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# Effect of organic soil amendments on damping-off of lettuce caused by *Corticium praticola*

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SUMMARY

Ten organic amendments were added to unsterile soil which was contaminated 14 days later with *Corticium praticola* and sown with lettuce seeds. Substantial increases in final stands of seedlings were obtained with grass meal, bran and wood cellulose. Corn and barley meal, linseed cake and fish meal decreased final stands; molassine meal, potato starch and peptone had relatively little effect. Seedlings grown with wood cellulose were very chlorotic and stunted.

Up to 30% of lettuce seeds sown in soil which, 180 days earlier, had been amended with corn meal and contaminated with *C. praticola* became colonized by the fungus. None was colonized in unamended soil or in soil amended with grass meal. Ninety days after amendment and contamination fewer seeds were colonized in soil amended with grass meal than in unamended soil. The amendment of soil with grass meal was as effective as thiram seed treatment in protecting lettuce seedlings against *C. praticola* and grass meal was particularly effective in reducing both the numbers of seedlings attacked and the survival of the fungus in the soil.

## INTRODUCTION

Biological methods for controlling diseases caused by soil-borne plant pathogens are attractive for a number of reasons. First, there is the encouraging fact that soil-borne pathogens grow in association with a great variety of other micro-organisms before they infect underground parts of plants, and it might be possible to modify the balance in favour of those that are antagonistic to the pathogens. Then there is the consideration that many diseases caused by soil-borne pathogens are not amenable to more orthodox control by the use of chemicals. Furthermore, there is the general desirability that where possible and practicable, control based on methods that use chemicals should be replaced by others that do not.

*Rhizoctonia solani* Kühn is an important soil-borne pathogen that causes serious losses in a wide variety of crops, usually in the seedling or young plant stage of growth. Much research has been directed at its control by biological methods. This research, in common with similar work with other pathogens, has been along two main lines. First, there is the use of micro-organisms known to be antagonistic to the pathogen in

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culture (Wood, 1950). Significant control has been obtained with this method only in the quite abnormal conditions of 'sterile' soil. The method is usually ineffective in soil in which the natural populations of micro-organisms have not been drastically decreased in one way or another (Allen & Haenseler, 1935; Weindling, 1932; Weindling & Fawcett, 1936).

The aim of the second method is to exploit the potential of members of the indigenous microflora to act as antagonists to the pathogen, usually by adding various substances (amendments) to the soil. With *R. solani* and other soil-borne pathogens, this method has been more successful (Davey & Papavizas, 1959, 1960; Papavizas & Davey, 1960; Weindling & Fawcett, 1936; Wood, 1950). It was the one chosen for the work described here in which we used *Corticium praticola* Kotila, a pathogen close to *R. solani* and which may well be the cause of much of the damage attributed to *R. solani* in the United Kingdom. In earlier work (de Silva, 1963) it had been found to be more virulent than isolates of *R. solani* from lettuce, the host plant used in this work.

## MATERIALS AND METHODS

The isolate of *Corticium praticola* used was from the Imperial College culture collection and was descended from an isolate originally obtained from lettuce (Flentje, 1956). It was re-isolated in pure culture after passage through lettuce seedlings; stock cultures were grown on the following medium: glucose 1·0, $NaNO_3$ 0·2, $KH_2PO_4$ 0·1, $MgSO_4.7H_2O$ 0·05, agar 2·0, g/100 ml.

Cultures for contaminating soil were grown on corn meal 4·0 g, washed sand 196·0 g, water 20 ml, in 500 ml conical flasks, autoclaved for 2 h at 15 p.s.i., cooled and seeded with eight disks (5 mm diameter) from the edge of 3–4-day-old agar cultures. Corn meal cultures were incubated at *c.* 24 °C for 2 to 3 wk and shaken periodically to disperse mycelia and sclerotia. They were then passed through a 3 mm sieve and well mixed before adding to soil. Rates of contamination are given as g wet culture per g dry weight of soil.

Soil came from the upper 15 cm of a sandy loam in a garden at Imperial College, Silwood Park, Ascot, Berks. It was air dried, passed through a 6 mm sieve and stored in large galvanized steel containers with loosely fitting lids.

Lettuce plants, cv. All the Year Round, were grown from seed (Sutton and Sons Ltd) with a germination rate throughout the work of *c.* 98–100% on moist paper.

The main experimental unit was an earthenware pot (7·5 cm upper diameter), soil level 1·5 cm below the rim and containing 20 seeds evenly spaced 5 mm below the soil surface. Soil was brought to a water content suitable for germination and cultures of the pathogen were added 24 h before seeds were sown. Pots were covered with a sheet of transparent Polythene to decrease water loss before emergence. Thereafter, water was added periodically to levels that allowed normal plant growth in the controls. Each treatment consisted of four replicates except where otherwise stated. Pots were randomized on greenhouse benches.

Pots were placed in a glasshouse in which the temperature did not fall below 16 °C and did not often exceed 20 °C.

Organic materials used to amend soil were obtained as commercial products (W. A.

Lidstone Ltd, Slough, Berks). Rates of application are given as g fresh weight per g dry weight of soil.

In the controls, emergence of seedlings, as appearance of the plumule above the soil surface, was complete 6 to 8 days after sowing seed. If infection occurred seeds and seedlings were killed either before or after emergence. *C. praticola* was assumed to be the cause of death when its characteristic brown infection cushions were seen by microscopic examination of the seed or seedlings (de Silva, 1963). Results are usually expressed as the final stand of seedlings 28 days after sowing seed. Seedlings were rarely killed after this period.

## RESULTS

### *Expt* 1. *Effect of amendments on seedling survival*

In the absence of the pathogen the final stand normally exceeded 80% of seed sown and in eight of the ten treatments it exceeded 90% (Table 1).

Table 1. *Effects of* Corticium praticola* *on numbers of healthy lettuce seedlings after 2 wk incubation with various organic amendments*

| Control No *Corticium praticola* No amendment | Amendment | Contaminated with 3% *C. praticola* culture % amendment | | | |
|---|---|---|---|---|---|
| | | 0 | 0·5 | 1·0 | 2·0 |
| 91·3 ± 2·5 | Grass meal | 46·3 ± 6·8 | 78·8 ± 2·4 | 68·8 ± 7·2 | 26·3 ± 10·9 |
| 97·5 ± 1·4 | Corn meal | 61·3 ± 2·4 | 27·5 ± 11·0 | 2·5 ± 1·4 | 0·0 ± 0·0 |
| 92·5 ± 4·3 | Barley meal | 52·5 ± 9·6 | 18·8 ± 7·5 | 32·5 ± 3·2 | 13·8 ± 5·8 |
| 98·8 ± 1·2 | Molassine meal | 47·5 ± 5·9 | 48·8 ± 5·5 | 42·5 ± 14·8 | 52·5 ± 6·5 |
| 80·0 ± 7·3 | Linseed cake | 53·8 ± 2·4 | 51·3 ± 12·9 | 41·3 ± 8·2 | 0·0 ± 0·0 |
| 93·8 ± 3·1 | Bran | 42·5 ± 10·9 | 55·0 ± 5·4 | 57·5 ± 6·6 | 62·5 ± 1·4 |
| 93·8 ± 2·4 | Fish meal | 26·3 ± 6·5 | 25·0 ± 5·4 | 35·0 ± 3·5 | 0·0 ± 0·0 |
| 81·3 ± 3·5 | Peptone | 11·3 ± 3·1 | 22·5 ± 3·2 | 28·0 ± 8·3 | 13·8 ± 4·7 |
| 90·0 ± 3·5 | Potato starch | 41·3 ± 7·2 | 36·3 ± 4·7 | 32·5 ± 3·2 | 32·5 ± 7·8 |
| 90·0 ± 5·7 | Wood cellulose | 38·8 ± 1·3 | 51·3 ± 6·9 | 46·0 ± 6·2 | 62·5 ± 5·2 |

Figures are mean (%) final stands of four replicates.
* Added to soil 24 h before sowing seeds.

In contaminated unamended soil the final stand was usually about half that in the controls. In two of the treatments it was much less, in the range 11 to 26%. There were no controls in which amended soil alone was used because other experiments had shown that phytotoxicity caused by amendment had abated after 2 wk and that germination and final stands of seedlings were always about equal to those in ordinary soil.

There were substantial increases in final stands with grass meal (0·5%) and wood cellulose (2·0%). There were considerably fewer seedlings with corn and barley meal, linseed cake and fish meal at the higher levels of amendment. Molassine meal, bran, potato starch and peptone had no significant effect.

Grass meal increased final stands at the lowest level used but not at 2·0%. In contrast, wood cellulose gave better stands at higher levels though here the differences were not so striking. Also, with wood cellulose, although there were more seedlings,

they were abnormal in appearance and very stunted. Ten seedlings were taken at random from each wood cellulose treatment 4 wk after sowing seed. Roots were collected after washing seedlings on a 3 mm sieve. The lengths of primary roots were measured from tips to base of hypocotyl. Shoot length was measured from the base of hypocotyl to the apex of the longest leaf and leaf areas were measured with an Eel Unigalvo leaf area measuring machine. Dry weights were taken after drying to constant weight at 80 °C (Table 2).

Table 2. *Effects of wood cellulose on growth of lettuce seedlings*

| Soil treatment | Mean shoot length (cm) | Mean root length (cm) | Mean leaf area ($cm^2$) | Mean dry wt (mg) |
|---|---|---|---|---|
| No *Corticium praticola* | 10·75 | 7·56 | 9·44 | 14·11 |
| *Corticium praticola* (Cp) | 9·71 | 6·57 | 8·96 | 13·01 |
| Cp + 0·5 % cellulose | 4·87 | 5·99 | 1·36 | 3·31 |
| Cp + 1·0 % cellulose | 3·39 | 5·01 | 1·16 | 2·72 |
| Cp + 2·0 % cellulose | 3·07 | 5·10 | 0·44 | 2·35 |

Figures are means (%) for ten seedlings.

Table 3. *Effects of* Corticium praticola, *grass meal and corn meal on numbers of lettuce seedlings surviving after 4 wk*

| | | Contaminated with 3 % *C. praticola* culture % amendment | | | |
|---|---|---|---|---|---|
| Soil only | Amendment | 0 | 1·0 | 2·0 | 3·0 |
| 86·3 ± 3·7 | Grass meal | 35·0 ± 8·8 | 78·8 ± 4·3 | 55·0 ± 18·1 | 27·5 ± 9·2 |
| 90·0 ± 3·5 | Corn meal | 25·0 ± 9·4 | 0·0 ± 0·0 | 1·3 ± 1·3 | 6·3 ± 3·2 |

Figures are mean (%) final stands of four replicates.

Wood cellulose did not much affect the length of roots but drastically decreased lengths of shoots, areas of leaves and dry weights of the seedlings which were also strikingly chlorotic. Surviving seedlings left in the soil for some weeks hardly grew any more and most of them died.

### *Expt 2. Comparison of grass and corn meals*

In this experiment a special study was made of the effects of amendment with grassmeal or corn meal. Final stands of seedlings after 4 wk are shown in Table 3. In contaminated unamended soil only 35 % seedlings survived (86 % in the controls) but amendment with 1 % grass meal increased survival to 79 %. In contrast, corn meal at three concentrations decreased survival from 25 % to 0 %, 1 % and 6 %.

### *Expt 3. Survival of* Corticium praticola *in soil*

Seedlings surviving for about 4 wk were removed from the pots of soil used in Expt 2. Two pots per treatment were then kept uncovered on glasshouse benches for 90 days and two for 180 days and watered periodically to keep moisture contents at levels that would have been suitable for growth of seedlings. The survival of the pathogen was assessed by baiting soil with forty lettuce seeds per pot, 5 mm deep

and removing samples of ten (five from each of two pots) after 12, 36 and 60 h. The forty seeds were evenly spaced and their positions marked with pins so that individual seeds could be recovered without dislodging the others. To test for the presence of *Corticium* each seed was washed in six changes of 15 ml sterile water, dried between sterile filter paper and plated on a medium containing glucose 1·0, peptone 0·5, $KH_2PO_4$ 0·1, $MgSO_4.7H_2O$ 0·05, agar 2·0, g/100 ml, 30 ppm streptomycin and 33 ppm rose bengal (Martin, 1950).

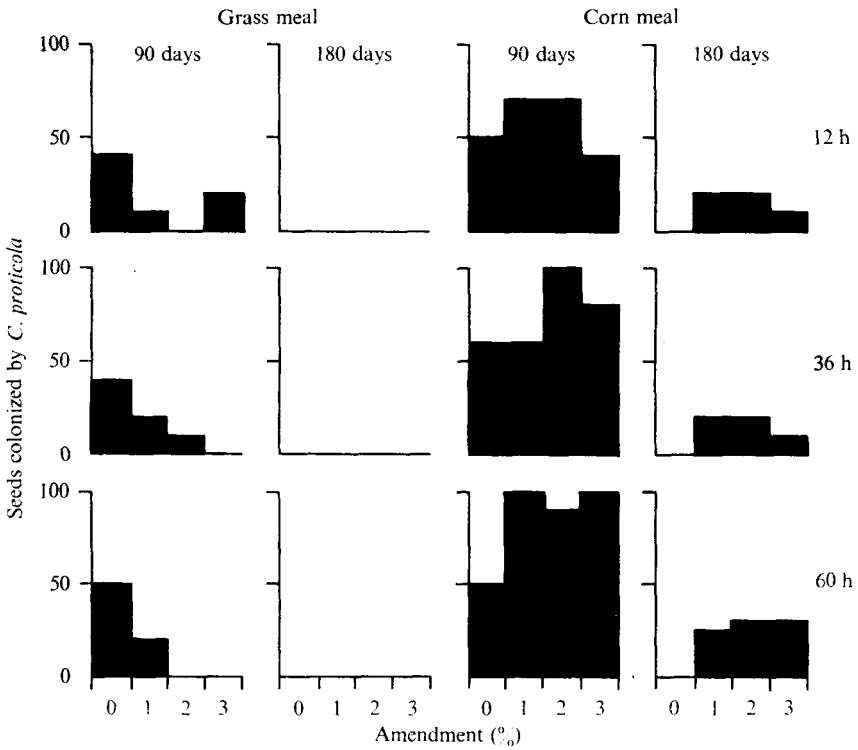


Fig. 1. Persistence of *Corticium praticola* in unamended soil and in soil amended with grass meal or corn meal. All soil contaminated with *C. praticola*, stored for 90 or 180 days and sown with lettuce seed. Ordinates, seed colonized by *C. praticola* as % seed sown, 12, 36 and 60 h after sowing.

After 90 days storage of the soil about half the seeds in unamended soil yielded the pathogen. More seeds did so in soil amended with 1 and 2% corn meal after 12 h, with 2 and 3% after 36 h and with 1, 2 and 3% after 60 h (Fig. 1).

Amendment of the soil with 2% grass meal and storage for 90 days substantially decreased the number of seeds yielding *C. praticola*. With 3% amendment, very few did so. This suggested that the pathogen had been almost eliminated from the soil.

After 180 days storage of the soil, *C. praticola* was not recovered from seed in unamended or grass meal amended soil whereas with corn meal there was *c.* 10–20% recovery at each level of amendment. In the soils stored for 180 days the twenty-five

seeds remaining in each pot were allowed to germinate and all but a few of those in soil that was unamended or amended with grass meal gave healthy seedlings (Table 4). In soil amended with corn meal *c.* 10% of seeds did not grow and *c.* 20% of those that did were killed after emergence.

All the seedlings that died after emergence had characteristic brownish infection cushions of *C. praticola* and each gave colonies of the pathogen when plated on agar.

Table 4. *Percentages of lettuce seedlings surviving 28 days after sowing in soil which had been amended and contaminated with* 3% C. practicola *culture 180 days previously*

| Uninfested and unamended soil | | | Contaminated with 3% *C. praticola* culture % amendment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | | 1·0 | | 2·0 | | 3·0 | |
| E | F | Amendment | E | F | E | F | E | F | E | F |
| 100 | 100 | Grass meal | 98 | 98 | 100 | 100 | 100 | 100 | 98 | 98 |
| 98 | 98 | Corn meal | 100 | 100 | 90 | 64 | 92 | 74 | 90 | 70 |

Figures are means (%) of two replicates each of twenty-five seeds.
E, emergence; F, final stand.

Table 5. *Control of established* Corticium praticola *by grass meal treatment*

| | Mean final stand of seedlings (%) | | | | | |
|---|---|---|---|---|---|---|
| | Uninfested soil | | Infested soil | | | |
| Series* | No amendment | Plus 2% grass meal | No amendment | Plus 1% grass meal | Plus 2% grass meal | Seeds thiram treated |
| 1 | 91·0±3·6 | 4·0±2·4 | 5·0±3·2 | 10·0±4·6 | 9·0±4·3 | 80·0±5·4 |
| 2 | 95·0±1·4 | 95·0±1·5 | 36·0±3·8 | 96·0±2·4 | 95·0±2·4 | 70·0±5·6 |

* Series 1, sown 2 wk after soil infestation and 24 h after amendment; series 2, sown 4 wk after soil infestation and 14 days after amendment.

### *Expt 4. Control of established parasite by grass meal treatment*

The results of the previous experiments indicated that although *C. praticola* would not persist in the natural soil used in this work, it would do so if the soil was amended with corn meal. Use was made of this fact to determine whether grass meal could control established mycelium of the parasite.

Soil was treated with 0·5% corn meal and infested with 3% *C. praticola* culture on corn meal/sand medium. The soil was then thinly spread in a metal tray in the greenhouse, watered occasionally and mixed every 7 days for 2 wk. Uninfested soil was similarly treated. The uninfested soil was then divided into two lots and one was amended with 2% grass meal. The infested soil was divided into four portions; one was amended with 1% grass meal, another with 2% grass meal and two were left unamended. There were thus six lots of soil, each of which was enough to fill eight pots. Four pots of each lot of soil were sown with lettuce seeds 24 h after the amendments had been added (series 1) and four were sown 2 wk later (series 2). One set of four pots containing infested soil with no amendment was sown with seeds dusted with thiram seed dressing

to compare its effect with that of grass meal. The healthy seedlings in each pot were counted 4 wk after sowing.

The low number of surviving seedlings in the uninfested soil to which grass meal had been added shortly before the seeds were sown (Table 5, series 1) shows that the grass meal was causing phytotoxicity at this time. Its effects on the attack by *Corticium* could thus not be assessed. When the seeds were sown 2 wk later, however, the phytotoxicity had disappeared and it was apparent that the presence of grass meal had completely eliminated the effect of the fungus although this was still capable of causing the loss of *c.* 60% of the seedlings in the unamended soil. There were more survivors in the soil treated with grass meal than among those plants grown from seeds treated with thiram and it was also noted that in the presence of grass meal the seedlings emerged more quickly and grew to a greater size than in unamended soil. It was apparent that the presence of grass meal was controlling the effect of the mycelium of the pathogen after it had become established in the soil.

## DISCUSSION

Earlier work on the effect of organic amendments on disease caused by *Rhizoctonia solani* has indicated that those with a high C/N ratio hardly ever increase disease whereas those with a low C/N ratio usually do (Davey & Papavizas, 1959, 1960; Maier, 1959; Papavizas & Davey, 1960). In our experiments, the C/N ratios of various organic materials used were not determined but grass meal and wood cellulose decreased disease, whereas corn meal, barley meal, linseed cake and fish meal reduced the numbers of plants and potato starch and peptone had little effect. Although wood cellulose decreased disease it also caused seedlings to be chlorotic and stunted, possibly through nitrogen deficiency following multiplication of cellulolytic micro-organisms in the amended soil.

The decrease in disease caused by grass meal is of more interest. At higher levels, and in the first 2 wk after treatment of soil, seedlings were damaged, probably in ways similar to those described by Patrick, Toussoun & Snyder (1962) and Toussoun & Patrick (1963) who showed that phytotoxins accumulating after degradation of organic amendments delayed or prevented seed germination. The effects of such toxins declined a few weeks after soil treatment. The fact that there were large increases in survival when seeds were sown 2 wk after amendment with grass meal at 1% dry weight of soil suggests that this would not be a wholly impractical method of control especially if the grass meal were incorporated into soil in a narrow zone where seeds would be sown later. Another advantage of grass meal is that it also increased the rate at which populations of the pathogen decreased in the absence of the host plant. Further studies on amendment with grass meal as a method of disease control seem justified.

The results with corn meal indicated the care that is needed in selecting amendments for biological control which encourage the survival of the pathogen long after it has disappeared from unamended soil.

The grass meal treatment appeared to be somewhat better than thiram seed treatment. Although the seeds treated with thiram showed almost complete emergence,

about 30% of the seedlings damped-off later, presumably because the seed treatment gave inadequate protection to the hypocotyl. On the other hand, the grass meal, being mixed with the entire soil, may have given more general seedling protection.

## REFERENCES

ALLEN, M. C. & HAENSELER, C. M. (1935). Antagonistic action of *Trichoderma* on *Rhizoctonia* and other soil fungi. *Phytopathology* **25**, 244–252.

DAVEY, C. B. & PAPAVIZAS, G. C. (1959). Effect of organic soil amendment on *Rhizoctonia* disease of snap beans. *Journal of Agronomy* **51**, 493–496.

DAVEY, C. B. & PAPAVIZAS, G. C. (1960). Effect of dry manure plant materials and nitrogen on *Rhizoctonia solani* in soil. *Phytopathology* **50**, 522–525.

DE SILVA, R. L. (1963). A comparative study of the pathogenicity of three strains of *Rhizoctonia solani* Kühn. Ph.D. Thesis, University of London.

FLENTJE, N. T. (1956). Studies on *Pellicularia filamentosa* (Pat.) Rogers. 1. Formation of the perfect stage. *Transactions of the British Mycological Society* **39**, 343–356.

MAIER, C. R. (1959). Effect of certain crop residues on bean root rot pathogens. *Plant Disease Reporter* **43**, 1027–1030.

MARTIN, J. P. (1950). Use of acid, rose bengal and streptomycin in the method for estimating fungi. *Soil Science* **69**, 215–232.

PAPAVIZAS, G. C. & DAVEY, C. B. (1960). *Rhizoctonia* disease of bean as affected by decomposing green materials and associated microflora. *Phytopathology* **50**, 516–522.

PATRICK, Z. A., TOUSSOUN, T. A. & SNYDER, W. C. (1962). Substances injurious to plants from decomposing plant residues. *Phytopathology* **52**, 24–25.

TOUSSOUN, T. A. & PATRICK, Z. A. (1963). Effects of phytotoxic substance from decomposing plant residues on root-rot of bean. *Phytopathology* **53**, 265–270.

WEINDLING, R. (1932). *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology* **22**, 837–845.

WEINDLING, R. & FAWCETT, H. S. (1936). Experiments in biological control of *Rhizoctonia* damping-off. *Phytopathology* **24**, 1142.

WOOD, R. K. S. (1950). The control of diseases of lettuce by the use of antagonistic organisms. 1. The control of *Botrytis cinerea* Pers. *Annals of Applied Biology* **38**, 203–216.