**EPPO Datasheet: *Verticillium nonalfalfae 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**

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| **Preferred name:** *Verticillium nonalfalfae hop strains***Authority:** Inderbitzin, H.W. Platt, Bostock, R.M. Davis & K.V. Subbarao**Taxonomic position:** Fungi: Ascomycota: Pezizomycotina: Sordariomycetes: Hypocreomycetidae: Glomerellales: Plectosphaerellaceae**Other scientific names:** *Verticillium albo-atrum hop strains* Reinke & Berthold**Common names in English:** verticillium wilt of hop[view more common names online...](https://gd.eppo.int/taxon/VERTAH/)**EPPO Categorization:** A2 list**EU Categorization:** RNQP (Annex IV)[view more categorizations online...](https://gd.eppo.int/taxon/VERTAH/categorization)**EPPO Code:** VERTAH | 1704.jpg[more photos...](https://gd.eppo.int/taxon/VERTAH/photos) |

**Notes on taxonomy and nomenclature**

Verticillium wilt of hop (*Humulus lupulus*) is caused by the soil-borne fungi *Verticillium nonalfalfae* and *V. dahliae*. In temperate regions, *V. nonalfalfae* is the most frequently found species and is responsible for the most severe outbreaks in hop crops. 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. albo-atrum* 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. albo-atrum* sensu lato pathogenic to hop are now considered to be *V. nonalfalfae*. Previously, *V. alfalfae* and *V. nonalfalfae* were respectively referred to as the alfalfa and non-alfalfa pathotypes of *V. alboatrum* (Barbara and Clewes, 2003), and have also long been recognized as two genetically distinct groups named *V. albo-atrum* group 1 and group 2 (EFSA, 2014). This complex taxonomic history 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. nonalfalfae* has a larger host range including cultivated plants such as alfalfa (*Medicago sativa*), celery (*Apium graveolens*), cotton (*Gossypium hirsutum*), cucumber (*Cucumis sativus*), *Petunia* sp., potato (*Solanum tuberosum*), spinach (*Spinacia oleracea*), tomato (*Solanum lycopersicum*) (Inderbitzin and Subbarao, 2014). Over the past decades, *V. nonalfalfae* has been identified as the causal agent of wilt and mortality of the highly invasive tree-of-heaven *(Ailanthus altissima*) and studied as a potential biological control agent (Schall and Davis, 2009; Kasson *et al.*, 2015; Lechner *et al.*, 2023).

**Host list:** *Humulus lupulus*

**GEOGRAPHICAL DISTRIBUTION**

Considering the complex taxonomic history of *Verticillium* species, there is uncertainty around the geographical distribution of *V. nonalfalfae*. Nevertheless, based on data provided by Interbitzin *et al.* (2011, 2013) and Inderbitzin and Subbarao (2014), the presence of hop strains of *V. nonalfalfae* has been reported from the USA, Europe and New Zealand. Strains which are aggressive on hops have been documented in the United Kingdom (Chambers *et al.*, 1985), Slovenia (Radišek *et al.*, 2003), and more recently in Germany, in the Hallertau area (Bavaria) which is a major hop-growing area (Seefelder *et al.*, 2009; Maurer *et al.*, 2013).

 **EPPO Region:** Belgium, Germany, Poland, Serbia, Slovakia, Slovenia, United Kingdom (England) **North America:** United States of America (Oregon) **Oceania:** New Zealand

 **BIOLOGY**

**Life cycle, population dynamics and climatic thresholds**

*Verticillium nonalfalfae* is a soil-borne fungal pathogen which survives as resting mycelium in the soil and on diseased plant debris, on the soil surface or incorporated into it. Mycelium or conidia are able to infect plants through healthy or wounded roots and spread through the xylem vessels. Colonization is enhanced by the production of spores spreading through the plants by the transpiration flow and by their subsequent germination (Cregeen *et al.*, 2015). The fungus colonizes the plant’s entire vascular system, causing plant stunting and wilting, vascular browning and foliar chlorosis and necrosis (Kunej *et al.*, 2020). On hops, the rate and severity of disease development are inversely related to soil temperature. After infection, xylem vessel lumens and pit chambers are frequently plugged with gum and pectin, and the interior cell walls are often coated with gum, suberin and, occasionally, pectin. Such deposits probably impede lateral water flow and, together with the disruption in xylem vessel element differentiation, the blockage of mature vessels by immature parenchyma and the destruction of metaxylem vessel elements, result in malfunctioning of xylem (EFSA, 2014). On hop, Talboys and Wilson (1970) found, over a 15-year-study, that Verticillium wilt caused by *V. nonalfalfae* was severe on tolerant cultivars in years with mean soil temperatures of 12.5–13.0 °C at 20 cm depth during mid-April to late June, and was much reduced when the mean soil temperature increased to 14.4–15.0 °C. Comparatively severe disease developed on susceptible cultivars over the entire hop-growing period, including the warmer season. Moreover, the rate and severity of disease development were inversely related to soil temperature.

In view of the biological features outlined above, Verticillium wilt is essentially a soil-borne or debris-borne disease (and this is certainly so for hops). In areas where *V. nonalfalfae* is widespread, very little consideration has been given to other possible means of transmission. However, the recent spread of Verticillium wilt of lucerne in North America has led to interest in other pathways (e.g. on insects; Harper and Huang, 1984), which, although it is expected to be an unlikely (rarely occurring) pathway, might introduce the disease into new areas, despite phytosanitary measures taken against the predominant means of transmission.

In general, the biology of *V. nonalfalfae* is similar to that of *V. alfalfae*, *V. albo-atrum* sensu stricto, and *V. dahliae*. The fungus survives for longer periods on soil particles or plant remnants because it forms resting structures, and these withstand adverse environmental conditions (Wilhelm, 1955).

**Host specificity**

The occurrence of different virulent strains or pathotypes of *V. nonalfalfae* has been well documented in hops. The first record of hop infections was in England in 1924, which caused mild vascular wilt disease (Radišek *et al.*, 2006). A few years later, in 1933, more severe outbreaks appeared, known as progressive (lethal) wilt (Sewell and Wilson, 1974). The appearance of these two wilt syndromes was attributed to pathogen virulence, the sensitivity of hop cultivars and ecological factors (Sewell and Wilson, 1974; Radišek *et al.*, 2006). In general, strains of *V. nonalfalfae* which are specific to hops have classically been described as either ‘mild’ or ‘lethal’. The mild wilt varies in intensity from year to year and rarely causes plant death, whereas lethal wilt is less influenced by seasonal climatic variations and causes very severe symptoms with rapid plant withering (Radišek *et al.*, 2006; Flajšman *et al.*, 2017). In England, one mild (M) and three types of lethal (PV1, PV2, and PV3) *V. nonalfalfae* isolates have been identified by using virulence testing on different sets of hop cultivars (Sewell and Wilson, 1974). Lethal *V. nonalfalfae* isolates were geographically limited to English hop-growing regions until 1997, when lethal outbreaks were registered in Slovenia (Radišek *et al.*, 2006). Virulence testing and molecular analysis of *V. nonalfalfae* hop isolates collected in all Slovene hop-growing regions identified two pathotypes, which were designated PG1 (mild) and PG2 (lethal) (Radišek *et al.*, 2003).

Later it was recognized (1) that there is a continuum of variation between less and more severe strains, and (2) that this variation exists in all hop-growing areas in the United Kingdom and Slovenia (Radišek *et al.*, 2006). Thus, a high association with virulence was observed in hop isolates. Cluster analysis confirmed that all lethal hop isolates were genetically different from mild hop isolates. The lethal hop isolates from England and Slovenia expressed the same virulence phenotype, although they showed a different AFLP pattern, whereas the mild hop isolates formed two subgroups, to which isolates clustered irrespective of geographical location (Radišek *et al.*, 2003; 2006; Flajšman *et al.*, 2017). It is thus probable that the differences in the English and Slovene isolates’ arose in the presence of a specific set of cultivars, relate specifically to them, and are of no particular significance elsewhere yet. These data suggest multiple origins of *V. nonalfalfae* hop isolates, and the possible appearance of new virulent isolates in the future in other hop growing regions (Radišek *et al.*, 2006).

**DETECTION AND IDENTIFICATION**

**Symptoms**

*Verticillium nonalfalfae* is differentiated into fluctuating (mild) and progressive (lethal) pathotypes depending on the virulence of the isolates (Maurer *et al.*, 2013). Plants infected with the lethal pathotypes wither and often die, whereas hops infected with the mild pathotypes tolerate the disease. The symptoms of Verticillium wilt on hops caused by *V. nonalfalfae* vary depending on the pathogenicity of the fungal strain and the sensitivity of the cultivar. Susceptible hop cultivars can suffer from severe symptoms (e.g., leaf chlorosis and necrosis) and also have complete dieback of rootstock if infected by a highly virulent strain of *V. nonalfalfae* (Kunej *et al.*, 2021).

Affected plants usually occur in scattered groups but they may be distributed throughout the hop garden. 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 late 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 fluctuating (mild) type, which is most commonly seen. With more aggressive strains and susceptible cultivars (a combination which will occur more and more rarely in practice), symptoms can be of the more severe ‘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 (EPPO, 2020). On leaves, infection is usually first apparent from the end of May onwards and 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 (Berne *et al.*, 2020; EPPO, 2020). 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 and they eventually turn black (Berne *et al.*, 2020; EPPO, 2020; Deketelaere *et al.*, 2017). Plants which survive the following winter often produce only a few weak bines the next season, and these soon develop symptoms and die.

**Morphology**

The most conspicuous character of *Verticillium* spp. is verticilliate conidiophores, which can be observed under a dissecting microscope, and conidia. Further identification of *V. nonalfalfae* and *V. dahliae* is based on formation of resting structures, macroscopically visible as a darkening of the cultures after 1–2 weeks of incubation and conidia (Smith, 1965; EPPO, 2020). Examination of darkened cultures under microscope (100 x magnification) should reveal the nature of the resting structures: resting mycelia in the case of *V. nonalfalfae* and microsclerotia in the case of *V. dahliae* (EPPO, 2020).

*Verticillium nonalfalfae* typically has a smooth-walled hyphae, 1.5–3 μm wide. Conidiogenous cells are phialides, arranged in (1–) 2–6 whorls along conidiophores. Conidiophores are erect, branched, or unbranched, hyaline, with a dark swollen base, sometimes up to 11 μm wide. Phialides are borne on verticillately branched conidiophores, arranged in 2–6 whorls along conidiophores, usually 2–5 phialides in each whorl. Conidia are hyaline, smooth-walled, cylindrical with rounded apices to oval, allantoid at times, which occur singly at the apices of phialides, mainly one-celled but occasionally one-septate, (4.0–) 6.0 (–10.5) x (2.5–) 3.0 (–3.5) µm. Dark resting mycelium consisting of brown pigmented thick-walled hyphae up to 9 µm wide, straight or curved, arranged solitary or in aggregates (hyphal ‘knots’ but no microsclerotia) (Smith, 1965; Inderbitzin *et al.*, 2011; EPPO, 2020).

See also Smith (1965), Hawksworth and Talboys (1970), and EPPO (2020).

**Detection and inspection methods**

*Verticillium nonalfalfae* 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 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 nonalfalfae* 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 (EPPO, 2020), which promotes the production of resting structures. This method takes up to 2 weeks. Key morphological characteristics are the production of the resting structures: resting mycelia and absence of microsclerotia in the case of *V. nonalfalfae* and dark microsclerotia in the case of *V. dahliae* (EPPO, 2020). *Verticillium nonalfalfae* is morphologically indistinguishable from *V. alfalfae* and *V. albo-atrum* sensu lato but differs in host range and DNA characters (EPPO, 2020). Specific molecular methods (conventional and real-time polymerase chain reaction [PCR] tests) have been developed for the differentiation of *V. albo-atrum* sensu stricto, *V. alfalfae* and *V. nonalfalfae* (Inderbitzin *et al.*, 2013; 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. PCR tests with specific primers for the diagnosis of *V. dahliae* and *V. nonalfalfae* 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 (EPPO, 2020). No effective soil test is available for *V. nonalfalfae*.

**PATHWAYS FOR MOVEMENT**

As a soil-borne fungus, *V. nonalfalfae* is not readily dispersed over long distances. Debris of infected host plants may serve as a principal means of the pathogen’s survival and can act as reservoir of inoculum, by supporting conidia production. From crop debris, the fungus can infect roots that come into contact with it. Once established in an area, *V. nonalfalfae* can spread within a field or between fields passively through the transport of infested soil by water or wind, the dissemination of infected host plant debris by wind or transport via insects (Berlanger *et al.*, 2005; EFSA, 2014; Berne *et al.*, 2020). *Verticillium nonalfalfae* can also be spread over short or long distances by human-mediated movements of contaminated seeds, infected plant debris during the process of cultivation or after harvesting, infested soil by vehicles, machinery, hop poles, agricultural equipment, shoes or infested potting soil for nursery production (Inderbitzin *et al.*, 2013; EFSA 2014; Berne *et al.*, 2020). Hop strains of *V. nonalfalfae* are most likely to be moved into new areas by transport of soil and hop plants for planting.

**PEST SIGNIFICANCE**

**Economic impact**

*Verticillium nonalfalfae* generally causes wilt disease of hops, which can be severe when more aggressive strains attack susceptible cultivars. In this way, the pathogen almost eliminated hop cultivation in South-East England in the 1940s, when a few severe outbreaks appeared (Sewell and Wilson, 1974), but selection of wilt-tolerant cultivars along with the application of various agronomic measures enabled production to be sustained. Lethal outbreaks were also recorded in Slovenia in particular in the Savinja Valley causing significant economic damage where more than 180 ha of hop gardens were affected (Radišek *et al.*, 2006). The emergence of strains able to attack tolerant cultivars has been considered to be the main threat for the hop industry in England (Talboys, 1987).

**Control**

Overall, Verticillium wilt caused by *V. nonalfalfae* on hop 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 (Radišek *et al.*, 2006, Klosterman *et al.*, 2009) and no single disease control measure is efficient enough if applied individually. The application of integrated management strategies that combine selection of planting site, disease risk assessment, cultural practices, such as crop rotation and manipulation of fertility and irrigation, use of disease-free 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 are essential for hops (Jeger *et al.*, 1996; Jiménez-Díaz *et al.*, 2006; Klosterman *et al.*, 2009; EFSA, 2014) and may reduce disease incidence and severity, but they do not eliminate *V. nonalfalfae*.

**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. Other host commodities could also be a pathway of introduction of the pathogen into new areas, as populations of *V. nonalfalfae* 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. nonalfalfae*, 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. 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 1982 and revised in the two editions of 'Quarantine Pests for Europe' in 1992 and 1997, as well as in 2019. 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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