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SOIL INOCULATION FOR LEGUMINOUS PLANTS.

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SOIL INOCULATION FOR LEGUMINOUS PLANTS.

BY J. F. DUGGAR.

GENERAL OBJECT OF THESE EXPERIMENTS.

A summary of this bulletin is given on page 483.

The subject of maintaining the fertility of the land very closely concerns every tiller of the soil, whether cotton planter, tobacco grower, grain farmer, livestock breeder, or horticulturist. This bulletin bears on the question of soil improvement. It approaches that subject by explaining how, in experiments recently conducted at this station, we were able to largely increase the yields of certain soil-improving plants, which plants are also valuable for forage.

NITROGEN COLLECTING PLANTS.

Nearly every intelligent Southern farmer recognizes the fact that a crop of cowpea vines turned under at the proper time adds greatly to the fertility of the soil. The superiority in this respect of cowpeas over crabgrass, ragweed, etc., is generally recognized. Both cowpeas and weeds are helpful in so far as they add vegetable matter, which, when rotted, improves the mechanical condition of the soil and enables it to retain increased quantities of water in a season of drought.

If both are beneficial, then why the superiority of cowpeas? The answer is found in the fact, not known until comparatively recent years, that cowpeas and related plants have the power, not possessed by the majority of plants, to draw a large part of their nitrogen from the air instead of being entirely limited in growth by the amount of nitrogen in the soil or fertilizer.

Among all flowering plants 'this power of utilizing the free nitrogen of the air is found only in those which the botanist classifies as belonging to the natural order *Leguminosæ.* Hence the term leguminous plants or legumes so generally used in the sense of nitrogen-collecting plants.

In the plowing under of non-leguminous plants no fertilizing element is added to the soil, these in decaying restoring no more nitrogen, phosphoric acid and potash than they abstracted from the soil in growing. This is also true in regard to the phosphoric acid and potash in leguminous plants. But legumes in decaying afford more nitrogen than they took from the soil, that portion of their nitrogen supply which they obtained from the air being a distinct gain to the soil in which the leguminous plant is buried.

In the class of leguminous plants are embraced all clovers, peas, vetches, beans, lespedeza, melilotus, alfalfa, and a multitude of others, both wild and cultivated.

The plowing under of the entire plants of this class, and in some cases the turning under of the roots and stubble alone, enriches the soil by increasing the nitrogen supply. This nitrogen, if purchased in the form of cotton seed meal or commercial fertilizers, would cost 12 to 18 cents per pound.

While the leguminous plants do not need to be fertilized with nitrogenous manures, they do require a liberal supply of phosphoric acid and potash, either in the soil or fertilizer. The growth of leguminous crops will not make decidedly richer soils that are deficient in mineral elements, unless the good effects of leguminous plants are supplemented by applications of phosphates and potash compounds. These mineral fertilizers are needed by leguminous plants and by the non-leguminous staple crops that follow them in the rotation. The market price of the mineral constituents of fertilizers is considerably less than half that of nitrogen, hence the economy of liberally supplying legumes with mineral fertilizers in order that they may the more heavily draw on the air for nitrogen.

For forage, as well as for green manuring, leguminous plants rank above grasses and other forage plants, being richer in nitrogenous material and producing a richer manure.

ROOT TUBERCLES.

By examining the roots of thrifty leguminous plants one will find numerous enlargements or swellings, varying in size from that of a mustard seed to that of a pea, or sometimes grouped together in a rough mass more than half an inch in diameter. (An illustration of young enlargements or tubercles on the roots of hairy vetch plants is given in figure 4.) The larger and more abundant these tubercles or root nodules are, the greater the activity of the plant in appropriating the nitrogen of the air.

Without these tubercles a leguminous plant cannot profit by atmospheric nitrogen, and has no advantage over non-leguminous plants, as the gasses, cotton, etc. If it has tubercles on its roots a leguminous plant is able to use the free nitrogen of the air. The air comes in contact with the tubercles under the surface, for air circulates in all cultivated soils. The exact means by which these tubercles place a supply of nitrogen at the disposal of leguminous plants is not thoroughly understood and a discussion of the theories bearing on that phase of the subject is uncalled for here. It is enough to say that these tubercles may be regarded as houses in which dwell multitudes of germs, or more specifically bacteria or bacteria like bodies, separately invisible except under a microscope of high power; and that these are the agencies by which atmospheric nitrogen reaches the higher plant. On the decay of the tubercle great numbers of these micro-organisms are left in the soil ready to cause tubercles on the next crop of the same legume.

Nearly every genus of leguminous plants has its own specific or "adapted" germ, which, if present in the soil or on the seed sown, is able to cause the development of these tubercles and to secure to the plant the consequent advantages. As a general rule, to which there are exceptions, the germ which induces the growth of tubercles on one legume is unable, at least temporarily, to produce tubercles on plants belonging to other genera; for example, the germ which causes tubercles on clover is impotent on vetch. Hence for the thrifty growth of a given legume, say clover, the soil must contain the corresponding form of germ life; if this "clover germ" is absent, the only way to successfully grow clover on poor and medium soils is to use liberal quantities of nitrogenous fertilizers, an expensive practice. If the proper germs are present and if all other conditions are favorable, as drainage, proper mechanical condition, and a sufficiency of phosphoric acid, potash and lime, clover plants will thrive and be largely independent of soil nitrogen, drawing a large part of their supply from the atmosphere.

Are most soils naturally stocked with the micro-organisms necessary to the thrifty growth of every kind of cultivated leguminous plant? Our experiments, of which a part are recorded in this bulletin, show that in some southern soils the germs essential to the thrifty growth of certain legumes are wanting or else present in insufficient number. Their absence from many southern soils explains why the opinion is so prevalent in some localities that the clovers are not suited to the extreme South.

SOIL INOCULATION.

Inoculating the soil, as the term is used here, consists in supplying some material containing the germs necessary to cause the growth of root tubercles.

We do not hesitate to compensate for a deficiency of phosphoric acid or potash or even of nitrogen by applying fertilizers. It is just as logical to apply the requisite germs as to provide the essential fertilizing elements, for really in supplying the germs we are in effect feeding the plant on nitrogen.

Soil inoculation may be effected either by applying the material containing the germs directly to the soil or by placing the seed before planting in contact with the inoculating material. Both methods were used in these experiments.

WINTER GROWING LEGUMES.

Only a few cultivated legumes are extensively grown in the South and indeed the majority of farmers of this State who grow leguminous crops of any kind confine themselves to the cowpea. The value of this plant in southern agriculture cannot easily be over estimated and the acreage in cowpeas should be extended far beyond present limits. As a summer crop no substitute for the cowpea could be named which would give such general satisfaction as a renovating plant.

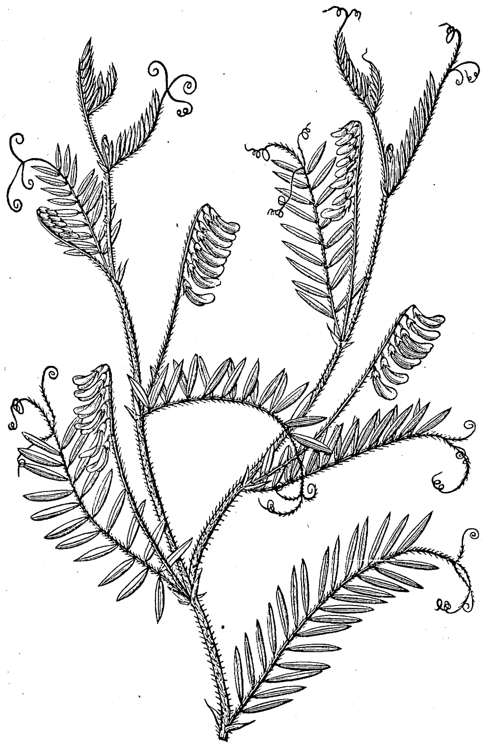

Fig. 1. Hairy vetch. (F. Lawson-Scribner, U. S. Dept. of Agriculture, Divison of Agrostology, Circular No 2.)

But winter leguminous plants are also needed. They not only add vegetable matter and nitrogen when plowed under in the spring, but they also, while growing, prevent the winter rains from washing the soil and from leaching out the most valuable fertilizing material.

With the aim of securing these advantages numerous renovating plants have been tested here and especial attention has been given to hairy vetch and crimson clover which are regarded as the most promising of the winter growing legumes.

HAIRY VETCH (*Vicia villosa*).

Hairy vetch is a vinelike annual plant, the slender branches sometimes growing eight feet long. Sown in September or October, it is ready for cutting the following April or May. If not supported, the vines form a dense mat of fine stems and leaves 18 to 30 inches thick. If rye is sown thinly with the seed of hairy vetch the vetch plants climb to considerable height on the rye straw and are more easily harvested.

Hairy vetch is valuable for turning under as a green manure, for green forage, for hay and for pasturage. Both here and at the Mississippi Experiment Station it was found a most excellent plant to furnish winter grazing on Bermuda grass sod. The main objection heretofore mentioned to the general culture of hairy vetch is the cost of the seed. Our seed purchased this season from Peter Henderson, New York city, cost $4.50 per bushel. At least one bushel of seed per acre is desirable when sown broadcast for hay or pasturage, to which for hay might be added one or two pecks of rye or one bushel of winter oats. Doubtless much thinner seeding in drills would suffice when only seed is wanted. The high price of seed need not prohibit the culture of hairy vetch, for the seed can be grown on the farm after a start is made with purchased seed.

According to our experiments, another obstacle to vetch culture will be found in the absence from many soils of the germ that produces tubercles on vetch plants. For this condition the experiments indicate a remedy.

FIELD EXPERIMENTS WITH HAIRY VETCH.

October 17, 1896, four plots, each one-fortieth acre, lying side by side, were sown with seed of hairy vetch. The soil was very poor. Preceding crops were Kafir corn for forage in 1896, and oats in 1895. So far as could be learned this field had never borne any other leguminous crop than cowpeas and no cowpeas since 1894.

The plowing and harrowing necessary in preparing a seed bed was the same for all plots. The fertilizer contained no nitrogen; on each plot were applied 400 pounds per acre of acid phosphate and 120 punds per acre of sulphate of potash. Every plot was seeded at the rate of 30 quarts per acre, sown broadcast and covered with a smoothing harrow and roller. On plots 1 & 3 the seed received no treatment. The seed for plots 1 & 4 was treated as follows before sowing: The seed was dipped in water into which there had been stirred and allowed to settle earth from a lawn, once a garden spot, where common vetch (*Vicia sativa*) had for several years in succession made a thrifty growth. At the time of taking the earth for this purpose the young plants of common vetch were about two inches high and already they showed the tubercles on the roots which are characteristic of vigorous leguminous plants. The seed for plots 2 & 4, after being moistened in this material, was sown and immediately covered, the harrow and roller running across all plots in covering the seed.

Early in December the more vigorous growth on the inoculated plots was noticeable. By January 1, the difference was striking even at a distance of a hundred yards. The treated plants were of a luxuriant green, the untreated plants decidedly brownish and smaller than the others. At that date, two and one half months after planting, average plants were dug from both series of plots and photographed. (See figures 3 & 4.) On the inoculated plots practically all the plants had clusters of tubercles on the roots. On plot 1, untreated, no tubercles had formed on the roots. The difference grew greater and greater, the plants without tubercles making scarcely any growth after February, while the inoculated plants grew without interruption.

May 20, 1897, all plots were cut. A scythe was used on the inoculated plots, but on plot 1, untreated, the plants were so small that they had to be cut with a sickle and picked up one by one. The inoculated plants were then in the height of bloom, had formed some immature pods, and constituted a mass of green about 15 inches thick, most of the branches being about 3 feet long. On plot 1 there were few branches over 8 inches long and there were fewer branches per plant than on the other plots.

The green weights were taken immediately after cutting, May 20, and the dry weights June 21, after thorough curing and one month's storage of the hay.

The results calculated per acre were as follows:

Yield per acre of hairy vetch without and with inoculation.

Hairy vetch.	Green forage.	Cured hay.
	Lbs.	*Lbs.*
Not inoculated (Plot 1)	900	232
Inoculated (Plot 2)	9136	2540

Comparing the yields on plots 1 & 2 we find that with inoculation the yield was over ten times as great as without inoculation, the increase in hay being 995 per cent.

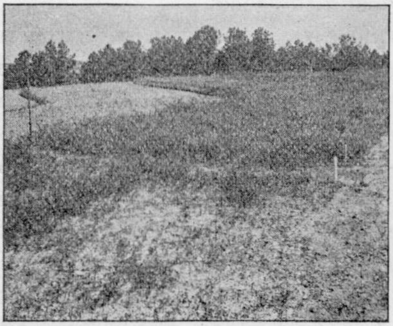

Fig. 2. The foreground shows the plot not inoculated (P 1); the background, the inoculated plot (P 2).

Figure 2 shows the appearance of plots 1 & 2, a short time before cutting; the light colored and nearly bare fore-ground is the uninoculated plot, while the more luxuriant growth toward the background and beyond the stakes represents the inoculated plot.

The yields of only plots 1 & 2 are given in above table, for only this pair of plots represents fairly the gain in favor of inoculation. Lot 3 was so situated that a thin sheet of surface water from plot 4 flowed over it. The effect was to bring sufficient germs from the treated plot to inoculate about half the plants on plot 3. That about half of these plants had been thus accidentally inoculated was suspected during the spring from the irregular growth on plot 3. When cut most of the unthrifty plants on plot 3 had attained a length of only about 8 inches and were devoid of turbercles. The other plants, accidentally inoculated, had branches 3 feet long and an abundance of tubercles.

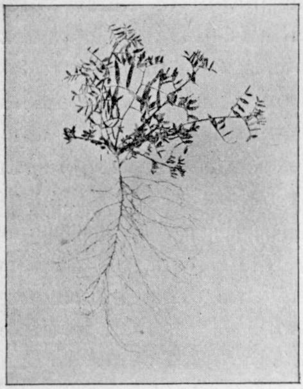

FIG. 3. Hairy vetch plant, Jan. 1, from plot not inoculated.

FIG. 4. Hairy vetch plant, Jan. 1, from inoculated plot.

The actual yield of thoroughly cured hay were 1,036 pounds per acre with partial inoculation (plot 3) and 2,184 with careful inoculation of the seed before planting (plot 4).

It should be added that after cutting the vines the soil under the thicker stuble on plot 2 was in better mechanical condition, more friable and less hard, than under the comparatively bare surface of plot 1, where there had been no inoculation. This same favorable effect of a fair growth of hairy vetch on the mechanical condition of the soil was noted in another field. The yield on this latter field where the plant had been grown for several years in succession and where there was an abundant supply of tubercles was 17,765 pounds per acre of green forage, which when cured, afforded 4,174 pounds of hay.

That the soil of plot 2 was improved by the stubble of hairy vetch is apparent now (August 20) when the German millet sown after vetch on all plots is notably greener and larger on the inoculated than on the untreated vetch plots.

POT EXPERIMENTS IN THE GREENHOUSE.

The object of these experiments was to ascertain whether five different soils from neighboring farms would be helped by inoculation to produce vigorous plants of hairy vetch, Canada field pea, crimson clover, alfalfa, lupins, lespedeza and cowpeas. These pot experiments were planned to run parallel with similar experiments on a large scale in the field. However the artificial inoculating material, which had to be opened when the pot experiments were begun, had fermented and lost its vitality before outdoor planting could be commenced.

Ordinary unglazed flower pots, fresh from the factory, were used, the large, small and medium sizes holding respectively 14, 8, and 3 pounds of soil. The only fertilizer used was high grade acid phosphate, one-half ounce for each large and medium pot and one-fourth ounce for each small pot. In the bottom of the large and medium pots was spread one and one-half ounces of cotton seed hulls and one-half ounce in each small pot. After planting, each pot was covered with a layer of cotton seed hulls, half an inch thick, to prevent compacting the soil in watering the plants.

Five different upland soils were used in the pots. The soils, all taken from the surface, were as follows:

Soil A.—From an upland rocky field, with reddish loam soil, about 3 miles southwest of Auburn. This field had been cleared about twenty years and had been in cotton most of that time. It had certainly not borne a crop of legumes in 5 years, and was believed to have never had any cultivated leguminous plant on it. It was of more than average fertility, last year's cotton crop being estimated at one-half bale per acre on this field, with the usual light fertilization.

Soil B.—Surface soil from a sandy cotton field, cleared about 5 years before, and which had never borne a crop of cultivated leguminous plants. This field is about 2 miles southeast of Auburn, and apparently less fertile than the preceding.

Soil C.—Sandy soil from woodland of mixed pine and hardwood, adjoining the field from which Soil B. was taken. The coarse litter on the surface was raked away before taking the sample, which, however, contained much organic matter in a fine state of division.

Soil D.—Loamy soil, of poor quality, from a pasture on the Station Farm where for two years Japan clover (*Lespedeza striata*), also called lespedeza and old field clover, had grown thickly for two years. The old lespedeza plants and about an inch of the surface soil were removed before taking the sample.

Soil E.—From the immediate vicinity of decayed cowpea roots in a poor sandy soil on the Station Farm, where this plant was grown in 1896 and at intervals in previous years.

The pots were arranged in pairs, every condition being alike in the two pots, except that the seed or soil of one pot was inoculated and given an odd number. The even numbered pots were not inoculated.

Inoculation was effected as follows:

One-half bottle of imported nitragin or germ fertilizer (a gelatinous material containing immense numbers of specific living germs) was diluted with well water and sprinkled on about 25 pounds of earth, and one gill of this earth was then mixed with the upper soil of the odd numbered pots. A bottle of "clover nitragin" was used for the pots intended to be inoculated for clover, and a bottle of "vetch nitragin" for the inoculated vetch pots. The earth used in preparing the earthy inoculating material was in all cases taken from the same field as Soil B.

The inoculating material for cowpeas was "homemade," consisting of 6 ounces of Soil E., from around old cowpea roots, applied to each large odd numbered pot that was intended for cowpeas. Likewise the inoculating material for lespedeza consisted of 2 ounces of Soil D., from a lespedeza pasture.

Crimson clover and lespedeza had to be replanted March 13; the other seeds were planted February 24–28, equal quantities of seed being used in the inoculated and not inoculated series. The pots were placed in a greenhouse and watered with boiled or rain water. Equal quantities of water were supplied to inoculated and not inoculated plants, although this was a hardship on the larger inoculated plants. All plants suffered from insufficient or infrequent watering and from the attacks of the red spider.

Except alfalfa and lespedeza, all plants on April 17 were reduced to a uniform stand in the treated and untreated series. The vetch, Canada field pea, lupin, and crimson clover plants were harvested May 24, the remainder June 11, except the alfalfa. The dirt was washed from the roots with a jet of water. Weighings of thoroughly dried tops and roots were made June 24–26, on chemical balances.

POT EXPERIMENTS WITH HAIRY VETCH.

Hairy vetch seed was sown February 24 in eight medium sized flower pots, containing four different soils. One pot containing each soil was left without inoculation and given an even number. The other pot of each pair, designated by an odd number, was inoculated as before described with a small amount of earth previously treated with clover (Trifolium) nitragin. In addition the seed were also dipped in a solution of this nitragin.

The superior growth on most soils of the plants which were inoculated may be seen by reference to figures 5 & 6.

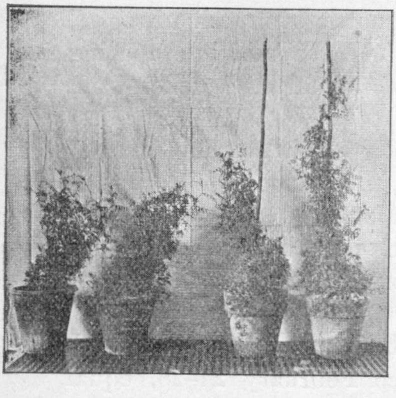

FIG. 5. Haiy vetch.
A 10, not inoculated.
A 9, inoculated.
C 8, not inoculated.
C 7, inoculated.

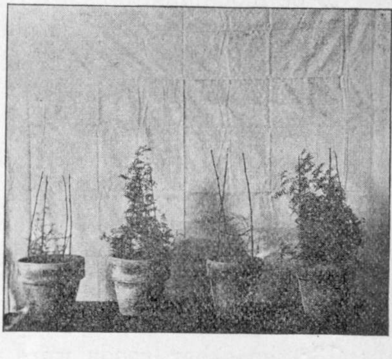

FIG. 6 Hairy vetch.
D 8, not inoculated.
D 7, inoculated.
E 10, not inoculated.
E 9, inoculated.

The tops of the vetch plants were cut and the dirt carefully washed from the roots May 24. After thorough drying, both tops and roots were weighed, with the results shown in the appendix to this bulletin.

For the sake of clearness only the increase in the yields of tops, roots, and entire plant are given here, the results being expressed in percentages :

Gain in percentages from inoculating hairy vetch grown on different soils.

Soil.	SOIL FROM	Increase attributable to inoculation.		
		Tops.	Roots.	Entire plant.
		Per cent.	*Per cent.*	*Per cent.*
A	Cotton field, 20 years cleared.	21	65	38
C	Woodland....................	25	69	38
D	Lespedeza pasture...........	577	69	186
E	Cowpea field................	928	252	466

The above table shows that on all soils there was a large gain in both tops and roots as the result of inoculation. For these four soils the average increase from inoculation was 86 per cent. in tops, 92 per cent. in roots and 89 per cent. in total product, using the weights given in the appendix as a basis of comparison.

Examination of the roots from all pots showed that in every inoculated pot there was an abundance of tubercles. In the pots not inoculated the case was quite different; the untreated plants growing in Soils D. and E. had not a single tubercle, which deprivation of atmospheric nitrogen is sufficient to account for the extremely slight growth on those two poor soils. The untreated plants on the richer woodland soil fared better, as did also those in soil A., for in this latter case a number of tubercles developed on the untreated as well as on the treated plants. Even under these circumstances inoculation was beneficial.

POT EXPERIMENTS WITH CANADA FIELD PEA (*Pisum arvense*).

This plant is scarcely distinguishable in appearance from the English or garden pea. In Canada, and in the northern part of the United States, it is grown for grain and forage, both being very nutritious. Our tests in the field indicate that is not so valuable here as is hairy vetch. The results of inoculation with this plant are regarded as equally applicable to the English pea. Only new garden spots seem to need inoculation for English peas.

Canada field peas were grown in four soils, there being four pairs of medium-sized pots. One pot of each pair was treated with inoculation earth prepared with vetch (Vicia) nitragin, pea nitragin not being at hand. The seed were not dipped in a solution of nitragin, as the vetch seed had been.

From a very early period of growth the inoculated plants took the lead, growing taller and having larger leaflets of a darker green color than the corresponding untreated plants. Their superiority in size May 19, a few days before harvesting, may be seen by reference to figures 7, 8, 9 and 10.

The weights of thoroughly dried tops and roots gave the following percentage gains for inoculation :

Gain in percentages from inoculating Canada field peas grown on different soils.

Soil.	SOIL FROM—	Increase attributable to inoculation.		
		Tops.	Roots.	Entire plant.
		Per cent.	*Per cent.*	*Per cent.*
A	Cotton field, 20 years cleared.	165	—15*	58
B	Cotton field, 5 years cleared..	268	139	199
C	Woodland	151	495	275
D	Lespedeza pasture..........	234	94	156

* Loss.

From the above table it is apparent that the increase in tops, attributable to inoculation, was in no case less than 151 per cent.

Averaging the actual yields on all soils, the average increase for Canada field peas with inoculation was 197 per cent. in tops, 87 per cent. in roots and 138 per cent. in total product.

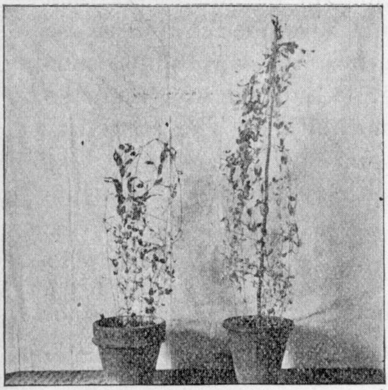

FIG 7. Canada field peas,
A 8, not inoculated.
A 7, inoculated.

FIG. 8. Canada field peas.
B 8, not inoculated.
B 7, inoculated.

FIG. 9. Canada field peas.
C 6, not inoculated.
C 5, inoculated.

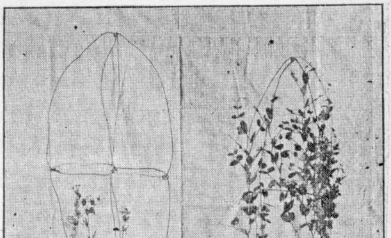

FIG. 10. Canada field peas.
D 6, not inoculatbd.
D 5, inoculated.

Plants growing in the inoculated pots produced an abundance of tubercles. The plants without inoculation developed no tubercles in Soils B. and D., and only one small group of tubercles in Soil C., and this, occurring near the surface, was doubtless caused by accidental inoculation from without.

In Soil A., which for many years had been free from leguminous plants, an abundant supply of tubercles developed without treatment. It should be noted here that without inoculation tubercles were developed in Soil A. on hairy vetch, Canada field peas, crimson clover, lupins, cowpeas and lespedeza. In no other soil tested was there so general an infection without inoculation. The regularity with which tubercles developed unaided in a soil so long free from legumes is in accord with results of experiments in Germany.

CRIMSON CLOVER (*Trifolium incarnatum*).

This is an annual plant, which, sown in September or October, matures early in the following May, and, if thrifty, may be used as pasturage, forage or green manure early in April in time to cause no interference with late planted crops.

Since sufficient seed for one acre (1 peck) can be bought for about 80 cents, cost of seed does not constitute an objection to crimson clover as in the case of hairy vetch. There is reason to believe that crimson clover will prove the most useful of all soil-improving plants for the cotton planter owning suitable soil. Yet this plant has heretofore failed in a very large percentage of the communities of Alabama in which it has been tried. During the past season crimson clover was tested under the writer's supervision on more than thirty farms in different parts of the State. In the great majority of instances it failed. Typical crimson clover roots from nearly all of these localities were examined by the writer and the dwarfed plants in almost every case showed either the entire absence of tubercles or more frequently the presence of an inadequate number of quite small tubercles.

The failure of crimson clover on the Station Farm the past season was complete. This was true whether the land was well or ill prepared, whether the stand of plants was thick or thin. Few plants grew over 3 inches high, or had flower stems longer than 6 inches. If thrifty, the growth should have been considerably above one foot.

On examining the roots this failure could be easily accounted for. No nitrogenous fertilizer or manure was applied to crimson clover and no tubercles developed on the roots except along certain roadsides where small tubercles appeared late in the season. The failure was apparently due to nitrogen-hunger, which can be overcome, as shown below.

POT EXPERIMENTS WITH CRIMSON CLOVER.

Our pot experiments were intended to ascertain whether this cause of failure could be overcome by inoculation. The results justified the expectation. However, the yields were extremely small in pots for the following reasons: (1) The small size of most of the pots used for crimson clover; (2) planting in March instead of in September; (3) the attacks of red spiders in the greenhouse, and (4) insufficient and infrequent watering, causing the death of some of the plants and necessitating the close of the experiment while the plants were very young. These causes, singly or together, also reduced the yields of other plants growing in pots.

The plants when cut had been growing only about 10 weeks, or about one-third the usual time required for growth.

As inoculating material clover (Trifolium) nitragin was used, mixed with Soil B., as previously explained.

May 24, about 10 weeks after planting the seed, the little plants were harvested, many of them, especially among the larger uninoculated plants, being already nearly or quite dead, as the result of insect injury and of deficiency in the water supply.

FIG. 11. Crimson clover.
B 2, not inoculated.
B 1, inoculated.
E 2, not inoculated.
E 1, inoculated.

The weights taken after thorough drying, showed the following gains from inoculation :

Gain in percentages from inoculating crimson clover grown on different soils.

Soil.	SOIL FROM—	Increase attributable to inoculation.		
		Tops.	Roots.	Entire plant.
		Per cent.	*Per cent.*	*Per cent.*
A	Cotton field, 20 years cleared.	72	70	71
B	Cotton field, 5 years cleared..	130	7	74
C	Woodland..................	150	506	326
E	Cowpea field (av. 4 pots).....	609	198	379

Here the increase in tops attributable to inoculation was in no case less than 72 per cent. Averaging the actual yields on all soils, the average increase from inoculating crimson clover was 158 per cent. in tops, 128 per cent. in roots and 146 per cent. in total product.

That the inoculated plants were more vigorous than those not inoculated may be seen by reference to figure 11, which shows typical differences between the two classes of plants. This superiority of the inoculated plants was not due to the entire absence of tubercles on the plants that were not artificially inoculated. Plants in these pots were found to have tubercles, and this was true in all soils; these tubercles, however, were evidently not so active and efficient in promoting growth as were those on the plants which had been artificially inoculated. Apparently the germs causing the tubercles in the even-numbered pots were accidentally introduced in watering the plants, (see Note 1 in the Appendix). If so, then we should expect tubercles to develop later in the pots not intentionally inoculated, and this later development may be the explanation of the lesser efficiency of these tubercles.

The results of inoculating crimson clover, although obtained with plants that had made only a fraction of their normal growth, give encouragement to the hope that this plant may be made to thrive in many soils where it has failed heretofore, in default of inoculation. No other winter growing renovating plant seems so well fitted to the needs of the cotton farmer as crimson clover. Its successful growth as a catch-crop in winter would greatly improve the soil, and thereby materially increase the profits of the cotton planter.

Probably in those localities in which red or other clover is generally and successfully grown the soil is already inoculated with the necessary organisms and artificial inoculation is superfluous for clover of any kind.

POT EXPERIMENTS WITH ALFALFA (*Medicago sativa*).

Alfalfa was grown only in Soil A., the one which for a long time had borne no legumes. Only two pots, each containing 14 pounds of earth were used. The amount of boiled or rain water was the same for both pots until after May 19, when the photograph shown in figure 12 was taken.

After that time the inoculated plants, which were several times larger than the others and which consequently had suffered when confined to the limited amount of water needed by the smaller untreated plants, were given larger amounts of water than the others, as their greater size required.

Alfalfa seed under treatment were planted in both pots February 26, 1897. Before planting, the upper layer of soil of pot A 15, had been inoculated as follows :—

Seed of bur clover (*Medicago maculata*) harvested in Starkville, Miss., the preceding spring were placed in a sifter and the adhearing dust sifted out February 26, 1897. Of this dust, one-fourth ounce was incorporated with the upper portion of the soil of pot A 15. The soil of pot A 16 was not treated. Even as early as April 5, only 35 days after planting the seed, the inoculated plants were twice as tall as those not inoculated. The photograph shown in figure 12 was taken May 19. Later, with a liberal supply of water and removal from greenhouse to the open air, the inoculated plants made a steady growth until July 22, when the plants in both pots were cut.

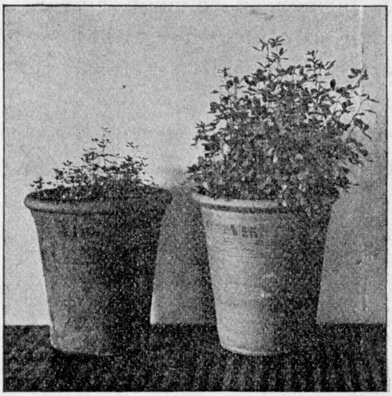

FIG. 12. Alfalfa.
A 16, rot inoculated.
A 15, inoculated.

At that date the inoculated plants averaged 15 inches high while the average height in the other pot was only about 5 inches. A number of the inoculated plants were in bloom and a few seed pods had formed. Not a sign of blooming was visible on the untreated plants.

The results of the first cutting, expressed in apothecaries' grains, were as follows :

Results of inoculating alfalfa with dust from bur clover.

	Green material.	Cured hay.	Increase in hay attributable to inoculation.
	Grains.	*Grains.*	*Per cent.*
Not inoculated......	174.5	40	....
Inoculated.........	965.5	174.4	336

After the first cutting the inoculated plants put out shoots more vigorously than the others. Of the untreated plants about half died, without throwing out new sprouts.

At the present writing (August 20), there promises to be in the second cutting fully as much difference in favor of the inoculated pot as there was at the first cutting.

As the roots were not washed out, but left to continue growth, it cannot be stated whether or not the uninoculated plants had tubercles. The appearance of the plants suggests that there has been no general infection of the roots of the inoculated plants, but that tubercles are abundant on the treated plants.

POT EXPERIMENTS WITH WHITE LUPINS (*Lupinus albus*).

This plant was grown only on the soil that had been for many years free from leguminous plants. Here the tubercles developed without, as well as with, inoculation, and there was no gain in yield of either roots or tops as the result of inoculation with lupin nitragin.

POT EXPERIMENTS WITH LESPEDEZA (*Lespedeza striata*).

Seed of lespedeza, also known as Japan clover, old field clover, and wild clover, were planted in small pots on all soils. The inoculating material for each odd-numbered pot was 2 ounces of Soil D from a lespedeza pasture. In no case could the eye detect any difference between the growth of treated and untreated plants. In every soil, whether inoculated or not treated, tubercles developed in abundance.

Only the tops were weighed, and these showed no difference in yield attributable to inoculation. Apparently the germs required to cause the growth of tubercles on lespedeza were present in all of these soils, either through the previous growth of native species of lespedeza, or carried thither in the dust blown from the old fields, pastures, and roadsides, which in this locality as so generally covered with this plant, or else carried into all pots in the form of dust adhearing to the seeds that were planted.

POT EXPERIMENTS WITH COWPEAS (*Vigna catjang*).

Cowpeas were grown in large pots on all soils. The inoculating material used was for each odd numbered pot 6 ounces of Soil E., from a field where cowpeas grew in 1896 and where this plant always develops an abundance of tubercles. At no period of growth could there be seen any marked difference in growth of plants on treated and untreated plots.

The results show no advantage in inoculating cowpeas on any of these soils, a result that is not strange in view of the fact that cowpeas are so generally grown in this locality and that the dust from cowpea fields, probably containing the necessary micro-organisms, has been scattered far and wide by the wind.

METHODS AND COST OF INOCULATION.

Inoculating material is of two general kinds, (1) the prepared article, which is for sale in bottles under the name nitragin or germ fertilizer, and (2) the earth from around the roots of mature leguminous plants of the same or closely related kind as the plant to be inoculated.

The nitragin used in these experiments was purchased from Lucius and Bruening, Hoechst on Main, Germany. through their American agents, Victor Koechl & Co., 79 Murray street, New York city. The cost was $1.25 per bottle, plus express from New York. The manufacturers' directions state that one bottle is sufficient for five-eighths of an acre. Adding 10 per cent. to the price in New York to cover express from that point, the cost would be $2.20 per acre for nitragin.

This outlay, while it would doubtless often prove profitable, is greater than the writer is prepared to advise for large areas. This is in view of the fact that cheaper, although apparently somewhat less effective, methods of inoculation can be adopted. Inoculation as applied to the field of hairy vetch noted on page --, cost nothing except labor. A better method consists in spreading broadcast a ton or two per acre of earth from an old clover field on land intended for clover, the same quantity of earth from an old vetch field for a new seeding of vetch, and so for other leguminous plants.

If no field of red or crimson clover is convenient, spots of low white or creeping clover (*Trifolium repens*), or of Carolina clover (*T. Carolinianum*) may perhaps be found in pasture or lawn, and the earth of these spots used as inoculating material for red or crimson clover.

In a similar manner one wishing to sow hairy vetch in a field where no similar plant had recently been grown could use earth from around the roots of common vetch, or even from that part of the garden where English peas have recently grown and developed tubercles. For alfalfa, earth from a spot where alfalfa, bur clover, or black medic (*Medicago lupulina*) had grown might be used.

Having once started on the farm a small plot of the desired leguminous plant and insured the abundant production of tubercles on plants growing there, the soil of this small spot could be used in future years as inoculating material for a number of acres.

If earth for inoculation purposes were scare, partial inoculation could doubtless be effected by simply using enough earth to sow with the seed, dampening the seed to cause the earth to adhere. Extreme dryness or long exposure to bright sunlight injures or destroys the germs that cause tubercles on leguminous plants. Hence the inoculating material should be worked in deep enough to come in contact with the moist soil. The spreading and harrowing in of the earth should be thoroughly done. For this work cloudy days, or some other time than the middle of the day, and prompt covering of the inoculating material have been recommended.

CROPS AND SOILS THAT NEED NO INOCULATION.

Not every leguminous plant requires inoculation in order to produce tubercles. Many soils are naturally supplied with the tubercle-producing germs through the growth of wild leguminous plants. Moreover where a given legume is extensively grown there is probably a wholesale inoculation of surrounding soils by means of the wind, which carries the germ-laden dust. This seems the explanation of the fact that on no soil has the writer been able to find cowpeas free from tubercles, and many observations have been made, all in localities where the culture of this plant is general. The seed of certain legumes may also be the means of conveying the necessary germs to a soil lacking the appropriate form of germ life. This seems the true explanation of the fact that lespedeza (and bur clover from unhulled seed) have developed tubercles in all soils where we have thus far tested them. Seeds of both these plants are borne in close contact with the ground where particles of the germ-laden soil easily lodge upon the burs or seed coats.

Even though few or no tubercles may be produced the first year where a rare legume is grown for the first time, there is an increase in the number of tubercles from year to year if the same legume continues to occupy the land. Hence we should not look upon inoculation as needing to be repeated, but as a procedure useful only or chiefly in the first year's growth of a rare legume.

Experimenters have been studying the tubercles of leguminous plants for only a few years and much is yet to be learned about every phase of this subject. Some of the readers of this bulletin can aid in gathering facts relative to the distribution of tubercle-producing organisms in this State by sending to the writer specimens of the roots of the less generally grown leguminous plants, as clover, alfalfa, beggar weed, soja bean and vetch, labelling the specimens with the sender's address and writing fully in regard to the crops previously grown on the land from which the specimen is taken. Many observations will perhaps help to answer the very practical question "What classes of soils in Alabama would be benefitted by inoculation for clover, vetch, etc."

SUMMARY.

Cowpeas, clovers, vetches and other plants, known as leguminous plants or legumes, when plowed under enrich the soil by adding nitrogen, a large proportion of which they have obtained from the atmosphere.

The enlargements or tubercles on the roots are the means by which the free nitrogen of the air is made available to leguminous plants. If these tubercles are wanting on a leguminous plant, the nitrogen of the air is unavailable to that plant, just as it is to cotton, grass, and all other higher plants that are not leguminous.

On some soils these normal tubercles do not develop on the roots of such legumes as have never before been grown in that locality. The growth of tubercles can be induced by adding certain germs to the soil, a process which is called inoculation.

In Alabama the cowpea is the most generally satisfactory renovating plant for summer growth. There is also need

for winter growing leguminous plants, such as hairy vetch and crimson clover, to occupy the land in winter between two sale crops, in order to decrease washing and leaching of the soil and to add vegetable matter rich in nitrogen.

Hairy vetch, without fertilizer, yielded on a field where this plant had been repeatedly grown 17,765 pounds of green forage or 4,174 pounds of hay per acre.

On a field where hairy vetch had never before been grown and where the fertilizer applied contained phosphoric acid and potash but no nitrogen, the yield was only 235 pounds of hay per acre; on an adjoining and similar plot, the seed for which were inoculated with earth from an old vetch field, the yield of hay was 2,540 pounds, an increase of 995 per cent following a treatment which cost nothing except a small amount of labor. On the inoculated plot the plants were well supplied with tubercles, while on the plot not treated the plants were bare of tubercles.

A germ fertilizer, intended to make available the free nitrogen of the air by inducing the growth of tubercles on legumes grown in soils not already stocked with the proper forms of germ life, was purchased in Germany and used in Auburn in pot experiments with hairy vetch, Canada field peas and crimson clover.

Inoculation with this germ fertilizer or nitragin greatly increased the yields of all of these plants as compared with untreated plants.

This increase in the weight of inoculated plants, after thorough drying, was as follows:

Hairy vetch, increased by 89 per cent.

Canada field peas, increased by 138 per cent.

Crimson clover (young plants), increased by 146 per cent.

Lupins (tested on only one soil), not increased.

Germ fertilizer prepared for vetch, was effective on Canada field peas.

Inoculation material procured without cash outlay acted like nitragin, and greatly increased the yields of hairy vetch and alfalfa.

Soil from a field where a given leguminous plant has recently been successfully grown is an effective inoculating material for the same kind of plant when first sown in a soil not already naturally supplied with the required form of germ life.

The dust adhering to the seed of bur clover was an effective inoculating material for alfalfa; the increase in the first cutting of alfalfa hay following this inoculation was 336 per cent.

Inoculation for cowpeas and lespedeza was apparently unnecessary in the soils used in these experiments. In or near all of these soils these two plants have been growing for years. Hence we may infer that these soils have been previously inoculated by germ-laden dust or by some other natural agency.

In a soil which for many years had borne no leguminous plants, tubercles developed without intentional inoculation on hairy vetch, Canada field peas, crimson clover and lupins, as well as on cowpeas and lespedeza. This soil was more nearly independent of inoculation than any other soil tested, and yet even on this soil the increase in the weight of inoculated plants over plants not inoculated was 38 per cent. with hairy vetch, 58 per cent. with Canada field peas, and 79 per cent. with crimson clover.

Many soils are naturally inoculated as regards the most commonly grown leguminous plants and hence are not benefitted by artificial inoculation.

APPENDIX.

NOTE 1.—As it was impossible to obtain sufficient distilled or thoroughly sterilized water it was necessary to use part of the time boiled water and part of the time rain water. So far as was possible without special facilities for this kind of work, precautions were taken to reduce the chances of accidental infection. When rain water was used it was caught from the greenhouse roof after the latter had been washed by the first portion of the rainfall.

Apparently the inoculation of all the even-numbered pots of crimson clover was accidental, a suspicion which was confirmed as regards Soil E., by the fact that crimson clover plants growing in this soil in the field produced no tubercles. The suspected source of accidental inoculation of the clover plants was the boiled water. This was taken from a well in a pasture containing some tubercle-bearing plants of creeping clover. The water was boiled and cooled in this pasture, and germ-laden dust may have been blown into the water while cooling.

The fact that all the plants in some of the untreated pots of vetch and Canada field peas were devoid of tubercles indicates that the germ required to induce the growth of tubercles on these two plants was not present in the water applied.

NOTE 2.—The following table gives the number of plants left in each pot after thinning, amount and kind of soil in pot, and yields of air dry tops, roots, and entire product of plants grown in pots. All pots containing cowpeas and lespedeza were carried entirely through the experiment, but the results appearing so uniform and other duties claiming the experimenter's attention, the weights of the roots of these two plants and also some of the weights of the tops of the cowpeas were omitted:

	No. of soil and pot.	Lbs. soil in pot.	No. of plants left.	Yield of air dry material in apothecaries' grains. Tops.	Roots	Entire plant.
				Grains.	*Grains.*	*Grains.*
Hairy vetch, inoc	A 9	8	9	141.6	114.2	255.8
" " not "	A 10	8	9	116.6	68.7	185.1
" " inoc	C 7	8	8	191.1	96 5	289.6
" " not "	C 8	8	8	154 1	57.0	211.1
" " inoc	D 7	8	9	71.8	60 2	132.0
" " not "	D 8	8	9	10.6	35.5	46.1
" " inoc	E 9	8	5	144 0	92.1	236.1
" " not "	E 10	8	5	14.0	27.7	41.7
Canada f. peas, inoc	A 7	8	7	181.5	86.3	267.8
" " " not "	A 8	8	7	68.4	101.1	169.5
" " " inoc	B 7	8	9	148.0	107.4	255.4
" " " not "	B 8	8	9	40.2	45 0	85.2
" " " inoc	C 5	8	10	84.6	113.0	197.6
" " " not "	C 6	8	10	33.6	19.0	52.6
" " " inoc	D 5	8	9	74.7	54.7	129.4
" " " not "	D 6	8	9	22 3	28.1	50.4
Crimson clover, inoc	A 3	3	1	29 3	21.8	51.1
" " not "	A 4	3	1	17.0	12.8	29.8
" " inoc	B 1	14	6	190.0	75 0	265 0
" " not "	B 2	14	6	82.5	69 6	152.1
" " inoc	C 3	3	5	57.0	37.1	84.1
" " not "	C 4	3	5	22.8	6.1	28.9
" " inoc	E 1	14	13	153 6	56.6	209.6
" " not "	E 2	14	13	29.3	38 2	67.5
" " inoc	E 3	3	5	35.7	21.9	57.6
" " not "	E 4	3	5	4.0	4.9	8.9
White lupins, inoc	A 17	14	18	112.0	82.5	194.5
" " not "	A 18	14	18	140.0	96.7	236.7
Alfalfa, 1st cut, inoc	A 15	14	....	276.6	....	....
" not "	A 16	14	....	40.	....	....
Cowpeas, (Backwoods) inoc	A 5	14	7	119.0	31.0	150.0
" " not "	A 6	14	7	108.8	58 0	166.9
" " inoc	B 5	14	8	....	....	....
" " not "	B 6	14	8	....	....	....
" " inoc	C 1	14	10	....	....	....
" " not "	C 2	14	10	....	....	....
" " inoc	D 1	14	7	91.5	43.6	135.1
" " not "	D 2	14	7	95	55.8	150 8
" " inoc	E 5	14	5	....	....	....
" " not "	E 6	14	5	....	....	....
Lespedeza striata, inoc	A 19	3	....	42 5	....	....
" " not "	A 20	3	....	36 3	....	....
" " inoc	B 9	3	....	29.	....	....
" " not "	B 10	3	....	16.9	....	....
" " inoc	C 9	3	....	37.0	....	....
" " not "	C 10	3	....	50.8	....	....
" " inoc	D 9	3	....	32.7	....	....
" " not "	D 10	3	....	27.0	....	....
" " inoc	E 11	3	....	22.0	....	....
" " not "	E 12	3	....	24.3	....	....

NOTE 3.—That dust from unhulled bur clover was able to inoculate alfalfa plants is not strange in view of the fact that both belong to the same genus, and that bur clover seed are borne near the ground and not harvested until after the vines are thoroughly dead, when the burs are swept up with brooms, thus mixing considerable earth with the seed.

The most unexpected point in this experiment is that the organism adhearing to perfectly dry bur clover seed retained its vitality from the time of harvesting bur clover (May or June) until late in the following October. The bag containing one bushel of bur clover seed was kept in a dark, airy storage room about three-fourths of the time, and in a very light room the rest of the time.

Our experiments in the field had previously shown that bur clover seed, without inoculation, developed tubercles on all soils tested, and that these tubercles first appeared in immediate contact with the old seed-bur.

NOTE 4.—The average increase for all soils resulting from inoculation has been calculated by comparing directly the weight of tops, roots, and entire product of inoculated and uninoculated plants, and not by averaging together the percentages given respectively on pages 471, 472 and 476.

In calculating this average for crimson clover, it was thought best to make allowance for the smaller size of some of the pots of crimson clover.