EFFECT OF SOIL AMENDMENTS ON NUMBERS OF SOIL MICROORGANISMS AND ON THE ROOT ROT–*FUSARIUM* WILT COMPLEX OF PEAS

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[*Manuscript received April* 16, 1969]

Summary

The possibility of controlling *Fusarium* and *Pythium* in soil by blood and bone meal supplement, and the effect of this and other amendments on the root rot–*Fusarium* wilt complex of peas was investigated in laboratory and glasshouse experiments. Blood and bone meal was found to stimulate the growth not only of bacteria and actinomycetes, but also of *Fusarium* and *Pythium*. When crude chitin was added to soil, the numbers of *Pythium* increased. It was shown that in liquid cultures *P. ultimum* is able to utilize chitin as a nutrient source.

Blood and bone meal added to soil at the time of planting did not significantly decrease wilting of peas, but the fungicide Dexon decreased wilting and significantly increased the height of pea plants.

I. INTRODUCTION

In studies of the effect of various nutrients on mycolysis in soil, Bumbieris and Lloyd (1967) found that numbers of bacteria increased in soils supplemented with glucose, peptone, or blood and bone meal. Actinomycete numbers remained constant when glucose was added to soil but increased with peptone or blood and bone meal supplements. Fungal hyphae subsequently added to such soils lysed faster than in untreated soil. The effect of peptone, and blood and bone meal on bacterial and actinomycete numbers is thus similar to that of chitin which has been reported as stimulating the numbers of these microorganisms in the rhizosphere (Khalifa 1965).

As blood and bone meal is a widely used fertilizer the previous work has now been extended by studying the effect of this and other soil amendments on the number of bacteria, actinomycetes, and *Fusarium* and *Pythium* spp. in naturally infested soil, and the effect of such amendments on the root rot–*Fusarium* wilt complex of peas.

II. MATERIALS AND METHODS

(*a*) *Soil*

The soil used in these investigations was taken from a field near Renmark in S.A. where peas had been grown for five consecutive years prior to 1963, and which was also used by Kerr (1963) for his investigations of the root rot–*Fusarium* wilt complex of peas. The soil contains 7·4% clay and is referred to as "light sandy loam" by Kerr (1964) who gives its moisture characteristics. Numbers of microorganisms were estimated using the dilution plate method. Nutrient agar with 20 μg/ml Mycostatin (Squibb) was used for bacteria, and chitin agar (Lingappa and Lockwood 1962) for actinomycetes. The selective medium of Kerr (1963) was used for

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Fusarium spp. and that of Vaartaja and Bumbieris (1964) for *Pythium* spp. Estimates of numbers of microorganisms per gram of air-dry soil were as follows: bacteria, $8 \cdot 4 \times 10^6$; actinomycetes, $3 \cdot 0 \times 10^6$; *Fusarium* spp., $2 \cdot 0 \times 10^3$; and *Pythium* spp., 40.

(*b*) *Soil Amendments*

The soil was first sieved through a 2-mm sieve and its moisture adjusted to 10% (pF about 2·3) by means of a fine water spray and constant mixing. This moisture level was maintained for the duration of all experiments. In the various tests blood and bone meal was added at the rate of 0·5% (w/w), crude chitin at the rate of 1·0% (w/w), and the fungicide Dexon (5% granular) at the rate of 0·1% (w/w). Untreated controls were included in all tests. For the sake of convenience blood and bone meal will be referred to as BB throughout this paper.

In laboratory tests the amended soils were kept in open glass beakers at room temperature (about 23°C), and changes in microbial numbers were estimated at 3-weekly intervals.

For glasshouse experiments the prepared soils were transferred to 4-in. pots and 10 seeds of the pea cultivar Greenfeast were planted in each pot. To determine changes in microbial numbers, three pots of each treatment were removed at intervals and their contents sieved through a 2-mm sieve and thoroughly mixed before estimating microbial numbers.

(*c*) *Control of Root Rot*–Fusarium *Wilt Complex of Peas*

For these experiments the amended soils were transferred to 6-in. pots (about 2 kg per pot), and five seeds of Greenfeast peas were planted in each pot. The treatments were replicated three times. Extent of wilt was assessed by measuring the portion of plant wilted from soil level upwards and expressing it as a percentage of the height of the plant.

III. Experimental Details and Results

(*a*) *Changes in Microbial Numbers in Laboratory Tests*

In the laboratory test, changes in the numbers of microorganisms in soils supplemented with BB and chitin were compared with those in untreated controls. After 3 weeks numbers of *Pythium* had increased more (difference significant at $P < 0 \cdot 01$) in soils supplemented with BB, or with chitin, than in the control soil [Fig. 1(*a*)]. At the end of the experiment the numbers of *Pythium* in the BB-supplemented soil were also higher ($P < 0 \cdot 05$) than in the other treatments [Fig. 1(*a*)]. Numbers of *Fusarium* increased slightly in BB-supplemented soil, but did not increase with chitin supplement during the first 9 weeks of the experiment. After 12 weeks *Fusarium* numbers were higher ($P < 0 \cdot 01$) in the control than in the supplemented soils [Fig. 2(*a*)].

Numbers of both bacteria and actinomycetes [Figs. 3(*a*) and 4(*a*)] increased considerably more in the BB-supplemented than in the control soil (differences significant at $P < 0 \cdot 05$ for bacteria after 3 weeks, and at $P < 0 \cdot 01$ for actinomycetes after 6 weeks). Numbers of bacteria and actinomycetes were not estimated in chitin-supplemented soil.

(*b*) *Changes in Microbial Numbers in Glasshouse Tests*

This experiment was carried out to determine whether an effect similar to that observed in the laboratory would persist in soil supplemented with BB in the presence of growing pea plants.

Only two treatments were used: (1) control, and (2) soil supplemented with BB. The soils were prepared 3 weeks before planting and stored in plastic bags to allow microbiological changes to take place.

Numbers of *Pythium* increased in both soils during the 3 weeks storage but especially so with the BB supplement [Fig. 1(*b*)]. Three weeks after peas were planted the numbers of *Pythium* in the controls had increased more ($P<0{\cdot}01$) than in the BB-supplemented soils. During the following 4 weeks numbers of *Pythium* decreased

Fig. 1

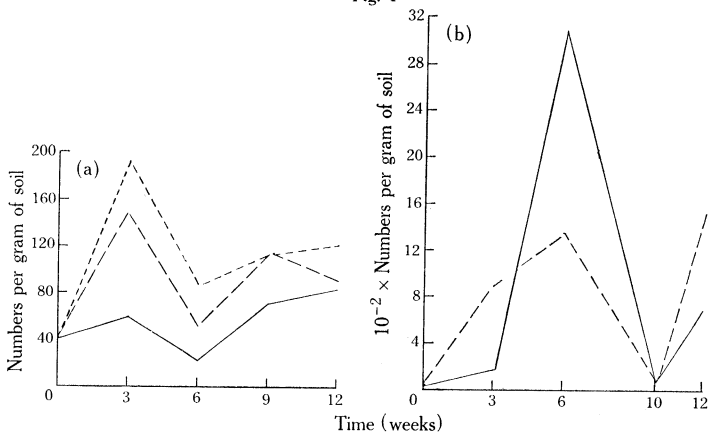
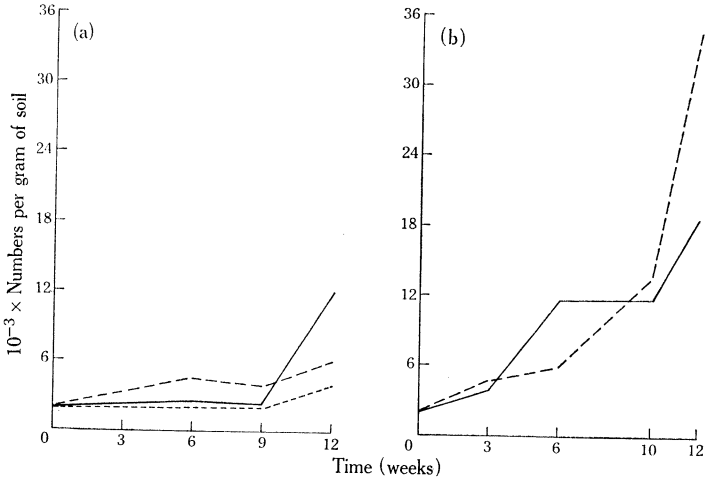


Fig. 2



Figs. 1 and 2.—Changes in *Pythium* (Fig. 1) and *Fusarium* numbers (Fig. 2) in laboratory (*a*) and glasshouse tests (*b*). Peas planted at 3 weeks in glasshouse experiments. ——— Control. — — BB supplement. – – – Chitin supplement.

sharply in both treatments but increased again during the last 2 weeks of the experiment [Fig. 1(*b*)]. Numbers of *Fusarium* 3 weeks after peas were planted were higher ($P<0{\cdot}01$) in the controls than in the BB-supplemented pots. However, during the last weeks of the experiment *Fusarium* numbers increased considerably in the BB-supplemented pots but less so in the control pots [Fig. 2(*b*)].

Bacterial numbers also increased very markedly ($P<0{\cdot}01$) during the first 3 weeks after planting of peas in the BB-supplemented pots, then decreased during the following 4 weeks but increased again sharply during the last 2 weeks of the experiment. In the control pots the bacterial numbers increased rather gradually during the 12 weeks of the experiment [Fig. 3(*b*)]. An increase ($P<0{\cdot}01$) in the numbers of actinomycetes in the BB-supplemented soil during the 3-week storage period was followed by a gradual decrease during the remaining period of the experiment. The numbers of these organisms in the controls remained practically unchanged during the 12 weeks of the experiment [Fig. 4(*b*)].

Fig. 3

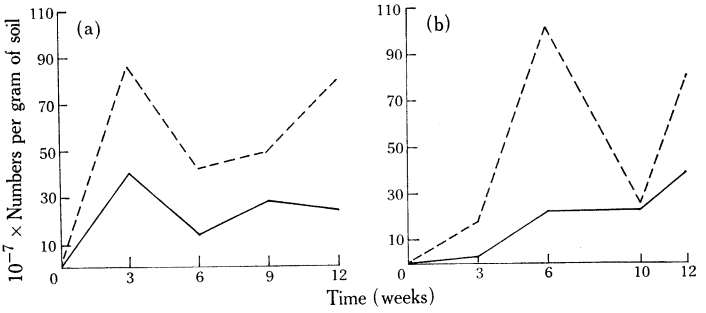
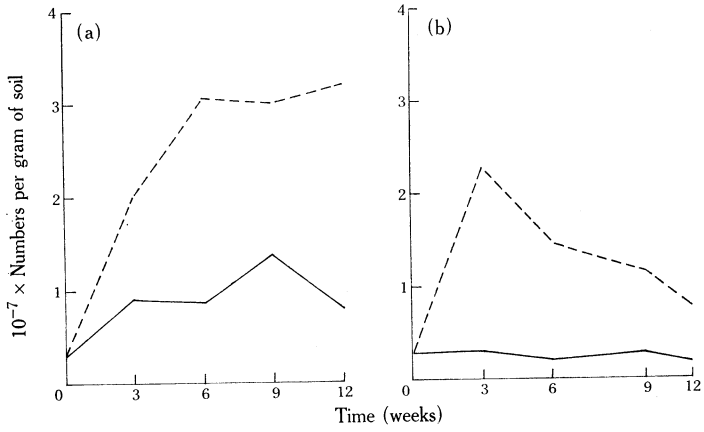


Fig. 4



Figs. 3 and 4.—Changes in bacterial (Fig. 3) and actinomycete numbers (Fig. 4) in laboratory (*a*) and glasshouse tests (*b*). Peas planted at 3 weeks in glasshouse tests. —— Control. — — BB supplement.

(*c*) *Effect of Dexon on* Pythium *and* Fusarium *spp.*

Because both BB and chitin supplements stimulated *Pythium* spp. in soil in the laboratory test [Fig. 1(*a*)], and BB stimulated these fungi in the glasshouse test [Fig. 1(*b*)], it was decided to investigate the effect of the fungicide Dexon, which is specific for pythiaceous fungi (Kerr 1963), on the numbers of *Pythium* and *Fusarium* spp. in a glasshouse experiment. The experiment was carried out as described for the previous test.

Figure 5(*a*) shows that, while the numbers of *Pythium* increased considerably in the control pots during the first 3 weeks after planting of peas, the corresponding increase in the Dexon-treated pots was only slight (difference significant at $P<0{\cdot}01$). The numbers of *Fusarium* increased only slightly in both treatments during the first 6 weeks but during the second half of the experiment their numbers increased very considerably in the Dexon-treated pots ($P<0{\cdot}01$) [Fig. 5(*b*)]. Dexon was not toxic to pea plants at the concentration used.

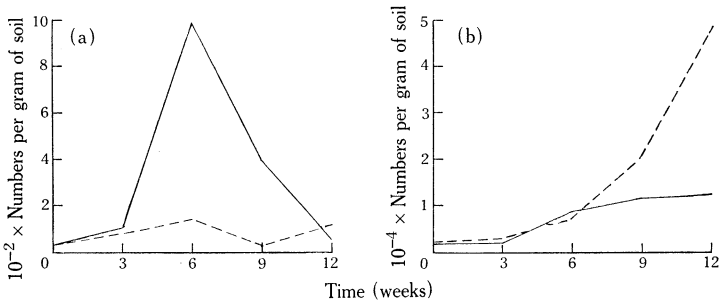


Fig. 5.—Effect of Dexon on numbers of *Pythium* (*a*) and *Fusarium* spp. (*b*) planted in glasshouse experiments. ——— Control. — — Dexon addition. Peas planted at 3 weeks.

(*d*) *Utilization of Chitin by* Pythium ultimum

Because of the considerable increase in the numbers of *Pythium* in the chitin-supplemented soil [Fig. 1(*a*)] the ability of these fungi to utilize chitin as a nutrient source was investigated. *P. ultimum* Trow. was chosen as the test organism because of its association with the root rot–*Fusarium* wilt complex of peas (Kerr 1963).

In this experiment washed and macerated mycelium of *P. ultimum* was inoculated into 500-ml flasks containing 100 ml of one of the following two media:

Medium A—0·8 g $NaNO_3$, 0·4 g KH_2PO_4, 0·2 g KCl, 0·2 g $MgSO_4.7H_2O$, trace of $FeSO_4$, and 400 ml distilled water;

Medium B—as medium A, but containing in addition 1 g (dry weight) colloidal chitin prepared according to Lingappa and Lockwood (1962).

Dry weights of the mycelia grown in each of the two media (at 25°C) were determined after a period of 2 weeks. The treatments were replicated four times.

As the colloidal chitin did not pass through filter paper when separating fungal mycelium from culture medium, the dry weight of mycelium was calculated as the difference between total weight and the sum of the known dry weights of filter paper and colloidal chitin. In this way any possible losses of chitin during filtering would have decreased the obtained mycelial weight, and would not invalidate the results. On the other hand, any colloidal chitin adhering to the surface of fungal hyphae was not included in the dry weight of mycelium.

When the fungus was grown in two different media the mean dry weights of four replicates of the harvested mycelia were as follows: medium A (no carbon source), 0·90 mg; and medium B (with 1 g colloidal chitin), 21·50 mg (difference significant at

$P < 0 \cdot 01$). Similar results were obtained when the experiment was repeated. When *Fusarium solani* f. *pisi* was grown in the same two media, the mean dry weight obtained from medium A was 0·87 mg and that obtained from medium B was 1·46 mg.

(*e*) *Effect of Soil Amendments on the Root Rot–*Fusarium *Wilt Complex of Peas*

In the previously described experiments the increase in the numbers of *Fusarium* in both BB- and chitin-supplemented soils was not significant during the first 9 weeks [Figs. 2(*a*) and 2(*b*)]. However, numbers of *Pythium* increased considerably in both soils [Figs. 1(*a*) and 1(*b*)], while Dexon prevented an increase in the numbers of these fungi [Fig. 5(*a*)].

The possibility of controlling the root rot–*Fusarium* wilt complex of peas by means of these materials singly and in combination was further investigated. The following treatments were used in this experiment: (1) control; (2) soil treated with Dexon; (3) soil amended with chitin; (4) soil amended with chitin and Dexon; (5) soil amended with BB; and (6) soil amended with BB and Dexon.

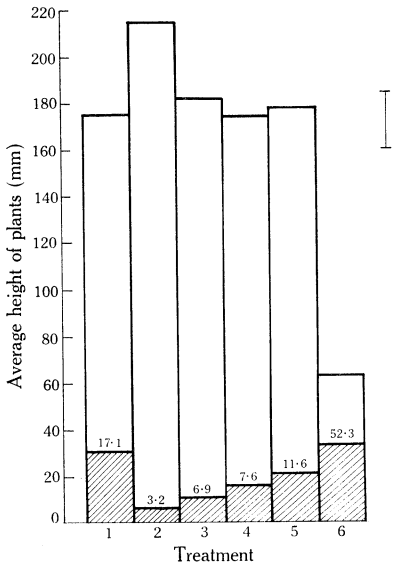


Fig. 6.—Effect of soil amendments on pea plants. Treatments defined in Section III(*e*). Shaded areas indicate average extent of wilting as a percentage of the total plant height [least significant difference ($P = 0 \cdot 05$) = 8%]. Vertical bar indicates least significant difference at the 1% level for heights of plants.

Figure 6 shows the effect of soil amendments on the growth of peas planted at the time of preparation of soil, and the extent of wilting assessed 47 days after planting.

Fusarium oxysporum was isolated from the lower parts of stems of plants from control, BB-supplemented, and BB+Dexon-supplemented pots.

IV. Discussion

Blood and bone meal supplement to soil stimulated the growth of bacteria and actinomycetes, and also *Fusarium* and *Pythium* spp. in both laboratory and glasshouse tests. However, the increase of *Fusarium* numbers was not as marked as that of *Pythium* numbers, and at one stage in the glasshouse experiment their numbers in BB-supplemented pots were lower than in the controls [Fig. 2(*b*)]. This may be explained by the very high numbers of bacteria and actinomycetes present in the soil at

this stage [Figs. 3(*b*) and 4(*b*)]. On the other hand, as previously suggested (Bumbieris and Lloyd 1967), fungi are able to grow and compete with other microorganisms provided that suitable nutrients are present in sufficient quantities. This explains the concurrent increase in the numbers of all four groups of microorganisms, especially in the glasshouse test where abundant nutrients would be provided by the growing pea plants. The greater increase in both *Pythium* and *Fusarium* numbers with BB supplement in the presence of pea plants (Figs. 1 and 2) may have been due to an additive effect of root exudates present in the rhizosphere. Thus BB supplement failed to control *Fusarium* and *Pythium* spp. in soil.

Crude chitin added to soil did not allow *Fusarium* numbers to increase for the first 9 weeks of the laboratory experiment [Fig. 2(*a*)] but stimulated the growth of *Pythium*. Stimulation of *P. aphanidermatum* (Edson) Fitzpatrick in soil supplemented with chitinous materials was reported by Singh and Pande (1965) who argued that such stimulation was not likely to be caused by the ability of the fungus to use chitin as a nutrient source. Domsch (1960) reported that certain phycomycetes grew well on chitin substrate. The present work shows that *P. ultimum* is able to utilize chitin as a nutrient source in liquid cultures, and that the increase in *Pythium* numbers in chitin-supplemented soil may at least partly be due to such ability.

BB supplement to soil did not significantly decrease wilting of peas under the existing experimental conditions (Fig. 6). The poor growth of plants in treatment 6 where blood and bone meal was combined with Dexon (Fig. 6) may have been caused by accumulation of toxic materials produced by the chemical interaction between blood and bone meal and Dexon.

The significant suppression of wilting by chitin supplement (Fig. 6) agrees with the earlier results of Mitchell and Alexander (1961) who studied the effect of various amendments, and direct application of lytic soil bacteria, on *Fusarium* diseases of beans and radishes. These authors further showed that chitin stimulated actinomycetes much more than it stimulated bacteria, and proposed that actinomycetes are the primary group of organisms associated with fungal suppression (Mitchell and Alexander 1962). However, in this work Dexon was more effective than chitin in controlling the root rot–*Fusarium* wilt complex of peas, and also increased the height of plants. This, and also the suppression of *Pythium* numbers by Dexon [Fig. 5(*a*)] emphasizes the importance of *Pythium* in the root rot–*Fusarium* wilt complex of peas, and supports the observations of Kerr (1963), and Escobar, Beute, and Lockwood (1967).

V. Acknowledgments

The author gratefully acknowledges the helpful advice given by Dr. A. Kerr during the course of these investigations, and in preparation of the manuscript. Thanks are also due to Mrs. L. Wichman for preparation of diagrams.

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