 ISSN 0250-8052. DOI: 10.1111/epp.12301

Thousand cankers disease in Europe: an overview

L. Montecchio[1], M. Vettorazzo[2] and M. Faccoli[3]

[1]*Department of Land, Environment, Agriculture and Forestry (TeSAF), University of Padova, Viale dell'Università 16, I-35020, Legnaro (PD), (Italy); e-mail: montecchio@unipd.it*

[2]*Veneto Region, Depertment for Agriculture and Rural Development, Phytosanitary Service, Via Longhena 6, I-30175, Marghera (VE), (Italy)*

[3]*Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, I-35020, Legnaro (PD), (Italy)*

Walnut (*Juglans regia* L.) is traditionally present in most European countries as an ornamental tree, and in Southern Europe in particular it is grown for both fruit and wood. Since the 1980s, to supply the increasing demand for walnut timber, large areas of southern and central Europe, from France to Hungary, have been planted with black walnut (*Juglans nigra* L.) to provide wood for furniture production. The fungus *Geosmithia morbida* and its vector *Pityophthorus juglandis*, causing the thousand cankers disease of walnut in the USA in the last 2 decades, were recently reported in Europe (in Italy) on both walnut species. Thousand cankers disease can have a high negative impact on the landscape and economy of many agricultural and forest areas. Following a detailed pest risk analysis performed by EPPO in 2015, both organisms were included in the EPPO A2 List of pests recommended for regulation as quarantine pests. The main biological, epidemiological and monitoring aspects of thousand cankers disease and its status in Europe are reported.

Introduction

Thousand cankers disease is a complex disease caused by the pathogenic fungus *Geosmithia morbida* Kolarík, Freeland, Utley & Tisserat (Ascomycota, Hypocreales). Its vector is the walnut twig beetle *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae, Scolytinae). Since the mid-1990s the disease has been responsible for widespread mortality of walnut species in the USA (Zerillo *et al.*, 2014). In 2013, both *G. morbida* and *P. juglandis* were recorded for the first time in Europe in the Veneto Region (North-Eastern Italy) on symptomatic black walnuts (*Juglans nigra*). In 2014 both pests were also found on English walnut (*Juglans regia*) (Montecchio & Faccoli, 2014; Montecchio *et al.*, 2014).

Thousand cankers disease is considered to be a serious threat to *Juglans* spp. in the entire area in which they are grown within the EPPO region, as the distribution of susceptible walnut species and hybrids (naturally present, cultivated for fruit or timber production, or grown as ornamental trees) frequently overlaps in a geographical continuum. Moreover, the vector has long-distance dispersal ability, and trade in walnut commodities in the EPPO region is not regulated.

The vector

Pityophthorus juglandis (Fig. 1g) is a bark beetle species native to Mexico and the South-Western USA (southern California, Arizona and New Mexico); it has recently become introduced into many American states. Adults are very small, 1.5–1.9 mm long, yellowish-brown, about three times as long as wide, with, characteristically, 4–6 concentric rows of asperities on the anterior slope of the pronotum; each row is usually broken near the median line of the pronotum. Adults reproduce in the phloem of walnut trees, boring short galleries where they lay eggs (Faccoli, 2015). Larvae are white, C-shaped, legless and typical of bark beetles, with a reddish-brown head capsule. Pupae are white, exarate with distinguishable body parts. Teneral adults are yellowish-brown and soft before they darken to a reddish-brown and their elytra harden (Blackman, 1928).

A detailed description of the biology of *P. juglandis* in Italy is given in Faccoli *et al.* (2016); these authors report that adult beetles are caught in traps from mid May to late October. Overwintered adults fly from mid May (when the mean air temperature is about 18°C), and first-generation adults emerge about 7–9 weeks later, at the beginning of July. Finally, second-generation adults emerge in late summer (the end of September). Walnut twig beetles mainly overwinter as adults under the bark of host trees infested the previous autumn. Temperature lethal effects were investigated in laboratory experiments by Peachey (2012) and Luna *et al.* (2013) through direct exposure of infested logs to different thermal regimes.

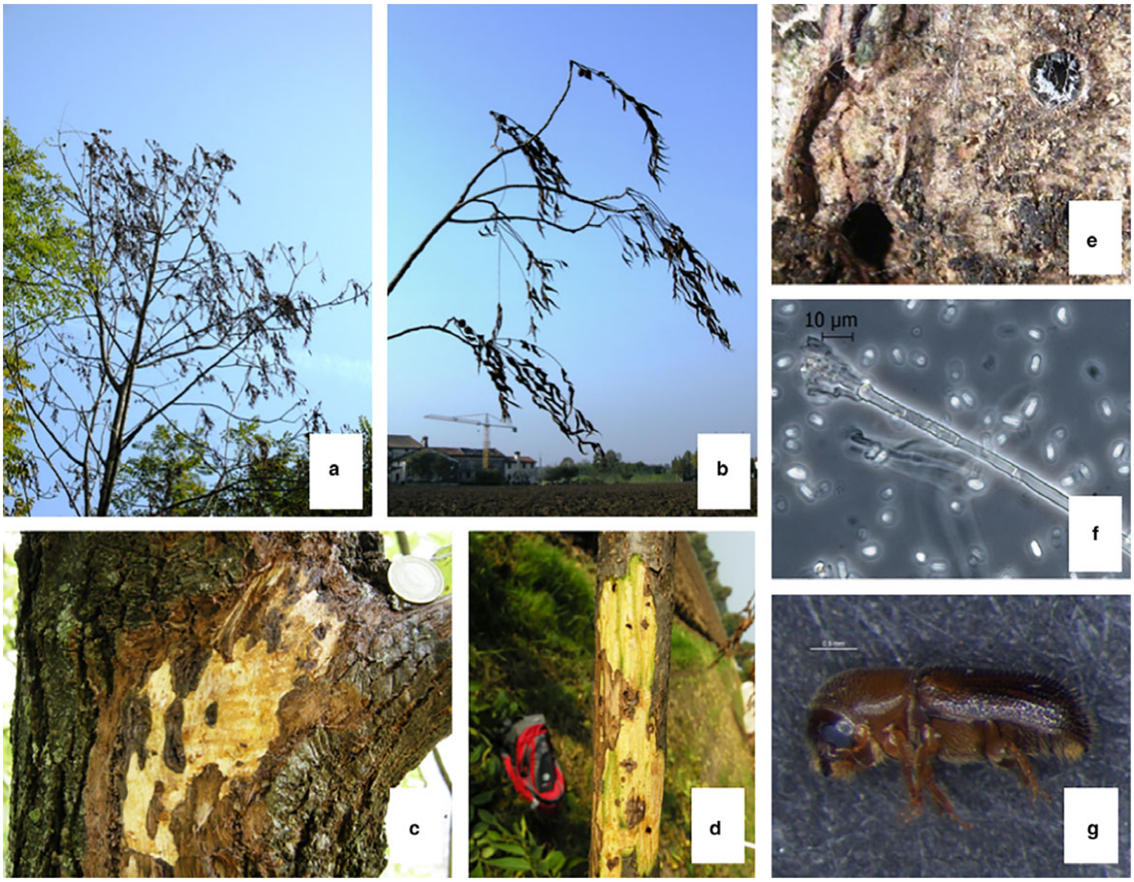

Fig. 1 Main symptoms of thousand cankers disease: (a) canopy dieback; (b) flag-like wilting; (c), (d) subcortical cankers along trunk and branches; (e) holes of walnut twig beetles with emerging mycelium; (f) *Geosmithia morbida*; (g) *Pityophthorus juglandis*.

The pathogen

Geosmithia morbida was described in 2011 as the first plant pathogen among *Geosmithia* species (Kolarik *et al.*, 2011).

Once carried into the bark tissues by its vector *P. juglandis*, the fungus grows within and around the entry holes, producing coalescing cankers (Fig. 1c,d), reducing the functionality of both cambium and phloem and leading to the death of distal parts of the tree.

For diagnostic confirmation the fungus should be cultured on potato dextrose agar (PDA) at 26–28°C for 5–7 days from woody samples collected from canker edges or from emerging *P. juglandis* (Tisserat *et al.*, 2011; Cranshaw & Tisserat, 2012). The fungus produces hyaline to whitish mycelium and verticillate conidiophores (Fig. 1f) usually in slow-growing, sublobate and planar colonies. Among isolates, different colony morphologies can be distinguished by different pigmentation (whitish to brownish), edge shape (smooth to uneven) and growth speed (Montecchio *et al.*, 2015b). The particular morphological features of both mycelium and colonies are detailed in Kolarik *et al.* (2011). In case of uncertainty the identity of the fungus can be confirmed by sequencing the internal transcribed spacer (ITS) region of the rDNA using the primers ITS1 or ITS5 and ITS4 (Cranshaw & Tisserat, 2012; Zerillo *et al.*, 2014).

Pathogenic strains of *Fusarium solani* may also be isolated from cankers very similar to those caused by thousand cankers disease, but their morphological features clearly differ from those of *G. morbida* (Montecchio *et al.*, 2015a).

The hosts

Susceptible hosts belong to the genera *Juglans* and *Pterocarya* (*J. ailantifolia*, *J. californica*, *J. cinerea*, *J. hindsii*, *J. major*, *J. mandshurica*, *J. microcarpa*, *J. mollis*, *J. nigra*, *J. regia*, *J. hindsii* × *J. regia*, *J. nigra* × *J. hindsii*, *J. cinerea* × *J. ailantifolia*, *J. nigra* × *J. regia*, *P. fraxinifolia*, *P. rhoifolia* and *P. stenoptera*; Seybold *et al.*, 2012a; Serdani *et al.*, 2013; Utley *et al.*, 2013). Furthermore, susceptibility and intensity of symptoms varies among species (with *J. nigra* being the most susceptible) and individuals of the same species (Tisserat *et al.*, 2011; Freeland *et al.*, 2012; Utley *et al.*, 2013).

Symptoms

Non-specific symptoms can appear several years after infestation, manifesting in a gradual yellowing, wilting and flag-like wilting of leaves and dieback of twigs and branches (Fig. 1a,b); in the most susceptible genotypes, death of the tree occurs 3–5 years after the first symptoms appear (Tisserat *et al.*, 2009).

Macroscopic symptoms of thousand cankers disease are detectable at an early stage on small-diameter (approximately 15 mm; Seybold *et al.*, 2012b) twigs. These consist of feeding wounds or entrance and exit holes produced by infected walnut twig beetles that release mycelial propagules, allowing fungal colonization of the neighbouring subcortical tissues. This gives rise to small subcortical dark brown to black cankers, from a few millimetres up to 10–20 cm in size, elongated lengthwise along the stem (Fig. 1c,d). There are usually multiple infestations of the same branch, due to both the feeding behaviour and reproduction of *P. juglandis*, resulting in multiple, coalescing cankers girdling the branch, and eventually in the macroscopic symptoms reported above, spreading downward and often causing the death of the tree. A lower percentage of non-merging cankers, equidistant from neighbouring ones and frequently exhibiting darker edges (due to incompatibility reactions between isolates, which are probably associated with the presence of a mycovirus) can be observed (Montecchio *et al.*, 2015b).

Further pictures of *P. juglandis* and *G. morbida*, as well as of the symptoms of infestation, can be found in Cranshaw & Tisserat (2008), Seybold *et al.* (2012a) and Utley *et al.* (2013).

Epidemiology

The fungus is regularly vectored by *P. juglandis*. *Geosmithia morbida* is a wound pathogen and the vector transports fungal propagules and inoculates them into the bark tissues.

Human-assisted movement of infested bark, wood with bark (i.e. round wood, processing residues, firewood) and plants for planting can easily contribute to the spread of thousand cankers disease over long distances and across geographical barriers. This could explain the presence of a US strain (Montecchio & Faccoli, 2014) in the first European outbreak located in North Italy a few kilometres from a sawmill importing walnut logs from North America.

Preliminary investigations through spore trapping and laboratory trials demonstrated that *G. morbida* can produce (Fig. 1e) and release conidia from walnut twig beetle exit holes, which could spread at least over short distances (Montecchio L. & Fanchin G., unpublished data). Air-dispersed conidia from infected walnuts to recently pruned fruit orchards growing close by cannot be excluded as a spread pathway.

Survey guidelines

Detection of thousand cankers disease is very difficult in the early stages of the infestation, and is generally possible only if external symptoms are visible. Unfortunately, this happens several years after infestation. Surveys need to be performed during the vegetative season, as the upper part of the canopy shows symptoms that are important for fungal detection. Yellowing and flagging of leaves or abnormal thinning and dieback of the canopy are not specific to this disease but are important features to look for. Collection of symptomatic twigs or branches with *P. juglandis* holes or subcortical galleries and cankers, noticeable by gently peeling back the bark, is an essential procedure. Then, samples of approximately 2 cm diameter and 10–15 cm long with visible symptoms collected from living branches may be easily taken, transferred into moist chambers, incubated at room temperature for approximately 7 days and observed under the microscope. Quite frequently, conidiophores of *G. morbida* will appear inside or around the walnut twig beetle holes. Exit holes and galleries from dead twigs and branches may be confused with those of other organisms, and microscopic or molecular identification is required.

Black multi-funnel traps (Lindgren) baited with the male-produced aggregation pheromone composed of 3-methyl-2-buten-1-ol (commercially available) are used to detect the occurrence of *P. juglandis* (survey) or follow an already known population (monitoring) (Seybold *et al.*, 2012a; Faccoli *et al.*, 2016). *Pityophthorus juglandis* has also been trapped by yellow sticky panels on walnut trees and by sticky clear panels stapled to walnut trunks, but captures were very low and incidental. Attempts to increase captures using walnut wood, pityol and other compounds useful in trapping some other walnut bark beetles did not increase capture of *P. juglandis*. Other types of traps may be used as well (sticky-coated and other barrier-type traps), but they are neither easy to use nor convenient (Seybold *et al.*, 2012a). Evidence of a female-produced pheromone has also been reported (Cranshaw & Tisserat, 2012), but this pheromone is not effective for trapping. Appropriate locations for trapping are sites with dieback walnut plantations, sites containing wood waste, firewood lots, saw mills, plantations close to international ports and airports or previously infested trees. Traps can be used in both private and public sites, including residential areas, private gardens, public parks and roads in urban areas, walnut plantations, orchards, riparian areas, etc. (USDA, 2016). Traps should be located about 5–6 m from the main stem and the branches, and about 2 m from the ground to avoid *P. juglandis* infesting healthy trees (Seybold *et al.*, 2012a). Traps may more easily detect beetles than a physical survey of branches: when beetle populations are low, few branches are affected and signs are not easily recognizable (Cranshaw & Tisserat, 2012).

In Europe, pheromone-baited traps can be deployed whenever adult *P. juglandis* are active (from May to

October, when the mean air temperature exceeds 18°C; Faccoli *et al.*, 2016). Traps are checked every 7–14 days, and lures replaced approximately every 2–3 months, depending on the temperature at the site (USDA, 2016). Detection of *P. juglandis* in a new region across a large area (survey) needs a much lower density of traps (Faccoli *et al.*, 2016) than the density needed to assess the extension of an already known population (USDA, 2016).

A process for visual survey and data collection is detailed in USDA (2016).

Control

No official protocols are available at present for treating (prophylactically or curatively) thousand cankers disease in trees.

Tests (still in progress) performed both *in vitro* and on naturally infected black walnut trees identified an injectable, non-registered mix of pesticides that was able to kill more than 90% of the insects and the fungus for at least 12 months (Montecchio *et al.*, unpublished).

Heat treatment of infected logs at 48°C (measured 1 cm below the cambium) for 40 min was demonstrated to be effective against *G. morbida* (Mayfield *et al.*, 2014).

Actions taken and infestation status in Italy

In 2014 the Veneto Region issued an official 'Decree of compulsory control' (Regione del Veneto, 2014) indicating the infested areas (periodically updated according to new reports) and a list of phytosanitary measures to be implemented, as follows:

- Prohibition of movement outside the infested areas of plants for planting of *Juglans* and *Pterocarya* with a diameter above 10 mm and wood products of the same genera (including felling and pruning residues and bark), except (a) wood squared to remove entirely the bark, the phloem layer and the first xylem rings, and (b) wood treated to reach at least 60°C at the first xylem rings for at least 45 min (higher than in Mayfield *et al.*, 2014, as a precautionary measure).
- A survey by the Phytosanitary Service of the Veneto Region of nurseries producing plants for planting of *Juglans* and *Pterocarya* in the demarcated zones, and obligation of nurseries to keep a register of plant movements.

Results

Since 2013, the Phytosanitary Service of the Veneto Region has been performing a detailed field survey. In 2015, a survey was also carried out in 50 sites scattered within the regional territory outside the demarcated areas (Fig. 2a). From each tree showing possible symptoms, a woody sample was collected and submitted to laboratory analysis at the University of Padova, but no samples tested positive for *P. juglandis* or *G. morbida*.

Results of the 2015 survey indicated that there is no evidence that thousand cankers disease has spread in the Veneto Region beyond the boundaries of the current demarcated areas, although dispersal of *P. juglandis* was detected in 4 new sites (Fig. 2a).

According to official reports, *G. morbida* and/or *P. juglandis* were recently detected in Italy in the following neighbouring regions (Fig. 2b):

- Lombardy, 2014. *Pityophthorus juglandis* trapped in the Province of Mantova. *Geosmithia morbida* not detected, symptoms of thousand cankers disease lacking.
- Friuli-Venezia Giulia, 2015. Two *P. juglandis* adults trapped in the Province of Pordenone. *Geosmithia morbida* not detected, symptoms of thousand cankers disease lacking.
- Piedmont, 2015. Both *P. juglandis* and *G. morbida* detected in two sites close to the Province of Torino. Visible symptoms of thousand cankers disease.

Conclusion

Thousand cankers disease can potentially spread in the EPPO region in areas where walnut trees occur and, due to the thermophilic character of the fungus, this disease is expected to move mainly southward from the current outbreaks.

Eradication of thousand cankers disease and *P. juglandis* from Italy is very difficult because of the large area where the pathogen, its vector and the host trees are present.

Containment of spread seems to be more realistic, but this requires stringent measures, including early detection based on specific and intensive surveys of both *G. morbida* (sampling and isolation from *P. juglandis*, bark beetle holes, cankers, logs consignments) and *P. juglandis* (pheromone traps).

Detailed inspections at points of entry into the European Union, and regulation of at least the most relevant commodities (i.e. round wood, firewood, bark, plants for planting) would help prevent further introductions and dispersal. Unfortunately, there are still many gaps in our knowledge about the ecology and biology of both insect and disease; further research, particularly on European walnut species and hybrids, and integrated pest management practices are of crucial importance for developing effective management strategies in the EPPO region.

Maladie des mille chancres en Europe : état des lieux

Le noyer (*Juglans regia* L.) est présent traditionnellement dans la plupart des pays européens comme arbre ornemental. Il est plus particulièrement cultivé dans la partie sud de la zone pour ses fruits et son bois. En outre, afin de répondre à la demande croissante en bois de noyer pour la production de meubles, des surfaces importantes du sud et du centre de l'Europe, allant de la France à la Hongrie, ont fait l'objet depuis les années 1980 de plantations de noyers noirs (*Juglans nigra* L.). Le champignon *Geosmithia morbida* et son vecteur *Pityophthorus juglandis,* responsables de la

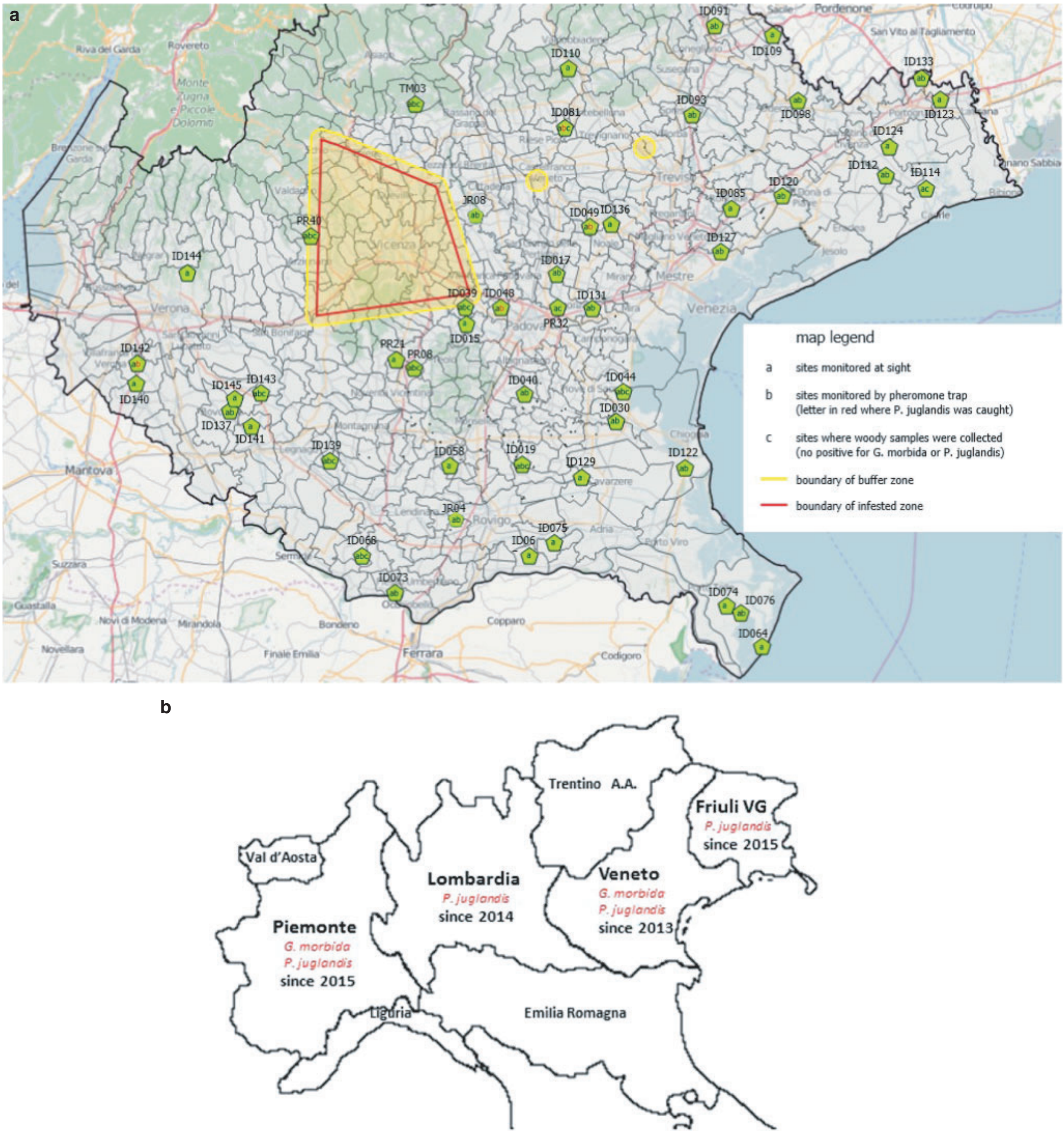

Fig. 2 Status of thousand cankers disease in Italy: (a) demarcated areas in the Veneto Region according to a 2015 survey (infested area = 70 138 ha; buffer zone (2 km width) = 25 611 ha); (b) presence of *Pityophthorus juglandis* and *Geosmithia morbida* in Italy.

maladie des mille chancres aux Etats-Unis lors des 20 dernières années, ont été observés en Europe (Italie) sur ces deux espèces de noyers. La maladie des mille chancres pourrait avoir un impact important sur le paysage et l'économie de nombreuses zones agricoles et forestières. A la suite d'une analyse de risque phytosanitaire (ARP) réalisée par l'OEPP en 2015, ces deux organismes ont été ajoutés à la Liste A2 de l'OEPP, liste des organismes nuisibles recommandés pour réglementation en tant qu'organismes de quarantaine. Les principales propriétés biologiques, épidémiologiques, ainsi que des indications pour la surveillance de la maladie des mille chancres sont présentées dans cet article. Il est fait un état des lieux de son statut en Europe.

Заболевание «тысячи язв» в Европе. Общий обзор

Грецкий орех (*Juglans regia* L.) традиционно присутствует в большинстве европейских стран как

декоративное дерево, и, главным образом, в южной части региона, выращивается для производства орехов и древесины. Кроме того, с тем чтобы ответить на растущий спрос на ореховую древесину для обеспечения древесиной производства мебели, с 1980-ых годов крупные зоны южной и центральной Европы, от Франции до Венгрии, были засажены черным орехом (*Juglans nigra* L.). Гриб *Geosmithia morbida* и его переносчик *Pityophthorus juglandis,* вызывающие заболевание «тысячи язв» ореха в США в течение двух последних десятилетий, недавно были зарегистрированы в Европе (в Италии) на обоих видах ореха. Заболевание «тысячи язв» оказывает сильное отрицательное воздействие на пейзаж, а также на экономику многих сельскохозяйственных и лесных районов. После проведения ЕОКЗР в 2015 г. детального АФР оба вредных организма были включены в список А2, вредных организмов, рекомендуемых ЕОКЗР для регулирования в качестве карантинных. В статье рассматриваются основные биологические, эпидемиологические и мониторинговые аспекты заболевания «тысячи язв», а также его статус в Европе.

References

Blackman MW (1928) The genus *Pityophthorus* Eichh. In North America: a revisional study of the Pityophthori, with descriptions of two new genera and seventy-one new species. *Bulletin of the New York State College of Forestry at Syracuse University* **25**, 5–183.

Cranshaw W & Tisserat N (2008) Pest alert Walnut twig beetle and Thousand Cankers Disease of Black walnut. http://www.ext.colostate.edu/pubs/insect/0812alert.pdf [accessed on 4 April 2016].

Cranshaw W & Tisserat N (2012) Questions and answers about Thousand Cankers Disease of walnut. Department of Bioagricultural Sciences and Pest Management, Colorado State University. http://bspm.agsci.-colostate.edu/files/2013/03/Questions-and-Answers-Revision-April-2012.pdf [accessed on 4 April 2016].

EPPO (2015). Pest Risk Analysis for Thousan cankers disease (*Geosmithia morbida* and *Pityophthorus juglandis*). www.eppo.int/quarantine/pest_risk_analysis/PRA_intro.htm [accessed on 4 April 2016].

Faccoli M (2015) *European Bark and Ambrosia Beetles: Types, Characteristics and Identification of Mating Systems*. WBA Handbooks 5, Verona (IT).

Faccoli M, Simonato M & Rassati D (2016) Life history and geographical distribution of the walnut twig beetle, *Pityophthorus juglandis* (Coleoptera: Scolytinae), in southern Europe. *Journal of Applied Entomology*. doi: 10.1111/jen.12299.

Freeland E, Cranshaw W & Tisserat N (2012) Effect of *Geosmithia morbida* isolate and temperature on canker development in black walnut. *Plant Health Progress*. doi:10.1094/PHP-2012-0618-01-RS.

Kolarik M, Freeland E, Utley C & Tisserat N (2011) *Geosmithia morbida* sp. Nov., a new pathogenetic species living in symbiosis with the walnut twig beetle (*Pityophthorus juglandis*) on Juglans in USA. *Mycologia* **103**, 325–332.

Luna EK, Sitz RA, Cranshaw WS & Tisserat NA (2013) The effect of temperature on survival of *Pityophthorus juglandis* (Coleoptera: Curculionidae). *Environmental Entomology* **42**, 1085–1091.

Mayfield AE, Fraedrich SW, Taylor A, Merten P & Myers SW (2014) Efficacy of heat treatment for the Thousand Cankers Disease vector and pathogen in small Black walnut logs. *Journal of Economic Entomology* **107**, 174–184.

Montecchio L, Faccoli M, Short D, Fanchin G, Geiser D & Kasson MT (2015a) First Report of *Fusarium solani* phylogenetic species 25 associated with early stages of Thousand Cankers Disease on *Juglans nigra* and *Juglans regia* in Italy. *Plant Disease* **99**, 1183.

Montecchio L, Fanchin G, Berton V & Scattolin L (2015b) Vegetative incompatibility and potential involvement of a mycovirus in the Italian population of *Geosmithia morbida*. *Phytopathologia Mediterranea* **54**, 465–476.

Montecchio L & Faccoli M (2014) First record of thousand cankers disease *Geosmithia morbida* and walnut twig beetle *Pityophthorus juglandis* on *Juglans nigra* in Europe. *Plant Disease* **98**, 696.

Montecchio L, Fanchin G, Simonato M & Faccoli M (2014) First record of thousand cankers disease fungal pathogen *Geosmithia morbida* and walnut twig beetle *Pityophthorus juglandis* on *Juglans regia* in Europe. *Plant Disease* **98**, 1445.

Peachey E (2012) Studies on the Walnut Twig Beetle (WTB), *Pityophthorus juglandis*, in relation to its association with *Geosmithia morbida*, its survival in felled logs, and its sensitivity to temperature extremes. *Thesis for the Degree of Master of Science. Department of Bioagricultural Sciences and Pest Management*. Colorado State University, 75 pp.

Regione del Veneto (2014) *Bollettino ufficiale*. XLV, 83, 26 agosto 2014.

Serdani M, Vlach JJ, Wallis KL, Zerillo M, McCleary T & Tisserat NA (2013) First report of *Geosmithia morbida* and *Pityophthorus juglandis* causing thousand cankers disease in butternut. *Plant Health Progress*. doi:10.1094/PHP-2013-1018-01-BR.

Seybold SJ, Colema TW, Dallar PL, Dar NL, Graves AD, Pederso LA *et al.* (2012a) Recent collecting reveals new state records and geographic extremes in the distribution of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae) in the United States. *Pan-Pacific Entomologist* **88**, 277–280.

Seybold SJ, Dallara PL, Hishinuma SM & Flint ML (2012b) Detecting and identifying the walnut twig beetle: Monitoring guidelines for the invasive vector of Thousand cankers disease of walnut. UC IPM Program, University of California Agriculture and Natural Resources. www.ipm.ucdavis.edu/thousandcankers [accessed on 4 April 2016].

Tisserat N, Cranshaw W, Leatherman D, Utley C & Alexander K (2009) Black walnut mortality in Colorado caused by the walnut twig beetle and thousand cankers disease. *Plant Health Progress*. doi:10.1094/PHP-2009-0811-01-RS.

Tisserat N, Cranshaw W, Putnam ML, Pscheidt J, Leslie CA, Murray M *et al.* (2011) Thousand Cankers Disease is widespread in black walnut in the western United States. *Plant Health Progress*. doi:10.1094/PHP-2011-0630-01-BR.

USDA (2016) Thousand Cankers Disease survey guidelines for 2016. United States Department of agriculture: Forest Service (FS) and Plant Ptotection and Quarantine (PPQ). https://www.aphis.usda.gov/plant_health/plant_pest_info/tcd/downloads/TCD_Survey_Guidelines.pdf [accessed on 4 April 2016].

Utley C, Nguyen T, Roubtsova T, Coggeshall M, Ford TM, Grauke LJ *et al.* (2013) Susceptibility of walnut and hickory species to *Geosmithia morbida*. *Plant Disease* **97**, 601–607.

Zerillo MM, Ibarra Caballero J, Woeste K, Graves AD, Hartel C, Pscheidt JW *et al.* (2014) Population Structure of *Geosmithia morbida*, the Causal Agent of Thousand Cankers Disease of Walnut Trees in the United States. *PLoS ONE* **9**, e112847.