

EPPO Datasheet: *Clavibacter insidiosus*

Last updated: 2024-01-15

IDENTITY

Preferred name: *Clavibacter insidiosus*

Authority: (McCulloch) Li et al.

Taxonomic position: Bacteria: Actinobacteria: Micrococcales: Microbacteriaceae

Other scientific names: *Aplanobacter insidiosum* McCulloch, *Clavibacter michiganensis* subsp. *insidiosus* (McCulloch) Davis et al., *Corynebacterium insidiosum* (McCulloch) Jensen, *Corynebacterium michiganense* pv. *insidiosum* (McCulloch) Dye & Kemp

Common names: bacterial blight of lucerne, bacterial root rot of lucerne, bacterial wilt of lucerne, vascular wilt of lucerne

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EPPO Categorization: A2 list

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EU Categorization: RNQP ((EU) 2019/2072 Annex IV)

EPPO Code: CORBIN



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Notes on taxonomy and nomenclature

In 1984 Davis *et al.* proposed the genus *Clavibacter* and moved several *Corynebacterium* species, including *Corynebacterium michiganense*, into this new genus. This movement changed the name of *Corynebacterium michiganense* subsp. *insidiosum* into *Clavibacter michiganense* subsp. *insidiosum* which was later corrected to *Clavibacter michiganensis* subsp. *insidiosus* (Zgurskaya *et al.*, 1993). In 2018 Li *et al.* elevated this subspecies to species rank based on whole genome data.

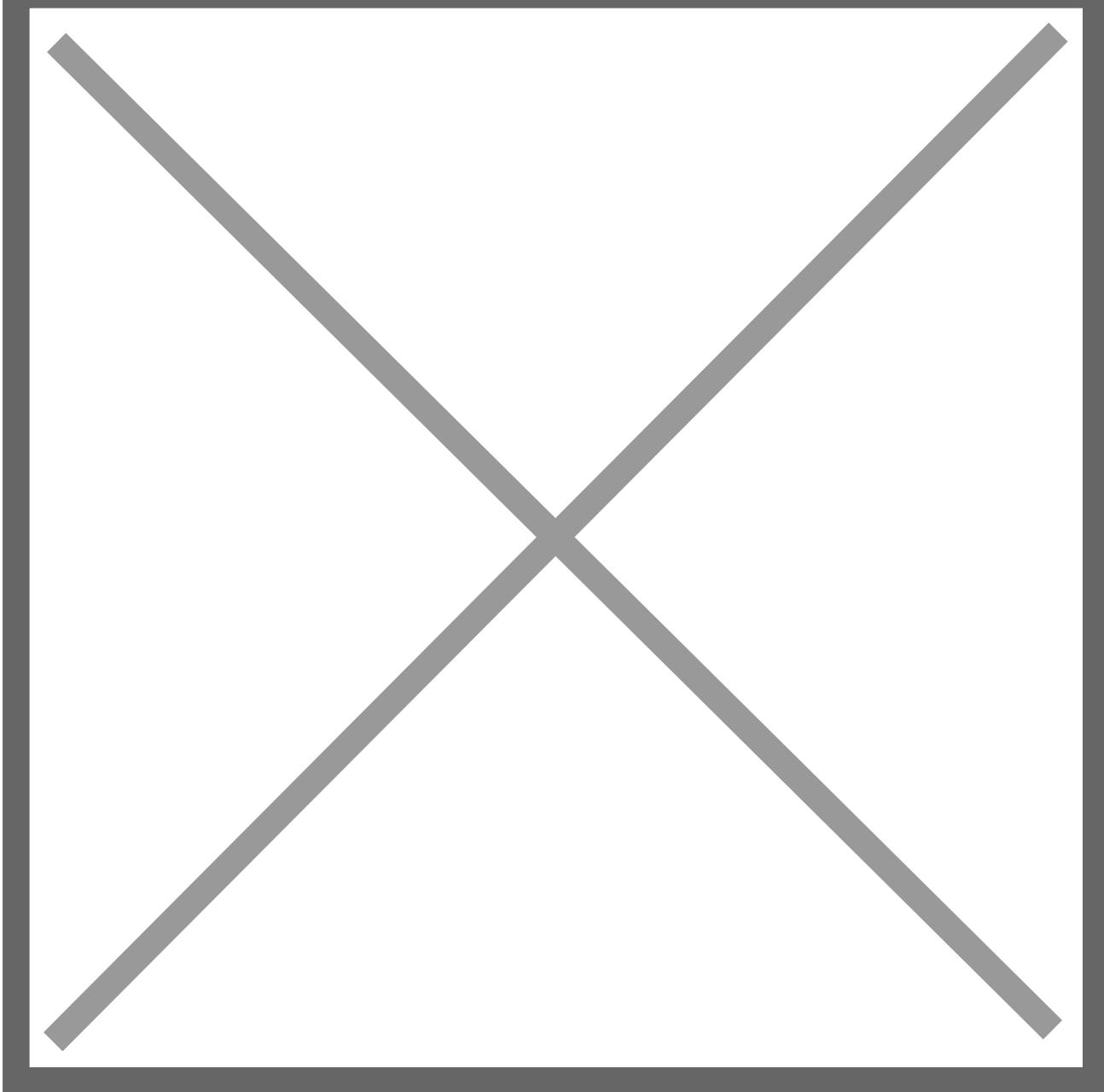
HOSTS

The main host of *C. insidiosus* is *Medicago sativa* (lucerne or alfalfa). Other *Medicago* species such as *M. falcata* and *M. truncatula* have been reported as natural hosts of this bacterium as well. *C. insidiosus* has also been found on *Melilotus albus*, *Onobrychis viciifolia*, *Lotus corniculatus* and *Trifolium* sp. (Bradbury, 1986; Lu *et al.*, 2015). On inoculation, *C. insidiosus* can induce symptoms in several other *Medicago* species. A report of *C. insidiosus* on *Ze a mays* is considered doubtful (Bradbury, 1986).

Host list: *Lotus corniculatus*, *Medicago falcata*, *Medicago sativa*, *Medicago truncatula*, *Melilotus albus*, *Onobrychis viciifolia*, *Trifolium* sp.

GEOGRAPHICAL DISTRIBUTION

Lucerne plants with symptoms caused by *C. insidiosus* were first reported in Illinois and Wisconsin in 1924 rapidly followed by reports from other states throughout the USA, indicating that the pathogen was already widespread before this first report (Jones, 1925; Jones and McCulloch, 1926). During the remainder of the 20th century, the pathogen was found in the most important lucerne production areas in the USA and Canada, and was also detected in other continents. In several countries with historical findings of *C. insidiosus*, the disease no longer occurs (e.g. in Brazil, Canada, South Africa). In the EPPO region, findings were mainly sporadic and since the 1980s, the disease is either no longer found or no significant outbreaks have been reported.



EPPO Region: Kazakhstan, Lithuania, Poland, Russian Federation (Central Russia, Southern Russia), Tunisia, United Kingdom

Africa: Tunisia

Asia: Iran, Islamic Republic of, Kazakhstan, Saudi Arabia, Turkmenistan

North America: Mexico, United States of America (Arkansas, California, Iowa, Kansas, Kentucky, Maryland, Minnesota, Montana, Nebraska, Nevada, New York, North Dakota, Ohio, Oklahoma, Oregon, South Dakota, Texas, Utah, Washington, Wisconsin)

Oceania: Australia (New South Wales, South Australia, Tasmania, Victoria), New Zealand

BIOLOGY

The bacterium can enter its host through wounds, such as mowing wounds, wounds caused by freezing and thawing, or feeding wounds caused by nematodes or insects. *C. insidiosus* usually spreads from plant to plant in water. Following entry into the plant, the bacterium is able to spread rapidly through the vascular system. *C. insidiosus* is thought to be primarily a disease of the perennial plant parts and as such it colonizes the taproot and crown of lucerne plants. From there it can spread to newly formed stems (EFSA, 2014; Hunt, 1971; Jones and McCulloch,

1926; Koehler and Jones, 1932).

In inoculation experiments, symptoms can be observed after three weeks (Hale, 1972). In the field, the disease is rarely observed in one year old plants. In the second year after sowing, symptoms may become visible on the above ground parts but it usually takes three years for a field to become severely affected (Koehler and Jones, 1932). In the laboratory and on solid growing media, *C. insidiosus* grows best at 23 °C, but in the field disease incidence has been shown to be greater at 16 °C compared to 24°C and 28°C. Abundant soil moisture aids the progress of the disease (EFSA, 2014; Jones and McCulloch, 1926; Koehler and Jones, 1932).

Overwintering generally occurs in the roots and crowns of diseased plants. Bacteria remain viable for 10 years in lucerne stems stored at 20-25°C, but they survive only poorly in non-sterile soils (Carroll and Lukezic, 1971; Cormack, 1961; Koehler and Jones, 1932).

DETECTION AND IDENTIFICATION

Symptoms

Within lucerne crops, symptomatic plants may be scattered throughout the field or found in patches. Lower wetter parts of the field are often affected first. Symptoms are often inconspicuous in the first year and affect the lucerne crop rather uniformly, hence the 'insidious' species name. Mild symptoms include leaf-mottling and cupping or upward curling of leaf margins, with some reduction in plant height. Moderate infection additionally leads to a proliferation of the stems, giving a witches' broom effect. In severe infections, young plants or newly formed stems are only a few centimeters high, have thin and spindly stems, leaflets are small and thicker, often distorted and show marginal or entire bleaching, and ultimately plants usually die. In the field, severely affected plants can be easily spotted due to their yellow green color and stunted growth. When drought stress occurs, infected plants only show enhanced wilting, compared to healthy plants. In the absence of, or in addition to aerial symptoms, there is a yellow to pale-brown discolouration of the young woody root tissue at the junction of the cortex and vascular cylinder. This is visible when peeling off the cortex or cutting the tap-root (Close and Mulcock, 1972; Jones, 1925; Jones and McCulloch, 1926; Koehler and Jones, 1932).

Morphology

C. insidiosus is an aerobic, Gram-positive, capsulated, non-motile rod, 0.4-0.5 x 0.7-1.0 µm, which does not produce chains. Agar colonies are generally pale-yellow, round or amorphous, smooth, glistening, flat or slightly raised. Characteristic blue pigment granules occur irregularly in cultures on media high in available sugars after 6-8 days at 21°C. DNA G+C content of the type strain (LMG 3663^T) is 72.7% (Close and Mulcock, 1972; Li *et al.*, 2018; McCulloch, 1925).

Detection and inspection methods

The EPPO Standard on diagnostics PM 7/99 (2) (EPPO, 2022) provides comprehensive information on the available methods for detecting *C. insidiosus* in both symptomatic plant material and seeds. It also provides information on isolation and identification methods. Laboratory analysis should be performed on cut sections of the main root or on seeds. For detection of *C. insidiosus* with immunofluorescence, two antisera are suggested and some performance characteristics are provided. For detection with PCR, two conventional PCR tests (Samac *et al.*, 2017; Ward *et al.*, 2008) and two real-time PCR tests (Samac *et al.*, 2017; Ward *et al.*, 2008) are suggested, with the conventional PCR by Ward *et al.* (2008) being suggested only for plant material and not for seed testing. In addition to these immunofluorescence and PCR tests, DNA barcoding (Zaluga *et al.*, 2011), BOX-PCR (Louws *et al.*, 1998) and MALDI-TOF MS (Zaluga *et al.*, 2011) can be used for identification.

PATHWAYS FOR MOVEMENT

Short distance spread of *C. insidiosus* mainly occurs through the movement of water (or water droplets) containing the pathogen. The spread by contaminated machinery and especially mowers appears to be important. Mowing

lucerne crops when the foliage is wet creates ideal conditions for bacterial dissemination since both infected and non-infected plants are wounded and the mower spreads infected plant material and contaminated water droplets over the field (Jones and McCulloch, 1926; Koehler and Jones, 1932; University of Illinois Department of Crop Sciences, 1988). There have also been reports of local spread by insects and nematodes, and the presence of nematodes in the field favors infection (Hawn, 1963; Hunt, 1971; K?dela *et al.*, 1984).

Long distance spread can occur through contaminated seed lots. *C. insidiosus* can be present on particles present in the seed lot (e.g. plant debris), on the surface of the seeds or inside the seeds. The exact role of these seed contaminations in new outbreaks is uncertain as data on the transmission of the bacterium from seed to seedling is generally missing. In experiments, the percentage of symptomatic plants that transmitted the bacterium to its seeds was 7%, which is relatively low (EFSA, 2014; Samac *et al.*, 1998).

PEST SIGNIFICANCE

Economic impact

In the past, *C. insidiosus* caused serious damage with major economic impact on lucerne crops in countries including the USA, Canada and New Zealand (Hale and Close, 1974; Koehler and Jones, 1932; Peake and Cormack, 1955). However, at the moment in most parts of the world the presence of the pathogen is limited and its impact is strongly reduced. Canada is now free of the pathogen and in the USA major impact is no longer observed. In the European Union the impact of *C. insidiosus* is also low. In Australia growers have reported that even infected fields remain highly productive (Ophel-Keller, 2005). However, in some parts of the world, e.g. Iran, the pathogen still caused damage in lucerne fields (Heidari and Khodakaramian, 2012).

Control

The most important strategy to control *C. insidiosus* is the use of resistant lucerne cultivars. In the USA and Canada such cultivars have already been available for decades and their use has probably led to the strong reduction of damage caused by *C. insidiosus*. In Europe, lucerne cultivars with reduced susceptibility have also been developed (EFSA, 2014; Kozová *et al.*, 2003; Víchová and Kozová, 2004). Other practices that can help to reduce the risk of damage due to *C. insidiosus* are: avoiding poorly drained soils, harvesting young stands before old ones, harvesting fields with symptoms last, steam cleaning equipment with steam between fields, mowing only when foliage is dry, growing other crops for two or three years before reseeding lucerne, reducing injuries to crowns, and using pathogen-free seed from regions where the bacterium is absent (EFSA, 2014; University of Illinois Department of Crop Sciences, 1988).

Phytosanitary risk

C. insidiosus has not been reported in most EPPO countries where lucerne is grown, even though there are suitable climate and soil conditions in these areas. The use of resistant cultivars and strict phytosanitary measure have likely strongly reduced the risk of spread and establishment of this pathogen (EFSA, 2014).

PHYTOSANITARY MEASURES

Seeds should come from areas known to be free of *C. insidiosus*. Seeds of lucerne from areas where *C. insidiosus* occurs should come from a field which, along with adjacent fields, was found free from *C. insidiosus* during the last growing season, and where no lucerne was grown during the 3 years prior to the sowing of this crop. Alternatively, seeds should come from cultivars which are considered highly resistant to *C. insidiosus* (EPPO, 2019).

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Datasheet history

This datasheet was first published in the EPPO Bulletin in 1982 and revised in the two editions of 'Quarantine Pests for Europe' in 1992 and 1997, as well as in 2024. It is now maintained in an electronic format in the EPPO Global Database. The sections on 'Identity', 'Hosts', and 'Geographical distribution' are automatically updated from the database. For other sections, the date of last revision is indicated on the right.

CABI/EPPO (1992/1997) *Quarantine Pests for Europe (1st and 2nd edition)*. CABI, Wallingford (GB).

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