PRE-EMERGENCE ROTTING OF PEAS IN SOUTH AUSTRALIA

II. FACTORS ASSOCIATED WITH THE SOIL

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Summary

Pre-emergence rotting of wrinkle-seeded peas in the field in South Australia is usually due to attack by *Pythium ultimum* within 96 hr of planting. *Fusarium* spp. follow *Pythium* closely in invading the seed but are not a primary cause of rotting.

Pythium attack generally occurs at the cotyledonary node and rapidly spreads up the shoot and radially into the cotyledons. *Pythium* occasionally attacks the young shoot and spreads down the seedling axis.

The percentage of seeds attacked increases with increase of soil moisture level from wilting point to field capacity.

It is suggested that attack of seedlings may be preceded by diffusion of materials from the seed into the surrounding soil which causes prolific growth of *Pythium* and leads to infection. Soil moisture may influence diffusion of material from the pea seed rather than have a direct effect on *Pythium* activity.

Smooth-seeded peas are susceptible to attack by *Pythium* but are seldom seriously affected under field conditions.

I. Introduction

Wrinkle-seeded varieties of peas often give thin strands and poor yields in South Australia as a result of pre-emergence rotting. While part of this rotting may be attributed to factors inherent in the seed (Flentje 1964), percentage emergence in the field was often significantly lower than expected on the basis of laboratory germination tests. Furthermore the percentage emergence of one seed sample in a soil varied with time of planting. These differences indicated that factors associated with the soil as distinct from those inherent in the seed were causing pre-emergence rotting. This paper records the investigations of these soil factors.

II. Literature Survey

It has been generally accepted that pre-emergence rotting of wrinkle-seeded peas is due to invasion of germinating seed by soil-borne fungi shortly after sowing. *Pythium* is commonly suggested as the main cause of the rotting (Hull 1937; McNew 1943*a*, 1943*b*; Hutton 1944; Angell 1950; Jacks 1961). *Fusarium* spp. have also been suggested as the main cause of the rotting (Brett, Dillon Weston, and Booer 1937; Padwick 1938) but some of this work is open to criticism (Baylis 1941) because the surface-sterilizing agents used on the seed prior to isolation would probably kill

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Pythium and reveal only fungi, such as *Fusarium*, which are more resistant. *Rhizoctonia*, also mentioned (Sharvelle and Shema 1941) as a possible cause, appears to be less important than *Pythium* or *Fusarium*.

Soil moisture has been shown by several workers to influence the incidence and severity of the rotting. Hull (1937) and Baylis (1941) investigated time of sowing in the field and showed that rain shortly after sowing increased the percentage of seedlings rotted. Jones (1931) carried out similar investigations in the greenhouse and simulated the effect of rain by applying half or an inch of water at different intervals after sowing. The percentage of seedlings rotted was highest with 1 in. of water applied immediately after planting but analysis of the results is difficult in the absence of data on penetration of water into the soil. Angell (1952) obtained results similar to those of Jones. McNew (1943*a*, 1943*b*) made a more critical analysis by germinating peas in flats filled with soil at different moisture levels and enclosing them in oil cloth to retain constant moisture levels. He showed that the percentage of rotted seedlings was significantly higher at the higher moisture levels. In these investigations, however, the data on soil moisture are not critical enough to allow a detailed analysis of the influence of this factor.

Ledingham (1946) found no correlation between soil moisture and pre-emergence rotting, but suggested that soil temperature might be important. Jones (1931), McNew (1943*a*, 1943*b*), and Hutton (1944) have also suggested that temperature is important.

No critical work has been carried out on the influence of soil type, texture, or pH, although several workers (e.g. Hutton 1944) point out that rotting is more severe in lighter sandy soil than in heavier clay and clay loam soil. Angell (1950) has shown that liming and other factors influence rotting caused by *Pythium*.

There is some evidence that smooth-seeded peas, in contrast to the wrinkle-seeded peas, show little or no pre-emergence rotting even at the high soil moistures (McNew 1943*a*, 1943*b*).

III. Experimental and Results

(*a*) *Field Experiments*

Pre-emergence rotting due to soil factors was measured by sowing replicate lots of 1000 seeds in field plots, counting seedlings which emerged, and comparing this with the percentage laboratory germination (Flentje 1964). Variation in the influence of the soil factors was studied by sowing at different times throughout autumn, winter, and spring, recording soil moisture, soil temperature, and rainfall from sowing to emergence, and comparing the percentage emergence under the differing conditions. To follow germination and pre-emergence rotting during any one sowing, lots of 100 seeds were removed from one of the replicates at 48-hr intervals and examined.

Three experiments were carried out, one in 1944 and one in 1945 at the Waite Institute, near Adelaide, the soil being a red-brown silt loam with a wilting point of approximately 8% and a field capacity of approximately 21%, and one in 1945 at Mundalla, near Bordertown, S.Aust., the soil being a grey sandy loam with a wilting point of approximately 4% and a field capacity of approximately 16%. Each experiment was laid out as 24 plots in a 6 by 4 rectangular pattern showing six replicates across and four times of sowing down, time of sowing being randomized independently within each of the six replication strips. Seeds were sown approximately $1\frac{1}{2}$ in. apart at a depth of $1\frac{1}{2}$–2 in. through a small two-hoe drill designed for the purpose and employing normal drill cups to allow for any abrasive injury to seeds which might be experienced in commercial practice.

Soil temperature records at the 1-in. level were available for the Waite Institute experiments only. Soil moisture was determined by taking 50 soil samples, each approximately $\frac{1}{2}$ kg, at $1\frac{1}{2}$–2 in. depth over the sown plots. These were taken 24 hr after sowing only at Mundalla but were taken at 2–3-day intervals over the period from sowing to emergence at the Waite Institute. Daily rainfall records were available at both places.

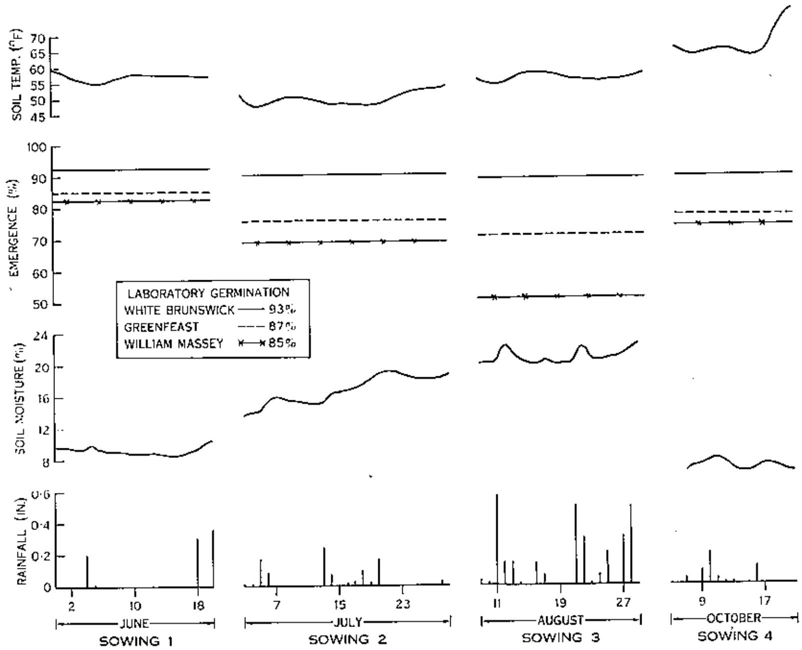

Fig. 1.—Percentage laboratory germination and percentage field emergence together with soil moisture, soil temperature, and rainfall data for samples of White Brunswick, Greenfeast, and William Massey peas sown at four different times in 1945 at the Waite Institute.

Fifteen seed samples, five of each of the wrinkle-seeded varieties Greenfeast and William Massey and five of the smooth-seeded variety White Brunswick, were sown. In the laboratory germination tests, seed wound through the two-hoe drill was used to eliminate drill injury as a possible variable. Lots of 200 seeds from each sample were planted on agar to determine whether seed-borne pathogens were present; in no case were any detected.

As the results in each year in the Waite Institute experiment were similar, those for 1945 are presented. Furthermore as the results for all seed samples within each variety were similar, those for only one sample of each are presented below, the same samples being presented for the Waite Institute (Fig. 1) and Mundalla

(Table 1) experiments. Four sowings were made at the Waite Institute, but only three were practicable at Mundalla.

In each of the field experiments several results appeared to be consistent, namely:

(1) Percentage emergence of the smooth-seeded variety White Brunswick in any planting did not differ significantly from the percentage laboratory germination, indicating there was no significant amount of pre-emergence rotting in this variety due to soil factors.

(2) Percentage emergence of wrinkle-seeded varieties in all but one planting was significantly lower than the percentage laboratory germination, indicating that a significant amount of pre-emergence rotting due to soil factors occurred.

TABLE 1

PERCENTAGE FIELD EMERGENCE OF WHITE BRUNSWICK, GREENFEAST, AND WILLIAM MASSEY PEAS TOGETHER WITH SOIL MOISTURE AND RAINFALL DATA FOR THREE SOWINGS IN 1945 AT MUNDALLA

Date of Sowing	Soil Moisture Content at Sowing (%)	$10^2 \times$ Daily Rainfall (in.) for 14 Days after Sowing	Emergence (%)		
			White Brunswick	Greenfeast	Massey
26. vi.45	13–14	1, –, –, 4, –, –, 4, 30, 12, –, –, –, –, –	88·2	75·2	67·8
31.vii.45	11–12	–, 5, –, –, –, 60, 8, 5, 6, 20, 26, 5, –, –	87·2	79·0	69·6
19. x.45	1–2	–, –, –, 19, 108, –, 20, 3, –, 40, –, –, –, –	88·1	66·2	51·2

(3) Percentage emergence appeared to vary inversely with either the soil moisture or amount of rainfall immediately after sowing.

(4) There was no obvious relationship between percentage emergence and soil temperature.

In the lots of 100 White Brunswick seeds removed at intervals of 48 hr, a number corresponding with the laboratory germination percentage germinated and continued vigorous growth to emergence. In the wrinkle-seeded varieties after 48 hr there was a healthy germination of clean seeds equivalent to the laboratory germination percentage. After 96 and 144 hr, however, the seeds were readily divided as set out in Table 2 into three groups:

(i) Seeds with a vigorous seedling axis, showing no rotting and with only loosely adhering soil.

(ii) Seeds with a well-developed radicle but whose cotyledons were enclosed in a "ball" of soil and fungal mycelium. When the soil was washed away the seedling axis showed a water-soaked lesion at the cotyledonary node extending up into the plumule and radiating into the cotyledons.

(iii) Seeds with poorly developed or no radicle and enclosed in a ball of soil as in group (ii). These seeds were rotted around the periphery of the cotyledons as well as in the embryo area.

The number of seedlings in group (i) corresponded with the percentage emergence for that sowing, those in group (ii) corresponded with the difference between percentage emergence for that sowing and percentage laboratory germination, and those in group (iii) were constant and corresponded with the percentage that failed in the laboratory germination. In samplings after 144 hr, it was difficult to distinguish between groups (ii) and (iii) because of the rapid progress of the rotting.

For different sowings of any seed sample the number of seeds in group (iii) at the 96-hr sampling was relatively constant, but those in groups (i) and (ii) varied significantly. The results for the 1945 Waite Institute experiment in Table 2 illustrate this relation.

TABLE 2

DATA FROM THE FIRST THREE SOWINGS IN 1945 OF WILLIAM MASSEY SEED IN WAITE INSTITUTE SOIL

	Laboratory Germination (%)	Field Emergence (%)	Difference (%)	Separation of Seeds 96 hr after Sowing		
				Class (i) (%)	Class (ii) (%)	Class (iii) (%)
First sowing	85	82·5	2·5	81	5	14
Second sowing	85	69·5	15·5	71	12	17
Third sowing	85	51	34	51	33	16

The results from the fourth sowing in Waite Institute soil in 1945, however, did not fit the above pattern. Germination of seeds was much slower and, although after 96 hr, seeds corresponding with those in group (iii) above and showing definite rotting could be distinguished, the remainder were free from adhering soil or rotting, but were in different stages of germination. When emergence counts were made subsequently there were many seeds fully swollen, unrotted, but with little or no seedling axis developed. It appears that the moisture level of 6–8% for the fourth sowing is the minimum for pea seed germination; slight variation in the seeds or in soil moisture allowed some to develop and to emerge while others failed.

(b) *Pot Culture Experiments*

As soil moisture appeared to be an important environmental factor influencing the amount of pre-emergence rotting in the field, this was investigated under controlled conditions in pot experiments.

Soil taken from the plot areas of the field experiments at the Waite Institute and Mundalla was used. After thorough mixing, the soil from each area was divided into several lots and the moisture level of each adjusted to give a graded series from near wilting point to near field capacity.

This was achieved by watering an area *in situ* with a sprinkler, covering it for 24–48 hr with a tarpaulin, then removing the top 6 in. of soil. After subdivision into several lots, these were allowed to dry out to the appropriate levels, the level being measured by frequent samplings. Small increases in the moisture levels were obtained by spraying the soil with the required amount of water through an atomizer spray, the fine spray reducing the possibility of the soil aggregating in lumps.

The pot experiments were prepared using 9-in. diameter enamel pots each containing 5 kg of soil; William Massey and White Brunswick peas were tested, five replicate pots of 30 seeds planted $1\frac{1}{2}$–2 in. deep being used for each cultivar, soil type, and moisture level. The pots were capped with waxed brown paper to retain a constant soil moisture level and incubated at 18–20°C. Emergence counts were made after 2 weeks.

In addition two other pot series of graded soil moistures prepared with Waite Institute soil which had either been autoclaved at 2 atm for 2 hr or treated with 5% formalin at the rate of 4·5 litres/250 kg soil. William Massey and White Brunswick seeds were planted in each pot series.

In both the autoclaved and the formalin-treated Waite Institute soil there was no significant difference between percentage emergence and percentage laboratory germination at any soil moisture level for either pea variety.

In both the untreated Waite Institute and Mundalla soils the percentage emergence of the William Massey seed decreased significantly as the percentage soil moisture increased. The percentage emergence of White Brunswick peas showed little difference, however, being significantly lower only at the highest moisture level near field capacity. The results are shown in Figure 2.

Twenty seeds of each variety were removed from a pot of each treatment above after 48, 96, and 144 hr and divided into groups (i), (ii), and (iii) as in the field experiment. The results with seed of each variety in the untreated soils were similar to those obtained in the field. In the autoclaved or formalin-treated soil, however, there were no seeds in group (ii) at any soil moisture level.

The appearance of germinating William Massey seeds removed from the pots of autoclaved, formalin-treated, and untreated Waite Institute soil at different moisture levels after 96 hr is shown in Plate 1, Figure 1.

These results confirm the indications from the field experiments that soil moisture exercises a marked influence on the emergence of wrinkle-seeded peas, particularly over the range of moisture levels midway between wilting point and field capacity which are commonly regarded as ideal for pea seed germination. The absence of rotting in the autoclaved or formalin-treated soil indicates that the rotting in untreated soil is due to soil organisms and not a direct effect of soil moisture.

The smooth-seeded peas showed no significant difference between percentage emergence and percentage laboratory germination at the lower moisture levels, but at the highest moisture level the emergence was significantly lower, indicating that pre-emergence rotting, not observed in the field, occurred in the pots. A percentage of White Brunswick seedlings removed from the high moisture level pots had been attacked on the seedling axis between the cotyledonary node and the plumule and had failed to emerge. This percentage corresponded with the difference in percentage emergence between high and low soil moisture levels.

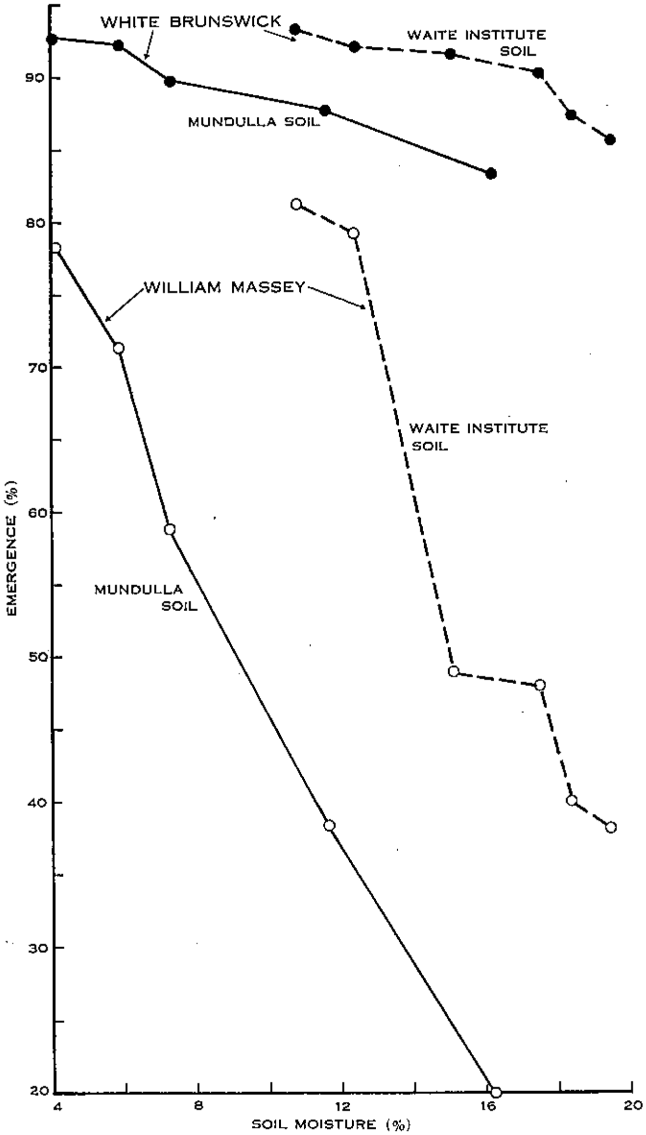

Fig. 2.—Percentage emergence of White Brunswick and William Massey peas at different soil moisture levels in pots of Mundalla and Waite Institute soils.

(c) *Isolation of Fungi from Germinating Seedlings*

After examination the germinating seeds, taken at 48-hr intervals from both field and pot experiments, were washed for a few minutes in running tap water to remove all adhering soil. They were then immersed in one volume of sterile tap water to which was added two volumes of 1 : 1000 mercuric chloride which was mixed by shaking. The seeds were allowed to stand in it for 2 min, then washed in three changes of sterile distilled water, dried thoroughly in sterilized blotting paper, and planted on potato–dextrose–agar. Where the seedling axis was well developed, the cotyledons and seedling axis were plated separately, otherwise the whole seedling was plated as a unit.

Before deciding on this procedure, the following methods for treating the seeds prior to isolation were tested:

(1) 1, 2, 3, or 4 min in 1 : 1000 mercuric chloride;

(2) 25 min in 3% hydrogen peroxide;

(3) 20 min in a solution of 10 g commercial calcium hypochlorite in 140 ml water.

None of these treatments proved as satisfactory as that described earlier. Treatments (2) and (3) were apparently insufficient to remove surface contaminants and isolation plates were rapidly overgrown by *Mucor* and *Rhizopus*. Treatments included in (1) overcame this difficulty but were too severe. Seeds known to have been invaded with *Pythium* frequently gave rise to colonies of *Fusarium* only as this fungus was apparently more resistant to the mercuric chloride.

The majority of seeds removed 48 hr after seeding when treated with 1 : 1500 mercuric chloride and planted on agar continued to develop with only occasional growth of *Aspergillus*, *Chaetomium*, *Penicillium*, *Mucor*, and some bacterial colonies. A small percentage gave rise to colonies of *Pythium* and these seeds failed to develop further. A percentage corresponding with the percentage of seeds that failed to develop satisfactorily in the laboratory germination test failed to develop when planted on agar and showed a prolific growth of *Mucor*, *Rhizopus*, and other saprophytic organisms.

Seeds removed after 96 hr which were separated into the three groups [(i), (ii), and (iii) as previously described] were maintained in these groups when planted on agar. Seeds of group (i) when planted on agar gave rise to occasional colonies of *Aspergillus*, *Penicillium*, *Chaetomium*, *Mucor*, and a variety of bacteria. Seeds of group (ii) in almost every case yielded colonies of *Pythium*. Less frequently *Fusarium* spp. and still less frequently *Mucor* spp. were associated with the colonies of *Pythium*. Seeds of group (iii) yielded a prolific growth of *Mucor*, *Rhizopus*, *Aspergillus*, *Penicillium*, and various bacteria.

Seeds removed at the intervals after 96 hr were also maintained in the three groups described above with approximately the same numbers in each class as in the 96-hr sample. Those in group (i) when planted on agar again gave rise to occasional colonies of the same organisms as those from the 96-hr sample. Those in groups (ii) and (iii) were almost completely rotted after 144 hr and when planted on agar gave rise to occasional colonies of *Pythium* and *Fusarium*, but the plates were rapidly overgrown by *Mucor* and *Rhizopus* and had a prolific development of bacterial colonies.

It appeared from these results that the seeds in group (i) were those capable of emergence, but which rotted due to attack by soil-borne organisms. The probable causes of the rotting were *Pythium* spp. or *Fusarium* spp. or both. Several subcultures of *Pythium* and several of *Fusarium* were taken and maintained.

One isolate of *Pythium* was identified as *P. polymorphon*; the remainder were all *P. ultimum*. One culture of each species was maintained for pathogenicity testing. Two species of *Fusarium* occurred regularly. These were identified as *F. solani*, the more common one, and *F. roseum* "Culmorum".

The seeds from the fourth sowing in Waite Institute soil in 1945 when planted on agar gave rise to occasional colonies of saprophytic organisms. *Pythium* was not obtained from any of these seeds and many of the seeds which, although fully swollen, were ungerminated and unrotted after 6–8 days showed no growth of organisms on the agar. This supports the hypotheses advanced earlier that the reduced emergence in this sowing was due to the restricted moisture available and was not related to the pre-emergence rotting occurring in other sowings.

(*d*) *Pathogenicity Tests of Isolated Fungi*

The pathogenicity of *P. ultimum*, *F. solani*, and *F. roseum* "Culmorum", singly and together, and the pathogenicity of *P. polymorphon* was tested by inoculating previously autoclaved Waite Institute soil with a suspension of mycelium of these organisms in sterilized tap water and then planting seeds in the inoculated soil.

The fungi were grown separately in 500-ml conical flasks each containing 150 ml of potato–dextrose solution. *Fusarium* was grown for 14 days and *Pythium* spp. for 7 days at 25°C. The resulting mycelial mats from six flasks of each fungus were filtered off, washed in sterile tap water, and suspended in 500 ml sterile tap water by stirring for 2 min in a Waring Blendor. For the combination of *Fusarium* and *Pythium* six mats of each were suspended together in 500 ml sterile tap water. The suspensions were added to 30-kg lots of dried autoclaved soil and after thorough mixing the moisture level of the soil was adjusted to 18% on an oven-dry basis. Control soil was prepared in the same way except that sterile tap water was used instead of the mycelial suspensions. The seven soils so obtained were held separately in closed containers for 10 days to allow the *Pythium* and *Fusarium* to develop in the inoculated soils. After 10 days each lot of soil was remixed and five 9-in. diameter enamel pots were planted from each lot of soil in the following manner:

Soil (3 kg) was added to each pot and levelled. Thirty Greenfeast peas were spaced equidistantly over the surface and a further 2 kg of soil was added to cover the seeds to a depth of 2 cm. Pots were then capped with a waxed brown paper lid which reduced moisture loss to a negligible amount. The series of 30 pots was then incubated at 20°C and emergence counts were made after 14 days. Soil moisture determination at the end of the experiment showed that the moisture level had not fallen more than 1% in any pot. The results are set out below:

Inoculum:	Control	*F. roseum*	*F. solani*	*P. ultimum*	*P. polymorphon*	*P. ultimum* + *F. roseum*	*P. ultimum* + *F. solani*
Emergence (%):	96	94	95	17	18	19	15

Fusarium spp. by themselves apparently had no effect on emergence under these conditions. Both species of *Pythium* caused a significant reduction in emergence.

Fusarium spp. combined with *Pythium* caused no greater reduction than *Pythium* alone.

A further pathogenicity test was prepared along the lines previously described but employing a range of soil moistures from just above wilting point to just below field capacity and using autoclaved Waite Institute soil for the control compared with *Pythium*-inoculated autoclaved soil. A series with untreated Waite Institute soil was also included to compare emergence under these conditions with that in similar soil autoclaved and inoculated with *Pythium*. The results are set out in Table 3.

TABLE 3

PERCENTAGE EMERGENCE OF WILLIAM MASSEY SEED IN WAITE INSTITUTE SOIL WHICH WAS UNTREATED, AUTOCLAVED, AND AUTOCLAVED AND INOCULATED WITH PYTHIUM

Soil Moisture Content (%)	Emergence (%)		
	Autoclaved Soil	Autoclaved Soil + *Pythium*	Untreated Soil
17·9	83·7	21·2	46·5
14·5	82·1	40·4	53·6
11·8	84·0	73·1	84·2
9·5	81·3	80·2	83·1

The appearance of seedlings from the autoclaved and *Pythium*-inoculated autoclaved soils at the four moisture levels is illustrated in Plate 1, Figure 2.

(*e*) *Occurrence of* Pythium *in South Australian Soils*

Three other soils were obtained from areas in South Australia where peas were being grown commercially. In each case soils were taken from sites which had been under cultivation for many years, but where, as far as was known, peas had not previously been planted. The localities and descriptions of the soils are set out in Table 4.

TABLE 4

DATA FOR DIFFERENT SOUTH AUSTRALIAN SOILS USED IN PEA EMERGENCE STUDIES

Locality	Soil Type	Wilting Point (as % moisture)	Field Capacity (as % moisture)
Kalangadoo	Black rendzina clay	9·8	28·4
O'Halloran Hill	Grey-black calcareous clay	10·6	29·2
Port Pirie	Red sandy loam	3·1	14·3

A pot experiment was prepared in which Greenfeast peas were grown in each soil at a graded series of moisture levels from wilting point to field capacity. The Waite Institute soil as used earlier was included as a reference. A second sample of Waite Institute soil similar in characteristics but which was under natural pasture and had never been cultivated was also included in the experiment to obtain some indication of the effect of previous cropping on the incidence of *Pythium*. The percentage emergence in each soil over the range of soil moisture levels used is shown in Figure 3.

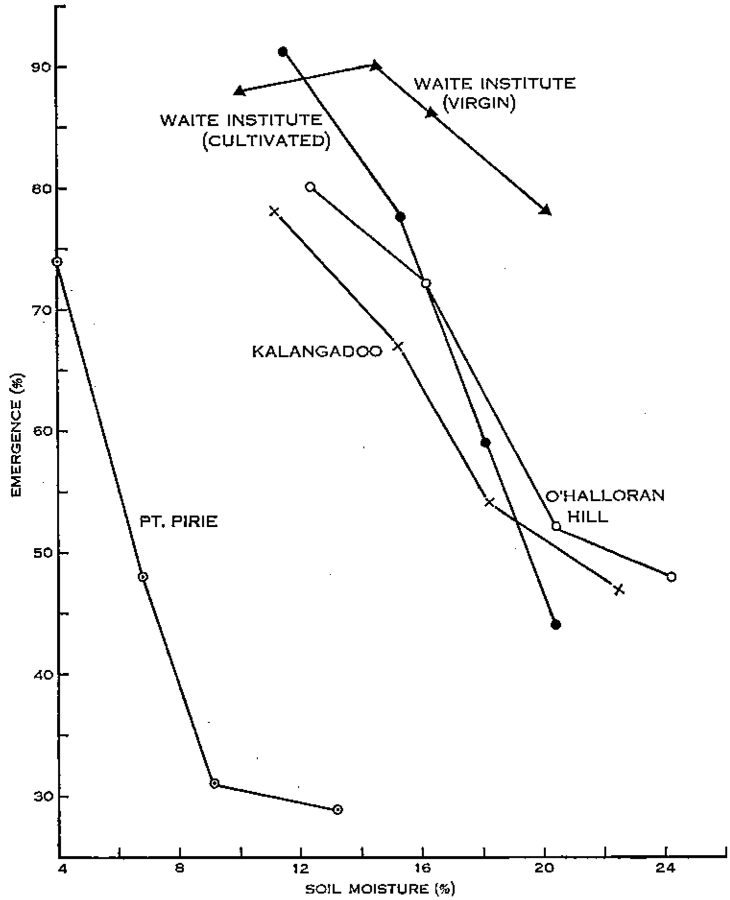

Fig. 3.—Percentage emergence of Greenfeast peas in five South Australian soils at different moisture levels.

Isolations were made from peas removed at intervals of 48 hr from additional pots prepared for this purpose. Except for the virgin Waite Institute soil, the results were similar to those already obtained from the Mundalla and Waite Institute soils, emergence decreasing significantly in each soil with increasing moisture level. The decreases in emergence were paralleled by increasing numbers of seeds showing a "balling" of soil and rotting of the cotyledons and seedling axis after 96 hr. Isolations from such seed yielded *Pythium* in every case and less frequently *Fusarium* spp.

In the virgin Waite Institute soil the emergence was significantly lower only at the highest moisture level near field capacity, indicating much less pre-emergence rotting than in cultivated Waite Institute soil. However, at this moisture level occasional seeds showed a "balling" of soil and rotting of the seedling axis and these always yielded *Pythium* when planted on agar.

IV. Discussion

The major cause of pre-emergence rotting of wrinkle-seeded peas in the field in South Australia appears to be *Pythium* spp. Although all isolates of *Pythium* proved to be *P. ultimum*, except for one of *P. polymorphon*, it is not known whether this indicates that *P. ultimum* is especially pathogenic to peas or that this species is the most common one in the soils investigated. *Pythium* spp. are apparently distributed widely in agricultural soils in South Australia, occurring even in a virgin soil; the population of *Pythium* spp., however, is lower in the virgin than in cultivated soils.

The common occurrence of *Fusarium* spp. in isolations made 144 hr or later after planting or when surface-sterilization is too drastic suggests that earlier workers who did not take these factors into account may have been in error in attributing the rotting to *Fusarium* spp.

Pythium attack usually occurred from 48–96 hr after planting, at the point of attachment of the seedling axis to the cotyledons. It spread rapidly up into the shoot, which was completely susceptible, and radially and out through the cotyledons. Only occasionally was there an original attack just prior to emergence through or just below the growing point of the shoot.

Smooth-seeded peas are apparently susceptible to attack as approximately 10% of seedlings rotted in Waite Institute and Mundalla soils at moisture levels near field capacity, but the attack occurred through the growing point of the shoot and percentage rotting was much less than with the wrinkle-seeded varieties. This was not observed in field experiments, but occurred in pot experiments.

Soil moisture level markedly influences the percentage of seeds rotted. Over the lowest third of the range in soil moisture, from wilting point to field capacity, there was no significant rotting; it increased steeply over the middle third of the range, but showed little further increase over the top third to field capacity. The increases of up to 10% in pre-emergence rotting, with only 1% increase in soil moisture over the middle third of the range from wilting point to field capacity, makes it difficult to interpret the results of previous investigations in which detailed information on soil moisture is not presented.

The influence of soil moisture could be along one of the following lines:

(1) *Pythium* may be restricted in its growth through the soil at lower moisture levels which still permit germination and emergence of peas.

(2) The physiology of the germinating pea may be altered with increasing moisture level. Germination was more rapid and the appearance of the testa was different at the higher moisture levels.

Indirect evidence from the above experiments suggests that the influence of moisture is not directly on the growth of *Pythium* through soil. As the shoot of the pea seedling is apparently susceptible to attack it is surprising that the tip was seldom invaded directly if the main influence of soil moisture was on the ability of *Pythium* to grow through the soil. Similarly the pot experiments showed that the smooth-seeded peas are susceptible, as 15% were rotted at the high soil moisture levels, but such attack was absent in the field or at a slightly lower moisture level in the same pot experiment when wrinkle-seeded peas were attacked.

There is more direct evidence, however, that effect of soil moisture is on the germinating pea itself. The "balling" with soil of the wrinkle-seeded peas at the higher moisture levels, mentioned by previous workers (Hull 1937; Baylis 1941), is due to a prolific growth of *Pythium* mycelium which holds the soil firmly to the seed. This occurred between 48 and 96 hr after planting, before the rotting was far advanced, so it can hardly be explained by growth of the fungus out from the point of attack into the surrounding soil. It appears more likely that water-soluble materials may diffuse out from the germinating pea and stimulate the growth of *Pythium* in the surrounding soil.

The smooth-seeded peas may escape attack because they lack such diffusible materials suggested for wrinkle-seeded peas.

While rotting of wrinkle-seeded peas increased markedly with increasing soil moisture, even at levels close to field capacity a percentage of seeds showed no sign of rotting. Examination of seeds removed at intervals of 48 hr after sowing indicated that if no attack by *Pythium* had occurred within the first 96 hr then no attack took place at all. This suggests either that host factors influencing the attack by *Pythium* operate only over the first 96 hr, or that the seed itself is variable and the factors do not operate in some seedlings; these seedlings then escape infection, as suggested for the smooth-seeded peas.

An investigation of the interaction between *Pythium* and the wrinkle- and smooth-seeded peas in relation to soil moisture has been carried out and will be described in Part III of this series (Flentje and Saksena 1964).

V. Acknowledgment

I wish to acknowledge my deep indebtedness to the late D. B. Adam for his supervision and critical encouragement during this investigation.

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Explanation of Plate 1

Fig. 1.—Appearance of germinating William Massey peas removed from pots of autoclaved, formalin-treated, and untreated Waite Institute soil after 96 hr at different moisture levels.

Fig. 2.—Appearance of germinating William Massey peas removed from autoclaved and autoclaved plus *Pythium*-inoculated Waite Institute soil at different moisture levels.

PRE-EMERGENCE ROTTING OF PEAS IN SOUTH AUSTRALIA. II

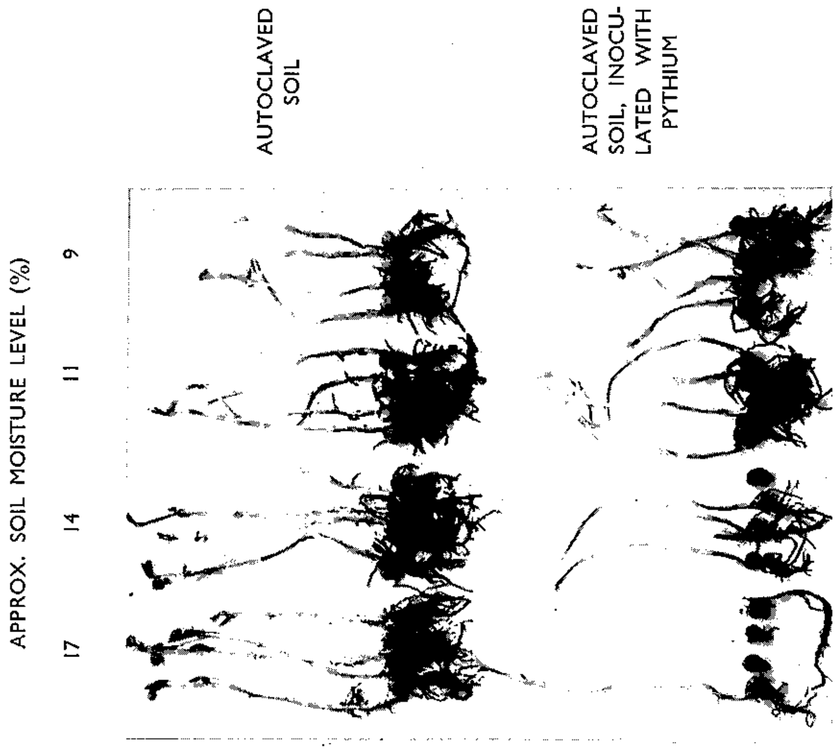

Fig.2

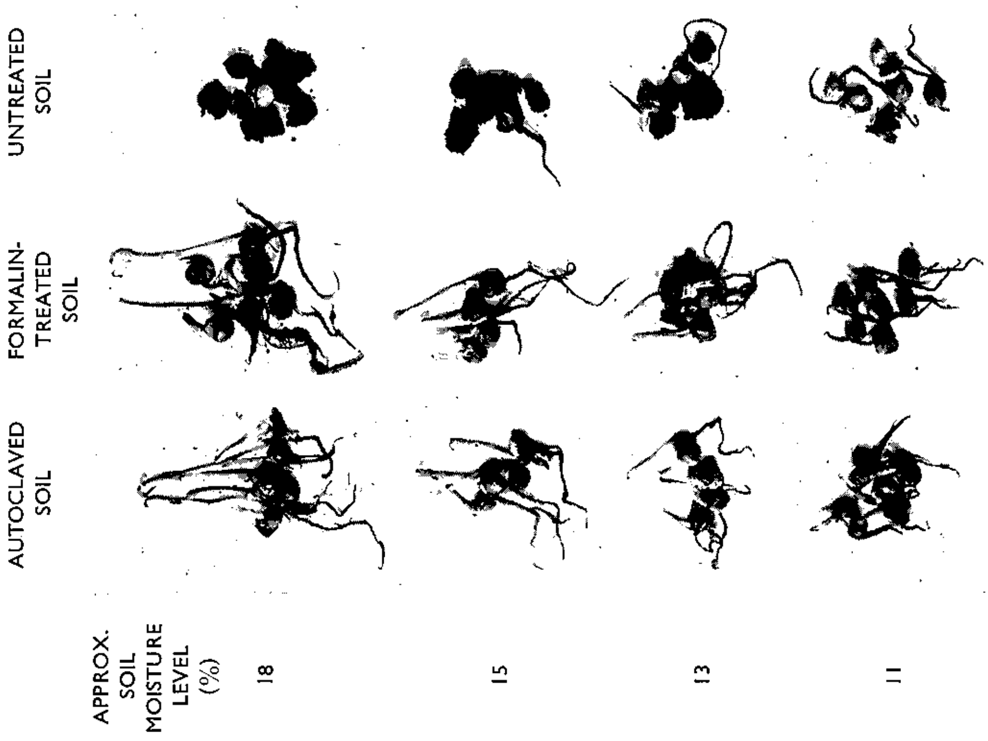

Fig. 1