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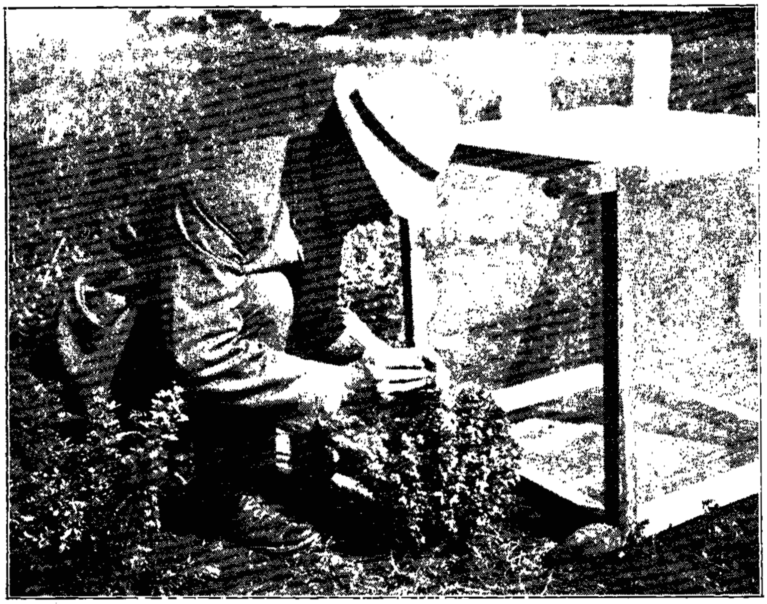
ALFALFA BREEDING: MATERIALS and METHODS



Hand pollination in the field.

By
H. F. ROBERTS AND GEO. F. FREEMAN.

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ALFALFA BREEDING: MATERIALS AND METHODS.

BY HERBERT F. ROBERTS *and* GEORGE F. FREEMAN.

SUMMARY.

1. The range of variation in alfalfa plants and the opportunities presented to the breeder for improvement of races of alfalfa by selection. Page 83.

2. The possibility of breeding frost- and drought-resistant strains of alfalfa, and the methods pursued by this Station to this end. Page 89.

3. A method for determining the relative transpiration of different types of alfalfa plants. Page 90.

4. A method for obtaining new races by hand pollination. Page 99.

5. A method not hitherto described or published for originating new races with great rapidity by a rapid modification of the hand pollination method. Page 102.

THERE exists, within the knowledge and investigation of the writers of this bulletin, no report upon experiments concerned with the breeding of alfalfa, presenting the possibilities involved in the improvement of the crop through the introduction of close-pollinated pure races of superior plants.

In the spring of 1906 the Department of Botany of this Station took up the problem. An area was planted to first-grade alfalfa seed from a single package, marked "Montana," in hills standing 18 x 18 inches each way. No evidence of variation appeared in the seeds that would not appear in any and all samples of alfalfa seed that we have seen. After the seedlings started, they were thinned to one in each hill. A similar plot, treated in a similar manner, was planted with seed of the so-called Turkestan alfalfa. The seed of this sort was distinctly different in mass appearance from the other, being of a markedly grayish cast, instead of having the usual golden-yellow color with a faint greenish tinge, typical of the seed of ordinary alfalfa.

From the outset, as the seedlings gained in size, the most marked and striking differences manifested themselves among the individual plants of the different plots, in respect to habit of growth, form, size and color-shade of the leaves, density of the foliage, size and succulence of the stems, and later in the color of the flowers; all of which characters were sufficiently obvious to strike the eye of the most casual and untrained observer; while in addition, other distinctive characters appeared in the plants, such as would usually command the attention of the botanist only. These differences, in a word, comprehended practically every possible organ of the plant, and were distributed with substantial impartiality over the whole of the two plots concerned. That the differences in question were not due to the influence of soil conditions is evidenced by the fact that plants of the widest dissimilarity were found side by side, with amazing frequency, all over the field.

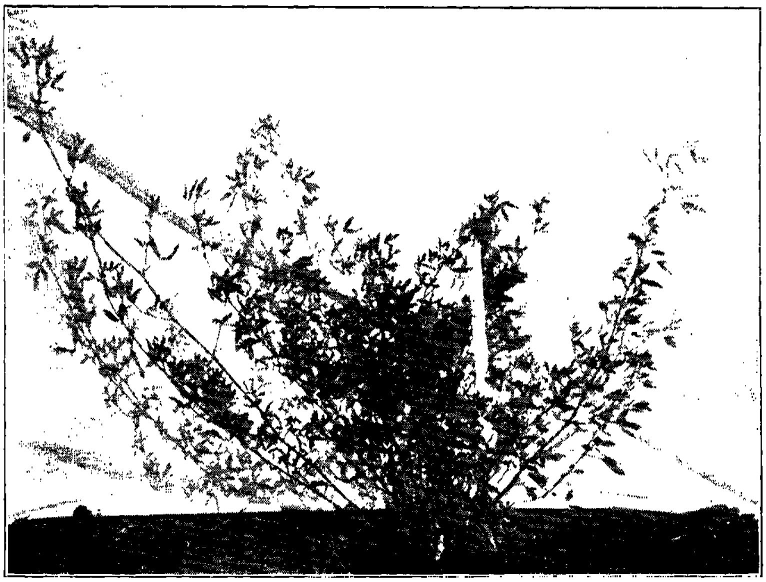

PLATE I.—Plant No. 61; plant upright, loose, open in habit; stems long; leaves set far apart, narrow. Compare with Plate III.

The effect to the eye, of substantial uniformity in alfalfa plants as seen under average field conditions, is thus shown to be due to close planting in the field, whereby individual differences are obscured and swallowed up in the general mass.

The question at once arose in the minds of the writers hereof, whether the variants alluded to above might not, magnified into pure races of their types, be found to represent great differences in commercial value in respect to forage yield, resistance to drought, to exceptional low temperatures, and to other adverse environmental factors. At the outset, 100 plants were selected, marked with stakes and numbered. These plants covered the greatest possible variety of types. From time to time, as the plants developed and came into bloom, others were added for various reasons, bringing the total of selected plants to date (February, 1908) up to 189. In order to give some conception of the material afforded for selection, some of these more striking types are illustrated in plates I, II, III and IV.

PLATE II.—Upright, stiff, dense, bushy habit; stems large, succulent; leaves set well together, broad. Compare with Plate IV.

It has been found advisable at the outset, in the selection of distinctive types for further study, to make a brief statement of the salient characters. In later work these concise descriptions were found invaluable in case of the occasional misnumbering of stakes, or of other accidents. It is therefore recommended to breeders that the preliminary survey of the material selected be accompanied by descriptions of a qualitative nature sufficient to serve for identification. Following are appended some characteristic examples from the records, of these preliminary descriptions, to which are added (table I) further examples from our records of more exact estimates of the characteristics of the plants. These more detailed analyses need not be made of all of the plants selected, but of those which are intended for further experiments immediately.

PLATE III.—No. 80; habit dense; foliage broad, closely set. Compare with Plate I. This plant has 20 per cent. greater forage value than No. 61. See Table I.

Following these it was deemed advisable to proceed so far as practicable with a more exact analysis of the morphological characters of the selected plants. This need not cover at once the entire series selected, but should proceed *pari passu* with all quantitative studies. Every plant selected for hand pollination, transpiration studies, special studies in variation and heredity, etc., should be subjected to a close scrutiny and analysis of the characters shown by the principal organs. While such descriptions have no high scientific value, they are indispensable for purposes of record, as all experienced breeders of plants who are compelled to work with very many individuals will admit. The following table gives some descriptive analyses of this nature from our records. These preliminary surveys of the plants are further serviceable for thoroughly and slowly acquainting the experimenter with all the visible details of his material, will suggest correlations, will build up mental images of great value, and, in a word, still bring the operator into a condition of at-homeness with the forms, which will save many subsequent hasty examinations and will exclude many speculations. Each investigator will pursue his own method, but the writers believe that a compulsory close examination of a wide series of forms within the group being studied, as is implied by the following examples, will be worth vastly more than a casual and a superficial survey, as a foundation of experience for future quantitative work.

No. 49. Dense, upright, well branched; leaves narrow, lanceolate, light green.

No. 50. Tall, gray-green; leaves oval to ovate; flowers dark purple.

No. 52. Habit very loose, open and spreading; foliage sparse; leaves narrow; flowers pale lilac.

No. 56. Habit sprawling, low; foliage rather sparse; leaflets pale green, very long, linear or elliptical to oblanceolate.

No. 58. Habit tall, upright; leaflets large, oval, pale green.

No. 69. Habit low, spreading, but not vine-like; leaves rather small, lanceolate.

No. 75. Habit stiff, upright, stocky, stems reddish; leaves narrow, oblanceolate, dark green, very sparingly pubescent.

No. 96. Plant of very luxuriant, succulent growth; leaves long, broad, light green, frequently with supernumerary leaflets.

No. 97. Plant of sparse but not succulent habit; leaflets narrow, long-acuminate, distinctly gray-green. Plant stands beside No. 96, 18 inches distant.

No. 99. Plant of dense succulent habit; leaflets very round-oval to orbicular, light green.

GENERAL HABIT.

Here the individual variations are endless. Some plants are very loose and open in habit, willowy, with long lithe stems, and with leaves set far apart by the extended internodes. Sometimes the stems of such plants are upright, as in plate I, but in other cases the stems are sprawling and prostrate at the outset in the seedling stage, spreading over the ground like trailing vines.

Some of the plants have a dense, bushy habit, stiff and upright, with leaves set well together, and with stems large and succulent in some cases, or large and woody in others. (Plate II.)

Some species, while possessing the dense upright habit, have very short stems, while others have exceedingly long but fine, wiry stems. Amongst all these general differences due to the habit and character of the stems, there comes in a wilderness of subdivisions of type due to variation in the leaf organs. It should be emphasized that these differences in habit were apparent in the seedlings. That they are induced by variations

TABLE I.

No.	Date harvested.	Habit.	Stems. Size.	Stems. Texture.	Stems. Color.	Stems. Degree of pubescence.	Leaves. Length.	Leaves. Width.	Leaves. Type shape.	Leaves. Degree of pubescence.
							mm	mm.		
6	Oct. 17	Mostly erect	Small	Somewhat succulent	Green	Slight	23	14	3-10	Slight to medium
29	Oct. 18	Spreading	Medium	Slightly woody	Green	Dense	23	14	3-10	Rather Dense
38	Oct. 18	Nearly erect	Medium	Slightly succulent	Green, and stem purple at base	Medium	26	13	72-10	Medium
61	Oct. 5	Spreading	Medium to large	Rather woody	Green, but red or purple at base	Medium to dense	30	10	4	Medium
64	Oct. 1	Spreading	Small	Slightly woody	Green	Slight	18	6.5	4	Medium
66	Oct. 2	Spreading	Medium	Slightly succulent	Green	Medium	25	9	4-10	Medium
67	Oct. 1	Spreading	Large	Slightly succulent	Green	Medium	32	15	72-10	Medium
80	Oct. 9	Spreading	Medium	Slightly succulent	Green	Medium	30	13	10	Medium to slight
89	Oct. 16	Distinctly erect	Large	Slightly woody	Green, slightly purple at base	Slight	25	10	72-10	Medium
91	Oct. 8	Spreading	Small	Slightly succulent	Green	Medium or slight	23	9	4-1	Medium
92	Oct. 16	Spreading	Medium	Slightly succulent	Rather grayish-green	Dense	27	16	1-10	Dense

in soil conditions seems too remote for serious consideration, in view of the substantial uniformity of the soil throughout the plots, and the fact that the most various types are found side by side.

LEAF CHARACTERS.

The stems of alfalfa are numerous, coming from a shortened perennial woody axis called the "crown." On the primary axes the principal leaves are borne, which, by virtue of their greater size, give stamp and character, as a rule, to the plant. These primary leaves may or may not subtend numerous secondary axes, bearing smaller leaves, which may be few or numerous, and which may or may not subtend still smaller branches, but which, as a rule, correspond closely in general type to the prin-

TABLE I—CONTINUED.

Stipules. Relative size	Stipules. Margin	Stipules. Apex	Stipules. Type	Total weight of plant	No. stems	Weight of stems	No. leaves	No. leaves per stem	Per centage ratio of leaves to entire plant	Stage of plant
Small	Serrate toward margin	Long acuminate	4	127.7	60	53.1	2,100	35	58.4	Buds not yet showing, very slightly frosted.
Medium	Serrate	Long acuminate	10	54.3	38	17.9	1,296	34	67.0	Buds scarcely showing.
Medium to small	Serrate at base	Long acuminate	4	108.5	53	45.0	1,487	28	58.6	Buds just appearing, very slightly frosted.
Medium	Serrate	Long acuminate	4	93.5	23	47.7	1,305	40.8	49.0	Beginning to flower.
Small	Serrate	Long acuminate	4	76.2	62	29.1	2,542	41	61.8	Just beginning to flower.
Medium	Serrate	Long acuminate	4	150.6	67	67.1	2,500	37	62.3	Buds just appearing.
Medium	Entire	Acuminate	4	127.8	36	61.6	1,390	39	51.8	Just beginning to flower.
Medium	Deeply serrate	Long acuminate	4	185.5	58	76.7	2,660	45.8	58.6	Beginning to flower.
Medium	Serrate	Long acuminate	4	69.7	25	32.1	945	37.8	33.8	Buds just appearing.
Medium	Serrate	Acuminate	44	130.0	75	47.7	2,171	28.9	63.3	Buds scarcely showing.
Medium	Serrate	Long acuminate	4	109.8	56	41.8	1,130	20.1	61.8	Buds scarcely showing.

cipal axes. It is found that the leaves of alfalfa vary widely in depth of color. Some plants have a distinctly gray or glaucous, often a silvery, appearance; others have very dark green and others very light green leaves. Leaf color is quite uniform over the entire plant, and differences in this respect among the plants are marked and striking. Plants with any of these color types may have pubescence (often very dense) on either or both sides of the leaf, or the leaves may be absolutely glabrous. Pubescence, while apparently not so uniform a character over an entire plant as color, is yet distinctive and marked. It should be observed that plants of the silvery-gray cast may be entirely smooth or glabrous, so that this color

PLATE IV.—Plant low, spreading habit, dense; leaflets broad, closely set. Compare with Plate II.

characteristic is by no means dependent upon the possession of light-reflecting hairs.

In respect to size and form, a most bewildering and amazing range of types presents itself, many of which include features of form and otherwise of the leaflets, too elusive to be described or even recognized in the case of a single stem, but the multiplication of which over the entire plant is often such as to leave a well-defined and sharp mental image of the plant as a whole. This enables the eye easily to differentiate it from another individual, a single stem of which would be indistinguishable from any single stem of the former.

Leaving aside, however, such minute differences, there is a remarkable series of widely different and distinctive types. Such a series is shown in plate V.

It will be seen that the leaflets fall roughly into two general classes, according as they are rounded or oblong. From plants of almost perfectly rounded orbicular leaflets of very small size, we find a continuous series to plants with very large leaflets of the same general type. From very short oblong leaflets we trace a similar series to leaflets of very great length, and all along we find a subseries of leaflets of any width, from narrow linear to broadly ovate but more or less acuminate.

PLATE V.—A few striking leaf types of *Medicago sativa.*

That differences in the leaf characters and in the general habit of the plants may be associated with facts of economic value, will easily suggest itself from a comparison of the leaves of plants Nos. 1 and 61, of Nos. 3 and 4, of Nos. 9 and 10, all in plate V. In plant No. 1, for instance, a stem of given length bears more leaves than No. 61, and the stems are more succulent. The broad-spreading stipules of No. 1, not observed in any other plant in the field, will be noted as characteristic. It will be interesting to compare further the totally different types of plants represented by Nos. 61 and 80, the former with few, narrow, small leaflets (plate I), the latter (plate III) with broad leaflets closely set, giving a dense habit that contrasts notably with the scanty, open foliage of the former. Referring to table I, in the next to the last column, it will be noted that the leaves of No. 61 constitute but 49 per cent. of the total green weight of the plant, as against 58.6 per cent. of leafage for No. 80, or of 67 per cent. for No. 29—a difference in favor of the two latter of 10 per cent. and 16 per cent. respectively.

Whether these great differences in apparent forage value, as judged by the leaf-bearing capacities of different alfalfa plants, are fixed characters which descend to the offspring, will be ascertained by our later investigations. That these differences do exist in individuals is clear, and that they are not temporary responses to local growth conditions seems certain.

VARIATION IN COLOR OF FLOWERS.

The prevailing color effect of alfalfa flowers, as seen in a mass in the field, verges on a dark blue. Individually, the flowers are of a light lavender hue, dashed with lines of deep lavender. Frequently the ground color is dark lavender with rather faint lines of darker shade. Another color is a deep purple with a reddish tinge and with dark purple lines. Plants of a distinct blue occur, having keels either blue, or yellow tipped with blue. Total absence of the color-determining character seen in white-flowered variants of blue-flowered types, such as in the white-flowered forms of our native *Verbena stricta, Ruellia ciliosa,* and others, seems also to occur, although rarely, with *Medicago sativa.* More frequently, such white-appearing flowers are seen on closer scrutiny to have a tinge of purple, blue or yellow. A few plants have been noted with flowers of a dark "smoky" blue, tinged with yellow, green or purple, and having keels purple or yellow tipped with purple. In the fields under investigation were plants bearing flowers with greenish-yellow standards, sometimes with a smoky bluish tinge, and shading off into the color of the flowers last described.

The color characters in and of themselves are of no consequence to the farmer, but they suggest possible correlations with characters of economic value, and therefore compel scientific investigation.

PHYSIOLOGICAL CHARACTERS.

That the differences apparent in alfalfa plants extend further to physiological characters was demonstrated very early in the spring of 1907 with reference to resistance to low tem-

PLATE VI.—General appearance of field, from American-grown seed, after the freeze of March 23, 1907.

peratures. On March 23 a severe freeze followed upon an extended period of warm weather, during which time the alfalfa plants had been growing rapidly, were about six inches high, and very succulent. On the date mentioned the temperature fell to 19 degrees F. There was observed the greatest imaginable difference between the plots of the so-called Turkestan and American alfalfa, taken as a mass, in respect to their degree of susceptibility to frost. The American alfalfa was badly injured, and the whole field took on a brown appearance after the frost-killed stems had commenced to dry. (Plate XI.)

On the other hand, the Turkestan plot, taken as a whole, was little or not at all injured. It should be stated that the two plots adjoined, and were of the same elevation; the Turkestan plot sloping slightly to the west, while the other sloped rather more decidedly to the east. It was observed, however, while the mass injury was as stated, that there were numerous exceptions. Whereas the Turkestan plot remained, as a whole, green, some of the plants were killed completely to the ground, while others were partially killed. The majority were, as stated, not injured at all, or very slightly so. (Plates VII and VIII.) Occasionally, of the two plants side by side, one was killed to the ground while the other remained uninjured.

In the American alfalfa plot, although the overwhelming majority of the plants were badly injured, there were occasional individuals that escaped injury completely. As in the Turkestan plot, the plants that were affected in many instances stood side by side with those that were not.

The severity of the freeze in question was such that, in general, farmers over the affected region were obliged to abandon the first cutting of alfalfa, and mow the ground in order to stimulate the plants to make a vigorous second growth. The desirability of propagating frost-resistant plants, other things being equal, is too obvious for comment. Some two weeks after the frost, and after the frost-killed stems had turned brown, notes were taken upon the plants in the two plots, which are summarized in tables II and III.

TABLE II.

	Turkestan.	American.
Number of plants counted	711	743
Per cent. of plants untouched by frost, uninjured	10.8	0.7
Per cent. of plants showing tips of one-fifth stems killed, practically uninjured	28.5	5.6
Per cent. of plants showing five or more stems badly frozen back, others green to tip, injury rather severe	58.5	54.2
Per cent. of plants having all stems killed to the ground but sprouting from base, injury very serious	2.2	38.7
Per cent. of plants apparently killed	0.0	0.8

TABLE III.

	Turkestan.	American.
Per cent. of plants practically escaping injury	39.3	6.3
Per cent. of plants slightly injured	58.5	54.2
Per cent. of plants seriously injured	2.2	39.5

Plants in both fields showing perfect frost resistance were marked, those exhibiting promising qualities otherwise being set apart for future breeding purposes.

RELATIVE TRANSPIRATION.

Alfalfa races intended to be grown in semiarid regions under limited or no irrigation are desirable. Land in semiarid, unirrigated districts, usually worth from less than five dollars, to ten dollars per acre, may be enormously enhanced in value by alfalfa growing. An attempt has been made, by the importation of supposedly drought-resistant strains, to achieve this result, not, however, with entire success. The problem of

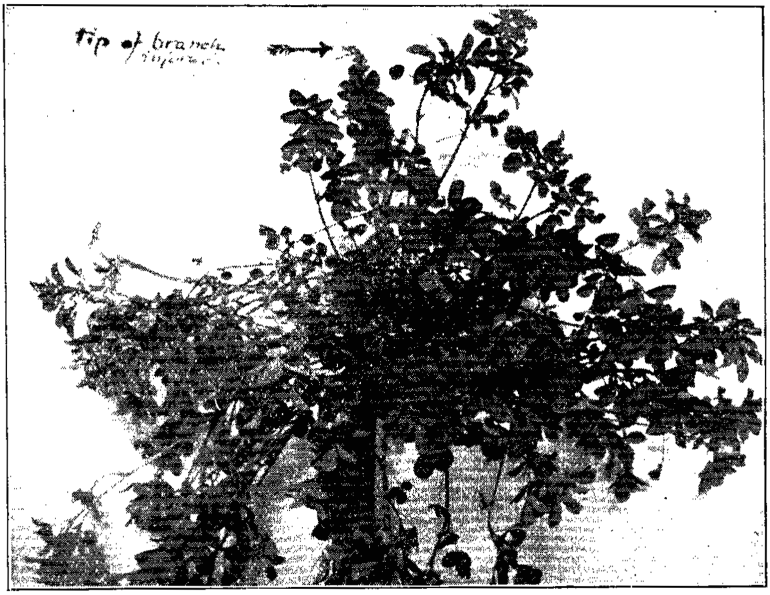

PLATE VII.—Plant from Turkestan alfalfa plot, showing almost no injury. Tip of branch at top center of plate is shown slightly injured.

obtaining a drought-resistant race lies probably in securing plants with a minimum transpiration rate. Some of the strains of Turkestan alfalfa introduced into this country, which were native and already adapted to a region of very limited rainfall, have here proved more or less successful in this regard. In this way, indeed, the importation of already established races, native to a dry habitat, is desirable practice when the extreme of drought-resistance is to be sought. Nature has in this case marked out the problem, by having left surviving in their respective habitats the most resistant races. The extremely drought-resistant races, however, like Turkestan alfalfa, are apt to be less succulent and to yield less forage to the acre than the races growing naturally in the regions of adequate rainfall. It is desirable, therefore, in alfalfa breeding, to investigate these more richly producing races, in search of individuals of higher specific drought-resistance, to be the foundation of strains which will combine this characteristic with succulence and high yield of forage.

Clearly, drought-resistance in a plant depends either upon its effectiveness in gathering water from the soil, or its effectiveness in retaining it when gathered, or both. The most ob-

PLATE VIII.—Plant from Turkestan alfalfa plot showing greater degree of injury than the one in Plate VII. The two plants grew five feet apart.

vious and simple method of attacking the question of the drought-resistance of alfalfa plants is apparently by determination and comparison of the amounts of water they individually give off in transpiration from the leaves in the same length of time. The one having the lowest transpiration rate would be altogether likely to be the best suited for a dry region or for withstanding such occasional droughts as occur in all localities.

Without radical modification, however, no method for measuring transpiration has as yet been devised which can satisfactorily be used in this work. Cut stems, when placed in water, do not transpire at the rate normal to the same plants growing upon their own roots, nor even at a rate sensibly proportionate to it. Since the alfalfa plants under experiment are destined to become mother plants for seed, they cannot be taken up and grown in pots, and their transpiration rates ascertained by weighing the potted plants. The polymeter method, as devised by Dr. W. A. Cannon, of the Desert Botanical Laboratory, Tucson, Ariz., has many good features, and might be utilized. It possesses the disadvantage, however, of furnishing a constantly increasing relative humidity in the air surrounding the plant, which retards evaporation, and renders difficult the comparison of transpiration rates in the different plants. The method and apparatus herein described, overcome most of the difficulties involved, and seem likely to become highly useful in the study of transpiration in its relation to plant breeding. This method of measuring transpiration may be said to be only a modification and a new adaptation of those used by Ganeau de Lamartière[1] and E. and J. Verschaffelt,[2] in that air is drawn over the plant in the same manner, and the transpired moisture is collected in the U-tubes containing hygroscopic substances which are not contained in the vessel with the plant, but which are connected in the same aspiration series, so that the air, after passing through the evaporating cylinder, is next passed through the U-tubes. A different absorbent is used, however, and the apparatus is, moreover, adapted for measuring the transpiration of plants on their own roots. The essential point of difference, nevertheless, lies in the condition of the air as supplied to the plant. While in the method here used normal air is supplied, both of the above investigators first completely dried the air before allowing it to reach the plant. Now this is detrimental, for the reason that perfectly dry air, coming in contact with the leaves of a plant, will cause the stomata to close, and thereby the transpiration will be reduced below normal.

Plate X shows the apparatus as set up in the field. It consists essentially of a glass cylinder, 20 cm. long and 3 cm. in diameter, constituting a transpiration chamber into which a branch of the plant is inserted; two U-tubes for holding phosphorus pentoxide, and a large 19½-litre demijohn to be used as an aspirator. The whole series is connected by rubber tubing so that as the water flows from the bottle, a constant current of

1. Ganeau de Lamartière, L., "Récherches Physiologiques sur les feuilles developpés a' l' ombre et au Soleil"; VI Transpiration. Revue gén. de. Botanique, Tom IV, 1892, p. 529.

2. Verschaffelt, E. en J., "De transpiratie der planten in Koolzuurorije"; Genootschap "Dodonaeea" te Gent, Jaarg. II, 1890, p. 305.

For both the above citations, see, also, Burgerstein, "Die Transpiration der Pflanzen," 1904, pp. 16-56.

PLATE IX.—Two plants in Turkestan alfalfa plot growing 18 inches apart. One killed to the ground, the other absolutely uninjured, after the freeze of March 23, 1907.

air is drawn into the transpiration cylinder over the leaves of the plant, and thence into the U-tubes, where both the water of the normal air and that given off by the plant are absorbed by the P_2O_5. The transpiration cylinder is fitted with a cork at the bottom, which contains two holes, one to accommodate the stem of the plant, the other for a small intake tube for the outside air. The cork is cut in halves so that it may be fitted around the stem of the plant before the latter is inserted into the cylinder. The cork in the top is also provided with two holes, one for the insertion of a thermometer, and another for the outflow tube. The U-tubes are provided with ground glass stop-cock stoppers, and with suitable intake and outflow side tubes. Before going to the field, the U-tubes are carefully filled with the P_2O_5, resting in alternate layers with glass wool, which supports the pentoxide and yet allows free passage of the air through the tubes. The U-tubes are then weighed, the stop-cocks being kept carefully closed so that no air may reach the pentoxide between the time of weighing and the setting up of the apparatus. It is well before going into the field to fill a number of tubes in this manner, in order that several runs may be made without returning to the laboratory. For operating, the apparatus is set up as above described, care be-

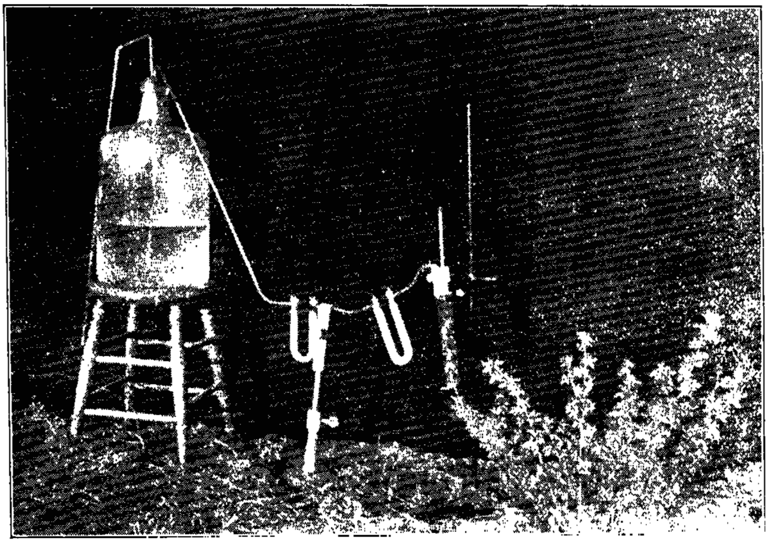

PLATE X.—Transpiration apparatus set up for operation in the field.

ing taken that all of the connecting joints are tight; the aspirator is then filled with water and the stop-cocks turned so as to prevent the passage of air. The last operation is to insert the plant into the cylinder and start the water flowing from the aspirator. When the water ceases to flow, the exact time is noted, the stop-cocks are turned off in the U-tubes, and the stem clipped from the plant at the point of its entrance into the cylinder. The series is then disconnected, and the plant-shoot kept in the cylinder until it is ready for weighing and the determination of the leaf areas. Now since, as above mentioned, the increase in weight of the P_2O_5 tubes includes both the moisture contained in the normal air that has passed through them and the moisture given off by the plant during the same time, it becomes necessary, in order to determine the latter, to ascertain first the amount of the former factor. This may be done by means of the relative humidity method with a sling psychrometer, or wet and dry bulb thermometer, or by setting up another aspirator series with the tubes operating as in the transpiration apparatus itself, but omitting the plant, thus determining directly the moisture content of the normal air. The latter method is preferred, for the reason that it is direct and does not require corrections for altitude, barometric pressure, etc., and, moreover, with but little additional labor the extra apparatus can be set up and operated during the same time that the transpiration series is in operation. If the apparatus be of the same size, it will merely be necessary to subtract the increase in weight of the normal air series from that of the transpiration series in order to obtain the total transpiration of the plant-shoot for the given time. Then $60\left(\frac{q}{a \times t}\right)$ = the transpiration rate per hour, where q is the quantity of water transpired, a the leaf area involved, and t the time-duration of the experiment in minutes. The area of leaves enclosed in the transpiration cylinder, the volume of the cylinder, and the amount of air drawn through by the aspirator were in the proportions of 1 : 5 : 500. The flow of water through the aspirator was so regulated that each transpiration experiment lasted approximately an hour. The air was thus completely changed within the cylinder every thirty-six seconds. The rise in humidity in the cylinder, due to transpiration from the plant, can therefore easily be controlled, either by regulating the rapidity of the flow of air through the cylinder or by changing the amount of leaf surface enclosed.

After a few trials, however, one will learn to estimate sufficiently closely the capacity of the apparatus so as not to overcrowd it. The latter condition is indicated by the collection of drops of moisture on the cylinder, showing that the air within has reached the saturation point. This condition may be remedied either by increasing the rate of flow of water through the aspirator, or by diminishing the amount of leaf surface enclosed. The same U-tubes may be used repeatedly without refilling, so long as a part still contains dry P_20_5. Care should be taken to set up the tube in the same way each time, *i. e.,* to have the air enter and pass out of the tube in the same direction. The reason for this is that the phosphoric acid, after being formed from P_20_5+water, will itself take up water. If, then, the tube be turned, so that dry air, on leaving the tube, passes over this phosphoric acid, which has previously taken up an excess of water, it will itself take up water from the acid and the tube will lose in weight.

In order to test the practicability of this method in the field, two alfalfa plants were chosen that were growing within about four feet of one another and which showed marked differences in form and texture of the leaves. They had previously been cut at the same time, and the stems were therefore of the same age, and in the same stage of growth: *i. e.,* just beginning to bloom. Both were vigorous plants, but No. 67 was somewhat larger than No. 64. No. 67 had few, large, thick stems, and large, thick, bluish-green leaves. No. 64, on the other hand, was diffuse in its branching habit, the stems were small and inclined to be willowy, while the leaves were small, numerous, thin, and of a lighter green.

Three simultaneous experiments were made on these two plants, in order to determine whether any constant difference could be shown to exist in their transpiration rates per sq. cm. of leaf surface. The following table gives the details of this work. The column marked "control" contains the data derived from the apparatus which was set up to measure the quantity of water in the normal air.

An examination of table IV shows a constantly higher transpiration rate for No. 64 over No. 67. This difference, however, was very variable. Unfortunately a larger leaf area was taken each time for No. 67: so much *so* that the relative humidity in its cylinder was higher than that of No. 64 in the first two experiments. This fact would place No. 67 at a disadvantage in these two experiments, since the higher humidity would retard its transpiration rate to a greater relative degree. Had it not been, therefore, for experiment 3, in which, notwithstanding the greater leaf area of No. 67, the total transpiration of No. 64 was greater, thereby causing a greater relative humidity in its cylinder, the series would have seemed inconclusive. This third experiment, however, confirms the results in the other two, by showing that plant No. 67 may even overcome the disadvantage of a higher relative humidity and still transpire nearly twice as much per sq. cm. of leaf surface. This would indicate, therefore, that plant No. 64 had a greater drought-resisting ability, and that it would be the better plant to select for a dry region. The preliminary results from transpiration experiments show that the relative drought resistance of different pure races of alfalfa plants can be determined to a high degree of probability.[3]

3. Further details concerning the method and apparatus used herein for determining relative transpiration in plants are given in an article by Prof. Geo. F. Freeman, appearing shortly in the *Botanical Gazette,* entitled "A method for the quantitative determination of transpiration in plants."

TABLE IV.

Plant No.	Date of experiment, Sept., 1907	Experiment began	Experiment ended	Time in minutes	Total gain of P_2O_5 tubes, mg.	Amount of air used, liters	Amount of water in air used, mg.	Net gain of P_2O_5 tubes, mg.	Relative humidity of external air	Relative humidity of air in cylinder	Total number of leaves	Total area of leaves, sq. cm.	Loss per hr. per sq. cm. of leaf surface	Temperature inside of cylinder	Temperature outside of cylinder
EXPERIMENT I.															
		P. M.	P. M.											C	C
64	21	3:50	4:50	60	375	19.5	130	245	28.7%	77.5%	33	22.28	10.9	26.5°	25°
67	21	3:51	4:55	64	418	19.5	130	288	28.7%	86.4%	14	32.46	8.3	26.5	25
Control	21	3:48	4:51½	63½	130	19.5	......	......	......	......	......	......	......	......	......
EXPERIMENT II.															
		A. M.	A. M.												
64	23	10:16	11:26	70	324	19.5	148	175	28.0%	59.8%	12	17.13	8.7	28.5	28
67	23	10:22	11:29	67	353	19.5	148	205	28.0%	65.3%	14	41.98	4.3	28.5	28
Control	23	10:14	11:15	61	148	19.5	......	......	......	......	......	......	......	......	......
EXPERIMENT III.															
		P. M.	P. M.												
64	23	4:13	5:29	77	422	19.5	126	295	25.3%	80.4%	29	21.25	10.9	28	27
67	23	4:16	5:40	84	415	19.5	126	289	25.3%	79.1%	11	33.81	6.1	28	27
Control	23	4:10	5:14	68	126	19.5	......	......	......	......	......	......	......	......	......

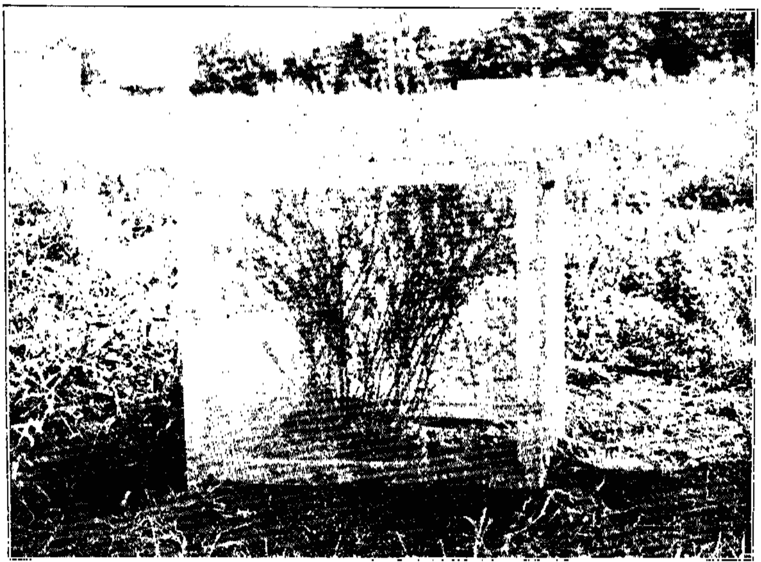

PLATE XI.—Showing cage and manner of enclosing alfalfa plant for hand pollination. A cage of 27½ x 27½ x 24 inches is the smallest convenient size.

PROPAGATION OF NEW RACES.

This concludes a general survey of the chief salient differences in habit and character of the alfalfa plants under our observation, expressed in qualitative and descriptive terms. It will perhaps serve to call attention to the opportunities presented for selection. The preliminary survey being made, the plants selected are marked, as our next problem becomes one of securing pure races of these distinctive types.

This can be accomplished in one of two ways—by vegetative propagation by cuttings from selected plants, or by close pollination. The former method as applied to alfalfa seems to have been first conceived by Dr. B. T. Galloway, chief of the Bureau of Plant Industry, United States Department of Agriculture, and described for the first time by J. M. Westgate.[4] It was finally elaborated in the experiments of Westgate and Oliver.[5] This method has advantages where the rapid propagation of proved superior plants is desired. In the work of the writers

4. "A method of breeding a strain of alfalfa from a single individual." American Breeders' Association, vol. II, p. 65.

5. "The application of vegetative propagation to leguminous forage plants," Bul. 102, part IV, Bureau of Plant Industry, U. S. Department of Agriculture, March 23, 1907.

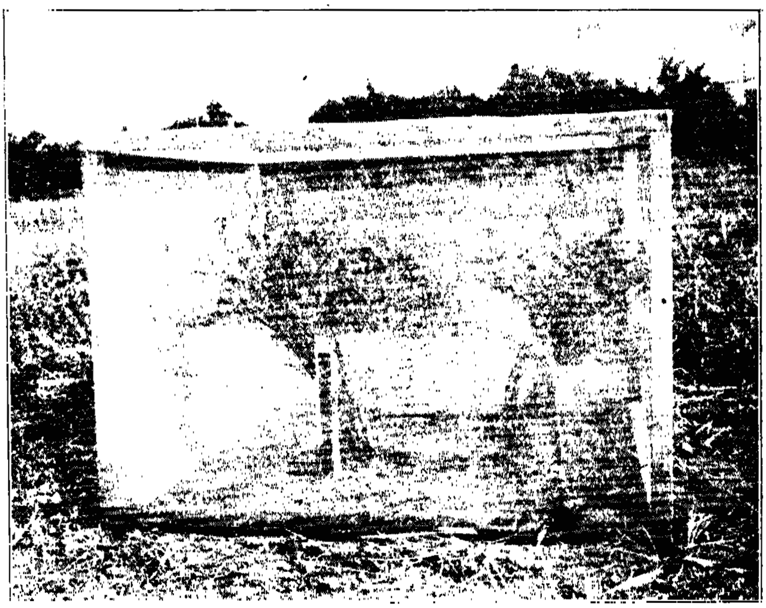

PLATE XII.—Large cage, showing enclosure of several alfalfa plants.

it was found that extensive and exhaustive field studies were necessary to ascertain what and how many of the characters selected for might be heritable. By close-pollinating numerous plants of distinctive types many pure races can be originated at once, and a greater number of progeny obtained for purposes of the study of heredity and variation at less labor and expense than by the method of vegetative propagation,

The flower of alfalfa is rather an advantageous one for hand-pollinating purposes. The two wings have projecting processes which overlap, and assist in holding down the curved, spring-like column formed by the united group of stamens which enclose the pistil. A set of interlocking processes for the keel, further assist in forming this spring-trap arrangement. When an insect of sufficient weight alights upon the keel, it depresses the latter, together with the enclosing wing petals; the trigger-like processes are pushed down past the upcurved column of the pistil and stamens, releasing them, and allowing the whole column to spring up with considerable explosive force against the erect standard. At the time of pollination, the style with the stigma has grown up above the stamens, and when released the stigma precedes the stamens, striking the insect's body first, in case the latter rests upon the keel, bearing its deposit of pollen brought from another flower. The burst anthers in turn dust off a new deposit of pollen as they are driven past the insect, which is thus equipped with a fresh supply of pollen to become available for the next flower. Sometimes the shaking of the flower stems by the wind, or by the pelting of the rain, may accomplish the same result. Self-fertilization may be secured also by visits of insects not yet loaded with pollen, which may, by setting off the explosive mechanism, bring about self-pollination. Since the pollen is shed before the stamen-pistil column is released, it happens that the stigma is already partly covered with pollen. Nevertheless, self-fertilization seems to occur but seldom in enclosed plants protected from insect visits.

The explosive mechanism of the alfalfa flower has long been known, having been discovered as early as 1832 by A. P. De Candolle.[6]

In 1894, Burkill[7] found it impossible to make seeds set in the unexploded flower, even though pollen were in contact with the stigma. He considers this fact to be due to the circumstance that the stigma does not become receptive to the pollen until its cells are injured by violent contact with some object. In proof he adduces the fact that he had caused unexploded flowers to set seed by pinching the stigma, by cutting off the tip of the keel, or by rubbing the stigma with a stiff brush. It appears therefore probable that insects secure the fertilization of alfalfa flowers largely by accidental injury to the stigma while endeavoring to cause the probosis to enter; or else by exploding the flowers and causing the stigma to be dashed against the standard, the necessary amount of injury may be accomplished to enable the pollen to become effective, in which case it may either be the already present pollen of the same flower, or foreign pollen brought by the insect that is utilized.

In Burkill's experiment referred to, impact of the stigma upon the standard, following upon the release of the trigger

6. Physiologie, II, p. 548.

7. "On the fertilization of some species of *Medicago L.* in England"; I. H. Burkill, Proceedings of the Cambridge Philosophical Society, vol. VIII, pp. 142, 143, 1892-'95.

mechanism by means of some instrument, resulted in the setting of the seed in thirty-five per cent. of the cases.

In the experiments at this Station, the results obtained by artificially releasing the trigger mechanism by hand, thereby bringing the stigma into violent contact with the standard, as in Burkill's case, gave exceedingly varying results, as will be seen by reference to table V, which will be discussed later.

Experiments hitherto are singularly at variance regarding the ability of *Medicago* to set seed when enclosed in cages and insects excluded. Kirchner[8] obtained no seed in these cases, and Fruwirth[9] in occasional plants obtained one or two pods containing from one to three seeds. Henslow,[10] on the other hand, obtained in such plants, in comparison with plants grown in the open, an abundance of seeds, in a percentage ratio of 77 of the former to 100 of the latter

In our experiments, several plants were selected for a study of this problem. At the time of their selection all of the plants in the open field were setting seed more or less freely. For the sake of comparison some plants which were apparently weak, and others which were apparently strong in seeding capacity, were selected and enclosed in frame cages covered with wire screen, as shown in plates XI and XII. The soil was well hoed around the edges of the cages so as to close all openings and exclude the visits of any insect large enough to pollinate the flowers.

At the time of their selection the plants were already setting seed, and consequently could be judged roughly as to their relative seed-bearing tendencies. On this basis they have been designated in the table as "strong" and "weak" seeders.

The above table indicates that, as a rule, those plants that set seed liberally outside were strong in seeding capacity when enveloped within cages, and *vice versa*. Nos. 89 and 90 gave a heavy crop of seeds. Some of the differences exhibited above may well be ascribed to individual variability in respect to seed-bearing capacity. The fact that pronounced individual differences exist in this respect was even more strikingly

8. "Ueber die Wirkung der Selbstbestäubung bei ben Papilionaceen," Naturwissenschaftliche Zeitung für Land und Forstwirtschaft." Heft 1-3, 1905.

9. "Die Züchtung der Landwirtschaftlichen Kulturpflanzen," vol. III, 189—.

10. Transactions of the Linnæan Society, Botany, 1877, p. 317.

PLATE XIII.—Method of manipulating alfalfa flowers in hand pollination. The spatula rests upon the keel, which is depressed, allowing the column of stamens and pistil to fly upward against the standard.

brought out in a series of experiments in which plants in cages were pollinated by hand. In one series of these the flowers were counted, in order to determine the percentage setting seed out of the total of flowers pollinated. In another series the flowers were pollinated by a rapid method, in which counting was impracticable. In both cases the plants were selected on account of some promising or distinctive characters

TABLE V.

Plant No.	Apparent seeding ability of the plant.	No. of stems.	Total weight of the plant, grams.	Total number of pods.	No. of pods with seeds.	Per cent. of pods bearing seeds.	Total number of seeds.	Average number of seeds per pod.	No. of seeds per 10 grams weight of plant.
69	Strong	32	156.75	544	25	4.6	27	1.08	1.72
71	Weak	9	71.25	2	1	50.0	1	1.00	0.14
89	Strong	21	103.88	391	140	35.8	214	1.64	20.57
90	Strong	16	121.13	606	187	30.85	257	1.37	21.24
91	Weak	56	313.50	113	21	18.58	23	1.09	0.73
92	Strong	17	178.13	95	37	38.93	59	1.59	3.31
70	Strong	17	103.88	414	20	4.80	25	1.25	2.40

rendering them desirable as mother plants for breeding purposes. For this reason it was desired to obtain from each as large an amount of close-fertilized seed as possible. In table VI appear the results from a series of plants in which countings were made.

TABLE VI.—ALFALFA CLOSE-POLLINATION EXPERIMENT.

Serial number of plant..........	Total number of flowers pollinated..........	Total weight of plant, grams....	No. of stems per plant..........	Total number of pods..........	Per cent. of pods bearing seeds....	Total number of seeds..........	Average number of seeds per pod..	Per cent. of flowers setting pods..	No. of seeds per 10 grams total weight... .....
1..........	2,515	330	38	648	5.24	52	1.53	25.77	1.6
3..........	2,096	91	55	133	....	3	1.50	6.34	0.3
4..........	3,389	200	55	985	7.20	87	1.22	29.00	4.3
6..........	1,926	17	59	106	1.90	5	2.50	5.50	2.9
9..........	3,127	63	32	784	17.40	136	1.10	25.07	21.6
11..........	1,721	85.5	26	1,126	40.00	709	1.53	65.42	82.0
37..........	1,576	57	28	753	26.16	310	1.57	47.77	54.4
47..........	490	57	65	50	2.00	1	1.00	10.20	0.1
66..........	10,048	242.25	31	2,403	24.84	726	1.21	23.81	30.0
93..........	1,494	57	33	631	6.81	50	1.16	42.23	8.8
95..........	6,354	1,938	38	2,124	....	874	....	33.42	45.0
65..........	3,421	114	37	141	4.25	6	1.00	41.21	0.5
108..........	2,858	106.875	23	1,618	23.55	600	1.57	56.61	56.0
67..........	3,241	135.375	18	1,304	25.46	406	1.22	43.32	30.1
64..........	2,320	102.6	39	1,278	8.70	142	1.27	55.08	14.0
109..........	1,069	14	8	311	57.87	239	1.32	29.09	170.7
97..........	1,407	37	20	608	16.94	128	1.24	43.21	34.6
98..........	1,627	64.13	8	779	7.30	70	1.22	41.12	10.9
99..........	3,680	85.5	21	1,534	38.72	910	1.53	41.68	106.4
38..........	1,822	114	18	279	58.77	236	1.44	15.31	20.7
29..........	942	35.63	9	272	4.41	14	1.17	28.87	3.6
13..........	1,416	74	36	3	66.66	2	1.00	.21	0.3
14..........	122	191	51	141	7.80	16	1.45	*115.00	0.8
17..........	198	50	13	75	12.00	12	1.33	37.88	2.4
110..........	3,497	77	23	1,390	22.67	506	1.61	39.46	65.7
111..........	2,589	....	19	973	11.51	127	1.31	37.58	....

* A very vigorous plant on which but few flowers were pollinated by hand. The excess of pods set, over the number of flowers pollinated, is explained by the fact that a large number of flowers set seed without hand pollination. See Table V.

From this table it will appear that the plants under experiment possess marked differences, not only in seed-bearing tendency in general, but even in the number of seeds produced per individual pod. There appears, moreover, to exist no constant relation between the number of flowers pollinated and the number of seeds produced. Although from the experiments exhibited in table VI it is likely that some seed would have been obtained from the enclosed plants without hand pollination, yet the considerable crop of seeds secured would never have been possible without the assistance of the means employed, as is evidenced by the fact that in the case of plants which produced a heavy crop of seeds when hand pollinated, where there were flower clusters left unpollinated the flowers on these soon dropped without setting seed. An interesting result was obtained in the case of No. 93, mentioned in table VI. The stems of the plant were separated as they stood into two portions, on one of which the flowers were hand pollinated, while on the other they were not. Both portions were included under a wire cage to prevent adventitious visits of insects. Comparison of the results appears in the following table:

According to the results above it is seen that, reducing to

TABLE VII.

	Flowers not hand pollinated.	Flowers hand pollinated.
Number of stems..	17	11
Total weight (grams)	178	78
Number of clusters of pods	62	76
Total number of pods	95	288
Average number of pods per cluster	1.53	3.79
Number of pods bearing seeds	37	97
Per cent. of pods bearing seeds	38.93	33.68
Average number of seeds per pod	1.59	1.20
Total number of seeds secured	59	118
Number of seeds per 10 grams weight of the plant	3.31	15.13

TABLE VIII.

No. of plant	No. of color-type of flower*	Date of harvesting seed	Date of harvesting plant	Total weight of plant	Number of stems	Number of pods	Number of pods with seeds	Per cent. of pods with seeds,	Number of seeds	Average number of seeds per pod,	Number of seeds per 10 grams wt. of plant
112	6	Sep. 9	Sep. 9	24.8	28	22	10	45.4	11	1.10	4.4
113	5	" 9	" 9	17.1	18	85	42	49.4	49	1.17	28.6
114	5	" 9	Oct. 5	17.0	9	74	29	39.2	47	1.62	27.6
115	2	" 9	Sep. 9	14.2	11	91	29	31.8	38	1.31	26.7
116	4	" 9	" 9	19.0	6	176	54	30.6	64	1.18	33.6
117	1	" 9	" 9	35.6	16	352	168	47.7	231	1.37	64.8
118	10	" 9	Oct. 5	60.4	49	313	131	41.8	182	1.39	30.1
119	3	" 9	" 5	21.4	7	50	22	44.0	23	1.04	10.7
120	3	" 9	" 5	34.0	10	151	42	27.8	79	1.88	23.2
121	6	" 9	" 5	50.0	14	33	6	18.2	8	1.33	1.6
122	3	" 9	" 5	39.4	32	235	113	48.1	179	1.58	45.4
123	1	" 9	" 5	21.6	17	319	215	67.4	296	1.37	137.0
124	1	" 9	Sep. 9	21.3	18	172	95	55.2	121	1.27	56.8
125	5	" 9	Oct. 5	23.0	13	151	66	43.7	98	1.48	42.6
126	3	Oct. 5	" 5	44.4	14	254	108	42.5	248	2.29	55.8
127	2	Sep. 9	Sep. 9	9.5	9	69	24	34.8	28	1.17	29.4
128	3	" 9	Oct. 5	15.6	12	120	59	49.1	80	1.35	51.2
129	4	" 9	" 5	41.6	19	81	32	39.5	43	1.34	10.3
130	9	" 9	Sep. 9	14.2	13	33	19	57.5	32	1.68	22.5
131	4	" 9	Oct. 5	40.4	20	47	15	31.9	17	1.15	4.2
132	6	" 9	" 5	38.0	35	156	64	41.0	102	1.59	26.8
133	5	" 9	" 5	16.0	17	82	43	52.4	53	1.23	33.1
134	4	" 9	" 5	48.8	15	416	243	58.4	419	1.72	83.8
135	2	" 9	" 5	26.0	16	67	1	1.5	1	1.00	0.4
136	8	" 9	" 5	12.2	16	60	37	61.6	65	1.75	53.3
137	3	" 9	" 5	22.8	25	142	53	37.3	75	1.41	32.9
138	3	" 9	" 5	13.6	9	36	13	36.1	19	1.36	13.9
139	4	" 9	" 5	19.4	18	99	23	23.2	31	1.34	15.9
140	3	" 9	" 5	8.0	5	16	9	56.2	18	2.00	22.5

*These numbers refer to water-color sketches in the possession of the department.

a common basis, the hand-pollinated portion of the plant produced 60 per cent. more seeds than the unpollinated portion, while its productivity per gram weight of the green plant was disproportionately greater, amounting in this case to 457 per cent.

A further series of plants were pollinated by hand, but the hand-pollinated flowers were not counted. The results of these experiments are exhibited in table VIII. Here, again, the green weight of the plant is taken as a basis of comparison of their relative seed production. It appears from these results that the individual variability of the plants in respect to seed-forming capacity was no less than in the previous case. The plants of this series were growing on the field of a private owner to the north of the College land.

For the purpose of studying the relative effects of close and cross-fertilization on the vigor of the offspring, several promising plants were selected, and divided by setting the edge of the wire cage across the middle of the plant, thereby enclosing the half on which the flowers were to be hand pollinated within the cage and leaving the remaining half standing outside the cage, freely accessible to insect visitors. Table IX enables a comparison to be made of the effectiveness of the two methods of pollination, so far as seed production is concerned.

From the above table it appears that far more pods and even more pods containing seeds were produced on the hand-pollinated series, but that a greater number of seeds per pod

TABLE IX.

Plant No.	Method of pollination.	Weight of stems, grams	No. of stems	No. of pods	No. of pods producing seeds	Per cent. of pods producing seeds	No. of seeds produced	Average number of seeds per pod	No. of seeds per 10 grams wt. of plant
29	Insects	49.87	11	255	30	11.76	61	2.03	12.2
	Hand	35.63	9	272	12	4.41	14	1.17	3.9
38	Insects	103.88	12	327	91	27.82	164	1.80	15.7
	Hand	114.00	18	279	164	58.77	236	1.44	20.7
97	Insects	28.50	8	239	65	27.19	67	1.03	23.5
	Hand	37.00	20	608	103	16.94	128	1.24	34.6
98	Insects	85.50	11	449	228	50.78	451	1.96	52.7
	Hand	64.13	8	779	57	7.30	70	1.22	10.9
109	Insects	14.00	6	198	67	33.83	96	1.43	68.5
	Hand	14.00	8	311	180	57.87	239	1.32	170.7
Summary	Insects	281.75	48	1,468	481	32.76	839	1.74	29.7
	Hand	264.76	63	2,249	516	22.49	687	1.33	25.9

PLATE XIV.—Showing method of grasping the flowers in rapid hand pollination.

were produced in the insect-pollinated series, and, as a matter of fact, more seeds per plant on this account. Taking the green weight of the plant again as a basis of comparison, the insect-pollinated series gave 29.7 seeds per each 10 grams of plant weight, while the hand-pollinated section produced slightly less—25.9 seeds per 10 grams of weight of the plant. This difference in averages is, however, slight, and becomes of even less moment in view of the still greater individual differences in the plants and the small number of plants included in the experiment. It is really surprising, as a matter of fact, that hand-pollination should approximate so closely to the results following the visits of insects. The essential point, however, lies not in the actual number of seeds produced as the result of hand pollination, *but in the fact that the seeds are of* *pure lineage and known origin,* including no unknown parental characters, as in the case of the seeds from insect-pollinated plants. In the breeding of alfalfa it is indispensable to work solely with close-pollinated plants, as in no other way can the desired characters in the selected mother plants be preserved intact in the offspring.

In the process of hand-pollination of alfalfa flowers, the plants selected must first be enclosed within screen-wire cages of convenient size. We employ cages of two sizes, measuring 27½" x 27½" x 24" and 51½" x 51½" x 36", respectively, using the latter often to enclose as many as four plants at once. The earth must be carefully heaped about the base on the outside, to prevent insects from crawling under, and every time the cage is replaced after lifting, care must be taken to see that no stray branch of a plant is pinioned down outside the cage. Two methods of pollination may now be followed. In case it is desired to keep a record of the number of flowers pollinated, the method illustrated in plates XI and XII is employed. Some part of a blunt metal instrument, such as the point of a dull knife or narrow spatula, or even a wooden toothpick, is pressed downward upon the keel. This releases the trigger mechanism, and the column of stamens and pistil flies up, striking the stigma against the face of the standard. The pollen, being somewhat sticky, adheres in masses to the instrument, which, being used on subsequently pollinated flowers of the same plant, renders the deposition of pollen upon the stigmas increasingly probable. In hand pollination, therefore, there is a considerable degree of cross-pollination likely among the different flowers on the same plant, which, however, is of no especial importance, since the characters which a plant has are usually rather evenly distributed over the different parts of the plant body. After finishing the pollination of the flowers on any one plant the instrument used must of course be carefully cleansed before proceeding to the next. After a little practice one person can by this method pollinate about 10,000 flowers a day. The plants should be gone over every other day, and all the flowers pollinated as they successively come into flower. There is no difficulty in distinguishing from the others the flowers that have been pollinated, since the column of the stamens and pistil always remains pressed against the standard after having been once released; and, furthermore, the floral organs wither soon after fertilization, while the unpollinated flowers will continue in fresh condition for several days longer. If, however, they are not then pollinated the flowers become flaccid, and the spring mechanism ceases to operate. Where, however, it is desired to pollinate many flowers rapidly, without reference to the exact number concerned, another and more rapid method, not hitherto published or described, may be followed. A flower cluster, when in full bloom, is taken between the thumb and the first and second fingers, and subjected to a light pressure, while at the same time it is slightly rolled. By this operation the keels of all of the flowers which are in the proper stage will be made to burst, setting free the stamens and pistils and covering the stigmas with pollen. One soon learns by practice the manner of exerting the desired pressure, and how much to roll the cluster in order to obtain the desired result without injuring the cluster by bruising it. While no exact counts have been made, comparing the two methods as to results, the writers are satisfied that this method is quite as effective as the other, while being from fifteen to twenty times as fast. Plate XIV shows the position of the hands when pollinating by this rapid method. Of course it is needless to say that the fingers should be carefully freed from pollen in passing from one plant to another. By the method last described, one person should be able to pollinate at least 200 or 300 plants a day.

Our conclusions are, that *Medicago sativa* is a species having a wide range of variation, amounting, perhaps, to numerous elementary species; that plants of alfalfa in ordinary fields show this exceedingly great variation in type, whether due to the existence of many elementary species, to numerous hybrids, or to an extensive fluctuating variability. We find that there is abundant opportunity for improvement by selection and close-pollination of superior races, and have selected and pollinated such a series, obtaining therefrom enough seed in the season of 1907 to start 1000 close-bred plants of about twelve distinctive types of alfalfa. That this method is superior to methods of vegetative propagation for the breeding of alfalfa there seems to be no doubt.