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THE INFLUENCE OF CERTAIN FUNGI ON THE GERMINATION OF PEAS.

by

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THE INFLUENCE OF CERTAIN FUNGI ON THE GERMINATION OF PEAS.

A. INTRODUCTION.

The rotting of pea (Pisum sativum) seed in the course of germination, resulting in a poor initial stand, is fairly extensively recorded in the American literature, and appears to be a problem of considerable importance both to the canning (Starr,25) and market-gardening (Horsfall et al.,9) industries. While it has been recorded and studied to some extent in England, the extent of its occurrence here is still uncertain. The main object of most of the American investigators has been the control of the disease, but some attention has been paid to its cause.

Haenseler (8) repeatedly isolated forms of Pythium from pea seed that had failed to germinate, and demonstrated that in steam sterilised soil a fungus of this genus could cause complete failure of germination. Croisier (5) and Richards (22) showed that Rhizoctonia Solani could prevent the emergence of peas, and Jones and Linford (14) that Pythium de Baryanum could do likewise. Species of Fusarium have been mentioned by Starr (25) as responsible in part for the disease. The experiments that have been conducted by other workers under controlled conditions do not suggest, however, that members of this genus destroy peas in the first stages of growth. Croisier (5) found that the majority of a large number of seed-borne Fusaria investigated by him reduced emergence little, if at all, and that

very few of them approached Rhizoctonia Solani in activity under the experimental conditions, which he considered to be very favourable for the Fusaria. A perusal of the tables presented by workers who have studied the members of this genus responsible for the wilt (Snyder, 24) and stem and root -rot ~~Fusaria~~ (Haenseler, 8 ; Jones, 12) of peas, and the root-rot caused by Aphanomyces euteiches (Jones and Drechsler, 13) shows that these fungi did not reduce emergence in sterilised soil. The more recent American writers (Horsfall et al., 9 ; Anderson et al., 1) regard the disease as an ordinary case of damping-off due to species of Pythium and Rhizoctonia, the first mentioned authors particularising P.ultimum and R.Solani.

In England Hull (11) isolated a phycomycete and a Fusarium from peas which had failed to come up in pots under laboratory conditions, and found that the former prevented emergence the more effectively under those conditions. In similar circumstances Brett et al. (2) record the isolation of a Fusarium, Padwick (20) of Penicillium, Fusarium and Botrytis. Padwick demonstrated that several species of Fusarium, Botrytis cinerea, Helminthosporium sativum and a species of Sclerotinia could reduce or inhibit emergence, but he did not show that any of these fungi could be obtained from seed which had rotted under field conditions. A large number of Fusaria tested by him did not affect emergence. No study has been made in England of the fungi present in typical diseased material from the field. In Denmark a Pythium type of fungus (6) and in Holland Pythium de Baryanum (Buisman, 4) have been found capable of reducing the emergence of peas.

Defects in the seed used are an obvious cause of poor germination. In America seed naturally infected with Ascochyta Pisi, A.pinodella and Mycosphaerella pinodes was found by Linford and Sprague (17) and by Jones (15) to germinate less readily than clean seed. Croisier (5) however, showed that emergence was not reduced in soil heavily infected artificially with A.Pisi, a result similar to that obtained by Sattar (23) and by Padwick (20) in England, while Sattar and Hull (11) have shown that dipping seeds in spore suspensions of the fungus is likewise ineffective. Spores of M.pinodes and A.pinodella similarly applied did, however, prevent emergence (Sattar). These fungi can only be important when the seed is heavily infected or planted in fields rich in infective debris. Croisier (5), as stated before, has shown that some degree of pathogenicity may be possessed by seed-borne Fusaria, and he also records Rhizoctonia Solani on seed. The apparently physiological disease Marsh Spot was found by Lacey (18) and by Pethybridge (21) to reduce germination only when it was very severe, but Pethybridge considered that its effect might be greater under conditions more unfavourable to germination than those of his experiments.

Cold wet soils showed an increased amount of the disease in America according to Haenseler (8) and Jones (16), and Haenseler considered that it was favoured by a heavy soil. In England Hull (11) has made similar observations in respect of soil moisture. A species of Pythium studied by Haenseler caused complete failure of emergence when the water content of the soil ranged from 60-80% of saturation but its pathogenicity was less in

drier soils. In respect of temperature however, the work of Richards (22) with Rhizoctonia Solani indicates a rather restricted action of this fungus on peas at temperatures of and below 9.5°C. Jones and Linford (14) suggest an even higher minimum (16°C.) for Pythium de Baryanum, since they found it to be abundant in soils in Wisconsin in which it was not recorded as a cause of seedling injury to peas. They consider that most peas raised in Wisconsin pass through the seedling stage when the soil temperature is below this figure. These observations draw attention to the inadequacy with which the temperature factor has been treated in studying the action at least of Pythium and Fusarium on germinating peas. Wrinkled varieties proved more susceptible to the disease than smooth in the work of Jones (16) and Hull (11).

Several attempts have been made to improve the percentage emergence of peas by the application of organic mercury dusts to the seed. The control of the disease obtained with these in America by Jones (16) and in England by Hull (11), Brett et al.,(2) and Ogilvie and Mulligan (19), though often substantial, seems inferior to that recorded in America by Anderson et al. (1) and Horsfall et al. (9) with cuprous oxide. This has been used with considerable success in England (unpublished data kindly supplied by Mr.L.Ogilvie). Horsfall (10) found zinc oxide to be ineffective, and Gilgut and Guba (7) considered it to be inferior to the proprietary organic mercurial Semesan. In much of Horsfall's work soil infected with Pythium ultimum was used.

The object of the present research has been to determine the fungi responsible for the failure to emerge of pea seed sown in the early spring in England, to investigate the factors which determine the severity of the disease, and to test possible control measures.

B. EXPERIMENTAL METHODS.

1. Field Experiments.

In the early spring of 1937 and again in 1938 regular sowings of the two varieties of pea principally used by Hull were made at the Imperial College Field Station in Slough (Bucks.). Here both a fairly light and a fairly heavy soil are available, the latter being comparatively low-lying.

Each sowing consisted of four randomised blocks each containing one row under each treatment. Sixty seeds were sown per row at a depth of one to two inches and about two inches apart. Of these, ten, marked out at the time of planting, were removed during the course of the experiment for examination and for isolation of the fungi present.

Daily records of soil temperature at a depth of one and a half inches were kept, and the soil moisture was determined weekly by the following method. One sample of the top three inches of soil was removed at each of

four fixed points with a tubular sampler. These cores were mixed and the amount of water in the composite sample estimated in triplicate. The results are expressed as percentages of the saturation capacity, which was determined by the Rothamsted method as 50% and 65% of the dry weight of the light and heavy soils respectively. Throughout this work the temperature used for drying has been 105°C.

The seedlings were counted weekly from the time that the bulk of them had appeared until no further increase was observed in the figures. Emergence continued over several weeks when the soil was cold, the latest plants to appear being most commonly retarded by damping-off of the plumule before emergence. The greatest count recorded for any row was taken as the final figure for that row, the number of plants that died after emergence having been too small to affect the results.

Destruction of the seed by mice was avoided by continual trapping. Birds, however, interfered with some of the later sowings in 1938. The results given below are only from replicates which have not been in any way damaged, and cases where the full number has not been used are indicated.

No frost injury was observed.

2. Laboratory Experiments.

A description of the methods used in laboratory work can be given more conveniently when the experiments themselves are described.

C. THE EFFECT OF FIELD CONDITIONS AND OF VARIETY ON THE EMERGENCE OF UNTREATED SEED.

It is evident from the accompanying graphs (Figs.1 and 2) that the spring of 1937 was a wet one. The light soil was above 50% and the heavy above 55% of saturation practically from the beginning of February until the middle of April, and for considerable periods these figures were substantially exceeded. In the following year, however, the light soil was between 6-13% drier in February, and fell rapidly below 50% of saturation at the end of that month, becoming very dry by the end of April. Conditions in the heavy soil in February 1938 were similar to what they had been in the preceding year, but subsequently the water content in 1938 fell gradually below 55% of saturation whereas in 1937 it rose well above it. Experience suggests that the weekly determinations were perhaps a little infrequent as the heavy soil showed the effect of mid-week rains on some occasions when they had largely dried out from the light by the date of sampling.

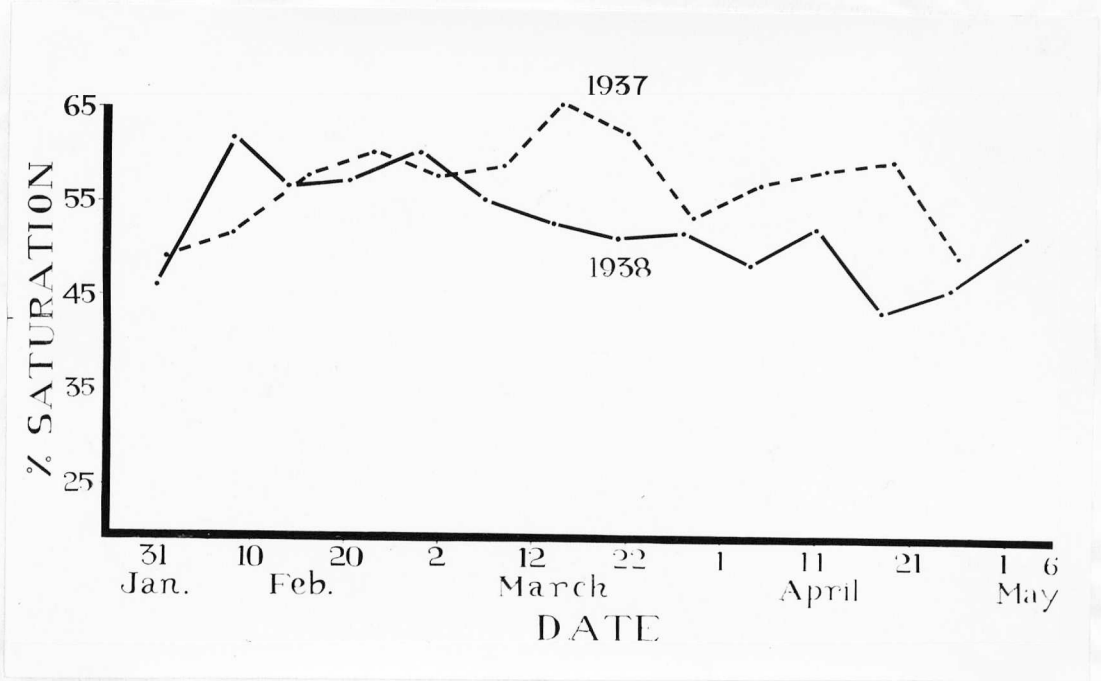


Fig.1. Soil moisture records on the heavy soil.

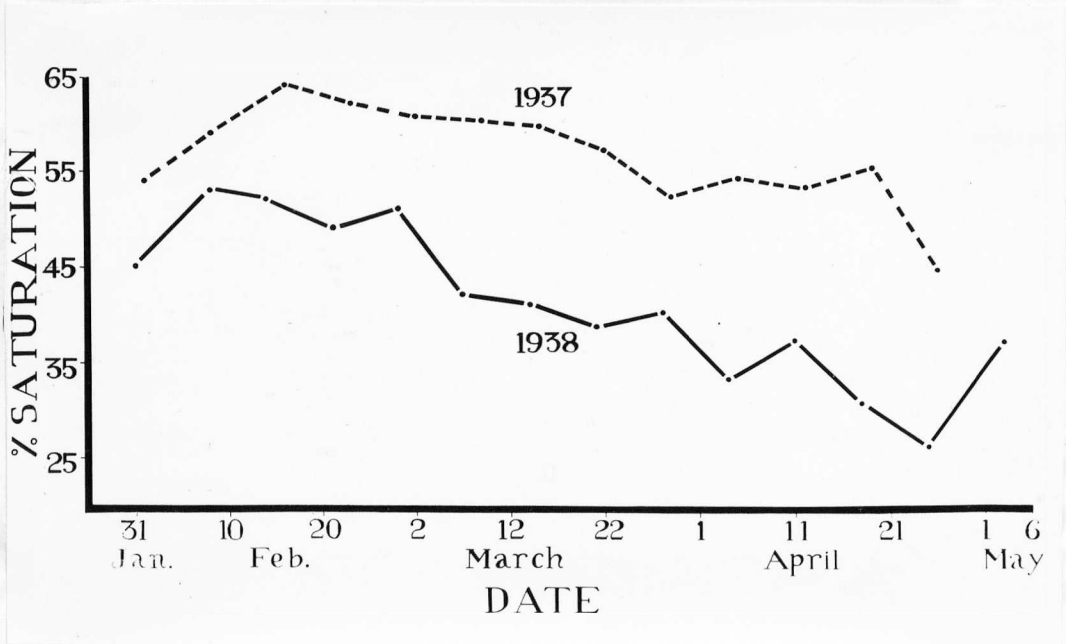


Fig.2. Soil moisture records on the light soil.

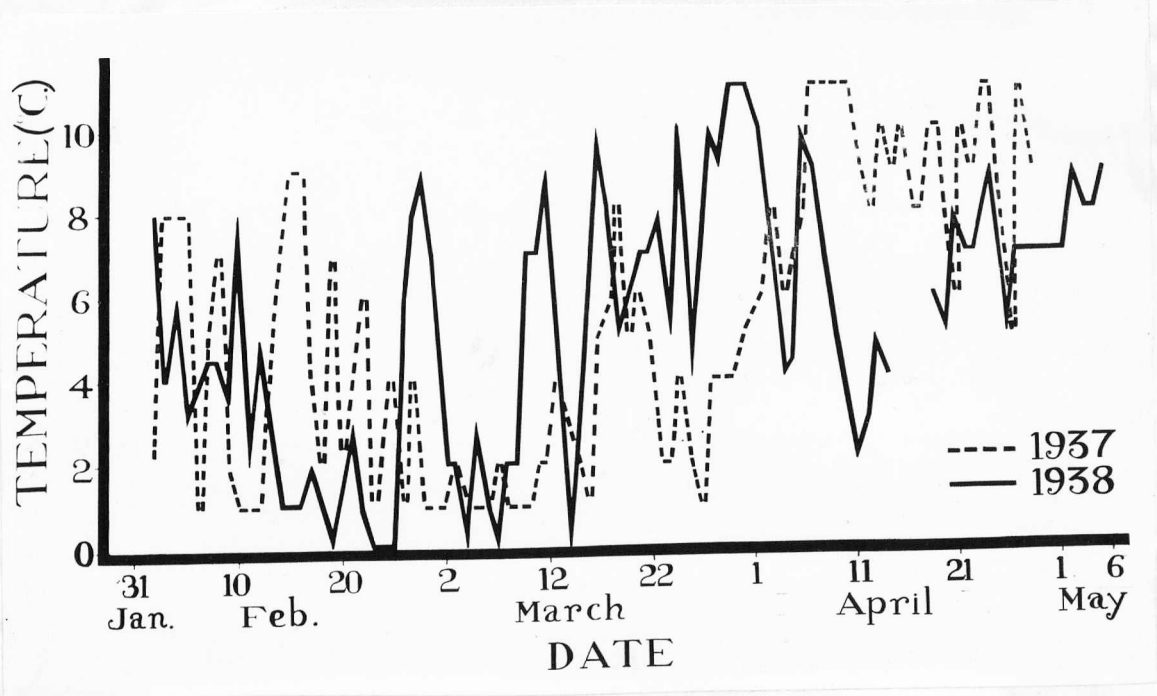


Fig.3. Soil temperatures at a depth of 1.5 inches at 8 a.m.

The soil temperatures (Fig.3) fall naturally into four periods as is shown in Table I. (1) From Feb.2-13 they fluctuated considerably in both years, but the average values were approximately equal. (2) From Feb.14-25 they remained much the same in 1937 as in the preceding period, but were lower in 1938. (3) From Feb.26 until April 3 the temperatures were nearly always higher in 1938 than on the corresponding days in the previous year, the average figure in the first case being higher and in the second lower than it had been before. (4) From April 4 until the records ceased

Table I.

Average soil temperatures at 8 a.m. °C.

Period	1937	1938
Feb.2-13	4.0	4.8
" 14-25	4.8	1.1
" 26-Ap.3	3.1	6.3
Ap.4-29	9.0	6.1

temperatures remained much as before in 1938, but in 1937 they rose substantially, exceeding as a rule the value attained in the following year.

The maximum percentage emergence of untreated seed plotted against the date of sowing is given for the light soil in Fig.4 and for the heavy soil in Fig.5.

The most striking difference evident on inspection of these graphs is in the amount of disease present in the two years. In 1937 both varieties germinated well for all sowing dates, but in 1938 the emergence was initially poor, more especially with Gradus peas, but improved steadily later in the season.

Such small fluctuations in emergence as there were in 1937 (between 66-86% with Pilot and between 75-91% with Gradus) were not always in the

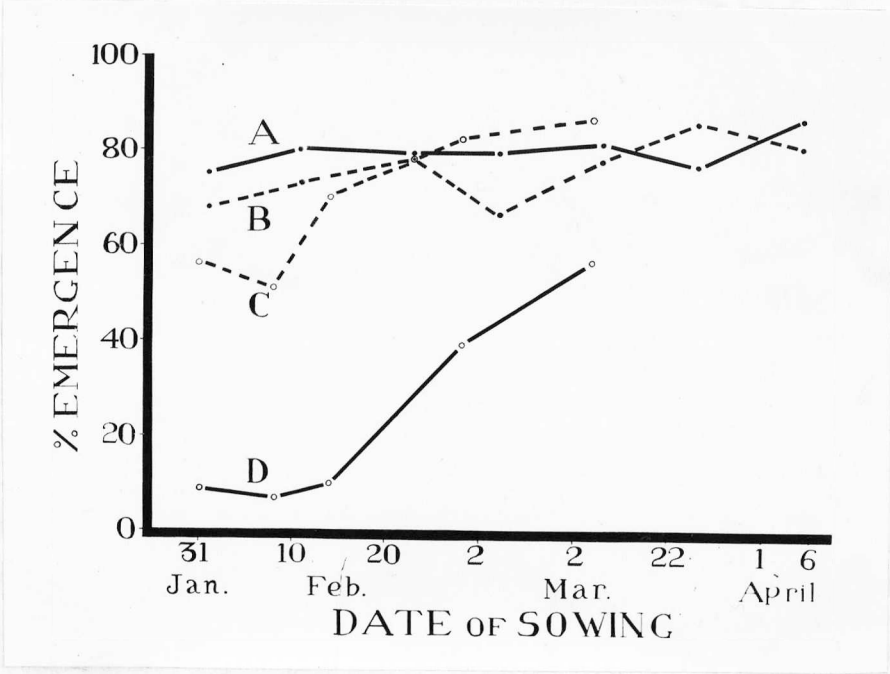


Fig.4. Maximum percentage emergence of untreated seed on the light soil.

A Gradus 1937 B Pilot 1937

C Pilot 1938 D Gradus 1938

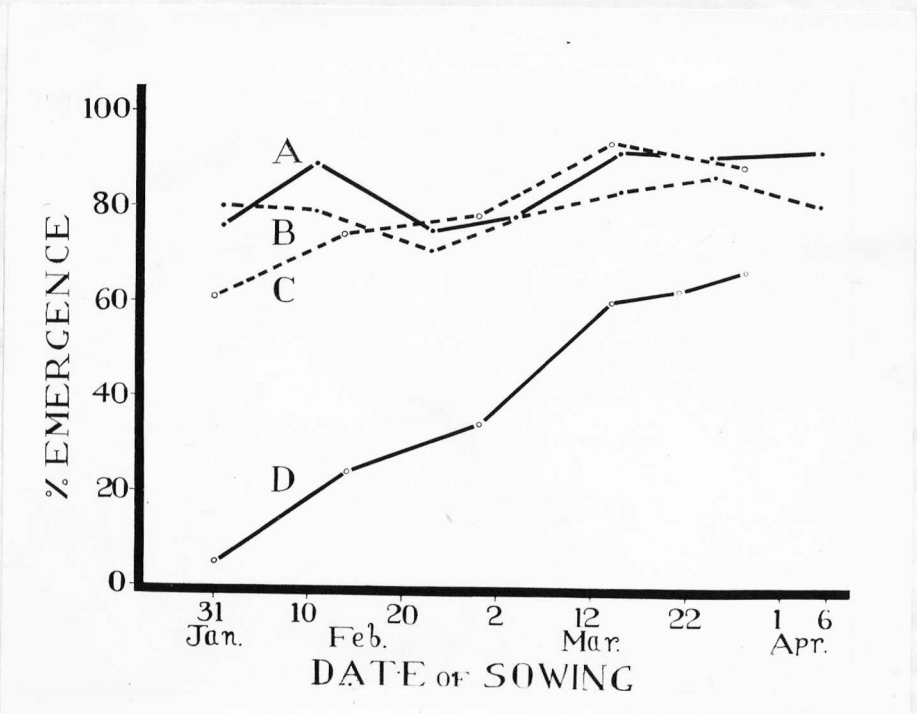


Fig.5. Maximum percentage emergence of untreated seed on the heavy soil.

Lettering as in Fig.4.

same direction on both varieties, and cannot be related to changes in soil moisture or soil temperature. In the light of the work of Hull(11) and of the results obtained in the following year it is evident that the conditions, in particular those of soil moisture, were favourable throughout the season for the development of the disease. A suggested explanation of its low intensity will be offered later.

In 1938, under conditions of temperature and soil moisture initially comparable, especially in the case of the heavy soil, with those of the preceding year, the intensity of the disease was at first high, particularly with Gradus peas. A steady rise in the percentage emergence is however, seen in all sowings made from the middle of February onwards, and it can be correlated with a falling soil moisture. Though this was not actually reduced before March, none of the sowings emerged before the middle of this month, and it is obvious that the later the date of sowing the shorter the period in wet soil. However, the soil temperature was on the average higher from the end of February onwards, so that the effects of these two factors cannot be clearly separated. Though in the later part of the season the soil in 1938 was very much drier than it was at any time in 1937, the germination of the Gradus seed was never as good as it had been on any occasion in the preceding year.

Soil type was not without effect on emergence. There are eleven sowings of each variety available for comparison in this respect, a total of twenty-two in all. The calculation of the standard errors of the differences between the pairs of means in the usual way shows that there

were only five cases in which the difference approached or reached the 5% point of significance according to the table of t given by Fisher (6A). These are shown in Table II. It is noteworthy that in all these cases the emergence was better on the heavy soil, and that the germination of Gradus peas first showed improvement in 1938 on this soil. In the remaining cases, where the difference fell well short of significance, the emergence of Gradus peas was greater on the heavy soil on four occasions, and greater on the light soil the same number of times. With Pilot peas it was greater on the heavy soil on seven occasions, and on the light soil only twice. The balance of this evidence does, then, support the view that there is a tendency, more obvious at some times than at others, for emergence to be greater on the heavy soil.

Table II.

A comparison of the emergence of peas in light and in heavy soil.

Date of sowing	Variety	% Emergence Heavy soil	Light soil	Diff.	5% Point
1937					
Feb.1	Pilot	79.5	67.0	12.5	16.42
" 23	Pilot	77.5	65.5	12.0	11.58
March 15	Gradus	91.0	80.5	10.5	15.12
" 23	Gradus	89.5	76.0	13.5	12.54
1938					
Feb.14	Gradus	23.5	9.5	14.0	12.14

In every sowing in 1938 the emergence of the Gradus seed was much lower than that of the Pilot, the best results with the former being comparable with the worst with the latter. In the preceding year, however, differences which approached or reached significance lay with one exception in the opposite direction. These are given in Table III. The form of statistical analysis adopted was that which will be described in the section dealing with the control of the disease. It will be shown later that this inferior germination of the Pilot variety was in all probability due to imperfections in the sample of seed, that is to say to a cause largely irrelevant to the present research.

Table III.

A comparison of the emergence of Pilot and Gradus varieties in 1937.

Date of sowing	Soil	% Emergence Gradus	Pilot	Diff.	z (5% point 1.1577)
Feb. 1	Light	74.5	67.0	+ 7.5	1.1558
" 11	Light	79.5	72.5	+ 7.0	1.0788
" 11	Heavy	89.0	78.5	+ 10.5	1.0460
March 4	Light	78.5	65.5	+ 13.0	1.4773
" 15	Heavy	91.0	82.5	+ 8.5	0.9420
" 25	Light	76.0	84.5	- 8.5	1.1872
April 5	Heavy	91.0	80.0	+ 11.0	1.8718

D. THE NATURE OF PRE-EMERGENCE INJURY.

As previously stated ten seeds of each row, whether treated or untreated, were assigned for mycological investigation in the early stages of growth. Half of these were dug up when the radicles had just grown out, that is to say one to three weeks after planting according to the speed of germination, the remainder after two to six weeks when the first plants appeared above the ground. A healthy seed was readily found, but the soil adhered to a rotted one so that it was easily overlooked. Indeed, by the time of the second sampling part of the seed had often completely disappeared, this being, presumably, such of it as had begun to decay at a very early stage.

In all, some 4,500 seeds were sown for inspection in this way. Some of the data accumulated from the examination of untreated seed are given in Table IV. The figures are ~~the~~ here percentages of the number of seeds sown.

Table IV.

Untreated pea seed dug up in the course of germination.

Variety	Av. emerg.	Recovd.	Healthy	Stunted	Rotted undevd.	Plumule rotted	Radicle rotted	Both rotted	Animal injury
1937									
Pilot	77	94	76	3	5	3	2	2	3
1937									
Gradus	81	96	80	1	2	6	1	0	6
1938									
Pilot	75	92	67	0	3	5	0	1	16
1938									
Gradus	36	80	41	3	20	4	4	5	3

In 1937 the results of both samplings are included but in 1938 those of the first sampling only, since, especially with Gradus, a great deal of seed escaped detection at the time of the second. As has been explained above the missing fraction in the figures presented can safely be regarded as being nearly all rotted seed. The average emergence of the remaining fifty seeds in each row is also given in the table. In classifying the embryos primary consideration has been given to the state of the seedling axis. Lesions involving the cotyledons only were so common, especially when the seed germinated slowly, that they have been ignored except when they caused a stunting of the axis.

If columns two and four of Table IV are compared it will be seen that there was a substantial agreement between the average emergence and the number of healthy embryos in the sampling strips. In view of the fact that in 1938 the figures refer to the first samplings only, it is evident that with Gradus seed nearly enough disease was present at this early stage to account for the proportion of the seed that failed. This conclusion is supported by the result of the second sampling most of the material in which was healthy or very much decayed. The discrepancy between the number of healthy Pilot embryos and the average emergence was probably due to the survival of much of the seed damaged by animals.

The type of injury which caused most damage to the Gradus seed in 1938, and which also predominated in the Pilot seed of 1937, was a very early rotting of the axis (and often of the cotyledons as well) occurring when it had grown little or not at all. This class of material would, moreover,

constitute the bulk of that which could not be found. It is possible that it is at this stage that pea seed is most susceptible since Jones (16) found that, whereas watering of the seed-bed at the time of planting reduced germination, the effect was negligible if the water was added a day later. His work was done at 60°F. when germination would begin in a fairly short time, but different fungi might be concerned in the disease at that temperature.

The examination of seed which had received various dust treatments did not prove very instructive. The same types of injury were found as with untreated seed, their frequency being greater or less according as the treatments were beneficial or the reverse. The very early rotting of the seed again predominated even with the most beneficial of the treatments. This means that the seed decayed when largely or wholly enclosed within the dust-coated testa.

E. THE FUNGI PRESENT IN DISEASED MATERIAL.

From the preceding section some impression can be gained of the quantity and nature of the material available for mycological investigation. Since the rotting of the cotyledons did not necessarily prevent emergence there was a likelihood of obtaining comparatively non-pathogenic fungi from them, a point well illustrated by the list of isolations given by

Padwick (20). In selecting material for isolations only that in which the axis itself was attacked has been taken. When the amount of disease was small it was possible to utilise all the available seed both from treated and untreated rows, excluding of course that which was excessively decayed. When, however, there was an abundant supply only a proportion, usually ten seeds of each variety, could be handled. This was selected without discrimination as to the soil type from which the seed came, or as to whether it had been treated or not, as it was evident at an early stage that no important differences would be found.

The following methods were used throughout both seasons. After the embryos had been well washed in running water the seedling axes were separated from the cotyledons and vigorously shaken in a test-tube in twelve changes of sterile water. They were then placed on slopes of plain agar and incubated at 20°C. The cotyledons, after the removal of any excessively decayed portions, were dipped in 95% alcohol, flamed, placed on 0.5% tartaric acid agar and incubated at 20°C. By these methods a fungus was obtained from 80-100% of the axes, and from most of the cotyledons when they were also diseased. The fungi were sub-cultured on to plain agar and an inoculum from the back of the plate transferred to a tube of potato or cornmeal agar. This procedure, which is recommended by Brown (2), prevented losses from bacterial contamination.

A note was made of the type of injury shown by the embryos and of the date of each isolation. Apart from the fact that some fungi seem to attack the cotyledons only, there is no evidence that any particular

species is associated with any special type of injury, or that any great change occurred in the fungi present as the season advanced. The results are therefore given in summarised form, as in Table V.

Table V.

Fungi isolated from peas sown in the field.

Species	From seedling axis 1937		1938		From cotyledons 1937		1938	
	Pilot	Gradus	Pilot	Gradus	Pilot	Gradus	Pilot	Gradus
Pythium spp.	61	58	37	79	58	20	6	28
Mucor sp.	0	0	1	1	0	0	16	55
Ascochyta Pisi	14	0	0	2	48	0	1	3
" pinodes	1	0	0	0	1	0	0	0
" pinodella	1	0	0	0	0	0	0	0
Pleospora sp.	8	0	0	0	24	0	0	0
Fusarium spp.	2	0	2	3	4	2	9	26
Penicillium spp.	0	0	0	0	10	4	9	11
Gymnoascus sp.	0	0	0	0	3	2	7	3
Trichoderma spp.	0	0	0	0	3	0	1	9
Botrytis sp.	0	0	0	0	8	0	0	0
Rhizoctonia Solani	0	0	0	0	0	1	0	0
Miscellaneous	2	0	0	0	4	5	4	1

This table is a list of the cultures obtained by the methods of isolation previously given from approximately ~~250~~ 300 rotted seedling axes or parts thereof, and from their accompanying cotyledons when these were in a suitable state. In general not more than one isolate has been obtained from the same cotyledon, and there has been invariably only one from each axis.

The seedling axes yielded very large numbers of cultures of Pythium and practically nothing else. Pythium spp. also preponderated in the isolations from cotyledons in 1937 but in 1938 were in large measure replaced by a species of Mucor. This fungus was absent in the preceding year, and since, as will be shown later, it was not seed-borne, its presence suggests a change in the soil flora. It was virtually confined to cotyledons, and it will be shown later that it was not pathogenic, while Pythium spp. were strongly so.

The species of Ascochyta showed a restricted distribution in being practically confined to the Pilot seed in 1937 and the Gradus in 1938. The Pleospora sp. occurred only in the former batch. This is explained by the fact that these fungi were seed-borne as will be demonstrated later. A.Pisi and Pleospora sp. were the only fungi other than Pythium to be detected in diseased axes to any extent.

The amount of Fusarium present was on the whole small, its only substantial occurrence being in the cotyledons of Gradus in 1938. The only other genus at all well represented was Penicillium and this was restricted to the cotyledons. These isolates have not been studied further in this

work, but Padwick (20) found that the species of this genus tested by him did not reduce emergence.

In view of the importance attached to Rhizoctonia Solani as a cause of seedling injury to peas in America its virtual absence from this list is noteworthy.

It will be remembered that the material from which Pythium was so abundantly isolated was thoroughly washed with sterile water, but not treated with any definite antiseptic. In view of the observation made by Jones and Linford (14) and by Buisman (4) that species of Pythium could be isolated with almost equal frequency from healthy and from diseased pea roots if they were not surface sterilised, it was thought desirable to try the effect of surface sterilising seedling axes with antiseptics. The great sensitivity of Pythium mycelium to these is noted by both the authors cited. The treatment considered necessary to kill superficial hyphae is apt to destroy the internal Pythium hyphae as well.

If the seedling axes were treated with 0.1% mercuric chloride and subsequently washed in three changes of sterile water Pythium alone was again obtained, but if the immersion was longer than 45 seconds the number of isolates was reduced. The occasional ones that were obtained even with three minutes immersion were Pythium.

Padwick (20), who isolated from cotyledons only, surface sterilised these by immersion for two to three minutes in 0.1% silver nitrate followed by a rinse in a saturated salt solution, and plated the material out on

potato-dextrose agar. This method was employed on seedling axes of Gradus peas in 1938 with the following results, ten seeds being taken in each case.

Mar.14. 10 seeds washed only - Pythium 8 ; no fungus 2.

" " treated silver nitrate - A.Pisi 2, Fusarium 1 ; no fungus 7.

Mar.28. " " washed only - Pythium 9 ; no fungus 1.

" " treated silver nitrate - Mucor 2, Fusarium 2 ;no fungus 5.

Apr.4. " " washed only - Pythium 10.

" " treated silver nitrate - A.Pisi 8 ; no fungus 2.

This method completely eliminated Pythium from the axes the bulk of which then gave no fungus, the remainder Mucor, Fusarium and Ascochyta. The large amount of Ascochyta obtained on April 4 is at variance with the other results and was perhaps due to the discharge of a pycnidium after surface sterilisation. With cotyledons however, the same fungi were obtained as when the material is flamed, including an occasional Pythium.

In order to determine which of these various fungi were actually carried on the seed samples used, twenty seeds of each batch were washed in twelve changes of sterile water, and twenty were treated for three minutes with 0.1% mercuric chloride followed by three changes of sterile water. The skins, cotyledons and axes were placed separately on plain agar. Table VI shows the fungi which were present and the number of seeds in one or more parts of which each one occurred. A seed rarely contained more than one species, or showed a clearly visible lesion. Samples of fifty seeds were

also examined separately for Marsh Spot.

Table VI.

Number of seeds infected in a sample of forty.

Species	1937		1938	
	Pilot	Gradus	Pilot	Gradus
Ascochyta Pisi	18	0	0	4
" pinodes	0	0	0	1
" pinodella	1	0	0	0
Pleospora sp.	6	0	0	0
Penicillium spp.	4	11	0	2
Aspergillus sp.	0	0	0	7
Miscellaneous	2	2	0	0
Marsh spot(50 seeds)	4	0	0	1

The table shows that the Pilot seed in 1937 was heavily infected with Ascochyta Pisi. This and the Pleospora were obtained several times from the cotyledons and axis. The Marsh spot lesions were small. The Gradus seed in this year, however, carried little else but Penicillium and this came almost wholly from the skins, and provided the only instance in which the number of isolations was substantially reduced by the use of mercuric chloride. The Pilot seed in 1938 was clean, and the amount of Ascochyta in the Gradus seed was small.

The species of Aspergillus present in Gradus seed in 1938 was the only seed-borne fungus which did not occur among the isolations made from diseased material from the field (Table V). It would then appear to be the least important of them all. The fact that, in the list of isolations from the field, species of Ascochyta were virtually confined to the samples of seed which carried them, shows that they were not present, or at least not active, in the soil.

The isolates believed to be species of Pythium were plated onto a single batch of cornmeal agar and classified on the nature of the fruiting present into eight classes.* All these, but no new ones were recognised again in the following year. They are listed below together with the criteria by which they were known and the number of isolates referred to each. The taxonomic work to which reference has principally been made is that of Matthews (18A).

Class A. Abundant sexual organs which agreed well with those of P.ultimum.
Number of isolates :- 1937, 57 ; 1938, 7.

Class B. Sexual organs and conidia agreeing well with those of P.de Bary-
" C. anum. Three classes were originally made in this group on the
" D. basis of the relative abundance of oogonia and conidia, and the proportion of the former which were intercalary. Subsequent experience showed no reason for retaining these distinctions.
Number of isolates :- 1937, 25 ; 1938, 32.

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* Representative cultures of these have been submitted to Mr.C.G.C. Chesters of the Botany Department, University of Birmingham for his opinion.

Class E. Oogonia few and extremely large, approximately 45 μ in diam. Number of isolates :- 1937, 2 ; 1938, 0.

Class F. Conidia alone present, spherical, more or less abundant especially superficially, tending to become highly granular and to develop a thickened wall. Average diameter about 20 μ. Number of isolates :- 1937, 82 ; 1938, 52.

Class G. Conidia alone present, spherical, few and comparatively large often exceeding 30 μ in diameter. Number of isolates :- 1937, 14 ; 1938, 32.

Class H. Conidia alone present, spherical intermediate in size and number between Class F and Class G. Number of isolates :- 1937, 13 : 1938, 20.

Class I. Sterile Phycomycetes closely resembling members of the preceding classes in general cultural characters. Number of isolates :- 1937, 2 ; 1938, 7.

Unclassified :- 1937, 22 ; 1938, 0.

Representative cultures were inoculated onto sterilised carrot and grown in large vessels of water in the hope of inducing the development and discharge of sporangia, so that the genus might be identified by its formal criterion of producing its zoospores in a vesicle. No indication of

zoospore formation was however obtained, whereas the asexual reproductive bodies which appeared here as on agar, had often been seen to produce germ tubes on the latter medium. It was assumed therefore that the fungi were conidia-producing members of the genus Pythium, a conclusion supported by the fact that it was possible to refer those cultures which produced the sexual stage to species within that class. A possible exception is the unimportant group E.

It will be seen that in both years the most abundant form was Class F. In 1937 there was also a substantial amount of Class A, but in the following year there was very little of this, its place as the second most abundant form being taken by Class G.

The cultures selected for further experiments were purified by hyphal-tipping. Bacteria were difficult to eliminate from fungi of this genus but these cultures appeared clean even on neutral bouillon agar.

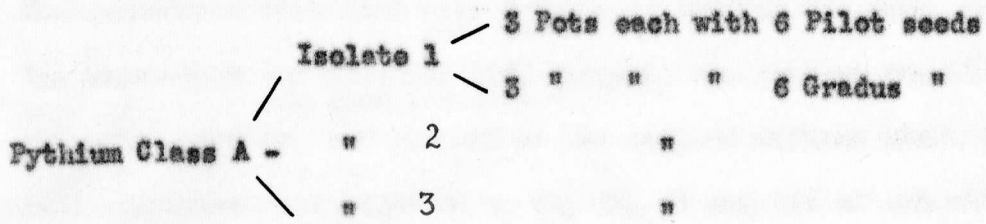
Species of Ascochyta were identified on quaker-oat agar as recommended by Jones (15).

F. THE PATHOGENICITY OF ISOLATES UNDER CONTROLLED CONDITIONS.

An endeavour has been made to test the pathogenicity of the more frequent isolates under conditions approaching those which obtained in the

field. The following details of technique were observed in all the experiments. Soil-borne fungi were grown at 20°C. for 10-17 days on a sterilised mixture of 95 parts by weight of soil and five of cornmeal. The inoculum was stirred into the whole bulk of the soil used for potting at the rate of 4-5%. The controls received a similar amount of uninoculated medium. Containers were sterilised by heat or with formalin. Soil was sterilised, inoculated and potted at a water content of approximately 40% of saturation, the time allowed in the autoclave being one to three hours at twenty pounds pressure.

In the first experiment the six chief classes of Pythium were used, and it was designed according to the following scheme :-



Similarly with Classes B, C, D, F, G and controls.

The seed was of the 1937 batch and surface sterilised with mercuric chloride. The soil was watered to 84% of saturation and held at 3-5°C. in a refrigerator. In the course of the experiment the water content fell less than 10%. The percentage emergence was recorded after seven weeks, and was as follows

	Pilot	Gradus
Controls	73.5	56.8
Inoculated	3.6	3.9

All the cultures used inhibited emergence almost or quite completely. The odd plants that did appear in the inoculated pots were fairly uniformly distributed among them. The imperfect germination of the controls, especially with Gradus, is believed to be due to the excessive wetness of the soil. Some evidence in support of this view is given by the next experiment to be described.

In a test of the effect of soil moisture and temperature on the pathogenicity of Pythium in sterilised soil a single isolate of Form F and one of Form A were used. The soil had a saturation capacity of 60%, and the containers were tins each capable of holding 600 grms. of dry soil. The seed, which was from the 1938 samples, was surface sterilised with mercuric chloride, and planted at the rate of fifteen seeds per tin. The soil moistures were adjusted to 40, 55, 70 and 85% of saturation and were not allowed to fall more than 6% between waterings. Three replicates were used throughout.

One set of tins was placed in an unheated frame (in February) the other in a heated laboratory. The soil temperature at 9 a.m. in the former was usually 4-5°C. and in the latter 11-12°C.

The accompanying graphs (Figs.6 and 7) and plates I and II show the average emergence ~~per cent~~. Excluding the results at 85% of saturation

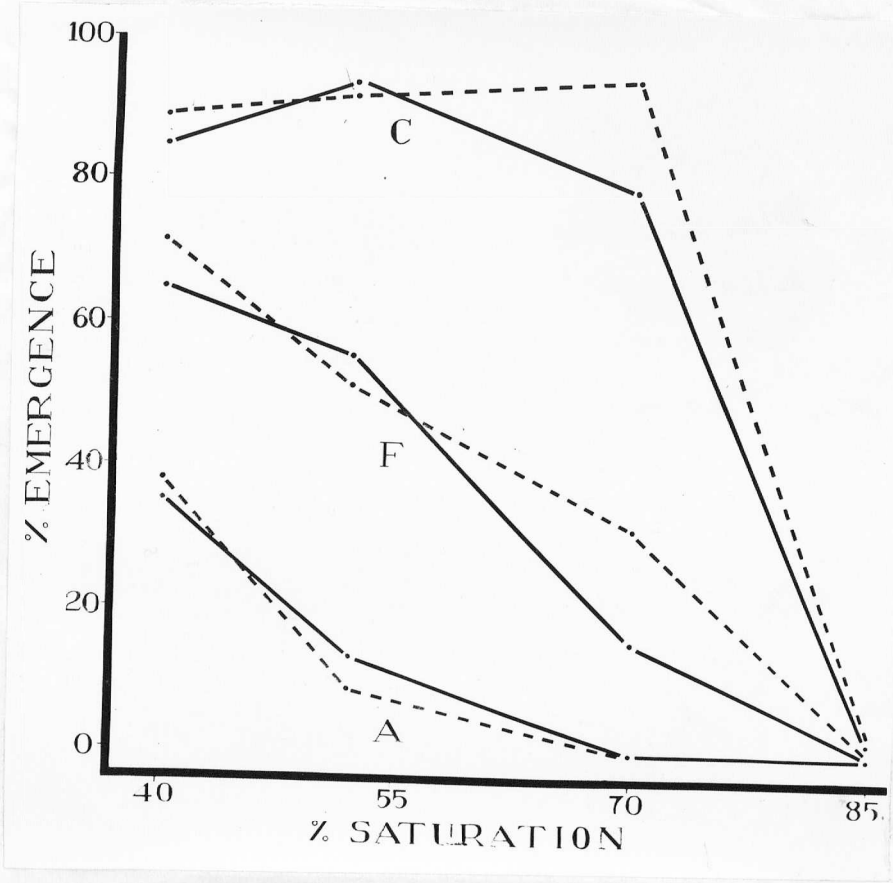


Fig.6.

Pilot seed.

Percentage emergence of peas at different soil moistures.

C Control.

F Pythium F.

A " A.

Solid line Lower temperature

Broken line Higher temp.

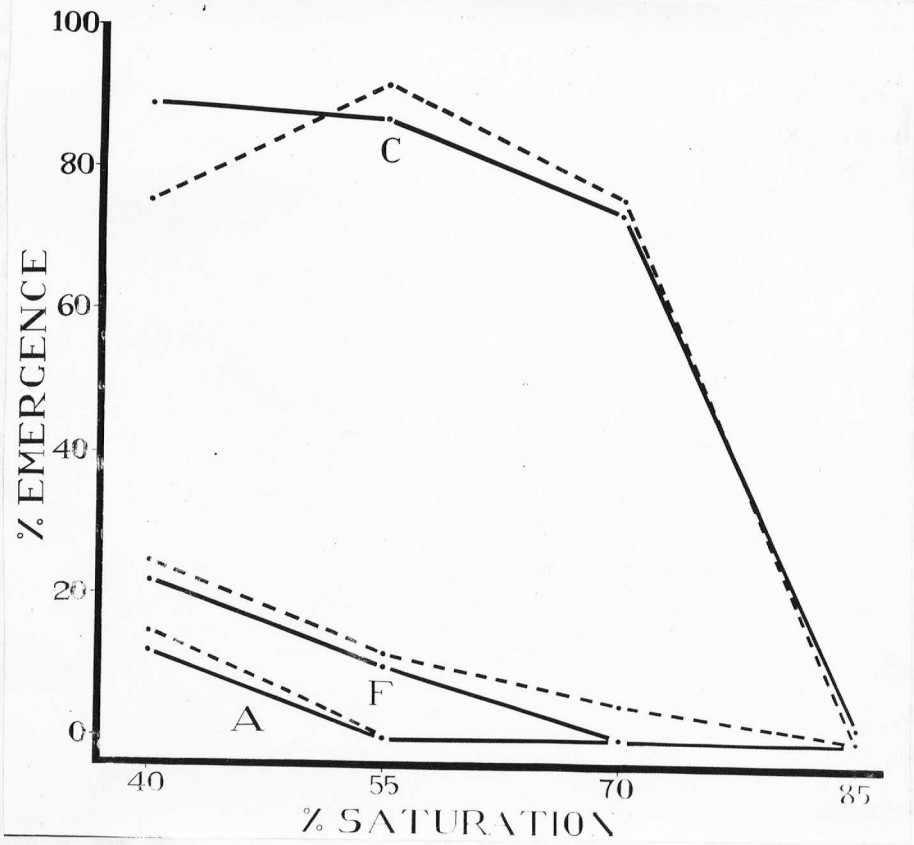


Fig.7.

Gradus seed.

the following points are consistently indicated (a) that the difference in soil temperature did not materially affect the pathogenicity of either of the fungi or the germination of the controls (b) that emergence with both fungi bore a strongly marked inverse relationship to soil moisture whereas controls did not show this (c) that the isolate of Pythium A was the more pathogenic at all soil moistures (d) that Gradus was more susceptible than Pilot throughout.

The failure of emergence in the controls at 85% of saturation calls for some discussion. The quantity of water originally added to reach this figure was fully absorbed, but, when moistened to this extent, the soil packed down with handling of the tins, so that it became saturated. As, in the open field,soil moisture has not exceeded 65% it seems unlikely that physiological failure of the seed through excessive wetness of the soil has occurred there. Imperfect germination in the controls of other pot experiments may however be due to this cause.

Two attempts were made to demonstrate the pathogenicity of Pythium in unsterilised soil. In the first, in Dec.- Jan. 1938, heavy soil freshly obtained from Slough, and permitted to dry only sufficiently for it to be sieved, was used. It was inoculated at the rate of 6% with three isolates of each of the three most abundant Pythium forms of 1937, the cultures being ten days old. The seed was from the 1938 samples and was sown at the rate of 15 seeds per 5-inch pot. The soil moisture was originally 85%, but as water loss from different pots was very variable, they were subsequently watered weekly by standing them in about one and a half inches of water for

about eight hours. They were placed in the open and covered with sheets of glass. There were nine control pots per variety and, as all the isolates of each Pythium type behaved similarly, nine replicates per fungus.

Table VII.

Average percentage emergence in pots of unsterilised soil.

	Control	Pythium F	Pythium A	Pythium H
Gradus	8.0	0.7	6.0	1.3
Pilot	73.3	57.1	64.5	63.7
Diff. from control		16.2	8.8	9.6
5% Point		17.2	13.9	13.9

In view of the poor germination of the Gradus controls little effect from the inoculations could be expected. With Pilot emergence was reduced in every case, but not significantly. An inoculum of 6% was apparently too light under the circumstances.

A second attempt was made on the light soil at Slough with Pythium F. In each of three plots ten-foot drills were inoculated with a culture nine days old on a mixture of sand and bran (four parts to one by weight). This was stirred into the bottom of the drill at the rate of 400 or 800 grms. wet weight per row. The controls received equivalent amounts of uninoculated sterilised medium. The treatments were randomised in the plots in

the usual way. The rows were one foot apart.

Table VIII.

Average percentage emergence in the field.

	Pilot		Gradus	
	Control	Pythium F	Control	Pythium F
400 grms. inoculum	64.7	78.7	22.0	28.6
800 " "	75.3	73.3	19.3	23.3

The most substantial difference between control and inoculated rows was with 400 grms. of inoculum and Pilot seed, and this is in the opposite direction from what would be expected. An analysis of variance of the type to be described later does not show this to be significant (Z = 1.2355, 5% point = 1.4592). The soil moisture (Fig.2) was about 42% at the time of sowing (March 7th,1938), and later fell. If reference is made to Figs. 6 and 7 it will be seen that the figures above are actually fairly close to those for the emergence in sterilised soil inoculated with this fungus at 40% of saturation, which suggests that the soil was already very highly infested with Pythium so that an intensification of the disease with further additions of the fungus was unlikely.

Though Sattar (23), Padwick (20) and Hull (11) have found that artificial inoculations of Ascochyta Pisi do not reduce emergence under greenhouse or laboratory conditions, it was thought desirable to try the effect of this fungus in a very wet cold soil. The experiments were similar to the one first described with Pythium, the same soil and containers, and soil moisture (84%) being used. The tumblers were, however, covered with glass and placed out of doors, the time of year being January. The seed was thoroughly washed with sterile water and then dipped in strong spore suspensions of Ascochyta made from cultures 23 days old on quaker-oat agar. All but one of the isolates were newly obtained from Pilot seed. Four isolates of Pleospora, one a recent one, three of Fusarium and three of Pythium form F were also included in the experiment. As the Pleospora spored poorly a disc from the edge of a young culture on potato extract agar was placed beneath each seed. Pythium and Fusarium, being soil fungi, were inoculated into the soil in the usual way. Of the Fusaria (d) and (e) were obtained from Pilot embryos and (f) from a cotyledon. They appeared to be distinct species. There were three replicates, but where all the cultures behaved similarly a single average figure is given for them. The results obtained are presented in Table IX.

Table IX.

Average emergence per tumbler of six Pilot seeds.

Fungus	No. of tumblers	Average emergence
Control	21	4.5
Pythium F	9	0.2
Fusarium (d)	3	2.7
" (e)	3	4.0
" (f)	3	4.0
Pleospora	12	4.8
Ascochyta Pisi	3	4.3
"	3	4.3
"	3	2.7
"	3	3.0
"	3	1.3

It is obvious that two of the Fusarium, two of the Ascochyta and all the Pleospora isolates did not reduce emergence. The remainder had some effect but were not in any way comparable with Pythium. However the application of the fungus to the outside of the seed is not the same as a natural infection which often includes the cotyledons and seedling axis, and it is considered that Ascochyta and Pleospora were probably responsible for the emergence of Pilot seed being inferior to that of Gradus in 1937, perhaps in part indirectly through increasing its susceptibility to Pythium.

In the above experiment a parallel series of tumblers contained Gradus seed but, apparently through the severity of the conditions, emergence was so poor in the controls that the results were abandoned.

A final experiment in sterilised soil was conducted primarily to test the pathogenicity of the species of Mucor which had appeared so abundantly among the isolations from cotyledons in 1938 (Table V). Two isolates from seedling axes and one from a cotyledon were used. Three forms of Fusarium (Form B from a cotyledon, Forms C and D from seedling axes) and three isolates of Ascochyta Pisi (two of which had been obtained from Gradus axes ~~embryos~~ surface sterilised with silver nitrate) were also tested. The seed used was from the 1938 samples and was surface sterilised with mercuric chloride. The soil-borne fungi Mucor and Fusarium were inoculated into the soil in the manner previously adopted, in the case of Ascochyta the seed was dipped in strong spore suspensions of the fungus. The soil moisture was kept at 60% of saturation and the temperature maintained at 5 - 7°C. in an ice-chest. As a test of the suitability of the experimental conditions for the incidence of the disease tins of unsterilised soil planted with Gradus seed were included. In this case seed was taken from both the 1937 and the 1938 samples to see if any difference in their disease resistance could be detected.

Three replicates were used, each involving fifteen seeds, but as the isolates of Mucor and Ascochyta did not differ among themselves a single figure is given for each species in Table X in which the results of this experiment are presented.

Table X.

Percentage emergence of peas.

Fungus	No.of replicates	% Emergence Pilot	Gradus
Control	9	83.7	68.2
Mucor sp.	9	84.4	75.6
Ascochyta Pisi	9	78.5	60.0
Fusarium Form B	3	0.0	0.0
" " C	3	86.7	71.1
" " D	3	75.6	71.1
		Gradus 1937	Gradus 1938
Unsterilised soil	3	0.0	2.2
Sterilised soil	3	60.0	68.2

Table X shows clearly that only Fusarium Form B, which was very highly pathogenic, had any marked effect upon emergence. The results in unsterilised soil show that the experimental conditions were suitable for a very high incidence of the disease. They show also that the 1937 sample of Gradus seed, while still germinating quite well in sterilised soil, was quite as susceptible as that of 1938.

The pathogenicity of every fungus which has occurred to a substantial extent among the isolations from diseased material has been tested under conditions highly favourable for the disease in one or more of the foregoing

experiments, the results of which may now conveniently be brought together in tabular form as follows :-

Species	No.of isolates tested	Effect on emergence
Pythium spp.	18	Inhibited emergence almost completely
Fusarium spp.	6	One inhibited emergence, five had little or no effect
Mucor sp.	3	No effect
Ascochyta Pisi	8	Little or no effect
Pleospora sp.	4	No effect

It may be concluded, then, that the only fungi capable of causing the disease which were isolated with any degree of frequency from the diseased embryos obtained from the field were species of Pythium.

G. CONTROL OF THE DISEASE.

1. Experimental.

Two seed dressings have been tried as control measures, one the proprietary organic mercurial used by Hull, the other a brand of red copper oxide which was one of the most effective of those tried by Anderson et al (1).

The amount of both these compounds that would adhere to dry peas was determined by shaking 50 grms. of seed for five minutes with the dust, separating off what was not taken up, and shaking for a further two minutes in a clean dry tube to remove any very loosely held powder. Actually the effect of this second shaking was negligible. The effect of wetting the seed with solutions of glycerine and soft soap and applying the dressing after it had dried was also tried. Table XI shows the amount of dust in grms. taken up per 100 grms. of seed when shaken with the quantities of the fungicides given in the heading of the table.

Table XI.

Adhesive power of dusts.

Sticker	Dressing retained with			
	Mercurial 0.5 grms.	Mercurial 1.0 grms.	Cuprous oxide 0.5 grms.	Cuprous oxide 1.0 grms.
None	0.12 - 0.22		0.36	0.52
10% Glycerine	0.34	0.58	-	-
20% "	0.44	0.88	-	-
40% "	0.44	0.90	-	-
10% Soft soap	0.36	0.40	-	-
20% " "	0.48	0.76	-	> 1.0

The stickers did not prevent the dressings from working evenly through

the sample. They provide, then, satisfactory means of increasing the load of dust carried by the seed. No difference was observed in the capacity of Pilot, Gradus or an unknown dried pea of retaining the mercurial compound.

Throughout both seasons 0.5% mercurial applied with 20% soft soap as sticker was employed as a control measure. To test the possible toxicity of this to peas a preliminary experiment was set up in tumblers of unsterilised light soil from Slough watered to 55% of saturation and maintained at 20° C. Six seeds were sown per tumbler and there were five replicates. The results are presented in Table XII.

Table XII.

Percentage emergence after seven days.

Variety	Control	Soft soap only	Soft soap + 0.5% mercurial
Pilot	26.7	20.0	83.3
Gradus	23.3	10.0	86.7

This result is at variance with all work at low temperatures in showing an equal susceptibility to disease in both varieties, and a very high level of control with this treatment. It indicates that very misleading results might be obtained by working at temperatures dissimilar to those

under which the disease is prevalent in nature. The fungi concerned were not determined.

The results obtained in field experiments were subjected to a simple form of statistical analysis whenever a difference occurred which gave promise of significance. A typical example is given below.

Maximum emergence of peas sown at Slough on Jan.31,1938, in light soil.

	Pilot			Gradus		
Block	Control	Treated	Mean	Control	Treated	Mean
1	32	40	36	2	4	3
2	28	36	32	3	7	5
3	24	30	27	3	7	5
4	27	34	30·5	9	6	7.5
Mean	27.75	35.0	31.38	4.25	6.0	5.13

Pilot

	Degrees of freedom	Sum of squares	Mean square	S.D.	$\log_e$S.D.	Z
Block	3	83.38	27.79	5.3	+1.66771	+1.63726
Treatment	1	103.68	103.68	10.1	+2.31253	+2.28208
Error	3	2.82	0.94	0.97	0.03045	
Total	7	189.88				

The 5% point for $n_1 = 1$ & $n_2 = 3$ from Fisher's (6A) table of Z = 1.1577

" " " " $n_1 = 3$ & $n_2 = 3$ " " " " " = 1.1137

There have, then, been significant effects for both block and treatment. A similar analysis was done for Gradus when warranted by the figures.

In 1937 0.5% of mercurial was the only treatment tried. The results are given in Table XIII. This shows that in both varieties and on both soils the dust generally improved emergence. Three times with Pilot and once with Gradus the increase is significant, but rather through the smallness of the error than through its own magnitude. In no case is there a significant reduction of germination with the method of analysis adopted though on Feb.11 one is consistently shown. At no time does the emergence of the controls leave a very great deal of room for improvement.

The treatment did not delay germination or reduce the vigour of the shoots but the checking of the radicle observed by Hull was noted. Other roots however developed freely to supercede it.

In 1938 this treatment was used again with the results shown in Table XIV. This table shows that, while the treatment was at first beneficial (the increase fails to reach significance only with Gradus on the heavy soil), it subsequently became harmful. This change is seen in the second sowing with Pilot but not until the third with Gradus. Unfortunately damage by birds necessitated reducing the number of replicates in the three last sowings on the heavy soil and abandoning the final sowing on the light soil. In the case of the sowing of Mar.14th, however, the differences are large and the two replicates agree well, so that it is reasonable to suppose that significant reductions of emergence similar to those on the light soil would have been obtained. The final sowing on the heavy soil shows no

Table XIII.

Maximum percentage emergence of peas treated with mercurial dust in 1937.

Light soil.

Date of sowing	Pilot Treated	Pilot Control	Pilot Diff.	Pilot Z(5% point =1.1577)	Gradus Treated	Gradus Control	Gradus Diff.	Gradus Z(5% point =1.1577
Feb.1	67.5	67.0	0.5		76.5	74.5	2.0	
" 11	65.5	72.5	-7.0		68.5	79.5	-11.0	0.6431
" 23	83.0	77.5	5.5		79.0	78.5	0.5	
Mar.4	77.0	65.5	11.5	1.2164	81.0	78.5	2.5	
" 15	85.5	77.0	8.5	1.7130	88.5	80.5	8.0	0.5468
" 25	75.5	84.5	-9.0 ~~-7.5~~		84.0	76.0	8.0	
Ap.5	88.0	79.5	8.5	0.6118	92.5	85.0	7.5	1.5911

Heavy soil.

Feb.1.	85.0	79.5	5.5		80.5	76.0	4.5	
" 11	71.5	78.5	-7.0		78.5	89.0	-10.5	1.0460
" 23	78.0	71.0	7.0	1.2040	78.0	75.0	3.0	
Mar.4	83.0	77.5	5.5		81.0	78.0	3.0	
" 15	84.0	82.5	1.5		88.0	91.0	-3.0	
" 25	85.5	85.5	0.0		91.5	89.5	2.0	
Ap.5	82.0	80.0	2.0		85.0	91.0	-6.0	

Table XIV.

Maximum percentage emergence of peas treated with mercurial dust in 1938.

Light soil.

Date of sowing	Pilot Treated	Pilot Control	Pilot Diff.	Pilot Z(5% point = 1.1577)	Gradus Treated	Gradus Control	Gradus Diff.	Gradus Z(5% point = 1.1577)
Jan.31	70.0	55.5	+14.5	2.2821	12.0	8.5	+3.5	0.0000
Feb.14	59.5	70.0	-10.5	1.0082	22.0	9.5	+12.5	1.6554
" 28	77.0	82.0	-5.0	0.4643	29.5	38.5	-9.0	0.4643
Mar.14	73.0	86.0	-13.0	1.4307	35.0	56.0	-21.0	1.4307
Heavy soil.								
Jan.31	73.5	60.5	+13.0	1.2588	9.5	5.0	+4.5	0.7885
Feb.4	67.0	73.5	-6.5	0.2392	29.0	23.5	+5.5	-0.2098
" 28[a]	67.0	78.0	-11.0	-	29.0	34.0	-5.0	-
Mar.14[a]	75.0	93.0	-18.0	-	33.0	60.0	-27.0	-
" 28[b]	88.0	88.0	0.0	-	55.5	66.0	-10.5	-

a = 2 replicates, b = 3 replicates.

deleterious effect from treatment with Pilot, and a substantially reduced one with Gradus. With the latter this is largely due to plants which appeared in the treated rows after the rains at the end of April, but with the former emergence was virtually complete before the soil moisture increased.

Certain other treatments were tried in season 1938. On Feb.8th on the light soil a comparison was made of the effectiveness of the maximum loads of mercurial and of cuprous oxide which the seed would carry without a sticker

(0.2% and 0.5% respectively). These were applied simply by shaking the seed with plenty of dust and removing the excess. The results are given in Table XV.

Table XV.

Maximum percentage emergence.

Variety	Control	Mercurial	Cuprous oxide	Z (1% point =1.1955)	Sign.diff. (5% point)
Pilot	50.5	69.5	84.0	1.8222	9.3
Gradus	7.0	15.0	38.0	2.3668	5.2

Since the error is evenly distributed it may be stated from the above analysis that there was a significant improvement with both varieties with the mercurial. However the stand with cuprous oxide was substantially better than with the mercurial, but with Gradus was still inferior to that given by untreated Pilot seed. Rotted seedling axes from these plots gave an unusually large proportion of Pythium de Baryanum.

The sowings of Feb.28 and Mar.14 on the heavy soil included three treatments in addition to the standard 0.5% mercurial (Table XVI). Since the replication was reduced by birds the results cannot be shown to be significant but they are consistent. It may be noted that 0.3% of cuprous oxide is the amount recommended by the makers for peas. All treatments were harmful except 0.2% mercurial.

Table XVI.

Maximum percentage emergence.

Variety	Date of sowing	Control	Mercurial 0.2%	Mercurial 0.5%	Cuprous oxide 0.3%	Cuprous oxide 0.5%
Pilot	28/2/38	78.0	77.0	67.0	-	71.0
"	14/3/38	93.0	-	75.0	77.0	75.0
Gradus	28/2/38	34.0	31.0	29.0	-	34.0
"	14/3/38	60.0	-	33.0	36.0	27.0

On Mar.21st three levels of cuprous oxide and three of mercurial were tried on Gradus seed on the heavy soil. The sticker employed where necessary was 20% soft soap. The results are given in Table XVII.

Table XVII.

Maximum percentage emergence.

Control	Mercurial 0.2%	Mercurial 0.5%	Mercurial 0.75%	Cuprous oxide 0.2%	Cuprous oxide 0.5%	Cuprous oxide 1.0%	Z (1% point = .6950)	Sign.Diff. (5% point)
61.5	62.5	42.5	32.5	56.5	50.0	23.5	1.5074	9.8

The error is sufficiently evenly distributed to state without further analysis that the two heavier dressings of both preparations were deleterious

and that the effect was greater with the larger quantity.

One experiment was carried out in pots out of doors in Feb. - Mar. The soil which had been used in testing the pathogenicity of Pythium in unsterilised soil was employed, after being well mixed, and the pots watered as in that experiment. There were six replicates each containing fifteen seeds. The results are given in Table XVIII.

Table XVIII.

Maximum percentage emergence.

		Control	Mercurial 0.2%	Mercurial 0.5%	Mercurial 0.75%	Cuprous oxide 0.5%
Pilot	Emergence	63.3	82.2	88.8	85.6	78.8
	Treatment-Control		18.9	25.5	22.3	15.5
	5% Point		21.7	17.8	19.9	19.4
Gradus	Emergence	0	8.9	5.6	2.2	6.7

This table shows that the emergence of Pilot seed was improved by all treatments, the improvement being greatest and significant with the two heavier mercurial dressings. None of the treatments however succeeded in enabling the Gradus seed to give rise to more than a few odd seedlings. Pythium was isolated with the usual frequency from the embryos of rotted seed of both varieties from pots specially set aside for the purpose.

2. Discussion of Control Measures.

The most striking feature of the above experiments is the change from a beneficial to a negligible or decidedly harmful effect shown in the field sowings in 1938 by both cuprous oxide and the mercurial. It might be contended that at all times dressings kill the physiologically weaker seed and preserve the remainder, and that it is only when the disease is very pronounced in the controls that this leaves a balance in favour of seed treatment. The increased emergence obtained in 1937 with 0.5% mercurial even when the controls germinated very well, and the fact 0.5% cuprous oxide gave better emergence on Feb.8 (especially with Pilot) than it did later make this hypothesis untenable. It is evident then that the harmful action which certain of the treatments exhibited later was negligible or absent, not merely obscured, at the beginning of the season. An obvious reason for this change is the drying out of the soil, an explanation which agrees with Horsfall's (9) statement that drought makes cuprous oxide harmful, and the experience of Brett et al (2) that organic mercurials were harmful in the field during dry weather, but not in simultaneous greenhouse experiments where water was abundantly supplied. Dressings of as much as 0.5% cuprous oxide and 0.75% mercurial can then be used in a wet soil without fear of reducing emergence should the incidence of disease prove to be slight.

There is some suggestion that the harmful effect of treatments is

bound up with the activity of the pathogen. Thus, though probably more material was taken for isolation from treated than from untreated rows, a high percentage of Pythium was still obtained from embryos when treatments were being deleterious. There is also the peculiar fact that the bad effect disappeared from the final sowing of Pilot before the soil moisture rose. The point of practical value involved is that misleading results might be obtained in experiments conducted to test the phytocidal effect of treatments in soils not abundantly infected with pathogenic fungi.

As regards the relative merits of different levels of treatment of the two dusts 0.2% mercurial has the advantage of being harmless in dry soil, but in wet soil it was substantially inferior to 0.5% cuprous oxide in the field, but not in pots.

The fungicidal effectiveness of 0.2% or 0.3% of cuprous oxide was not tested under conditions of high soil moisture, but the results suggest that in dry soil they were somewhat harmful, especially the latter. The only direct comparison of different levels of mercurial in a wet soil was in pots, and this indicates that 0.5% is best, but indirect comparison of the early field sowings of 1938 reveals no substantial superiority of this quantity over 0.2%. The level of control given by the mercurial has in general been rather inferior to that obtained by Hull (11).

H. DISCUSSION.

The most striking feature of the work detailed in the preceding sections is the marked contrast in the amount of disease present in the two seasons studied.

Previous authors, whose work is cited in the introduction, have concluded that a high soil moisture is favourable to the disease. This view is strongly supported both by the writer's field observations made during the spring of 1938 and by the results of laboratory experiments with species of Pythium which are presented in this work (Figs. 6 and 7). The spring of 1937 can, then, be regarded as one in which the disease was almost absent under conditions which were in general ~~most~~ more favourable for its occurrence than they were in the following season when it was extremely severe. There would seem to be only two possible explanations of this - (a) that the seed used in 1937 was far more susceptible than that used in 1938 ; (b) that the causal organisms were almost absent from the soil in 1937 and very abundant in 1938.

The first explanation is rendered unlikely by the fact that two varieties of seed have been used and both have shown poorer germination in the second year, at least in the earliest sowings. There is moreover no reason to suspect the 1938 seed. It was practically free from fungi to which any possible significance could be attached, and from Marsh-spot, and

it was capable of quite good germination in sterilised soil under conditions otherwise similar to those of the field. In an experiment which was carried out testing this point the Gradus seed of 1937 proved highly susceptible to the disease. The possibility of its having been altered by a year's storage, however, cannot be disposed of decisively.

In respect of the second explanation the fact that in 1938 the disease was found to be very severe on all the plots used is significant. Some of this had carried peas in 1937 and some had not. In the period intervening between the two seasons as far as is known no uniform cultural treatment had been given to all the plots. It seems, then, unlikely that either the presence of a previous pea crop or any other detail of cultivation was responsible for the multiplication of the causal organisms of the disease. Instead it is suggested that it was through some general climatic cause that the soil conditions preceding the spring of 1937 were unfavourable for the development of the species of Pythium concerned, while those preceding the spring of 1938 were highly favourable.

Garrett (7) mentions a season in South Australia in which the take-all disease of wheat failed to reach epidemic proportions in a spring which was very favourable to its occurrence. He suggests as an explanation for this that the causal organism (Ophiobolus graminis) had been largely eliminated from the soil by the action of antagonistic bacteria, which the combination of an exceptionally high soil moisture with a high soil temperature in the preceding summer had allowed to come strongly into play.

In order to see whether any similar explanation might be advanced in the present instance an examination was made of the rainfall records of the Kew Observatory. No very striking difference between the years 1936 and 1937 was evident however. In view also of the lack of information as to the soil and other conditions which might govern the persistence and multiplication of species of Pythium it seems that the question must be left over for further investigation. In any case it is clearly evident that the comparative wetness of any one spring is not in itself a measure of the amount of disease that will be present.

The problem insofar as it has been studied in this work has been simply one of pre-emergence damping-off caused by species of Pythium. An exception might be made in the case of the Pilot seed of 1937 in which seed-borne species of Ascochyta and Pleospora may have played some part.

While some measure of control has been obtained both with mercurial and cuprous oxide seed dressings neither of these has been sufficiently effective to raise the emergence of the highly susceptible Gradus variety to the level of that of untreated Pilot seed. It would appear that the selection of a suitable variety is the main step in circumventing the disease. It may not, however, be possible to find among the more palatable wrinkled peas such a level of resistance as this smooth variety shows. The work of selection might be considerably expedited by employing at laboratory temperatures rather dry sterilised soil inoculated with Pythium.

The danger of applying certain seed treatments when the soil is dry

has been clearly indicated. On the other hand a spring as dry as that of 1938 very rarely occurs, so that the danger of phytocidal damage is probably not of serious moment.

I. SUMMARY.

1. The failure of garden peas to emerge in early spring has been studied over two seasons.

2. While the conclusion of earlier workers that the disease is favoured by a high soil moisture has been confirmed in respect of observations made within a given season, it has been shown that the comparative wetness of any one spring is not in itself a measure of the amount of disease which will be present.

3. Of the two varieties of pea used the smooth one (Pilot) proved substantially more resistant than the wrinkled (Gradus).

4. The bulk of the seed which failed to emerge was attacked at a very early stage of germination.

5. A variety of fungi were isolated from the cotyledons of diseased

embryos, but species of Pythium were obtained from almost every seedling axis.

6. Of the various isolates tested the species of Pythium and in one instance a species of Fusarium were the only ones capable of inhibiting emergence in sterilised soil under conditions otherwise comparable with those of the field. The disease is accordingly attributed principally to species of Pythium.

7. The species of Pythium concerned were conidia-producing forms. Such of the isolates as developed sexual organs proved referable to Pythium de Baryanum and P.ultimum.

8. Some measure of control has been obtained with a proprietary mercurial seed-dressing but this has proved inferior to cuprous oxide. Dosages of these substances which were beneficial in wet soil gave considerable seedling damage under drier conditions. The use of a resistant variety seems to be the better way of circumventing the disease.

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K. EXPLANATION OF PLATES.

Plate I. The effect of two forms of Pythium on the emergence of Pilot peas in sterilised soil at water contents ranging from 40-85% of saturation.

Plate II. The effect of two forms of Pythium on the emergence of Gradus peas in sterilised soil at water contents varying from 40-85% of saturation.

PLATE I.



CONTROL.



PYTHIUM F.

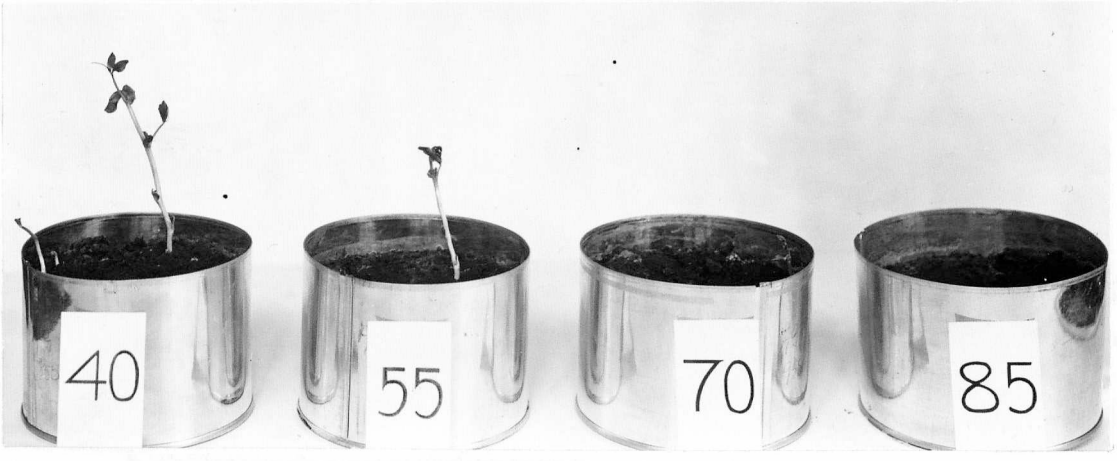


PYTHIUM A.

PLATE II.



CONTROL.



PYTHIUM F.



PYTHIUM A.

A PHYSIOLOGICAL STUDY OF THE PATHOGENICITY OF SEVERAL SPECIES OF SCLEROTINIA

Part I. A comparison of the pathogenicity of *Botrytis cinerea*, Pers., *B. Tulipae* (Libert.), Hopkins and *B. elliptica* (Berkeley), Cooke on apple, tulip and lily.

Part II. The physiological basis of the difference in pathogenicity on apple between *Sclerotinia fructigena*, Pers., *S. fructicola* (Wint.), Rehm and *Botrytis cinerea*, Pers.

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PART I. A COMPARISON OF THE PATHOGENICITY OF BOTRYTIS CINEREA, PERS., B.TULIPAE (LIBERT.), HOPKINS AND B.ELLIPTICA (BERKELEY), COOKE, ON APPLE, TULIP AND LILY.

A. INTRODUCTION.

It is well known that Botrytis cinerea Pers. has a fairly wide range of hosts which it attacks with different degrees of activity. Thus Wilson (20) has shown that the conidia of the fungus are capable of attacking directly perfectly healthy stems and leaves of Vicia Faba. Brooks (3), however, found that the spores were unable to form lesions on healthy unwounded lettuce leaves, and Salam (15) subsequently confirmed this by showing that attack on this host is initiated in dead and moribund tissue, the mycelial inoculum established therein then invading healthy parts. The observations of Pape (13) and of Gleisberg (7) suggest that this mode of attack is probably of wide occurrence. They record several instances of active parasitism by this fungus being initiated through debris adherent to the host. Apples, the host selected for B.cinerea in the work to be described, were found to be infected usually through abrasions by Faes and Tonduz (6), but Horne (11) recorded that it was able to enter through the lenticels. In autumn this fungus is abundant as a saprophyte or weak parasite on a great variety of dead or moribund plant tissue.

B.cinerea occurred once among a large number of isolations from primary lesions on tulip made by Beaumont et al. (1). Van Poeteran (14) thought it possible, however, that this species was the cause of a severe outbreak of disease in tulips in Denmark, and it was recorded on tulip bulbs by Kawamura (12).

The presence of B.cinerea on lilies has been recorded by Cotton (5), by Taylor (17) and by van Beyma and van Hell (2). The first two authors regarded it as a saprophyte. Van Beyma and van Hell undertook a series of experiments to test its pathogenicity to lilies. They found that an agar inoculum of a strain of B.cinerea from lettuce was unable to infect unwounded leaves of L.candidum, but as B.elliptica failed to do so in the same experiment it is possible that the conditions were at fault. On wounded leaves it proved a weak parasite, as it did not spread beyond the infected leaves, while a fully pathogenic culture of B.elliptica eventually killed the plant. These authors found that mycelial inocula of two strains of B.cinerea isolated from lilies, one with, and one without sclerotia, could attack wounded or unwounded leaves of L.umbellatum, but they did not regard them as comparable in pathogenicity with van Hell's strain of B.elliptica. They experimented also with a strain of B.elliptica which was about as feeble a pathogen on lilies as was B.cinerea.

Botrytis Tulipae(Libert.),Hopkins is the causal organism of tulip fire. The fungus itself and the disease for which it is responsible have been described by Hopkins (10) in America by Beaumont et al. (1) in England, and by Westerdijk and van Beyma (19) in Holland. According to these authors the

conidia are capable of attacking directly healthy flowers, stems and leaves of tulips. Hopkins found that uninjured bulbs could be infected by conidia, but Beaumont et al. (1) state that they are attacked only if they are very susceptible, heavily infected or bruised.

Hopkins did not succeed in infecting lilies with B.Tulipae but, save that he used a mycelial inoculum, he gives no experimental details. Apart from tulip he obtained positive results only on narcissus and crocus. Beaumont et al. consider that published reports of B.Tulipae crossing to other hosts and other species of Botrytis infecting tulips are to be doubted in view of the difficulty of distinguishing between allied species of the genus. B.Tulipae has not been recorded on lilies. Schnellhardt and Heald (16) inoculated it into Jonathan apples and found it, in common with B.Fabae and B.Trifolii, to be only weakly parasitic.

Botrytis elliptica(Berkeley),Cooke is the causal organism of the Botrytis disease of lilies. The most comprehensive accounts both of this and of the pathogen are those of Guterman (9) from America, of Ward (18) and Wright (21) from England, and of Westerdijk and van Beyma (19) and van Beyma and van Hell (2) from Holland. According to these authors the conidia are able to attack directly healthy flowers, stems and leaves of lilies. Guterman and Cotton (5) mention the absence of bulb lesions.

B.elliptica does not appear to have been recorded on any host other than lily. An attempt by Beaumont (1) to infect tulips with sclerotia of the fungus was unsuccessful. He gives no experimental details.

The work to be described in this paper was carried out with the intention of discovering to what extent each of these three species of Botrytis is capable of attacking the hosts proper to the other two. This has been done with the object both of defining more clearly the problem in selective parasitism which they would seem to present, and of ascertaining the probability of the hosts studied being attacked in the field by the species not usually associated with them.

B. MATERIAL AND METHODS.

The method of approach adopted in this work was to study fairly fully the action of a single strain of each species which showed a high degree of pathogenicity on its usual host. No difficulty was experienced in obtaining a satisfactory culture of B.Tulipae. The strain of B.cinerea used was more active than any of six others tested on apple, including two which had been found occurring naturally on that host. It is interesting to note that the selected strain had been obtained from a lily stem. A suitable strain of B.elliptica was not at first forthcoming, so several have been employed to some extent in this work, namely two isolated by the writer from diseased lilies and three from the collection of the Centraalbureau voor Schimmelcultures, Baarn. The purity of all the cultures, was insured at the beginning by taking a single spore or hyphal tip. Table I shows the source of the isolates used.

Table I.

Cultures of Botrytis employed.

Species		Source
B.cinerea		Lilium sp. - from a stem, autumn, 1937.
B.Tulipae		Tulip - isolated spring, 1937.
B.elliptica	BE 1	Lilium sp. - from a capsule, autumn, 1937.
"	Cotton	Centraalbureau voor Schimmelcultures, Baarn.
"	Solberg	
"	van Hell	
"	BE 2	Lilium sp. - from stem, spring, 1938.

The apples used were Bramley's Seedling variety which had been kept in cold store. The tulips were of the William Pitt variety, one of those listed by Beaumont et al.(1) as among the most susceptible to B.Tulipae. The lily species was L.candidum which is one of the most susceptible species to B.elliptica according to Guterman (9) and to Cotton (5). The plants were raised in pots in unheated frames at the Chelsea Physic Garden. In each experiment a single pot of plants was subjected to each treatment. As three bulbs were planted per pot this meant that there were 3 replicates.

In the introduction it was pointed out that a factor predisposing to attack is contact of healthy tissue with infected debris. In order to simulate such conditions a series of experiments was carried out with inocula of mycelium in nutrient agar. Again since the natural spread in the field is presumably due to the dispersal of spores by wind and rain, a similar series was carried out with spore suspensions.

Agar inocula. When an agar inoculum was used it was taken from the edge of a rapidly growing culture on quaker-oat agar and was 2-3 c.mm. in volume. The method described by Granger and Horne (8) was employed throughout in inoculating apples, each fruit being infected at different points with all three fungi. In the case of lilies and tulips the point of inoculation was cleaned with alcohol, and afterwards covered with a cotton-wool swab moistened with distilled water. This was not allowed to become

dry during the experiment. If a wound was made this was done by raising or removing a thin flap of tissue 1-2 sq. mm. in area.

Spore inocula. An obstacle encountered in this phase of the work was the difficulty experienced in obtaining spores of two of the species. B.cinerea yielded an abundant supply of spores after about fourteen days when grown on quaker-oat or Dox's agar, and the spore suspensions used in this work were always obtained in this way.

B.Tulipae, as was discovered by Beaumont et al.(1), spores fairly well on Dox's agar in sunlight. This however cannot be depended upon, and artificial light proved a poor substitute. Accordingly in any experiment with B.Tulipae recourse was had to spores produced on tulip plants which had been inoculated in an earlier experiment. This method was justified in practice by the freedom of the control plants from infection and by repeated checking of the identity of the parasite. This was readily effected by measurement of spore size, the character by which the species of Botrytis used are most easily distinguished.

B.elliptica is not recorded as sporing in culture with the exception of the mention by Wright (21) of bizarre types of spore produced on quaker-oat agar. The writer is indebted to Dr.C.E.F.Guterman of Cornell University for the information that he has obtained spores by placing grown cultures on potato-dextrose agar at about 60°F. with the lid of the Petri-dish

raised, but this method did not succeed with strain BE 1 or any of those from Baarn. Strain BE 1 was cultured on a very wide range of media under various conditions of lighting and temperature, but spore production occurred only when rotted apple tissue was subjected to desiccation by leaving it in a partially uncovered dish, or where scraped agar cultures were similarly treated. It was far too sparse and erratic, however, to serve for more than to identify the fungus. This method did not succeed with cultures from Baarn. Strain BE 1 did not spore on the host, and of those from Baarn only the rather feebly pathogenic strain of Cotton did, and that but sparsely. It is interesting to note that van Hell's strain had spored freely on infected plants at Baarn eight years previously. The long period in culture had apparently led to loss of this character but not, as will be shown, to loss of pathogenicity.

When spore suspensions were used to infect lilies and tulips it was found necessary to wipe the latter with a damp swab to prevent the spray from running off. The lilies and tulips infected with the same fungus were placed together under one large belljar, the pots being plunged in ashes in a shaded corner out of doors.

Every major result was confirmed by re-isolation of the fungus, or by measuring the spores produced. Suitable controls were included in all experiments, and in no case did these show any extraneous infection.

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C. EXPERIMENTAL.

(1) Experiments with agar inocula.

A preliminary experiment employing agar inocula on wounded surfaces was set up on Feb.11 when the lilies were 6-10 inches high, and the tulips only 4 inches high. The plants were placed in an unheated frame during cold weather for 20 days. The apples were incubated at 20°C, and the amount of estimated after five days. The results of this experiment are given in Table II. In this, and in subsequent tables in this sub-section, the measure of intensity of attack used is the number of grams of rot in the case of apples, and the greatest diameter in millimetres of the lesion in the case of lilies and tulips. If the agreement between the replicates was reasonably close a single average figure is given in the table, but if there was considerable variation two or more average values are presented, each derived from the number of lesions which were comparable in size. Each average figure is accompanied by this number in brackets.

Table II.

Lesions produced by an agar inoculum on a wounded surface.

Fungus	Lily		Tulip		
	Leaf	Stem	Leaf tip	Leaf base	Apple
B.cinerea	(3) 9.7	(6) 7.8	(2) 8.5 (1) 0	(2) 8.5	(5) 30.5
B.Tulipae	(3) 12.3	(4) 24.8	(3) 23.3	(3) 36.0	(5) 1.9
B.elliptica BE 1	(3) 22.0	(6) 12.3	(3) 9.3	(3) 6.7	(5) 3.7

Table II shows that some measure of attack was secured in every case, but it was substantially greatest in amount with each fungus on its usual host. There is one marked exception to this in the attack of B.Tulipae on lily stems, which was greater than that of B.elliptica. When the swabs were removed and the plants left in a frame all the lesions dried out except those of B.Tulipae, which continued to grow and proved fatal to both lilies and tulips.

A similar experiment was set up on March 4th, but it was conducted under much warmer conditions in a slightly heated greenhouse. In an endeavour to find a more parasitic strain of B.elliptica three cultures from Baarn were included. The apples were again incubated at 20°C. The results, as given in Table III, were recorded after five days.

Table III.

Lesions produced by an agar inoculum on a wounded surface.

Fungus	Lily		Tulip		
	Leaf	Stem	Leaf tip	Leaf base	Apple
B.cinerea	(6) 11.3	(6) 5.6	(3) 16.7	(2) 7.5	(5)36.0
B.Tulipae	(6) 23.7	(5) 20.7	(3) 25.0	(3) 30.0	(5) 2.1
B.elliptica BE 1	(6) 23.0	(6) 15.0	(3) 14.0	(1) 13.0	(5) 2.8
" Solberg	(6) 20.7	(6) 11.2			
" Cotton	(6) 27.5	(6) 17.5			
" van Hell	(6) 35.8	(6) 28.3			

It is evident that the substantial change in temperature did not alter the comparative pathogenicity of the species previously used, and also that van Hell's strain of B.elliptica was, compared with the others, highly pathogenic to lilies, being much more active than B.Tulipae. Actually it had completely severed all the infected stems. A feature of the attack of B.elliptica on tulips was the presence of long strips of dead tissue from which no fungus could be isolated. These extended above and below the lesion near the tip of the leaf, and were noted again in other experiments with this strain, with van Hell's, and also with B.cinerea, but never with B.Tulipae. When the swabs were removed and the plants placed in a frame the lesions on the lily stems continued to develop save in the case of B.cinerea, and of two of the plants inoculated with B.elliptica strain BE 1.

On April 13th an experiment was set up to determine the ability of agar inocula to infect unwounded lilies and tulips with the results given in Table IV. At the same time wounded tulips were inoculated with van Hell's strain of B.elliptica and with strain BE 1 to see if the greater pathogenicity of the former on lily would be reflected in tulip. The experiment was conducted in a cool greenhouse, and a wound inoculation of B.elliptica of van Hell served for comparison with the conditions of the preceding experiment. The apples were again incubated at 20°C., and by omitting B.cinerea it was possible to leave these for 11 days, the duration of the remainder of the experiment.

Table IV.

Lesions produced by an agar inoculum on an unwounded surface.

Fungus	Lily		Tulip		Apple
	Leaf	Stem	Leaf	Stem	(wounded)
B.cinerea	(4) 0 (2) 1.0	(6) 0	(2) 32.5 (1) 1.0	(2) 0 (1) 1.0	-
B.Tulipae	(6)26.7	(4) 9.5 (2) 1.0	(3) 25.3	(3)14.3	2.7
B.elliptica BE 1	(5)17.8 (1) 1.0	(6) 0	(3) 5.7	(3) 0	19.6
" van Hell	(6)28.0	(4) 1.0 (1)10.0 (1) 0	(2) 17.0 (1) 3.0	(2) 0 (1) 1.0	0.5
" " (wounded)	(6)40.0	(6)24.3	(3) 22.3	(3) 6.0	
" BE 1(wounded)	-	-	(3) 5.0	(2) 0 (1) 6.0	

Lesions which are recorded as having had a diameter of 1.0 mm. were very small rust-coloured flecks. These were covered and left for a further five days, but only those caused by B.Tulipae on lily stems developed.

It is evident that B.Tulipae succeeded in establishing active lesions on leaves and stems of both lily and tulip, but its stem lesions on tulip were larger than those on lily. In contrast B.cinerea was active only on tulip leaves. The B.elliptica strains attacked the leaves of both hosts. Their failure to attack lily stems, with the exception of one good lesion from

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van Hell's strain, is remarkable, but they were no more active on tulip stems. The inoculations with and without wounds suggest that the more pathogenic strain on lily was also the more active on tulip, while on apple it virtually failed to attack. The longer incubation period demonstrated clearly that B.elliptica BE 1 which was ~~completely~~ comparatively inactive on lily was more pathogenic on apple than B.Tulipae, a result suggested by the previous experiments.

Three experiments were set up with agar inocula on wounded dormant lily bulb scales. These were placed in dishes lined with wet blotting paper and incubated at 20°C. for a fortnight. The results were highly variable in all cases, there being sometimes no attack. The largest lesions were 8-12 mms. across, and were very dry and grew very slowly. It was evident that none of the fungi possessed any special capacity for attacking this part of the lily under the existing conditions.

(2) Experiments with spore suspensions.

On April 30th, when some of the tulips were coming into flower, tulips and lilies were sprayed with spore suspensions in water of B.cinerea and B.Tulipae. In this, and in nearly all experiments of this type, B.elliptica could not be used because spores were not available. The progress of the experiment was as follows :

B.cinerea - profuse spotting of tulip leaves after 3 days but no attack on stems even after 10 days ; lilies unaffected after 10 days apart from some rather doubtful spots on a few of the young leaves.

B.Tulipae - attack of tulips as for B.cinerea after 3 days but later developing on stems ; by the tenth day stems and leaves were heavily coated with pustules of the fungus ; on lilies the terminal leaf buds of all the plants were attacked, together with small lesions on the stems and leaf bases where water had been held.

This experiment was repeated with B.cinerea on an unknown variety of tulips which had been grown out of doors. Some of the plants were flowering. After two days every leaf was spotted, the attack being quite as vigorous as on greenhouse tulips sprayed at the same time.

When spore suspensions were made up in a weak nutrient (1% turnip extract) the attack of B.cinerea on outdoor tulips was rendered more severe. This was not evident in the early stages, but large lesions arose after five days at the base of several of the leaves and rotted the stem. These were absent from plants sprayed with a suspension of the spores in water. Such treatment did not, however, enable B.cinerea to produce any definite lesions on lilies, nor was the attack of B.Tulipae on this host intensified, except that the number of lesions on the young stems was rather more.

The results just described with spore suspensions of B.cinerea and B.Tulipae were, with one exception, in agreement with those previously

obtained for mycelial inocula. That exception was the failure of spore inocula of B.Tulipae to attack mature lily leaves even when a nutrient was supplied. In view of the fact that the spore suspension appeared to dry out too soon for attack to take place, steps were taken to maintain infection drops for a longer period. Large drops of spore suspension were placed singly on dry lily leaves so that they would not spread, and swabs of cotton-wool, boiled in and well rinsed with distilled water, were dipped in the spore suspension and wrapped round the leaves. After three days both methods had resulted in the formation of lesions in every case.

It was thought that the delay exhibited by B.Tulipae in attacking lily leaves might be due to the exosmosis of materials which checked spore germination, a possibility demonstrated by Brown (4) for B.cinerea on certain leaves. Using Brown's technique the writer could not, however, detect any difference either with B.cinerea or with B.Tulipae, between the germination of conidia in distilled water and that in water which had lain on lily or tulip leaves for 24 or for 72 hours. Spores of B.cinerea always germinated more vigorously than those of B.Tulipae under the same conditions.

When the experiments described above had been completed some lily stems severely attacked by B.elliptica which was sporing freely were received. A spore suspension from this source, together with the strains of B.cinerea

and B.Tulipae previously used, was sprayed onto tulips and inoculated into apples. The tulips comprised the outdoor ones previously used and material in flower from a florist. The bulbs or stems were placed in jars of water. The B.elliptica was also sprayed onto lilies. The progress of attack was as follows :

B.cinerea - profuse spotting of tulip flowers in 24 hours ; leaves spotted within two days and stems within four ; active rotting of apple.

B.Tulipae - attack of tulip leaves, flowers and stems as with B.cinerea but the lesions on flowering stems more pronounced ; slight attack on apple.

B.elliptica - attack on all parts of tulip as with B.cinerea, the characteristic large spores being freely produced. Leaves of L.candidum attacked within two days, no attack on apple.

D. DISCUSSION.

The main result arising from the foregoing experiments is that each of the three species is in general most active on the host plant with which it is typically associated. Specialisation is, however, not absolute, and there is one exception to the general statement given above which will be discussed later.

The converse relationship is most pronounced between B.cinerea and B.elliptica with respect to their host plants apple and lily. Wounded Bramley Seedling apples are highly resistant to strains of B.elliptica which are actively pathogenic to L.candidum, whereas a strain of B.cinerea which parasitises apple tissue does not attack unwounded leaves or stems of lily, though some attack results when these are wounded. It is interesting in this connection to note that the strain BE 1 of B.elliptica, which is feebly pathogenic to lily, attacks apples fairly actively. In fact the order of pathogenicity of all the fungi used in this work is the reverse on apple to what it is on lily.

Tulip shows a less specialised behaviour inasmuch as it is in substantial measure susceptible to all three species of fungi. In the experiments with agar inocula the intensity of attack on tulips of B.cinerea and B.elliptica never equals that of B.Tulipae, and this species is alone in being able to attack the unwounded base of tulip stems, a position where, according to Beaumont et al. (1) it very commonly forms lesions in the field. However definite lesions are produced by B.cinerea and B.elliptica in the form of agar inocula on the lamina of wounded or unwounded tulip leaves, and the spore suspensions of these fungi caused spotting of the leaves and flowers of tulips at maturity. This happens, even when the tulips have been raised out of doors, with as much rapidity as with B.Tulipae. B.cinerea and B.elliptica could also form lesions on the flower stems. In one

experiment, at least, these leaf and flower lesions grew at a pace comparable with those of B.Tulipae. No experiments with spore suspensions were performed with tulips at an early stage of development, but as the lesions produced with agar inocula of B.cinerea in the earliest experiments were substantially smaller than those of B.Tulipae, there is an indication that the spotting, if any, would be substantially less aggressive at this stage with B.cinerea than with B.Tulipae.

B.Tulipae similarly showed a less marked degree of specialisation. As a parasite of lily it was inferior, when applied as an agar inoculum in a wound, to van Hell's strain of B.elliptica, and when applied as a spore suspension it took longer to form lesions than did strain BE 2. Nevertheless, given a sufficiently long period favourable for infection, even the conidia were capable of establishing the fungus in both leaves and stems, and a lesion once formed did not need the continuation of exceptionally favourable conditions to be aggressive. The vigour of B.Tulipae on lily is shown clearly in Table IV in which agar inocula of this fungus proved to be more pathogenic on unwounded lilies than van Hell's strain of B.elliptica, which was the most active of the "lily" strains available. It should be remembered, however, that the latter fungus, having been long maintained in culture, had probably lost some of its original virulence. On apple B.Tulipae is a feeble pathogen, but both spores and mycelial inocula could

produce a definite lesion when placed in a wound. The ability of the fungus to establish itself through the lenticels was not investigated.

In the light of the results obtained in this work an attempt can be made to estimate the importance to each host plant of the two fungal species not usually associated with it. In addition to pathogenicity the possibility of infection being transferred from one host to another under the usual conditions of cultivation must be considered.

As parasites of apple B.elliptica and B.Tulipae must be of negligible importance. The first species, or at least strains of it with a typical measure of pathogenicity on lilies, cannot attack apples ; the second species, in addition to being a rather feeble parasite on apples has little opportunity of infecting this host since the trees are not in fruit in the tulip season.

Lilies are sufficiently susceptible to B.Tulipae to be extensively damaged by it given a sufficiently long period favourable to infection. If tulips and lilies are cultivated side by side there would be opportunities of cross-inoculation inasmuch as the growing season of most lilies would overlap that of tulips by one or two months, viz. April to June or thereby (The species L.candidum has agrowing period which includes all the growing period of tulips). The significance of B.Tulipae to the grower of lilies is, however, reduced by the fact that the two host plants are not as a rule grown on any large scale, and on commercial nurseries would not as a rule

be grown side by side.

B.cinerea as a possible parasite of lily has the dangerous feature of being very common on a great variety of hosts. On the other hand the evidence obtained in this work is that B.cinerea is a very weak parasite of lily. It must be remembered, however, that the strain used was chosen specifically on account of its activity on apple, and that there is a distinct suggestion that the vigour of a Botrytis isolate on apple is inverse to its vigour on lily. It is quite possible, therefore, that other isolates of B.cinerea might prove to be much more active on lily. Hence the significance of B.cinerea to the lily must be left open.

Tulips, at least in the flowering stage, can be spotted on the flowers and leaves with almost as much rapidity by B.elliptica and B.cinerea as by B.Tulipae. In view, however, of the fact that many species of lilies are in leaf for a small part only of the tulip season, and, moreover, that lilies are not common plants, the importance of B.elliptica to tulips is probably small. B.cinerea, on the other hand, is very common in the spring on lettuces and crucifers, and should have adequate opportunity of infecting tulips. It would seem likely, then, that some of the spotting of tulip flowers and leaves is due to this fungus. It is possible that under fairly dry conditions the lesions caused by B.cinerea might be more liable to cease development than those of B.Tulipae. The former species might then be responsible for the small non-aggressive spots recorded by Beaumont et al. (1) as being very

common on tulips. These authors found that typically no fungus could be isolated from these spots, and considered that this was because the causal organism, regarded by them as being B.Tulipae had died. The possibility of its being B.cinerea is worth investigation.

E. SUMMARY.

1. Strains of B.cinerea, B.Tulipae and B.elliptica fully pathogenic on apple, tulip and lily respectively have been shown to be more active on their usual host than on those proper to the other species, and conversely the most pathogenic species on any one host has been the species usually associated with that host.

2. The descending order of pathogenicity on apples was B.cinerea, B.elliptica (strain weakly parasitic on lily), B.Tulipae, B.elliptica (strain actively pathogenic on lily). The last member of this series failed to form a definite lesion even when inoculated into a wound on the fruit.

3. This same series represented the ascending order of pathogenicity of lilies. The first member of this series could form lesions on lily stems and leaves only if they were wounded.

4. Tulips proved at the flowering stage to be almost equally susceptible to all three species, but at an earlier stage B.Tulipae was markedly the most active.

5. On the basis both of pathogenicity and of opportunities for infection it is considered likely that B.cinerea may be of substantial importance as a cause of spotting of tulips, but that the other species are not likely to occur to any extent except on their usual host.

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PART II. THE PHYSIOLOGICAL BASIS OF THE DIFFERENCE IN PATHOGENICITY ON APPLE BETWEEN SCLEROTINIA FRUCTIGENA, PERS., S. FRUCTICOLA (WINT.), REHM., AND BOTRYTIS CINEREA PERS.

A. INTRODUCTION.

The object of this work was to study certain aspects of the physiology of fungi showing different degrees of pathogenicity on apple fruit with a view to discovering what features of metabolism were primarily responsible for these differences. Preliminary experiments led to the selection of Sclerotinia fructigena, S. fructicola and Botrytis cinerea as being fungi which are closely related systematically, and which show well-marked differences in their pathogenicity to apple.

Several workers have made observations which tend to show that differences in pathogenicity between various apple-rotting fungi are largely due to differences in response to the nutrients available in the host tissue. Seth (20) found that the rate of spread of several species and strains of Phomopsis, Cytosporina and Diaporthe on synthetic media resembling apple in composition was directly correlated with pathogenicity. Horne (14,15) has stressed the importance of acid and of nitrogen content of the fruit in

relation to the speed at which it is invaded by fungi, and Vasudeva (22) has shown that the rate of attack of apples by S.fructigena is increased by the addition of a nitrogenous substance to the inoculum. Muskett et al (18) demonstrated that there was a close correlation between the rate of spread of Cytosporina ludibunda in apple and that on autoclaved apple pulp.

In the case of S.cinerea (S.fructicola) as a parasite of plums, however, the part played by the mechanical characteristics of the fruit has been stressed by ~~Davison and Willaman~~ Willaman et al. (23). These authors discovered that resistant plums had in general a higher crude fibre content, higher pentosan content, tougher skin and firmer flesh than susceptible ones. A correlation between the resistance of potato tissue to mechanical puncture and its resistance to decay by Pythium de Baryanum was claimed by Hawkins and Harvey (13).

The importance of ability to produce a macerating enzyme in relation to relative pathogenicity was investigated by Harter and Weimer (12) in the case of species of Rhizopus showing different degrees of parasitic activity on sweet potato. Although the fungi varied considerably in their ability to produce the enzyme there was no parallelism between enzyme production and pathogenicity.

The work described in this paper is of a preliminary nature. An attempt has been made to investigate the importance of two factors only,

namely ability to produce a macerating enzyme on an apple substrate, and the intrinsic growth rate of the fungi on the nutrients present in apple.

B. MATERIALS AND METHODS.

The apples employed in this work were of the Bramleys' Seedling variety. For any one experiment fruit was available from trees which had received the same cultural treatment and were on the same root-stocks, and the various consignments used during the work differed only in the matter of root-stock.* The pH of the expressed juice of the apples was tested repeatedly with a B.D.H. Capillator and was always found to be approximately 2.8.

The fungi employed were a recent isolate of Sclerotinia fructigena, Pers. from apple, a culture of Sclerotinia fructicola (Wint.), Rehm obtained from an apple in New South Wales by Harrison, and a recent isolate of Botrytis cinerea, Pers. According to Harrison (10) the Australian form of Sclerotinia fructicola is to be regarded as identical with the American fungus now known by that name, but formerly referred to as S. cinerea (Bon.), Schröter.

Spores of B. cinerea and S. fructicola were obtained from cultures on

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*The writer is indebted to Mr. A.C. Painter of the East Malling Research Station for selecting and storing the fruit.

quaker-oat agar incubated at 20°C., those of S.fructigena from cultures on potato-mush agar grown at room temperature in continual artificial light.

Further details of experimental procedure will be given more conveniently when the experiments themselves are described.

C. EXPERIMENTAL.

(1) The comparative pathogenicity of the fungi employed.

The difference in pathogenicity in apple shown by the three fungi used is exemplified by the results set forth in Table I. In this experiment mycelial inocula in agar of the three species were inserted equatorially at different points on each of ten apples, and the fruit was incubated at 20°C. for four days. The weights of rotted tissue were then determined. The method of inoculation employed was that described by Granger and Horne (8).

This table shows that in every fruit S.fructigena produced more rapid decay than B.cinerea, while S.fructicola showed an intermediate degree of activity. The occasional instances in which the usual order of pathogenicity was not maintained, e.g. Fruit 9, are doubtless due to the fact that individual apples may vary in resistance at different points, as has been

shown by Gregory and Horne (9).

Table I.

Weights of rotted tissue in grams.

Fruit		S.fructigena	S.fructicola	B.cinerea
1		20.4	10.4	5.0
2		16.8	13.2	3.4
3		15.2	6.0	1.9
4		15.3	9.3	7.2
5		14.8	9.4	4.5
6		10.1	5.8	0.9
7		19.0	9.6	1.9
8		14.2	8.5	3.3
9		9.3	10.2	2.0
10		12.4	7.8	5.8
	Mean	14.8 ±1.12	9.0 ±0.69	3.6 ±0.62

Every consignment of apples received was tested in this way. The relative pathogenicity of the fungi was always the same. The result was found to be independant of whether a mycelial or a spore inoculum was used.

(2) Attempts to prepare macerating enzymes.

As a first line of approach to the problem an attempt was made to study the rate of production and the activity of any macerating enzyme which the fungi might produce when grown on apple.

Tests for the presence of such enzymes were made in the following preparations :-

(1) In the juice expressed from lesions on apples and clarified by centrifuging. This was tested (a) directly (b) after an attempt had been made to concentrate and partially purify any enzyme present by treatment with alcohol. The clarified juice was added to three times its volume of 95% alcohol and the mixture was allowed to stand overnight. The precipitate formed was then collected and dried in vacuo over calcium chloride. It was ground when it was very dry without the addition of sand, and, for testing purposes, added to distilled water at the rate of 2½%. This was twice the strength used by Menon (16) when testing alcoholic precipitates. From the table given by Davison and Willaman (7) it is evident that small quantities of the macerating enzyme of Rhizopus tritici (termed by them protopectinase) can remain in solution on the addition of alcohol until a concentration of above 90% of alcohol is reached. It is realised that the method of precipitation employed might involve the loss of part of the macerating enzyme, or even of the whole of it if it was present in a very low

concentration, but the precipitates obtained with further additions of alcohol proved so slight that it was not considered practicable to prepare them in sufficient quantity for testing.

(2) In cultures of the fungi made by Brown's plate method (1). A suspension was made of 1 cc. of spores in 100 cc. of the juice expressed from apple pulp which had been frozen. This was neither autoclaved nor filtered, and contained considerable amounts of suspended matter. The suspension was spread thinly on glass plates and left at room temperature for two and a half days. Tests were then made for macerating enzyme (a) directly in the culture fluid (b) in the precipitate obtained from this with alcohol as in 1(b) above (c) in the mycelium produced by the germination of the spores. This was washed in water, dried in vacuo over calcium chloride, and ground with an equal weight of silver sand. For test purposes this powder was added to distilled water at the rate of 6.5%, the strength used for such preparations by Muhleman (17) and by Menon (16).

(3) In cultures of the fungi made on thin slices of apple. Slices 2-4 mms. thick were dipped in a suspension of 1 cc. of spores in 100 ccs. of distilled water, and left on the glass plates employed in method (2) above for two and a half days. Tests were then made for the presence of macerating enzyme (a) directly in the juice expressed from the rotted apple slices (b) in the precipitate obtained from this with alcohol as in 1(b) above.

The method employed to detect macerating enzyme has been to place three discs of potato tuber tissue $\frac{1}{8}$ mm. in thickness in three ccs. of the preparation to be tested, and to incubate them at 20°C. A drop of toluene was usually added to check bacterial development. A control was set up in a similar quantity of the preparation in which any enzyme had been deactivated by heating to 70°C. This gives a safe margin over the temperature of 65°C. at which Brown (1) found the deactivation of the macerating enzyme obtained from B.cinerea to be almost instantaneous. The experiment was always discontinued before there was any indication of softening in the control discs. In cases where ground mycelium or an alcoholic precipitate was digested in water for the purpose of being tested any undissolved material was not filtered off. This procedure, which is recommended by Harter and Weimer (11) allows full opportunity for solution of any enzyme present.

Apple discs one millimetre in thickness have also been employed extensively in testing, but owing to the poor coherence of this tissue, a point which will be discussed further later, it has been impossible to regard with confidence any results so obtained.

Table II gives the results of tests with potato discs of the various classes of preparation already described. In some cases more than one separate preparation has been made. This table shows that B.cinerea, the least pathogenic of the three fungi, has proved to be the only species capable of producing a demonstrable amount of macerating enzyme when grown on

Table II.

The time required in hours for the maceration of potato discs.

Preparation	S. fructigena	S. fructicola	B. cinerea
1.(a) Juice from 4-5 days old lesion on apple (2 preps.)	>40	>40	>40
" " 6-8 " " " on apple	>48	>48	>48
(b) Alc.ppt.from juice from 6-8 day old lesions	>60	>60	>60
2.(a) Apple juice in which spores had germinated	--	>24	>24
" " " " " "	>41	>41	41
" " " " " "	>48	--	48
(b) Alc. ppt. from 2(a)	--	>60	>60
(c) Extract of mycelium produced in apple juice	>60	>60	48
" " " "	--	>36	18
" " " "	--	--	18
3.(a) Juice from apple slices on which spores had germinated	>54	>54	54
(b) Alc. ppt. from 3(a)	>60	>60	12

an apple substrate. Insofar as the methods employed may be relied upon as a measure of it, relative activity in the production of macerating enzyme cannot then be the factor determining relative pathogenicity. Failure to demonstrate the presence of the enzyme in juice expressed from lesions of B.cinerea on apple

may be due to its secretion being confined to the young hyphae, so that it is only in preparations such as Nos. 2 and 3 where the proportion of young mycelium is greatly increased that the presence of the enzyme can be demonstrated by the methods employed.

It is convenient at this point to review the results of other investigators who have studied the production of macerating enzymes by the fungi used here. It is well known that B.cinerea produces a macerating enzyme on a wide range of media. The only attempt to prepare one from a culture on apple appears to be that of Menon (16) who obtained an active preparation from cultures 10 days' old on autoclaved plugs of apple tissue. In respect of the ability of S.fructigena and S.fructicola (S.cinerea) to produce a macerating enzyme the literature is somewhat contradictory. The results of the various workers are assembled in tabular form below :

Author	Fungus	Medium	Mode of culture	Age of culture (days)	Enzyme present in	Enzyme absent from
Davison & Willaman(7)	S.cinerea S.fructigena	prune juice	flasks	2		hyphae
"	S.cinerea	"	Brown's plate method	1		hyphae & medium
Muhleman(17)	S.cinerea	"	flasks	3 - 5	hyphae	medium & alc. ppt. from medium
Burges(4)	S.fructigena	"	flasks(?)	-	hyphae, medium(v. little),alc. ppt.from medium	
Menon(16)	S.fructigena	apple & other extracts	flasks	-		hyphae & medium
		turnip extract	Brown's plate method	5	hyphae & alc.ppt. from medium	
		autoclvd. apple, potato & turnip	plugs of tissue	10	alc.ppt.from medium	

It would appear from these results that the production of macerating enzyme in demonstrable quantity by these two species of Sclerotinia depends a good deal on the conditions of culture. All the authors cited above have used methods which are somewhat different from those of the present work, and which in no case approximate so closely to the conditions prevailing in parasitic attack. Such

demonstrations of the production of enzyme as have been made, then, cannot be regarded as directly opposed to the results of the writer's experiments.

Obviously the most direct test for the presence of enzymes capable of macerating apple tissue would be made by employing apple discs. It has already been stated that they were not satisfactory, however, for the detection of slowly acting enzymes, at least. Discs cut from mature apples possess little coherence, and showed a tendency to soften when placed in some liquids which did not contain enzyme. While several workers have employed apple discs, this tendency does not seem to have passed unnoticed. Davison and Willaman (7) state that "apple tissue seemed slightly macerated by oxalates, but this effect may be only a slight hydrolysis or softening due to water present". Vasudeva (22) took as an end point when using apple discs in enzyme tests the time when the tissue had fallen into a mush. Such obvious maceration has not been seen in any of the preparations obtained in this work. Muhleman (17), on the other hand, seems to have found apple a perfectly satisfactory test tissue, but he used green fruit, which probably has greater coherence.

The tendency to disintegrate was found to be most pronounced in extracts of hyphae, and comparatively slight in liquid expressed from lesions. It was accordingly suspected of being an osmotic effect - the result of the cells swelling and bursting apart when water was absorbed from hypotonic solutions. To test this theory 15 discs, $\frac{1}{2}$ mm. thick, were cut from five

different apples and divided among the following treatments : (a) placed in a covered dish (b) immersed in distilled water (c) in apple juice (d) in Mc Ilvaine's buffer pH 2.8 prepared according to the formula of Clark (5). This was the pH of the apple juice, and was a 0.12 molecular solution (e) in a 0.5 molecular cane sugar solution. This was found by Barger's method, which is described by Buller (3), to be approximately equal in osmotic pressure to apple juice. In order to ensure rapid entry of the solutions into the tissue the discs were evacuated under a filter pump. After one hour the discs in water and in the buffer had lost coherence, but those in apple juice and in sugar solution and those merely kept in a damp chamber were perfectly sound, and retained some measure of coherence even after 24 hours.

This result was confirmed by placing 0.2 cc. of each of the liquids listed above on the surface of blocks of apple tissue 2.5 cm. in diameter and leaving them in covered dishes for 24 hours. The liquid remaining was then removed along with any softened apple tissue, and its weight determined as follows.

Water 0.46 gms. Buffer 0.57 gms. Juice 0.23 gms. Sugar 0.21 gms. These figures are the average of six determinations. As these weights show, nothing more than the fluid originally added was removed from the blocks in the case of juice and sugar solution, but the buffer and the water had had a distinct disintegrating effect on the surface of the blocks.

These results show that apple tissue, at least when the apple is mature will disintegrate in pure water, and in solutions of low osmotic pressure which contain no enzyme.

3. The intrinsic growth-rate of the fungi on apple.

In order to discover to what extent the difference in pathogenicity might be due merely to a difference in response to the nutrients available in the host tissue, an attempt has been made to compare the growth of the selected fungi on living with that on dead apple tissue.

Eighteen apples were halved from stalk to eye in a sterile way, and the cut surfaces sealed with paraffin wax. Half of each apple was either frozen or chloroformed, the other half serving as a control. The freezing was carried out by placing the halves in a large glass chamber, sealing it, and submerging it completely for 24 hours in a mixture of ice and salt. This was changed once. The halves to be chloroformed were left overnight in a desiccator into the bottom of which a little chloroform had been poured. The frozen half-apples were inoculated as soon as they had thawed along with the corresponding unfrozen halves, a separate apple being used for each fungus, so that there were three replicates. The chloroformed halves were allowed to remain freely exposed to the air for three days before inoculation. To check excessive loss of water the fruit was then wrapped in

grease-proof paper, and left at room temperature for six days.

The frozen fruit was exceedingly soft and brown, and the areas occupied by the fungi were indistinguishable either by colour or texture from the rest of the fruit. The chloroformed fruit was, however, firm and rubbery resembling very much in appearance and in texture apple tissue which has been rotted by S.fructigena or S.fructicola. The area of chloroformed apple which had been invaded by these fungi was thus indistinguishable from the remainder. In the case of B.cinerea, however, the fungus brought about a substantial softening of the tissue, producing a lesion similar in texture to that produced on a living apple, which, as is well known, is considerably softer than a Sclerotinia lesion. These observations lend support to the view that macerating enzymes are active to some extent in lesions formed by B.cinerea on apple, but do not play a great part, if indeed they are present at all, in lesions of S.fructigena and S.fructicola.

As most of the lesions could not be scraped out and weighed, the wax was removed from the half-apples, and a cut made passing from the core towards the point of inoculation to avoid carrying any hyphae into the uninvaded tissue. The amount of spread of the fungus was then recorded by direct measurement, the edge of the lesion being located where necessary by examining small portions of tissue under the microscope for the presence of hyphae. The results are given in Table III. In some cases the fungi were found to have reached the cut surface of the half-apple.

Table III.

Radial growth in millimetres of fungi on living and on dead apple tissue.

Fungus	Control	Chloroformed	Control	Frozen
S.fructigena	>28	15	>28	-
	>25	14	>25	>25
	>23	20	>23	20
S.fructicola	20	17	>28	nil
	20	18	>25	almost nil
	>20	10	23	6
B.cinerea	20	13	19	2
	14	13	19	nil
	24	14	20	5

Chloroforming of the tissue retarded the growth of all the fungi. Traces of the chemical remaining in the fruit probably account for this effect. Freezing was even more deleterious to the growth of the fungi except for S.fructigena, which was practically unaffected by it. Interference with aeration through flooding of the intercellular spaces was probably the inhibiting factor.

In view of the failure of the preceding methods to produce satisfactory results an attempt was made to compare radial growth of the fungi in apple with that in unautoclaved apple juice. The flesh of five apples was frozen and the juice was expressed and centrifuged. An attempt was then made to sterilise it by passing it through five thicknesses of Whatman No.42 filter paper. A Seitz bacterial filter was found to clog very quickly, and, because of the acidity of the juice, the removal of bacteria was not considered necessary. The filtrate was poured into petri-dishes (9 cms. internal diameter) at the rate of about 15 ccs. per plate, and the plates were inoculated with a very small portion of mycelium in agar. There were five replicates. Five apples were simultaneously inoculated with all three species of fungi. The radial growth of the fungi in millimetres after three days is given in Table IV. The method of sterilising employed for the juice was not successful, but contaminating cultures did not appear until the third day.

Table IV.

Radial growth in millimetres of fungi on living apple and in apple juice.

Species	Av.radial growth in apple	Av.radial growth in juice
S.fructigena	17	15
S.fructicola	12	14
B.cinerea	8	19

While the growth of the species of Sclerotinia did not differ greatly in the juice from what it was in the fruit that of B.cinerea was much greater in the former medium. This result suggests the conclusion that the growth rate of B.cinerea on apple is determined in considerable measure by its ability to penetrate and kill the tissue, and that that of the species of Sclerotinia is not. The experiment is open to criticism, however. The medium cannot be regarded as containing all the nutrients available in the fruit, and the conditions of oxygen and carbon dioxide tension are probably very much altered. Results in which more confidence could be placed might be obtained by using unsterilised apple pulp.

D. DISCUSSION.

No production of macerating enzyme was demonstrated when S.fructigena and S.fructicola were grown on apple, but B.cinerea gave positive results. Enzymes can, however, be deactivated rapidly by some tissues. Brown (2) records a striking instance of this in the case of macerating enzyme of B.cinerea placed on blocks of sub-turgid potato tissue. The possibility of enzyme being produced by the species of Sclerotinia when they are grown on

apple, but disappearing too rapidly to be detected by the usual methods, cannot then be dismissed.

The relative firmness of the rot caused by S.fructigena and S.fructicola does, however, suggest that there is far less cell wall disturbance with these fungi than with B.cinerea. Indeed, the differences in texture between various apple rots would seem to find a very probable explanation in the extent to which macerating enzymes are active. With the very soft rots produced by species of Penicillium it is possible, according to Nobécourt (19), to demonstrate macerating activity in the crude juice expressed from lesions. This suggests that the enzyme is more abundantly produced here than in the firmer Botrytis lesions with which no such demonstration could be made by the writer. In such firm lesions as those of S.fructigena and S.fructicola macerating enzyme is, perhaps, entirely absent. Alternative explanations which have been offered for the firmness of Sclerotinia lesions are that the stout hyphae impart firmness (Valleau, 21), or that a hydrophilic gel of calcium pectate is precipitated by the fungus (Willaman, 24).

A comparison of the growth-rates of the fungi in apple juice with that in living tissue suggests that there may be some substantial difference between the mode of parasitic attack by the species of Sclerotinia and that by B.cinerea. The growth of B.cinerea appears to be retarded in the fruit,

possibly because it is dependent on the previous destruction of the tissue by enzyme. The Sclerotinias, on the other hand, grow through living apple at a rate comparable with their intrinsic rate in an apple cultural medium. One may suggest, therefore, that the hyphae advance almost unimpeded through the well-developed intercellular spaces of the fruit. The method of penetration of fruit tissues by S.fructigena and S.fructicola (S.cinerea) does not seem to have been studied of recent years but, according to Valleau (21), Behrens and Schellenberg concluded that the hyphae were exclusively intercellular, an opinion which he himself endorsed. Cooley (6), on the other hand, considered that S.cinerea passed as readily through the cells as between them. Valleau considered that enzyme was produced which split the middle lamella, but its existence was denied by the previous workers.

Should macerating enzyme be entirely absent, and the hyphae at least initially intercellular, it seems necessary to postulate the secretion of some toxin to bring about the death of the host cells. The examination of fungal extracts for the presence of such a compound is a line of work which calls for attention. In view of the unsatisfactory nature of apple tissue as a test material, the evidence on this point may have to be obtained indirectly.

E. SUMMARY.

1. *Sclerotinia fructigena* is more pathogenic on Bramley's Seedling apples than *Botrytis cinerea*. *S.fructicola* shows an intermediate degree of pathogenicity.

2. Only *B.cinerea* is capable of secreting a demonstrable quantity of macerating enzyme when grown on an apple substrate.

3. On unautoclaved apple juice the growth-rate of the species of *Sclerotinia* is similar to that in the fruit, but that of *B.cinerea* is over twice as fast.

4. It is suggested that differences in texture of apple rots is due to differences in the extent to which macerating enzymes are active in them.

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