THE ROOT ROT–*FUSARIUM* WILT COMPLEX OF PEAS

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Summary

At least four fungal pathogens are involved in the root rot–*Fusarium* wilt complex of peas which is a serious problem following intensive cropping of peas in South Australia. The pathogens are *Fusarium oxysporum* f. *pisi* race 2 Snyder & Hansen, *F. solani* f. *pisi* Snyder & Hansen, *Pythium ultimum* Trow, and *Ascochyta pinodella* L. K. Jones. In susceptible pea cultivars there is a marked interaction between *F. oxysporum* and *P. ultimum*. *P. ultimum* alone causes initial stunting from which plants gradually recover; *F. oxysporum* alone probably causes little damage; both fungi together cause initial stunting followed by severe wilt symptoms about 6 weeks after sowing and death 2 weeks later. The importance of *F. solani* and *A. pinodella* has not been fully determined, but they probably cause only minor damage.

Conventional methods of soil inoculation were found unsatisfactory and techniques involving soil cultures of *Fusarium* spp. and sand–maize meal cultures of *P. ultimum* were developed, which enabled stable populations of the pathogens to be established in virgin soil. By varying the amount of inoculum, populations could be readily adjusted.

The effect of cropping with peas on soil populations of *F. oxysporum*, *F. solani*, and *Pythium* spp. was studied.

I. INTRODUCTION

In River Murray Irrigation Settlements of South Australia peas are grown extensively for the fresh market, being sown towards the end of summer (February–March) and harvested during early winter (May–June). Originally peas were grown between young fruit trees, but more recently open acres have been sown, although in most cases the land will eventually become orchards. For a number of reasons, there is no satisfactory alternative cash crop to peas, with the result that peas are sown on the same land each year, in some cases for 8 consecutive years. Very good yields are generally obtained for the first 2 years, followed by a marked reduction in the third and later years, until after four or five consecutive crops, pea production becomes uneconomic.

Preliminary investigations showed that wilt caused by *Fusarium oxysporum* f. *pisi* race 2 Snyder & Hansen was widespread and probably important in reducing yield, but glasshouse and field trials with wilt-resistant pea cultivars indicated that other pathogens must be involved. In cropped soil the resistant cultivars grew better than the susceptible ones, but plants were stunted and yield unsatisfactory.

The work presented here is an account of later investigations.

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II. Materials and Methods

(a) *Soils*

Four soils from near Renmark were used in the investigation, two taken from a field which had been cropped with peas for 5 consecutive years, and two from virgin scrub adjacent to the cropped field. Of the two cropped soils, one was taken from towards the top of a sandy rise and the other was a heavier soil from a lower lying area of the same field. The two virgin soils also varied in texture and were collected from sites about 20 yd from a fence enclosing the cropped field.

The soils will be referred to as "cropped light", "cropped heavy", "virgin light", and "virgin heavy". All were passed through a 2-mm sieve before being used for experimental work. The pH values, physical components, and total nitrogen contents of the four soils were determined (Table 1).

Table 1

SOME PHYSICAL AND CHEMICAL PROPERTIES OF FOUR RENMARK SOILS

Soil	pH	Total Nitrogen (%)	Mechanical Analysis (%)		
			Sand	Silt	Clay
Virgin light	7·1	0·040	96·9	0	3·0
Cropped light	7·3	0·044	94·8	1·0	4·0
Virgin heavy	8·0	0·064	88·8	6·1	5·1
Cropped heavy	7·5	0·095	84·4	8·2	7·4

(b) *Pea Cultivars*

Three pea cultivars were used: Greenfeast, which is susceptible to *Fusarium* wilt, and BC4/F6 and New Era, which are resistant. BC4/F6 is similar to Greenfeast in agronomic characters, but is homozygous for resistance to *F. oxysporum* f. *pisi* races 1 and 2. It was bred at the Waite Agricultural Research Institute by Mr. M. V. Carter by a backcross technique with Greenfeast as the recurrent parent and New Era as the donor.

(c) *Microbiological Techniques*

Suitable techniques had to be devised for measuring soil populations of *F. oxysporum*, *F. solani*, and *Pythium* spp. Both cropped soils had a relatively high population of fast-growing fungi including species of *Mucor*, *Actinomucor*, and *Trichoderma* and selective media had to be used to inhibit fast-growing fungi. The media found suitable and used throughout were:

(1) *Basal Medium*

$NaNO_3$	2·0 g	Sucrose	30·0 g
KH_2PO_4	1·0 g	Yeast extract (Difco)	0·5 g
KCl	0·5 g	Davis agar	15·0 g
$MgSO_4.7H_2O$	0·5 g	Distilled water	1000·0 ml
$FeSO_4$	0·01 g		

(2) *Medium used in measuring populations of* Fusarium *spp.* (Fusarium *medium*)

Basal medium *plus*	
Streptomycin sulphate (Glaxo)	50 p.p.m.
Pentachloronitrobenzene	100 p.p.m.
Rose bengal	60 p.p.m.

(3) *Medium used in measuring populations of* Pythium *spp.* (Pythium *medium*)

Fusarium medium *plus*	
Mycostatin (Squibb)	100 units/ml

Additions to the basal medium were incorporated just prior to pouring. For measuring *Fusarium* populations, soil-dilution plates (1 in 500 or 1 in 1000) were used and colonies of *F. oxysporum* and *F. solani* were identified after 5 days' incubation at 25°C. Sometimes plates were left for a further 2 days in diffuse daylight to assist identification. The *Pythium* medium is similar to that used by Singh and Mitchell (1961) except that mycostatin replaces pimaricin. For the assessment of *Pythium* populations, soil dilutions of 1 in 10 or 1 in 20 were used and colonies counted after 2 days. Short exposure of the plates to diffuse daylight after incubation for 24 hr prevents further growth of most *Pythium* colonies and must be avoided.

(*d*) *Methods of Soil Inoculation*

During the investigation it was required to inoculate virgin soil with *F. oxysporum*, *F. solani*, and *P. ultimum* so that the resultant populations of these fungi in inoculated soil were similar to those in cropped soil. To do this it was necessary to determine the survival of fungi in virgin soils following different methods of inoculation. For *F. oxysporum* three methods of inoculation were tested in both heavy and light virgin soils: firstly a microspore suspension, obtained by growing the fungus for 4 days in liquid basal medium, was sprayed on soil which was then thoroughly mixed; secondly a sand–maize meal (5% maize meal) culture and thirdly a soil culture of the fungus were mixed with soil, using 2% inoculum in both cases. Populations of *F. oxysporum* were measured 2 and 4 weeks after inoculation and thereafter at monthly intervals for 5 months (Fig. 1). There was a sharp initial drop in population following inoculation with a spore suspension or sand–maize meal culture whereas the population was relatively constant over a 6-month period following inoculation with a soil culture. There was no significant difference in survival of the fungus in the two soils.

Virgin light soil was inoculated with *P. ultimum* using sand–maize meal cultures at the levels of 2% and 0·5% (w/w) and survival following the latter was determined. Two weeks after inoculation the *Pythium* population was about 700 propagules per gram of soil and it varied between 500 and 700 over a 6-month period.

The survival of *F. solani* following soil inoculation was not determined, but is likely to be similar to that of *F. oxysporum*.

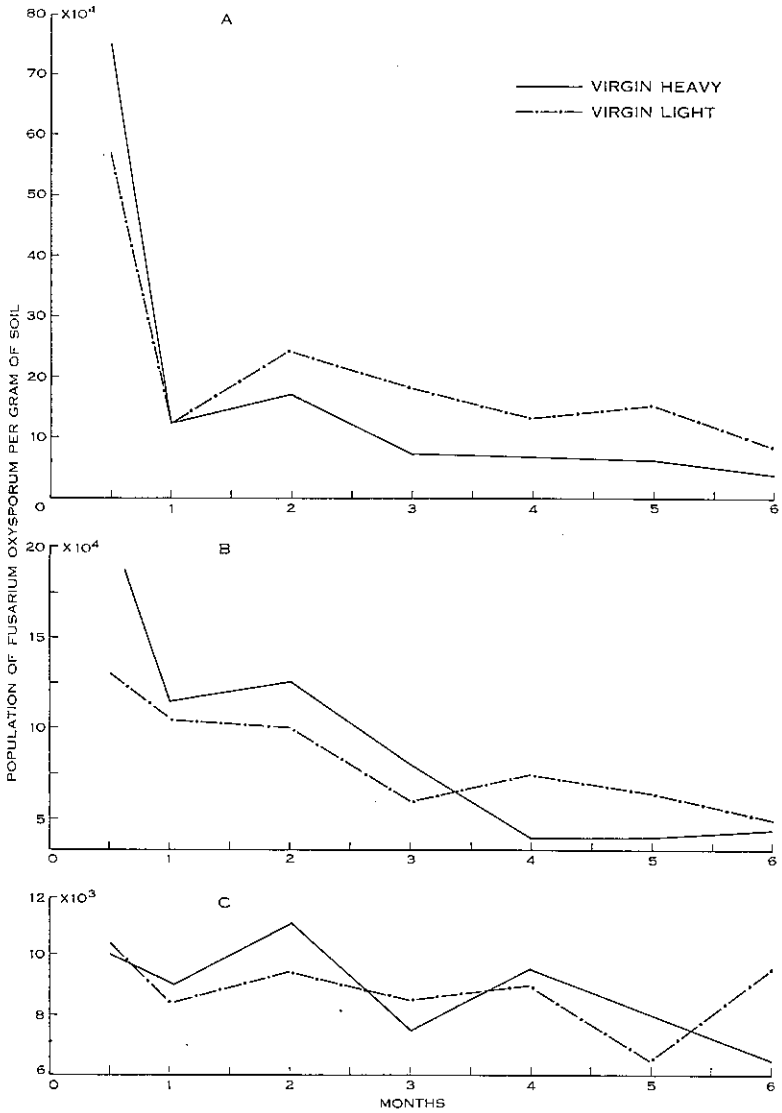

Fig. 1.—Survival of *F. oxysporum* in light and heavy virgin soils following inoculation with a microspore suspension (*A*), 2% sand–maize meal inoculum (*B*), and 2% soil inoculum (*C*).

During the investigation soil cultures were used to inoculate soils with *F. oxysporum* and *F. solani*, and sand–maize meal cultures to inoculate soils with *P. ultimum*.

(e) *Pathogenicity Testing*

To test the pathogenicity of fungal isolates to peas, two methods of inoculation were used. With profusely sporing fungi, such as *F. oxysporum* and *F. solani*, agar plates were inoculated with the isolates to be tested and on the same day the required number of peas were sown in coarse, washed, and sterilized river sand. After 10–14 days the seedlings were removed from the sand, their roots washed and cut, and then dipped in a spore suspension prepared from the fungal cultures. The seedlings were then transplanted into either sand or potting soil. With non-sporing or sparsely sporing fungi such as *F. roseum*, *Pythium* sp., and *Ascochyta pinodella*, potting soil was inoculated with sand–maize meal cultures of the fungi (2% inoculum) and seedlings 10–14 days old were then transplanted into the inoculated soil.

III. Experimental and Results

(a) *The Pathogens*

Frequent fungal isolations were made from roots and stems of both wilt-susceptible (cv. Greenfeast) and resistant (cv. BC4/F6) plants 0–6 weeks old which were growing in cropped light and heavy soils. Seeds were treated with "Tetroc" (tetrachloro-*p*-benzophenone) to prevent damping-off. The most frequent isolates were *Pythium* sp., *F. oxysporum*, *F. solani*, and less frequently *A. pinodella*.

Pythium sp. was isolated from rotted root tips of both cultivars 1 week and later after sowing, from brown lesions, very often at the junction of tap root and side root, and from discoloured vascular tissue of both roots and stem bases. Vascular discoloration appeared 4 weeks after sowing, was much more pronounced in susceptible than in resistant cultivars, and was associated with yellowing and death of lower leaves. *Pythium* sp. was isolated much more frequently from plants growing in light soil.

Frequent attempts were made to induce the *Pythium* isolates to fruit. Sporangia were formed profusely on many substrates and germinated by germ tubes, but oogonia and antheridia were rare. The few antheridia observed were sessile and all isolates were tentatively identified as *P. ultimum* (Middleton 1943).

F. oxysporum was consistently isolated from the same tissues that yielded *P. ultimum* except that it was infrequently isolated from discoloured vascular tissue of cv. BC4/F6. In addition it alone was isolated from discoloured vascular tissue of cv. Greenfeast from ground level upwards, 6 weeks after sowing.

Black lesions developed on stem bases and on the tops of tap roots 3 weeks after sowing, being more common in heavy than in light soils. *F. solani* was isolated frequently and *A. pinodella* less frequently.

The pathogenicity was determined of representative cultures of the fungi isolated. *P. ultimum* produced an extensive root rot and moderate stunting of seedlings, but little discoloration of lower leaves and no vascular browning. Most isolates of *F. oxysporum* induced typical wilt symptoms in wilt-susceptible cv. Greenfeast and had no effect on cv. New Era or cv. BC4/F6; others were non-pathogenic or caused only slight to moderate stunting. Isolates of *F. solani* also

showed a variation in pathogenicity, some causing an extensive brown root rot, others intense blackening of roots and stem bases, and yet others were non-pathogenic. *A. pinodella* caused moderate to slight stunting and marked blackening of roots and stem bases. All other fungi tested were non-pathogenic.

(*b*) *Populations of Fungi in Virgin and Cropped Soils*

The marked reduction in yield of peas in the field is associated with intensive cropping. A comparison of the populations of *Pythium* spp., *F. oxysporum*, and *F solani* in cropped and virgin soils is given in Table 2. The data are compiled from all relevant measurements during the investigation. Species of *Pythium* could not be readily distinguished on soil plates, but there appeared to be more than one. The population of *A. pinodella* in soil could not be estimated.

TABLE 2
FUNGAL POPULATIONS (PROPAGULES PER GRAM) IN VIRGIN AND CROPPED SOILS

Soil	*Pythium* spp.	*F. oxysporum*	*F. solani*	Total Fungi
Virgin heavy	26	184	333	23,000
Cropped heavy	343	833	4856	49,900
Virgin light	16	30	83	14,500
Cropped light	97	583	2183	40,200

It is clear that cropping has a marked effect on the three fungi, their populations being from 6 to 26 times higher in cropped soil. Total fungal populations were also increased by cropping but must be considerably higher than recorded in Table 2 because the medium used was highly selective.

(*c*) *The Relative Importance of* P. ultimum, F. oxysporum, *and* F. solani

(i) *Chemical Treatment of Cropped Soils.*—The relative importance of the three pathogens on growth of peas was studied in a glasshouse experiment involving two pea cultivars (Greenfeast, New Era) and two soil fungicides ("Dexon", specific for pythiaceous fungi, and "Mylone", a non-specific fungicide).*

Cropped light soil was divided into three equal parts and one part was treated with "Dexon" (100 lb active ingredient per acre), one part with "Mylone" (400 lb active ingredient per acre), and one part was not treated. Cropped heavy soil was divided into two parts and one treated with "Dexon" and the other untreated. The five soils were added separately to 10 enamel undrained pots (4 kg per pot) and the moisture contents adjusted to pF 2. The pots were covered

* "Dexon" is manufactured by Bayer, Ltd. and "Mylone" by Union Carbide. Both were supplied by Henry York & Co. Pty. Ltd., Australia.

with plastic and left for 3 days, then the plastic covers were removed and the pots left for a further 2 weeks, the moisture contents of the soils being adjusted every second day. Half the pots (five for each soil–treatment combination) were sown with cv. Greenfeast peas, half with cv. New Era. Seed was treated with "Tetroc" and 10 seeds sown per pot. After emergence the number of seedlings per pot was reduced to five and rain-water was added as required to replace water lost.

The effect of treatment on growth of peas was noticeable after 1 month. After 6 weeks all peas of both cultivars growing in untreated light soil were stunted, and had dead or yellow lower leaves. A week later similar symptoms, although less severe, were apparent on plants growing in heavy untreated soil. After 10

TABLE 3

DRY WEIGHTS (G) OF TWO PEA CULTIVARS GROWN IN HEAVY AND LIGHT CROPPED SOILS WHICH WERE UNTREATED OR TREATED WITH THE SOIL FUNGICIDES "DEXON" AND "MYLONE"

Means of five pots

Cultivar	Heavy Soil		Light Soil		
	Untreated	Treated with "Dexon"	Untreated	Treated with "Dexon"	Treated with "Mylone"
Greenfeast	4·6	12·6	3·2	9·4	8·5
New Era	8·6	16·2	6·3	13·6	14·9
Treatment means*	6·6	14·4	4·8	11·5	11·7

* Least significant differences between treatment means are 1·8 (5% level), 2·4 (1% level), and 3·2 (0·1% level).

weeks the effect of both "Dexon" and "Mylone" treatments was very marked. Plants in treated soils were vigorous, green, and generally healthy, while those in untreated soils were stunted, with dead lower leaves; many Greenfeast plants in light soil were dead. Plate 1, Figure 1, shows the effect of "Dexon" treatment.

Although most of the plants in "Dexon"-treated soil were healthy, some wilted and died during flowering. Six out of 50 Greenfeast and 7 out of 50 New Era plants wilted and died. Symptoms of wilt were not typical of those induced by infection by *F. oxysporum*, infected plants having an extensive black root rot, presumably due to infection by *F. solani*, although no isolations were made.

Eleven weeks after sowing, the plant tops were harvested and dry weights were determined (Table 3). In the statistical analysis there was no significant interaction between treatment and cultivar, and treatment means only were compared. The difference between the growth of peas in "Dexon"-treated soils and untreated soils was significant at the 0·1% level. Growth of peas in heavy soil

was significantly better than that in light soil even although the populations of *Pythium* spp. and *F. solani* were higher in the heavy soil (cf. Table 7). There was no significant difference between the effects of "Dexon" and "Mylone", indicating that non-pythiaceous fungi are relatively unimportant in reducing yield. The difference in yield between New Era and Greenfeast is probably mainly due to a difference in rate of maturity of the two cultivars, New Era maturing earlier than Greenfeast.

The effect of "Dexon" treatment was so great that it was considered necessary to check the specificity of the fungicide, both in agar and in soil. In basal agar medium "Dexon" was incorporated at the rates of 0, 10, 50, 250, and 1000 p.p.m., and four plates of each were inoculated separately with disks of agar inoculum of *P. ultimum*, *F. oxysporum*, and *F. solani*. Colony diameter of *P. ultimum* was

TABLE 4
COLONY DIAMETERS (MM) OF THREE FUNGI GROWN ON AGAR CONTAINING VARIOUS CONCENTRATIONS OF "DEXON"

Fungus	Concentration of "Dexon" (p.p.m.)				
	0	10	50	250	1000
P. ultimum	90	22*	0	0	0
F. oxysporum	53	60	59	62	62
F. solani	56	60	58	57	48

* Very sparse growth.

measured after 2 days when the controls had reached the peripheries of the petri dishes; colony diameters of *Fusarium* spp. were measured after 5 days (Table 4). The specific action of "Dexon" is apparent. *P. ultimum* was markedly inhibited by 10 p.p.m. "Dexon" and there was no growth at higher concentrations. Growth of *F. oxysporum* was stimulated by "Dexon" at all concentrations and *F. solani* was slightly inhibited only at 1000 p.p.m.

It is possible, however, that after "Dexon" is added to soil its specificity may alter. "Dexon" was thoroughly mixed with cropped soils both light and heavy, at a rate equivalent to 100 lb active ingredient per acre. Two weeks after treatment a bioassay similar to that described by Pinck, Soulides, and Allison (1961) was carried out. Glass cylinders, 19 mm external diameter, were gently heated and placed on top of agar plates (*Fusarium* medium) seeded with either *P. ultimum*, *F. oxysporum*, or *F. solani*. To seed the agar, spore suspensions were used for the two *Fusarium* spp. and a soil culture for *P. ultimum*. Two cylinders were placed on each plate, one being filled with 1 g of "Dexon"-treated soil and one with 1 g of untreated soil. The plates were examined after 2 days.

In the plates seeded with *P. ultimum* there was a wide zone of inhibition around the "Dexon"-treated soil but not around the untreated soil. There was no inhibition of the two *Fusarium* spp.

These results support the view that in the previous pot trial "Dexon" controlled only *Pythium* spp. and did not inhibit *F. oxysporum* or *F. solani*.

(ii) *Inoculation of Virgin Soil.*—The results of the previous pot experiment indicated that *Pythium* spp. were more important than either *F. oxysporum* or *F. solani* in causing poor growth of peas in heavily cropped soils. There is circumstantial evidence, however, that there may be an interaction between *Pythium* spp. and *F. oxysporum*. In the isolation of pathogens from diseased tissue, the two fungi were frequently isolated from the same or similar lesions and in inoculation studies the damage to peas following inoculation of soil with pure cultures of *P. ultimum* did not closely resemble the symptoms on peas grown in cropped soils. Perhaps the most significant evidence is that wilt-susceptible plants grown in cropped soil develop symptoms typical of *Fusarium* wilt and *F. oxysporum* is readily isolated.

The possibility of interactions between all three pathogens was investigated by using virgin light soil which has a low pathogen population and adding the pathogens individually and in combination so that the resulting populations were similar to those in cropped light soil.

Although the populations of the three pathogens in cropped light soil had been determined previously (Table 2), the ratio of pathogenic to non-pathogenic races of the two species of *Fusarium* was not known. To estimate this ratio, the pathogenicity to peas of all cultures of *F. oxysporum* and *F. solani* which had been isolated directly from light cropped soil was determined. Of 97 isolates of *F. oxysporum* 63 (65%) were pathogenic and of 8 isolates of *F. solani* 2 (25%) were pathogenic. In addition the pathogenicity of *F. oxysporum* from both light and heavy virgin soil was determined. None of 8 isolates from light virgin soil was pathogenic but 5 out of 9 from heavy virgin soil produced typical wilt symptoms in inoculated plants. There is either a native pathogenic population in this soil or it has become infested from the neighbouring crop, possibly by wind-blown soil.

Virgin light soil was inoculated with *P. ultimum* and with both pathogenic and non-pathogenic strains of *F. oxysporum* and *F. solani* so that the resulting populations and the ratios of pathogenic to non-pathogenic strains of *Fusarium* spp. were similar to those in cropped light soil. Soil cultures of *Fusarium* spp. in which the fungal populations had been determined, and sand–maize meal cultures of *P. ultimum* (1·0 g inoculum per kilogram soil) were used. The experiment involved seven inoculation treatments: (1) untreated, (2) *F. oxysporum*, (3) *P. ultimum*, (4) *F. solani*, (5) *F. oxysporum* and *P. ultimum*, (6) *F. oxysporum* and *F. solani*, and (7) *F. oxysporum*, *P. ultimum*, and *F. solani*. Each treatment was applied to 40 kg air-dry soil which was adjusted to pF 2 and then added to 10 enamel undrained pots, half being sown with cv. Greenfeast peas and half with cv. BC4/F6. The use of the latter variety in place of cv. New Era was to minimize variations caused by a difference in time of maturity. All seed was treated with

"Tetroc" before sowing. After seedling emergence the pots were watered with rain-water as required.

P. ultimum alone caused obvious stunting of both cultivars 4 weeks after sowing, but plants gradually recovered. *F. oxysporum* alone caused no damage to cv. BC4/F6 plants and damage to cv. Greenfeast plants was apparent only after the onset of flowering and pod development when 17 out of 25 plants developed symptoms of wilt. With these two fungi combined, however, all cv. Greenfeast plants wilted and died before flowering commenced, whereas all cv. BC4/F6 plants were alive, although stunted. Plate 1, Figures 2 and 3, shows the appearance of plants just before harvesting.

TABLE 5

NUMBER OF PLANTS (CV. GREENFEAST) SHOWING ABOVE-GROUND SYMPTOMS OF INFECTION BY F. OXYSPORUM OR F. SOLANI

25 plants per treatment

Symptoms	Days after Sowing	Inoculation Treatment						
		Control	*F. oxysporum*	*P. ultimum*	*F. solani*	*F. oxysporum* + *P. ultimum*	*F. oxysporum* + *F. solani*	*F. oxysporum* + *F. solani* + *P. ultimum*
F. oxysporum infection	49	0	2	0	0	13	0	13
	59	0	3	2	0	25	1	25
	69	0	6	2	0	25	8	25
	79	0	17	2	0	25	12	25
F. solani infection	49	0	0	0	8	0	2	0
	59	1	0	0	15	0	3	0
	69	1	0	0	18	0	6	0
	79	1	0	0	20	0	10	0

After disease symptoms appeared the amount of water lost by each pot over 2-day periods was measured by weighing, and the results are presented graphically in Figure 2 for treatments involving *P. ultimum* and *F. oxysporum*. The water lost by control pots is taken as 100% for each 2-day period. Figure 2 illustrates clearly the initial low water uptake of wilt-susceptible plants affected by *P. ultimum* and their gradual recovery unless *F. oxysporum* is also present, in which case the water uptake is drastically reduced. *F. oxysporum* alone had no significant effect on the water uptake of plants at this stage. The two pathogens singly had a similar effect on wilt-resistant plants but in combination the water uptake gradually increased relative to the control plants.

The number of plants showing above-ground symptoms of infection by *F. oxysporum* or *F. solani* was recorded at 10-day intervals from 7 weeks after sowing

until just before harvesting (Table 5). In soil inoculated with *F. oxysporum* alone the number of cv. Greenfeast plants showing symptoms of infection by this fungus increased during this period and at the end of the experiment 68% were infected.

After 12 weeks, above-ground parts of plants were harvested and dry weights measured (Table 6). Analysis of variance showed a significant (0·1% level) difference between both cultivars and treatments and also a significant (1·0% level) interaction between cultivar × treatment. The interaction between *F. oxysporum* and *P. ultimum* was not quite significant at the 5% level. *F. solani* had a contrasting effect on the two cultivars causing serious damage to Greenfeast, but none to BC4/F6. This effect is difficult to explain because in the breeding of BC4/F6 there was no intentional selection for resistance to this fungus. Moreover, in soil inoculated with *F. solani* there was severe blackening of stem bases of all plants of both cultivars.

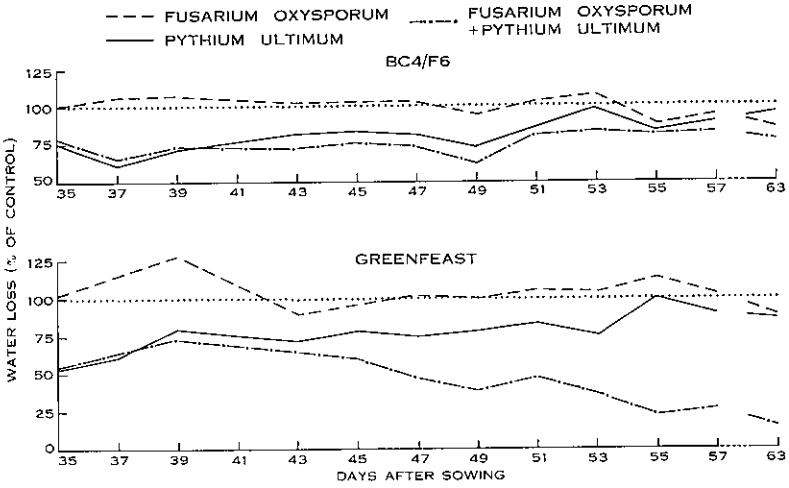

Fig. 2.—Water uptake by cultivars Greenfeast and BC4/F6, growing in virgin soil inoculated with *F. oxysporum* and *P. ultimum* singly and together.

(*d*) *Influence of Cropping and Chemical Treatments of Soil on Fungal Populations*

In the two previous pot experiments the populations of *Pythium* spp., *F. oxysporum*, and *F. solani* were estimated both before sowing and after harvesting. The results of the "Dexon" experiment are given in Table 7. The values for fungal populations following "Mylone" treatment are not given because pathogens were not recorded from most pots. There was one notable exception where, after harvesting, the soil in one pot had more than 1000 propagules of *Pythium* per gram, the highest value recorded for any soil. Presumably the soil had been recontaminated with *Pythium* and with relatively few organisms to compete with, the *Pythium* population increased rapidly.

A statistical analysis of Table 7 showed clearly that:

(1) Before sowing, heavy soil had a higher population of *Pythium* spp. and *F. solani* than light soil whereas the population of *F. oxysporum* was not significantly different in the two soils.

(2) "Dexon" had no effect on initial populations of the three pathogens, even although pre-sowing assessments were made 2 weeks after treatment. After harvesting, however, the population of *Pythium* spp. was significantly lower in "Dexon"-treated soils than in untreated soils, and also significantly lower than the initial population.

(3) There was a much higher population of *Pythium* spp. in untreated soils after harvesting than before sowing and the post-harvest population of *F. oxysporum* was higher than the initial population in both treated and untreated soils. The population of *F. solani* was the same after harvesting as before sowing.

TABLE 6

DRY WEIGHT (G) OF TWO PEA CULTIVARS GROWN IN VIRGIN SOIL INOCULATED WITH P. ULTIMUM, F. OXYSPORUM, AND F. SOLANI, BOTH SINGLY AND IN VARIOUS COMBINATIONS

Means of five pots. Least significant differences are: 3·02 (5% level), 4·02 (1% level), 5·25 (0·1% level)

Cultivar	Inoculation Treatment						
	Control	*F. oxysporum*	*P. ultimum*	*F. solani*	*F. oxysporum* + *P. ultimum*	*F. oxysporum* + *F. solani*	*F. oxysporum* + *P. ultimum* + *F. solani*
Greenfeast	12·3	8·7	8·9	6·4	2·7	8·0	2·5
BC4/F6	11·3	9·7	10·0	13·7	8·1	12·0	6·8

(4) Cultivars had little influence on the pathogenic populations, although there were interactions (just significant at the 5% level) between time × cultivar for both *Pythium* spp. and *F. solani* and a similar-order interaction between soil × cultivar for *Pythium*. The importance of these interactions is not known.

In the second pot experiment using virgin soil inoculated with the three pathogens in various combinations, the pre-sowing and post-harvest populations are given in Table 8. All soils not inoculated with a particular fungus are combined whether or not they were inoculated with other fungi.

Before sowing, the populations of *Pythium* spp. and *F. solani* were similar to those in light cropped soil and that of *F. oxysporum* approximately three times higher.

In inoculated soil only *Pythium* spp. increased markedly with cropping. In uninoculated soil the populations of all fungi were higher after harvesting than

before sowing, but both *Fusarium* species were still much lower than in the inoculated soils. With *Pythium*, however, after harvesting there was as high a population in the uninoculated as in the inoculated soils.

IV. Discussion

Conventional methods of inoculating soil with pathogenic fungi usually involve mixing with soil a fungal spore suspension or a fungal culture grown in sand–maize meal or similar substrates (Johnson *et al.* 1959). With *F. oxysporum* both these methods were tested and resulted in an initial rapid drop in population and a more or less stable population only after 3 or 4 months. By using a soil culture, however, where the fungal propagules are probably chlamydospores, the resulting population was stable and approximately the same as the number of

Table 7

POPULATIONS (PROPAGULES PER GRAM OF SOIL) OF PYTHIUM SPP., F. OXYSPORUM, AND F. SOLANI BEFORE SOWING PEAS AND AFTER HARVEST

Means of five soil samples and duplicate plates

Fungus	Soil Treatment	Cultivar	Before Sowing		After Harvesting	
			Heavy Soil	Light Soil	Heavy Soil	Light Soil
Pythium spp.	Untreated	Greenfeast	278	81	836	414
		New Era	237	61	870	357
	"Dexon"-treated	Greenfeast	300	87	91	22
		New Era	306	51	191	26
F. oxysporum	Untreated	Greenfeast	800	300	2000	1600
		New Era	300	600	1200	700
	"Dexon"-treated	Greenfeast	500	300	1400	1100
		New Era	400	300	800	1000
F. solani	Untreated	Greenfeast	5200	1700	3900	1600
		New Era	4200	1700	3100	4000
	"Dexon"-treated	Greenfeast	3800	2300	1900	1300
		New Era	4100	1400	3000	2800

propagules added. If the number of propagules in the soil culture is known, a soil can be inoculated to bring the population of *F. oxysporum* (and also *F. solani*) to a predetermined level. With *P. ultimum*, sand–maize meal cultures appear to be suitable for soil inoculation. The amount of inoculum required to bring the *P. ultimum* population of virgin light soil to that in cropped light soil was found to be approximately 1·0 g per kilogram soil, as opposed to conventional rates of between 10 and 50 g inoculum per kilogram soil.

The results of both pot experiments indicate clearly that in a wilt-susceptible cultivar there is a marked interaction between *Pythium* spp. and *F. oxysporum* f. *pisi* race 2. The evidence that "Dexon" inhibits only *Pythium* spp. is very strong and yet treating soil with "Dexon" controls *Fusarium* wilt. The second pot experiment involving virgin soil inoculated with the two fungi both alone and together provides further evidence for an interaction. Fifty-nine days after sowing only three plants out of 25 growing in soil inoculated with *F. oxysporum* alone showed symptoms of wilt while all 25 were wilted in soil inoculated with both *F. oxysporum* and *P. ultimum*. This is illustrated in Figure 2 which shows water loss from plants growing in soils inoculated with the two fungi singly and in combination. The final dry weight values for this experiment did not show a significant interaction between the two fungi, but this is not surprising because by the end of the experiment there was a high population of *Pythium* spp. in all soils, whether inoculated or not.

TABLE 8

POPULATIONS (PROPAGULES PER GRAM OF SOIL) OF PYTHIUM SPP., F. OXYSPORUM, AND F. SOLANI BEFORE SOWING PEAS AND AFTER HARVEST

Soil Treatment	Before Sowing			After Harvesting		
	Pythium spp.	*F. oxysporum*	*F. solani*	*Pythium* spp.	*F. oxysporum*	*F. solani*
Not inoculated	22	25	83	279	200	375
Inoculated	80	1900	1983	248	2113	1583

Nematodes have frequently been reported (McGuire, Walters, and Slack 1958; Labruyere, Den Ouden, and Seinhorst 1959; Thomason, Erwin, and Garber 1959) to increase the susceptibility of plants to *Fusarium* wilt, but this appears to be the first report of an interaction between *Pythium* spp. and *F. oxysporum*. The most likely explanation is that *F. oxysporum* does not readily infect healthy, undamaged roots and that infection of roots by *Pythium* spp. promotes infection by *F. oxysporum*. A similar explanation has been proposed by Robertson (1959) for the interaction between *Pythium* sp. and *Rhizoctonia lamellifera* in blast disease of oil-palm seedlings.

There was no evidence that *F. solani* protected peas from infection by *F. oxysporum* as reported by Buxton and Perry (1959) or of a population interaction between these two fungi as reported by Worf and Hagedorn (1961). These workers were dealing with highly artificial conditions and it appears that their findings do not apply under more natural conditions.

From the work reported here it is difficult to assess the importance of *F. solani* in affecting yield of peas. Perhaps the best evidence is from the cropped soil treated with "Dexon" where 7 out of 50 wilt-resistant cv. New Era plants

ROOT ROT–FUSARIUM WILT COMPLEX OF PEAS

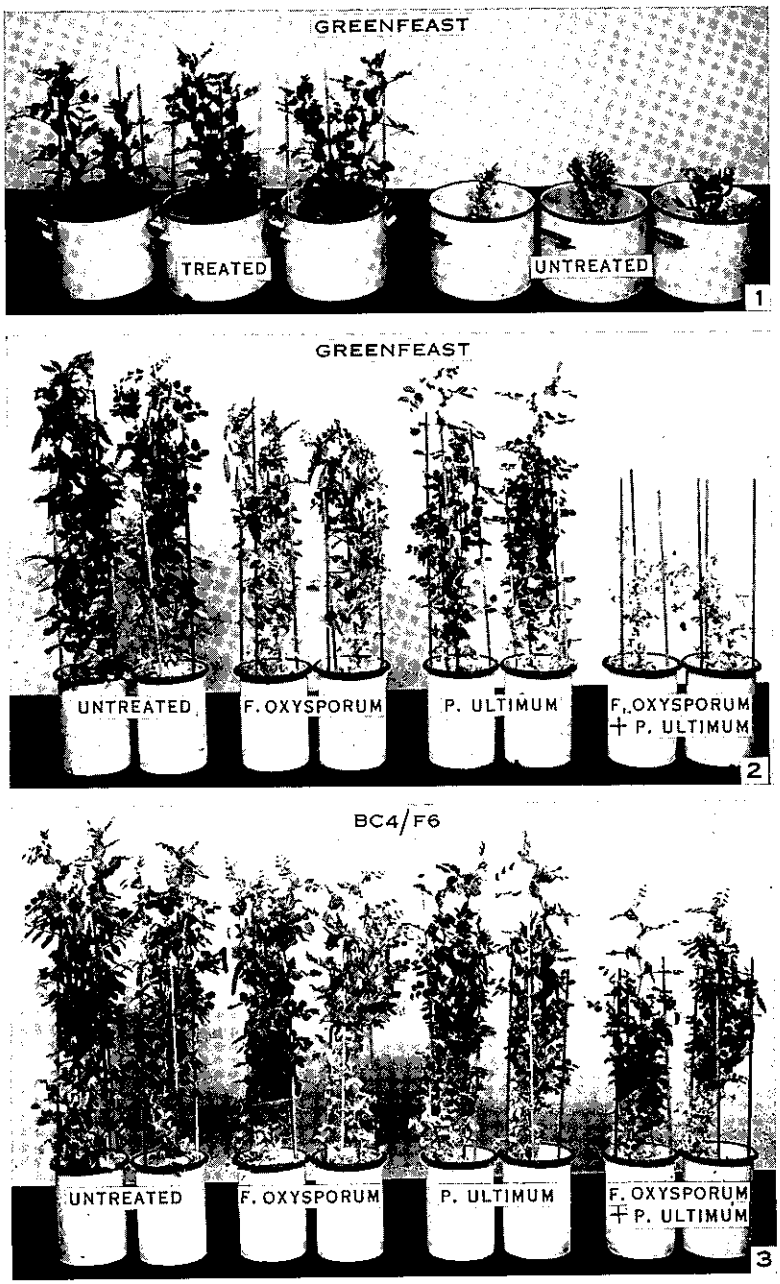

Fig. 1.—Effect of treating cropped light soil with "Dexon" on growth of cv. Greenfeast peas.

Figs. 2 and 3.—Effect of inoculation of cv. Greenfeast (Fig. 2) and cv. BC4/F6 (Fig. 3) peas grown in virgin light soil with *F. oxysporum* and *P. ultimum* either singly or in combination.

were killed, presumably by *F. solani*. In the experiment with inoculated virgin soil, *F. solani* significantly reduced yield of cv. Greenfeast, but not of cv. BC4/F6 which is difficult to explain because no intentional selection for resistance to *F. solani* was made in the breeding programme of cv. BC4/F6. More work is required to determine the nature and importance of these effects produced by *F. solani*.

Cropping with peas has a marked influence on the populations of all three pathogens studied. This is clear from Table 2 where the fungal populations of natural cropped and virgin soils are compared. It is also clear from Tables 7 and 8 showing pre-sowing and post-harvest populations in soils used in pot experiments. The critical population levels of the three fungi, above which disease becomes serious and below which an economic crop can be grown have not yet been established. It seems unlikely that the *Pythium* population would often be below this critical level because after growing one crop of peas in virgin soil in pots, it was nearly three times higher than the *Pythium* population of natural soil cropped with peas for 5 consecutive years.

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