Plant Disease • 2020 • 104:2377-2390 • https://doi.org/10.1094/PDIS-08-19-1770-RE

e-**X**tra*

The Identification of a New Species, *Diaporthe humulicola,* a Pathogen Causing Diaporthe Leaf Spot on Common Hop

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Abstract

Common hop, *Humulus lupulus*, is a commercially important crop in the United States, with an increasing number of hop yards being established in the Northeast. In 2018, a new fungal disease was observed at two research hop yards in Connecticut. This new pathogen affected all hop cultivars being grown and caused leaf spots and browning of cones. The causal organism was isolated and Koch's postulates were performed to confirm pathogenicity. The disease symptoms were similar to the previously described Phoma wilt; however, morphological and phylogenetic analyses placed the causal organism as a new species of *Diaporthe*. We propose the name *Diaporthe humulicola*. The disease increased under hot, humid conditions (around 24°C and 90% relative humidity), which prevail during the summer in the northeastern United States as well as other parts of the country. An in vitro preliminary assessment of fungicide sensitivity revealed that pyraclostrobin and boscalid inhibited *D. humulicola* growth in culture and should be further assessed for field efficacy against this new disease of hop. The proper identification and monitoring of this pathogen will be important to inform hop growers of this new threat.

Keywords: Coelomycetes, Diaporthaceae, *Humulus lupulus*, new pathogen, *Phomopsis*

Common hop, *Humulus lupulus* L., is grown commercially for production of hop cones used predominately for preservation and flavoring in beer and, to a lesser extent, for personal care products and some medicines (Mahaffee et al. 2009; Stevens and Page 2004; Zanoli and Zavatti 2008). The United States leads the world in hop production with acreage primarily in the Pacific Northwest region, including Oregon, Washington, and Idaho (George 2018). In the past 10 years, there has been a resurgence in hop cultivation in the northeastern United States to meet the demand for local ingredients from the growing craft brew industry and requirements for labeling as a local product. The Connecticut Agricultural Experiment Station initiated a hop research project in 2013 that consisted of creating two hop yards, one in Windsor, CT and one in Hamden, CT, to be managed similarly to commercial hop. Each year since plant establishment, hop plants were evaluated for presence and severity of fungal diseases and insect pests (Allan-Perkins et al. 2019b). Common diseases present in the Northeast have been similar to the Pacific Northwest. We have found that the most prevalent disease in this region is downy mildew. Powdery mildew is an equally destructive hop disease (Mahaffee et al. 2009) but has only been reported from one hop yard in Windsor, CT (J. LaMondia, personal communication) and one hop yard in Colchester, CT (Allan-Perkins et al. 2019a).

In July and August 2018, lesions were detected on hop leaves at both of the Connecticut Agricultural Experiment Station hop yards. Leaf spot symptoms were ellipsoid, brownish-gray lesions, often with white rings, sometimes with chlorotic margins (Figs. 1A and B). Leaf spot lesions were not delimited by veins and often were associated with leaf margins (Figs. 1A and B). Lesions were detected on eight cultivars: Fuggle, Willamette, Hallertauer, Chinook, Galena, AlphAroma, Perle, Newport, Saaz, and Summit. On bines of the cultivar Saaz, cones presented with brown margins on the bracts in addition to infected leaves (Fig. 1C). Symptoms were similar to those described for Phoma wilt, caused by *Phoma exigua* Desm. (Mahaffee et al. 2009). Infected leaves had dark pycnidia producing ellipsoid eguttulate conidia, similar to that of *P. exigua* (Mahaffee et al. 2009). This pathogen has been reported to cause disease on hop in China, Europe, and New Zealand (Mahaffee et al. 2009; Radisek et al. 2008). Metagenomic analysis of hop plants in Slovenia found *Phoma* spp. present on symptomatic and asymptomatic plants, along with other fungal species such as *Alternaria*, *Fusarium*, and *Sclerotinia* spp. (Jakse et al. 2015). In Canada, *Phoma* spp. are considered occasional pathogens on hop (Grant and Filotas 2014). Within the United States, *Phoma* spp. have been reported as causing secondary infection of hop cones, leading to browning, in Vermont (Darby 2017).

P. exigua, synonym of *Boeremia exigua* var. *exigua*, has previously been called *Phyllosticta decidua* Ellis & Kellerm. 1883, which was reported as causing disease on hop in Wisconsin in 1944 (Greene 1944) and in Iowa in 1929 (Gilman and Archer 1929; USDA 1960). *Phoma herbarum* was reported on hop in Spain and China (Farr and Rossman 2019; Gonzalez Fragoso 1917; Zhuang 2005). Other *Phoma* spp. reported as causing infection on hop are *P. aliena* (syn. *Didymella aliena*) in the Netherlands (Boerema et al. 2004) and *P. macrostoma* in Poland (Farr and Rossman 2019; Mulenko et al. 2008).

The objective of this study was to determine the identity of the *Phoma*-like pathogen causing disease on hop in Connecticut. Morphological identification as well as molecular analyses were used to determine the identity of the fungus. Koch's postulates were performed to confirm the pathogenicity of the isolated fungus on hop leaves. Fungicide efficacy was tested for three fungicides registered for use on hop in Connecticut that have been reported to have efficacy for *Phoma* and *Phomopsis* spp. on other crops. The results of this study will provide important information for hop growers on diseases present in the northeastern United States and potential control strategies.

Materials and Methods

Field identification, collection, and culturing. Hop bines planted in Windsor, CT at the Connecticut Agricultural Experiment Station

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Funding: United States Department of Agriculture Agricultural Marketing Service Grant Number 16-SCBGP-CT-0012.

*The ***e*-X**tra logo stands for "electronic extra" and indicates that one supplementary figure and one supplementary table are published online.

The author(s) declare no conflict of interest.

Accepted for publication 24 March 2020.

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Valley Laboratory were observed to have lesions on leaves on 31 July 2018. Leaves from cultivars AlphAroma, Newport, Saaz, Willamette, Fuggle, Galena, and Chinook and a cone from the Saaz plant were collected and brought into the laboratory. The edge of one lesion from each leaf or cone was excised from the tissue, surface sterilized in 10% bleach solution for 30 s followed by a rinse for 30 s in sterile water, and plated onto half-strength potato dextrose agar (1/2PDA). Cultures were grown at 20°C with a cycle of 12 h of light and 12 h of darkness. Hyphal tip isolations were performed for each original culture on 1/2PDA. Conidial suspensions were made using sterile water to create streak plates for isolation on 1/2PDA. Plugs from hyphal tip isolations and solitary colonies from streak plates were transferred to 1/2PDA slants for long-term storage. Additional leaf samples were pressed for long-term storage and the remaining leaf samples were split and half frozen and half refrigerated for further use.

On 7 August 2018, similar lesions were observed on hop in Hamden, CT at the Connecticut Agricultural Experiment Station Lockwood Farm. All cultivars were affected. Leaves from cultivars AlphAroma, Newport, Cascade, Summit, Sterling, and Brewer's Gold were collected and brought to the laboratory. Leaves were processed as described for those collected from Windsor, CT. In May 2019, similar lesions were observed on hop in Windsor and Hamden, CT and leaves were processed as previously described. By the end of August, all cultivars displayed symptoms; however, Cascade bines showed the fewest symptoms. Hop yield loss due to disease was not assessed. Cascade and Newport yield increased at Windsor Farm from 2017 to 2018 but decreased at Lockwood Farm E. Allan-Perkins, K. Mauer, and J. A. LaMondia, *unpublished*). Summit yield also increased at Windsor but cones were not collected at Lockwood Farm due to poor cone quality (E. Allan-Perkins, K. Mauer, and J. A. LaMondia, *unpublished*). AlphAroma yield was lower at Lockwood Farm in 2018 than 2017 and cones were not collected at Windsor farm due to poor quality (E. Allan-Perkins, K. Mauer, and J. A. LaMondia, *unpublished*).

Isolates from AlphAroma (leaf) in Windsor, Saaz (cone) in Windsor, and Newport (leaf) in Hamden were used for molecular identification and denoted as CT2018-1, CT2018-2, and CT2018-3, respectively. CT2018-1 was used as the holotype and identified morphologically. Plugs from fungal isolates CT2018-1 were placed individually in 1.5-ml centrifuge tubes with 1 ml of sterilized distilled water in triplicate and submitted to the UAMH Centre for Global Microfungal Biodiversity at the University of Toronto (UAMH Collection ID 12076). Temperature and precipitation data were collected by the Connecticut Agricultural Experiment Station weather stations (HOBO U30 with S-THB-M002 12 Bit Temp/RH Sensor and S-RGB-M002 Rain Gauge; Onset Computer Company, Bourne, MA, U.S.A.) in Windsor and Lockwood, CT. In early September, all hop cultivars at both locations showed symptoms with nearly 100% of bines affected.

Morphological identification. Leaf lesions were observed under an Olympus SZ11dissecting scope for presence of pycnidia (Fig. 2). Pycnidia were excised from the leaf lesion, soaked in 50% ammonia solution for 4 h, placed into 50% gum arabic solution, frozen using a BFS-3MP Freezing Stage (Physitemp Instruments LLC, Cliften, NJ, U.S.A.), and sliced with a Microm HM310 microtome (Thermo Scientific, Waltham, MA, U.S.A.) at 10-μm thickness. Four sliced pycnidia were measured and photographed using an Axiocam 506 color camera mounted to a Zeiss Imager M2 compound microscope with differential interference contrast (Carl Zeiss AG, Oberkochen, Germany) with the Zeiss software ZEN (Fig. 2b). Additional pycnidia (Fig. 2c), conidiophores (Fig. 2d), and conidia (Figs. 2e and f) were measured using an Olympus BX40 compound microscope (Olympus Life Sciences, Tokyo, Japan) and an Olympus BH2 compound microscope, for a total of 30 of each from three different isolates. Means, standard deviations of the measurements, and 95% confidence intervals of means were calculated using the Data Analysis package in Microsoft Excel for Mac (v. 16.16.13; Microsoft Corporation, Redmond, WA, U.S.A.).

DNA extraction, amplification, and sequencing. Genomic DNA was extracted from fungal cultures of isolates CT2018-1, CT2018-2, and CT2018-3 grown on 1/2PDA using the ZR Fungal/Bacterial DNA MicroPrep Kit (Zymo Research, Irvine, CA, U.S.A.) according to manufacturer's instructions. The resulting DNA was subject to PCR using oligonucleotides V9G or ITS5 with ITS4 or LR1 for the internal transcribed spacer (ITS) region of ribosomal DNA (Van den Ende and de Hoog 1999; Vilgalys and Hester 1990; White et al. 1990), EF1-728F and EF1-986R for translation elongation factor α-1 (EF1α) (Carbone and Kohn 1999), CYLH3F and H3-1b for histone (HIS) (Crous et al. 2004; Glass and Donaldson 1995), CAL288F and CAL737R or CAL235F and CAL-2Rd for calmodulin (CAL) (Carbone and Kohn 1999; Groenewald et al. 2013; Quaedvlieg et al. 2012), LROR and LR7 for the large ribosomal subunit (28S) (Rehner and Samuels 1994; Vilgalys and Hester 1990), fRPB2-5F2 and fRPB2-7cR RNA polymerase II subunit (RBPS2) (Liu et al. 1999), and TUBUF2 and TUBUR1 (Kroon et al. 2004) for β-tubulin (TUB) amplification. The parameters for the PCR protocol were 94°C for 3 min, 94°C for 30 s, 45°C for 30 s, and 72°C for 2 min, repeat 40 times; and 72°C for 7 min with 50°C as annealing temperature for the HIS PCR.

The resulting PCR products were purified using QIAquick PCR Purification columns (Qiagen, Valencia, CA, U.S.A.) and DNA concentrations were determined on a NanoDrop Lite Spectrophotometer (Thermo Scientific). The PCR products were sequenced using the following oligonucleotides: ITS by ITS1-5 and LR1 (Vilgalys and Hester 1990; White et al. 1990); LSU by LROR, LR5, LR3R, LR3B, and LR7 (Li et al. 2017; Vilgalys and Hester 1990; White et al. 1990), EF1α by EF1-728F and EF1-986R; HIS by CYLH3F and H3-1b (Crous et al. 2004; Glass and Donaldson 1995), CAL

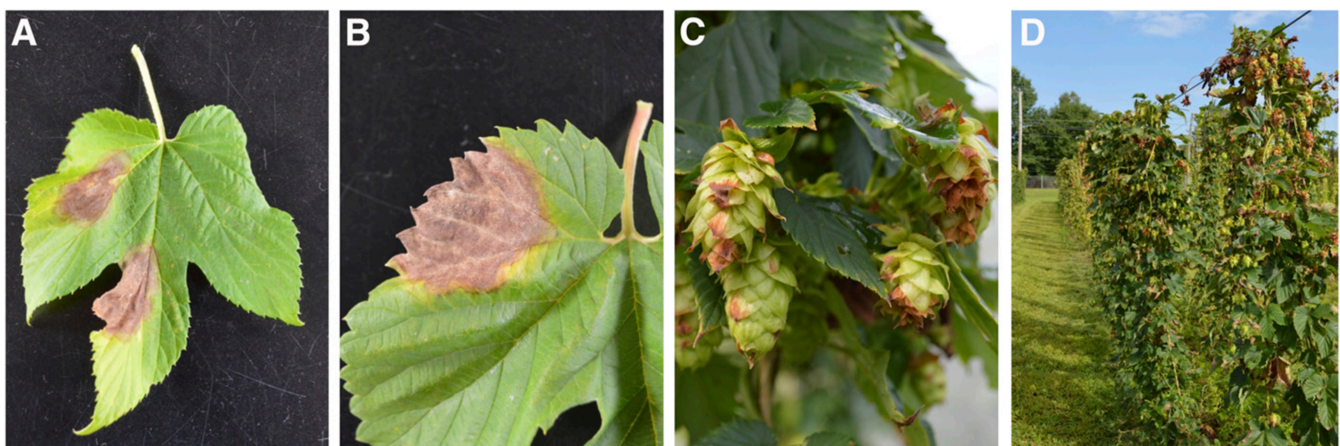


Fig. 1. Diaporthe leaf spot caused by *Diaporthe humulicola* on **A** and **B,** leaf tissue; **C,** cone tissue; **D,** affected hop bines of common hop *(Humulus lupulus)* at Valley Laboratory Farm in Windsor, CT.

by CAL288F, CAL737R, CAL235F, or CAL-2Rd (Carbone and Kohn 1999; Groenewald et al. 2013 Quaedvlieg et al. 2012); and β-tubulin by TUBUF1, TUBUR2, T12, and T22 (Kroon et al. 2004; O'Donnell and Cigelnik 1997). All DNA sequencing was performed at the W. M. Keck Biotechnology Resource Laboratory, Yale School of Medicine (New Haven, CT, U.S.A.). DNA sequence information was deposited to GenBank, accession numbers of the taxa and isolates that were newly sequenced in the study are listed in Table 1, and sequences are listed in Supplementary Table S1.

Alignment and phylogenetic analyses. Sequences for each gene were aligned using Molecular Evolutionary Genetics Analysis (MEGA-7) across computing platforms (Kumar et al. 2016). Pairwise distances were calculated among the isolates using the maximum-likelihood model. Sequences from the type specimens were compared against nucleotide sequences in the NCBI GenBank database using the nucleotide BLAST search algorithm.

DNA sequences were obtained from NCBI GenBank for the ITS region of ribosomal DNA for *Phoma* spp. previously reported as causing disease on hop, representative species of the genus *Diaporthe*, and additional members of the family Diaporthaceae (Table 2). These sequences were aligned with the ITS sequences for three isolates of the new putative species using ClustalW within MEGA-7. A phylogenetic tree was created using maximum-likelihood analysis with 1,000 bootstrap replicates in MEGA-7 (Fig. 3).

To better place the CT2018 fungal isolates, a five-gene tree was created using ITS, EF1α, TUB, HIS, and CAL. These loci had the most representative sequences in the NCBI GenBank for *Diaporthe* spp. Sequences used by Gomes et al. (2013) to resolve the *Diaporthe* phylogeny were downloaded from GenBank (Table 3) and aligned with the Connecticut isolates using the online program MAFFT (https://mafft.cbrc.jp/alignment/server) using default settings (Katoh and Toh 2008) for each locus individually. The alignments were manually edited to remove large gaps and to be of equal lengths for all loci using the program MEGA-7. The alignments were combined using the online interface *FaBox* (https://users-birc.au.dk/palle/php/fabox/alignment_joiner.php) (Villesen 2007). Bayesian inference was analyzed for the combined dataset of ITS, EF1α, TUB, HIS, and CAL sequences with MrBayes3.2.6 (Ronquist et al. 2012). Four Markov chains were used for four runs from random starting trees for 2 million generations. Tree sampling frequency was 1,000 generations. The first 1/10 of generations were discarded as burn-in. A majority-rule consensus tree of all remaining trees was calculated. Branches that received Bayesian posterior probabilities of 0.95 were set as significantly supported. Phylogenetic trees were drawn with TreeGraph2 (Stöver and Müller 2010). Sequence

Table 1. GenBank accessions for Connecticut *Diaporthe humulicola* isolates

Isolate	Locus	Accession number
CT2018-1	Calmodulin	MN180204
CT2018-2	Calmodulin	MN180205
CT2018-3	Calmodulin	MN180206
CT2018-1	Translation elongation factor 1α	MN180207
CT2018-2	Translation elongation factor 1α	MN180208
CT2018-3	Translation elongation factor 1α	MN180209
CT2018-2	β Tubulin	MN180210
CT2018-1	β Tubulin	MN180211
CT2018-3	β Tubulin	MN180212
CT2018-1	Histone 3	MN180213
CT2018-2	Histone 3	MN180214
CT2018-3	Histone 3	MN180215
CT2018-1	RNA polymerase II subunit	MN180216
CT2018-2	RNA polymerase II subunit	MN180217
CT2018-3	RNA polymerase II subunit	MN180218
CT2018-1	Internal transcribed spacer	MN152927
CT2018-2	Internal transcribed spacer	MN152928
CT2018-3	Internal transcribed spacer	MN152929
CT2018-1	Large ribosomal subunit	MN152977
CT2018-2	Large ribosomal subunit	MN152978
CT2018-3	Large ribosomal subunit	MN152979

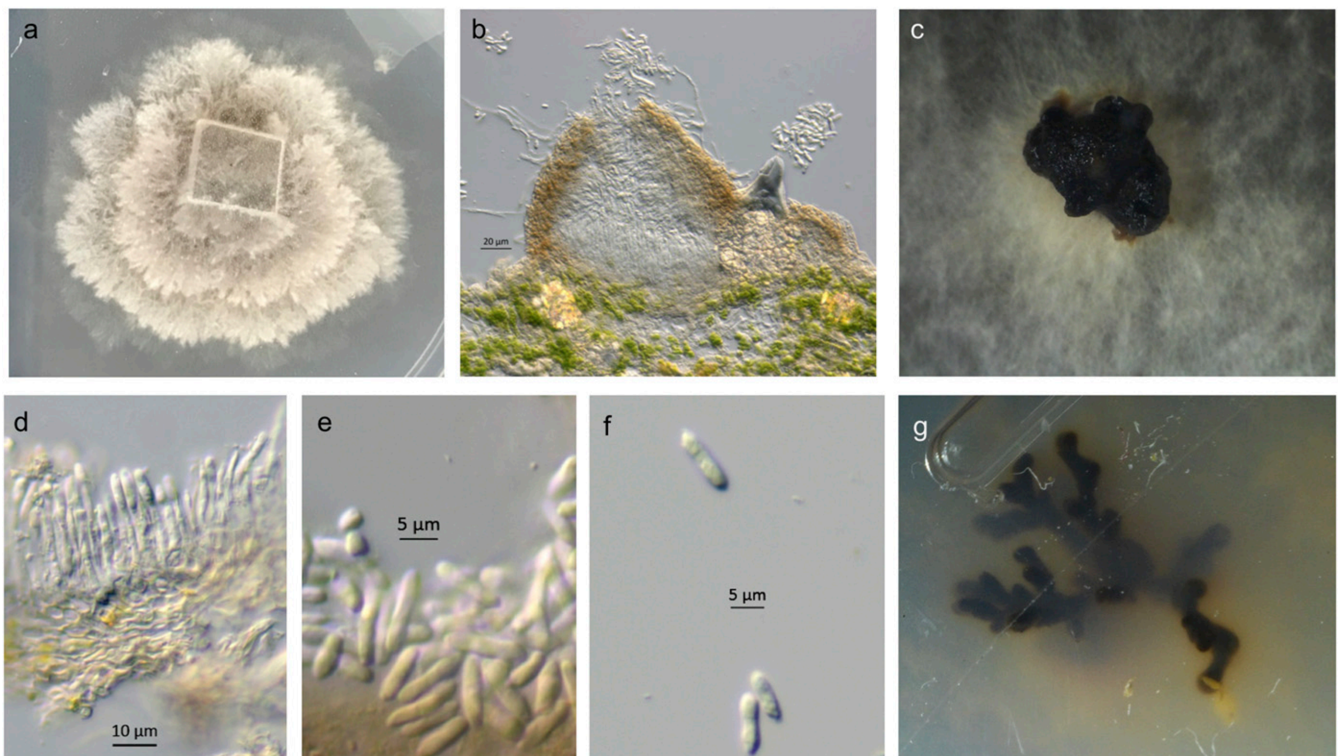


Fig. 2. *Diaporthe humulicola* (Holotype UAMH 12076) **a,** growing on half-strength potato dextrose agar (1/2PDA); **b,** a pycnidium on leaf tissue of common hop (*Humulus lupulus*); **c,** pycnidium growing on 1/2PDA; **d,** conidiophores lining the pycnidial wall of a pycnidium on leaf tissue of common hop; **e** and **f,** conidia on an infected leaf of common hop; and **g,** rhizomorph-like sclerotia in which a number of pycnidia were embedded produced by isolate CT2018-3.

alignments for the genus placement and placement with the *Diaporthe* trees were submitted to TreeBASE and accessed online (https://treebase.org/treebase-web/search/study/anyObjectAsRDF.rdf?namespacedGUID=TB2:S25003).

Koch's postulates. Strap cuttings were taken from hop plants in June 2018 and maintained in the greenhouse with ambient light. In September 2018, conidia were harvested from the hyphal tip culture of the CT2018-1 isolate by adding approximately 1 ml of sterile water to the plate and dislodging conidia with a sterile glass rod to make a conidial suspension. Then, 5 ml of a conidial suspension (2×10^6 conidia/ml) was sprayed on each strap cutting for a total of six plants. Two negative control plants received 5 ml of sterile water. The strap cuttings were then placed in clear plastic bags and evaluated for disease presence after 7 days. Leaf lesions were excised from infected

Table 2. Sequences of *Phoma* spp. and members of the order Diaporthales used in phylogenetic analysis for genus determination of *Diaporthe humulicola*

Species	Isolate	Type	Substrate	ITS[a]	Reference
Boeremia exigua var. *exigua*	CBS 141361	–	*Veronica officialis*	KY550229	Michel et al. 2018
B. exigua var. *exigua*	CBS 431.74	–	*Solanum tuberosum*	FJ427001	Aveskamp et al. 2009
Diaporthe acaciarum	CBS 138862	T	*Acacia tortilis*	KP004460	Crous et al. 2014
D. anacardii	CBS 720.97	T	*Anacardium occidentale*	NR_ 111841	Gomes et al. 2013
D. arecae	CBS 161.64	T	*Areca catechu*	KC343032	Gomes et al. 2013
D. betulina	CFCC 52560	T	*Betula albo-sinensi*	MH121495	Yang et al. 2018b
D. citri	CBS 135422	T	*Citrus sp.*	KC843311	Udayanga et al. 2014b
D. eres	AR5193, CBS:138594	T	*Ulmus laevis*	KJ210529	Udayanga et al. 2014a
D. ganjae	CBS 180.91	T	*Cannabis sativa*	KC343112	Gomes et al. 2013
D. inconspicua	CBS 133813	T	*Maytenus ilicifolia*	KC343123	Yang et al. 2017
D. nothofagi	BRIP 54801	T	*Nothofagus cunninghamii*	JX862530	Tan et al. 2013
D. perjuncta	CBS 109745	T	*Ulmus glabra*	NR_ 147527	Gomes et al. 2013
D. ravennica	MFLUCC 15-0479	T	*Tamarix sp.*	KU900335	Thambugala et al. 2017
D. rosae	MFLUCC 17-2658	T	*Rosa* sp.	MG828894	Wanasinghe et al. 2018
D. sambucusii	CFCC 51986	T	*Sambucus williamsii*	KY852495	Yang et al. 2018b
D. terebinthifolii	CBS 133180; LGMF914;	T	*Schinus terebinthifolius*	KC343216	Gomes et al. 2013
D. unshiuensis	ZJUD 52, CGMCC3.17569	T	*Citrus unshiu*	KJ490587	Yang et al. 2018b
D. velutina	LC 4421	T	*Neolitsea* sp.	KX986790	Gao et al. 2017
D. garethjonesii	MFLUCC 12-0542A	T	–	KT459423	Dissanayake et al. 2015
Diaporthella corylina	CBS 121124	–	*Corylus* sp.	KC343004	Gomes et al. 2013
Diaporthosporella cercidicola	CFCC 51994	–	–	KY852492	Yang et al. 2018a
D. cercidicola	CFCC 51995	–	–	KY852493	Yang et al. 2018a
D. cercidicola	CFCC 51996	–	–	KY852494	Yang et al. 2018a
Diaporthostoma machili	CFCC 52100	T	*Machilus leptophylla*	MG682080	Fan et al. 2018
D. machili	CFCC 52101	–	*M. leptophylla*	MG682081	Fan et al. 2018
Didymella macrostoma	KP 00116	–	*Pyrus communis*	MG791816	K. T. K. Pham, R. Berghuis, and M. Wenneker, unpublished
Ophiodiaporthe cyatheae	HMH-2013 YMJ 1364	T	*Cyathea lepifera*	JX570889	Fu et al. 2013
Phaeocytostroma megalosporum	CBS 284.65	–	*Oryza sativa*	FR748045	Lamprecht et al. 2011
P. ambiguum	CPC 17072	–	*Zea mays*	FR748037	Lamprecht et al. 2011
P. ambiguum	CPC 17071	–	*Z. mays*	FR748036	Lamprecht et al. 2011
P. plurivorum	CBS 113835	–	*Helianthus annus*	FR748046	Lamprecht et al. 2011
P. sacchari	CBS 275.34	–	–	MH855512	Vu et al. 2019
Phoma aliena	CBS 379.93	–	*Berberis* sp.	GU237851	Aveskamp et al. 2010
P. aliena	CBS 877.97	–	*Buxus sempervirens*	GU237910	Aveskamp et al. 2010
P. aliena	ICMP 6602	–	*Actinidia deliciosa*	KT309949	P. R. Johnston and D. Park, unpublished
P. exigua	ICMP 15330	–	–	EU573008	Irinyi et al. 2009
P. exigua	IHRB 2PEX	–	*Humulus lupulus*	EF136399	Radisek et al. 2008
P. exigua var. exigua	CBS 431.742	–	*H. lupulus*	EF136400	Radisek et al. 2008
P. herbarum	EF68d	–	–	KT355016	Unpublished
P. herbarum	CBS 615.75	T	*Rosa multiflora*	FJ427022	Aveskamp et al. 2009
P. macrostoma	IMI 299239	–	*H. lupulus*	DQ474110	W. M. Pitt, K. L. Bailey, Y.-B. Fu, and G. W. Peterson, unpublished
P. macrostoma	ICMP 6803	–	*Lolium perenne*	KT309987	P. R. Johnston and D. Park, unpublished
P. macrostoma	ICMP 7033	–	*Trifolium fragiferum*	KT310027	P. R. Johnston and D. Park, unpublished
Phomopsis conorum	CBS 587.79	–	*Pinus parviflora* var. *pentaphylla*	KC343153	Gomes et al. 2013
Phomopsis emicis	BRIP 45089a	T	*Emex australis*	JF957784	Udayanga et al. 2011
P. fukushii	BRIP 45089b	–	–	JQ619898	Udayanga et al. 2012
P. fukushii	CBS 116953	–	*Pyrus pyrifolia*	KC343147	Gomes et al. 2013
Phomopsis tuberivora	CBS 268.32	T	*Solanum tuberosum*	JF957785	Udayanga et al. 2011
Pustulomyces bambusicola	MFLUCC 11-0436	T	Bamboo	KF548664	Dai et al. 2014
Stenocarpella macrospora	CBS 117560	–	*Z. mays*	FR748048	Lamprecht et al. 2011
S. maydis	CBS 117558	–	*Z. mays*	FR748051	Lamprecht et al. 2011
Valsa ambiens	CFCC 89894	–	*Pyrus* sp.	KR045617	Fan et al. 2014

[a] GenBank accession number for internal transcribed spacer.

plants and plated onto 1/2PDA and incubated at ambient laboratory conditions for 7 days. Cultures were identified morphologically.

Fungicide efficacy. In order to determine possible control measures for this pathogen in the field, the fungicides trifloxystrobin (Bayer Crop Science, Flint, NC, U.S.A.), pyraclostrobin and boscalid (Pageant Intrinsic; BASF, Morrisville, NC, U.S.A.), and pyraclostrobin (Insignia Intrinsic SC; BASF) were tested for their ability to inhibit *D. humulicola* growth in vitro. Trifloxystrobin and the pyraclostrobin and boscalid mix (as Pristine; BASF) are currently registered for use on hop and listed as providing control for *Phoma* and *Phomopsis* spp. on other crops. To determine whether the combination of boscalid and pyraclostrobin is needed to control the disease, we also included the pyraclostrobin-only fungicide, although it is not registered for use on hop. We calculated fungicide rates

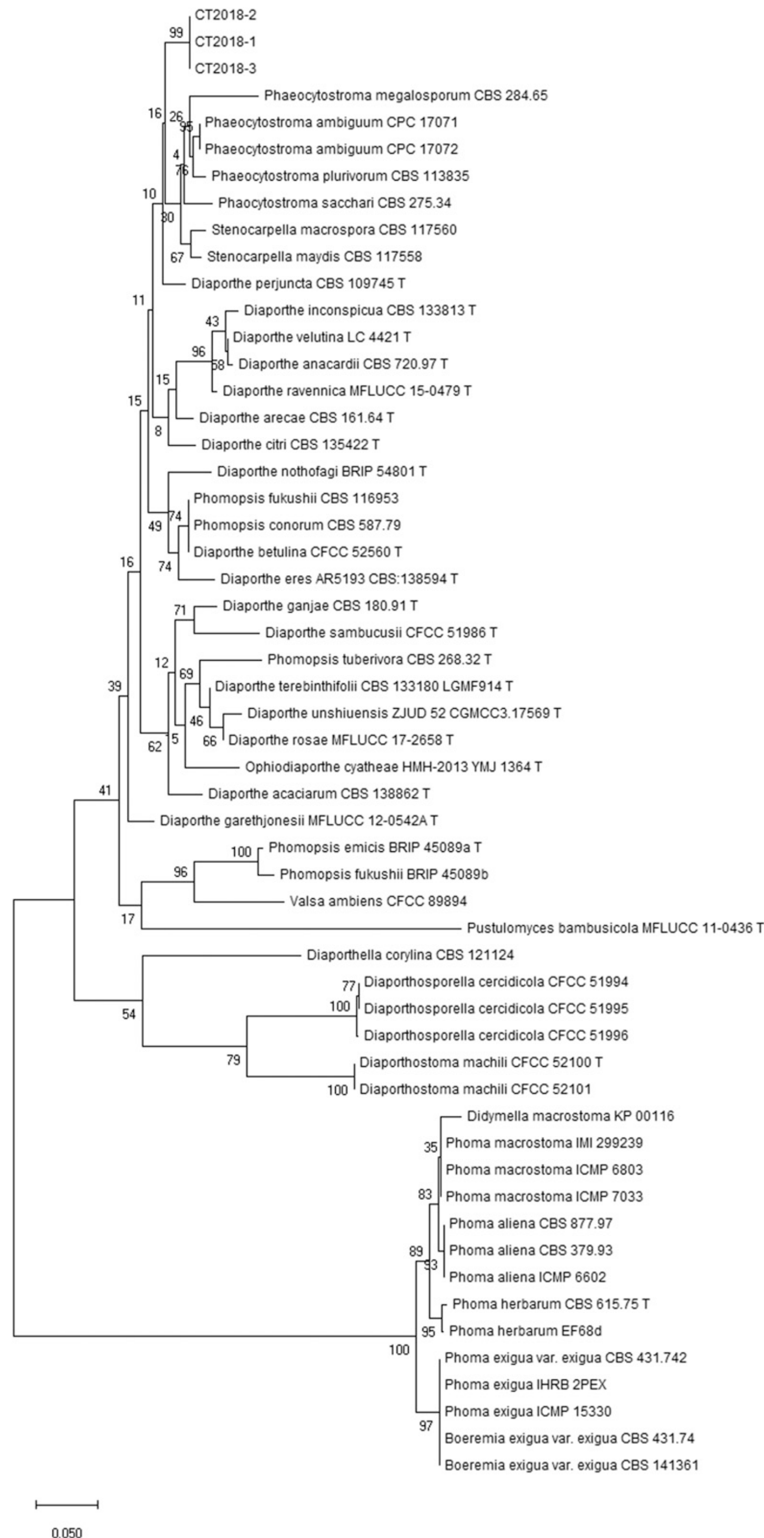


Fig. 3. Maximum-likelihood tree of *Diaporthe humulicola* isolates CT2018-1, CT2018-2, and CT2018-3 with members of Diaporthales and *Phoma* spp. using the internal transcribed spacer (ITS) unit locus of ribosomal DNA. Bootstrap values of resampling with 1,000 replicates are reported at the noses.

Table 3. Sequences of Diaporthaceae species used in phylogenetic analysis (Gomes et al. 2013) for species placement of *Diaporthe humulicola*[a]

Sequence name	Isolate	GenBank accession numbers[b]				
		ITS	EF1α	TUB	CAL	HIS
D. acaciigena	CBS 129521; CPC 17622 T	KC343005	KC343731	KC343973	KC343247	KC343489
D. acerina	CBS 137.27	KC343006	KC343732	KC343974	KC343248	KC343490
D. alleghaniensis	CBS 495.72; ATCC 24097 T	KC343007	KC343733	KC343975	KC343249	KC343491
D. alnea	CBS 146.46	KC343008	KC343734	KC343976	KC343250	KC343492
D. alnea	CBS 159.47	KC343009	KC343735	KC343977	KC343251	KC343493
D. ambigua	CBS 114015; STE-U 2657; CPC 2657 T	KC343010	KC343736	KC343978	KC343252	KC343494
D. ambigua	CBS 117167; STE-U 5414; CPC 5414	KC343011	KC343737	KC343979	KC343253	KC343495
D. ambigua	CBS 127746; IMI 395956	KC343014	KC343740	KC343982	KC343256	KC343498
D. ampelina	CBS 111888; ATCC 48153; STE-U 2673; CPC 2673	KC343016	KC343742	KC343984	KC343258	KC343500
D. ampelina	CBS 114016; STE-U 2660; CPC 2660; PV F98-1 T	AF230751	AY745056	JX275452	AY745026	–
D. ampelina	CBS 114867; STE-U 4708; CPC 4708	KC343017	KC343743	KC343985	KC343259	KC343501
D. ampelina	CBS 267.80; STE-U 2671; CPC 2671	KC343018	KC343744	KC343986	KC343260	KC343502
D. amygdali	CBS 111811; STE-U 2632; CPC 2632	KC343019	KC343745	KC343987	KC343261	KC343503
D. amygdali	CBS 115620; FAU 1005	KC343020	KC343746	KC343988	KC343262	KC343504
D. amygdali	CBS 126679 T	KC343022	KC343748	KC343990	KC343264	KC343506
D. amygdali	CBS 126680	KC343023	KC343749	KC343991	KC343265	KC343507
D. arctii	CBS 136.25	KC343031	KC343757	KC343999	KC343273	KC343515
D. arecae	CBS 161.64 T	KC343032	KC343758	KC344000	KC343274	KC343516
D. arecae	CBS 535.75	KC343033	KC343759	KC344001	KC343275	KC343517
D. arengae	CBS 114979; HKUCC 5527 T	KC343034	KC343760	KC344002	KC343276	KC343518
D. aspalathi	CBS 117168; STE-U 5420; CPC 5420	KC343035	KC343761	KC344003	KC343277	KC343519
D. aspalathi	CBS 117169; STE-U 5428; CPC 5428 T	KC343036	KC343762	KC344004	KC343278	KC343520
D. aspalathi	CBS 117500; STE-U 5408; CPC 5408	KC343037	KC343763	KC344005	KC343279	KC343521
D. australafricana	CBS 111886; STE-U 2676; CPC 2676 T	KC343038	KC343764	KC344006	KC343280	KC343522
D. australafricana	CBS 113487; STE-U 2655; CPC 2655	KC343039	KC343765	KC344007	KC343281	KC343523
D. batatas	CBS 122.21	KC343040	KC343766	KC344008	KC343282	KC343524
D. beckhausii	CBS 138.27	KC343041	KC343767	KC344009	KC343283	KC343525
D. brasiliensis	CBS 133183; LGMF924; CPC 20300 T	KC343042	KC343768	KC344010	KC343284	KC343526
D. brasiliensis	LGMF926; CPC 20302	KC343043	KC343769	KC344011	KC343285	KC343527
D. carpini	CBS 114437; UPSC 2980	KC343044	KC343770	KC344012	KC343286	KC343528
D. caulivora	CBS 127268; Dpc1 T	KC343045	KC343771	KC344013	KC343287	KC343529
D. caulivora	CBS 178.55; ATCC 12048; Alfaro 243	KC343046	KC343772	KC344014	KC343288	KC343530
D. celastrina	CBS 139.27	KC343047	KC343773	KC344015	KC343289	KC343531
D. chamaeropis	CBS 454.81	KC343048	KC343774	KC344016	KC343290	KC343532
D. chamaeropis	CBS 753.70	KC343049	KC343775	KC344017	KC343291	KC343533
D. cinerascens	CBS 719.96	KC343050	KC343776	KC344018	KC343292	KC343534
D. citri	CBS 199.39	KC343051	KC343777	KC344019	KC343293	KC343535
D. citri	CBS 230.52	KC343052	KC343778	KC344020	KC343294	KC343536
D. citri	LGMF946; CPC 20322	KC343053	KC343779	KC344021	KC343295	KC343537
D. convolvuli	CBS 124654; DP 0727	KC343054	KC343780	KC344022	KC343296	KC343538
D. crataegi	CBS 114435; UPSC 2938	KC343055	KC343781	KC344023	KC343297	KC343539
D. crotalariae	CBS 162.33	KC343056	KC343782	KC344024	KC343298	KC343540
D. cuppatea	CBS 117499; STE-U 5431; CPC 5431 T	KC343057	KC343783	KC344025	KC343299	KC343541
D. cynaroidis	CBS 122676; CMW 22190; CPC 13180 T	KC343058	KC343784	KC344026	KC343300	KC343542
D. decedens	CBS 109772; AR 3459	KC343059	KC343785	KC344027	KC343301	KC343543
D. decedens	CBS 114281; UPSC 2957	KC343060	KC343786	KC344028	KC343302	KC343544
D. detrusa	CBS 109770; AR 3424	KC343061	KC343787	KC344029	KC343303	KC343545
D. detrusa	CBS 114652; UPSC 3371	KC343062	KC343788	KC344030	KC343304	KC343546
D. detrusa	CBS 140.27	KC343063	KC343789	KC344031	KC343305	KC343547
D. elaeagni	CBS 504.72	KC343064	KC343790	KC344032	KC343306	KC343548
D. endophytica	CBS 133811; LGMF916; CPC 20292 T	KC343065	KC343791	KC344033	KC343307	KC343549
D. endophytica	LGMF911; CPC 20287	KC343066	KC343792	KC344034	KC343308	KC343550
D. endophytica	LGMF919; CPC 20295	KC343067	KC343793	KC344035	KC343309	KC343551
D. eres	CBS 375.61	KC343088	KC343814	KC344056	KC343330	KC343572
D. eres	CBS 422.50	KC343089	KC343815	KC344057	KC343331	KC343573
D. eres	CBS 439.82; BBA P-407; IMI 162181a T	KC343090	KC343816	KC344058	KC343332	KC343574
D. eugeniae	CBS 444.82	KC343098	KC343824	KC344066	KC343340	KC343582
D. fibrosa	CBS 109751; AR 3425	KC343099	KC343825	KC344067	KC343341	KC343583
D. fibrosa	CBS 113830; UPSC 2117	KC343100	KC343826	KC344068	KC343342	KC343584
D. foeniculacea	CBS 123208; Di-C004/5 T	KC343104	KC343830	KC344072	KC343346	KC343588
D. foeniculacea	CBS 123209; Di-C004/4 T	KC343105	KC343831	KC344073	KC343347	KC343589
D. foeniculacea	CBS 187.27T	KC343107	KC343833	KC344075	KC343349	KC343591
D. ganjae	CBS 180.91; ILLS 43621 T	KC343112	KC343838	KC344080	KC343354	KC343596
D. gardeniae	CBS 288.56	KC343113	KC343839	KC344081	KC343355	KC343597

(*Continued on next page*)

[a] Sequence names are the currently accepted species name determined by Gomes et al. (2013). Extype cultures are denoted by the letter T.
[b] ITS = internal transcribed spacer, EF1α = translation elongation factor 1-α, TUB = β-tubulin, CAL = calmodulin, and HIS = histone.

Table 3. (*Continued from previous page*)

Sequence name	Isolate	GenBank accession numbers[b] ITS	EF1α	TUB	CAL	HIS
D. helianthi	CBS 344.94	KC343114	KC343840	KC344082	KC343356	KC343598
D. helianthi	CBS 592.81 T	KC343115	KC343841	KC344083	KC343357	KC343599
D. cf. *heveae* 1	CBS 852.97	KC343116	KC343842	KC344084	KC343358	KC343600
D. cf. *heveae* 2	CBS 681.84	KC343117	KC343843	KC344085	KC343359	KC343601
D. hickoriae	CBS 145.26 T	KC343118	KC343844	KC344086	KC343360	KC343602
D. hongkongensis	CBS 115448; HKUCC 9104; AT 646 DF 24 T	KC343119	KC343845	KC344087	KC343361	KC343603
D. hordei	CBS 481.92	KC343120	KC343846	KC344088	KC343362	KC343604
D. impulsa	CBS 114434; UPSC 3052	KC343121	KC343847	KC344089	KC343363	KC343605
D. impulsa	CBS 141.27	KC343122	KC343848	KC344090	KC343364	KC343606
D. inconspicua	CBS 133813; LGMF930; CPC 20306 T	KC343123	KC343849	KC344091	KC343365	KC343607
D. inconspicua	LGMF922; CPC 20298	KC343124	KC343850	KC344092	KC343366	KC343608
D. inconspicua	LGMF931; CPC 20307	KC343125	KC343851	KC344093	KC343367	KC343609
D. infecunda	CBS 133812; LGMF906; CPC 20282 T	KC343126	KC343852	KC344094	KC343368	KC343610
D. infecunda	LGMF908; CPC 20284	KC343127	KC343853	KC344095	KC343369	KC343611
D. infecunda	LGMF912; CPC 20288	KC343128	KC343854	KC344096	KC343370	KC343612
D. juglandina	CBS 121004; DP 0659	KC343134	KC343860	KC344102	KC343376	KC343618
D. longispora	CBS 194.36 T	KC343135	KC343861	KC344103	KC343377	KC343619
D. lusitanicae	CBS 123212; Di-C001/5 T	KC343136	KC343862	KC344104	KC343378	KC343620
D. lusitanicae	CBS 123213; Di-C001/3	KC343137	KC343863	KC344105	KC343379	KC343621
D. manihotia	CBS 505.76	KC343138	KC343864	KC344106	KC343380	KC343622
D. mayteni	CBS 133185; LGMF938; CPC 20314 T	KC343139	KC343865	KC344107	KC343381	KC343623
D. megalospora	CBS 143.27	KC343140	KC343866	KC344108	KC343382	KC343624
D. melonis	CBS 435.87	KC343141	KC343867	KC344109	KC343383	KC343625
D. melonis	CBS 507.78 T	KC343142	KC343868	KC344110	KC343384	KC343626
D. musigena	CBS 129519; CPC 17026 T	KC343143	KC343869	KC344111	KC343385	KC343627
D. neilliae	CBS 144.27	KC343144	KC343870	KC344112	KC343386	KC343628
D. neoarctii	CBS 109490; GB 6421; AR 3450 T	KC343145	KC343871	KC344113	KC343387	KC343629
D. nobilis	CBS 113470; DAOM 226800	KC343146	KC343872	KC344114	KC343388	KC343630
D. nobilis	CBS 116953; NZ-26	KC343147	KC343873	KC344115	KC343389	KC343631
D. nobilis	CBS 116954; NZ-27	KC343148	KC343874	KC344116	KC343390	KC343632
D. nomurai	CBS 157.29	KC343154	KC343880	KC344122	KC343396	KC343638
D. novem	CBS 127269; 5-27/3-1	KC343155	KC343881	KC344123	KC343397	KC343639
D. novem	CBS 127270; 4-27/3-1 T	KC343156	KC343882	KC344124	KC343398	KC343640
D. novem	CBS 127271; 5/27/3-3	KC343157	KC343883	KC344125	KC343399	KC343641
D. oncostoma	CBS 100454	KC343160	KC343886	KC344128	KC343402	KC343644
D. oncostoma	CBS 589.78	KC343162	KC343888	KC344130	KC343404	KC343646
D. oncostoma	CBS 809.85	KC343163	KC343889	KC344131	KC343405	KC343647
D. oxe	CBS 133186; LGMF942; CPC 20318 T	KC343164	KC343890	KC344132	KC343406	KC343648
D. oxe	CBS 133187; LGMF936; CPC 20312	KC343165	KC343891	KC344133	KC343407	KC343649
D. oxe	LGMF915; CPC 20291	KC343166	KC343892	KC344134	KC343408	KC343650
D. padi var. *padi*	CBS 114200; UPSC 2569	KC343169	KC343895	KC344137	KC343411	KC343653
D. padi var. *padi*	CBS 114649; UPSC 3496	KC343170	KC343896	KC344138	KC343412	KC343654
D. paranensis	CBS 133184; LGMF929; CPC 20305 T	KC343171	KC343897	KC344139	KC343413	KC343655
D. perjuncta	CBS 109745; ARSEF 3461; AR 3461 T	KC343172	KC343898	KC344140	KC343414	KC343656
D. perseae	CBS 151.73	KC343173	KC343899	KC344141	KC343415	KC343657
D. phaseolorum	CBS 113425	KC343174	KC343900	KC344142	KC343416	KC343658
D. phaseolorum	CBS 116019; STAM 30	KC343175	KC343901	KC344143	KC343417	KC343659
D. phaseolorum	CBS 116020; STAM 31	KC343176	KC343902	KC344144	KC343418	KC343660
D. pseudomangiferae	CBS 101339 T	KC343181	KC343907	KC344149	KC343423	KC343665
D. pseudomangiferae	CBS 388.89	KC343182	KC343908	KC344150	KC343424	KC343666
D. pseudophoenicicola	CBS 176.77	KC343183	KC343909	KC344151	KC343425	KC343667
D. pseudophoenicicola	CBS 462.69 T	KC343184	KC343910	KC344152	KC343426	KC343668
D. pustulata	CBS 109742; AR 3430	KC343185	KC343911	KC344153	KC343427	KC343669
D. pustulata	CBS 109760; AR 3535	KC343186	KC343912	KC344154	KC343428	KC343670
D. pustulata	CBS 109784; AR 3419	KC343187	KC343913	KC344155	KC343429	KC343671
D. raonikayaporum	CBS 133182; LGMF923; CPC 20299 T	KC343188	KC343914	KC344156	KC343430	KC343672
D. rhoina	CBS 146.27	KC343189	KC343915	KC344157	KC343431	KC343673
D. saccarata	CBS 116311; STE-U 3743; CPC 3743 T	KC343190	KC343916	KC344158	KC343432	KC343674
D. schini	CBS 133181; LGMF921; CPC 20297 T	KC343191	KC343917	KC344159	KC343433	KC343675
D. schini	LGMF910; CPC 20286	KC343192	KC343918	KC344160	KC343434	KC343676
D. sclerotioides	CBS 296.67; ATCC 18585; IMI 151828 T	KC343193	KC343919	KC344161	KC343435	KC343677
D. sclerotioides	CBS 710.76; PD 76/674	KC343194	KC343920	KC344162	KC343436	KC343678
D. scobina	CBS 251.38	KC343195	KC343921	KC344163	KC343437	KC343679
D. sojae	CBS 100.87	KC343196	KC343922	KC344164	KC343438	KC343680
D. sojae	CBS 116023; STAM 35	KC343198	KC343924	KC344166	KC343440	KC343682

(*Continued on next page*)

Table 3. (*Continued from previous page*)

Sequence name	Isolate	GenBank accession numbers[b]				
		ITS	EF1α	TUB	CAL	HIS
D. sojae	CBS 659.78; NRRL 13656	KC343201	KC343927	KC344169	KC343443	KC343685
Diaporthe sp. 1	CBS 119639; B 11861	KC343202	KC343928	KC344170	KC343444	KC343686
Diaporthe sp. 2	LGMF947; CPC 20323	KC343203	KC343929	KC344171	KC343445	KC343687
Diaporthe sp. 2	LGMF932; CPC 20308	KC343204	KC343930	KC344172	KC343446	KC343688
Diaporthe sp. 3	CBS 287.29	KC343205	KC343931	KC344173	KC343447	KC343689
Diaporthe sp. 5	CBS 125575	KC343207	KC343933	KC344175	KC343449	KC343691
Diaporthe sp. 6	CBS 115584; HKUCC 7784; AT 7	KC343208	KC343934	KC344176	KC343450	KC343692
Diaporthe sp. 7	CBS 115595; HKUCC 10129	KC343209	KC343935	KC344177	KC343451	KC343693
Diaporthe sp. 7	CBS 458.78	KC343210	KC343936	KC344178	KC343452	KC343694
Diaporthe sp. 8	LGMF925; CPC 20301	KC343211	KC343937	KC344179	KC343453	KC343695
D. stictica	CBS 370.54	KC343212	KC343938	KC344180	KC343454	KC343696
D. subordinaria	CBS 101711	KC343213	KC343939	KC344181	KC343455	KC343697
D. subordinaria	CBS 464.90	KC343214	KC343940	KC344182	KC343456	KC343698
D. tecomae	CBS 100547	KC343215	KC343941	KC344183	KC343457	KC343699
D. terebinthifolii	CBS 133180; LGMF914; CPC 20290 T	KC343216	KC343942	KC344184	KC343458	KC343700
D. terebinthifolii	LGMF907; CPC 20283	KC343217	KC343943	KC344185	KC343459	KC343701
D. terebinthifolii	LGMF909; CPC 20285	KC343218	KC343944	KC344186	KC343460	KC343702
D. toxica	CBS 534.93; ATCC 96741 T	KC343220	KC343946	KC344188	KC343462	KC343704
D. toxica	CBS 535.93	KC343221	KC343947	KC344189	KC343463	KC343705
D. toxica	CBS 546.93	KC343222	KC343948	KC344190	KC343464	KC343706
D. vaccinii	CBS 122115; FAU 590	KC343226	KC343952	KC344194	KC343468	KC343710
D. vaccinii	CBS 122116; DF 5022	KC343227	KC343953	KC344195	KC343469	KC343711
D. vaccinii	CBS 160.32; IFO 32646 T	KC343228	KC343954	KC344196	KC343470	KC343712
D. vexans	CBS 127.14	KC343229	KC343955	KC344197	KC343471	KC343713
D. viticola	CBS 113201; STE-U 5683; CPC 5683 T	KC343234	KC343960	KC344202	KC343476	KC343718
D. viticola	CBS 759.95	KC343242	KC343968	KC344210	KC343484	KC343726
D. viticola	CBS 794.96	KC343243	KC343969	KC344211	KC343485	KC343727
D. woodii	CBS 558.93	KC343244	KC343970	KC344212	KC343486	KC343728
D. woolworthii	CBS 148.27	KC343245	KC343971	KC344213	KC343487	KC343729
Diaporthella corylina	CBS 121124; AR 4131	KC343004	KC343730	KC343972	KC343246	KC343488

(micrograms per milliliter) for testing based on label rates for *Phoma* and *Phomopsis* control for trifloxystrobin and pyraclostrobin and boscalid mix (Table 4). For pyraclostrobin, we calculated rates that would be equal to the concentration of pyraclostrobin in the mixed fungicide (Table 4). Fungicides were added to 1/2PDA agar to obtain the desired concentrations in micrograms per milliliter. *D. humulicola* cultures were grown on 1/2PDA from the CT2018-1 and CT2018-3 isolates. Plugs taken from the *D. humulicola* cultures with a 5-mm core borer were placed in the center of the fungicide-amended plates in replicates of four and onto four unamended (untreated control) plates. Cultures were grown at 22°C in complete darkness for 96 h. The diameter of the cultures was measured at two perpendicular angles. Relative mycelial growth (RMG) was calculated as growth of fungicide-amended culture divided by growth on unamended (untreated control) media. The experiment was repeated twice for trifloxystrobin at the original calculated concentrations. For pyraclostrobin and boscalid and pyraclostrobin alone, concentrations were reduced due to complete growth inhibition at all original concentrations and subsequently repeated twice (Table 4).

Results

Field conditions. The growing season in 2018 had similar air temperatures at both locations (Table 5). When symptoms appeared in July at Windsor, the average temperature was 24.4°C, with 23.9°C in August at both Windsor and Hamden. Average monthly rainfall was greater in July and August compared with May and June at both locations, coinciding with the appearance of symptoms (Table 5). In 2019, leaf spots were observed following wet and warm weather conditions at both locations.

Symptoms in the field. Symptoms occurred from the spring to summer and appeared to be small dark spots on the leaves, often water soaked in the early stage, and expanded into ellipsoid, brownish-gray lesions, often with white rings, or with chlorotic margins. In late summer, dark-brown to black pycnidia developed in the lesions on the adaxial side under moist conditions. Milky-colored conidial masses oozed out from the ostioles under humid or wet conditions. Leaf spot lesions were not delimited by veins and often associated with leaf margins (Fig. 1). As disease progressed, cones became infected, showing dark reddish-brown margins of bracts (Fig. 1C).

Koch's postulates test. Inoculated plants displayed leaf lesions with brownish-gray lesions with white concentric rings, whereas the untreated controls did not display symptoms. Cultures resulting from isolations from inoculated plant leaf lesions had identical appearance to *D. humulicola* on 1/2PDA and microscopic examination found identical conidia.

Phylogenetic analyses. All three isolates had identical ITS sequences. BLAST analysis of the ITS region placed the CT2018-1 isolate as a member of the genus *Diaporthe*, with the closest GenBank accession being an unidentified *Diaporthe* sp. isolate CLJ-1 (accession number LC373144) isolated from *Cinchona ledgeriana* L. at 96.55% identity. For the EF1α locus, the three isolates shared identical sequences with the closest BLAST match as *Diaporthe anacardii* (Early & Punith.) R. R. Gomes, C. Glienke, & Crous, accession number MK442692, at 81.44% identity. For TUB, sequences from all three isolates had identical sequences and the nearest BLAST hit was an unidentified *Phomopsis* sp. strain Pho08 (accession number HQ586907) isolated from *Vitis vinifera* L. at 95.75% identity. All three isolates shared identical HIS locus sequences, with the closest BLAST match being *D. eres* Nitschke, accession number MG516978. At the CAL locus, all three isolates presented with sequence variation. Pairwise distances were 0.63% for CT2018-1 and CT2018-2, 0.16% for CT2018-1 and CT2018-3, and 0.16% for CT2018-2 and CT2018-3. The closest match revealed by a BLAST search was *D. perjuncta* Niessl., accession number KC343414, at 83.2% identity. The 28S locus sequence was identical for all three isolates and the closest BLAST match was *Stenocarpella maydis*

(Berk.) B. Sutton, accession number KP164561, at 99.06% identity. The three isolates were not identical for the *rpb2* gene sequences, with pairwise distances of 1.4% between CT2018-1 and CT2018-2, 0.87% for CT2018-1 and CT2018-3, and 0.52% for CT2018-2 and CT2018-3. A BLAST search of CT2018-1 was most similar to *Phomopsis viticola* (Sacc.) Sacc. isolate PhoCT2L (currently accepted name: *D. ampelina*), accession number HQ446836, isolated from a *Vitis* sp. at 92.73% sequence identity.

A maximum-likelihood phylogenetic tree of *Phoma* spp. and Diaporthaceae species placed *D. humulicola* isolates CT2018-1, CT2018-2, and CT2018-3 within *Diaporthe*, Diaporthaceae, Diparothales and separated out the *Phoma* spp. and *Didymella macrostoma* (Mont.) Qian Chen & L. Cai with 100% bootstrap support (Fig. 3).

Maximum-likelihood and Bayesian analyses showed that *D. humulicola* clearly separated from the outgroup, *Diaporthella corylina* Lar. N. Vassiljeva, as well as *Diaporthe acaciigena* Crous, Pascoe & Jacq. Edwards, *D. pustulata* Sacc., *D. amygdali* (Delacr.) Udayanga, Crous & K. D. Hyde, *D. australafricana* Crous & Van Niekerk, *D. viticola* Nitschke, *D. cynaroidis* Marinc. M. J. Wingf. & Crous, *D. beckhausii* Nitschke, *D. heveae* Petch, and *D. toxica* P. M. Will., Highet, W. Gams & Sivasith. (Fig. 4; Supplementary Fig. S1). All three isolates of *D. humulicola* grouped together with 100% significance (Fig. 4). *D. humulicola* clustered most closely (98.4%) with *D. ambigua, D. longispora, D. scerlotiodies, D. mayteni, D. raonikayaporum, Diaporthe.* sp. 2, *D. angelica, D. subordinaria, D. arctii, D. neoarctii, D. cuppatea, D. lusitanicae, D. novem, D. infecunda, D. batatas, D. citri, D. sojae, Diaporthe* sp. 1, *D. convolvuli, D. endophytica, D. phaseolorum, D. melonis, D. helianthin, D. hordei, D. vexans, D. megalospora, D. schini, D. tecomae, D. terebinthifolii, D. ganjae, D. manihota, D. oxe, Diaporthe* sp. 3, *D. paranensis, D. brasiliensis,* and *Diaporthe* sp. 5 (Fig. 4).

Taxonomy. The results of the molecular analysis and observations of morphological characteristics in planta and in culture support the conclusion that all three isolates—CT2018-1, CT2018-2, and CT2018-3—are a single, new species of *Diaporthe*. Two subcultures of CT2018-3 produced rhizomorph-like sclerotia in which a number of pycnidia were embedded with sporulation structures similar to the pycnidia that were produced in cell culture and on leaf tissue (Fig. 2g). The rhizomorph structures were observed after 5 1/2 months of growth on 1/2PDA at ambient room temperature.

Diaporthe humulicola E. B. Allan-Perkins, D. W. Li, N. P. Schultes & J. A. LaMondia sp. nov. MycoBank number MB832379. Sexual state: undetermined. Conidiomata*:* pycnidial, solitary or aggregated, conical to globose or flask-like, brown to dark brown, up to 267 μm diam. on leaves and 1,250 μm on 1/2PDA, subepidermal in leaf and petiole tissues, erumpent through surface, unilocular (Fig. 2b). Ostiole: present. Wall: parenchymatous, textura angularis. Conidiophores: reduced to conidiogenous cells, hyaline, unbranched, not septate, growing along all walls of pycnidium, (17.2) 21.7–26.6 (29.5) × (1.2) 1.4–2.7 (3.7) μm (mean ± standard deviation [SD]: 24.7 ± 2.9 × 2 ± 0.6, $n = 30$) (Fig. 2d). Conidiogenous cells*:* enteroblastic, monophialidic, determinate, unbranched, hyaline, smooth, tubulate, cylindrical, (2.5) 3.4–6.4 (7.4) × (1.2) 2.0–3.3 (4.9) μm (mean ± SD: 4.9 ± 1.5 × 2.6 ± 0.7, $n = 30$) (Fig. 2d). α Conidia*:* hyaline, eguttulate, rarely 1 to multiguttulate, 1-celled, smooth, cylindrical or clavate with obtuse ends, some constricted in the middle, occasionally becoming dumb-bell shaped, (3) 4.4–11.6 (15) × (2.5) 2.7–5.4 (7.5) μm (mean ± SD: 8 ± 3.6 × 4.1 ± 1.3, $n = 30$) (Fig. 2e and f). β and γ Conidia: not observed.

Culture characteristics: Colony grown on 1/2PDA. Irregular form, flat elevation, undulate margin, white and brown-gray bands, pycnidia sparse and irregularly dispersed over agar surface (Fig. 2a).

Holotype: U.S.A., Connecticut, Windsor, 41°51′0.65″N, 72°39′38.16″W, *Humulus lupulus* cultivar AlphAroma leaf, 31 July 2018, E. B. Allan-Perkins, UAMH 12076 (= CT2018-1). Holotype specimen is a living specimen being maintained via lyophilization at UAMH Centre for Global Microfungal Biodiversity, The Gage Research Institute, University of Toronto, Toronto, Canada.

Etymology: Latin; *Humulus* referring to host genus, *Humulus*, and -cola, ones that grow on.

Additional materials examined: USA, Connecticut, Windsor, 41°51′0.65″N, 72°39′38.16″W, *Humulus lupulus* cultivar Saaz cone, 31 July 2018, E. B. Allan-Perkins, (CT2018-2). U.S.A., Connecticut, Hamden, 41°24′20.34″N, 72°54′26.78″W, *Humulus lupulus* cultivar Newport, 7 August 2018, M. Salvas, (CT2018-3).

Host/distribution: from *Humulus lupulus* in Windsor and Hamden, Connecticut, U.S.A.

Comments: In addition to the holotype described above, conidiophores, conidiogenous cells, and conidia were measured from two additional isolates. For isolate 2018-3 (isolated from leaf of cultivar Newport collected in Hamden, CT), conidiophores measured (14.8) 17.7–24.5 (29.5) × (1.8) 1.9–2.7 (3.1) μm (mean ± SD: 21.1 ± 3.4 × 2.3 ± 0.4, $n = 30$), conidiogenous cells measured (4.9) 7.4–12.1 (16.0) × (1.8) 2.1–3.7 (6.2) μm (mean ± SD: 9.7 ± 2.4 × 2.9 ± 0.8, $n = 30$), and α conidia measured (6.2) 7.3–10.2 (12.3) × (2.5) 2.5–4.4 (5.4) μm (mean ± SD: 8.8 ± 1.5 × 3.4 ± 0.9, $n = 30$). For isolate 2018-9 (isolated from leaf of cultivar AlphAroma collected in Hamden, CT), conidiophores measured (11.1) 14.4–22.4 (24.6) × (1.8) 1.8–3.1 (4.9) μm (mean ± SD: 18.4 ± 4.0 × 2.5 ± 0.7, $n = 30$), conidiogenous cells measured (2.5) 5.9–11.1 (14.8) × (1.8) 2.2–3.9 (6.2) μm (mean ± SD: 8.5 ± 2.6 × 3.0 ± 0.8, $n = 30$), and α conidia measured (6.9) 7.8–10.6 (12.3) × (2.5) 3.0–4.9 (6.2) μm (mean ± SD: 9.2 ± 1.4 × 3.9 ± 1.0, $n = 30$). According to both morphological characteristics and phylogenetic analyses (Fig. 3), all three isolates belong to *D. humulicola*.

D. humulicola shows similar symptoms on common hop as *Phoma exigua* and *P. aliena* but its morphology lacks scleroplectenchyma in pycnidia and molecular analysis shows that it is a member of the genus *Diaporthe.*

Table 5. Weather conditions in Windsor and Hamden, CT hop yards during *Diaporthe humulicola* infection of hop in 2018

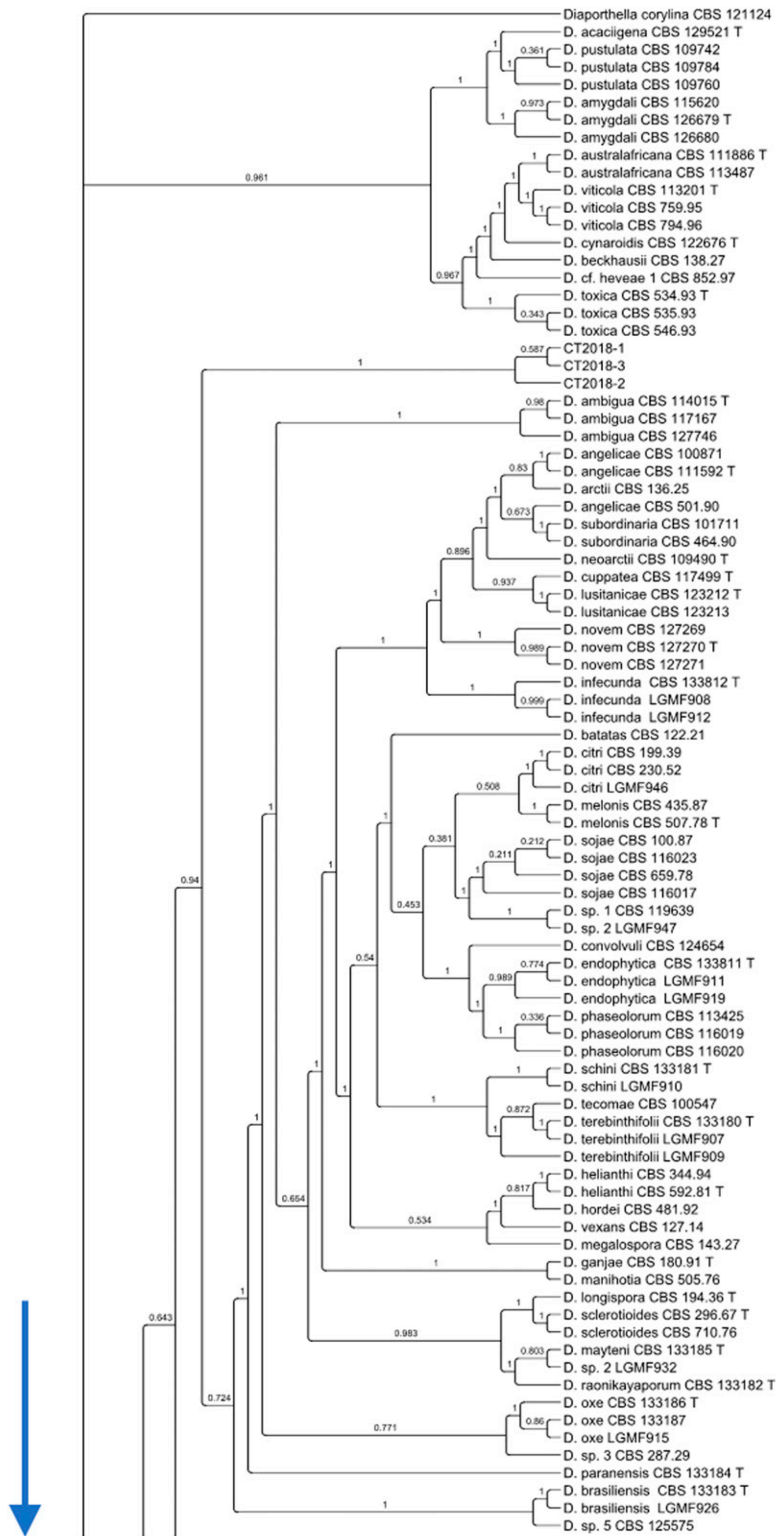
	Temperature (°C)		Monthly rainfall (cm)	
Month	**Windsor**	**Hamden**	**Windsor**	**Hamden**
March	3.09	3.33	16.52	16.65
April	6.84	6.99	37.55	44.90
May	17.96	17.03	15.29	22.06
June	20.17	19.66	22.58	26.32
July	24.36	23.74	30.26	29.94
August	23.89	23.90	42.39	25.87
September	19.08	19.36	48.45	65.03

Table 4. Fungicide concentrations used for in vitro analysis of *Diaporthe humulicola* fungicide sensitivity

Parameters	Trifloxystrobin (Flint)	Pyraclostrobin + Boscalid (Pageant)	Pyraclostrobin (Insignia)[a]
Recommended rate	1 oz/15–30 gal	8–12 oz/100 gal	NA
Percent active ingredient	50.0	12.8[b]	20.0
Active ingredient (μg/ml)	249.67	76.70–115.05	NA
Concentrations tested (μg/ml)	10, 100, 250	40, 80, 120	25, 50, 75
Concentrations tested in repeated experiments (μg/ml)	NA	20, 10, 1	12, 6, 0.6

[a] Calculated concentrations to be tested to equal pyraclostrobin in micrograms per milliliter in Pageant. NA = not available.
[b] Percent active ingredient for pyraclostrobin only.

Anamorphic states of some taxa of *Diaporthe* are trimorphic and develop α, β, and γ conidia (Chi et al. 2007). A number of species of *Diaporthe* (*Phomopsis*) develop only α conidia (Chi et al. 2007; Gomes et al. 2013; Sutton 1980; Yang et al. 2018b). Among the species with only α conidia, the ones similar to *D. humulicola* with unbranched conidiophores include *D. acericola* Dissan., Camporesi & K. D. Hyde, *D. alangii* C. M. Tian & Qin Yang, *D. alleghaniensis* R. H. Arnold, *D. bicincta* (Cooke & Peck) Sacc., *D. bohemiae* Guarnaccia, Eichmeier & Crous, *D. brasiliensis* R. R. Gomes, Glienke & Crous, *D. celastrina* Ellis & Barthol., *D. cichorii* Dissan., Camporesi



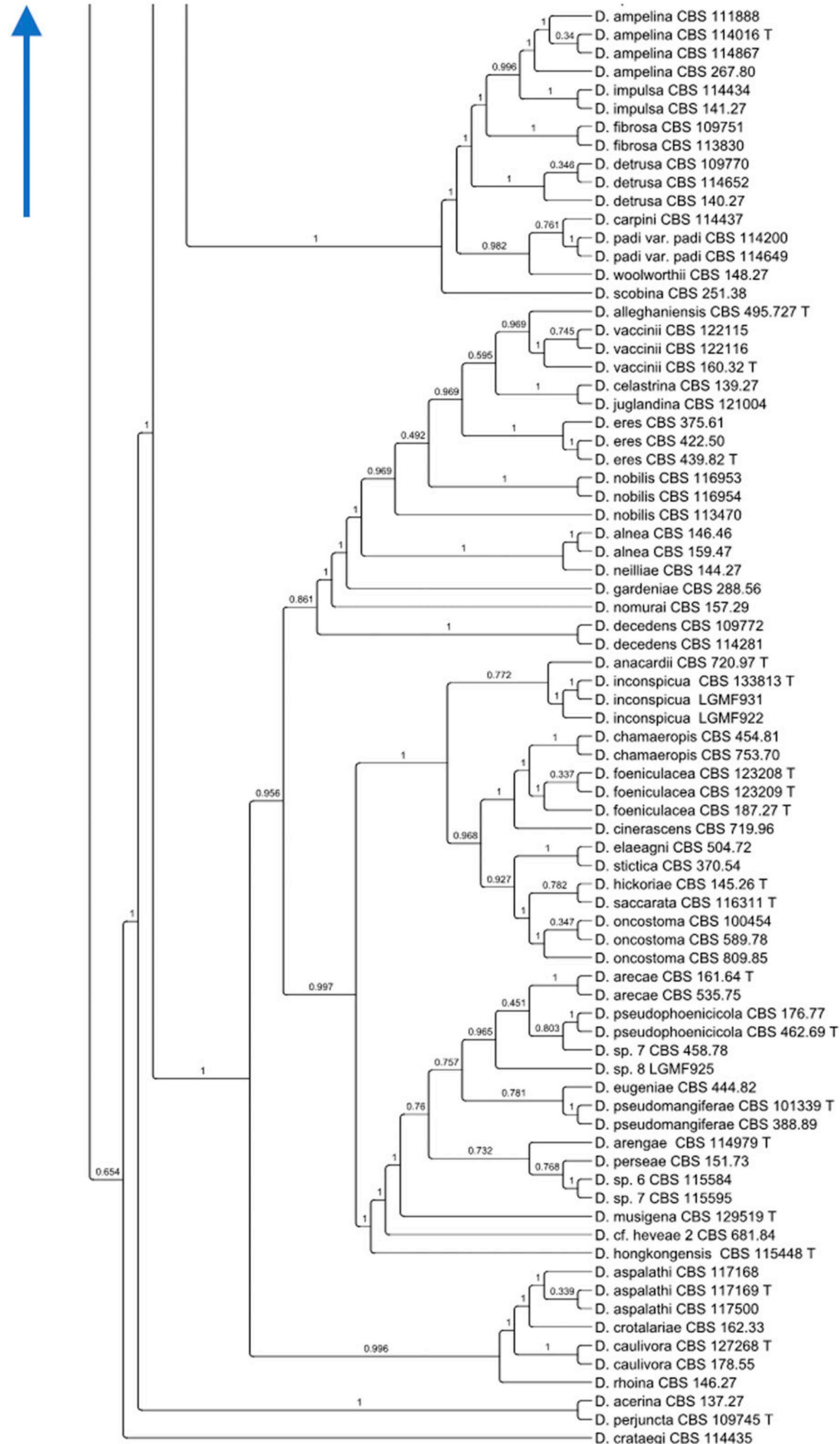
(*Continued on next page*)

Fig. 4. Phylogenetic tree of *Diaporthe humulicola* isolates CT2018-1, CT2018-2, and CT2018-3 with members of Diaporthales based on Bayesian inference analyzed for the combined dataset of internal transcribed spacer, translation elongation factor α-1, β-tubulin, histone, and calmodulin sequences. Tree sampling frequency was 1,000 generations. Branches that received Bayesian posterior probabilities of 0.95 were set as significantly supported. *Diaporthella corylina* was included as the outgroup.

& K. D. Hyde, *D. conica* C. M. Tian & Qin Yang, *D. dorycnii* Dissan., Camporesi & K. D. Hyde, *D. eres*, *D. helicis* Niessl, *D. hungariae* Guarnaccia, Armengol & K. Z. Váczy, *D. kadsurae* C. M. Tian & Qin Yang, *D. longicolla* (Hobbs) J. M. Santos, Vrandečić & A. J. L. Phillips, *D. melonis* Beraha & M. J. O'Brien, *D. pulla* Nitschke, and *D. sojae* Lehman, (Chi et al. 2007; Dissanayake et al. 2017; Gomes et al. 2013; Guarnaccia et al. 2018; Sutton 1980; Udayanga et al. 2015; Yang et al. 2018b). The conidial shape, size, and guttation will differentiate these species from *D. humulicola* (cylindrical or clavate, some constricted in the middle, occasionally becoming dumb-bell shaped, 4.0 – 12.2 × 2.2 – 5.0 μm). Phylogenetic relationships further showed that these taxa were different from *D. humulicola* (Fig. 4).

Several species such as *Phomopsis abdita* (Sacc.) Traverso, *P. lantanae* (M. E. A. Costa & Sousa da Câmara) B. Sutton, and *P. terminaliae* (Henn.) B. Sutton share some morphological characteristics with *D. humulicola* (Chi et al. 2007; Sutton 1980) but do not have molecular data available for phylogenetic analysis. *P. terminaliae* has large α conidia (10–15 × 3–4 μm) to separate it from *D.*

Fig. 4. (*Continued from previous page*)



humulicola. *P. abdita* has α conidia fusiform to ellipsoid, 2–4 guttulate, 8–10 × 2 μm and *P. lantanae*, fusiform, biguttulate, 6.5–9 × 2.5 μm, which will differentiate the two from *D. humulicola* by the shape and guttation.

D. humulicola is distinct from its closest relative from phylogenetic analysis, *D. brasilensis*, based on the latter having septate conidiophores and conidia being generally smaller than those of *D. humulicola* and having a rounded apex compared with the obtuse ends of *D. humulicola* conidia.

Fungicide efficacy. All three fungicides reduced fungal growth in vitro compared with unamended plates (Figs. 5 and 6). Trifloxystrobin had the lowest inhibition of *D. humulicola* growth, with average RMG of 54% for CT2018-1 and 23% at 10 μg/ml (Fig. 5). Pyraclostrobin combined with boscalid inhibited all growth at concentrations at and above 10 μg/ml (Fig. 6). At 1 μg/ml, CT2018-3 was inhibited completely but CT2018-1 had an average RMG of 2.73% (Fig. 6). Growth of CT2018-3 was completely inhibited with pyraclostrobin alone at 12 μg/ml (Fig. 7). For pyraclostrobin at 0.6 μg/ml, CT2018-1 had an average RMG of 4.99% and CT2018-3 had an average RMG of 2.04% (Fig. 7). CT2018-1 tended to grow better under all fungicide concentrations compared with CT2018-3 (Figs. 5, 6, and 7).

Discussion

We have demonstrated the presence of a new pathogen, *D. humulicola*, on common hop in Connecticut discovered in field plots in both 2018 and 2019. The pathogen results in symptoms similar to Phoma wilt on hop, including similar leaf lesions and browning of cones (Mahaffee et al. 2009). *Phoma* spp. have been implicated in causing minor infections of hop plants in the United States and as a more destructive pathogen in Europe, New Zealand, and China. More recently, *Phoma* spp. have been implicated in causing loss of hop cones due to browning late in the season in Vermont (Darby 2017). However, both morphological and phylogenetical analyses revealed that *D. humulicola* is distinct from *Phoma* spp. The latter are characterized by pycnidia that contain scleroplectenchyma, which was not observed for *D. humulicola.* Phylogenetically, *Phoma* belongs to Pleosporales, Dothideomycetes, while *Diaporthe* is placed within Diaporthales, Sordariomycetes (MycoBank 2019). Of the species observed on hop, *P. exigua, P. aliena*, *P. herbarium*, and *P. macrostoma* differ in morphology based on the lack of scleroplectenchyma in *D. humulicola* and that the latter has much larger conidia than those of the three *Phoma* spp. Molecular analysis based on NCBI Blast results and phylogenetic analyses clearly demonstrate that the new pathogen on hop is a member of the genus *Diaporthe*.

Although 28S blast results found the closest match to be *S. maydis*, we do not believe that *D. humulicola* is a member of this genus. *S. maydis* has pigmented α conidia and is mostly restricted to maize (Crous et al. 2006; Gao et al. 2017; Lamprecht et al. 2011). Phylogenetically, it is placed within the family Diaporthaceae. However, *D. humulicola* has hyaline conidia and, based on our multigene tree, does not group with *S. maydis*. Within the family Diaporthaceae, phylogenetic analysis placed *D. humulicola* 98.4% support with *D. ambigua, D. longispora, D. scerlotiodies, D. mayteni, D. raonikayaporum, Diaporthe* sp. 2, *D. angelica, D. subordinaria, D. arctii, D. neoarctii, D. cuppatea, D. lusitanicae, D. novem, D. infecunda, D. batatas, D. citri, D. sojae, Diaporthe* sp. 1, *D. convolvuli, D. endophytica, D. phaseolorum, D. melonis, D. helianthin, D. hordei, D. vexans, D. megalospora, D. schini, D. tecomae, D. terebinthifolii, D. ganjae, D. manihota, D. oxe, Diaporthe* sp. 3, *D. paranensis, D. brasiliensis*, and *Diaporthe* sp. 5 but was supported as its own taxa with 100% support and distinct morphological features.

Many members of the genus *Diaporthe* (anamorph *Phomopsis*) are endophytes, saprobes, or pathogens of plant species worldwide (Gomes et al. 2013). Although some are host-specific pathogens, many have wide host ranges and they cause a variety of diseases, including cankers, leaf spots, dieback, rot, wilt, and blights (Gomes et al. 2013). Through Koch's postulates, it was demonstrated that *D. humulicola* causes leaf lesions on common hop. Field isolations showed that this organism was also present on cones. Based on the leaf spots characteristic of this disease, we propose the disease name of Diaporthe leaf spot.

The ability of *D. humulicola* to infect many hop cultivars and at two different locations shows the potential for this to become a major pathogen on hop in Connecticut. Weather conditions conducive for disease seem to be hot and humid weather, which often occurs in July and August in the northeastern United States. The early observance of this pathogen in May 2019, where it was not present in the spring in the same hop yards in 2018, may suggest that it overwintered in the

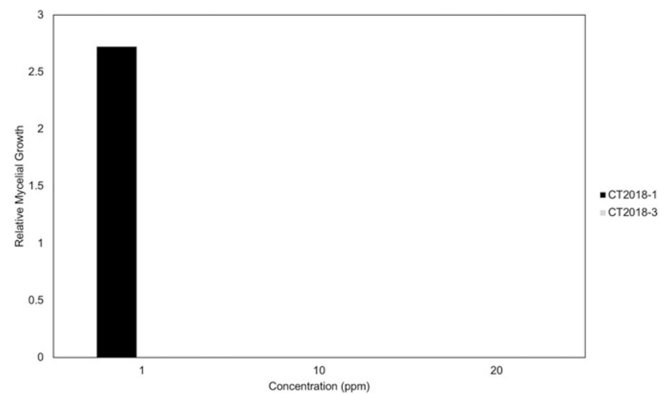


Fig. 6. Average relative mycelial growth of *Diaporthe humulicola* isolates CT2018-1 and CT2018-3 on pyraclostrobin and boscalid fungicide-amended plates (representing two replicate experiments).

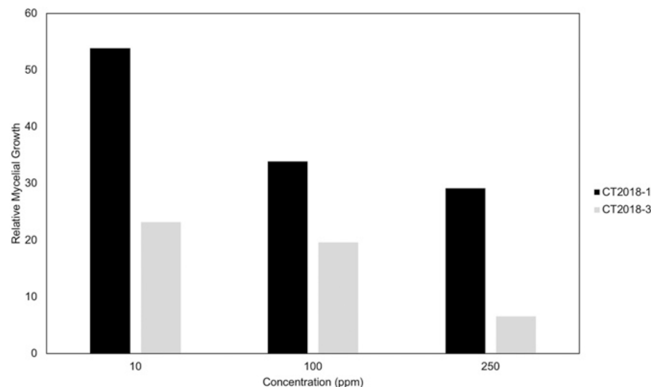


Fig. 5. Average relative mycelial growth of *Diaporthe humulicola* isolates CT2018-1 and CT2018-3 on trifloxystrobin fungicide-amended plates (representing two replicate experiments).

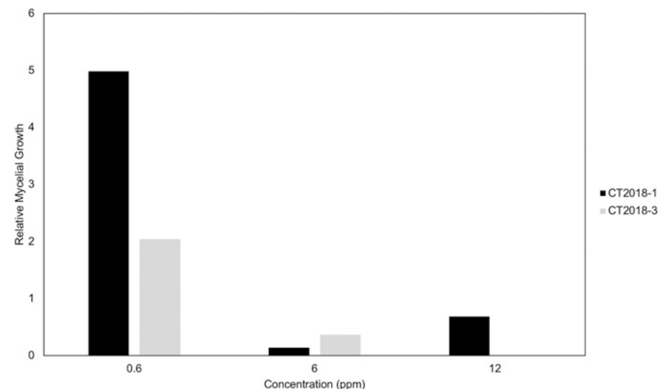


Fig. 7. Average relative mycelial growth of *Diaporthe humulicola* isolates CT2018-1 and CT2018-3 on pyraclostrobin fungicide-amended plates (representing two replicate experiments).

hop yard and was able to cause infection early in the season. Fungicides applied when these weather conditions are predicted may reduce the severity of Diaporthe leaf spot. In vitro analysis of fungicide susceptibility indicated that pyraclostrobin, boscalid mixed with pyraclostrobin, and trifloxystrobin all reduce *D. humulicola* growth in culture. Future studies to determine the effective concentration for 50% inhibition of growth for each fungicide and the correlation of those with field efficacy need to be conducted in order to provide growers with fungicide recommendations. Further research is needed on alternative hosts and the disease cycle in order to develop management strategies for disease control. The high similarity of symptoms of Diaporthe leaf spot to Phoma wilt warrant further research into whether these two diseases are caused by *D. humulicola* and *Phoma* spp. or if they are caused by the same pathogen. The identification of *D. humulicola* in Connecticut is important to hop growers in the region because proper diagnosis and treatment may prevent hop cone loss. The spread of this pathogen into other hop-growing areas will need to be monitored as conducive weather conditions occur in hop-growing regions in the United States such as the Midwest. Although the Northeast is not a major hop-growing region, the movement of *D. humulicola* into the Midwest or Pacific Northwest could have large implications for hop cone production because the United States is the world's leader in supplying hop.

Acknowledgments

We thank J. Preste and R. Cecarelli for maintenance of hop yards and M. Salvas for assisting with field collections, culturing, and Koch's postulates.

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