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SOME FACTORS ASSOCIATED WITH POOR EMERGENCE AND GROWTH OF LUCERNE SEEDLINGS IN SOILS FROM 'RUNOUT' LUCERNE CROPS

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ABSTRACT

In a series of glasshouse experiments, emergence and growth of seedlings of lucerne (*Medicago sativa* L.) cv. Wairau were studied in soils collected from run-out lucerne crops at nineteen locations in the major lucerne-growing regions of New Zealand. Observations were made on the incidence of seedling failure before and after emergence and growth. The effect of amendment with lucerne residues on emergence and growth was also noted. There was a wide range of seedling emergence among soils and in the amount of seedling loss due to *Pythium* spp. before and after emergence. Seedling emergence and growth were significantly increased after the soil was treated with an air-steam mixture at 60^0 for 30 minutes. It is concluded that many of the soils tested contained organisms which were inhibitory to the early growth of lucerne plants. Addition of lucerne residues to the soils significantly reduced emergence and growth of seedlings. Since old lucerne roots can be a source of fungi pathogenic to lucerne it is recommended that such material be allowed to rot away completely before resowing lucerne.

Additional Key Words: Medicago sativa, Pythium spp., soil pasteurisation, pre- and post-emergence losses, root residues

INTRODUCTION

Establishment of lucerne (*Medicago sativa* L.) throughout New Zealand, measured as a percentage of viable seed sown, is generally poor even under favourable agronomic conditions (Wynn-Williams, 1982) and there are occasional crop failures brought about by pathogens such as *Pythium* spp. (Falloon and Skipp, 1982). When lucerne is sown into old lucerne fields difficulties in establishment frequently occur and the new crop has low numbers of plants and unthrifty growth. In overseas studies, toxic compounds arising from lucerne residues (Klein and Miller, 1980; Webster *et al.*, 1967), and pathogenic fungi (Damirgi *et al.*, 1978), have been suggested as the cause.

This paper reports on a series of glasshouse experiments on factors affecting emergence and growth of 'Wairau' lucerne in soils collected from unthrifty, poor producing ('run-out') lucerne crops in the major lucerne growing areas of New Zealand. There were three objectives:

1) to determine whether the establishment of lucerne in soil from unthrifty lucerne stands is influenced by the disease status of the unthrifty crop;
2) to assess the effects if micro-organisms (especially fungi) on the emergence and growth of lucerne seedlings;
3) to measure the effects of lucerne residues added to soil on emergence and growth of seedlings.

MATERIALS AND METHODS

Soils

Soil was collected at 19 locations throughout New Zealand from crops of 'Wairau' lucerne where the incidence of major diseases such as bacterial wilt (*Corynebacterium insidiosum*) and verticillium wilt (*Verticillim albo-atrum*) was high enough to cause a decline in production (Table 1). In several locations soil was also collected from pasture adjacent to the lucerne crop. The soil samples were stored at 1^0 C. The pH of each soil was measured on three separate samples of 10g dried soil added to 25ml of distilled water and allowed to stand for 24-36 hours before reading.

Experiment 1

The purpose of this experiment was to measure emergence and post-emergence losses of lucerne seedlings in soils from 16 lucerne crops and from pastures adjacent to 6 of those crops. Thirty seeds (laboratory germination, 90%) of 'Wairau' were sown into 3kg of soil held in black polyethylene planter bags. There were three replicates for each of the 22 soils. The planter bags were set out on glasshouse benches in three randomised complete blocks. The soil was kept moist by regular watering. The seedlings which had emerged and had their cotyledons open were counted at 7, 10, 25, and 49 days after sowing. Isolations for fungi were made from seedlings which collapsed after emerging.

Experiment 2

This experiment was designed to assess the effects of fungi in the soil on emergence and growth of lucerne. Emergence and growth of seedlings were compared in untreated and in pasteurised (Baker, 1970) soils. Samples of 15 lucerne soils and 4 pasture soils from Canterbury and Taupo were treated with aerated steam at 60^0 C for 30 minutes in an apparatus built to the design and specification of Aldrich and Nelson (1969). This treatment kills fungi and bacteria and has a minimal effect on the chemical properties of the soil (Baker, 1970; Dawson *et al*, 1965).

Untreated and pasteurised samples of each soil were placed in separate planter bags and 30 seeds of 'Wairau' sown into each bag. There were three replicates of each soil/treatment combination. The planter bags were set out in three randomised, complete blocks on glasshouse benches. Seedling emergence was recored 19 days after sowing. The plants were lifted from the soil after seven weeks and the lengths of the roots and shoots of all plants in each bag recorded. After measurement, the root systems were washed free of soil, surface sterilised for 30 seconds in 1% sodium hypochlorite, rinsed in sterile distilled water and pieces 2-3mm in length placed on a medium of prune agar (Difco) for the isolation of root-inhabiting fungi.

Table 1. Location, soil, age of crop and soil pH for the 19 unthrifty lucerne crops which were sampled for this study.

Location	Soil	Age of crop	soil pH	
		(years)	lucerne	pasture
T17 Wairakei Res. Stat.	Wairakei sand	9	5.0	5.1
T20 Maroa	Oruanui sand	>5	5.3	
T21 Wairakei	Taupo sandy loam	n.r.	5.2	
T24 Broadlands	Whenuroa sand	8	5.8	
T25 Broadlands	Whenuroa sand	6	5.3	4.8
T28 Oruanui	Waipahihi sand	4	5.5	
T29 Tirohanga	Ngakuru-Taupo sandy loam	2	5.2	
T30 Tirohanga	Ngakuru-Taupo sandy loam	>5	4.7	
T31 Tirohanga	Ngakuru-Taupo sandy loam	7	4.8	4.6
T29 Tirohanga	Ngakuru-Taupo sandy loam	6	5.4	4.7
C36 DSIR, Lincoln	Wakanui loam	7	6.5	
C36 DSIR, Lincoln	Wakanui loam	7	6.1	
C38 Winchmore	Lismore stony silt loam	7	6.4	
C39 Greenpark	Motukarara silt loam	7	6.1	
C40 Dunsandel	Lismore v. stony silt loam	7	5.9	
O41 Cromwell	Lochar sandy loam	4	5.3	5.5
O45 Cromwell	Becks	n.r.	6.4	
048 Maniototo	Middlemarch fine sandy loam	n.r.	5.7	
051 Maniototo	Middlemarch fine sandy loam	n.r.	5.6	6.0

n.r. = not recorded

Experiment 3

The effects on emergence and subsequent growth of lucerne seedlings of lucerne residues added to soil were assessed in this experiment. Lucerne residues in the form of dried, ground root tissue from plants from a ten year-old crop were mixed at several different concentrations with soil which was untreated or pasteurised. The amounts of lucerne residues added were chosen to simulate 0, ½, 1, 2, and 4 times the concentrations of lucerne residues found in the top 20cm of soil after a mature lucerne crop had been ploughed and cultivated by rotary hoeing or discing. Calculations on the amount of root tissue present were based on several assumptions:

1) the plant population would be 30 plants/m2,
2) root tissue per plant would be 62.5g (dry weight),
3) the root tissue would be incorporated into the top 20 cm of soil during cultivation.

Thirtyfive seeds (laboratory germination, 80%) of 'Wairau' were sown in planter bags containing 3 kg of untreated or pasteurised soil to which 0, 12½, 25, 50, or 100g of lucerne roots were added. The bags were set out on glasshouse benches in five randomised, complete blocks. Fifteen days after sowing the number of dead and healthy seedlings were counted. Fungal isolations were made from the dead and dying seedlings. Eight weeks after sowing all surviving seedlings in each bag were removed and counted and the shoot length and dry weight of 10 plants were measured.

Analysis

Analysis of variance on either raw or transformed data, was carried out for each experiment.

RESULTS

Experiment

The number of seedlings emerging seven days after sowing varied widely among the soils, ranging from 6 - 18. Laboratory testing of seed viability gave an expected emergence of 27 seeds per pot. In 17 of the 22 soils some seedlings collapsed and died between 7 and 49 days after sowing and this loss tended to be the greatest for soils with the highest counts for seedling emergence at seven days (Figure 1). There were four soils (T25a, T28, C39, and O41) with low seedling emergence at seven days and for two of these, T25a and O41, this reflected pre-emergence failure. The other two soils, T28 and C39, were unusual because seedling

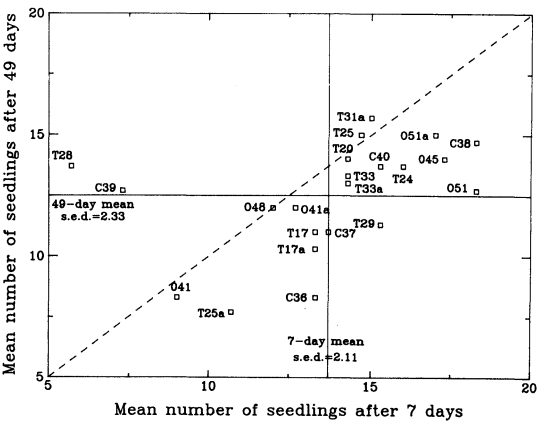

Figure 1. Relationship between the number of lucerne seedlings emerging 7 days after sowing and the number of plants successfully established 49 days sowing in soils collected from lucerne crops and adjacent pasture (a) in Canterbury (C), Otago (O), and Taupo (T). The broken line represents the situation where there is no difference between the 7-day and 49-day figures for a soil. The data for the lucerne crop T31 are missing.

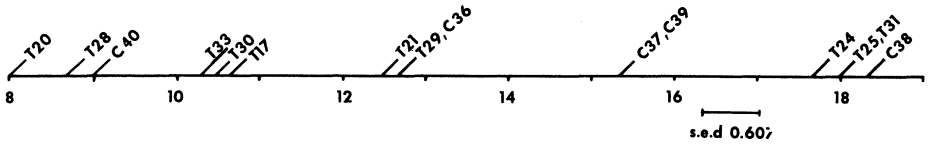

Figure 2. Emergence from 30 seeds of 'Wairau' lucerne in untreated lucerne soils from Canterbury (C) and Taupo (T) 19 days after sowing.

Table 2 Mean number of seedlings from 30 seeds of Wairau lucerne 19 days after sowing in a lucerne soil from an adjacent pastures[1].

Sampling location	Lucerne	Pasture soil
T17	10.7	8.7
T25	18.0	10.7*
T31	18.0	12.0*
T33	10.3	16.3*

[1] Starred figures are significantly ($P<0.05$) different from the corresponding figure for lucerne soil.

emergence was initially delayed and then between day 7 and day 49 approximately doubled to become slightly greater than the mean. The variation in pH from 4.7 - 6.5 among soils did not appear to affect the seedling numbers (Table 1). *Pythium* spp. were the only fungi isolated from seedlings which had collapsed after emergence.

Experiment 2

As for experiment 1, seedling emergence in untreated lucerne soils varied widely, and pots contained 8 - 18 healthy seedlings 19 days after sowing (Figure 2). Had all the viable seed sown emerged pots would have contained 24 seedlings. In all soils tested one to three seedlings died after emergence.

Soil pasteurisation significantly ($P<0.01$) improved seedling emergence in 10 of the 15 lucerne soils tested (Figure 3). There were differences in emergence between untreated soils from lucerne and adjacent pasture for three of the four locations where this contrast was available. Emergence was significantly greater ($P<0.05$) in the soil from lucerne crops at two of these locations and at one location it was greater in soil from the adjacent pasture (Table 2). Pasteurisation significantly improved seedling emergence in soils under lucerne and pasture for three of the four locations and the absence of any interaction between location and soil or soil treatment suggested that the differences between soil from lucerne crops and adjacent pastures were not affected by pasteurisation.

The comparison of shoot growth of plants in pasteurised and untreated soils from lucerne crops revealed that there was a significant ($P<0.05$) positive response to pasteurising in eight soils, and a significant negative response in one soil (Figure 4). There was a significant ($P<0.05$) positive response of root growth of plants in pasteurised soil from five localities (Figure 4). Examination of a scatter diagram showed that there was no obvious relationship between emergence and plant growth.

Fusarium spp. were commonly isolated from roots of seedlings grown in untreated soils, but there was no evidence of necrosis. *Monodictys* spp., *Penicillium* spp., and *Trichoderma* spp. were common on roots of plants grown in pasteurised soils.

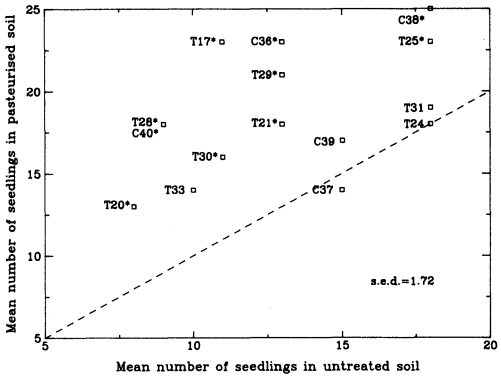

Figure 3. Comparison of the number of lucerne seedlings 19 days after sowing in untreated, natural soils and in the same soils treated to remove soil-borne pathogens by heating them to 60^0 for 30 minutes (pasteurising). The broken line shows the situation where pasteurising has no effect; soils where there was a significant ($P<0.01$) reponse to pasteurising are marked with an *. Thirtyfive seeds were sown.

Table 3 Mean emergence and growth of lucerne seedlings from 35 seeds of 'Wairau' sown in untreated and pasteurised soil amended with lucerne residues.

	Amt. of lucerne residue (g)					Approx standard errors of means[1]
	0	12.5	25	50	100	
Number of seedlings 15 days after sowing						
Pasteurised soil:	31.9	12.9	10.0	8.9	14.5	1.6 - 3.9
Untreated soil:	12.9		7.8		4.5	
Number of plants 8 weeks after sowing						
Pasteurised soil:	31.3	11.7	11.0	14.3	18.8	1.7 - 3.6
Untreated soil:	13.5		11.5		6.5	
Shoot length (cm) 8 weeks after sowing						
Pasteuried soil:	14.0	9.7	9.7	12.3	14.0	1.0 - 1.3
Untreated soil:	12.5		8.6		10.5	
Dry weight per plant (mg) 8 weeks after sowing						
Pasteurised soil;	310	180	200	250	260	20 - 40
Untreated soil:	180		150		130	

[1] Tabulated means are back-transformed after square root or log transformation applied for analysis of variance. The given standard errors apply approximately to the smallest and largest means respectively; other means have intermediate standard errors.

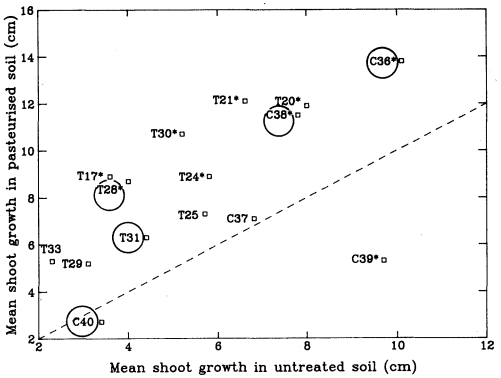

Figure 4. Mean shoot growth of lucerne seedlings in pasteurised soil (heated to 60^0 C for 30 minutes) compared with mean shoot growth in untreated, natural soil 7 weeks after sowing. The broken line shows the situation where pasteurising has no effect; soils where there was a significant (P<0.05) response to pasteurising are marked with an *. Soils marked with a circle showed significantly (P<0.05) greater root growth in pasteurised soil.

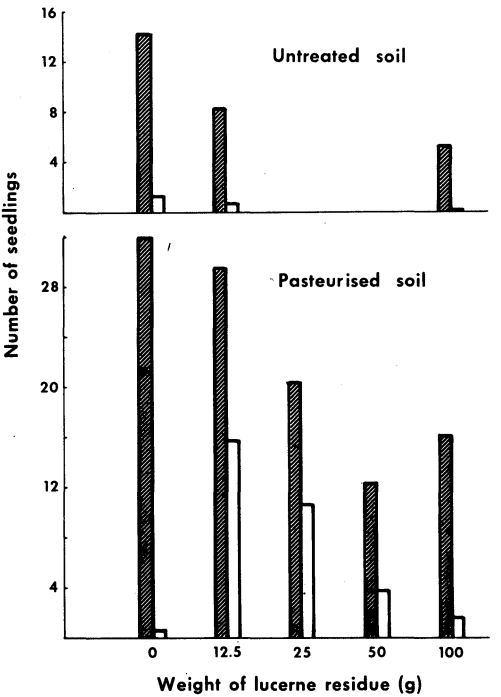

Figure 5. Total number of lucerne seedlings that emerged over 15 days after sowing (■) and the number that collapsed post-emergence (□), when sown in untreated or pasteurised soil amended with different concentrations of lucerne residue.

Experiment 3

Results are summarised in Table 3. Amendment of soil with lucerne residues significantly reduced the number of healthy seedlings present in both pasteurised and untreated soil 15 days after sowing. The reduction was greater in pasteurised soil, where the addition of lucerne residues reduced seedling numbers significantly (P<0.001) from 31.9 in the non-amended soil to a mean of 11.6 seedlings for the amended soils. The addition of lucerne residues to untreated soil significantly (P<0.05) reduced seedling numbers from 12.9 to a mean of 6.1 plants in the two amended soils. In pasteurised soil all 4 concentrations of lucerne residue reduced the number of seedlings similarly, but in untreated soil only the 100g amendment significantly reduced seedling numbers. The proportion of seedlings collapsing after emergence decreased as the amount of lucerne residues increased (Figure 5). There were only slight differences in seedling numbers in all treatments at 15 days and at 8 weeks after sowing.

Eight weeks after sowing shoot length and plant dry weight were measured on ten plants from each planter bag. There was no effect of the number of plants per planter bag on plant growth. Consequently the simple, unweighted plot means were used in the analyses of variance. In pasteurised soil, the length of the shoots were significantly (P<0.05) reduced by amendment with 12.5 and 25g of lucerne residue but were similar to non-amended soil at the two higher concentrations. In non-amended, untreated soil, shoot lengths were similar to those in the non-amended, pasteurised soil and the addition of lucerne residue produced similar effects to those in pasteurised soil. Plant dry weights were more variable than plant numbers or shoot lengths. Plant weights in pasteurised soil, were significantly (P<0.05) reduced by the two lower concentrations of lucerne residue but were not significantly less than the non-amended control at the two higher concentrations. In non-amended, untreated soil plant weights were significantly (P<0.05) lower than those in the corresponding pasteurised soil (in contrast to the result for shoot lengths), and addition of lucerne residue produced no significant changes.

DISCUSSION

In all the untreated lucerne soils tested the potential establishment of viable seed was reduced by at least 25%. Post-emergence losses of seedlings represented between 5 and 15% of viable seed. Losses after emergence were directly attributable to infection of the seedlings by *Pythium* spp. The comparison of emergence in lucerne and pasture soils indicated that the presence of 'decline' diseases in the lucerne crop was not related to poor emergence. In experiment two there was significantly poorer emergence in soil from pastures than in soil from 'runout' lucerne taken from two locations (Table 2). The lack of a relationship between soil pH and emergence was, perhaps, surprising but the short term nature of the experiments meant that any adverse effects of low pH, such as poor nodulation, did not become evident.

For ten of the fifteen soils taken from lucerne stands seedling emergence was increased by pasteurising the soil (Exp. 2) and in eight of these soils less than 50% of the viable seed sown emerged in the untreated soil. Since the pasteurising treatment reduced the numbers of micro-organisms including fungi and other potential pathogens (Baker, 1970) it appears that for many of the soils there was a microbially induced reduction in emergence and early survival. The organisms directly involved were not determined but is is likely that *Pythium* spp. were major causes of the pre-emergence failures as well as the post-emergence losses (Blair, 1971; Close *et al.*, 1982., Falloon and Skipp, 1982).

Pasteurisation improved emergence in three of four pasture soils and differences between soils from the pasture and adjacent lucerne were not affected by the treatment. This suggests that there were non-microbial factors which also affected emergence.

In addition to the beneficial effect on seedling emergence, pasteurising soil increased shoot growth of seedling in seven of the eight soils which had high pre-emergence losses. This suggests that microorganisms in those soils were also restricting seedling growth. Fungal isolations from the roots of seedlings growing in untreated soils showed that *Fusarium* spp. were common, and these fungi may be responsible for restricting the growth of seedlings, even in the absence of root damage.

Amendment of soil with lucerne root tissue significantly reduced the number of healthy seedlings by inducing both pre- and post-emergence losses (Table 3, Figure 5). Pre-emergence losses increased when the amount of lucerne residue, added to the pots, was increased although in pasteurised soil this response appeared to have reached a limit when around 100g of residue was added. The collapse of seedlings after emergence, however, decreased as the amount of residue was increased. There would appear to be no simple explanation for this but is is possible that high pre-emergence failure ensured that only very vigorous seedlings emerged, and that these seedlings were less susceptible to post-emergence attack by pathogenic fungi. The effects of the lucerne residues on seedlings probably occurred because of the presence in them of pathogenic fungi, such as *Pythium* spp., and/or enhanced activity of these organisms in the amended soil. The possibility that toxic compounds in the residues were responsible for the reduction in seedling numbers appears unlikely since, in pasteurised soil, there was no difference in the number of healthy seedlings in soil amended with either 12.5g or 100g of lucerne roots. Similarily, there was no evidence that the growth of established seedlings was affected by any toxicity associated with the lucerne residues.

The evidence presented in this study supports the conclusion that pathogenic fungi were responsible for poor emergence and establishment of lucerne seedlings in many of the lucerne soils tested. Since old lucerne roots can serve as reservoirs of inoculum of fungi pathogenic to lucerne it is advisable to allow sufficient time for this tissue to rot away completely before attempting to resow old lucerne land back to lucerne. *Pythium* spp. clearly are associated with reduced emergence in many soils and treating lucerne seed with a suitable fungicide, such as metalaxyl, before sowing will provide excellent protection against these fungi (Falloon & Skipp 1982). Wherever major decline diseases have been identified in the old lucerne crop a new cultivar which has resistance should be used.

CONCLUSIONS

1. Poor establishment of lucerne seedlings in soils from 'runout' lucerne stands was not related to the presence of diseases in the 'runout' crop.
2. There was a microbially induced reduction in emergence and survival of seedlings in most soils tested.
3. Lucerne residues added to soil reduced the number of healthy seedlings.

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