THE EFFICIENCY OF LEGUME INOCULATION FOR ARIZONA SOILS

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By

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A THESIS

Presented to the faculty of the University of Arizona as a part of the Requirements for the Degree of Master of Science.

Presented May 15, 1922.

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Introduction

A few years ago several companies handling commercial cultures of Bacillus radicicola began a more or less extensive advertising campaign for their products. At that time no data had been obtained as to the actual need of Arizona soils for artificial inoculation. The Agronomy Department of the University of Arizona outlined experiments in inoculation work which were carried on at the various experiment stations in the state. In addition to the field work detailed pot experiments were conducted at the University at Tucson. Not only was the excess yield, or lack of same, due to inoculation investigated, but the possibilities for increased root growth with its extra manurial value and the possibilities for increased nitrogen content of tops, roots and soil were studied.

The soils of the arid Southwest are very different from those of the other sections of the United States and as yet little is known as to bacterial action in these soils. No attempt has been made in the present study to investigate the habits of the bacteria themselves further than an investigation of the effect of the symbiotic nitrogen-fixing Bacillus radicicola upon the growth and nitrogen content of the various legumes studied. The methods used and the results obtained are recorded in this publication.

FIELD TESTS

Salt River Valley Experiment Farm

The soil upon this farm is a tight textured clay loam soil. In the fall of 1919 ten one-acre plats were planted to Hairy Peruvian alfalfa, some of the plats being planted with inoculated seed and others receiving no treatment. Soil from an old alfalfa field was scattered on one of the acres. At no time could a noticeable difference in growth be observed. The yields for the years 1920 and 1921 show that the four untreated acres averaged 132 more pounds to the acre per cutting than did the plats which had received the inoculation.

Table I. Inoculation studies on a ten-acre field of alfalfa on the Salt River Valley Experimental Farm near Mesa, Arizona.

No.	Border Treatment	Yield cured hay per acre per average cutting		
		1920	1921	Average for two years
		Lbs.	Lbs.	Lbs.
W57	none	1475	2056	1775
W58	none	1799	2196	1997
W59	Westrobac inoculation	1653	1760	1706
W60	Westrobac inoculation	1725	2607	2166
W61	Farmogerm inoculation	1903	2292	2097
W62	Soil transfer	1946	2385	2165
W63	Mulford inoculation	1729	2262	1995
W64	Mulford inoculation	1868	2271	2069
W65	none	2335	2850	2592
W66	none	2348	2271	2259
	Average of the four untreated acres	1987	2343	2165
	Average of the six inoculated acres	1804	2263	2033

Examinations of the roots of the alfalfa plants at several times during the season showed that nodules were present in approximately equal abundance regardless of whether the seed had been artificially inoculated or not. This land had never grown any legumes previous to the present planting of alfalfa at least since the University of Arizona took charge of it in 1915.

Table 2. Inoculation studies with cowpeas at the Salt River Valley Experiment Farm near Mesa, Arizona.

Treatment	Yield per acre threshed seed		
	1920	1921	Average for two years
	Lbs.	Lbs.	Lbs.
Farmogerm inoculation	222	105	163.5
Mulford culture inoculation	225		
Westrobac inoculation	294		
Not inoculated	165	76	120.0

There was a rank growth of vines in every case both on the inoculated areas and upon the uninoculated areas. Seed did not set in sufficient quantities on any of the borders to pay for the threshing. Red Kipper cowpeas were used for these tests.

A careful examination of the roots has shown that during the hot months of the year when cowpeas are ordinarily growing for green manuring purposes very few or no nodules are developed, (1) either when the seed has been inoculated, or when the seed has not been inoculated.

This same thing has been noted with soybeans; namely, that root nodules common to legumes and so necessary before the plants

can utilize the atmospheric nitrogen provided by the Bacillus radicicola are not present during the middle of the summer in the hot climate of the Salt River Valley.

Sulphur Spring Valley Dry Farm

During the early part of the growing season of 1919 there was no noticeable difference between the inoculated and the uninoculated plants in the tests conducted at the Sulphur Spring Valley Dry Farm. Nodules were easily found on the roots of all of the varieties on both the inoculated and the uninoculated plants. About fifteen days before harvest several of the inoculated plats showed gains over those not inoculated and at harvest time had gained 15.7 percent.

The season of 1920 was a very dry one and all yields were so small that comparative data were unsatisfactory. The inoculated plats made a gain of but 3.4 percent that season over the plats not inoculated.

Grasshoppers and rabbits worked upon the legume plots so badly during the season of 1921 that the results are not comparable.

From the data obtained as well as from the appearance of the plats before being damaged by drought, insects and rabbits during the last two seasons, indications are very strong that artificial inoculation is needed for maximum yields upon this farm. The soil is a very poor range soil with considerable sand in it. All of the legumes were grown each year upon plats that had had no legumes growing on them in previous years.

Table 3. Inoculation tests at Sulphur Spring Valley Dry Farm

Crop	Treatment	Yields per acre		
		1919	1920	1921
		Forage	Seed	Seed
Early Bird Velvet beans	inoc.	1375		
	not inoc.	1320		
Chinese Velvet beans	inoc.	1375		
	not inoc.	1100		
Black Eye cowpea	inoc.	2090	88	
	not inoc.	1760	82	
Red Ripper cowpea	inoc.	1155		451
	not inoc.	1265		407
New Era cowpeas	inoc.	2695		
	not inoc.	2475		
Mammoth Yellow soybeans	inoc.	770		
	not inoc.	770		
Pink beans	inoc.		104.5	220
	not inoc.		99	352
Red Ripper cowpeas	inoc.		71.5	
	not inoc.		71.5	
Taylor cowpeas	inoc.		38.5	
	not inoc.		44	
Groit cowpeas	inoc.			385
	not inoc.			429
Pinto beans	inoc.			102
	not inoc.			187
Tepary beans	inoc.			407
	not inoc.			517
Average gain or loss of inoculated plats		15.7%	3.4%	-17.7%

Yuma Mesa Farm

Cultivation was first begun upon a portion of the experiment station located upon the Yuma mesa in the spring of 1921. Vetch was used as a cover and green manure crop that first season in the young citrus orchard. Most of the seed was inoculated with a commercial culture prior to planting, but some received no treatment. There was a marked difference between the growth of the inoculated plants and those not inoculated, the inoculated plants making several times the growth that those not inoculated made. Not only was the growth greater, but as the season progressed many of the plants not inoculated died. An examination of the roots showed that the inoculated plants had a much heavier root system and a greater abundance of nodules than did the plants which had received no inoculation. Practically all of the plants which survived on the area not inoculated had some root nodules present which is conclusive proof that the Bacillus radicicola is present to some extent in this raw desert land, but not in sufficient quantities to allow maximum inoculation of legumes and artificial inoculation must be resorted to.

A mixture of vetch and rye was planted in the same orchard in the spring of 1922. A part of the area was planted with inoculated seed and a part with seed not inoculated. At the present time, May 1, the growth of vetch is appreciably thicker where inoculated than where no inoculation was given, even though both areas had a heavy crop of inoculated plants plowed under last year. The soil in this orchard is very uniformly sandy and

devoid of much organic matter. One year's growth of inoculated legumes did not put the soil into condition to grow a maximum crop of vetch the next year without another inoculation.

Fig. 1.-Vetch in the citrus orchard of the Yuma Mesa Farm. Borders on left inoculated; on right not inoculated. Picture taken April 6, 1921.

Fig.2.-Vetch on the Yuma Mesa Farm. Border on left inoculated; on right not inoculated.Picture taken April 28,1921.

Fig.3.-Vetch and rye on the Yuma Mesa Farm. First crop on land. Border on left inoculated; on right not inoculated. Picture taken May 6, 1922.

Table 4. The effect of inoculation upon the dry weight of the tops, roots and nodules of common vetch grown on the experiment station upon the Yuma mesa. Average of five representative plants from each area. Plants collected May 6, 1922.

Soil and treatment	Dry weight in grams		
	Tops	Roots	Nodules
Cropped last year with inoculated vetch.			
Inoculated this year	4.744	2.92	.173
Not inoculated this year	7.162	1.451	.286
Cropped for first time this year.			
Inoculated	4.228	1.492	.138
Not inoculated	2.283	1.01	.216

An examination of Table 4 will show that although the artificially inoculated plants made a much heavier root growth, the weight of the nodules upon the plants not inoculated was on an average greater than that upon the inoculated plants. It was also noticeable that the nodules were on an average larger upon the plants not inoculated than upon those inoculated. On the area cropped for the first time this year, most of the plants which were not inoculated died after a few weeks. The ones which did survive had plenty of nodules but were the best plants upon the area. In every other case average plants were taken.

A marked difference can be seen in the appearance of the citrus trees grown on the area, which had a good crop of green manure plowed under last year, and those grown on the area with the uninoculated vetch, which made little growth last year for green manure. The leaves on the former are dark green in color while those of the latter are a much lighter green.

POT EXPERIMENTS

Pot experiments were conducted at the University during the growing seasons of 1921 and 1922, not only in order to determine the possibility of increasing the yields of legumes by inoculation, when grown upon such soils as are found in the vicinity of Tucson, but also to determine the effect of inoculation upon the nitrogen content of the tops and roots of the plants and of the soil upon which the plants grew. If the plants had been grown in the field it would have been exceedingly difficult to have obtained fair samples of the soils for analysis.

Methods.--Three different soils were used: A sandy loam virgin soil from the Rillito River bottom; a similar soil, but under cultivation with a good rotation system for a number of years; and a sandy desert soil which had never been under cultivation. These soils were all carefully screened through a one millimeter screen and thirteen kilograms put into each pot, all pots having been previously coated on the inside with paraffin.

Two trenches, each about a foot deep, were dug in the screen garden used for the work. In the bottom of each trench a platform, having twelve holes in which to hold the pots, was placed in such a manner that the bottoms of the pots were prevented from coming into contact with the surrounding soil and perhaps become inoculated with bacteria from foreign soil. Had the pots been placed upon the ground, bacteria if present could easily have entered through the drainage holes in the bottoms of the pots. Since the pots used were not glazed the paraffin coating served to prevent the entrance of bacteria through the pores of the walls. After the pots containing the soils were in place on the platforms, dirt was carefully packed around them completely filling the trenches above the platforms, leaving the pots protruding two or three inches above the surface of the ground. One set of twelve pots which was to be planted with inoculated seed was placed in a different section of the garden, approximately fifty feet from the other set which was not to be inoculated. This was done to avoid accidental inoculation as much as possible.

Inoculation material was obtained from the U. S. Department of Agriculture and the seeds planted in the inoculated pots were all uniformly treated. The pots were planted on April 12, 1921 as follows:

Hairy Peruvian Alfalfa

Virgin soil, Rillito River bottom	4 pots
Virgin soil, Rillito River bottom, sterilized	2 "
Cultivated Rillito River bottom soil	4 "
Virgin desert soil	4 "

Whippoorwill Cowpeas

Virgin soil, Rillito River bottom	6 "

Ito San Soybeans

Virgin soil, Rillito River bottom	4 "

One half of these pots were planted with inoculated seed and the other half with seed which had not been inoculated.

Results.--In spite of the care that was used to prevent accidental inoculation commensurate with the equipment available, some outside inoculation did occur as evidenced from the fact that the pot of alfalfa plants whose seeds had not been inoculated and grown in sterilized soil*contained a few root nodules. These pots of sterilized soil were used for the purpose of ascertaining the occurrence of accidental contamination as well as a check upon the vitality of the inoculation material used. In the case of the soybeans which had not been inoculated, no root nodules could be found. In all other instances some root nodules were found on both the inoculated plants and upon those not inoculated. The nodules were much more abundant, however, upon the plants in the inoculated pots than they were upon the plants not receiving the inoculation.

*Sterilized in autoclave for 1 hour at 20# pressure.

The inoculated plants reached maturity in every instance 5 to 7 days before those not inoculated.(2) All of each variety were harvested at the same time and dry weights taken. In the case of the alfalfa, three cuttings were made during the season from the pots containing the inoculated plants and but two in those which had not been inoculated since the third cutting did not grow sufficiently to warrant cutting. After the last cuttings were made samples of the soils were taken and the roots carefully washed from the soil upon a screen. This same procedure was used with the soybeans and cowpeas, cuttings being made just before the formation of seed with the exception of two pots of cowpeas, which were allowed to remain until seed had ripened before harvesting.

There was a decided gain in weight of dry matter in the tops of the inoculated plants over those not inoculated in every instance. In the case of the alfalfa this difference was greatest in the plants grown upon desert soil, the gain being almost 65 percent. The next greatest gain was on the sterilized virgin river bottom soil, the gain here being 59 percent. The same soil not sterilized before planting produced plants which made a gain of 55 percent due to inoculation, while the gain upon the cultivated soil was 58 percent. This indicates that, while inoculation was of great value in each case, it was of greatest value on the poorer desert soil as far as gain in dry matter was concerned.

The increase in dry matter due to inoculation was more pronounced in the first cuttings in each instance with the exception of the plants grown on the sterilized soil, where

the increase was greater with the second cutting by 20 percent. This indicates that bacterial action was on the increase in the pots which had not received inoculation to such an extent that they were becoming more nearly like those which had been given the benefit of the inoculation.(3)

The Bacillus radicicola must have been present in the original soils to such an extent that when the alfalfa was planted and water applied they soon multiplied and by the time the second cuttings of alfalfa were made they had made quite a gain in abundance over the number present when the first cuttings were made. The percent of gain due to the inoculation averaged 62 percent at the first cutting with the three soils, while at the second cutting it averaged but 40 percent. This conclusion is further substantiated by the fact that in the case of the sterilized soil the increase due to inoculation at the first cutting was 31 percent and at the second cutting 51 percent. While the sterilizing undoubtedly killed all of the soil bacteria present in the soil at the time, accidental inoculation did occur afterwards as evidenced by the presence of root nodules in the pot, which did not receive the artificial inoculation. This accidental contamination was so small that the bacteria seemingly did not increase nearly so rapidly in the sterilized soil as they did in the same soil not sterilized and where bacteria were present in more abundance before any artificial inoculation was done.

Table 5. The effect of inoculation upon the dry weight and upon the nitrogen content of the tops of alfalfa plants.

Soil	Treatment	Cutting No.	Dry Weight: Grams per pot	Dry Weight: Percent gain or loss	Nitrogen: Percent	Nitrogen: Percent gain or loss	Nitrogen: Lbs. gain or loss per ton
Virgin Rillito River bottom	inoc.	1	11.50	54.36	2.57		
	not inoc.	1	7.45		2.33	.24	4.8
	inoc.	2	12.00		2.38		
	not inoc.	2	8.75	37.14	2.59	-.21	-4.2
	inoc.	3	1.65				
	not inoc.	3	none				
	inoc.	total	25.15	55.24			
	not inoc.	"	16.20				
Virgin Rillito River bottom sterilized	inoc.	1	9.25		2.46		
	not inoc.	1	7.05	31.20	1.99	.47	9.4
	inoc.	2	9.10		2.60		
	not inoc.	2	6.00	51.66	2.51	.09	1.8
	inoc.	3	2.4				
	not inoc.	3	none				
	inoc.	total	20.75				
	not inoc.	"	13.05	59.00			
Cultivated Rillito River bottom	inoc.	1	11.03		2.48		
	not inoc.	1	6.60	67.12	2.38	.10	2.0
	inoc.	2	8.05		2.35		
	not inoc.	2	6.05	33.05	2.40	-.05	-1.0
	inoc.	3	0.9				
	not inoc.	3	none				
	inoc.	total	19.98				
	not inoc.	"	12.65	57.85			
Virgin Desert east of campus	inoc.	1	10.75		2.64		
	not inoc.	1	6.48	65.89	2.42	.22	4.4
	inoc.	2	10.20		2.68		
	not inoc.	2	6.80	50.00	2.56	.12	2.4
	inoc.	3	0.9				
	not inoc.	3	none				
	inoc.	total	21.85				
	not inoc.	"	1328	64.53			

The nitrogen content of the alfalfa tops was slightly greater in every instance in the first cuttings of the inoculated plants than in those not inoculated. This was also true of the second cuttings of inoculated plants on the two soils which responded to inoculation the best; namely, the desert soil and the sterilized river bottom soil. With both the virgin and the cultivated river bottom soils which had not been sterilized, the nitrogen content of the second cuttings of plants, which had not been inoculated, showed a slightly higher nitrogen content than those which had been inoculated.(4)

Fig.4.-Inoculated alfalfa growing in cultivated Rillito River bottom soil.

Fig.5.-Alfalfa plants not inoculated growing in cultivated Rillito River bottom soil.

Table 6. The effect of inoculation upon the dry weight and upon the nitrogen content of the roots of alfalfa plants.

Soil	Treatment	Dry Weight		Nitrogen	
		Grams per pot	Percent gain or loss	Percent	Percent gain or loss
Virgin Rillito River bottom	inoc.	38.25	31.89	1.68	-.24
	not inoc.	29.00		1.92	
Virgin Rillito River bottom sterilized	inoc.	24.50	42.44	2.19	.16
	not inoc.	17.20		2.03	
Cultivated Rillito River bottom	inoc.	42.85	110.04	1.61	-.49
	not inoc.	20.40		2.10	
Virgin Desert east of campus	inoc.	30.40	52.38	1.68	-.42
	not inoc.	19.95		2.10	

In every instance the dry weight of the roots of the inoculated alfalfa plants was greater than that of those not inoculated, being less with the sterilized soil than with any of the others. The nitrogen content of the roots was greater in the uninoculated plants than in those inoculated, with the exception of the sterilized soil in which instance there was a gain of .16% in the inoculated over the uninoculated plants. This can be explained by the fact that while the third cutting or aftermath was large enough to harvest on the inoculated plants, it was not large enough on the uninoculated plants. The few leaves that did grow on the plants not inoculated were weighed and analyzed with the roots, thus raising the percent of nitrogen materially. There was practically no aftermath on the uninoculated plants grown on the sterile soil and as a consequence the nitrogen content in this instance was lower than it was in the roots of the inoculated plants grown on sterile soil.

Table 7. The effect of inoculation of alfalfa upon the nitrogen content of the soil.

Soil	Treatment	NITROGEN					
		Percent at beginning	Percent at close	Percent gain or loss due to inoculation	Percent gain or loss each treatment	Grams gain or loss soil plus roots per pot	Percent gain or loss soil plus roots
Virgin Rillito River bottom	inoc	.066	.063		-.003	.25	2.92
	not inoc	.066	.063	none	-.003	.166	1.94
Virgin Rillito River bottom sterilized	inoc	.066	.063		-.003	.146	1.71
	not inoc	.066	.064	.001	-.002	.089	1.04
Cultivated Rillito River bottom	inoc	.066	.068		.002	.949	11.06
	not inoc	.066	.065	.003	-.001	.298	3.48
Virgin Desert east of campus	inoc	.032	.040		.008	1.55	37.27
	not inoc	.032	.039	.001	.007	1.33	31.94

The nitrogen content of the cultivated Rillito River bottom soil was the same as that of the virgin soil from the Rillito River bottom, probably due to the fact that nearly all of our virgin soils are poor in humic nitrogen and a good rotation system will at least keep the soil from becoming any more deficient and it is very easily possible to increase the nitrogen content materially as indicated in the last column of Table 7.

The desert soil contained a trifle less than one-half as

much nitrogen at the beginning of the experiment as did the river bottom soils. The desert soil made considerable gain in nitrogen by growing either the inoculated or the noninoculated alfalfa over what it had in the beginning. This was not true with the other soils, excepting when inoculated alfalfa was grown in cultivated river bottom soil, in which instance it gained .002 percent. This would amount to 40 pounds of nitrogen per acre and does not take into consideration the nitrogen contained in the roots. When the nitrogen in the roots was added to that of the soils at the end of the experiments, all showed a gain with the desert soil far in the lead, the pots containing the inoculated plants having made a gain of over 37 percent. In each case the gain in nitrogen of the soil and roots combined was greater with the inoculated plants.

Table 8. The effect of inoculation upon the dry weight and upon the nitrogen content of the tops of soybeans and cowpeas.

Crop	Treatment	Dry Weight		Nitrogen		
		Grams per pot	Percent gain or loss	Percent	Percent gain or loss	Lbs. gain or loss per ton
Soybeans cut before formation of seed	inoc.	20.25		1.21		
	not inoc.	9.40	115.4	1.08	.13	2.6
Cowpeas cut before formation of seed	inoc.	38.05		2.74		
	not inoc.	15.45	146.2	1.92	.82	16.4
Cowpeas cut after formation of seed	inoc.	203.40		2.21		
	not inoc.	16.80	1110.0	2.13	.08	1.6

Fig. 5a. The effect of inoculation upon the growth of cowpeas. The two pots upon the right are inoculated while those on the left are not.

Fig.5b. The effect of inoculation upon the growth of soybeans. The two pots upon the right are inoculated while those upon the left are not.

The results obtained with soybeans (5) and cowpeas which were grown only upon the virgin river bottom soil were practically the same as with those of the alfalfa, (See tables 4,5,and 6). The principal difference was that there was a gain of .002 percent of nitrogen in the soil itself with the growing of the cowpeas, while with the alfalfa and the soybeans there was a loss of from .002 to .004 or from 40 to 80 pounds of nitrogen per acre. This loss was more than made up by the addition of the nitrogen contained in the roots in the case of the alfalfa, but with the soybeans there was still a loss of nitrogen in the combined roots and soil, this loss being .72 percent with the soil growing the inoculated plants and 4.89 percent with the soil growing the plants not inoculated. The roots of the inoculated soybean

Table 9. The effect of inoculation upon the dry weight and upon the nitrogen content of the roots of soybeans and cowpeas.

Crop	Treatment	Dry Weight		Nitrogen	
		Grams per pot	Percent gain or loss	Percent	Percent gain or loss
Soybeans cut before formation of seed	inoc.	11.60		1.71	
	not inoc.	8.75	32.57	1.14	.57
Cowpeas cut before formation of seed	inoc.	29.76		1.50	
	not inoc.	16.88	76.3	1.50	none
Cowpeas cut after formation of seed	inoc.	26.10*		1.41*	
	not inoc.	16.00*	57.22	.92*	.49

*Roots not washed out of the soil immediately upon harvesting the cowpeas. The roots were partially decayed when finally washed and analyzed. In all other instances the roots were taken from the soil within twenty-four hours after harvest of the tops.

plants had no nodules whatever,(6) while all of the other plants, both inoculated and not inoculated, had nodules, the nodules being more numerous on the inoculated plants. The gain in nitrogen when that in the roots was added to that in the soil was much greater with the cowpeas than with the alfalfa, being 8.23 percent with the inoculated cowpeas and 5.98 with those not inoculated, while the gain was but 2.92 percent and 1.94 percent respectively with the alfalfa.(7)

Table 10. The effect of inoculation of soybeans and cowpeas upon the nitrogen content of the soil.

Crop	Treatment	NITROGEN						
		Percent at beginning	Percent at close	Percent gain or loss due to inoc.	Percent gain or loss each treatment	Grams gain or loss soil plus roots per pot	Percent gain or loss soil plus roots	Lbs. gain or loss per acre
Soybeans cut before formation of seed	inoc.	.066	.064		-.002	-.062	- .72	-9.5
	not inoc.	.066	.062	.002	-.004	-.42	-4.89	-64.4
Cowpeas cut before formation of seed	inoc.	.066	.068		.002	.71	8.23	108.9
	not inoc.	.066	.068	none	.002	.51	5.98	78.2
Cowpeas cut after formation of seed	inoc.	.066	.068		.002	.63	7.32	96.6
	not inoc.	.066	.68	none	.002	.41	4.81	62.9

The weight of the rrots of the alfalfa was greater than the combined weights of the various cuttings of the tops of the alfalfa in every case, averaging 60.5 percent of roots and 39.5 percent of tops. With the cowpeas the weight of the tops averaged 61.02 percent, while that of the roots averaged 39 percent of the total weight. The root systems of the soybeans averaged 42 percent and the tops 57.7 percent. This difference in the weights of the root systems of various legumes emphasizes the fact that when growing a crop for green manuring purposes the amount of top growth is not always the biggest factor in its value as a soil improver.

Table 11. The effect of inoculation upon the percent of roots and tops of alfalfa, cowpeas and soybeans. Dry weight.

Crop	Treatment	PERCENT	
		Tops	Roots
Alfalfa*			
Virgin soil Rillito river bottom	inoc.	39.6	60.4
	not inoc.	35.8	64.2
Virgin soil Rillito river bottom	inoc.	45.8	54.2
	not inoc.	43.1	56.9
Cultivated soil Rillito river bottom	inoc.	31.6	68.4
	not inoc.	38.3	61.7
Virgin soil Desert east of campus	inoc.	41.9	58.1
	not inoc.	39.9	60.1
Cowpeas**			
Cut before formation of seed	inoc.	56.3	43.7
	not inoc.	47.8	52.2
Cut after formation of seed	inoc.	89.7	11.3
	not inoc.	50.3	49.7
Soybeans**	inoc.	63.6	36.4
Cut before formation of seed	not inoc.	51.8	48.2

*All cuttings of the alfalfa tops are included. In the case of the cowpeas and soybeans but one cutting was made.
**Virgin Rillito river bottom soil unsterilized was the only soil used with the cowpeas and soybeans.

Table 12. The amount of nitrogen taken from the soil and that taken from the air for the use of the plants.

Crop	Treatment	Grams in tops	Grams in roots	Total in plants	Grams in soil at beginning	Grams in soil at end	Loss or gain in soil	Grams taken from soil by plants	Grams taken from air by plnats	Percent taken from air
Alfalfa										
Virgin soil R.R. bottom	inoc.	.62	.64	1.26	8.58	8.19	-.39	.39	.87	69
	not inoc.	.40	.56	.96	8.58	8.19	-.39	.39	.57	58
Virgin soil R,R. bottom steril.	inoc.	.53	.54	1.07	8.58	8.19	-.39	.39	.68	64
	not inoc.	.35	.35	.70	8.58	8.32	-.26	.26	.44	63
Cultivated R.R. bottom soil	inoc.	.48	.69	1.17	8.58	8.84	.26	none	1.43	125
	not inoc.	.30	.43	.73	8.58	8.45	-.13	.13	.60	82
Virgin Desert soil	inoc.	.58	.51	1.09	4.16	5.2	1.04	none	2.13	195
	not inoc.	.33	.42	.75	4.16	5.07	.91	none	1.66	221
Cowpeas										
Cut before formation seed	inoc.	1.04	.45	1.49	8.58	8.84	.26	none	1.75	117
	not inoc.	.30	.25	.55	8.58	8.84	.26	none	.81	147
Cut after formation seed	inoc.	4.50	.37	4.87	8.58	8.84	.26	none	5.13	105
	not inoc.	.36	.15	.51	8.58	8.84	.26	none	.77	151
Soybeans										
Cut before formation seed	inoc.	.25	.20	.45	8.58	8.32	-.26	.26	.19	42
	not inoc.	.10	.10	.20	8.58	8.06	-.52	.52	.0	0

The cowpeas obtained a greater percent of nitrogen from the air than did either the alfalfa or the soybeans. In the case of the soybeans more nitrogen was obtained from the soil than from the air, the inoculated plants having obtained 42 percent from the air and the remainder from the soil. With the cultivated river bottom soil, the inoculated plants of alfalfa obtained more than enough nitrogen for its own use from the air, as was also true of both the inoculated and the uninoculated plants grown on desert soil. With the alfalfa the inoculated plants in all cases, excepting when grown on desert soil, obtained a larger percent of its nitrogen from the air than did the plants not inoculated.

Table 13. The effect of applying inoculation material at different rates upon the weight in grams of the tops and roots of alfalfa.

Treatment	Tops	Roots	Total
Soil not sterilized, seed not inoculated	.722	.318	1.040
Soil sterilized *			
seed not inoculated	.388	.614	1.002
seed given normal inoculation	.255	.201	.456
seed given 10 times normal inoculation	.433	.393	.826
seed given 20 times normal inoculation	.791	.700	1.491

Twelve pots containing uniform virgin Killito River bottom soil were planted to alfalfa, the seed of which had been inoculated at different rates in order to ascertain the effect of an excess application of the inoculation material. Nitrosoil inoculum was used for this test, but it was not as fresh as it should have

*Sterilized in autoclave for 1 hour at 20# pressure.

been.(8) The nodules upon the roots of the alfalfa grown in the soil not sterilized were much larger as well as more numerous than those upon the roots of any of the other treatments, excepting that in the case of the pots receiving 20 times the normal application of inoculation material, the nodules were as numerous, but were very small. The nodules resulting from bacterial action of the bacteria already in the soil were many times larger in every instance than were those resulting from bacterial action of the bacteria applied in the inoculation

Fig.6. The effect of applying inoculation material at different rates. 1. Soil not sterilized, plants not inoculated; 2. Soil sterilized, plants not inoculated; 3. Soil sterilized, plants given normal inoculation; 4. Soil sterilized, plants given 10 times normal inoculation; 5. Soil sterilized, plants given 20 times normal inoculation.

material. This has been noted in practically all of the inoculation experiments conducted by the Experiment Station during the last few years.

A study of table 13 will show that inoculation did not increase the weight of the tops or the total plant weights with the normal application or with the 10 times normal application of inoculation material, but did increase the weights with an application of 20 times the normal. These results emphasize the necessity of using the amount of inoculation material recommended by the companies which put out the material, when making pot tests. The tendency is to use enough for an acre or so upon a few pots.

Fig.7. The difference between natural and artificial inoculation upon the formation of root nodules. The nodules upon the roots of the two plants on the right were formed through the action of bacteria already present in the soil, while the nodules upon the roots of the two plants at the left were formed by bacteria introduced in commercial inoculum and are too small to be seen in a photograph, in most cases being smaller than a pinhead.

Summary

Inoculation has not proven beneficial at the Salt River Valley Experiment Farm with alfalfa.

In most instances inoculation has been beneficial on the poor soil of the Sulphur Spring Valley Dry Farm.

Increase in yield has been marked with vetch upon the exceedingly sandy soil of the Yuma Mesa Farm. This increase was greater the first year of cultivation than the second. A very noticeable increase was obtained, however, with inoculated vetch following inoculated vetch as compared with vetch not inoculated following inoculated vetch.

The weight of the nodules upon the roots of the vetch which had not been inoculated was greater than of those which had been inoculated, while the number of nodules per plant was less.

Inoculation hastened maturity from 5 to 7 days with alfalfa grown in pots at the University.

The weights of the tops and of the roots of all legumes worked with in the pot tests were increased materially by inoculation.

Inoculation increased the weight of nitrogen in the tops of the alfalfa from 1 to 9 pounds per ton depending upon the soil used. The gain in nitrogen content of the cowpea hay was 16 pounds per ton and but 2.6 pounds per ton with soybeans.

A distinct gain in the nitrogen content of the soil itself was obtained by the growing of alfalfa either inoculated or not inoculated, when grown upon the poor desert soil or upon the

Rillito River bottom soil which had been in cultivation for a number of years. With the other two soils there was a loss.

When the nitrogen content of the roots was added to that of the soil, there was a gain in every instance with the alfalfa of from 1.04 percent in the case of the virgin Rillito River bottom soil to 37.27 percent with the desert soil. Cowpeas grown upon the virgin soil of the Rillito River bottom brought about a gain of from 6 to 8 percent while soybeans incurred a loss of from .72 to 4.89 percent.

Inoculation increased the percent of roots to tops with alfalfa but had the opposite effect with cowpeas and soybeans.

Nodules formed upon the roots of vetch anf of alfalfa by the action of bacteria already present in the soil, are as a rule larger than those formed by the bacteria in commercial cultures.

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