Trans. Brit. mycol. Soc. 40 (2), 177-186 (1957).

PLEOSPORA STRAMINIS, P. RUBELLOIDES AND P. RUBICUNDA: THREE FUNGI CAUSING PURPLE-STAINING OF DECAYING TISSUES

By JOHN WEBSTER

Botany Department, University of Sheffield

(With 3 Text-figures)

Pleospora straminis, previously unrecorded for Britain is described. P. rubelloides comb.nov. is described and compared with the related P. rubicunda on the host and in culture.

Herbaceous and woody debris decaying in damp situations, frequently shows purple staining. Various fungi such as *Leptosphaeria* spp., *Ophiobolus rubellus* and *Lophiotrema* sp. are sometimes present, but three species of *Pleospora* have been found associated with the purple stain. Since there has been some confusion in the nomenclature of these fungi, they are described below:

PLEOSPORA STRAMINIS Saccardo, Michelia, 1, 407, 1879 (Fig. 1)

= P. obliqua Wehmeyer, Mycologia, 43, 579, 1951.

Several collections of this fungus on grasses, are in the Sheffield Herbarium:

Herb. Sheffield number	Host	Locality	Date
225	Wheat stubble	Londesborough, nr. Market Weighton, Yorks	26. vi. 49
226	Barley stubble	King's Lynn, Norfolk	7. viii. 49
227	Barley stubble	King's Lynn, Norfolk	7. viii. 49
82 i	Dactylis glomerata	Lindrick Common, nr. Worksop, Notts	15. vii. 50
1046	Wheat stubble	Bolton Percy, York. (coll. W. G.	17. iii. 51

The following description is based on specimen 225 (see Fig. 1).

Perithecia, single beneath leaf sheath or stem, associated with a purple coloration; black, thin-walled, globose, surrounded at the base by sparse black hyphae, neck papillate, projecting; $240-390\,\mu$ in diameter. Asci, broadly club-shaped, rounded at the tip, tapering to a short stalk below, bitunicate, 3-8 spored, $130-192\times23-32\,\mu$ interspersed by branched filiform 'paraphyses'.

Ascospores, biseriate in the upper portion of the ascus, uniseriate below, oblong-elliptical with rounded ends, the upper half of the spore larger than the lower, with nine to eleven transverse septa, constricted at the septa,

especially at the middle, and with numerous longitudinal septa; golden-

yellow in colour, brown with age; $26-34 \times 10-12 \mu$.

Cultures. On potato-dextrose agar the fungus grew slowly (2.5 cm. in 6 weeks at room temperature), producing a pink colour in the medium, the colonies becoming black at the margins. Perithecia were formed in 4 weeks in cultures started from either single ascospores or asci, and were

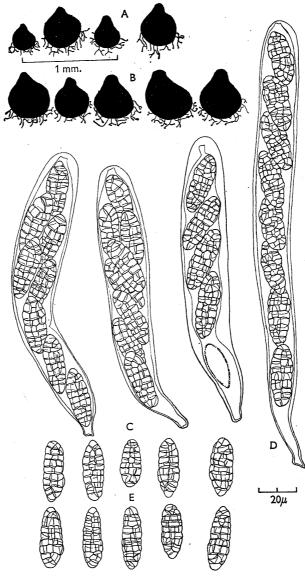


Fig. 1. Pleospora straminis. A, outlines of perithecia from stubble; B, outlines of perithecia from culture on P.D.A.; C, asci from the host; D, ascus from culture; E, ascospores from the host (Herb. Sheffield 225.).

frequently larger than those on stubble, measuring 330–450 μ in diameter. The asci were also larger, 155–230 × 20–27 μ .

For measurements of asci and ascospores from other collections see Table 1.

Wehmeyer (1951) has re-described *P. straminis* as *P. obliqua*. Material from specimen 255 was at first identified by him as *P. obliqua*. Later, having seen authentic material of *P. straminis* (which I had been unable to obtain), he agreed that specimen 255 was identical with *P. straminis*. There is a curious discrepancy in the measurements given for this fungus, however. Saccardo's measurements (see Table 1) are appreciably larger than those given by Berlese from the type, which match more closely those from the present collections.

PLEOSPORA RUBICUNDA Niessl., Verh. naturforsch. Ver. Brünn, 14, 1876. (Fig. 2)

Several collections of this fungus have been made, mainly on various kinds of plant debris near the sea. The following description is based on Herb. Sheffield no. 366 on Artemisia (?), Wolferton Marshes, Norfolk,

6 August 1948.

Perithecia, purple to black in colour; seated on purple-stained areas of tissue, developing beneath the surface, later semi-erumpent with about half the perithecium exposed, subglobose or elongated parallel to the long axis of the host, up to 400μ in diameter; neck papillate, ostiole often slit-like; perithecial wall uneven in thickness, $40-50\mu$ thick above, about 30μ thick below. Asci, bitunicate, numerous, broadly club-shaped to cylindrical, rounded above, tapering to a stalk below, up to 8-spored, $170-240 \times 16-22\mu$; separated by numerous branched 'paraphyses'. Ascospores, biseriate in the broader portion of the ascus, uniseriate below, crescent-shaped when viewed laterally, with 9-13 transverse septa and one or more longitudinal septa in the middle cells, tapering and rounded at the ends, constricted at the septa, especially in the middle; yellow to brown in colour, $30-40 \times 8-12\mu$.

Cultures. Ascospores germinated readily on agar media producing germ tubes, apparently from any cell. Cultures started from single ascospores and single asci were transferred to slopes of oat or potato-dextrose agar. The fungus grew slowly, forming colonies about 5 cm. in diameter in 2 months, with a characteristic purple colour in the medium and low pale aerial mycelium. Pycnidia of the *Phoma* type were found in 2-month-old cultures. The pycnidia were up to $500\,\mu$ in diameter, subglobose, wall thin $(10-15\,\mu)$ black, composed of dark interwoven hyphae. The sporophores form a palisade of hyaline skittle-shaped cells lining the wall and abstrict spherical to broadly elliptical hyaline pycnospores about $2\,\mu$ in diameter. Masses of pycnospores exude as flesh-coloured drops from the ostioles of the pycnidia. Perithecia have been found only once in culture (a multi-ascospore isolate of specimen 1723). When discovered, these perithecia were in poor condition and contained collapsed and distorted ascospores.

Pycnidia. Pycnidia resembling those found in culture have been found associated with perithecia. They are globose, submerged at first, later more

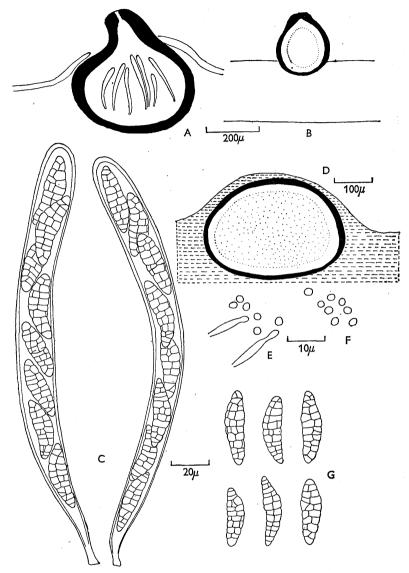


Fig. 2. Pleospora rubicunda. A, L.S. host stem and perithecium; B, L.S. host stem and pycnidium; C, asci from the host; D, section of culture showing pycnidium; E, pycnospores and sporophores from the host; F, pycnospores from culture; G, ascospores from the host. (A, C, D, G, from Herb. Sheffield 336; B, E, from Herb. Sheffield 1752B.)

superficial, up to 200μ in diameter, thin-walled and containing numerous spherical or elliptical pycnospores about 2μ in diameter. When pycnospores from the host are transferred to agar media, they germinate after about 1 week, giving rise to cultures which resemble those derived from ascospores.

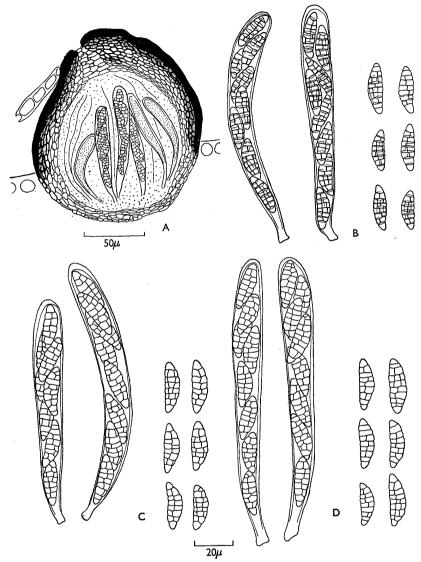


Fig. 3. Pleospora rubelloides. A, L.S. wheat stem and perithecium; B, asci and ascospores; C, asci and ascospores from the host; D, asci and ascospores from culture on oat agar. (A, C, D, from Herb. Sheffield 1722; B from the type specimen of Sphaeria rubelloides.)

Other collections. I have collected the fungus in Britain, France and Denmark only in maritime habitats, on the following hosts: Artemisia (?), Lepidium latifolium and Halimione portulacoides, and on various fragments of unidentified debris washed up by the sea, including woody plants. Niessl's original collection from Brünn was on Sambucus ebulus, but the fate of this specimen is not known. The dimensions of some recent collections and the type are listed in Table 2.

Table 1. Dimensions (in μ) of perithecia, asci and ascospores of Pleospora straminis

ascospores	Culture	1	1	$24 - 37 \times 9 - 12$	1	l	$30-36 \times 11-13$	
Dimensions of ascospores	Host	$40-45 \times 16-18$	$34-38 \times 12-14$	$26 - 34 \times 10 - 12$	$30-41 \times 11-13$	$29-37 \times 10.5-12.5$	$25-34 \times 10-14$	
1	ĺ			-27			-56	
ithecia Dimension of asci	Host	$140 - 150 \times 25 - 30$	$120 - 130 \times 24 - 28$	$130 - 192 \times 23 - 32$	120–190×20–26	$120-170 \times 20-24$	$104 - 140 \times 20 - 27$	
f perithecia	Host Culture	1	I	330-450	1	I	350-650	
Diameter of	Host	330	300-350	240-390	360-460	180-250	240–260	
	Specimen or authority	Saccardo, 1879	Type (Berlese, 1900)	Sheffield 225	Sheffield 226	Sheffield 227	Sheffield 821	

Table 2. Dimensions (in μ) of perithecia, asci and assospores of Pleospora rubicunda

		Diameter of		Dimensions of	Transverse
Specimen or authority	Host	perithecia	Dimensions of asci	ascospores	septa
Niessl, 1876	Sambucus ebulus	350	$140-160 \times 18-22$	$30-33 \times 11-13$	10-11
Type (Berlese, 1900)	Sambucus ebulus	250-350	$110-130 \times 14-18$	$26 - 32 \times 8 - 10$	12-15
Sheffield 366*	Artemisia?	300-400	$170-240 \times 16-22$	$30-40 \times 8-12$	9 - 13
Sheffield 855	Unidentified	250-320	$130-200 \times 18-20$	$29 - 36 \times 9 - 11$	7-10
Sheffield 856*	Unidentified	250-400	$170-200 \times 18-20$	$30-40 \times 9-12$	9-10
Sheffield 857	Unidentified	300-400	200 X 20-22	$30-40 \times 9.5 - 12$	9-10
Sheffield 86i	Unidentified	300-400	$150-180 \times 20-22$	$34-41 \times 10-12$	9-10
Sheffield 863	Halimione portulacoides	350	$150-170 \times 18-22$	$34-40 \times 9-11$	8-11
Sheffield 1674*	Unidentified	320-400	$180 - 190 \times 18 - 20$	$28 - 32 \times 9 - 10$	9-10
Sheffield 1723*	Spartina?	400	$130 - 180 \times 18 - 20$	$35-46 \times 10-12$	9-11
Sheffield 1727*	Unidentified	320-350	$210-270 \times 20-24$	$30-44 \times 8-13$	11-6
Sheffield 1752*	Lepidium latifolium	290-320	$154 - 166 \times 20 - 24$	$32-40 \times 9-10$	9-10

* Produced Phoma pycnidia in culture.

Pleospora rubelloides (Plowright ex Cooke) comb.nov. (Fig. 3)

= Sphaeria rubelloides Plowright ex M. C. Cooke, Grevillea, 5, 120, 1877. Leptosphaeria rubelloides (Plowright) Sacc., Sylloge Fungorum, 2, 77, 1883. Pleospora minor Wehmeyer, Amer. J. Bot. 39, 242, 1952.

Cooke (1877) described this fungus as follows:

'Sphaeria rubelloides. Plow. Perithecia scattered, soon exposed, mostly seated on reddish stains; asci lanceolate, sporidia fusiform, multiseptate (about 9) yellow. On putrid culms of Triticum repens, King's Lynn (C.B.P.) Sporidia '03 × '007 mm.' Examination of Plowright's specimens in Herb. Kew. (on Triticum repens, Ap. 1876 Sea Bank, King's Lynn), showed that the fungus is a *Pleospora*. Unfortunately, since the description makes no mention of longitudinal septa, Saccardo (1883) compiled it as a Leptosphaeria. Berlese (1894) added support to this transfer by citing it as a synonym of L. culmiphraga (Fr.) Ces. & de Not. Wehmeyer (1952) has since redescribed the fungus under the name Pleospora minor. Curiously, his description is based on a later collection of Plowright's from King's Lynn, labelled P. rubicunda Niessl. Plowright evidently was confused between his Sphaeria rubelloides and Pleospora rubicunda. In 1877 Phillips & Plowright listed 'Sphaeria (Pleospora) rubicunda Niessl. South Wootton. May, 1877. Sporidia 35–10 mk. On Juncus. Differing from Sp. rubelloides, Plow., Grevillea, Vol. v, p. 120, in the larger and muriform sporidia.' This specimen (Plowright Sph. Britt. 3, no. 70), however, matches the type material of S. rubelloides. A later specimen (Plowright Sph. Britt. 3, no. 71) labelled 'Sphaeria (Pl.) rubicunda Niessl Lynn 1879, Sp. rubelloides, Plow. on putrid grass', also matches S. rubelloides. Collections by Plowright on rotten wood, Brandon, Norfolk, 1879, in Cooke's Herbarium at Kew are also of S. rubelloides, although labelled Pleospora rubicunda. P. rubicunda and Sphaeria rubelloides are, however, distinct, and in view of the confusion, the type specimen of S. rubelloides is redescribed.

Perithecia, black, up to 250μ in diameter, globose or somewhat elongated parallel to the stem, subepidermal, piercing the epidermis by a small papillate ostiole, later raising the epidermis and becoming almost superficial; seated on purple-coloured patches on the decaying stems. Asci, numerous, club-shaped, tapering at the base to a short stalk, rounded at the apex, bitunicate, usually 8-spored; $94-120 \times 12-16\mu$. Ascospores, uniseriate in the lower portion of the ascus, irregularly biseriate above; yellow to brown in colour, crescent-shaped when viewed laterally, with one face flattened, the other sharply curved, tapering at the ends, with six to nine transverse septa (usually seven) and a variable number of longitudinal septa in all but the end cells; slightly constricted at the transverse septa, especially at the

middle: $20-26 \times 6-8\mu$.

Other collections. Over fifty other collections have been made on various hosts in Britain, France and Denmark. The fungus is common during the summer on old straw and grasses rotting under damp conditions, but is also found on other plant debris, especially in estuaries, salt marshes and sand dunes, along with the related Pleospora rubicunda. The fungus has been collected on the following hosts: Agropyron repens, A. junceiforme, Ammophila

arenaria, Arctium minus, Dactylis glomerata, Elymus arenarius, Festuca arundinacea, Melilotus altissima, Scirpus maritimus and on wheat and barley stubble. The range of variation is naturally greater than that found in the type specimen, and the dimensions of perithecia, asci, ascospores on the host and in culture are listed below (Table 3). In certain collections, the size of the ascospores is appreciably greater than in the type collection (e.g. $26-34 \times 7-10\mu$ in specimen 212, and $26-36 \times 9-11\mu$ in specimen 1743). The number of transverse septa may also be greater. However, the size of ascospores obtained in cultures from such collections, approximates more

closely to that of the type.

Cultures. Ascospores transferred to agar media germinate overnight, producing a germ tube from any cell. The fungus is homothallic and forms perithecia readily within 2 months on oat agar slopes. The characteristic purple coloration is formed in the medium as a secretion of the hyphae, which are colourless. A low-growing felt of white aerial mycelium covers the surface of the medium and the perithecia, which are formed within 2 months, are frequently in contact with the glass of the culture vessel. The perithecia are globose with a short blunt neck, and may be larger than those found on the host, measuring up to 500μ . Similarly, the asci may be longer (up to 180μ in culture, compared with up to 140μ on the host). The dimensions of the ascospores formed in culture show less variation, however, than those on the host. No other type of fructification has been seen in cultures of P. rubelloides.

DISCUSSION

The three fungi described above are clearly related to each other. Berlese (1888) pointed out that 'La Pleospora rubicunda è affine alla Pleospora straminis'. P. rubicunda and P. rubelloides are, perhaps, more closely similar. Whilst most collections of P. rubicunda can generally be separated from the majority of collections of P. rubelloides by the larger perithecia, longer and wider asci, and larger ascospores with more numerous transverse septa, none of these characters is completely reliable due to the overlap in the range of dimensions in the two species. Indeed, certain collections here assigned to P. rubelloides (e.g. 212 on Agropyron repens and 1743 on Ammophila arenaria) with rather large spores or wide asci, have been determined only after recourse to cultures. The absence of a *Phoma* stage in culture and the production of perithecia in single ascospore cultures, have been taken as evidence supporting this determination, and this is further supported by the more normal size of ascospores in culture in specimen 212. The existence of such 'intermediate' collections raises the question whether we should consider the two 'species' as a single more variable one. At present the only reliable criterion separating the two species is the production of a 'Phoma' stage in cultures assigned to P. rubicunda. Perithecia have been found only once in P. rubicunda in a multi-ascospore culture, and have never been observed in cultures derived from single ascospores, whilst in P. rubelloides perithecia are formed readily in single ascospore cultures. This may imply that whilst P. rubelloides is homothallic, P. rubicunda is heterothallic, but the evidence on this point is not completely satisfactory.

Table 3. Dimensions (in μ) of perithecia, asci and ascospores of Pleospora rubelloides

septa	Host ture	- 6-9	8-ro —	1-6-2	8-12 7-9	6-2 6-2	6-2 2	6-8 7-9	7-9 7-8	7-8 7-9	8-10 8-10	8-6 6-6	6-2 6-2	2-6 2-6	7-8 7-9
of spores	Culture	1	and the same of th	!	$22-27 \times 6 \cdot 5 - 8$	$22 - 28 \times 6 \cdot 5 - 9$	$22 - 30 \times 7 - 10$	$22 - 30 \times 6 \cdot 5 - 9$	$22 - 26 \times 6 \cdot 5 - 8$			$25 - 30 \times 8 - 10$	$27 - 32 \times 8 - 10$	$24 - 32 \times 8 - 9$	$26 - 32 \times 7 - 9$
Dimensions of spores	Host	$20 - 26 \times 6 - 8$	$24 - 33 \times 7.5 - 10$	$22 - 28 \times 6 - 8$	$26 - 34 \times 7 - 10$	$23 - 28 \times 7 - 8$	20-22×7	$20-26 \times 6 \cdot 5-8$	$22 - 25 \times 7 \cdot 5 - 8$	$24 - 28 \times 7 - 9$	н	$22 - 28 \times 7 - 8$		$21-28 \times 6 \cdot 5 - 7$	$20-26 \times 7-8$
s of asci	Culture	J	I	l		$106 - 112 \times 14 - 15$	$114-120\times16-18$	$120 - 150 \times 13 - 18$			120-140 × 20-22		$110-150 \times 14-18$	$120-160 \times 14-16$	$130-140 \times 14-18$
Dimensions of asci	Host	$94-120 \times 12-16$	$116-130 \times 17-21$	$86 - 130 \times 13 - 17$	$98-126 \times 15-19$	$86 - 108 \times 16 - 17$	$80-90 \times 14-16$	$94-120 \times 15-16$	$120-140 \times 15-16$	120-130×14-16	$110-150 \times 20-24$	$100-120 \times 14-16$	$100-115 \times 15-16$	$100-114 \times 14-16$	$100-110\times13-16$ $130-140\times14-18$
Diameter of perithecia	Culture	1	1	1	300-350	300500	300-400	200-350	200-380	250	200-290	250-350	200-300	250-300	
Diameter o perithecia	Host	250	250	200-300	200-320	200-240	160-200	200-250	200-240	200-250	250-300	200-250	200-250	280-350	250-300
	Host	Agropyron repens	Putrid grass	Rotten wood (Brandon)	Agropyron repens	A. repens	A. repens	Wheat straw	Festuca arundinacea	Melilotus altissima	Ammophila arenaria	Scirpus maritimus	Elymus arenarius	Festuca arundinacea	Barley straw
	Specimen	Type	Sph. Britt. III, 71	Herb. M. C. Cooke	Sheffield 212	Sheffield 850	Sheffield 853	Sheffield 1722							1751

I am grateful to the Director of the Royal Botanic Gardens, Kew, for permission to study specimens, and to Dr R. W. G. Dennis for helpful discussion. To Prof. L. E. Wehmeyer my thanks are due for examining certain collections. The work has been assisted by a grant from the University of Sheffield Research Fund.

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(Accepted for publication 21 February 1956)