaminant (nursery material and other matters from horticultural trade)
c) Transport-Stowaway (nests transported in container/bulk, including sea freight, airfreight, train, etc.)
d) Transportation of habitat material (soil, vegetation, wood, …)
e) Food contaminant (including of live food)

See question 1.1 for details.

Qu. 2.2a. Transport-Stowaway (Hitchhikers in or on airplane). We also considered arrivals by any vehicular means from invaded areas outside the PRA area whose journey time would not exceed a few days.

Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

[5] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf
[6] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

RESPONSE	unintentional	CONFIDENCE	high

Response:
This concerns only newly-mated queens without workers.

Qu. 2.3a. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year? including the following elements: • discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway. • an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication • if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	moderately likely	CONFIDENCE	low

Response:
Most new colonies are established by queens aided by a group of workers (dependent colony foundation), which decreases the probability of entry of lone queens. However, the entry of queens that originated from areas where *W. auropunctata* reproduces by nuptial flight must be considered. This is the case in the native range and at least in the Galápagos Islands (Meier 1994 cited in Harris et al. 2005) in the invaded range, although independent colony foundation was not demonstrated.

See Q1.3a for more details on species reproduction, propagule pressure, and the volume of movements along this pathway.

Qu. 2.4a. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	likely	CONFIDENCE	high

Response:
Considering the small size of *W. auropunctata* queens (< 3mm) and the queens' hiding behaviour when attempting to start a new colony, their entry into the risk assessment area undetected is likely.

Qu. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	likely	CONFIDENCE	high

Response:
Although *W. auropunctata* is a tropical species, studies demonstrated a shift in population thermotolerance in the native range (Orivel et al. 2009; Chifflet et al. 2018). Populations can exist in habitats with very different climatic conditions. In the native range the annual temperature remains stable at values below 30°C and humidity never drops below 80% in natural habitats, whereas in invaded human-modified habitats, temperatures may reach 40°C and humidity may drop to 50%. This is confirmed by the establishment of an invasive population in Israel that has much harsher conditions (colder in winter, and warmer and drier in summer) (Vonshak et al. 2009) and by the recent southern expansion of native populations in Argentina (Rey et al. 2012; Chifflet et al. 2018). Workers start to forage at 6°C (Coulin et al. 2019), which increases its chances of entry during the most appropriate months of the year.

Sexuals are produced throughout most of the year (Passera 1994) and reproduction of ant queens can occur over several months and commodities along with which ants can be imported into Europe throughout the year.

Qu. 2.6a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	likely	CONFIDENCE	medium

Response:
Many airports in the Mediterranean region are surrounded by suitable habitats including irrigated gardens and parks. Indeed, this species as an invasive ant simply requires soil as a substrate in which to establish a nest and has been found to occur in diverse degraded habitats with a wide range of climatic conditions (see section A4 for a more comprehensive description of the species habitat requirements). The recent invaded area in Spain is not different from other areas in the Mediterranean region, which supports the likelihood of queens' transfer to suitable habitats.

Qu. 2.7a. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	likely	CONFIDENCE	medium

Response:
The likelihood is scored likely because the number of queens travelling through this pathway is expected to be relatively low and the duration of the transportation would not favour the survival of the queens.

Qu. 2.2b. Transport-Contaminant (nursery material and other matters from horticultural trade) **Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?**

RESPONSE	unintentional	CONFIDENCE	high

Response:
This concerns both fully developed colonies (with many active workers) and newly-founded nests (nucleus of workers and a queen that left the nest to start a new colony) transported in nursery material for the horticultural trade. Newly-founded colonies can also be formed by queens transported in ships before the nursery material arrives at destination. However, independent colony foundation has never been observed in *W. auropunctata* despite observations of nuptial flights.

Whilst entry is almost always unintentional, *W. auropunctata* was intentionally introduced and released in cacao plantations in Cameroon to biologically control pest insects, particularly Hemiptera (Wetterer et al 1999).

Qu. 2.3b. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	likely	CONFIDENCE	low

Response:
There are very limited data on ant nests arriving through the horticultural trade in Europe. At least some nests have entered in Europe (Spain), New Zealand, Australia, US and several Caribbean and Pacific islands.
Considering this pathway as one of the main sources of introduction, it is likely that a large number of colonies will enter in the risk assessment area along this pathway. Millions of plants arrive with soil or in pots (with substrates) from infested areas (Southern US, Mexico, Caribbean islands and China) every year in Europe and, although the soil/substrate is supposed to be sterile, infestation by ants can occur just before or during transport.

Qu. 2.4b. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	likely	CONFIDENCE	medium

Response:

Fully developed nests can be detected despite the workers being tiny and light brown to golden brown, making them harder to detect in the soil. However, newly-founded colonies of few queen(s) and workers in the soil/substrate can easily enter undetected.

Qu. 2.5b. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
The horticultural trade is active throughout the year and populations of *W. auropunctata* both in native and invaded areas show pre-adaptation to temperate climatic conditions (see Q2.5a).

Qu. 2.6b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Potted plants and plant materials are likely to be transported outdoors in gardens, which may adjoin a suitable habitat. It is expected that suburban and urban habitats are most at risk at the beginning of an invasion. This is supported by the high propensity of *W. auropunctata* to invade urban areas even in its native range (Vonshak et al. 2010; Chifflet et al. 2018).

Qu. 2.7b. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Given the high numbers of horticulture items imported into Europe each year from infested areas, the probability of entry along this pathway is high. Since these ants have an affinity for nesting at tree bases and in potted plants, they are easily spread between plant nurseries. When contaminated plants are purchased and planted, the ants are likely to enter into the environment.

Qu. 2.2c. Transport-Stowaway (nests transported in container/bulk, including sea freight, airfreight, train, etc.) **Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?**

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This section includes travelling colonies that are not directly associated with the horticultural trade. Virtually any article of commerce can host hitchhiking nests of all sizes and ages,

including newly-founded and fully developed colonies. This section considers a wide range of articles such as sea containers, vehicles (incl. used car parts), machinery, building material, packaging materials, bark, aquaculture material and used electrical equipment.

Qu. 2.3c. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?
including the following elements: • discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway. • an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication • if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:
There are very limited data on ant nests entering in Europe. At least some nests have entered Europe (Spain), New Zealand, Australia, US and several Caribbean and Pacific islands. Sea containers and all articles of commerce cited in Q2.2c were scored by Harris et al. (2005) as presenting a high likelihood of introduction for nests.

Propagule pressure may be high since the number of incidents are likely to be high and the fact that the number of workers in a polygynous colony can reach around 5000 workers/m^2 in areas where it is abundant (Clark et al. 1982).

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 2.4c. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Many of these commodities are not carefully inspected at the endpoint. While established nests may be obvious despite the workers being very small, newly-founded colonies are often inconspicuous. Therefore newly-founded colonies with few queen(s) and workers could easily enter undetected in the risk assessment area.

Qu. 2.5c. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Commodities that can carry *W. auropunctata* are introduced to the risk assessment area throughout the year and populations from both native and invaded areas show pre-adaptation to temperate climatic conditions (see 2.5a). Therefore it is likely that the organism enters into the environment during the months of the year the most appropriate for establishment.

Qu. 2.6c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Several of the potential commodities and items in which nests can hide can be transported to suitable habitats since the ant particularly likes disturbed habitats, which are found everywhere, specifically in urban and semi-urban habitats (e.g. harbours, airports, private households, train stations). This is confirmed by the high propensity for *W. auropunctata* to invade urban areas even in its native range (Vonshak et al. 2010; Chifflet et al. 2018).

Qu. 2.7c. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Given the high numbers and types of containers, commodities and items that can be associated with *W. auropunctata*, this pathway can be considered as having a high likelihood of entry, as determined by Harris et al. (2005). However, contrary to the horticulture pathway, the final destination of some items (e.g. vegetables or fruits or electrical equipment) may decrease the likelihood of release in nature.

d) Qu. 2.2d. Transportation of habitat material (soil, vegetation, wood, …)

Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This concerns both fully developed colonies (with many active workers) and newly-founded nests (nucleus of workers and a queen that have left the nest to start a new colony) transported

in soil or vegetation during the movement of habitat material. Newly-founded colonies can also be formed by queens transported in ships before the shipment arrives at destination. However, independent colony foundation has never been observed in *W. auropunctata* despite observations of nuptial flights.

Whilst entry is almost always unintentional, *W. auropunctata* was intentionally introduced in cacao plantations in Cameroon to biologically control pest insects, particularly Hemiptera (Wetterer et al 1999).

Qu. 2.3d. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	unlikely	CONFIDENCE	low

Response:
There are very limited data on ant nests arriving through the horticultural trade in Europe. At least some nests have entered in Europe (Spain), New Zealand, Australia, US and several Caribbean and Pacific islands.
Considering the low probability of habitat transfer from overseas into Europe, it is unlikely that a large number of colonies will enter into the environment in the risk assessment area along this pathway.

Qu. 2.4d. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	likely	CONFIDENCE	medium

Response:
The probability of detection will be negatively correlated to the volume of soil of vegetation transported. Fully developed nests might be detected despite the workers being tiny and similar in colour to many soils. A newly-founded colony of a queen(s) and workers in the soil/substrate can easily enter undetected.

Qu. 2.5d. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	likely	CONFIDENCE	high

Response:
Such trade may be active throughout the year and populations of *W. auropunctata* both in native and invaded areas show pre-adaptation to temperate climatic conditions (see Q2.5a).

Qu. 2.6d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	likely	CONFIDENCE	high

Response:
Soil and vegetation are likely to be transported to gardens, which may adjoin a suitable habitat. It is expected that suburban and urban habitats are most at risk at the beginning of an invasion. This is supported by the high propensity of *W. auropunctata* to invade urban areas even in its native range (Vonshak et al. 2010; Chifflet et al. 2018).

Qu. 2.7d. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	likely	CONFIDENCE	low

Response:
Wasmannia auropunctata can be transported in large amount along this pathway as soil or vegetation are highly suitable habitats. However, the propagule pressure is unknown outside of the trade of plants for planting, the probability of the transfer of habitat (soil and vegetation) from both the native and introduced ranges into Europe along this pathway might be low.

e) Qu. 2.2e. Food contaminant (including of live food) **Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?**

RESPONSE	unintentional	CONFIDENCE	high

Response:
This concerns both newly-founded nests (nucleus of workers and a queen that left the nest to start a new colony) and solitary queens transported with food (e.g. fruits and vegetables). Newly-founded colonies can be formed by queens transported in ships before the nursery material arrives at destination. However, independent colony foundation has never been observed in *W. auropunctata* despite observations of nuptial flights.

Qu. 2.3e. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year? including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:

There are very limited data on ant nests entering into Europe. At least some nests entered into the environment in Spain and also in Israel in the Mediterranean basin. In the Netherlands, *W. auropunctata* was intercepted between two to five times during import inspections at the Plant Protection Service (Boer & Vierbergen 2008) but colonies have never been observed into the environment.

The number of organisms entering into the environment will directly depend on the number of organisms introduced into the RA. Ants are not listed as quarantine pests in the EU and, therefore, records rarely appear in the national and international lists of intercepted pests. However, millions of tons of fruits and vegetables arrive in Europe from infested areas (South America, Central America, Southern US, Caribbean islands, Pacific islands and South Asia) every year and, although they are supposed to be washed, infestation by ants can occur just before or during transport. *Wasmannia auropunctata* has been intercepted at ports and airports in US on Yam tubers, *Zea mays* and *Zingiber officinale*. Interceptions on food sources represent 34% of the total records in US.

Qu. 2.4e. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Newly-founded nests may be detected despite the workers being tiny and light brown to golden brown, making them harder to detect in the soil. Newly-mated queens can easily enter undetected.

Qu. 2.5e. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
This trade is active throughout the year and populations of *W. auropunctata* both in native and invaded areas show pre-adaptation to temperate climatic conditions (see Q2.5a).

Qu. 2.6e. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Food is likely to arrive in warehouses, which may adjoin a suitable habitat. It is expected that suburban and urban habitats are most at risk at the beginning of an invasion. This is supported by the high propensity of *W. auropunctata* to invade urban areas even in its native range (Vonshak et al. 2010; Chifflet et al. 2018).

Qu. 2.7e. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:
Since these ants can nest in disturbed areas, they can easily find a suitable habitat nearby. When contaminated food is stored or purchased, the ants are likely to enter into the environment.

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

Qu. 2.8. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in current conditions and specify if different in relevant biogeographical regions. Provide a thorough assessment of the risk of entry into the environment in relevant biogeographical regions in current conditions.

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
The species has been already recorded/intercepted in Europe and it is likely that this will happen again, most likely via contaminated soil in the horticultural trade and/or as stowaway with container/bulk imports in sea or air freights.

Qu. 2.9. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in foreseeable climate change conditions and specify if different in relevant biogeographical regions. Thorough assessment of the risk of entry in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if likelihood of entry is likely to increase or decrease for specific pathways.

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Climate change will not change the risk of introduction or likelihood of entry based on the specified active pathways.

3 PROBABILITY OF ESTABLISHMENT

Important instructions:
• For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established.

Qu. 3.1. How likely is it that the organism will be able to establish in the risk assessment area based on the history of invasion by this organism elsewhere in the world (including similarity between other abiotic conditions within it and the organism's current distribution)?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
It is likely that *W. auropunctata* will establish colonies in the risk assessment area. An established population was already recorded in southern Spain (Espadaler et al. 2018). The species is also present in Israel under harsher climatic conditions (Vonshak et al. 2009; Vonshak et al. 2010). Despite these contrasting abiotic conditions, the Israeli populations display nesting and foraging behaviour similar to that observed in tropical and subtropical areas (Vonshak et al. 2010). The population in Israel originates from a population that had extended its distribution south in Argentina under a temperate climate (Rey et al. 2012). This population seems to be pre-adapted to lower temperatures, which increases the likelihood of colony establishment in the risk assessment area. The origin of the European population in Spain has to be determined to confirm this hypothesis.

Bertelsmeier et al. (2015a), using a climate matching model (Maxent) based on present distributions, mapped suitable areas globally for 15 of the worst invasive ant species (incl. *W. auropunctata*) (Annex I). They showed that around 5% of the European continent is presently suitable for *W. auropunctata*. However, this model seems to be more conservative than the Maxent model developed by Federman et al. (2013) (Annex II). In this later model, irrigation was included as a variable. This corrected model predicted a larger suitable area in Europe, including the continental biogeographic region that is absent from Bertelsmeier et al. (2015a). Beckmann et al. (2019) found that both the Mediterranean and the Atlantic regions are at risk of species establishment (Annex IV).

Urbanisation is another key factor that determines the success of invasive ants' establishment (Holway et al. 2002). *Wasmannia auropunctata* is highly competitive in such habitats (Orivel et al. 2009; Vonshak et al. 2010), and there is little doubt that it will find suitable urban areas, even under northern latitudes to establish colonies.

Qu. 3.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?

RESPONSE	**moderately widespread**	**CONFIDENCE**	**high**

Response:
Wasmannia auropunctata prefers disturbed habitats, which are found everywhere in Europe. However, even though some populations are more thermotolerant (Rey et al. 2012), as a tropical species it needs elevated temperatures to complete its life cycle, which may limit its distribution to the Mediterranean and Atlantic regions, at least in natural areas. Climatic records show that it can survive in areas with minimum temperatures ranging from 8°C to 22.7°C and maximum temperatures ranging from 29°C to 39.7°C, as well as a maximum of 12 months with less than 15 mm precipitation (Vonshak et al. 2010). The critical thermal maximum for both workers and queens is around 42°C and their critical thermal minimum is around 3.7°C (Coulin et al. 2019).

Qu. 3.3. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area?

RESPONSE	**N/A**	**CONFIDENCE**	**high**

Response:
Wasmannia auropunctata does not require another species for establishment.

Qu. 3.4. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Wasmannia auropunctata is an ecologically successful dominant ant both in disturbed and protected ecosystems in areas to which it has been introduced. *Wasmannia auropunctata* appears to be highly competitive compared with other invasive ant species. *Wasmannia auropunctata* was ranked first during competitive confrontations with six other highly invasive ants under laboratory conditions (Bertelsmeier et al. 2016). This is confirmed by the massive impacts it has on other ants in nature (Jourdan 1997; Holway et al. 2002; Vonshak et al. 2010; Berman et al. 2013a).

In several suitable areas it will have to face the competition with two invasive species, the Argentine ant *Linepithema humile* and *Tapinoma magnum*. These species are highly competitive (Blight et al. 2010; Blight et al. 2014) and confrontations will be asymmetric as they both already form supercolonies of many hundred thousand individuals. However, *W. auropunctata* was superior to the Argentine ant under laboratory confrontations (Bertelsmeier et al. 2015b; Bertelsmeier et al. 2016). The Argentine ant is largely distributed along the Mediterranean coast from Portugal to Italy through Spain and France. Moreover, these competitor species have a more temperate distribution and may have a competitive advantage over *W. auropunctata* in those parts of the risk assessment area. Nonetheless, where these competitive species are not present, establishment may easily occur.

Qu. 3.5. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	likely	CONFIDENCE	medium

Response:
No species of the genus *Wasmannia* are native to Europe, no specialist natural enemies of *Wasmannia* are known to occur in Europe. Thus, establishment in Europe is only likely to be hindered by other ant species and possibly generalist predators that may prey on individual queens.

Qu. 3.6. How likely is the organism to establish despite existing management practices in the risk assessment area?

RESPONSE	likely	CONFIDENCE	medium

Response:
No specific management practices are in place against invasive ants in the wild in Europe. Eradication of single nests is straightforward in buildings but much less so outdoors. However, some eradication programmes have succeeded at a local scale, such as over 2ha on Santa Fe Island (Galapagos) (Hoffmann et al. 2016). An invasive population has been successfully eradicated over 21 ha in the Galapagos Islands after a nine months period of treatments (Causton et al. 2005).

Qu. 3.7. How likely are existing management practices in the risk assessment area to facilitate establishment?

RESPONSE	very unlikely	CONFIDENCE	high

Response:
There have been no management practices applied in the risk assessment area but conventional management practices to date should not facilitate establishment (Hoffmann et al. 2016).

Qu. 3.8. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	likely	CONFIDENCE	medium

Response:
The eradication of *W. auropunctata* outdoors is difficult, especially when populations reach high densities of nests and individuals within those nests. Only killing of the queens will eradicate the population, which requires the use of toxinS with a delayed action to reach that queens that are protected inside the nest.

An invasive population has been successfully eradicated over 21 ha in the Galapagos Islands after a nine months period of treatments (Causton et al. 2005). This is the largest successful eradication campaign. However incipient colonies can be successfully eradicated (Hoffmann et al. 2016).

Qu. 3.9. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area? including the following elements: • a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the Union • an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the Union. If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**Very likely**	**CONFIDENCE**	**high**

Response:
Mikheyev et al. (2008) showed that the probability of establishment increases with propagule pressure in *W. auropunctata* although others factors such as local biotic and abiotic conditions may determine establishment success.

Independent nest founding is considered highly unlikely (Ulloa-Chacon 1990, cited in Harris et al 2005). Colonies mainly reproduce through budding by which a group of workers leave the nest with a queen to start a new colony within a few metres, in both the invaded areas and in native urban areas (Chifflet et al. 2018). This limits the success of a lone queen in colony foundation. Indeed, in this type of reproduction, the likelihood of unaided queens starting a new colony is very low (Hölldobler and Wilson 1977).

Despite *W. auropunctata* normally having single queen (monogynous) populations in the native range, clonal polygynous forms are mainly found both in introduced areas and in native disturbed habitats. The polygynous form can more easily establish because the higher number of queens increases reproduction potential, especially in the critical early stages of establishment.

In polygynous populations in the introduced range, the density of nests is more than 100 times higher than in native natural habitats. The number of queens and workers in a polygynous nest can vary enormously, from 35 to 90 queens per m^2 and from 500 to 2,500 workers per m^2 in New Caledonia (Orivel et al. 2009). In the Galapagos Islands, Clark et al. (1982) estimated the number of workers at 5,000 individuals/m^2 in areas where it is abundant.

Sexuals are produced throughout most of the year (Passera 1994) and can reproduce under varying climatic conditions.

The division of labour, i.e. the existence of reproductive caste, enabled ants to become ecologically dominant invertebrates in terrestrial habitats, with a high success rate of reproduction and dispersal. For example in the case of the Argentine ant, *Linepithema humile*, it was shown that as few as 10 workers and a queen are sufficient for a colony to grow quickly (Hee et al. 2000; Luque et al. 2013).

Qu. 3.10. How likely is the adaptability of the organism to facilitate its establishment?

RESPONSE	**Very likely**	**CONFIDENCE**	**high**

Response:
Wasmannia auropunctata is one of the most widespread invasive ants. Despite it being considered a tropical ant, its southern expansion in its native range and as well as its presence in Spain and Israel highlights its adaptability to various climatic conditions (Vonshak et al. 2009; Rey et al. 2012; Chifflet et al. 2018). This adaptability is evident from laboratory cold-tolerance tests which showed that workers from populations established in Israel survived significantly better and recovered faster than populations from northern part of its distribution (native and introduced areas) (Rey et al. 2012).

Wasmannia auropunctata favours environments that are associated with humans, but it can colonise both open and closed habitats (Orivel et al. 2009; Chifflet et al. 2018). However, several factors can constrain establishment of this species. Humidity is required for the survival of the species and may be a key factor in defining suitable habitats (Federman et al. 2013).

Qu. 3.11. How likely is it that the organism could establish despite low genetic diversity in the founder population?

RESPONSE	**Very likely**	**CONFIDENCE**	**high**

Response:
Most invasive ants, which are among the most invasive insects worldwide, establish following the entry of single nests or queens (Holway et al. 2002). This is the case in *W. auropunctata* whose population in Israel originates from one queen and one male genotype, that reproduce clonally (Vonshak et al. 2009). A similar pattern has been seen in Hawaï and in central Africa where single-clone introductions gave rise to the vast majority of local infestations (Mikheyev et al. 2009).

Its invasive success is highly associated with its particular reproductive system. Some populations have a classical haplodiploid reproductive system in which diploid females (i.e. queens and workers), are produced via sexual reproduction, whereas haploid males develop from unfertilised eggs through arrhenotokous parthenogenesis (Foucaud et al. 2007). In other populations, both queens and males are clonal (Fournier et al. 2005) but differ in their mode of reproduction. Diploid queens reproduce through automictic thelytokous parthenogenesis, a system showing strongly reduced recombination rates (Foucaud et al. 2010b) by which new reproductive females (gynes) are genetically identical to their mother.

Interestingly, this reproductive system is strongly associated with the type of habitat. Sexual populations are usually not numerically dominant (i.e. with low density of workers, brood, queens and nests), and establish mostly in natural environments with little or no human disturbance (e.g. primary or secondary forests), whereas clonal populations are usually numerically dominant (i.e. with high density of workers, brood, queens and nests) and colonise human-modified habitats (Foucaud et al. 2009). Therefore, low genetic diversity does not seem to be a barrier to establishment.

Qu. 3.12. If the organism does not establish, then how likely is it that casual populations will continue to occur? Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
If queens fail to lay eggs, it is however likely that populations will continue to occur because of recurrent introduction events. As shown with interception data from countries such as US (Bertelsmeier et al. 2018), New Zealand (Harris et al 2005), *W. auropunctata* is intercepted at ports of entry but not with a high frequency. However, the recent detection of established populations in Israel and Spain suggests a non-negligible rate of propagule pressure.

Qu. 3.13. Estimate the overall likelihood of establishment in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided. Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the Union.

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
If we consider the invaded area in the risk assessment area, the maximum scores are given as a recent establishment of an active population in southern Spain (Espadaler et al. 2018) confirms the certainty of *W. auropunctata* establishing populations in the risk assessment area.

However, if we consider the uninvaded area, the scores decrease to likely with a medium confidence level as the predicted area covered by suitable conditions is restricted.

In the Mediterranean biogeographical region, establishment under current conditions is likely at least in urban areas (Spain, France, Italy, Greece, Croatia). Also, both the southern Atlantic (Southern France, Northeast of Spain and entire coast of Portugal) in the Mediterranean region and parts of Ireland and west of France are considered to be potentially susceptible (Bertelsmeier et al. 2015a Annex I; Beckmann et al. 2019 Annex IV). However, all these predicted suitable areas are restricted and cover a very limited area in the risk assessment area.

When considering irrigation, suitable areas cover a larger part of the risk assessment area including biogeographic region (Federman et al. 2013, see annex II).

Qu. 3.14 Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided. Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk. With regard to climate change, provide information on • the applied timeframe (e.g. 2050/2070) • the applied scenario (e.g. RCP 4.5) • what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods) The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
Under foreseeable climate change, the overall area suitable for *W. auropunctata* will not significantly decrease in the future (according to Bertelsmeier et al. 2015a and Beckmann et al. 2019, Annexes I and IV respectively). *Wasmannia auropunctata* may shift from a southern distribution to a Northwest distribution. Whereas suitable areas are expected to decrease in Portugal, Spain, Italy, Greece, they will increase in Ireland and UK in the model developed by Bertelsmeier et al. (2015a). Beckmann et al. (2019) found an increase in suitable areas under foreseeable climate change in the Mediterranean, Atlantic and Black Sea Biogeographical regions (Annex IV).

4 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of entry section. In other words, intentional anthropogenic “spread” via release or escape (“jump-dispersal”), should be dealt within the entry section. However, as repeated releases contribute to the spread of the target organism in the risk assessment area, the relevant pathway(s) should be briefly discussed here too, with an explicit reference to the entry section for additional details.

Qu. 4.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

The description of spread patterns should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minor	CONFIDENCE	high

Response:
Polygynous colonies spread by “budding”, i.e. queens disperse only short distances over land and take workers with her to start a new colony. Such a strategy does not allow a rapid spread but does allow increased nest densities by increasing survival rates of queens and colonies. Such a pattern is currently observed in the newly infested area in southern Spain (Espadaler et al. 2018).

New colonies can also be founded by winged queens, capable of flying long distances. However, although winged queens have been captured in the invaded range (see above), independent queens have not been observed founding new colonies (Causton et al. 2005).

The question is scored “minor” because it is very likely to spread more slowly by natural means than by human assistance.

Qu. 4.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (List and comment on each of the mechanisms for human-assisted spread and provide a description of the associated commodities.)

including the following elements:

- a list and description of the anthropogenic spread mechanisms of the species in relation to the environmental conditions in the Union.

- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

RESPONSE	major	CONFIDENCE	high

Response:
Human assisted pathways of spread include the agricultural and horticultural trade of plants, plant materials, and soil/substrate as well as other movements of commodities and these are frequent and large. This species reproduces mostly or entirely by nest budding rather than nuptial flights, and its natural long-range dispersal is limited (Lubin 1984). Therefore, *W. auropunctata* spreads in its non-native range primarily through human activities (Holway et al. 2002), such as transfer of plants, soil, food packaging, logs, and wood products (Lubin 1984; Roque-Albelo and Causton 1999; Wetterer and Porter 2003; Wetterer 2013).

Qu. 4.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways with an indication of their importance and associated risks (e.g. the likelihood of spread in the Union, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host). Where possible details about the specific origins and end points of the pathways shall be included.
- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- All relevant pathways should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used.

Pathway name:
a) Transport-Contaminant (Contaminant nursery material)
b) Transport-Stowaway (Container/bulk, including road transport, sea freight, airfreight, train, etc.)
c) Transportation of habitat material (soil, vegetation, wood, …)
d) Food contaminant (including of live food)
e) Unaided (Natural dispersal)

See question 1.1 for details.

a) Transport-Contaminant (Contaminant nursery material)

Qu. 4.3a. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	CONFIDENCE	high

Response:

Qu. 4.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year? including the following elements: • an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication • if appropriate, indicate the rate of spread along this pathway • if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very likely	CONFIDENCE	high

Response:
Within Europe, movements of potted plants are unrestricted. Soil/substrate in potted plants is a favourite medium for nesting (see introduction and entry sections above). Thus, newly founded nests or parts of fully developed nests could easily be moved. Other horticultural material such as mulch, hay and other plant material can also harbour ant nests.

Polygynous nests include many queens and may contain thousands of workers. Ant nests might get onto the pathway in large numbers as contaminants of horticultural materials including soil.

The peculiar, almost unique, reproductive caste system of these eusocial insects can facilitate the development of viable colonies. For example, in the case of the Argentine ant, *Linepithema humile*, it was shown that as few as 10 workers and a queen are sufficient to originate a colony (Hee et al. 2000; Luque et al. 2013).

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 4.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	likely	CONFIDENCE	high

Response:
A newly founded nest or parts of fully developed nests are able to survive transport and storage. The introduction of a population of *W. auropunctata* in Israel, which is believed to originate from south America, illustrates its capacity to travel over long distance (Vonshak et al. 2009).

Colonies of the ant *Temnothorax rugatulus* can survive for several months without food resources (Rueppell and Kirkman 2005).
Likelihood of survival is high, nevertheless will decrease with increasing travel duration. Multiplication of a colony (production of sexuals and reproduction) during spread within the EU cannot be ruled out, but is rather unlikely.

Qu. 4.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Horticultural plants and products and soils/substrates are not systematically treated before translocation within the EU (directive 2000/29/CE) (see management annex for treatments before introduction into Europe).

Qu. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Fully developed nests could be quite visible even though workers are small (<2mm). In contrast, newly-founded nests with few queen(s) and workers can easily travel undetected in soil or other horticultural products.

Qu. 4.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
Potted plants and plant materials are often planted or stored in, or close to, highly suitable habitats, such as gardens, parks, road sides, etc. It is expected that the distribution of these media will facilitate occurrences in urban, suburban and agricultural habitats.

Qu. 4.9a. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**moderately rapidly**	**CONFIDENCE**	**medium**

Response:
We consider this pathway as the most likely pathway of spread of *W. auropunctata* within Europe. A similar conclusion has been made for New Zealand (Harris et al 2005).

The rate of spread will depend on the internal volume of trade within Europe.

For comparison, accidental transportation by humans has resulted in rates of spread of 10.50 km/yr in the case of *S. invicta* into uninvaded areas of the USA (Ross and Trager 1990).

b) Transport-Stowaway (Container/bulk, including road transport, sea freight, airfreight, train, etc.)

Qu. 4.3b. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	CONFIDENCE	high

Response:
Virtually any article of commerce can host hitchhiking ants within nests of all sizes and ages, including newly-founded and fully developed nests. A free volume of 10ml should be sufficient for an incipient colony composed by a queen and a dozen of workers. There are very many transported items (e.g. vehicles (incl. used car parts), machinery, building material, agricultural equipment packaging materials, bark, used electric equipment, non-agricultural soil, sand, gravel) that are suitable to carry nests and are grouped here together.

Qu. 4.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very likely	CONFIDENCE	high

Response:
There are very limited data on ant nests translocated within the EU. Polygynous nests include many queens and may contain thousands of workers. Ant nests might get onto transported items in large numbers as stowaways.

For the Argentine ant, *Linepithema humile*, it was shown that as few as 10 workers and a queen are sufficient for a colony to grow quickly (Hee et al. 2000; Luque et al. 2013).

The likelihood of reinvasion after eradication is identical to the likelihood of spread in the first place.

Qu. 4.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	likely	CONFIDENCE	high

Response:
The likelihood of colony survival is high, but will decrease with increasing travel duration. Post introduction distances and hence transport periods are likely to be relatively short. Multiplication of a colony during spread within the EU cannot be ruled out, but is rather unlikely.

Qu. 4.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	likely	CONFIDENCE	high

Response:
Most potential commodities that can carry ants or nests are not managed to limit ant spread.

Qu. 4.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	likely	CONFIDENCE	high

Response:
Fully developed nests are quite visible. In contrast, newly-founded nests with few queen(s) and workers can easily travel undetected in most potential transported items.

Qu. 4.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	very likely	CONFIDENCE	medium

Response:
Several of the potential commodities and items in which nests can hide can be transported to suitable outdoor habitats since the ant particularly likes disturbed soils, which are found everywhere, specifically in urban, semi-urban and agricultural habitats.

Qu. 4.9b. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	rapidly	CONFIDENCE	medium

Response:

Given the high numbers and types of commodities and items that can be associated with *W. auropunctata*, this species has the potential to spread rapidly in the RA area through this pathway.

The rate of spread will depend on the internal volume of trade within Europe.

c) Transportation of habitat material (soil, vegetation, wood, …)

Qu. 4.3c. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	CONFIDENCE	high

Response: There should be no intentional spread of this species along this pathway.

Qu. 4.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very likely	CONFIDENCE	high

Response:
Within Europe, movements of habitat (e.g. soil and vegetation) are unrestricted. Soil/substrate is a favourite medium for nesting as the species can nest in the soil. Thus, newly founded nests or parts of fully developed nests could easily be moved. Other habitat material such vegetation, can also harbour ant nests.

Polygynous nests include many queens and may contain thousands of workers. Ant nests might get onto the pathway in large numbers as contaminants of habitat material.

The peculiar reproductive caste system of these eusocial insects can facilitate the development of viable colonies. For example, in the case of the Argentine ant, *Linepithema humile*, it was shown that as few as 10 workers and a queen are sufficient to originate a colony (Hee et al. 2000; Luque et al. 2013).

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 4.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
A newly founded nest or parts of fully developed nests are able to survive transport and storage. Likelihood of survival is high, nevertheless will decrease with increasing travel duration even if this pathway might concern only transfer over short distances (within member states). Multiplication of a colony (production of sexuals and reproduction) during spread within the EU cannot be ruled out, but is rather unlikely.

Qu. 4.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
There is no specific regulation along this pathway as invasive ants are not listed as pests.

Qu. 4.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Both fully developed nests and newly-founded nests with few queen(s) and workers can easily travel undetected in soil or vegetation as this pathway can involve large volumes of habitat material.

Qu. 4.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
Habitat materials are often deposited in, or close to, highly suitable habitats, such as gardens, parks, road sides, etc. It is expected that the distribution of these media will facilitate occurrences in urban, suburban and agricultural habitats.

Qu. 4.9c. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**moderately rapidly**	**CONFIDENCE**	**medium**

Response:
We consider this pathway as a likely pathway of spread of *W. auropunctata* within Europe. However, the transfer of habitat materials may occur mainly over short distances within a country which will limit the rate of spread within the risk assessment area.

d) Food contaminant (including of live food)

Qu. 4.3d. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	CONFIDENCE	high

Response: There is unlikely to be any intentional spread along this pathway.

Qu. 4.4d. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	likely	CONFIDENCE	low

Response:
Within Europe, movements of food sources are unrestricted. Newly founded nests or newly-mated queens, although independent colony foundation has never been observed in the introduced range of the species, could easily be moved.

The peculiar reproductive caste system of these eusocial insects can facilitate the development of viable colonies. For example, in the case of the Argentine ant, *Linepithema humile*, it was shown that as few as 10 workers and a queen are sufficient to originate a colony (Hee et al. 2000; Luque et al. 2013).

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 4.5d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	Very likely	CONFIDENCE	high

Response:
A newly founded nest or new-mated queens are able to survive transport.

The likelihood of survival is high, nevertheless this will decrease with increasing travel duration. Multiplication of a colony (production of sexuals and reproduction) during spread within the EU cannot be ruled out, but is rather unlikely.

Qu. 4.6d. How likely is the organism to survive existing management practices during spread?

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:
Fruits and vegetable are often washed before shipment but there are no known existing management practices under current regulations during transport and storage. Fruits and vegetables can be infested after treatment either before departure or during transport. There is little information available on management during transport or its efficacy.

Qu. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
A newly-founded colony of a queen(s) and workers or a solitary queen can easily arrive undetected.

Qu. 4.8d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
Food sources are likely to be transported indoors in warehouses, which may adjoin a suitable habitat. It is expected that suburban and urban habitats are most at risk at the beginning of an invasion. This is supported by the high propensity of *W. auropunctata* to invade urban areas even in its native range (Vonshak et al. 2010; Chifflet et al. 2018).

Qu. 4.9d. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**moderately rapidly**	**CONFIDENCE**	**medium**

Response:

We consider this pathway as a likely pathway of spread of *W. auropunctata* within Europe. The rate of spread will depend on the internal volume of trade within Europe.

For information, accidental transportation by humans has resulted in rates of spread of 10.50 km/yr in the case of *S. invicta* into uninvaded areas of the USA (Ross and Trager 1990).

e) Unaided (Natural dispersal)

Qu. 4.3e. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	CONFIDENCE	high

Response: N/A

Qu. 4.4e. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very likely	CONFIDENCE	high

Response:
Spread by budding includes a large number of workers and few queens that is sufficient to originate a viable population. This type a reproduction increases nests densities but limits the distance of spread to a few meters (Hölldobler and Wilson 1990).

The likelihood of reinvasion after eradication is identical to the likelihood of spread in the first place.

Qu. 4.5e. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very likely	CONFIDENCE	high

Response:
Dispersion by budding increases queen survival compared to the low life expectancy of independent colony foundation.

Qu. 4.6e. How likely is the organism to survive existing management practices during spread?

RESPONSE	very likely	CONFIDENCE	high

Response:
There are no management practices currently in place.

Qu. 4.7e. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	likely	CONFIDENCE	high

Response:
Low ant densities (e.g. small newly-founded nests) often remain undetected for longer periods. However, spread will mainly occur from well-established nests, which would be more noticeable and spread should be detected earlier.
The fact that *W. auropunctata* has a painful sting, and is highly likely to be found in close association with urban areas, people should aid early detection of its presence, even if its initial establishment goes unnoticed (Espadaler et al. 2018).

Qu. 4.8e. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	very likely	CONFIDENCE	high

Response:
Dispersion by budding is limited (less than 300m/year) (Holway et al. 2002) increasing the chances of individuals to find suitable habitats. This is particularly true in *W. auropunctata* which is a true generalist species that is able to invade both open and closed habitats.

Qu. 4.9e. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	very slowly	CONFIDENCE	high

Response:
The rate of spread is relatively low in polygynous colonies that reproduce by budding (below 300m per year, Hölldobler & Wilson 1990). In Spain, the new population has spread across 5ha approximately in more than 5 years (Espadaler et al. 2018). Expansion rates of *W. auropunctata* vary from 73 m/year in Gabon (Walsh et al. 2004) and up to 500 m/year at Galápagos Archipelago (Lubin 1984).

End of pathway assessment, repeat Qu. 4.3 to 4.9. as necessary using separate identifiers.

Qu. 4.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	**very difficult**	**CONFIDENCE**	**high**

Response:
It will probably be very difficult to physically contain the species. Its spread will be constrained by climate, habitat suitability and competition from other invasive species. If *W. auropunctata* become established in a European region, quarantine measures could be put in place to restrict the risk of medium to long-distance spread, e.g. through nursery stock, as in USA for *S. invicta.*

Qu. 4.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible). Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the Union.

RESPONSE	**moderately rapidly**	**CONFIDENCE**	**high**

Response:
Based on the low ecoclimatic suitability in Europe, we can estimate that it will spread unaided to all potentially suitable biogeographical regions, but slower than in tropical and sub-tropical regions. However, recent studies confirmed the southern expansion of its native range, highlighting its capacity of adaption increasing potentially its suitable range in the risk assessment area.

Its spread will occur mainly through human transport but its distribution will be indirectly constrained by climate, habitat suitability and competition from other dominant ants (invasive and native).

The rate of spread will also depend on the internal volume of trade within Europe.

Qu. 4.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible). Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	**moderately rapidly**	**CONFIDENCE**	**high**

Response:
Climate change will not significantly increase the potential or rate of spread directly, even if it is expected to increase the distribution range to north-western Europe (Bertelsmeier et al. 2015a) (Annex I). Beckmann et al. (2019) found an increase in suitable areas under foreseeable climate change in the Mediterranean, Atlantic and Black Sea Biogeographical regions (Annex

IV). Climate change may facilitate population growth with subsequently increasing potential for spread.

5 MAGNITUDE OF IMPACT

Important instructions:
• Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed. • Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change). • Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)

Biodiversity and ecosystem impacts

Qu. 5.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?
including the following elements: • Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems • impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	**Major**	**CONFIDENCE**	**high**

Comment:
Wasmannia auropunctata is one of the most harmful invasive ant species worldwide. Indeed, the environmental impacts of *W. auropunctata* seem to be more pronounced than those of other invasive ants, except maybe the Red Imported Fire Ant *Solenopsis invicta* (Lowe et al. 2000; Holway et al. 2002). The severity of impact is most likely to relate to the population densities achieved.

Environmental impacts caused by the ant in the invaded range excluding the risk assessment area are multiple:

- <u>Impacts on fauna</u>:

The invasion of *W. auropunctata* is systematically followed by a reduction of biodiversity initially through a major decrease in ants and other invertebrates (Lubin 1984; Jourdan 1997; Vonshak et al. 2010; Berman et al. 2013a,b). In addition to dominating many ant communities numerically, *W. auropunctata*, leads to a systematic eradication of almost 100% of the native species (Orivel et al. 2009; Berman et al. 2013a,b). The effects of competition and predation may alter the invertebrate community even if the establishment of *W. auropunctata* at a site increased the total biomass of ant predators. Ant abundance, species richness, and community composition were all significantly affected by *W. auropunctata* density in Israel (Vonshak et al. 2010). Population density of *W. auropunctata* also affected two other ground arthropod groups, spiders, and beetles.

Foraging ants also prey on and are a severe threat to vertebrates. The venomous sting of *W. auropunctata* may give it a greater ability to subdue vertebrate and large invertebrate prey. For example, it has significantly reduced population sizes of an endemic skink in New Caledonia (Jourdan et al. 2001). It also impacts hatchlings of the Melanesian scrub fowl in northern Melanesia (Wetterer and Porter 2003). However, no other studies that quantified impacts of *W. auropunctata* on vertebrate populations were found.

- <u>Impacts on plants</u>:

The impact on wild plants has been less studied than that on animals or cultivated plants. *Wasmannia auropunctata* interferes with seed dispersal of myrmecochorous plants by reducing dispersal distances, and leaving seeds exposed on the soil surface (Ness and Bronstein 2004). *Wasmannia auropunctata* rarely bury the myrmecochorous seeds they encounter, instead leaving them exposed on the soil surface. Seeds displaced by the little fire ant are typically displaced only short distances and sometimes workers ingest the elaiosome *in situ* but do not move the seed itself. These exposed seeds are not protected from fire or mammalian seed predators, and likely have less access to nutrients than do buried seeds or seeds deposited in ant nests.

As with other invasive ants, *W. auropunctata* is attracted to plants because of their carbohydrate-rich resources or it is attracted to by honeydew-producing herbivores (Ness and Bronstein 2004). In the native range, *W. auropunctata* they can provide protection to some plants by protecting it from molesting herbivores (De La Fuente and Marquis 1999). The presence of *W. auropunctata* benefits the plant: ant-visited plants grew significantly higher than ant-excluded plants (De La Fuente and Marquis 1999).

Qu. 5.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?
Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	**Minor**	**CONFIDENCE**	**Low**

Comment:
Because the species has only one established population in Europe, there is only one current study of its impact on biodiversity. Espadaler et al (2018) reported an absence of native ants in the invaded area. However, a specific study monitoring the population dynamics of *W. auropunctata* and its the associated impacts is needed to increase the level of confidence.

Qu. 5.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?
See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	**Major**	**CONFIDENCE**	**Low**

Comment:
It is likely that, if *W. auropunctata* spreads in the Mediterranean biogeographical region, the impact on native biodiversity, in particular on arthropods, and small vertebrates may be major to locally massive and similar to that it is observed in presently invaded areas elsewhere. These impacts would be at least similar to those of *Linepithema humile*, which is already spreading in the risk assessment area and threatens arthropods, seed dispersal and sometimes vertebrates (Holway et al. 2002; Alvarez-Blanco et al. 2017).

Qu. 5.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?
including the following elements: • native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives • protected sites impacted, in particular Natura 2000 • habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats • the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comment:
N/A Because the species has only one established population in an urban area in Europe, and there is no current study of its impact on biodiversity (except on the ant fauna) and related ecosystem services. The current urban invaded area in Spain is not under any protected legislation.

Qu. 5.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?
including the following elements: • native species impacted, including red list species and species listed in the Birds and Habitats directives • protected sites impacted, in particular Natura 2000

- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	major	CONFIDENCE	low

Comment:
Wasmannia auropunctata can inhabit a wide range of habitats, open perturbated habitats to primary humid forest (Berman et al. 2013a). It is a threat to both invertebrates and vertebrates. In the risk assessment area, it will preferentially invade the Mediterranean biogeographic region which has the highest conservation value in the risk assessment area (Medail and Quezel 1999).

Therefore, many natural habitats of high conservation value, and their status, in suitable areas would be threatened by the ant. Some of them could be N2000 habitats. Some of them could be N2000 habitat, such as sea dunes of the Mediterranean coast (code 22), natural grasslands (code 61) and semi-natural dry grasslands and scrubland facies (code 62).

Ecosystem Services impacts

Qu. 5.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of relevant services use the CICES classification V5.1 provided as an annex.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	major	CONFIDENCE	medium

Comment:
Provisioning-Nutrition: Foragers tend honeydew-producing homoptera, especially mealybugs, including root-feeding species. Souza et al. (2009) found that Homoptera were higher in areas of cacao plantations dominated by *W. auropunctata*. Homopteran tending often increases pest populations and can reduce crop seed set and yields. The presence of *W. auropunctata* was also associated with an increase in pest crop abundance in Solomon islands subsistence gardens (Fasi et al. 2013). The presence and abundance of *W. auropunctata* therefore has the potential to inflict considerable crop loss in these rural subsistence gardens.

Regulating-Seed dispersal: *Wasmannia auropunctata* may interfere with seed dispersal activities of native ant species and therefore reduce the distribution of viable seeds (Ness and Bronstein 2004). They leave the seeds exposed on the soil surface, may ingest the elaiosome but fail to move the seed or they may move the seed shorter distances than the native ants they displace. These exposed seeds are not protected from fire or mammalian seed predators, and likely have less access to nutrients than do buried seeds or seeds deposited in ant nests.

<u>Regulating-Pest and disease Control</u>: *W. auropunctata* may interfere with beneficial insects that exert biocontrol activities in modified habitats. Although it has been introduced in Gabon to control agricultural pests, it is now no longer used because of its health impact. They were associated with higher pests abundance in cacao plantation and in subsistence gardens in Solomon islands (Souza et al. 2009; Fasi et al. 2013).

<u>Cultural-Physical use of landscapes</u>: *Wasmannia auropunctata* is a social nuisance in infested areas. *W. auropunctata* colonies are common around urban areas and are considered urban pests in many countries (see Harris et al 2005; Wetterer and Porter 2003). It could disrupt lifestyles, particularly outdoor activities that have a greater risk of contact with ants (e.g., picnics, gardening). Ant control would be necessary within a heavily infested area to allow such activities to continue.

Qu. 5.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 5.6.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comment:
N/A. Because the species has only one newly established population in Europe, no study has evaluated its current impact on ecosystem services yet.

Qu. 5.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 5.6.

RESPONSE	**Major**	**CONFIDENCE**	**medium**

Comment:
It is likely that, if *W. auropunctata* finds suitable habitats and climates for its development in the Mediterranean biogeographical region, the impact on ecosystem services may be major to potentially locally very strong and similar to that observed in presently invaded areas outside the EU.

Economic impacts

Qu. 5.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management. • Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	**major**	**CONFIDENCE**	**medium**

Comment:
Wasmannia auropunctata is considered to be an economically important pest ant in some introduced areas however, data on the overall estimate of economic losses are unavailable.

Bueno and Fowler (1994) found that *W. auropunctata* was “the most consistently found native species” of ants found in Brazilian hospitals and “in inner portions of the hospitals, only the exotic species, and *W. auropunctata*, are consistently present.” *Wasmannia auropunctata* was the only native ant species not easily controlled with conventional insecticides. In southern Bahia, Delabie et al. (2006) found *W. auropunctata* in 12 of 100 houses inspected.

In many areas, *W. auropunctata* is a significant horticultural pest. It stings field labourers and they may be unwilling to pick fruit in infested areas (Smith 1965, cited in Harris et al. 2005). It enhances populations of honeydew producing homopterans, which are a pest in their own right and damage their host plant by sucking sap and encouraging the build-up of sooty mould (e.g., cocoa and citrus in Brazil (Souza et al. 2009), and citrus in Puerto Rico (Michaud and Browning 2006)). The association between *W. auropunctata* and Homoptera may increase the occurrence of diseases, including viral and fungal infections, and in turn it increases the cost associated with agricultural pest management.

Fasi et al. (2016) showed that the presence of the little fire ant affects gardening activities by reducing time spent working effectively, influencing decisions about where to make gardening plots, discouraging children’s participation, and changing traditional gardening practices.

A notable success in the early 2000s was the eradication of *W. auropunctata* from Marchena Island (Galapagos, 22 ha) (Causton et al. 2005). The eradication programme has cost approximately US$183,423, and a further US$136,000 was required for monitoring over the next four years. The total projected cost of removing *W. auropunctata* from one hectare of infested area was estimated in 2004 to be US$15,584 (Causton et al. 2005).

A recent study simulated the costs of decreasing or increasing management efforts to control *W. auropunctata* on the Hawaiian Islands (Lee et al. 2015). Since its introduction in the 1990s it has spread to over 4000 locations on the island of Hawaii and has been found in isolated locations on Kauai, Maui, and Oahu Islands. This study demonstrated that increased management expenditures can suppress infestations; reduce spread between sectors; and decrease long-term management costs, damages, and stings.

Increased management effort has a significant impact on the number of Little Fire Ant sting incidents. Under current management, people on the island of Hawaii will suffer 2.3 billion sting incidents over 35 years. Their pets will endure 0.9 billion sting incidents over 35 years. With efforts to suppress Little Fire Ant populations, under least cost management during the next 35 years people and pets will suffer fewer sting incidents, down to 94 million for people and 9 million for pets. Management effort has a significant impact on costs and damages. In the next 35 years the cost of Little Fire Ant under current management will balloon to $6.1 billion. With efforts to suppress Little Fire Ant populations, under least cost management, net costs drop to $51 million, a substantial savings to the local economy.

An immediate expenditure of $8million in the next 2–3 years plus follow-up prevention, monitoring, and mitigation treatments will yield $1.210 billion in reduced control costs, $129 million in lowered economic damages, 315 million fewer human sting incidents, and 102 million less pet sting incidents over 10 years. Over 35 years, the benefits would include $5.496 billion in reduced control costs, $538million less economic damages, 2.161 billion fewer human sting incidents, and 762 million fewer pet sting incidents (Lee et al. 2015).

Qu. 5.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found". Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comments:
N/A Because the species has only one established population in Europe, there is no current cost of damage available but research costs (travel expenses and materials) have been incurred as a result of its arrival.

Qu. 5.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.10.

RESPONSE	moderate	CONFIDENCE	low

Comments:
It is likely that, if *W. auropunctata* spreads in the Mediterranean and Atlantic regions, the economic costs may be locally moderate to major, and similar to that observed in presently invaded areas elsewhere. Economic damages are sector-specific and vary with the size and extent of the infestation. Economic damages are based on estimated mean impacts from *W. auropunctata* and assumed to increase with level of infestation (Motoki et al. 2013). However, its extent and strength depend on the densities of ants, and are as such very difficult to estimate considering the uncertainty related to habitat/climatic suitability.

Qu. 5.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high

Comments:
N/A Because the species has only one established population in Europe, there is no current cost associated with managing this ant.

Qu. 5.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.12.

RESPONSE	moderate	CONFIDENCE	low

Comments:
It is likely that, if *W. auropunctata* spread in the Mediterranean and Atlantic regions, the management costs may be locally moderate to major, and similar to that observed in presently invaded areas elsewhere. However, its extent is very difficult to estimate considering the uncertainty related to habitat/climatic suitability.

Social and human health impacts

Qu. 5.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on • illnesses, allergies or other affections to humans that may derive directly or indirectly from a species; • damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure; • direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species. Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	Major	CONFIDENCE	medium

Comments:
Wasmannia auropunctata is a social nuisance in infested areas. Colonies are common around urban areas and are considered an urban pest in many countries (Harris et al 2005).
This ant has a painful sting that may cause injury to humans and domestic animals (Harris et al 2005). The sting may produce an immediate, intense pain followed by red swelling.

Reports of widespread blindness in both humans and mammals caused by *W. auropunctata* stings deserve particular attention. The sting can cause irreversible corneal lesions leading to blindness (Rosselli and Wetterer 2017).

Bueno & Fowler (1994) found that *W. auropunctata* was “the most consistently found native species” of ants found in Brazilian hospitals and “in inner portions of the hospitals, only the exotic species, and *W. auropunctata*, are consistently present.” *Wasmannia auropunctata* was the only native ant species not easily controlled with conventional insecticides.

Qu. 5.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area. • In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	moderate	CONFIDENCE	low

Comments:
It is likely that, if *W. auropunctata* spread in the Mediterranean and Atlantic regions, the social impact, including health impacts, may be moderate to potentially locally strong, and similar to that observed in presently invaded areas elsewhere.

Other impacts

Qu. 5.16. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?

RESPONSE	minimal	CONFIDENCE	high

Comments:
W. auropunctata is not known for being used as food or feed.

Ants have been observed carrying pathogens however up to date no transmission to humans or food contaminations have been recorded (Alharbi et al. 2019).

Qu. 5.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high

Comments:
N/A - No other impacts were found.

Qu. 5.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	major	CONFIDENCE	medium

Comments:
There are no specialist natural enemies of *Wasmannia* spp. in Europe because there is no species of the genus *Wasmannia* in Europe. Thus, only generalist natural enemies of ants may affect the ant and these are highly unlikely to regulate (control) populations.

Qu. 5.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided. Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	major	CONFIDENCE	medium

Comments:

It is likely that, if *W. auropunctata* spread in the Mediterranean and Atlantic regions, the overall impacts, may be locally major, and similar to that observed in presently invaded areas elsewhere. There are strong indications that the species has already caused a decrease in local biodiversity in the risk assessment area (Spain), at least in the ant fauna and people are already complaining against its sting (Espadaler et al. 2018).

Qu. 5.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	major	CONFIDENCE	low

Comments:

The extent of the impacts is very difficult to estimate considering the uncertainty related to habitat/climatic suitability. However, climate change will not decrease the extent of the impacts.

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**	**COMMENT**
Summarise Introduction*	**likely**	**medium**	The species has been already recorded/intercepted in Europe and it is likely that this will happen again, specifically with contaminated soil in the horticultural trade and/or as stowaway with container/bulk imports in sea or air freight. Climate change is not changing the risk of introduction based on the mentioned active pathways. Queens may enter the risk assessment area through the ant market on the internet. This pathway should be considered in the future if the webmarket of ants is not sufficiently regulated.
Summarise Entry*	**likely**	**high**	The species has been already recorded in Europe in the environment and it is likely that this will happen again, most likely via contaminated soil in the horticultural trade and/or as stowaway with container/bulk imports in sea or air freights. Climate change is not changing the likelihood of entry in the environment based on the mentioned active pathways.
Summarise Establishment*	**very likely**	**high**	The species is already established in the risk assessment area. Based on global species distribution models, *W. auropunctata* could become established in almost all countries around the Mediterranean Sea, with both the Atlantic Coast from Spain to Portugal and the Adriatic coast of Italy. Less than 2% of Europe is and will be suitable under

			climate change in the future to 2080. Predictions on the geographic extent of potential establishment indicate a slight increase in suitable areas.
Summarise Spread*	**moderately rapidly**	**high**	Based on the low ecoclimatic suitability in Europe, we can estimate that it will spread unaided to all potentially suitable biogeographical regions, but slower than in tropical and sub-tropical regions. Recent studies confirmed the southern expansion in its native range, highlighting the adaptive capacity of the species, which might increase its suitable range in the risk assessment area in the future. Its spread will occur mainly through human transport but its distribution will be indirectly constrained by climatic suitability and competition from other dominant ants (invasive and native). Climate change will not significantly increase the potential or rate of spread.
Summarise Impact*	**major**	**low**	It is likely that, if *W. auropunctata* spread in the Mediterranean and Atlantic regions, the overall impacts, may be locally major, and similar to that observed in presently invaded areas elsewhere. However, its extent is difficult to estimate considering the uncertainty related to habitat/climatic suitability. If only limited areas in the Mediterranean and Atlantic biogeographical regions will be suitable for the ant, impacts will be largely restricted to these zones.

Conclusion of the risk assessment (overall risk)	**high**	**medium**	*Wasmannia auropunctata* is one of the most damaging invasive ants and one of the most successful at invading and colonizing new areas. There is no doubt that the species can enter Europe through a variety of pathways, but its establishment and impact would be constrained by climate, habitat suitability and competition from other already established invasive ant species. It will have environmental, economic and social impact in some areas of Southern Europe, but the extent of its potential distribution remains unclear.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:
Yes if recorded, established or invasive
– if not recorded, established or invasive
? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.
For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

EU Member States and the United Kingdom (Based on Bertelsmeier et al. 2015a and Beckmann et al. 2019)

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria					
Belgium					
Bulgaria					
Croatia			Yes	Yes	
Cyprus					
Czech Republic					
Denmark					
Estonia					
Finland					
France			Yes	Yes	
Germany	Yes				
Greece			Yes	Yes	
Hungary					
Ireland			Yes	Yes	
Italy	Yes		Yes	Yes	
Latvia					
Lithuania					
Luxembourg					
Malta					
Netherlands	Yes				
Poland					
Portugal			Yes	Yes	
Romania					
Slovakia					
Slovenia					
Spain	Yes	Yes	Yes	Yes	Yes
Sweden					
United Kingdom	Yes		Yes	Yes	

Biogeographical regions of the risk assessment area (Based on Bertelsmeier et al. 2015a and Beckmann et al. 2019)

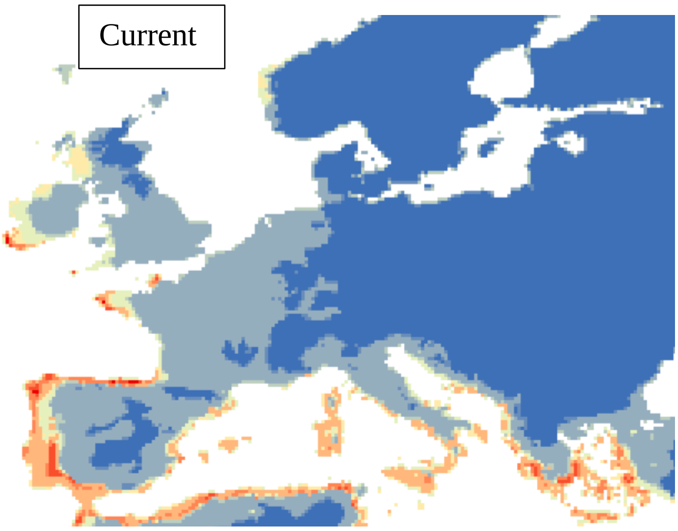
	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine					
Atlantic	Yes		Yes	Yes	
Black Sea				Yes	
Boreal					
Continental					
Mediterranean	Yes	Yes	Yes	Yes	Yes
Pannonian					
Steppic					

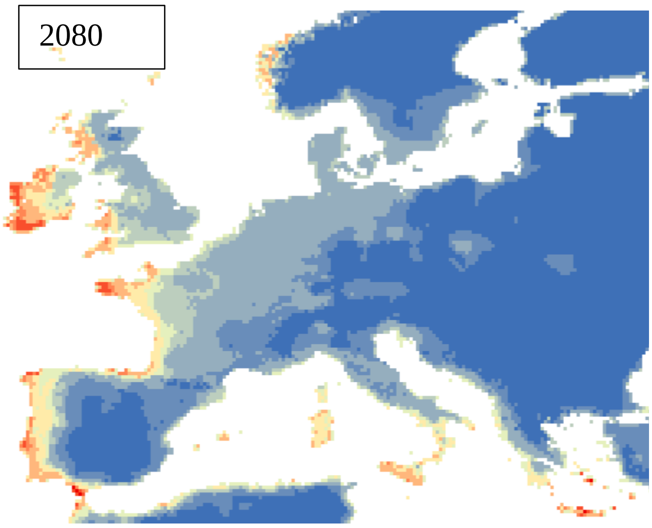
Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea					
Black Sea					
North-east Atlantic Ocean					
Bay of Biscay and the Iberian Coast					
Celtic Sea					
Greater North Sea					
Mediterranean Sea					
Adriatic Sea					
Aegean-Levantine Sea					
Ionian Sea and the Central Mediterranean Sea					
Western Mediterranean Sea					

ANNEX I Species distribution models under current and future (2080) climatic conditions

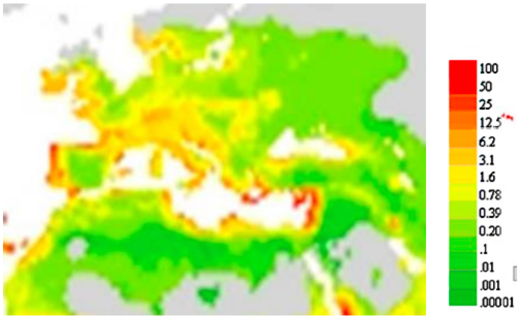
(source : Bertelsmeier et al 2015a). To consider a range of possible future climates, Bertelsmeier et al. (2015a) used downscaled climate data from three GCMs: the CCCMA-GCM2 model; the CSIRO MK2 model; and the HCCPR-HADCM3 model (GIEC 2007). Similarly, they used the two extreme SRES: the optimistic B2a; and pessimistic A2a scenario. They predicted an expansion of the potential range of *W. auropunctata* but the proportion of regions scored with a high suitability index (over 0.7) decreases. This method is based on the assumption that the species' niche remains unchanged when extrapolations are made in space (new potential distribution) and time (future climate scenarios). Occurrence points from both the invaded and native ranges were included to the full set of climatic conditions under which the species can persist because for invasive species in novel environments niche shifts can occur leading to differences with the native shift.





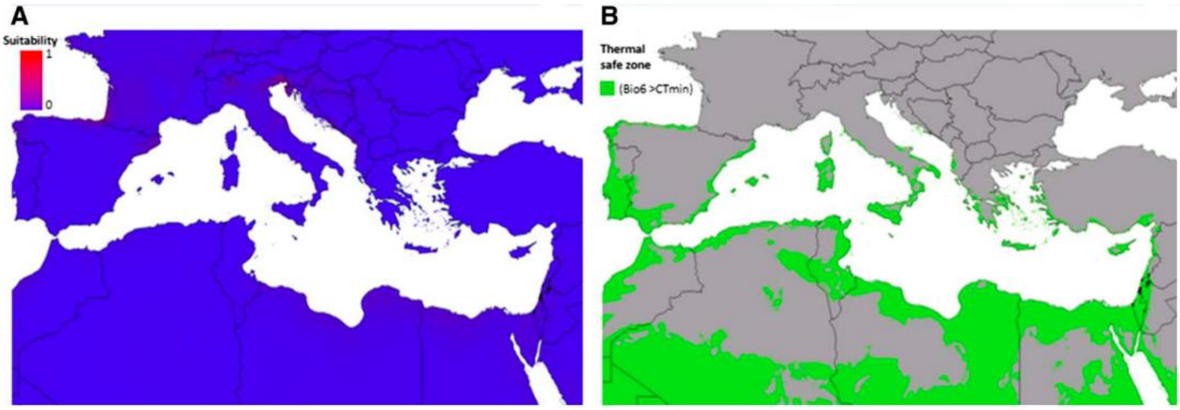
ANNEX II Species distribution models under current climatic conditions

(source : Federman et al. 2013). They used the Maxent model to predict potential invasion and establishment of *W. auropunctata*. Bioclimatic variables were obtained from the WorldClim dataset. These variables were derived from the monthly temperature and rainfall values, in order to generate biologically meaningful variables. The bioclimatic variables represent annual trends, seasonality, and extreme or limiting environmental factors. Yearly reference evapo-transpiration was obtained from the database of the Food and Agriculture Organization of the United Nations (FAO)



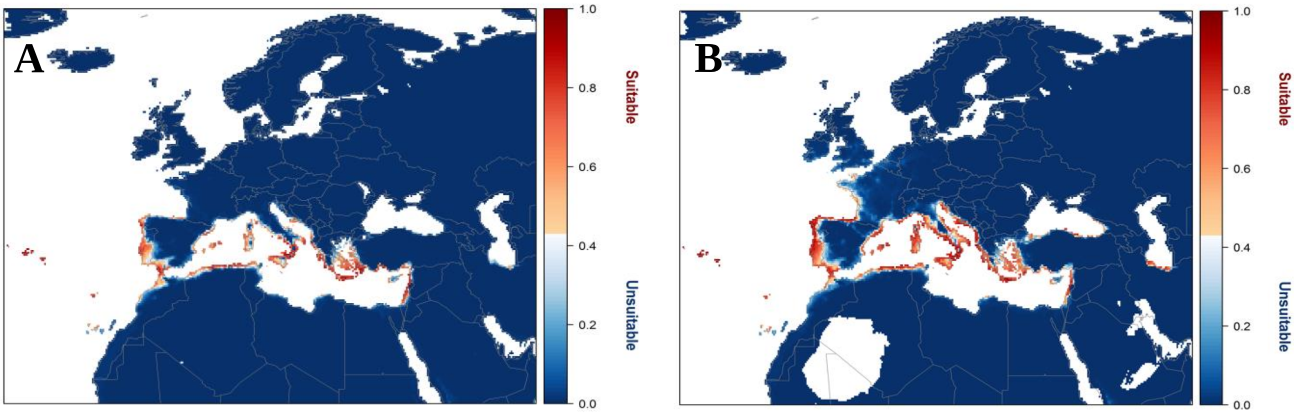
ANNEX III Climatic suitability and thermal safe zone maps (only Mediterranean basin)

a Maxent result with its habitat suitability index.
b Thermal safe zone map, in green regions with « minimum temperature of the coldest month » (Bio6) hotter than the lowest CTmin (4.2°C). *Wasmannia auropunctata* presences are depicted in black dots (source: Coulin et al. 2019). Coulin et al. (2019) analysed 19 bioclimatic variables related to temperature and precipitation at 30 arc-seconds resolution available from WorldClim. After variables selection, the remaining variables were analysed with *W. auropunctata* clade B native range presence data to fit a SDM using the Maxent procedure. To explore the link between the thermo-physiological constraints and the SDM, the lower CTmin measured in their study was evaluated by analysing the latitudinal change of the minimum temperature of the coldest month (Bio6) and its effect on the probability of presence.



ANNEX IV Projected (A) current suitability for *Wasmannia auropunctata* establishment in Europe and the Mediterranean region and (B) in 2070s under climate change scenario RCP4.6

(source: Beckmann et al 2019). To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip55m). Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats, we used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, nighttime lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was ln+1 transformed for the modelling to improve normality.



ANNEX V Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Possible	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX VI Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Biodiversity and ecosystem impact**	**Ecosystem Services impact**	**Economic impact (Monetary loss and response costs per year)**	**Social and human health impact, and other impacts**
	Question 5.1-5	*Question 5.6-8*	*Question 5.9-13*	*Question 5.14-18*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[7]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread,

[7] Not to be confused with "no impact".

	ecosystem effects			severe, long-term, irreversible health effects.

ANNEX VII Scoring of Confidence Levels

(modified from Bacher et al. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	**Description**
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence *and/or* Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area *and/or* Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous *and/or* The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred *and/or* Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty *and/or* The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) *and* Impacts are recorded at a comparable scale *and/or* There are reliable/good quality data sources on impacts of the taxa *and* The interpretation of data/information is straightforward *and/or* Data/information are not controversial or contradictory.

ANNEX VIII Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES "classes")
Provisioning	**Biomass**	**Cultivated *terrestrial* plants**	Cultivated terrestrial plants (including fungi, algae) grown for nutritional purposes; Fibres and other materials from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a source of energy *Example: negative impacts of non-native organisms to crops, orchards, timber etc.*
		Cultivated *aquatic* plants	Plants cultivated by in- situ aquaculture grown for nutritional purposes; Fibres and other materials from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an energy source. *Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.*
		Reared animals	Animals reared for nutritional purposes; Fibres and other materials from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide energy (including mechanical) *Example: negative impacts of non-native organisms to livestock*
		Reared *aquatic* animals	Animals reared by in-situ aquaculture for nutritional purposes; Fibres and other materials from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an energy source *Example: negative impacts of non-native organisms to fish farming*
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for nutrition; Fibres and other materials from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a source of energy *Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)*
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for nutritional purposes; Fibres and other materials from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a source of energy *Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)*
	Genetic material from all biota	**Genetic material** from plants, algae or fungi	Seeds, spores and other plant materials collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to breed new strains or varieties;

			Individual genes extracted from higher and lower plants for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
	Water[8]	**Surface water** used for nutrition, materials or energy	Surface water for drinking; Surface water used as a material (non-drinking purposes); Freshwater surface water, coastal and marine water used as an energy source *Example: loss of access to surface water due to spread of non-native organisms*
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for drinking; Ground water (and subsurface) used as a material (non-drinking purposes); Ground water (and subsurface) used as an energy source *Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.*
Regulation & Maintenance	**Transformation** of biochemical or physical inputs to ecosystems	**Mediation of wastes or toxic substances** of anthropogenic origin by living processes	Bio-remediation by micro-organisms, algae, plants, and animals; Filtration/sequestration/storage/accumulation by micro-organisms, algae, plants, and animals *Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics*
		Mediation of nuisances of anthropogenic origin	Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure) *Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.*
	Regulation of physical, chemical, biological conditions	**Baseline flows and extreme event** regulation	Control of erosion rates; Buffering and attenuation of mass movement; Hydrological cycle and water flow regulation (Including flood control, and coastal protection); Wind protection; Fire protection *Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.*
		Lifecycle maintenance, habitat and gene pool protection	Pollination (or 'gamete' dispersal in a marine context); Seed dispersal; Maintaining nursery populations and habitats (Including gene pool protection) *Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries*
		Pest and disease control	Pest control; Disease control

[8] Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

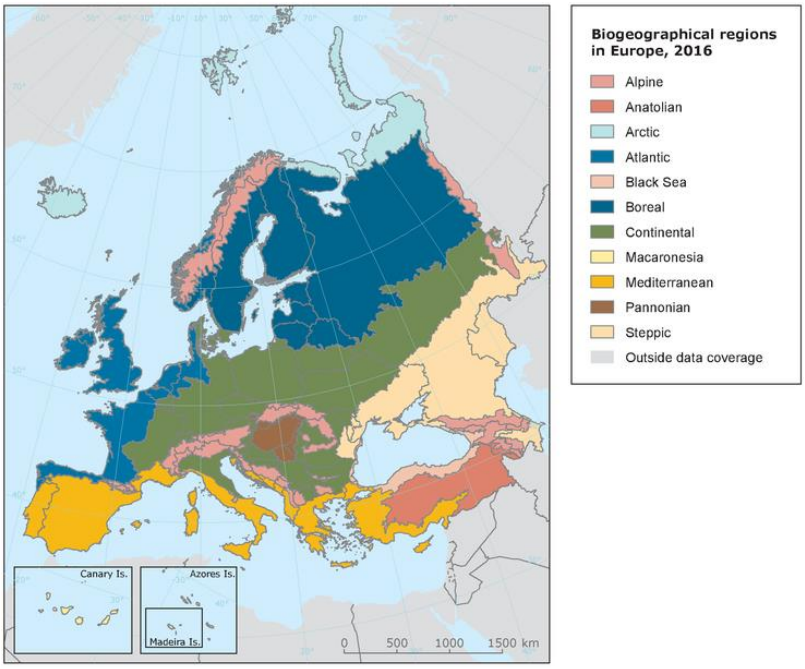
			Example: changes caused by non-native organisms to the abundance and/or distribution of pests
		Soil quality regulation	Weathering processes and their effect on soil quality; Decomposition and fixing processes and their effect on soil quality *Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality*
		Water conditions	Regulation of the chemical condition of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes *Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication*
		Atmospheric composition and conditions	Regulation of chemical composition of atmosphere and oceans; Regulation of temperature and humidity, including ventilation and transpiration *Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)*
Cultural	**Direct, in-situ and outdoor interactions** with living systems that depend on presence in the environmental setting	**Physical and experiential** interactions with natural environment	Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through active or immersive interactions; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.*
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living systems that enable education and training; Characteristics of living systems that are resonant in terms of culture or heritage; Characteristics of living systems that enable aesthetic experiences *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance*
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	**Spiritual, symbolic** and other interactions with natural environment	Elements of living systems that have symbolic meaning; Elements of living systems that have sacred or religious meaning; Elements of living systems used for entertainment or representation *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning*
		Other biotic characteristics that have a **non-use value**	Characteristics or features of living systems that have an existence value; Characteristics or features of living systems that have an option or bequest value *Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.*

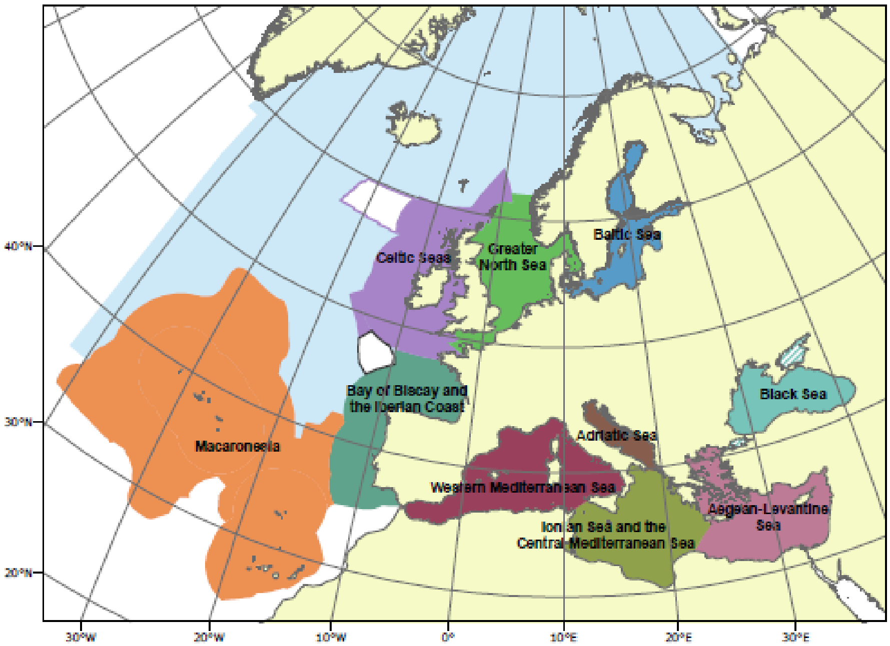
ANNEX IX EU Biogeographic Regions and MSFD Subregions

See https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 , http://ec.europa.eu/environment/nature/natura2000/biogeogregions/

and

https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf





ANNEX X Delegated Regulation (EU) 2018/968 of 30 April 2018

see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968