SOME OBSERVATIONS ON A SMUT DISEASE OF OATS

A thesis presented by

JOHN THOMAS MILLS, B.Sc. (HONS.)

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From the Department of Botany, Imperial College of Science and Technology, Field Station, Silwood Park, Sunninghill, Berkshire.

ABSTRACT

The mechanism of infection of oat plants by *Ustilago avenae* has been investigated under the optimal conditions of 20°C. and 20% soil water holding capacity. The coleoptiles and first internodes of seedlings grown from contaminated seeds were often directly penetrated by promycelia. Penetration tubes and associated structures are described. The mycelium in the coleoptiles and first internodes was mainly intracellular, but in the first leaf and deeper tissues it was mainly intercellular. The mycelium reached the growing point *via* the third or fourth leaf base, 14-17 days after sowing, and before the elongation of the internodes; later it was carried upwards by the growth of the internodes.

Spore formation in the ovary and in some vegetative tissues was studied. The spores were formed by the segmentation of sporiferous mycelium.

Some of the factors influencing spore dispersal and flower infection were investigated. Turbulence was apparently the main agent of dissemination. Rain was important only when the panicles contained many spores, and in the process of flower infection.

Some of the factors which influence infection were investigated. In time and depth of sowing experiments the highest infection obtained was 20%. Initially, high moisture and later, low temperature limited infection. Sowings at 2.5 and 5.0 cm. deep produced comparable infection probably because the times for emergence and the lengths of the susceptible periods were similar.

In sand culture experiments, plants at first had 7 days under optimal conditions for infection. Later, smutted plants were produced in treatments receiving complete nutrients and in those deficient in nitrogen, calcium, or potassium.

Gibberellic acid and maleic hydrazide applied as seed soaks or as seedling sprays did not influence smut infection. Maleic hydrazide, however, stimulated spore germination on agar.

Control of the disease was obtained by treating contaminated seed for two hours with the liquid organomercurials, Ceresan 100 and Chipcote 75.

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INTRODUCTION

The smut diseases of oats are caused by two fungi, Ustilago avenae (Pers.) Rostr. loose smut, and Ustilago kolleri Wille, covered smut. Loose smut is generally more striking because of the masses of dark brown spores that replace the glumes. Both species can infect the young seedling and flowers; occasionally the embryo is infected (Gage, 1927).

Swinburne (1960) has described in detail the path of the hyphae of Tilletia caries from the seed to the growing point in bunt of wheat; Kolk (1960) and Western (1936) did the same for U.avenae and oats. The present work repeats that of Kolk and Western but particular attention has been given to the path of the mycelium from the coleoptile to the first leaf, and to the relation between the mycelium and anatomy of the host.

There is little information in the literature on the factors affecting the dispersal of U.avenae spores, and the natural infection of the flowers. It was decided to carry out spore trapping in a plot containing smutted panicles in order to investigate natural infection of the flowers and the effect of rain and turbulence on spore dispersal.

During the decade 1920-30 there were many investigations on infection by artificial inoculation of flowers with spores and the determination of the nature and position of the overwintering inoculum. Conflicting results were obtained so this problem has been re-investigated. A few experiments were carried out on the control of the disease using flower and seed inoculated oats and liquid organo-mercurials.

Reed (1936) has stated that if the mycelium reaches the growing point, post emergence conditions apparently do not alter the resultant infection. Some pre-and post emergence factors and their influence on the disease have been studied with particular reference to the path of the mycelium in the plant and the host morphology.

PART I. STUDIES ON THE CHLAMYDOSPORES

INTRODUCTION

It is important in critical work on the loose or covered smut of oats to know the genetical purity of the parasite and the host. A mixture of U.avenae and U.kolleri can occur in the same panicle as a complex inoculum. Hybridization is known, the host often producing a range of symptoms. In addition numerous physiological races of the smuts exist with widely varying pathogenicities.

The first part of this work deals with the identity of the isolates used, the effects of different storage conditions on the viability of the chlamydospores and with the effect of different environmental conditions on germination. In addition, the various stages in spore germination in agar were followed in detail in order that comparison could be made later with spore germination on the host.

A. IDENTITY OF THE INOCULA

Review of Literature

The fungus causing the covered smut of oats has been known during the past seventy years by the following names:-

...Ustilago kolleri Wille (1893); Ustilago levis (Kellerm. and Swing.) Magnus (1894); and Ustilago hordei (Pers.) Lagerh. (Fischer 1953); while the fungus causing the loose smut of oats has been known as Ustilago avenae (Pers.)Rostr. (1890). In this thesis the names U.kolleri and U.avenae will be used when reference is made to the covered and loose smut of oats respectively, except when reference is made to work specifying a particular synonym when the synonym will be used. The glumes as well as the rest of the panicle are attacked by loose smut. The spores are echinulate and 4-8 (average 6) μ in diameter. In covered smut there is no attack on the glumes, the spores are smooth and 7-11 (average 8.5) μ in diameter, somewhat larger than in loose smut (Ainsworth and Sampson, 1950). Under the electron microscope U.avenae spores appear closely echinulate whereas those of U.kolleri are completely smooth. (Hille and Brandes, 1956).

In the field, it is often difficult to separate the two species by the symptoms they cause (Popp and Hanna, 1935) as heads of the covered smut type often contain echinulate spores. However, when Popp and Hanna (1935) inoculated plants with two monosporidial cultures of U.avenae of opposite sex echinulate chlamydospores were produced whereas similar inoculations with U.levis produced smooth chlamydospores. The appearance of the spore wall is therefore genetically determined and can be regarded as

...reliable in differentiating the two species. Symptom expression however is much more complex (Popp and Hanna, 1935; Holton, 1959) because it depends on interactions between the host, parasite and the environment.

Popp and Hanna (1935), obtained hybrids between U.avenae and U.levis by crossing monosporidial lines of opposite sex. The spores produced were echinulate. Dominance of the echinulate factor was proved by back crossing the hybrids with U.levis and U.avenae. Popp and Hanna (1935) think that hybridization almost certainly occurs in nature.

The following experiments were conducted to investigate the specific nature of the inocula used in the thesis.

Methods

Spore inocula were obtained from two places. Collection 1 (to be called C1) showing typical loose smut symptoms on S.147 oats was from Aberystywth, Wales. Collection 2 (C2) also on S.147 was from Jealotts Hill, Berkshire, and showed typical covered smut symptoms. Both Collections were obtained in January 1960.

C1 and C2 were propagated on Anthony and S.147 oats at Silwood Park; spores were collected on July 1st, 1961. These spores and those from eight oat smut collections belonging to the Commonwealth Mycological Institute (C.M.I.) at Kew were measured and examined in the following

...way. One hundred spores from each collection were measured after mounting in lactophenol. The results are shown in Table 1.

Results

In Table 1 collections C3-C10 are of material deposited at the Commonwealth Mycological Institute, Kew; and C1 and C2 the inocula under investigation.

The collections most like U.kolleri are C3, C4, C5 and C6. The glumes are not completely replaced by spores and the spores are larger and smooth. Collection C10 most approaches U.avenae, the spores are echinulate and the glumes are replaced by spores.

The glumes are not completely replaced by spores in collections C7, C8 and C9; these are symptoms caused by U.kolleri. But the spores are echinulate and smaller; these are features typical of U.avenae. The collections C7, C8 and C9 are similar to C2 on S.147 oats from Jealotts Hill. Fig.1 is a photograph of a typical range of symptoms obtained with collection C2 on S.147 oats in the greenhouse. The two panicles on the right of the photograph have spores mainly at the base of the glumes. This is a typical feature of covered smut. The panicle in the centre has more smut on the glumes but the glumes are not replaced by spores. The two panicles on the left of the photograph still have glumes but they have almost

TABLE 1.

Spore measurements and morphology of Collections C1-10, and gross host symptoms.

No. of Collection	Site	I.M.I No.	Host	Symptom Type	Spore Morphology	Size of spores range (AV.)μ	Classification C.M.I.
C1	Ascot, Berkshire	92,729	Avena Sativa (Anthony)	A	echinulate	3.5-7.0(5.0)μ	A
C1	"	92,730	A.sativa S.147	A	"	3.5-5.5(5.0)μ	A
C2	"	92,732	A.sativa Anthony	A	"	3.5-7.0(5.5)μ	A
C2	Jealott's Hill, Berkshire	92,731	A.sativa S.147	K	"	3.5-5.5(5.0)μ	A
C3	Aberystywth	32,362	A.brevis	K	smooth	7.0-9.0(8.5)μ	K
C4	"	32,347	A.sativa	K	"	7.0-11.0(8.5)μ	K
C5	Hadfield Plots N. Zealand	10,951	Oats	K	"	5.5-9.0(7.0)μ	K
C6	Lahore India	39,457	A.sativa	K	"	5.5-9.0(7.5)μ	K
C7	Otago, N.Zealand	51,988	Oats	K	echinulate	5.5-7.0(6.5)μ	A
C8	Lusaka N.Rhodesia	68,170	A.sativa	K	"	3.5-7.0(5.0)μ	A
C9	Athens Greece	37,866	Oats	K	"	3.5-7.0(5.5)μ	A
C10	Cranleigh Surrey	39,450	Oats	K	"	5.5-7.0(6.0)μ	A

A = U.avenae K = U.kolleri

Fig. 1. Ustilago avenae collection C2 on S.147 oats showing the range of Ustilago kolleri type symptoms. (x ½ approx.).

...been replaced by spores and so closely approach U.avenae in symptoms.

Collections C1 on Anthony and S.147, and C2 on Anthony caused U.avenae symptoms because most of the panicles have glumes replaced by spores. Many panicles have a few small remains of glumes. The spores are all echinulate and smaller.

Discussion

Popp and Hanna (1935) have stated that the appearance of the spore wall is a reliable character for differentiating the two species. The spores in C1 and C2 are small and echinulate and are, therefore, U.avenae. However, hybrids between U.avenae and U.kolleri also have echinulate spores, this being a dominant allele. The collections C1 and C2 are thus either U.avenae and or hybrids between U.avenae and U.kolleri.

B. IDENTIFICATION OF THE PHYSIOLOGIC RACES

Review of Literature

In America, Reed (1940) identified 29 races of U.avenae using seven varieties of oats as differentials. Holton and Rodenhiser (1948) using 10 varieties retested some of Reed's races together with other collections and differentiated only 15 races, designated A1-15. Cherewick (1951) added two differentials, Mabel and Beacon, to those of Holton and Rodenhiser (1948) and found 23 races. Later Hansing (1954) and Luke et al (1961) using Holton and Rodenhiser's differentials and extra varieties identified six races (designated A16-22). Reed (1940) described 14 races of U.kolleri. This figure was revised by Holton and Rodenhiser (1948) to seven races. Later Cherewick(1951) using Holton and Rodenhiser's (1948) differentials and Mabel and Beacon found ten races of U.kolleri.

In England, Williams and Verma (1954) tested eight oat smut cultures using Holton and Rodenhiser's (1948) differentials. They were able to identify tentatively two races of U.avenae and four races of U.kolleri. They concluded that Holton and Rodenhiser's (1948) differentials were unsatisfactory for the identification of oat smut races in England.

The following experiments were conducted in order to

...identify the physiological races in C1 and C2.

Methods.

Spores of C1 and C2 propagated on S.147 oats were tested on differential varieties kindly sent by Dr. Cherewick from Winnipeg. These were Holton and Rodenhiser's (1948) differentials except that Lelina was replaced by Atlantic; they were all spring varieties.

Field Experiments

Seed of each variety was inoculated by the partial vacuum technique (Zade, 1928) (described later in Part II). It was then sown as follows: 2g. of each variety treated with C1, and C2 (separately) were sown per 6ft. row on 26th February;and repeated on 8th March, 1961. Guard rows of wheat were thickly sown between each oat variety and around each replicate. The final estimate of smut was made on the 17th July, 1961.

Greenhouse Experiments

Two experiments, using only C1, were sown in the greenhouse in November, 1961 and January, 1962. The same differentials and inoculation technique were used as in the field experiments. Seventy seeds of each variety were sown; ten seeds per five inch pot. There was no control of temperature.

The schemes devised by Holton and Rodenhiser (1948) and Luke et al (1961) for rating the amount of disease were used; the results obtained are presented in Table 3.

Results

Collection C2 was tested only in the field and Anthony was the sole variety to show infection (Table 2). Smutted ears were produced in only one replicate of Anthony. In this replicate the Holton and Rodenhiser scheme (1948) gives a susceptible rating for Anthony and the scheme of Luke et al (1961) an intermediate rating.

In the field, only Anthony, Black Dimond, Victory and Gothland produced smutted ears. The results however were inconclusive; the number of smutted plants varied per replicate. Results from the greenhouse experiments show that Black Dimond and Gothland are resistant, and Anthony and Victory are susceptible, to C1. (Table 3)

Discussion

It is apparent from the results in Table 2 and 3 that the differential varieties give very different reactions when inoculated with C1 and C2; these collections could be different physiologic races. The ratings for C1 and C2 differ from those of the American races A1-15. (Holton and Rodenhiser, 1948). Unfortunately, they cannot be compared with the results obtained by Reed

TABLE 2.

Smut infection on differential varieties by Collections 1 and 2 in field experiments.

COLLECTION C1.

No.	Variety	Replicate	Total No. Plants	Total No. Ears	Number Smutted: Plants	Number Smutted: Ears	% Smut: Plants	% Smut: Ears	Ratings *	Ratings **
1	Anthony	1	22	59	0	0	0	0	R	R
		2	40	66	19	54	48	89	S	S
2	Black Dimond	1	13	28	1	1	8	4	R	R
		2	16	27	1	2	6	7	R	R
3	Victory	1	25	55	0	0	0	0	R	R
		2	23	47	6	14	27	30	S	I
4	Gothland	1	29	61	0	0	0	0	R	R
		2	18	34	1	1	6	3	R	R

Varieties MONARCH FULGHUM BLACK MESDAG CAMAS, NICOL, and ATLANTIC did not show smut.

COLLECTION C2.

No.	Variety	Replicate	Total No. Plants	Total No. Ears	Number Smutted: Plants	Number Smutted: Ears	% Smut: Plants	% Smut: Ears	Ratings	
1	Anthony	1	7	22	†2	5	29	23	S	I
		2	21	50	0	0	0	0	R	R

Varieties BLACK DIMOND, VICTORY, GOTHLAND, MONARCH, FULGHUM, BLACK MESDAG, CAMAS, NICOL and ATLANTIC did not show smut.

* Rating based on Holton and Rodenhiser (1948)

** Rating based on Luke et al (1961)

S = >10% smut; R = <10% smut. S = >30%; R = <10%, I = 10-30%

S = susceptible, R = resistant, I = intermediate.

† Smut present on 6th and 7th leaves of both plants.

TABLE 3.

Smut infection on differential varieties in greenhouse experiments. Collection I only.

No.	Variety	Replicate	Total no. Plants	Total no. Ears	Number Smutted: Plants	Number Smutted: Ears	% Smut: Plants	% Smut: Ears	Ratings *	Ratings **
1	Anthony	1	20	19	19	19	95	100	S	S
		2			NOT SOWN					
2	Black Dimond	1	39	14	0	0	0	0	R	R
		2	62	42	0	0	0	0	R	R
3	Victory	1	15	10	10	10	67	100	S	S
		2	44	43	32	32	73	74	S	S
4	Gothland	1	43	29	0	0	0	0	R	R
		2	67	58	1	1	1	1	R	R
7	Black Mesdag	1	34	25	2	2	6	8	R	R
		2	46	44	1	1	2	2	R	R

The differential varieties MONARCH, FULGHUM, CAMAS, NICOL and ATLANTIC did not show any smut.

* Rating based on Holton and Rodenhiser (1948)

** Rating based on Luke et al (1961)

...(1940), or Luke *et al* (1961) as they used other differential varieties.

Williams and Verma, (1954) found that Holton and Rodenhiser's (1948) differentials were inadequate for the identification of the English oat smut races. With one collection, they obtained however, 3% smut on Anthony; the other varieties were resistant. This result is similar to that obtained with C2 (Table 2); possibly they could be the same race.

The results obtained confirm that the present differential varieties are inadequate and that more are needed before races of oat smut in the United Kingdom can be identified.

C. VIABILITY OF THE CHLAMYDOSPORES AND SPORE GERMINATION

Review of Literature

There are three nuclear states in the life cycle of the oat smuts, the diploid, haploid and dikaryotic. The nature chlamydospore, or the teliospore (Fischer and Holton, 1957) is diploid. The sporidia and other products of spore germination are haploid. The parasitic mycelium and the young chlamydospores are dikaryotic. When the chlamydospores are mature, the two nucleii fuse and produce a single diploid nucleus. A meiotic division occurs in the germinating spore or in the promycelium (Cherwick, 1953).

Spore germination on agar at 22°C. (Ainsworth and Sampson, 1950) results in 1-2 promycelia per spore, the promycelia having 1-3 septa. Each promycelial cell usually produces one haploid nucleus. U.avenae produces sporidia abundantly in culture at temperatures ranging from 5°C. to nearly 30°C. (Western, 1937) Sporidia are abstricted at the apex of the promycelium, and at the septa. (Ainsworth and Sampson, 1950). The sporidia may produce secondary sporidia by budding (Lutman, 1910). The sporidia are usually of two opposite sexual groups. When two sporidia of opposite sex come into close proximity they often become united by a fusion hypha and from it a dikaryotic

...hypha often develops. Adjacent promycelial segments also often fuse by a fusion tube, the so called 'buckle joint'. A dikaryotic infection hypha is often produced from this structure (Lutman, 1910).

Swinburne (1960) using spores of Tilletia caries, bunt of wheat, found evidence of better germination in the light than in the dark.

Fischer (1936) has shown that the upper age limit of viability for U.avenae spores from herbarium material was 13 years. However, many workers (Sampson, 1928; Kondo 1961) have observed that the viability of U.avenae spores falls off within a year when stored at room temperature. Sampson (1928), working with U.levis and U.avenae spores showed that viability depends on the state of maturity of collection. Storage conditions also have an important effect on viability. Noble (1934) found a direct relationship between the relative humidity under which Urocystis tritici spores were stored and longevity of the spores. Storage at low temperatures generally prolongs viability. Malik (1959) showed that spores of Ustilago nuda stored at 0°C. in a desiccator lived longer than those stored at room temperature (15.5 - 21.0°C). More recently, Kondo (1961) studies the effect of various temperatures on the storage of lyophilized (freeze dried under vacuum) U.avenae spores. A high percentage of lyophilized spores, stored at 5°C. and - 15°C. for one

...year, retained their viability.

Methods

Only spore suspensions in sterile water were used and viability was tested in two ways; in hanging drop cultures or by spreading the suspension over the surface of 2% tap water agar in Petri-dishes. Generally 4 to 5 plates were used per treatment. Counts of germination were made 48hrs. after incubation at 20°C 36cm. under a battery of five 5ft. 80w. tubes. The average light intensity at the level of the Petri-dishes was 516ft.candles, the range was 250-675ft. candles. A slight "greenhouse effect" was evident within the plates. The internal temperature was 22°C., 2°C. higher than the temperature of the room.

In experiments I and II described below; smutted plants were used bearing panicles of C1 at stages 4 and 5 (see Table 4). The spores were shaken off, sieved and thoroughly mixed before use.

Experiment I. To study the effect of illumination on spore germination at 20°C.

In a preliminary experiment it was found that 48 hrs. was long enough for spore germination and that afterwards production of sporidia was so great that germination was difficult to determine. Suspensions of spores on agar

...were put in a constant temperature room at 20°C. in the light or in the dark. After 48hrs., 200 spores approximately were counted per plate. Some of the plates in the light were examined after 48hrs. and also after 4 and 7 days for the different stages in spore germination. The results of percentage germination in the light and dark are presented in Table 5.

Experiment II. To study the effect of storage temperature and time on spore viability.

200mg. of spores were put into each of seventy 7.5x 2.5cm. tubes. The corks were sealed by dipping them in cooling paraffin wax. Large tubes were used to provide oxygen for the spores in storage. Twelve tubes were placed in incubators at each of the following temperatures; 4, 8, 20, 25 and 33°C. (all in the dark), and 18°C. (in the light). At intervals of 4, 8, 12, 18, 24 and 44 weeks one tube at each temperature was removed and the viability tested as in Experiment I, above. 200 spores from each of five plates were counted.

Experiment III. To determine viability of spores from smutted panicles in different stages of development.

Oat seed was inoculated with spores of C2 by the

...partial vacuum technique. Plants were grown in the greenhouse and a scheme was devised (Table 4) for describing the stage of development of the smutted panicles. This scheme is based on Sreeramulu's work on U.nuda (1956). Spores were collected from smutted panicles in stages 1-5 of development by placing the panicle in an envelope and tapping the panicle slightly. The spores obtained were sieved through muslin and suspended in sterile water. A drop of this suspension was put on a coverslip and left as a hanging drop for 19hrs. Six hanging drop cultures of spores from each panicle were made and at a particular stage of maturity, three panicles were examined.

Results

The data in Table 5 shows that spore germination is greater in the light than the dark. Only one replicate was tested in the dark. It was decided to germinate spores only in the light in later tests.

After two days many spores had produced promycelia in the light. The promycelia usually had three cells. Many of the adjacent promycelial segments had fused (Fig.2). The fused promycelia, on average 3.5 by 20-25μ were often detached from their parent spores. Production of sporidia was seen in all the plates. The sporidia, 3.5 by 7-12μ were abstricted from the promycelial segments. Detached and germinated sporidia were very common. In Fig.2 can be seen a fusion between the germination tubes of two sporidia. After 4 days in the light, many of the sporidia have

TABLE 4.

Stages of development of oat panicles smutted by Ustilago avenae (Adapted from Sreeramalu, 1956)

Stage 1.	Smutted panicle apparent as seen through flag leaf when held up against the light.
Stage 2.	Panicle emerging from 7th leaf sheath.
Stage 3.	Panicle clear of sheath, stalk elongating; spore masses still compact.
Stage 4.	Spore masses drying, spores beginning to disperse.
Stage 5.	Nearly half of spore masses on each panicle dispersed. Fibrous material in panicle becoming apparent.
Stage 6.	More than half of spore masses dispersed; more fibrous material visible.
Stage 7.	Most of the spores have been dispersed; mainly fibrous material remains.

Results

TABLE 5.

The effect of illumination on spore germination at 20°C. (Experiment I).

Experiment	Plate	Total Spores	Germinated Spores	% Germination	
LIGHT					
1	1	227	72	32	
	2	212	63	30	
	3	199	101	51	
	4	311	83	27	
	5	205	91	44	average 36%
2	1	202	90	45	
	2	228	71	31	
	3	209	75	36	
	4	209	76	41	average 38%
DARK					
1	1	228	22	10	
	2	198	17	8	
	3	207	15	7	
	4	243	31	12	
	5	206	27	13	average 10%

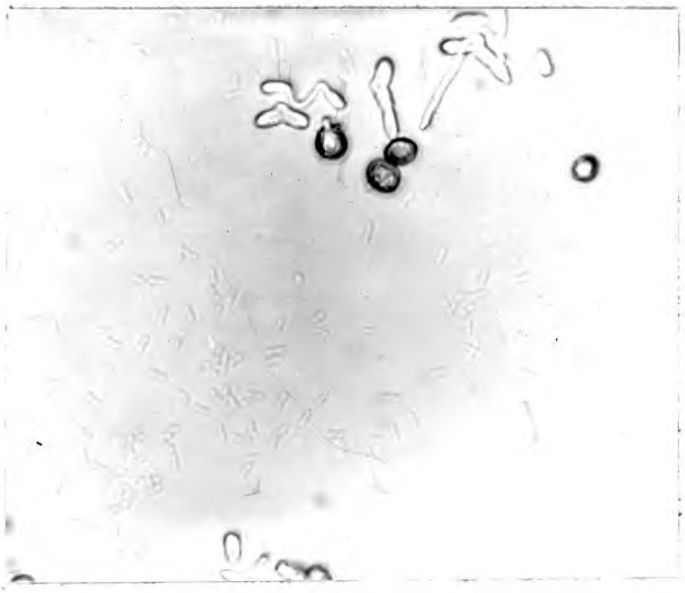

Fig. 2. Spore germination after 2 days in the light showing fused sporidia and fused promycelia both attached to and detached from the spores (Bacterial contamination also present.) x 640.

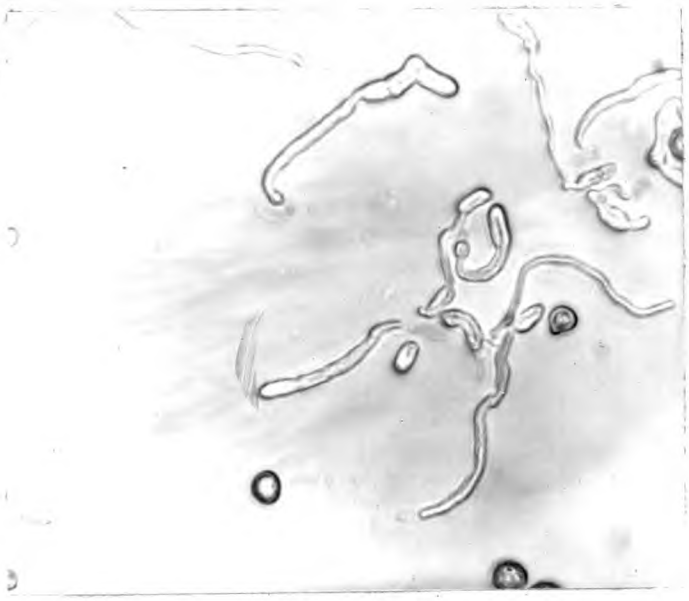

Fig. 3. Spore germination after 4 days in the light showing fused sporidia, germinating sporidia and a 'tube' arising from a detached fused promycelium. x 640.

...produced secondary sporidia by budding and often infection tubes were seen arising from the fused sporidial germination tubes. The terminal cell of promycelia often produced an extension (Fig.3). After 7 days, the sporidia of the sporidial fusions do not stain with cotton blue. Only the terminal portion of the infection tube takes up stain because of the presence of protoplasm which has moved from the sporidia and fusion tube. The infection tube is now 50-100μ in length.

In the dark, after 2 days, the germinating spores had produced sporidia, sporidial fusions and fused promycelia similar to the germinating spores after 2 days in the light.

Table 6 is a summary of the results obtained from Experiment II. After three months the viability of the spores has fallen to a very low level at all temperatures of storage. There is apparently least variation in viability at low temperatures (4 and 8°C). In viability tests spores produced promycelial fusions, sporidia and sporidial fusions irrespective of storage temperature.

In Experiment III, no counts were made of the germination of spores in the hanging drop cultures but it was seen that spores taken from smutted panicles at Stages 1 and 2 had not germinated after 19hrs. Some spores taken at Stage 3 did germinate and the proportion increased greatly when panicles at Stages 4 and 5 were used.

TABLE 6.

Changes in the viability of chlamydospores at different storage temperatures with time (Experiment II).

Time in Weeks	TEMPERATURE °C. 4	8	18	20	25	33	% Spore Germination
0	37	37	37	37	37	37	
4	22	28	31	18	16	18	
8	22	19	10	13	14	12	
12	2	7	6	2	6	11	
18	1	1	3	3	1	0	
24	2	1	1	0	0	6	
44	1	2	0	0	0	LOST	

Discussion

Swinburne (1960) found there was greater germination of T.caries spores in the light than in the dark. There also appears to be increased germination of U.avenae spores in the light. Germination on agar at 20°C produces many sporidia and sporidial fusions. Most of the infection tubes appear to arise from fused sporidia and not from the fusion of promycelial segments. Possibly the extensions of the promycelial terminal cells may also function as infection tubes. These structures and the products of spore germination on agar will be referred to later.

Sampson (1928), has studied the conditions affecting the viability of oat smut spores in storage. Several collections of U.avenae were harvested from the field from panicles only just emerged from their leaf sheaths. Most of the germination curves obtained showed a steady fall from 35-40% to zero after 14-36 weeks. However, one curve had an ascending portion, due to after ripening, then a fall to zero. However, it is apparent from Table 6 that there is no period of after ripening in Collection C1. A progressive loss of spore viability occurs irrespective of storage te mperature; there is a slight indication of enhanced viability at lower temperatures.

Sampson (1928) concluded that viability is largely dependent on the stage of maturity of the spores at

...harvest. The low figures of 35 and 40% germination in Experiments I and II could be explained by the immaturity of the spores at harvest. Spore viability appears to vary with the stage of the panicle as can be seen from Experiment III. The spores must attain maturity very quickly.

These preliminary experiments stress the importance of harvesting the spore inoculum at the correct time.

PART II. THE MECHANISM OF INFECTION

Introduction

Woolman, (1930), working with T.caries found that the development of the fungus in the host could be divided into three phases. Firstly, the entrance into and development within the epidermal layer of cells. During this time the fungus is gram negative. Secondly development in the deeper tissues of the coleoptile and in the first and second leaf sheaths; here it is gram positive. Thirdly, development in the young leaf blades, nodes, internodes and the growing point when it is also gram positive. He suggested that the change from gram negative to gram p ositive coincided with the fungus becoming parasitic on the host. U.avenae is similar to T.caries in being a seedling infecting smut which enters the young seedling at germination. In Part II infection will be dealt with in two stages. The first deals with the establishment of a stable parasitic relationship with the host. This includes spore germination on the surface of the host, how hyphae penetrate and the reactions of the host to penetration. The second stage deals with the later stages of parasitism, the passage of mycelium from coleoptile to the growing point and ovary and in particular whether mycelium did or did not

...pass the gap between the coleoptile and the first leaf and whether mycelium could be left behind by the plant. In addition, the method of spore formation was studied.

Terminology

The following terms will be used henceforward:

Ungerminated grain

GRAIN = caryopsis = embryo + endosperm + testa + pericarp

SEED = embryo + endosperm + testa

PERICARP = fruit wall (surrounding testa)

TESTA = seed coat (surrounding embryo + scutellum)

HULL = husk = lemma + palea

HULLED OATS = embryo + endosperm + test. + pericarp + lemma + palea

DEHULLED OATS = embryo + endosperm + testa + pericarp - (lemma + palea)

Young seedling and adult plant

COLEOPTILE and FIRST LEAF - Boyd (1931) states the coleoptile is equivalent to the first foliage leaf and the first leaf is in reality the second plumular leaf. However, in this thesis the terms 'coleoptile' and 'first leaf' have been retained for the coleoptile and first foliage leaf, respectively.

COLEOPTILE NODE - This is the third node and the one from which the coleoptile arises.

FIRST INTERNODE = MESOCOTYL - The first internode is the region between the coleoptile attachment and the first crown node. Mer (1957) uses the term 'mesocotyl' for the internode which extends during early seedling development. According to McCall (1934) this is the first internode. In order to avoid confusion, McCall's interpretation has been adopted.

THE CROWN - This is the term given to the group of closely associated nodes constituting the distal part of the primary seedling axis. The first crown node is the basal node of the crown from which diverges the first foliage leaf. In oats, the first crown node is the fourth node.

GROWING POINT REGION - This region includes the stem apex and associated leaf initials.

APICAL GROWING POINT - The extreme tip of the apex, distal to the youngest leaf initial.

THE FLAG LEAF - In oats, generally the flag (last) leaf is the seventh leaf, but sometimes there is an eighth leaf.

A. GENERAL MATERIALS AND METHODS

Onward, a spring variety and S147 a winter variety were used. After January 1961, Onward was largely replaced by S147 as mycelium in Onward was not clearly seen in sections. The viability of the seed was checked periodically by placing samples under Copenhagen tubes and examining for germination after 7 days. Only seed with a viability greater than 90% were used. The spore inoculum used was C1 collected from panicles in stages 4 and 5 (Table 4) by dislodging spores into envelopes which were then sealed and stored in a closed plastic box at 0°C. Before the spores were used in inoculation experiments the viability was tested on agar as described in Part I. Only spores with a viability greater than 30% were used.

John Innes No.1 compost was used for greenhouse sowings. The sand used was washed silica sand from Double Arches Pit No.21, Leighton Buzzard.

There was no control of greenhouse temperature, the average daily range over the winter period was 6-29°C. and the mean daily temperature 18°C. Additional lighting was provided by four 400w. mercury vapour lamps (Philipps) 4ft. apart and 2ft. above the tops of the plants for 18 hours a day.

Mildew (Erysiphe graminis) and aphis species mainly

...Rhopalosiphum insertum (Walker)were troublesome. The mildew was controlled by 'Karathane' smoke bombs (Murphy Chemical Co.) used at night, twice a week. The aphis were controlled by monthly fumigations with B.H.C. 'Murfum' (Murphy Chem.Co.). In the field, frit fly (Oscinella frit.L) caused many 'dead hearts' in the early summer, but no control was used. Many panicles were seen with symptoms of 'blasting' (a non-parasitic disease) in greenhouse and field.

Methods of Inoculation

Dehulling Technique - The method used was that of Kolk (1930) as modified by Brandwein (1937). Dehulled oats were placed in an envelope with sufficient spores to dust thoroughly. The grain was then sown groove downward, 2.5cm. deep in sand moistened to 20% of its water holding capacity contained in paraffin-waxed paper cups 7.5cm. diam. and 4cm. deep. The cups were covered with lids and placed in an incubator at 20°C. When the coleoptile had emerged about 4 days after sowing, the lids were removed and the cups transferred to the laboratory at 18-20°C. The seedlings were transplanted into compost in the greenhouse when the first leaf had emerged 1cm. beyond the coleoptile sheath. The seedlings were transplanted into bituminous

...painted tins 20cm. diam and 12.5cm. deep.

Partial Vacuum Method - The technique was first used by Zade (1928). Fig.4 is a photograph of the apparatus. Approximately 100g. of hulled oats were immersed in 10ml. of an aqueous spore suspension in a screw top bottle(A). which was loosely stoppered and placed in a desiccator B. The desiccator B was evacuated for 20min, the pressure was then suddenly released and as a result the spores were sucked under the hulls. The bottle was then removed, excess water poured off and the oats spread out to dry at room temperature (18-20°C) for 12hrs. They were then stored at 2°C for 24hrs. and sown in sand as described in the dehulling technique.

Hulled Dusted Technique - In this technique hulled oats were dusted with spores. This technique was only occasionally used.

Table 7 shows that in 1960 both the partial vacuum and dehulling techniques gave high percentages of infection in the greenhouse.

It was decided at the end of 1960 to use mainly the partial vacuum technique in 1961 owing to the ease of inoculation. The highest figure obtained in 1961 for percentage infection by this technique was 87%.

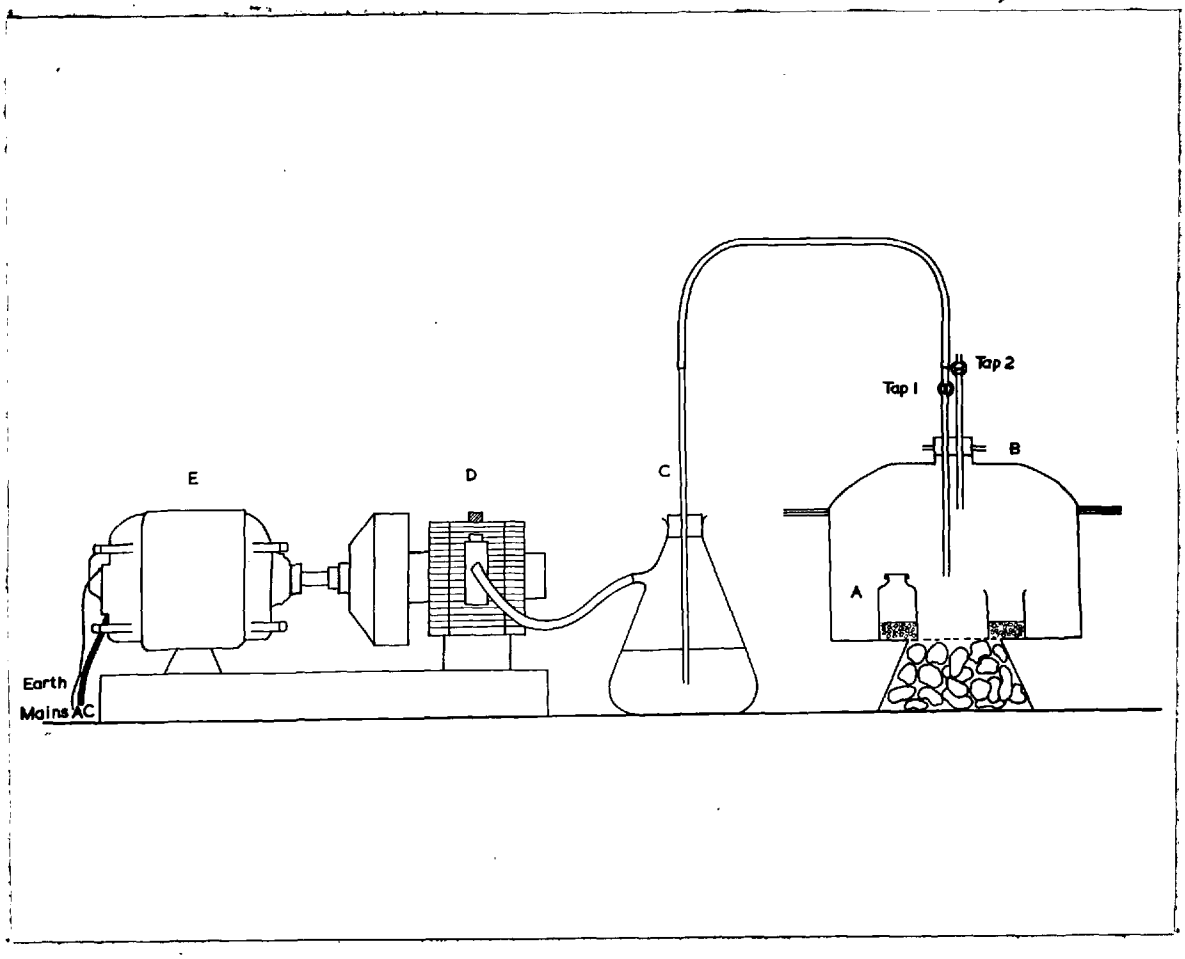

Fig. 4. The Partial Vacuum Method of inoculating oat seed with smut spores (After Zade, 1928).

TABLE 7.

Comparison of inoculation techniques showing the percentages of infection obtained in 1960 and 1961.

SEED	INOCULATION TECHNIQUE				
	FIELD			GREENHOUSE	
	Hulled Dusted	Dehulled Dusted	Partial Vacuum	Dehulled Dusted	Partial Vacuum
1960 Onward	10	20	*4	62	40
1961 Onward	11	-	-	-	47
S 147	17	62	23	73	87

* Many plants died from frit fly attack.

B. STUDIES OF SPORE GERMINATION ON THE HOST SURFACE.

Review of Literature

The dikaryophase can be initiated in several ways. Fusion can occur between promycelial cells, between sporidia or between any two compatible haploid cells (Fischer and Holton, 1957). Direct penetration of the host by germ tube (promycelium) has been observed in the oat smuts, but how the dikaryophase is established is not known. Brandwein (1944) demonstrated direct penetration by U.kolleri spores dusted on to dehulled oats. He found 922 invasions of which 390 were directly from chlamydospores. Western (1937) reported direct penetration of the stigmas of oat flowers by spores of U.avenae. He also described the production of sporidia, fusions between sporidia and penetrations from these fusions. Brandwein (1944) stated that few sporidia are seen on inoculated dehulled oats. Huttig (1931) and Western (1937) both state that temperature can effect the products of spore germination.

There follows an account of investigations made on spore germination and penetration of the host, and the effect of temperature on these processes.

Results

Experiment I - Spore germination and penetration.

... Dehulled S147 oats were dusted with spores and sown in sand. After 48hrs. pieces of the coleoptile were mounted in glycerine outer epidermis uppermost and examined under a x 90 objective to determine the stage of spore germination. Table 8 is a summary of the results.

In the first series of experiments, 49 from a total of 95 penetrations were caused directly by the promycelium. The remainder were either visible only as penetration tubes or the origin of the penetrating structure could not be determined because of masking by tangled hyphae from the promycelium or from the bridge between promycelial segments. No sporidia or fused sporidia were seen.

In the second series of experiments, direct penetration from the promycelium also formed a substantial proportion of the total. On 4.2.61 six of the 21 penetrations were grouped together. Possibly this was because penetration occurred at intervals from external mycelium.

Experiment II - The effect of temperature on the stages of spore germination using dehulled oats.

The procedure was the same as in Experiment I. The containers were each sown with five grains and kept at 0, 10, 15, 20 and 30°C., 2 containers at each temperature. One set of seed was examined after 5, the other after

TABLE 8.

Penetration of coleoptiles of dusted, dehulled S 147 oats.

Series I

Date inoculated	No. seedlings examined	No. penetrations seen	Penetrations direct by promycelium	Penetrations from fused hyphae or unknown cause
3.2.61	10	20	9	11
4.2.61	10	24	11	13
5.2.61	10	37	13	24
6.2.61.	10	30	8	22
7.2.61.	10	33	8	25
TOTALS		144	49	95

Series II

25.1.62.	10	8	3	5
29.1.62.	10	5	1	4
31.1.62	10	9	0	9
4.2.62.	10	26	5	21*
5.2.62.	20	31	8	23
TOTALS		79	17	62

* 6 penetrations in a group.

...10 days. Pieces of the coleoptile, first internode and pericarp were examined as in Experiment I.

At 0°C the seed had swollen but the coleoptiles or radicles were not visible. Spore germination on the pericarp was very low; two spores with 1μ germ tubes were seen after 5 days and several spores with 10μ germ tubes after 10 days.

At 10°C, the coleoptile had an average length of 2.5mm. after 5 days; many germinated spores were seen on the coleoptile and pericarp.

After 10 days many promycelial fusions still attached to their parent spores were present on the coleoptile and pericarp. No sporidia were seen.

The coleoptile after 5 days at 15°C. was 14mm. and the first internode 4mm. long. On the pericarp two fused promycelia detached from their parent spores and many germinated spores were seen. At 10 days, a 40μ germ tube was seen on the pericarp but no sporidia were seen on pericarp or coleoptile.

At 20°C.after 5 days, the coleoptile was 7mm. long and the first internode 18mm. long. On the pericarp spores were present with germ tubes 3.5 x 5-8μ. By 10 days many promycelial fusions were evident usually still attached to their parent spores. Some promycelia had no cell contents in the segment nearest the spore (this will be referred to again later). Some sporidia,

...3.5 x 6-9μ, were seen at 5 and 10 days on the surface of the pericarp.

After 5 days at 25°C. many promycelia with fused segments were attached to their parent spores. Several promycelia with thin first segments were seen on the pericarp. No sporidia were observed at 5 or 10 days.

At 30°C. the coleoptile was 22mm. long and the first internode 11mm. long after 5 days; there were fewer germinated spores on the pericarp than at 25°C. Sporidia or promycelial fusions were not observed at 30°C. Several promycelia with distorted first segments as described at 25°C. were observed at 5 but not at 10 days. The coleoptile and first internode had only a few germinated spores.

Experiment III - The effect of temperature on the stages of spore germination using glumed oats.

S147 hulled oats with their glumes were inoculated with C1 by the partial vacuum technique and dried for 24hrs. at 18-20°C. in the laboratory. They were then placed on moistened filter paper, 5 per Petri-dish at 0, 5, 8, 20, 25 and 30°C. for 5 and 10 days. The coleoptiles, glumes and pericarp were examined in lactophenol and cotton blue.

No oats had germinated at 0°C. after 10 days. Ungerminated spores were present on the pericarp and the

...glumes. A few spores with germ tubes 1-3μ long (Fig.5A) were present after 10 days.

At 5°C. oat germination had occurred only after 10 days but germinated spores were present on the glumes and pericarp after 5 and 10 days. The promycelia sometimes had narrow tips and fused segments. Occasionally the promycelia were elongate, the maximum length seen was 26μ. No sporidia were observed.

At 8°C. oat germination had occurred only after 10 days, the coleoptiles (average length 5mm.) were hidden by the pales. Spore germination had occured on the glumes and pericarp but on the coleoptiles only a few germinated spores were seen. After 5 days the germ tubes were approximately 16-23μ long, by the 10th day they were 26-30μ long. The promycelia often had narrow tips and fused segments (Fig.5B). No sporidia were seen.

At 20°C. all the oats had germinated after 5 days, many germinated spores were present on the glumes and pericarp but fewer on the coleoptile. Fusions between promycelial segments were common; some of the first segments were without contents. Some sporidia (6.5 x 3.5μ long) were seen on the glumes but they were less common on the pericarp and coleoptile. After 10 days fused promycelia were often present, generally detached from their parent spores (Fig.5C).

At 25°C. the stages of seed and spore germination

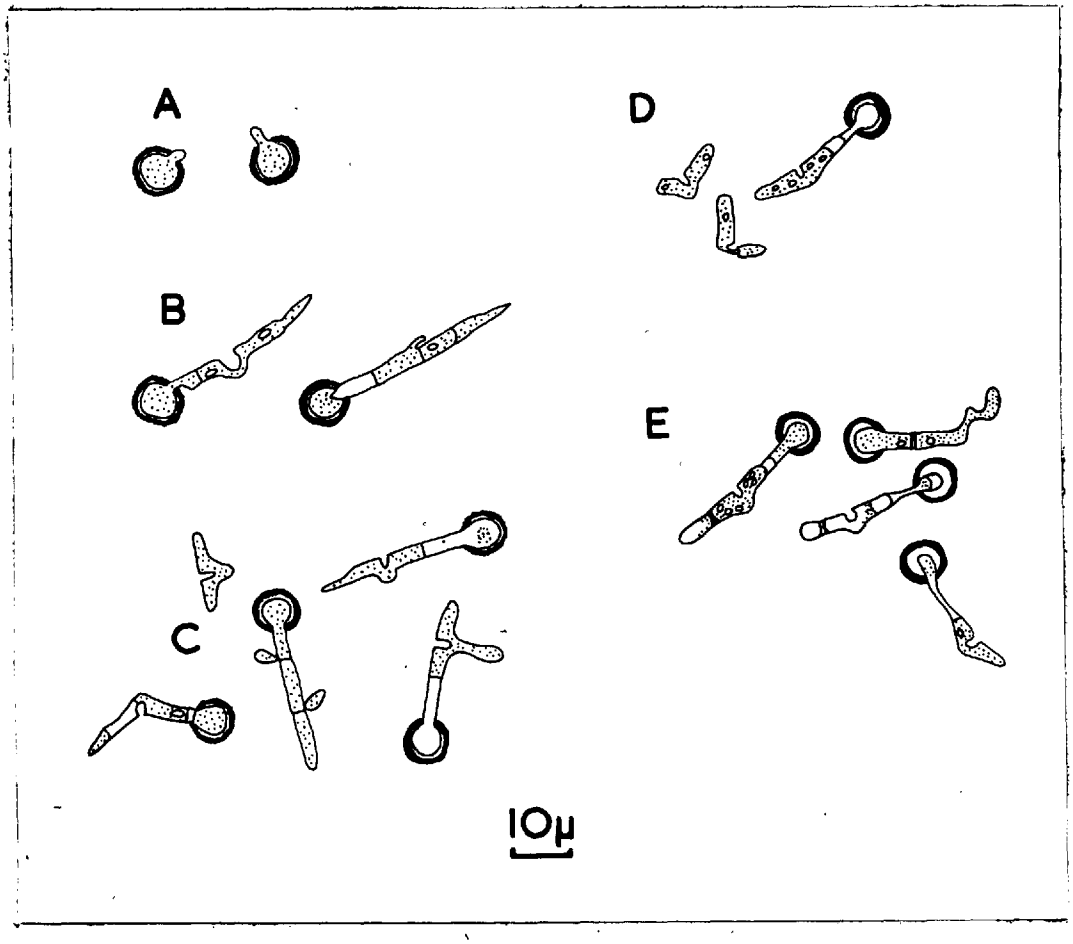

Fig. 5. Spore germination on moistened glumes of inoculated S.147 oats after 10 days. A, B, C, D and E refer to temperatures of incubation 0, 8, 20, 25 and 30°C.

...were very similar to those at 20°C. In addition, however, several sporidial fusions were observed on the glumes after 5 days. Fig.5D is a diagrammatic representation of detached and attached fused promycelia and fused sporidia on the glumes after 10 days. There is often a close similarity of form between some detached fused promycelia and fused sporidia.

Spore germination on the glumes after 5 and 10 days at 30°C. differed from 20 and 25°C. in that no sporidia were seen and the promycelia were often abnormal (Fig.5E). The first promycelial segment was often thinner and did not stain in cotton blue. The promycelial fusions were common but the promycelia did not detach from the parent spores. No spore germination was observed on the pericarp or coleoptile.

Discussion

In the first part of the thesis attention has already been drawn to the abundance of sporidia produced from spored on agar at 20°C. There was a difference in the products of spore germination on the surface of the host. A few sporidia were seen only in the experiments at 20 and 25°C. on dehulled (Experiment II) and glumed (Experiment III) oats, and were not seen at all in the experiments to determine the stage of spore germination responsible for penetration (Experiment I). It is apparent therefore

...that sporidia are of secondary importance in the penetration of oat grains. This confirms the results of Brandwein (1944) who found with U.kolleri 52 penetrations from fused sporidia out of a total of 532 penetrations. Huttig (1931) and Western (1937) have both suggested that the products of spore germination vary with temperature. The temperature experiments on oats inoculated by different techniques (Experimens II and III) confirm these observations. Different stages were predominant at different temperatures, notably production of sporidia and detached promycelia at 20 and 25°C., and 'deformed' promycelial segments at 30°C.

In Experiment I, penetrations direct from promycelia form an important proportion of the penetrations of the host. This verifies the observations of Western (1937) and Brandwein (1944). The larger number of penetrations seen in the first series of Experiment I is probably due to the greater viability of the sample of spores as the spores used in series 2 were known to have poor viability. It is possible that the dikaryophytic state is achieved in direct penetrations by later fusion of mycelium within the host, perhaps by fusion with mycelium derived from a promycelial fusion. Fusions of the mycelium from direct penetrations with other mycelium within the host were sought, but not observed.

C. STUDIES OF PENETRATION AND HOST PARASITE RELATIONS

Review of Literature

Spore germination occurs on the surface of the coleoptile and first internode. Kolk (1930) found the holes through which U.avenae entered the seedling were the same diameter as the hyphae. However, Tisdale (1917) using Fusarium lini and flax states that the diameter of the opening made by a hyphae is somewhat less than the regular diameter of the hypha itself. Swinburne (1960) has shown that penetration holes caused by T.caries often occur in lines probably due to to external mycelium penetrating at intervals. Woolman (1930) also with T.caries found 75% of the points.of attack occured in the lower 2. 5cm. of the coleoptile. He thought that the hyphae often penetrated between the cells and then entered the lumen of one of the adjacent cells. Brefeld (1895) did his classical experiments with Ustilago carbo (U.avenae) on oats to investigate the period of susceptibility in seedlings. He found the seedlings remained susceptible until the first leaf had emerged 1cm. beyond the coleoptile sheath.

Woolman (1930) studying penetration by T.caries of susceptible wheat varieties found that the first indication of an attack on the cell wall of the epidermis

...is an epidermal thickening. When seen in tangential sections this appears as a circular spot sometimes exhibiting zonation. Woolman thinks this thickening is due to gelatinization of the epidermis between the cuticle and plasma membrane. Both Kolk (1930) using U.avenae and Western (1936) with U.kolleri think that the membrane is invaginated rather than penetrated. A conical protuberance was seen by Woolman (1930) on the inside of the epidermis just beneath the point of attack of the hypha. When the hypha increases in size the conical portion becomes globular. Finally it becomes an elongated cylindrical sheath 8-12μ in diameter, enclosing the hypha which has a diameter of 1μ or less. Sheathing is characteristic of many smut fungi, for example, Malik (1959) describes sheathing of U.nuda and Woolman (1930) of T.caries. Fellows (1928) studying Ophiobolus graminis on wheat describes similar structures which he has termed 'lignitubers'. The formation of the infection tube for U.avenae apparently proceeds along similar lines to the development of the haustoria in the Erysiphaceae (Smith, 1900). The sheath, passing across the epidermal cell often widens to form a 'funnel' (Kolk, 1930). Western (1936) using U.kolleri examined sheaths microchemically and found they were not of lignin or of cellulose. Woolman (1930) also thinks the sheath is not of cellulose as it has an intense grass positive

...reaction. In resistant varieties of oats Western (1936) has shown that the formation of similar sheaths can increase the resistance of the plant to a particular strain of the smut.

Methods

It was decided to investigate in detail the phases of penetration and host parasite relations in a susceptible variety, and to compose penetration in some susceptible and resistant varieties. All the oats were inoculated with C1 by the dehulling technique then sown in sand at 20% water holding capacity in a 20°C. incubator. After 5 days the containers were removed to the laboratory. The sand was kept at the original moisture content. The susceptible variety was S.147 and the resistant varieties Camas and Nicol. These had been found to be resistant to C1 in physiologic race experiments.

Penetration and host parasite relations in a suceptible variety.

Ten seedlings were examined daily 2-8 days after sowing. The pericarp, coleoptile, first leaf, and first internode were examined by the lactophenol technique. Specimens were heated for 10min. in hot lactophenol then transferred to cotton blue in lactophenol for 5min. Finally the specimens were washed in cold lactophenol

...to remove excess stain and mounted, outer epidermis uppermost, in glycerine. The pericarp was stripped from the dorsal surface of the grain. In addition, each day 5-10 additional seedlings were treated in the following way. The coleoptile and first internode were fixed in formo-propriono-alchol (F.P.A.) for 20min. under vacuum in a desiccator. After dehydration the material was embedded in 52°C. paraffin wax, sectioned at 10-12μ and stained in Johansen's Quadruple Stain (Johansen 1940).

Penetration in susceptible and resistant varieties.

Thin strips from the base of the coleoptile were removed, some were mounted in glycerine, others were cleared first by the lactophenol technique. This was done at intervals from 34 to 136 hours after sowing.

Results

After 2 days surface preparations were made of the pericarp and coleoptile (2mm. long). Germinating spores were present on the surface and intracellular mycelium within the pericarp. Many of the spores had promycelia with fused segments (promycelial fusions). Some of these had become detached from their parent spores and in one instance a hypha had arisen from the fusion bridge between the segments. Only one pericarp penetration was seen, and it had originated from the apical cell of a

...promycelium. Sheaths were usually present around the intracellular mycelium. Within the cells the sheathed mycelium was 3.5μ in diameter; within the cell walls the mycelium and sheaths increased in diameter to 6.5μ forming diamond shaped structures. In the outer layers of the epidermis funnel shaped structures were often seen arising from points of penetration, they were formed by a sheathing of the penetrating hyphae. The funnels or 'penetration tubes' will be described later in more detail.

A few germinated spores were present on the surface of 2 day old coleoptiles but there was no development of internal mycelium. One spore had produced a promycelium with a narrow apical region similar to the structures in Fig.5C. Only occasional penetrations were observed mainly direct from the apical cells of promycelia. For ease of description this type of penetration will be called "penetration direct from the promycelium". The coleoptile cells usually possess non-vacuolate cytoplasm at 2 days old. In one instance a penetration tube was seen (Fig.6A) apparently invaginating the cytoplasm; the tube had not reached the far wall of the outermost cell and was surrounded by deeply staining cytoplasm. The internal diameter of the penetration hole (Fig.6A) could be seen by focussing downwards from above inside the hole. At first the sheath surrounding the penetration tube could not be seen but there was visible an approximately

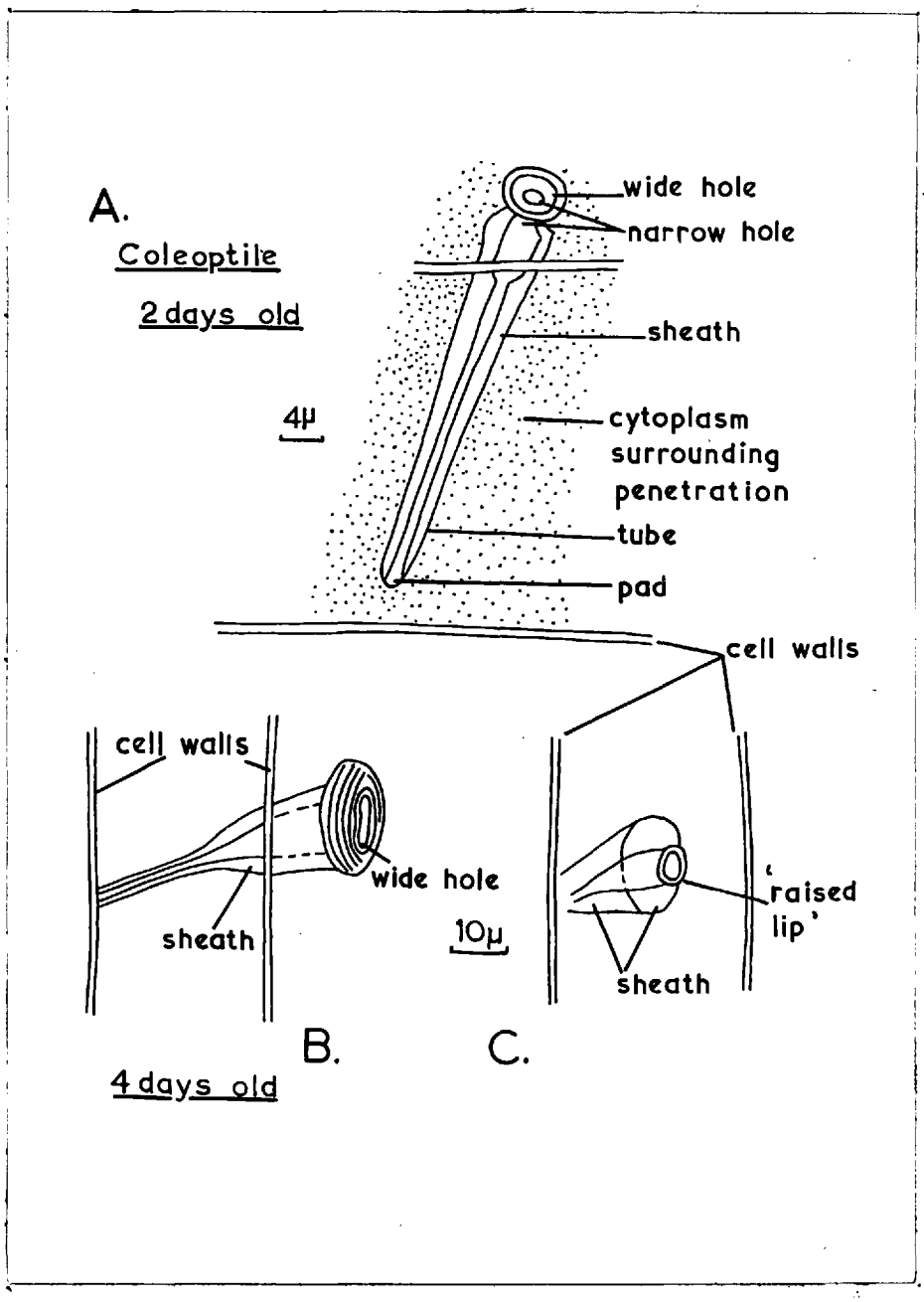

Fig. 6. Coleoptiles showing, A early penetration at 2 days, and B and C penetration holes and sheaths after 4 days. Drawn with the aid of a camera lucida.

...circular penetration hole of large diameter with a swollen rim. Beneath this the hole was circular but of smaller diameter and the sheath surrounding the invading hypha visible. In deeper layers the hole was successively triangular, kidney-shaped and finally circular. At the lower end of the penetration tube there was a small protuberance or swelling (Fig.6A). Coleoptiles sectioned at 2 days confirmed that penetration holes and tubes but not internal mycelium were present.

In seedlings 3 days old many fused promycelia both attached to and detached from their spores adhered to the pericarp; several penetrations and much intracellular branched mycelium was observed in the pericarp. A limited amount of intracellular mycelium and occasional penetration holes (Fig.7) were seen in the coleoptile (4mm. long). Sectioned coleoptiles showed mycelium in the inner epidermis parallel to the first leaf space. Specimens of the first internode examined by surface preparation showed fewer penetrations and less mycelium than the coleoptile, but, in sectioned material, mycelium was prevalent in the first internode.

After 4 days, intracellular mycelium was prevalent in the first internode in surface preparations and sections : 40% of sectioned seedlings had mycelium in the first internode. Several penetration holes were observed one of which was the same diameter as the

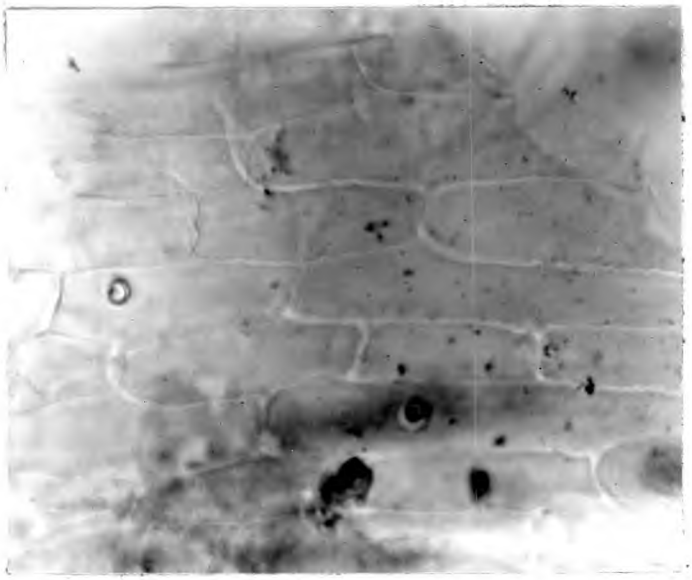

Fig. 7. Penetration holes in the coleoptile of a seedling 3 days old. x640

Fig. 8. Penetration hole and sheathing in the coleoptile of a seedling 4 days old x 640.

...penetrating hypha. In sectioned material 20% of the plants had mycelium in the third node and in the first leaf base. The coleoptile, 10mm. long, was above ground level but still enclosed the first leaf. Much intracellular and some intercellular mycelium was present in the coleoptile tissues. Many penetration tubes, frequently at an oblique angle to the surface, were seen half way between the coleoptile tip and node; often they were in groups and occasionally in straight lines. Sometimes an external hypha could be found still attached to the hypha within the host (Fig.9). The sheaths surrounding the invading hyphae were widest at the point of entry; usually there was a sharp decrease in diameter of the sheath across the outermost cells of the coleoptile(Fig. 9). Penetration was studied in detail in surface preparations (Fig.6, 8 and 9). At first there was only an indistinct outline of the penetration sheath; the penetration hole was large in diameter (4μ), and bordered by a prominent lip. Below the large hole there was often a smaller hole usually the same diameter as the penetrating hypha. The sheath was now clearly visible and sometimes appeared to be laid down concentrically (Fig.6B). At a deeper level the hole was triangular in shape but there was no evidence for collapse of the penetration tube. Later the sheath and mycelium narrow, the mycelium appearing kidney-shaped in cross-section. Not all

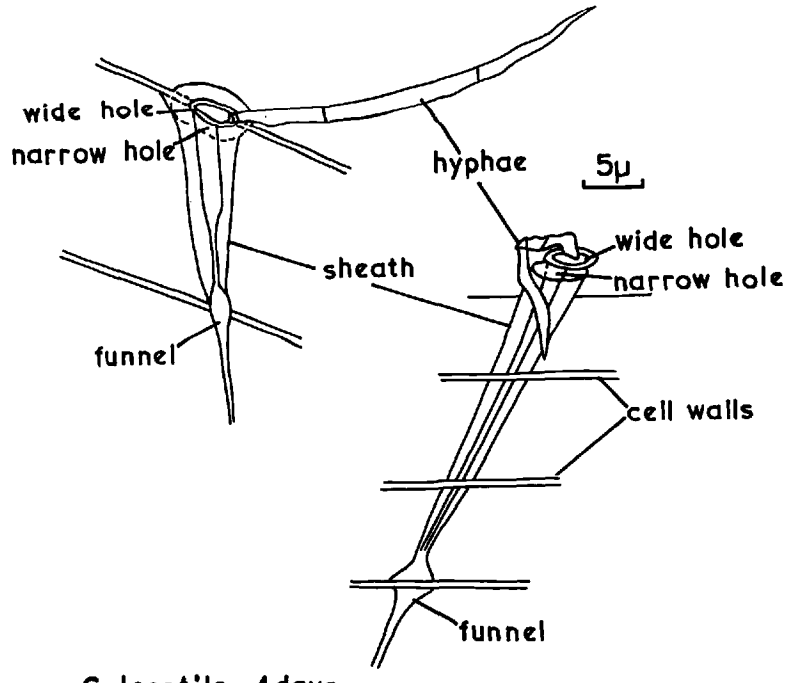

Fig. 9. Coleoptiles 4 days old showing details of the penetration holes, associated mycelium and sheathing, drawn with the aid of a camera lucida.

...penetrations were as described above; many had only one penetration hole and others a vesicle below the cuticle (Figs. 6C and 8).

After 5 days, the coleoptile was 18mm. long and still enclosed the first leaf. Much intracellular mycelium was present in the pericarp, coleoptile and first internode. In sectioned material, 50% of the seedlings had mycelium in the third node and third leaf base but only 20% mycelium in the upper first leaf.

Intracellular mycelium was prevalent in the pericarp, first internode (23mm.) and coleoptile (22mm. long) at 6 days. Intercellular mycelium was seen in the deeper layers of sectional coleoptiles. Sheathed intracellular mycelium was studied in the coleoptile; within the cells the diameter was 3.5μ but increased to 6.5μ across the cell walls forming diamond shaped structures. A stage in the development of one of these structures was observed; it consisted of a funnel-shaped structure with its base adjacent to the cell wall. The sheathed mycelium in the cells is often of varying diameter and surrounded by cytoplasm (Fig.10). The hypha inside the sheath was usually thin in diameter and did not readily stain. Occasionally within the cells short intracellular branches were seen arising from the main hyphae. The relations between host and parasite never appeared to be unusual, at all times the host nucleus appeared normal.

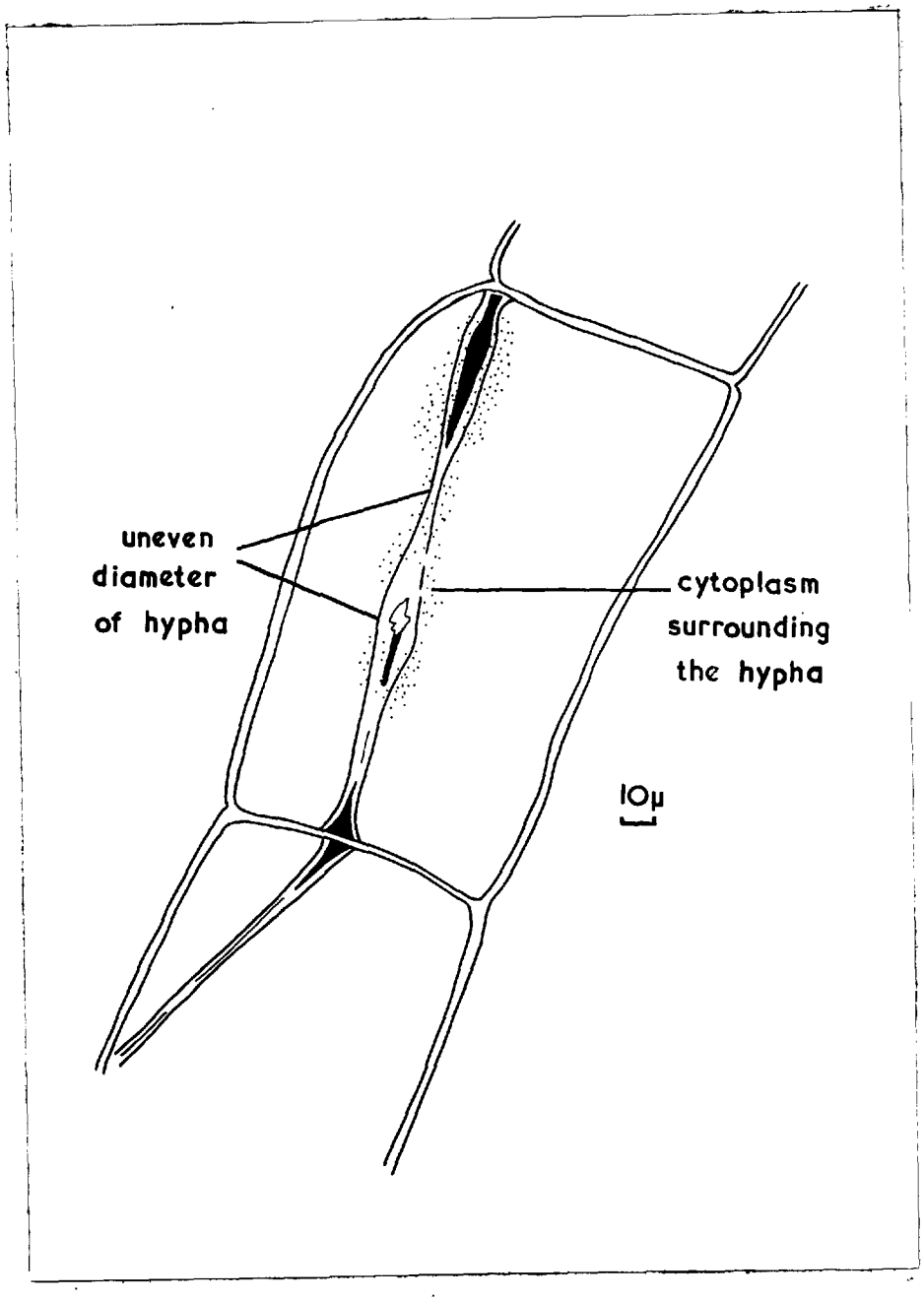

Fig. 10. Coleoptile of seedling 6 days old showing mycelium. The nucleus has been omitted.

Comparison of coleoptile penetration in susceptible and resistant varieties.

The earliest invasion of S.147 coleoptiles seen was as an invagination of the cytoplasm by a cone-shaped tube (similar to Fig.6A), 37 hours after inoculation. No very youhg stages were observed. By 38 hours, many germinating spores were present including a promycelium 27μ long with a narrow tip. By 136 hours (5.5 days) after inoculation much intracellular mycelium with small intracellular branches were prevalent throughout the coleoptile.

The first penetration tubes were observed in Camas and Nicol coleoptiles after 37 hours. By 46 hours both varieties had many penetration tubes similar to those in S.147. By 136 hours intracellular mycelium was prevalent in both Nicol and Camas. A piece of mycelium 420μ long was seen in Nicol. Mycelium in the coleoptiles of the resistant varieties had similar characteristics to that in S.147; the older portions of the mycelium were of unequal diameter and did not take up stain.

Discussion

Brandwein (1940) using Monarch oats and U.levis found that 36-42 hours after inoculation was the effective period of infection. The first penetration tube seen in S.147 coleoptiles was 37 hours after inoculation with U.avenae. There is thus a close correspondence with

...the times described by Brandwein.

The interpretation of the penetration holes and sheathing is complicated by possible artifacts. Penetration holes one above the other (Figs. 6 and 9) were often seen. They may be due to the invading hypha entering at an oblique angle (Fig.9) or they may be due to the promycelium and the narrow tip causing holes of differing diameter. Possibly the diameter of the large hole is due to stretching of the outermost layers in cell elongation. The penetration tube with pad at the base (Fig.6) is reminiscent of haustorical development in the Erysiphaceae (Smith 1900). No microchemical tests were made on the sheaths. Their origin and nature were not determined as this aspect has been dealt with by Western (1936).

Mycelium was often abundant in the inner epidermis of the coleoptile of older seedlings. It seems possible that this layer can act as a barrier and this point will be reviewed later. The variations in diameter of the hyphae in 6 day old coleoptiles (Fig.10) are similar to those figured by Kolk (1930). It has been suggested the variations could be due to swelling prior to disintegration or perhaps as a result of stretching in the cells (Kolk, 1930).

Coleoptile penetration and development of the mycelium in the resistant varieties Nicol and Camas

...was similar to that in S.147 Western (1936) using a susceptible and a resistant oat and several strains of loose and covered smut has enumerated 5 grades of resistance. The type of resistance exhibited by Camas and Nicol is not of the most severe type described by Western (which resulted in non-penetration of the coleoptile) It is a less severe type permitting penetration and establishment of mycelium in the coleoptile but preventing it from reaching the growing point and producing smutted panicles.

D. THE PATH OF THE MYCELIUM IN DEEPER TISSUES

Review of Literature

In the past there has been some debate on the path of oat smut mycelium from the coleoptile to the first leaf. Butler (1918) thought that mycelium passed from the coleoptile to the base of the first leaf in a channel among the vascular tissues of the third node. Kolk (1930), in inoculated oats 2.5 days old, has described and figured mycelium of U.avenae in the gap between coleoptile and first leaf. However, Western (1936), did not find mycelium of U.kolleri in this position.

Kolk(1930) found that U.avenae mycelium was mainly intracellular in the coleoptile, as the fungus proceeded deeper into the host the mycelium became increasingly intercellular until in the growing point all the mycelium was intercellular. She observed that mycelium in the older vacuolated cells was usually found in the layer of cytoplasm around the periphery of the cell. There was no evidence of any abnormal changes in the growth and behaviour of the host cells or nucleii. However, Walter (1934) found that mycelium of Ustilago zeae in maize plants was attracted towards the host cell nucleii.

Oat smut mycelium (U.kolleri) was reported by Lutman (1910) to be in the growing point 10-12 days after

...sowing. Western (1936) found that U.avenae mycelium was present earlier than 21 days; Kolk (1930) by 30 days and Brandwein (1937) also in 30 days (U.avenae and U.kolleri).

Gage (1927) using both oat smuts found that in mature smutted plants mycelium was most abundant in the nodes; it was mainly intracellular. This is contrary to the observations of Butler (1918), and Roesch (1926). In the internodes, mycelium was sparse and the hyphae long and unbranched, in the nodes the hyphae were short, convoluted and much branched. These findings confirm those of McAlpine (1910). Gage did not find any evidence for mycelial degeneration in the lower portions of the culms.

The next section is an account of the path of the mycelium from the coleoptile to the growing point. The way in which the mycelium passes from the coleoptile and first internode to the first leaf has been studied in detail.

Methods

Path of the mycelium from coleoptile to first leaf

All the seed was inoculated by the dehulling technique. The first series of harvests were made 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 days after inoculation, later harvests were after 2, 3, 4 and 5 days and were

...restricted to seedlings with an intact coleoptile sheath. This was done to avoid the possibility of contamination by spores in the coleoptile first leaf space. About 5 plants were fixed per harvest. The material was an inch long cut three-quarters of an inch above, and including the growing point. Fixation was in F.P.A. under reduced pressure for 20min., the material was then left in the fixative for a further 24hours. Specimens were then dehydrated in tertiary butyl alcohol, embedded in wax (M.Pt. 54°C.), sectioned at 10-12μ on a rocking microtome and stained with Johansen's Quadruple Stain. A total of 35 seedlings were sectioned.

Path of mycelium from coleoptile to growing point.

Inoculation was by the partial vacuum and de-hulling techniques with S.147 oats. There were two sowings of 15 tins for each inoculation technique and they were made 6 weeks apart in September-October 1961. Some of the plants were kept to maturity to determine the amount of infection before sectioning; the remaining plants were harvested at intervals, stored, and if the infection in the sowing was high, later sectioned. The time of harvesting was determined by the stage of growth of the plants. Ten plants were harvested at each of the following growth stages. These were the middle first, second, third, fourth, fifth and late

...sixth, leaf stages. The cutting, fixing, embedding and staining methods used were as described above. Camera lucida drawings of sections were obtained as described by Western (1936). A median section was first drawn and on this drawing was included all the mycelium seen under the low power in the median section and in the two sections on either side of it. The drawing therefore was an indication of the mycelium in five serial sections.

The internodes, and nodes examined were only taken from plants possessing smutted panicles. The internodes and nodes were sectioned 12μ thick by means of a freezing microtome and the sections stained with cotton blue and mounted in glycerine.

The nucleii of parasitic mycelium were stained with Heidenhain's Hematoxylin. The material was first embedded in 54°C. wax then sectioned.

Results

Path of the mycelium from coleoptile to first leaf.

Green stained penetration hyphae were present in the outermost layers of the coleoptiles 2 days old. A few short pieces of thick red intracellular mycelium were present in the first internode beneath the third node. There were no extensive development of mycelium. The cells in the lower part of the coleoptile and first leaf were meristematic.

Three days after sowing the cells of the first leaf were still meristematic but elongation was occurring in coleoptile cells. Six plants were sectioned, 4 had mycelium in the coleoptile and 2 in the first internode. Penetration points and green sheathed intracellular mycelium some of which appeared to be surrounded by cytoplasm, were seen in the coleoptiles. One plant had red intracellular mycelium in the cells at the junction of the coleoptile and first leaf and much red intercellular mycelium in the inner epidermis of the coleoptile.

After 4 days the five seedlings sectioned had mycelium in the outer layers of the coleoptile. Two seedlings had purple intercellular mycelium in the inner layers and two seedlings had mycelium in the first internode and another in the third node. Intercellular mycelium was seen in the first leaf base of one seedling but none in the space between the coleoptile and first leaf.

All the eight seedlings sectioned 5 days after sowing had mycelium in the coleoptile and first internode. In the outer layers of the coleoptiles the mycelium was green and intracellular and in the first internodes it was mainly red and intracellular. Four of the seedlings had intra- and intercellular mycelium in the third node and red intercellular mycelium in the first leaf base (Fig.11). In one instance a long piece

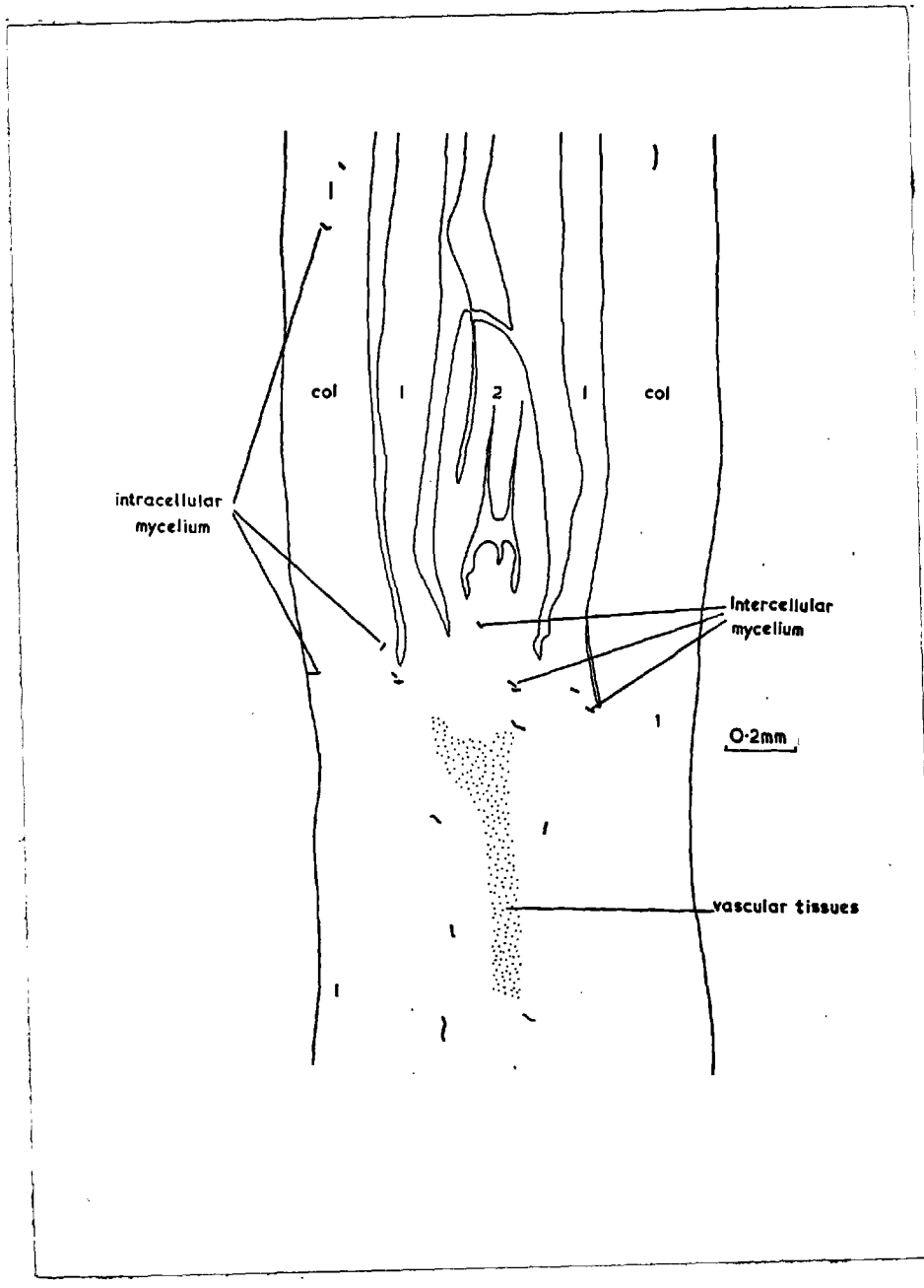

Fig. 11. Camera lucida drawing of L.S. crown region of 5 days old seedling.

...of red intercellular mycelium was present in the first leaf just above the growing point.

Only one seedling was sectioned 6 days after sowing. The cells of the coleoptile and of the first leaf above the growing point were still elongating. Mycelium was present in the cells of the coleoptile first leaf junction, in the base of the first leaf and in the tissues between the fourth node and the growing point. The mycelium was stained red in older cells but purple in the meristematic tissues. No mycelium was seen in the upper levels of the first leaf or in the gap between the coleoptile and first leaf.

The account that follows refers to sectioned plants sown on 24th October 1961; occasional reference will however be made to an earlier sowing. The harvests do not quite correspond in leaf stage (Table 9). Both sowings were inoculated by the dehulling technique and gave similar percentages of infection (83 and 89%), at maturity.

Path of the mycelium from the coleoptile to growing point.

The first harvest was made at the middle first leaf stage, 9 days from sowing. Much intracellular mycelium is present in the coleoptile and first internode; Fig.12 shows mycelium with small branches in a thick L.S. of the coleoptile. Intercellular mycelium is

TABLE 9.

Results of sectioning the first three harvests.

Sowing 1 (14.9.61.)

Leaf stage at Harvest	Age (Days)	No. examined	Below G.Point	In G.Point Region	In Apical G.Point
2nd Leaf(early)	12	3	0	33	0
3rd Leaf(early)	17	3	0	0	100
3rd Leaf(middle)	21	4	0	0	100

% smut at maturity = 83 (40 observations)

Sowing 2 (24.10.61.)

1st Leaf(middle)	9	5	100	0	0
2nd Leaf(middle)	14	9	55	11	33
3rd Leaf(middle)	20	9	11	11	77

% smut at maturity = 89 (38 observations)

Fig. 12. Intracellular mycelium in the coleoptile (L.S.) x 640.

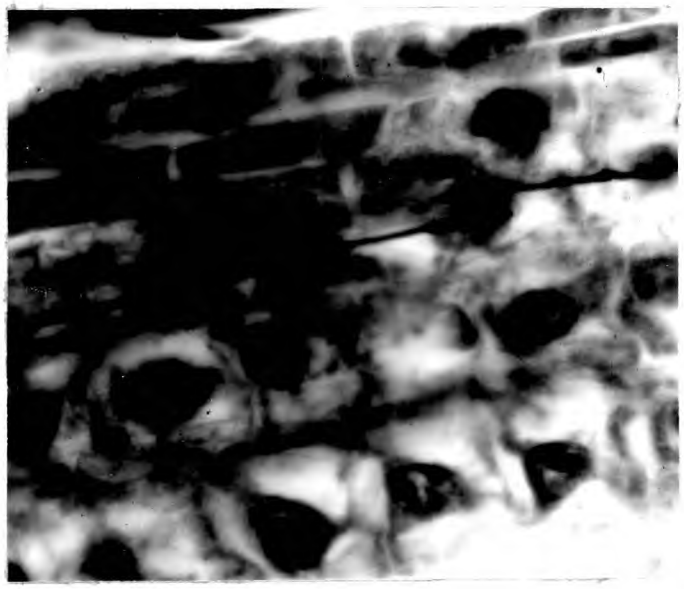

Fig. 13. Intercellular mycelium in the lower 6th leaf (L.S.) x 720.

...present in the inner layers of the coleoptile, first leaf base, second internode and second leaf base (Fig.14), but not in the growing point region or apical growing point. The mycelium is generally stained pink in the older cells and purple in meristematic tissues. No mycelium was observed in the coleoptile first leaf space.

The second harvest was made at the middle second leaf stage, 14 days after sowing; nine plants were sectioned. The intercellular mycelium had invaded the third leaf bases and the tissues near the growing point. Fifty-five percent of the plants had mycelium below the growing region, 11% in the growing point region and 33% in the apical growing point. Figs. 15, 16 and 17 are photographs of an L.S. showing mycelium in the apical growing point. There is a fan-shaped spread of mycelium. In Fig. 15 intercellular mycelium is present in the tiller bud in the axil of the coleoptile.

Fig.18 is a photograph of a camera lucida drawing of a plant in early third leaf, sectioned longitudinally. Intercellular mycelium is present in the tiller bud in the axil of the first leaf. The mycelium is probably derived from the first leaf base.

The third harvest was at the middle third leaf stage after 20 days, 9 plants were sectioned. Seventy seven percent of the plants had intercellular mycelium in the apical growing point, 11% in the growing point

...region and 11% just below the growing point. Fig.19 is a photograph of an L.S. showing typical intercellular mycelium below the apical growing point.

Tillers were generally infected by mycelium from the subtending leaf; the tillers in the axils of the coleoptiles, first leaves and second leaves were infected after 14, 17 and 21 days respectively.

In subsequent harvests, at the middle 4th, 5th and early 6th leaf stages, mycelium was present in most of the apical growing points.

Table 9 is a summary of the results obtained from sectioning the first three harvests of the two sowings inoculated by the dehulling technique.

In Figs. 13, 17 and 19 the intercellular mycelium varies in form. Fig.13 shows a long piece of unbranched purple stained mycelium in the lower sixth leaf. Where the mycelium crosses a cell wall it widens forming a diamond shaped structure. In Figs. 17 and 19 the mycelium is not long and straight but small and irregular.

Some nodes were sectioned separately; the mycelium was mainly intracellular but intercellular mycelium was also present. Fig. 21 is a diagrammatic representation of the distribution of mycelium in an L.S. of the 6th node of an infected plant; the mycelium was not observed to favour any particular tissue. Fig.20 is

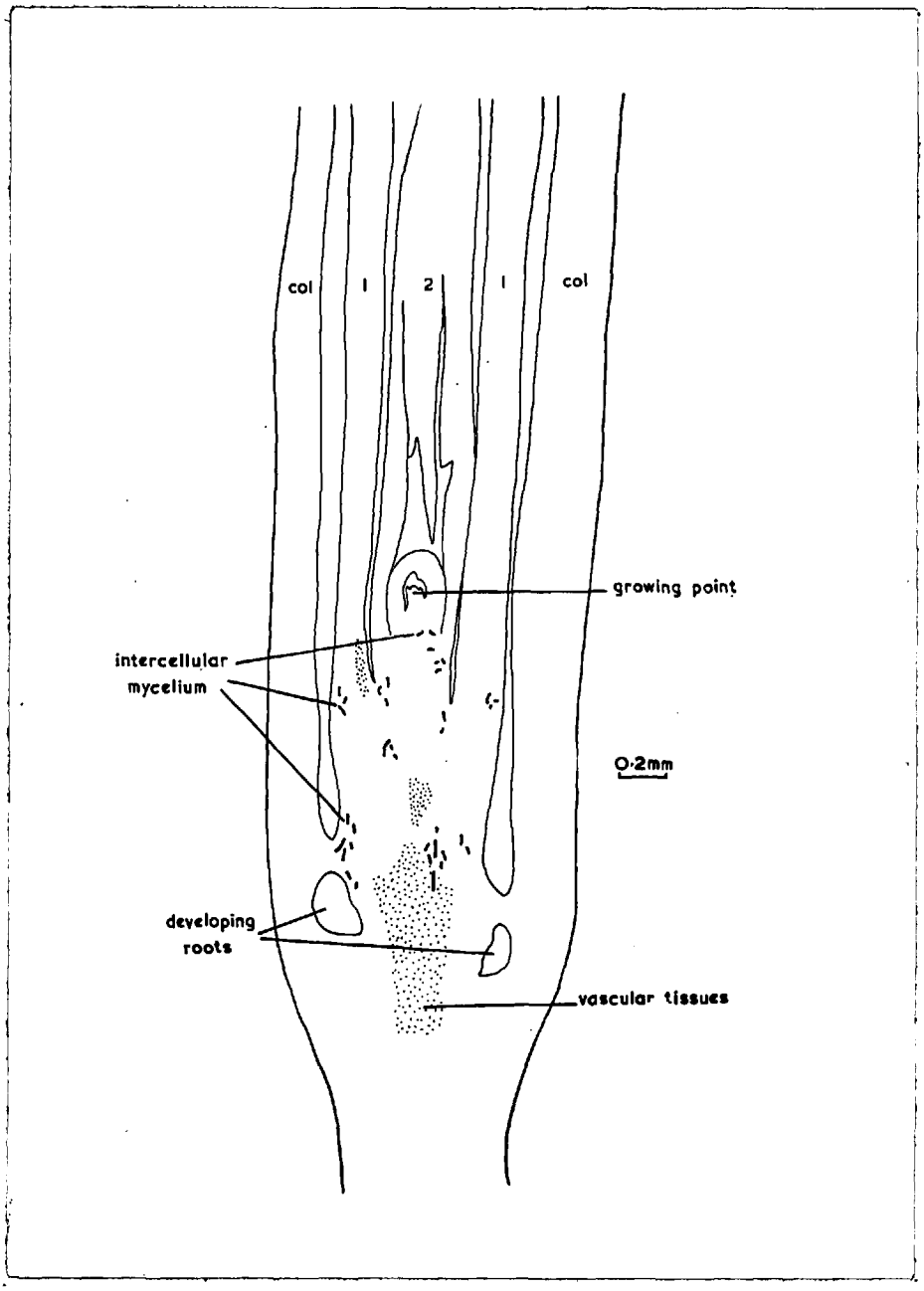

Fig. 14. Camera lucida drawing of L.S. crown node of 9 days old seedling, showing the distribution of the hyphae.

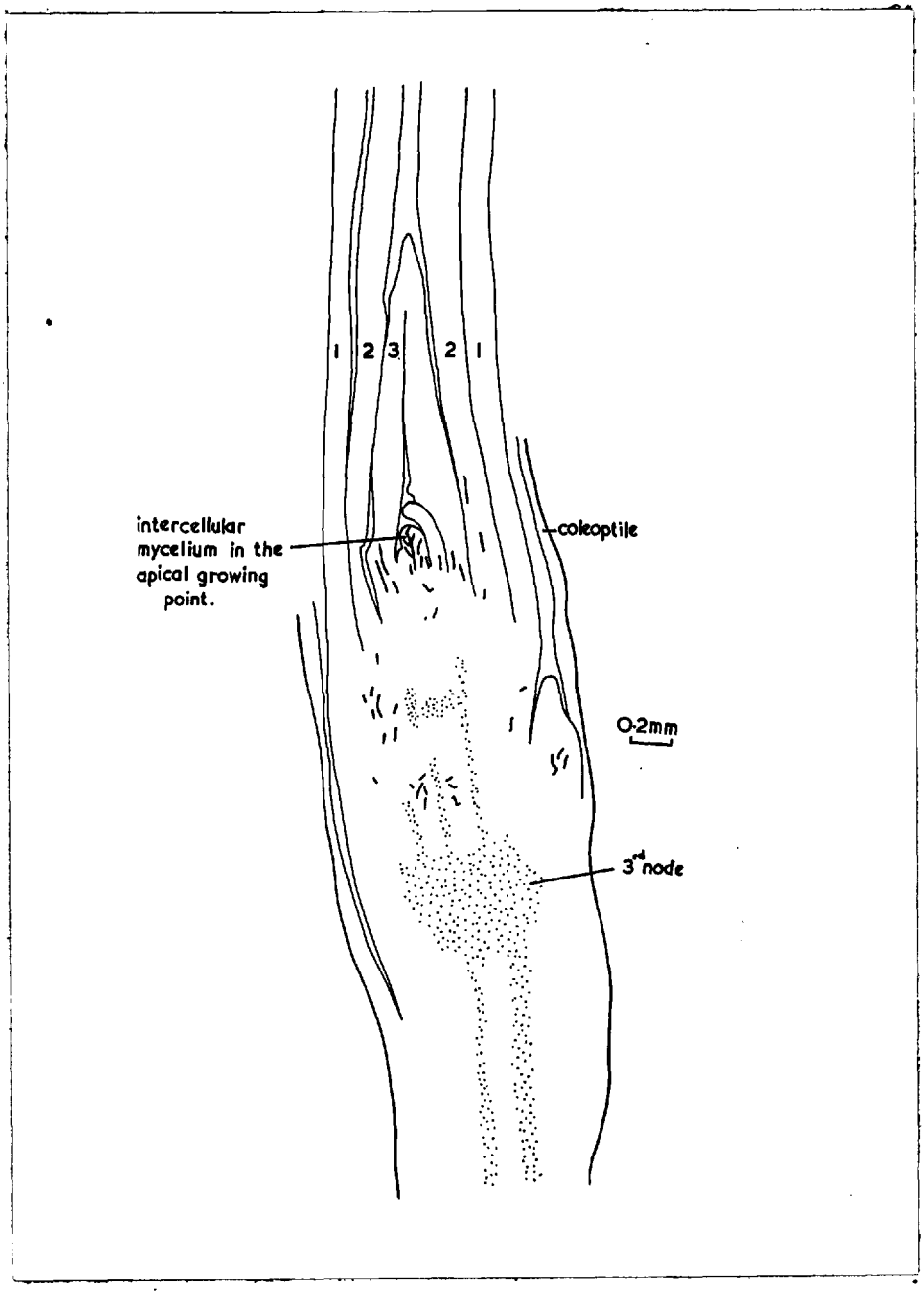

Fig. 15. Camera lucida drawing of L.S. section of seedling 14 days old, showing mycelium in the apical growing point.

Fig. 16. Intercellular mycelium in the apical growing point of seedling 14 days old. Same seedling as Figure 15. x 640.

Fig. 17. Intercellular mycelium in the apical growing point. Detail from figure 16. x 1520

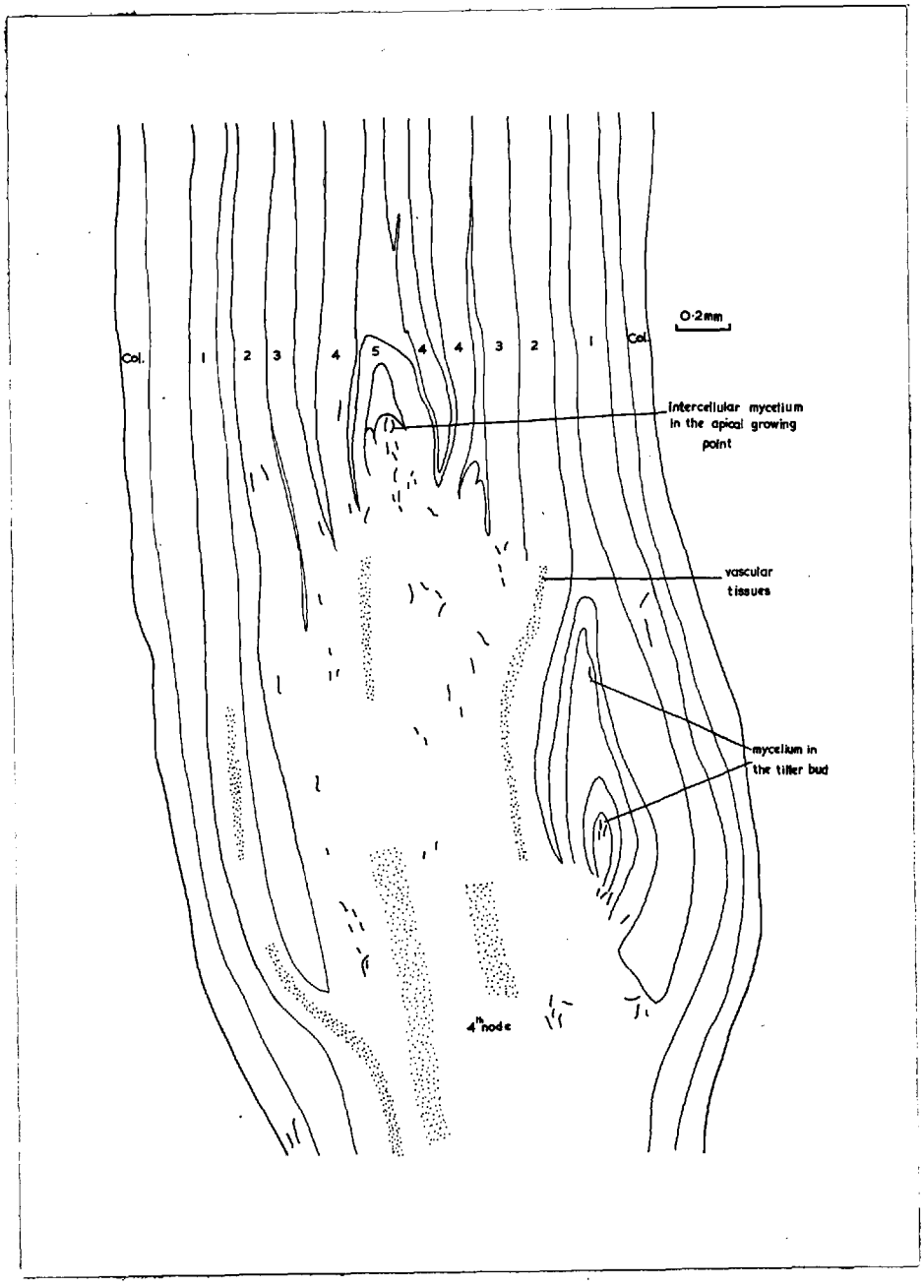

Fig. 18. Camera lucida drawing of L.S. crown node region of seedling 17 days old, showing mycelium in the tiller bud.

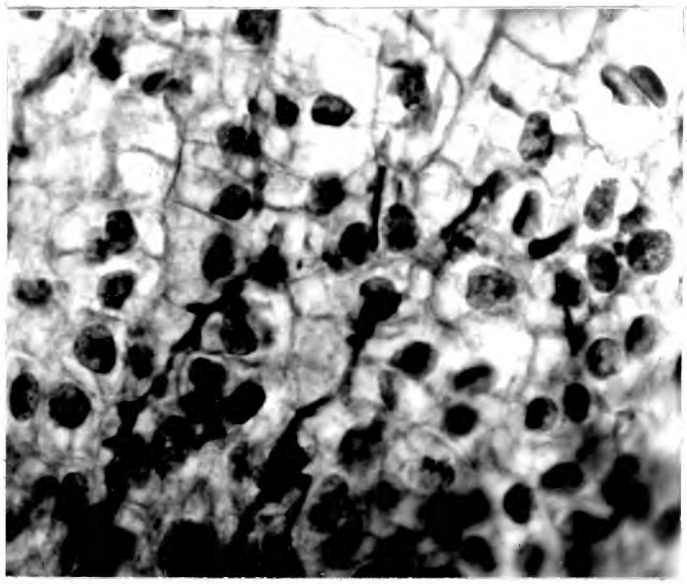

Fig. 19. Intercellular mycelium below apical growing point of seedling 21 days old. x 640.

Fig. 20. Intracellular mycelium and spores in the 9th node of plant with smutted panicle. x 160.

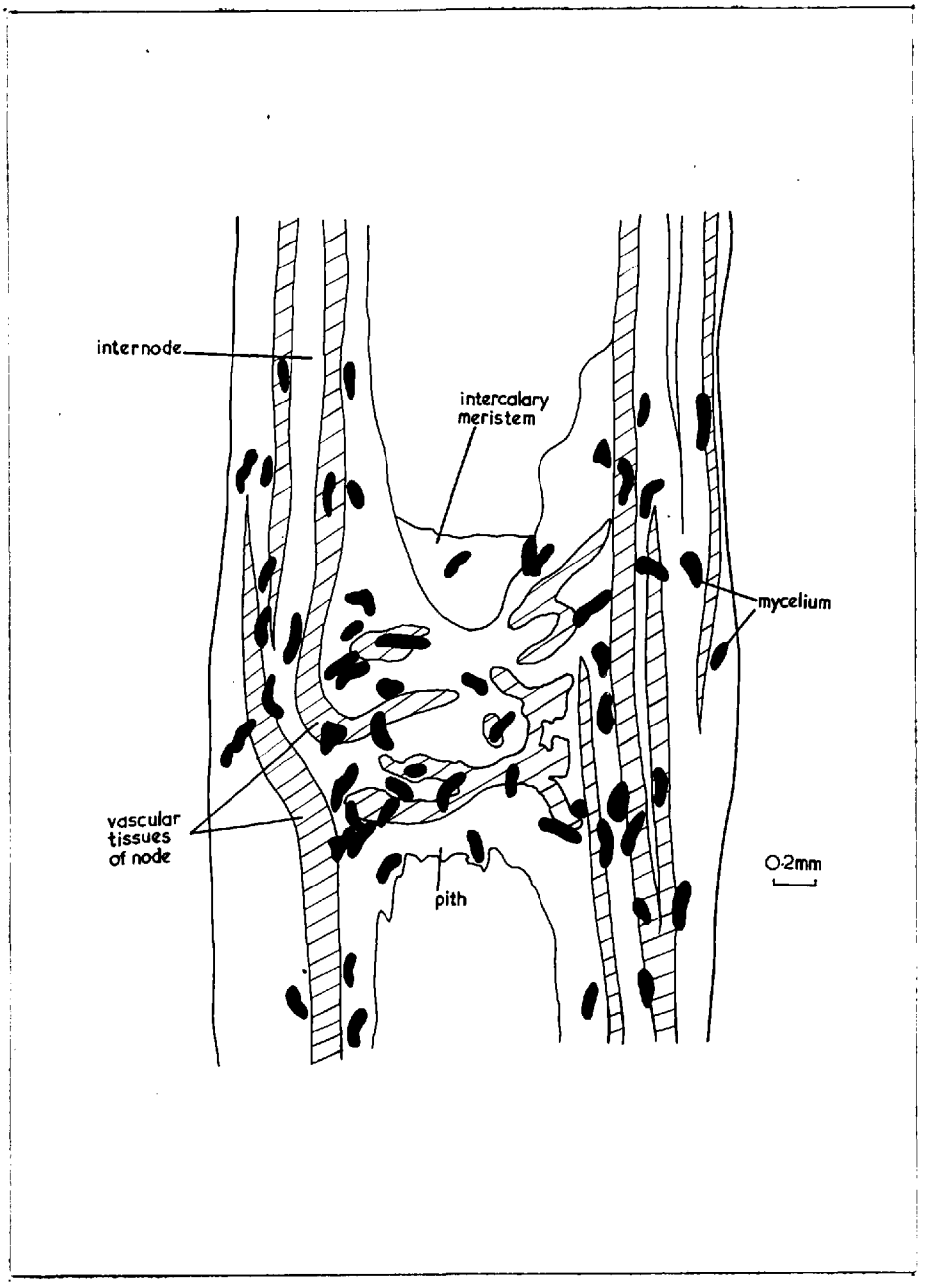

Fig. 21. Diagrammatic representation of 6th node of plant with smutted panicle showing the distribution of mycelium in the node.

...a photograph of intracellular mycelium and spores in the ninth node. The formation of spores in the nodes will be described and discussed later. The intracellular mycelium was of two types; some was thicker and more convolute. The nucleii did not stain easily. Generally, the thin mycelium was binucleate with the nucleii approximately 1.5μ in diameter. The thicker mycelium appeared to be multinucleate.

Discussion

Mycelium is often prevalent in the inner epidermis of the coleoptile. Possibly this region may act as a barrier (Fellows, 1928) preventing mycelium crossing the gap between coleoptile and first leaf.

No evidence has been found for mycelium crossing the coleoptile first leaf gap but observations have been made on the alternative route via the tissues joining the coleoptile and first leaf. Mycelium was often seen in the cells at the junction between the two tissues also the first appearance of mycelium in the first leaf is not in the upper part but at the base. These facts and the absence of mycelium in the gap indicate that mycelium does not cross the gap, supporting the view of Western (1936). The drawing given by Kolk (1930) of mycelium in the gap is not convincing as there is no continuity with mycelium in the coleoptile or first leaf tissues.

...In conclusion, mycelium reaches the first leaf either from the first internode through a gap in the vascular tissues of the third node as suggested by Butler (1918) and/or from the coleoptile via the junction between the tissues of coleoptile and first leaf. No evidence was obtained to prove or disprove Butler's theory.

Internodes in the oat are of two types, those that do and those that do not elongate (short internodes). The short internodes develop during the vegetative stage of the plant before the initiation of the panicle. The internodes that elongate develop during the reproductive stages (Bonnett, 1961). The short group begins at the coleoptile node and extends upward to include the internodes above the first, second and sometimes the third foliage leaf. If the mycelium can reach the growing point region before the elongation of the short internodes the panicles will become smutted. The fungus cannot be outgrown by the plant as it is carried passively upwards by the elongated internodes. Optimal conditions for infection prevailed in the two sowings so the fungus was able to reach the growing point region before the elongation of the internodes., resulting in high (83-89%) percentages of smut at maturity. The other 17-11% of the plants did not have smutted panicles. This could be explained in several ways. There could have been in these plants increased growth causing earlier elongation

...of the internodes or perhaps they were more resistant to infection. In addition, infection may have been affected by conditions in the microenvironment of the oats.

Mycelium was first observed in the growing point region in the early second leaf stage (12 days) and in the apical growing point by the middle second leaf (14 days). These times are in close agreement with those found by Lutman (1910) who observed mycelium of U.levis in the "growing point" after 10 days, and Western (1936) who found mycelium of U.avenae in the apical growing point before 21 days.

The variation in form of the intercellular mycelium could be explained by the nature of the cells adjacent to the mycelium. In non-meristematic regions the mycelium is long and unbranched, whereas in meristematic tissues the mycelium is short, thick, and branched. The type of cell has an affect on the size of the intercellular spaces and these modify the form of the mycelium.

Differences in staining action and position of the mycelium were seen as the fungus passed from the coleoptile to the growing point. Woolman (1930), and Swinburne (1960) noted a similar phenomenon with T.caries. Generally, the staining reactions were the same as found by Swinburne who also used Johansen's stain. There was however no intracellular mycelium in the first and second leaves the mycelium being intercellular.

...Beyond the inner epidermis of the coleoptile there was usually no sheathing of mycelium. There is a close parallel between the staining reactions of the mycelium of U.avenae and those obtained by Woolman with T.caries. His first phase of infection, the entrance into and development within the epidermal host cells in which the staining reaction is gram negative is probably represented by the green intracellular mycelium in the coleoptile of U.avenae. The onset of the gram positive second phase is probably represented by the change of the intracellular U.avenae mycelium from green to red. The change from gram negative to positive is stated by Woolman to coincide with the onset of parasitism. The third phase, also gram positive, could be represented in U.avenae by the change from red intra to red interceullar mycelium occurring approximately at the junction of the coleoptile and first leaf. It is in this region that the sheathing becomes very greatly diminished. However, in Woolman's scheme the third phase is exclusively intercellular but in U.avenae there is intracellular mycelium in the nodes.

E. SPORE FORMATION IN THE HOST

Review of Literature

One of the first to describe spore formation in Ustilago spp. was Fischer von Waldheim (1869) who noticed a tendency for spores to develop in chains. McAlpine (1910) found the spores of U.avenae were often formed in isolated groups which eventually coalesced. In the individual groups very minute colourless spores were towards the centre and dark coloured mature spores collected towards the periphery.

Lutman (1910) found the sporulating mycelium of U.levis was swollen, intercellular and multinucleate. Gelatinization of the mycelium occurred, afterwards it broke up to produce small angular segments some of which had two nuclei, others only one. The segments later changed their shape, and rounded off to form spores. Wang (1934) has shown that the multinucleate mycelium of U.avenae aggregates into fundamental masses of mycelium before spore formation. Later the mycelium becomes binucleate and spores are produced by segmentation. Wang found that the mycelial masses were first formed in the pericarp, then in the ovary. Malik (1959) found that the spores of U.nuda in barley spikes were formed by the segmentation of sporiferous hyphae.

Typical cereal inflorescence smuts commonly produce spores in vegetative tissues, notably the leaves (Fischer and Holton, 1957). Bartholomew and Jones,(1923), and McKay(1936) report sporulation of U.avenae in linear pustules on the leaves. Sampson (1929) has reported the same phenomenon with U.kolleri. McKay observed in greenhouse and field experiments that the smut was generally confined to the top leaf which was frequently twisted in to a spiral at the tip.

Spore formation in the nodes has been seen in wheat infected with T.caries (Swinburne 1960), and barley infected with Ustilago hordei (Grasso, 1953). Swinburne found that the spores were formed along the length of the pith of the last internode (flower stalk). There were no external signs of them.

Kavanagh (1961) has investigated the effect of temperature on spore formation in the loose smuts of barley and wheat. Infected plants grown at 18°C. produced normal spikes; at 24°C some spikes were normal but there was some reduction in spore formation, and at 29°C few or no spores were formed. Kiesling (1962) has carried out similar temperature experiments with U.hordei, growing inoculated plants at 16, 20, 24 and 28°C. All the inoculated plants grown at 24°C were infected; sori developed in the leaves of infected plants grown at 20, 24 and 28°C., but only head sori developed at 16°C.

The following section is an account of spore formation and distribution in the ovaries and vegetative tissues of plants infected by U.avenae.

Methods.

Onward and S.147 were inoculated with C1 by the dehulling and partial vacuum techniques and sown 2.5cm. deep in John Innes compost. The greenhouse temperature was uncontrolled; over the experimental period there was a daily mean temperature of 18°C. and a daily range from 6-29°C. The plants were harvested at intervals. Several methods were used for examining the smutted panicles.

1) Material containing the young panicle was cut into 3cm. lengths and fixed in F.P.A. in a pressure desiccator. Air bubbles were removed from the material by evacuation. After dehydration in tertiary butyl alcohol the material was embedded in 54-57°C wax. Sections were cut with a rocking microtome at 8-12μ and stained with Johansen's Quadruple Stain (1940).

2) Fresh material of developing panicles was sectioned with a freezing microtome. The sections were stained in cotton blue in lactophenol and mounted in glycerine.

3) Growing points were dissected out with needles and the length of the young panicles measured. The staining technique used was the same as 2.

The distribution and formation of spores was also studied in the leaves and culms. Sori on the leaves were sectioned with a freezing microtome. The same instrument was used for sectioning the nodes and internodes of five S.147 plants possessing smutted panicles.

Results.

Developing panicle

The first indication of the differentation of the panicle is the appearance of single lateral alternate projections arising in the axils of the leaf initials beneath the apex of the growing point. Young panicles 1mm. long (Fig.22) have scattered intercellular mycelium through all the tissues. In panicles 1.5cm. long (Fig.23) spore formation has occurred in patches (fundamental masses). The oldest spikelets at the top of the panicle are more developed and have more smut mycelium and spores than the younger spikelets at the base. The first macroscopic evidence of spore formation is usually seen when the panicles are 2cm. long. Infected panicles have a whitish - light brown colour. Later, in panicles 3cm. long, the colour changes to a blue - brown at 4cm. to a light brown. The fundamental masses have by now increased in size and filled the ovary with spores. The stages of spore formation can be seen in almost any infected panicle 1-2cm. long (Fig.23); Figs. 23-28 are

...of the same panicle. At first the relations between parasite and host appear normal. Mycelium can often be seen in close proximity to the nucleii, including the nucleii dividing by mitosis (Fig.24). Later the intercellular mycelium, 3μ in diameter, appears to aggregate at the corners of the cells increasing the intercellular spaces. Changes occur in the nucleus when the mycelium is in close proximity; it could not, however, be determined whether the mycelium actually touched the nucleus. The nucleus initially stains prominently but gradually, staining becomes less distinct. The nucleoli become reduced to small granules and finally the nuclearplasm diminishes then disappears. The mycelium forms groups of hyphae generally about 30μ in diameter. These become gelatinized and increase in size to about 30-80μ. The mycelium becomes thinner until it is approximately 1μ wide. Regular pieces about 1 x 1μ become split off and these later develop into spores (Figs. 25, 26 and 28). The regular pieces afterwards become triangular then more rounded in shape. They enlarge in size and the bounding wall differentiates into two layers, the intine and extine walls of the spore. The oldest spores were always observed in the centre of the fundamental masses. The mature spores stained purple and the mycelium red with Johansen's Quadruple Stain.

The anthers, pales and glumes were still visible in

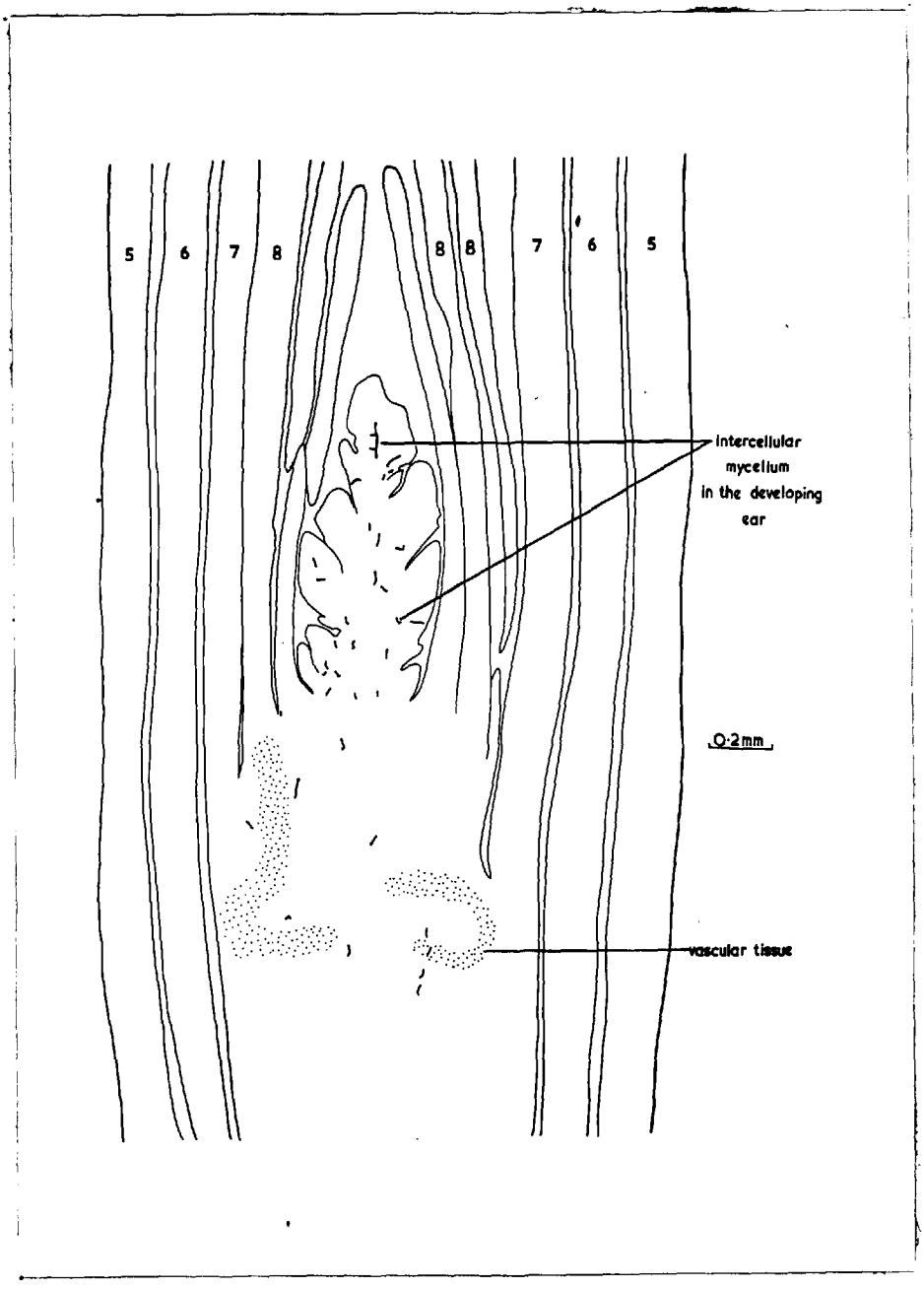

Fig. 22. Camera lucida drawing of L.S. section of a developing panicle 1cm. long showing the distrubution of mycelium

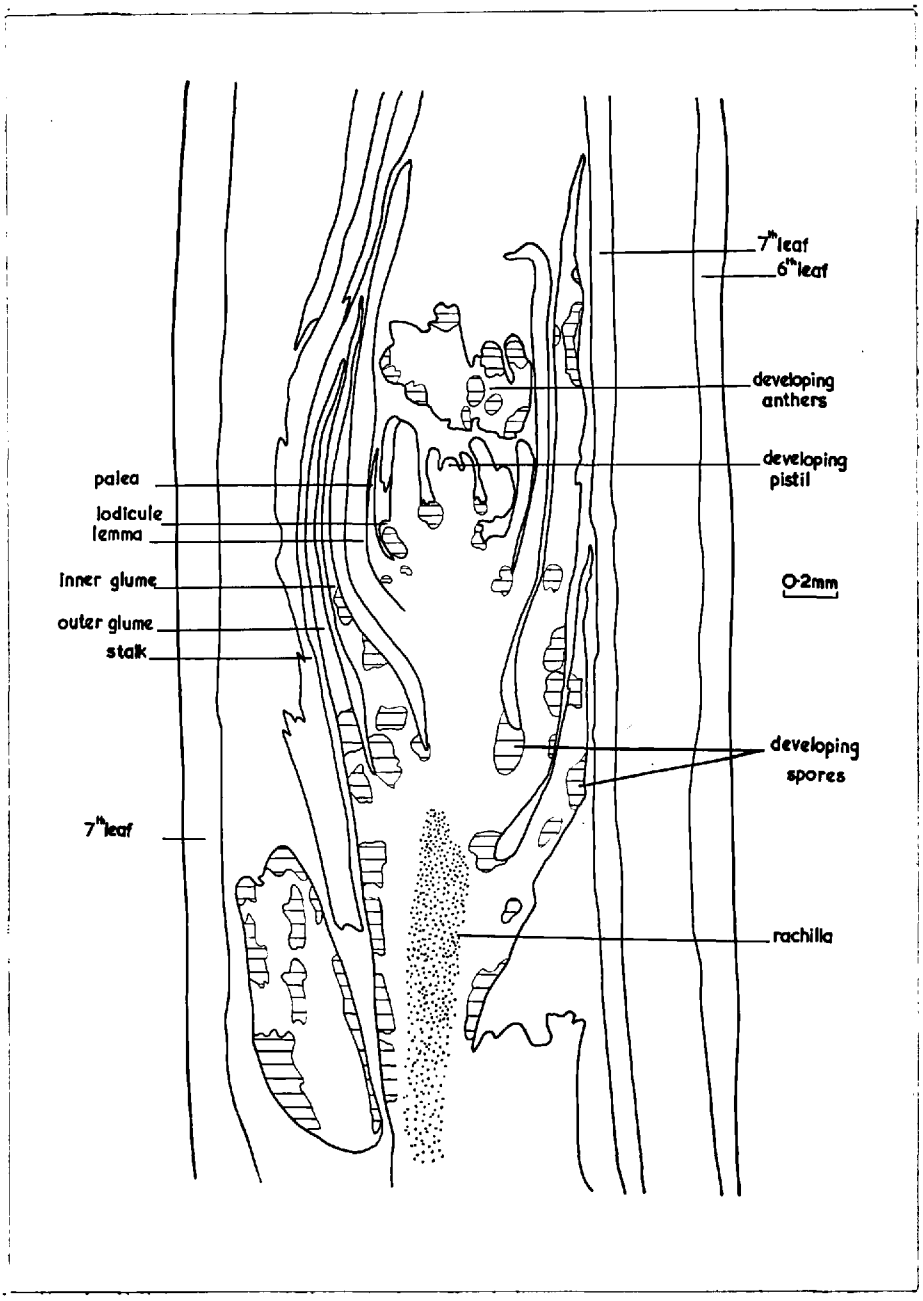

Fig. 23. Camera lucida drawing of L.S. developing panicle showing the areas of spore development.

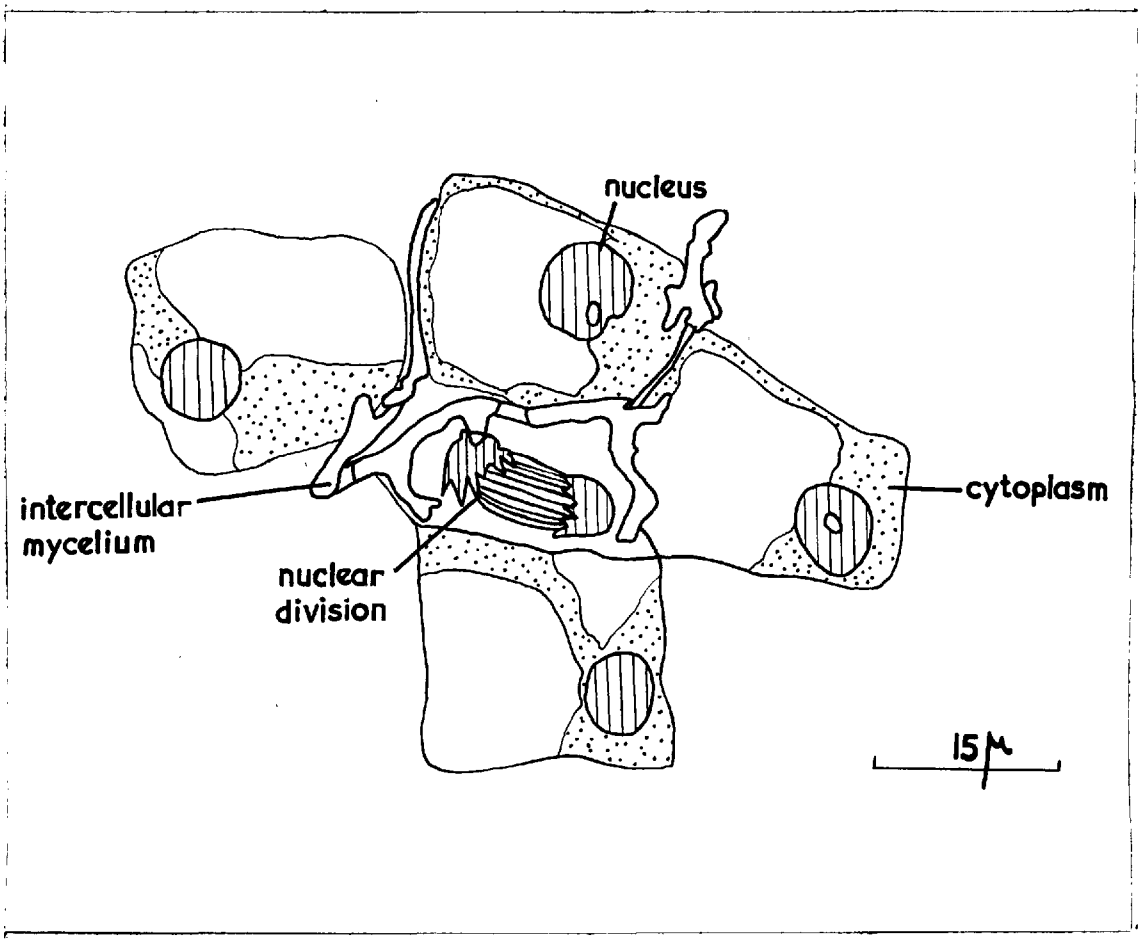

Fig. 24. Camera lucida drawing detail of L.S. ovary showing the proximity of the mycelium to the dividing nucleus.

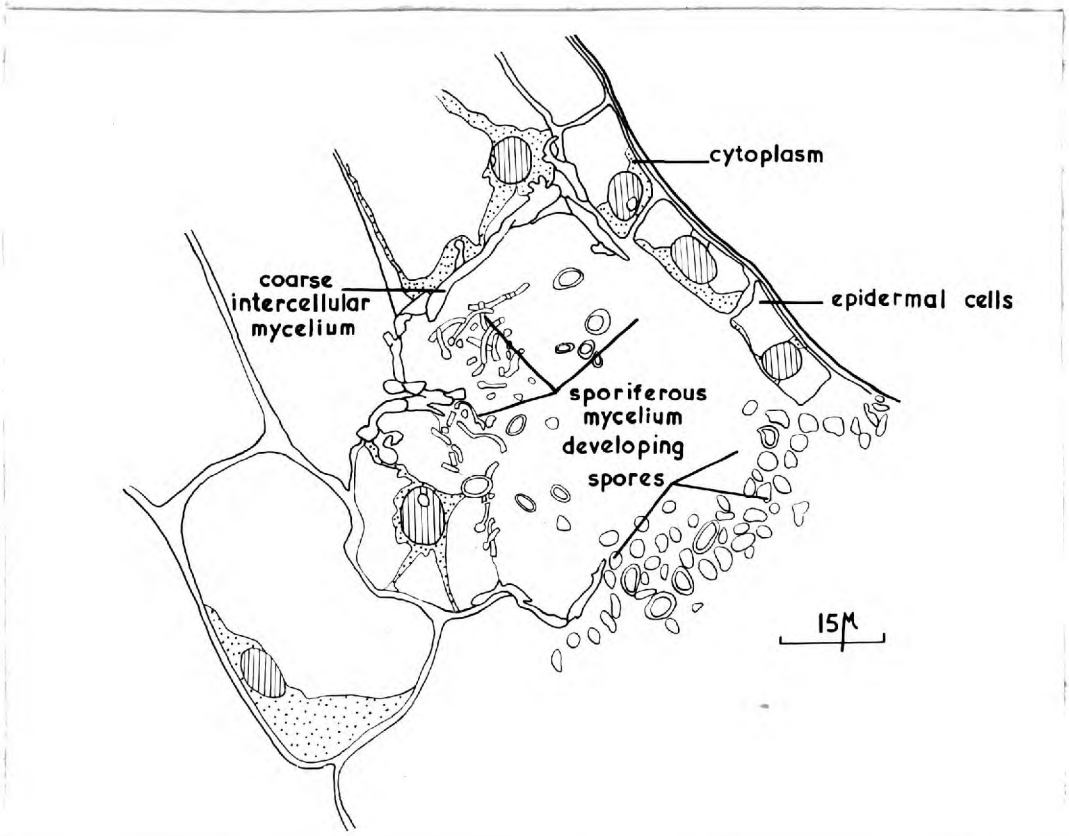

Fig. 25. Camera lucida drawing of L.S. ovary showing spore formation.

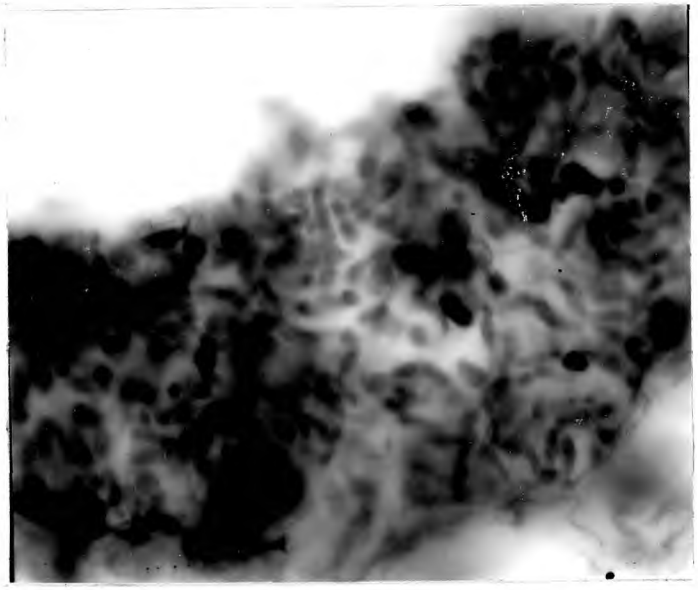

Fig. 26. Sporiferous mycelium from the same ovary as figure 25. x 1520.

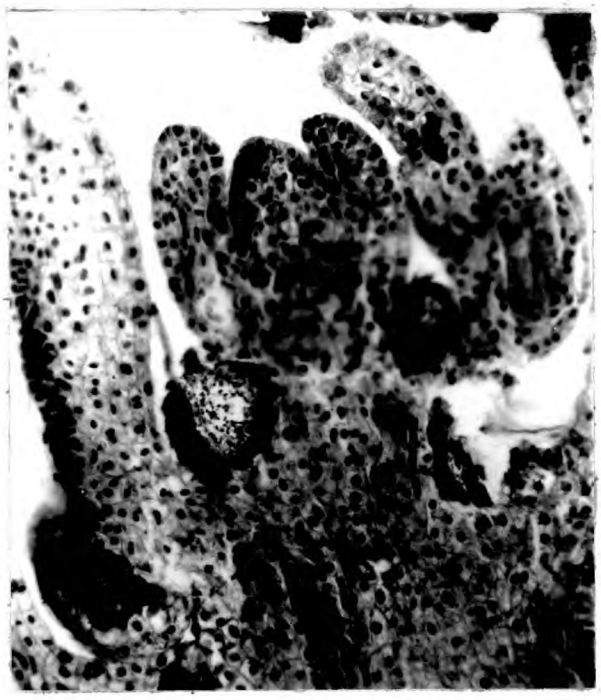

Fig. 27. L.S. ovary showing pockets of developing spores x 160.

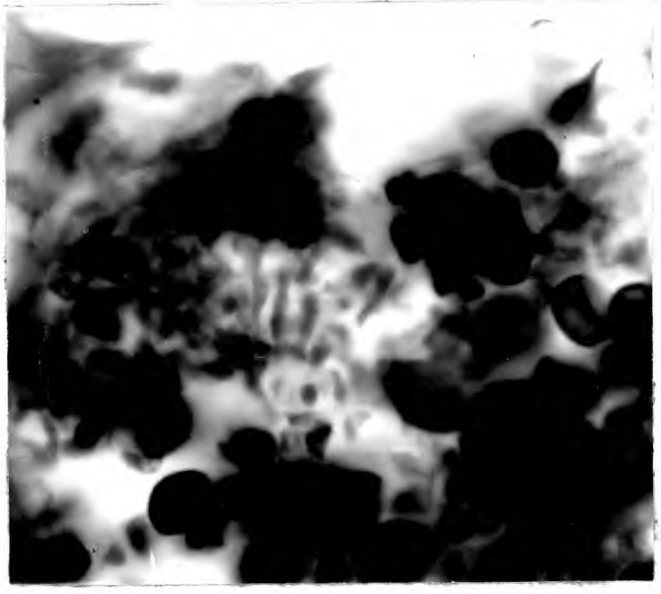

Fig. 28. Detail spore formation in ovary x 1520

...panicles at a late stage (Fig.23). There appears to be no preference for destroying any particular part of the flowers although spore formation did seem to favour the edge of tissues. Spore formation in S.147 and Onward panicles was by the segmentation of sporiferous hyphae.

Leaves

Spores were observed externally on the leaves of Onward and S.147 oats inoculated with C.1 in the greenhouse. Smutted leaves were much more common on S.147 than on Onward. Sori were also seen on the 6th and 7th leaves of Anthony oats inoculated with C2 and sown in the field. In all instances the smutted panicle was enclosed by the 7th leaf and a twisting of the flag leaf often occurred making the emergence of the panicle difficult or impossible. The sori on the 7th leaves were above the panicle and visible externally as longitudinal stripes between the veins. Sometimes the leaf tissues split giving the leaves a shredded appearance. Sections of the 7th leaves showed that the sori were formed in the mesophyll and partly in the upper and lower epidermis. The method of spore formation was the same as in the panicles. The amount of intercellular mycelium, 3μ in diameter, increases and enlarges the intercellular spaces destroying the cells in order to make more room for spore production. The mycelium becomes thin, splits up and eventually spores are formed. The areas of spore production are found at

...intervals in the mesophyll, these regions later meet and long sori are produced. When the epidermis is fractured the spores are exposed and when both the upper and lower epidermis are fractured the leaf splits giving a shredded appearance.

Nodes and internodes

In some S.147 plants spores were seen in the 9th node and in the flower stalk. The spores were formed mainly in the top part of the node (Fig. 20), in the parenchyma below the upper epidermis and in the parenchyma between the vascular strands. No large sori was formed; the spores were produced in whole series of adjacent cells by the segmentation of sporiferous mycelium, mainly intracellular in position. The cell walls did not break down but the cell nucleii were destroyed. In the internodes, most of the spores were aggregated in sori mainly situated in the sub-epidermal parenchyma. Some sori however, were present in the parenchyma between the vascular bundles. In the greenhouse, one example was seen of longitudinal striae visible externally on the flower stalk. The mycelium in the internodes was inter- and intracellular. The spores were apparently produced from the intracellular mycelium.

Discussion

These observations on the sporulation of U.avenae in oats agree with the findings of Wang (1934). The

...spores are formed by the segmentation of sporiferous mycelium. Probably the reason why spores were not formed in a particular floret tissue was because of the general distribution of the mycelium (Fig.22). The occurrence of spores on the leaves may be due to the effect of temperature or other environmental factors. The amount of smut infection and its expression on the leaves of wheat and barley has been shown by Kavanagh (1961) to be influenced by certain temperatures. However, both the genetic constitution of the fungus and the plant environment can alter the gross symptoms (Popp and Hanna, 1935).

PART III. STUDIES ON NATURAL AND ARTIFICIAL FLOWER INFECTION, SPORE DISPERSAL AND DISEASE CONTROL.

Introduction

The advent of accurate spore trapping apparatus in the last decade has made possible precise studies of the air spora. A spore trap has been devised by Hirst (1952) by the use of which the numbers of spores deposited at known times can be compared with meteorological data. An important factor in the dispersal of smut spores is turbulence but so far this has not been investigated in smutted crops probably because of the lack of suitable instruments. A dew balance has been devised recently by Hirst (1957) for measuring surface wetness and turbulence within crops.

There are two kinds of flower infection in the cereal smuts, the embryo and flower infecting smuts. In the first category the embryo is infected from spores alighting in the flowers, examples are the loose smut of wheat, Ustilago tritici, and the loose smut of barley U.nuda. In the flower infecting smuts e.g. U.avenae and U.kolleri, spores alight in the flowers but the embryo is rarely, if ever, infected. A controversy has continued for a number of years as to which is the most

...important source of inoculum for seed infection in flower inoculated oats. One school of thought favours resting mycelium within the glumes (Zade, 1922), another favours resting mycelium in the pericarp (Gage, 1927) and yet another for ungerminated spores on the glumes (McKay, 1936). The spores and mycelium beneath the hull are difficult to control by fungicides. During the last decade there has been much interest in liquid organomercurial fungicides. These are volatile compounds the vapour of which penetrates beneath the oat hulls killing the inoculum.

The first section is mainly a comparison between the flowering stages in healthy plants and the amount of spores present at corresponding times in smutted panicles. The second section deals with data obtained from the simultaneous operation of a spore trap and a dew balance within an oat crop possessing panicles smutted with U.avenae. Information was obtained on the diurnal and seasonal periodicity of the spores and how the spore load fluctuated with wind velocity, turbulence and rainfall. In addition information was sought on natural infection within the crop. In the third and fourth sections information is presented on the source of inoculum in artificially flower inoculated oats and on the use of liquid organomercurials on flower and seed inoculated oats under greenhouse and field conditions.

TERMINOLOGY

The terminology for describing the parts of the oat inflorescence are taken mostly from Bonnett (1961). The most important terms are listed below:

Palea - The inner flowering glume, next to the spikelet axis

Lemma - The outer flowering glume, exterior to the palea

Floret - This consists of the palea, lemma and flower; at maturity it is equivalent to the 'seed' of the oat.

Flower - Each flower consists of 2 lodicules, 3 stamens and the pistil.

Spikelet - Several florets, usually 3, enclosed in a pair of glumes, the inner and outer glumes.

Panicle - The whole inflorescence consisting of many spikelets.

Rachilla - The stalk bearing the florets, the central axis of the spikelet

Pedicel - The stalk bearing the spikelets.

A. RELATION OF THE FLOWERING PERIOD TO SPORE DISPERSAL.

Review of Literature

Sreeramalu (1956) studied the growth of the host plant and the period of smut dissemination in a barley crop infected with U.nuda. He was able to show the close coincidence of the flowering phase of the host and the maximal dispersal period of the smut spores. The next section deals with similar work with U.avenae.

Methods

Growth stages of uninfected and infected S.147 plants showing smut were compared following the method of Sreeramalu. The method of sampling was to examine the same ears at 3 day intervals. The scheme (Table 10) used for describing the flowering stages of the host was based on Feeke's Scale (Large 1954). The method of classifying the diseased panicles was based partly on that devised by Sreeramalu for U.nuda (Table 4).

TABLE 10.

Growth stages of the non-smutted oat plants.

(Feeke's Scale - Adapted from Large 1954)

Stage in Feeke's Scale	Description of growth stage
9	Ligule of last leaf just visible.
10	Sheath of last leaf completely grown out, ear swollen but not yet visible
10.1	First ears just visible, ears escaping through split in sheath
10.2	Quarter of heading process completed
10.3	Half of heading process completed
10.4	Three-quarters of heading process completed
10.5	All ears out of sheath
10.5.1	Beginning of flowering
10.5.2	Flowering complete to top of ear
10.5.3	Flowering over at base of ear
10.5.4	Flowering over, kernel watery ripe
11.1	Milky ripe

Results

Table 11 is a summary of the results obtained from a comparison of the growth stages of smutted and non-smutted panicles in the greenhouse. The flowering stages of the non-smutted plants are stages 10.5 to 10.5.3 of the Feeke's Scale. They occurred from the 4-9th of March. Maximal spore dissemination occurred in the stages 4, 5 and 6 of the scale in Table 4 also during the period 4-9th March. It is apparent that the stage of maximal dispersion of the

TABLE 11

Comparison of growth stages of smutted and non-smutted panicles in the greenhouse.

Date	Predominant stages in the Feeke's Scale	No.of non-smutted ears exam.	No. of smutted ears exam.	% of the number counted in each dispersal stage of the smutted ears						
				1	2	3	4	5	6	7
March 1st	10.3	56	64	44	30	23	3	0	0	0
March 4th	10.4-10.5.1	55	64	25	9	26	39	1	0	0
March 7th	10.5-10.5.1	54	64	2	25	6	64	8	0	0
March 9th	10.5.4	53	65	0	5	6	25	52	12	0

...spores coincides with flowering in the healthy panicles. The data in Table 11 also shows that the smutted ears have emerged earlier than the healthy ears. The experiment

...was carried out in an artificial greenhouse environment but the results bear out other observations made in the field. There is apparently a close coincidence of the flowering stage and the maximal dispersion of spores in U.avenae as well as in U.nuda.

B. STUDIES ON SPORE DISPERSAL AND NATURAL INFECTION.

Review of Literature

Spores of Ustilago spp. are mainly dispersed by wind but rain, animals and man are also instrumental in a comparatively minor way (Fischer and Holton, 1957). An automatic suction trap was developed by Hirst (1952) for estimating the concentration of different kinds of spores in the air at any given time. It is possible to correlate these concentrations with meteorological data at the time and site of trapping. Hirst (1952) has shown that this instrument has a trapping efficiency of not less than 60% for U.avenae spores.

On dry days, in agricultural or urban areas, Ustilago spp. spores exhibit a diurnal periodicity with a maximum in the afternoon and minimum in the early hours of the morning (Hirst, 1953; Sreeramalu, 1956; Hamilton, 1957). Gregory and Sreeramalu (1958) however found that maximum numbers of smut spores were trapped at 10.30 a.m. over an estuary. The maximal numbers of spores recorded in a cubic metre of air (m^3) are 60,000 at noon is a smutted barley crop (Sreeramalu, 1956), and 23, 800 over arable land (Hamilton, 1957).

Rain removes the spores from the air (Hirst, 1953; Hamilton, 1957). Spore clumps are normally prevalent

...on the slides. Hirst (1953) suggested it was these clumps which were washed out of the air, thus removing many spores. Wind velocity and turbulence are of great importance in dispersal (Hirst, 1953; Sreeramalu, 1956). Hamilton (1957) correlated the numbers of spores caught with meteorological data and found that gustiness of the wind, sunshine and high barometric pressure were the factors which promoted increased spore dispersal. A possible correlation was also found with relative humidity; increased R.H. was often associated with a decrease in spore numbers. Maximum spore numbers were caught at 21-24°C.

Methods

A Hirst spore trap was used from 20th June - 14th July 1961 to obtain a continuous record of the U.avenae spores dispersed from a smutted crop of oats. The trap was placed in the crop to swamp sources of the morphologically similar Ustilago perennans on Arrenatherum elatius present on nearby grassland. The crop was sown in a vegetable garden, the spore trap and a Hirst dew balance (Hirst, 1957) being placed in the centre of the plot (Fig.29). In the plot, rows 1-3 were Onward and rows 4-8 S.147 oats. The oats in rows 2, 5 and 7 were dehulled and dusted with spores of C1; the remainder of the rows

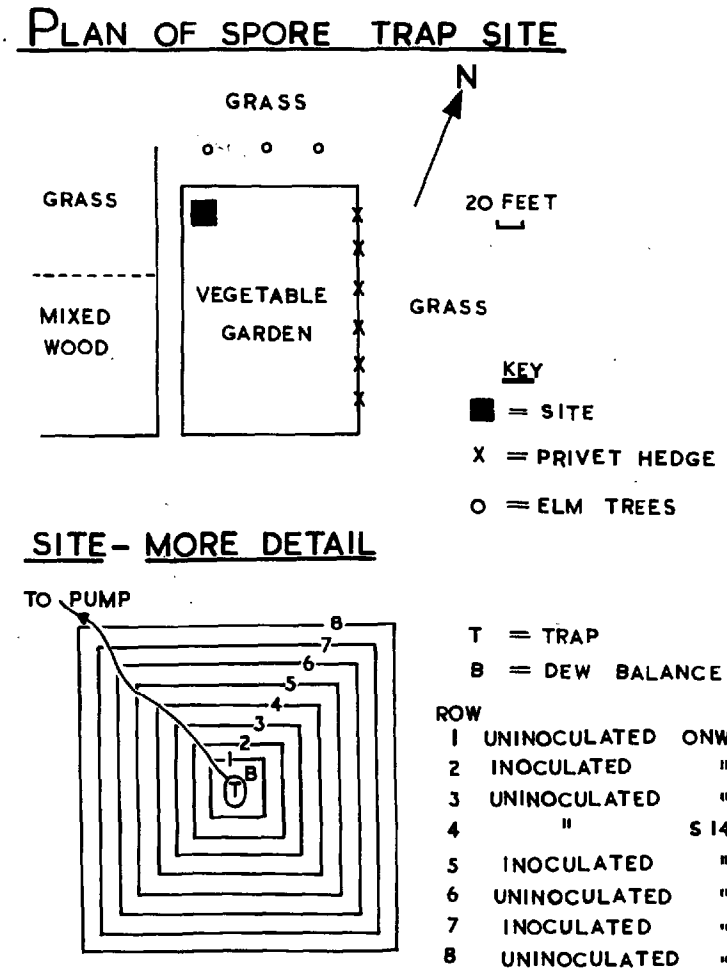

Fig. 29. Plan showing location of spore trap and details of the experimental plot.

...were of uninoculated and hulled oats. All the oats were sown 2.5cm. deep on the 22nd February 1961.

In early June plants which impeded the movement of the wind vane were removed. The orifice of the trap was set so that it was level with the top of the crop, approximately 1m. above ground level. The first smutted ears appeared on the 19th June. Spore trapping commenced on the 20th June but unfortunately a fault developed in the trap which was not corrected until the 25th June. The slides and the dew balance charts were changed about 7 p.m. daily. Spore trapping was terminated on the 14th July, 1961.

Macroscopic observations were made of leaves and florets of uninoculated plants adjacent to smutted plants. Some material was fixed in F.P.A. for 48 hours then preserved in 50% alcohol. Harvests were made on the 17th July and at maturity on the 8th August. On the same occasions other material was collected and preserved dry. Some was sown in the greenhouse before and after the winter 1961 to investigate the percentage natural infection and the possible resting period of the spores. Both fixed and dried material was examined for resting stages of the lactophenol technique.

Preparation of the Hirst spore trap slides.

About 55mm. of slide was coated with a 10% aqueous solution of Solvar and allowed to dry. A thin film of molten vaseline containing 10% paraffin wax was then

...applied to the Solvar coated area. The exposed slides were covered with 2.0x5.5cm. coverslips. No mounting medium was used.

Examination of the slides

It was decided to count the spores at hourly intervals on traverses across the slide at right angles to the direction of slide movement (short traverses). The traverse was examined under the H.P. objective of a binocular research microscope with a field of view of 300μ. The counting of the spores was facilitated by a graticule inserted in one eyepiece. The slides examined were selected after reference to meteorological data. In each short traverse there are 40 views of 300μ. When the spore numbers numbers were medium or low all the spores in the 40 fields were counted, but if the numbers were very high only the spores in the 2nd, 12th, 27th and 37th views were counted. The total of the 4 readings was than averaged, and multiplied by 40 to give an approximate number of the spores in the short traverse.

Presentation of results

All the results were transposed into the number of smut spores per cubic metre of air. The number of spores counted on a traverse of known area can readily be converted into an estimated number per cubic metre from the known suction rate (0.6m.^{3}/hr.), the cross-sectional dimensions of the orifice (2 x 14mm.) and the rate of

...movement of the slide (2mm./hr.).

Calculation of conversion of number of spores counted to number per cubic metre (Adapted partly from Sreeramalu,1956).

Fraction scanned at each traverse:

Rate of movement of slide = 2mm./hr.

Length of trace = 24 x 2 = 48mm.

Effective length of trace = 48 - 2 = 46mm.

Width of scanning traverse = 300μ

At each traverse, fraction of the total deposit scanned = $\frac{300}{46 \times 1000\mu} = \frac{1}{153}$

Volume of air sampled

Suction rate = 10 litres/min.

Volume of air sampled = 10x60x24litres/day

= 14.4 m^3/day

1 traverse scans $\frac{1}{153}$ x 14.4m^3 = .094$m.^3$

To convert the number counted to number per cubic metre

multiply by $\frac{1}{.094} = \underline{10.6}$

This figure however does not account for the trap not being 100% efficient; the number of spores should be greater. Also there is another factor to be borne in mind. The Hirst spore trap, unlike other traps, has a flow rate not automatically adjustable to the wind speed. To obtain the true number of spores caught per $m.^3$ of

... air a correction factor has to be applied dependent on the wind speed. If the wind speed is low, isokinetic conditions prevail in the trap (Gregory, 1961) and a correction factor is then not necessary. According to Geiger (1950) the wind velocity is reduced to a very low value in the crop. In this work no correction factor was applied to the number of spores estimated per $m.^3$ of air.

Meteorological data

The data was mainly obtained from the meteorological site at Silwood Park. The wind velocity figures used however are from South Farnborough 11 miles S.S.W. of Silwood. The daily mean values of temperature and relative humidity were recorded by a bimetallic thermograph and hair hygrograph respectively in a Stevenson screen 1.2m. above ground level. The amount of sunshine was obtained from a Campbell-Stokes sunshine recorder and the radiation from a Robitzsch actinometer. Rainfall data was from a Dines tilting-siphon recorder and wind speed from a Dines pressure-tube anemograph.

Plotting the graphs

The meteorological data was plotted on the graphs at two hour intervals with the exception of the rainfall figures which were plotted hourly. The data on the graphs for radiation and surface wetness are plotted in arbitary units. These graphs (Figs. 33-39) are copies of the original charts obtained from the instruments but much

...reduced in scale. The number of spores per $m.^3$ varied enormously; the range was from 21 to 238,000 spores. To accomodate this range of values on one scale on all seven graphs a log scale was used but the salient peaks of spores became too diminished. It was then decided to use two scales for the graphs, some with a unit of $5x10^3$ and others with a unit of $5x10^4$.

RESULTS AND DISCUSSION OF THE RESULTS

Figs. 33-39 show data for the spore numbers per $m.^3$ of air sampled and the corresponding meteorological conditions on selected days in June and July. The meteorological conditions and their possible effect on spore dissemination will be considered separately.

Rain - Rainfall was recorded on 3 days, June 26th and July 12th and 13th. The rain fell on the 26th June (Fig.33) at 6.40 a.m. (1.5mm.) and between 1.45p.m. and 3p.m. (0.9mm.). The first period of rain increased the number of spores caught on the slide from 2000 to 36,800/$m.^3$. By 9a.m. the number of spores had fallen to 5000. There was a similar rise and fall after the second period of rain. Initially the number was 3,900 which rose to 36,600 then appeared to fall. The peaks of spores after rain have also been described by Sreeramalu (1956) for U.nuda in barley. The increases in spore numbers caught on the slide are probably because of the rain removing loose

...spores from the panicles and possibly washing spores from the air. In between the wet periods the plants probably dried out making available more dry spores which were dispersed by the second period of rain. The drying is indicated by the fall in the surface wetness curve (top graph in Fig.31), and the amounts of sunshine and increased radiation (Fig.33). The rain which fell on the 12th July (Figs. 38 and 39) and 13th July (Fig.39) did not produce peaks of spores as on the 26th June (Fig.33). The absence of peaks cannot be explained by lack of spores in the panicles as in Fig.39 there is a large peak of 10,000 spores/m^3. The number is probably due to turbulence in the crop as indicated by the vertical lines in the corresponding graph for surface wetness. Turbulence will be considered later. Possibly in the early stages of the ears (June 26) the spores were easily dislodged by rain; in more mature panicles the spores were more readily dispersed by turbulence and not by rain.

The spores were often seen in clumps of 20-300 spores. Hirst (1953) suggested the clumping of spores is a means by which spores could be removed quickly from the air by rain. However, no noticeable increase in numbers of clumps was observed after rain.

Turbulence - The dew balance indicates the amount of surface wetness and also gives a measure of turbulence within the crop. Above the main part of the apparatus

...(Fig.30) is a cylinder of expanded polystyrene attached to a vertical rod. The cylinder is delicately balanced and becomes depressed by down draughts. The turbulence is indicated by sharp vertical lines in the surface wetness curve generally from 9 a.m. to 6 p.m. Figure 31 is a photograph of two dew balance graphs. In the lower graph turbulence has occurred from 10 a.m. to 6 p.m., no turbulence is shown in the lower graph. There is a large difference in the spore numbers trapped on the days 29th-30th June (Fig.35) and 30th June - 1st July (Fig.36) which could be related to the amount of turbulence. The meteorological data for wind velocity, temperature, relative humidity, radiation and sunshine are approximately the same in Figs.35 and 36. There is however more pronounced turbulence on the 30th June (Fig.35) corresponding with the large peak of 96,000 spores/m^3. On July 1st (Fig.36) there is a smaller peak of spores (16,000/m^3) and correspondingly less turbulence than in Fig.35. In the Figs.33-39 there is a close coincidence of turbulent conditions and dispersal of spores.

Temperature, Relative Humidity, Radiation and Sunshine. - In the Figs. 33-39 it is difficult to separate the effects of these meteorological factors in spore dispersal. Generally high temperatures and high amounts of radiation and sunshine are associated with low relative humidity. Also these factors are all often associated with high

...turbulence. The radiant heat may dry the spores quickly and make it easier for them to be dispersed. It could also be associated with turbulence; the radiant heat could heat the ground layer in the crop producing bubbles of air and these may rise more or less vertically to considerable heights carrying their spore load (Gregory, 1952). There appears to be no correlation between high temperature and the maximal dispersal of spores. The maximum spore number per cubic metre observed was on the 28th June (Fig.34) when the temperature was 19°C. On many other days of less spore dissemination the temperature was higher (29-32°C).

Wind Velocity - The data for wind speed in Figs. 33-39 indicate only the direction of the prevailing wind in the general neighbourhood. No measurements were made of wind velocity at panicle level. It seems probable that wind speed is low in the crop (Geiger, 1950). Possibly it would be best to attempt a correlation between high wind speed and spore dispersal. Hamilton (1957) found there was an increased number of Ustilago spp. spores present with increased gustiness of wind on a roof at South Kensington, London. The highest wind recorded at South Farnborough was 9.5 m/sec. on July 13th (Fig.39). It is unfortunate that the highest wind speed should occur when most of the spores had been dispersed. There is in Fig.39 an apparent relation between high wind speed and a peak of spores.

At the same time as the peak however there are also turbulent conditions. It is thus difficult in Fig.39 to separate the effects of high wind speed and turbulence on spore dissemination.

Diurnal and Seasonal Periodicity - There was a well marked seasonal periodicity on the days June 27-28 (Fig.34), June 29-30(Fig.35) June 30-July 1 (Fig.36) and July 10-11 (Fig. 37). The numbers of spores were very low from midnight to 6 a.m. Generally from 6 a.m. to 9 a.m. there was a gradual rise in spore numbers and from 11 a.m. to 3 p.m. a peak. Figure 32 is a photograph of spores deposited on the spore trap slide in the early afternoon of June 27-28. Generally after 3 p.m. the spore numbers fluctuated but gradually fell to a low number by 7 p.m. There was then a continual decline in spore numbers until midnight. The maximum number of spores 238,700 was trapped at 11 a.m. on 28th June (Fig. 34) but most spores were generally dispersed in the early afternoon. Hirst (1953), Sreeramalu (1956) and Hamilton (1957) also found maximal spore numbers in the afternoon and minimal numbers in the early hours of the morning. The maximum number of spores found by Sreeramalu in a smutted barley crop was 60,000/m^3.

A seasonal periodicity of spores over the experimental period was also apparent (Table 12). The first smutted ears appeared on the 19th June and all the smutted heads were visible on the 30th June. Both the figures for the

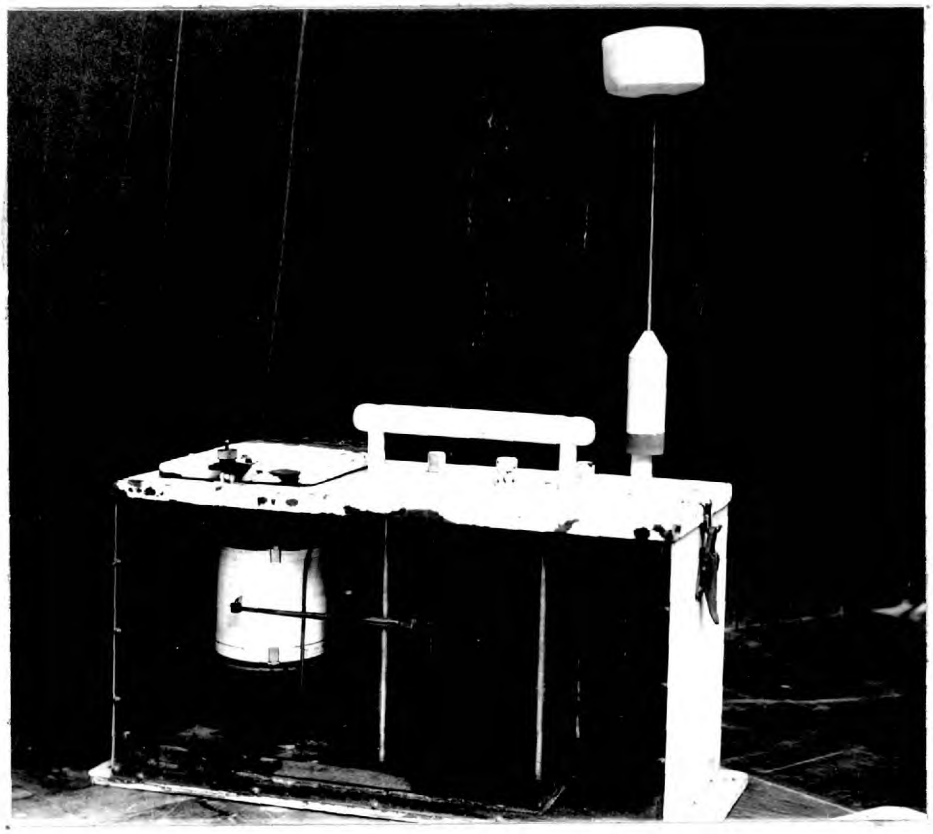

Fig. 30. The Hirst Dew Balance.

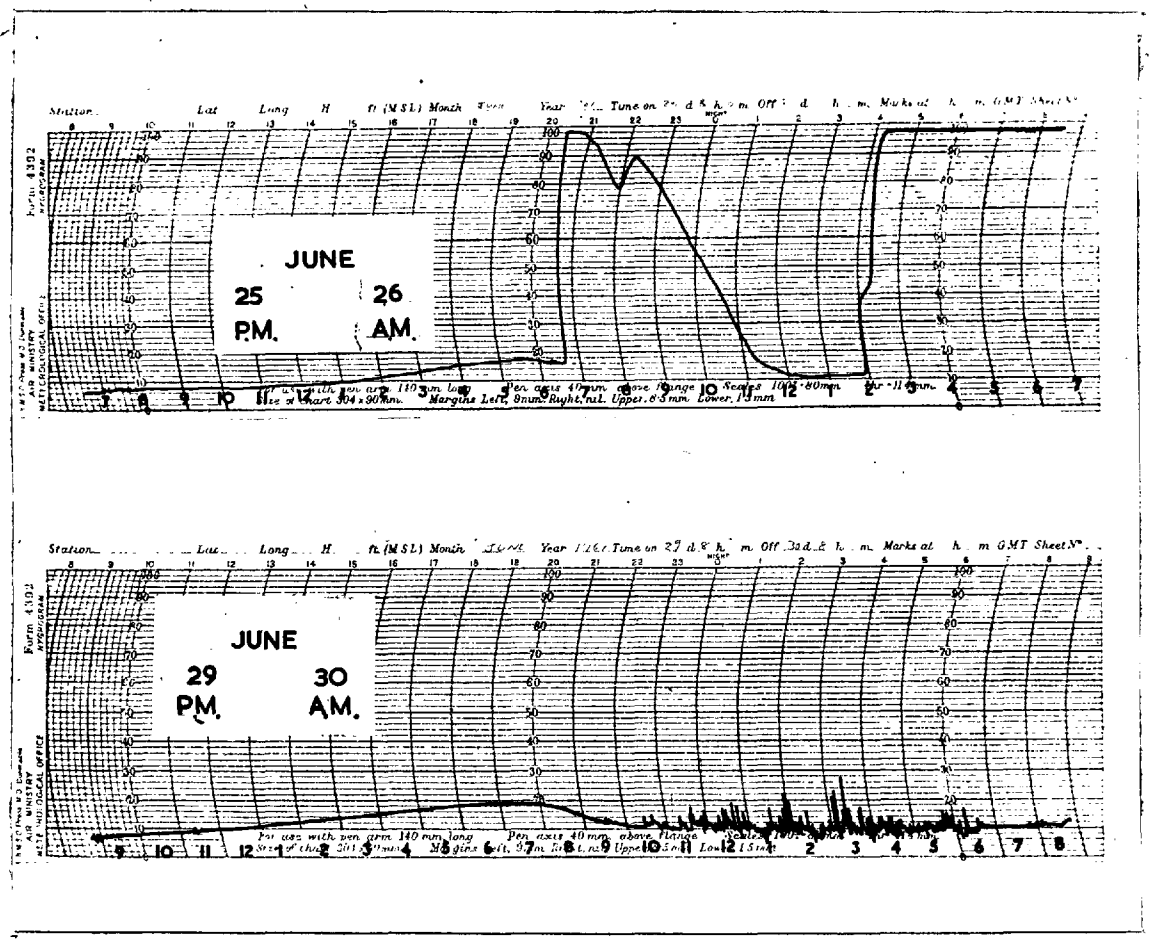

Fig. 31. Two charts from the dew balance within the experimental plot, June 1961.

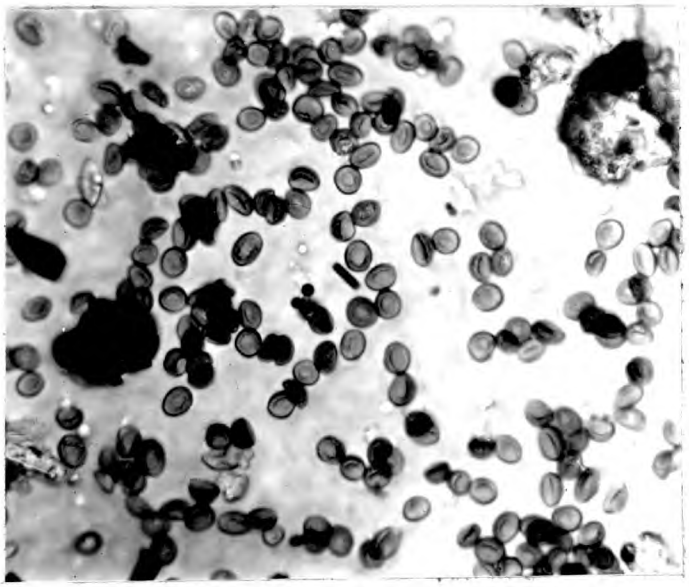

Fig. 32. Spore deposit on slide in early afternoon of June 27-28. x 640.

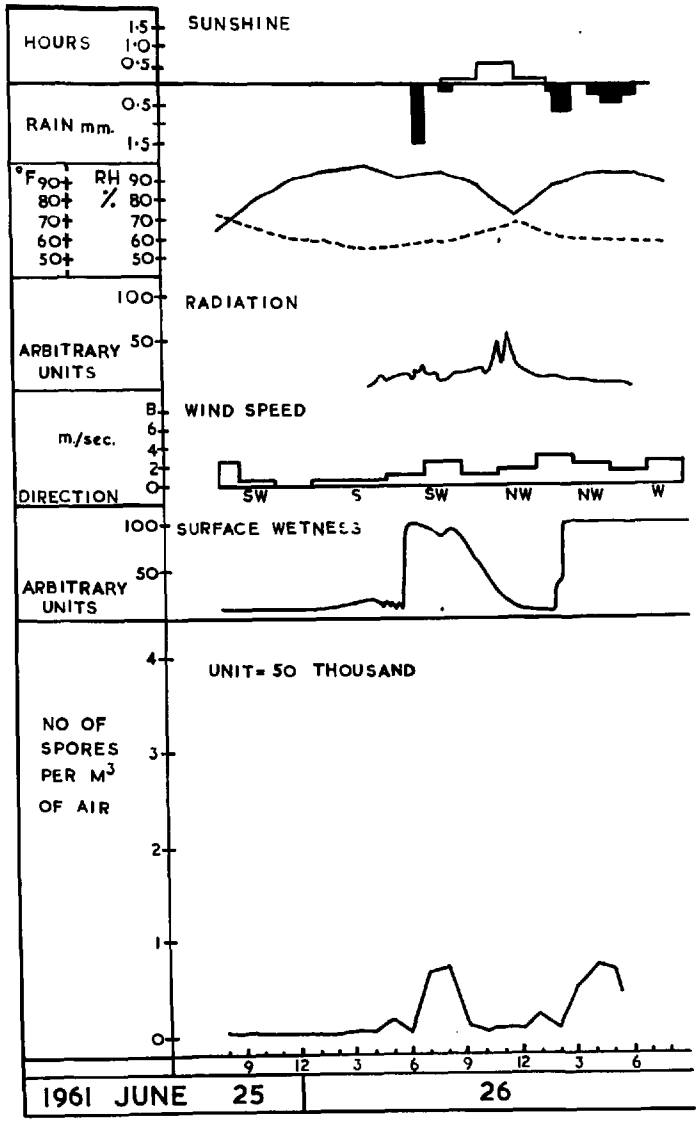

Fig. 33. Changes in spore concentration in relation to weather conditions on June 25th and 26th 1961.

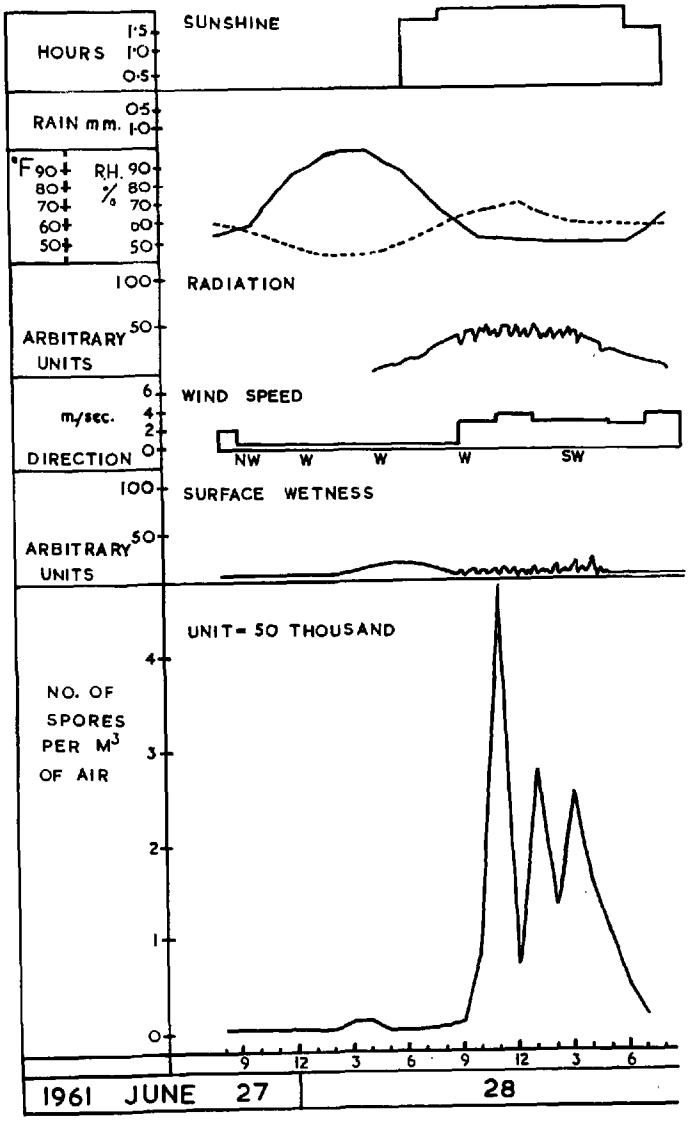

Fig. 34. Changes in spore concentration in relation to weather conditions on June 27th and 28th 1961.

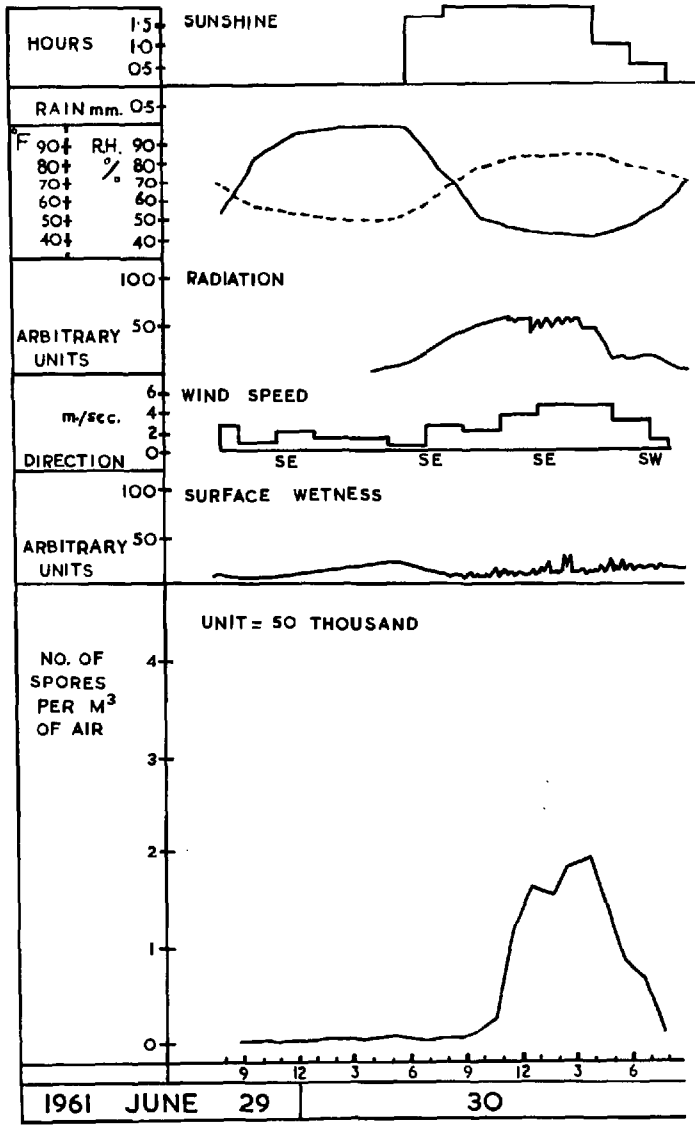

Fig. 35. Changes in spore concentration in relation to weather conditions on June 29th and 30th 1961.

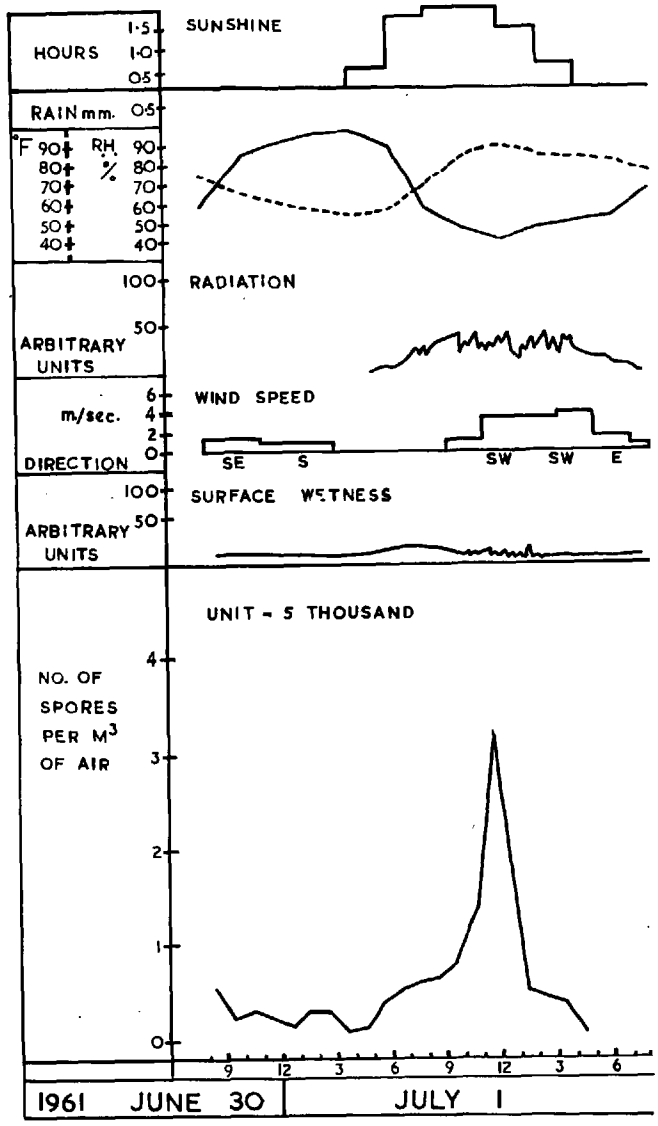

Fig. 36. Changes in spore concentration in relation to weather conditions on June 30th and July 1st 1961.

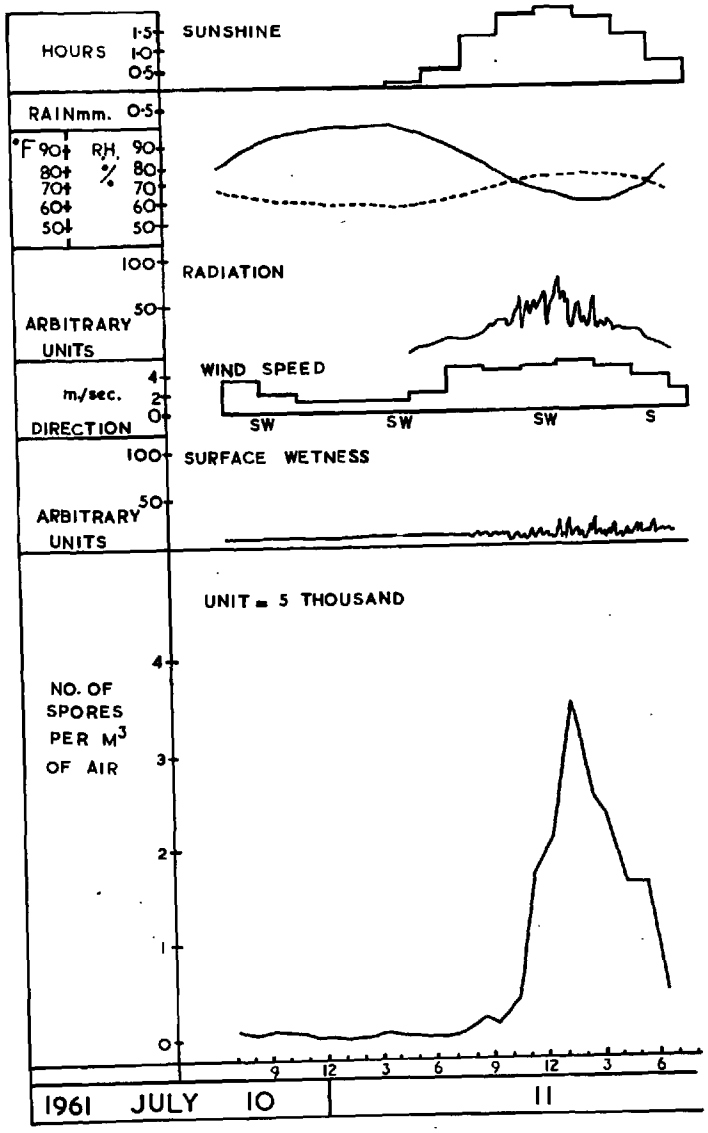

Fig. 37. Changes in spore concentration in relation to weather conditions on July 10th and 11th, 1961.

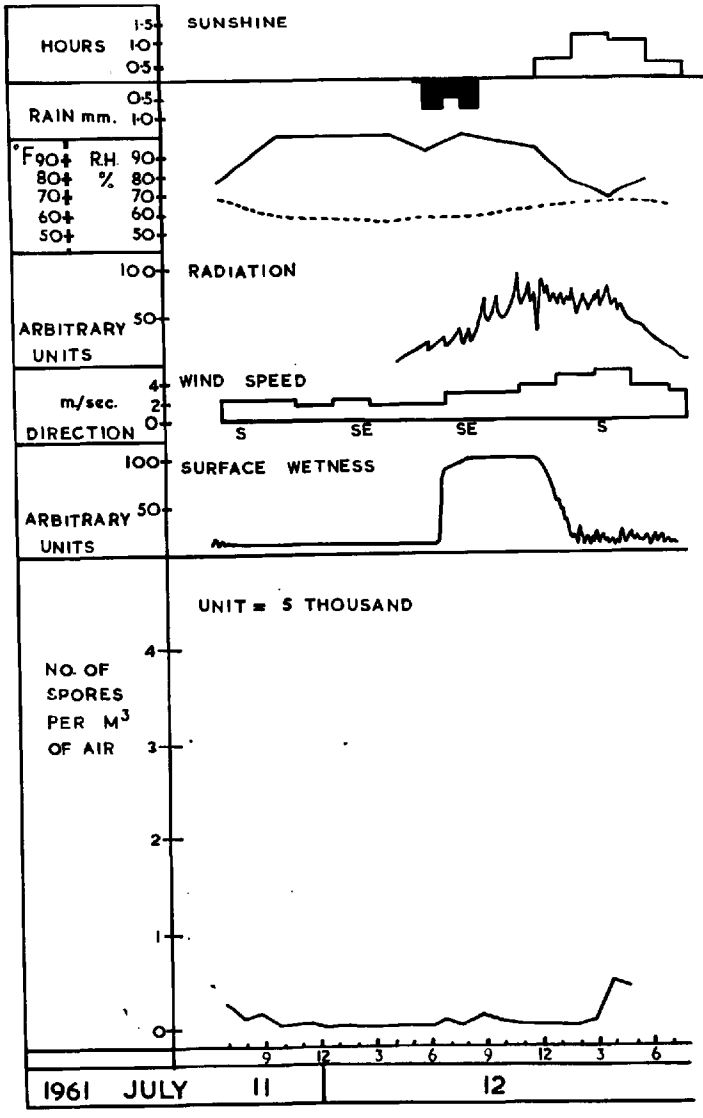

Fig. 38. Changes in spore concentration in relation to weather conditions on July 11th and 12th 1961.

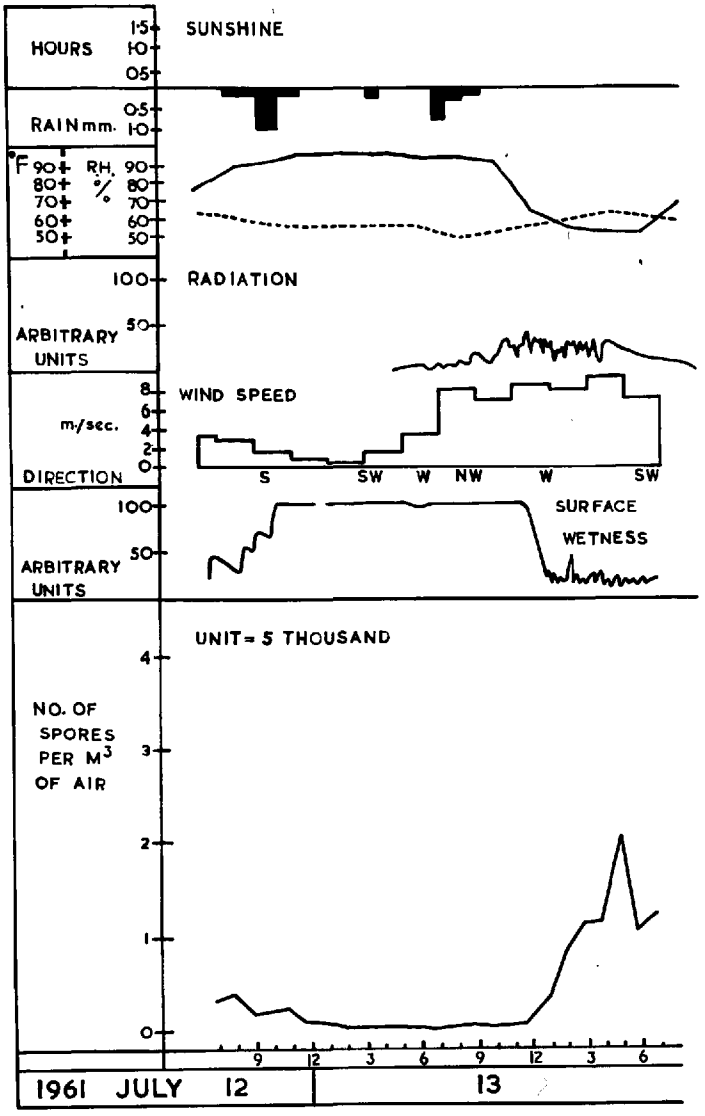

Fig. 39. Changes in spore concentration in relation to weather conditions on July 12th and 13th 1961.

...average number of spores per cubic metre over a 24 hour period and the maximum number of spores per cubic metre indicate that a high level of spore concentration is present in the crop only 6 days after the emergence of the first smutted ears. After 9 days a peak is reached then the numbers fall gradually.

TABLE 12.

Periodicity of spores and associated meteorological conditions over the experimental period (Nos. are to nearest 50).

Date	No.of Smutted Ears	Max.no. of Spores per M^3.	Av.no.of Spores /M^3 over the 24hrs.	Weather on Trapping Day.	Weather On Previous Day.
19 June	3	-	-	-	-
20 June	7	-	-	-	-
25/26 June		36,800	10.650	Rain	Dry
27/28 June		238,700	36,450	Dry,turbulent	Rain
29/30 June	68	96,650	25,750	Dry,turbulent	Dry
30June-1July		16,200	3,150	Dry,turbulent	Dry
10/11 July		17,900	3,750	Dry,turbulent	Dry
11/12 July		2,450	500	Rain	Dry
12/13 July		10,300	1,950	Rain, wind	Rain

It would appear that most of the spores are dispersed within 3 weeks of the emergence of the first ears. Sreeramalu (1956) and Hamilton (1957) have also found that the seasonal period for the dissemination of Ustilago spp.

...spores is approximately from the middle of June to early July.

Natural Infection - The final percentages of smut in the experimental plot, from inoculated seed, were estimated on June 30th 1961. The results are presented in Table 13 below.

TABLE 13.

Final percentages of smut in the experimental plot from seed inoculated oats.

Row in Plot	Variety	Number Smutted Plants	Number Not Smutted	% Smut	Notes
5	S.147	34	274	12.5	Most smut on N. & E. of plot
7	S.147	30	302	10.0	Most smut on S.&W. of plot
2	Onward	4	22	18.0	-

Macroscopic observations were carried out on the foliage and florets of plants on the north side of the plot. No spores were seen on the foliage of the plants in row 8 although the plants were often in contact with the smutted panicles in row 7. In rows 6,5 and 4 all the plants had spores on the stem, florets and 6th and 7th leaves.

Some panicles from rows 4-8 on the north side of the plot were preserved in 50% alcohol on the 30th June 1961 and examined by surface preparation in June 1962. Further

...harvests were made on 17th July 1961. The glumed oats were preserved dry in sealed bottles and sown in the greenhouse before and after the winter 1961-2. In June 1962 some remaining dry material was examined by surface preparation. The percentage smut produced from naturally infected material is presented in Table 14.

Examinations by the lactophenol technique were only made on harvested material of naturally infected oats which had produced smutted plants. The material was from row 3 of Onward and row 5 of S.147 (Table 14).

Many ungerminated spores were present on the glumes of S.147 [illegible] fixed on 30th June 1961. Only 5 smut spores had germinated on the glumes. Gemmae-type resting structures were produced by separation of the promycelial segments from three of the spores. Several Altenaria and Helminthosporium spp. spores had germinated on the glumes producing short pieces of mycelium. On the pericarp, anthers and stigmas the smut spores were mainly ungerminated. One spore however, had produced a germ tube (3μ long) on a stigma.

The dry material was first soaked for 52 hours in water to facilitate removal of the pericarp. S.147 material from row 5 and Onward from row 3 generally had ungerminated smut spores, and spores of Helminthosporium spp. on the pales, pericarp, anthers and stigmas. Only 3 smut spores had germinated, all on one stigma.

TABLE 14.

Percentage smut from naturally infected seed from the experimental plot sown before and after the Winter, 1961.

Series 1. Sown 25th July, examined 4th December 1961.

Plot Row Number	Variety	Number of mature Plants	Total Smutted	% Smut
3 (no glumes)	Onward	5	0	0
3 (+ glumes)	Onward	13	0	0
5 (no glumes)	S.147	19	0	0
5 (+ glumes)	S.147	15	0	0
6 (no glumes)	S.147	13	0	0
6 (+ glumes)	S.147	17	0	0
7N(no glumes)	S.147	13	0	0
7N(+ glumes)	S.147	19	0	0
7E(no glumes)	Onward	11	0	0
7E(+ glumes)	Onward	20	0	0
8 (no glumes)	S.147	12	0	0
8 (+ glumes)	S.147	17	0	0

Series 2. Sown 8th March, examined 16th May 1962.

3 (no glumes)	Onward	38	2	5.3
5 (no glumes)	S.147	16	1	6.2
6 (no glumes)	S.147	15	0	0.0
6 (no glumes)	S.147	26	0	0.0
7E(no glumes)	Onward	35	0	0.0
7N(no glumes)	S.147	14	0	0.0
8 (no glumes)	S.147	22	0	0.0

The varieties S.147 and Onward are not cleistogamous; under suitable climatic conditions the pales open widely and as has been observed, spores are found within the flowers. The smut infection produced is very low 0-6%. Sampson (1929) however, tested 71 naturally infected samples and found 61 of them produced less than 5% smut and the highest smut infection in a naturally infected sample was 33%. She observed occasional chlamydospores but the infection was mainly caused by a slender, branched frequently budding mycelium under the pales.

Most of the spores seen were ungerminated, this is in contrast to the observation of Diehl (1925) who found spores of U.avenae.alighting on the ovary of the blossoming oat flower germinated at once, except in very dry years. However Diehl used artificial inoculation and mature spores. The 10 days during the flowering period of the S.147 and Onward were not very dry. The R.H. had an average value of 73% (range 37-98) and the average temperature of 17°C. (range 6-28°C.) over the 10 day period. Tapke (1931) using U.nuda and barley found at 56-85% R.H. 95% infection could be obtained and at 11-30% R.H. only 21% infection. The temperature in the experiments had a range of 19-33°C. It is improbable in the Silwood experiments that the climatic conditions were unsuitable for spore germination. The poor germination of the spores probably can be related to their immaturity at the time of dispersal. If the

...spores were immature at dispersal they would quickly lose their viability (Sampson 1928). The amount of natural infection was so low that the nature and source of the overwintering inoculum could not be determined. The infection could have been produced from an occasional ungerminated spore or spores which had germinated in the late summer to produce overwintering mycelium. The site of the overwintering inoculum was not the glumes as the oats were deglumed before sowing.

General Discussion

Several shortcomings are evident in the arrangement of the dispersal experiment, notably the site of the trap and the lack of instrumentation within the crop. Continuously recording thermocouples can be modified in various ways (Waterhouse, 1955) for measuring temperature relative humidity and wind speed in crops. Smith (1960) has compared the temperatures in a Stevenson screen using mercury-in-glass thermometers and in a wheat field using thermocouples and found that the screen values tended to be lower by day and higher by night.

It is difficult to separate the effects of wind speed and turbulence within the crop. If thermocouples(hot-wire anemometers) had been set up in the crop in conjunction with the dew balance a clearer idea would have been obtained of the effect of the two components. Waterhouse

...(1955) has shown that there is considerable wind in the top of a grass crop, however wind speed in cereals may well differ from grassland. The plot (Fig.29) was unfortunately near trees and probably they may have exaggerated the effects due to turbulence. Large eddies appreciable as gusts may have been caused by air flowing over the trees. The resulting frictional disturbance can shake plants and sweep eddies unusually deeply into crops (Hirst, 1959). Gregory (1952) has suggested that bubbles of air containing spores rise from heated vegetation and pass to the upper atmosphere. Probably this process is helped by turbulent conditions. Once the spores are in the upper atmosphere long distance wind dispersal occurs. Possibly when the spores are not released in bubbles of air the spore concentration decreases with increased distance from the source (Stepanov, 1935).

Rain can effect the transport of spores in several ways notably in washing spores from the atmosphere, dispersing spores from smutted heads and in the movement of spores up plants by rain splash. McCully et al (1956) released fluorescent non-wettable dust during rainstorms from aeroplanes flying at 150m. and found that moderate rain (2mm./hr.) falling for 6-7hr. cleansed 90% of the dust particles 1-10μ in diameter from the atmosphere. Spores of U.perennans, which are morphologically indistinguishable from U.avenae, were almost certainly

...present in the atmosphere and both species would be washed down and caught on the slides. This cleansing action by rain evidently acts as a complementary process with long distance wind dispersal. Rain can disperse spores from smutted panicles onto the surrounding foliage and oat flowers by an impinging action. Much work has been done (Engel, 1955; Frazer and Dombrowski, 1962) on the movement of water particles after a waterdrop hits a surface using high speed photography. A raindrop hitting a glass slide at high impact undergoes an outward radial flow of very high velocity as a result of the collision (Engel, 1955). Frazer and Dombrowski (1962) figure small (c.50μ) droplets detaching from the periphery of the main drop. It is possible that the impinging action of raindrops at terminal velocity falling onto smutted panicles full of spores would act in a similar way. Spores on the surface of minute drops of water or even dry spores in clumps could be thrown outwards onto florets of uninfected panicles or onto the foliage of surrounding plants. However, the falling waterdrop hits a non-resilient slide at right angles, whereas a raindrop hitting a resilient panicle will be approximatley in the same plane. Engel (1955) has photographed waterdrops at terminal velocity hitting sand in a Petri-dish. A crown of upward moving splash and sand grains were produced by the impact. Perhaps the sand grains can be compared to spores in

...panicles replete with spores and hit by raindrops at terminal velocity. In this work it has not been possible to separate the cleansing and impinging actions of rain. Gregory (1961) has suggested that certain spores present on the lower leaves of plants can be carried upwards onto leaves by rain-splash; perhaps this process is also instrumental with U.avenae spores. It is evident that the meteorological factors involved in the transport of U.avenae spores act in a complex supplementary and complementary way.

The results from naturally inoculated material are difficult to explain. There was very little spore germination on the floral parts. Probably the low natural infection was produced from mature spores which had overwintered without losing their viability or from overwintering resting stages produced from spores which had germinated in the late summer. The ungerminated spores present on the floral parts were probably immature as the conditions of R.H. and temperature were suitable for germination. The stages of flowering of the host have been shown to coincide with the maximal dispersal stage of the smutted panicles in the greenhouse, and Sreeramalu (1956) has observed the same phenomenon with U.nuda and barley in the field. During the flowering period of the oats mature spores were available from June 28th - July 11th (Figs.34-37) as shown by the spores numbers dispersed by turbulent conditions. A possible reason why some of the mature

...spores did not alight on the flowers is that the conditions for transport were unsuitable. It is most likely that rain is the agent of transport. Rain fell on the 12th July. (Fig.38) but there was no corresponding peak of spores because rain cannot dislodge spores from very old panicles.

If rain is the transporting agency in natural infection most of the spores on the preserved material would result from the action of the rain on the 25th and 26th June (Fig.33). At this period the spores were immature as the first smutted panicle was observed on the 19th June. The next period of rain was on the 12th July (Fig.38), by which time all the loose spores had been dislodged. In support of the rain transport hypothesis; Batts (1956) noted that there was much more infection by barley loose smut in wet years than dry years, and Malik (1959) by dropping spore suspensions of U.nuda at near terminal velocity into oat flowers was able to obtain considerable infection. Also, Jensen (1888) noted that oats dipped in spore charged water gave 29% smut while those dusted with dry spores resulted in no infection.

C. ARTIFICIAL FLOWER INFECTION.

Review of Literature

Falck (1908) observed germination of U.avenae spores in oat flowers. He examined the florets after blossom inoculation and found that some of the spores had germinated on the stigmas. Later in the season mycelium was observed on the inner surface of the glumes and also penetrating the outer layers of the caryopsis.

There has been a difference of opinion as to which source of inoculum is most effective in causing seed infection. Zade (1922) and his co-workers, Arland(1924), Diehl (1925) and Roesch (1926) hold the view that the resting mycelium within the glumes is chiefly responsible for infection. Gage (1927) believes the mycelium that penetrates the style of the flower and hibernates in the pericarp of the seed more often intiates the attack. Occasionally Gage (1927) found resting mycelium in the young embryo. McKay (1936) concluded that most of the infection was due to ungerminated spores within the glumes and not to resting mycelium. Rusch (1958) found that U.avenae overwintered as spores between the glumes and caryopsis.

Probably flower infection has been studied most in the embryo infecting smuts. Freeman and Johnson (1909) noticed the importance of the stage of flowering of barley

...and infection by U.nuda spores. They obtained maximum infection after inoculation during the period between full anthesis and the beginning of the development of the fertilized ovary. Tapke (1931) working with Ustilago tritici and Little Club wheat investigated the effect of R.H. at the time of inoculation. He obtained 94% infection at 60-85% R.H., and only 22% infection when the flowers were inoculated at 11-30% R.H. Similar results were obtained with U.nuda and barley. Malik (1959) inoculated barley ears at different stages of anthesis by various techniques. He found inoculation at particular stages of flowering produced more smut in certain techniques. In the partial-vacuum technique most smut was produced at pre-anthesis and anthesis. Malik also dropped spore suspensions of U.nuda onto flowers of susceptible and resistant barley varieties. Susceptible varieties became smutted but the resistant varieties produced no smut. Malik suggests the non-development of smut in the resistant varieties is because of their relatively cleistogamous habit of flowering.

The next section is an account of the nature and development of smut in inoculated oat flowers and how the stages of smut observed can be related to the infection produced on sowing the inoculated oats, and how the infection produced from the sowings varies with the stage of flowering at inoculation.

Methods

In the spring of 1960 three plots of 10 rows of Onward were sown in the field. In spring 1961 Onward and S.147 were sown in five plots each of ten rows. In June of each year the panicles were inoculated at pre- anthesis, anthesis and post-anthesis. To determine the stage of flowering florets from the middle of the panicle were examined before inoculation. The following inoculation methods were used.

1) The hypodermic needle technique - In 1960 this method was used but quickly abandoned in favour of (2) below. Only two panicles were inoculated. The technique was originally described by Poehlman (1945). A hypodermic needle (2.5cm. 25 gauge) was attached to a 25ml. plastic wash bottle filled with spore suspension. By piercing the lemmas and squeezing the bottle a few drops of the suspension were injected into the floret. Disadvantages quickly found were that it was difficult to distinguish between inoculated and uninoculated florets and the method consumed too much time.

2) Partial vacuum technique This was originally devised by Moore (1936) and modified by Oort (1939). The panicles are inserted into an inoculation chamber A (Fig.40). The stalks are protected by sponge rubber facing the split-rubber bung B. By applying suction with a pump D spore suspension in a reservoir C is carried into A. The tap

...E is closed when A is almost full and the pump depressed 12 times. Partial vacuum created by the pump expands the florets and when the vacuum is released the spores in suspension are drawn into the flowers. By opening taps E and F the spore suspension falls back into the reservoir C. In Fig.40, I indicates a wooden support 2m. long embedded in the ground, H a wire cage containing C attached to the support. An additional reservoir G was added to the apparatus in 1961 to prevent spore suspension falling into the pump. A modified foot pump was sometimes used instead of the hand pump. The modifications employed were a reversed washer on the piston and a reversed ball bearing valve. The advantage of the foot pump was that both hands were free for operation of the apparatus during inoculations. It was found that up to 10 panicles could be inoculated at a time. They were then tagged with plastic covered wire of various colours each colour denoting the stage of flowering at inoculation. In 1960 460 panicles were inoculated and 652 in 1961.

Panicles were harvested 2, 5, 10, 15, 20, 25 and 30 days after inoculation and at maturity for each of the inoculations at pre-anthesis, anthesis and post-anthesis. Four panicles were cut for each harvest, labelled, fixed in F.P.A. for 22-24 hours and stored in 50% alcohol. In addition some of the inoculated panicles were preserved dry at maturity. These and the fixed panicles were

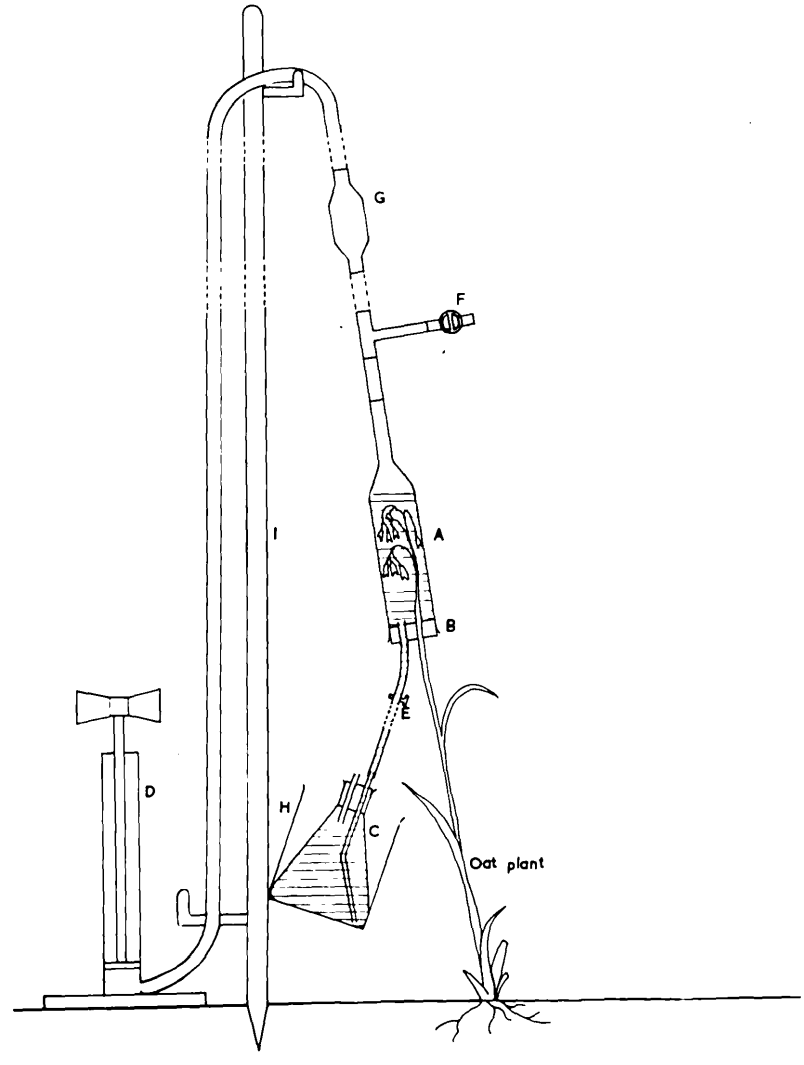

Fig. 40. Partial vacuum apparatus for the inoculation of oats with loose smut.

...examined by surface preparations. Some of the mature dried panicles were later sown in order to determine the efficiency of the inoculations.

In the winter of 1960 surface preparations of florets were made from panicles inoculated at all stages of anthesis. It was decided in the winter of 1961 to examine only the stored inoculated panicles which had produced smut in later sowings. The glumes, pericarp, stigma and anthers of fixed and dried material were examined by the lactophenol technique. The dried material was first soaked for 20 hours to facilitate removal of the pericarp. Some of the florets inoculated at anthesis were examined for mycelium in the embryo using the techniques of Simmonds (1946).

The flower inoculated oats were sown mainly in the greenhouse. The only field sowing was of Onward inoculated at anthesis in June 1960.. Before sowing the oats were deglumed. There were three sowings of 1961 flower inoculated material in the greenhouse. S.147 and Onward inoculated at all stages of flowering and with and without glumes were sown before and after the winter 1961 on the 2nd August 1961 and the 22nd January 1962. There were 12 treatments, 4 pots per treatment. The third sowing was on the 14th September 1961, the material was deglumed S.147 inoculated at anthesis.

Results

In the sowings before and after the winter 1961 smut was produced only in the later sowing from Onward inoculated at pre-anthesis (glumed) and anthesis(deglumed) The S.147 plants in the earlier sowing were unfortunately lost before th smut could be estimated and the S.147 plants in the later sowing did not come to maturity. The highest infection (8%) was produced on S.147 sown on the 14th September. The smut produced in the sowings (Table 15) is similar to the 5-6% smut obtained by natural infection (Table 14). Gage (1927) obtained 58-67% smut by dusting dry U.avenae spores onto oat blossoms and Malik (1959) found 6-22% U.nuda on barley inoculated by the Partial Vacuum technique. All the smut obtained by flower inoculation was from sowings of material inoculated at pre-anthesis and anthesis (Table 15). This confirms Malik's observations with U.nuda.

Flower inoculated 1960 Onward material was examined in December 1960 and February 1961. Examinations were made of 20 spikelets of the dried material, originally inoculated at pre-anthesis anthesis and post-anthesis. Some ungerminated spores were present on the glumes and there were a few spores on the pericarp, anthers and stigmas. No germinated spores, resting mycelium or 'gemmae'(dumb-bell-shaped pieces formed from the mycelium) were seen. Mycelium and spores of Altenaria spp. and

...Helminthosporium spp. were often observed on the glumes and pericarp and mycelium, possibly of Verticillium spp. on the anthers.

TABLE 15.

Summary of smut infection obtained from sowings of oats flower inoculated in 1960 and 1961.

Variety	Stage & date of of inoculation	Place of Sowing	Stage & Date of Sowing	Total Plants in ear	Smutted Plants in ear	% Smut
Onward	Anthesis 19June 1960	Field	Deglumed 12April 1961	131 157	1 3	1 2
Onward	Pre-Anthesis 16June 1961	Green-house	Glumed 22Jan.1961	28	1	4
Onward	Anthesis 29June 1961	"	Deglumed 22Jan.1962	37	1	3
S.147	Anthesis 26June 1961	"	Deglumed 14Sept.1961	36	3	8

After 3 months in dry storage no mycelium was observed in the embryos of 165 mature Onward oats flower inoculated in June 1960.

Fixed 1960 material was examined at 20, 25 and 30 days after inoculation and at maturity. Increasing contamination of the spikelets was observed with age. Ungerminated spores were present on many of the glumes particually at the tips. Occasionally spores produced small (2-5μ) germ tubes on the glumes and stigmas but not on the anthers

...or pericarp. There was no development of gemmae or resting mycelium.

The 1961 inoculated material was examined in June 1962. Fresh and fixed material of S.147 inoculated at anthesis and fresh material of Onward inoculated at pre-anthesis and anthesis were examined. A few gemmae were present on the glumes of S.147 material inoculated at anthesis, 20 days after inoculation. The gemmae were similar to those figured by Gage (1927) and Sampson (1929). They were 4-6 x 2μ in length, often produced buds and were usually found on the wings of the inner glumes. They appeared to be formed from small pieces of mycelium or germ tubes. There was no large development of mycelium. Spores of U.avenae were prevalent on the glumes, stigmas, anthers and pericarp. Germinated spores were occasionally seen on the glumes producing promycelia with 3 cells. Many other fungi were present on the parts examined. The mycelium of Alternaria spp. often produced structures ressembling gemmae 2-4μ in length. In addition, plate-like structures as figured by Hyde and Galleymore (1951) were seen on the glumes and pericarp.

Only ungerminated smut spores were observed on the glumes, anthers, stigmas or pericarp of Onward inoculated at anthesis or pre-anthesis. Many resting stages and mycelium of Alternaria spp. and other fungi were present, particually on the glumes and pericarp.

The temperature and R.H. figures for the 10 day periods after the flower inoculations in June 1960 and 1961 are summarized in Table 16. The maximum temperature, 32°C (90°F.) occurred on only one occasion.

TABLE 16.

R.H. and temperature data for the 10 day periods after inoculation of S.147 and Onward in 1960 and 1961.

Variety and Year	STAGE AT INOCULATION											
	Pre-Anthesis				Anthesis				Post-Anthesis			
	Temp.°C. Av.	Max.	R.H.% Av.	Min.	Temp °C. Av.	Max.	R.H. % Av.	Min.	Temp °C Av.	Max.	R.H. % Av.	Min
Onward 1960	18	27	79	36	17	26	78	36	14	22	76	40
Onward 1961	16	27	75	45	17	32	73	36	17	32	74	36
S.147 1961	16	27	75	45	17	32	73	37	17	32	73	36

R.H. = Relative humdity.

Discussion

The infection produced from artificial inoculations in 1960 and 1961 (Table 15) was low. There were many ungerminated spores in surface preparations of fresh and fixed floret material. It is probable that the low infection can be related to the viability of the spores

...or to the meteorological conditions during and after inoculation.

Diehl (1925) dusted spores onto flowers and found they germinated promptly on the moist stigmas. If the weather was cool and moist sporidia were produced, and if warm and dry the spores germinated directly into germ tubes. Only in very dry years did spores on the ovary remain ungerminated. The temperatures during and after inoculation in 1960 and 1961 (Table 16) were favourable to spore germination. It is likely that some of the spores could have germinated even at the maximum temperature of 32°C. In Part II there is evidence of spore germination at 30°C. and Arland (1924) has found some spore germination on the flowers at temperatures greater than 30°C. The relative humidities were also favourable (Table 16). Tapke (1931) observed germination of U.nuda spores on the flowers after 3 days at 11-33% R.H. A more likely reason for the poor germination and hence the low infection was the use of immature spores which quickly lost their viability.

Some spores on the glumes were observed to have germinated after 20 days with the production of gemmae but no development of mycelium. The maximum infection, 8%, was from hulled oats without glumes suggesting that the sources of infection are mycelium, gemmae or ungerminated spores on the pericarp or pales. However, no mycelium or

...gemmae were observed on the pericarp suggesting that most of the infection is from ungerminated spores remaining viable over the winter on the pericarp or pales. The results of spore germination and infection are so low however that the above observations cannot be used to support the hypothesis of Zade (1922), Gage (1927) or McKay(1936).

Increasing amounts of Altenaria and Helminthosporium mycelium were observed in 1960 and 1961 on material from 20 days after inoculation. In addition, in dried mature florets plate-like resting structures were seen similar to those described by Hyde and Galleymore (1951). Gaumann (1950) p.95 has described the mycelium of Helminthosporium gramineum on barley and U.avenae on oats as 'Saprophytic transitional infection'. The mycelium is present on the surface of the grains or glumes and lies dormant over the winter enhancing the chance of infection of the seedling when the grain germinates. In the saprophytic competition of a substrate there is often antagonism between the saprophytes. The Helminthosporium and Altenaria mycelium on the oat grains was vigorous in growth and may possibly have had an adverse effect on the germination of the spores or development of the mycelium of U.avenae.

D. CONTROL EXPERIMENTS

Review of Literature

Prior to 1930 control measures for oat smuts used mainly formaldehyde, and copper carbonate dusts. In the next 20 years these were largely replaced by organomercurial dusts. By 1950 early work had been done on liquid organomercurials. Today they are becoming increasingly important, their main advantage over the dusts is their ease of use and application. In the last decade several investigators have studied the effect of the length of storage period and vapour action of liquid organomercurials on oats possessing inoculum beneath the palea and lemma (sub-hull inoculum).

Commercially seed is stored from several days to many months after treatment before sowing. Hansing (1953) found that if inoculated oat seed was stored for only one day after treatment with Panogen less smut resulted than if it was sown immediately after treatment. He also found non-volatile fungicides gave less control of oat smut. Arny and Leben (1954) found that Panogen vapour gave adequate control of Helminthosporium victoriae present beneath the oat hulls after exposure of one day only, and part of the Panogen vapour was shown to penetrate beneath the hull.

In an effort to standardize fungicide tests, the American Phytopathological Society (1944) described conditions to be fulfilled for testing dust seed treatments.

...These conditions have been fulfilled in the following tests on liquid seed treatments.

Methods

The fungicides used were all liquid organomercurials. They consist of an active principle containing organically combined mercury and an inert filler. The following were used:-

1. Aabiton — The active ingredient is 1.43% methyl mercury benzoate containing 0.85% mercury. Aabiton is manufactured by the Berk Chemical Company.
2. Agrosol — This is manufactured by Plant Protection Ltd. (I.C.I.); the firm has stated that the chemical formulation should not be published.
3. Ceresan 100 — The active ingrediants are ethyl mercury 2, 3-dihydroxy propyl mercaptide 3.10%, and ethyl mercury acetate 0.67%; total metallic mercury is 2.30%. The manufacturers are Dupont and Company.
4. Chipcote 75 — The active ingredient is methyl mercury nitrile 1.85%. The product has the equivalent of 1.5% metallic mercury and is produced by the Chipman Chemical Company.

The following rates of application were used per

...30g. of oats:-

Aabiton	:	0.06g.	(Volume/Weight)
Agrosol	:	0.09g.	" "
Ceresan 100	:	0.06g.	" "
Chipcote 75	:	0.06g.	" "

A special technique was used for applying the fungicide. A 1ml. pipette attached to a metal syringe was used for measuring the fungicide. The liquid was then added to the side of a bottle lying at an angle of 45°. The cap of the bottle was then replaced and the bottle rolled on a level surface. In this way an even distribution of fungicide was obtained on the inner surface. Deglumed oats were then added and the bottle again rolled to give the oats an even coating of fungicide. The treated seed was poured through a wide necked funnel into envelopes and then sown.

Some of the oats were seed inoculated and others flower inoculated, by partial vacuum.

Experiment 1. Field experiments, April 1961, with flower inoculated oats.

Onward oats were flower inoculated at Anthesis with C1 in June 1960 and subsequently stored in the laboratory. Two series of experiments, each six treatments were sown on 12th April 1961 at Silwood in randomised plots. Each plot consisted of five 6ft. rows, 15g. of oats per plot. The six treatments were uninoculated untreated control,

...inoculated untreated control, inoculated treated with Aabiton, treated with Agrosol, treated with Ceresan 100, and treated with Chipcote 75. The oats were sown two hours after treatment. The percentages of smut were estimated on 2nd August 1961.

Experiment II. Greenhouse experiment, September 1961, with flower inoculated oats.

The oats were S.147 flower inoculated at anthesis in June, and sown on 14th September 1961. One series of experiments was sown with the same treatments as Experiment I. except for the omission of the treatment uninoculated, untreated. Each treatment consisted of fourteen 5ins. pots, 5 seeds per pot. The pots were randomised and put under lights on a 16 hour day. The percentage smut was estimated on 4th December 1961.

Experiment III. Greenhouse experiments, December 1961, and March 1962 with seed inoculated oats.

The oats used were S.147 inoculated with C2. Five treatments were used as in Experiment II, each consisting of twenty-five 5ins. pots; 8 seeds per pot. Two series of experiments were sown, on the 21st December 1961 and the 10th March 1962. In each series, thirteen days after sowing, counts were made of seedling emergence. Estimates of the percentage smut were made on the 7th May 1962 for series I and II respectively.

Results

No smutted plants were produced in the fungicide treated plots of Experiment I and there was only a low level of infection in the inoculated, untreated controls. Series 1 of the inoculated untreated plots produced 0.8% smutted plants and 1.2% smutted tillers, and series 2, 1.9% smutted plants and 1.0% smutted tillers.

Infection in Experiment 2 was also low (Table 17), 7.7% smut was produced from the total plants in ear. The Agrosol treatment was the only fungicide treatment to produce smut, totalling 4.0% of the total plants in ear.

TABLE 17.

The effect of various fungicides on flower inoculated S.147 oats in the greenhouse(Experiment 2)

Treatment(all inoculated)	Total Plants	Smutted Plants in Ear	Not Smutted in Ear	Doubtful *	% Smut Total Plants	% Smut Total Plants in Ear
Control Untreated	66	3	36	27	4.5	7.7
Aabitom	63	0	44	19	0.0	0.0
Agrosol	68	2	47	19	2.9	4.0
Ceresan 100	47	0	27	20	0.0	0.0
Chipcote 75	53	0	39	14	0.0	0.0

* Many dead plants present, death due to blasting.

TABLE 18.

The effect of various fungicides on seed inoculated S.147 oats in the greenhouse (Experiment 3).

Series I Treatment (All Inoculated)	Total Plants	Plants in ear		Plants Not in Ear	% Smut	
		Smutted	Not Smutted		Of Total Plants	Of Plants in Ear
Control Untreated	154	134	8	12	87.0	94.4
Aabiton	170	.5	138	27	2.9	3.5
Agrosol	193	18	154	21	9.3	10.5
Ceresan 100	183	3	157	23	1.6	1.9
Chipcote 75	167	1	150	16	0.6	0.7
Series 2						
Control Untreated	162	133	20	9	75.9	87.6
Aabiton	175	31	119	24	17.8	20.7
Agrosol	187	17	146	24	9.1	10.4
Ceresan 100	170	1	131	38	0.6	0.8
Chipcote 75	183	3	147	33	1.6	2.0

The smut infection produced in the controls of both series of Experiment 3 was very high (Table 18). There appears to be some stimulation of seedling emergence in all the fungicide treatments compared with the controls (Table 19). The best control after 2 hours soaking with fungicide is evidently from Ceresan 100 and Chipcote 75 (Table 18). Two hours soaking with Agrosol and Aabiton

TABLE 19.

The effect of fungicides on seedling emergence in Series 1 and 2 of Experiment 3.

Treatment (All Inoculated)	Total Seeds Sown per Series	% Emergence Series 1	Series 2
Control Untreated	200	85.5	89.0
Aabiton	200	85.5	98.5
Agrosol	200	99 5	99.0
Ceresan 100	200	97.0	97.0
Chipcote 75	200	92.5	98.0

...gave less control. No tillers were present in series 1 or 2.

Discussion

The low infection produced from flower inoculated oats in Experiment 1 and 2 is probably explained by the use of immature spores for the flower inoculations. The high infection in Experiment 3 was because spores of high viability were used and excellent conditions, 20°C. for 3 months, for seedling infection prevailed in the greenhouse throughout the experiment.

An increase in emergence of fungicide treated oats compared with the untreated controls has also been reported

...by Hansing (1953).

The reason why the percentage infection is high (0.5-10.0%) in treated plots is partly because the seeds were only soaked for 2 hours in the fungicides and also because of the technique of inoculation. The period of soaking prior to sowing was only 2 hours, in commercial practice it would be much longer, from one week to several months. Purdy (1958) has pointed out the desirability of using naturally infected oats for fungicide tests as the partial vacuum technique causes such high infection that even the best materials were ineffective. There was a difference in control however between the four fungicides even after 2 hours. Arny and Leben (1954) found there was a difference in vapour action between 2 liquid organomercurials Panogen and Ceresan M. The Panogen vapour quickly penetrated beneath the hulls and gave adequate control of sub-hullinoculum of Helminthosporium victoriae mycelium after an exposure of one day. The Ceresan M penetrated more slowly and required an exposure time of 8 days for comparable results. It is probable that the difference in control between the four fungicides is related to the differences in vapour action of the chemicals. The vapour of Ceresan 100 and Chipcote 75 is apparently taken up more quickly under the hull than the vapour of Aabiton and Agrosol.

PART IV. FACTORS AFFECTING INFECTION

INTRODUCTION

Reed (1936) has stated that if U.avenae mycelium reaches the growing point, post-emergence conditions cannot alter the resultant infection. This has been confirmed with bunted wheat plants in sand culture by Purdy (1961). Recently, the effect of temperature on smut infection in the post-emergence phase has been investigated. In several cereal smuts it has been found that the post-emergence temperature can alter the resultant infection (Tapke, 1940; Kavanagh, 1961). There is conflicting evidence on the influence of post-emergence factors on smut in the inflorescence (Kiesling, 1962).

It was decided to investigate several factors which have been reported to influence infection in the pre- and post-emergence phases. The factors chosen were, depth and time of sowing, nutrients in sand culture, and plant growth substances. It was hoped to correlate the results obtained with the morphology of the host and the movement of the fungus in the plant as described in Part II.

A. THE INFLUENCE OF DEPTH AND TIME OF SOWING ON SMUT INFECTION.

Review of Literature

Moldenhauer (1927) using oats dusted with U.avenae spores noted that there was increased smut with increased sowing depth. Jones and Nasr (1940), and Swinburne (1960) obtained similar results with T.caries. Jones and Nasr state that deep sowing seems to operate in smut diseases by delaying the rupture of the coleoptile and consequent onset of resistance in the seedling. The length of the first internode of oat seedlings varies with the depth of sowing of the seed (Bonnett, 1961).

Generally the highest infection is given by low soil moisture and high temperature. Bartholomew and Jones (1923) found that optimal infection of oats by U.avenae occurred at 18-20°C (64-66°F.) These temperatures coincide with the optimum for germination and growth of the fungus and the host. Johnston (1927) found the greatest infection of oats by U.levis and U.avenae was produced at 17-19°C. (62-66°F.) and at soil moistures of less than 30% water holding capacity. In Sampson's (1929) experiments the temperature was relatively constant and moisture was the critical factor.

The next section is an account of investigations made on the effect of the depth of sowing on the morphology of oat seedlings and the effect of meteor-

...ological conditions on disease development by monthly sowings in the field.

Methods

The field sowings were carried out at Silwood from October 1960 to March 1961 at approximately monthly intervals. Onward and S.147 hulled oats were dusted with C2 at the beginning of the experiment and subsequently stored. Four grammes of oats were sown per row one and two inches deep in plots consisting of five 6 ft. rows. Two plots were sown of each variety and at each sowing depth. Altogether eight plots per sowing date were sown. Counts were made of the emergence per row 5 weeks after sowing and the number of smutted plants and tillers per row at maturity. The meteorological data was taken from the site at Silwood. The figures for the daily average air temperature, four inch soil temperature and rainfall were averaged for the 10 day period after each sowing.

The variation in morphology of the seedlings with sowing depth was investigated in two series of sowings of Onward and S.147 hulled oats in the greenhouse. Series I, of Onward, was set up on the 2nd October, 1960, and series II, of S.147, on 30th May, 1962. The Onward oats were sown 1 or 2 inches deep and the S.147 1, 2 and 3 inches. Ten oats were sown per tin. Harvests of 20 seedlings per depth were made at intervals from the

...third to the nineteenth days. Measurements were made of the coleoptile from the tip to the node, the first internode, and the lengths of the first and second leaves above the coleoptile tip. In Series I the emergence per day was also calculated.

Results.

Field Sowings - The percentage smut produced was generally low. The maximum, 21% smut, was from the 1 inch deep sowing of S.147 on the 27th March 1961.

The change in sowing depth from 1 to 2 inches did not markedly influence the percentage of smutted plants (Fig.41 and 42). Generally Onward produced less smut than S.147.

The October sowings produced low infection, varying from 1-3%. Both the temperature and rainfall were relatively high in the 10 day period after sowing (11°C. and 76mm. of rain). It is probable that the high moisture was the factor limiting infection, as spores can germinate at 11°C (Fig. 5).

No smutted plants were produced from the November and December sowing and there was less than 1% smut from the January sowing. There was a fall in temperature and rainfall in the 10 day period after each of these sowings. The average temperatures of the periods had a range of 7-3°C. and the rainfall from 45.0 - 9.0mm. Possibly in

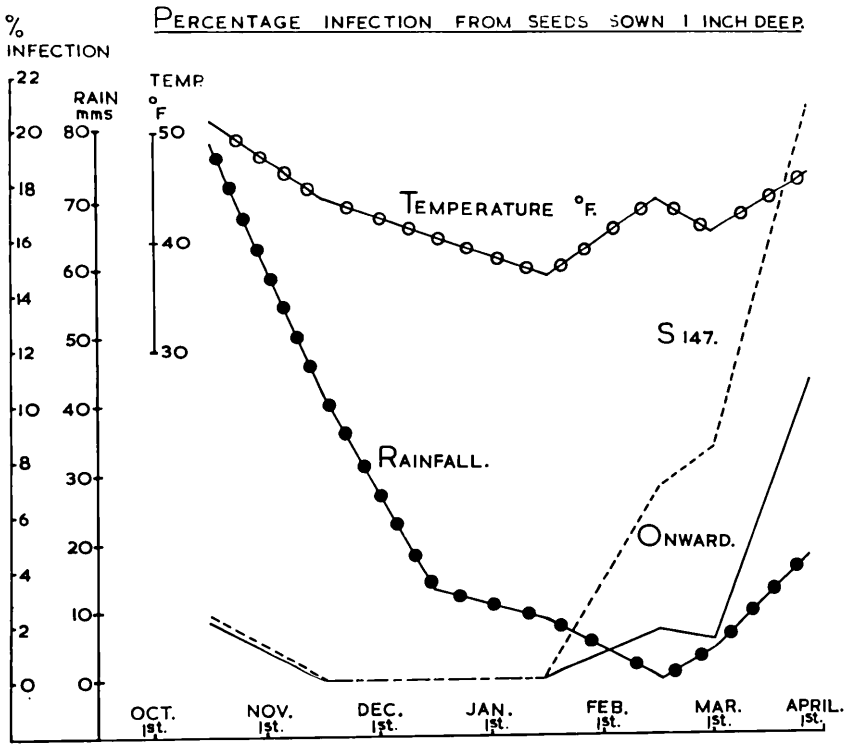

Fig. 41. The percentage infection from monthly sowings of Onward and S 147 oats dusted with C2 and sown linch deep in the field.

---- Infection produced on S 147.

——— Infection produced on Onward.

The data for temperature and rainfall are the averages for the 10 day period after each sowing

—●●●— Rainfall

—000— Temperature

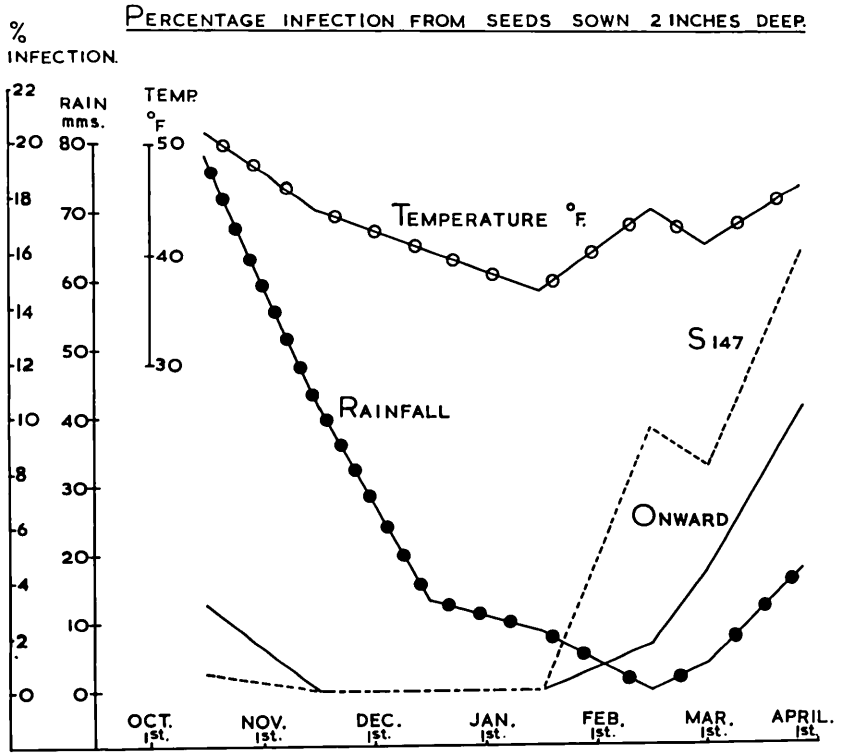

Fig. 42. The percentage infection from monthly sowings of Onward and S.147 oats dusted with C2 and sown 2 inches deep in the field.

- - - Infection produced on S.147

—·—·— Infection produced on Onward

The data for temperature and rainfall are the averages for the 10 day period after each sowing.

—●—●—●— Rainfall

—o—o—o— Temperature

...the November sowing high rainfall (40mm.) was the limiting factor and in the December and January sowings, low temperature (5-3°C).

The 13th February and 27th March sowings produced 2-10% and 11-21% smut respectively. The corresponding temperatures were 7 and 8°C and the rainfall 0.5 and 19.0mm. Compared with the October sowing, the temperature was lower and the rainfall much less. Temperature was probably the limiting meteorological factor.

There was poorer emergence of Onward seedlings compared with S.147 but no relation between decreased emergence and sowing depth in either variety (Table 20). Both the varieties had low emergence in the later sowings from February onwards because of bird damage.

TABLE 20.

Emergence of seedlings counted 5 weeks after each sowing.

Date of Sowing	Variety and Depth of Sowing			
	S.147 1in.	On. 1in.	On. 2in.	S.147 2in.
	Percentage Emergence			
18 October 1960	87	61	57	81
16 November 1960	72	44	43	75
15 December 1960	86	34	19	77
13 January 1961	83	24	18	86
13 February 1961	54	27	39	65
1 March 1961	55	13	38	76
27 March 1961	21	15	24	25

Greenhouse sowings - In the first series of sowings, of Onward, the shallow sown seedlings emerged on the 6th day. The deeper sown emerged on the 8th day at a greater emergence rate per day. By the 12th day there was 100% emergence in the two sowings. The lengths of the coleoptiles, first internodes and first and second leaves were measured and some of the data are shown graphically(Fig. 43).

The growth rates of the coleoptile and first internode were greater in the seedlings sown 2 inches deep. In Fig. 43 a dotted line indicates the critical 1cm. level (Brefeld, 1895) of the first leaf above the coleoptile sheath. In the 1 inch sowing this level is reached 8 days after sowing, and in the 2 inch sowing only 15 hours later. Table 21 is a summary of similar measurements of S.147 seedlings (Series II sowing). The critical 1cm. level in the 1 and 2 inch sowings is reached on day 6 but in the 3 inch sowings it was not until 2 days later. The period of susceptibility to infection is much greater, therefore, in the 3 inch sowing; the length of the period is about the same in the one and two inch sowings.

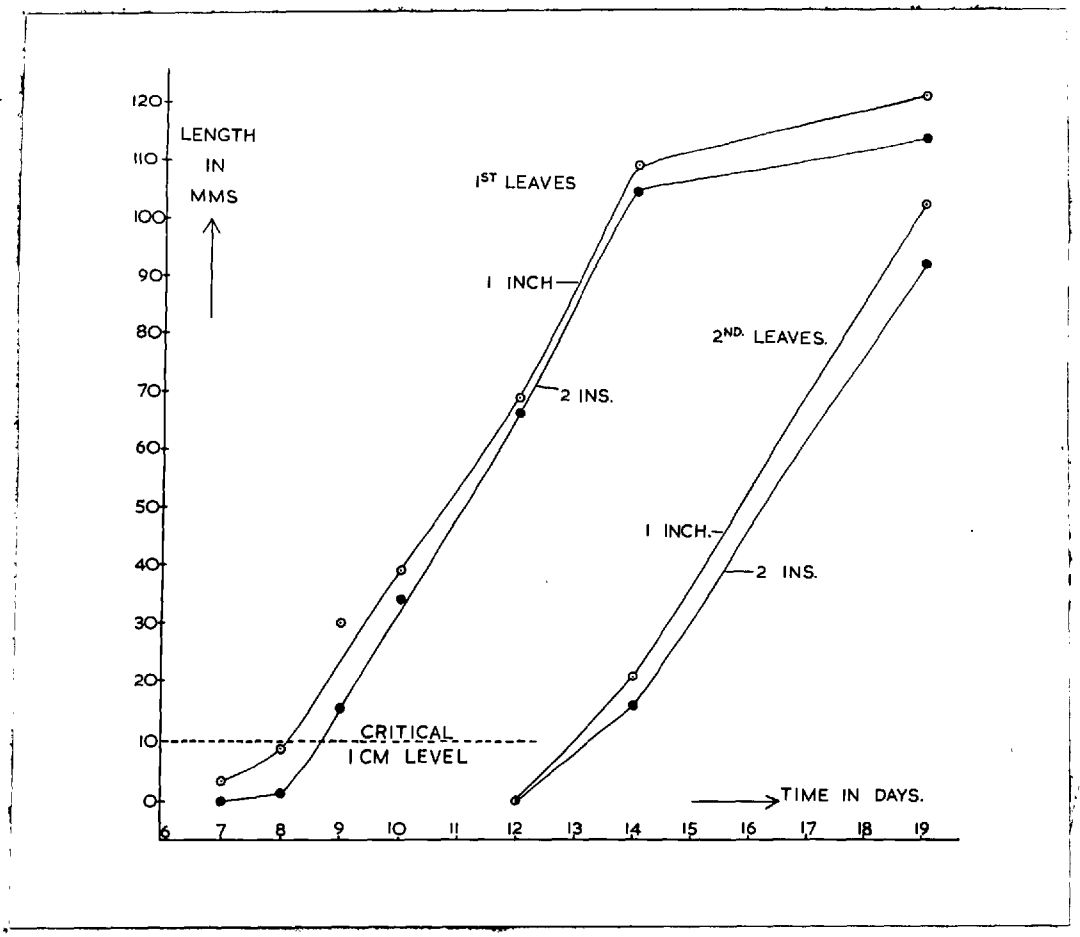

Fig.43. The variation in length of the first and second leaves of Onward seedlings sown one and two inches deep.

TABLE 21

Lengths of the first leaves above the coleoptile sheath of S.147 seedlings sown 1, 2 and 3 inches deep.

Day After Sowing	Depth of Sowing (Inches)		
	1	2	3
	Length in mm. *		
4	0	0	0
5	4.5	0	0
6	15.1	11.4	0
7	33.5	26.6	0
8	42.0	37.1	10.3
10	64.0	63.6	33.8

* Figures are averages of 20 observations.

Discussion

There is variation in the pre- and post emergence environmental conditions with the time and depth of sowing. Reed and Faris (1924) studied pre-emergence conditions and found that the temperature, moisture and pH of the soil were interdependent factors and that their interaction determined whether nfection took place. Bartholomew and Jones (1923) have established that optimal infection is attained with low moisture and medium temperature (18-20°C). In the 1960-61 field sowings the average temperature recorded for the 10 day period after a field sowing was only 3-7°C. with a maximum of 11°C. The soil temperature

... after the field sowings was thus too low for optimal infection. Hecke (1909) has emphasized the threefold importance of temperature, namely as a direct effect on the germination of the smut spores and seed, on the duration of the susceptible stage of the host plant, and on the passage of the fungus to the growing point. No evidence is available from the field sowings of the influence of post-emergence environmental conditions on infection. Tapke (1940) has observed that prolonged cold conditions after seedling emergence may result in a marked reduction in the incidence of oats loose smut. However, there were no long very cold periods after the 1960-61 field sowings.

Summarizing, the low infection in the field sowings was probably because of the unsuitable pre-emergence environmental conditions for spore germination. The growth of the plant is reduced under these conditions increasing the length of the susceptible period. However little smut was produced because of the lack of germinated spores. The optimal temperatures for germination of fungus and host have been shown by Hecke (1909) to be very similar. In October and November soil moisture was the factor limiting infection. When the soil dried out in the spring the soil temperature was too low for optimal infection and only 20% smut was produced. It is probable

...the infection produced from the sowings would have been considerably increased by dehulling (Sampson 1929).

Similar amounts of smut were produced from the 1 and 2 inch sowings unlike the 1.5 and 3 inch sowings of wheat dusted with bunt spores (Swinburne, 1960). He found there was increased infection in the deep sowings. In the 3 inch deep sowings of uninoculated S.147 the first leaf attains the critical 1cm. length above the coleoptile sheath 2 days later than the 1 and 2 inch sowings. In the 3 inch sowings the fungus would have more time to penetrate the coleoptile before it becomes resistant to penetration. Secondly, the elongation of the internodes, which push the growing point away from the coleoptile, occurs later so that there is more time for the fungus to reach the growing point region. In the 1 and 2 inch field sowings, therefore, the periods of susceptibility and consequently the smut produced are approximately the same.

B. THE INFLUENCE OF NUTRIENTS IN SAND CULTURE ON SMUT INFECTION.

Review of Literature

Sand culture experiments were used by Millikan (1939) to test the effect of various elements on infection of wheat with flag smut (Urocystis tritici Koern.). Excess calcium was found to increase incidence and deficiencies of calcium, or of nitrogen, or potassium to decrease the disease.

Swinburne (1960) did similar work with T. caries. He investigated the effect of calcium, potassium, nitrogen and phosphate on disease development. Absence of nitrogen, potassium, or phosphate caused a decrease in bunt, but if the nutrients were withheld only until elongation of the internodes, then calcium or nitrogen deficiencies were found to increase bunt.

Purdy (1961) investigated wheat bunt development in relation to post-infection environment. Deficient and excess amounts of nitrogen or potassium or phosphorus or calcium failed to increase or decrease bunt.

Methods

S 147 oats inoculated with C1 by the partial vacuum technique were sown in two series of experiments. Each series consisted of 16 tins, four tins per treatment, four treatments per replicate. The tins were bituminous

... painted, 50 cm. long by 20 cm. diam. and pierced at the base. The silica sand which was used came from Messrs. Arnolds Quarries, Pit no.21 Leighton Buzzard and had been washed at the pit. A layer of glass wool covered the holes to prevent loss of sand.

The oats were sown in sand in glass dishes and watered occasionally with distilled water. When the seedlings had emerged about 2 cm. they were transplanted, 8 seedlings per tin. The tins were arranged on a greenhouse bench under mercury vapour lamps kept on for 16 hours a day. The temperature during the experimental periods was uncontrolled, the average daily range was 6-30°C.

The four treatments consisted of nutrient solutions made up from the formulations of Hewitt (1952). The solutions chosen were those deficient in nitrogen, potassium, calcium and a complete medium containing the ions for normal plant growth. The concentrations of the stock solutions and the constituents of the nutrient solutions are given in Table 22. The nutrient solutions were added at the rate of 250 ml. per tin every other day, the solutions being applied at the periphery of the tin to avoid damage to the root anchorages. The first application was given at half the normal strength, subsequent additions were at normal strength. After 65 days all the plants were given tap water.

TABLE 22

Salt concentrations in stock and nutrient solutions from Hewitt, 1952.

Salt	Conc.of stock (g./100ml.)	No. of stock to make 2 litres of nutrient solution			
		C.M.	-K.	-Ca.	-N.
KNO_3	20.2	3.34	0	3.34	0
$NaNO_3$	34.0	0	1.66	3.34	0
$Ca(NO_3)_2$ anhyd.	32.8	3.34	3.34	0	0
K_2SO_4	8.7	0	0	0	6.66
$CaSO_4.10H_2O$	34.4 *	0	0	0	3.34
$MgSO_4 7H_2O$	18.4	4	4	4	4
$NaH_2PO_4.2H_2O$	20.8	2	2	2	2
* Weight/Volume Suspension					
Ferric citrate	2.45	2	2	2	2
$MnSO_4.4H_2O$	0.0223	2	2	2	2
$CuSO_4.5H_2O$	0.024	2	2	2	2
$ZnSO_4.5H_2O$	0.029	2	2	2	2
H_3BO_3	0.186	2	2	2	2
$(NH_4)_4MO_7O_2.4H_2O$	0.0035	2	2	2	2

C.M. = Complete Medium

-K = Medium deficient in potassium

-Ca = Medium deficient in calcium

-N = Medium deficient in nitrogen

Results

Series I

Inoculated S 147 oats were sown 4 cm. deep in sand on 15th January 1961 and transplanted into tins five days later.

Thirteen days after sowing the plants lacking nitrogen had thinner second leaves and were smaller than the plants in the other treatments.

After 24 days the nitrogen deficient plants were in third leaf, the potassium deficient plants were in the early fourth leaf and the other treatments between the late fourth to early fifth leaf stages.

After 28 days reddish-mauve stripes had appeared on the first leaf sheaths of plants not supplied with nitrogen and the tips of the first and second leaves were withered in the potassium deficient plants. Tillers were present only in the complete and calcium deficient treatments.

After 36 days there were many dead second and third leaves in the potassium deficient plants. The younger leaves were not as broad as the leaves in the complete treatment.

The first ears to emerge were in the nitrogen deficient treatment at 59 days. Only the topmost floret was present in the two plants in ear. Wilting had occurred in the last leaf of the main tillers of the calcium deficient plants. In appearance the plants were

...bushy and a mottled yellow-green colour. The texture of the leaves was very rough compared to the leaves of the plants in the complete medium.

After 80 days, 18 of the nitrogen deficient plants had panicles but none were smutted. Many of the calcium deficient plants had primary tillers with dead growing points. The plants had a bushy appearance because of the non-elongation of some internodes. In addition to the secondary tillers, a number of small green tillers had arisen at the base of many plants. Many of the plants lacking potassium had withered leaves with dead tips.

After 122 days plants in ear were present in all the treatments. In the complete nutrient treatment there were 36 panicles and in the potassium deficient 18, derived from primary and secondary tillers. The calcium deficient plants had 16 panicles derived solely from the secondary tillers as all the primary tillers were dead. The nitrogen deficient plants had produced 36 panicles mainly with one or two florets at the top of the panicle. The root systems of the nitrogen and potassium deficient plants were much less developed than the other treatments. The most root development was in the complete medium. There were, however, no smutted panicles produced in Series I. .

Series II

Inoculated S 147 oats were sown on 11th February, 1961 and transplanted 5 days later.

Nine days after sowing the nitrogen and potassium deficient plants were already noticeably smaller than the complete nutrient treatment. After 15 days the plants lacking nitrogen were blue-green in colour and the first leaves of these plants and of the potassium deficient plants possessed red tips.

After 54 days many of the growing points in the calcium deficient plants were dying or dead and the leaves were harsh in texture. Some intervenal chlorosis and blotchy patches were apparent on the leaves.

After 65 days there were five panicles in the nitrogen deficient treatment, two of which were smutted (Fig. 44). Many of the primary tillers of calcium deficient plants had reddish-mauve upper leaves and dead growing points.

After 99 days two smutted and three non-smutted panicles were present in the complete treatment. There were 4 smutted out of a total of 16 panicles in the nitrogen treatment. In the calcium deficient plants all the primary tillers were dead but the secondary tillers had in one instance produced a smutted ear. As in Series I, many small green tillers were present at the base of the plants.

Fig. 44. A nitrogen deficient plant of S.147 with a smutted panicle, 65 days old. x ½ approx.

Figure 45 is a photograph of Series II taken 115 days after sowing.

On the 126th day the experiment was terminated; the results obtained are presented in Table 23. All

Table 23. The effect of various nutrients in sand culture on inoculated S 147 oats, 115 days after sowing.

Treatment.	No.of Plants.	Av.Ht. cm.	Av. no. Tillers per Plant.	Root System	No. Smutted Plants.	% Smut
Complete	25	35	3.0 (Range 0-5)	extensive	11	44
No Nitrogen	27	13	1.5 (Range 0-3)	poor	5	19
No Calcium	8	14	10.0 (Range 0-18)	fair	2	25
No Potassium	28	20	2.7 (Range 0-4)	poor	7	25

the treatments had produced smutted panicles.

Fig. 45. Inoculated S.147 oats 115 days old in sand culture. Treatments from left to right are; complete medium, complete medium deficient in potassium, complete medium deficient in nitrogen, complete medium deficient in calcium.

Discussion

The deficiency symptoms were similar in appearance in plants with or without smutted panicles.

The nitrate deficient plants were the first to ear, confirming the observations of Reed (1936). They were also the first to produce symptoms, 1-2 weeks after sowing. Calcium deficiency however was not apparent until the 8th week. Probably some calcium was derived from the seed and from the sand.

Reed (1936) has concluded that the path of the smut mycelium does not appear to be substantially modified by differences in the rate of the post-emergence plant growth. He grew inoculated oats under optimal conditions for infection and transplanted the seedlings into sand 6-7 days after emergence. They were then grown with and without sodium nitrate and illumination. In all the treatments there was very high infection. The results from the second S 147 sowing bear out Reed's observations. The lack of smutted plants in the first S 147 sowing can probably be explained by poor spore viability.

If the inoculated oats are grown under optimal infection conditions for 7 days the fungus will be in the first or even the second leaf base (See Section on the Mechanism of Infection). At transplanting there could have been a slight slackening of seedling growth

... probably to the advantage of the fungus. Finally the nutrient treatments did not immediately affect plant growth, again favouring the fungus. It is, therefore, highly probable that many of the growing points possessed mycelium. Once in the growing point the mycelium is carried up passively by the elongation of the internodes and excess and deficient amounts of various elements. will apparently not affect the mycelium and the expression of symptoms. This has recently been demonstrated by Purdy (1961) with wheat seed inoculated with T. caries spores. The non smutted plants in the second S 147 sowing can probably be explained by some of the fungus not reaching the growing point before elongation of the internodes.

C. THE INFLUENCE OF GROWTH SUBSTANCES ON SMUT INFECTION.

Review of Literature

Schoene and Hoffman (1949) investigated some of the properties of maleic hydrazide (M.H.) and found that it had a pronounced but temporary inhibitory effect on the growth of many plants. Forsyth and Samborski (1958) produced a breakdown in the resistance of Khapli wheat to Puccinia graminis by applying M.H. to the soil before inoculation. Nair (1958) using wilt resistant flax decreased their resistance by spraying inoculated soil with M.H. In in vitro experiments M.H. stimulated growth of the pathogen. Richardson (1959) sprayed soil with M.H. and 2,4-dichlorophenoxyacetic acid (2,4-D.) before root inoculation of tomatoes with Fusarium. Generally, M.H. increased the severity of the wilt. The M.H. and 2,4-D. were found to affect resistance by acting on the host, not the fungus.

Stowe and Yamaki (1957) state that stem elongation is the most typical and striking plant response to treatment with gibberellic acid (G.A.). Brian et al (1954), and Stowe and Yamaki (1957) both state that G.A. has not affected several bacteria and fungi in tests. Farrar (1958) controlled U.avenae at concentrations of 500 and 1000 p.p.m. G.A. applied as a seed soak or as seedling spray. The G.A. was in 95% ethyl alcohol solution.

...Swinburne (1960) found no control of T.caries with 500 and 1000 p.p.m. G.A. applied as a seed soak or seedling spray. Mantle (1960) found no control of Ustilago tritici. using 1000p.p.m. G.A. applied to the seeds or seedlings. The frequency of sporulation on the flag leaf of plants from seeds soaked in G.A. suggests that growth of U.tritici is stimulated.

The next section is an account of experiments made with inoculated seeds and infected seedlings treated with M.H. and G.A. in various concentrations. Studies were also made on the effects of the growth substances on disease development.

Materials and Methods

Gibberellic acid powder was dissolved in polyethylene glycol 400 (P.E.G. 400) to give a $4x10^3$p.p.m. solution. This was diluted with water. The G.A. was kindly supplied by Plant Protection Ltd. and the P.E.G. 400 by the Shell Chemical Co.Ltd. A 50% ethyl alcohol solution was used for spraying plants in the field.

A 4×10^3p.p.m. maleic hydrazide solution was made up in hot water and then diluted with water. Two drops of a wetting agent, Tween 80, were added to every 100ml. of M.H.

In all the experiments the seed was inoculated with C2 by the partial vacuum technique. The oats were dried,

...stored in the refrigerator and then treated with the solution of growth substance in the evacuation apparatus (Fig.4) at the rate of 3ml. per 60 seeds. The oats were evacuated 20mins., dried on blotting paper and then sown. Untreated but inoculated oats were soaked in water. The seedlings were usually sprayed in the third leaf stage. Seedlings sprayed with M.H. were left in a damp chamber for 3 days as the G.A. was in non-aqueous P.E.G. 400 solution. This procedure was unnecessary with G.A. sprayed seedlings. Estimations of plant height, measured from ground level to topmost node, and the percentage smut at maturity were made in both greenhouse and field experiments. In the greenhouse the oats were sown 4cm. deep in tins of compost. The temperature was uncontrolled and the lights arranged so that there was 16 hours of light a day. The field experiments were sown at Silwood in a sandy loam.

Spore germination tests were carried out on 2% plain agar. The solutions of growth substances were added by sterilized pipette to 25ml. quantities of cooling sterilized agar. The final concentrations of growth substances were 50, 100, 500, 1000 p.p.m. Each petri-dish contained 12.5ml. of the agar-growth substance mixture. The control plates contained agar only. Spores of C2 in sterile water were sprayed onto the agar using an atomiser. There were four plates per treatment. The plates were illuminated by 5 80w. neon tubes suspended 37.5cm. above the experi-

...ment in a 20°C constant temperature room. Spore germination was assessed after 48hrs. About 2000 spores were randomly counted. Measurements were made in each treatment of germ tube lengths.

Results

Experiment I. G.A. applied as a seed soak in greenhouse and field.

Two series of experiments were sown in the greenhouse. Series 1, of Onward, was sown on 23rd October 1960 and series 2, of S.147, on 20th January 1961. Estimations were made of the emergence per day and total emergence of the seedlings.

TABLE 24.

The emergence of S.147 seedlings per day and total emergence at 12 days after the seeds had been soaked in water or 500 or 1000p.p.m. G.A.

Days After Sowing	Inoculated Seed No. G.A.	Inoculated Seed + 500 p.p.m.G.A.	Inoculated Seed + 1000 p.p.m. G.A.	Uninoculated Seed No. G.A.
6	0	0	0	0
7	3	18	21	10
8	27	20	19	30
9	6	7	11	10
10	0	0	0	0
11	0	0	1	0
12	1	1	0	0
Emergence as % seed sown	62	77	87	83

G.A. treated seedlings emerged more quickly than untreated (Table 24). The lengths of the coleoptiles and first and second leaves were measured after 15 days. The coleoptiles and first leaves were generally longer and the second leaves were usually shorter in treated compared with untreated plants. Most of the first leaf elongation occurred in the leaf sheath not in the lamina (Fig. 46). The apparent shorter length of the second leaves of treated plants is probably because part of the leaf is obscured by the large first leaf sheaths. The G.A. treated plants were floppy and more brittle than the untreated. The percentage smut and average heights of the plants were recorded at maturity (Table 25).

TABLE 25.

Average plant heights and percentage smut from two greenhouse sowings using seeds soaked with G.A. or water.

Treatment	Series	Total Plants	Total Smutted	Smut on Flag	% Smut	Average Heights Cm. Smutted	Average Heights Cm. Non Smutted
Uninoculated No. G.A.	1	46	0	0	0	0	38.4
	2	47	0	0	0	0	28.4
Uninoculated + 500p.p.m.G.A.	1	48	0	0	0	0	38.1
	2	NOT SET UP					
Inoculated No. G.A.	1	45	11	0	24	37.3	45.2
	2	34	5	0	15	24.0	30.3
Inoculated + 500p.p.m.G.A.	1	39	13	0	33	38.1	49.1
	2	42	9	5	21	24.2	32.8
Inoculated + 1000p.p.m.G.A.	1	NOT SET UP					
	2	47	8	4	17	27.0	28.8

Fig. 46. S 147 seedlings, seed soaked with G.A. showing extension of the first leaf sheaths 11 days after sowing. Treatments from left to right are; uninoc. no G.A., uninoc + G.A., inoc + G.A. and inoc. no G.A.

Experiment II. G.A. applied as a seedling spray in the greenhouse and field.

There were 2 greenhouse sowings; series 1, of Onward was sown on 19th October 1960, and series 2 of S.147 on 18th January 1961. Measurements and observations were made on the seedlings before and after spraying.

The Onward seedlings were first examined 6 days after spraying. The third leaf sheaths had extended considerably reducing the visible length of the fourth leaf. Later the fourth internode of treated plants had extended (average length 75mm.) more than the untreated (average length 38mm.) Six weeks after spraying the G.A. treated plants were taller but more spindly than the unsprayed. Many treated plants were prostrate but some had regained a vertical position by bending at the nodes. There was little variation in the number of nodes and much variation in the heights of mature plants sprayed with G.A. and water (Table 27). There was no control of smut. Smutted flag leaves were observed on plants treated and not treated with G.A.

There was no control of smut by G.A. and little apparent difference in plant height between the treatments with and without G.A. (Table 25). There was some evidence for increased smut on the flag leaves in the G.A. treatments. The number of tillers and nodes were compared in the S.147 treatments but no differences were found. The smutted plants of S.147 and Onward were usually smaller than the plants not exhibiting smut symptoms.

The field sowing on 19th March 1961 consisted of 4 plots of 5 rows, each six feet in length and containing 3.5g. of S.147 oats. The treatments and results are presented in Table 26. The final estimate was made on 19th July 1961. No control was achieved in the field confirming the greenhouse observations.

TABLE 26.

Average plants heights and percentage smut from seeds soaked with G.A. or water and sown in the field.

Treatment	Total Plants	Total Tillers	Total Smutted Plants	Total Smutted Tillers	% Smut Total Plants	% Smut Total Tillers	Av. Ht. Cm.
Uninoculated	35	183	-	-	-	-	34.2
Inoculated	35	137	7	32	20	23	30.4
Inoculated + 500p.p.m. G.A.	28	77	8	21	29	27	29.2
Inoculated + 1000p.p.m. G.A.	21	91	6	12	29	13	25.8

TABLE 27.

Plant heights, number of nodes and percentage smut from two greenhouse sowings.

Treatment	Series	Total Plants	Total Smutted	Smut on Flag	% Smut	Av.Ht.Cm. Non Smut	Av.Ht.Cm. Smut	No.of Nodes 3	4	5	6
Uninoculated	1	33	0	0	0	32	0	2	17	14	0
	2	41	0	0	0	35	0	0	32	9	0
Uninoculated + G.A. 1000 ppm.	1	38	0	0	0	45	0	0	15	22	1
	2		NOT	SET	UP						
Inoculated	1	34	16	0	47	36	28	2	12	20	0
	2	37	8	6	22	27	23	4	24	9	0
Inoculated + G.A. 500 ppm.	1		NOT	SET	UP						
	2	48	3	1	10	36	25	3	21	22	2
Inoculated + G.A. 1000 ppm.	1	32	12	0	38	46	43	0	2	26	4
	2	52	8	5	17	36	26	5	22	22	3

A field experiment was also sown; the plants were sprayed in the 6th leaf stage on 19th June 1961. The results are summarized in Table 28. At the end of July the tips of the panicles of the sprayed plants were 30cm. taller than the panicle tips of the unsprayed plants. The greater extension mainly occurred at the base of the flower stalk above the last node. The lower portion of the flower stalk was a very light green colour in both G.A. sprayed and unsprayed plants; but in the sprayed plants

...this region was much longer. As the plant height is measured up to the last node the large differences in height are not apparent in Table 28. There were many dead plants in the sowing due to an attack by frit fly. Some of the surviving plants, however did become smutted. This suggests that there was no control of smut by spraying with G.A. in the sixth leaf stage.

Experiment III. M.H. applied as a seed soak in greenhouse and field.

There were 3 sowings in the greenhouse at 2 monthly intervals. Each sowing had untreated inoculated and untreated uninoculated treatments as controls. The first series was sown on 22nd February 1961 and consisted of 2 M.H. treatments 500 and 1000 ppm., the second series had 2000 and 4000ppm. and the third series all four treatments from 500-4000ppm. M.H. Two drops of Tween 80 were added to each 100ml. of M.H. solution as a wetting agent. Measurements and observations on the plants were made as in the G.A. seed soak experiments.

There were similar results for seedling emergence in the three series. The data in Table 29 is from the third sowing. It is apparent that increasing concentration of M.H. causes decreased emergence.

The treated plants 19 days after sowing were a darker green and less developed than the untreated. Some of the plants in the 2000 and 4000 ppm. treatments were very dwarfed with a basal rosette of leaves. The untreated plants were in the late 3rd to early 4th leaf and the plants in the 4000ppm. treatment in the early 3rd leaf. The measurements and observations made at maturity are summarised in Table 30. There appears to be increased tillering with increased M.H. concentration. There was no control of smut. This was confirmed by the results of a field experiment. Four plots were sown on the 19th March 1961, 5 rows per plot. 3.5g. of S.147 per row. The M.H. concentration used were 1000 and 2000ppm. At maturity the inoculated control gave 23% smut, the 1000 p.p.m. treatment 25% and the 2000 p.p.m. 29.5% smut.

Experiment IV. M.H. applied as a seedling spray in the greenhouse and field.

There were 3 sowings in the greenhouse at 2 monthly intervals, the first sowing was on the 22nd February 1961. The controls and concentrations of M.H. used were the same as in the Experiment III. The M.H. was sprayed onto the seedlings in the 3rd leaf, the controls were sprayed with water.

There was no control of smut and the average heights of the plants and number of tillers per plant were similar in each treatment(Table 31). A field experiment was also sown of similar design to that in Experiment III. Plants in the 5th leaf were sprayed with M.H. on June 4, 1961. There was no control of smut. The inoculated control treatment produced 14% smut, the 1000ppm. M.H. 26%, and the 2000ppm. M.H. 15% smut.

TABLE 29.

The emergence of S.147 seedlings per day and total emergence at 11 days after the seeds had been soaked in water or various concentrations of M.H.

Day After Sowing	TREATMENTS					
	Uninoc.	Inoc.	Inoc. + 500 ppm.	Inoc + 1000 ppm.	Inoc. + 2000 ppm.	Inoc. + 4000 ppm.
5	2	4	6	4	2	0
6	14	16	17	10	10	4
7	12	10	6	6	4	1
8	8	5	5	1	1	2
9	0	1	2	1	0	3
10	0	0	0	3	0	0
11	1	1	0	0	0	0
Emergence as % seed sown	92.5	92.5	90.0	62.5	42.5	25.0

TABLE 30.

Plant heights average number of tillers and percentage smut from 3 greenhouse sowings using seeds soaked with M.H. or water.

Treatment	Series	Total Plants	Total Smutted	Smut on Flag	% Smut	Av.no. of Tillers per Plant	Av.Ht. Plants cm.
Uninoculated	1	48	0	0	0	2.2	24.4
	2	53	0	0	0	2.0	-
	3	32	0	0	0	1.3	18.3
Inoculated	1	52	17	9	32.7	2.4	23.7
	2	56	5	0	8.9	2.2	-
	3	30	6	0	20.0	1.4	18.6
Inoculated + 500ppm. M.H.	1	54	21	5	38.8	3.2	26.8
	3	31	7	0	22.6	1.4	16.4
Inoculated + 1000ppm. M.H.	1	40	14	2	35.0	3.2	23.7
	3	23	6	0	26.1	1.8	17.0
Inoculated + 2000ppm. M.H.	2	29	2	0	3.4	3.5	-
	3	16	4	0	25.0	2.8	16.7
Inoculated + 4000ppm. M.H.	2	13	4	0	30.7	5.5	-
	3	8	1	0	12.5	5.5	16.2

TABLE 31.

Plant heights, average number of tillers and percentage smut from three greenhouse sowings.

Treatment	Series	Total Plants	Total Smutted	Smut On Flag	% Smut	Av.no.of Tillers per Plant	Av.Height cm.
Uninoculated	1	52	0	0	0	1.5	21.7
	2	37	0	0	0	3.5	-
	3	32	0	0	0	1.8	20.3
Inoculated	1	53	10	3	18.9	1.9	18.6
	2	36	5	0	13.9	3.4	-
	3	27	8	0	29.6	1.8	19.9
Inoculated	1	52	11	2	21.1	3.6	18.1
+ 500ppm.M.H.	3	24	5	0	20.8	1.9	23.0
Inoculated	1	54	20	12*	37.0	3.7	19.9
+ 1000ppm.M.H.	3	29	7	0	24.1	1.9	22.8
Inoculated	2	37	1	0	5.4	3.4	-
+ 2000ppm.M.H.	3	27	6	0	22.2	1.9	19.4
Inoculated	2	39	4	0	10.2	3.4	-
+ 4000ppm.M.H.	3	24	5	0	20.8	2.1	19.8

* Including 2 plants with smut on the 6th leaves.

TABLE 32.

Chlamydospore germination on agar containing increasing concentrations of M.H.

Treatment p.p.m.M.H.	Series	% Germination Av.of 4 plates	Mean % Germination	Mean % Germination (after angular transformation)
0	1	27		
	2	6	20.3	25.7
	3	28		
100	1	27		
	2	9	17.3	24.0
	3	16		
500	1	53		
	2	56	49.7	44.7
	3	40		
1000	1	66.		
	2	54	61.7	51.7
	3	65		
2000	1	77		
	2	70	71.3	57.7
	3	67		

Significant Difference S.D. at $P_{.05}$ = 0.05 = 10.1
S.D. at $P_{.01}$ = 0.01 = 14.4

Experiment V. The effect of M.H. on spore germination on agar.

The concentrations of M.H. used in the agar - M.H. mixtures were 100, 500, 1000 and 2000ppm. The experiment was repeated three times; the results obtained are summarised in Table 32. It is apparent that M.H. is stimulatory at certain concentrations. The length of the germ tubes and the numbers of sporidia and fused promycelia increased with increasing M.H. concentration (Table 33) At 1000 and 2000 ppm. the protoplasm often moved to the tip of the germ tube. In some examples the basal cell of the promycelium was normal but the second cell had divided into three long branches.

TABLE 33.

The effect of M.H. on length of germ tubes and stages of germination. Data is from Series 3.

Treatment M.H. ppm.	Germ Tube Length Av.in μ	Germination Stages.
0	9.0	No sporidia, few fused promycelia
100	13.0	Very few sporidia, some fused promycelia.
500	20.0	Some sporidia, many promycelial fusions.
1000	36.0	Many sporidia, many promycelial fusions, a few long threads.
2000	33.0	Very great number sporidia promycelial fusions, many long threads.

Discussion

Farrar (1958) controlled U.avenae with G.A. and suggested that the control is effected in 2 ways. Firstly, germination is speeded up in seed soak experiments giving less time for infection to occur, and secondly, by stimulating plant growth, the plant is able to outgrow the fungus. In the present experiments no control was achieved with G.A. and there was little time difference between emergence in the G.A. treatments and the controls. No evidence for the action of G.A. on spore germination was obtained but Stowe and Yamaki (1957) have stated that there has been little evidence for any stimulatory or inhibitory effect of G.A. on fungi. The fungus is probably already in the growing point of many plants at the time of spraying. The G.A. causes increased extension of the internodes which push the apex upwards; ultimately smutted panicles are produced. The small difference in time for emergence in the seed soak experiments does not appear to affect the percentage smut at maturity. The G.A. probably acts on the pectic substances of the cell walls including the pectates of the middle lamella which is the location of the smut mycelium in deeper tissues. It is probable the fungus can move more easily in G.A. treated plants. (Swinburne, 1960). This could help to explain the appearance of

...smutted plants in the seed soak treatments despite the inhibitory effect of the G.A. solvent. The P.E.G. 400 probably reduced spore germination in the seed soak treatments but any germinated spores would have had an easier progress through the plant.

The M.H. treatments and controls produced similar smut percentages in the seed soak and seedling spray experiments, but M.H. in vitro stimulated spore germination. Application of M.H. as a spray does not apparently affect the smut in the growing point because smutted plants are produced. In seed soak experiments very high M.H. concentrations decreased emergence and seriously affected plant growth. The reduced growth would favour the fungus. The susceptible period is longer and the plant is less likely to outgrow the fungus. The mode of action of M.H. in the plant is complex; apparently it has an affect on the carbohydrate balance and probably other metababolic systems (Audus, 1959). How this affects the path of the fungus in the plant is unknown.

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APPENDICES

APPENDIX 1 The formulation of John Innes no.1 compost.

7 parts of loam
3 parts of peat
2 parts of sand

To each bushel add ¼lb. of John Innes base fertilizer and ¾oz. of chalk.

APPENDIX 2. Johansen's Quadruple Staining Technique(Johansen 1940)

1. Bring slides down to 70% alcohol.
2. Stain in methyl cellosolve - 50% alcohol Safranin solution for 24-48 hours.
3. Rinse in tap water.
4. Stain in 1% aqueous Methyl Violet 2B for 10-15mins.
5. Rinse in tap water.
6. Rinse for 15 seconds in a mixture of equal parts 95% alcohol methyl cellosolve and tertiary butyl alcohol.
7. Immerse for 10-15 mins. in Fast Green F.C.F. solution.
8. Rinse briefly in a mixture of equal parts of 95% alcohol and tertiary butyl alcohol plus about 0.5% glacial acetic acid.
9. Stain for 3 mins. in Orange G. in methyl cellosolve - 95% alcohol solution.
10. Rinse briefly in a wash composed of one part each of clove oil, methyl cellosolve, and 95% alcohol.
11. Rinse in a wash of equal parts clove oil, absolute alcohol
12. Rinse in 2 changes of xylol and mount in Canada balsam.

APPENDIX 3. Heidenhain's Iron Hematoxylin Staining Technique (after Wang 1934, and Johansen 1940).

1. Remove paraffin with xylol for 10mins.
2. Pass through a mixture of equal parts of xylol and absolute alcohol for 10 mins.
3. Pass into a mixture of equal parts of absolute alcohol and ether plus 1% celloidin for 3 mins.
4. Remove slides, keep in air until the sections become opaque but not completely dried out.
5. Plunge into 70% alcohol for 5mins.
6. Pass through 35% alcohol into water.
7. Wash thoroughly with water, rinse in distilled water.
8. Mordant in 3% Iron Alum solution for approximately 2 hours.
9. Wash thoroughly in running water for 5 mins., rinse in distilled water.
10. Stain in 0.5% Heidenhain's Iron Hematoxylin for 2 hours.
11. Wash off excess stain with water.
12. Destain in 2% Iron Alum Solution. Control destaining on a glass plate under the microscope, time approximately 10mins.
13. Wash in running water for at least 1 hour.
14. Dehydrate in 50, 70, 95% alcohols allowing at least 5 mins. in each.
15. Leave in absolute alcohol-xylol 1:1 mixture for 5 mins.
16. Wash in 2 changes of xylol, 5 mins. in each.
17. Mount in Canada balsam.