STUDIES ON THE PATHOLOGY, SEXUALITY, CYTOLOGY AND SPORE FORMS OF GRAMINICOLOUS SEPTORIA SPECIES

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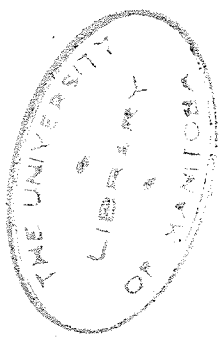

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ABSTRACT

PART 1

SEXUAL AND ASEXUAL SPORE FORMS OF CERTAIN SPECIES OF SEPTORIA ON CEREALS AND GRASSES AND THE PATHOGENICITY OF THE SPECIES

Single macropycnidiospores assignable to Septoria avenae Frank were isolated from wheat, barley, oats, rye and grasses in Canada. Cultures established from the single spores produced pycnidia and macrospores and a 3-septate Leptosphaeria which agreed well with L. avenaria Weber. Single ascospores or entire asci assignable to L. avenaria Weber were isolated from wheat, oats, barley and grasses. Cultures established from them produced perithecia and ascospores, and pycnidia and macrospores which conformed to S. avenae Frank. No infection was obtained on oats or rye, only a trace on barley and a few of the grasses, and slight infection on wheat. The collections, therefore, were assigned to L. avenaria f. sp. triticea T. Johnson. No microspores were found on field material, and none were produced in culture.

Single macrospores of S. avenae (oat-infecting) and S. nodorum (wheat-infecting) were isolated from oats and wheat respectively. Cultures established from them produced macrospores and microspores. Microspores of the former were also found in the field, and the connection with the macrospores was established. Pathogenicity tests confirmed that the former is vigorously pathogenic on oats, the latter on wheat. No other cereal or grass was infected. No perithecia were produced in culture.

Also studied culturally and tested for pathogenicity were isolates of S. avenae f. sp. anthoxanthi from Anthoxanthum odoratum, and 2 isolates from

Agropyron intermedium and Festuca elatior which conformed to S. nodorum but were non-wheat-infecting. An isolate from rye, provisionally designated as Septoria sp., was not vigorously pathogenic on oats, wheat, barley or rye, although it did produce a slight infection on wheat. Macrospores and microspores were produced abundantly in culture, but no perithecia.

Evidence was presented and discussed that L. avenaria f. sp. triticea, which is slightly pathogenic on wheat, but abundant on mature leaves of wheat and on the senescent parts of cereals and some grasses, is a plurivorous forma specialis, and that S. avenae (oat-infecting) and S. nodorum (wheat-infecting) have very specific host ranges.

The International Rules of Botanical Nomenclature were interpreted to mean, in the case of S. avenae Frank, that when the formae speciales were erected the type automatically became S. avenae Frank f. sp. avenae. This citation was used henceforth in place of S. avenae (oat-infecting).

PART II

STUDIES ON THE PERFECT STAGE

Investigations were undertaken to determine whether perithecial production could be induced in S. avenae f. sp. avenae and S. nodorum (wheat-infecting) by growing them on a variety of media and by mating the respective isolates in all possible combinations. No perithecia were obtained in the cultures.

It was concluded that homothallism operates in L. avenaria f. sp. triticea because cultures established from single ascospores from nature and from culture produced perithecia and viable ascospores in culture, and because perithecia-producing lines could be obtained by transference of single ascospores for up to 5 generations. An abnormal, viable ascospore found in one culture from wheat was described.

The world distribution of 3-septate Leptosphaeria species and the possibility of some of these being identical with L. avenaria f. sp. triticea was discussed.

PART 111

ACCESSORY SPORE FORMS IN SEPTORIA AND THEIR ROLE IN THE LIFE CYCLE

Young oat plants, mature oat plants and stubble at the end of the growing season and stubble which remained in the field during the winter were checked for the spores of S. avenae f. sp. avenae. Abundant macrospores were found on young plants and in some cases on stubble at the end of the growing season. Microspores were not found on young plants, but in some cases they occurred with the macrospores on plants at the end of the growing season, and they occurred abundantly on the overwintering stubble. In those cases where the macro- and micro-forms occurred in the same pycnidium, only very few spores intermediate in size were found.

Microspores began to germinate on agar medium after approximately 40 hours and continued to germinate up to 96 hours. Cultures established from single microspores produced the macro- and sometimes the micro-form on certain media and produced typical disease lesions on oats which in turn produced pycnidia and macrospores. Infection on oats by microspores was only obtained when plants were held in a humid atmosphere for up to 10 days. The role of the microspores in the overwintering of the organism was discussed.

Microspores of S. nodorum (wheat-infecting) and S. oudemansii were obtained in culture, and cultures which were established from single microspores produced pycnidia and macrospores which, in the former case, were pathogenic on wheat.

Low temperatures were found to favour the production of microspores and high temperatures to favour the production of macrospores. Various temperatures did not induce microspore formation in L. avenaria f. sp. triticea or Phaeoseptoria poae.

The role of the microspores in the life cycles of the fungi was discussed.

PART IV

CYTOLOGY OF THE SPECIES UNDER STUDY

Immature, mature and germinating macrospores and ascospores of the species under study were found to have one nucleus per cell, all the nuclei in a spore being originally derived from one nucleus. Nuclei were fairly evenly spaced along germ tubes and were never clustered at the tip. Microspores were found to be uninucleate. Ascospores were distinctly delimited at the 16-nucleate stage of the ascus, and could separate from the ascus remains and the other ascospores at this stage if the ascus wall was broken.

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INTRODUCTION

Many species of *Septoria* have been described on the Gramineae but only those causing disease of economic crops such as wheat and oats have been studied in any detail; the other species have received little attention save from the taxonomic point of view.

In some cases the plant species listed as hosts to a species of *Septoria* belong to widely separated genera, and even to different tribes. This has occurred particularly in the case of *Septoria avenae* and *Septoria nodorum*. More information is required, by cross-inoculation tests, as to whether all members of these and other species are in fact plurivorous and capable of infecting many different plant species, or whether the fungal species consist of *formae speciales*, or groups of morphologically similar individuals whose parasitic abilities are limited to certain hosts.

Various Ascomycetes have been recorded as being associated with *Septoria* species on the Gramineae, but only in a few cases has the connection between the perfect and imperfect stages been established. Accessory asexual spore forms have been described for some species on field material, but only once has the second spore form been studied in culture, and the connection with the *Septoria* stage established. The role played by the sexual and asexual spore forms in the overwintering or the oversummering of the organisms has been little investigated.

This study is concerned primarily with *Septoria avenae*, and to a less extent with *S. nodorum*. Investigations have been made to determine whether members of each species differ in their ability to parasitize graminaceous hosts, in their ability to produce a perfect stage, in the production of asexual spore forms and in the role of the various spore forms in the life cycle of the species.

PART 1

SEXUAL AND ASEXUAL SPORE FORMS OF CERTAIN SPECIES OF SEPTORIA ON CEREALS AND GRASSES AND THE PATHOGENICITY OF THE SPECIES

REVIEW OF LITERATURE

The genus Septoria

The history of the genus Septoria has been reviewed by Wakefield (68), Sprague (61, 62) and Rogers (51). Discussion has centred on the validity of Septoria Sacc. of 1884 versus Septoria Fr. of 1828, Septaria Fr. ex Kunze and Phleospora Wallr. A proposal to conserve Phleospora against Septoria was rejected at the Seventh International Congress at Stockholm (Rogers, 52). The writer follows most of the above authorities by referring all true pycnidial species to Septoria Fr. em. Sacc., accepting Phleospora as a separate genus with incomplete pycnidia.

Frandsen (21) proposed provisionally to unite a small group of Septoria species with "bacterioid Phyllosticta-like conidia" in a section Microseptoria within the genus.

Sprague (61, 62), Johnson (31) and Shaw (56) discussed various aspects of the genus in relation to Stagonospora, Ascochyta and Hendersonia Berk., particularly in relation to the length:width ratio of the spores and to the cultural characteristics. The intergrading of these genera and the lack of easily-recognised generic boundaries has probably been responsible for the same organism being assigned to different genera at different times.

In the three cases where the connection between the asexual and sexual stages of graminicolous species has been established, the perfect stage has been a Leptosphaeria (L. avenaria Weber, 1922, L. avenaria f. sp. triticea Johnson, 1947 and L. nodorum Müller, 1952). Unsubstantiated as yet, because the connections have not been demonstrated, are the reports that other

species of Leptosphaeria and of Metasphaeria, Sphaerella and Sphaerulina are associated with species of Septoria on the Gramineae.

Septoria avenae Frank

In 1895 S. avenae sp. nov. was described by Frank in Germany on oats (cited by Weber, 69). It was distinct from S. graminum var. c avenae Desm. of 1847, which is now known as S. tritici f. avenae (Desm.) Sprague (Sprague, 60, 62).

Weber (69) reported S. avenae in Wisconsin in 1921 but considered that thus far it seemed to be of negligible economic importance. He obtained the ascigerous stage in culture and named it Leptosphaeria avenaria sp. nov.

Cobb (11), Carne and Campbell (9), Noble et al. (45) and Brittlebank[i] recorded S. avenae and Septoria sp. on oats in Australia, but Shaw (56) found both S. avenae and S. tritici f. avenae present, and it is not known to which organisms the early records refer.

Drayton (19) and Bisby et al. (7) recorded S. avenae and an associated Leptosphaeria in Manitoba and Saskatchewan. Frandsen (21) recorded S. avenae on oats in Denmark.

Meehan and Murphy (39) reported that S. avenae was responsible for browning and lodging of oats in Iowa in 1948, and that perithecia of the ascigerous stage were occasionally present in advanced lesions. Poole and Murphy (48) recorded the reaction of oat varieties to S. avenae, and also reported on a toxic substance produced by the organism which produced leaf blotch and black stem symptoms when sprayed on to oats. Because they considered that stem infection was the most important phase, they proposed that the common name of the disease be changed from "speckled leaf blotch" to "black stem". Goto (24), Derick (14) and Huffman (25) reported the results of inoculation tests on varieties in the greenhouse and/or the field reactions under natural conditions.

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i Brittlebank, C.C. Catalogue of Australian Fungi. 1940. Unpublished.

Sprague (62, 63) listed S. avenae as occurring on species of Agrostis, Arrhenatherum, Calamagrostis, Festuca, Glyceria, Oryzopsis, Schizachne, Scolochloa and Trisetum montanum.

Stanton (65) reported the widespread and destructive occurrence of a kernel blight phase on oats in the United States. Simons and Murphy (58) found that the organism was not transmitted on infected seed. Poole and Murphy (49) noted that the disease increased in Iowa from 1949 onwards. They found that there was a significant positive correlation between reaction to the leaf blotch and black stem phase of the disease.

Johnson (32) investigated the cultural characteristics of S. avenae. Cultures established from field collections gave wild type colonies of rather uniform appearance, but subculturing on potato-sucrose agar led to the production of a great variety of variants. He found small pycnidia and microspores in some cultures, the microspores being capable of germination, but unable to infect oat seedlings in greenhouse tests. No perithecia were found in culture.

Johnson (31), Sprague (62) and Shaw (56) pointed out the similarity of S. avenae and Stagonospora arenaria Sacc. This latter species has been recorded on many grasses and its spores are about the same size as those of S. avenae f. sp. triticea. Sprague is maintaining St. arenaria as a distinct species until more information is available.

Shaw (56) recorded S. avenae in Australia on Avena sterilis. No infection was obtained in inoculation tests of species of Agropyron, Anthoxanthum, Arrhenatherum, Dactylis, Elymus, Festuca, Hordeum, Secale, Stipa and Triticum, but other species of Avena proved susceptible. No perithecia were obtained in culture. Noble and Montgomerie (44) reported the organism in Scotland, where it has caused leaf spots, culm breaking and kernel infection.

In Canada the disease has been assuming importance in the East, where culm infection has been so heavy that crops have lodged. A moderately heavy infection occurred at Agassiz, B.C. in 1953, but in Alberta infection is still recorded as trace to slight (Conners, 12).

Formae speciales of Septoria avenae

Johnson (31) recorded that he had found pycnidia and spores conforming rather closely to those of S. avenae on wheat and barley from 1942 onwards in Canada. Occasionally, perithecia of Leptosphaeria were found on the leaves. Similar perithecia were found in cultures established from pycnidiospores, and pycnidia and spores developed in cultures established from ascospores. He found that the organism was weakly parasitic on wheat and would not infect oats, and described it as L. avenaria f. sp. triticea f. sp. nov., the pycnidial stage being S. avenae f. sp. triticea. Meehan and Murphy (39) recorded this special form in the United States, and Shaw (56) recorded it in New Zealand and Australia from 1950 onwards. Both pycnidia and perithecia were found on leaves and glumes, and on nodes and internodes of stubble. The connections were confirmed culturally.

Shaw (56) described S. avenae f. sp. anthoxanthi f. sp. nov.[i] on Anthoxanthum odoratum. This form did not infect oats, wheat, the other cereals or certain grasses, and was distinct culturally from the other form.

Septoria nodorum (Berk.) Berk.

Weber (70) summarized the history of the species since its description by Berkeley in 1845. Shaw (56) summarized the work from 1922 to 1952, including reports on varietal reactions, presence in wheat seed, inoculation tests on grasses and its world occurrence. Sprague (62) and Sprague and Fischer (64) recorded S. nodorum on 27 species of 13 genera of the Gramineae.

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i This description has not been published as yet, but the citation is given in this form hereafter for convenience

Alfaro (1) found perithecia associated with the disease, and although they were immature and the genetical connection could not be demonstrated, he considered that they were of the genus Leptosphaeria. Chona and Munjal (10) recorded that perithecia were found associated with the disease in India, but as the work was still in progress, no definite statement was made. Müller (41) described Leptosphaeria nodorum sp. nov. in Germany, the perfect stage of S. nodorum.

MATERIALS AND METHODS

Pycnidiospores assignable to Septoria avenae Frank and asci and ascospores assignable to Leptosphaeria avenaria Weber were isolated from wheat, barley, oats, rye and grasses in Canada. Pycnidiospores assignable to S. avenae were isolated from Anthoxanthum odoratum from Australia. For comparison with the above, isolations were made of pycnidiospores of S. nodorum from wheat and grasses in Canada and from wheat in England, pycnidiospores of S. oudemansii from Poa pratensis and pycnidiospores of Stagonospora meliloti from Melilotus alba in Canada.

Cultures were established from the above, and were i) maintained by mycelial transfer on potato sucrose agar (P.S.A.), and ii) subcultured by means of single spores, either asexual or sexual, which were isolated on P.S.A., transferred to P.S.A. slopes, and subcultured on to various media, particularly sterile lemon leaves. Most cultures lost the ability to sporulate if maintained by mycelial transfer.

The isolates were tested on a variety of media, including P.S.A., sterile lemon leaves, maize grain on cob, asparagus stems, alfalfa stems, wheat grains, oat straw, wheat straw, carrot and potato agar, ground oat hull agar and an agar medium with different proportions of carbon and nitrogen source, following Nitimargi (23). Johnson (32) found that pycnidial production was abundant on sterile lemon leaves, and this was confirmed. Perithecial production was best on this medium, and on some samples of sterile asparagus stem and maize grain on cob.

The source of the collections, the spore forms present and the type of spore originally isolated to establish the cultures are given in Tables 1-Vl. In Tables 1-Vl only those collections are listed from which isolations were made and pathogenicity tests were carried out.

The wheat used in the pathogenicity tests was "Gaza", although

subsidiary tests on other varieties, mainly "Marquis", "Lee" and "Little Club" were also carried out; the oats were "Victory" and/or "Red Rustproof", the barley was "Vantage", and the rye was "Prolific" and/or "Rosen".

The grasses were established from seed taken from the diseased plants whenever possible, and failing this, from seed obtained from the Botany Unit, Ottawa, and other sources, or from clones from the field.

The inoculum was either in the form of spores from the field or culture, these being sprayed on to the plants, or macerated mycelium which was applied to the leaves with an inoculating needle. Unless stated otherwise, plants were incubated for 48 hours.

RESULTS

Septoria avenae (oat-infecting)

The isolates shown in Table 1 conformed in morphology and septation to S. avenae Frank, were all isolated from oats, and were vigorously pathogenic on oats, producing lesions in about 4 days if the inoculum was mycelium, and in about 7 days if the inoculum was spores. The isolates did not infect wheat, barley, rye or the grasses as shown in Table Vll.

Mass pycnidiospore or mass mycelial cultures rarely, if ever, produced pycnidia on P.S.A. Single micro- and sometimes single macrospore cultures occasionally did. Pycnidia and macrospores formed abundantly on sterile lemon leaves, the spores often exuding in pink cirri in situ. All the isolates were capable of producing microspores in culture on certain media under certain conditions (see Part lll) except Accessions 3 and 35 which ceased sporulating shortly after being isolated.

Macrospores were found in abundance in lesions on growing oats, but were not found late in the autumn or the winter. Microspores were not found on growing oats; they were only recorded on material collected in the autumn and winter. Both spore forms occurred on some collections made in early September (see Table 1 and Part lll).

No isolate in Table 1 produced the perfect stage in culture, even on a variety of media. (For further attempts to produce the perfect stage see Part ll). Some isolates from oats, shown in Table lll, which would not reinfect oats, and which produced the perfect stage in culture, were assigned to Leptosphaeria avenaria f. sp. triticea, (see next section).

It is to be noted that in the 3 cases where Leptosphaeria perithecia were found on senescent oat parts, cultures established from the asci and ascospores failed to reinfect oats. The collections were consequently assigned to L. avenaria f. sp. triticea (see next section and Table lll).

The size and the septation of macrospores of several isolates of S. avenae (oat-infecting) and of other isolates of the group under study are shown in Table VIII.

Leptosphaeria avenaria f. sp. triticea.

The isolates shown in Tables II, III and IV conformed in morphology and septation to S. avenae but were not pathogenic on oats, and therefore were not the oat-infecting S. avenae.

The isolates were not vigorously pathogenic on wheat and so could be easily distinguished from S. nodorum, as indeed they could be so distinguished on morphological grounds.

Culturally, the isolates could be distinguished from S. avenae f. sp. anthoxanthi and S. avenae (oat-infecting). The special form anthoxanthi is very slow-growing on P.S.A. and produces sparse pycnidia on that medium. S. avenae (oat-infecting) grows quicker, but is still slower than S. avenae f. sp. triticea.

Of the 40 isolates, 21 of the 26 established from macrospores produced perithecia in culture, the one isolate established from surface-sterilized barley seed produced perithecia, and 12 of the 13 established from asci or ascospores produced perithecia, although a few isolates did so less abundantly than the others. Therefore, of the 40 isolates, 34 produced perithecia and ascospores in culture which conformed to Leptosphaeria avenaria Weber, and which, because of their inability to infect oats, and slight ability to infect wheat, were assigned to L. avenaria f. sp. triticea.

Of the 13 cultures established from entire asci or ascospores, 9 produced pycnidia and macrospores in culture which conformed to S. avenae, and which, because of their inability to infect oats, and slight ability to infect wheat, were also assigned to L. avenaria f. sp. triticea.

Of the 6 cultures not forming perithecia, 3 (isolates 75 from

wheat, 156A from barley and 81 from Hordeum jubatum) did not produce pycnidia either, although small black initials were found on some media. The other 3 (isolates 71 from wheat, 159 from rye and 132 from barley) produced pycnidia, although very sparingly. The inability of the cultures to produce either perithecia or pycnidia was possibly caused by growing them on media which were unsatisfactory for the production of fruiting bodies. This happened particularly in the beginning of the study.

No microspores were found in any of the cultures on any medium.

It is evident that the isolates from grasses in Table IV are either very similar to or identical with L. avenaria f. sp. triticea, for the following reasons: their asexual spores conform to S. avenae and S. avenae f. sp. triticea (the former, as pointed out by Johnson (31), being sometimes a little longer than the latter; their sexual spores conform to L. avenaria; they are not pathogenic on oats, and they either failed to infect or only very slightly infected wheat and the hosts from whose senescent parts they were isolated.

Because of the plurivorous nature of the special form triticea, it follows that when collections which conform to S. avenae are examined, they should not be assigned to any of the special forms (even though, for example, they are found on mature oat leaves), without the appropriate inoculation tests being carried out.

Johnson (31) pointed out that the special form triticea has low parasitic vigour, and this is confirmed in the present study. While slight infection was obtained on "Gaza", only trace infection occurred on "Marquis" and "Lee" and none at all on "Little Club".

Infection of young leaves was noted a few times in the field. Early in the summer of 1954 young wheat crops in Saskatchewan and Manitoba were examined for leaf spots. Only a few lesions were found, and these were

in conjunction with leaf rust pustules. In Plate 1, Fig. 1, a leaf rust pustule is shown, surrounded by necrotic tissue. In Plate 1, Fig. 2, the same pustule is shown, with pycnidia in the surrounding tissue. The pycnidia in the other lesions contained spores of S. avenae f. sp. triticea. The occurrence of the pustules and lesions together might have been coincidental, but on the other hand, it is possible that the entry of this special form into the young, vigorously-growing leaf is facilitated by the rust infection.

Septoria nodorum (wheat-infecting)

Parallel tests were made with isolates of S. nodorum for comparative purposes, since this species is morphologically the nearest to S. avenae. As shown in Table V, isolates from wheat whose spores conformed to S. nodorum were all vigorously pathogenic on wheat. No infection was obtained on oats, barley or rye, or the grasses shown in Table VII. Shaw (56) had reported previously that the host range of the wheat-infecting S. nodorum is confined to wheat.

Only rarely were pycnidia produced on P.S.A., but abundant pycnidia with macrospores were produced on sterile lemon leaves, the spores occasionally exuding in pink cirri in situ. Microspores were produced in culture (see Part III), but no perithecia were obtained. (For further attempts to produce the perfect stage see Part II).

Miscellaneous Septoria species (Table VII)

The morphology, size and septations of the spores of Accs. 59 from Agropyron intermedium and 63 from Festuca elatior conform to S. nodorum, but are distinct from the wheat-infecting strain, since wheat is immune to them. No infection was obtained on oats, barley, rye or the hosts from which they were isolated. Both isolates produced pycnidia and macrospores in culture, but no microspores or perithecia. It is possible that the

isolates belong to a plurivorous strain, since morphologically similar individuals have been found on the senescent parts of many grasses, as listed by Sprague (62) and Sprague and Fischer (64). They are provisionally placed in S. nodorum, but their inability to infect wheat is stressed.

The spores of Acc. 160 from rye range from 19.5-33 x 2.5-3u, with mainly 3, sometimes 1, septa. Pycnidia and macrospores were produced abundantly in culture, and also microspores on sterile lemon leaves, and on P.S.A. when the cultures were established from single microspores. (See Part III). No perithecia were obtained in culture. The colonies differed slightly from those of S. nodorum, S. avenae (oat-infecting) and S. avenae f. sp. triticea. The isolate caused only a trace infection on wheat, and did not infect oats, barley, rye or grasses shown in Table VII. The isolate does not appear to be S. secalis, whose macrospores are nearer those of S. avenae in size, and whose microspores are given by Sprague (62) as 10 x 0.5u. The microspores of Acc. 160 were 3-4 x 1u. Comparison of Acc. 159 from rye with this accession indicates that while the former can be assigned to L. avenaria f. sp. triticea without hesitation, the latter is more difficult to place. Its inability to form the perfect stage, and ability to form microspores, distinguishes it from f. sp. triticea. Its spores range in size between those of S. nodorum and S. avenae, but it is unable to attack wheat vigorously like S. nodorum, or oats like S. avenae. It is provisionally designated Septoria sp.

S. avenae f. sp. anthoxanthi was vigorously pathogenic on Anthoxanthum odoratum, but non-infective on wheat, oats, barley, rye, or the grasses in Table VII. Cultures on P.S.A. are extremely slow-growing when compared with those of S. avenae (oat-infecting) and L. avenaria f. sp. triticea. No perithecia were obtained in culture, and no microspores were

found either in culture or on infected leaves.

Acc. 68 from Poa pratensis was assigned to S. oudemansii. It is culturally distinct from S. nodorum, the colonies being white, turning grey and black, but not carbonaceous. Morphologically, it is very close to S. nodorum. No perithecia were obtained, but microspores were produced on some media (see Part III).

Stagonospora meliloti produced macrospores and possibly microspores in culture (the macrospores being variable and grading into small spores) but no perithecia.

TABLE I

Septoria avenae (oat-infecting)

Summary of spore forms on field material and in culture, and of pathogenicity tests

Acc.	Host	Locality	Original isolate	Spore forms[i] Nature macro	Nature micro	Nature asco	Culture macro	Culture micro	Culture asco	Pathogenicity[ii] on wheat	oats	barley	rye
2	oats	Ottawa, Ont.	macro	+			*+	*		-	++	-	-
3	"	Eastern Canada	"	+						-	++	-	-
29	"	Charlottetown, P.E.I.	"	+			*+	*		-	++	-	-
31	"	Normandin, Que.	"	+			*	*		-	++	-	-
32	"	Kentville, N.S.	"	+				*		-	++	-	-
35	"	Ste. Anne de la Pocatiere, Que.	"	+			+			-	++	-	-
47	"	Agassiz, B.C.	"	+			*	*+		-	++	-	-
76	"	Lennoxville, Que.	"	+			*+	*		-	++	-	-
133	"	Charlottetown, P.E.I.	"	+			+	+		-	++	-	-
134	"	Ottawa, Ont.	micro	+	+		+	+		-	++	-	-
169	"	Charlottetown, P.E.I.	"		+		+	+		-	++	-	-
171	"	Normandin, Que.	"	+	+		+	+		-	++	-	-
172	"	Ottawa, Ont.	"		+		+			-	++	-	-
174	"	Ottawa, Ont.	"		+		+			-	++	-	-
175	"	Ottawa, Ont.	"		+		+	+		-	++	-	-
176	"	Charlottetown, P.E.I.	"		+					-	++	-	-
177	"	Normandin, Que.	"		+								

i In this and all subsequent tables macro refers to macropycnidiospores, micro to micropycnidiospores, and asco to ascospores

ii Susceptibility is shown as ++, slight susceptibility as +, trace as tr, immunity as -

* Spores obtained in mated cultures, details in Part III

TABLE II

Leptosphaeria avenaria f. sp. triticea

Summary of spore forms on field material and in culture, and of pathogenicity tests

Acc.	Host	Locality	Original isolate	Spore forms in Nature			Spore forms in Culture			Pathogenicity on wheat	oats	barley	rye
				macro	micro	asco	macro	micro	asco				
7	wheat	Leduc, Alta.	macro	+			+		+	+	-	-	-
8	"	Ste. Agathe, Man.	"	+					+	+	-	tr	-
44	"	Lacombe, Alta.	"	+					+	+	-	-	-
50	"	Gladstone, Man.	"	+					+	+	-	tr	-
68	"	Macdonald College, Que.	"	+					+	+	-	-	-
69	"	L'Assomption, Que.	"	+			+		+	tr	-	-	-
71	"	Charlottetown, P.E.I.	"	+			+			+	-	-	-
73	"	Lennoxville, Que.	"	+					+	tr	-	tr	-
75	"	Lennoxville, Que.	"	+						tr	-	tr	-
129B	"	Edmonton, Alta.	"	+					+	tr	-	-	-
157	"	Scott, Sask.	"	+			+		+	tr	-	tr	-
27	"	Lac du Bonnet, Man.	entire asci			+	+		+	+	-	tr	-
50	"	Gladstone, Man.	entire asci			+			+	+	-	tr	-
94	"	Morden, Man.	asco			+	+		+	tr	-	-	-
154	"	Carmel, Sask.	asco			+	+		+	tr	-	-	-

TABLE III

Leptosphaeria avenaria f. sp. triticea (cont.)

Summary of spore forms on field material and in culture, and of pathogenicity tests

Acc.	Host	Locality	Original isolate	Spore forms						Pathogenicity on			
				Nature			Culture						
				macro	micro	asco	macro	micro	asco	wheat	oats	barley	rye
56	barley grain	Edmonton, Alta.	mycelium						+	+	-	-	-
70	barley	Normandin, Que.	macro	+					+	+	-	-	-
156A	barley	Kelvington, Sask.	"	+						tr	-	-	-
132	barley	Charlottetown, P.E.I.	entire asci			+	+			-	-	-	-
42	rye	Charlottetown, P.E.I.	macro	+					+	+	-	-	-
45	"	Creston, B.C.	"	+			+		+	+	-	-	-
46	"	L'Assomption, Que.	"	+					+	+	-	-	-
159	"	Scott, Sask.	"	+			+			+	-	tr	-
11	oats	Darlingford, Man.	macro	+			+		+	+	-	-	-
141	"	Gilbert Plains, Man.	"	+			+		+	tr	-	-	-
142	"	Roblin, Man.	"	+			+		+	tr	-	-	-
24	oats	Ottawa, Ont.	entire asci			+	+		+	+	-	-	-
162	"	Fort Garry, Man.	asco			+	+		+	tr	-	-	-
172	"	Ottawa, Ont.	"			+	+		+	+	-	-	-

TABLE IV

Leptosphaeria avenaria f. sp. triticea (cont.)

Summary of spore forms on field material and in culture, and of pathogenicity tests

Acc.	Host	Locality	Original isolate	Spore forms Nature macro	nicro	asco	Culture macro	micro	asco	Pathogenicity on wheat	oats	barley	rye	x
57.1	Agropyron subsecundum	Ottawa, Ont.	asco entire asci			+	+		+	+	-	tr	-	
58.1	Agropyron trachycaulum	Fort Garry, Man.	macro	+		+			+	+	-	-	-	
58.2	Agropyron trachycaulum	Fort Garry, Man.	entire asci	+		+			+	tr	-	-	-	
136	Agropyron sp.	Gilbert Plains, Man.	macro	+					+	tr	-	-	-	
168	Calamagrostis canadensis	Lac du Bonnett, Man.	asco			+	+		+	tr	-	-	-	
12	Elymus canadensis	La Riviere, Man.	macro	+			+		+	+	-	-	-	-
22	Elymus canadensis	Kings Park, Man.	"	+			+		+	tr	-	-	-	-
62	Elymus virginicus	Ottawa, Ont.	"	+					+	+	-	-	-	-
81	Hordeum jubatum	Fort Garry, Man.	"	+						-	-	-	-	-
96	Hordeum jubatum	Riding Mts., Man.	asco			+			+	tr	-	-	-	-
65.1	Poa pratensis	Fort Garry, Man.	asco entire asci			+			+	+	-	-	-	-

x Hosts from which the isolates were originally isolated

TABLE V

Septoria nodorum (wheat-infecting)

Summary of spore forms on field material and in culture, and of pathogenicity tests

Acc.	Host	Locality	Original isolate	Spore forms in Nature			Spore forms in Culture			Pathogenicity on wheat	oats	barley	rye
				macro	micro	asco	macro	micro	asco				
1	wheat	Rothamsted, Eng.	macro	+				*		++	-	-	-
30	"	Scott, Sask.	"	+			*+	*		++	-	-	-
37	"	Edmonton, Alta.	"	+				+		++	-	-	-
54	"	Edmonton, Alta.	"	+			*	*		++	-	-	-
67	"	Normandin, Que.	"	+			*	*+		++	-	-	-
72	"	Fredericton, N.B.	"	+			*	*		++	-	-	-
92	"	Plum Coulee, Man.	mycelium							++	-	-	-
98	"	Lilyfield, Man.	"							++	-	-	-
99	"	Lilyfield, Man.	macro	+			+	+		++	-	-	-
100	"	Oakbluff, Man.	"	+			+			++	-	-	-
158	stubble wheat	Fredericton, N.B.	"	+			+			++	-	-	-
170	"	Normandin, Que.	"	+						++	-	-	-

* Spores obtained in mated cultures

TABLE VI

Miscellaneous *Septoria* species

Summary of spore forms on field material and in culture, and of pathogenicity tests

Acc.	Host	Locality	Species	Original isolate	Spore forms in Nature			Spore forms in Culture			Pathogenicity on wheat	oats	barley	rye	x
					macro	micro	asco	macro	micro	asco					
59	*Agropyron intermedium*	Ottawa, Ont.	*S. nodorum* (non-wheat infecting)	macro	+			+			-	-	-	-	-
63	*Festuca elatior*	Ottawa, Ont.	"	"	+			+			-	-	-	-	-
160	rye	Charlottetown, P.E.I.	*Septoria* sp.	"	+			+	+		tr	-	-	-	
80	*Anthoxanthum odoratum*	Mt. Wilson, Australia	*S. avenae* f. sp. *anthoxanthi*	"	+			+			-	-	-	-	++
88	*Poa pratensis*	Granite Lake, Ont.	*S. oudemansii*	"	+			+	+		-	-	-	-	tr
130	*Melilotus alba*	Fort Garry, Man.	*Stagonospora meliloti*	"	+			+	+(?)						

x Hosts from which the isolates were originally obtained

TABLE VII

The results of pathogenicity tests of some isolates on cereals and grasses

Pathogenicity on	*S. avenae* (oat-infecting) Acc. 134	*S. avenae* f. sp. *anthoxanthi* Acc. 80	*Leptosphaeria avenaria* f. sp. *triticea* Acc.168	*S. nodorum* Acc. 99	*Septoria* sp. Acc. 160
Agropyron intermedium	-	-	-	-	-
A. tenerum	-		-	-	-
Agrostis alba	tr				-
Anthoxanthum odoratum	-	++	-	-	-
Calamagrostis epigeios	-				-
Elymus canadensis	-	-	-	-	-
E. virginicus	-	-	-	-	
Festuca elatior		-	-	-	-
Hordeum jubatum	-	-	-	-	-
Phleum pratense			-	-	
Poa compressa	-				-
P. pratensis	-			-	-
"Victory oats	++	-	-	-	-
"Gaza" wheat	-	-	tr	++	tr
"Prolific" rye	-	-	-	-	-
"Vantage" barley	-	-	-	-	-

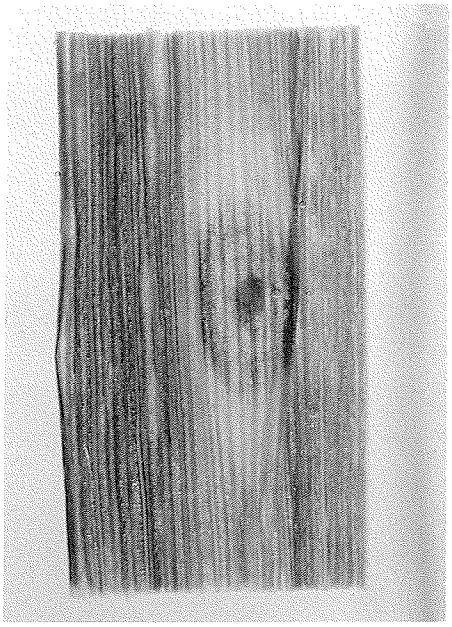
TABLE VIII

Size and septation of pycnidiospores of some isolates from cereals and grasses

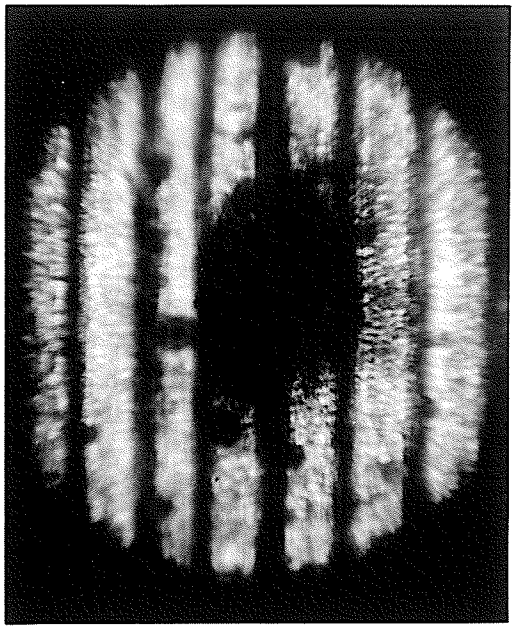
Acc.	Isolate	Source	*	Septations	Size of macropycnidiospores u
2	S. avenae (oat-infecting)	Oats	C	3	33.5-46 x 2.5-3
176	" " "	"	C	3(4)	31 -45 x 2.5-3
80	S. avenae f.sp. anthoxanthi	Anthoxanthum odoratum	N	3	28 -35 x 3 -3.5
8	L. avenaria f.sp. triticea	wheat	N	3	32 -35 x 2.5-3
159	" "	rye	N	3	29.5-39 x 2 -2.5
142	" "	oats	N	3	28 -39 x 2.5
58	" "	Agropyron trachycaulum	N	3(2,1)	28 -36.5 x 2.5
136	" "	Agropyron sp.	N	3	28 -39 x 2.5-3
168	" "	Calamagrostis canadensis	C	3(4)	31 -40.5 x (2)2.5(3)
12	" "	Elymus canadensis	N	3(1)	29.5-36.5 x 2.5-3
22	" "	Elymus canadensis	N	3	25 -39 x 2.5-3
62	" "	Elymus virginicus	C	3(1,2)	26.5-33.5 x 2.5-3
81	" "	Hordeum jubatum	N	3	25 -36.5 x 2.5-3
160	Septoria sp.	rye	C	3(1)	19.5-32 x 2 -2.5
99	S. nodorum (wheat-infecting)	wheat	C	1(3)	14 -19.5 x 2 -2.5
59	S. nodorum (non-wheat-infect.)	Agropyron intermedium	N	1(2,3)	15 -24 x 2 -2.5
63	" " " "	Festuca elatior	N	1(2,3)	14 -21 x 2 -2.5
88	S. oudemansii	Poa pratensis	N	1(2)	14 -18 x 1.5-2

* Spores from Nature (N) or Culture (C)

PLATE 1



1



2

Fig. 1 Leaf rust pustule on wheat leaf, surrounded by necrotic tissue. Leaf fixed in acetic-alcohol (3:1), cleared in alcohol and mounted in cotton blue lactophenol. x 8

Fig. 2 Same pustule and lesion as above. Pycnidia of S. avenae f. sp. triticea in the necrotic area. x 40

DISCUSSION

It appears from previous work and from this study that the species S. avenae Frank is divisible into groups according to differences in pathogenicity, production of the perfect stage and of accessory spore forms, each group having slight differences in asexual spore morphology.

The differences are summarized as follows:

	Pathogenic on:	Spore forms:		
S. avenae (oat-infecting)	Oats	macro	micro	-
S. avenae f.sp. anthoxanthi	Anthoxanthum odoratum	macro	-	-
Leptosphaeria avenaria f. sp. triticea	Slightly pathogenic on wheat, found abundantly on mature wheat leaves and sheaths, and on senescent parts of oats, barley, rye and certain grasses	macro	-	asco

It is possible that the perfect stage of S. avenae (oat-infecting) may be found again, and that microspores are produced by the 2 special forms under certain conditions. It is also possible that collections from grasses whose asexual spores conform to S. avenae Frank will be found which are unable to produce perithecia, or which have perithecia not conforming to L. avenaria Weber, or which are vigorously pathogenic on certain grasses. If and when these are found, new special forms will need to be erected, or they will be known under the name of the new perfect stage.

Article 34 of the International Code of Botanical Nomenclature (Lanjouw, 35) states: "The description of a subordinated taxon which does not include the nomenclatural type of the higher taxon automatically creates a second subordinated taxon of the same rank which has as its nomenclatural type the type of the higher taxon!! Also, Article 35 states:

"If any infra-specific taxon which includes the nomenclatural type of the epithet of the next higher taxon is to be mentioned by a subdivisional name, that name must repeat the epithet of the higher taxon unaltered, but, contrary to Article 55, without citation of an author's name."

This is interpreted to mean, in the case of S. avenae, that, with the erection of the special forms triticea and anthoxanthi, the type becomes S. avenae Frank f. sp. avenae automatically, and the perfect stage, when it is found again, L. avenaria Weber f. sp. avenaria.

The position then is as follows:

S. avenae Frank f. sp. avenae	Pathogenic on oats
S. avenae Frank f. sp. anthoxanthi[i]	Pathogenic on A. odoratum
Leptosphaeria avenaria G.F. Weber f.sp. triticea T. Johnson (imperfect S. avenae Frank f.sp. triticea T. Johnson	Slightly pathogenic on wheat, found on senescent parts of cereals and grasses

Henceforth, throughout this study, the oat-infecting S. avenae is referred to as S. avenae f. sp. avenae.

The intra-specific relationships between the formae speciales and the specific relationships between S. avenae, S. nodorum and S. oudemansii are discussed in Part II, together with a consideration of their phylogeny in regard to sexuality and parasitism.

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i Refer footnote on page 5

PART II

STUDIES ON THE PERFECT STAGE

REVIEW OF LITERATURE

The perfect stage of S. avenae Frank

In 1869 Auerswald recorded an Ascomycete on oats with hyaline ascospores measuring 15-16 x 3.5-4u, and described it as Leptosphaeria avenae sp. nov. (Cited by Weber, 69). It was transferred to Metasphaeria by Saccardo (53) because of the hyaline spores.

Weber (69) isolated single pycnidiospores from diseased oats and 4 months later found perithecia in both P.D.A. and oat meal agar cultures established from them. He isolated single ascospores and pycnidia developed in the cultures in 3 weeks and perithecia about 3 weeks later. Using ascospores developed in culture, inoculations were made on to oat seedlings, and typical Septoria infections resulted in about 2 weeks. Weber considered that this was the ascigerous stage of S. avenae, that it was distinct from Auerswald's L. avenae, the ascospores being 23-28 x 4.5-6u, and described it as L. avenaria sp. nov.

In England Moore (40) found oat plants with a Leptosphaeria on the leaf sheaths. The material was examined at Harpenden and by Mr. W. E. Mason but was not definitely identified. Of the species described it most closely resembled "L. avenae Auersw. ex Wint." The ascospores were 0-3 septate, hyaline within the perithecium, honey-coloured in mass outside and measured 14-18 x 2.5-4u.

Drayton (19) and Bisby et al. (7) recorded a Leptosphaeria associated with S. avenae on oats in Manitoba and Saskatchewan. Meehan and Murphy (39) stated that perithecia of the ascigerous stage of Leptosphaeria avenaria Weber were occasionally present in advanced lesions on oats in Iowa. Johnson (32) did not find perithecia of

S. avenae in the field or in culture in Canada. Shaw (56) did not find perithecia in the field or in culture in Australia.

Leptosphaeria avenaria f. sp. triticea

Johnson (31) described L. avenaria f. sp. triticea on wheat and less commonly on barley and rye in Canada. The ascigerous stage agreed very well with that reported by Weber for oats, but Johnson's organism would not infect oats and was mildly pathogenic on wheat. The ascospores measured (16)19-25(28) x 4-6u. The asexual stage showed very little difference, if any, from the asexual stage of S. avenae.

Meehan and Murphy(39) recorded this special form in the United States. Shaw (56) recorded it on material from New Zealand and Australia from 1950 onwards. The isolates failed to infect oats and the connection between the perfect and imperfect stages was confirmed.

The perfect stage of S. nodorum

Weber (70) stated that in 1904 Voglino cultured S. nodorum and found perithecia in certain cultures that contained ascospores which were hyaline and one-septate, and which he thought was a Sphaerella. However, Weber mentioned that there seemed to be some question of the maturity of the ascospores. In 1919 Davis found some perithecia associated with pycnidia of S. nodorum which were 3-septate and hyaline, and which he placed in the genus Sphaerulina. Weber found perithecia associated with pycnidia of S. nodorum. The ascospores were 3-septate, of a yellow-olivaceous colour, 20-30 x 5-6u and were assigned to the genus Lepto-sphaeria. Weber thought Davis' material was probably immature. The relation between S. nodorum and the Leptosphaeria could not be proved by Weber, since the ascospores were not viable (Weber, 70).

Alfaro (1) found perithecia associated with S. nodorum in Spain and although they were immature, considered they were probably a Leptosphaeria.

Chona and Munjal (10) found perithecia on dried-up leaves and sheaths affected with S. nodorum in India. The perithecia contained 3-septate ascospores, olive yellowish in colour, measuring 15-20 x 3-4u. In 20 single ascospore cultures one developed typical pycnidia of S. nodorum. No definite statement was made since the work was still in progress.

Shaw (56) found perithecia of Leptosphaeria intermingled with S. nodorum pycnidia in Australia, but cultures established from ascospores produced pycnidiospores of L. avenaria f. sp. triticea in culture. No perithecia were obtained in cultures of S. nodorum established from pycnidiospores.

Müller (1952) described Leptosphaeria nodorum sp. nov., the perfect state of S. nodorum in Germany. The perithecia were 160 x 250u, asci 60-70 x 9u, and ascospores 3-septate, subhyaline to faint yellow, tapering at both ends and measuring 20-26 x 4u. Cultures of the Leptosphaeria produced abundant spores of S. nodorum, and just as severe symptoms were produced in the same time, when wheat was inoculated with the Leptosphaeria as when inoculated with S. nodorum.

Other species of Leptosphaeria recorded on cereals

L. herpotrichoides, whose ascospores have 6-8 septa and which is not known to have an imperfect stage, is well known culturally and is distinct from the group under study. L. culmifraga, with 7-9 septa, is given as a synonym of L. herpotrichoides (Anon., 3). L. nigricans, whose ascospores have 5 septa, is well-known culturally, Hughes (26) having shown that its imperfect stagewas a Phaeoseptoria with 7-16 distinct septa. L. secalis, with 4-7 septa, was recorded on rye in Austria (Saccardo, 53).

L. eustomoides Sacc. (Saccardo, 53), with spores 20 x 4.5u and 3 septa, was recorded on an Andropogon in Italy. A fungus of this name has

recently been used by Zogg (74) in Switzerland, in mixed infections on wheat. Müller (41) made some observations on L. tritici and then rejected its attribution to L. eustomoides.

L. tritici (Gar.) Pass. was recorded by Saccardo (53) on wheat in Italy, with asci 48-50 x 15-16u, and 3-septate ascospores measuring 18-19 x 4.2-5.5u. Heald, in 1906 (cited by Stevens and Hall, 66), recorded Leptosphaeria leaf blight, caused by L. tritici (Gar.) Pass., in Nebraska, and noted that the lower leaves were killed early in the season, but that large losses were not usual. L. tritici was recorded in England in 1932 and 1938. (Anon., 3).

No information is available regarding the host range and cultural characteristics of this species. The following notes have appeared in the R.A.M.:

In 1923 Small (59) reported it causing a leaf spot of wheat in Uganda. In 1926-27 Garbowski (22) recorded it on wheat, rye and oats in Poland. In 1936 Săvulescu et al. (54) noted that L. tritici was transmitted to wheat in autumn from self-sown plants in Rumania. From 1948 onwards Zogg et al. (76, 77) noted that cereals in Switzerland sustained heavy damage from leaf-shrivelling fungi such as S. nodorum, S. tritici, L. tritici and S. avenae. In 1953 Allison (2) recorded L. tritici on wheat in Iraq.

Müller (41) compared L. tritici with L. nodorum Müller. He distinguished the former by the size of the ascospores, 16-20 x 4u as against 20-26 x 4u for L. nodorum, by the less pointed ends, the deeper colour and the less obvious constriction between the second and third cell, and by the lack of conidial formation.

ATTEMPTS TO INDUCE THE FORMATION OF THE PERFECT STAGES OF S. AVENAE F. SP. AVENAE AND S. NODORUM (WHEAT-INFECTING)

No perithecia developed in any culture of S. avenae f. sp. avenae on any of the media listed under "Materials and methods". The cultures were maintained at various conditions of light and temperature.

In case this special form is heterothallic, the isolates, from 7 localities in Eastern Canada and from British Columbia, were mated in every combination, but no perithecia were obtained.

No perithecia developed in any culture of S. nodorum (wheat-infecting) on any media under various conditions of light and temperature. The isolates from 8 localities in Canada and one from England were mated in every combination, but no perithecia were obtained.

The non-production of perithecia in the above may be due to the genetic inability of these isolates to produce them. Müller (41), discussing the perfect stage L. nodorum, stated "Es besteht auch die Möglichkeit, dass ein Teil der vorhandenen Stämme von Septoria nodorum überhaupt das Vermögen zur Bildung der Hauptfruchtform verloren hat." The same could apply to S. avenae f. sp. avenae.

On the other hand, the isolates may all belong to the same compatibility strain of heterothallic organisms, even though they were drawn from different localities, as occurs in Gibberella cyanogena where the strains are isolated geographically (Gordon, 23). Weber (69), however, found that perithecia developed in single ascospore cultures of L. avenaria.

The third possibility is that the media used were unsatisfactory for the expression of the perfect stages, if the isolates had the genetic ability to produce them. Weber used P.D.A. and oat meal agar, Shaw (56) used P.D.A. and in this study, P.S.A. and ground oat hull agar as well as other media. Müller did not record the medium on which he obtained perithecia of L. nodorum.

HOMOTHALLISM OF LEPTOSPHAERIA AVENARIA WEBER F. SP. TRITICEA

In order to determine whether heterothallism or homothallism operates in this organism, cultures were established as follows:

1. Single ascospores were isolated from perithecia on material from the field, or from perithecia produced in cultures which had originally been established from ascospores, asci, pycnidiospores or mycelium, and the cultures were examined for perithecia.

When evidence for homothallism was obtained, the isolates were subjected to the further test:

2. Lines were carried on by isolating single ascospores, obtaining perithecia, reisolating single ascospores and so on, up to 5 generations to date.

1. Production of perithecia in cultures established from single ascospores

As shown in Table IX, 124 single ascospores were isolated (by the method outlined in "Materials and methods, ii") and perithecia with mature, pigmented ascospores formed in 89 of the cultures established from them. Twenty-four of the cultures which did not form perithecia with ascospores produced black bodies with no distinguishable contents. Eleven cultures were sterile.

The number of cultures producing perithecia would probably have been higher if some cultures had not been subcultured on to a certain batch of asparagus stems. Perithecia had been produced abundantly on asparagus stems in preliminary tests, so some of the ascospores isolated in the beginning of the work were subcultured on to it. Only 2 cultures of Accs. 7 and 22 produced perithecia out of 14 on asparagus stems, whereas 16 out of 18 produced perithecia on sterile lemon leaves, all the cultures having been established from single ascospores which were isolated at the same time. The cultures which had been sterile on asparagus stems

were subcultured on to sterile lemon leaves on 3 different occasions and once on to another batch of asparagus stems, but still no perithecia were obtained, as is usual if transfer is made by mycelium.

The single ascospore cultures which did not produce perithecia were mated in all combinations on sterile lemon leaves, but no perithecia were obtained.

The above data strongly suggested that this organism is homothallic, and that those cultures established from single ascospores which did not produce perithecia, were probably sterile only because of unsuitable media which either prevented perithecia from forming asci or spores, or prevented perithecial formation altogether.

2. Lines carried on by single ascospores

Shown in Table X are selections of 4 lines, all maintained by transference of single ascospores. Perithecia with viable ascospores were obtained in each transfer to date, i.e., up to the fifth generation. Of 40 single ascospore cultures established, 34 have formed perithecia.

The tendency of many fungal cultures to become non-sporulating if maintained by mycelial transfer is well known. Isolates of this organism showed the same tendency, and it was generally the practice to maintain the cultures by transference of single spores. Jinks (30) found that even when this was done, sporulation of Aspergillus nidulans gradually decreased.

Parallel transfers were made of Acc. 168 by means of i) single ascospores, ii) single pycnidiospores and iii) mass mycelial transfer. Both pycnidial and perithecial production decreased quickly when cultures were maintained by mass mycelial transfer. Lines maintained by transfer of single macrospores and ascospores were showing a slight decrease in perithecial production at the fifth generation.

The total number of single ascospore cultures established in the 2 procedures (1 and 2) was 164, of which 123 formed fertile perithecia.

It is to be noted that no difference in behaviour was recorded for isolates either from different sources (wheat, rye, oats and grasses) or from different localities in several distinct geographical areas.

As shown in Part IV, the ascospores were originally uninucleate, and not binucleate as in Neurospora tetrasperma, Pleurage anserina and Gelasinospora tetrasperma which are secondarily homothallic, each ascospore containing both compatibility factors.

It is concluded that L. avenaria f. sp. triticea is homothallic, mycelium from a single haploid ascospore having the ability to produce fertile perithecia.

Abnormal Ascospore

While examining perithecia, asci and ascospores in a culture of L. avenaria f. sp. triticea from wheat (Acc. 94), an abnormal ascospore was noted. The ascus was of the usual shape, with crozier remains visible at the base, but instead of the normal content of 8 ascospores, only one ascospore was present.

The ascospore walls were clearly delimited at the sides, but not quite so clearly at the top and bottom of the ascus, where a little epiplasm was visible. Eight very distinct septa could be counted and 2 indistinct ones, and the spore was slightly pigmented.

The abnormal ascospore, and other normal ascospores within and outside asci were in a water mount under a cover glass; so the spores were checked for germination over a period of 20 hours. After 1½ hours the first germ tube appeared from the abnormal ascospore, and the second one hour later. By 20 hours, many germ tubes had appeared and were growing vigorously. (Figs. 3 and 4; Plate 2, Figs. 5, 6 and 7).

Attempts to isolate hyphal tips were unsuccessful, because they were appressed to the cover glass and slide, and were torn during the removal of the glass. The culture which produced the abnormal spore has been maintained but no further abnormalities have been found.

Abnormal ascospores have been recorded a few times in the literature. Dodge (15) cited the occurrence in Bulgaria inquinans and Humaria rutilans, and recorded (16) that in backcrosses involving N. tetrasperma, the asci formed tended to produce one or two very large spores, one perithecium having a majority of asci with one or two giant spores. He obtained germination of the giant spores, and noted that there was no special tendency towards development of asci with large spores in the resulting cultures. Zickler (cited by Dodge and Appel, 17) found that by treating spores with chloral hydrate he could induce the formation of giant ascospores which would nevertheless germinate. Dodge was of the opinion, however, that these were indurated aborted asci. He was also of the opinion that high temperatures during certain critical stages in the development of the asci of Neurospora may yield indurated ascus abortion without the intervention of the dominant lethal I.

Dowding (18) recorded giant ascospores in Pleurage anserina, and Dr. W. L. Gordon (unpublished) has recorded them in Fusarium species. Pontecorvo (47) recorded that in diploid lines of Aspergillus nidulans 16-spored asci occurred, sometimes a few with 8 and, very rarely, one with any number of spores formed with a single giant one to 16.

In the case of the abnormal ascospore reported here, some combination of environmental factors was probably responsible, a combination which has not been repeated to date.

TABLE IX

Single ascospore cultures forming perithecia (*L. avenaria* f. sp. *triticea*)

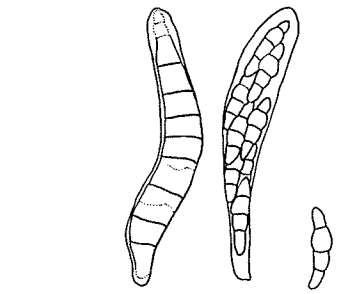
Acc.	Source of isolate: Host	Source of isolate: Locality	Originally isolated as	No. of single ascospores isolated	No. of single ascospore cultures forming perithecia	No. of single ascospore cultures forming initials only
7	wheat	Leduc, Alta.	macro	18	9	6
8	wheat	Ste. Agathe, Man.	"	6	6	
69	wheat	L'Assomption, Que.	"	7	7	
73	wheat	Lennoxville, Que.	"	8	5	2
94	wheat	Morden, Man.	asco	6	5	1
154	wheat	Carmel, Sask.	"	3	3	
46	rye	L'Assomption, Que.	macro	8	4	2
162	oats	Fort Garry, Man.	asco	5	5	
172	oats	Ottawa, Ont.	"	8	8	
22	*Elymus canadensis*	Kings Park, Man.	macro	12	7	4
168	*Calamagrostis canadensis*	Lac du Bonnet, Man.	asco	6	6	
57	*Agropyron subsecundum*	Fort Garry, Man.	entire asci	12	8	1
65	*Poa pratensis*	Fort Garry, Man.	" "	18	15	3
96	*Hordeum jubatum*	Riding Mnts., Man.	asco	6	1	5
				124	89	24

TABLE X

Production of perithecia in lines maintained by single ascospores (*L. avenaria* f. sp. *triticea*)

Acc.	No. of ascospores isolated	No. of ascospore cultures forming perithecia	No. of generations represented
172	12	10	4
94	11	11	4
73	11	8	4
168	6	5	5
Total	40	34	

3



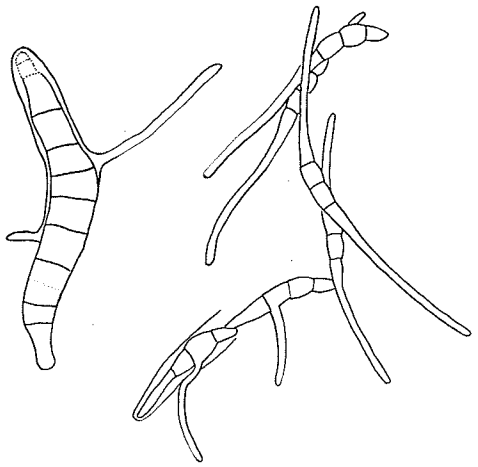


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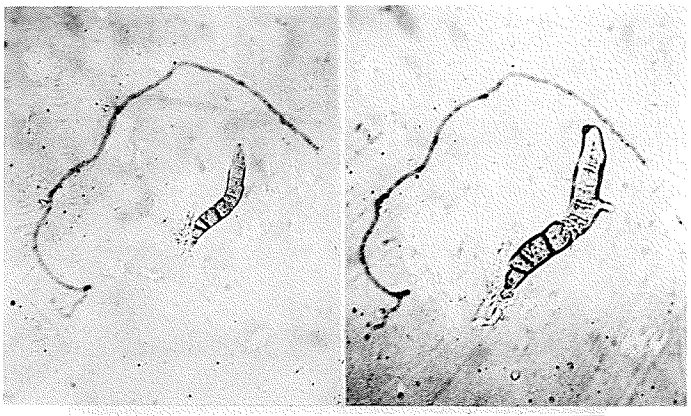
Fig. 3 Abnormal ascospore of L. avenaria f. sp. triticea drawn with the aid of the camera lucida from living unstained material. Showing ascus wall, epiplasm and septate ascospore. For comparison is shown a normal ascus and an ascospore. x 700

Fig. 4 Abnormal germinating ascospore with 2 germ tubes, and normal germinating ascospores. After about 3 hours. x 700

PLATE 2



5 6

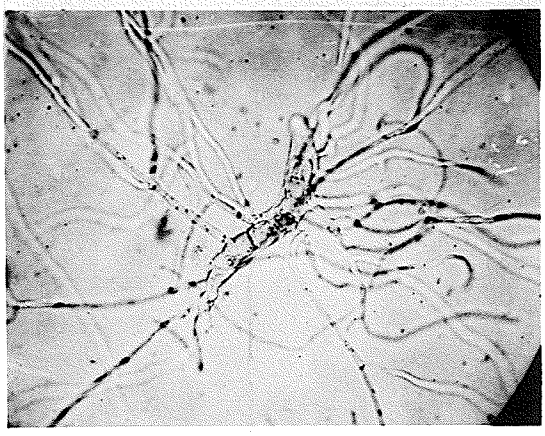


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Fig. 5 Abnormal ascospore of L. avenaria f. sp. triticea produced in culture, showing septations. Living, unstained material mounted in water. x 150

Fig. 6 Germinating abnormal ascospore with one germ tube. After about 1½ hours. x 300

Fig. 7 Germinated abnormal ascospore with many germ tubes and hyphae. After 20 hours. x 300

DISCUSSION

Johnson discussed S. avenae f. sp. triticea in relation to S. triticola Lobik and Stagonospora arenaria. Nothing further can be added to his remarks except to point out that, at least in some cases, collections on grasses with spores conforming to S. avenae, which, on their morphology could possibly also be assigned to Stagonospora arenaria, have now been shown to have a perfect stage, conforming to Leptosphaeria avenaria f. sp. triticea.

At present the f. sp. triticea is known to occur in Canada, the United States, New Zealand and Australia. However, various species of 3-septate Leptosphaeria have been recorded from other cereal-growing areas of the world, as summarized in Table XI, and the question arises as to whether some of these records refer to the organism identified here as L. avenaria f. sp. triticea.

It is possible that many of the records in the table refer to the same organism. On the other hand, some of the records for "L. tritici" might in reality refer to the perfect stage of Septoria nodorum or even to other species.

The question of the identity and world distribution of the perfect stages will be resolved if future work takes into account the following requirements:

Morphological characters which are used to distinguish species of Leptosphaeria, such as size of perithecia and ascospores, clustering of fruit bodies, colour and morphology of ascospores, should be maintainable through culture, infection and reisolation;

Asexual spore forms should be determined on media known to support sporulation; otherwise lack of conidial formation need not necessarily mark the species as a non-asexual-spore producer;

TABLE XI

Comparison of 3-septate *Leptosphaeria* species recorded on cereals

Species	Authority	Host	Locality	Ascospore measurements u	Ascospore characteristics	Asexual spore form & measurements u	
L. avenae Ausw. (*M.avenae* Ausw.) Sacc.	cited by Weber	oats		15-16	hyaline	No information	
L. avenae Ausw. ex Wint	Moore	oats	England	14-18 x 2.5-4	(0-3 septa (hyaline-(honeycoloured	No information	
L. avenaria Weber	Weber	oats	U.S.	23-28 x 4.5-6	light yellow to slightly olivaceous	*S. avenae*	25-45 x 3-4
L. tritici (Gar.) Pass.	Saccardo	wheat	Italy	18-19 x 4.2-5.5		No information	
L. tritici (Gar.) Pass.	R.A.M. records	wheat oats rye	(Nebraska, Iraq (Eng., Uganda, (Poland, Rumania (Switzerland	no measurements given		No information	
L. tritici (Gar.) Pass.	Müller	wheat	Germany	16-20 x 4	brown	No conidia	
L. nodorum Müller	Müller	wheat	Germany	20-26 x 4	pointed ends, sub-hyaline to light yellow	*S. nodorum*	No measurements given
Leptosphaeria sp.	Alfaro	wheat	Spain	no measurements given		(associated with (*S. nodorum*	No measurements given
Leptosphaeria sp.	Chona & Munjal	wheat	India	15-20 x 3-4	yellowish olive	associated with *S. nodorum*	No measurements given
L. avenaria f.sp. *triticea* Johnson	Johnson	wheat barley rye	Canada	(16)19-25(27)x (3.9)4.5-5.5(6)	light yellow	*S. avenae* f.sp. *triticea*	(18)26-42(53)x (2.3)2.8-3.5(4.2)
L. avenaria f.sp. *triticea* Johnson	Shaw	wheat	Australia	16-24 x 3-4	light yellow	*S. avenae* f.sp. *triticea*	(20)24-36 x (2)2.5-3(4)
L. avenaria f.sp. *triticea* Johnson	this study	wheat rye oats barley grasses	Canada	range as above J & S	light yellow	*S. avenae* f.sp. *triticea*	range as above J & S

Measurements of asexual spores would distinguish, in most cases, whether the organism was S. nodorum or S. avenae f. sp. triticea. It is possible that in the past some collections were assigned to S. nodorum when they were S. avenae f. sp. triticea;

Pathogenicity tests on wheat and oats would distinguish between S. nodorum, S. avenae f. sp. triticea and S. avenae f. sp. avenae.

The determination of the intra-specific relationships between the formae speciales, and of specific relationships between S. avenae, S. nodorum and S. oudemansii has not been considered hitherto.

The mechanical difficulties operating against hybridization is this group are apparent; one of the special forms is homothallic and the other special forms and species do not produce the perfect stage, either with ease or at all. However, homothallic species have been crossed (Henrard with Aspergillus nidulans, cited by Olive (46), Edgerton and his colleagues with Glomerella cingulata (Wheeler, 71) and Pontecorvo (47) with A. nidulans,) so that this itself need not necessarily exclude the possibility of hybridization.

If the special forms and species which do not produce the perfect stage are, in fact, strains of heterothallic species whose compatibility types are either separated geographically, or one or the other of them has been lost, then it is still possible that perithecia might form if there were close relationships between any of the forms or species, if opposite mating types were brought together.

With this in view, hybridization studies have been commenced, but so far no perithecia have been produced by matings of S. nodorum with S. oudemansii, S. avenae f. sp. avenae with Septoria sp. (Acc. 160) from rye, or S. avenae f. sp. anthoxanthi with Septoria sp. (Acc. 160) from rye.

Information on the relationships in the whole group might make

possible an understanding of the phylogeny of the members.

Savile (55) considered that it was probable that the first successful fungi were parasitic and that the heterotrophic forms have been repeatedly derived from them.

Jackson (28), Buller (8), Whitehouse (73) and Raper (50) are of the opinion that most existing homothallic species are probably derivatives of heterothallic species. Dodge (16) considered that self-fertility in an ascospore was a later development than self-sterility, and Jackson (27) considered that the present evolutionary tendency in rusts is towards self-fertility.

On the basis of the above considerations the group under study might represent 2 divergent lines of development. One line (S. avenae f. sp. avenae, S. nodorum and S. oudemansii) now has macro and micro spore forms and little or no ability to form the perfect stage, and its members differ a little from each other in spore size and in hosts parasitized. The other line has macro asexual spores, is sexually self-fertile and is, to a large extent, heterotrophic.

As well as hybridization studies, the types of mutations obtained spontaneously and by induction might give some indication of the line of development in these groups.

PART III

ACCESSORY SPORE FORMS IN SEPTORIA AND THEIR ROLE IN THE LIFE CYCLE

REVIEW OF LITERATURE

Creager (13) found that a Phoma-form was connected with the Stagonospora-form of Stagonospora curtisii on Narcissus. Jones and Weimer (34) found that a Phoma-form occurred as well as the macro-form of Stagonospora meliloti (Leptosphaeria pratensis) on Melilotus alba. The microspores germinated but failed to infect M. alba. From tests at various temperatures they concluded that low temperature was required for the development of the Phoma-form. Erwin (20) did not obtain microspores in cultures of Stagonospora meliloti on alfalfa stems kept at 7 temperatures between 5° and 30°C.

Frandsen (21) reported microspores in some species of Septoria on the Gramineae and proposed that a division of the genus be made on this character. Sprague (62) recorded spermatia-like microspores of S. avenae on Glyceria and microspores of 7 other species of Septoria on various grasses, viz.: S. infuscans on species of Agropyron and Elymus, S. jaculella on Bromus spp., S. oudemansii on Poa spp., S. passerinii on species of Hordeum, Hystrix and Sitanion, S. secalis on Secale cereale, S. secalis var. stipae on species of Agrostis and Stipa, S. tenella on Festuca spp., and S. tritici on Secale cereale and species of Triticum.

Johnson (32) reported small pycnidia containing microspores in cultures of S. avenae. The microspores germinated and cultures derived from them could produce macrospores, although no infection on oats was obtained. He found that microspores only were produced in cultures at 5-7°C, while both spore forms occurred at 20-22°C, and concluded that low temperature, while favourable to microspore formation, tends to have an inhibitory effect on macrospore formation.

In 1951 Dr. Mary Noble notified Dr. T. Johnson by letter that she had found spermatium-like microspores in ground oat hull cultures and oatmeal agar cultures of S. avenae in Scotland. Shaw (56) found microspores of S. tritici on mature plants under conditions of rising temperature in the field in Australia, and later (unpublished) on mature plants under conditions of falling temperature in the field in Canada.

MICROSPORES IN S. AVENAE F. SP. AVENAE

The occurrence of microspores on field material and in culture

Fields of standing diseased oat stubble were examined in Ottawa in September 1953. About 6-8" of the stems remained, and had the "black stem" symptom. In many cases the second node from the base was covered with pycnidia in a manner reminiscent of S. nodorum (Plate 3, Fig. 8). The pycnidia contained either macropycnidiospores, macro- and what was tentatively taken to be micro-spores, or solely microspores. Plate 4, Fig. 9 shows some of the exuded contents of a pycnidium. Some intermediate spores were present in pycnidia with the 2 spore forms, but these were always greatly in the minority. Such intermediate types are shown in Plate 4, Fig. 9. Germination of the small spores was not obtained at the time, so that the connection with the macrospores could not be established. On the circumstantial evidence of being found in the same pycnidium, however, it was thought that the small spores were the microspores of S. avenae f. sp. avenae.

In April and May, 1954, cultures from macrospores which had been mated in all possible combinations on sterile asparagus stems in an attempt to produce the perfect stage were found to be producing pycnidia with microspores. As the microspores also occurred in some of the unmated controls, it was considered that the mating as such was not responsible for their production. The microspores were single celled, hyaline, rod shaped with rounded ends, slightly curved, measured 2.5-5 x lu and agreed well with those described by Johnson.

As shown in Table XII, 32 cultures were established from single microspores, first on P.S.A., and then subcultured after about 5-6 days on sterile lemon leaves. Pycnidia and macropycnidiospores formed abundantly on sterile lemon leaves and even on P.S.A. (the original isolates only very rarely

produced pycnidia and macrospores on the latter medium). One culture produced both macro- and micro-spores. Mycelium and spores from the cultures were inoculated on "Red Rustproof" oats and lesions similar to those produced by the macrospores appeared in about 4-5 days. The oat plants were covered with glass chimneys to raise the humidity, and pycnidia and macrospores were later found in many lesions. Some of the lesions were surface-sterilized and plated on P.S.A. and typical S. avenae f. sp. avenae colonies were recovered. Subsequently, microspores were found in cultures of other isolates from macrospores, as shown in Table 1.

In the summer of 1954 arrangements were made for diseased oats from Eastern Canada to be forwarded to Winnipeg, and oats received from the Uniform Rust Nurseries were also examined. The first specimens from Eastern Canada had many pycnidia with macrospores on the leaves, but oats received from Ottawa, Ont., at the end of August had pycnidia with microspores on the sheaths. Later in the season microspores were found on leaves and particularly on the nodes of diseased oats from Ontario, Prince Edward Island and Quebec. Single microspores were isolated, cultures established and taken through the full procedure for identification and infection as shown in Table XIII.

At the end of the season Mr. R. A. Derick of the Cereal Division, Ottawa, on request, kindly lifted from the soil about 3 dozen oat plants (stubble bases) and placed them in flats at Ottawa. The plants were left in the open without protection in the snow in as near natural conditions as possible. About one-third of the plants were lifted and sent to Winnipeg on 31-12-54 (Acc. 174), and another one-third on 17-1-55 (Acc. 175). The balance will be forwarded at the end of the winter, together with plants which remained in the soil for the whole period.

In February and March stubble from fields which had been severely diseased with S. avenae f. sp. avenae was forwarded from Charlottetown, P.E.I., and Normandin, Que. This stubble had remained in the field since harvest, and was lifted from under several feet of snow. (Accs. 176 and 177).

Many of the nodes of these plants (i.e., stubble bases) were covered with pycnidia (Plate 3, Fig. 8). No macrospores were found at all, but there were abundant microspores. Some stems were found with elongated pycnidia up to 180u long on the internodes, often in longitudinal rows corresponding to the chlorenchyma. The full identification and infection procedure was carried out with 53 single microspore cultures from this material, as shown in Table XIII.

Germination of the microspores and infection of oat plants

The microspores germinated very slowly on P.S.A. and on plain agar, swelling first until oval in shape, then producing a germ tube at one end, usually followed by another germ tube from the other end. A germinated macrospore with abundant mycelium and some swollen and germinated microspores after approximately 44 hours on P.S.A. are shown in Plate 4, Fig. 10.

Macrospores of S. avenae germinate on P.S.A. and in water in about 6 hours. No germination of the microspores, however, was obtained in liquid media such as water, dilute potato juice and sucrose solution, dilute dextrose solution , straw decoction or soil extract solution.

In preliminary tests on P.S.A. some germ tubes were noted at 42 hours but microspores were still germinating at 96 hours.

The germination of the microspores on the field material which was kept in the laboratory at room temperature was tested in November and January. Also tested were the microspores of Acc. 172, portion of which was hung outside the laboratory under natural conditions, and microspores

from the stubble (Acc. 174). The viability of the macrospores of Accs. 133 and 171 was tested at the same time. In the tests 100 microspores or 100 macrospores were examined at random, and the spore was considered "germinated" if a germ tube was discernible. Difficulty was experienced in scoring "ungerminated" spores at 96 hours, because of their small size in comparison with the swollen, germinated spores and the mycelium.

The results of the tests are shown in Table XLV. Because of the uncharacteristic morphology of the spores, single microspores were isolated in every test and taken through the full procedure of identification and infection, 50 single spore isolations being made to-date. Microspores from all collections were viable in the January test, no germation lower than 69% being obtained. The viability will also be tested in March.

It is to be noted that germination does not begin until about 40 hours, and that it proceeds during the next few days.

The germination of the microspores was tested on P.S.A. at 6-7°, 13-14°, 21-22° and at 30-31°C. Spores at 21-22° germinated normally, only a few at 13-14° even after 96 hours, and none at 6-7° or 30-31°. The spores which had been held at 6-7° and 13-14° germinated normally when the plates were transferred to room temperature, but no germination was obtained of the spores held at 30-31° and later transferred to room temperature.

Microspores from the field and from culture failed to infect oat plants when sprayed in a water suspension on to the leaves or applied with an inoculating needle, and incubated for 48 hours (as is usual for macrospore infection). No infection took place when spores were suspended in potato juice and sucrose solution, dilute dextrose solution, straw decoction and soil extract and inoculated on to plants which were incubated for 48 hours.

However, when it was found that germination on agar had often not

begun at 48 hours, the times of incubation were lengthened. As germination was obtained on plain agar, it was considered that either humidity, oxygen or surface tension or a combination of these factors and not nutrition as such, was involved.

Infection of oat plants was subsequently obtained when spores were suspended in Tween 80-albumin solution, which was suggested and made available by Dr. R. Hawirko of the Microbiology Department, (Tween 80 being a commercial product consisting chiefly of a polyoxyethylene derivative of sorbitan monooleate). The plants were kept in a humid atmosphere for 10 days after inoculation. Infection was also obtained when plants were inoculated with spores in 1% Tween 80 solution, 0.2% sodium lauryl sulphate (both made available by Dr. Hawirko) and in water, as long as a high humidity was provided for 10 days. From these preliminary experiments it appears as if the humidity is the important factor. A shorter period than 10 days might suffice, and this is being determined.

Some of the lesions which developed as a result of microspore infection were surface-sterilized and plated on P.S.A. Typical colonies of S. avenae f. sp. avenae were obtained which in turn were vigorously pathogenic on oats.

Overwintering of S. avenae f. sp. avenae.

In discussing the mode of overwintering of the organism, Weber (69) noted that he had tied detached oat leaves containing pycnidia and macrospores to a stake in the field at Wisconsin, and tested the viability every two weeks. The germination was found to be 90% even on 1st April, i.e., 6 months later.

Machacek and Wallace (38) did not find S. avenae which infected oats among the species of Septoria isolated from wheat, oats and barley from 8 seed-inspection districts across Canada during 1939-43, and S. avenae was not isolated from cereal seed subsequent to this (Machacek et al., 37).

Stanton (65) reported the occurrence of a kernel blight phase of the oat organism in several northern states of the United States in 1952, but Simons and Murphy (58) found that the fungus was not transmitted through infected seed. Noble and Montgomerie (44) reported on the disease in Scotland, and stated that recent work at East Craigs showed that the disease is seed borne.

Weber (69) showed that macrospores in pycnidia on detached, diseased leaves will remain viable throughout the winter, and therefore they could be a means of overwintering. However, the stubble samples received from Eastern Canada during this study did not have any macrospores, only abundant microspores. This is no guarantee, of course, that no macrospores at all occurred on overwintering stubble in Eastern Canada--quite possibly they did. It is considered, however, that the microspores, on account of their abundance and their infective ability, warrant considerable attention as overwintering agents and spring inoculum.

Future work should endeavour to establish the carry-over, if any, in the seed, the occurrence of the macro- and micro-forms on the stubble, and the proportion of these spore forms to each other under different environmental conditions in different localities.

TABLE XII

Spore forms produced in cultures derived from microspores of S. avenae f.sp. avenae from culture, and the infectivities of the cultures

Acc.	No. of microspores isolated	P.S.A.	Sterile lemon leaves	Reaction of "Red Rustproof" oats	Oats in humid atmosphere
2 x 31	14	macro	macro	Susceptible	macro
2 x 29	6	macro	macro	"	macro
76 x 47	6	macro l with micro	macro	M. sus.	
133	6	macro	macro	Susceptible	macro

TABLE XIII

Spore forms produced in cultures derived from microspores of S. avenae f. sp. avenae from nature, and the infectivities of the cultures

Acc.	Date	Source	Isolated from	No. of micro-spores isolated	P.S.A.	Sterile lemon leaves	Reaction of "Victory" oats *
2	9.53	Ottawa, Ont.					
134	31. 8.54	Ottawa, Ont.	sheaths	6	macro	macro	Sus.
169	28. 9.54	Charlottetown, P.E.I.	leaves	6	(macro (micro	macro	Sus.
169	"	Charlottetown, P.E.I.	nodes	8	macro	macro	Sus.
171	28. 9.54	Normandin, Que.	sheaths	8	(macro (micro	macro	Sus.
172	12.10.54	Ottawa, Ont.	nodes	9	macro	macro	Sus.
174	31.12.54	Ottawa, Ont.	nodes	4	macro	macro	Sus.
175	17.1 .55	Ottawa, Ont.	internodes	8	macro	macro	Sus.
176	25. 2.55	Charlottetown, P.E.I.	internodes	4	macro	macro	Sus.
177	10. 3.55	Normandin, Que.	nodes				

* Many lesions produced pycnidia and macrospores when placed in more humid atmosphere, e.g., under glass chimneys

TABLE XIV

Germination (%) of macro- and micro-spores of S. avenae f. sp. avenae and the results of identification and infectivity tests

	Acc.			22.11.54							24.1.55						
				Hours					No. of single spores isolated	Reaction on Victory oats	Fruiting bodies on PSA & s.l.l.	Hours					No. of single spores isolated	Reaction on Victory oats	Fruiting bodies on PSA & s.l.l.
				6	24	48	72	96				6	24	48	72	96			
macro spores	133	leaves	PSA	100					3	Sus	P*+macro	64							
	133	"	H_2O									79							
	171	sheaths	PSA	100								96							
micro spores	134	sheaths	PSA	0	0	80	88	92	2	Sus	P+macro	0	0	60	86	93	4	Sus	P+macro
	169	nodes	"	0	0	75	92	97	2	Sus	P+macro	0	0	58	65	81	4	Sus	P+macro
	169	leaves	"	0	0	90	95	100	2	Sus	P+macro	0	0	74	86	89	4	Sus	P+macro
	171	sheaths	"	0	0	84	98	98	2	Sus	P+macro	0	0	65	91	94	2	Sus	P+macro
	172	nodes	"	0	0	88	96	100	2	Sus	P+macro	0	0	64	84	96	8	Sus	P+macro
	172	nodes (outside)	"	0	0	78	98	100	2	Sus	P+macro	0	0	40	52	69	4	Sus	P+macro
	172	nodes	H_2O	0	0	0	0	0											
	174	nodes (from under snow 31.12.54)	PSA									0	0	63	76	91	4	Sus	P+macro
	175	nodes (from under snow 17.1.55)	"									0	0	58	63	78	8	Sus	P+macro
		"	H_2O									0	0	0	0	0			

* P refers to pycnidia

PLATE 3

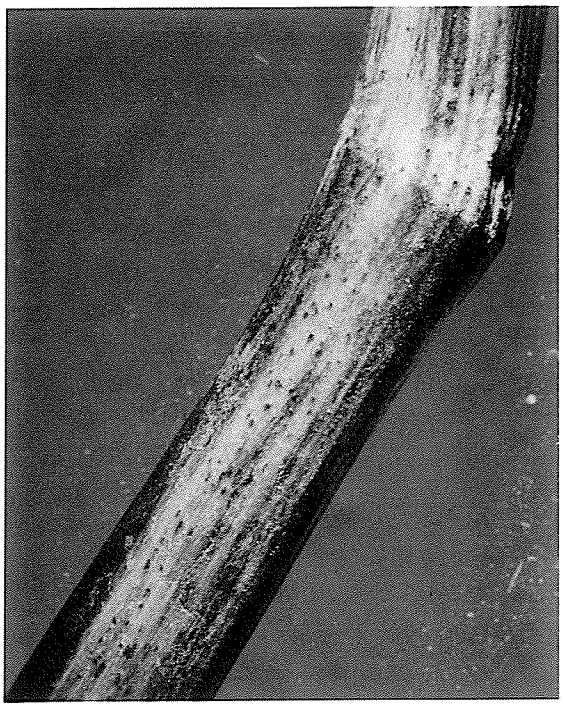

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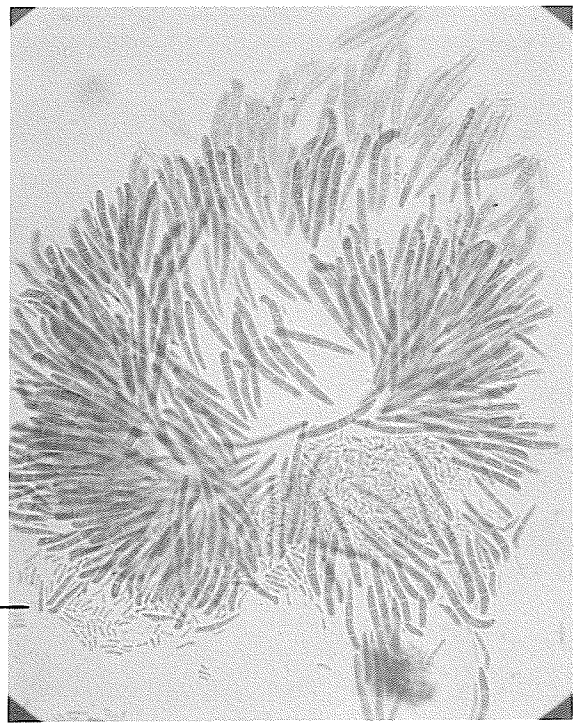
Fig. 8 Portion of oat stem which had remained in the field until 17.1.55, with pycnidia of S. avenae f. sp. avenae on node and internode. Microspores are contained in the pycnidia. x 6

PLATE 4



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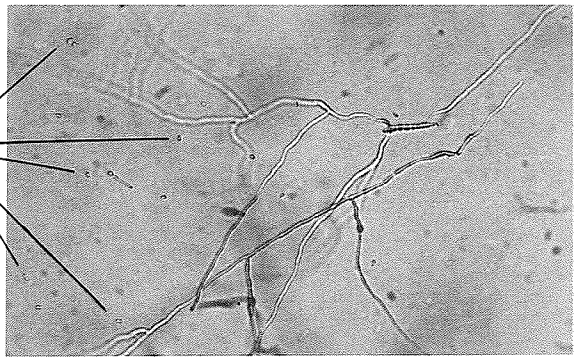

10

Fig. 9 Some of the contents of a pycnidium of S. avenae f. sp. avenae from a node of oat stubble from the field 3.9.53, showing macrospores (3-septate), a few intermediate spores (0-1 septate), and bacterioid microspores (0-septate). Mounted in cotton blue lactophenol. x 400

Fig. 10 Germinated macrospores of S. avenae f. sp. avenae with abundant hyphae, and germinating, swollen microspores with germ tubes just appearing. Living material photographed on P.S.A. after 44 hours. x 120

MICROSPORES IN OTHER SPECIES

In April, 1954, pycnidia with microspores were found in cultures of S. nodorum (wheat-infecting) on sterile maize grain, the isolates having been mated in all combinations in an attempt to obtain the perfect stage. Pycnidia and microspores were also found in unmated controls. The microspores were single celled, hyaline, rod-shaped with rounded ends and measured 2.5-5 x 0.8-1u. Subsequently, pycnidia with microspores were found in cultures of Acc. 99 (see Table XV).

Altogether 33 single microspores were isolated, and cultures established on P.S.A., then subcultured on to sterile lemon leaves, inoculated on to "Marquis" or "Little Club" wheat, and, in some cases, the resulting lesions were surface-sterilized and plated on P.S.A. In other cases the inoculated plants with lesions were covered with glass chimneys and later pycnidia and macrospores were found in some of the lesions. The pycnidia which are produced on leaves in this way seldom have heavily pigmented walls, but are well formed.

No microspores have previously been recorded in S. nodorum.

Pycnidia with microspores were also found in cultures of S. oudemansii on sterile lemon leaves. They were hyaline, rod-shaped, and measured 2.5-4 x 0.8-1u. Pycnidia with macrospores were also present. Single microspores were isolated and taken through the usual procedure, including inoculation of Poa pratensis, which showed a trace infection, and of wheat, which was immune.

Microspores were also found in cultures of Septoria sp. (Acc. 160) from rye on sterile lemon leaves held at 14°C, and were isolated as above. Cultures from single microspores on P.S.A. at room temperature produced abundant micro- and few macro-spores, whereas the cultures on sterile lemon leaves at room temperature produced abundant macro- and few micro-spores.

The results of the above are summarized in Table XV.

TABLE XV

Spore forms produced in cultures derived from microspores of various isolates, and the infectivities of the cultures

Acc.	Isolate	No. of single microspores isolated	Reaction of wheat	Subsequent treatment and spore forms
30 x 72	S. nodorum (wheat-infecting)	13	"Marquis" Susceptible	Surface-sterilized lesions plated on P.S.A. and colonies typical of S. nodorum obtained Wheat lesions in humid atmosphere produced some pycnidia and macrospores
72 x 67	"	8		Colonies on P.S.A. typical of S. nodorum
54 x 67	"	6		Colonies on P:S.A. typical of S. nodorum
99	"	6	"Little Club" Susceptible	Colonies on P.S.A. typical of S. nodorum
88	S. oudemansii	10	-	Abundant pycnidia with macro and micro on P.S.A. Slight infection on Poa pratensis
160	Septoria sp.	12	trace on "Gaza"	Colonies on P.S.A.: 2 with macro 1 " macro and micro 9 " micro Colonies on sterile lemon leaves: 1 with macro and micro 11 " macro

EXPERIMENTS TO DETERMINE THE FACTORS RESPONSIBLE FOR MICROSPORE FORMATION

As mentioned previously, Jones and Weimer (34) considered that low temperature was required for the development of the Phoma-form of Stagonospora meliloti, and Johnson (32) obtained results with S. avenae which indicated that low temperature, while favourable to microspore formation, tended to have an inhibitory effect on macrospore formation.

In order to obtain more information on the role of temperature in macro- and micro-spore formation, isolates which had been found to be microspore producers, such as S. avenae f. sp. avenae, S. nodorum (wheat-infecting), S. oudemansii and Septoria sp. (Acc. 160), were sub-cultured on to sterile lemon leaves from 4-5 day old cultures on P.S.A. derived from single macrospores. Also treated in this way were isolates of L. avenaria f. sp. triticea, Hendersonia sp., Phaeoseptoria poae and Stagonospora meliloti. Duplicate cultures were held at 5-6°, 12-13° and 21-22°C and were read (by sampling the fruiting bodies formed and determining the spore forms present) 4 times over a period of 2 months. The results are summarized in Table XVI.

No microspores were produced by the L. avenaria f. sp. triticea isolates at any of the 3 temperatures, and asexual and sexual spores were only produced at the highest temperature. The Phaeoseptoria and Hendersonia isolates produced only macrospores, although the latter isolate, at the 2 lowest temperatures, produced small, oval-shaped cells in the pycnidia along with the macrospores. The Stagonospora meliloti isolate produced very variable macrospores grading into spores as small as 3u. The spores of this isolate were not tested for viability.

The microspore-producing isolates all had the same tendency, viz., mainly macrospore production at high temperature and mainly microspore

production at low temperature.

Shaw (56) considered that the physiology of the host influenced microspore production in S. tritici, and an attempt was made in this study to determine if the C:N ratio of the substrate influenced the production of the spore forms of the isolates, as Nitimargi (43) found for the A and B spores of species of Phomopsis. Following this worker, a basal medium was prepared of asparagine, 2 gm; magnesium sulphate, 0.75 gm; potassium phosphate, 1.25 gm; agar, 15 gm and water, 1000 cc. To 4 aliquots of this medium were added 32 gm, 16 gm, 2 gm and 0.5 gm of sucrose respectively, and practically the same series of isolates as was used in the temperature test was subcultured on to it.

Vegetative growth decreased throughout the series with decrease of carbohydrate, but, as was generally found on P.S.A., no fruiting bodies at all were produced, except by Acc. 88 (S. oudemansii), which formed pycnidia and macrospores throughout the series. As mentioned in the first part of this study, these species will only sporulate on certain media; so if this physiological aspect is to be investigated in the future, another medium will be necessary which will permit both sporulation and adjustment of the C:N ratio as desired.

From the temperature experiment it can be concluded that, on the media used, the macro-form predominated over the micro-form at the higher temperature, while the micro-form predominated at the lower, in those strains which are genetically capable of dimorphism. Variation in temperature failed to induce microspore formation in isolates of L. avenaria f. sp. triticea or Phaeoseptoria poae.

TABLE XVI

Spore forms produced by various isolates at different temperatures

Acc.	Isolate	5-6°C	12-13°C	21-22°C
88	S. oudemansii	- *	micro	macro
47	S. avenae f. sp. avenae	- *	micro	macro
133	"	micro	micro	macro
134	"	micro	micro	micro & macro
99	S. nodorum (wheat-infecting)	-	micro	macro
160	Septoria sp.	-	micro & macro	macro
80	S. avenae f. sp. anthoxanthi	-	macro	macro
142	L. avenaria f. sp. triticea	-	-	macro & ASCOSPORES
154	"	-	-	macro & ASCOSPORES
157	"	-	-	macro & ASCOSPORES
111	Hendersonia sp.	macro	macro	macro
86	Phaeoseptoria poae	macro	macro	macro

* at 2°C

SPECIES OF PHOMA AND PHYLLOSTICTA PREVIOUSLY RECORDED ON CEREALS AND GRASSES

It was considered that species with microspores may have been already described as distinct species of Phoma or Phyllosticta, especially since the microspores often occur unaccompanied by the macrospores.

Phyllosticta avenophila Tehon and Daniels was recorded on Avena sativa in the United States by Sprague (62), but the spores were oval to elliptic, and measured 6.5-8 x 3-4u and are distinct from the microspores of S. avenae f. sp. avenae.

Lobik (36) described Phyllosticta avenae sp. nov. on dry leaves of Avena sativa in Russia. It had small spores, 4.2-7.2 x 1.6-2u, bacterioid and hyaline, and there is a possibility that these were microspores of the oat organism. No information on parasitic ability was given.

Phoma rhaetica sp. nov. was described by Zogg (75) on dead haulms of wheat, barley and rye in Switzerland. It was weakly pathogenic on wheat, but as the spores measured 6-11 x 2-3u it seems distinct from the microspores of S. nodorum.

Sprague (62) recorded Phyllosticta owensii Sprague on Dactylis glomerata and Panicum capillare with non-septate spores, bacillar, 2.3-4.6 x 1-1.4u. Also recorded was Phyllosticta anthoxella Sprague on Anthoxanthum odoratum with aseptate, bacillar, hyaline spores 5-9 x 1-1.6u. A species of Phoma causing blacking of stems with small pycnidia containing spermatia-like microspores measuring 4-6 x 0.5-0.8u was also recorded by Sprague on species of Festuca.

Further work with these species should establish whether they are autonomous or microspore stages of other species.

DISCUSSION

The results of this study, and the work of Johnson and Noble, have shown that isolates of S. avenae f. sp. avenae, S. nodorum, S. oudemansii and Septoria sp. (Acc. 160) have the genetic ability to produce microspores, and do so with ease on certain media at certain temperatures. On the other hand, L. avenaria f. sp. triticea lacks this ability to produce microspores but produces the perfect stage with ease.

Weber (69) showed that the macrospores of S. avenae (oat-infecting) on detached leaves can overwinter, and in this study evidence has been presented that the microspores are able to survive the winter under the conditions occurring in Eastern Canada.

The occurrence and viability of macrospores and ascospores of L. avenaria f. sp. triticea on stubble exposed in the field in Manitoba will be determined in the spring. Mature perithecia of this special form were found on cereals and grasses before winter, and on stubble in the spring, in 1954, so probably the perithecia can act as overwintering bodies. However, it should be noted that the perithecia produced in culture do not have a long maturation period, nor do the ascospores from the field and from culture need any special treatment for germination. The situation is different in some other organisms where the perithecia mature slowly during the overwintering, and only reach maturity when their hosts become available in the spring. In the southern hemisphere the perithecia of this special form were found on the leaves and awns of standing crops at the beginning of summer, and on stubble at the beginning of winter. Ascospores from these perithecia were viable, so the special form apparently also has the ability to oversummer as perithecia (Shaw, 56). In this case it seems as if the physiological state of the host is a more important factor than temperature in inducing the formation of the second spore form, viz., the ascospores.

A somewhat similar situation holds in the case of the microspores of

S. tritici. In Australia they were noted on mature plants in the late spring under conditions of rising temperature, and in Canada, in 1954, they were noted on mature plants in the autumn under conditions of falling temperature. Here again it is probably the physiological state of the host which is more important than the temperature.

However, it is evident that temperature does affect the type of spore form produced by S. avenae f. sp. avenae and the other microspore-producing species under study.

One interesting aspect of the microform of the oat-infecting strain is the much greater length of time required before the spores begin to germinate and before the majority of spores have germinated. They take approximately 6-7 times longer (i.e. about 40 hours) to begin to germinate than the macrospores do, and continue to germinate progressively over a similar period (about 40-50 hours). How this fits in with the environmental conditions occurring in nature in Eastern Canada is not yet apparent.

Accessory spore forms occur in other species of fungi, and in many cases they are smaller than the other form, and approach the figure of minimal area, i.e., they are nearly spherical or oval. Variously called micro-conidia, microspores, spermatia or Phoma-forms, they have been recorded, e.g., in species of Mycosphaerella, Guignardia, Neurospora, Gelasinospora, Nectria, Phyllachora, Rhytisma, Stromatinia (Sclerotinia), Peziza, Phialea, Trichothecium, Polythrincium and Sporocybe, and are well known in some rusts. Exceptions to the spherical or oval figures do occur, however, e.g., the small, rod-shaped microspores of the species under study (whose length:width ratio is about 3-5:1), the small filiform microspores of the filiform-macrospore species of Septoria, e.g. S. tritici, and the filiform stylo- or B spores of Phomopsis. In those cases where the nuclear condition has been determined, the microspores or spermatia have been uninucleate. In the above, and, in many cases, widely-separated species, there appear to have been

parallel evolutionary trends towards the production of a spore form to give the greatest number of units for the least possible volume.

Another aspect is worthy of consideration. Some of these microforms can germinate, and perpetuate the organism, e.g., the microspores of S. avenae f. sp. avenae. Johnson (32) found no evidence to suggest that these microspores could act as spermatia. The microspores of Neurospora sitophila, on the other hand, have the ability to germinate and also the ability to act as spermatia, i.e., they can pass on their nucleus without the production of a germ tube. Other microspores have no ability to germinate but can act as spermatia, e.g., those of Gelasinospora adjuncta, although fertilization in this species can be accomplished without them, by means of hyphal fusions. In Puccinia graminis, fertilization is only rarely accomplished without spermatia. In other organisms, e.g., Stromatinia gladioli, fertilization can only be accomplished by spermatia.

In a great majority of the cases, however, the function of the microspores or spermatia-like spores has not been demonstrated. Most of them have never been germinated. In many cases, the large-spored form, which is parasitic on living hosts, is succeeded by the small-spored form, which in turn is succeeded by the perfect stage which is also the overwintering body. One exception to this sequence is that described for 2 species of Pycnopeziza by White and Whetzel (72), where pycnidia, spermagonia and apothecia occurred either intermingled on the same substratum or segregated in nearby groups, and matured at precisely the same time.

The factors determining the production of the various spore forms, or their sequence, are, with few exceptions, unknown. (Not considered here are those cases which are genetically unable to produce various types of spores, e.g., Neurospora crassa, in which induced mutants have been obtained whose inability to produce microspores depends on 2 mutant

alleles (Barratt and Garnjobst, 6)). The coincidence of the spermatia-like forms with the end of the growing season and the onset of winter has tended to associate their occurrence with falling temperature and/or the physiological changes accompanying either host maturity or the process of change from living host to non-living substrate.

Barnett and Lilly (4,5) studied the factors affecting the asexual reproduction of Choanephora cucurbitarum, and found that the production of conidia and sporangia in culture was influenced by thiamine and sugar in the medium, and by temperature, light, aeration and humidity. Nitimargi (43) found that different proportions of A, B and C spores were formed by species of Phomopsis as the C:N ratio of the media was altered.

It is to be noted that, in the case of S. avenae f. sp. avenae, intermediate-sized spores rarely occur. There appears to be 2 stable spore sizes, large and very small, determined, perhaps, by the physiological state of the thallus, or, perhaps, by purely physical factors such as decrease in temperature which increases surface tension which in turn might govern the size of budding spores. The slowness of the germination of the microspores might be due to their physiological state or to purely physical factors.

More information is obviously required concerning the factors responsible for the formation of accessory spore forms in fungi, in nature and in culture, and the role of the spore forms in the life cycle of the species.

PART IV

CYTOLOGY OF THE SPECIES UNDER STUDY

REVIEW OF LITERATURE

The nuclear condition of the species under study has not been determined previously, save for the work of Shaw (57), who showed that macrospores of S. avenae (oat-infecting), S. avenae f. sp. triticea and S. nodorum contain one nucleus per cell, so that the number of nuclei per spore equals the number of cells.

MATERIALS AND METHODS

The material used in this study included macrospores and ascospores when immature, mature and after germination, microspores produced in culture and in nature, mycelium from culture, paraphyses and asci from the time of the fusion nucleus to the delimiting of the ascospores.

The procedures used were as follows:

1. Pycnidia and perithecia were placed directly into 2% aceto-orcein in 60% acetic acid, crushed, warmed and examined immediately or stored at a low temperature overnight and examined on the following day.

2. Spores were allowed to exude in water on slides, or perithecia were crushed in water and as much of the wall removed as possible, or mycelium was added directly to the water. The water was allowed to evaporate at once, or after certain periods of time in germination studies. The material was hardened with methanol and stained with Giemsa after acid hydrolysis in N HCl at 60°C as given in detail by Shaw (57), except that ethanol was replaced by methanol at the suggestion of Dr. W. Jacobson of the Strangeways Research Laboratory, Cambridge, England, and Canada Balsam was used for mounting instead of Euparal. According to Murray et al. (42), Tulasne and Vendreley (67) and Jacobson and Webb (29) Giemsa demonstrates the distribution of desoxyribonucleic acid.

RESULTS

The macropycnidiospores of the isolates under study were found to have one nucleus per cell, thus confirming the previous report. Immature spores which are still unicellular have one nucleus per spore.

It was found that when the spore begins to germinate, the germ tube is at first anucleate. Then the nucleus in the cell of the spore divides; one daughter nucleus appears in the germ tube at a little distance from the cell of the spore, and the other daughter nucleus remains in the cell of the spore. If another germ tube issues from the same cell, the latter daughter nucleus divides and supplies the nucleus for the second germ tube.

As the germ tube grows the nuclei in it are spaced at fairly regular intervals along the hyphae. The nuclei never appear clustered at the tip as reported for some other organisms, e.g., Fusarium culmorum (Jones, 33). In the older hyphae, which are strongly septate, there is one nucleus per cell. In the hyphal tips the septations are often difficult to see, but the nuclei are still spaced about the same distance apart. The cytoplasm of the tip of germ tubes from spores germinated in water is homogeneous compared with the vacuolated condition of the older part of the germ tube and the spore. A germinating spore of S.nodorum is shown in Plate 5, Fig. 14.

The microspores were found to be uninucleate, as shown in Plate 5, Fig. 11.

The paraphyses, which are multicellular, have one nucleus per cell. The cytoplasm in the paraphyses appears to stain less with aceto-orcein than does the cytoplasm in the spores or the mycelium.

The cell wall of the mature ascospore is pigmented, and difficulty was experienced in staining the nuclei of mature ascospores with aceto-orcein, and even with Giemsa after acid hydrolysis at 60°C there was some

difficulty. However, near-mature spores (as judged by the development of pigment) and spores which have just commenced to germinate, have one nucleus per cell. The germ tube is first devoid of a nucleus, then one appears at a little distance from the tip and from the original cell, as shown in Plate 5, Fig. 13. As the germ tube lengthens the nuclei in it are spaced at fairly regular intervals along the hyphae. They were never found clustered at the tip.

The major nuclear happenings were followed in the asci from the time of the fusion nucleus in the primary ascus to the maturation of the ascospores. No attempt was made to follow individual chromosomes through meiosis, on account of their small size. The number appeared to be 10 or 12, which might mean a haploid number of 5 or 6; no reliance, however, can be placed on this figure.

The fusion nucleus is large compared with the nuclei in the mycelium. In the asci with 2 and 4 nuclei, the nuclei are arranged along the long axis of the ascus. The spindles of the 8 nuclei at a later stage are slightly oblique to the long axis, and the spindles of the 16 nuclei at a still later stage are more oblique again. From the proximity (as shown in the material stained with aceto-orcein) of the 2 nuclei in each of the 8 pairs at the 16-nucleate stage (especially of the pairs at the tip of an ascus) and from the density of the cytoplasm around the pairs, it is concluded that the nuclei of an ascospore are derived from only one of the original 8 nuclei.

At the 16-nucleate stage the oblique ascospores are already delimited, and if the ascus wall is broken at this stage, the immature ascospores separate from each other and the ascus wall with ease. In Plate 5, Fig. 12, an ascospore with one septum and with one nucleus in each cell can be seen quite free from the ascus cytoplasm. Each nucleus then divides (as

shown in Plate 5, Fig. 12, where a one-septate spore is visible with 4 nuclei) and a septum forms between the 2 pairs, so that the near-mature ascospore has one nucleus per cell.

It is concluded that the microspores are uninucleate, and the macrospores and ascospores have initially only one nucleus per cell. Therefore, if lines producing micro- and macro-spores, and lines producing macrospores and ascospores, are maintained by the transference of single spores, it is unlikely that variations arising in the cultures of these lines could be attributed to heterocaryosis.

PLATE 5

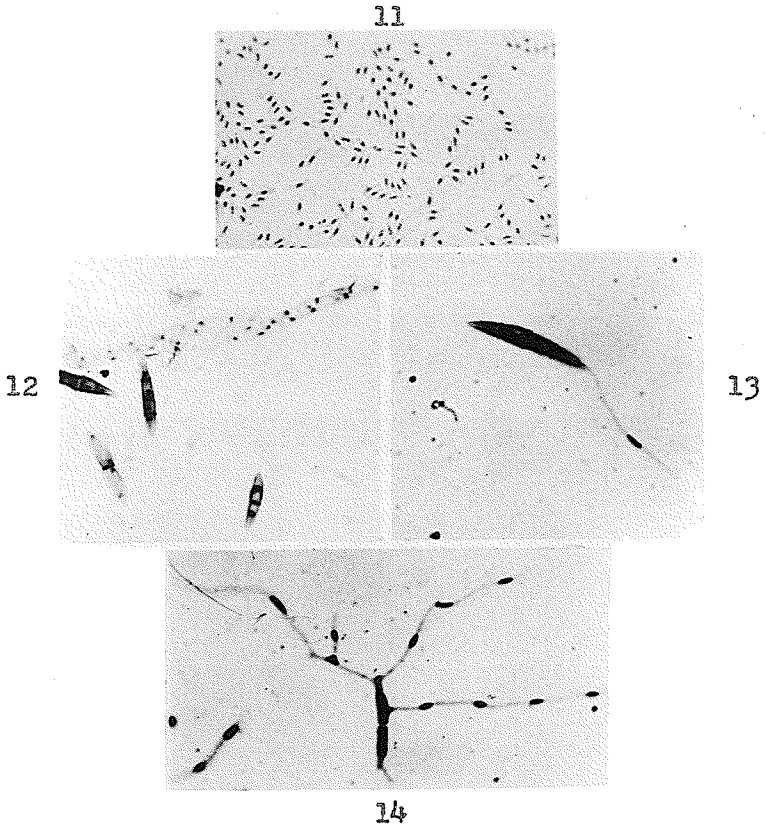

Fig. 11 Microspores of S. avenae f. sp. avenae from nature, showing one nucleus per spore. Stained with Giemsa after acid hydrolysis. x 620

Fig. 12 Immature ascospores of L. avenaria f. sp. triticea, showing one-septate spore with 2 nuclei, and 1-septate spore with 4 nuclei, and 3-septate spores with 4 nuclei. Stained with Giemsa after acid hydrolysis. x 620

Fig. 13 Mature, germinating ascospore of L. avenaria f. sp. triticea showing one nucleus in the germ tube and one nucleus per cell in the spore. Stained with Giemsa after acid hydrolysis. x 620

Fig. 14 Germinating macrospore of S. nodorum, with nuclei in the germ tubes and one nucleus per cell in the spore. Stained with Giemsa after acid hydrolysis. x 620

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