DACTULIOCHAETA, A NEW GENUS FOR THE FUNGUS CAUSING RED LEAF BLOTCH OF SOYBEANS

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ABSTRACT

The generic name Dactuliochaeta is established to accommodate Pyrenoachaeta glycines, the causal agent of red leaf blotch of soybeans and its synanamorph Dactulioaphora glycines. The fungus is redescribed and illustrated from isolates originating from sclerotia on Neotonia wightii or soybean (Glycine max) leaves or that were sieved from soil in Zambia, Africa. The genus is characterized by setose sclerotia which germinate to form mycelium and then setose pycnidia and/or pycnidia and sclerotia on their surfaces. The inner pycnidial walls are lined with ampulliform to lageniform, phialidic conidiogenous cells that often are perichinally thickened at the conidiogenous locus and bear uninucleate, hyaline conidia.

Key Words: Dactulioaphora, Pyrenoachaeta, sclerotia, soybeans, taxonomy.

The causal organisms of red leaf blotch of soybeans [Glycine max (L.) Merr.] was named Pyrenoachaeta glycines Stewart (Stewart, 1957). Aspects of the disease, including the disease cycle, geographic distribution, and severity in soybean fields in Africa were reviewed by Hartman et al. (1987).

Sutton (1980) characterized the genus Pyrenoachaeta de Not. as having immersed, thin-walled, setose pycnidia, and ellipsoid, asperate conidia produced enteroblastically from phialides borne on long, filiform conidiophores that are branched at the base. Schneider (1976) revised the genus Pyrenoachaeta and recognized 11 species, all with conidiophores bearing integrated, acropleurogenous phialides. The type specimen of Pyrenoachaeta glycines (BPI 20705 from Ethiopia) was studied by Hartman and Sinclair (1987) and by Schneider (1973), who noted P. glycines was similar to Phoma Sacc. in that its conidia are formed from small conidiogenous cells lining the inner pycnidial wall rather than borne on elongated conidiophores. Stewart's (1957) original description of P. glycines did not include information on conidiophore morphology or conidial ontogeny. No teleomorph is known.

The fungus has a synanamorph, Dactulioaphora glycines Leakey (Datnoff et al., 1986), first described by Leakey (1964) from collections on Neotonia wightii (Arnott) Lackey [Glycine javanica auct. mult. non L.; = G. wightii (Arn.) Verde.] (Lackey, 1977) and G. max. Leakey placed the fungus in the Mycelia Sterilia with three other Dactulioaphora spp. Leakey (1964) and Mukiihi (1969) reported that Dactulioaphora spp. reproduced by forming sclerotia from cup-shaped sclerotioaphores that remained attached to the host. These are visible as rings of thick-walled brown mycelium surrounding circular, fractured cells from which sclerotia separate (Leakey, 1964).

Datnoff et al. (1986) showed that D. glycines was the sclerotial state of P. glycines and found pycnidia of P. glycines on herbarium specimens of D. glycines (IMI 59853b and 89782b). This material was referred to by Leakey (1964) in the original description.

This paper describes in detail the morphological characteristics of both anamorphic states and, based on this information, a new genus is established for the fungus.

MATERIALS AND METHODS

Sclerotia from infected soybean leaves were collected from York Farms, a commercial farm in Lusaka Province, Zambia and coded Y1–2. Soybean field soil was collected at Mpongwe, Copperbelt Province, Zambia and sieved for sclerotia. Isolates were coded M1–23. Sclerotia were obtained from N. wightii leaves collected at Mt. Makulu, Zambia and coded NW1–6. One isolate was obtained from Zimbabwe as a mycelial culture and coded ZW1.

Sclerotia were freed of host tissue or soil using forceps under a dissecting microscope. Sclerotia were dipped in 10% Clorox for 30 sec, rinsed in sterile distilled water, and plated on water agar
(WA). Mature pycnidia formed in 20-day-old cultures on WA. Pycnidial ooze was transferred to sterile water and a drop of the resulting suspension smeared over 9-cm diam WA culture plates. Single-germinated conidia were transferred to WA within 24 h to initiate single-oidium isolates. Isolates were maintained in the dark on malt extract agar (MEA) slants at 5 C.

Soybean seedlings were grown either in the laboratory under 16 h fluorescent light (75 EM\(^{-2}\) SEC\(^{-1}\)) at 25 ± 2 C or in a growth chamber under 12 h fluorescent (125 EM\(^{-2}\) SEC\(^{-1}\)) light–dark cycles at 20 ± 2 C. Unifoliolate leaves (growth stage VI) were detached and 10-mm diam leaf disks were cut from the center of a leaf using a #9 cork borer. Leaf disks were placed on moistened filter paper (Whatman #2) inside of 9-cm diam culture plates. A 1–3 mm plug was cut from the margin of 10-day-old colonies of isolate M1, N2, Y2, and ZW1 that had grown on WA. Mycelial plugs were placed on the center of each leaf disk. Leaf disks were maintained under 12 h fluorescent (50 EM\(^{-2}\) SEC\(^{-1}\)) light–dark cycles at 20 C. This procedure was replicated three times and repeated twice with observations made 5–10 times over a 2-month period. Freehand sections of pycnidia and sclerotia that formed on the leaf disks were made with and without freezing the tissue with “instant chill” (Tech Spray, Amarillo, Texas). Sclerotia collected from detached leaf disks were pooled and 30–40 sclerotia were plated on WA or other substrates to observe germination. Ten sclerotia were crushed in sterile WA between glass slides and individual cells were smeared over water using coverslips to observe their germination or mounted in lactophenol on glass slides as semipermanent mounts for further observation.

Herbarium material deposited by Stewart (BPI 20705) was studied and 50 conidia were measured for comparison with sizes of fresh conidia produced by our isolates. Pycnidial ooze was collected from 20-day-old inoculated leaf disks and suspended in water. The size of conidia from five isolates (n = 500) was recorded. The diam of pycnidia, and the length, width, and septation of setae were based upon the range of three isolates (n = 90). The diameter of sclerotia and cells of sclerotia along with the length, width and septation were based upon the range of two isolates (n = 60). Observations and micrographs were made with a Nikon SMZ-10 dissecting microscope, and an Olympus BHC for bright-field and a BHS for interference-contrast microscopy. Morphological measurements were made at ×1250 for conidia, cells of sclerotia, and setae and at ×250 for pycnidia and sclerotia. Nuclei of conidia were stained with DAPI following outlined procedures (Jacobs, 1987). Representative isolates were deposited with ATCC and ILLS, 46163 G. max, 46164 G. max, and 46165 N. wightii.

RESULTS

Dactuliochaeta Hartman et Sinclair, gen. nov.

Fungus anamorphosis.


Sclerotia disseita vel aggregata, in hospitis superficie superficialia, sclerotiophiroms mutarumuris visibilibus, obscure brunnceae nigrescentia, sphaerica vel depressa, setosa; sclerotiorum murus crassus, cellulis interioribus subhyaliniis, crasse tunicatis parenchymaticis. Setae sclerotiorum copiosae, rectae, acuminatae, laevigate, crasse tunicatae, setae; apicibus pallide brunnneis.

SPECIES TYPE: Dactuliochaeta glycines (Stewart) Hartman et Sinclair.

Mycelium composed of branched, septate, hyaline, smooth, hyphae. Pycnidia usually solitary, sometimes aggregated, erumpent, semi-immersed to superficial when mature on host material or immersed in agar, subhyaline to brown, globose to subglobose, mostly uniloculate, infrequently multilocular; setose ostiole usually single and circular. Wall of pycnidium composed of an outer layer of thick, yellow to brown, tangentially flattened cells, and a 2–3-cell-thick inner layer of subhyaline cells. Pycnidial setae straight, most abundant around ostiole, sparse on lower half of pycnidium, acuminate, smooth,
thick-walled, aseptate or 1–2 septate, dark brown, apices light brown, rounded or obtuse. Conidiogenous cells monopodial, from the inner cells of the pycnidial wall, hyaline to subhyaline, discrete, ampulliform to lageniform, sometimes proliferating percurrently. Conidia ellipsoid with obtuse apices, aseptate, hyaline, smooth, uninucleate.

Sclerotia solitary or aggregated, superficial on host surface to black, dark brown to black, spherical to oblate, setose; outer cells of sclerotia thick, pigmented; inner cells subhyaline, thick-walled, parenchymatous. Sclerotial setae copious, straight, acuminate, smooth, thick-walled, setate; apices pale brown; basal cells dark. Sclerotio phores ring-like, dark brown, erumpent, persistent on host material.

Dactuliochaeta glycines (Stewart) Hartman et Sinclair, comb. nov.


Colonies on MEA woolly, pink to orange-red to brown. Reverse brown to black in the center 3/4 of colony with the outer 1/4 red to pink with white to tan margins. Colony 16 mm diam at 20 C, 17 mm after 7 days at 25 C. Colonies on oatmeal agar red, turning green to blue-green with NaOH. Mycelium composed of septate, branched, hyaline, smooth hyphae. Pycnidia solitary, immersed to semi-immersed in host tissue or agar media, growing out from the surface of sclerotia. Pycnidia subhyaline to brown, globose to subglobose, 87–298 μm diam, usually uniloculate; ostiole surrounded by unbranched, thick-walled, straight to slightly flexuous, aseptate to septate, smooth, pale brown, acuminated setae, 29–102(164) μm long, 3.6–10.9 μm wide towards the base, originating from outer pycnidial wall cells, with apices subacute to acute, subhyaline (Figs. 1, 4, 9); ostiole usually single, circular, 10–20 μm diam, surrounded by thick-walled cells (Figs. 4, 5). Pycnidial wall 3–7 μm thick, composed of an outer layer, 1–2 cells wide; cells brown, thick-walled; and inner layer two cells wide; cells thin-walled, subhyaline, isodiametric or somewhat elongated, pseudoparenchymatous (Fig. 8). On various media, morphological sizes and shapes of some structures may vary. For example, pycnidia on WA agar may be immersed and multiosiolate (Fig. 6).

Conidiogenous cells monopodial, formed from the innermost cells of the pycnidial wall, hyaline, discrete, ampulliform to lageniform, 4–10 μm long, sometimes proliferating percurrently (Figs. 11–18). Conidiogenous cells periclinally thickened at primary conidiogenous locus (Figs. 13, 14). Sterile hyphae often present, arising from the conidiogenous cell layer, 6–23 μm in length (Figs. 16–19). Conidia entero ballistic, ellipsoid to obovate, aseptate, hyaline, smooth, uninucleate, multiguttulate, (3.3)–8.8 × 1.4–3.5(–3.9) μm (Figs. 2, 3). Conidia flesh colored in mass, usually-produced in a slimy matrix, germinating readily on WA.

Sclerotia usually singular, sometimes lobed, forming epicuticularly, often juxtaposed with sclerotio phores visible at maturity (Figs. 21–24, 31, 43–49, 51, 52). Sclerotia oval to oblate when mature, 96–357(–408) μm (Figs. 31, 39–51). Wall of sclerotia brown, 2–4 cells thick, internally composed of thick-walled, parenchymatous cells, 4.1–6.4–15.5 μm diam (Figs. 36–39, 42). Sclerotial setae straight, 0–1 septate, acuminate, smooth, 5–36 μm long, 2–7 μm wide at the base; apices hyaline to pale brown and base dark brown (Figs. 53, 54). Sclerotia develop from primordia consisting of 5–10 barrel-shaped cells (Figs. 32–35).

Sclerotia on WA germinate within 24 h. Several types of germination were recorded: 1) eruptive mycelial germination from one end (Fig. 40); 2) mycelial germination throughout many cells; 3) pycnidial eruption from one, two, or

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Figs. 1–10. *Dactuliochaeta glycines*. 1. Cross-section through a pycnidium with two locules, ×250. 2. Ellipsoid conidia, ×2500. 3. DAPI-stained uninucleate conidia, ×2500. 4. Top view of a pycnidium showing ostiole, setae, and dark peripheral cells, ×1650. 5. Cross-section of ostiolar region, ×2500. 6. Pycnidium developed on WA with multiple ostioles, ×61. 7. Immature pycnidium that had arisen from a sclerotium, ×250. 8. Cross-section of pycnidium showing thin outer wall layer, ×250. 9. Sclerotium (left) and pycnidium (right) on a soybean leaflet, ×71. 10. Cross-section of a pycnidium (left) that developed from a sclerotium (right), ×355. Figs. 1, 2, 5, 7, 8, 10 by differential interference contrast microscopy. Figs. 4, 6 by bright-field microscopy. Fig. 9 by macrophotography.
often three lateral positions on a sclerotium (Figs. 4, 7, 10, 20, 41, 42); and 4) sclerotium formation on top of an already mature sclerotium (Fig. 50). On host tissue new sclerotia may develop either on a newly formed sclerotiphore or be borne Within an already existing sclerotiphore, or on top of an existing sclerotium (Figs. 21–30). Only 1–2% of the individual inner cells of sclerotia were observed to germinate on WA (Fig. 37). Those that germinated formed colonies with pycnidia and sclerotia.

Collections Examined: On G. max and N. wightii, Jimma Ethiopia, 15.IX.85, R. B. Stewart (BP1 20705), holotype and isotype. Isolates from G. max leaves were collected 15.III.85 from York Farms, Lusaka Province; from N. wightii leaves from Mt. Makulu, Lusaka Province, 7.V.85, and from soil 12.IV.85 at Mpongwe, Copperbelt Province, Zambia (ATCC-6453, 64514, 64515, 64516, 64517; and IILS 46163, 46164 G. Max; 46165 N. wightii). An isolate from soybean, Rattray Arnold Research Station, Zimbabwe, II.83, Department of Crop Science, University of Zimbabwe, Harare.

Discussion

Dactuilochoaeta glycines is similar to species of Paraphoma Morgan-Jones & White (1983) and P. Sacc. in producing enteroblastic, ellipsoid, aseptate conidia from monophialidic conidiogenous cells which line the inner surface of the pycnidial wall. Its conidiogenous cells differ from those of these two genera, however, in that they sometimes proliferate percurrently, thereby producing one to several secondary conidiogenous loci. Dactuilochoaeta also differs from Paraphoma by having thin-walled pycnidia, and differs from Paraphoma, Phoma, and Pyrenochoa by the production of pycnidia from sclerotia.

In this study, sizes of pycnidia, setae, and conidia fall closely within the range described by other researchers for Pyrenochoa glycines (Table I), although Levy (1987) reported that one of his isolates had significantly longer setae and smaller conidia compared to other isolates. Dactuilochoaeta glycines also is similar to Dactuliophora spp., but those species reproduce solely by sclerotia released from cup-shaped sclerotiphores on the living host.

The size of sclerotia in this study range from 96–357 µm, which is within that originally described by Leakey for Dactuliophora glycines (Table II). However, sclerotia with larger diam were observed in several isolates when grown

Figs. 11–20. Dactuilochoaeta glycines. 11, 12. Simple ampulliform conidiogenous cells. 13. Elongated conidiogenous cell with annellations (arrow) due to percurrent proliferation. 14. Conidiogenous cell with annellations (arrows). 15. Conidiogenous cells arising from a single locus. 16. Sterile hypha (left) and conidiogenous cell (right). 17. Lageniform conidiogenous cells structured on top of each other and percurrent proliferation of conidiogenous cell (arrow). 18. Elongated conidiogenous cell with sterile hypha. 19. Hypal extension within a pycnidium. 20. Cross-section of pycnidium formed on a sclerotium illustrating three distinct layers, conidiogenous cells (CC), bottom of pycnidial wall (PW), and sclerotial cells (S). Figs. 11–13 and 15–20 by differential interference contrast microscopy, ×2500. Fig. 14 by bright-field microscopy.

Figs. 21–38. Dactuilochoaeta glycines. 21–24. Series of developing sclerotia within a sclerotiphore (top) adjacent to another sclerotiphore (bottom). 21. ×78, 22. 24 h later, ×78. 23. 48 h later, ×78. 24. 60 h later, ×52, 25–27. Series of developing sclerotia within a sclerotiphore at 1-day intervals, 28. ×112, 29. ×61, 30. ×52, 31. Pycnidia (P), sclerotia (S) and immature sclerotia on sclerotipheres (IMS) formed on a leaflet, ×47, 32–35. Initials and development of a sclerotium on WA over a 2-day period, ×354, ×354, ×177, ×415, respectively. 36. Squash mount illustrating inner hyaline cells of sclerotium and pigmented ring cells, ×330. 37. Germination of an inner sclerotal cell, ×825. 38. Internal cells of a sclerotium with double wall layer (arrow), ×999. Figs. 21–31 by macrophotography, 32–37 by bright-field microscopy, and 38 by differential interference contrast microscopy.

Figs. 39–51. Dactuilochoaeta glycines. 39. Cross-section through sclerotium 24 h after germination, showing development of a pycnidium (P) and secondary sclerotium (SS), ×177, 0. Germinated sclerotium with eruptive mycelial germination from one end, ×47. 41. Germinated sclerotium (bottom) with a young pycnidium (top), ×56. 42. Germinated sclerotium with four lateral pycnidia, ×177, 43, 44. Lobed sclerotium moved to illustrate one sclerotiphore, ×52, 45, 46. Development of a sclerotium adjacent to an existing sclerotium; note the older sclerotium was moved to illustrate its sclerotiphore (arrow), ×52, 47. A turned-over sclerotium showing the concave underside along with part of the host material (arrow), ×65. 48. Formation of a sclerotium (left) adjacent to a mature sclerotium (right), ×52, 49. Young (above) and matured (below) sclerotium, ×65. 50. Formation of a secondary sclerotium from a sclerotium formed on WA from a colony derived from a single sclerotial cell, ×52, 51. Two sclerotia developed side by side, ×52. Figs. 39–42 by bright-field microscopy. Figs. 40, 41, 43–51 by macrophotography.
either at low temperatures or inoculated onto different hosts, which may explain in part the larger sizes reported by Levy (1987).

Mukiibi (1969) reported that sclerotal initials of *Dactuliotheca tarri* Leakey developed in culture from a single hyphal tip. Recognizable sclerotal primordia consisted of 5–10 barrel-shaped cells (5–6 μm) with dense contents. Primary sclerotia were caducous and subsequent ones were borne on sclerotioaphores in acropetal succession. We have found a similar situation with sclerotia of *Dactuliotheca glycines*. However, this and other reports by Datnoff et al. (1986) and Levy (1987) showed that sclerotia germinated to form mycelia, pycnidia or sclerotia on the outer surface of sclerotia. Neither Leakey (1964) nor Mukiibi (1969) reported culturing *Dactuliotheca glycines*. Leakey (1964) described the sclerotia of *D. glycines* as densely hispidulous; however, the figure legend showed *D. glycines* as non-hispidulous and *Dactuliotheca elongata* Leakey as hispidulous. All of the sclerotia examined by us were found to be densely hispidulous and formed epicuticularly. Sclerotioaphores were visible when sclerotia were mature, unless the sclerotia were borne within already existing sclerotioaphores. The

### Table I

<table>
<thead>
<tr>
<th>Source</th>
<th>Pycnidia diameter</th>
<th>Setae</th>
<th>Conidia</th>
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<tr>
<td></td>
<td></td>
<td>Length</td>
<td>Basal width</td>
</tr>
<tr>
<td>Datnoff et al., (1986)</td>
<td>69–283</td>
<td>9–109</td>
<td>2.5–6.0</td>
</tr>
<tr>
<td>Levy (1987)</td>
<td>98–284</td>
<td>20–242</td>
<td>2.5–5.9</td>
</tr>
<tr>
<td>Hartman*</td>
<td>87–298</td>
<td>29–102(–164)</td>
<td>3.6–11</td>
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* Diameter of pycnidia, setae length and width based upon the range of three isolates (n = 90). Length and width of conidia based upon the range of five isolates (n = 500).
TABLE II

<table>
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<tr>
<th>Source</th>
<th>Sclerotia diameter</th>
<th>Cell diameter</th>
<th>Length</th>
<th>Width</th>
<th>Number of septa</th>
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<td>8–12</td>
<td>15–25</td>
<td>4–0</td>
<td>0–1</td>
</tr>
<tr>
<td>Datnoff et al. (1986)</td>
<td>50–311</td>
<td></td>
<td>11–27</td>
<td>2–5</td>
<td></td>
</tr>
<tr>
<td>Levy (1987)</td>
<td>170–720</td>
<td></td>
<td>8–90</td>
<td>3–5</td>
<td>0–2</td>
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<tr>
<td>Hartman*</td>
<td>96–357(–480)</td>
<td>(4.1–)6.4–15.5</td>
<td>5–36</td>
<td>2–7</td>
<td>0–2</td>
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</tbody>
</table>

* Diameter of sclerotia and cells, length, width and septation of setae based upon the range of two isolates (n = 60).

+ Data not given.

sclerotia were either caducous with more sclerotia formed from the sclerotioophore or secondary sclerotia developed on the sclerotial surface.

The genus *Echinochondrium* Samson et van der Aa, another member of the Mycella Sterilia, closely resembles the sclerotial state of *Dactulichocheta glycines* and *Dactulichophora* spp. in morphology and size. Samson and van der Aa (1975) compared the types of *Echinochondrium* and *Dactulichophora* and concluded that they differed because of the presence of cup-shaped sclerotioophores in the latter. For *Dactulichocheta*, sclerotia produced in culture or immature sclerotia on leaves do not always have an associated sclerotioophore. Most significant is the unique association of the pycnidial and sclerotial states in *Dactulichocheta glycines*. Because sclerotia are not readily produced in culture, researchers at first did not recognize that they represented states of the same fungus. The genus is probably not monotypic and it is quite likely that as more of the species of *Dactulichophora* are studied or other species of *Echinochondrium* are found, they too may have a pycnidial state similar to *Dactulichocheta*.

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