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Factors affecting stand and nodulation of treated and nontreated forage legumes

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FACTORS AFFECTING STAND AND NODULATION OF TREATED AND NONTREATED FORAGE LEGUMES

by

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INTRODUCTION

Establishment of small seeded legumes in the field is of major concern to the farmers of Iowa. The number of seeds planted is up to ten or more times the number of plants that are ultimately established. One possible reason for poor establishment of these legumes if the seed and seedling rots incited by several species of Pythium (5, 7, 9, 22, 23, 24, 28, 37, 60). The role of these diseases undoubtedly depends upon several factors such as abundance and pathogenicity of the causal fungi in the soil, susceptibility of the host, soil moisture and temperature.

Two small seeded legumes are relatively new to Iowa agriculture, i.e. lespedeza (Lespedeza stipulacea Maxim) and birdsfoot trefoil (Lotus corniculatus L.). Lespedeza is a self-reseeding annual which is readily established without extreme care in original seeding or soil preparation. Seedings of birdsfoot trefoil, on the other hand, frequently result in failure of the stand establishment. The possible role of seed and seedling rot by Pythium in failures of trefoil seedings has not been evaluated.

Chemical treatment of small seeded legumes for the control of seed and seedling rot is not widely practiced. Seed treatment has increased original seedling stand in most cases but there is conflicting evidence that final field stand is increased. (4, 9, 18, 25, 26, 28, 30, 34, 61, 62).

Furthermore, very little is known about the limitations of environmental factors upon the effectiveness of seed treatment.

It has been suggested that seed treatment chemicals are deleterious to nodule bacteria commonly applied at the time of planting or already present in the soil (2, 14, 33, 55, 57). On the other hand, there is some evidence that the fungicides used for seed treatment are not inhibitory to nodule development if inoculation with nodule bacteria is carried out at the time of planting or if treated seeds are planted in soil in which the proper nodule bacteria are already present (1, 6, 7, 10, 28, 31, 33, 49, 50, 61, 62).

Usual seed treatment of legumes and other seeds consists in application of fungicide for protection of the germinating seed but not in quantities sufficient to protect seedlings from infection by soil-borne fungi. The application of large amounts of fungicides to legume seeds in a manner similar to that of pelleting onion seeds with Arasan (44, 52, 53, 54) is so far known to have been tried only by Kernkamp (35, 36). Conceivably, such pelleting of legume seeds with a substantial amount of fungicide might measurably improve the final stand.

REVIEW OF LITERATURE

Pythium debaryanum Hesse has been reported to cause extensive damage to forage legumes (Beach, 5; Buchholtz, 9; Hey, 27; Jackobs, 28; Kilpatrick et al, 37; Kreitlow et al, 39; McDonald, 45; Nakayama, 51). Halpin et al (23, 24) working with alfalfa, sweet clover, red clover and ladino observed that *P. debaryanum* Hesse, *P. ultimum* Trow, *P. splendens* Braun and *P. irregulare* Buisman were the principal cause of seed rotting, while *P. rostratum* Butler, *P. arrhenomanes* Drechsler and *P. paroecandrum* Drechsler were slightly pathogenic. Among the pathogenic species *P. debaryanum* and *P. ultimum* caused somewhat more seed rotting than *P. splendens* and *P. irregulare*. Furthermore they pointed out that of the four legumes tested, alfalfa and sweet clover were most susceptible, red clover the least, and ladino was intermediate. The difference in susceptibility between alfalfa and sweet clover was negligible. Gregory et al (22) found that *P. debaryanum* and *P. ultimum* caused much damping off of alfalfa. They and McDonald (45) observed that the resistance of alfalfa seedlings to damping off increased with age. Straby and MacNeill (60) have reported that *P. ultimum* caused extensive damping off of red clover in sandy soil. Jacks (29) observed damping off of alfalfa in the field to be due mainly to *P. ultimum*.

Pythium remains alive and virulent for a long time in wet soil but loses its virulence quickly in dry soil (Beach, 5; Gerdemann, 17; Graham et al, 20; 21; Kadow and Anderson, 31; Kreitlow et al, 39). In experiments by Jones (30) germination of pea seed was greatly reduced by application of water immediately after planting but affected little if water was applied 24 to 48 hours after planting. On the contrary, McNew (46) found more disease if the application of water was delayed because the ungerminated pea seeds remain in contact with soil fungi for longer time before emergence.

Temperature plays an important role in seed rot and seedling necrosis of many crops. The lower temperatures which are unfavorable to host as well as parasite are, therefore, less favorable to disease development (Leach, 41). Temperatures below 15° C and above 30° C are generally less dangerous in respect to development of seedling disease because the Pythium is checked or grows slowly at such temperatures (Beach, 5; Buchholtz, 7; Gerdeman, 18; Halpin et al, 23; 24; Hanson, 26; Kadow and Anderson, 31). Ledingham (42) has reported that the effect of temperature is partly dependent on host and that garden and field peas differ in their temperature requirement. Nakayama (51) observed severe attack by P. debaryanum and P. ultimum on alfalfa with moderate watering at 8°-10° C as well as at 25°-30° C. On the contrary, Jackobs (28) found no significant differences in emergences of alfalfa, red clover and sweet clover in the range of 7°-22° C.

Leach (41) has concluded that "in all combinations of host and parasite, the ratio between the coefficient of velocity of seedling emergence and growth rate of the pathogen is inversely related to the severity of infection." That is, the disease is severe when the ratio, (growth rate of host)/(growth rate of pathogen) is less than 1. The probability of disease is reduced as the ratio increases from 1 to 4 and there is little or no disease if the ratio is above 4. This indicates why there may be more seed rotting of high temperature crops at low temperatures and vice versa. Graham et al (20, 21) have reported that with ladino, seed rotting was severe at 80°-94° F and damping off was severe at 59°-73° F. With lespedeza they found that seed rotting was not so high at 52°-66° F but was greater at 59°-87° F. None of the fungi caused damping off at 94° F. In results somewhat different from those of Leach, they could not correlate consistently the ratio, (velocity of emergence)/(growth rate of the pathogen), either with seed rotting or with damping off.

Relation of soil acidity to a seedling disease of alfalfa was first studied by Buchholtz (7). It was found that damping off was greater in acid soil than on similar neutral soil (Buchholtz, 5, 7). Kreitlow et al (39), on the other hand, reported that the soil reaction had very little effect on emergence of seedlings. Kadow and Anderson (31) found that the soil type and acidity were not important factors in either seed rotting or damping off of tomato and spinach in the greenhouse, and under field conditions the disease was more prevalent in heavier soils high in humus than in sandy soils. Fulkerson (16) suggested that the soils from different locations might have organisms varying in amount and pathogenicity. On this basis, he suggested that rich soil may give higher stand than poor soil from the same vicinity, but this may not be true for soils from different locations.

Jones (30) observed that treating of pea seeds with Uspulun and Semesan consistently resulted in higher germination irrespective of the effects of moisture and temperature. Contrary to this, Buchholtz (9) did not recommend seed treatment due to inconsistent increases in stands of alfalfa and to possible toxic effects of fungicides on nodule bacteria. He reported (7) increases in stand of alfalfa in field from 8 to 145 per cent depending upon the degree of infestation of soil. Vlitos and Preston (62) have found Phygon and Arasan to increase the stand of alfalfa significantly. Phygon was found to be superior to Arasan while Ceresan M proved to be injurious to all the legumes that were treated. Ledingham (42) reported that Ceresan M and Semesan were slightly better than Arasan, Spergon and DuBay 1452 F. In greenhouse and in field tests Kernkamp (35, 36) found consistent increases in stands of alfalfa, but increases in stands of red clover and sweet clover were not consistent. Mead (47) and Jacks (29) have reported that seed treatments did not completely prevent damping off. Sherwin et al (58) observed more consistent increases in stand from seed treatment with Arasan than from seed treatment with either New Improved Ceresan or Spergon. Furthermore, they observed that treated seeds responded better at 25° C than at any other temperature. On the other hand, Hanson (26) reported that fungicides increased the stand of alfalfa, sweet clover and red clover regardless of temperature.

Fulkerson (16) found Leytosan P and Ceresan M to be promising fungicides and suggested that Vancide 51 applied as a spray to the soil and planting treated seeds may be beneficial at least in the greenhouse. A seed dressing of Leytosan P was reported by Straby and MacNeill (60) to be effective against Pythium ultimum.

Hanson (25) found no significant increase in stand of alfalfa, red clover, or sweet clover from seed treatment with Arasan, Phygon or Vancide 51, in silt loam, loamy sand or sand. In muck soil the stand was increased by these fungicides. Other fungicides, namely Ceresan, Fermate, New Improved Ceresan, Semesan Jr., and Spergon did not increase the stand significantly in Miami silt loam.

Kernhamp (35) observed response to seed treatments by alfalfa, red clover and sweet clover under conditions adverse for germination or with seed of poor quality. Athow and Caldwell (4) found that treatment with Arasan improved emergence of poor quality pea seed, with significant increases in yield in some instances. Athow (3) recently found that the poor germinating as well as better germinating seeds of alfalfa and red clover were equally benefited by seed treatment.

Allison and Torrie (1) observed that in the field no fungicide significantly increased the stand of alfalfa, sweet clover, red clover, alsike, white clover and strawberry clover. In a field test Tyler et al (61) found no increase in stand or yield of alfalfa, trefoil and red clover from treatment with Arasan, Phygon or Orthocide-75. Athow (3) reported significant increases in stand from treatment of some seed lots of alfalfa and red clover, but no significant increases in yield. Seed samples of a given legume have been reported to respond differently to seed treatment (Kernkamp, 34; 35; Mead, 47).

In laboratory tests, Allison and Torrie (1) observed no injurious effects of New Improved Ceresan, Arasan and Spergon on germination and seedling stands of alfalfa, sweet clover, red clover, alsike, white clover and strawberry clover. Though Spergon retarded germination, it did not reduce the total germination of strawberry clover and ladino seeds. There was no retarding effect of Spergon on alsike, red clover, alfalfa and white clover. In humus soil in greenhouse and in field they found no damping off of strawberry clover and ladino, but the total emergence was significantly reduced in compost known to be infested with damping off organisms. Kreitlow et al (39) observed no injurious effects of New Improved Ceresan, Spergon, Arasan, Semesan or Cuprocide on germination of alfalfa and red clover in steamed soil, but treatments increased seedling stands in soil infested with *Pythium*.

Gerdemann (17) found no significant increase in stand from seed treatment in wet or dry soils. Treatments, which seemed to be harmless in wet soils were found to be toxic in dry soil. Arasan and lower doses of Ceresan M increased the stand of sweet clover in the field under dry conditions. Most of the treatments had no injurious or beneficial effects on alfalfa and the effects were similar in dry and wet soils.

Nakayama (51) flooded pots planted with treated and nontreated seeds of alfalfa at various times after planting and for varying lengths of time (24 to 72 hours). The stand from treated and nontreated seeds was less when the pots were flooded 36 hours after planting than when flooded immediately after planting or 72 hours after planting. The injury was more as the flooding time was increased. Dimond and Horsfall (13) have reported that organic fungicides are more effective in areas with low rainfall than in those of high rainfall. Kreitlow et al (39) observed that increase in soil moisture increased damping off but the stand increased with seed treatment even in saturated soil.

Mercurial fungicides have been reported to be more toxic and injurious to seedlings than other fungicides (Gerdemann, 17; Hanson, 25; Mead, 47; Vlitos and Preston, 62). Kreitlow et al (39) and Leukel (43) found Ceresan M as effective as New Improved Ceresan but less toxic.

Chilton and Garber (11), Kreitlow (38) and Rose (56) have reported that seed treatments increase the seedling stand of legumes and suggested that a proper fungicide may advantageously be applied as a seed treatment in some cases.

Duggar (14) recorded that treatment of peanut seed with disinfectants but without addition of legume nodule bacteria reduced nodule formation. Buchholtz (7) found nodules on roots of plants grown from treated and inoculated seed. Kadow et al (32) have suggested that if the legume bacteria are well established in the soil then treated seeds may be grown in it without interference with nodulation, but the soil should not be allowed to become too acid. Appleman (2) observed that the seeds of peas and soybeans treated with Semesan and inoculated with nodule bacteria, when planted, developed seedlings with satisfactory nodules. Cuprocide prevented nodulation and Ceresan reduced nodulation on canning peas but not on soybeans. Burton and Erdman (10) showed that cultures of *Rhizobium leguminosarum* mixed with Spergon when applied to pea seeds incited good nodule development in spite of some killing and inhibitive effects of Spergon on the nodule bacteria. Allison and Torrie (1) found no inhibition of nodules with either New Improved Ceresan, Arasan or Spergon. Miller (49) reported that seedlings from shelled seeds of peanut treated with Spergon showed no lack of nodulation. Kernkamp (33) found nodules equally well developed on soybean seedlings from seed treated with Spergon, New Improved Ceresan or Semesan Jr. and planted in nonsteamed field soil regardless of infestation by nodule bacteria or time of infestation. However, in steamed soil Spergon inhibited nodulation.

Vlitos and Preston (62) and Kernkamp (34, 36) found no inhibition of nodulation when seed treatments were followed by inoculation with nodule bacteria. On the other hand Vlitos and Preston observed more nodules on seedlings from treated than from nontreated seed in case of alfalfa and red cow-pea. Brentzel (6) suggested that the inoculum should be applied not more than an hour before sowing as the Spergon and Arasan are not inhibitory to nodule bacteria within this time. Milthorpe (50) observed that good nodules were formed on all plants from seed sown immediately after inoculation, irrespective of whether or not the seed was treated with fungicide. If the interval between inoculation and planting was up to two hours, then nodules were developed on roots from seeds treated with Spergon. Ceresan, Cuprocide and Tetrachloro-p-benzoquinone inhibited the development of nodules when seeds were sown two hours after inoculation. No nodules were developed on roots from treated seeds planted six hours after inoculation. Jacks (29) found that Dichlone, Captan, Thiram and Ferbam were not toxic to _R. meliloti_ when the seed was sown immediately after infesting with nodule bacteria.

Ruhloff and Burton (57) found that Arasan and Arasan SF were more toxic to species of _Rhizobium_ than Phygon, Spergon and Spergon DDT SL. Spergon had a toxic effect on alfalfa, pea and bean nodule bacteria. Phygon was moderately toxic to species of _Rhizobium_ except that of beans. Peterson and Buchholtz (55) have reported that Ceresan M was highly inhibitory to _R. meliloti_ and _R. japonicum_ in culture; Spergon was least toxic and Arasan intermediate. Mead (47) has concluded that nonmercurial fungicides were least toxic to bacterial inoculum.

Pelleting onion seeds with Arasan, Fermate and Thiosan to control smut was described by Newhall (52, 53). The field survey of smutted seedlings showed that the smutted seedlings from pelleted seeds and formaldehyde treatment were 24 per cent less than from nontreated seeds. He reported also (54) that Tersan and Orthocide were effective as pelleting materials. The Michigan method described by Linn, as reported by Linn and Newhall (44), results in large, hard, uniformly spherical pellets. They found neither better control nor higher stand from the Michigan method than from the inexpensive New York method of pelleting described by Newhall. On the contrary, the results were in favor of the New York method. The role of methocel sticker on the effectiveness of Arasan has been determined by Gorenz and Walker (19). They have found that the stand of onion seedlings though high is variable with 1/10 lb Arasan to 1 lb of seed without sticker, while 1/4 lb Arasan to 1 lb of seed in conjunction with sticker significantly increased the stand and the results were uniform.

Kernkamp (36) pelleted the seeds of alfalfa, red clover and sweet clover with 10 per cent fungicide and 5 per cent aqueous solution of methyl cellulose. He obtained better control of damping off with Arasan and Spergon than with other fungicides. Dow 9 B was extremely injurious to all legumes. However, in the field he found (35, 36) no advantage for pelleting over the usual seed treatment.

EXPERIMENTS

Isolation and Identification of Pythium Species Associated with Seed Rotting

Isolates of *Pythium* were taken from rotted seeds of alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), birdsfoot trefoil (*Lotus corniculatus* L.), corn (*Zea mays* L.) and milo (*Sorghum vulgare* L.) collected from soil in the greenhouse bench. The rotted seeds were first thoroughly washed with distilled water. The washed seeds were placed under sterile solidified 1.5 per cent Bacto-agar medium, which was about 3mm thick, in sterile Petri plates. Bacto-agar is slightly more acidic than unpurified agar and has less digestible free organic matter, which limits multiplication of bacteria and growth of fungi other than *Pythium*. Since it is almost transparent, the plates can be examined under microscope at 100X without being opened, thus minimizing air borne contaminations.

Placement of rotted seeds under the agar was accomplished by first lifting a side of the agar with a small spatula under aseptic conditions and placing with sterile forceps the rotted, washed seed at the bottom of the Petri plate, then removing the spatula and allowing agar to resume position on Petri plate on top of seed. Two seeds were thus placed on opposite sides of each plate. If the seeds were infected by *Pythium*, the hyphae grew through the agar and reached the upper surface within 24 hours at room temperature, leaving behind bacteria and nematodes that might have been present on or in the seeds. Fungi other than *Pythium* grew more slowly; hence the transfer of hyphal tips usually resulted in pure cultures. Isolation plates were examined after 3 to 5 days and it was observed that most of the cultures developed either spherical bodies or sporangia, oogonia and antheridia in the agar. The organisms thus examined were classified according to Middleton (48) as under:

Pythium sp.	135
P. mamillatum Meurs	21
P. rostratum Butler	20
P. aphanidermatum (Edson) Fitzpatrick	6
P. graminicolum Subramaniam	4
P. catenulatum Matthews	3
Total	189

Of these 189, two isolates each of *Pythium* sp. and *P. mamillatum* and one isolate each of *P. rostratum*, *P. aphanidermatum*, *P. catenulatum* and *P. graminicolum* were maintained as pure cultures on Bacto-agar for further observations and experimentation. This stock of cultures was supplemented with *P. debaryanum* isolated by Dr. A. L. Hooker from corn embryo tissue, *P. torulosum* Coker and Patterson isolated by Dr. L. H. Tiffany from roots of blue grass, and isolates 98A and 98B taken by Dr. E. F. Vestal from roots of corn.

In a preliminary test for determining the pathogenicity of these cultures, the seeds of alfalfa, red clover, sweet clover, white clover and trefoil were planted on four or five days old agar plate cultures. Pathogenicity of the cultures was determined on the basis of non-germinated and germinating seeds which were rotted before the hypocotyl elongated. Among the twelve isolates tested, Pythium aphanidermatum, P. debaryanum and Pythium sp. were found to be highly pathogenic. Hence they were preferred to other cultures for testing degree of susceptibility of the forage legumes.

The isolates of Pythium sp. which had not developed oogonia and antheridia on isolation plates were grown on potato dextrose, corn meal, oat meal, lima bean and carrot agar and plates of Bacto-agar with a seed of linseed, kenaf or hemp. In all such preparations only spherical bodies* were formed. These structures were suspended in a water drop and incubated at about 10° to 15° C for 1 to 4 hours and then examined at room temperature. No vesicle or zoospores were observed; on the other hand, these bodies were found always to germinate by one or two germ tubes. These isolates are, therefore, referred to as Pythium sp.

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*According to Middleton (48), "asexual reproductive bodies which do not give rise to spores cannot be termed sporangia."

Isolates were taken from rooted seeds collected during the course of experimental work by the method described before. Among these isolates Pythium sp. was predominant in all soils (Table 1), except that from rotted seeds in Wabash loam from Warren County, P. aphanidermatum was isolated almost exclusively. P. aphanidermatum has been isolated from Wabash loam from Warren County and Webster loam from Ames, both of which are rich in organic matter. These soils differ in pH, the former being slightly acidic and latter being slightly alkaline. P. ultimum was abundantly isolated from Webster loam, with pH 7.4, Clarion loam, with pH 6.1 and from the greenhouse soil mixture, with pH 6.9, and to a lesser extent from Tama silt loam, with pH 5.6. These four soils varied widely in pH.

It is interesting to note that P. debaryanum, which is generally considered to be the principal cause of seed and seedling rots, was isolated much less frequently than Pythium sp., P. aphanidermatum and P. ultimum. P. irregulare was isolated almost exclusively from seeds collected from Tama silt loam, with pH 5.6. P. torulosum and P. paroecandrum were least isolated and only from seeds collected from Tama silt loam with pH 5.6 and Clarion loam, with pH 6.1, respectively.

Table 1. Frequency of Pythium species in isolations from seeds planted in soil samples* from central Iowa

Source of soil sample	Ames		Warren County			Greenhouse mixture	Total
Soil type	Clarion loam	Webster loam	Tama silt loam		Wabash loam		
pH	6.1	7.4	5.6	5.7	5.8	6.9	
Pythium sp.	14	14	14	25	1	42	110
P. ultimum	14	10	5	-	1	9	39
P. aphanidermatum	1	8	-	-	26	-	35
P. debaryanum	2	1	1	4	1	7	16
P. irregulare	-	-	8	-	-	1	9
P. torulosum	-	-	1	-	-	-	1
P. paroecandrum	1	-	-	-	-	-	1
Other fungi	1	-	-	-	-	1	2
Total	33	33	29	29	29	60	213

*Other details regarding soil samples are in Section II.

Susceptibility of Seven Legume Species to *Pythium* on Petri Plate Cultures

The following small seeded legumes are grown for forage in Iowa: alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), alsike clover (*T. hybridum* L.), white clover (*T. repens* L.), yellow sweet clover (*Melilotus officinalis* (L.) Lam.), birdsfoot trefoil (*Lotus corniculatus* L.) and Korean lespedeza (*Lespedeza stipulacea* Maxim.). Two seed lots of each of these crops with good germination were supplied by the Iowa State College Seed Laboratory, which also provided the results of the laboratory germination test of each seed lot. These seed lots were used in subsequent experiments concerned with comparison of susceptibility of these seven legumes to seed rotting and seedling necrosis under a variety of environmental conditions, with possible control by seed treatments and with possible deleterious effects of seed treatments on nodulation.

Statistical analysis

Most of the statistical analyses of data have been according to the methods described by Snedecor (59). Multiple range tests have been by the method described by Duncan (15). Standard errors for all interactions have been determined by methods described by Cochran and Cox (12).

Relative susceptibility to seed rotting

One hundred seeds of a given seed lot were planted on non-nutrient agar in a Petri plate in which there was four or five days growth of *Pythium*. Such plates were then incubated at room temperature for five days, after which extent of rotting of each seed was recorded, by number as follows:

Healthy........................	1
Small necrotic spot on radicle...	2
Radicle $<$ .5 cm necrotic........	3
Radicle $>$ .5 cm necrotic........	4
Entire seed rotted...............	5
Hard seeds......................	1

The experiment consisted of two replications. Each replicate contained fourteen "plots", one for each seed lot. A "plot" was comprised of a Petri plate each of *Pythium* sp., *P. aphanidermatum*, *P. debaryanum* and no-culture. These four "treatments" were randomized within "plots" and "plots" were randomized within replications.

The average seed rotting indices for two replications of two seed lots of the seven legumes for each of the three Pythium species and the no-culture check are presented in Table 2. The analysis of variance presented in Table 3 showed that the differences among the legumes was highly significant. The mean indices of each legume were further

Table 2. Seed rotting indices of two seed lots of seven legumes on Petri plate cultures of three Pythium species

	Seed lots	Pythium sp.	P. aphanidermatum	P. debaryanum	No culture (check)
Alfalfa	1	3.61	3.54	3.65	1.17
	2	3.33	3.47	3.53	1.17
Red clover	1	2.85	3.20	3.10	1.15
	2	3.01	3.09	3.08	1.26
Sweet clover	1	3.41	3.33	3.39	1.13
	2	4.39	4.42	4.41	1.46
Alsike clover	1	3.10	3.07	3.28	1.16
	2	3.55	3.62	3.58	1.43
White clover	1	4.03	3.93	4.16	2.77*
	2	3.12	3.13	3.08	1.34
Trefoil	1	3.43	3.28	3.32	2.29*
	2	3.16	3.18	3.38	1.88*
Lespedeza	1	2.23	3.44	2.90	1.46
	2	2.55	3.42	3.26	1.54

*High seed rotting index seems to be due to delayed germination.

tested by multiple range test to determine the relative susceptibility of the legumes (Table 4). It is evident that of the seven legumes sweet clover and white clover were equally and significantly most susceptible, red clover and lespedeza were equally and significantly least susceptible, and

Table 3. Analysis of variance of seed rotting indices of seven legumes on Petri plate cultures of three Pythium species

Source of variations	Degrees of freedom	Mean square
Replicates	1	382.58
Legumes	6	10620.21**
Seed lots within legumes	7	11844.72**
Error (a)	13	175.52
"Treatments"	3	245867.25**
"Treatments" x legumes	18	3610.04**
"Treatments" x seed lots within legumes	21	456.60
Error (b)	42	104.84

**Significant at 1 per cent level.

alfalfa, alsike and trefoil were nearly equal and intermediate in susceptibility.

Since the "treatments" x legumes interaction was highly significant (Table 3), the mean indices of each legume for individual Pythium species were further tested by multiple range test to determine the relative susceptibility of legumes to individual Pythium species. The test (Table 5) showed that lespedeza was significantly least susceptible to Pythium sp. and sweet clover was significantly most susceptible to Pythium sp. Red clover was significantly more susceptible than lespedeza but was significantly less susceptible than trefoil, alsike, alfalfa, white clover and sweet clover.

Table 4. Comparisons among mean seed rotting indices of seven legumes, by multiple range test

a) Shortest Significant Ranges:

P:	(2)	(3)	(4)	(5)	(6)	(7)
R_p(5%):	10.13	10.63	10.92	11.09	11.19	11.29

b) Results:

Legumes:	R.c.	Les.	Als.	Alf.	Tref.	W.c.	S.c.
Means:	259.44	260.19	285.06	293.44	299.06	319.63	324.38

NOTE: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.

R.c. - Red clover
Les. - Lespedeza
Als. - Alsike clover
Alf. - Alfalfa
Tref. - Trefoil
W.c. - White clover
S.c. - Sweet clover

Table 5. Comparisons among mean seed rotting indices of legumes for three Pythium species and no-culture check, by multiple range test

a) Shortest Significant Ranges :

P:	(2)	(3)	(4)	(5)	(6)	(7)
R_p(5%):	31.66	33.32	34.32	35.09	35.64	36.20

b) Results:

Pythium sp.	Legumes:	Les.	R.c.	Tref.	Als.	Alf.	W.c.	S.c.
	Means:	239.25	293.00	329.50	333.00	347.50	357.50	390.25
P. aphanidermatum	Legumes:	R.c.	Tref.	Als.	Les.	Alf.	W.c.	S.c.
	Means:	315.00	323.25	334.75	343.50	349.75	353.25	387.75
P. debaryanum	Legumes:	Les.	R.c.	Tref.	W.c.	S.c.	Als.	Alf.
	Means:	308.00	309.25	335.00	362.25	390.00	393.00	559.25
Check (No culture)	Legumes:	Alf.	R.c.	S.c.	Als.	Les.	W.c.	Tref.
	Means:	117.25	120.50	129.50	129.50	150.00	205.50	208.50

NOTE: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

Table 6. Comparisons among mean seed rotting indices for three Pythium species, by multiple range test

a) Shortest Significant Ranges:

	(2)	(3)	(4)
P:	(2)	(3)	(4)
$R_p(5\%)$:	20.71	21.79	22.44

b) Results:

Cultures:	No culture	P. sp.	P. deb.	P. aph.
Means:	151.54	327.14	343.82	343.89

(327.14, 343.82 and 343.89 are underscored by the same line.)

NOTE: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.

P. sp. - *Pythium* sp.
P. deb. - *Pythium debaryanum*
P. aph. - *Pythium aphanidermatum*

The differences among the mean indices of trefoil, alsike, alfalfa and white clover were intermediate and not significant.

Similarly red clover was significantly less susceptible to *P. aphanidermatum* than white clover and sweet clover. The differences among the mean indices of red clover, trefoil, alsike, lespedeza and alfalfa, and among the mean indices of trefoil, alsike, lespedeza, alfalfa and white clover did not differ significantly less susceptible than sweet clover.

Alfalfa was significantly most susceptible to *P. debaryanum*, and lespedeza and red clover were significantly least susceptible. The differences between the mean indices of trefoil and white clover, and among the mean indices of white clover, sweet clover and alsike were not significant and they were intermediate in susceptibility.

Since differences among the "treatments" was highly significant (Table 3), the "treatments" mean indices for legumes were tested by multiple range tests. The analysis (Table 6) indicated that the three Pythium species were significantly pathogenic to all legumes. Although P. aphanidermatum and P. debaryanum appear to be more pathogenic than Pythium sp., the differences among the mean indices for the three cultures were not significant.

Comparisons between seed rotting indices for the two seed lots of each legume indicated that the differences between seed rotting indices of the two seed lots of sweet clover, alsike and white clover were highly significant, the differences between seed rotting indices of the two seed lots of trefoil and lespedeza were significant at the 5 per cent level, and for alfalfa and red clover there was not a significant difference between the two seed lots.

Relative susceptibility to seedling necrosis

Clorox treated seeds of the two seed lots of each legume were germinated on sterile moist filter paper in Petri plates. At the first leaf stage, five seedlings from each seed lot were transferred aseptically to the surface of five day old agar plate cultures of three Pythium species and of no-culture agar plates. While transferring the seedlings all possible care was taken to avoid injury to seedlings. After three days each seedling was scored for amount of disease, as follows:

Healthy....................	1
Root tip necrotic..........	2
Entire root necrotic.......	3
Entire root and part of hypocotyl necrotic....	4
Entire seedling necrotic...	5

There were two replications, each consisting of seven split plots according to legumes. Each split plot contained four sub-split plots for four "treatments" (three Pythium species and no-culture check). Each sub-split plot was one of two seed lots of the same crop. Sub-split plots were randomized within the split plot and split plots were randomized within the replicate.

The average necrosis indices for two replicates of two seed lots of the seven legumes and for each of three Pythium species are presented in Table 7. The analysis of variance presented in Table 8 showed that the differences among the seedling necrosis indices of legumes were significant at the 5 per cent level. The mean indices for the seven legumes were further tested by multiple range test to determine their relative susceptibility seedling necrosis (Table 9). The

Table 7. Seedling necrosis indices for two seed lots of seven legumes on Petri plate cultures of three Pythium species

	Seed lots	*Pythium* sp.	*P. aphanidermatum*	*P. debaryanum*	No culture (check)
Alfalfa	1	3.5	4.7	4.2	1.0
	2	2.6	3.1	3.6	1.5
Red clover	1	3.4	5.0	4.4	1.1
	2	2.7	4.3	3.6	1.0
Sweet clover	1	4.2	5.0	2.7	1.0
	2	4.4	4.9	4.6	1.6
Alsike clover	1	3.5	4.7	4.1	1.4
	2	3.3	3.4	4.4	1.1
White clover	1	3.9	4.9	4.2	1.0
	2	3.1	4.6	3.2	1.2
Trefoil	1	3.7	4.9	3.6	1.0
	2	3.7	4.6	4.5	1.0
Lespedeza	1	2.3	3.4	3.0	1.5
	2	2.4	3.1	2.4	1.1

test showed that the mean index for lespedeza was significantly lower than those for any other legume except alfalfa, the mean indices for sweet clover were significantly higher than for any other legume and the mean indices for alfalfa and red clover, for red clover, alsike and white clover, and for alsike, white clover and trefoil were nearly equal and intermediate.

Table 8. Analysis of variance of seedling necrosis indices of seven legumes on Petri plate cultures of three Pythium species

Source of variations	Degrees of freedom	Mean square
Replicates	1	18.08
Legumes	6	54.03*
Error (a)	6	8.27
"Treatments"	3	1323.01**
"Treatments" x legumes	18	16.06
Error (b)	21	14.29
Seed lots within legumes	7	23.63*
Seed lots within legumes x "treatments"	21	9.78
Error (c)	28	9.81

*Significant at 5 per cent level.

**Significant at 1 per cent level.

Although there was no significant "treatment" x legumes interaction, the mean index for each legume for each "treatment" was compared to determine the relative susceptibility of each legume to individual Pythium species. The comparisons (Table 10) showed that the legumes did not differ significantly in their susceptibility to individual Pythium species. However, lespedeza ranked lowest in susceptibility to all three Pythium species, sweet clover ranked most susceptible to *Pythium* sp. and *P. aphanidermatum*, and alsike ranked most susceptible to *P. debaryanum*. The other legumes were intermediate.

Table 9. Comparisons among mean seedling necrosis indices of seven legumes, by multiple range test

a) Shortest Significant Ranges:

P:	(2)	(3)	(4)	(5)	(6)	(7)
R_p(5%):	0.83	0.87	0.89	0.90	0.91	0.92

b) Results:

Legumes:	Les.	Alf.	R.c.	Als.	W.c.	Tref.	S.c.
Means:	15.00	15.12	15.94	16.19	16.31	16.87	17.75

NOTE: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

Table 10. Comparisons among mean seedling necrosis indices of seven legumes for three Pythium species and no-culture check, by multiple range test

a) Shortest Significant Ranges:

P:	(2)	(3)	(4)	(5)	(6)	(7)
R_p(5%):	10.76	11.31	11.60	11.86	12.04	12.19

b) Results:

Pythium sp.	Legumes:	Les.	Alf.	R.c.	Als.	W.c.	Tref.	S.c.
	Means:	11.75	15.25	15.25	17.00	17.50	18.50	21.50
P. aphanidermatum	Legumes:	Les.	Alf.	Als.	R.c.	W.c.	Tref.	S.c.
	Means:	16.25	19.50	20.25	23.25	23.75	23.75	24.75
P. debaryanum	Legumes:	Les.	S.c.	W.c.	Alf.	R.c.	Tref.	Als.
	Means:	13.50	18.25	18.50	19.50	20.00	20.25	21.25
Ck (no culture)	Legumes:	Tref.	R.c.	W.c.	Alf.	Als.	S.c.	Les.
	Means:	5.00	5.25	5.50	6.25	6.25	6.50	6.50

NOTE: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

Table 11. Comparisons among mean seedling necrosis indices for three Pythium species, by multiple range test

a) Shortest Significant Ranges:

P:	(2)	(3)	(4)
R_p(5%):	6.41	6.74	6.91

b) Results:

Cultures:	No culture	P. sp.	P. deb.	P. aph.
Means:	5.89	16.68	18.75	21.64

NOTE: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.

The differences among the effects of "treatments" were highly significant and there was no significant interaction, "treatments" x legumes (Table 8). The multiple range test (Table 11) showed that the three Pythium species were significantly pathogenic. Although the differences among the mean indices for these three species were not significant, relatively, Pythium sp. was least pathogenic, P. aphanidermatum was most and P. debaryanum was intermediate.

The difference in indices between seed lots within legumes were significant at the 5 per cent level (Table 8). The differences between the indices for the two seed lots of alfalfa, red clover and sweet clover were significant at the 5 per cent level, while for trefoil, alsike, white clover and lespedeza there were no significant differences between indices for the two seed lots.

Stands of Seven Legumes from Treated and Nontreated Seeds Planted in Various Soils

Portions of the two seed lots of alfalfa, red clover, sweet clover, alsike, white clover, trefoil and lespedeza were treated with Ceresan M (N-(ethylmercury)-p-toluenesulfonanilide) as a dry dust. The fungicide was applied liberally for maximum adherence to the seeds and the excess screened off. For each experiment seeds were taken out and counted two or three days before planting.

In sand in greenhouse bench

The greenhouse bench from which were collected the rotted seeds for the original isolations of *Pythium* (page 15), was the site for a planting of treated and nontreated seeds of the fourteen seed lots. One hundred treated and one hundred nontreated seeds of a given seed lot were planted half inch deep in rows two feet long and three inches apart. Seedling stands were counted three weeks after planting.

The experiment contained four replications. Each replicate was divided into fourteen randomized split plots, one for each seed lot. The split plot consisted of two rows, one with treated and one with nontreated seeds of the same seed lot.

Mean numbers of seedlings of the seven legumes for the four replicates are recorded in Table 12. The analysis of variance (Table 13) showed that the differences among seedling stands of seven legumes were highly significant, the increase in seedling stands due to treatment was highly significant, and the interaction, treatment x legumes was highly significant. Therefore, the data were further analyzed by multiple range test (Table 14). The test showed that responses to seed treatment by sweet clover and alfalfa were nearly equal and significantly greater than for the other five legumes. The response to seed treatment by red clover was significantly greater than the responses to seed treatment by white clover and trefoil. There were no significant differences among the responses to seed treatment by white clover, trefoil and lespedeza, among the responses by trefoil, lespedeza and alsike, and among the responses by lespedeza, alsike and red clover.

For stands from nontreated seed (Table 14) the mean stand of lespedeza was significantly higher than sweet clover, alfalfa, red clover, alsike and trefoil. There was no significant difference between the mean stands of white clover and lespedeza. The mean stands of sweet clover and alfalfa were nearly equal and significantly lower than stands of red clover, alsike, trefoil, white clover and lespedeza.

For stand from treated seeds (Table 14) the mean stand of lespedeza was significantly greater than mean stands of

Table 12. Mean numbers of seedlings from the treated and nontreated seeds of the two seed lots each of seven legumes planted in sand bench in greenhouse

	Seed lots	Laboratory germination test		Seedling stand from	
		Normal sprouts %	Hard seeds %	Treated seeds	Nontreated seeds
Alfalfa	1	96	1	64.0	36.7
	2	84	7	78.0	52.5
Red clover	1	90	3	76.0	66.5
	2	79	13	75.5	63.5
Sweet clover	1	87	2	71.0	54.5
	2	79	10	45.0	15.0
Alsike clover	1	88	9	75.2	75.5
	2	90	4	73.0	54.5
White clover	1	88	2	64.5	68.0
	2	80	17	68.2	77.7
Trefoil	1	67	25	51.5	54.0
	2	91	3	80.5	83.5
Lespedeza	1	91	4	90.0	91.0
	2	90	3	85.5	77.7

all other legumes. The mean stand of sweet clover was significantly lower than the mean stands of alfalfa, alsike, red clover and lespedeza. There were no significant differences among the mean stands of sweet clover, trefoil and

Table 13. Analysis of variance of stands from treated and nontreated seeds of seven legumes planted in sand bench in greehouse

Source of variation	Degrees of freedom	Mean square
Replicates	3	292.67
Legumes	6	2393.86**
Seed lots within legumes	7	1378.24**
Error (a)	39	73.90
"Treatments"	1	2313.22**
"Treatments" x legumes	6	611.95**
"Treatments" x seed lots within legumes	7	93.71*
Error (b)	42	33.85

*Significant at 5 per cent level.

**Significant at 1 per cent level.

white clover and among the mean stands of trefoil, white clover, alfalfa, alsike and red clover.

Further analysis by nonorthogonal comparisons of seedling stands from treated and nontreated seed of individual legumes (Table 15) showed that the increases in seedling stands of alfalfa and sweet clover due to treatment were highly significant and the increases in stands of red clover and alsike due to treatment were significant at the 5 per cent level. The decreases in stand of white clover and trefoil and the increase in stand of lespedeza due to treatment were not significant.

Table 14. Comparisons among mean stands from treated and nontreated seeds of seven legumes planted in sand bench in greenhouse, by multiple range test

a) Shortest Significant Ranges:

P:	(2)	(3)	(4)	(5)	(6)	(7)
R_p(5%):	11.87	12.49	12.86	13.16	13.36	13.57

b) Results:

treated -	Legumes:	S.c.	Tref.	W.c.	Alf.	Als.	R.c.	Les.
	Means:	58.00	66.00	66.38	71.00	74.13	75.75	87.75
nontreated -	Legumes:	S.c.	Alf.	R.c.	Als.	Tref.	W.c.	Les.
	Means:	34.75	44.63	65.00	65.00	68.75	72.88	84.38
	Legumes:	W.c.	Tref.	Les.	Als.	R.c.	S.c.	Alf.
	Diff.*:	-6.50	-2.75	3.37	9.13	10.75	23.25	26.37

NOTE: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

*Difference between mean seedling stands from treated and nontreated seeds of seven legumes.

Table 15. Nonorthogonal comparisons between seedling stands from treated and nontreated seed of seven legumes planted in sand bench in greenhouse

	Number of seedlings from:		Difference	Divisor	S.S./M.S.
	Treated seeds	Nontreated seeds			
Alfalfa	568	357	211	16	2782.06**
Red clover	606	520	86	16	462.25*
Sweet clover	464	278	186	16	2162.25**
Alsike clover	593	520	73	16	333.06*
White clover	531	583	-52	16	169.00
Trefoil	528	550	-22	16	30.25
Lespedeza	702	675	27	16	45.56

*Significant at 5 per cent level.

**Significant at 1 per cent level.

The stands from seed lots within legumes were different at the 1 per cent level of significance, and the interaction, treatment x seed lots within crops was significant at the 5 per cent level (Table 13).

The differences between stands from the two seed lots of each legume were highly significant except for red clover, white clover and lespedeza. The difference between the stands from the two seed lots of lespedeza was significant at the 5 per cent level, while the differences between the stands of the two seed lots of red clover and white clover were not significant.

<u>In samples of Clarion loam and Webster loam from Ames</u>

Two soil samples, one of Clarion loam, pH 6.1, and the other of Webster loam, pH 7.4, were collected from the Ash Avenue plots of the Botany and Plant Pathology Department, Ames.

Half the soil of each sample was steamed in autoclave at 15 lbs. pressure for two hours. Next day, steamed and nonsteamed portions of the two samples were placed in steamed three inch pots. In each pot were planted 50 treated and 50 nontreated seeds of a given seed lot. The seed lots so planted were the two each of alfalfa, red clover, sweet clover, alsike, white clover, trefoil and lespedeza. Seedling stands were counted three weeks after planting.

The experiment was arranged in two plots, one for each soil type. Each plot was comprised of two sub-plots, steamed and nonsteamed. A sub-plot consisted of four replications. Each replicate contained fourteen randomized split plots (seed lots). Each split plot was a pot partitioned into two parts, one part planted with treated seeds and the other planted with nontreated seeds of the same seed lot.

The mean numbers of seedlings for seven legumes for the four replicates are presented in Table 16. The analysis of variance (Table 18) showed that the difference between seedling stands in the two soils was not significant. The seedling stands in steamed soil were highly significantly greater than the stands in nonsteamed soil. Since the variation in stands of the seven legumes was highly significant and since there was no significant interaction, seed treatment x legumes, the mean seedling stands of the legumes were compared by multiple range test (Table 18). The test showed that the mean stand of lespedeza was significantly higher than mean stands of all other legumes. The mean stands of alfalfa and red clover were nearly equal and significantly higher than mean stands of sweet clover and alsike. There were no significant differences among the mean stands of trefoil, white clover and alfalfa, between the mean stands of alfalfa and red clover, between the mean stands of sweet clover and alsike, and between the mean stands of alsike and trefoil. The mean stand of sweet clover was significantly lower than all other legumes except alsike.

Although there was no significant interaction, seed treatment x legumes, comparisons of mean stands of legumes from treated and nontreated seeds (Table 19) showed that increase in stand from seed treatment was somewhat greater for alfalfa and red clover than for the other legumes.

Table 16. Mean numbers of seedlings from the treated and non-treated seeds and non-steamed Clarion loam and Webster loam from Ames

	Seed lot	Laboratory germination test		Mean see			
				Clarion loam; pH 6.1			
				Nonsteamed		Steamed	
		Normal sprouts, %	Hard seeds, %	Treated	Nontreated	Treated	Nontreat
Alfalfa	1	96	1	22.0*	15.5	38.5	39.0
	2	84	7	27.2	20.0	37.7	34.0
Red clover	1	90	3	36.0	36.2	35.0	34.0
	2	79	13	30.2	20.5	31.5	30.7
Sweet clover	1	87	2	17.7	18.2	32.7	31.7
	2	79	10	9.0	6.7	23.2	18.5
Alsike clover	1	88	9	16.2	11.2	22.0	25.0
	2	90	4	22.0	16.5	15.5	18.2
White clover	1	88	2	23.2	17.2	28.5	28.7
	2	80	17	32.5	28.2	28.7	26.7
Trefoil	1	67	25	15.2	13.0	27.7	26.5
	2	91	3	22.2	8.7	35.5	37.7
Lespedeza	1	91	4	34.7	38.0	36.0	36.0
	2	90	3	39.2	32.7	33.5	35.0

* Mean of 50 seeds planted in four replicates.

ted and non-treated seeds of the two seed lots of seven legumes planted in steamed
r loam from Ames

Mean seedling stands in:						
larion loam; pH 6.1			Webster loam; pH 7.4			
ed	Steamed		Nonsteamed		Steamed	
reated	Treated	Nontreated	Treated	Nontreated	Treated	Nontreated
.5.5	38.5	39.0	14.2	11.0	39.0	38.5
20.0	37.7	34.0	11.0	5.7	38.5	37.7
36.2	35.0	34.0	29.5	22.7	44.2	44.2
20.5	31.5	30.7	16.5	13.2	30.7	29.0
.8.2	32.7	31.7	15.2	19.2	30.7	28.7
6.7	23.2	18.5	8.0	1.7	21.0	21.7
1.2	22.0	25.0	21.0	25.2	24.7	26.2
6.5	15.5	18.2	24.7	11.2	25.5	25.2
7.2	28.5	28.7	15.5	17.2	29.0	30.7
8.2	28.7	26.7	21.0	28.7	29.2	27.0
3.0	27.7	26.5	18.2	24.0	25.0	24.5
8.7	35.5	37.7	20.5	29.5	27.2	26.5
8.0	36.0	36.0	41.0	33.0	43.5	42.7
2.7	33.5	35.0	22.7	26.5	38.0	33.0

Table 17. Analysis of variance of stands from treated and nontreated seeds of seven legumes planted in steamed and nonsteamed Clarion loam and Webster loam from Ames

Source of variation	Degrees of freedom	Mean square
Soils	1	110.01
Steaming	1	10569.14**
Soils x steaming	1	424.33
Error (a)	12	95.23
Legumes	6	1947.59**
Seed lots within legumes	7	615.53**
Legumes x soils	6	156.38
Legumes x steaming	6	687.30**
Legumes x steaming x soils	6	350.78**
Soils x seed lots within legumes	7	77.38
Steaming x seed lots within legumes	7	88.23
Steaming x soils x seed lots within legumes	7	90.87
Error (b)	156	93.78
Treatment	1	270.32**
Treatment x soils	1	92.89
Treatment x steaming	1	139.51
Treatment x steaming x soils	1	122.22
Treatment x legumes	6	22.62
Treatment x seed lots within legumes	7	28.80
Treatment x legumes x soils	6	40.83
Treatment x legumes x steaming	6	32.23
Treatment x legumes x soils x steaming	6	42.04
Treatment x soils x seed lots within legumes	7	33.83
Treatment x steaming x seed lots within legumes	7	22.27
Treatment x steaming x steaming x seed lots within legumes	7	60.48
Error (c)	168	36.40

**Significant at 1 per cent level.

Table 18. Comparisons among mean seedling stands of seven legumes planted in steamed and nonsteamed Clarion loam and Webster loam from Ames, by multiple range test

a) Shortest Significant Ranges:

P:	(2)	(3)	(4)	(5)	(6)	(7)
R_p(5%):	3.35	3.53	3.65	3.74	3.81	3.86

b) Results:

Legumes:	S.c.	Als.	Tref.	W.c.	Alf.	R.c.	Les.
Means:	19.03	20.67	23.89	25.78	26.86	29.97	35.20

NOTE: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

Table 19. Comparisons between mean seedling stands from treated and nontreated seed of seven legumes planted in steamed and nonsteamed Clarion loam and Webster loam from Ames

	Mean number of seedlings from		Differences
	Treated seeds	Nontreated seeds	
Alfalfa	28.53	25.19	3.34
Red clover	31.41	28.53	2.88
Alsike clover	21.47	19.87	1.60
Sweet clover	19.72	18.34	1.38
Lespedeza	35.78	34.62	1.16
White clover	25.97	25.59	0.38
Trefoil	23.97	23.81	0.16

The comparisons between the mean seedling stands of legumes in steamed and nonsteamed soils (Table 20) showed that the increased stand of alfalfa in steamed soil was significantly higher than the increase in stand of all the other legumes in steamed soils. The difference between the increases in stand of sweet clover and trefoil were nearly equal and significantly higher than the increase in stands of lespedeza and alsike in steamed soils. The increase in stands of red clover and white clover in steamed soil were intermediate.

Table 20. Comparisons among mean seedling stands of seven legumes in steamed and nonsteamed Clarion loam and Webster loam from Ames, by multiple range test

a) Shortest Significant Ranges:

P:	(2)	(3)	(4)	(5)	(6)	(7)
R_p(5%):	5.07	5.35	5.53	5.65	5.76	5.84

b) Results:

steamed -	Legumes:	Als.	S.c.	W.c.	Tref.	R.c.	Les.	Alf.
	Means:	22.81	26.06	28.59	28.84	34.31	36.91	37.87
nonsteamed -	Legumes:	S.c.	Alf.	Als.	Tref.	W.c.	R.c.	Les.
	Means:	12.00	15.84	18.53	18.94	22.97	25.62	33.50
	Legumes:	Les.	Als.	W.c.	R.c.	Tref.	S.c.	Alf.
	Differences:*	3.41	4.28	5.62	8.69	9.90	14.06	22.03

NOTE: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

*Differences between mean stands of legumes from steamed and nonsteamed soils.

Comparisons between seedling stands from the two seed lots of individual legumes showed that the differences in stands from seed lots were highly significant for red clover and sweet clover. The difference in stands from seed lots of lespedeza was significant at the 5 per cent level. There were no significant differences between the stands from the two seed lots of alfalfa, alsike, white clover and trefoil.

<u>In two samples of Tama silt loam and one of Wabash loam from Warren County</u>

Three soil samples, one of Wabash loam, with pH 5.8, and two of Tama silt loam, with pH 5.6 and pH 5.7 respectively, were collected from distantly located fields near Indianola, Warren County.

Treated and nontreated seeds of the seven legumes were planted in steamed and nonsteamed portions of the three samples, in an experiment similar to the one with the Clarion loam and Webster loam samples from Ames. Seedling stands were counted three weeks after planting.

The experiment was arranged in three plots, one for each soil sample, with each plot composed of two sub-plots, steamed and nonsteamed. Treated and nontreated seeds of the fourteen seed lots were planted in four replicates in each sub-plot.

The mean numbers of seedlings of seven legumes for the four replicates are recorded in Table 21. The analysis of variance (Table 22) showed that the differences among the seedling stands in three soil samples were highly significant. The interaction, soils x steaming was also highly significant. The comparisons of mean seedling stands in steamed and nonsteamed soils (Table 23) showed that the difference between seedling stands in steamed and nonsteamed was significantly greater for Tama silt loam, with pH 5.7 than for Wabash loam, with pH 5.8, and that this difference was greater for Wabash loam, with pH 5.8 than for Tama silt loam, with pH 5.6.

The differences among the seedling stands of the seven legumes were highly significant (Table 22), and since the interaction, legumes x treatment was not significant, the mean seedling stands of the legumes were compared by multiple range test (Table 25). The test showed that the mean seedling stand of lespedeza was significantly higher than the mean seedling stands of the other legumes. The mean seedling stands of sweet clover and trefoil were nearly equal and significantly less than white clover, red clover and lespedeza. The mean seedling stands of alfalfa, alsike and white clover were intermediate.

The interaction, legumes x steaming was highly significant (Table 22). The comparisons between mean seedling stands in steamed and nonsteamed soil (Table 25) showed that

Table 21. Mean numbers of seedlings from treated and non-treated seeds of the t
Tama silt loam and Wabash loam from Warren county.

	Seed lots	Laboratory germination test		Tama silt loam; pH 5.6				Mea T
				Nonsteamed		Steamed		Nons
		Normal sprouts, %	Hard seeds, %	Treated seeds	Non-treated seeds	Treated seeds	Non-treated seeds	Treate seeds
Alfalfa	1	96	1	29.7*	19.7	43.5	43.0	15.7
	2	84	7	30.0	19.0	40.5	40.5	29.5
Red clover	1	[illegible]	3	43.5	40.7	45.0	43.5	38.5
	2	7[illegible]	13	32.0	31.5	33.7	34.2	25.7
Sweet clover	1	87	2	37.5	35.0	36.0	36.0	26.0
	2	79	10	27.2	17.7	28.0	28.0	15.7
Alsike clover	1	88	9	38.5	34.7	40.0	38.7	32.2
	2	90	4	33.0	31.5	29.2	29.7	21.2
White clover	1	88	2	33.7	33.2	33.5	34.7	22.5
	2	80	17	38.0	40.5	39.0	38.5	39.7
Trefoil	1	67	25	31.7	29.2	33.0	32.5	15.2
	2	91	3	43.5	32.7	41.5	41.5	15.5
Lespedeza	1	91	4	42.7	38.0	39.7	40.7	40.0
	2	90	3	43.7	37.0	40.2	41.2	30.2

* Mean of 50 seeds planted in four replicates.

-treated seeds of the two seed lots of seven legumes planted in steamed and nonsteamed
nty.

; pH 5.6		Mean number of seedlings in: Tama silt loam; pH 5.7				Wabash loam; pH 5.8			
Steamed		Nonsteamed		Steamed		Nonsteamed		Steamed	
reated seeds	Non-treated seeds	Treated seeds	Non-treated seeds	Treated seeds	Non-treated seeds	Treated seeds	Non-treated seeds	Treated seeds	Non-treated seeds
43.5	43.0	15.7	5.5	42.5	44.5	25.5	6.2	29.0	30.2
40.5	40.5	29.5	8.7	40.5	39.2	15.5	7.5	37.7	38.2
45.0	43.5	38.5	26.0	42.5	42.7	42.7	29.0	40.7	39.7
33.7	34.2	25.7	6.0	31.5	29.7	24.2	11.5	22.2	22.7
36.0	36.0	26.0	11.7	38.2	38.7	14.0	5.2	37.0	36.5
28.0	28.0	15.7	0.5	28.7	27.5	10.7	3.0	29.5	29.2
40.0	38.7	32.2	17.0	40.5	41.0	32.0	27.2	34.0	34.0
29.2	29.7	21.2	8.5	21.0	22.7	31.2	21.2	22.0	21.7
33.5	34.7	22.5	6.5	36.2	36.7	20.5	7.2	34.0	35.2
39.0	38.5	39.7	26.2	40.0	40.5	35.7	29.5	30.7	30.2
33.0	32.5	15.2	8.0	33.5	34.2	18.2	14.2	26.2	27.5
41.5	41.5	15.5	9.2	40.5	40.7	13.2	8.2	34.5	33.2
39.7	40.7	40.0	17.7	42.5	41.5	42.5	33.5	34.2	35.0
40.2	41.2	30.2	19.7	43.5	44.0	26.5	21.7	41.5	42.5

Table 22. Analysis of variance of seedling stands from treated and nontreated seed of seven legumes planted in Tama silt loam and Wabash loam from Warren County

Source of variation	Degrees of freedom	Mean square
Soils	2	5109.88**
Steaming	1	20926.34**
Soils x steaming	2	2958.70**
Error (a)	18	112.99
Legumes	6	1430.51**
Legumes x soils	12	91.58
Legumes x steaming	6	949.14**
Legumes x steaming x soils	12	144.82
Seed lots within legumes	7	1558.70**
Seed lots within legumes x soils	14	82.36
Seed lots within legumes x steaming	7	305.23**
Seed lots within legumes x steaming x soils	14	133.00
Error (b)	234	94.20
Treatments	1	3501.72**
Treatments x soils	2	303.46**
Treatments x steaming	1	3658.67**
Treatments x legumes	6	32.30
Treatments x seed lots within legumes	7	13.10
Treatments x soils x steaming	2	319.50**
Treatments x soils x legumes	12	22.35
Treatments x soils x seed lots within legumes	14	23.27
Treatments x steaming x legumes	6	32.75
Treatments x steaming x seed lots within legumes	7	12.42
Treatments x steaming x soil x legumes	12	20.44
Treatments x steaming x soil x seed lots within legumes	14	16.01
Error (c)	252	23.75

**Significant at 1 per cent level.

Table 23. Comparisons among mean stands of seven legumes in steamed and nonsteamed Tama silt loam and Wabash loam from Warren County, by multiple range test

a) Shortest Significant Ranges:

P:	(2)	(3)
R_p(5%):	2.98	3.13

b) Results:

steamed -	Soils:	Wabash loam (pH 5.8)	Tama silt loam (pH 5.7)	Tama silt loam (pH 5.6)
	Means:	32.48	37.33	37.36
nonsteamed -	Soils:	Tama silt loam (pH 5.7)	Wabash loam (pH 5.8)	Tama silt loam (pH 5.6)
	Means:	19.26	20.65	33.78
	Soils:	Tama silt loam (pH 5.6)	Wabash loam (pH 5.8)	Tama silt loam (pH 5.7)
	Differences:*	3.58	11.83	18.07

NOTE: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

*Differences between mean stands of legumes from steamed and nonsteamed soils.

Table 24. Comparisons among mean stands of seven legumes planted in Tama silt loam and Wabash loam from Warren County, by multiple range test

a) Shortest Significant Ranges:

	(2)	(3)	(4)	(5)	(6)	(7)
P:	(2)	(3)	(4)	(5)	(6)	(7)
R_p(5%):	2.76	2.91	3.00	3.07	3.13	3.17

b) Results:

Legumes:	S.c.	Tref.	Alf.	Als.	W.c.	R.c.	Les.
Means:	24.92	27.41	28.43	29.29	31.78	32.50	36.68

NOTE: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.

Table 25. Comparisons among mean stands of seven legumes in steamed and nonsteamed Tama silt loam and Wabash loam from Warren County, by multiple range test

a) Shortest Significant Ranges:

P:	(2)	(3)	(4)	(5)	(6)	(7)
R_p(5%):	5.08	5.36	5.54	5.67	5.78	5.85

b) Results:

steamed -	Legumes:	Als.	S.c.	Tref.	R.c.	W.c.	Alf.	Les.
	Means:	31.23	32.79	34.87	35.71	35.77	39.12	40.56
nonsteamed -	Legumes:	S.c.	Alf.	Tref.	Als.	W.c.	R.c.	Les.
	Means:	17.04	17.73	19.94	27.35	27.79	29.29	32.79
	Legumes:	Als.	R.c.	Les.	W.c.	Tref.	S.c.	Alf.
	Differences:*	3.87	6.42	7.77	7.98	14.94	15.75	21.40

NOTE: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

*Differences between mean stands of legumes from steamed and nonsteamed soils.

the difference between mean stands in steamed and nonsteamed soil was significantly greater for alfalfa than for all other legumes except sweet clover. The differences in stand between steamed and nonsteamed soil for sweet clover and alfalfa and for sweet clover and trefoil were nearly equal in both cases and all were significantly higher than for alsike, red clover, lespedeza and white clover.

The difference between stands from treated and nontreated seeds and the interaction, seed treatment x soils were highly significant (Table 22). The comparisons among the mean stands from treated and from nontreated seeds in three soils (Table 26) showed that the greater differences between the stands in three soils due to seed treatment were not significant. However, the increase in seedling stand due to seed treatment was highest in Tama silt loam, with pH 5.7, lowest in Tama silt loam, with pH 5.6 and intermediate in Wabash loam, with pH 5.8. The comparisons in Table 27 indicated that the seed treatment significantly increased the stand of legumes in all three soils.

Although there was no significant interaction, legumes x seed treatment, comparisons among mean seedling stands of legumes from treated and nontreated seeds (Table 28) showed that increase in stands from seed treatment was somewhat greater for alfalfa, red clover and sweet clover than for the other legumes.

Table 26. Comparisons among mean stands from treated and nontreated seeds of seven legumes planted in Tama silt loam and Wabash loam from Warren County

a) Shortest Significant Ranges:

P:	(2)	(3)
R_p(5%):	4.33	4.56

b) Results:

treated -	Soils:	Wabash loam (pH 5.8)	Tama silt loam (pH 5.7)	Tama silt loam (pH 5.6)
	Means:	28.79	31.77	36.71
nontreated -	Soils:	Wabash loam (pH 5.8)	Tama silt loam (pH 5.7)	Tama silt loam (pH 5.6)
	Means:	24.34	24.82	34.42
	Soils:	Tama silt loam (pH 5.6)	Wabash loam (pH 5.8)	Tama silt loam (pH 5.7)
	Differences:*	2.49	4.45	6.95

NOTE: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

*Differences between mean stands from treated and nontreated seeds planted in three soils.

Table 27. Nonorthogonal comparisons between seedling stands from treated and nontreated seeds of seven legumes in Tama silt loam and Wabash loam from Warren County

	Seedling stands from:		Difference	Divisor	S.S./M.S.
	Treated seeds	Nontreated seeds			
Tama silt loam (pH 5.6)	4112	3855	257	224	294.86**
Tama silt loam (pH 5.7)	3558	2780	778	224	2702.16**
Wabash loam (pH 5.8)	3225	2726	499	224	1111.61**

**Significant at 1 per cent level.

Comparisons between seedling stands from the two seed lots of individual legumes showed that the differences in stands from seed lots of red clover, sweet clover, alsike and white clover were highly significant. The differences in stands from the seed lots of trefoil was significant at the 5 per cent level. There were no significant differences between the stands from the two seed lots of alfalfa and lespedeza.

Table 28. Comparisons between mean seedling stands from treated and nontreated seeds of seven legumes planted in Tama silt loam and Wabash loam from Warren County

	Mean number of seedlings from:		Differences
	Treated seeds	Nontreated seeds	
Alfalfa	15.82	12.60	3.22
Red clover	17.60	14.90	2.70
Sweet clover	13.70	11.22	2.48
Lespedeza	19.48	17.20	2.28
Alsike clover	15.61	13.68	1.93
White clover	16.82	14.96	1.86
Trefoil	14.45	12.96	1.49

In greenhouse soil at three temperatures and at two moisture levels

Three inch pots were filled with nonsteamed greenhouse soil. In each pot were planted 50 treated and 50 nontreated seeds of a given seed lot. As in other experiments there were fourteen seed lots, two each of seven small seeded legumes. After planting, the pots were kept in greenhouses at 16°, 21°, and 27° C. The pots were watered by two methods, one by the usual method of watering once each day (later referred to as moderate watering); the second method was to keep the soil saturated with water by keeping the pot in clay dishes filled with water (later referred to as saturated watering). Watering was started immediately after planting the seeds. The seedling stands were counted four weeks after planting.

Main plots in experiment were the three respective temperatures, 16°, 21° and 27° C. Each main plot consisted of two sub-plots, moderate watering and saturated watering. Each sub-plot was comprised of four replications. Each replicate contained fourteen (seed lots) randomized split plots. Each split plot was a pot partitioned into two parts, one part planted with treated seeds and the other with nontreated seeds of the same seed lot.

The mean numbers of seedlings of seven legumes for the four replicates are presented in Table 29. The analysis of variance (Table 30) showed that the differences among the stands at three temperatures and the greater stand with moderate than with saturated watering were significant at the 1 per cent level. The individual comparisons (Table 31) showed that stands with moderate watering at 16° and 21° C. were significantly greater at the 1 per cent level. The difference between stands with moderate and saturated watering at 27° C. was not significant.

The interaction, temperature x watering was highly significant (Table 30). Comparisons of relative differences (Table 32) showed that the differences between mean stands

Table 29. Mean numbers of seedlings from treated and non-treated seeds of the t
incubated at three temperatures and at two moisture levels.

	Seed lots	Laboratory germination test: Normal sprout %	Laboratory germination test: Hard seeds %	16°C. Moderate watering: Treated	16°C. Moderate watering: Non-treated	16°C. Saturated watering: Treated	16°C. Saturated watering: Non-treated	Mod Tre
Alfalfa	1	96	1	36.7*	22.0	33.7	10.7	19.
	2	87	7	35.7	18.2	27.2	8.7	8.
Red clover	1	90	3	42.5	38.0	31.7	19.5	41.
	2	79	13	33.2	22.7	24.2	5.5	31.
Sweet clover	1	87	2	28.2	16.2	17.7	6.7	10.
	2	79	10	23.7	5.7	12.0	1.2	13.
Alsike clover	1	88	9	37.0	23.5	36.0	21.5	34.
	2	90	4	35.2	30.5	26.2	14.2	33.
White clover	1	88	2	31.0	12.5	28.5	13.7	16.
	2	80	17	41.7	32.7	36.5	34.0	34.
Trefoil	1	67	25	27.2	25.5	21.2	10.0	18.
	2	91	3	44.5	17.7	29.7	11.5	25.
Lespedeza	1	91	4	44.0	30.2	26.0	16.0	42.
	2	90	3	26.5	18.5	37.2	17.5	22.

* Mean of 50 seeds planted in four replicates.

d seeds of the two seed lots of seven legumes planted in greenhouse soil,
evels.

	21°C.				27°C.			
ed watering	Moderate watering		Saturated watering		Moderate watering		Saturated watering	
Non-treated	Treated	Non-treated	Treated	Non-treated	Treated	Non-treated	Treated	Non-treated
10.7	19.0	4.7	5.7	2.5	18.2	0.5	6.7	3.2
8.7	8.2	0.5	11.5	4.2	16.0	1.2	17.2	0.7
19.5	41.7	15.2	10.2	6.5	23.2	9.7	26.2	8.0
5.5	31.7	12.0	12.7	1.2	15.0	4.0	16.0	5.0
6.7	10.7	1.2	5.0	1.0	4.2	0.2	15.0	3.2
1.2	13.0	1.5	5.0	0.2	17.5	1.2	4.0	1.0
21.5	34.7	10.7	32.7	7.0	16.2	1.5	33.5	13.7
14.2	33.5	12.2	11.2	5.5	4.5	0.0	19.5	9.2
13.7	16.7	7.0	11.7	1.0	11.2	2.5	11.7	7.7
34.0	34.7	26.0	32.5	14.7	28.5	15.5	28.5	22.5
10.0	18.0	6.2	10.7	2.0	6.7	0.5	9.5	7.0
11.5	25.7	7.7	18.5	6.5	19.7	8.7	13.5	6.5
16.0	42.0	24.5	35.2	15.2	32.0	11.2	15.2	16.2
17.5	22.7	9.5	3.2	1.0	18.0	3.2	5.2	6.2

Table 30. Analysis of variance of seedling stands from treated and nontreated seeds of seven legumes planted in greenhouse soil, incubated at three temperatures and at two moisture levels

Source of variation	Degrees of freedom	Mean square
Temperature	2	15561.66**
Watering	1	3785.75**
Temperature x watering	2	1611.56**
Error (a)	18	92.14
Legumes	6	2099.61**
Legumes x temperature	12	128.84
Legumes x watering	6	256.02*
Legumes x watering x temperature	12	184.16*
Seed lots within legumes	7	1753.80**
Temperature x seed lots within legumes	14	127.54
Watering x seed lots within legumes	7	133.20
Watering x temperature x seed lots within legumes	14	107.04
Error (b)	234	102.83
Treatments	1	24012.34**
Treatments x temperature	2	150.08
Treatments x watering	1	297.33*
Treatments x legumes	6	69.56
Treatments x seed lots within legumes	7	151.24*
Treatments x temperature x watering	2	204.20*
Treatments x temperature x legumes	12	88.65
Treatments x temperature x seed lots within legumes	14	57.79
Treatments x watering x legumes	6	30.32
Treatments x watering x seed lots within legumes	7	45.81
Treatments x temperature x watering x legumes	12	102.80
Treatments x temperature x watering x seed lots within legumes	14	60.62
Error (c)	252	61.28

*Significant at the 5 per cent level.

**Significant at the 1 per cent level.

Table 31. Nonorthogonal comparisons between stands of seven legumes with saturated and moderate watering at three temperatures

	Seedling stands with:		Difference	Divisor	S.S./M.S.
	Saturated watering	Moderate watering			
16° C.	2318	3207	-889	224	3528.22**
21° C.	1100	1968	-868	224	3363.50**
27° C.	1329	1167	162	224	117.16

**Significant at the 1 per cent level.

with moderate and saturated watering at 16° and 21° C. were nearly equal and significantly larger than the difference between mean stand with moderate and saturated watering at 27° C.

The differences among the seedling stands of the seven legumes were highly significant (Table 30). Individual comparisons (Table 33) showed that the mean stand of sweet clover was significantly lower than the mean stands of all other legumes, the mean stands of red clover, alsike, lespedeza and white clover were nearly equal and significantly greater than the mean stands of sweet clover, alfalfa

Table 32. Comparisons among mean stands of seven legumes with saturated and moderate watering at three temperatures, by multiple range test

a) Shortest Significant Ranges:

P:	(2)	(3)
$R_p(5\%)$:	2.69	2.83

b) Results:

saturated watering -	Temperatures:	21° C.	27° C.	16° C.
	Means:	9.82	11.87	20.70
moderate watering -	Temperatures:	27° C.	21° C.	16° C.
	Means:	10.42	17.57	28.63
	Temperatures:	27° C.	21° C.	16° C.
	Differences*:	-1.45	7.75	7.93

NOTE: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.

*Differences between mean stands with saturated and moderate watering at three temperatures.

and trefoil. The mean stands of alfalfa and trefoil were nearly equal and intermediate.

The interaction, legumes x watering was significant at the 5 per cent level (Table 30). The comparisons among the mean stands of legumes with moderate and saturated watering (Table 34) showed that the differences between stands with saturated and moderate watering for lespedeza and red clover

Table 33. Comparisons among mean stands of seven legumes planted at three temperatures with two watering levels, by multiple range test

a) Shortest Significant Ranges:

	(2)	(3)	(4)	(5)	(6)	(7)
P:	(2)	(3)	(4)	(5)	(6)	(7)
R_p(5%):	2.91	3.07	3.17	3.24	3.71	3.35

b) Results:

Legumes:	S.c.	Alf.	Tref.	R.c.	Als.	Les.	W.c.
Means:	8.18	13.07	14.80	19.01	19.59	19.87	20.98

NOTE: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

Table 34. Comparisons among mean stands of seven legumes with saturated and moderate watering, by multiple range test

a) Shortest Significant Ranges:

	(2)	(3)	(4)	(5)	(6)	(7)
P:	(2)	(3)	(4)	(5)	(6)	(7)
R_p(5%):	5.29	5.58	5.77	5.90	6.02	6.09

b) Results:

saturated watering -	Legumes:	S.c.	Alf.	Tref.	R.c.	Les.	Als.	W.c.
	Means:	6.02	11.04	12.23	13.92	16.21	19.21	20.27
moderate watering -	Legumes:	S.c.	Alf.	Tref.	Als.	W.c.	Les.	R.c.
	Means:	10.33	15.17	17.37	19.98	21.69	23.54	24.10
	Legumes:	Als.	W.c.	Alf.	S.c.	Tref.	Les.	R.c.
	Differences*:	0.77	1.42	4.13	4.31	5.14	7.33	10.18

NOTE: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

*Differences between the mean stands of legumes with saturated and moderate watering.

Table 35. Nonorthogonal comparisons between stands of seven legumes from saturated and moderate watering

	Seedling stands with:		Difference	Divisor	S.S./M.S.
	Moderate watering	Saturated watering			
Alfalfa	725	530	195	96	396.09**
Red clover	1157	668	489	96	2490.84**
Sweet clover	496	289	207	96	446.34**
Alsike clover	959	922	37	96	14.26
White clover	1041	973	68	96	48.16
Trefoil	834	587	247	96	635.51**
Lespedeza	1130	778	352	96	1290.67**

**Significant at the 1 per cent level.

were nearly equal and significantly higher than the differences between stands with saturated and moderate watering for alsike and white clover. The differences between mean stands with saturated and moderate watering for alfalfa, sweet clover and trefoil were nearly equal and intermediate.

The comparisons between stands with moderate and saturated watering for the seven legumes (Table 30) showed that the higher stands of alfalfa, red clover, sweet clover, trefoil and lespedeza with moderate watering than the stands with saturated watering were highly significant. The greater stands of alsike and white clover with moderate watering than the stands with saturated watering were not significant.

Since seed lots within legumes differed significantly (Table 30), the seedling stands from the two seed lots of individual legumes were compared. The differences between stands from two seed lots were significant for red clover, alsike, white clover, trefoil and lespedeza. There were no significant differences between stands from the two seed lots of either alfalfa or sweet clover.

<u>In Clarion loam in the field</u>

Treated and nontreated seeds of the seven legumes were planted half inch deep, 100 seeds per row, in rows two feet long and six inches apart in Clarion loam soil at the Ash Avenue plots of the Botany and Plant Pathology Department, Ames. Seedling stands were counted four weeks after planting.

The planting was in four replications. Each replicate contained fourteen split plots, one for each seed lot. Each split plot was of two rows, one with treated and one with nontreated seeds of the same seed lot. The treatments were randomized in the split plot and the split plots were randomized within the replication.

Mean numbers of seedlings of seven legumes from the four replications are recorded in Table 36. The analysis of variance (Table 37) showed that the differences among the stands of the legumes were highly significant. Comparisons among mean stands of seven legumes (Table 38) showed that the mean stand of alfalfa was significantly greater than the mean stands of all other legumes, the mean stand of trefoil was significantly lower than the mean stands of all other legumes. The mean stands of alsike, white clover and lespedeza were nearly equal and significantly greater than the mean stand of trefoil but less than the mean stands of sweet clover, red clover and alfalfa. The mean stands of sweet clover and red clover were nearly equal and significantly less than the mean stands of alfalfa.

The increase in stand due to treatment was highly significant. The interaction, treatment x legumes was highly significant. Multiple range test of the interaction (Table 39) showed that the differences between stands from treated and nontreated seeds of alfalfa, red clover and sweet clover due to treatment were nearly equal and significantly higher than the differences between stands from treated and nontreated seeds of lespedeza and trefoil. The differences between stands from treated and nontreated seeds of alsike and white clover were significantly higher than the

Table 36. Mean numbers of seedlings from treated and nontreated seeds of seven legumes planted in Clarion loam in the field

	Seed lots	Laboratory germination test		Mean number of seedlings from:	
		Normal sprouts %	hard seeds %	Treated seeds	Nontreated seeds
Alfalfa	1	96	1	50.7	31.5
	2	84	7	40.7	32.7
Red clover	1	90	3	38.0	25.7
	2	79	13	28.0	11.2
Sweet clover	1	87	2	40.5	24.0
	2	79	10	26.5	11.2
Alsike clover	1	88	9	19.5	11.0
	2	90	4	18.7	7.0
White clover	1	88	2	18.0	9.7
	2	80	17	26.2	11.7
Trefoil	1	67	25	8.7	5.7
	2	91	3	7.5	12.0
Lespedeza	1	91	4	16.7	14.0
	2	90	3	13.0	22.5

difference between the stands from treated and nontreated seeds of lespedeza.

The nonorthogonal comparisons of the stands from treated and nontreated seeds of individual legumes (Table 40) showed that the increases in stands of alfalfa, red clover, sweet clover, and white clover due to treatment were highly significant. The difference between stands from treated and nontreated seeds of alsike was significant at the 5 per cent level. The stands from treated and nontreated seeds of lespedeza and trefoil were not significantly different.

The comparisons showed that the stands from seed lots within crops differed significantly only in the case of red clover and sweet clover.

Table 37. Analysis of variance of seedling stands from treated and nontreated seeds of seven legumes planted in Clarion loam in the field

Source of variation	Degrees of freedom	Mean squares
Replications	3	
Legumes	6	1626.18**
Seed lots within legumes	7	223.94**
Error (a)	39	66.96
Treatments	1	2152.51**
Treatments x legumes	6	235.76**
Treatments x seed lots within legumes	7	57.76
Error (b)	42	44.24

**Significant at the 1 per cent level.

Table 38. Comparisons among mean stands of seven legumes planted in Clarion loam in the field

a) Shortest Significant Ranges:	P:	(2)	(3)	(4)	(5)	(6)	(7)	
	R_p(5%):	5.86	6.17	6.35	6.50	6.60	6.70	
b) Results:	Legumes:	Tref.	Als.	W.c.	Les.	S.c.	R.c.	Alf.
	Means:	8.50	14.06	16.44	16.56	25.56	25.75	39.00

NOTE: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.

Table 39. Comparisons among mean stands from treated and nontreated seeds of seven legumes planted in Clarion loam in the field

a) Shortest Significant Ranges:

P:	(2)	(3)	(4)	(5)	(6)	(7)
R_p(5%):	11.70	12.31	12.68	12.96	13.17	13.37

b) Results:

treated -	Legumes:	Tref.	Les.	Als.	W.c.	R.c.	S.c.	Alf.
	Means:	8.13	14.87	19.13	22.13	33.00	33.50	45.75
nontreated -	Legumes:	Tref.	Als.	W.c.	S.c.	Les.	R.c.	Alf.
	Means:	8.87	9.00	10.75	17.62	18.25	18.50	32.13
	Legumes:	Les.	Tref.	Als.	W.c.	Alf.	R.c.	S.c.
	Differences*:	-3.38	-0.74	10.13	11.38	13.62	14.50	15.88

NOTE: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

*Differences between mean seedling stands from treated and nontreated legumes.

Table 40. Nonorthogonal comparisons between stands from treated and nontreated seeds of seven legumes planted in Clarion loam in the field

	Seedling stands from:		Differ- ence	Divi- sor	S.S./M.S.
	Treated seeds	Nontreated seeds			
Alfalfa	366	257	109	16	742.56**
Red clover	264	148	116	16	841.00**
Sweet clover	268	141	127	16	1008.06**
Alsike clover	153	72	81	16	410.06*
White clover	177	86	91	16	517.56**
Trefoil	65	71	-6	16	2.25
Lespedeza	119	146	-27	16	45.56

*Significant at the 5 per cent level.

**Significant at the 1 per cent level.

Stands and Nodules on Seedlings from Pelleted Alfalfa Seeds

Seeds of alfalfa were pelleted with Arasan by a method somewhat similar to the methods described by Newhall (52, 53), Linn and Newhall (44) and Kernkamp (36). Five grams of seed were placed in each of eight bottles. The seeds in the first bottle were treated with Arasan by slurry method and in second bottle were treated with Ceresan M dust by the method previously described. To each of the remaining six bottles was added 5 drops of 5 per cent aqueous solution of methyl cellulose. The bottles were shaken well to distribute the methyl cellulose uniformly on the seeds. Then 2.5 per cent, 5.0 per cent, 10.0 per cent, 20.0 per cent and 40.0 per cent of seed weight of Arasan, each amount having been made to 40.0 per cent of seed weight by addition of talc, were added to five of the six bottles. The seeds were pelleted one or two days before planting and stored in tightly closed bottles to avoid excessive drying and eventual scaling off of the fungicide.

<u>In nonsteamed greenhouse soil</u>

Nonsteamed greenhouse soil was mixed with "Nitragin"* at the rate of 1 teaspoonful in sufficient soil to fill four four inch pots. One hundred seeds of a given treatment were planted half inch deep in one pot. Number of seedlings per pot were counted 5 days after planting when most of the seeds had germinated. Four weeks later, all remaining seedlings were taken out of each pot and the soil removed from the roots first by shaking and finally by washing in a manner which resulted in minimum disturbance of root nodules. The

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*Nitragin is the trade name of the bacterial inoculum of nodule bacteria supplied by The Nitragin Co., Inc., Milwaukee, Wisconsin.

healthy seedlings in each pot and number of nodules on them were counted.

The experiment comprised four replications. Each replicate contained 8 randomized plots, one for each treatment.

The means of the original seedling stands, final stands of healthy seedlings and nodules per plant are presented in Table 41. The analysis of variance (Table 42) showed that the differences in stands between treatments, between observations and the interaction, treatment x observation were highly significant. The highly significant interaction indicated the failure of the treatments to behave uniformly in the two observations. Multiple range test (Table 43) indicated that the differences between mean stands from two observations for 40 per cent, 20 per cent, and 10 per cent were significantly less than the differences in mean stands between two observations for Arasan slurry treated, 2.5 per cent Arasan pelleted and nontreated seeds. The reduction in stands of seedlings between observations for seeds treated with Ceresan M and for seeds pelleted with 5 per cent Arasan were nearly equal and intermediate.

The mean seedling stands in first observation for all treatments were nearly equal and significantly greater than the mean seedling stand from nontreated seeds. The mean seedling stands in first observation for all the treatments varied within limits.

Table 41. Mean number of seedlings of alfalfa in two observations, and nodules per plant, from seeds pelleted with Arasan at various dosages and planted in nonsteamed greenhouse soil infested with nodule bacteria

	Mean number of seedlings		Nodules per plant
	5 days after planting	35 days after planting	
Arasan 2.5% (Pellet)	72.50	36.00	3.60
" 5.0% "	74.75	54.25	3.74
" 10.0% "	79.00	72.75	4.50
" 20.0% "	77.25	73.00	2.60
" 40.0% "	74.50	77.50	2.91
" (slurry)	71.25	27.00	3.57
Ceresan M (Dry dust)	71.00	47.00	3.70
Nontreated (Check)	47.25	16.50	3.79

Table 42. Analysis of variance of stand of alfalfa in two observations from seeds pelleted with Arasan in various dosages and planted in nonsteamed greenhouse soil infested with nodule bacteria

Source of variation	Degrees of freedom	Mean squares
Replicates	3	21.73
Treatments	7	1938.56**
Error (a)	21	123.09
Observations	1	6683.07**
Error (b)	3	18.89
Observation x treatments	7	559.06**
Error (c)	21	83.42

**Significant at the 1 per cent level.

Table 43. Comparisons among mean stands of alfalfa from various treatments in two observations, planted in nonsteamed greenhouse soil, by multiple range test

a) Shortest Significant Ranges:

P:	(2)	(3)	(4)	(5)	(6)	(7)	(8)
R_p(5%):	18.23	19.16	19.71	20.08	20.38	20.57	20.69

b) Results:

Original stand:

Treatments:	nontreated	C.M.	slurry	2.5%	40%	5%	20%	10%
Means:	47.2	71.0	71.2	72.5	74.5	74.7	77.2	79.0

Final stand:

Treatments:	nontreated	slurry	2.5%A.	C.M.	5%	10%	20%	40%
Means:	16.5	27.0	36.0	47.0	54.2	72.7	73.0	77.5

Treatments:	40%	20%	10%	5%	C.M.	nontreated	2.5%	slurry
Differences*:	-3.0	4.2	6.3	20.5	24.0	30.7	36.5	44.2

NOTE: Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

*Differences between mean stands from original and final stands.

Nodules happened to be most abundant on seedlings from seed pelleted with 10 per cent Arasan (Table 41) but in general nodules were developed with all treatments. Since there was great variation in nodule development no statistical analysis has been attempted. It is interesting to note that nodule development was more abundant on seedlings from seeds pelleted with 40 per cent Arasan than on seedlings from seeds pelleted with 20 per cent Arasan.

<u>In steamed soil in the greenhouse</u>

Greenhouse soil was steamed at 15 lb pressure for two hours. Next day a portion of the soil was infested with nodule bacteria at the rate of one teaspoonful of "Nitragin" in an amount of soil sufficient to fill four three inch pots. Thirty-two three inch pots were filled with infested soil. In four of these pots were planted 50 alfalfa seeds from one of the eight treatments listed in previous section. Another 32 three inch pots were filled with steamed non-infested soil. In four of these pots were planted 50 seeds from one of the eight treatments which seed had in addition been infested with nodule bacteria. Thus the infestation with nodule bacteria was accomplished by two methods, one by infesting the soil, the other by infesting the seeds at the time of planting.

Six weeks after planting, the seedlings were removed from the pots and most of the soil was shaken from the roots without disturbing the nodules on them. Finally the roots were washed thoroughly to remove the remaining soil. The number of seedlings and the number of nodules were counted for each pot.

The experiment consisted of four replications. Each replicate contained two split plots according to two methods of infestations. Each split plot consisted of eight randomized plots, one for each treatment.

The mean number of seedlings per pot and nodules per plant from four replicates of the several treatments are recorded in Table 44. No statistical analysis has been attempted because stands in the steamed soil were uniform and there was great variation in nodulation between replicates of a given treatment and from plant to plant in the same pot.

Nodules happened to be most abundant on seedlings from seed pelleted with 20 per cent Arasan, but in general nodules were developed with all treatments. It appeared that overall, nodule development was somewhat more where seeds were infested with nodule bacteria prior to planting than where soil was infested with nodule bacteria.

In the field

Seeds of alfalfa and lespedeza were treated with Arasan by slurry method, with dry dust of Ceresan M and pelleted with 40 per cent Arasan. Treated seeds and nontreated seeds

Table 44. Mean number of seedlings of alfalfa and nodules per plant from seeds treated variously and planted in steamed soil, either soil or seeds infested with nodule bacteria

	Soil infested with nodule bacteria		Seeds infested with nodule bacteria	
	Mean number of seedlings	Nodules per plant	Mean number of seedlings	Nodules per plant
Arasan 2.5%	33.50	1.61	38.50	2.52
" 5.0%	39.50	1.41	37.75	2.23
" 10.0%	35.50	0.87	40.75	2.26
" 20.0%	37.75	2.28	37.75	3.00
" 40.0%	34.75	1.71	36.25	2.83
" (Slurry)	38.75	1.61	37.75	1.70
Ceresan M (Dry dust)	41.50	1.05	40.75	2.04
Nontreated (check)	38.00	1.77	38.00	2.96

were planted half inch deep, 100 seeds per row in rows two feet long and six inches apart in Clarion loam soil at the Ash Avenue plots of the Botany and Plant Pathology Department, Ames. Seedling stands were counted four weeks after planting.

The experiment consisted of four replications. Each replicate had two split plots according to two crops. Each split plot contained four randomized treatments.

Mean number of seedlings of alfalfa and lespedeza from four replicates are recorded in Table 45. The analysis of variance (Table 46) showed that the difference between stands

Table 45. Mean number of seedlings from treated, pelleted and nontreated seeds of alfalfa and lespedeza planted on Clarion loam in the field

	Treatments	Per cent Stand
Alfalfa	Slurry (Arasan)	22.75
	Ceresan M (Dry dust)	27.00
	Pelleting (40% Arasan)	34.75
	Nontreated	18.25
Lespedeza	Slurry (Arasan)	8.50
	Ceresan M (Dry dust)	7.50
	Pelleting (40% Arasan)	13.50
	Nontreated	6.50

Table 46. Analysis of variance of seedlings from treated, pelleted and nontreated seeds of alfalfa and lespedeza planted on Clarion loam in the field

Source of variation	Degrees of freedom	Mean squares
Replicates	3	101.03
Legumes	1	2227.78*
Error (a)	3	106.53
Treatments	3	196.36*
Treatments x legumes	3	39.36
Error (b)	18	45.20

*Significant at the 5 per cent level.

of alfalfa and lespedeza and the differences among the stands from treatments were significant at the 5 per cent level. The interaction, treatment x legumes was not significant.

The results in Table 45 showed that in the field pelleting increased the stand of alfalfa and lespedeza. The greater stands due to slurry and Ceresan M treatments for alfalfa and lespedeza were nearly equal to stands from nontreated seeds. The comparisons among the mean stands from treatments by multiple range test (Table 47) showed that the mean stand from pelleted seeds was significantly greater than mean stand from nontreated seeds. Though the mean stands from slurry treated and Ceresan M treated seeds were intermediate, none were significantly different from the mean stands from either nontreated or from pelleted seed.

Table 47. Comparisons of mean stands from treated, pelleted and nontreated seeds of alfalfa and lespedeza planted on Clarion loam, in the field, by multiple range test

a) Shortest Significant Ranges:

P:	(2)	(3)	(4)
$R_p(5)$:	9.98	10.48	10.79

b) Results:

Treatments:	Nontreated	Slurry	Ceresan M	Pelleting
Means:	12.37	15.62	17.25	24.12

NOTE: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.

DISCUSSION AND CONCLUSIONS

The overall relative susceptibility to seed rotting and seedling necrosis of the seven small seeded legumes tested in these experiments is recorded in Table 48, from least to most susceptible as follows: lespedeza, alsike clover, white clover, red clover, birdsfoot trefoil, alfalfa and sweet clover. This ranking is on the basis of mean seedling stands from nontreated seeds planted in nonsteamed soils.

The ranking is similar when it is based on differences in mean seedling stands from nontreated seeds planted in steamed and nonsteamed soils (Table 49) except for lespedeza, which on this basis showed slightly greater susceptibility than alsike and white clover.

Ranking of the seven legumes is also somewhat similar when based on disease indices in laboratory tests on Petri plate cultures (Table 50) except for alfalfa, which was ranked higher (third) and white clover, which was ranked lower (sixth). White clover seemed to be slow in germinating on Petri plate cultures, and for that reason may have showed more seed rotting. Alfalfa showed more resistance to seedling necrosis.

Seedling stands of the seven legumes in a field experiment were in an order quite different from those in greenhouse or laboratory tests. Alfalfa, red clover and sweet

Table 48. Mean seedling stands from nontreated seeds of seven legumes pla

	Average laboratory germination %	Sand bench	Rank	Clarion loam 6.1	Rank	Webster loam 7.4	Rank	Ta 5.6
Lespedeza	95.5	84.1	2	35.3	1	29.7	1	37
Alsike clover	92.5	90.0	1	14.3	5	18.2	4	33
White clover	89.0	72.9	3	22.7	3	23.3	3	36
Red clover	95.5	65.0	5	28.3	2	18.0	5	36
Trefoil	93.5	68.7	4	11.0	7	26.7	2	31
Alfalfa	93.0	44.6	6	17.7	4	8.3	7	19
Sweet clover	94.0	34.7	7	12.5	6	10.5	6	16

egumes planted in nonsteamed soil, in various experiments.

Rank	Tama silt loam 5.6	Rank	5.7	Rank	Wabash loam 5.8	Rank	Greenhouse soil temp.	Rank	Overall Mean	Rank
1	37.5	1	18.7	1	27.6	1	14.1	2	39.7	1
4	33.1	4	12.7	4	24.2	2	12.5	3	35.5	2
3	36.9	2	16.3	2	18.3	4	15.8	1	33.4	3
5	36.1	3	16.0	3	20.2	3	12.3	4	31.1	4
2	31.0	5	8.6	5	11.2	5	9.2	5	28.4	5
7	19.3	6	7.1	6	6.8	6	6.5	6	18.8	6
6	16.3	7	6.1	7	4.1	7	3.3	7	15.4	7

Table 49. Difference between the mean seedling stands from steamed and nonsteamed soils, planted with nontreated seeds of seven legumes in various experiments

	Difference in seedling stands in:											
	Clarion loam	Rank	Webster loam	Rank	Tama silt loam	Rank	Tama silt loam	Rank	Wabash loam	Rank	Overall Mean	Overall Rank
pH	6.1		7.4		5.6		5.7		5.8			
Alsike clover	7.7*	4	7.5	3	1.1	2	19.1	1	3.6	1	7.8	1
White clover	5.0	3	5.8	2	-0.2	1	21.0	3	14.2	4	9.2	2
Lespedeza	0.1	1	8.1	4	3.5	4	24.0	4	11.1	3	9.4	3
Red clover	4.0	2	17.3	6	2.7	3	20.2	2	11.0	2	11.0	4
Trefoil	21.2	7	-1.2	1	6.0	6	28.6	6	19.1	5	14.7	5
Sweet clover	12.6	5	14.7	5	5.6	5	27.0	5	28.7	7	17.7	6
Alfalfa	18.7	6	29.7	7	22.3	7	34.7	7	27.3	6	26.5	7

*Difference indicates susceptibility; the greater the difference, the greater the susceptibility.

Table 50. Mean indices of seed rot and seedling necrosis of seven legumes in Petri plate cultures

	Mean indices for:					
	Seed rot	Rank	Seedling necrosis	Rank	Overall Mean	Rank
Lespedeza	4.16*	2	2.40	1	3.28	1
Red clover	4.15	1	3.19	3	3.67	2
Alfalfa	4.69	4	3.02	2	3.85	3
Alsike clover	4.56	3	3.23	5	3.89	4
Trefoil	4.78	5	3.37	6	4.07	5
White clover	5.11	6	3.26	4	4.18	6
Sweet clover	5.19	7	3.55	7	4.37	7

*The greater the indices, the greater the susceptibility.

clover yielded better stands than other four legumes. This field planting was in late summer, in soil which had been dry and hot for a considerable time before planting. The seeds germinated during a period of continuous cool, wet weather. It is possible that most of the Pythium species in the soil might have died or become inactive during the hot dry period in summer, and that the wet, cool weather which followed was favorable for germination and seedling growth of alfalfa, red clover and sweet clover. This observation is in essential agreement with those of Beach (5), Buchholtz (9), Gerdemann (17), Graham et al (20, 21) and Kreitlow (38).

The experiments in which these seven legumes were compared constituted replicated comparisons involving two seed lots of each legume. Seed lots chosen were all such as to show 89 per cent or more of the normal sprouts plus hard seed in the Iowa State College Seed Laboratory test. Percentage germination of individual seed lots in steamed soil varied from 48.3 per cent by one lot of sweet clover to 79.9 per cent by one lot of red clover seed. Mean percentage germination in steamed soil of the two seed lots of each legume was as follows: lespedeza, 77.5; alsike, 65.0; white clover, 64.2; red clover, 69.4; trefoil, 63.6; alfalfa, 76.5; sweet clover, 57.8. It is evident that the seed lots of lespedeza, the most resistant legume, ranked highest, and the seed lots of sweet clover, the most susceptible legume, ranked lowest in germination in steamed soil. On the other hand, seed lots of alfalfa, which was ranked near to sweet clover in susceptibility, were nearly equal in germination in steamed soil to those of lespedeza, the most resistant legume. For the other four legumes, there was little variation between legumes in mean germination percentage of the two seed lots in steamed soil.

In the case of alsike, red clover, trefoil and sweet clover, one seed lot consistently germinated better than the other in steamed soil. Except for trefoil, in each case the seed lot germinating better in steamed soil also yielded better stands in nonsteamed soil.

In these experiments, high seed viability may have contributed slightly to the good stands of lespedeza and somewhat low seed viability to the poor stands of sweet clover. On the other hand, Halpin et al (24) have also rated sweet clover as highly susceptible to seed rot and seedling necrosis. In spite of some variation between legumes in seed viability, it still seems valid to suggest that among small seeded legumes, lespedeza ranks with alsike, white clover and red clover as resistant, and trefoil ranks with alfalfa and sweet clover as susceptible to seed rot and seedling necrosis by Pythium.

Of three Pythium species tested on agar plate cultures, P. aphanidermatum was found to be more pathogenic than P. debaryanum and Pythium sp. to germinating seeds of the seven legumes. It is perhaps pertinent that in Wabash loam soil, from which P. aphanidermatum was isolated almost exclusively, there were less healthy seedlings than in the other four field soil samples in which plantings were made, or in greenhouse soil. From the other four field soil samples, germinating seeds yielded Pythium sp. predominantly. Even so, in one of the two samples of Tama silt loam seedling stands were poorer than in the other. On the other hand, seedling stands were comparable in Clarion loam and Webster loam samples collected from the same location. Fulkerson (16) has suggested that rich soil may give higher stand than poor soil from one location but this may not be true for soils obtained from different locations because the soils from different locations might have organisms varying in amount and pathogenicity.

The variation in stands of seven legumes in soils with pH ranging from 5.6 to 7.4 showed that the soil pH had no influence on stands of legumes. The stands of legumes in Webster loam, with pH 7.4 and Clarion loam, with pH 6.1 were nearly equal. On the other hand, the stands of legumes in Tama silt loam, with pH 5.6 were significantly greater than the stands of legumes from Tama silt loam, with pH 5.7 and Wabash loam, with pH 5.8. The interaction, legumes x soils was not significant in soils from Ames or from Warren County which indicated that the effects of pH or other soil factors were essentially the same for all legumes. Kadow and Anderson (31) and Kreitlow et al (39) have suggested that soil reaction was not important in seed rotting and damping off.

In the greenhouse plantings at three temperatures disease was significantly greater at 21° and 27° C than at 16° C. That is, the higher temperature, which is generally favorable for the better growth of some of the pathogenic fungi, especially *Pythium*, is not favorable for the better growth of small seeded legumes. Leach (41) has shown that when the ratio, (growth rate of host)/(growth rate of pathogen) is less than 1 the disease is severe.

The stands of legumes were less with saturated watering than with moderate watering. It was observed that the germination was delayed for two to three days with saturated watering. It is possible that some pathogenic fungi, especially Pythium, might have been favored by the higher soil moisture from saturated watering. There were larger increases in stand due to treatment with moderate watering than with saturated watering.

The overall response to seed treatment by seven legumes tested in these experiments is recorded in Table 51, from least to maximum response by legumes, as follows: trefoil, white clover, lespedeza, alsike, sweet clover, red clover and alfalfa. This order is on the basis of difference between mean stands from treated and nontreated seeds planted in nonsteamed soils. The results indicated that whether in greenhouse or in field, irrespective of soil type, alfalfa, red clover and sweet clover most of the time responded more advantageously to seed treatment than did alsike, lespedeza, white clover and trefoil. Apparently favorable response to seed treatment was by the comparatively large-seeded legumes, namely alfalfa, red clover and sweet clover, which also in these experiments germinated more rapidly than the smaller seeded legumes, namely alsike, white clover and trefoil.

Table 51. Difference between the mean seedling stands from treated and
in various experiments.

	Sand bench	Rank	Clarion loam	Rank	Webster loam	Rank	Mean seedlings Tama silt lo	Rank	
pH			6.1		7.4		5.6		5.7
Trefoil	-2.7*	1	7.8	6	-7.3	1	6.6	6	6.7
White clover	-6.5	2	5.1	4	-4.7	2	-1.0	1	14.7
Lespedeza	3.3	3	1.6	2	2.1	4	5.7	4	16.3
Alsike clover	9.1	4	5.2	5	4.6	6	2.6	3	13.8
Sweet clover	23.2	6	0.8	1	1.1	3	6.0	5	14.7
Red clover	10.7	5	4.7	3	5.0	7	1.6	2	16.1
Alfalfa	26.3	7	6.8	7	4.2	5	10.5	7	15.5

* Difference indicates the response by legumes to seed treatment. (

:reated and nontreated seeds of seven legumes planted in nonsteamed soils,

ın seedlings from:								
'ama silt loam			Wabash		Greenhouse		Overall	
Rank		Rank	loam	Rank	(Temp.)	Rank	Mean	Rank
	5.7		5.8		6.9			
6	6.7	1	4.5	1	11.3	3	3.8	1
1	14.7	3	9.7	5	10.3	2	3.9	2
4	16.3	7	6.8	2	11.5	4	6.7	3
3	13.8	2	7.3	3	14.2	7	8.1	4
5	14.7	4	8.2	4	9.7	1	9.1	5
2	16.1	6	13.2	6	13.4	6	9.2	6
7	15.5	5	13.6	7	13.2	5	12.9	7

reatment. Greater the difference the greater the response.

Though lespedeza seed was comparable in size to the former it was slow in germination, responded less to seed treatment.

Alfalfa seed variously treated and pelleted when planted in greenhouse showed that there were significantly better stands of seedlings from seeds pelleted with either 10, 20 or 40 per cent Arasan than from seeds pelleted with 2.5 and 5.0 per cent Arasan, slurry treated with Arasan, or treated with Ceresan M dust. Poorest stand was from non-treated seed. In field trials, the stands from pelleted seeds of alfalfa and lespedeza were significantly greater than the stands from nontreated seeds. It seems reasonable to suggest that the higher dosages of Arasan applied in pelleting offered better protection of the germinating seeds and seedlings from infection by soil-borne fungi.

Nodules were developed on roots of legumes planted in steamed or nonsteamed soil regardless of whether bacterial inoculum was applied to seed or soil, even with high dosages of chemical seed treatment. In fact, nodulation was actually (not significantly) somewhat greater on seedlings from seeds pelleted with 10 and 20 per cent Arasan than on seedlings from nontreated seed, or seeds otherwise treated or pelleted with other dosages of Arasan. Vlitos and Preston (62) have suggested that seed treatment fungicides might have a stimulatory effect on nodulation.

Nodulation was somewhat greater on seedlings from seeds infested with nodule bacteria than on seedling from seeds planted in infested soil. This was true for seeds with or without chemical seed treatment. It would appear that the fungicide and the nodule bacteria simultaneously present on the seed were compatible at least for a short time.

Nodule development was slightly better on seedlings in bacteria-infested nonsteamed soil than on seedlings in infested steamed soil. This observation agrees with that of Kernkamp (36).

In general, these nodulation experiments tend to suggest that seed treatment fungicides do not "protect" the germinating seed and seedling from infection by nodule bacteria, at least not to an extent comparable to the protection afforded against the soil borne fungi which cause seed rot and seedling necrosis.

SUMMARY

Two seed lots of each of alfalfa, red clover, sweet clover, alsike, white clover, birdsfoot trefoil and lespedeza were tested for susceptibility to seed rot and seedling necrosis by Pythium in the laboratory and in greenhouse and field plantings.

Seeds were placed on four or five day old agar plate cultures of _P. debaryanum_, _P. aphanidermatum_ and unidentified _Pythium_ sp. By means of seed rotting indices, sweet clover and trefoil were found to be most susceptible to these three species of Pythium. Lespedeza and red clover were least susceptible, alsike and alfalfa were intermediate; white clover appeared to be susceptible, probably due to delayed germination.

Small seedlings of the seven legumes were planted on four or five day old agar plate cultures of the same three species of _Pythium_. By means of seedling necrosis indices, lespedeza, red clover, alfalfa and alsike were determined to be least susceptible and sweet clover, white clover and trefoil to be most susceptible to these three species of _Pythium_.

Treated and nontreated seeds of the seven legumes were planted in two samples of Tama silt loam, with pH 5.6 and pH 5.7, respectively, in Clarion loam, with pH 6.1, in Wabash loam, with pH 5.8, and in Webster loam, with pH 7.4.

According to numbers of seedlings from nontreated seed, overall susceptibility of the seven legumes, from least to most, was as follows: lespedeza, alsike, white clover, red clover, trefoil, alfalfa and sweet clover. This ranking is also somewhat similar to those based on disease indices in laboratory tests on Petri plate cultures. Hence this order seems to be natural.

In a field planting, seedling stands of the seven legumes were in an order quite different from those in greenhouse tests; stands of alfalfa, red clover and sweet clover were better than of the other four legumes.

Of the three *Pythium* species tested on agar plate cultures, *P. aphanidermatum* was found to be more pathogenic than *P. debaryanum* and *Pythium* sp. to germinating seeds of the seven legumes. *P. aphanidermatum* was isolated almost exclusively from Wabash loam, on which there were very poor stands in the greenhouse. From the other four soil samples, germinating seed yielded *Pythium* sp. predominantly. Even so, seedling stands were poorer in one of the two samples of Tama silt loam than in the other. On the contrary, seedling stands were comparable in Clarion loam and Webster loam samples collected from the same location.

The variation in legume seedling stands in these experiments was not correlated with variation in soil pH. The stands in Webster loam, with pH 7.4 and Clarion loam, with pH 6.1 were nearly the same. On the other hand the stands in Tama silt loam, with pH 5.6 were significantly greater than the stands in Tama silt loam, with pH 5.7 and in Wabash loam, with pH 5.8.

In the greenhouse plantings at three temperatures, with moderate and saturated watering, disease was significantly greater at 21° and 27°C than at 16°C. The stands of legumes were less with saturated watering than with moderate watering. There were larger increases in stand due to treatment with moderate watering than with saturated watering.

The overall response to seed treatment by seven legumes tested in these experiments indicated that whether in greenhouse or in field, irrespective of soil type, alfalfa, red clover and sweet clover most of the times responded more advantageously to seed treatment than did alsike, lespedeza, white clover and trefoil.

Germination of the fourteen seed lots was comparable in seed laboratory tests. Although there was some variation between seed lots and between legumes in germination in steamed soil, such variation was not as great as in unsteamed soil, and not consistently in the same direction and magnitude. Therefore, the small variation between seed lots in viability was considered not to be responsible for differences between legumes in these experiments.

Alfalfa seeds variously treated and pelleted when planted in the greenhouse showed that there were significantly better stands of seedlings from seeds pelleted with either 10, 20 or 40 per cent Arasan than from seeds pelleted with 2.5 and 5.0 per cent Arasan, slurry treated with Arasan, or treated with Ceresan M dust. Poorest stands were from nontreated seed. In a field trial, the stands from pelleted seeds of alfalfa and lespedeza were greater than the stands from slurry treated (Arasan), Ceresan M treated (dust) and nontreated seeds.

Nodules were developed on roots of the legumes planted in steamed or nonsteamed soil regardless of whether bacterial inoculum was applied to seed or soil, even with high dosages of chemical treatment. In fact, nodulation was actually somewhat greater from seeds pelleted with 10 and 20 per cent Arasan than on seedlings from nontreated seed, or seeds otherwise treated or pelleted with other dosages of Arasan.

Nodulation was somewhat greater on seedlings from seeds infested with nodule bacteria than on seedlings from seeds planted in bacteria-infested soil. This was true for seeds with or without chemical seed treatment.

Nodule development was slightly better on seedlings in bacteria-infested nonsteamed soil than on seedlings in bacteria-infested steamed soil.

In general, these experiments tend to suggest that seed treatment fungicides do not "protect" the germinating seed and seedling from infection by nodule bacteria at least to an extent comparable to the protection afforded against the soil-borne fungi which cause seed rot and seedling necrosis.

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