European Food Safety Authority

EFSA Journal 2014;12(6):3721

SCIENTIFIC OPINION

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

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The European Commission requested the EFSA Panel on Plant Health to perform the pest categorisation for *Clavibacter michiganensis* subsp. *michiganensis* (Cmm). The agent responsible for vascular tomato wilt and canker is the clearly defined and valid gram-positive taxon *C. michiganensis* subsp. *michiganensis*. This pathogen can be accurately identified based on a range of sensitive and specific methods. Tomato (*Solanum lycopersicum*) is the main host, but peppers (*Capsicum annum* and *C. frutescens*) are also naturally susceptible to Cmm. These host plants are cultivated throughout Europe and conditions are conducive to disease development in open fields in southern Europe and in greenhouses. The disease is present in many EU Member States. Outbreaks are rare but usually severe. It causes a range of symptoms on the aerial parts of plants, including the fruits. Detection methods are available for any type of plant material either presenting symptoms or symptomless. Seed testing has proven to be a good control option by discarding contaminated seed lots. Despite tomato seed production being done under strict sanitation using recommended practices, seed contamination still occurs occasionally. Contaminated seeds and transplants are responsible for long-distance dissemination of the pathogen. Under conducive conditions, even low levels of seed contamination can result in disease outbreaks. Cultivation practices can favour secondary spread of the bacterium and an increase in disease incidence both in greenhouse and in open-field crops. No effective biological or chemical control agents are registered for bacterial canker in Europe. Cmm meets all criteria defined in International Standard for Phytosanitary Measures (ISPM) 21. Cmm meets all ISPM 11 criteria, although it has been observed in 16 EU Member States. The outbreaks are usually severe but sporadic.

KEY WORDS

pest categorisation, *Clavibacter michiganensis* subsp. *michiganensis*

[1] On request from the European Commission, Question No EFSA-Q-2012-00807, adopted on 22 May 2014.

[2] Panel members: Richard Baker, Claude Bragard, Thierry Candresse, Gianni Gilioli, Jean-Claude Grégoire, Imre Holb, Michael John Jeger, Olia Evtimova Karadjova, Christer Magnusson, David Makowski, Charles Manceau, Maria Navajas, Trond Rafoss, Vittorio Rossi, Jan Schans, Gritta Schrader, Gregor Urek, Johan Coert van Lenteren, Irene Vloutoglou, Stephan Winter and Wopke van der Werf. Correspondence: alpha@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Tomato Bacteria: Marie-Agnès Jacques, Michael John Jeger, Charles Manceau, Jan Schans, Emilio Stefani and Jan Martin van der Wolf for the preparatory work on this scientific opinion, and the hearing expert Dani Shtienberg, and EFSA staff Svetla Kozelska and Tomasz Oszako 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. *michiganensis* (Smith) Davis et al. EFSA Journal 2014;12(6):3721, 29 pp. doi:10.2903/j.efsa.2014.3721

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

SUMMARY

Following a request from the European Commission, the EFSA Panel on Plant Health (hereafter the Panel) was asked to deliver a scientific opinion on the pest categorisation of *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) for the European Union (EU) territory.

The Panel performed the pest categorisation for Cmm following the guiding principles and steps presented in the EFSA guidance on the harmonised framework for pest risk assessment (EFSA PLH Panel, 2010) and as defined in the International Standard for Phytosanitary Measures (ISPM) No 11 (FAO, 2013) and ISPM No 21 (FAO, 2004).

After consideration of the evidence, the Panel reached following conclusions.

Identity of the pest

Cmm is the causative agent of bacterial canker of tomato. The organism under assessment is a clear, distinguished taxonomic entity and can be accurately identified based on a range of discriminatory methods. Cmm can cause a wide variety of symptoms on host plants, which include (unilateral) wilting, stem cankers, chlorotic leaf spots, desiccation of leaf margins and bird's eye spots on the fruits. Some symptoms may be confused with those caused by other organisms.

Presence in the risk assessment area

The pathogen is found in 16 EU Member States, but, with the exception of Greece and Italy, the population is transient, with restricted distribution or low prevalence; outbreaks have been found only in some years and in specific areas. Only Greece reports that the pathogen is widespread. In Denmark, Estonia, Finland, Ireland, Malta, Portugal, the Slovak Republic, Sweden, Iceland and Norway the disease has never been reported or the pathogen has been eradicated. No data are available from Croatia and Luxembourg.

Regulatory status

The pathogen is listed in Council Directive 2000/29/EC, Annex II A II, as a harmful organism, known to occur in the Community and relevant for the entire Community, whose introduction into, and spread within, all Member States shall be banned if present on certain plants or plant products. Measures regulating the import into and movement within the EU of potentially infested host plants include special requirements with respect to Cmm for specified plant material, prohibition of import for specified plants from specified third countries and official control of host plant material produced within the EU for use by professional producers of plants and fruits.

The Panel notes that there are no regulatory special requirements in place with respect to Cmm for the movement within the EU of seeds of *Capsicum*. This may increase the probability of spread of the pathogen on this commodity.

Potential for establishment and spread in the risk assessment area

Tomato, the most important host of Cmm is one of the major vegetable crops in Europe that is grown in all Member States. Other natural hosts of Cmm are pepper and some solanaceous weeds, but in these hosts Cmm is found only sporadically. Epiphytic populations of Cmm on a broad range of plants can be detected in the event of disease outbreaks and these may play a role in secondary spread.

The pathogen can occur in both glasshouses and field crops. The environmental conditions in southern Europe are particularly favourable for disease expression in the field, as the optimal growth temperature for Cmm is between 24 °C and 28 °C. Although the pathogen has been found in many EU Member States, infections occur only sporadically.

The pathogen is seed borne and seed is considered to be the major means of long-distance dispersal. The pathogen can survive for years on seed, and a low inoculum dose of a few cells can result in

transmission from seed to seedling. A few infection loci can lead to outbreaks. Transplants can also be a primary infection source and can serve as a means of long-distance dispersal. At production sites, tomato volunteer plants and infected soil and crop debris, in which Cmm can survive, are recognised as a source of inoculum. Cultivation practices including clipping and pruning contribute considerably to the rapid spread of the pathogen in a crop.

Control is mainly based on prevention and exclusion. Detection methods are available for any type of plant material, whether presenting symptoms or symptomless. In Europe, seed production is done under strict sanitation controls using recommended practices to avoid seed contamination. Seed testing has proven to be a good control option as contaminated seed lots are discarded. Extraction of seed from fruit debris using fermentation and acid treatments reduces Cmm populations, but internal infections cannot be eliminated by seed treatments. No methods or chemical control agents are available that effectively control Cmm in infected crops. There are no commercial cultivars available with resistance or an acceptable level of tolerance.

Potential for consequences in the risk assessment area

The pathogen is considered to be one of the most important bacterial pathogens of tomato and pepper and can be very destructive. Infections often result in high yield losses; in several cases losses of between 50 % and 100 % have been reported. However, growers and the seed industry are putting considerable efforts into preventing the introduction and dissemination of Cmm. Production systems involving integral testing of tomato seed and transplants using validated protocols are used by the tomato seed companies and nurseries. These largely exclude the introduction and spread of Cmm by propagation material. This has resulted in a considerable reduction in crop damage and may be considered an effective way of controlling the disease.

Cmm meets the following ISPM 11 criteria:

Identity of the pest: The identity of the pest is clearly defined.

Presence or absence in the risk assessment area: Cmm has been observed in 16 EU Member States. However, the outbreaks are sporadic but usually severe.

Regulatory status: The pest is under official control.

Potential for establishment and spread in the risk assessment area: The risk assessment area has ecological/climatic conditions, including those in protected cultivation, that are suitable for the establishment and spread of the pest, and host species are present in the risk assessment area.

Potential for economic consequences (including environmental consequences) in the risk assessment area: The pathogen is considered to be one of the most important bacterial pathogens of tomato and pepper and can be very destructive.

Cmm meets all criteria defined in ISPM 21. Cmm is a seed-borne bacterium and can be present in plants for planting (seed and transplants), which has considerable impact on the intended use of those plants.

No major uncertainties were identified within the pest categorisation.

TABLE OF CONTENTS

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.l).

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.

Arabic mosaic virus, Tomato black ring virus, Raspberry ringspot virus, Strawberry latent ringspot virus, Strawberry crinkle virus, Strawberry mild yellow edge virus, *Daktulosphaira vitifoliae* (Fitch), *Eutetranychus orientalis* Klein, *Parasaissetia nigra* (Nietner), *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis *et al*., *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, *Didymella ligulicola* (Baker, Dimock and Davis) v. Arx, and *Phytophthora fragariae* Hickmann var. *fragariae* are regulated harmful organisms in the EU. They are all listed in Annex II, Part A, Section II of Council Directive 2000/29/EC, which means that they are organisms known to occur in the EU and whose introduction into and spread within the EU is banned if they are found present on certain plants or plant products.

Given the fact that these organisms are already locally present in the EU territory and that they are regulated in the EU since a long time, 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. 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.

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).

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 Arabic mosaic virus, Tomato black ring virus, Raspberry ringspot virus, Strawberry latent ringspot virus, Strawberry crinkle virus, Strawberry mild yellow edge virus, *Daktulosphaira vitifoliae* (Fitch), *Eutetranychus orientalis* Klein, *Parasaissetia nigra* (Nietner), *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis *et al*., *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, *Didymella ligulicola* (Baker, Dimock and Davis) v. Arx, and *Phytophthora fragariae* Hickmann var. *fragariae*, for the EU territory.

For each organism EFSA is asked to identify risk management options and to evaluate their effectiveness in reducing the risk to plant health posed by the organism. EFSA is also requested to provide an opinion on the effectiveness of the present EU requirements against those organisms, which are laid down in Council Directive 2000/29/EC, in reducing the risk of introduction of these pests into, and their spread within, the EU territory.

Even though a full risk assessment is requested for each organism, in order to target its level of detail to the needs of the risk manager, and thereby to rationalise the resources used for its preparation and to

[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.

speed up its delivery, EFSA is requested to concentrate in particular on the analysis of the present spread of the organism in comparison with the endangered area, the analysis of the observed and potential impacts of the organism as well as the availability of effective and sustainable control methods.

The European Commission amended further the Terms of reference through a new request regarding 38 plant pests listed in the Annexes of the EC Directive 2000/29/EC (ARES (2014)970361) as follows:

"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* subsp. *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."

ASSESSMENT

1. Introduction

1.1. Scope and purpose

In this opinion, the EFSA Panel on Plant Health (hereafter the Panel) produced a pest categorisation for *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), as requested by the European Commission (ARES (2014)970361). In the conclusions of this opinion the Panel summarises the main findings. The pest risk assessment area is the territory of the European Union (hereinafter referred to as the EU) with 28 Member States (hereinafter referred to as EU MSs), restricted to the area of application of Council Directive 2000/29/EC, which excludes Ceuta and Melilla, the Canary Islands and the French overseas departments.

2. Methodology and data

2.1. Methodology

The Panel performed the pest categorisation for Cmm following the guiding principles and steps presented in the EFSA guidance on the harmonised framework for pest risk assessment (EFSA PLH Panel, 2010) and as defined in International Standard for Phytosanitary Measures (ISPM) No 11 (FAO, 2013) and International Standard for Phytosanitary Measures No 21 (FAO, 2004).

In accordance with the Harmonised framework for pest risk assessment in the EU (EFSA PLH Panel, 2010), this work is initiated as result of the review or revision of phytosanitary policies and priorities. As explained in the background of the EC request, the objective of this mandate is to provide updated scientific advice to the European risk managers for their evaluation of whether these organisms listed in the Annexes of the Directive 2000/29/EC still 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 be deregulated. Therefore, to facilitate the decision making process, in the conclusions of the pest categorisation, the Panel addresses explicitly each criterion for quarantine pest according to ISPM 11 (FAO, 2013) but also for regulated non quarantine pest according to ISPM 21 (FAO, 2004) and includes additional information required as per the specific terms of reference received by the EC.

2.2. Data

2.2.1. Literature search

A literature search on Cmm was conducted at the beginning of the mandate. As the same species is sometimes mentioned under synonyms (see section 3.1.1), the most frequent synonyms, along with the most usually used common names, were used for the literature search consulting the ISI Web of Knowledge database. Further references and information were obtained from experts and from citations within the references. Searches were also carried out on the Internet.

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 Panel sent a short questionnaire on the current situation at the country level, based on the information available in the European and Mediterranean Plant Protection Organization (EPPO) Plant Quarantine Data Retrieval system (PQR) (EPPO, online) to the National Plant Protection Organisation (NPPO) contacts in all the EU Member States (in January 2013, with answers received up to March 2013). In some cases, supplementary information was also sought for clarification. A summary of the answers received is presented in Table 1.

In order to obtain information on the distribution of the main host plants, the EUROSTAT database was consulted.

3. Pest categorisation

3.1. Identity and biology of the pest

3.1.1. Taxonomy

Clavibacter michiganensis subsp. *michiganensis*, originally described under the name *Bacterium*, is the causal agent of bacterial wilt and canker of tomato. After several modifications to its nomenclature (see Synonyms below), the revision of gram-positive plant pathogenic bacteria nomenclature undertaken by Davis et al. (1984) led to the definition of the genus *Clavibacter*, containing *C. michiganensis* and four other species. Following the reclassification of the four other species, *C. michiganensis* is currently *michiganense* (Smith, 1910), is the only species in the genus *Clavibacter* (Saddler and Kerr, 2012).

Based on phenotypic and biochemical features, genetic markers and specific hosts (Saddler and Kerr, 2012), *C. michiganensis* is subdivided into six subspecies. All strains of *C. michiganensis* pathogenic on tomatoes are grouped in the subspecies *michiganensis*. Based on phylogenetic analysis, pathogenic strains isolated from tomato seeds and plant parts can be further differentiated from non-pathogenic look-alike *C. michiganensis* strains that colonise the same habitat (Jacques et al., 2012). Based on colony morphology, pathogenicity and genetic markers (16S rRNA and internal transcribed spacer sequences), pepper strains are distinct from tomato strains (Yim et al., 2012).

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

Name:
Clavibacter michiganensis subsp. *michiganensis*

Synonyms:
Bacterium michiganense (Smith, 1910), *Pseudomonas michiganense* (Smith) Stevens, 1913, *Aplanobacter michiganense* (Smith, 1914), *Phytomonas michiganensis* (Smith) Bergey et al., 1923, *Erwinia michiganense* (Smith) Jensen, 1934, *Mycobacterium michiganense* (Smith) Krasil'nikov, 1941, *Corynebacteriurn michiganense* (Smith, 1910) Jensen, 1934 (Approved Lists, 1980), *Corynebacterium michiganense* pv. *michiganense* (Dye and Kemp, 1977), *Corynebacterium michiganense* subsp. *michiganense* (Carlson and Vidaver, 1982).

Taxonomic position:
C. michiganensis: Kingdom *Bacteria*; Phylum *Actinobacteria*; Class *Firmibacteria*. The genus *Clavibacter* was designed to accommodate the plant pathogenic coryneform bacteria, the cell wall peptidoglycan of which contains 2,4-diaminobutyric acid as dibasic amino acid (Davis et al., 1984); they are strictly aerobic gram-positive rods that do not produce endospores.

Common names used in English-speaking countries are bacterial canker of tomato, bird's eye of tomato fruit and vascular tomato wilt.

Within *C. michiganensis* subsp. *michiganensis*, no infradivisions such as pathovars or races have been proposed, making this subspecies a relatively homogeneous pathogen. It was first reported in 1910 from Michigan in the USA (Smith, 1910) and soon after, in 1914, in Italy (Lamichhane et al., 2011). Cmm is closely related to other *C. michiganensis* subspecies, which were also isolated for the first time in the USA. However, this provides no definitive evidence of the geographical origin of the pathogen.

3.1.2. Disease cycle

3.1.2.1. Inoculum sources

The primary inoculum sources for Cmm are contaminated seed (Strider, 1969; Fatmi et al., 1991; De Leon et al., 2011; EPPO, 2013a) and contaminated transplants (Ricker and Riedel, 1993; see also Figure 1). The threshold for a disease outbreak is low, as one infected seed in 10 000 is capable of initiating an epidemic (Chang et al., 1991). Contaminated seeds harbouring population sizes as small as 10–100 colony-forming units (cfu) per seed often lead to disease (Hadas et al., 2005). Infected seedlings are often asymptomatic, leading to undetected spread of the pathogen during cultural practices in nurseries (Chang et al., 1991; Gitaitis et al., 1991). Contaminated tomato debris, contaminated alternative hosts and tomato volunteers may also serve as a primary inoculum source (Chang et al., 1992).

From a primary inoculum source, plants may grow with epiphytic populations of Cmm that may develop, or hydathodes may guttate fluid with high densities of Cmm; both may serve as inoculum to cause secondary infections. The severity of secondary spread is greatly influenced by cultural practices such as grafting and environmental conditions (Chang et al., 1992; Carlton et al., 1998).

Bacterial canker agent can survive in seeds for long periods (Bryan, 1930; Strider, 1969). Survival in contaminated debris in soil depends on soil type (Moffett and Wood, 1984;) but can last up to two years on infested debris at the soil surface (Gleason et al., 1991).

The relative importance of each inoculum source depends mainly on environmental conditions and the cultivation system. It has been emphasised (Shtienberg Dani, personal communication 28 April 2014, Department of Plant Pathology and Weed Research, ARO, the Volcani Center, Bet Dagan, Israel,) that contamination of a crop through Cmm-contaminated debris may not be efficient, but there are contradictory reports indicating that contaminated debris should be considered as a contaminant (Moffett and Wood, 1984; Gleason et al., 1991).

3.1.2.2. Infection

Cmm infections occur through two main sites of entry, i.e. hydathodes (Carlton et al., 1998) and wounds (Huang and Tu, 2001). Epiphytic Cmm cells become suspended in guttation droplets and, upon being drawn back into the leaf of the contaminated guttation droplets, the pathogen becomes distributed along the margin of the leaflet, and symptoms of marginal necrosis develop (Carlton et al., 1998). There is no clear indication in the literature that stomata can serve as sites of entry for Cmm in leaf tissues. This bacterium can also enter through broken trichomes (Bryan, 1930; Kontaxis, 1962; Layne, 1967), pruning wounds (Carlton et al., 1994; Chang et al., 1991) and wounded roots (Kendrick and Walker, 1948, cited by Strider, 1969). Infection of seeds occurs through the vascular route but also after penetrating the ovary wall or floral parts (Medina-Mora et al., 2001; Tancos et al., 2013).

The period between infection and symptom expression varies considerably, ranging from 7 to 84 days, and is determined by the temperature, plant age, soil characteristics and the nutrient status of the plant (Gleason et al., 1993). Populations densities need to exceed 10^7 cfu/g of fresh weight before symptoms are expressed (Chang et al., 1992). More particularly, the incubation period is longer if more tolerant cultivars are used, if inoculation densities are lower and if the temperatures are above or below than the optimum of 25 °C. Conditions decreasing the incubation periods also favour an increase in disease severity (Gleason et al., 1993).

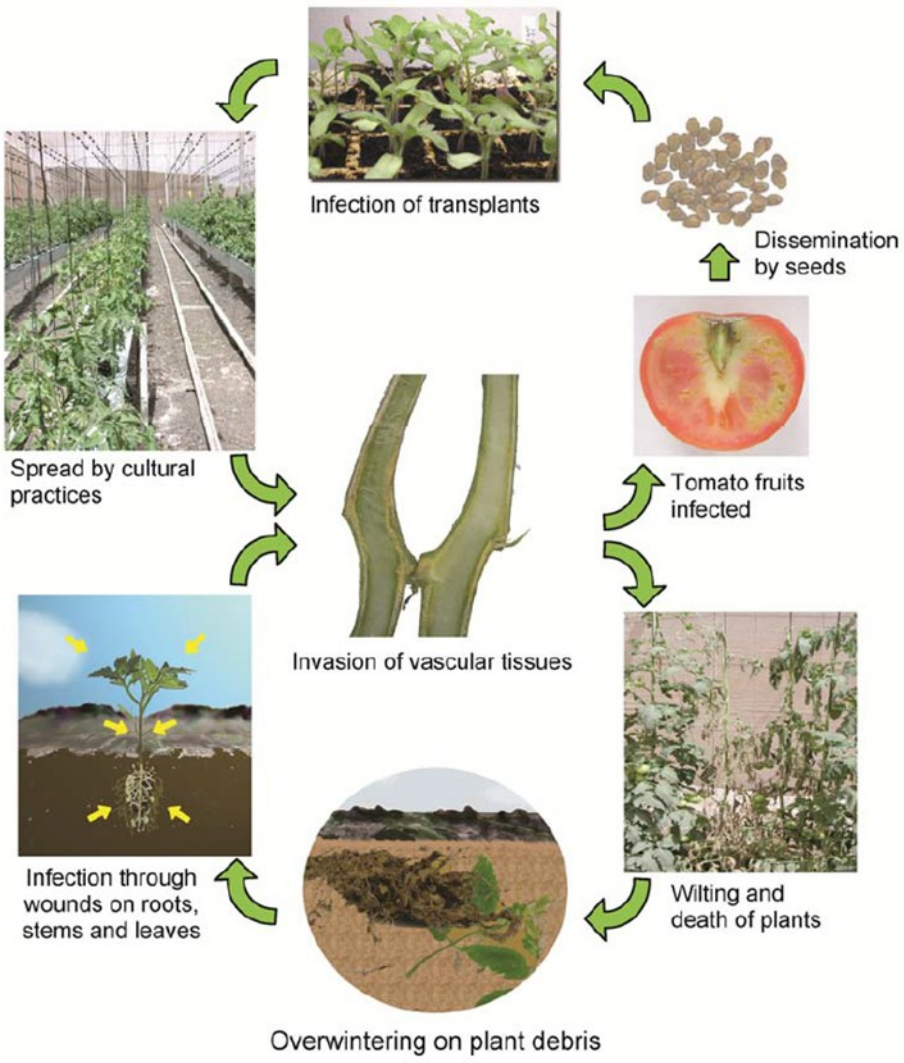

Figure 1: Disease cycle of *Clavibacter michiganensis* subsp. *michiganensis* on tomato plants (from De Leon et al., 2011)

3.1.2.3. Symptomatology

Symptoms caused by Cmm on host plants are of two main types depending on whether the infection is systemic or superficial. Some symptoms of Cmm may be confused with those caused by *Ralstonia solanacearum*, *Fusarium* spp. or *Verticillium* spp. Distinctive characters are listed by Strider (1969).

Following systemic infection of tomato plants grown in a greenhouse, leaves wilt at high temperatures. Interveinal pale green water-soaked areas then appear on leaves that rapidly desiccate, giving rise to a scorched appearance. Under high temperature (25–30 °C) and evapotranspiration stress, entire leaves and finally whole plants wilt and desiccate within a few days. During the early stages of disease development, unilateral wilting of leaflets and leaves is common. Canker lesions develop on the stem and the plant dies. Under less favourable conditions for the pathogen, wilting may be delayed. The vascular tissues of stems of wilted plants usually appear dark yellow to brown. The pith may collapse. Wilting may occur early on grafted plants (Xu et al., 2010) but usually develops later at plant maturity. However, in the field, wilting is not frequent. Desiccation of the edge of the leaflets is seen mainly on lower leaves and then the plant slowly desiccates. At an advanced stage, small whitish pustules appear on leaf veins and petioles. Brown stripes that appear on stems and petioles may form cankers. When infection occurs at a late stage of plant development, plants can survive and generate fruits. Fruits may

fail to develop and fall or ripen unevenly. They can appear marbled with longitudinal chlorotic streaks and internal bleaching of vascular and surrounding tissues. Systemically infected fruits yielding infected seeds may also appear healthy. No systemic infection has been reported in pepper (Lai, 1976).

In tomato, and also in pepper, the pathogen can cause spots on leaves, petioles, peduncles and fruits as a result of a local infection. Foliar symptoms occasionally include small, white, blister-like spots, and, more commonly, marginal necrosis of leaflets, also referred to as "firing" symptoms. Typical bird's eye symptoms on fruit are found infrequently in tomato glasshouse crops and even less commonly in open-field crops. They are also found on pepper fruits (Volcani et al., 1970). These spots have a dark brown centre, which becomes raised, and are surrounded by a distinct white halo (Ehring and Griesbach, 1985).

Tomato and pepper seeds are most commonly symptomless while carrying the pathogen. Only occasionally do seeds present symptoms. Contamination of seeds may occur during seed extraction (Strider, 1969). It should be mentioned that, in comparison with tomato isolates, pepper isolates showed limited pathogenicity on tomato and higher pathogenicity on pepper (Yim et al., 2012).

Based upon spotting of the cotyledons and/or wilting or vascular discolouration following inoculation, aubergine has been considered as a host for Cmm (Hassan et al., 1968; Stamova and Sotirova, 1987). However, there is a lack of reports of natural occurrences of Cmm on aubergine and consequentially a lack of descriptions of naturally occurring symptoms.

3.1.3. Detection and identification of the pest

A full range of detection methods has been developed for Cmm on symptomatic and asymptomatic plant samples (EPPO, 2013a). Whatever the type of plant sample (leaf fragment, stem, seeds) to be analysed, the sample extracts are tested for the pathogen either directly (immunological methods) or after plating on semi-selective media or by DNA extraction. Appropriate controls, including spiking of plant extracts with predetermined Cmm densities, are proposed in the EPPO diagnostic standards EPPO (2013a), and by the International Seed Federation (ISF, 2014) to check for false-negative results that could result from difficulties in recovering Cmm owing to the presence of other microorganisms or inhibitory compounds in plant extracts. After incubation of inoculated plates, colonies with suspected Cmm morphology are further identified using confirmatory identification tools such as pathogenicity tests (EPPO, 2013a), immunofluorescence (Franken et al., 1993), or polymerase chain reaction (PCR) with specific primers (Dreier et al., 1995; Santos et al., 1997). As an alternative to classic PCR, a BIO-PCR (biological amplification followed by PCR) method was proposed (Hadas et al., 2005). Recently, revised detection methods were recommended by international organisations (International Seed Testing Association and EPPO) especially for seed testing. Concerning seed testing, there is particular concern on sample and subsample sizes. First, the sample has to be representative of the seed lot and, second, the sample size to be analysed should allow with reasonable probability the detection of contamination rates that could give rise to epidemics. Hence, ISF (2014) recommended a minimum sample size of 10 000 seeds with a maximum subsample size of 10 000 seeds. Apart from the extraction step, which is clearly specific for seeds, other steps can be adapted to any type of plant sample to test for the presence of Cmm (EPPO, 2013a; ISF, 2014).

Specific and reliable PCR tests are available for the identification of Cmm. Comparative testings were done for five PCR identification tests and an immunofluorescence (IF) assay, using a large (197 strains) worldwide collection of Cmm and other, non-pathogenic *C. michiganensis* (Jacques et al., 2012). While none of the IF or the five PCR tests generated false-negative results, only one test (gene encoding the protein two-component system sensor kinase, Ptssk,; Berendsen et al., 2011) produced no false-positive result. The Ptssk test produced results that totally matched pathogenicity test results, even for weakly pathogenic strains, for which signals were obtained. No signal was obtained for other *C. michiganensis* strains non-pathogenic on tomato. This is one of the two recommended tests in the EPPO protocol (EPPO, 2013a). This test is combined with the test using primers PSA-8/R proposed by Pastrik and Rainey (1999) in the ISF (2014) method.

It is debatable if tests should exclude avirulent and weakly pathogenic strains of Cmm. These strains are occasionally found after partial deletion of a chromosomal pathogenicity island, or after losing entire plasmids carrying pathogenicity genes (Jacques et al., 2012). Plasmids can be lost under stress conditions, e.g. after seed treatment at elevated temperatures. Reversion to pathogenic variants cannot be entirely ruled out, and therefore tests should preferably detect all Cmm strains irrespective of whether they are positive in pathogenicity tests or not. However, it is clear that detection tests should not give positive signals for the phylogenetically distant non-pathogenic *Clavibacter* strains.

Several tests have been proposed to confirm the identity of strains; they include abrasion such as in the cotyledon assay (EPPO, 2013a), injection into the main stem of plantlets of inoculum (EPPO, 2013a; ISF, 2014), or inoculation of developing roots of axenically grown plants (Vieira Lelis et al., 2014). The invasive tests do not provide information on the ability of strains to ingress into plant tissues by natural openings and may therefore overestimate strain virulence. However, they provide a good indication of the strain's ability to infect plants through wounds.

3.2. Current distribution of the pest

3.2.1. Global distribution

Cmm is widely distributed, being reported from all five continents (Figure 2).

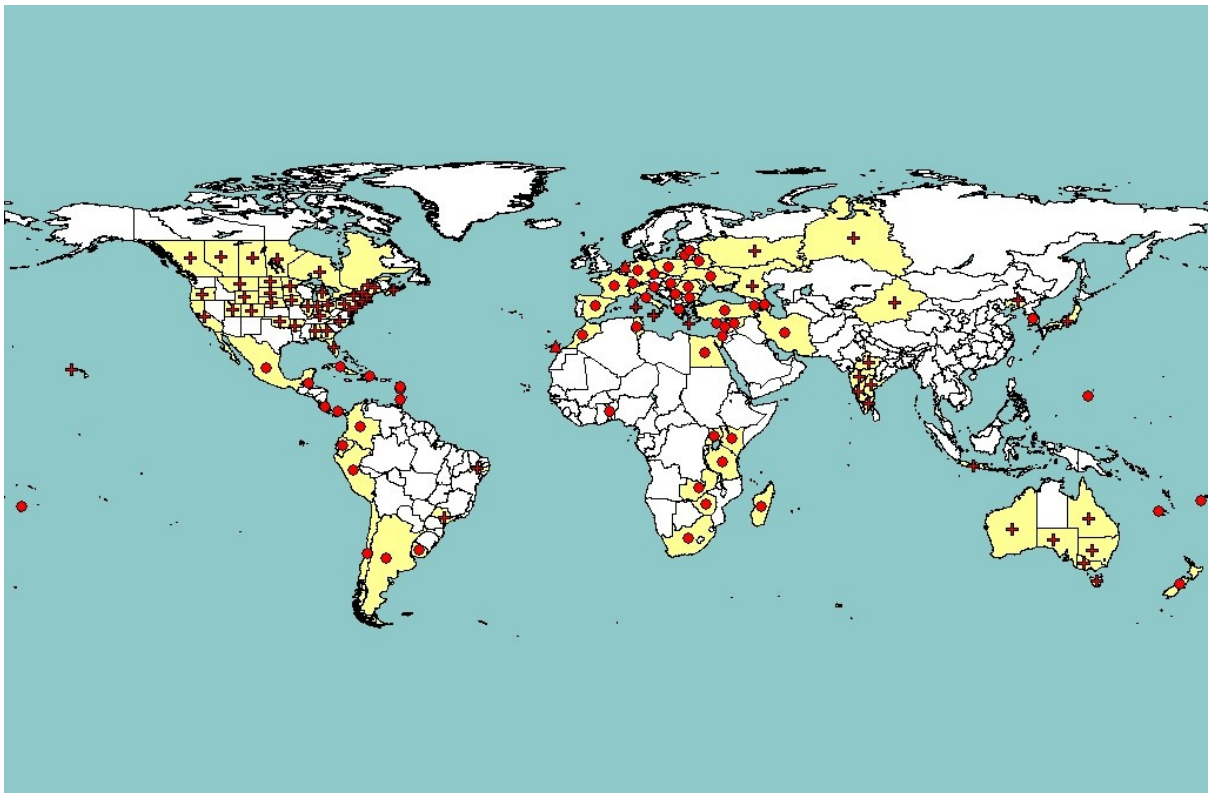

Figure 2: Global distribution of *Clavibacter michiganensis* subsp. *michiganensis* (extracted from EPPO PQR (2014, version 5.3.1.), accessed on 12 March 2014). Red circles represent pest presence as national records, red crosses pest presence as subnational records and red triangles transient pest presence (note that this figure combines information from different dates, some of which could be out of date)

3.2.2. Distribution in the risk assessment area

As indicated by the answers to a questionnaire sent by EFSA to Member States, the presence of Cmm is reported in 16 countries (Austria, Belgium, Bulgaria, Cyprus, the Czech Republic, France, Germany, Greece, Hungary, Italy, Latvia, the Netherlands, Poland, Romania, Slovenia and Spain) (Table 1). However, with the exception of Greece and Italy, these countries indicate that the presence is limited to a transient population under the process of being eradicated, a few occurrences only or a restricted distribution. Data on the presence or absence of the organism are not available in Croatia and Luxembourg. Outbreaks are usually severe but sporadic.

Table 1: The current distribution of *Clavibacter michiganensis* subsp. *michiganensis* in the risk assessment area, based on answers received from the 28 EU Member States, Iceland and Norway up to March 2013

Member State	Current situation
Austria	**Transient**, under eradication
Belgium	**Present,** at low prevalence, only in protected cultivation, under eradication
Bulgaria	**Present**, restricted distribution
Croatia	– (no data at NPPO)
Cyprus	**Present**, restricted distribution
The Czech Republic	**Present**, restricted distribution
Denmark	**Absent**, pest eradicated
Estonia	**Absent**, no pest record
Finland	**Absent**, confirmed by survey
France	**Present**, restricted distribution
Germany	**Present**, few occurrences
Greece	**Present**, wide spread
Hungary	**Present**, restricted distribution
Ireland	**Absent**, pest eradicated
Italy	**Present**, no details
Latvia[(a)]	**Present**, few occurrences
Lithuania	**Absent**, pest eradicated
Luxembourg[(a)]	–
Malta	**Absent**, not known to occur
Poland	**Present**, few occurrences
Portugal	**Absent**, few occurrences were eradicated
Romania	**Present**, restricted distribution
Slovak Republic	**Absent**
Slovenia	**Present,** only in protected cultivation
Spain[(a)]	**Present**, few occurrences
Sweden	**Absent**, pest eradicated
The Netherlands	**Transient**, under eradication
The United Kingdom	**Absent**, pest no longer present
Iceland	**Absent**, no records
Norway	**Absent**, pest eradicated

(a): When no information was made available to EFSA, the pest status in the European and Mediterranean Plant Protection Organization Plant Quarantine Data Retrieval system was used.
–: No information available

3.3. Host range and EU distribution of main host plants

3.3.1. Host range

Tomato is by far the most important host of Cmm, but natural infections have also been found on *Capsicum annuum* and *C. frutescens* (Strider, 1969; Lai, 1976; Moffett and Wood, 1984; Latin et al., 1995; Lewis-Ivey and Miller, 2000; Yim et al., 2012), and several solanaceous weeds (e.g. *Solanum nigrum*, *S. douglasii* and *S. triflorum*) (Bradbury, 1986). Among other solanaceous plants, aubergine (*S. melongena*) is susceptible upon artificial inoculation (Thyr et al., 1975). Initial reports and suspicions on the susceptibility of potato (*S. tuberosum*) to Cmm could not be confirmed (Strider, 1969). Several solanaceous and non-solanaceous plants, including *Datura stramonium*, *Chenopodium album* and *Amaranthus retroflexus*, have been identified as reservoirs for epiphytic survival and spread (Chang et al., 1992). The significance of these epiphytic populations is not fully understood, although they appear to contribute to infections through pruning wounds (Carlton et al., 1994). Stamova and Sotirova (1987) claim to have produced leaf wilt by artificial inoculation of maize, wheat, barley, rye and other plants with Cmm, but this has not been confirmed by others (EPPO, 2013a; CABI, 2014).

3.3.2. EU distribution of main hosts

Tomato is one of the most important vegetable crops in Europe: apart from a few countries, it is widely cultivated, both in a protected environment and in the open (Hucorne, 2012). Pepper is also an important vegetable, although its area of production is less extensive. Table 2 shows the production areas for tomatoes and pepper (*C. annuum*) in the EU Member States in 2012.

Table 2: Area of production in 1 000 ha of tomatoes and peppers in 2012, as extracted from the EUROSTAT database (crops products – annual data (apro_cpp_crop)) on 18 March 2014

Country	Tomatoes	Tomatoes for fresh consumption under glass or high accessible covers	Red peppers	Red peppers under glass or high accessible covers
Austria	0.2	0.2	0.2	0.1
Belgium	0.5	0.5	0.1	0.1
Bulgaria	3.4	0	3	0
Croatia	0.4	0.1	1	0.6
Cyprus	0.2	–	–	–
The Czech Republic	0.4	0	0	0
Denmark	0	0	0	0
Estonia	0	0	–	–
Finland	0.1	0.1	0	0
France	5.2	2	0.5	0
Germany	0.3	0.3	0.1	0.1
Greece	16	2.8	4.3	1
Hungary	1.8	0.4	2	–
Ireland	0	0	0	0
Italy	91.9	6.4	9	2.3
Latvia	0	0	0	0
Lithuania	0.6	0	0	0
Luxembourg	0	0	–	–
Malta	0.3	–	–	–
The Netherlands	1.7	1.7	1.3	1.3
Poland	13.1	2.2	2.5	1.1
Portugal	15.4	1	1.4	0.1
Romania	29.8	1.4	11.6	0.3
Slovakia	0.5	0	0.3	0
Slovenia	0	0	0	0
Spain	48.6	18.5	17.4	10.7

Country	Tomatoes	Tomatoes for fresh consumption under glass or high accessible covers	Red peppers	Red peppers under glass or high accessible covers
Sweden	0	0	0	0
The United Kingdom	0	0	0	0
EU-28	230.4	37,6	54.7	17.7

–: Data not available.

3.4. Regulatory status

3.4.1. Legislation directly addressing the pest

The pathogen is regulated as a harmful organism in the EU and is listed in Council Directive 2000/29/EC in the following section:

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
2. *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis et al.	Plants of *Lycopersicon lycopersicum* (L.) Karsten ex Farw., intended for planting

3.4.2. Legislation addressing hosts of the pest

It is prohibited to import plants intended for planting, other than seeds, of host plant species of Cmm from third countries, other than European and Mediterranean countries (Council Directive 2000/29/EC, Annex III part A (13).

Annex III, Part A - Plants, plant products and other objects the introduction of which shall be prohibited in all Member States

Description	Country of origin
13. Plants of *Solanaceae* intended for planting, other than seeds and those items covered by Annex III A (10), (11) or (12)	Third countries, other than European and Mediterranean countries

Special requirements with respect to Cmm have been formulated in Council Directive 2000/29/EC for import of seeds of tomato, originating in non-EU countries, into EU Member States (Annex IV, Part A, Section I) and for movement of seeds of tomato, originating in the EU, within the EU (Annex IV, Part A, Section II).

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
48. Seeds of *Lycopersicon lycopersicum*	Official statement that the seeds have been obtained by means of

(L.) Karsten ex Farw.	an appropriate acid extraction method or an equivalent method approved in accordance with the procedure referred to in Article 18(2), AND (a) either the seeds originate in areas where *Clavibacter michiganensis* ssp. *michiganensis* (Smith) Davis et al., *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye and *Potato spindle tuber viroid* are not known to occur; OR (b) no symptoms of diseases caused by those harmful organisms have been observed on the plants at the place of production during their complete cycle of vegetation; OR (c) the seeds have been subjected to official testing for at least those harmful organisms, on a representative sample and using appropriate methods, and have been found, in these tests, free from those harmful organisms.

Section II — Plants, plant products and other objects originating in the Community

Plants, plant products and other objects	**Special requirements**
27. Seeds of *Lycopersicon lycopersicum* (L.) Karsten ex Farw.	Official statement that the seeds have been obtained by means of an appropriate acid extraction method or an equivalent method approved in accordance with the procedure referred to in Article 18(2), AND (a) either the seeds originate in areas where *Clavibacter michiganensis* ssp. *michiganensis* (Smith) Davis et al. or *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye are not known to occur; OR (b) no symptoms of diseases caused by those harmful organisms have been observed on the plants at the place of production during their complete cycle of vegetation; OR (c) the seeds have been subjected to official testing for at least those harmful organisms, on a representative sample and using appropriate methods, and have been found, in these tests, free from those harmful organisms.

The Panel notes that, according Annex II, Part A, Section II, the introduction of Cmm into, and its spread within all Member States, shall be banned if present on plants of tomato intended for planting. This ban is addressed by Annex III, Part A (13), Annex IV, Part A, Section I (48) and Annex IV, Part A, Section II (27). For the introduction of Cmm into the EU via tomato seeds, this ban is covered by Annex IV, part A, section I (48). However, no special requirements have been formulated to prevent the introduction into the EU of Cmm, when present on seeds of *Capsicum* spp. The absence of such special requirements may increase the probability of spread within the EU of this pathogen.

Plants intended for planting, other than seeds of *Solanaceae*, and seeds of tomato, originating in the EU, are listed in Annex V, Part A, Section I (2) of Council Directive 2000/29/EC. This means that a plant passport is required for movement of these plants within the EU if they are produced by

producers whose production and sale is authorised to persons professionally engaged in plant production, that is, producers of *Solanaceous* plants and fruits.

Plants for planting, other than seeds of *Solanaceae*, and seeds of tomato, prepared and ready for sale to the final consumer (hobby gardeners), do not require a plant passport for movement within the EU, provided that it is ensured by the responsible official bodies of the Member States that their production is clearly separate from that of other products.

Plants intended for planting, including seeds, of *Capsicum* spp. and of tomato, originating outside the EU, are listed in Annex V, Part B, Section I (1) of Council Directive 2000/29/EC. This means that these plants must be subject to a plant health inspection in the country of origin or the consignor country before being permitted to enter the EU, and they must be accompanied by a phytosanitary certificate.

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—Plants, 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.

2.2. Plants of *Solanaceae*, other than those referred to in point 1.3, intended for planting, other than seeds.

2.4. Seeds of *Helianthus annuus* L., *Lycopersicon lycopersicum* (L.) Karsten ex Farw. and *Phaseolus* 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 [.....], *Capsicum* spp., […], *Lycopersicon lycopersicum* (L.) Karsten ex Farw. [.....].

3.5. Potential for establishment and spread in the risk assessment area

3.5.1. Availability of suitable host plants (outdoors, in protected cultivation or both)

The main host plant of Cmm, i.e. tomato, is grown in greenhouses almost everywhere in the EU (Table 2). Open-field tomato cultivation is a common practice in southern EU Member States. Some alternative hosts of Cmm are widely distributed or cultivated throughout EU Member States either in the open field or in greenhouses. This is especially the case for *C. annuum*, *C. frutescens* and *S. nigrum*.

3.5.2. Suitability of environment

The minimum, optimum and maximum temperatures for growth and survival of Cmm are 1 °C, 24-28 °C and 35 °C, respectively (Strider, 1969). Cmm is highly resistant to desiccation compared with many other plant pathogenic bacteria and can survive for at least eight months on glass. It also remains viable on seeds and dried planting material for many years (Dhanvantari and Brown, 1993; Van der Wolf et al., 2012). In dried soil, the planktonic cells of the pathogen will remain viable for seven but not as long as eight months (Strider, 1969). A strain of *C. michiganensis* isolated from peanut was highly resistant to ultraviolet light. This resistance is possibly associated with the pigments produced (Jacobs and Sundin, 2001).

The disease progresses rapidly between 24 and 32 °C (Strider, 1969). In addition, the disease develops more rapidly in soil at a water-holding capacity (WHC) of 80 % (which is optimal for growth of tomatoes) than at a WHC of 40 % or 100 %, at a low light intensity, in young plants (Sharabani et al, 2013), under high nutrient conditions, in sandy rather than organic soils (Strider, 1969) and at high rather than low relative humidity (Xu et al., 2012). When leaves of tomato plants were spray inoculated, distinct symptoms presented at a high humidity of 87–97 %, slight symptoms at 75–85 %, and no symptoms at 50–70 % (Basu, 1966).

For tomato field crops, the climatic conditions in southern Europe are favourable for disease development. In glasshouses, tomatoes are typically grown at a temperature of 25 °C, which is optimal for the development of bacterial canker. Cmm is not found in Scandinavian countries, but that may be partly a result of the smaller tomato production areas in these countries (see Table 2). Cmm is regularly reported in EU Member States indicating that, once this pest is introduced, conditions for its establishment are suitable.

3.5.3. Cultural practices

Cultivation measures may contribute to the spread of the disease, by dissemination of the pathogen, by wounding the plants and by the establishment of conditions favouring symptom expression. In the cultivation of processing tomatoes, seedlings are clipped with rotary mowers to ensure a uniform stand. This practice has resulted in a huge increase in disease incidence. Other measures favouring symptom development are overhead irrigation, chemical sprays, handling of transplants during harvest and transport, clipping and pruning to remove leaves or shoots that have developed from axillary buds and de-leafing and -suckering. Grafting on rootstocks and clipping to produce two stems have favoured the dissemination and spread of the pathogen, as these practices produce wounds on both rootstock and scion (Chang et al., 1992; Xu et al., 2010). Wounding favours infection, but Cmm can also enter the plants via natural openings such as hydathodes and those that occur during lateral root formation (Kontaxis, 1962, Sharabani et al., 2013).

The probability of infection is dependent on the inoculum dose. In greenhouse studies with stab-inoculated plants, densities of 10^3 cfu/ml, equivalent to a few cells, caused the disease on a susceptible variety (Foster and Echandi, 1973). In the case of leaf inoculations, a minimum Cmm concentration of 10^6 cfu/ml was required for symptom expression, independently of whether leaves were injured or not (Basu, 1966). Using a contaminated grafting tool, 100 % infection of rootstocks and scions was found at inoculum doses of 10^6 cfu/ml or higher, 44 % and 88 % at 10^4 cfu/ml, but none at 10^2 cfu/ml (Xu et al., 2010). In nutrient film culture of tomato plants, disease incidence was also dose dependent. For plants with unwounded roots 10^6 cfu/ml of nutrient solution were required to establish an infection, and for plants with wounded roots 10^3 cfu/ml were required (Van Vaerenbergh et al., 1985).

3.5.4. Control methods

Although several chemical and biological control agents have shown some effect on Cmm, no chemical compounds and biocontrol agents are registered in the EU to control Cmm in plants (Werner et al., 2002). Control is further hampered because of a lack of resistant or highly tolerant cultivars (Chang et al., 1992; Werner et al., 2002; De Leon et al., 2011). Control is mainly based on seed

treatments and on hygiene and cultivation measures reducing the risks of introductions and dissemination.

3.5.4.1. Plant propagation material

During seed production, seed treatments to remove fruit debris such as seed fermentation and treatments with hydrochloric and sulphuric acid can result in a considerable decrease in the percentage of infected seeds, but it is generally accepted that internally infected seeds cannot be cured (Strider, 1969; Dhanvantari, 1993; De Leon et al., 2011). The same is true for physical treatments of seeds, such as hot water treatments (Grondeau and Samson, 1994). An effective control of the disease during production of plants for planting requires management of the entire production chain.

Recently, through a collaborative action of the seed industry, inspection services and NPPOs, a system has been developed for "good seed and plant practice" (GSPP) to prevent tomato seed and plant lots from being infected by Cmm. The GSPP system can be accessed at www.gspp.eu/. For this, seed and seedling production locations are isolated from the potentially contaminated environment. The system is based on:

1. Prevention of infection by managing the main risk factors. Water used at the production site must be free of Cmm, contact infection from people working at the production site must be prevented by wearing protective clothing and disinfection procedures, all propagation material should have been produced under GSPP standards and the absence of Cmm on any materials used at the production site must be guaranteed. If required, materials should be disinfected.

2. Constant monitoring during the growing season of both seeds and young plants must guarantee the absence of Cmm.

3. Seed should be tested before delivery of all seed lots using seed tests approved by GSPP.

4. Production under GSPP standards should be monitored by independent audits.

The system is already used by 18 seed companies in Europe.

3.5.4.2. Fruit production

Management of the disease during fruit production is mainly based on the use of Cmm-free plantlets, rotation, removal of plant debris, soil disinfection by steaming, hygiene and cultivation practices that prevent introduction and spread of the disease.

3.5.5. Spread capacity

3.5.5.1. Role of seed

Seed is the main long-distance means of dissemination, and it is generally believed that global dissemination has occurred through infected tomato seed (Strider, 1969). Cmm also contaminates pepper seeds, and it has been reported that the detection rate of Cmm in imported seeds in Korea during quarantine inspections has recently increased (Yim et al., 2012). In the USA the use of infected seeds resulted in disease outbreaks in fields, in which the disease had never occurred. The incidence of infection of tomato seeds varies from < 1 % to 97 % (Strider, 1969; Chang et al., 1991; Dhanvantari and Brown, 1993). The number of Cmm cells can be up to 10^4 cfu per seed (Hadas et al., 2005; Van der Wolf et al., 2012; EPPO, 2013a). During storage, populations on seed decline, but the rate may depend on the seed lot, the storage conditions and to what extent deep-seated infections are present in the seed (Dhanvantari, 1993; Van der Wolf et al., 2012). Low densities of bacteria of 5–25 cfu per seed can result in transmission from seed to seedling (Kaneshiro and Alvarez, 2003; Van der Wolf et al., 2012). Densities higher than 600 cfu per seed can result in non-germinability of seed (Alvarez Anne, personal communication, March 2014, Plant and Environmental Protection Sciences, University of Hawaii at Manoa) and highly infected seed may therefore have a limited significance in the

epidemiology of bacterial canker. Even a seed infection incidence of 0.01 % (1 in 10 000) may result in approximately 100 infection foci per hectare and lead to epidemics if conditions are favourable for secondary spread (Chang et al., 1991; Gitaitis et al., 1991, Gleason et al., 1993). Depending on the environment, the transmission of Cmm from seed to seedling can vary from 0.25 % to 100 % (Strider, 1969; Tsiantos, 1987; Dhanvantari, 1989; Chang et al., 1992; Dhanvantari and Brown, 1993; Van der Wolf et al., 2012).

In summary, seed infection incidences can vary but often are low as a consequence of seed treatments to remove fruit debris and, less importantly, as a result of storage of seed. The efficiency of transmission from seed to seedling, however, is often high and low incidences of infected seeds with a low number of viable cells per seed can result in epidemics.

3.5.5.2. Role of plants for planting

Plants for planting can carry Cmm but remain symptomless, as reported by Gitaitis et al. (1991). From 24 000 transplants sampled from 24 commercial fields in Georgia (USA) only three were latently infected. The low infection incidence may explain why outbreaks occur only sporadically. After artificial inoculation of transplants the bacteria rapidly colonised the plants and developed infections within two days, but no symptoms were observed for 17 days. Seedlings grown from contaminated seeds can release high densities of bacteria of up to 10^7 cfu/ml via guttation (Sharabani et al., 2013). These bacteria can be dispersed during cultural practices which inflict wounds, including clipping, debudding, pruning, grafting and topping, all common practices in tomato cultivation (Chang et al., 1991). Symptoms were observed for the first time 14–20 days after transplanting (31–40 days after clipping). Every 0.1 % increase in infected seedlings resulted in an increase of 10 % of systemically infected plants. The pathogen was further spread when plants were mixed in bundles after harvesting. Symptoms due to this carry-over contamination were seen 35–42 days after transplanting.

3.5.5.3. Role of crop debris

Cmm can survive for more than two years in crop residues on the soil surface but for a shorter time when buried in the soil (Chang et al., 1992, Gleason et al., 1993). Trevors and Finnen (1990) found that survival can be strain dependent. Cmm has been found in overwintered crop residues in different climate zones (Farley, 1971). The persistence of Cmm in soil is remarkable, because the pathogen is sensitive to many antibiotics, including those produced by other bacteria and actinomycetes found in soil (Strider, 1969), and also to some secondary metabolites produced by plants such as essential oils (Van der Wolf et al., 2008). From bacteria isolated from soil or the rhizosphere, 10 % and 80 %, respectively, of the bacteria tested produced antimicrobial compounds against Cmm (Boudyach et al., 2001). Obviously, survival time is long enough to establish infections on seedlings in the following season if crop rotation is not practised. The role of crop debris as a primary source of inoculum has been supported by studies using molecular fingerprinting techniques, showing that similar haplotypes were found in symptomatic plants from the same location in different years (Kleitman et al., 2008; De Leon et al., 2009; Kawaguchi et al., 2010).

3.5.5.4. Seeds and planting material versus crop debris

The recent occurrence of bacterial canker in European regions where the disease had never been known previously indicates that the risks of new introductions through the use of infected seeds and/or planting material are still considerable (De Leon et al., 2011). However, in areas were the pathogen was already established, it was likely that infected crop debris served as the primary infection source in follow-on crops. The relative importance of these primary infection sources is illustrated by the following examples.

In Cyprus, the pathogen was probably introduced for the first time via infected seed produced in Israel (Ioannou et al., 2000). In the Canary Islands, the pathogen was found for the first time in 2002 and displayed low polymorphism, indicating a recent and limited number of introductions (De Leon et al., 2009). The disease was probably introduced via infected seed. In Serbia, outbreaks were found in the

period between 2006 and 2008. Use of genetic fingerprinting methods indicated that in some cases the pathogen survived in greenhouses but also that new introductions occurred via seeds or plants for planting (Milijašević-Marčić et al., 2012). Similar results were found in studies with strains from Israel isolated between 1998 and 2005 (Kleitman et al., 2008). In Turkey, using strains from outbreaks between 2005 and 2009 from different production fields, strains could be divided into five genetic clades, indicating multiple introductions probably via seeds or plantlets, but in other cases strains were genetically related indicating local spread (Baysal et al., 2011). In Michigan (USA), outbreaks were recorded in 1997 and 1998. The disease was found in plant beds at the same production location in two subsequent years and the haplotype isolated was identical (Quesada-Ocampo et al., 2012). It was concluded that plant debris was the source of inoculum, despite the fact that the soil was fumigated. The problem with bacterial canker was not eliminated after the growers started to use indoor plantlets instead of outdoor plantlets. In other cases it was suggested that the outbreaks were seed borne as the possibility of other infection sources could be largely eliminated. In recent years in Japan, bacterial canker has emerged in commercial greenhouses in some areas (Kawaguchi et al., 2013). The strains isolated in different years from the same greenhouse and location belonged to one haplotype, indicating that infections originated from the previous greenhouse population (Kawaguchi et al., 2010). This was confirmed by other studies on the spatiotemporal distribution of disease, in which a scattered pattern of clusters of diseased plants was observed, indicating residual plant debris in soil as the primary inoculum (Kawaguchi et al., 2013).

3.5.5.5. Other means of dispersal

Cmm is spread by splashing water, during tying, staking and harvesting, during spraying with pesticides and on clothes during crop handling, particularly following guttation and where free water is available. In nutrient film technique (NFT) culture of tomatoes growing in recirculating nutrient solution, it was found that levels of 10^3–10^6 cfu/ml were sufficient to initiate infections, independent of the plant development stage but dependent on whether roots were damaged or not (Van Vaerenbergh et al., 1985; Griesbach and Lattauschke, 1991). The presence of Cmm in the nutrient solution used in NFT culture was believed to be a result of a release of cells from the plants rather than of bacterial growth in the nutrient solution (Van Vaerenbergh et al., 1985). The efficiency of transfer may be dependent on the environmental conditions. However, in similar experiments with NFT culture in the Netherlands, in which infected plants were positioned in the centre of a table, there was no transfer from infected to Cmm-free plants (Van der Wolf et al., 2012).

In tomato field crops, aerosols, insects and wind-driven rain might be a source of dispersal for Cmm, but little information is available. No spread of Cmm was found with the green peach aphid (*Myzus persicae*), onion thrips (*Thrips tabaci*), spotted cucumber beetles (*Diabrotica undecimpunctata*) and tarnish plant bugs (*Lygus lineolaris*) (Ark, 1944, cited by Strider, 1969). In studies by Chang et al. (1992) in a field crop planted in Illinois (USA), the distance over which Cmm was spread from a point inoculum was only several metres over a period of 2.5 months. This indicates that long-distance spread by means other than seed and planting material is unlikely.

Cmm can be detected epiphytically on hosts, including volunteer tomato plants (Strider, 1969; Gleason et al., 1991, 1993; Chang et al, 1992). Levels of detection on tomato were up to 10^9 cfu/g, on solanaceous hosts up to 10^5 cfu/g and on non-hosts up to 10^3 cfu/g of fresh weight when they were planted near to artificially inoculated, symptomatic plants. On tomato, higher concentrations were found on young leaves, more susceptible cultivars and under high-moisture conditions (Strider, 1969; Gleason et al., 1993). The role of the epiphytic populations in the epidemiology of the pathogen is not clear, but it is likely that these are involved in secondary spread.

3.6. Potential for consequences in the risk assessment area

3.6.1. Pest effects on host plants

Disease outbreaks of bacterial canker in tomato are sporadic, but the consequences can be considerable in both greenhouse and field crops. The pathogen poses a constant threat to tomato production because it is seed borne, it persists for a relatively long time in association with crop debris from where it can infect (wounded) roots, it is readily spread during production practices in tomato cultivation, it becomes rapidly systemic, there are no curative treatments available, and there is no tolerance of practical importance in commercial cultivars, with plants often dying following infection.

3.6.1.1. Field crops

In the EU territory, several incidences of bacterial canker in field crops have been described that resulted in considerable losses. In Cyprus, the disease was found for the first time in 1998 with incidences of up to 90 %. In 2010, for the first time in Italy, widespread outbreaks of bacterial canker were found. More than 300 ha of tomato fields were affected, with disease incidences ranging from 70 % to 100 %, resulting in severe losses (Lamichhane, et al., 2011).

Outside the EU territory, high losses due to bacterial canker in tomato field crops have been reported. In the USA, epidemics occurred from the 1930s up to the 1980s, resulting in yield losses of up to 80 % for individual growers and 10 % regionally (Gleason et al., 1993). In the 1950s the pathogen almost destroyed the tomato industry in North Carolina (Strider, 1969). Seeds were often found to be the primary source of infection, but transmission from infected plant debris to seedlings also resulted in yield losses of up to 27 % (Gleason et al., 1993). In East Africa, high losses of up to 80 % for individual farmers were found in field crops (Strider, 1969). In the south-west of India, infected production fields were found during surveys, with disease incidences between 25 % and 48 %, indicating that the disease can also occur in hot climates. In Turkey in 2011, high losses and a disease incidence of almost 100 % were found in commercial fields (Baysal et al., 2011). Disease outbreaks were associated with the use of infected seeds (Umesha, 2006).

Yields can also be affected by secondary spread of the pathogen. For plants in a field crop infected during clipping, yield losses of as much as 46 % were found due to systemic infections (Chang et al., 1992). Yield loss is dependent on the time of inoculation. For trellised tomatoes in a field crop losses were approximately 90 % if artificially infected seeds were used, 100 % if inoculated during transplanting, approximately 99 % if inoculated at the first pruning, and 60 % if inoculated at the first flowering, whereas no effect was found if infection occurred during the first harvest (Dullahide et al., 1983). The damage can be further influenced by interactions with other organisms. Disease is, for example, more severe in the presence of *Meloidogyne incognita* (De Moura et al., 1975).

3.6.1.2. Glasshouses

In greenhouse crops, secondary spread will not always result in yield losses, even if symptoms appear (Ricker and Riedel, 1993). However, in the EU territory regular outbreaks of bacterial canker in glasshouses resulting in economic damage have been reported. In 2013, Cmm was found on six farms in Italy with a total glasshouse surface of 16 000 m². It was suspected that infected seeds or substrate was the cause (EPPO, 2013b). Although in the Netherlands the incidence of Cmm-infected crops has gradually declined, incidental infections still occur. In 2014, suspected tomato plants were found on two fruit production company sites (www.ippc.int). Outside the EU, high losses in glasshouse crops were reported in Canada in 1965 (Strider, 1969).

3.6.2. Environmental consequences

There are no observed consequences of bacterial canker of tomato for the environment. Indeed, there is no chemical treatment in use in EU Member States to control this disease, and no potential indirect consequences for the environment are suspected.

3.7. Conclusions on the pest categorisation

Identity of the pest

Cmm is the causative agent of bacterial canker of tomato. The organism under assessment is a clear, distinguished taxonomic entity and can be accurately identified based on a range of discriminatory methods. Cmm can cause a wide variety of symptoms on host plants, which include (unilateral) wilting, stem cankers, chlorotic leaf spots, desiccation of leaf margins and bird's eye spots on the fruits. Some symptoms may be confused with those caused by other organisms.

Presence in the risk assessment area

The pathogen is found in 16 EU Member States, but, with the exception of Greece and Italy, the population is transient, with restricted distribution or low prevalence; outbreaks have been found only in some years and in specific areas. Only Greece reports that the pathogen is widespread. In Denmark, Estonia, Finland, Ireland, Malta, Portugal, the Slovak Republic, Sweden, Iceland and Norway the disease has never been reported or the pathogen has been eradicated. No data are available from Croatia and Luxembourg.

Regulatory status

The pathogen is listed in Council Directive 2000/29/EC, Annex II A II, as a harmful organism, known to occur in the Community and relevant for the entire Community, whose introduction into, and spread within, all Member States shall be banned if present on certain plants or plant products. Measures regulating the import into and movement within the EU of potentially infested host plants include special requirements with respect to Cmm for specified plant material, prohibition of import for specified plants from specified third countries and official control of host plant material produced within the EU for use by professional producers of plants and fruits.

The Panel notes that there are no regulatory special requirements in place with respect to Cmm for the movement within the EU of seeds of *Capsicum*. This may increase the probability of spread of the pathogen on this commodity.

Potential for establishment and spread in the risk assessment area

Tomato, the most important host of Cmm is one of the major vegetable crops in Europe that is grown in all Member States. Other natural hosts of Cmm are pepper and some solanaceous weeds, but in these hosts Cmm is found only sporadically. Epiphytic populations of Cmm on a broad range of plants can be detected in the event of disease outbreaks and these may play a role in secondary spread.

The pathogen can occur in both glasshouses and field crops. The environmental conditions in southern Europe are particularly favourable for disease expression in the field, as the optimal growth temperature for Cmm is between 24 °C and 28 °C. Although the pathogen has been found in many EU Member States, infections occur only sporadically.

The pathogen is seed borne and seed is considered to be the major means of long-distance dispersal. The pathogen can survive for years on seed, and a low inoculum dose of a few cells can result in transmission from seed to seedling. A few infection loci can lead to outbreaks. Transplants can also be a primary infection source and can serve as a means of long-distance dispersal. At production sites, tomato volunteer plants and infected soil and crop debris, in which Cmm can survive, are recognised as a source of inoculum. Cultivation practices including clipping and pruning contribute considerably to the rapid spread of the pathogen in a crop.

Control is mainly based on prevention and exclusion. Detection methods are available for any type of plant material, whether presenting symptoms or symptomless. In Europe, seed production is done under strict sanitation controls using recommended practices to avoid seed contamination. Seed testing has proven to be a good control option as contaminated seed lots are discarded. Extraction of seed

from fruit debris using fermentation and acid treatments reduces Cmm populations, but internal infections cannot be eliminated by seed treatments. No methods or chemical control agents are available that effectively control Cmm in infected crops. There are no commercial cultivars available with resistance or an acceptable level of tolerance.

Potential for consequences in the risk assessment area

The pathogen is considered to be one of the most important bacterial pathogens of tomato and pepper and can be very destructive. Infections often result in high yield losses; in several cases losses of between 50 % and 100 % have been reported. However, growers and the seed industry are putting considerable efforts into preventing the introduction and dissemination of Cmm. Production systems involving integral testing of tomato seed and transplants using validated protocols are used by the tomato seed companies and nurseries. These largely exclude the introduction and spread of Cmm by propagation material. This has resulted in a considerable reduction in crop damage and may be considered an effective way of controlling the disease.

Cmm meets the following ISPM 11 criteria:

Identity of the pest: The identity of the pest is clearly defined.

Presence or absence in the risk assessment area: Cmm has been observed in 16 EU Member States. However, the outbreaks are sporadic but usually severe.

Regulatory status: The pest is under official control.

Potential for establishment and spread in the risk assessment area: The risk assessment area has ecological/climatic conditions, including those in protected cultivation, that are suitable for the establishment and spread of the pest, and host species are present in the risk assessment area.

Potential for economic consequences (including environmental consequences) in the risk assessment area: The pathogen is considered to be one of the most important bacterial pathogens of tomato and pepper and can be very destructive.

Cmm meets all criteria defined in ISPM 21. Cmm is a seed-borne bacterium and can be present in plants for planting (seed and transplants), which has considerable impact on the intended use of those plants.

No major uncertainties were identified within the pest categorisation.

DOCUMENTATION PROVIDED TO EFSA

1. Request to provide a scientific opinion on the risk to plant health of 13 regulated harmful organisms, for the EU territory. Ref. Ares(2012)880155—19/07/2012. Submitted by the European Commission, DG SANCO (Directorate General for Health and Consumers).

2. Request to provide a scientific opinion on the risk to plant health of 38 regulated harmful organisms, for the EU territory. Ref. Ares(2014)970361—28/03/2014. Submitted by the European Commission, DG SANCO (Directorate General for Health and Consumers).

REFERENCES

Ark PA, 1944. Studies on bacterial canker of tomato. Phytopathology, 34, 394–400.

Basu PK, 1966. Conditions for symptomatological differentiation on bacterial canker, spot, and spec on tomato seedlings. Canadian Journal of Plant Science, 46, 525–530.

Baysal Ö, Mercati F, Ikten H, Çetinkaya Yıldız R, Carimi F, Aysan Y and da Silva JAT, 2011. *Clavibacter michiganensis* subsp. *michiganesis*: tracking strains using their genetic differentiations by ISSR markers in Southern Turkey. Physiological and Molecular Plant Pathology, 75, 113–119.

Berendsen SMH, Koenraadt H, Woudt B and Oosterhof J, 2011. The development of a specific real time TaqMan for the detection of *Clavibacter michiganensis* subsp. *michiganensis*. Presented at the American Phytopathological Society–International Plant Protection Convention Meeting, Honolulu, Hawaii, 6–10 August 2011.

Boudyach EH, Fatmi M, Akhayat O, Benizri E and Ait Ben Aoumar A, 2001. Selection of antagonistic bacteria of *Clavibacter michiganensis* subsp. *michiganensis* and evaluation of their efficiency against bacterial canker of tomato. Biocontrol Science and Technology, 11, 141–149.

Bradbury JF, 1986. Guide to plant pathogenic bacteria. CAB International, Wallingford, UK, 332 pp.

Bryan MK, 1930. Studies on bacterial canker on tomato. Journal of Agricultural Research, 41, 825–851.

CABI (CAB International), 2014. Crop protection compendium. CAB International, Wallingford, UK. Available online: www.cabi.org/cpc/

Carlson RR and Vidaver AK, 1982. Taxonomy of *Corynebacterium* plant pathogens, including a new pathogen of wheat, based on polyacrylamide gel electrophoresis of cellular proteins. International Journal of Systematic Bacteriology, 32, 315–326.

Carlton WM, Gleason ML and Braun EJ, 1994. Effects of pruning on tomato plants supporting epiphytic populations of *Clavibacter michiganensis* subsp. *michiganensis*. Plant Disease, 78, 742–745.

Carlton WM, Braun EJ and Gleason ML, 1998. Ingress of *Clavibacter michiganensis* subsp. *michiganensis* into tomato leaves through hydathodes. Phytopathology, 88, 525–529.

Chang R, Ries S and Pataky J, 1991. Dissemination of *Clavibacter michiganensis* subsp. *michiganensis* by practices used to produce tomato transplants. Phytopathology, 81, 1276–1281.

Chang RJ, Ries SM and Pataky JK, 1992. Local sources of *Clavibacter michiganensis* subsp. *michiganensis* in the development of bacterial canker on tomatoes. Phytopathology, 82, 553–560.

Davis MJ, Gillaspie AG, Vidaver AK and Harris RW, 1984. *Clavibacter* a new genus containing some phytopathogenic coryneform bacteria, including *Clavibacter xyli* subsp. *xyli* sp. nov., subsp. nov. and *Clavibacter xyli* subsp. *cynodontis* subsp. nov., pathogens that cause ratoon stunting disease of sugarcane and bermudagrass stunting disease. International Journal of Systematic Bacteriology, 34, 107–117.

De Leon L, Rodriguez A, Llop P, Lopez MM and Siverio F, 2009. Comparative study of genetic diversity of *Clavibacter michiganensis* subsp. *michiganensis* isolates from the Canary Islands by RAPD-PCR, BOX-PCR and AFLP. Plant Pathology, 58, 862–871.

De Leon L, Siverio F, Lopez MM and Rodriguez A, 2011. *Clavibacter michiganensis* subsp. *michiganensis*, a seedborne tomato pathogen: healthy seeds are still the goal. Plant Disease, 95, 1328–1339.

De Moura RM, 1995. Interaction of *Corynebacterium michiganense* and *Meloidigyne incognita* on tomato. Phytopathology, 65, 1332–1335.

Dhanvantari BN, 1989. Effect of seed extraction methods and seed treatments on control of tomato bacterial canker, Canadian Journal of Plant Pathology, 11, 400–408.

Dhanvantari, BN, 1993. Studies on bacterial spot of sweet pepper. Agriculture Canada, Ontario vegetable crop research. 2 pp. Available online: https://atrium.lib.uoguelph.ca/xmlui/bitstream/handle/10214/5389

Dhanvantari BN and Brown RJ, 1993. Improved seed treatments for control of bacterial canker of tomato. Canadian Journal of Plant Pathology, 15, 201–205.

Dreier J, Bermpohl A and Eichenlaub R, 1995. Southern hybridization and PCR for specific detection of phytopathogenic *Clavibacter michiganensis* subsp. *michiganensis*. Phytopathology, 85, 462–468.

Dullahide SR, Moffett ML, Heaton JB and Giles J, 1983. Effect of time of inoculation of *Corynebacterium michiganense* subsp. *michiganense* on yield of trellised tomatoes. Australian Plant Pathology, 12, 15–16.

EFSA PLH Panel (EFSA Panel on Plant Health), 2010. Guidance on a harmonised framework for pest risk assessment and the identification and evaluation of pest risk management options by EFSA. EFSA Journal 8(2):1495, 66 pp. doi:10.2093/j.efsa.2010.1495

Ehrig F and Griesbach E, 1985. Development of bird eye spots on tomato fruits—investigations by scanning electron microscopy. Archiv fur Phytopathologie und Pflanzenschutz, 21, 419–423.

EPPO (European and Mediterranean Plant Protection Organization), 2013a. EPPO Standards Diagnostic PM 7/42 (2) *Clavibacter michiganensis* subsp. *michiganensis*. EPPO Bulletin 43, 46–67.

EPPO (European and Mediterranean Plant Protection Organization), 2013b. Outbreaks of *Clavibacter michiganensis* subsp. *michiganensis* in Sardegna and Lazio (IT). EPPO Reporting Service No 05, 4–5.

EPPO (European and Mediterranean Plant Protection Organization), online. EPPO-PQR (Plant Quarantine Data Retrieval system). Available online: www.eppo.int/DATABASES/pqr/pqr.htm

FAO (Food and Agriculture Organization of the United Nations), 2004. ISPM 21: International standards for phytosanitary measures; pest risk analysis of regulated non-quarantine pests. FAO, Rome. Available at: https://www.ippc.int/sites/default/files/documents//1323945746_ISPM_21_2004_En_2011-11-29_Refor.pdf

FAO (Food and Agriculture Organization of the United Nations), 2013. ISPM 11: International standards for phytosanitary measures; Pest risk analysis for quarantine pests. FAO, Rome. Available at: https://www.ippc.int/sites/default/files/documents/20140512/ispm_11_2013_en_2014-04-30_201405121523--494.65%20KB.pdf

Farley JD, 1971. Recovery of *Corynebacterium michiganense* from overwintered tomato stems by the excised petiole inoculation method. Plant Disease Reporter, 55, 654–656.

Fatmi M, Schaad NW and Bolkan HA, 1991. Seed treatments for eradicating *Clavibacter michiganensis* subsp. *michiganensis* from naturally infected tomato seeds. Plant Disease, 75, 383–385.

Foster RL and Echandi E, 1973. Relation of age of plants, temperature, and inoculum concentration to bacteria canker development in resistant and susceptible *Lycopersicon* spp. Phytopathology, 63, 773–777.

Franken AAJM, Kamminga GC, Snyders W, Van Der Zouwen PS and Birnbaum YE, 1993. Detection of *Clavibacter michiganensis* ssp. *michiganensis* in tomato seeds by immunofluorescence microscopy and dilution plating. Netherlands Journal of Plant Pathology, 99, 125–137.

Gitaitis R, Beaver RW and Voloudakis AE, 1991. Detection of *Clavibacter michiganensis* subsp. *michiganensis* in symptomless tomato transplants. Plant Disease, 75, 834–838.

Gleason ML, Braun EJ, Carlton WM and Peterson RH, 1991. Survival and dissemination of *Clavibacter michiganensis* subsp. *michiganensis* in tomatoes. Phytopathology, 81, 1519–1523.

Gleason ML, Gitaitis RD and Ricker MD, 1993. Recent progress in understanding and controlling bacterial canker of tomato in eastern North America. Plant Disease, 77, 1069–1076.

Griesbach E and Lattauschke E, 1991. Ubertragung von *Clavibacter michiganensis* subsp. *michiganensis* in Tomaten_Hydroponikkulturen und Moglichkeiten zur Bekampfung des Erregers. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, 43, 69–73.

Grondeau C and Samson R, 1994. A review of thermotherapy to free plant materials from pathogens, especially seeds from bacteria. Critical Review in Plant Sciences, 13, 57–75.

Hadas R, Kritzman G, Klietman F, Gefen T and Manulis S, 2005. Comparison of extraction procedures and determination of the detection threshold for *Clavibacter michiganensis* subsp. *michiganensis* in tomato seeds. Plant Pathology, 54, 643– 649.

Hassan AA, Strider DL and Konsler TR, 1968. Application of cotyledonary symptoms in screening for resistance to tomato bacterial canker and in host range studies. Phytopathology, 58, 233–239.

Huang R and Tu JC, 2001. Effects of nutrient solution pH on the survival and transmission of *Clavibacter michiganensis* ssp. *michiganensis* in hydroponically grown tomatoes. Plant Pathology 50, 503–508.

Hucorne P, 2012. The actual distribution of crops in Europe. 36 pp. Available online: www.eppo.int/PPPRODUCTS/zonal_efficacy/zonal_map.pdf

Ioannou N, Psallidas PG and Glynos P, 2000. First record of bacterial canker (*Clavibacter michiganensis* ssp. *michiganensis*) on tomato in Cyprus. Journal of Phytopathology, 148, 383–386.

ISF (International Seed Federation), 2014. Method for the detection of *Clavibacter michiganensis* subsp. *michiganensis*. Version 4.1., January 2014. 10 pp. Available online: www.worldseed.org/cms/medias/file/TradeIssues/PhytosanitaryMatters/SeedHealthTesting/ISHI-Veg/Tomato_Cmm_Jan_2014.pdf

Jacobs JL and Sundin GW, 2001. Effect of solar UV-B radiation on a phyllosphere bacterial community. Applied and Environmental Microbiology, 67, 5488.

Jacques MA, Durand K, Orgeur G, Balidas S, Fricot C, Bonneau S, Quilévéré A, Audusseau C, Olivier V, Grimault V and Mathis R, 2012. Phylogenetic analysis and polyphasic characterization of *Clavibacter michiganensis* strains isolated from tomato seeds reveal that non-pathogenic strains are distinct from *C. michiganensis* subsp. *michiganensis*. Applied and Environmental Microbiology, 78, 8388–8402.

Kaneshiro WS, and Alvarez AM, 2003. Variability and survival of *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) in seed. Phytopathology, 93, 43.

Kawaguchi A, Tanina K and Inoue K, 2010. Molecular typing and spread of *Clavibacter michiganensis* subsp. *michiganensis* in greenhouses in Japan. Plant Pathology, 59, 76–83.

Kawaguchi A, Tanina K and Inoue K, 2013. Spatiotemporal distribution of tomato plants naturally infected with bacterial canker in greenhouses. Journal of General Plant Pathology, 79, 46–50.

Kendrick JB Jr and Walker JC, 1948. Predisposition of tomato to bacterial canker. Journal of Agricultural Research, 77, 169–186.

Kleitman F, Barash I, Burger A, Iraki N, Falah Y, Guido S, Weinthal D, Chalupowicz L, Gartemann K-H, Eichenlaub R and Manulis-Sasson S, 2008. Characterization of a *Clavibacter michiganensis* subsp. *michiganensis* population in Israel. European Journal of Plant Pathology, 121, 463–475.

Kontaxis DG, 1962. Leaf trichomes as avenues for infection by *Corynebacterium michiganense*. Phytopathology, 52, 1306–1307.

Lai M, 1976. Bacterial canker of bell pepper caused by *Corynebacterium michiganense*. Plant Disease Reporter, 60, 339–342.

Lamichhane JR, Balestra GM and Varvaro L, 2011. Severe outbreaks of bacterial canker caused by *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis et al. on tomato in central Italy. Plant Disease, 95, 221.

Latin R, Tikhonova I and Rane K, 1995. First report of bacterial canker of pepper in Indiana. Plant Disease, 79, 860.

Layne REC, 1967. Foliar trichomes and their importance as infection sites for *Corynebcaterium michiganense* on tomato. Phytopathology, 57, 981–985.

Lewis-Ivey ML and Miller SA, 2000. First report of bacterial canker of pepper in Ohio. Plant Disease, 84, 810–811.

Medina-Mora CM, Hausbeck MK and Fulbright DW, 2001. Bird's eye lesions of tomato fruit produced by aerosol and direct application of *Clavibacter michiganensis* subsp. *michiganensis*. Plant Disease, 85, 88–91.

Milijašević-Marčić S, Gartemann K-H, Frohwitter J, Eichenlaub R, Todorović B, Rekanović E and Potočnik I, 2012. Characterization of *Clavibacter michiganensis* subsp. *michiganensis* strains from recent outbreaks of bacterial wilt and canker in Serbia. European Journal of Plant Pathology, 134, 697–711.

Moffett ML and Wood BA, 1984. Survival of *Corynebacterium michiganense* subsp. *michiganense* within host debris in soil. Australian Plant Pathology, 13, 1–3.

Pastrik KH and Rainey FA, 1999. Identification and differentiation of *Clavibacter michiganensis* subspecies by polymerase chain reaction-based techniques. Journal of Phytopathology, 147, 687–693.

Quesada-Ocampo LM, Landers NA, Lebeis AC, Fulbright DW and Hausbeck MK, 2012. Genetic structure of *Clavibacter michiganensis* subsp. *michiganensis* populations in Michigan commercial tomato fields. Plant Disease, 96, 788–796.

Ricker MD and Riedel RM, 1993. Effect of secondary spread of *Clavibacter michiganensis* subsp. *michiganensis* on yield of northern processing tomatoes. Plant Disease, 77, 364–366.

Saddler, GS and Kerr EM, 2012. "Genus V. *Clavibacter*." In: Bergey's manual of systematic bacteriology, 2nd ed. Vol. 5: The Actinobacteria, Part A. Eds Goodfellow M, Kampfer P, Busse HJ, Trujillo ME, Suzuki K, Ludwig W and Whitman WB. Springer, New York, 877–883.

Santos MS, Cruz L, Norskov P, Rasmussen OF, 1997. A rapid and sensitive detection of *Clavibacter michiganensis* subsp. *michiganensis* in tomato seeds by polymerase chain reaction. Seed Science and Technology, 25, 581–584.

Sharabani G, Manulis-Sasson S, Borenstein M, Shulhani R, Lofthouse M, Chalupowicz L and Shtienberg D, 2013. The significance of guttation in the secondary spread of *Clavibacter michiganensis* subsp. *michiganensis* in tomato Greenhouses. Plant Pathology, 62, 578–586.

Smith EF, 1910. A new tomato disease of economic importance. Science, 803, 794–796.

Stamova L and Sotirova V, 1987. Reaction of different crops to artificial inoculation with *Corynebacterium michiganense* (E.F. Sm.) H.L. Jensen. Archiv fur Phytopathologie und Pflanzenschutz, 23, 211–216.

Strider DL, 1969. Bacterial canker of tomato caused by *Corynebacterium michiganense*. A literature review and bibliography. Technical Bulletin No 193, North Carolina Agricultural Experiment Station, Raleigh, North Carolina,110 pp.

Tancos MA, Chalupowicz L, Barash I, Manulis-Sasson S and Smart CD, 2013. Tomato fruit and seed colonization by *Clavibacter michiganensis* subsp. *michiganensis* through external and internal routes. Applied and Environmental Microbiology, 79, 6948–6957.

Thyr BD, Samuel MJ and Brown PG, 1975. New solanaceous host records for *Corynebacterium michiganense*. Plant Disease Reporter, 59, 595–598.

Trevors JT, Finnen RL, 1990. Introduction and recovery of *Clavibacter michiganensis* subsp. *michiganensis* from agricultural soil. Plant and Soil, 126, 141–143.

Tsiantos J, 1987. Transmission of *Corynebacterium michiganense* pv. *michiganense* by seeds. Journal of Phytopathology, 119, 142–146.

Umesha S, 2006. Occurrence of bacterial canker in tomato fields of Karnataka and effect of biological seed treatment on disease incidence. Crop Protection, 25, 375–381.

Van der Wolf JM, Birnbaum Y, Van der Zouwen PS and Groot SPC, 2008. Disinfection of vegetable seed by treatment with essential oils, organic acids and plant extracts. Seed Science and Technology, 36, 76–88.

Van der Wolf JM, Van der Zouwen PS, Ludeking D, Hamelink MR and Schenk M, 2012. Onderzoeksverslag 'Distributie van *Clavibacter michiganensis* subsp. *michiganensis* in tomatenplanten'. Plant Research International, onderdeel van Wageningen UR, Business Unit Bio-interacties en Plantgezondheid, Rapport 448. Wageningen, Netherlands, 1–31.

Van Vaerenbergh J, Jamart G and Kamoen O, 1985. Epidemiology of *Corynebacterium michiganense* in NFT tomato. Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent, 50/3a. 997–1013.

Vieira Lelis FM, Czajkowski R, de Souza RM, Ribeiro DH and Wolf JM, 2014. Studies on the colonization of axenically grown tomato plants by a GFP-tagged strain of *Clavibacter michiganensis* subsp. *michiganensis*. European Journal of Plant Pathology, 139, 53–66.

Volcani Z, Zutra D and Cohn R, 1970. A new leaf and fruit spot disease caused by *Corynebacterium michiganense*. Plant Disease Reporter, 54, 804–806.

Werner NA, Fulbright DW, Podolsky R, Bell J and Hausbeck MK, 2002. Limiting populations and spread of *Clavibacter michiganensis* subsp. *michiganensis* on seedling tomatoes in the greenhouse. Plant Disease, 86, 535–542.

Xu X, Miller SA, Baysal-Gurel F, Gartemann K-H, Eichenlaub R and Rajashekara G, 2010. Bioluminescence imaging of *Clavibacter michiganensis* subsp. *michiganensis* infection of tomato seeds and plants. Applied and Environmental Microbiology, 76 (12), 3978–3988.

Xu X, Rajashekara G, Paul PA, and Miller SA, 2012. Colonization of tomato seedlings by bioluminescent *Clavibacter michiganensis* subsp. *michiganensis* under different humidity regimes. Phytopathology, 102, 177–184.

Yim KO, Lee HI, Kim JH, Lee SD, Cho JH and Cha JS, 2012. Characterization of phenotypic variants of *Clavibacter michiganensis* subsp. *michiganensis* isolated from *Capsicum annuum*. European Journal of Plant Pathology, 133, 559–575.