Erysiphe trifolii Causing Powdery Mildew of Lentil (*Lens culinaris*)

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ABSTRACT

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The taxonomy of the powdery mildew fungus infecting lentil in the Pacific Northwest (PNW) of the United States was investigated on the basis of morphology and rDNA internal transcribed spacer (ITS) sequences. Anamorphic characters were in close agreement with descriptions of *Erysiphe trifolii*. However, teleomorphs formed chasmothecial appendages with highly branched apices, whereas *E. trifolii* has been described as producing flexuous or sometimes loosely branched appendages. Branched appendages have been described in *Erysiphe diffusa*, a fungus reported from species of *Lens*, *Glycine*, and *Sophora*, raising the possibility that the PNW fungus could be *E. diffusa*. Examination of morphological characters of an authentic specimen of *E. trifolii* from Austria determined that it included chasmothecial appendages resembling those seen in PNW specimens. Furthermore, ITS sequences from five powdery mildew samples collected from lentils in PNW greenhouses and fields from 2006 to 2008 were identical to one another, and exhibited higher similarity to sequences of *E. trifolii* (99%) than to those of any other *Erysiphe* spp. available in GenBank. Parsimony analysis grouped the lentil powdery mildew into a clade with *Erysiphe baeumleri*, *E. trifolii*, and *E. trifolii*-like *Oidium* sp., but indicated a more distant relationship to *E. diffusa*. In greenhouse inoculation studies, the lentil powdery mildew fungus did not infect soybean genotypes known to be susceptible to *E. diffusa*. The pathogenicity of *E. trifolii* on lentil was confirmed using modified Koch's postulates. This is the first report of *E. trifolii* infecting lentil. *E. diffusa* and *E. trifolii* have different host ranges, so the discovery of *E. trifolii* on lentil has implications both for determining species of powdery mildews on cool-season grain legumes, and in disease management.

Lentil (*Lens culinaris* Medik.) is a staple food crop in many developing countries. In the United States, lentil is an important rotational crop in cereal-based production systems in the Pacific Northwest (PNW) and in the Northern Great Plains. The crop faces several major biotic stresses which limit yield, including Ascochyta blight (caused by *Ascochyta rabiei* (Pass) Labr.), Botrytis gray mold (caused by *Botrytis cinerea* Pers. ex. Fr.), Fusarium root rot (caused by several *Fusarium* spp.), and Rhizoctonia root rot (caused by *Rhizoctonia solani* Kühn) (5,24).

Powdery mildew of lentil has been reported from various parts of the world including South Asia, the Middle East, the Mediterranean, East Africa, Eastern Europe, the former USSR, South America, and more sporadically, from North America (1). Although usually a minor disease, it can be severe on certain lentil cultivars and in some parts of the world, particularly

in India during January and February (1). Powdery mildew has been a persistent disease problem of lentil breeding materials in the greenhouse (6), and poses a threat to precious lentil breeding materials such as F1 plants.

Erysiphe pisi DC. is the powdery mildew pathogen that has been reported (often as *E. communis* auct. or *E. polygoni* auct.) on lentil from various parts of the world including Argentina, Chile, India, Italy, Jordan, Mexico, Romania, Sudan, Tanzania, and the former USSR (3,12,15). *Leveillula taurica* (Lév.) Arnaud has been reported from the former USSR (15). The species of *Erysiphe* attacking lentil often has been unclear, and some reports refer only to *Erysiphe* sp. or *Oidium* sp. (3,15). Recently, *Erysiphe diffusa* (Cook & Peck) U. Braun & S. Takam. was reported (as *Microsphaera diffusa* Cook & Peck) infecting lentils in Canada (4). The species name was assigned on the basis of dichotomously branched chasmothecial appendages (4).

Infections by *Erysiphe* species typically result in small white colonies on leaf surfaces. Lesions expand to cover entire leaf surfaces and pods. Mycelial growth and conidial production can be especially extensive at flowering. In the case of severe infections, leaves become chlorotic, then curled and necrotic prior to abscission. Yield decline may occur and plants some-

times die (1). Infection by *L. taurica* results in lesions of varying size on leaves and stems, with areas of infection displaying dense, felt-like mycelium.

The taxonomy of the species of powdery mildew infecting lentil in the United States has not been critically examined. This study was initiated to clarify which species of powdery mildew infects lentil in the PNW. Because lentil plants used in genetics and breeding research frequently are grown in greenhouses as well as in fields, this study included comparison of strains in the greenhouse with the lentil powdery mildew occurring in the field.

MATERIALS AND METHODS

Fungal samples. Seven samples of powdery mildews were used in this study (Table 1). Four samples (LGH06, LGH07, LGH07-119, and LGH N07) were collected from three separate greenhouses during 3 years (2006 to 2008). The greenhouses are separated by a minimum of 500 m. One sample (LSP07) was collected from a lentil field near Pullman, Whitman Co., WA. One sample (Soy08) of powdery mildew from wild soybean (*Glycine* sp.) was obtained from the USDA Soybean Germplasm Collection at the University of Illinois, Urbana. These samples were used for morphological and molecular studies. An authentic specimen of *E. trifolii*, WSP 70928, determined by U. Braun and originating from GZU Dupla Fungorum (27), was also used for morphological comparisons.

Morphological characterization. Symptomatic leaves of all the powdery mildew samples were examined under $\times 100$ to $\times 1,000$ using bright field microscopy (Carl Zeiss Model Axioskop 40, Oberkochen, Germany). Morphological characters evaluated included diameter of chasmothecia, chasmothecial appendages, number of ascospores per ascus, and lengths and widths of asci, ascospores, conidia, and conidiophore foot cells. At least 50 measurements for each character were taken per sample for comparison with descriptions of powdery mildew species recorded on lentil (8,9).

ITS sequencing and phylogenetic analysis. Total DNA was extracted from conidia and/or mycelia from infected lentil plants using the FastDNA kit (MP Bio-medicals, LLC, Solon, OH) as described by Chen et al. (11). Polymerase chain reaction (PCR) amplification of the ITS region from each isolate was performed using the

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primers ITS1 and ITS4 (34) or *Erysiphe*-specific primers designed based on conserved sequences of the ITS region of *Erysiphe* spp. available from GenBank. The *Erysiphe*-specific primers were EryF (5' TACAGAGTGCAGGCTCAGTCG 3') and EryR (5' GGTCAACCTGTGATC CATGTGACTGG 3'). The specific primers were helpful in avoiding the amplification of ITS regions of the host plant or other contaminating organisms. PCR reactions in 20-μl volume consisted of 2 units of *Taq* DNA polymerase (Promega, Madison, WI), 1× PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 40 ng of the template, and 10 to 20 pmol of each primer, and were subjected to the following temperature parameters: initial denaturation at 92°C for 10 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 30 s, extension at 72°C for 2 min, and final extension at 72°C for 10 min (Bio-Rad iCycler thermocycler, Bio-Rad Laboratories, Hercules, CA). A negative control without template DNA also was included in each set of PCR reactions.

Amplified DNA fragments were first cloned into plasmid pCR2.1TOPO (Invitrogen Corp., Carlsbad, CA) and transformed into One Shot Topo10 chemical competent cells by following the manufacturer's instructions. Each transformation mixture (50 to 100 μl) was spread and grown on Luria-Bertani (LB) agar plates containing 40 μg/ml of X-Gal (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside) and 50 μg/ml of kanamycin (Invitrogen) for blue-white colony selection. The LB medium contains 10 g of Bacto tryptone, 5 g of Bacto yeast extract, 10 g of NaCl, and

15 g of Bacto agar per liter of distilled water (pH 7.0). Positive colonies detected by PCR were grown overnight in LB broth containing 50 μg/ml kanamycin at 37°C. Plasmids were isolated using the Montage life science kit (Millipore Corporation, Bedford, MA) following the manufacturer's instructions. Plasmids containing inserts were further verified by restriction digestion with *Eco*RI restriction enzyme and separated on 1% agarose gel. Sequencing reactions were carried out directly with purified PCR product or with isolated plasmids using one of the six primers: EryF, EryR, ITS1, ITS4, M13F, and M13R. Nucleotide sequences were determined from both strands using an ABI PRISM 377 automatic sequencer (Applied Biosystems, Foster City, CA) at the Sequencing Core Facility of Washington State University. Sequences were used in BLAST searches against the GenBank database (<http://www.ncbi.nlm.nih.gov/BLAST>) to identify the most similar sequences available in the databases.

Sequence accessions in the GenBank with high similarity values to the sequences determined in this study were selected for phylogenetic analysis. These included sequences in the species of *E. baeumleri* (Magnus) U. Braun & S. Takam., *E. diffusa*, *E. pisi*, *E. trifolii*, and *Oidium* spp. (Table 2). One sequence (AB078807) of *E. glycine* was used as an outgroup according to Takamatsu et al. (30). Sequences were aligned using the ClustalW program (19). The alignment was refined with a word processing program, and then used in phylogenetic analysis. Phylogenetic analysis was performed

using the DNA Parsimony program of the PHYLIP package (16) available at <http://bioweb2.pasteur.fr/phylogeny/intro-en.html>. Each nucleotide was weighted equally and each deletion is treated as one step change.

Greenhouse inoculation and pathogenicity assay. Lentil cv. Crimson and two soybean genotypes, L84-2237 (Plant Introduction 547870) and cv. Harosoy (PI 548573), from the USDA Soybean Germplasm Collection at Urbana, IL were planted in the greenhouse. The soybean genotypes L84-2237 and Harosoy are known to be susceptible to *E. diffusa* (14,21). Twenty total lentil plants in five pots (four plants per 15-cm pot) and 16 total soybean plants of each genotype in four pots (four plants per 15-cm pot) were used in the experiment. Three-week-old plants were dusted copiously with conidia collected from infected lentil plants until the young leaves had a white powdery appearance. Plants were maintained in a greenhouse with a 16-h photoperiod, 19 to 23°C daytime temperature, 15°C at night, and watered daily at the base. Plants were fertilized with nutrient solution (20-10-20 N-P-K) twice a week through watering. Relative humidity was not controlled and fluctuated between 25 and 50%. Development of powdery mildew symptoms as evidenced by white powdery spots was observed and recorded at 2-day intervals for 3 weeks, then at weekly intervals until plants matured. The experiment was repeated once.

The pathogenicity bioassay described by Tiwari et al. (31) was used with some modifications. Seeds of lentil (cv. Crimson) were grown in a separate greenhouse

Table 1. Isolates of powdery mildews used in study

Isolate	Host	Location	Year of isolation	Chasmothecia production	Species	Embank accession
LGH06	Lentil	Greenhouse 12, Pullman, WA ^a	2006	No	<i>Erysiphe trifolii</i>	FJ378883
LGH07	Lentil	Greenhouse 12, Pullman, WA ^a	2007	No	<i>E. trifolii</i>	FJ378876
LGH07-119	Lentil	Greenhouse 119, Pullman, WA ^a	2007	Yes	<i>E. trifolii</i>	FJ378884
LGH07	Lentil	Plant Growth Facility, Pullman, WA ^a	2007	Yes	<i>E. trifolii</i>	FJ378882
LSP07	Lentil	Spillman Farm, Pullman, WA	2007	No	<i>E. trifolii</i>	FJ378881
Soy08	<i>Glycine</i> sp.	Urbana, IL	2008	No	<i>E. diffusa</i>	FJ378880
WSP70928	<i>Melilotus officinalis</i>	Steiermark, Austria	1984	Yes	<i>E. trifolii</i> ^b	N/A ^c

^a Greenhouse 12, Greenhouse 119, and Plant Growth Facility are three disconnected greenhouses separated by at least 500 m located on the campus of Washington State University, Pullman.

^b Species identity was determined by Braun (8,27).

^c Not available.

Table 2. Sequences used in phylogenetic analysis

Species	Host plant	Isolate	Country of origin	Sequence accession ^a	Reference
<i>Erysiphe baeumleri</i>	<i>Vicia amoena</i>	YNMH12360-12	Japan	AB015933	Takamatsu et al. (29)
<i>E. diffusa</i>	<i>Glycine</i> sp.	Soy08	USA	FJ378880	This study
<i>E. diffusa</i>	<i>Glycine max</i>	OIGma 10	Brazil	EF196675 (11)	Almeida et al. (2)
<i>E. glycine</i>	<i>G. max</i>	MUMH1462 (ascomata)	Japan	AB078807	Takamatsu et al. (30)
<i>E. pisi</i>	<i>Lathyrus latifolius</i>	UC1512315	USA	AF011306	Saenz and Taylor (26)
<i>E. trifolii</i>	<i>Lens culinaris</i>	LGH07-119	USA	FJ378884 (5)	This study
<i>E. trifolii</i>	<i>Trifolium pratense</i>	TPU-1546	Japan	AB015913 (5)	Takamatsu et al. (29)
<i>Oidium</i> sp. (<i>E. trifolii</i> -like)	<i>Vicia faba</i>	MUMH837	Japan	AB079854 (3)	Okamoto et al. (25)
<i>Oidium</i> sp. (<i>E. diffusa</i> -like)	<i>G. max</i>	MUMH791	Japan	AB078800 (10)	Takamatsu et al. (30)

^a Number of identical sequences available in GenBank indicated in parentheses.

where no powdery mildew was detected to obtain powdery mildew-free leaves. Leaves from 14-day-old plants were surface sterilized with 70% ethanol for 30 s followed by three serial washings with sterile distilled water (28) and were air-dried in a biological safety cabinet. Moist chambers were made with sterile wet filter papers inside sterile 9-cm-diameter petri dishes (33). Trimmed leaf petioles were immersed in sterile 1% sucrose solution inside a sterile 200- μ l micropipette tip with its narrow end sealed with a flame. A piece (4 $\times$ 4 cm) of sterile metal 200- μ m mesh was kept between the filter paper and the leaf (35) to prevent free moisture formation on the leaf surface, prolonging the greenness of the leaf tissue. Each petri dish setup included one lentil leaf, and leaflets were oriented abaxial surface facing upward. Abaxial surfaces of the leaves were inoculated using a fine paint brush covered with fresh conidia from greenhouse-grown lentil plants until the surfaces were perceptibly tinged with white color (20). The paint brush was disinfected by rinsing in 95% ethanol followed by air-drying between treatments. Noninoculated (mock inoculated) lentil leaves were kept as controls under the same conditions. Inoculated leaves and controls were incubated at room temperature under white fluorescent light with a 12-h photoperiod (33). Three replicate petri dishes per treatment were used. Powdery mildew symptom development was observed and recorded as presence or absence of powdery mildew pathogen growth for 2 weeks at 2-day intervals using a dissecting microscope. To confirm that the resultant colonies in the pathogenicity assay were caused by the conidia used in inoculation, modified Koch's postulates

were carried out. ITS sequences of the resultant colonies were determined and compared with the ITS sequence of the inoculum used in the pathogenicity assay. Small amounts of conidia from each of three leaves in three petri dish trials were used separately for DNA isolation. DNA was released from the conidial samples using the microwave method described by Ferreira and Glass (17) for the limited number of conidia available. About 500 fresh conidia produced on lentil leaves during the leaf bioassay were taken with an inoculation needle or loop and transferred into a 500- μ l Eppendorf tube. Conidia in the tube were subjected to microwave treatment for 5 min at the high power (setting of 10, 12,000W) in a domestic microwave oven (Kenmore, Elite). A beaker of water was kept inside the oven to absorb microwaves and to protect conidia in the samples from excess dryness. Thirty microliters of TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) was added to the tube, centrifuged at 12,400 RCF for 5 min, and 2 μ l of the resulting sample was used in 20- μ l PCR reaction mixture to amplify the ITS region. Temperature parameters for PCR amplification were the same as above with the exception of annealing at 55°C for 1 min. The methods for sequencing PCR products described above for field and greenhouse specimens were followed for DNA sequencing.

RESULTS

Morphological study. Infected lentil plants from the greenhouse and field initially exhibited symptoms of small white lesions on leaf surfaces (Fig. 1), expanding to cover entire leaf surfaces and pods at the later stages. Mycelial growth and sporula-

tion were especially extensive at flowering. Under severe infection, leaves became chlorotic, then curled and necrotic prior to abscission. Hyaline mycelium was found in scattered patches with abundant production of single, ellipsoid-cylindrical conidia (Fig. 2). Chasmothecia were scattered to gregarious, initially light yellow to tan and turning dark and rusty brown as they approached maturity (Fig. 3). Some chasmothecia were formed in colonies lacking conidia. Chasmothecial appendages were branched 3 to 6 times at the apex; branches were rather loose, diffuse, often deeply cleft with tips that were straight, not curved (Fig. 4). Flexuous appendages were on average 5.5 times as long as the chasmothecial diameters (Fig. 5). Mature chasmothecia contained 3 to 8 asci. Appressoria were lobed. Mature chasmothecia were separated easily from mycelial mats. Morphological characters were compared with those from standard descriptions of *Erysiphe* species previously recorded on lentils, as well as that for *E. trifolii* (Table 3). Conidia of the powdery mildew sample from a wild soybean (*Glycine* sp.) were ovoid and measured 28-36 $\times$

Fig. 1. Powdery mildew symptoms on a lentil leaf showing fresh white powdery growth.

Fig. 2. Conidia of *Erysiphe trifolii* found on lentil.

Fig. 3. Chasmothecia of *Erysiphe trifolii* on a lentil leaf.

12.5-16 μm . No chasmothecia were found on the samples from *Glycine* sp. The *E. trifolii* herbarium specimen displayed long flexuous chasmothecial appendages (Fig. 6), and 10 to 15% of the appendages were highly branched (3 to 6 times) at their apices (Fig. 7).

ITS sequences and phylogenetic analysis. PCR amplifications were successful with all fresh specimens including the microwave-treated conidia. Amplified products were about 650 bp long. DNA sequences from both strands were obtained. The full length of the ITS region (646 bp) was obtained for all the isolates from lentil, deposited in GenBank, and assigned accession numbers FJ378876, FJ378881, FJ378882, FJ378883, and FJ378884 (Table 1). ITS sequences were identical among all lentil powdery mildew samples. When the complete ITS sequence of lentil powdery mildew was used in a BLAST search, the sequences in GenBank with highest similarity (99.8%, one base-pair difference) were three identical se-

quences (AB079853 to AB079855) from *E. baeumleri*/*trifolii*-like *Oidium* sp. from three different hosts from Japan (25). The sequences in the GenBank with next highest similarity (99.4%, three base pair differences) were five identical sequences (AB015913, AB163926, AB167523, AB167524, and AF298542) from *E. trifolii* (13,22,29) and a sequence (AB015933) from *E. baeumleri* (29). The sequence of *E. pisi* accession AF011306 deposited by Saenz and Taylor (26) exhibited 97% similarity (14 base pair differences). The ITS sequence of the powdery mildew from wild soybean (deposited with GenBank as FJ378880) had 20 base pair differences (including one-base deletion) from the lentil powdery mildew sequence, and was identical to 21 GenBank sequence accessions (e.g., EF196675 and AB078800) deposited as *E. diffusa* or *Oidium* sp. from soybean and lupine from Brazil, Korea, Japan, and the United States (2,30).

The sequence alignment used in the analyses was 584 nucleotides in length.

There were 21 parsimony-informative sites. Parsimony analysis produced one most parsimonious tree with 113 steps. The sequence of lentil powdery mildew formed a single clade (monophyletic) with sequences of *Erysiphe baeumleri*, *E. trifolii*, and *E. trifolii*-like *Oidium* spp., and was distantly related to (paraphyletic) *E. diffusa* (Fig. 8). The powdery mildew sequence from the wild soybean was identical to and formed a separate clade with *E. diffusa* sequences in the GenBank (Fig. 8).

Greenhouse inoculations and pathogenicity assay. In the greenhouse inoculation studies, visual powdery mildew signs and symptoms started to appear on all inoculated lentil plants 6 days after inoculation, progressing to cover most of the leaves and part of the stems in a 2-week period after inoculation. No powdery mildew signs or symptoms appeared on any of the soybean plants during the entire growth period from the seedling stage to maturity. The same results were obtained in the repeated experiment.

Fig. 4. Terminal branching of chasmothecial appendages of *Erysiphe trifolii* on infected lentil.

Fig. 5. Long (about 6.5 times as long as the chasmothecial diameter) and flexuous chasmothecial appendages of *Erysiphe trifolii* on lentil. Branching pattern is visible near the tips of chasmothecial appendages.

Table 3. Morphological characterization of powdery mildew pathogens from lentil and wild soybean, and comparison with *Erysiphe diffusa*, *E. pisi*, and *E. trifolii* as described by Braun (8,9)

Character	Powdery mildew on lentil ^a	Powdery mildew on <i>Glycine</i> sp. ^a	<i>Erysiphe</i> species from Braun (8,9)		
			<i>E. pisi</i>	<i>E. trifolii</i>	<i>E. diffusa</i>
Conidia length $\times$ width	25-43 $\times$ 13-22	28-36 $\times$ 12.5-16	24-55 $\times$ 13.5-22	30-45 $\times$ 16-21	25-35 $\times$ 11-17.5
Conidiophore foot cell length $\times$ width	27-39 $\times$ 7-9	25-33 $\times$ 7.7-9 ^b	(15-)20-50(-70) $\times$ 6-10	(15-)25-38(-55) $\times$ 6.5-9	25-38 $\times$ 7.5-10
Chasmothecia (diam.)	(78-)83-130	N/A ^c	(80-)85-150	(80-)90-150(-180)	75-135
No. of asci/chasmothecia	3-6(-8)	N/A	(3-)4-8(-13)	3-12	4-10
Ascus (length $\times$ width)	(46-)57-75(-91) $\times$ 40-54(-58)	N/A	40-85 $\times$ 25-55	45-80 $\times$ 25-50	40-75 $\times$ 25-45
No. ascospores/ascus	1-5	N/A	(2-)3-6	(2-)3-5(-6)	3-6
Ascospore (length $\times$ width)	19-30 $\times$ (9-)12-19	N/A	(15-)18-25(-28) $\times$ 10-16.5	(15-)18-25(-28) $\times$ 10-16.5	16-24 $\times$ 9-15

^a Data were based on at least 50 measurements unless otherwise indicated, with extreme values in parentheses.

^b Data based on five measurements.

^c Not available.

In the pathogenicity assay with detached leaves, signs of powdery mildew infection began to be visible 7 days after inoculation. Sizes and shapes of conidia matched those of conidia used for inoculation. No chasmothecia developed on the lentil leaves used for the in vitro pathogenicity test during the 2-week period. Leaves of the mock-inoculated controls remained asymptomatic during the entire period. The ITS region of powdery mildew from all three replicates was successfully amplified with PCR, and two of the samples were sequenced. ITS sequences were identical to the sequence obtained from the conidia from greenhouse lentil plants and used in inoculation.

DISCUSSION

Several species names have been applied to powdery mildew fungi occurring on lentil. The most commonly reported name is *Erysiphe pisi* (often reported as *E. communis* auct. p.p. or *E. polygoni* auct. p.p.) from various parts of the world (1,6). *L. taurica* also occurs on lentil (15). Recently, *E. diffusa* was reported on lentil (4). In this report, we document the occurrence of *E. trifolii* as a causal agent of powdery mildew of lentil, based on comparison of morphological characters with an authentic specimen of *E. trifolii*, and parsimony analysis of ITS sequences with those deposited in GenBank by previous researchers. Discovery that *E. trifolii* can cause powdery mildew of lentil is significant in three aspects: (i) To our knowledge, it is the first report of *E. trifolii* causing powdery mildew of lentil. (ii) This report broadens the taxonomic concept of *E. trifolii* to include chasmothecial appendages with 3 to 6 times branched apices

similar to those reported for *E. diffusa*. (iii) It demonstrates the necessity of close examination of lentil powdery mildew before assuming species identity because the several *Erysiphe* species reported from lentil occur in differing host ranges and may differ significantly in their epidemiology.

Determination of species names for powdery mildews of legumes traditionally has been based on a few teleomorphic features, including chasmothecial appendage morphology (8,9). However, recent phylogenetic studies demonstrated that anamorphic features are more indicative of phylogenetic lineages than are teleomorphic features, and that anamorphic characters are of utility in species determination (10,13,18). Chasmothecial appendages traditionally used to distinguish genera are now used to help distinguish species (10).

Use of molecular characters, especially ITS sequence data, has given promising results for species determination in some powdery mildews (2,10,13,30). In this study, we used both ITS sequence data and morphological characters to determine the species of the lentil powdery mildew pathogen. Based on the anamorphic characters and ITS sequence data, the pathogen found on lentil was assigned to *E. trifolii*. However, it frequently displayed highly branched (3 to 6 times) chasmothecial appendages which were more similar to those of *E. diffusa* than to those illustrated for *E. trifolii* (8). This appendage branching pattern resembles that illustrated for *E. diffusa* f. *diffusa*, or sometimes even the more intricately branched pattern of *E. diffusa* f. *elongata* in Braun (8). Considering the length (about 6.5 times as long as the chasmothecial diameter) and flexuous

nature of their chasmothecial appendages (which are short and rigid for *E. diffusa*), these characters for our powdery mildew isolates on lentil plants are in good agreement with *E. trifolii* (U. Braun, *personal communication*).

Because *E. diffusa* was reported from *Glycine* spp. (2), morphological and molecular characteristics of a powdery mildew specimen from wild soybean were compared to those of lentil powdery mildew. The ITS sequence of wild soybean powdery mildew, with 20 nucleotide differences from the lentil powdery mildew ITS sequence, was identical to 23 GenBank accessions of *E. diffusa* and *E. diffusa*-like *Oidium* sp. In addition, conidia of the powdery mildew pathogen of wild soybean were in good agreement with those described for *E. diffusa* (8) and were shorter than those of the lentil powdery mildew pathogen (Table 2). Furthermore, the lentil powdery mildew did not infect soybean genotypes that are known to be susceptible to *E. diffusa*. Close examination of WSP 70928, an authentic specimen of *E. trifolii*, revealed the presence of *E. diffusa*-like branching pattern on about 10 to 15% of the chasmothecial appendages. After considering these morphological and molecular characters, the lentil powdery mildew pathogen species in this study was determined to be *E. trifolii*. It is more like *E. trifolii* than *E. baeumleri* because of its regular dichotomous branching patterns of the chasmothecial appendages (Fig. 4). *E. baeumleri* tends to produce irregular branching patterns according to the drawings of Braun (8). Further taxonomic studies and phylogenetic studies would be helpful because *E. trifolii* has been regarded as a complex of similar species

Fig. 6. Long and flexuous chasmothecial appendages of *Erysiphe trifolii* from the authentic specimen WSP 70928.

Fig. 7. Branching apices of chasmothecial appendage of *Erysiphe trifolii* from the authentic specimen WSP 70928.

consisting of *E. trifolii*, *E. baeumleri*, and *E. asteragali* DC. (8). The nature of this complex is yet incompletely determined (U. Braun, *personal communication*). It is possible that ITS sequences do not adequately differentiate *E. baeumleri* from *E. trifolii*, or the two taxa might actually be conspecific (J. Cunnington, *personal communication*). Given these latter considerations, and because these taxa have previously been referenced as the “*trifolii*” complex, we apply the name *E. trifolii* to the powdery mildew pathogen of our lentil samples.

Three lines of evidence are presented to document that *E. trifolii* can produce highly branched chasmothecial appendages. First, the powdery mildew samples with branched chasmothecial appendages from lentil yielded ITS sequences with higher similarity values in comparison with ITS sequences from *E. trifolii* than from *E. diffusa* or other *Erysiphe* species (Fig. 8); second, anamorphic features are most similar to the descriptions of *E. trifolii* and differ from those of *E. diffusa* (Table 2); third, the authentic herbarium specimen of *E. trifolii* also contained branched chasmothecial appendages (Figs. 7 and 8). As a result of this study, three *Erysiphe* species (*E. diffusa*, *E. pisi*, and *E. trifolii*) now are known to infect lentil. *E. trifolii* can be differentiated from *E. pisi* based on long and branched chasmothecial appendages, and *E. trifolii* can be differen-

tiated from *E. diffusa* by its production of comparatively larger conidia and long, flexuous chasmothecial appendages. In addition, the ITS sequences of the three species differ.

Lentil production is expanding into new areas (23). As a result of the expansion, new diseases or new pathogens may develop on lentil, and previous minor diseases may become economically important. Accurate determination of pathogen species has practical implications in managing the disease. The three species causing powdery mildew on lentil have different host ranges (3,7,32). Knowledge of the pathogen species causing the disease will help in determining cropping sequences and in selecting adjacent crops in managing powdery mildew of lentil.

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Fig. 8. Parsimony analysis of internal transcribed spacer (ITS) sequences showing close evolutionary relationship of powdery mildew of lentil with *Erysiphe baeumleri*, *E. trifolii*, and *E. trifolii*-like *Oidium* spp., and distantly related *E. diffusa*. Bootstrap values are indicated for the branches. Bold taxa indicate sequences determined in this study. Numbers of identical sequences in GenBank or in this study are indicated in parentheses. Vertical distances are for clarity and horizontal distances are drawn proportional to number of nucleotide changes.

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