The threat posed by invasive alien flatworms to EU agriculture and the potential for phytosanitary measures to prevent importation.

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TSSR 2021 01.02. The threat posed by invasive alien flatworms to EU agriculture and the potential for phytosanitary measures to prevent importation

Date of completion: 02/04/2021

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The threat posed by invasive alien flatworms to EU agriculture and the potential for phytosanitary measures to prevent importation

A report for the IUCN

by

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February 2021

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Summary

Invasive alien terrestrial flatworms are predators that can disrupt ecosystem functioning by feeding on native soil fauna. In such a way, invasive alien flatworms can also be indirect plant pests by feeding on beneficial species such as earthworms that contribute towards plant productivity. There are 28+ native species of terrestrial flatworm in Europe with more being identified every year due to renewed scientific interest and developments in taxonomy. However, these native flatworm species are mostly small and innocuous animals. There are 21+ alien terrestrial flatworm species in Europe (includes four species only found in the UK) and some of these are much larger and damaging predators. For example, the invasive alien New Zealand flatworm (*Arthurdendyus triangulatus*) can reduce earthworm biomass by 20% in pasture leading to a reduction in grass yield of 7%. *Arthurdendyus triangulatus* is currently restricted to the British Isles and Faroe Islands but another large flatworm species, *Obama nungara*, is spreading in continental Europe and could have similar impacts to *A. triangulatus*. The key pathway for introduction of alien flatworms is plants for planting (nursery stock), especially containerised plants with growing media. The flatworms opportunistically shelter in plant containers and are not associated with a particular plant product. Very few control measures against invasive alien flatworms have been tested. Potentially, the most promising approach is hot water treatment applied as a drench. Flatworms are cryptic, nocturnal species and difficult for plant inspectors to detect. However, they would potentially be an ideal target for the development of environmental DNA detection techniques. Invasive alien flatworms are also spreading elsewhere in the world but no countries have specific phytosanitary treatments targeted at such species.

1. Brief evaluation of the potential size of the threat posed by alien flatworms

Key points:

- Worldwide, there are 944 described terrestrial flatworm species
- Many more species remain to be described
- There are 28 (to possibly 43) terrestrial flatworm species native to Europe (EU27)
- There are at least 21 alien flatworm species recorded in Europe (EU27 = 17, 4 only from the UK)

1.1 Number of flatworm species or genera alien to Europe

The number of described terrestrial flatworm species (Platyhelminthes, Tricladida, Geoplanidae) is c. 944 in 67 genera (GBIF 2021; Turbellarian Taxonomic Database 2021), which fits with estimates of 800-900 in earlier publications (Sluys 1999; Sluys 2016; Sluys 2019; Tyler et al. 2006-2021). However, this is likely to be a substantial underestimate as these are cryptic soil-dwelling species with most found in biodiversity-rich and under-investigated sub-tropical and tropical forests. The majority of terrestrial flatworm species are found outside of Europe, with hotspots in South America, eastern Australia (incl. Tasmania), New Zealand, South-East Asia and Madagascar (Sluys 1998).

Of the five subfamilies of Geoplanidae (Bipaliinae, Microplaninae, Geoplaninae, Rhynchodeminae, Eudoxiatopoplaninae) two are native to Europe, namely the Microplaninae and the Rhynchodeminae (Álvarez-Presas et al. 2014), although for the latter subfamily its status as endemic is questionable (Álvarez-Presas et al. 2018). The number of native species in Europe was given as ten (one species undescribed) in the review by Jones (1998) (Table 1), who pointed out the paucity of terrestrial flatworm records from Europe due to a cessation of taxonomic studies in the 20th century. Subsequently, other recent studies have added at least 18 new species leading Mateos et al. (2017) to suggest on the basis of morphological and molecular analyses that there were considerably more microplanid species to be described from Europe. The details of the native flatworms found in EU27 countries are given in Table 1. Álvarez-Presas et al. (2018) estimate the current number of European microplanids as 43, presumably including unnamed species known only from molecular diagnostics. As planarian taxonomy continues to be resolved, this figure is likely to increase. None of the European flatworm species (nor earthworm species) have had their risk of extinction assessed according the IUCN Red List Categories and Criteria (IUCN 2012) at a global or European level.

Table 1. Native European terrestrial flatworm species, their distribution and key recent references. Please note, this effectively corresponds to an EU27 species list.

Species	European distribution	References
Geobenazzia tyrrhenica Minelli, 1974	Italy	Jones 1998
Microplana aixandrei Vila-Farré et al., 2008	Spain	Vila-Farré et al. 2008
Microplana astricta Sluys et al., 2017	Bulgaria	Mateos et al. 2017
Microplana cephalofusca Sluys et al., 2017	Bulgaria	Mateos et al. 2017
Microplana cingulata Sluys et al., 2017	Bulgaria	Mateos et al. 2017
Microplana fuscomaculosa Sluys et al., 2017	France, Spain, UK	Mateos et al. 2017
Microplana gadesensis Vila-Farré et al., 2008	Spain	Vila-Farré et al. 2008
Microplana giustii Minelli, 1976	Italy	Jones 1998
Microplana grazalemica Vila-Farré et al., 2008	Spain	Vila-Farré et al. 2008
Microplana groga Jones et al., 2008	Spain	Jones et al. 2008
Microplana henrici (Bendl, 1908)	Austria, Bulgaria, Bosnia-Herzegovina, France, Italy, Spain	Jones 1998; Mateos et al. 2017
Microplana humicola Vejdovsky, 1889	Austria, Czech Republic, Germany, UK. Probably Europe-wide, but under-recorded	Jones 1998
Microplana hyalina Vila-Farré & Sluys, 2011	Italy, Spain	Mateos et al. 2017; Vila-Farré et al. 2011
Microplana kwiskea Jones et al., 2008	Bulgaria, Germany, Italy, Poland, UK	Jones et al. 2008; Mateos et al. 2017; Sluys et al. 2016
Microplana lutulenta Álvarez-Presas et al., 2017	Bulgaria	Mateos et al. 2017
Microplana monacensis (Heinzel, 1929)	Monaco, Spain	Mateos et al. 2017; Vila-Farré et al. 2011
Microplana nana Mateos et al., 1998	Spain	Mateos et al. 1998; Sluys et al. 2016
Microplana nervosa Sluys et al., 2017	Spain	Mateos et al. 2017
Microplana pereraca Marcus & Marcus, 1959	Azores	Jones 1998
Microplana plurioculata Sluys et al., 2016	Spain	Sluys et al. 2016
Microplana polyposis Sluys et al., 2016	France	Sluys et al. 2016
Microplana pyrenaica (Graff, 1893)	France, Portugal	Jones 1998

Table 1. Native European terrestrial flatworm species, their distribution and key recent references (continued).

Species	European distribution	References
Microplana robusta Vila-Farré & Sluys, 2011	Spain	Sluys et al. 2016; Vila-Farré et al. 2011
Microplana scharffi (Graff, 1899)	Belgium, Ireland, Madeira, Monaco, European Turkey, UK.	Jones 1998
Microplana sparsa Sluys et al., 2017	Portugal	Mateos et al. 2017
Microplana terrestris (Müller OF, 1773)	Europe-wide	Jones 1998
Rhynchodemus howesi Scharff, 1900	Pyrenees	Jones 1998; Minelli 1977; Sluys et al. 2016
Rhynchodemus sylvaticus* (Leidy, 1851) *possibly introduced	Austria, Azores, Belgium, Bulgaria, Czech Republic, Ireland, France, Germany, Italy, Netherlands, Poland, Slovakia, Sweden, UK	Jones 1998

1.2 Number of alien species already introduced in Europe, with and without recorded impacts

The number of records of alien species introduced to Europe has increased in recent years due to greater awareness of invasive flatworms, citizen science approaches and increased taxonomic capability, often based on molecular techniques. Some of this interest has been driven by the potential economic impact of *A. triangulatus* in the UK and Ireland. Nevertheless, there remains significant gaps in our knowledge of flatworm distribution in Europe. Most recent work has been conducted in the UK, France, Spain and the Netherlands.

There are at least 21 alien flatworm species recorded in Europe (Table 2). As with native flatworms, this number is likely to increase as several species have yet to be identified. Land planarians are carnivorous so potentially have an impact on other soil dwelling invertebrates but aside from predation lists developed from observational studies, very little is known about the impact of most species of terrestrial flatworm on prey populations. In contrast to the majority of native European species which are generally small and discrete, most alien species are large animals and it is likely that their impact is high and related to their great size. However, their impact is not documented, except for *A. triangulatus* (Murchie & Gordon 2013). Impacts on soil fauna are easy to overlook and it is possible that even small flatworm species could be having a significant predatory impact on some soil fauna species. The basic ecology of both native and alien terrestrial flatworms is poorly known.

1.3 Number of species that are considered to be invasive alien species somewhere in the world

Winsor et al. (2004) lists c. 30 species (depending on taxonomy) that have been found outside their native range in other parts of the world apart from Europe, although many of these are not considered damaging (selected examples are given in Table 3). This figure has undoubtedly increased in the intervening years since this paper was published with the renewed interest in invasive alien flatworms and advances in taxonomy. It is of note that several European *Microplana* species have been found in the USA, demonstrating the capacity of these organisms to be transported to other countries. It is presumed that these largely historical introductions were with shipments of European plants and soil to America and before current phytosanitary precautions.

Table 2. Terrestrial flatworms alien to Europe, their distribution, impact and key recent references. This includes four species that have only been recorded in Europe in the UK (marked with an asterisk).

Species	European distribution	Native to	Impact	References
****Arthurdendyus albidus*** Jones & Gerard, 1999	UK	New Zealand	Predator of earthworms. Limited distribution. No evidence of impact stated in the literature.	Jones & Gerard 1999
****Arthurdendyus australis*** (Dendy, 1894)	UK	New Zealand	Predator of earthworms. Limited distribution. No evidence of impact stated in the literature.	Johns & Boag 2003; Jones 1998; Jones & Boag 2001
Arthurdendyus triangulatus (Dendy, 1895)	UK, Ireland, Faroe Islands	New Zealand	Predator of earthworms. Widespread distribution. Severe depletion of earthworm populations.	Blackshaw & Stewart 1992; Boag & Yeates 2001; Cannon et al. 1999; Murchie & Gordon 2013; Willis & Edwards 1977
Artioposthia exulans (Dendy, 1901) Fyfe, 1946	UK, Ireland	New Zealand	Predator of invertebrates. Limited distribution. No evidence of impact stated in the literature.	Jones & Fenwick 2018
Australoplana sanguinea alba (Jones, 1981)	UK, Ireland	Australia	Predator of earthworms. Widespread distribution. Potential for impact but much less so than *A. triangulatus*.	Boag et al. 1995; Jones 1998; Santoro & Jones 2001
Bipalium kewense Moseley, 1878	Worldwide distribution In hothouses in UK, France, Italy, Czech Republic Slovakia, Germany, Ireland, Spain, Austria, Belgium, Finland, Netherlands. In the wild in Southern France, Monaco, Portugal, Spain, Azores Islands	South-East Asia	Predator of earthworms. Evidence of impact on earthworm populations.	Justine et al. 2018; Winsor 1983

Table 2. Terrestrial flatworms alien to Europe, their distribution, impact and key recent references (continued)

Species	European distribution	Native to	Impact	References
Diversibipalium multilineatum (Makino & Shirasawa, 1983)	France, Switzerland, Italy	Japan	Predator of invertebrates. Limited distribution.	Justine et al. 2018; Mazza et al. 2016
***Diversibipalium* sp. "black"**	France Italy (J-L Justine unpubl.)	Asia	Limited distribution but high numbers reported in a field in Italy (J-L Justine unpubl.)	Justine et al. 2018
Caenoplana variegata (Fletcher & Hamilton, 1888) [syn. *Caenoplana bicolor*]	Netherlands, UK, Spain, France, Greece (Crete)	Australia	Predator of arthropods (woodlice, insects, myriapods, spiders), scavenger	Álvarez-Presas et al. 2014; de Waart 2016; Jones et al. 2020; Justine et al. 2020b; Vardinoyannis & Alexandrakis 2019
Caenoplana coerulea Moseley, 1877	UK (hothouse), France, Spain	Australia	Predator of arthropods. Limited distribution.	Álvarez-Presas et al. 2014; Breugelmans et al. 2012; Jones 1998; Jones 2005
Caenoplana decolorata Mateos et al., 2020	Spain, France	Australia	Predator of invertebrates. Limited distribution.	Justine et al. 2020b; Mateos et al. 2020
Dolichoplana striata Moseley, 1877 [syn. *Dolichoplana feildeni*]	Ireland (hothouse), Jersey, Germany, UK, Spain, France (hothouse) (J-L Justine unpubl.)	Sri Lanka or Indonesia	Earthworm predator. Some evidence of impact on earthworm beds.	Álvarez-Presas et al. 2014; Anderson 1986; Jones 1998; Winsor et al. 2004
Kontikia andersoni Jones, 1981	UK, Ireland	Indo-Pacific/?New Zealand	Unknown	Anderson 1986; Jones 1998; Winsor et al. 2004
Kontikia bulbosa Sluys, 1983	Portugal (Madeira) Spain (Canary Islands)	Unknown	Unknown	Jones 1998; Winsor et al. 2004
****Australopacifica atrata*** (Steel, 1897)	UK	Australia	Scavenger	Jones 2019

Table 2. Terrestrial flatworms alien to Europe, their distribution, impact and key recent references (continued)

Species	European distribution	Native to	Impact	References
****Parakontikia coxii*** (Fletcher & Hamilton, 1888) [syn. *Australopacifica coxii*]	UK	Australia	Limited distribution. No evidence of impact stated in the literature.	Boag & Yeates 2001; Jones 1998
Parakontikia ventrolineata (Dendy, 1892) [*syn. Kontikia ventrolineata*]	UK, France, Spain, Netherlands	Australia	Predator of invertebrates/scavenger. A pest in gardens because it dwells on fruits and vegetables.	Álvarez-Presas et al. 2014; de Waart 2016; Jones 1998; Jones et al. 1998; Justine et al. 2014a
Marionfyfea adventor Jones & Sluys, 2016	UK, Netherlands, France	?New Zealand	Limited distribution. No evidence of impact stated in the literature.	Jones & Sluys 2016
Rhynchodemus hallezi Graff, 1899	UK hothouse (doubtful)	Philippines	Unknown	Jones 1998
Obama nungara Carbayo et al., 2016	Guernsey, Italy, France, Spain, UK Austria (J-L Justine, unpubl) Germany (J-L Justine, unpubl.) Switzerland (AK Murchie, unpubl.)	Argentina, Brazil	Polyphagous predator of invertebrates, including earthworms and snails	Justine et al. 2020c; Soors et al. 2019; Lago-Barcia et al., 2015
Platydemus manokwari Beauchamp, 1963	France (hothouse). Never found in the wild in Europe.	New Guinea	Predator of snails. Serious impact on endemic snails in Pacific islands.	Justine et al. 2014b; Justine et al. 2015

Table 3. Selected terrestrial flatworm species that have invaded other (non-European) parts of the world (key reference Winsor et al. 2004)

Species	Country	Native to	Impact	References
Bipalium adventitium Hyman, 1943	USA, Canada	Asia	Predator of earthworms. Widespread distribution. Pest of commercial earthworm beds.	Fiore et al. 2004; Justine et al. 2019; Sluys 2016
Bipalium kewense Moseley, 1878	Worldwide, including EU27 (Table 2)	Vietnam	Predator of earthworms. Widespread distribution.	Justine et al. 2018; Winsor 1983a
Bipalium pennsylvanicum Ogren, 1987	USA	Asia	Predator of earthworms	Sluys 2016
Bipalium vagum Jones & Sterrer, 2005	Many tropical countries, including many Asian countries and Southern USA. Antilles Islands (Guadeloupe, Martinique, Saint Martin, Saint Barthélemy); French Guiana; La Réunion.	Asia	Predator of molluscs	Ducey et al. 2007; Justine et al. 2018; Sluys 2016
Dolichoplana striata Moseley, 1877	USA. Many tropical countries.	Indo-Malay region	Predator of earthworms. Pest of commercial earthworm beds.	Sluys 2016; Winsor et al. 2004
Parakontikia ventrolineata (Dendy, 1892)	USA	Australia	Generalist predator attacking molluscs and isopods, scavenger	Sluys 2016; Winsor et al. 2004
Platydemus manokwari Beauchamp, 1963	Many tropical countries, including Southern USA. Antilles Islands (Guadeloupe, Martinique, Porto Rico, and others). Found in a hothouse in France (Table 2).	New Guinea	Predator of molluscs. Impact on endemic species (Gerlach et al. 2020)	Justine et al. 2014b; Justine & Winsor 2020; Justine et al. 2015
Amaga expatria Jones & Sterrer, 2005	Bermuda, Antilles (Guadeloupe, Martinique)	Continental South America, precise country unknown	Predator of both earthworms and terrestrial molluscs. Possible impact on endemic species.	Jones & Sterrer 2005; Justine et al. 2020a

2. The impact of alien flatworms on plant health as indirect pests and on agriculture

Key points:

- Terrestrial flatworms are predatory and may eat beneficial species such as earthworms
- Indirect plant pests reduce plant productivity but do not feed directly on plants
- *Arthurdendyus triangulatus* reduces earthworm biomass by 20% leading to a predicted reduction in grass yield of 7%
- *Obama nungara* is established in continental Europe and could have a similar impact on earthworms as *A. triangulatus*
- Terrestrial flatworms may prey on pest slugs or snails and have been introduced elsewhere for biocontrol purposes but this has led to negative impacts on native species
- Flatworm contaminated consignments or products are likely to be rejected by consumers and traders

Indirect plant health pests are species which have an impact on crop production, even though they do not feed directly on plants themselves. Terrestrial flatworms are predators of other soil fauna and therefore their impact is mostly evaluated in terms of reducing biodiversity. Mostly this is through direct predation (or potentially competition with native species) but there is also some evidence of potential detrimental effects on vertebrates that inadvertently eat flatworms (Winsor, 1983b). Soil fauna are important components of soil ecosystem functioning, which is of direct benefit to agricultural production. Soil fauna feed on dead plant material and microflora, thus helping in the decomposition process and recycling of nutrients within the soil. Assessing the impact of flatworms on agricultural production has therefore two stages: first, determining the impact of the predatory flatworms on their soil fauna prey; and second, determining the value of the invertebrate prey to agricultural production.

The New Zealand flatworm, *Arthurdendyus triangulatus*, is an example of an indirect plant pest because it preys on earthworm species that benefit crop yield, but does not itself feed on plants (Murchie 2010; Sluys 2016). This may have important implications for the overall management of the problem, because as plant health legislation is usually dependent on pest risk assessments of direct economic losses, the impact of the flatworms does not fit easily into current legislative frameworks. In some initial discussions, it was even suggested by legislators that *A. triangulatus* should be considered a veterinary problem because it fed on animals. This shows that there is not a simple way to address the problem.

Arthurdendyus triangulatus is the most studied terrestrial flatworm in Europe in terms of its negative impacts on agriculture. Several studies have demonstrated a reduction in earthworm biodiversity and densities following introduction of *A. triangulatus*. The first of these was the study by Blackshaw (1989) in Northern Ireland, where a marked decline in earthworm number and biomass was noted during an earthworm enhancement experiment (1983-1987), and associated with an increase in numbers of *A. triangulatus*. Subsequent studies confirmed the capability of *A. triangulatus* to reduce earthworm populations (Blackshaw 1990; Blackshaw 1995; Blackshaw & Stewart 1992). Although *A. triangulatus* will prey on all lumbricid earthworm species presented to it (Stewart 1993), some earthworm species are more susceptible to flatworm predation than others. There are two reasons for this. The first is to do with the ease by which flatworms can access earthworms, with surface dwelling and vertical-burrowing earthworm species more vulnerable to predation than deeper, soil-dwelling species (Blackshaw 1995; Blackshaw & Stewart 1992; Boag et al. 1994; Lillico et al. 1996). Second, the capability of earthworm populations to absorb the impact of flatworm predation is dependent on the life history characteristics of the species, with larger, longer-lived and slow-reproducing species more vulnerable than smaller fast-reproducing species (Murchie & Gordon 2013). Consequently, anecic earthworm species, in particular *Lumbricus terrestris*, which have semi-permanent vertical burrows and are slow reproducing, are most at risk from *A. triangulatus* predation (Blackshaw 1995; Blackshaw & Stewart 1992; Jones et al. 2001). The contribution of individual earthworm species to crop production has not been well studied, but *L. terrestris* has been termed an ‘ecosystem engineer’ due to its ability to change soil characteristics and flora (Bohlen et al., 2004).

In a 4-year manipulation study, Murchie & Gordon (2013) sought to quantify the impact of *A. triangulatus* on earthworm populations in the field. They physically moved flatworms around a naturally-infested grass field site to create plots with different flatworm densities. This allowed them to regress earthworm biomass against flatworm densities. There was an overall negative relationship between flatworm density and earthworm biomass. Taking a field-observed flatworm density of 0.8 per m^2 as a baseline (Murchie et al. 2003), gave a reduction in total earthworm biomass of 20%. However, the majority of this was due to loss of anecic species, which suffered a 74% reduction in biomass. At 1.1 flatworms per m^2, the anecic earthworm biomass is reduced to zero.

Earthworms are considered beneficial soil organisms in agro-ecosystems and particularly so for permanent grass pasture. Their burrowing creates channels that aerate and drain the soil, whilst their feeding activities help to recycle plant nutrients (Edwards & Bohlen, 1996; Curry, 1994). The loss of earthworm species due to flatworm predation could have a number of negative effects. Lack of earthworm burrowing and collapse of old burrows could impede drainage leading to increased water-logging, greater prevalence of *Juncus* rushes and exacerbate livestock diseases such as liver fluke (*Fasciola hepatica*) which rely on wet pasture conditions (Alford 1998). These issues may become more important with climate change causing increased flooding events. Earthworms feed on dead plant material and lack of earthworms increases thatch formation at the soil surface (Clements et al. 1991). Thatch build-up has been associated with the presence of *A. triangulatus* (Blackshaw 1995). Furthermore, a layer of thatch at the soil surface may provide an ideal habitat for flatworms. In a mulch experiment in orchard plots, high densities of *A. triangulatus* (9.3 m^{-2}) were found under a thick layer of thatch (Murchie & Mac An tSaoir 2006), leading the authors to consider a 'vicious circle': *A. triangulatus* feeds on earthworms, lack of earthworms leads to build up of thatch, thatch proves to be an ideal habitat for flatworms.

The role of earthworms in recycling nutrients and their relationship with plant productivity is the major factor when assessing the impact of *A. triangulatus* predation on agriculture. It is notable that one of the best demonstrations of earthworms benefitting agricultural systems comes from New Zealand, where European earthworm species were introduced to pastures and increased grass yield by up to 29% (Stockdill 1982). *Arthurdendyus triangulatus* is not considered a pest in New Zealand agriculture, perhaps due to unfavourable conditions in pastures and differences in earthworm prey availability (Fraser & Boag 1998), along with natural controlling factors of flatworms in their native habitat. The benefit of earthworms to crop yield was quantified in a meta-analysis of 58 studies (van Groenigen et al. 2014). Overall, presence of earthworms increased crop yield by 25% and above ground biomass of pasture grasses of 24%. For the main pasture species, ryegrass, the figure was greater at a 34% increase in above ground biomass. Murchie (2018) used these figures to calculate the impact of *A. triangulatus* as a reduction in pasture biomass of 6.8%, based on a 20% reduction in earthworm biomass x 34% contribution of earthworms to ryegrass biomass. They do however caution that since the contribution of individual earthworm species is not factored in, the disproportionate impact of *A. triangulatus* predation on anecic species could lead to greater losses.

For other flatworm species, evidence of impact on agriculture is sparse. For the earthworm predators (e.g. *Bipalium* spp. and *O. nungara*), it is possible that they will have a similar impact to *A. triangulatus* being of a large size and similar ecology. There is some evidence from the US of invasive alien *Bipalium* spp. having an impact on commercial earthworm production (Dindal 1970; Sluys 2016). Much will depend on the habitats invaded, whether the flatworms can survive open farmland and the densities achieved. *Bipalium kewense* and *O. nungara* are found in the wild in continental Europe but most records are from gardens (Justine et al. 2018; Justine et al. 2020c). There is no evidence to date of significant invasion of agricultural land. This may be recorder bias with gardeners more likely to see and report unusual flatworms than farmers, who are often working in machinery remote from the soil. However, it may also reflect the different stages in the invasion process. The majority of early records of *A. triangulatus* were from gardens (Moore et al. 1998). In Northern Ireland, just 4% of grassland fields (n = 75) were found to have *A. triangulatus* in 1991; yet by 1998/99, 70% of the same fields contained *A. triangulatus* (Murchie et al. 2003). It was postulated that flatworms were initially moved around by the plant trade but once established in gardens they dispersed naturally to surrounding farmland. Given the high densities of *O. nungara* in some French gardens (Justine et al. 2020c), invasion of surrounding habitats is a virtual certainty.

For flatworm species that prey on soil invertebrates other than earthworms, it is more difficult to evaluate their impact on agriculture. This is because far less is known about the contributions of these prey species (molluscs, isopods and other soil-dwelling arthropods) to agricultural production. In risk-assessing the flatworm snail predator, *P. manokwari*, for Europe, Murchie & Beckmann (2020) considered that the main biodiversity risk was to endemic snail species and the main economic risk was to heliciculture (snail farming). Although gastropods undoubtedly play an important role in decomposition processes in natural habitats, there was little evidence of a benefit to agricultural production. Indeed, most references to gastropods in agriculture are as crop pests or vectors of livestock diseases. Flatworm predators of snails, such as *P. manokwari*, have been found to carry vertebrate parasites (*Angiostrongylus* spp.), which they have ingested whilst feeding on infected snails (Chaisiri et al. 2019).

In many tropical territories including islands in the Pacific, *P. manokwari* has been intentionally introduced in an attempt to limit the proliferation of the Giant African Snail, *Lissachatina fulica*, also an invasive alien species. The Giant African Snail eats many crops and clearly corresponds to the definition of a species which has a negative impact on agricultural production (Vogler & Beltramino 2013), and thus any species which eats this snail would be considered of positive effect on plant production. However, a recent study concluded that *P. manokwari* is not an effective biocontrol agent, but has major negative effects on native snail fauna (Gerlach et al. 2020). Since some flatworm species now introduced in Europe are predators of terrestrial molluscs, especially *O. nungara*, there is a potential danger that professionals or amateur gardeners will voluntarily spread it to limit the proliferation of snails and slugs; the effects of the predator flatworm on snails and slugs would be easily visible, while its effect on earthworms would remain unnoticed. Communication with the public will be essential for avoiding unscheduled initiatives for propagating the flatworms.

The final issue to consider is the effect of invasive flatworms on both local and international horticultural trade (Alford 1998). The presence of invasive flatworms in products bought from a garden centre or nursery could lead to reputational damage and subsequent loss of customers (Boag & Neilson 2014). There is also potential for contamination of foodstuffs. *Parakontikia ventrolineata* has a tendency to be found on fruit and vegetables within gardens in France and disgusts those wishing to consume the produce. If flatworm contamination is suspected, cross-border shipments are likely to be rejected.

3. Active pathways of introduction of flatworms into the EU (commodities, conditions for survival during transport etc.)

Key points:

- Flatworms are associated with trade in containerised plants
- They opportunistically shelter in plant containers
- They are not associated with a particular plant product
- Growing media and plant root balls are a buffered environment
- The greatest cause of mortality of flatworms during transit is likely to be desiccation
- Egg capsules (cocoons) may be a more robust life-stage than adults

The association of invasive alien flatworms with gardens, nurseries and botanic gardens implies that most species have been introduced into Europe via the plant trade and in particular containerised plants and plants for planting (Álvarez-Presas et al. 2014; Blackshaw & Stewart 1992; Sluys 2016). Flatworms can be embedded in the growing media in containers or sometimes within plant material itself. For example, a number of bright yellow flatworms (*Fletchamia* sp.) were discovered inside *Dicksonia antarctica* tree ferns imported from Australia, during a plant health inspection of a nursery in the UK (Cannon & Baker 2007; Matthews 2005). *Paraba multicolor*, a Brazilian species was intercepted in imported plants in Germany (Kraepelin, 1901). However, plant health interceptions of flatworms are relatively rare, with most initial findings following establishment in gardens and nurseries. Aside from the example of tree ferns, associations of flatworms with a particular commodity are largely speculative. The flatworms are not transported on a host plant like a phytophagous pest, but rather opportunistically shelter in the suitable habitat (a plant container) and are unintentionally transported and introduced. *Arthurdendyus triangulatus* was considered to have been unintentionally introduced into Northern Ireland from New Zealand with daffodil bulbs (Willis & Edwards 1977), but Blackshaw & Stewart (1992) thought that trade in roses was more likely. Boag & Neilson (2014) found *A. triangulatus* in a nursery in New Zealand that exported plants to Scotland and considered this a potential pathway. The recent finding of *Artioposthia exulans* in the UK was discussed by Jones & Fenwick (2018) who considered that the flatworm may have been transported from New Zealand in leaf structures of *Phormium* spp. (New Zealand flax). *Bipalium kewense* and *P. manokwari* have both been found in Europe in hothouses, where they have been introduced with exotic plants. This has been happening for a while. The name *kewense* comes from Kew Gardens in London, where *B. kewense* was first found and described in 1878. More recently, *P. manokwari* has been found in a hothouse in Caen, France, representing the sole record of this species in Europe (Justine et al. 2014b). However, it is unknown with what plants or shipments that flatworms were transited.

Flatworms may be transported in other material. There are reports of flatworms being moved around locally in topsoil, manure and soil on machinery. Flatworms appear to have an affinity with plastic on the soil surface, presumably because it provides a moist, smooth and compressed resting habitat. Flatworms have been moved stuck to the outside of plastic bags containing potting soil and fertiliser (J-L Justine, unpubl.), and on the plastic wrapping surrounding baled silage (Boag & Neilson 2014). It is thought that *A. triangulatus* may have been transported to the Faroe Islands with potatoes from Scotland. In the US, flowers from Mexico were found to contain *Parakontikia ventrolineata* (Sluys 2016). Contamination of fruit and vegetables is feasible with flatworms sheltering in plant material but they would be less likely to survive being more susceptible to desiccation during international transit.

Flatworms are soil-dwelling and therefore a plant container with moist growing media represents a protected and buffered environment. Active flatworms lack water-retaining physiology and are susceptible to desiccation. However, it would seem that the conditions necessary to transport live plant material are largely suitable for flatworms. In unfavourable dry conditions, flatworms may aestivate deeper within the soil in mucus lined chambers (Winsor et al. 2004), although given the size of a plant container, this might be unlikely. The susceptibility of flatworm egg capsules (cocoons) to desiccation is largely unknown and they may be a more resistant life-stage during transit. They would also be smaller and potentially more mechanically robust than the adults. There has been comparatively little research on this. Flatworms can survive without feeding for several months (Baird et al. 2005), so the availability of prey would not be a limiting factor. As flatworms are not associated with a particular host plant or goods, it is difficult to specify a definitive trade route. Imported plants from both flatworm's native and invaded habitats constitute a risk. This is thus a world-wide problem, given the potential sources of invasive alien flatworms within and outside of Europe. It is recognised that plants for planting (nursery stock) present a greater risk of pest introduction than other commodities, due to the increased likelihood of pest survival on plant and soil material, the subsequent use of the plants in the wider environment and the diversity of plants traded from many differing origins (EPPO 2012).

4. Any method available that is or could be applied during transport to prevent introductions

Key points:

- Potential treatments must balance flatworm mortality against phytotoxicity
- Control by temperature manipulation would depend on the flatworm species
- Fumigation would be a possible means of flatworm eradication in-transit but has not been tested. Pesticides used against nematodes would likely not be active against flatworms.

The consensus is that most alien flatworms have entered Europe through trade in containerised plants. The flatworms were sheltering in plant pots or in crevices in the plants themselves. The problem in treating the flatworms in-transit is to find measures that would kill them but not harm the living plants. As the number plant species involved in trade is large and diverse, this would need to be considered on a case-by-case basis looking the requirements of the plants and the flatworm species concerned.

Temperature manipulation, for example transit in cold storage may kill tropical flatworm species such as *Platydemus manokwari*, which are susceptible to lower temperatures. For *P. manokwari* temperatures below 18°C for 14 d resulted in increased mortality in the laboratory (Gerlach 2019). However, the opposite would be true for a cold-hardy species such as *Arthurdendyus triangulatus* from New Zealand, which would survive well at lower temperatures, e.g. 5°C (Blackshaw 1992). Conversely, temperatures above 20°C would be detrimental to *A. triangulatus* survival, but may not be practical for shipping plants due to increased need for watering and husbandry.

Fumigation is used as a phytosanitary measure to treat plants and soil. Previously, the most common fumigant used was methyl bromide but this was removed from use in the EU in 2010 due to environmental (ozone depletion) and human health concerns, although it may still be used in countries exporting to the EU, and for restricted plant health purposes in other countries including the US, China, India, Australia and New Zealand (Cox 2017; Eschen et al. 2015). Alternative fumigants have been trialled for plant health purposes and these include sulfuryl fluoride, phosphine and ethanedinitrile. We are not aware of any studies testing the effectiveness of chemical fumigants against flatworms. However, as fumigants are used for phytosanitary control of other soil-dwelling pests (e.g. nematodes) they may be a possibility for flatworm eradication providing phytotoxic effects were kept to a minimum and there are no human health concerns. However, the specific chemicals used would need to be trialled. In general, pesticides and medicines targeted against nematodes are not effective against flatworms.

Other possibilities for in-transit treatment are controlled atmosphere treatment and irradiation. Controlled atmosphere treatment involves lowering oxygen levels by pumping in CO_2, nitrogen or other gases into a sealed container. This is mainly used for pest control on delicate artefacts but has shown potential for control of insect pests on living plants (Held et al. 2001). There have been no studies on whether this would be a feasible option for flatworm control. Irradiation is an established phytosanitary procedure for fresh fruits and vegetables in the US, Australia and New Zealand (Hallman & Blackburn 2016). It is doubtful whether it would be useful on live plants due to the potential for damage to the product.

5. Any method available that could be applied at inspection points to detect flatworms in imported containerised plants and plants-for-planting

Key points:

- Current methods of detection are visual inspection and destructive sampling
- Flooding plant containers may expel adult flatworms (but not egg capsules)
- eDNA technology has great promise for detecting flatworms in plant containers

Due to their cryptic nature, detection of flatworms is problematic. The presence of mucus or slime on plant material or underneath plant pots may be indicative of flatworm presence but further examination would be necessary to confirm as native slugs and snails would also leave slime trails. Visual inspection is unlikely to be effective on its own. A proportion of plants would need to be destructively sampled by uprooting, hand-sorting and examination of root-balls and growing media.

It has been suggested that submerging plant pots in water overnight will force flatworms out from the growing media (B. Boag, pers. comm.) and this could be used by nurseries to check imported stock for flatworms. As far as we are aware this has not been trialled experimentally. Flatworms can also be expelled from soil using the chemical methods (e.g. a mustard solution) for sampling earthworms (Murchie & Gordon 2013). It is possible that adult flatworms could be expelled from growing media in the same way by applying a drench to the plant containers. However, these techniques will not work for egg capsules (cocoons) embedded in the soil as they are immobile. Also, the impact of submerging containers and drenches would need to be assessed in terms of their impact on the plant product. It may be that this would also constitute destructive sampling for a proportion of a consignment.

A possible novel alternative is to use environmental DNA (eDNA) techniques. As flatworms reside within the growing media in containers, they will shed mucus and faeces. Passing water through the growing media would enable the run-off to be screened for flatworm eDNA signatures using diagnostic PCR. This would have the advantage of being a minimally invasive technique and, depending on the sensitivity, could process a large number of samples by pooling the initial washings. Environmental DNA techniques are widely used for monitoring aquatic species, including invasive alien species (Darling & Mahon 2011; Dejean et al. 2012;

Larson et al. 2020). They have also been used to detect plant pathogens (*Phytophthora* spp.) in water and soil samples (Catala et al. 2015). The technique has been mooted as a portal inspection tool for marine invasive alien species (Borrell et al. 2017; Zaiko et al. 2015) and has recently been considered for plant health applications such as detecting invasive forest insects by using water sprays to collect eDNA from trees (Valentin et al. 2020). As flatworms produce mucus and are in an enclosed space within growing media, it seems that they would be an ideal target for eDNA detection following a wash through with water. However, the technique would need to be developed and trialled for both known and potentially unknown flatworm species.

6. Any method that could be used to minimise the survival of flatworms on such plants (e.g. heat treatment)

Key points:

- There are no approved pesticides for flatworm control and few studies on this topic
- Hot water immersion is a recognised phytosanitary procedure and the most likely means of disinfesting containerised plants

If necessary, pesticides can be applied post-import and post-inspection to eradicate plant health pests. However, there are very few studies on pesticidal control of invasive flatworms. Grassland pesticides were largely ineffective against *Arthurdendyus triangulatus*, with only the now-withdrawn organochlorine insecticide, gamma HCH, having an effect (Blackshaw 1996). The slug parasitic nematode (*Phasmarhabditis hermaphrodita*) was also not effective against *A. triangulatus* (Rae et al. 2005). It is possible that some of the drugs used to treat parasitic flatworms in veterinary or human medicine, such as praziquantel, could be active against free-living flatworms but this has not been tested. Most of these drugs are not approved for control of agricultural pests but there are a few examples of active ingredients used in both crop protection and endoparasite control, e.g. abamectin (although, it is not active against flukes and hence unlikely to be active against terrestrial flatworms).

Flatworms are sensitive to temperature. Hot water immersion is a recognised phytosanitary measure for control of nematodes and other pests and pathogens. For example, flower bulbs are immersed in water baths at temperatures above 40°C for several hours to eradicate quarantine nematode species such as *Ditylenchus dipsaci* and *Ditylenchus destructor* (EFSA PLH Panel 2014; Qiu et al. 1993). Hot-water treatment has been suggested as a means to kill invasive flatworms in plant containers (Blackshaw 1996; Sugiura 2008). Immersion of *A. triangulatus* in water at 30°C for 20 minutes, killed adults within 24 h, while immersion for only 5 minutes at 34°C killed adult flatworms within 1 h (Murchie & Moore 1998). For tropical flatworm species, the temperatures used will need to be higher. However, exposure to temperatures above 43°C resulted in 100% mortality of *P. manokwari* after 5 minutes (Sugiura 2008). This would still be within the range used for nematode control, although the effects on plants would need to be evaluated, as would the ability of hot water to penetrate the growing media. Justine et al. (2014b) suggested that a hot-water drench may be a more practical method of control than immersion for containerised plants. This was based on the work of Tsang et al. (2001) who developed a hot water drenching system to disinfest containerised plants of nematodes. There has been little work on the effects of hot water treatment on flatworm egg capsules. Some preliminary studies suggests that they are as susceptible to heat treatment as adults (A.K. Murchie, unpubl.).

7. Experiences and methods applied outside the EU (regarding preventing introductions, detection and control treatments), particularly in Australia, New Zealand and North America – noting effectiveness and costs if available

Key points:

- There are no specific phytosanitary measures applied elsewhere in the world against terrestrial flatworms
- Australia, New Zealand and North America only permit plants for planting to be imported with bare roots or in inert growing media
- The EU allows plants for planting to be imported with sufficient growing media to maintain them in transit subject to special requirements to ensure freedom from pests

We found no phytosanitary measures related specifically to invasive alien flatworms in North American, Australian or New Zealand legislation. For Australia and New Zealand, this is unsurprising as these countries have not experienced the same problems as Europe and have a more diverse native flatworm fauna that has acted as a source of invasive alien species rather than a recipient. In Australia and New Zealand, terrestrial flatworms have not achieved the same pest status compared to Europe. Where there are alien species (e.g. *Platydemus manokwari* in Australia, *Australoplana sanguinea alba* in New Zealand) their impact is not reported to be problematic, perhaps due to the presence of natural regulatory factors such as indigenous predators, competitors or prey resistance.

North America is similar to Europe in having smaller native flatworm species, and fewer species, than those found in Australasia, South East Asia and South America (see map in Sluys 1998), so large invasive alien species are notable. However until recently, invasive alien flatworms, mainly *Bipalium* spp. (Ducey et al. 2007; Fiore et al. 2004), have remained largely of scientific interest rather than provoking widespread concern. This may be because *B. kewense* has been established in North America since 1891 (Winsor 1983a), representing the 'old' introduced species as categorised by Justine et al. (2014b). Somewhat ironically, in North America invasion by European earthworm species such as *Lumbricus terrestris*, a species severely affected by *A. triangulatus* predation in Europe, has garnered much attention due to their effects on native habitats (Hendrix et al. 2008) and predatory flatworms have been mentioned, albeit cursorily, as potential biocontrol agents. However, the finding of *P. manokwari*, a snail predator, in Florida, USA has significantly raised the profile of invasive alien flatworms in North America with much media interest (Justine et al. 2015; Sluys 2016). Despite this, and clear recognition of the threat to biodiversity, *P. manokwari* is not a regulated species within Florida probably because it is so well established, being found now in 40 counties and other US states. This means that there are no restrictions in moving the species around the state, although for inter-state movement a permit would be required, if say flatworm infestation of soil was suspected (Florida Fish and Wildlife Conservation Commission 2021) but there seems to be no specific monitoring or controls in place. At the federal level in the USA, pests and harmful species are overseen by the Animal and Plant Health Inspection Service (APHIS), which is part of the U.S. Department of Agriculture. Although there is no specific mention of invasive alien flatworms in the Code of Federal Regulations, as predatory species, invasive alien flatworms would be governed by general regulations covering biological control agents (Sluys 2016).

One of the key differences that made the EU vulnerable to invasive alien flatworm incursion is that plants for planting were previously allowed to be imported in sufficient growing media to sustain them in transit, whereas other countries had stricter biosecurity approaches to growing media. Depending on the size of plants, substantial quantities of soil may have been involved, leading Eschen et al. (2015) to comment that 'in effect an entire ecosystem is being transplanted'. However, the current EU Commission Implementing Regulation (EU) 2019/2072 came into force in 2019 and stipulates the special requirements for growing medium attached to plants to sustain them. The special requirements (Annex VII) are summarised as follows:

a-i) the growing medium was free from soil and had not previously been used to grow plants,

a-ii) or was composed of peat or coconut fibre,

a-iii) or was subjected to fumigation or heat treatment,

a-iv) or was subjected to effective systems approach to ensure freedom from pests,

and stored in appropriate conditions to keep it free from quarantine pests, and since planting,

b-i) physical or hygiene measures have been taken to ensure freedom from Union quarantine pests

b-ii) or within two weeks prior to export, the soil has been completely washed off and plants may be replanted.

Following from Brexit, DEFRA (UK) have produced guidelines for UK exporters to the EU27 (DEFRA 2020). This provides practical measures to be taken to meet the requirements above. For b-i) this includes physical isolation from soil and other sources of contamination and specifies that the plants be maintained on raised benches or concrete and that pots with drainage holes should on a saucer or impermeable layer. Such approaches have been recommended to prevent contamination with invasive alien flatworms (EPPO 2001a,b). If the current procedures are followed precisely, this should minimise the likelihood of contamination with invasive alien flatworms but much depends on compliance, inspection and policing of these phytosanitary requirements.

Australia and New Zealand generally have the strictest phytosanitary regulations and consignments with soil are not permitted. It is preferred that plants are imported bare-rooted or, if necessary, in inert material to protect them during shipping (Australian Goverment 2021a; Australian Goverment 2021b; Biosecurity New Zealand 2012). Such measures clearly restrict the variety and the quantities of plants that can be imported and there is a cost to trade and for implementing biosecurity protocols. In the USA, importation of plants for planting is regulated under the Plant Protection Act by APHIS (Code of Federal Regulations, Part 319, Sub-part H

- Plants for Planting). Similar to Australia and New Zealand, plants growing in soil are not permitted, unless from Canada, but some other growing media may be allowed as listed in the Plants for Planting Manual (USDA 2017). Canada also does not permit plants rooted in soil with the exception of those from continental USA (Government of Canada 2015).

Twelve species of terrestrial flatworms have been introduced into Australia / Australian territories but these are likely historic introductions that pre-date current phytosanitary arrangements (L. Winsor, pers. comm.). Australia and New Zealand operate a ‘white list’ approach, i.e. only permitting commodities that have been assessed as safe, compared to a ‘black list’ approach in the EU, which targets specific pests and pathogens (Eschen et al. 2015). So although Australia and New Zealand do not specifically legislate against invasive alien flatworms, it would seem that their strict plant health biosecurity, in particular preventing the import of plants rooted in growing media, largely prevents the importation of invasive alien flatworms by acting on their primary pathway of introduction.

Some countries, for example Denmark, Sweden, Norway and Iceland, list invasive alien flatworms as quarantine pests (Boag & Yeates 2001; Cannon et al. 1999; Schrader & Unger 2003). Recent local regulations in the French territories in the Antilles have also sought to address the problem of invasive alien flatworm species. The French overseas departments can issue local regulations concerning the environment. It is noteworthy that Guadeloupe, Martinique and Saint Martin have very recently (2020) added several species of invasive alien flatworms on the list of species forbidden for “introduction into the territory, including transit under customs supervision, introduction into the natural environment, possession, transport, peddling, use, exchange, offering for sale, sale or purchase of live specimens” (Anonymous 2020a; Anonymous 2020b; Anonymous 2020c).

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