Mycosphaerella lupini sp. nov., a serious leaf spot disease of perennial lupin in Southcentral Idaho, USA

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Mycosphaerella lupini sp. nov., a serious leaf spot disease of perennial lupin in southcentral Idaho, USA

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Abstract: *Mycosphaerella lupini* is described as the teleomorph of *Thedgonia lupini,* the organism commonly associated with a leaf spot disease of perennial lupin in the U.S.A. The latter organism is also shown to be distinct from *Didymella lupini,* which is associated with a *Phoma* anamorph, and occurs predominantly on overwintered lupin stems. *Thedgonia lupini,* which is known to produce conidia in disarticulating chains, is shown to do so by sympodial as well as percurrent proliferation. The genus *Thedgonia* is accordingly emended to incorporate both types of proliferation of the conidiogenous locus. *Mycosphaerella lupini* is the first teleomorph linked to the anamorph genus *Thedgonia.*

Key Words: Ramularia, Thedgonia, Lupinus, systematics

INTRODUCTION

Perennial lupin (*Lupinus* spp.), particularly *L. argenteus* Pursh in the Sawtooth National Forest in Southcentral Idaho near Ketchum, are commonly infected by a cercosporoid fungus known as *Thedgonia lupini* (Davis) U. Braun (=*Ramularia lupini* Davis) (Farr et al., 1989; Braun, 1994). The disease is found on several lupin species, but in this study it was most prominent on *L. argenteus* and *L. sericeus* Pursh, which grow at elevations ranging from 1700 to 2500 m in the Sawtooth National Forest. Heavy infection is often observed to lead to premature defoliation. The brownish lesions vary from subcircular to irregular, 1–15 mm diam. Abundant hyaline conidia develop on yellowish sporodochia in the centers of lesions, and as the season progresses, black spermogonia develop around the edges of the necrotic lesions. Recently, a teleomorph was collected on overwintered leaves of *L. argenteus.* The teleomorph, which matures in spring, forcibly discharges its ascospores when the ascomata are wetted by rain or dew. Airborne ascospores appear to be an important source of primary inoculum for infection of foliage in the spring. In preliminary inoculation studies, ascospores were found to produce foliar lesions identical to those caused by conidia. In ejecting single ascospores onto artificial media, the asexual and spermogonial state were successfully induced in culture.

The teleomorph, which is a bitunicate ascomycete with hyaline, two-celled ascospores, is morphologically similar to *Didymella lupini* (Cooke & Harkn.) Berl. & Voglino, which was described from overwintered stems of *Lupinus* L. collected in California (Cooke and Harkness, 1884). To date the disease has only been associated with the anamorph of the pathogen (*T. lupini*), commonly still referred to as *R. lupini* (Farr et al., 1989). The latter fungus was recently treated by Braun (1994, 1995), who placed it in *Thedgonia* B. Sutton based on its hyaline, catenulate conidia with unthickened conidial hila, and its sympodial mode of proliferation of the conidiogenous cells. An examination of several specimens of the anamorph in the present study revealed them to have enteroblastic conidiogenesis, and conidiogenous cells to proliferate sympodially, but also percurrently, which is a mode of proliferation not previously reported for species of *Thedgonia.* Furthermore, although Sutton and Hennebert (1994) suspected *Thedgonia* to have a *Mycosphaerella* teleomorph, prior to this study no established associations were reported for *Thedgonia.* The aims of the present study were, therefore, to characterize this new anamorph/teleomorph relationship, to compare the teleomorph with *D. lupini,* and to reevaluate the anamorph genus *Thedgonia.*

MATERIALS AND METHODS

Samples of leaves of *L. argenteus* and *L. sericeus* exhibiting typical leaf spot symptoms were collected from different areas of the Sawtooth National Forest near Ketchum, Idaho at elevations between 1700 and 2500 m in Jul to Sep since 1990. Plant tissue with

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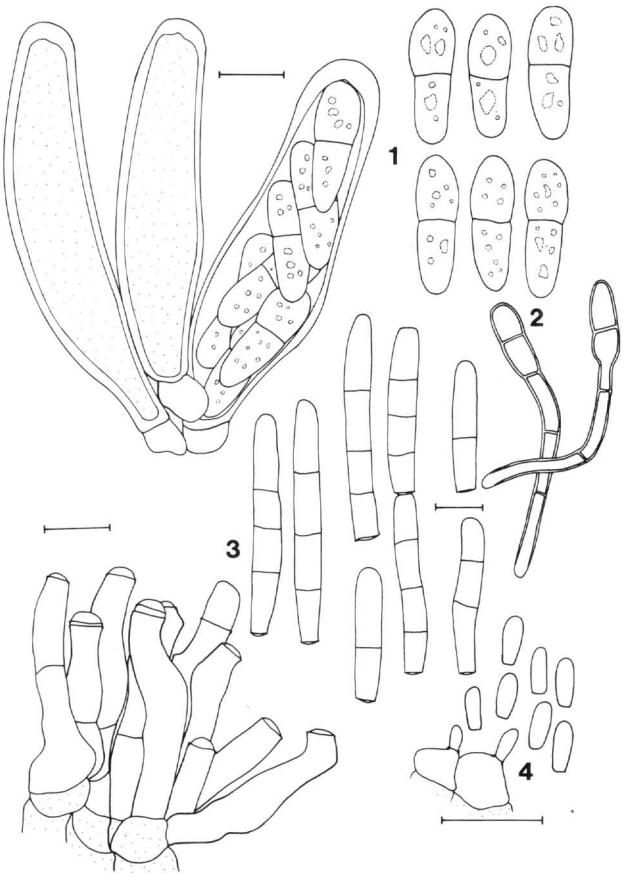
lesions were surface-disinfected in 0.25% NAOCl for 5 min, dried on paper towels and placed on 2% water agar (WA) and potato dextrose agar (PDA; Difco Laboratories, Detroit, Michigan) in 9-cm diam plastic petri dishes. Dishes were incubated at 21–24 C under fluorescent lights with a 12-h photoperiod. Hyphal tips or single spores were cultured on PDA or oatmeal agar (OMA; Difco Laboratories, Detroit, Michigan), dishes sealed with Parafilm, and incubated as explained above. Colony colors (top and bottom) were determined using the Methuen handbook of color (Kornerup and Wanscher, 1978). All measurements were made of fungal structures in vivo mounted in lactophenol. Thirty measurements of each structure were taken, whenever sufficient material was available; extremes are given in parentheses.

Symptomatic lupin leaves were placed in nylon-net bags (11 × 13 cm) with a 1.6-mm mesh permeable to air. In Oct, the bags were placed outdoors on the soil surface in the Sawtooth National Forest at Idaho, and at Pullman, Washington. The bags were collected the following Apr or May. Leaf tissue was examined microscopically for the presence of ascomata of the pathogen, and ascospores discharged following the procedure of Kaiser et al. (1997). Germinating ascospores were examined after 24 h, illustrated, then transferred to PDA and OMA and incubated as stated above.

The pathogenicity of single conidial and ascospore isolates from diseased leaves was tested on 20–40-d-old seedlings of *L. argenteus* and *L. sericeus* in greenhouse inoculation studies. Conidia were collected from PDA and OMA by flooding the dishes with sterile distilled water and gently scraping the colony surface with a bent glass rod. Conidial suspensions (1–5 × 10^5 conidia/mL) were sprayed on the foliage of the lupin seedlings. Control plants were sprayed with distilled water. Inoculated and control plants were incubated in moist chambers for 96 h, and then moved to a greenhouse bench at 18–22 C. Ascospores from overwintered lupin leaves were discharged downward onto the foliage of lupin seedlings maintained in moist chambers. Leaves containing ascomata were placed on the surface of WA petri dishes, and the dishes, supported by metal stands, inverted over the seedlings. After 72 h, the inoculated plants were removed from the moist chambers and placed on a greenhouse bench.

RESULTS

Taxonomy.—A comparison of the teleomorph of *T. lupini* with the type specimen of *D. lupini* revealed the two taxa to be morphologically distinct. Firstly, *D. lupini* occurs on overwintered stems, whereas *T. lupini* and its teleomorph are found on lupin leaves. The ascospores of *D. lupini* are also smaller (12–17 × 5–6.5 μm), and unequally septate, in comparison to those of the newly collected fungus, which are larger [(15–)17–20(–23) × (4.5–)5–6(–7) μm], and medianly septate. Lastly, a *Phoma* sp. was also found associated with *D. lupini,* which is distinct from *T. lupini,* the anamorph of this pathogen. Although the new fungus had pseudothecia similar to those of *D. lupini,* the former lacked pseudoparaphyses, which indicated that it would be better accommodated in *Mycosphaerella* Johanson.

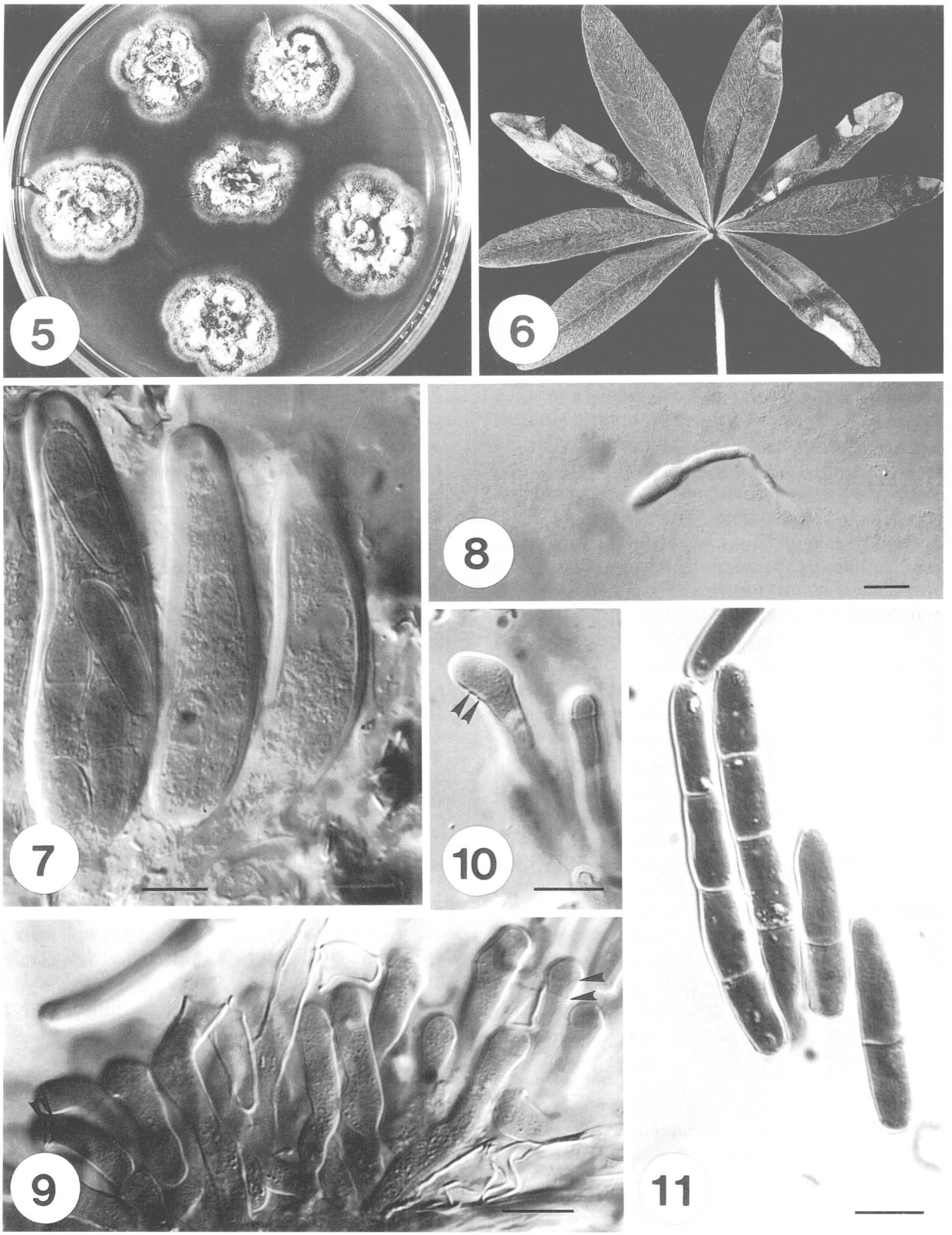


FIGS. 1–4. *Mycosphaerella lupini* and its anamorph *Thedgonia lupini* (type, BPI). 1. Asci and ascospores. 2. Ascospores germinating on water agar after 24 h. 3. Fasciculate conidiophores and cylindrical, catenulate conidia. 4. Spermatiogenous cells and spermatia. Bars = 10 μm.

Mycosphaerella lupini W.J. Kaiser et Crous, sp. nov. FIGS. 1–11

Anamorph. Thedgonia lupini (Davis) U. Braun, *Nova Hedwigia* 58: 204. 1994.

Ramularia lupini Davis, *Trans. Wis. Acad. Sci. Art. Lett.* 15: 777. 1907.



FIGS. 5–11. *Mycosphaerella lupini* and its anamorph *Thedgonia lupini* (type, BPI). 5. Colonies after 1 mo on oatmeal agar. 6. Field symptoms on a leaf of *L. argenteus*. 7. Asci and ascospores. 8. Ascospore germinating on water agar after 24 h. 9, 10. Conidiophores with apical, percurrent proliferations (arrows). 11. Cylindrical conidia. Bars = 10 μm.

Pseudothecia amphigena, nigra, subepidermalia, globosa, 100–130 μm diam. Asci fasciculati, bitunicati, late ellipsoidei, recti vel parum incurvati, 8-sporis, 50–60 × 11–18 μm. Ascosporae 3- multiseriatae, imbricatae, hyalinae, guttulatae, parietibus tenuibus, rectae ad parum curvatae, fusoideo-ellipsoideae, base obtusa et apice obtuso, mediano 1-septatae, ad septum constrictae, (15–)17–20(–23) × (4.5–)5–6(–7) μm.

Etymology. In reference to its host, *Lupinus.*

Leaf spots amphigenous, irregular to subcircular, 1–15 mm diam, light brown with an indefinite margin, often coalescing with age. Pseudothecia aggregated in black clusters on overwintered leaves, subepidermal, becoming erumpent, globose, 100–139 μm wide; apical papillate ostiole 10–15 μm diam; wall consisting of 3–4 layers of medium brown *textura angularis,* hymenium at base consisting of 1–2 layers of elongated, flattened, hyaline cells. Asci aparaphysate, fasciculate, bitunicate, subsessile, broadly ellipsoidal, straight or incurved, 8-spored, 50–60 × 11–18 μm. Ascospores tri- to multiseriate, overlapping, hyaline, guttulate, thin-walled, straight or slightly curved, fusoid-ellipsoidal with obtuse ends, widest in the middle of apical cell, medianly 1-septate, slightly constricted at septum, tapering towards both ends, but more prominently towards lower end, (15–)17–20(–23) × (4.5–)5–6(–7) μm. Spermogonia black, intermingled with pseudothecia, and similar in general morphology. Spermatiophores reduced to spermatiogenous cells. Spermatiogenous cells hyaline, ampulliform, 4–5 × 3–5 μm. Spermatia hyaline, smooth, subcylindrical with obtuse ends, 4–5 × 1.5 μm, not germinating on agar media. Mycelium internal, consisting of septate, branched, smooth, hyaline hyphae, 3.5–5 μm wide. Caespituli sporodochial, predominantly hypophyllous, yellow on lesions. Conidiophores densely aggregated in sporodochia, arising from the upper cells of a hyaline to pale yellow stroma up to 80 μm wide; conidiophores hyaline, smooth, 0–3-septate, subcylindrical, straight to geniculate-sinuous, unbranched, hyaline, smooth, tapering to flat-tipped apices with enteroblastic conidiogenesis, proliferating sympodially or 1–3 times percurrently near the apex, 10–30 × 3.5–5 μm. Conidia solitary, or in false chains when mature, hyaline, smooth, eguttulate, subcylindrical, apex obtuse to flattened, base truncate, more or less straight, 1–3(–5)-septate, (25–)35–50(–60) × (4–)5–6(–8) μm; hila inconspicuous with a minute marginal frill.

Cultures. Colonies erumpent on agar surface, irregular or sectored, with moderate, white aerial mycelium, and smooth, irregular margins, reaching 36 mm diam on PDA after 1 mo at 21–24 C with a 12-h fluorescent white light photoperiod per day, surface white to olive brown (4D1), and reverse grayish-yellow (4B5) to olive brown (4D3).

Hosts. Lupinus argenteus var. *argenteus, L. argenteus* var. *heteranthus* (S. Watson) Barneby, *L. argenteus* var. *holosericeus* (Nutt.) Barneby, *L. aridus* Douglas, *L. latifolius* J. Agardh, *L. nanus* Douglas ex Benth., *L. nootkatensis* Donn. ex Sims, *L. polyphyllus* Lindl., *L. polyphyllus* var. *humicola* (A. Nelson) Barneby, *L. sericeus* Pursh, *L. sulphureus* Douglas ex Hook., *Lupinus* sp.

Known distribution. USA. (Alaska, California, Idaho, Montana, Oregon, Texas, Utah, Washington, Wyoming).

HOLOTYPE. USA. IDAHO: Sawtooth National Forest, near Ketchum, elevation of 2600 m, latitude N 43° 46.045′, longitude W 114° 33.843′, overwintered leaves of *Lupinus argenteus,* 1988, *W.J. Kaiser* (BPI 806257, culture ex-type STE-U 1661–1663).

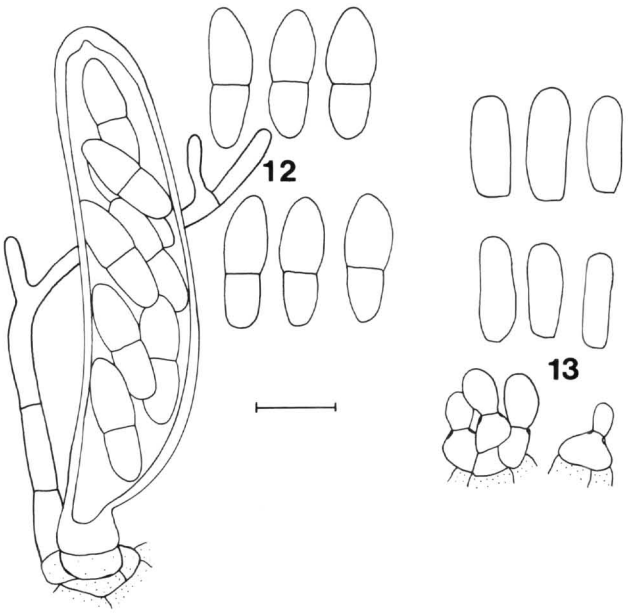
Additional specimens examined. USA. ALASKA: Homer, leaves of *Lupinus* sp., 6 Aug. 1948, *C.L. Lefebvre* (BPI 417815). IDAHO: Sawtooth National Forest, near Ketchum, leaves of *Lupinus argenteus,* 5 Apr. 1990, *W.J. Kaiser* (BPI 806258, teleomorph); Sawtooth National Forest, near Ketchum, elevation of 2600 m, latitude N 43° 46.045′, longitude W 114° 33.843′, leaves of *Lupinus argenteus,* 2 Jul. 1994, *W.J. Kaiser* (BPI 806259); Cache National Forest, 10 miles N.W. of Liberty, Bear Lake Co., *L. caudatus,* 26 Jul. 1965, *C.T. Rogerson* (BPI 417818). CALIFORNIA: Surf, on *L. nanus,* 9 Apr. 1938, *O.A. Plunkett* (BPI 417820). WASHINGTON: Spokane, leaves of a *Lupinus* sp., 6 Jul. 1905, *J.J. Davis* (BPI 417816, ISOTYPE of anamorph); Leavenworth, *L. aridus,* 23 Jun. 1933, *G.G. Hedgcock* (BPI 417817). WYOMING: Wolf Creek near Dayton, on *L. laxifolius* (?), 10 Jul. 1934, *C.R. Rollins* (BPI 417819).

Didymella lupini (Cooke & Harkn.) Berl. & Voglino, *Add. Syll. Fung.* I-IV: 88. 1886.

Sphaeria (*Didymella*) *lupini* Cooke & Harkn., *Grevillea* 13: 18. 1884. FIGS. 12, 13

Anamorph. Phoma sp. (by association, not proven in culture).

Pseudothecia embedded in overwintered stems, black, subepidermal, becoming erumpent, globose, to 200 μm diam, wall consisting of 2–3 layers of dark brown *textura angularis*; ostiole apical, 15–20 μm diam. Asci bitunicate, subsessile, lining the basal wall, ellipsoidal, straight or incurved, 8-spored, 40–80 × 12.5–17.5 μm. Pseudoparaphyses hyaline, subcylindrical, septate, branched, frequently constricted at septa, 2.5–4 μm; attached to apical part of the cavity, growing downwards, and becoming attached to the basal region. Ascospores bi- to triseriate, overlapping, hyaline, nonguttulate, thin-walled, straight, fusoid-ellipsoidal with obtuse ends, widest in the middle of the apical cell, unequally 1-septate, constricted at sep-



FIGS. 12, 13. *Didymella lupini* and its associated *Phoma*-like anamorph (type, BPI). 12. Ascus, ascospores and pseudoparaphysis. 13. Conidia and conidiogenous cells. Bar = 10 μm.

tum, 12–17 × 5–6.5 μm, apical cell 6–10 μm long, basal cell 3.5–6.5 μm long. Pycnidia black, subepidermal, intermingled between ascomata, similar in general morphology. Conidiophores reduced to conidiogenous cells. Conidiogenous cells hyaline, subcylindrical to ampulliform, 4–6 × 4–5 μm. Conidia hyaline, smooth, thin-walled, subcylindrical, apex obtuse, base bluntly rounded, 10–15 × 3.5–6 μm.

Hosts. Lupinus arboreus Sims, *Lupinus* sp.

Known distribution. USA. (California).

HOLOTYPE. USA. CALIFORNIA: Sacramento, stems of *L. arboreus,* Jun. 1880, *W. Johnson,* Harkness Coll. 2074 (BPI 629501).

Additional specimens examined. USA. CALIFORNIA: locality unknown, stems of *Lupinus,* Harkness Coll. 2074, BPI 800183 (presumed ISOTYPE); Los Angeles Co., Claremont, on stems on *Lupinus* sp., 1 Mar. 1909, *C.F. Baker,* Plants of Southern California, Baker 5223 (BPI 610986–610988).

Thedgonia B. Sutton emend Crous & U. Braun

Teleomorph. Mycosphaerella.

Phytopathogenic, causing leaf spots. Mycelium internal, consisting of hyaline or pale brown, smooth, septate, branched hyphae. Conidiophores fasciculate to sporodochial, arising from a stroma that can be substomatal, intraepidermal or erumpent; conidiophores subcylindrical, straight to curved, flexuous, geniculate-sinuous, simple or branched near the base, reduced to conidiogenous cells or septate, hyaline to subhyaline. Conidiogenous cells terminal, integrated, enteroblastic, proliferating sympodially with wide, unthickened, inconspicuous conidial scars, or 1–4 times percurrently near the apex. Conidia in simple or rarely branched, false chains (conidia with one attachment point), cylindrical, oblong, or broadly vermiform, didymosporous, phragmosporous or scolecosporous, euseptate, 1- to multiseptate, hyaline, smooth, hila truncate, flat, unthickened, inconspicuous, sometimes with a minute marginal frill.

Teleomorph and pathogenicity.—Ascomata of the *Mycosphaerella* teleomorph developed abundantly on the overwintered lupin leaves in the nylon-net bags. Single ascospore cultures of *M. lupini* produced only the anamorph and spermogonial state on PDA and OMA when incubated in culture. In inoculation studies *M. lupini* and *T. lupini* caused foliar lesions on *L. argenteus* and *L. sericeus* which were identical to those observed under field conditions. Lesions developed on inoculated leaves within 10–15 d after inoculation.

DISCUSSION

Several hyaline cercosporoid genera morphologically similar to *Thedgonia* have been linked to *Mycosphaerella,* namely *Cercosporella* Sacc., *Pseudocercosporella* Deighton and *Ramularia* Unger (Braun, 1995). Of these genera, *Ramularia* and *Cercosporella* have thickened conidial loci, and *Thedgonia* is most similar to *Pseudocercosporella,* except that it has conidia occurring in chains. When Sutton (1973) erected *Thedgonia* based on *T. ligustrina* (Boerema) B. Sutton, it was circumscribed as having phytopathogenic species with hyaline structures, and catenulate conidia that arise from sympodial conidiogenesis. The observation that most of the conidiogenous cells of *T. lupini* have inconspicuous, apical, percurrent proliferations are thus in contrast with published observations on other species of this genus. However, the cylindrical conidiogenous cells of the type species *T. ligustrina* corresponds with those of *T. lupini,* and suggests that percurrent proliferation could also be more common in other species of *Thedgonia* and that it is best to emend the generic description to incorporate this mode of conidiogenesis.

Thedgonia remains a well-delimited genus characterized by cylindrical, enteroblastic conidiogenous cells that proliferate sympodially and/or percurrently, producing cylindrical conidia that have one attachment point, and that occur in false, disarticulating chains. The occurrence of percurrent proliferation in *Thedgonia* is not unusual, as it has in the last few years also been observed to be common in many

species of other cercosporoid genera such as *Cercostigmina* U. Braun and *Pseudocercospora* Speg. Species formerly placed in *Pseudocercospora* that have percurrent proliferating conidiogenous cells and euseptate, scolecosporous conidia are presently accommodated in *Cercostigmina,* a genus intermediate between *Pseudocercospora* and *Stigmina* Sacc. Further molecular research into the separation of cercosporoid genera based on their mode of conidiogenesis, pigmentation and type of conidioma is presently underway.

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