SCIENTIFIC OPINION

Scientific Opinion on the pest categorisation of *Clavibacter michiganensis* subsp. *insidiosus* (McCulloch) Davis et al.[1]

EFSA Panel on Plant Health (PLH)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The European Commission requested EFSA's Panel on Plant Health to perform the pest categorisation for *Clavibacter michiganensis* subsp. *insidiosus*. The identity of the bacterium responsible for the bacterial wilt of lucerne is clearly defined. *C. michiganensis* subsp. *insidiosus* is present in only a few MSs in the EU and it is listed in the Annex IIAII of the Directive 2000/29/CE. Only sporadic disease outbreaks occur, and not in countries where lucerne production is of importance. The pathogen causes yield and quality loss only if susceptible cultivars are grown and conditions are favourable for disease expression. The pathogen is not reported in the main lucerne-producing MSs. There are no indications that in last decade the pathogen has a high impact on lucerne production in the EU, possibly because of the use of bacterial wilt-resistant varieties. *C. michiganensis* subsp. *insidiosus* is seed-borne and probably seed-transmitted, although with some uncertainty. The main pathway for long-distance dispersal of this pathogen is very likely via seeds, while machines and contaminated hay may also potentially play some role in the dissemination of the pathogen. The pathogen can be easily detected and identified on the basis of various microbiological and molecular tests and disease symptoms, including leaf mottling, reduction in plant height, and "witches' broom" syndrome. Effective management strategies are available and include the use of resistant cultivars and, probably, the use of pathogen-free seeds. Finally, the Panel concluded that severe consequences, in terms of yield and quality losses, are expected for lucerne only if bacterial wilt-susceptible varieties are grown and if weather conditions are conducive to the disease.

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KEY WORDS

Clavibacter michiganensis subsp. *insidiosus*, bacterial wilt of lucerne, pest categorisation, quarantine pest, regulated non-quarantine pest

[1] On request from the European Commission, Question No EFSA-Q-2014-00243, adopted on 27 November 2014.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Dir_2000_29_bacteria, Marie-Agnès Jacques, Charles Manceau, Emilio Stefani, Emilio Montesinos, Trond Rafoss and Jan Martin van der Wolf, for the preparatory work on this scientific opinion, and EFSA staff Svetla Kozelska for the support provided to this scientific opinion.

Suggested citation: EFSA PLH Panel (EFSA Panel on Plant Health), 2014. Scientific Opinion on the pest categorisation of *Clavibacter michiganensis* subsp. *insidiosus* (McCulloch) Davis et al. EFSA Journal 2014;12(12):3910, 30 pp. doi:10.2903/j.efsa.2014.3910

Available online: www.efsa.europa.eu/efsajournal

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The current European Union plant health regime is established by Council Directive 2000/29/EC[4] on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community (OJ L 169, 10.7.2000, p. 1).

The Directive lays down, amongst others, the technical phytosanitary provisions to be met by plants and plant products and the control checks to be carried out at the place of origin on plants and plant products destined for the Union or to be moved within the Union, the list of harmful organisms whose introduction into or spread within the Union is prohibited and the control measures to be carried out at the outer border of the Union on arrival of plants and plant products.

The Commission is currently carrying out a revision of the regulatory status of organisms listed in the Annexes of Directive 2000/29/EC. This revision targets mainly organisms which are already locally present in the EU territory and that in many cases are regulated in the EU since a long time. Therefore it is considered to be appropriate to evaluate whether these organisms still deserve to remain regulated under Council Directive 2000/29/EC, or whether, if appropriate, they should be regulated in the context of the marketing of plant propagation material, or be deregulated. The revision of the regulatory status of these organisms is also in line with the outcome of the recent evaluation of the EU Plant Health Regime, which called for a modernisation of the system through more focus on prevention and better risk targeting (prioritisation).

In order to carry out this evaluation, a recent pest risk analysis is needed which takes into account the latest scientific and technical knowledge on these organisms, including data on their agronomic and environmental impact, as well as their present distribution in the EU territory. In this context, EFSA has already been asked to prepare risk assessments for some organisms listed in Annex IIAII. The current request concerns 23 additional organisms listed in Annex II, Part A, Section II as well as five organisms listed in Annex I, Part A, Section I, one listed in Annex I, Part A, Section II and nine organisms listed in Annex II, Part A, Section I of Council Directive 2000/29/EC. The organisms in question are the following:

Organisms listed in Annex II, Part A, Section II:

- *Ditylenchus destructor* Thorne
- *Circulifer haematoceps*
- *Circulifer tenellus*
- *Helicoverpa armigera* (Hübner)
- *Radopholus similis* (Cobb) Thorne (could be addressed together with the IIAI organism *Radopholus citrophilus* Huettel Dickson and Kaplan)
- *Paysandisia archon* (Burmeister)
- *Clavibacter michiganensis* spp. *insidiosus* (McCulloch) Davis *et al.*
- *Erwinia amylovora* (Burr.) Winsl. *et al.* (also listed in Annex IIB)
- *Pseudomonas syringae* pv. *persicae* (Prunier *et al.*) Young *et al.*
- *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye
- *Xanthomonas campestris* pv. *pruni* (Smith) Dye
- *Xylophilus ampelinus* (Panagopoulos) Willems *et al.*
- *Ceratocystis fimbriata* f. sp. *platani* Walter (also listed in Annex IIB)
- *Cryphonectria parasitica* (Murrill) Barr (also listed in Annex IIB)
- *Phoma tracheiphila* (Petri) Kanchaveli and Gikashvili
- *Verticillium albo-atrum* Reinke and Berthold
- *Verticillium dahliae* Klebahn

[4] Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. OJ L 169/1, 10.7.2000, p. 1–112.

- Beet leaf curl virus
- Citrus tristeza virus (European isolates) (also listed in Annex IIB)
- Grapevine flavescence dorée MLO (also listed in Annex IIB)
- Potato stolbur mycoplasma
- *Spiroplasma citri* Saglio *et al.*
- Tomato yellow leaf curl virus

Organisms listed in Annex I, Part A, Section I:

- *Rhagoletis cingulata* (Loew)
- *Rhagoletis ribicola* Doane
- Strawberry vein banding virus
- Strawberry latent C virus
- Elm phloem necrosis mycoplasm

Organisms listed in Annex I, Part A, Section II:

- *Spodoptera littoralis* (Boisd.)

Organisms listed in Annex II, Part A, Section I:

- *Aculops fuchsiae* Keifer
- *Aonidiella citrina* Coquillet
- Prunus necrotic ringspot virus
- Cherry leafroll virus
- *Radopholus citrophilus* Huettel Dickson and Kaplan (could be addressed together with IIAII organism *Radopholus similis* (Cobb) Thorne
- *Scirtothrips dorsalis* Hendel
- *Atropellis* spp.
- *Eotetranychus lewisi* McGregor
- *Diaporthe vaccinii* Shear.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested, pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002, to provide a pest risk assessment of *Ditylenchus destructor* Thorne, *Circulifer haematoceps, Circulifer tenellus, Helicoverpa armigera* (Hübner), *Radopholus similis* (Cobb) Thorne, *Paysandisia archon* (Burmeister), *Clavibacter michiganensis* spp. *insidiosus* (McCulloch) Davis *et al, Erwinia amylovora* (Burr.) Winsl. *et al, Pseudomonas syringae pv. persicae* (Prunier *et al)* Young *et al. Xanthomonas campestris* pv. *phaseoli* (Smith) Dye, *Xanthomonas campestris* pv. *pruni* (Smith) Dye, *Xyîophilus ampelinus* (Panagopoulos) Willems *et al, Ceratocystis fimbriata* f. sp. *platani* Walter, *Cryphonectria parasitica* (Murrill) Barr, *Phoma tracheiphila* (Petri) Kanchaveli and Gikashvili, *Verticillium alboatrum* Reinke and Berthold, *Verticillium dahliae* Klebahn, Beet leaf curl virus, Citrus tristeza virus (European isolates), Grapevine flavescence dorée MLO, Potato stolbur mycoplasma, *Spiroplasma citri* Saglio *et al,* Tomato yellow leaf curl virus, *Rhagoletis cingulata* (Loew), *Rhagoletis ribicola* Doane, Strawberry vein banding virus, Strawberry latent C virus, Elm phloem necrosis mycoplasma, *Spodoptera littoralis* (Boisd.), *Aculops fuchsiae* Keifer, *Aonidiella citrina* Coquillet, Prunus necrotic ringspot virus, Cherry leafroll virus, *Radopholus citrophilus* Huettel Dickson and Kaplan (to address with the IIAII *Radopholus similis* (Cobb) Thorne), *Scirtothrips dorsalis* Hendel, *Atropellis* spp., *Eotetranychus lewisi* McGregor and *Diaporthe vaccinii* Shear., for the EU territory.

In line with the experience gained with the previous two batches of pest risk assessments of organisms listed in Annex II, Part A, Section II, requested to EFSA, and in order to further streamline the preparation of risk assessments for regulated pests, the work should be split in two stages, each with a specific output. EFSA is requested to prepare and deliver first a pest categorisation for each of these 38 regulated pests (step 1). Upon receipt and analysis of this output, the Commission will inform

EFSA for which organisms it is necessary to complete the pest risk assessment, to identify risk reduction options and to provide an assessment of the effectiveness of current EU phytosanitary requirements (step 2). *Clavibacter michiganensis* spp. *michiganensis* (Smith) Davis *et al.* and *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, from the second batch of risk assessment requests for Annex IIAII organisms requested to EFSA (ARES(2012)880155), could be used as pilot cases for this approach, given that the working group for the preparation of their pest risk assessments has been constituted and it is currently dealing with the step 1 "pest categorisation". This proposed modification of previous request would allow a rapid delivery by EFSA by May 2014 of the first two outputs for step 1 "pest categorisation", that could be used as pilot case for this request and obtain a prompt feedback on its fitness for purpose from the risk manager's point of view.

As indicated in previous requests of risk assessments for regulated pests, in order to target its level of detail to the needs of the risk manager, and thereby to rationalise the resources used for their preparation and to speed up their delivery, for the preparation of the pest categorisations EFSA is requested, in order to define the potential for establishment, spread and impact in the risk assessment area, to concentrate in particular on the analysis of the present distribution of the organism in comparison with the distribution of the main hosts and on the analysis of the observed impacts of the organism in the risk assessment area.

ASSESSMENT

1. Introduction

1.1. Purpose

This document presents a pest categorisation prepared by the EFSA Scientific Panel on Plant Health (hereinafter referred to as the Panel) for the species *Clavibacter michiganensis* subsp. *insidiosus* in response to a request from the European Commission.

1.2. Scope

The risk assessment area is the territory of the European Union (hereinafter referred to as the EU) with 28 Member States (hereinafter referred to as MSs), restricted to the area of application of Council Directive 2000/29/EC.

2. Methodology and data

2.1. Methodology

The Panel performed the pest categorisation for *C. michiganensis* subsp. *insidiosus* following guiding principles and steps presented in the EFSA Guidance on a harmonised framework for pest risk assessment (EFSA PLH Panel, 2010) and as defined in the International Standards for Phytosanitary Measures (ISPM) No 11 (FAO, 2013) and ISPM No 21 (FAO, 2004).

In accordance with the harmonised framework for pest risk assessment in the EU (EFSA PLH Panel, 2010), this work was initiated as result of the review or revision of phytosanitary policies and priorities. As explained in the background of the European Commission request, the objective of this mandate is to provide updated scientific advice to European risk managers to take into consideration when evaluating whether those organisms listed in the Annexes of Council Directive 2000/29/EC deserve to remain regulated under Council Directive 2000/29/EC, or whether they should be regulated in the context of the marketing of plant propagation material, or should be deregulated. Therefore, to facilitate the decision-making process, in the conclusions of the pest categorisation, the Panel addresses explicitly each criterion for a quarantine pest in accordance with ISPM 11 (FAO, 2013) but also for a regulated non-quarantine pest (RNQP) in accordance with ISPM 21 (FAO, 2004) and includes additional information required as per the specific terms of reference received by the European Commission. In addition, for each conclusion, the Panel provides a short description of its associated uncertainty.

Table 1 below presents the ISPM 11 (FAO, 2013) and ISPM 21 (FAO, 2004) pest categorisation criteria on which the Panel bases its conclusions. It should be noted that the Panel's conclusions are formulated respecting its remit and particularly with regards to the principle of separation between risk assessment and risk management (EFSA founding regulation[5]); therefore, instead of determining whether the pest is likely to have an unacceptable impact, the Panel will present a summary of the observed pest impacts. Economic impacts are expressed in terms of yield and quality losses and not in monetary terms, in agreement with EFSA guidance on a harmonised framework for pest risk assessment (EFSA PLH Panel, 2010).

[5] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31/1, 1.2.2002, p. 1–24.

Table 1: International Standards for Phytosanitary Measures ISPM 11 (FAO, 2013) and ISPM 21 (FAO, 2004) pest categorisation criteria under evaluation

Pest categorisation criteria	ISPM 11 for being a potential quarantine pest	ISPM 21 for being a potential regulated non-quarantine pest
Identity of the pest	The identity of the pest should be clearly defined to ensure that the assessment is being performed on a distinct organism, and that biological and other information used in the assessment is relevant to the organism in question. If this is not possible because the causal agent of particular symptoms has not yet been fully identified, then it should have been shown to produce consistent symptoms and to be transmissible	The identity of the pest is clearly defined
Presence (ISPM 11) or absence (ISPM 21) in the PRA area	The pest should be **absent from all or a defined part of the PRA area**	The pest is **present** in the PRA area
Regulatory status	If the pest is present but not widely distributed in the PRA area, it should be under official control or expected to be under official control in the near future	The pest is under official control (or being considered for official control) in the PRA area with respect to the specified plants for planting
Potential for establishment and spread in the PRA area	The PRA area should have ecological/climatic conditions including those in protected conditions suitable for the establishment and spread of the pest and, where relevant, host species (or near relatives), alternate hosts and vectors should be present in the PRA area	–
Association of the pest with the plants for planting and the effect on their intended use	–	Plants for planting are a pathway for introduction and spread of this pest
Potential for consequences (including environmental consequences) in the PRA area	There should be clear indications that the pest is likely to have an unacceptable economic impact (including environmental impact) in the PRA area	–
Indication of impact(s) of the pest on the intended use of the plants for planting	–	The pest may cause severe economic impact on the intended use of the plants for planting
Conclusion	If it has been determined that the pest has the potential to be a quarantine pest, the PRA process should continue. If a pest does not fulfil all of the criteria for a quarantine pest, the PRA process for that pest may stop. In the absence of sufficient information, the uncertainties should be identified and the PRA process should continue	If a pest does not fulfil all the criteria for a regulated non-quarantine pest, the PRA process may stop

In addition, in order to reply to the specific questions listed in the terms of reference, three issues are specifically discussed only for pests already present in the EU: the analysis of the present EU distribution of the organism in comparison with the EU distribution of the main hosts; the analysis of the observed impacts of the organism in the EU; and the pest control and cultural measures currently implemented in the EU.

The Panel will not indicate in its conclusions of the pest categorisation whether to continue the risk assessment process as it is clearly stated in the terms of reference that at the end the pest categorisation the European Commission will indicate if further risk assessment work is required following their analysis of the Panel's scientific opinion.

2.2. Data

2.2.1. Literature search

A literature search on *C. michiganensis* subsp. *insidiosus* was conducted at the beginning of the mandate. The search was conducted for the scientific name of the pest together with the most frequently used common names on the ISI Web of Knowledge database. Further references and information were obtained from experts, from citations within the references as well as from grey literature. The results of the EFSA procurement "Preparatory work for pest categorization of 3 bacteria listed in the annexes of the Council directive 2000/29/EC, contract number: NP/EFSA/ALPHA/2014/06-CT01" were also used.

2.2.2. Data collection

To complement the information concerning the current situation of the pest provided by the literature and online databases on pest distribution, damage and management, the PLH Panel sent a short questionnaire on the current situation at country level, based on the information available in the European and Mediterranean Plant Protection Organization (EPPO) Plant Quarantine Retrieval (PQR) system, to the National Plant Protection Organisation (NPPO) contacts of the 28 EU MSs, and of Iceland and Norway. Iceland and Norway are part of the European Free Trade Association (EFTA) and are contributing to EFSA data collection activities, as part of the agreements EFSA has with these two countries. A summary of the pest status based on EPPO PQR and NPPO replies is presented in Table 2.

Information on the distribution of the main host plants was obtained from Hucorne (2012), who retrieved data from the EUROSTAT database.

3. Pest categorisation

3.1. Identity and biology of *Clavibacter michiganensis* subsp. *insidiosus*

3.1.1. Taxonomy

Clavibacter michiganensis subsp. *insidiosus*, originally described under the name *Aplanobacter insidiosus* by McCulloch in 1925, is the causal agent of bacterial wilt of lucerne, also known as alfalfa. After several modifications of its nomenclature (see Synonyms, this section), the revision of gram-positive plant pathogenic bacteria nomenclature, undertaken by Davis et al. (1984), leads to the definition of the genus *Clavibacter* comprising *C. michiganensis* and four other species. Following the reclassification of the four other species, *C. michiganensis* is currently the only species of the genus *Clavibacter* (Saddler and Kerr, 2012).

Based on phenotypic and biochemical features, genetic markers and hosts specificity (Saddler and Kerr, 2012), *C. michiganensis* is subdivided into six subspecies. All strains of *C. michiganensis* pathogenic on lucerne are grouped in the subspecies *insidiosus*.

The organism being assessed is therefore a clear, distinguished taxonomic entity and the Panel refers to it with the following valid scientific name:

Name:

Clavibacter michiganensis subsp. *insidiosus*

Synonyms:

Corynebacterium michiganense subsp. *insidiosum* (McCulloch) Carlson and Vidaver, 1982

Corynebacterium michiganense pv. *insidiosum* (McCulloch) Dye and Kemp, 1977

Burkholderiella insidiosa (McCulloch 1925) Savulescu, 1947, cited by Alipour, 2013

Mycobacterium insidiosum (McCulloch) Krasil'nikov, 1941

Corynebacterium insidiosum (McCulloch) Jensen, 1934

Erwinia insidiosa (McCulloch) Bergey, 1930, cited by Jensen, 1934

Phytomonas insidiosa (McCulloch 1925) Bergey, 1930, cited by Alipour, 2013

Bacterium insidiosum (McCulloch 1925) (Stapp 1928)

Aplanobacter insidiosus McCulloch, 1925

Taxonomic position:

Domain: Bacteria; Kingdom: Procaryotae; Phylum: Actinobacteria; Class: Actinobacteria; Order: Actinomycetales; Family: Microbacteriaceae; Genus: *Clavibacter*; Species: *michiganensis*; Subspecies: *insidiosus*.

The genus *Clavibacter* was designed to accommodate the plant pathogenic coryneform bacteria. The cell wall of *Clavibacter* sp. contains peptidoglycan with 2,4-diaminobutyric acid as dibasic amino acid (Davis et al., 1984). *Clavibacter* species are strictly aerobic gram-positive rods which do not produce endospores.

The common English names for the disease are "bacterial wilt, blight, root rot" (EPPO, 1997). In French it is called "jaunissement bactérien de la luzerne" or "flétrissement bactérien de la luzerne", in German "bakterielle Luzernewelke" and in Spanish "podredumbre de la alfalfa".

3.1.2. Biology

3.1.2.1. Inoculum sources

There is no doubt that the bacterium is seed-borne (Cormack and Moffatt, 1956; Nemeth et al., 1991), but there is no absolute evidence that the disease is seed-transmitted. Data on translocation of *C. michiganensis* subsp. *insidiosus* from contaminated seed to plantlets are missing. The only indirect and partial evidence of seed transmission is the patchy distribution of diseased plants in naturally contaminated fields (Close and Mulcock, 1972), which could reflect the presence of only a few contaminated seeds throughout the seed lot or the presence of contaminated plant debris on or in the soil. Contamination of seeds is reported as infrequent. Samac et al. (1998) reported that less than 1 % of the seed produced in greenhouse and field trials contained the bacterium when harvested from artificially infected plants. Erwin and Kahn (1987), however, reported a seed contamination rate of 5 % in California, USA. Cormack and Moffatt (1956) observed *C. michiganensis* subsp. *insidiosus* in the vascular system of the floral rachis and pedicels. Bacteria were not found in flowers but were found in the vascular tissue of seed pods. Nevertheless, bacteria were infrequent in seed and confined to the aleurone layer of the endosperm.

The bacterium can persist in dried plant material for 8 to 10 years (Cormack, 1961). There is no information available on the role of dried hay and dried plant debris as inoculum sources.

It is not known if other natural hosts of *C. michiganensis* subsp. *insidiosus* can serve as inoculum sources. *C. michiganensis* subsp. *insidiosus* has been reported to naturally infect *Lotus corniculatus* (bird's-foot trefoil), *Medicago falcata* (yellow-flowered alfalfa), *Melilotus albus* (sweet clover), *Onobrychis viciifolia* (sainfoin) and *Trifolium* sp. (Bradbury, 1986). The importance of those naturally contaminated plants in the ecology of *C. michiganensis* subsp. *insidiosus* and the epidemiology of the alfalfa wilt is unknown.

3.1.2.2. Infection

According to Koehler and Jones (1932), bacterial wilt of lucerne is primarily a disease of the perennial part of the lucerne plant, i.e. the taproot and the crown. Infection occurs through stem and root wounds. The bacterium is carried by water and benefits from mowing, freezing and thawing wounds (Koehler and Jones, 1932). Feeding wounds, such as those made by nematodes and insects, also favour bacterial penetration in plants. Nematode transmission by *Ditylenchus dipsaci* has been shown (Hawn, 1963, 1971). Hawn (1963) indicated that the bacterium is carried on, rather than within, the body of the nematode. It was shown that the incidence of infection is increased in the presence of this nematode (Hawn and Hanna, 1967) and by *Meloidogyne hapla* (Hunt et al., 1971). However, there is no evidence that *M. hapla* is a carrier of *C. michiganensis* subsp. *insidiosus*. Kudela et al. (1984) reported that imagoes of *Sitona lineatus* can spread the bacteria between stands of lucerne.

Once inside the vascular system of the stems, the pathogen is translocated rapidly; in five hours it can move 9 cm (Jones and McCulloch, 1926).

It should be noted that *C. michiganensis* subsp. *insidiosus* is adversely affected by the presence of another lucerne pathogen, i.e. *Fusarium oxysporum* f. sp. *medicaginis* (Frosheiser and Barnes, 1978). This fungus synthesises a substance which inhibits the growth of *C. michiganensis* subsp. *insidiosus* (Johnson et al., 1982).

3.1.2.3. Incubation period

While the bacterium can be re-isolated from inoculated plants 10 to 14 days after inoculation, it takes longer (21 days after inoculation) for symptoms to appear (Hale, 1972). In field-grown plants, symptoms of the disease are rarely observed in seedlings or young plants, but normally only on plants three years old or older (Jones and McCulloch, 1926; Koehler and Jones, 1932).

Disease incidence is greater at moderate air temperature (16 °C) than at higher temperatures (24 and 28 °C) but a few symptoms develop at lower temperatures (Jones and McCulloch, 1926). At elevated temperature (_> 30 °C), plants die within 30 to 45 days after infection (Koehler and Jones, 1932). The temperature of the soil has a great impact on plant infection. Bacteria pass through the vascular tissue of the root, albeit slowly, even at a soil temperature as low as 9 °C, and readily at higher temperatures (12 or 15 °C) (Jones and McCulloch, 1926).

The progress of the disease is greatest in soils with abundant moisture (Koehler and Jones, 1932).

3.1.2.4. Symptomatology

In fields, infected plants are yellow–green in colour, exhibit stunted growth and are scattered throughout stands or grouped in patches (Close and Mulcock, 1972). The initial symptoms are leaf mottling together with a slight cupping or curling upwards of the leaflet margins associated with a reduction in plant height. A "witches' broom" syndrome can be observed as a result of stem proliferation. In cases of severe infection, plants present thin spindly stems and small distorted leaflets. Bleaching can be limited to the leaf margin or gain the entire leaf. Usually, infected plants die. Wilting of plants is not often seen. However, infected plants are more wilted than healthy plants in cases of high moisture stress. Root infection appears in the form of yellow–brown discoloration of the vascular

vessels (Koehler and Jones, 1932; Close and Mulcock, 1972). Symptoms in root, such as partial yellow to brown discoloration of the woody cylinder, can be seen in the absence of symptoms on aerial parts of the plants (Cormack et al., 1957). Seed production is limited in plants infected with *C. michiganensis* subsp. *insidiosus* (Close and Mulcock, 1972).

The wilt appearance of the plants is due, in part, to the production by *C. michiganensis* subsp. *insidiosus* of an extracellular phytotoxic glycopeptide (Ries and Strobel, 1972). Three exopolysaccharide components participate in the reduction of the water movement through pit membranes and plugging of mesophyll capillaries (van Alfen et al., 1987). Xylem colonisation is an important part of the disease as resistance is associated with vascular morphology (Cho et al., 1973).

There is no information on the symptomatology of plant hosts other than lucerne.

3.1.2.5. Survival

Overwintering generally occurs in the roots and crowns of diseased plants (Koehler and Jones, 1932) and this is favoured by low soil moisture and temperature (Nelson and Neal, 1974). The bacteria may survive on contaminated dry plant material for up to 10 years but not if it is left in contact with the soil (Cormack, 1961). Indeed, the ability of *C. michiganensis* subsp. *insidiosus* to compete with soil microflora seems to be low (Carroll and Lukezic, 1971; Nelson and Neal, 1974). The bacterium survives in seeds for up to three years (Erwin, 1990, reported in EPPO, 1997).

3.1.2.6. Disease cycle

The key steps of the disease cycle of bacterial wilt of lucerne can be summarised as follows: from the main primary inoculum source, that is contaminated seed and potentially contaminated plant parts, bacteria infect susceptible lucerne cultivars at root or crown penetration sites. Host colonisation and the appearance of symptoms are favoured by high soil temperature and moisture. The bacteria survive in dry plant materials and in seeds.

3.1.3. Intraspecific diversity

Within *C. michiganensis* subsp. *insidiosus*, no infradivisions, such as pathovars or races, have been proposed, making this subspecies a relatively homogeneous pathogen. The diversity of this pathogen remains poorly described. Kao et al. (1985) briefly reported that, despite a high degree of homogeneity in biochemical properties, two types of pigmentation (orange and blue) were recorded and considerable variations in plasmid content and virulence were mentioned. It is unknown whether variation in virulence between strains really exists or is the result of phenotypic variations induced by lyophilisation and storage conditions of strains (Víchová and Kozová, 2004). In BOX-PCR (a BOX - based repetitive extragenic palindromic polymerase chain reaction), a genetic fingerprinting method, nine strains of *C. michiganensis* subsp. *insidiosus* from different origins displayed little variation (Smith et al., 2001). Using multilocus sequence analysis (MLSA), based on three housekeeping genes, five *C. michiganensis* subsp. *insidiosus* strains from Poland, isolated in different years, were identical, indicating that the genetic diversity is low (Waleron et al., 2011).

3.1.4. Detection and identification

Protocols for sampling and detection of *C. michiganensis* subsp. *insidiosus* in symptomatic and asymptomatic plant tissues, including seeds, are available (EPPO, 2010).

3.1.4.1. Isolation via dilution plating

No selective media have been developed for *C. michiganensis* subsp. *insidiosus*, but dilution plating of plant material can be done on general media (EPPO, 2010). On yeast peptone glucose agar (YPGA), many strains produce an indigo diffusible pigment. The probability of successful isolation is much higher for symptomatic than for asymptomatic plants.

3.1.4.2. Serological methods

Other detection methods include enzyme-linked immunofluorescence assay (ELISA) and immunofluorescence cell staining (Nemeth et al., 1991; Kokošková et al., 2000).

3.1.4.3. Molecular techniques

Detection

Molecular techniques such as PCR and real time-PCR techniques can be used for specific detection of the pathogen in (symptomatic) plant material. A PCR assay developed by Samac et al. (1998) is capable of detecting fewer than five *C. michiganensis* subsp. *insidiosus* cells in a reaction mixture. The high sensitivity of the assay reflects the presence of multiple (30) copies of the target IS*1122* sequence in the pathogen. Pastrik and Rainey (1999), Borowicz (2001) and Ward et al. (2008) also produced conventional PCR assays targeting the internal transcribed spacer (ITS) region between the 16S and 23S rRNA genes to identify *C. michiganensis* subsp. *insidiosus* strains. For detection purposes, the sensitivity of the PCR assay was further improved by increasing the number of targets and diminishing the concentration of PCR-inhibiting substances by incubation of the seed macerates in a growth medium (Ward et al., 2008).

Three quantitative PCR assays have been described. Two tests target the ITS region between the 16S and 23S rRNA genes (Bach et al., 2003; Marefat et al., 2007) and the other test was developed by Ward et al. (2008) based on the primers proposed by Samac et al. (1998). These three quantitative PCR assays detect *C. michiganensis* subsp. *insidiosus* using DNA extracted from plant tissues.

Identification

For identification, genomic fingerprinting methods are used, such as BOX-PCR and MLSA, which allow differentiation of *C. michiganensis* subsp. *insidiosus* from other *C. michiganensis* subspecies (Smith et al., 2001, Waleron et al., 2011; Jacques et al., 2012).

3.2. Current distribution of *Clavibacter michiganensis* subsp. *insidiosus*

3.2.1. Global distribution

The first description of the disease was from Illinois and Wisconsin, USA, in 1925 (Jones, 1925), and was later followed by reports from others states in the USA (Jones and McCulloch, 1926). In North America, the disease has also been reported in Canada (from British Columbia to Quebec), and there have been unconfirmed reports in Mexico (EPPO, 1997). In South America, there have been unconfirmed reports of *C. michiganensis* subsp. *insidiosus* in Brazil and Chile (EPPO, 1997). In Asia, the bacterium is present in Iran (Heidari and Khodakaramian, 2012; Alipour, 2013), Saudi Arabia and Turkmenistan (EPPO, 1997). In Africa, both South Africa and Tunisia have reported disease caused by *C. michiganensis* subsp. *insidiosus* (EPPO, 1997). In Oceania, the disease was reported as far back as 1965 in Australia (Smith and Taylor, 1967) and since 1970 in New Zealand (Close and Mulcock, 1972).

Ward et al. (2008) reported that bacterial wilt is no longer a concern for lucerne production in Canada as a result of the widespread use of wilt-resistant cultivars.

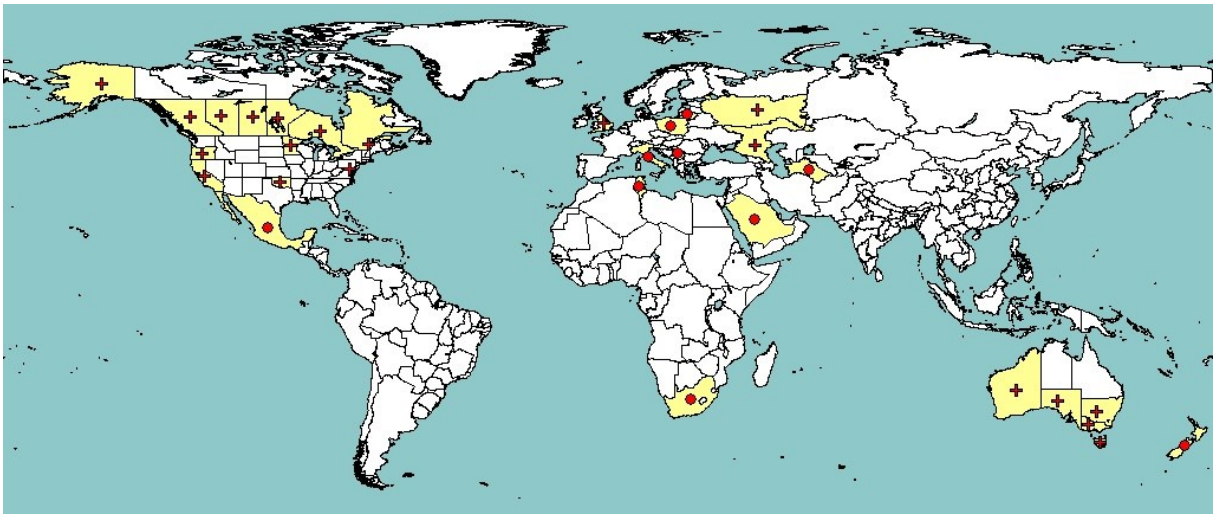

Figure 1: Global distribution of *Clavibacter michiganensis* subsp. *insidiosus* [extracted from EPPO PQR (2014, version 5.3.1), accessed on 4 July 2014]. Red circles represent pest presence as national records and red crosses pest presence as subnational records

3.2.2. Distribution in the EU

Currently, the pest is present in a few EU MSs, i.e. Estonia, Lithuania, Poland and the UK. Previous reports in other countries have not been confirmed. According to EPPO PQR, the pest was widely distributed in the 1990s in southern and central Russia. No more recent information is available.

Table 2: Current distribution of *Clavibacter michiganensis* subsp. *insidiosus* in the 28 EU MSs, Iceland and Norway, based on the answers received via email from the NPPOs or, in absence of reply, on information from EPPO PQR

Country	NPPO answer	NPPO Comments
Austria	**Absent,** no pest records	
Belgium	**Absent,** no pest records	
Bulgaria	**Absent**	
Croatia	**Absent,** no pest records	
Cyprus	–	
Czech Republic	**Absent,** pest no longer present	
Denmark	**Not known to occur**	
Estonia	**Present,** subject to official control	
Finland	**Absent,** no pest record	
France	–	
Germany	**Absent,** no pest records	
Greece [(a)]	—	
Hungary	**Absent,** pest eradicated	
Ireland	**Absent,** no pest record	
Italy	**Absent**	Reported once in the past, never officially confirmed. Not found in visual inspections in the last 30 years
Latvia [(a)]	–	
Lithuania [(a)]	**Present,** restricted distribution	

Country	NPPO answer	NPPO Comments
Luxembourg [(a)]	–	
Malta	**Absent,** no pest records	
Poland	**Present,** one occurrence in 2006	For 2009 to 2013, a total of 103 visual inspections were carried out by the SPHSIS on seed crops of *Medicago sativa*. In addition, 62 samples were laboratory tested. All samples were negative
Portugal	**No records**	
Romania [(a)]	**Absent,** pest no longer present	
Slovak Republic	**Absent,** no pest record	
Slovenia	**Absent,** no pest records	
Spain	**Absent**	
Sweden	**Absent,** not known to occur	
The Netherlands	**Absent,** no pest records	
United Kingdom	**Present,** in all parts of area	
Iceland [(a)]	—	
Norway [(a)]	—	

(a): When no information was made available to EFSA, the pest status in the EPPO PQR (2012) was used.
—, No information available.
EPPO PQR, European and Mediterranean Plant Protection Organization Plant Quarantine Data Retrieval System; NPPO, National Plant Protection Organisation; SPHSIS, Plant Health and Seed Inspection Service.

3.2.3. Vectors and their distribution in the EU

C. michiganensis subsp. *insidiosus* can be carried and disseminated by the nematode *Ditylenchus dipsaci* (Hawn, 1963, 1971). Hawn (1963) indicated that the bacterium is carried *on*, rather than *within*, the body of the nematode. Insects, such as imagoes of *Sitonia lineatus*, are also reported to be capable of spreading the bacterium (Kudela et al., 1984). However, these observations were not confirmed by others. Both *D. dipsaci* and *S. lineatus* are distributed throughout Europe.

3.3. Regulatory status

3.3.1. Council Directive 2000/29/EC

3.3.1.1. *Clavibacter michiganensis* subsp. *insidiosus*

C. michiganensis subsp. *insidiosus* is a regulated harmful organism in the EU and listed in Council Directive 2000/29/EC in Annex II, Part A, Section II, point 1 (see Table 3).

Table 3: *Clavibacter michiganensis* subsp. *insidiosus* in Annex II of Council Directive 2000/29/EC

Annex II, Part A	Harmful organisms whose introduction into, and spread within, all Member States shall be banned if they are present on certain plants or plant products	
Section II	Harmful organisms known to occur in the Community and relevant for the entire Community	
(b)	Bacteria	
	Species	Subject of contamination
1	*Clavibacter michiganensis s*pp. *insidiosus* (McCulloch) Davis et al.	Seeds of *Medicago sativa* L.

3.3.1.2. Regulated hosts of *Clavibacter michiganensis* subsp. *insidiosus*

C. michiganensis subsp. *insidiosus* has more potential hosts than those for which it is regulated in Annex IIAII (see section 3.4.1). Specific requirements of Annex IV and Annex V of Council Directive 2000/29/EC are presented in Table 4 only for the host plants and commodities regulated for *C. michiganensis* subsp. *insidiosus* in Annex IIAII.

Table 4: *Clavibacter michiganensis* subsp. *insidiosus* host plants in Council Directive 2000/29/EC

Annex IV, Part A	Special requirements which must be laid down by all Member States for the introduction and movement of plants, plant products and other objects into and within all Member States	
Section I	Plants, plant products and other objects originating outside the Community	
	Plants, plant products and other objects	Special requirements
49.1	Seeds of *Medicago sativa* L.	Official statement that: (a) no symptoms *Ditylenchus dipsaci* (Kühn) Filipjev have been observed at the place of production since the beginning of the last complete cycle of vegetation and no *Ditylenchus dipsaci* (Kühn) Filipjev has been revealed by laboratory tests on a representative sample; or (b) fumigation has taken place prior to export
49.2	Seeds of *Medicago sativa* L., originating in countries where *Clavibacter michiganensis* ssp. *insidiosus* Davis et al. is known to occur	Without prejudice to the requirements applicable to plants listed in Annex IV(A)(I)(49.1), official statement that: (a) *Clavibacter michiganensis* ssp. *insidiosus* Davis et al. has not been known to occur on the farm or in the immediate vicinity since the beginning of the past 10 years; (b) either—the crop belongs to a variety recognised as being highly resistant to *Clavibacter michiganensis* ssp. *insidiosus* Davis et al., or—it had not yet started its fourth complete cycle of vegetation from sowing when the seed was harvested and there was not more than one preceding seed harvest from the crop, or—the content of inert matter which has been determined in accordance with the rules applicable for the certification of seed marketed in the Community, does not exceed 0.1 % by weight; (c) no symptoms of *Clavibacter michiganensis* ssp. *insidiosus* Davis et al. have been observed at the place of production, or on any *Medicago sativa* L. crop adjacent to it, during the last complete cycle of vegetation or, where appropriate, the last two cycles of vegetation; (d) the crop has been grown on land on which no previous *Medicago sativa* L. crop has been present during the last three years prior to sowing
Section II	Plants, plant products and other objects originating in the Community	
	Plants, plant products and other objects	Special requirements
28.1	Seeds of *Medicago sativa* L.	Official statement that: (a) no symptoms of *Ditylenchus dipsaci* (Kühn) Filipjev have been observed at the place of production since the beginning of the last complete cycle of vegetation and that no *Ditylenchus dipsaci* (Kühn) Filipjev has been revealed by laboratory tests on a representative sample; or (b) that fumigation has taken place prior to marketing
28.2	Seeds of *Medicago sativa* L.	Without prejudice to the requirements applicable to the

	plants listed in Annex IV(A)(II)(28.1), official statement that: (a) the seeds originate in areas known to be free from *Clavibacter michiganensis* spp. *insidiosus* Davis et al.; or (b)—*Clavibacter michiganensis* ssp. *insidiosus* Davis et al. has not been known to occur on the farm or in the immediate vicinity since the beginning of the past 10 years, and—the crop belongs to a variety recognised as being highly resistant to *Clavibacter michiganensis* ssp. *insidiosus* Davis et al. or—it had not yet started its fourth complete cycle of vegetation from sowing when the seed was harvested, and there was not more than one preceding seed harvest from the crop, or—the content of inert matter which has been determined in accordance with the rules applicable for certification of seed was marketed in the Community, does not exceed 0.1 % by weight,—no symptoms of *Clavibacter michiganensis* ssp. *insidiosus* Davis *et al*. have been observed at the place of production or on any *Medicago sativa* L. crop adjacent to it, during the last complete cycle of vegetation or, where appropriate, the last two cycles of vegetation,—the crops has been grown on land on which no previous *Medicago sativa* L. crop has been present during the last three years prior to sowing
Annex V	Plants, plant products and other objects which must be subject to a plant health inspection (at the place of production if originating in the Community, before being moved within the Community—in the country of origin or the consignor country—if originating outside the Community) before being permitted to enter the Community
Part A	Plants, plant products and other objects originating in the Community
Section I	Plant, plant products and other objects which are potential carriers of harmful organisms of relevance for the entire Community and which must be accompanied by a plant passport
2	Plants, plant products and other objects produced by producers whose production and sale is authorised to persons professionally engaged in plant production, other than those plants, plant products and other objects which are prepared and ready for sale to the final consumer, and for which it is ensured by the responsible official bodies of the Member States, that the production thereof is clearly separate from that of other products
2.4	[...] — Seeds of *Medicago sativa* L., [...]
Part B	Plants, plant products and other objects originating outside the Community
Section I	Plants, plant products and other objects which are potential carriers of harmful organisms of relevance for the entire Community
1	Plants, intended for planting, other than seeds but including seeds of [...] *Medicago sativa* L.

3.3.1.3. *Clavibacter michiganensis* subsp. *insidiosus* carrier

D. dipsaci, acting as a carrier of *C. michiganensis* subsp. *insidiosus,* is currently listed in Council Directive 2000/29/EC in Annex II, Part A, Section II, point 4 (see Table 5).

Table 5: *Ditylenchus dipsaci* in Council Directive 2000/29/EC

Annex II, Part A	Harmful organisms whose introduction into, and spread within, all Member States shall be banned if they are present on certain plants or plant products	
Section II	Harmful organisms known to occur in the Community and relevant for the entire Community	
(a)	Insects, mites and nematodes, at all stages of their development	
	Species	Subject of contamination
4	*Ditylenchus dipsaci* (Kühn) Filipjev	Seeds [...] of *Medicago sativa* L.

3.3.2. Marketing directives

The host plants of *C. michiganensis* subsp. *insidiosus* that are regulated in Annex IIAII of Council Directive 2000/29/EC are explicitly mentioned in the following Marketing Directive:

- Council Directive 66/401/EEC[6] on the marketing of fodder plant seed

3.4. Elements to assess the potential for establishment and spread in the EU

3.4.1. Host range

Lucerne (*M. sativa*) is the main natural host of *C. michiganensis* subsp. *insidiosus*, but other species of the *Fabaceae* family are also considered natural hosts, including *Medicago* spp., such as the lawn plant black medick (*Medicago lupulina*), honey clover (*M. albus*), the fodder crops bird's-foot trefoil (*L. corniculatus*), common sainfoin (*O. viciifolia*) and *Trifolium* subsp. (Bradbury, 1986; EPPO, 1997). *C. michiganensis* subsp. *insidiosus* has been found to infect a number of *Medicago* spp. via artificial inoculation, including *M. dzawkhetica*, *M. glutinosa*, *M. hemicycla*, *M. marina*, *M. prostrata*, *M. sativa* var. *gaetula*, *M. sativa* var. *parviflora*, *M. sativa pilifera*, *M. sogdiana*, *M. suffruticosa*, *M. tianschanica* and *M. transoxana* (Bradbury, 1986). A record on *Zea mays* according to Bradbury (1986) should be regarded as doubtful. Bacterial wilt-like symptoms have been reported on lupin (*Lupinus albus*) in New Zealand, but the presence of *C. michiganensis* subsp. *insidiosus* as causative agent was not confirmed (Harvey et al., 1996).

3.4.2. EU distribution of main host plants

Lucerne, the main host plant, is widely grown in different regions of the EU, except for the northern MSs (Table 6). Production is, in particular, of economic importance in Italy, Romania, Spain, (northern) France and Hungary. The pathogen is not reported to be present in any of these countries (Table 2).

Table 6: Area of production in 1 000 ha for lucerne types of lucerne cultivated alone, of major economic importance, the principal of which is *Medicago sativa*, with *M. falcata*. (Source: Hucorne, 2012. Data on production areas have been retrieved from the EUROSTAT database. The mean of the years 2006–2010 have been calculated for each crop/country)

Country	Lucerne
Austria	13.0
Belgium	0.0
Bulgaria	70.7
Croatia	25.9
Cyprus	0.6

[6] Council Directive 66/401/EEC of 14 June 1966 on the marketing of fodder plant seed. OJ L 125, 11/07/1966, p. 2298–2308.

Country	Lucerne
Czech Republic	73.1
Denmark	4.3
Estonia	12.1
Finland	0.0
Northern France	217.5
Southern France	85.0
Germany	39.4
Greece	62.8
Hungary	137.2
Ireland	0.0
Italy	713.2
Latvia	0.0
Lithuania	5.0
Luxembourg	0.0
Malta	0.0
Netherlands	5.9
Poland	32.1
Portugal	0.0
Romania	322.6
Slovakia	52.4
Slovenia	1.4
Spain	241.8
Sweden	0.0
United Kingdom	0.0
EU-28	2 116.0

3.4.3. Analysis of the potential *Clavibacter michiganensis* subsp. *insidiosus* distribution in the EU

Only in a limited number of EU countries is the production of lucerne of economic importance. These countries include Italy, Romania, Spain, Hungary and (northern) France (Table 6). The pathogen was found in Italy and Romania, but findings were sporadic and there have been no reports since the 1980s. *M. albus* is present in most countries on the continent, although not in the UK or Ireland. Various *Medicago* species, including *M. aculeata*, *M. agrestis* and *M. arabica*, are widely distributed throughout the EU (Flora Europaea, online, data retrieved October 2014).

A temperate climate and soil with a high moisture content favour disease development, indicating that in most lucerne production areas the climate is suitable for the pathogen to develop.

3.4.4. Spread capacity

3.4.4.1. Spreading

Short-distance dissemination can occur through wind dispersion of soil and debris, via contaminated water and via animals (University of Illinois, 1988; EPPO, 1997). The pathogen is also readily spread by contaminated farm machinery. In particular, machines that causes wounds, such as mowers and tillage machines, are important in the dissemination of and infection with the pathogen (Jones and McCulloch, 1926). Frost cracks can also provide an entrance for the pathogen; the disease seems to be

more destructive after a severe winter (Koehler and Jones, 1932). Short-distance spread of *C. michiganensis* subsp. *insidiosus* in soil may also occur via nematode transmission by *D. dipsaci* (Hunt et al., 1971), and possibly by the beetle *S. lineatus* (Kudela et al., 1984).

Long-distance spread may occur via seed harvested from severely infected plants or possibly, although unlikely, by shipping of infected hay (EPPO, 1997). However, there is only indirect evidence for the spread with infected seed, as data on transmission from seed to seedling are missing. Therefore, the role of seed-borne inoculum in the spread and epidemiology cannot be determined adequately. Histological studies showed that the pathogen can be present on the surface of seeds as a contaminant or in the seed coat of mature seeds up to the aleurone layer of the endosperm (Cormack and Moffatt, 1956). The pathogen could be readily detected in seed disinfected with 2 % chlorine, indicating deep-seated infections. Furthermore, *C. michiganensis* subsp. *insidiosus* was detected in association with nematodes in seed lots or as a contaminant in seed debris (Cormack and Moffatt, 1956; Wood and Close, 1974; Erwin and Khan, 1987). It was estimated that, on average, 7 % of symptomatic plants transmitted *C. michiganensis* subsp. *insidiosus* to seed, whereas the incidence of infected seed in seed lots was between 2.5 and 8.7 % (Samac et al., 1998). The infection incidence was higher in plants exhibiting severe symptoms than in those with mild symptoms. Infected plants, in particular those yielding high densities of *C. michiganensis* subsp. *insidiosus* in the pedicels, tend to produce lower yields of seed (Cormack and Moffatt, 1956). The pathogen was more frequently isolated from coloured or shrivelled seeds than from normal seeds. From all infected plants grown in the greenhouse or in the field, an estimated 0.21 to 0.55 % of seed was infected. In other studies, the infection percentage was comparable; 2 % of the unripe, green seeds from artificially inoculated plants were infected compared with 5 % of the mature seeds (Erwin and Khan, 1987).

The risk of long-distance dispersal via contaminated hay is considered low. The pathogen may be present in hay, where it can survive for a long time, but hay is used as feed and the risk of infected hay being brought to fields used for cultivation of lucerne is considered as low.

3.4.4.2. Survival

The pathogen can overwinter in the roots and crowns of diseased plants (Jones and McCulloch, 1926). Normally, under field conditions the pathogen survives poorly free in soil, with a reported maximum survival time of 31 days (Nelson and Neal, 1974). Only in frozen soil and in dry soil (wilting point) was a long survival time, of up to 169 days, found (Nelson and Semeniuk, 1963). In excised lucerne roots buried in moist warm soil the pathogen could not be recovered after one month (Nelson and Neal, 1974). However, if excised roots were kept in dry soil or at a constant low temperature of –5 to 5 °C the pathogen could persist for 50 weeks (Nelson and Neal, 1974). In lucerne production areas soil temperatures will be higher and soil moisture conditions will fluctuate. It is therefore assumed that soil-borne inoculum may play a part in initiation of disease only if no proper crop rotation is applied.

The pathogen can survive in dried seed tissue for 10 years (Erwin, 1990). However, if infected plant material is pulverised and soaked in water, the bacteria will die after 12 hours (Cormack et al., 1957). This may be due to low persistence of the pathogen in water or interactions with other organisms developed in and released from the soaked plant material.

3.4.4.3. Conditions favouring disease development

In general, the disease develops slowly; the incubation time upon artificial inoculation is often between five weeks and three months (Close and Mulcock, 1972; Hale, 1972). The susceptibility of the seedling and rooted cuttings to the disease increases with age, with a maximum susceptibility at 7–10 weeks of age (Cormack et al., 1957). In New Zealand, outbreaks were more frequently found in irrigated stands (border dyke irrigation and sprinkler irrigation) than in dry-land stands (Hale and Close, 1974). This may be caused by dissemination via irrigation water, but the high humidity, due to irrigation, may also favour disease development.

Hunt et al. (1971) and Norton (1969) showed that the root-knot nematode, *M. hapla*, increased the incidence of bacterial wilt in lucerne to the same levels as cutting the root ball before inoculation of soil with *C. michiganensis* subsp. *insidiosus*. The presence of the nematode in the soil resulted in a 5 to 14 times higher bacterial wilt incidence in lucerne (Hunt et al. 1971). This effect was found independent of whether the bacteria or the nematode came first (Griffin and Hunt, 1972). The nematodes probably create infection courts for the bacteria in the roots, but they may also release nutrients for bacterial multiplication. Dissemination of *C. michiganensis* subsp. *insidiosus* with the root-knot nematode is less likely. In a four-year field experiment, no migration or transfer of the nematode from infected to non-infected plots, across 1 m aisles, was found (Norton, 1969). In addition, the presence of the stem and bulb nematode, *D. dipsaci*, increased the bacterial wilt incidence (Hawn, 1963). It was suggested that the nematode could transmit the bacteria, as numerous bacterial cells were observed on the nematode after artificial inoculation. This nematode was able to decrease bacterial wilt resistance in lucerne by causing physiological or biochemical change, but not in all cultivars, (Hawn and Hanna, 1967).

Disease incidence was significantly higher at soil temperatures above 20–28 °C than at 16 °C. In experiments conducted in the 1920s, in which five soil temperatures were tested, ranging between 10 and 30 °C, the highest disease incidence was found at 30 °C (Koehler and Jones, 1932). In the same studies, four soil moisture contents were tested, between 35 and 80 % of the water-holding capacity. The highest incidence of disease was found at the highest moisture content. The disease is rare in dry areas where annual rainfall is lower than 60 cm (Jones and McCulloch 1926). Disease development was found at 24 and 28 °C but not at 16 °C (Jones and McCulloch, 1926). However, temperatures as low as 9 °C allow multiplication of the pathogen and invasion of stems from infected roots (Jones and McCulloch 1926). In practice, the disease is more frequently observed under cool and moist weather conditions, in particular in poorly drained areas. The better the crop stands, the faster the disease progresses (Koehler and Jones, 1932). In an old thin crop, the disease progresses only slowly. Crown injuries can increase disease levels. Plants weakened by bacterial wilt are more susceptible to winterkill than are healthy plants (University of Illinois, 1988). The root system of the plants can be heavily infected without showing symptoms in the haulms, which risks the unnoticed spread of the pathogen (Jones and McCulloch, 1926).

3.5. Elements to assess the potential for consequences in the EU

3.5.1. Potential effects of *Clavibacter michiganensis* subsp. *insidiosus*

Reports from the USA indicate that bacterial wilt can have a major economic impact on lucerne production (Jones and McCulloch, 1926; Smith and Taylor, 1967; Kellock and Coster, 1968). In 1925, only 8 % of lucerne fields in Illinois in the USA were infected with *C. michiganensis* subsp. *insidiosus*, but by 1930 this had grown to 65 % (Koehler and Jones, 1932). In experiments with infected plants kept under different temperature and moisture conditions, a high percentage of plants were killed and the dry weights of remaining infected plants were reduced, on average, by 25–50 % (Koehler and Jones, 1932). If plants are diseased, they will not recover (Koehler and Jones, 1932). In several states, lucerne production became unprofitable after three to five years (Jones and McCulloch, 1926). In Canada, bacterial wilt became a major limiting factor in the production of irrigated lucerne in the 1950s (Peake and Cormack, 1955). In New Zealand, outbreaks of bacterial wilt resulted in significant reduction of hay yields and plant death. In particular, in irrigated areas, fields became unprofitable after two to three years (Hale and Close, 1974). In Australia, only the Gippsland region of south-eastern Victoria suffered significant crop loss (Smith and Taylor, 1967). In the EPPO region, the pathogen caused losses in the past, but exact figures are not available (EPPO, 1997).

Outside the EU, bacterial wilt incidences have decreased considerably in many countries. In New Zealand, bacterial wilt is limited (McSweeney and Dunbier, 1978). The release of new disease-resistant cultivars, however, has largely overcome the problem. In Australia, bacterial wilt caused significant losses of lucerne production in New South Wales between 1966 and 1986 but not thereafter, which can probably also be attributed to the use of resistant cultivars (Pilkington, 2003.

However, in some parts of the world, the pathogen is still responsible for significant crop losses, for example in the Hamedan province of Iran, the most important lucerne-producing region of the country (Heidari and Khodakaramian, 2012).

3.5.2. Observed impact of *Clavibacter michiganensis* subsp. *insidiosus* in the EU

The information in the questionnaire (Table 2) provided by National Plant Protection Services of the MS on the incidence of bacterial wilt, indicated that, currently, the disease is widespread only in the UK, where lucerne is not an important crop. In major lucerne production areas in EU, the disease has been eradicated. It is not known to what extent the use of resistant cultivars has contributed to the reduction of bacterial wilt outbreaks. It is possible that other management measures, including the use of pathogen-free seed and hygiene, have also resulted in a rapid decrease in the number of outbreaks.

3.6. Currently applied control methods in the EU

The currently applied control methods in the EU are very likely limited to use of resistant lucerne varieties. No more specific information is available.

General guidelines provided for lucerne production in the USA are applied for the management of the disease:

- The use of resistant lucerne varieties (Graham, 1960; Straley et al., 1974; Kao et al., 1985; Acharya, 2014), this is of high importance in control programmes.
- The use of pathogen-free seed, produced in regions where the pathogen is absent or not found, for at least 10 years.
- Cultivation practices. Mowing when the lucerne stands are dry. Rotation with other crops to help prevent the build-up of pests; in particular, infections with root-knot nematode should be prevented. Growing of other crops for two or three years before reseeding a field with lucerne. The use of grass in a mixture with lucerne will reduce disease development in a crop (Koehler and Jones, 1932). Fields should be kept free of volunteer lucerne plants and alternative hosts of the disease such as *Trifolium* spp. Proper drainage of soils will limit development of the pathogen. Deep tillage to aid decomposing pest-infested residues. Less intensive harvesting schedules to reduce the loss rate of susceptible plants. Harvesting of young stands before old stands, when using the same equipment, and harvesting fields showing wilt symptoms last. A high, balanced fertilisation based on a soil test to help maintain plant vigour (University of Illinois, 1988).
- Transfer of lucerne plant material (hay, fodder, seed and silage) from infected to non-infected areas should be avoided.
- Sanitation measures. Cleaning of equipment with steam before moving from field to field, especially where wilt is present.

3.7. Uncertainty

There are uncertainties about:

- The incidence and severity of outbreaks. Little information is available in the literature and what there is largely relies on data provided by the NPPOs. The frequency and intensity of the surveys are unknown.
- The use of bacterial wilt-resistant lucerne varieties in Europe. There are reports (e.g. from the Czech Republic) that, because of the development and introductions of new resistant varieties, bacterial wilt is now of only low importance in practice (Víchová and Kozová, 2004). These observations were confirmed by the low incidence of (reported) outbreaks since the 1970s. However, it is not known to what extent these resistant varieties are used.

- The role of seed in the transmission of the disease. The finding of infected seed lots and the patch-wise distribution of *C. michiganensis* subsp. *insidiosus* in naturally infected seeds are strong indicators for the seed-borne character of the pathogen. However, transmission from seed to seedling has not been proven.
- The role of seed from resistant cultivars in the spread of the bacterium. The infection incidence of seed from resistant cultivars has never been determined.
- The role of dried hay and dried plant debris as inoculum sources, as little information is available.
- The economic impact of (sporadic) outbreaks. Direct crop losses in the MSs or the cost of the statutory measures have never been determined, neither the costs due to statutory measures;.
- The control methods used in the EU specifically the use of resistant varieties, as little information is available.

CONCLUSIONS

The Panel summarises in Table 7 its conclusions on the key elements addressed in this scientific opinion in consideration of the pest categorisation criteria defined in ISPM 11 and ISPM 21 and of the additional questions formulated in the terms of reference.

Table 7: The Panel's conclusions on the pest categorisation criteria defined in the International Standards for Phytosanitary Measures No 11 and No 21 and on the additional questions formulated in the terms of reference (ToR)

Criterion of pest categorisation	Panel's conclusions on ISPM 11 criterion	Panel's conclusions on ISPM 21 criterion	List of main uncertainties
Identity of the pest	*Is the identity of the pest clearly defined? Do clearly discriminative detection methods exist for the pest?*		–
	The identity of the pest is unambiguous: *Clavibacter michiganensis* subsp. *insidiosus*. Various molecular tools for a precise identification of the pest are available		
Absence/presence of the pest in the risk assessment area	*Is the pest absent from all or a defined part of the risk assessment area?*	*Is the pest present in the risk assessment area?*	Uncertainty on the presence or absence of *C. michiganensis* subsp. *insidiosus* in EU MSs. Information on the frequency and intensity of surveys is lacking. There is no information available on the use of resistant cultivars which may mask symptom expression
	The pathogen is not known to occur in most of the EU countries	The pathogen is present in the UK, Estonia, Lithuania and Poland. No epidemics were recently reported in the EU	
Regulatory status	*In consideration that the pest under scrutiny is already regulated just mention in which annexes of 2000/29/EC and the marketing directives the pest and associated hosts are listed without further analysis. Indicate also whether the hosts and/or commodities for which the pest is regulated in AIIAI or II are comprehensive of the host range*		–
	The pest and the main associated hosts are under official control and regulated by the Directive 2000/29/EC (Annexes II-A-II, IV-A-I, IV-A-II, V-A-I, V-B-I) and by the Council Directive 66/401/EEC		

Criterion of pest categorisation	Panel's conclusions on ISPM 11 criterion	Panel's conclusions on ISPM 21 criterion	List of main uncertainties
	There are possible additional natural hosts (other species of the Fabaceae family, including *Medicago* spp., such as the lawn plant black medick (*Medicago lupulina*), honey clover (*Melilotus albus*), the fodder crops bird's-foot trefoil (*Lotus corniculatus*) and common sainfoin (*Onobrychis viciifolia*) and *Trifolium* subsp.) which are currently not regulated		
Potential for establishment and spread	*Does the risk assessment area have ecological conditions (including climate and those in protected conditions) suitable for the establishment and spread of the pest?* *Indicate whether the host plants are also grown in areas of the EU where the pest is absent* *And, where relevant, are host species (or near relatives), alternative hosts and vectors present in the risk assessment area?* Suitable climate and soil conditions are present in all EU MSs where lucerne is grown. Nevertheless, the pest is only (sporadically) found in Estonia, Lithuania, Poland and the UK. No reports are available of wild plant species (weeds) recognised as alternative hosts. These weeds can be found in most EU MSs	*Are plants for planting a pathway for introduction and spread of the pest?* *C. michiganensis* subsp. *insidiosus* is seed-borne but no information is available on the transmission from seed to seedling	Uncertainty on the transmission of *C. michiganensis* subsp. *insidiosus* by seed and contaminated hay. Uncertainty of role weeds in the epidemiology of the disease
Potential for consequences in the risk assessment area	*What are the potential for consequences in the risk assessment area?* *Provide a summary of impact in terms of yield and quality losses and environmental consequences* *C. michiganensis* subsp. *insidiosus* can cause serious losses in two- to three-year-old lucerne stands if susceptible varieties are used and conditions are favourable for disease development. However, recent reports on outbreaks are lacking possibly due to the use of resistant cultivars or by an improved seed health management	*If applicable is there indication of impact(s) of the pest as a result of the intended use of the plants for planting?* There are indications for dispersal of the pathogen via seed. However, since the introduction of resistant cultivars, no reports have been published on outbreaks as a result of the use of plants for planting	There is uncertainty on the incidence and severity of bacterial wilt in Europe as a consequence of a limited number of surveys

Criterion of pest categorisation	Panel's conclusions on ISPM 11 criterion	Panel's conclusions on ISPM 21 criterion	List of main uncertainties
Conclusion on pest categorisation	*C. michiganensis* subsp. *insidiosus* is reported in few MSs (the UK, Estonia, Lithuania and Poland). No epidemics were recently reported in the EU. Outside the EU, recent outbreaks have been reported only in Iran	*C. michiganensis* subsp. *insidiosus* is seed-borne and probably seed-transmitted, although definite data are lacking to support this point. No information is available on the role of contaminated plant debris and alternative hosts in the epidemiology of the disease. The pathogen may be more widespread than is suggested by data presented. The use of resistant cultivars may mask symptom expression and information is lacking on the frequency and intensity of surveys	Uncertainty mostly concerns the current distribution of *C. michiganensis* subsp. *insidiosus* in the EU because of the limited number of specific surveys and the potential use of resistant cultivars
Conclusion on specific ToR questions	*If the pest is already present in the EU, provide a brief summary of* – *the analysis of the present distribution of the organism in comparison with the distribution of the main hosts, and the distribution of hardiness/climate zones, indicating in particular if, in the risk assessment area, the pest is absent from areas where host plants are present and where the ecological conditions (including climate and those in protected conditions) are suitable for its establishment, and* *C. michiganensis* subsp. *insidiosus* is present in a restricted number of MSs in the EU (Estonia, Lithuania, Poland, the UK). Only sporadic disease outbreaks occur, and not in countries where lucerne production is of importance. The pathogen causes yield and quality loss only if susceptible cultivars are grown and conditions are favourable for disease expression. The pathogen is not reported in the main lucerne-producing MSs (Spain, Italy, France, Romania, Hungary) – *the analysis of the observed impacts of the organism in the risk assessment area* There are no indications that in last decade the pathogen has had a high impact on lucerne production in EU		Uncertainty mostly concerns the distribution of *C. michiganensis* subsp. *insidiosus* in the EU because of the limited number of specific surveys and the potential use of resistant cultivars

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ABBREVIATIONS

EFSA	European Food Safety Authority
EPPO	European and Mediterranean Plant Protection Organization
EPPO PQR	European and Mediterranean Plant Protection Organization Plant Quarantine Retrieval System
EU	European Union
ISPM	International Standards for Phytosanitary Measures
MS(s)	Member State(s)
NPPO	National Plant Protection Organisation
PLH Panel	Plant Health Panel
PRA	pest risk analysis
RNQP	regulated non-quarantine pest