PRE-EMERGENCE ROTTING OF PEAS IN SOUTH AUSTRALIA

III. HOST–PATHOGEN INTERACTION

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Summary

Pre-emergence rotting of peas by *Pythium* occurs during two stages of growth, namely when the radicle and plumule are breaking free of the testa and during elongation of the epicotyl prior to emergence.

Attack during the first stage occurs only with wrinkle-seeded peas and is due to exudation of sugar from the seed which stimulates *Pythium* growth. Sugar loss occurs during the first 24 hr of germination and together with pre-emergence rotting is markedly increased by damage to the testa.

Attack during the second stage occurs in both smooth and wrinkle-seeded varieties.

Soil moisture may influence pre-emergence rotting through its effect both on the amount of sugar lost and on the activity of *Pythium*.

I. Introduction

Pre-emergence rotting of wrinkle-seeded peas in the field in South Australia is largely due to attack by *Pythium ultimum* within 3–4 days after planting, when the plumule is breaking free of the cotyledons (Flentje 1964). Soil moisture level markedly influences rotting, the percentage of seeds rotted increasing significantly with increase in soil moisture between wilting point and field capacity. In the early stages of attack, *Pythium* grows prolifically in the soil surrounding each seed which suggests that it may be stimulated by materials diffusing from the germinating seed.

Smooth-seeded peas, although apparently susceptible to *Pythium*, were less frequently attacked and then through the hypocotyl or shoot tip. There was no evidence that *Pythium* was stimulated in the soil immediately surrounding the seed.

Although *Pythium* has been reported previously as the cause of rotting, and the influence of soil moisture has been demonstrated (Baylis 1941; McNew 1943; Angell 1952; Flentje 1964) no detailed investigation of the host–pathogen interaction has been reported. An investigation of this interaction is reported in this paper.

II. Experimental and Results

(a) *Exudation from Germinating Peas*

Bulk seed lots of the varieties William Massey and White Brunswick were screened to remove unsound seeds. Then 100-g samples of sound seed were washed

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in alcohol, three times in sterile distilled water, and surface-sterilized for 4 min in 0·1% mercuric chloride. After washing again in three changes of sterile distilled water the samples were reweighed to determine how much water they had absorbed, transferred to sterile 500-ml conical flasks, and sterile distilled water was added to make 300 ml the total of water added. A stream of filtered air was slowly passed through a sintered-glass bubbler set in the base of each flask. Under these conditions the pea seeds germinated at approximately the same rate as other 100-g samples on paper towelling held in the same 20°C constant-temperature room. Duplicate flasks of each pea variety were removed at intervals up to 52 hr, the unabsorbed liquid in each flask was measured, filtered through a bacterial filter, and stored at 1°C in sterile flasks.

Two 10-ml samples of unabsorbed liquid from each replicate flask were evaporated to determine the total soluble solids, and two similar samples were used to determine the sugar content. The pH of the remainder was measured, adjusted to 7·0, and five 15-ml samples were transferred to sterile 500-ml flasks which were then inoculated with 1-mm^3 pieces cut from the growing edge of a colony of *Pythium* on potato–dextrose–agar. The flasks were incubated at 25°C for 14 days, after which the mycelial mats were removed by filtering and their dry weights obtained.

A number of experiments of this type were carried out with some variations in technique but the results were substantially the same in each case. The greatest difficulty was in maintaining the liquids free of contamination. One procedure which was varied was the assay of the liquid by growing *Pythium* on 15-ml aliquots. In some experiments the liquid removed from the peas at different intervals was made up to the same volume as that of the first time of removal. In soil, however, seeds absorb water, and reduce the surrounding soil moisture level, so it was decided that a better indication of the interaction in soil would be obtained if the liquid removed from the peas was used directly without making it up to the same volume in each case.

In all experiments little material was exuded from smooth-seeded peas and two samplings were quite adequate, whereas in wrinkle-seeded peas considerable material was exuded and ten samples were taken. The results are shown in Figure 1.

The pH of the exudate from each pea variety fell from 5·6 after $3\frac{1}{4}$ hr to 4·8 after 52 hr. Of the soluble solids lost after 52 hr approximately 50% of those from William Massey and 10% from White Brunswick were sugar, approximately 90% of this being sucrose. The method of Munson and Walker (1906) was used for the sugar determination.

To compare the growth of *Pythium* on exudate from wrinkled and smooth-seeded peas with growth on synthetic media containing a known amount of sugar, exudates were obtained after 48 hr as described above. One 100-ml lot of each exudate was retained as collected, to a second 100 ml of each was added: asparagine 0·107 g, KH_2PO_4 0·100 g, $Na_2HPO_4.12H_2O$ 0·175 g, $MgSO_4.7H_2O$ 0·020 g, ferric citrate 0·004 g. Two lots of 100 ml of nutrient solution were made up containing the above materials plus 1·0 and 2·0 g dextrose respectively. The six solutions were then filtered through a bacterial filter, five lots of 15 ml from each solution were transferred to sterile 500-ml conical flasks, inoculated with *Pythium* as described above, and incubated at 25°C for 14 days. The dry weights of the *Pythium* mats grown on these solutions were obtained as described previously and the results are set out in the following tabulation:

Medium	Dry Weight (mg)	Medium	Dry Weight (mg)
Basal medium plus:		Exudate from smooth-seeded peas	5·1
1% Dextrose	50·8	Exudate from wrinkle-seeded peas	41·9
2% Dextrose	119·7		
Exudate from smooth-seeded peas	5·4		
Exudate from wrinkle-seeded peas	48·3		

Wrinkle-seeded peas, when germinated in water apparently exude, within the first 24 hr, materials which will support a growth of *Pythium* equivalent to that supported by a 1·0% solution of dextrose. It is reasonable to assume that such exudation occurs also in soil and that it would stimulate *Pythium* and lead to attack of the germinating seeds. Smooth-seeded peas apparently do not exhibit this phenomenon.

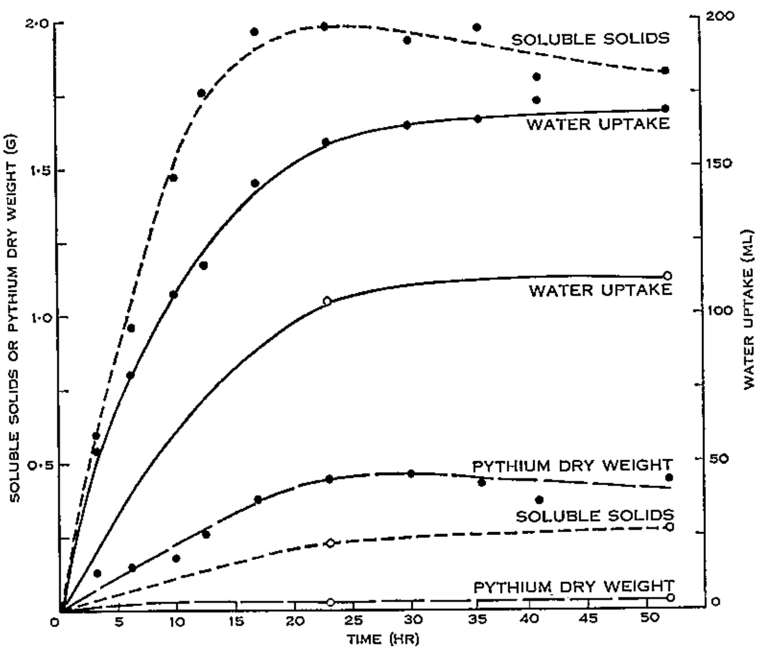

Fig. 1.—Water uptake, total soluble solids lost, and dry weight of *Pythium* grown on exudates from 100 g of William Massey (●) and White Brunswick (○) peas germinating for different times in distilled water.

(*b*) *Influence of Delayed Planting on Seed Rotting*

Since the loss of materials from wrinkle-seeded peas apparently occurs during the first 24 hr of germination, germination of seeds in water or sterile moist sand before planting in *Pythium*-infected soil should obviate the stimulation of *Pythium* and reduce the amount of seed rotting.

This possibility was examined by either soaking Greenfeast and White Brunswick seeds in water, with a stream of air bubbling through it, for periods from 4 to 24 hr,

TABLE 1

PERCENTAGE EMERGENCE OF GREENFEAST AND WHITE BRUNSWICK SEEDS TRANSPLANTED TO WAITE INSTITUTE SOIL AT 20% MOISTURE AFTER GERMINATING IN WATER OR MOIST SAND FOR DIFFERENT PERIODS

Method of Germination	Time to Transplanting	Percentage Emergence	
		White Brunswick	Greenfeast
Seeds soaked in water	0 hr	72	8
	4	72	5
	8	70	12
	12	71	18
	16	73	29
	20	73	41
	24	73	42
Seeds held in moist sand	1 day	70	46
	2	79	50
	3	91	56
	4	99	76
	5	100	93
	6		100

or holding them in sterile washed sand at 15% moisture level for periods from 1 to 7 days before transplanting them into Waite soil at 20% moisture level. Emergence

TABLE 2

SEEDLING DEVELOPMENT AT TRANSPLANTING FROM SAND TO WAITE INSTITUTE SOIL

No. of Days in Sand	Stage of Development	Greenfeast	White Brunswick
1	Seeds	Smooth and swollen. Testa unbroken	Swollen. Radicle breaking testa
2	Radicle	Breaking through testa	2–3 cm long
3	Radicle	Up to 1 cm	3–4 cm long
	Plumule	—	Up to 1 cm
4	Radicle	2–2·5 cm	5–7 cm
	Plumule	Up to 1 cm	2–4 cm
5	Radicle	4–5 cm	7–9 cm
	Plumule	2–3 cm	5–6 cm
6	Radicle	4–6 cm	—
	Plumule	3–4 cm	—

counts (Table 1) were made 10 days after soaking in water or planting in sand. Seedling development was recorded at each time of transplanting from sand (Table 2).

The data were analysed following angular transformation. With Greenfeast seed there was a significant increase in percentage emergence over seed soaked for 4 hr for each additional 8 hr up to 20 hr soaking in water. The next significant increases occurred in seeds transplanted after 4 and 5 days in moist sterile sand.

After emergence had been counted, the seedlings were removed from all pots. Seeds which failed to emerge when planted in Waite soil after soaking in water for less than 20 hr were heavily "balled" with soil held by a prolific growth of *Pythium.* These seeds had rotted before the plumule had properly emerged. On the other hand, those seeds which failed to emerge when planted after more than 20 hr in water or moist sand showed no "balling" with soil, but had been attacked either through the epicotyl or plumule.

With the White Brunswick seed there was a significant increase in percentage emergence with transplanting after 3 and 4 days of germination in moist sand only. None of the unemerged seedlings in any pots was "balled" with soil, but they had been attacked by *Pythium* through the epicotyl or plumule.

Table 3

PERCENTAGE EMERGENCE OF GREENFEAST AND WHITE BRUNSWICK PEAS IN WAITE INSTITUTE SOIL AT 20% MOISTURE CONTENT

Soil Treatment	Method of Planting	Percentage Emergence	
		White Brunswick	Greenfeast
Untreated	Separately	85	31
Autoclaved	Separately	98	99
Untreated	Together	31	27
Autoclaved	Together	97	98

These results confirm the hypothesis that wrinkle-seeded Greenfeast peas, during the first 24 hr of germination either in water or in moist sand, exude materials which stimulate a prolific growth of *Pythium,* but that smooth-seeded White Brunswick peas do not. The results also suggest, however, that at high soil-moisture levels *Pythium* is active in soil and will attack the epicotyl or plumule even without the stimulus of materials exuding from the seed. Attack at this later stage of seedling development was clearly seen only in those seedlings transplanted after exudation from the seed had apparently ceased. The period of susceptibility was approximately 2 days shorter for smooth than for wrinkled peas and this was associated with the more rapid elongation of the epicotyl and earlier emergence of the smooth-seeded peas (Table 2).

(c) *Effect of* Pythium *Population on Rotting*

Although both smooth- and wrinkle-seeded peas are susceptible to *Pythium* attack, the more severe rotting of wrinkle-seeded peas appeared to be due to the higher population of *Pythium* around the seeds brought about by the exudation of materials during germination.

This possibility was further examined in an experiment in which White Brunswick and Greenfeast peas were planted separately and together in both untreated and autoclaved Waite Institute soil at 20% moisture level. Where the two varieties were planted together the seeds were in pairs, touching, with the Greenfeast above and slightly to the side of the White Brunswick seed. Emergence counts were made after 10 days (Table 3). When planted separately in untreated soil the percentage rotting of White Brunswick seeds was significantly less than that of Greenfeast seeds. Where the seeds were planted together in pairs, if the Greenfeast seed was attacked the paired White Brunswick seed was also rotted, and the effect on emergence is shown in Figure 2. The paired White Brunswick seeds were partially balled with soil and the attack occurred earlier than for White Brunswick seeds planted separately. Thus an increased and probably more active population of *Pythium* surrounding the White Brunswick seeds increased the percentage rotting of these seeds to the same level as in Greenfeast.

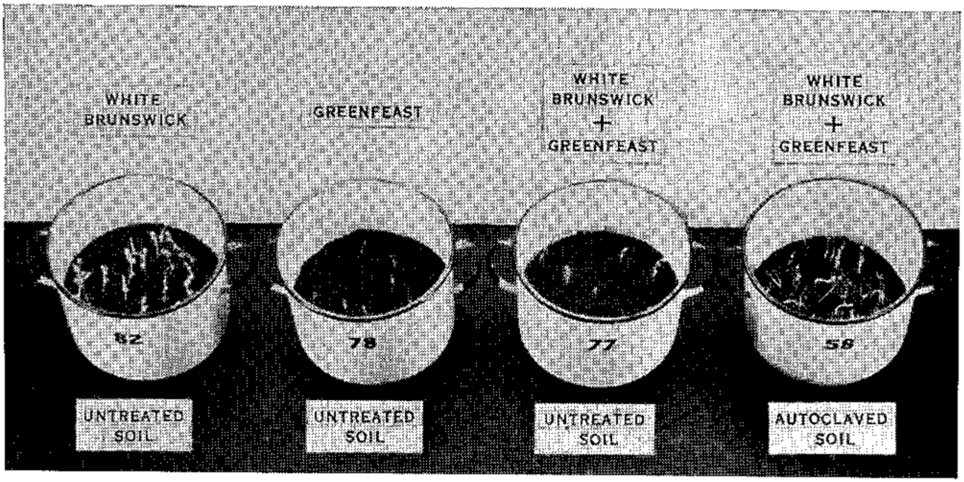

Fig. 2.—White Brunswick and Greenfeast peas grown separately and together in Waite Institute soil at 20% moisture content.

Previous experiments have given little information as to whether soil moisture influences rotting by its effect on the amount of exudation from seeds or by its effect on the growth of *Pythium* through soil. An analysis of this influence was attempted by varying the *Pythium* population in Waite soil.

Waite Institute soil was divided into three lots. To the first (Waite soil 1) distilled water was added at the rate of 500 ml per 30 kg soil. To the second and third lots (Waite soils 2 and 3) a suspension of washed *Pythium* mycelium in distilled water was added at the rate of 500 ml per 30 kg soil. The mycelial suspensions were prepared by growing *Pythium* at 25°C in 250-ml erlenmeyer flasks containing 50 ml of 1% potato–dextrose solution. After 5 days the *Pythium* mycelial mats were washed in sterile distilled water and suspended, using a Waring Blendor, in sterile distilled water at the rate of three mycelial mats per 500 ml water for Waite soil 2, and nine mats per 500 ml water for Waite soil 3.

After thorough mixing, each lot of soil was subdivided into six subsamples and the moisture levels of the subsamples were adjusted to give a graded series from near wilting point to near field capacity. The soils were then transferred to enamel pots, sown with Greenfeast peas, and capped with waxed brown paper. The percentage emergence in the three soils at different moisture levels is set out in Figure 3.

There was no significant difference in percentage emergence between soils 1 and 2 below the 16% moisture level, but percentage emergence was significantly lower in soil 3 at the 11% moisture level. Below 16% moisture and most readily seen in soil 3, failure of seedlings to emerge was largely due to attack of the epicotyl and plumule by *Pythium*. At 16% moisture and above, percentage emergence was significantly lower in soils 2 and 3 than in soil 1; seedlings which failed to emerge were balled with soil and invaded by *Pythium* before the radicle had extended.

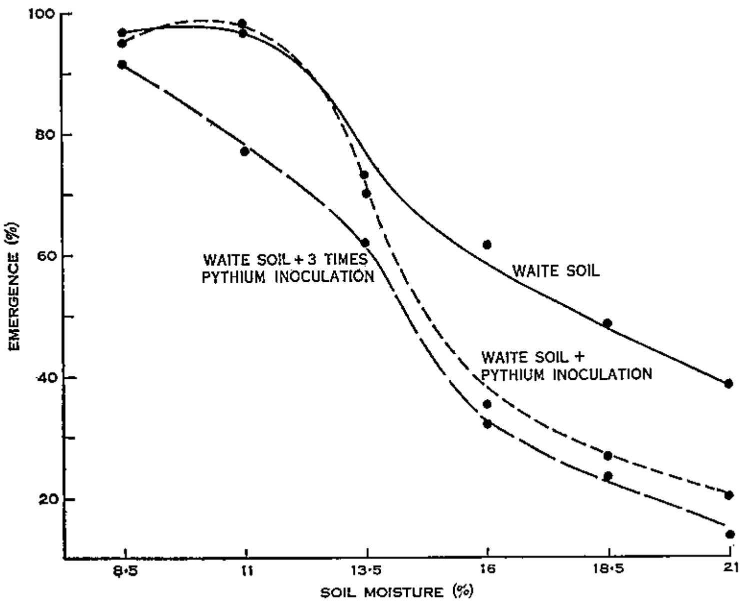

Fig. 3.—Percentage emergence of Greenfeast peas in Waite Institute soil at different moisture levels and with different amounts of *Pythium* inoculum added.

The results indicate that soil moisture influences both the growth of *Pythium* and the amount of exudation from seed. There was a significant increase in rotting between 8·5 and 13·5% moisture levels, especially in soil 3 but the attack was mainly on the epicotyl and plumule. Thus *Pythium* was increasingly active over this range, but not greatly stimulated around the cotyledons. Above 13·5% moisture, however, there was marked stimulation of *Pythium* growth around the cotyledons in all soils, suggesting that the exudate only became available in that range.

(*d*) *Relation of Testa Damage to Exudation and Rotting*

As the materials lost from wrinkle-seeded peas during germination influenced pre-emergence rotting so markedly, the role of the testa in exudation and rotting was examined.

The testa is known to suffer damage according to the harvesting conditions, particularly the type of machinery used in harvesting. The percentage of seed with cracked testas was determined by placing the seed in water at 75°C, allowing it to stand for 30 min, and counting those seeds in which the testa separated from the cotyledons (Hutton, personal communication). In commercial samples of smooth- and wrinkle-seeded peas 60–85% of seeds had cracked testas.

Two samples of Greenfeast peas were obtained from one crop; one sample was harvested by hand while the other was machine-harvested the same day. One subsample from the hand-harvested peas was retained untreated, the others were given one of the following treatments:

(i) Testa broken with a fine needle near the micropyle;
(ii) Testa broken with a fine needle opposite the micropyle;
(iii) Seed put through a commercial seed drill.

Table 4

Germination and emergence data for one crop of Greenfeast peas subjected to different harvesting and post-harvesting treatments

Sample Treatment	Seed with Cracked Testa (%)	Water Uptake (g/100 g seeds)	Total Soluble Solids Exuded per 100 g Seeds (mg)	Dry Weight of *Pythium* on Exudate from 100 g Seeds (mg)	Percentage Emergence in Waite Soil of Moisture Content:		
					18·0%	16·4%	12·6%
Hand-harvested	2	144	714	114	86·6	90·8	97·5
+crack near micropyle	100	112	1470	254	43·3	69·2	93·3
+crack opposite micropyle	100	104	2102	464	48·3	76·6	95·8
+put through drill	9	139	980	158	80·8	87·5	95·8
Machine-harvested	64	123	2064	446	24·8	30·0	75·8
+put through drill	65	120	2419	596	30·0	31·6	71·5

One subsample of the machine-harvested peas was retained without further treatment; the other subsample was put through a commercial seed drill. For each of the resulting six seed samples, the following information was obtained:

(1) Percentage of seed with cracked testas;
(2) Water uptake per 100 g seeds after germination for 48 hr at 20°C in 300 ml aerated distilled water;
(3) Total soluble solids exuded per 100 g seeds after germination for 48 hr at 20°C in 300 ml aerated distilled water;
(4) Dry weight of *Pythium* when grown for 14 days at 25°C on the exudate removed from germinating peas in (3) above;
(5) Percentage emergence in Waite Institute soil at three moisture levels.

The results are given in Table 4.

Hand-harvested seed with negligible cracking of the testa exudes much less material during germination and is less affected by pre-emergence rotting than machine-harvested seed. Deliberate cracking of the testa in hand-harvested seed affected both the amount of exudate and the percentage of seeds rotted; seed cracked away from the micropyle lost a greater amount of exudate but showed significantly less rotting at the 16·4% moisture level than did seed cracked near the micropyle. Machine-harvested seed (having 64% of seeds with a cracked testa) exuded approximately the same amount of material, but had a significantly higher percentage rotting at all moisture levels than the hand-harvested seed cracked away from the micropyle. The adverse affect of machine-harvesting must include other factors in addition to cracking of the testa. Passage through a drill had little effect on seed-coat cracking, increased the amount of exudate, but had no significant effect on pre-emergence rotting.

III. General Discussion

Pre-emergence attack of peas by *Pythium* occurs during two stages of growth, namely, about the time the radicle and plumule are breaking free of the testa and during the elongation of the epicotyl prior to emergence.

Attack during the first stage occurs with wrinkle-seeded peas only. There is considerable evidence that this is due to sugar, mainly sucrose, exuded from the seed during the first 24 hr of germination. There is direct evidence that such exudation, previously demonstrated by Bonner, Haagen-Smith, and Went (1939), occurs when the seed is soaked in water. There is indirect evidence from several experiments that the exudation occurs also in sand and soil at high moisture levels. There appears to be negligible exudation of sugar from White Brunswick seeds.

Several workers have shown that a considerable amount of sucrose accumulates in wrinkle-seeded peas prior to harvesting for canning, and Bonney and Fischbach (1945) found in dry harvested seeds that each of 13 wrinkle-seeded varieties contained approximately twice as much sucrose as the smooth-seeded Alaska. Such a difference in dry harvested seeds would hardly explain the much greater differences in amount of sugar lost by smooth- and wrinkle-seeded peas during the first 24 hr of germination as reported in the present investigation. There must be factors other than total amount of sucrose, probably in the germination process itself, to account for the differences between the different types of seed in sugar loss. Differences in the amount and type of stored starch (McCready *et al.* 1950) and of crude dextrin and other storage materials (Bonney and Fischbach 1945) suggest there may be different processes involved in germination and early growth of smooth- and wrinkle-seeded varieties which could influence the amount of sugar loss.

Although cracking of the testa of wrinkle-seeded peas greatly increased both sugar loss and pre-emergence rotting, deliberate cracking of the testa opposite the micropyle as compared with cracking near the micropyle increased sugar loss but not percentage rotting. The increased percentage rotting with the same amount of sugar exuded in machine-harvested seeds (64% with cracked testa) compared with the hand-harvested seeds cracked away from the micropyle (100% with cracked testa), indicates that factors other than sugar loss and cracking of the testa must also influence pre-emergence rotting.

Attack during the stage of elongation of the epicotyl occurred with seedlings from both smooth and wrinkled seed. The transplanting experiments indicate that rotting is more severe on seedlings from wrinkled than smooth seed, although the results of experiments in which seeds were planted together suggest that both types of seedlings are susceptible to *Pythium* attack. The fact that seedlings which emerged, particularly those transplanted after 3–5 days, showed no lesions on the epicotyl suggests that the period of susceptibility to *Pythium* is limited either because the plumule may also lose materials which stimulate *Pythium* growth or because the epicotyl becomes resistant once the plumule emerges and photosynthesis begins. This suggestion of a limited susceptibility period is supported by the increase in percentage emergence in both varieties with the increase from 1 to 5 or 6 days in time to transplanting, and the fact that no post-emergence damping-off occurred.

The effects, on pre-emergence rotting, of adding *Pythium* inoculum to soil suggest that soil moisture level may influence both *Pythium* activity and the amount of exudate from wrinkle-seeded peas. The influence on amount of exudation appears the more important, but further critical investigation is necessary to define the influence precisely. However, at lower moisture levels, the attack of epicotyl and plumule tissue in the absence of any obvious effect of exudates from the seedlings suggests that *Pythium* was actively growing through the soil and not just existing as dormant propagules such as oospores.

Although the effects of root exudates on the soil microflora are being increasingly investigated, the effects of seed exudates have received little attention. Schroth and Cook (1964) have recently demonstrated the marked influence which exudates from germinating bean seed have on *Fusarium solani* f. *phaseoli*. The exudates influence the germination of and growth from chlamydospores, the amount of exudate lost increasing with damage to the seed coat. Both bean and pea seeds, being large and with a considerable amount of stored protein and carbohydrate, may have more obvious effects on the soil microflora, but it would be profitable to examine the exudation from a wide range of seeds in respect of pathogenic organisms which invade seedlings at an early stage of growth, investigating, in particular, the influence of soil moisture.

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