This text is an integral part of the *EPPO Study on bark and ambrosia beetles associated with imported non-coniferous wood* and should be read in conjunction with the study

Pest information sheet
Bark beetle

PITYOPHTHORUS JUGLANDIS (COLEOPTERA: SCOLYTINAE)
walnut twig beetle

EPPO lists: Geosmithia morbida and its vector *Pityophthorus juglandis* were added to EPPO A2 List of pests recommended for regulation in 2015. *P. juglandis* and *G. morbida* are currently not regulated by EPPO countries (EPPO Global Database; EPPO, 2018). The assessment of potential risks results from a comprehensive PRA carried out by an EPPO Expert Working Group on PRA. The present datasheet is mostly based on the EPPO PRA and draft EPPO datasheet (EPPO, 2015a, 2015b), complemented with new data from references published since 2015.

PEST OVERVIEW

Taxonomy
Pityophthorus juglandis Blackman, 1928. Based on the analysis of various DNA sequences, Rugman-Jones *et al.* (2015) identified two clearly separated lineages, but conclude that further studies are needed to determine whether these correspond to two cryptic species. One lineage was responsible for recent expansion of the range of *P. juglandis* and the spread of *G. morbida* in North America (Rugman-Jones *et al.*, 2015).

Associated fungi
Geosmithia morbida (pathogenic). All known isolates are currently considered as one species (EPPO, 2015a citing others). Kolarik *et al.* (2017) identified four additional *Geosmithia* sp. in the galleries of *P. juglandis*. In addition, *Fusarium solani* has also been isolated from cankers on *Juglans*; its role in tree mortality is not known, although this fungus is known to be pathogenic to *J. nigra* and may contribute to canker development on the main trunk and tree mortality (EPPO, 2015a).

G. morbida and *P. juglandis* together cause thousand cankers disease, a disease that has been devastating on walnuts in the USA (EPPO, 2015a).

Morphology and biology (from EPPO, 2015b, except where a source is indicated)
Adults of *P. juglandis* are minute, 1.5-1.9 mm long and yellowish-brown in colour. At emergence, most *P. juglandis* adults fly to branches to mate and excavate new galleries for laying eggs, while some may remain in the trunk and expand overwintering cavities. Males excavate a small nuptial chamber under the bark, release sex pheromones, and are joined by a few females. Females create egg galleries under the bark, which are generally across the grain of the wood. Adults emerge through minute, round exit holes. Emerging adults carry on their cuticule spores of *G. morbida* that can create new infections when they tunnel into other branches or trunks to feed, reproduce or overwinter. After the tree starts showing foliar symptoms, enormous numbers of *P. juglandis* may emerge from the trees. In an extreme case in Colorado, 23 040 adults emerged from 2 logs of *J. nigra* (circa 6 adults emerging/cm^2 log surface). A single generation was observed to be completed in 7 weeks in logs at room temperature. In general, there are 2-3 overlapping generations, depending on climatic conditions (EPPO, 2015b). In Northern Italy, two overlapping generations were observed (Faccoli *et al.*, 2016).

In areas with cold winters (e.g. Colorado), *P. juglandis* overwinters as dormant adults in cavities in the bark of the trunk, and as larvae that may continue development during warm periods. In areas with mild winter (e.g. California), overwintering stages include larvae at all stages of development.

P. juglandis flight activity peaks at temperatures between 23-24ºC and ceases below 17-18ºC. Under experimental conditions, lower lethal temperatures (LT99) reported for adults and larvae differed in two studies (ca. -18 in one, below -22 C in the other; Luna *et al.* 2013; Peachey 2012). *P. juglandis* survived in infested trees in Colorado where temperatures reached −29°C in February 2011, suggesting that it could survive the winter in much of the native range of *J. nigra*, but may be limited in areas where temperatures regularly drop below the lower lethal temperature. The degree-days requirements for *P. juglandis* are unknown.

P. juglandis attacks standing living trees, including healthy trees. Entry and emergence holes are present on the bark on larger diameter branches (generally above 1.5 cm on *J. nigra*) or on stems. In caged experiments, entrance holes have been observed in *J. nigra* seedlings with basal diameter as small as 0.55 cm. *P. juglandis* was shown to infest logs in certain conditions (e.g. reinfesting treated logs). In Italy, cankers and holes were observed on 1-1.5 cm diameter twigs in the field; *G. morbida* can be artificially inoculated on *J. nigra* and *J. regia*, and create cankers in 5-10 mm diameter plants (2-year old). In a field based experiment to determine whether *P. juglandis* colonized young walnut nursery trees, and therefore whether those presented a risk of transporting the pest, Audley *et al.* (2017) found that even though some adults initiated colonization (entry holes, some galleries) on trees baited with a pheromone lure, there was no successful establishment nor any evidence of reproduction even in the largest diameter trees (the average diameter at the base of the smaller and larger trees was 1.8 cm and 2.4 cm, respectively).

In infected trees, *G. morbida* is located in the phloem and cambium, and grows within and around the feeding sites and galleries of *P. juglandis* where it was introduced, killing tissues and producing cankers. *G. morbida* does not produce deep staining of the wood, but it may reach the sapwood (superficially) at advanced stages of the disease, and may result in a brown to black discoloration of the sapwood.

P. juglandis does not show some characteristics typical of most bark beetles: there does not seem to be a close relationship between the health of the tree and susceptibility, and there is no evidence of the need of critical numbers to establish in a tree to overwhelm tree defences (EPPO, 2015a), although the insects do produce, and respond to, aggregation pheromones (Seybold *et al.* 2012a, 2012b).

Spread biology
The EPPO PRA (EPPO, 2015a) noted that the flight capacity of *P. juglandis* was unknown, but that other small bark beetles of similar size are capable of flying long distances (e.g. distances of 86 km noted for *Pityogenes chalcographus*). Recent results in a flight mill experiment in the USA (Kees *et al.*, 2017) indicated a maximum total flight distance of 3.6 km in 24h, and the mean and median distances were 372 m and 158 m, respectively. *P. juglandis* adults flew for 34 min on average within a 24-h flight trial. On the basis of the low flight capacity observed, the authors conclude that natural dispersal may only contribute marginally to spread, and expected that natural flight capacity is limited to not more than 3 or 4 km at the extremes (with an assumption that the beetle only flies for about 5 days), and that insects will remain somewhat localized at sites of introduction in the short term. However, the authors of this study noted that survival of the beetles (which had to be imported in Minnesota from California) was exceedingly low, possibly due to low air humidity or the absence of adult feeding (B. Aukema, pers. comm.). This could have somehow biased the tests' results.

Nature of the damage
Thousand cankers disease produces progressive crown dieback resulting in reduced growth rates and, in extreme cases, tree mortality. The fungus is not systemic but, with multiple infestations on the tree, multiple cankers coalesce, cutting off the supply of nutrients, which results in dieback of branches and the subsequent death of the tree. Timber quality can be affected by reduced growth and yield. Nut production/yield may be reduced in diseased trees or because of tree mortality (but there is no direct damage to nuts) (EPPO, 2015a). Other organisms may also contribute to tree decline during the last stages of the disease. Once crown symptoms become visible, death may occur within a few years (e.g. 2-5), with some trees having a slower dieback over many years.

Detection and identification

- *Symptoms.* Thousand cankers disease can result in crown symptoms and usually causes cankers on branches and trunks. At early stages of the disease, the only indications of damage are the entry holes of *P. juglandis*. The first symptoms in the crown are leaf yellowing, wilting of foliage and thinning, followed by twig and branch dieback. Dead leaves generally fall from declining branches. Branches fail to leaf out in spring. Symptoms only appear after considerable canker formation (probably after several years depending on the tree species and dimensions), and vary in different locations. Entry and emergence holes of *P. juglandis* can be observed on the bark on larger diameter branches (generally above 1.5 cm on *J. nigra*) or on stems but these are difficult to see when bark is deeply furrowed.
- *Trapping.* Pheromones and traps are available. Pheromone-baited branches are used in low population areas to sample for *G. morbida*. Detailed survey guidelines, including visual surveys and sampling, have been developed in the USA and references are given in EPPO (2015b). Recently, Blood *et al.* (2018) demonstrated that *P. juglandis* is not attracted to ethanol, but is attracted by volatiles from *J. nigra* and *G. morbida*, and is repelled by limonene.
- *Identification*. Morphological characters that allow distinguishing adults of *P. juglandis* from other *Pityophthorus* spp. have been published, as well as a screening aid to help differentiate *P. juglandis* from other bark beetles in trap samples or specimens collected from suspect walnut trees in the USA (LaBonte and Rabaglia 2012). *G. morbida* should be cultured for species confirmation. It can easily be identified based on morphological characteristics. Its identity can be confirmed by sequencing the rDNA ITS region. Recently, a PCR-based rapid molecular detection protocol for *P. juglandis* and *G. morbida* was proposed (Oren *et al.*, 2018).

Distribution (see Table 1)

P. juglandis is present in North America (USA and Mexico) and in Italy. In a few cases, either *G. morbida* or *P. juglandis* have been found, and the reasons are not fully elucidated yet.

In Italy, *P. juglandis* was first found in the Veneto region in 2013. The number of sites found to be colonized by the beetle in the Veneto region increased from 13 in 2014 to 29 in 2017 (Faccoli, pers. observation based on pheromone trap survey). It was found associated with *Geosmithia morbida* at a few sites. In 2014 it was also found in Lombardy. In 2015, *P. juglandis* and the associated fungus *G. morbida* were found in two black walnut (*Juglans nigra*) plantations in Piemonte region, municipality of Rondisone (TO), more than 320 km west of the first recorded site (Faccoli *et al.*, 2016a). In 2015, two adults were trapped in Friuli Venezia Giulia (Porcia, PN) and in the following year (2016) the species was found again in 4 different sites of the same region. The fungus *G. morbida* was not detected in Friuli Venezia-Giulia nor in Lombardia (Montecchio *et al.*, 2016).

Host plants (see Table 2)

The hosts of *P. juglandis* all belong to the family Juglandaceae, genera *Juglans* and *Pterocarya*. Based on observations in the *Juglans* collection of the USDA-ARS National Clonal Germplasm Repository in California, *P. juglandis* is considered to have the capacity to develop in all species of *Juglans* that it may encounter (Hishinuma *et al.*, 2016). Among *Juglans* hosts, *J. major* is considered to be a native host of *P. juglandis*, and *J. californica* may also possibly be an indigenous or native host (EPPO, 2015b). *Carya* species (also Juglandaceae) are not hosts of *P. juglandis* and *G. morbida* (EPPO, 2015b).

The susceptibility to thousand cankers disease varies between species and hybrids, and between trees of the same species. This is also the case for the most susceptible *J. nigra*, for which healthy trees may be found in areas severely impacted by the disease. *J. major* and *J. nigra* consistently appear as the least and most susceptible host species respectively. All other known *Juglans* hosts infested in the field or in collections, as well as hybrids, seem to fall in an intermediate category. *J. regia* (the most important species for the EPPO region), is susceptible but seems to present a wide intraspecific variation (EPPO, 2015b).

Known impacts and control in current distribution (all from EPPO, 2015b, except if another source is indicated)
In the USA, to date amenity trees have been most affected. However, the greatest potential impacts of thousand cankers disease are considered on timber production (primarily *J. nigra*) with additional losses to nut production (primarily *J. regia*). In one area of the USA, Boulder Colorado, where thousand cankers disease has been present for over a decade, the value of affected plants is estimated at approximately 3 million USD and over 60% of trees died within 6 years of the disease's original detection. Many municipalities and homeowners in the USA have already incurred costs associated with the loss of *Juglans* amenity plants due to tree removal and replacement costs, indirect effects on shade, heating/cooling, and added landscape value to property.

P. juglandis has been present in Southern California, but historically has not been an issue in cultivated *J. regia* until recent years. Certain rootstocks on which nut-producing *J. regia* may be grown (e.g. 'Paradox' a hybrid of *J. hindsii* x *J. regia*) are susceptible to thousand cankers disease. *J. regia* is considered to be less susceptible to thousand cankers disease than are some other *Juglans* species (e.g. *J. nigra*, *J. hindsii*), however, mortality, although not extensive, has been observed.

There are also costs incurred by government and universities associated with survey and detection, monitoring, public outreach, and development and implementation of interstate quarantines.

Social damage in the USA is currently due to death of amenity and garden trees, but losses of jobs are anticipated for the future.

In Italy, *P. juglandis* was found in Northern Italy (Veneto) since 2013 in 5 black walnut plantations. In the following years the pest was found in 13 (2014), 17 (2015), 26 (2016) and 29 (2017) new sites (M. Faccoli, pers. obs.). The damage, in term of number of infested trees, greatly varied according to the different plantations from a few trees to about 25-30% of the plants.

Control: No control methods are currently available to effectively protect individual trees from developing thousand cankers disease or to cure diseased trees. Research is actively conducted on control methods (chemical, biological control, semiochemical, resistant cultivars) of this recently-recognized pest complex. In laboratory and field trials, Castrillo *et al*. (2017) found that exposure of beetles to walnut logs sprayed with the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium brunneum* reduced populations, and have a potential use in integrated management. Blood *et al*. (2018) identified attractants and a repellent and concluded that it may be possible to use them in push-pull strategies, which remain to be developed.

POTENTIAL RISKS FOR THE EPPO REGION
Pathways
Entry
In Italy, the most likely pathway for introduction may have been fresh infested walnut wood and timber (with bark) from the USA. From genetic analysis, all the analysed populations appear to have derived from a single introduction (Faccoli *et al*., 2016). The EPPO PRA (EPPO, 2015a) stated that the likelihood of entry was high, especially on wood with bark of *Juglans* and *Pterocarya* and wood packaging material if ISPM 15 is not applied. From the biological point of view, wood is very favourable for entry, and there is trade of walnut roundwood and sawn wood, and also firewood, to a large number of EPPO countries where *Juglans* species are grown.
The likelihood of entry on wood chips and wood waste is considered low, but not excluded (EPPO, 2015a). Entry would require that individuals survive processing and transport, and transfer to a suitable host. The volume and frequency of trade are favourable to entry, but walnut is not likely to constitute a high percentage of any load of wood chips. *P. juglandis* would be more exposed to desiccation in chips than in wood, and transfer would require that wood chips are stored outdoors or used in particular conditions (mulch).
The likelihood of entry on bark on its own is considered as low, and no information could be found on existing trade (EPPO, 2015a).

The probably of entry would increase if trade volumes further increase and the pests further spread widely to Eastern USA, where the main walnut wood-producing States are located. From Italy, most production plantations of *Juglans* were established in the 1990s and the trees are well below dimensions that are usually used commercially; the current volumes of *Juglans* wood traded from Italy are therefore likely to be much lower than from the USA.
Plants for planting are also a very suitable entry pathway (EPPO, 2015a), if there is a trade. The recent results in Audley *et al.* (2017 - above) shows some temporary association of adults with small trees, although no offspring was produced in their experiments. Cut branches of *Juglans* and *Pterocarya* is considered a very unlikely pathway (unlikely to be traded, not mentioned in relation to possible pathways in the USA) (EPPO; 2015a).

Summary of pathways (uncertain pathways are marked with '?'):

- *wood (round or sawn, with or without bark, incl. firewood) of Juglans and Pterocarya*
- *non-coniferous wood chips, hogwood, processing wood residues (except sawdust and shavings)*
- *wood packaging material if not treated according to ISPM 15*
- *plants for planting (except seeds) of Juglans and Pterocarya*
- *bark of hosts?*

Spread (following introduction, i.e. within EPPO region)
P. juglandis flies and is expected to spread naturally from areas within the EPPO region where it becomes established. Natural spread has occurred in the USA, although human-assisted pathways are considered more important for spread and are critical for spread over long distances and across geographic barriers. The EPPO PRA estimated that natural spread from Italy was likely to happen towards Slovenia, Austria and Croatia in the East, and possibly to France in the West (further away). However, human-assisted spread will be the main means of spread in the EPPO region. An illustration of this is the finding of *P. juglandis* and *G. morbida* in Piemonte in 2015, more than 320 km from the first recorded site in 2013 (see above).

Establishment
P. juglandis and *G. morbida* have the potential to establish throughout the EPPO region where *Juglans* species occur, and climate was not considered a limiting factor in the EPPO PRA. Among the host plants of *P. juglandis*, at least *J. regia*, *J. nigra* (although a North American species, see below) and *J. mandshurica* occur naturally in the PRA area. These species as well as many other *Juglans* spp. are also grown commercially (for wood or nuts) and as amenity trees (parks, gardens). *Pterocarya* would presumably be grown as amenity trees. *J. regia* is the most widespread *Juglans* in the PRA area, used commercially for nut and wood production, and as amenity tree. *J. nigra* has been used as a forest tree since the 19th century and is acclimatized from Western Europe (including Italy) to Ukraine and Russia, through Central Europe. It is extensively planted for wood production in parts of central and eastern Europe, and is available for sale as ornamental. 11 *Juglans* spp. of various origins were recorded as being available for sale in nurseries in Europe, as well as five *Pterocarya* spp. including the known hosts.
P. juglandis is able to develop on healthy or stressed trees, and on cut trees. The current management practices in orchards and forests in the EPPO region were not considered to hinder its establishment. Finally, small populations are believed to be able to start an outbreak, although *P. juglandis* may be present in huge numbers in logs, which would help establishment of populations. There is a possibility that adults may be present all year round in the southern part of the PRA area. Finally, it has adapted to several *Juglans* species other than those present in its native range, and is very successful on some of them. It is very likely that it could move to yet other *Juglans* species.

Potential impact (including consideration of host plants)
All walnut trees in the EPPO regions are at risk in the long term. The greatest risk is to *J. regia* nut production, with secondary losses to timber (*J. regia*, *J. nigra*) and amenity plants. Environmental impacts may occur on walnuts in the wild, especially when they reach areas where those are important (e.g. sensitive environments, mountains, pure wild stands in Central Asia). The social impact may be locally high in areas of intensive plantation or orchards, and in areas where walnuts are an important

source of income for local populations (either collected from the wild, orchards or gardens). *P. juglandis* and *G. morbida* are likely to be more damaging (more generations of *P. juglandis*) in the Southern and Eastern parts of the EPPO region, where walnuts are also grown more widely. Faccoli *et al.* (2016) noted that given its widespread presence and rapid reproduction and dispersal, *P. juglandis* might quickly increase its abundance and distribution in Italy and other European countries, and that damage will probably increase in the near future, leading to a gradual decline of walnut health and a progressive reduction in the number of *J. nigra* plantations.

Table 1. Distribution (all records from EPPO Global Database, with additional references)

	Comments
EPPO region	
Northern Italy (Veneto, Lombardia, Piemonte, Friuli Venezia Giulia)	First found in Veneto in 2013, later in 3 other regions of Northern Italy. In Lombardia and Friuli Venezia Giulia, as of Montecchio *et al.* (2016), only *P. juglandis* had been found (trapped), not *G. morbida* and no symptoms of the disease had been observed.
North America	
Mexico	Only *P. juglandis* has been recorded, in the state of Chihuahua. These findings pre-date by many decades the discovery of thousand cankers disease and description of *G. morbida*. There are no known recent attempts to make dedicated collections of either organism in Mexico (EPPO, 2015a).
USA (Arizona, California, Colorado, Idaho, Indiana, Maryland, Nevada, New Mexico, North Carolina, Ohio, Oregon, Pennsylvania, Tennessee, Utah, Virginia, Washington)	In Indiana, only *G. morbida* had been found at the time of the EPPO PRA, but the presence of *P. juglandis* has later been confirmed (EPPO, 2018)

Table 2. Hosts

Family	**Species**	**Reference**
Juglandaceae	*Juglans ailantifolia*	EPPO, 2015a
Juglandaceae	*Juglans californica*	EPPO, 2015a
Juglandaceae	*Juglans cinerea*	EPPO, 2015a
Juglandaceae	*Juglans hindsii*	EPPO, 2015a
Juglandaceae	*Juglans major*	EPPO, 2015a
Juglandaceae	*Juglans mandshurica*	EPPO, 2015a
Juglandaceae	*Juglans microcarpa*	EPPO, 2015a
Juglandaceae	*Juglans mollis*	EPPO, 2015a
Juglandaceae	*Juglans nigra*	EPPO, 2015a
Juglandaceae	*Juglans regia*	EPPO, 2015a

Family	Species	Reference
Juglandaceae	*Juglans* hybrids, e.g. Paradox rootstock *J. hindsii* x *J. regia*, *J. nigra* x *J. hindsii*, *J. cinerea* x *J. ailantifolia*, *J. nigra* × *J. regia*	EPPO, 2015a
Juglandaceae	*Pterocarya fraxinifolia*	Hishinuma *et al.*, 2016
Juglandaceae	*Pterocarya stenoptera*	Hishinuma *et al.*, 2016
Juglandaceae	*Pterocarya rhoifolia*	Hishinuma *et al.*, 2016

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