EPPO Datasheet: *Verticillium dahliae hop strains*

Last updated: 2024-07-29

Only hop strains of *Verticillium dahliae* and *V. nonalfalfae* have been included on the EPPO A2 List of pests recommended for regulation as quarantine pests, considering the severity of the disease they may cause on this particular crop, and their limited geographical distribution. Within the European Union, both pathogens are currently listed as regulated non-quarantine pests (RNQPs) with a zero tolerance on hop plants for planting (EU, 2019).

IDENTITY

Preferred name: *Verticillium dahliae hop strains*
Authority: Klebahn
Taxonomic position: Fungi: Ascomycota: Pezizomycotina: Sordariomycetes: Hypocreomycetidae: Glomerellales: Plectosphaerellaceae
Common names: verticillium wilt of hop
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EPPO Categorization: A2 list
view more categorizations online...
EU Categorization: RNQP (Annex IV)
EPPO Code: VERTDH

more photos...

Notes on taxonomy and nomenclature

Verticillium wilt of hop (*Humulus lupulus*) is caused by the soil-borne fungi *Verticillium nonalfalfae* and *V. dahliae*. On hops grown in temperate regions, *V. nonalfalfae* is the most frequently found species and is responsible for the most severe outbreaks. Originally, *V. dahliae* was described by Klebahn (1913) from *Dahlia* sp. cv. Geiselher in Germany. In 2011, Inderbitzin *et al.* provided a new taxonomic framework for *Verticillium* species, their boundaries and evolutionary relationships, based on multigene phylogenetic analyses and morphological investigations. As a result, *V. alboatrum* sensu lato was split into three different species: 1) *V. alboatrum* sensu stricto, isolated from potatoes and potato soil; 2) *V. alfalfae* whose only known host is alfalfa (*Medicago sativa*); 3) *V. nonalfalfae* attacking a wide range of host plants, including hop (Inderbitzin *et al.*, 2011, 2013; Inderbitzin and Subbarao, 2014). In this new taxonomic concept, isolates of *V. alboatrum* sensu lato pathogenic to hop are now considered to be *V. nonalfalfae*. *Verticillium dahliae* was initially confounded with *V. alboatrum* sensu lato and then with *V. longisporum* but it is now confirmed to be a separate species (Inderbitzin *et al.*, 2011). This taxonomic confusion has introduced uncertainty about past data on both causal agents of Verticillium wilt of hop.

HOSTS

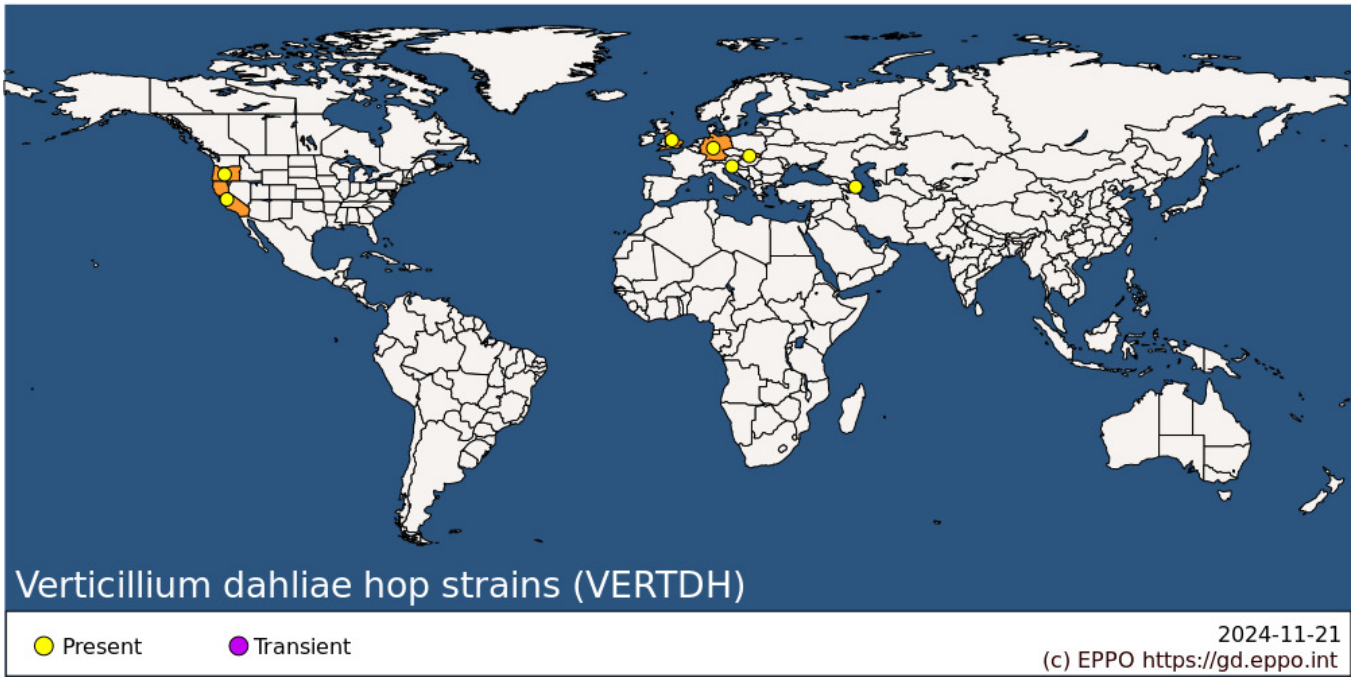
In this datasheet, the only host considered is hop (*Humulus lupulus*). However, as a species *V. dahliae* has a wide host range infecting more than 70 herbaceous and woody plant species. Its hosts include economically important arable, vegetable and fruit crops, such as almond (*Prunus dulcis*), apple (*Malus*), apricot (*Prunus armeniaca*), artichoke (*Cynara cardunculus*), *Brassica* spp., bell pepper (*Capsicum annuum*), grapevine (*Vitis vinifera*), hazelnut (*Corylus avellana*), olive (*Olea europaea*), peach (*Prunus persica*), plum (*Prunus domestica, P. salicina*), potato (*Solanum tuberosum*), quince (*Cydonia oblonga*), *Rosa* spp., sunflower (*Helianthus annuus*), strawberry (*Fragaria* x *ananassa*), and tomato (*Solanum lycopersicum*) (Inderbitzin and Subbarao, 2014).

Host list: *Humulus lupulus*

GEOGRAPHICAL DISTRIBUTION

Considering the complex taxonomic history of *Verticillium* species, and in particular the past confusion between *V. dahliae*

and *V. alboatrum* sensu lato, there is uncertainty around the geographical distribution of *V. dahliae*. As a species (including both hop and non-hop strains), *V. dahliae* has a wide geographical range (EPPO, 2024). Verticillium wilt of hop has generally been attributed to *V. nonalfalfae*, but *V. dahliae* has been isolated in some cases, as shown in the map below.



EPPO Region: Azerbaijan, Germany, Slovakia, Slovenia, United Kingdom (England)
North America: United States of America (California, Oregon)

BIOLOGY

Life cycle, population dynamics and climatic thresholds

Verticillium dahliae is a soil-borne fungal pathogen that colonizes the xylem vessels of hop plants, resulting in a vascular wilt disease. The fungus penetrates roots of a susceptible plant in the region of elongation and the cortex is colonized. From the cortex, the hyphae invade the xylem vessels where conidia are formed. Vascular colonization occurs as conidia are drawn up into the plant along with water. During colonization, *V. dahliae* produces and secretes hydrolytic enzymes that are involved in the degradation of the plant cell wall, thus facilitating the spread of the fungus. Due to fungal material and host reaction products, the vascular system becomes plugged, preventing water from reaching upper parts of the plant (Kunej *et al.*, 2020). Leaves and stems deprived of water soon begin to exhibit symptoms of wilting and foliar chlorosis. Symptoms of this disease are seen throughout the plant. As the diseased plant declines, the fungus produces microsclerotia which are released into the soil with the decomposition of plant material. When plant parts bearing microsclerotia are incorporated into the soil, aggregated microsclerotia are initially held together by the shoot tissue, but gradually, as the shoot decomposes, the microsclerotia separate. This is reflected in an apparent increase in soil inoculum density 1 or 2 years after the incorporation of infected plant material (Soesanto, 2000). The microsclerotia are long-lived and survive well over a range of soil moisture and temperature conditions, but lose viability most rapidly in wet, warm soil (Green, 1980; Soesanto, 2000). On hops, the rate and severity of disease development are inversely related to soil temperature. The level of *V. dahliae* inoculum in soil may fluctuate over time due to chemical inputs, tillage, cropping patterns, and prolonged soil flooding (Short *et al.*, 2015). Both exceptionally high and exceptionally low rainfall reduce the occurrence of the disease in hops (Talboys and Wilson, 1970; Soesanto, 2000). The fungus can survive for at least 14 years in the soil as microsclerotia (Short *et al*., 2015), which are dispersed through seed, vegetative planting material, soil, water, and agricultural equipment (Atallah *et al*., 2010). In view of the biological features outlined above, Verticillium wilt is essentially a soil-borne or debris-borne disease (and this is certainly the case for hops).

Host specificity

Verticillium dahliae exhibits significant variation in its impact on hop plantations. Although generally considered a generalist pathogen affecting multiple hosts, *V. dahliae* demonstrates population differentiation due to varying environmental conditions (Walker *et?al*., 2015). Previous studies of the population structure of *V.?dahliae* have revealed a population specific to tomato and no strong association between population structure and the host of origin of isolates on other hosts (Short *et al.*, 2015). These authors also revealed a lack of differentiation linked to geographical locations, despite extremely high genotypic diversity, therefore, the hypothesis of the local population expanding its host range was not substantiated (Atallah *et?al*., 2010). In hop-growing regions, there is a continuum of strain severity, with no distinct disease entity except in England, where a very virulent isolate was identified (Chambers *et al*., 1985; Atallah *et?al*., 2010; Berne *et al.*, 2020). Differences in aggressiveness between *V. dahliae* strains have been also reported in Germany (Zinkernagel, 1982). Elsewhere, hop wilt is always mild, and no special strains are known.

Understanding the genetic diversity within *V. dahliae* is crucial for managing hop wilt effectively. Advances in vegetative compatibility and molecular analyses have revealed higher genetic diversity than previously thought (Barbara and Clewes, 2003; Jiménez-Díaz *et al.*, 2006). *Verticillium dahliae* is categorized into six vegetative compatibility groups (VCGs), which are genetically distinct populations (Klosterman *et al.*, 2009). These VCGs help in identifying and deploying resistant hop cultivars and preventing pathogen spread (Jiménez-Díaz *et al.*, 2005; Klosterman *et al.*, 2009; Kombrink *et al.*, 2017).

VCGs are identified using spontaneous nitrate non-utilizing mutants, with VCG1, VCG2, and VCG4 further divided into subgroups A and B with subgroup A being more severe than subgroup B in each case (Klosterman *et al.*, 2009). While VCGs do not fully describe genetic diversity or recombination potential, they are valuable for understanding pathogen evolution and enhancing hop disease management strategies (Jiménez-Díaz *et al.*, 2005; Klosterman *et al.*, 2009; Kombrink *et al.*, 2017). This can aid in the deployment of resistant cultivars, preventing pathogen introductions and exploring the evolution of an agronomically important group of phytopathogens.

DETECTION AND IDENTIFICATION

Symptoms

The first foliar wilt symptoms appear approximately 20 days post inoculation and rapidly progress in susceptible varieties, whereas resistant hop varieties show no symptoms or mild symptoms (Berne *et al.*, 2020). The fungal biomass gradually increases in roots and stems of susceptible varieties, while in resistant plants, the colonization in roots is significantly less extensive, and fungal DNA in stems is barely detected (O'Brien *et al.*, 2012; Berne *et al.*, 2020). As the diseased plant undergoes senescence, the fungus produces resting structures that are released into the soil, where they remain dormant for several years in the absence of a host (Deketelaere *et al.*, 2017; Berne *et al.*, 2020). Symptoms are usually most prevalent and severe in wet seasons or in areas where the soil is excessively wet in summer. Disease intensity fluctuates from season to season; plants affected one year may look healthy the next year and for a number of seasons after that. The first symptoms on leaves usually appear in July or early August as a yellowing of the lower leaves, which gradually spreads to other leaves higher up the bine; only occasionally is the whole plant affected (EPPO, 2020). The lower leaves dry out, wither and may fall, while wedge-shaped necrotic areas may develop on the upper leaves. Bines often become swollen and externally may appear brown and corky. Notching or cutting the bine about 0.3–1.0 m from the base will reveal a characteristic light-brown discoloration of the internal woody tissues (Berne *et al.*, 2020; EPPO, 2020).

The symptoms described above are those of the 'mild' or 'fluctuating' type, which is most commonly seen. With more aggressive strains and susceptible cultivars, symptoms can be of the more severe or 'progressive' type. A new outbreak usually starts from one infected plant but, by the time it is noticeable, there are often several together in a patch. On leaves, infection is usually first apparent from the end of May onwards (EPPO, 2020). The bottom leaves on one or more bines turn yellow; this yellowing progresses upwards within a few days and, within a week, half or more of the leaves on affected bines may be yellow or dead. Other bines on the infected plant may also begin to show symptoms. A tiger-striping effect is infrequently found on the upper leaves. After 2–3 weeks, all the leaves are

dead and usually fallen, and plants often die before the end of the season. Bines rarely become swollen but do show the characteristic internal brown discoloration when cut. They eventually turn black (Deketelaere *et al.*, 2017; Berne *et al.*, 2020; EPPO, 2020). Plants which survive the following winter often produce only a few weak bines the next season, and these soon develop symptoms and die.

Morphology

Growth of *V. dahliae* shows as white, fluffy mycelium, which appears after an incubation period of 3–5 days. Conidia occur singly at the apices of phialides, mainly one-celled but occasionally one-septate, (3.5–) 6.5 (–13.5) x (2.0–) 3.0 (–4.5) µm (EPPO, 2020). They are hyaline, ellipsoidal to irregularly subcylindrical. Phialides are borne on the verticillately branched conidiophores, which are darkened at the base when grown on plant tissues. The species can be identified by the production of resting structures (after 1–2 weeks of incubation), macroscopically visible as a darkening of the cultures (EPPO, 2020). Primary identification of species depends on these resting structures, and *V. dahliae* produces dark brown to black microsclerotia arising from single hyphae by repeated budding and consisting of swollen, almost globular cells. This epidemiologically important resting structure (microsclerotia) varies in shape from elongate to irregularly spherical and is 25–50 (–100) µm in diameter (Inderbitzin et al., 2011; EFSA, 2014; EPPO, 2020). Two other *Verticillium* species, *V. longisporum* and *V. zaregamsianum*, can also form microsclerotia, but these species can be differentiated from *V. dahliae* by the larger size of their conidia (Inderbitzin *et al*., 2011). See also Hawksworth and Talboys (1970), Goud *et al.* (2003), and EPPO (2020).

Detection and inspection methods

Verticillium dahliae hop strains can be detected following the EPPO diagnostic protocol PM 7/78 (EPPO, 2020). The pathogen can be detected based on the typical symptoms which are known in Verticillium wilt of hops for both *V. dahliae* and *V. nonalfalfae* and in both fluctuating (mild) or progressive (lethal) disease forms. Typical symptoms include yellowing and wilting of the leaves, leading to interveinal necrosis and leaf drop, while affected bines exhibit swelling, rough epidermis, and brown vascular discoloration (EPPO, 2020).

Verticillium dahliae can easily be isolated from infected hosts by isolation from xylem of roots, stems, branches, twigs, and even leaves and seeds. Isolation of the fungus is made by growth on semi-selective medium (PLYA or PDA), which promotes the production of resting structures. Identification by this method takes up to 2 weeks. Key morphological characteristics are *V. dahliae's* production of resting structures (dark microsclerotia; see morphology) while *V. nonalfalfae* can be differentiated by the absence of microsclerotia and presence of dark resting mycelium (EPPO, 2020). The examination of darkened cultures can be done under microscope (100 x magnification), either in situ or squashed onto a slide should reveal the nature of the resting structures (EPPO, 2020).

Despite the possibility of identifying *Verticillium* species based on morphological characters, for reliable identification it is highly recommended that identification should be based on or confirmed by molecular methods, as morphological identification can be prone to errors, especially with atypical isolates lacking diagnostic structures. Specific PCR protocols with specific primers for diagnosis of *V. dahliae* and *V. nonalfalfae* on hop are available, and sequencing is described (EPPO, 2020). Fungal DNA should be extracted from mycelium taken from solid (PDA, PLYA) or liquid medium or using other appropriate standard methods, including commercial kits with protocols for filamentous fungi. Several PCR-based tests have been developed to detect *V. dahliae* inoculum in soil (EPPO, 2020).

PATHWAYS FOR MOVEMENT

The wide geographical distribution of *V.?dahliae* (including both hop and non-hop strains) contrasts with its lack of any obvious adaptation to airborne spread. Its sticky, unmelanized asexual spores (conidia) are likely prone to rapid desiccation and damage by UV radiation, and they are generally assumed to be of minimal importance in the spread of Verticillium wilt (Jiménez-Díazet *et al.*, 2006). However, several other characteristics of this fungus make it particularly amenable to spread through human-mediated intercontinental trade, especially its long-lived environmental stage (microsclerotia) (Fisher *et?al*., 2012). In addition to the survival of melanized microsclerotia in soil, plant debris and commercial crop seed, the fungus exhibits saprotrophic growth in the absence of host plants, and has the generalist ability to colonize trees, agronomic crops, and weeds. Microsclerotia remain viable in the soil for at least 14 years (Wilhelm, 1955), and serve as the primary source of inoculum which makes long-term

management in agriculture difficult.

Hop strains of *V. dahliae* are most likely to be moved into new areas by transport of soil and hop planting material.

PEST SIGNIFICANCE

Economic impact

Hop is susceptible to *V. dahliae* hop strains, which, however, are considered of minor importance compared to *V. nonalfalfae* hop strains (EFSA, 2014). *Verticillium dahliae* generally causes a mild disease of hops, which can however be severe when attacks susceptible cultivars. Hop wilt attributed to *V. dahliae* is moderately severe in Germany (Zinkernagel, 1982) noting differences in strain aggressiveness, and highly virulent in the United Kingdom according to Chambers *et al.* (1985), but elsewhere Verticillium hop wilt is always mild, and no special strains are known (Talboys, 1987; EFSA, 2014, EPPO, 2020).

Control

Overall, Verticillium wilt caused by *V. dahliae* hop strains is difficult to manage due to ineffective fungicides that do not suppress fungal colonization in plants, and its resting structures that are viable in the soil for many years (Klosterman *et al.*, 2009). No single disease control measure is efficient enough if applied individually. The implementation of integrated management strategies combining an appropriate selection of planting site, disease risk assessment (e.g. assessment of the available inoculum in the soil, determination of pathotypes/races/VCGs/strains present in the site, cropping history of the field, etc.), cultural practices, such as crop rotation and manipulation of fertility and irrigation, use of healthy planting material, including seeds, use of available resistant cultivars and sometimes pre-plant soil treatments, such as solarisation, that reduce the viability of the pathogen in soil (Jeger *et al.*, 1996; Jiménez-Díaz *et al.*, 2006; Klosterman *et al.*, 2009; EFSA, 2014) may reduce disease incidence and severity, but they do not eliminate *V. dahliae*.

Phytosanitary risk

Outbreaks of Verticillium wilt have been recorded on hops, causing substantial yield losses in parts of the EPPO region. Several countries are important hop producers (e.g. Germany, Czech Republic, Poland, and Slovenia). Once established in an area, this vascular and soil-borne disease is difficult to control and eliminate. The climatic conditions prevailing in the hop-growing areas of Europe do not appear to be limiting factors to Verticillium wilt (EFSA, 2014). Movements of potentially infected hop planting material and soil can readily spread the disease. *Verticillium dahliae* is a polyphagous species and has many more potential hosts than those for which it is regulated. Populations of *V. dahliae* are considered host-adapted rather than host specific, i.e. they display cross-pathogenicity but are more virulent to the host from which they were isolated.

PHYTOSANITARY MEASURES

To avoid the introduction and spread of hop strains of *V. dahliae*, it can be recommended that hop plants for planting should have been produced in a place of production known to be free from the pathogen or have been produced according to a certification scheme. Production sites of hop plants for planting should be isolated from hop gardens, and producers should keep records of cropping and soil borne disease history to demonstrate that *Verticillium* spp. have not been found during the last 5 years. Visual inspection during the growing season carried out at appropriate times should also confirm the absence of Verticillium wilt symptoms. Guidance on how to produce healthy and vegetatively propagated plants can be found in the EPPO Certification scheme for hop (EPPO, 2009).

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Datasheet history

This datasheet was first published in the EPPO Bulletin in the second edition of 'Quarantine Pests for Europe' in 1997, as *Verticillium* spp. on hops. 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.

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