been closed and the shields varied to suit the occasion. Specially prepared and analysed J. & J. cotton was the absorbent used. The absorbent was cut to the circumference of the cup and was weighed before and after application and the difference and time noted.

DESCRIPTION OF DEVICES:

Fig. I—Cross-section: A is the cup, B the absorbent and C the flange which fits to the surface.

Fig. II—Cross-section: A is the cup, B the absorbent, C the flange, D the neck of the cup and E a tube leading from the neck which can be secured to the same either by threads or other suitable means or can be made continuous with the cup. The purpose of the tube E is to produce pressure or vacuum in the chamber by its being connected to a pump or other device for that purpose.

Fig. III—Side view: A is the chamber, C the flange, D the neck of the cup, E the tube leading off from this neck and F are apertures in the side of the cup. In this cup is contained the absorbent. E is connected with a suction or pressure device. This method produces a circulation of air in the cup because of the apertures F. The cup may be constructed with an additional part so that the apertures can be closed and in that way its purpose will be identical with that of Figure II.

Fig. IV—Cross-section: A is the cup, B the absorbent, C the flange, E is a tube connected to a channelway G running around the cup and opening into the cup by means of slits. The tube E can be connected with a pressure or a vacuum device so as to produce negative or positive pressure in the cup or there may be holes in the top of the cup so that a circulation of air is produced. The top may be so constructed that holes in it can be opened or closed.

Fig. V—Side view: This is a side view of Fig. IV in which A is the cup, C the flange, G the channelway around the cup and F openings from it into the cup.

Fig. VI—Cross-section: A is the cup, B the absorbent, H an electrode imbedded in the absorbent and I the wires leading from the electrodes. The principle of this device is to measure the conductivity through the absorbent as the perspiration is being absorbed.

Fig. VII—Cross-section: A is the cup, B the absorbent, C the flange and J is a thermometer. The principle of the thermometer is to measure the temperature change in the absorbent. In this case the cup can be made of non-heat conducting material as desired.

The measurement of the amount of sweat in a given length of time and from a given area under similar and varying conditions is the aim of the above devices and procedures. The additional factor that the devices described are simple, easily applicable over a given area from which sweat is desired, and the estimations are not complicated should add to their practicability and assure their early usage. Certainly this procedure is less complex than many which are daily routine in the well equipped hospitals of to-day. In a later paper the author will discuss variation in the secretion of sweat under diverse conditions.

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DOUBLE PLATE METHOD USED FOR CULTURING TILLETIA LEVIS

A NEW procedure has been followed whereby pure cultures of *Tilletia levis* may more easily be made. The bottom of a petri dish is covered with a two per cent. potato dextrose agar and allowed to cool until all the moisture is gone from the top of the plate. Then the top of the dish is poured with a three per cent. non-nutrient agar somewhat cool. The agar is poured in the center of the lid until an area about one and one half inches in diameter is covered.

Streaks of sterilized smut spores are made across the non-nutrient agar with a loop needle. These are then incubated for ten days at 12° to 14° C.

As is well known, the sporidia of *Tilletia levis* are ejected from the sterigmata and in the double plate they fall to the nutrient agar below where they can be picked off singly or grown into multisporidial cultures free of contamination.

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SPECIAL ARTICLES

INBREEDING IN ALFALFA ESTABLISHES A HIGH DEGREE OF HOMOZYGOSITY[1]

A PREVIOUS report[2] of alfalfa-seed investigations at the Utah Station indicated that about 2 to 2.5 times as many pods were set when artificial tripping was practiced. A considerable number of pods developed from flowers which showed no evidence of having been tripped.

An inbreeding experiment of considerable proportions, the data from which are just now available[3] make clear that alfalfa in one of the famous seed-growing areas in the Uintah Basin, Utah, is much less heterozygous than has been thought. As conducted, the experiment did not establish what proportion of the seed pods developed under conditions of natural self-fertilization. Although this would be difficult to

[1] Contribution from Department of Agronomy, Utah Agricultural Experiment Station. Publication authorized by director, June 15, 1931.

[2] Carlson, J. W., "Artificial tripping of flowers in alfalfa in relation to seed production," *Jour. Amer. Soc. Agron.*, 22: 780–786 (1930).

[3] Carlson, J. W., and Stewart, G., "Alfalfa-seed production," Utah Agr. Exp. Sta. *Bul.* 226 (1931).

do on purely positive evidence, a good beginning has been made. After generations of inbreeding, however, a study of eight different plant characters in each of 154 inbred strains, fully 50 per cent. of progenies were practically homozygous for one or more of the characters. Such a degree of homozygosity makes evident a considerable amount of self-fertilization in the generations immediately preceding the self-fertilization.

The inbreeding was brought about by putting paper bags over flowering branches of the alfalfa plant before the blossoms had opened. Branches were selected on which the tips of the blooms were well out of the bud; any flowers that were mature enough to have begun to open were clipped off, thereby enclosing in the bag only those flowers which had had no opportunity for cross-pollination. Parent plants of the following varieties were grown in rows three feet apart and with plants 27 to 40 inches apart in the row: Utah Common, Dakota Common, Grimm, Grimm Saskatchewan 666, Hardigan, and Ontario Variegated. These varieties represent a wide range in size, coarseness, leafiness, and erectness.

Seed production under bags, as a rule, is relatively poor, but in seasons moderately favorable for seed-setting, some seeds are obtained from most plants on which five to ten bags have been placed. In 1927, when the bagging was done, a few plants failed to produce any seeds under bags even when seeding well in the open air. Of approximately 180 plants so bagged, seeds were obtained from 154, of which 88 produced 50 to 100 seeds under bags. In 1928, the selfed seed from each parent plant was seeded in a single row of 50 plants when there were enough seeds. When the number of seeds obtained was fewer than 50 all of them were sown, one in a place. There were a few rows with only one to five or six plants, though in most cases 10 to 40 plants were obtained.

When these first-generation plants from self-fertilized seeds bloomed for the first time, some of them were bagged in order to produce second-generation selfed seed, from which progeny rows were seeded in 1929.

In 1929 it was observed that of the progeny grown from selfed seed, plants in some rows were variable, fully as much so as were the commercial varieties, whereas the progeny in other rows seemed to be much more uniform in erectness, in height, in diameter of stem, in size and shape of leaves and in color of blossoms and foliage. Measurements were taken of 40 plants in each of the parent rows and of all the plants in the progeny rows. Five rows of the Utah Common variety were measured—two near the breeding plats and one each from three different parts of the field but some rods away. Data were taken from each plant as to height, width, angle of erectness, diameter of a main stem three or four inches above the ground, leaflet length, leaflet width, color of the blossoms and color of the foliage. The colors were read from a standard color chart and assigned numerical values with the help of an artist. The variability of each row was computed as the standard deviation and as the coefficient of variability.

The variability of the varieties is reported in Table 1 with the variability expressed in percentage of the mean values of each character studied. Though the mean values differed widely, only the variability is reported in order to show uniformity or lack of it.

TABLE 1

COEFFICIENTS OF VARIABILITY OF PLANT, STEM, LEAF AND BLOOM CHARACTERS OF ALFALFA VARIETIES WITH 40 PLANTS IN EACH ROW MEASURED

	Percentage						
	Plant height	Plant width	Angle of erectness	Diameter of stem	Length of stem	Width of leaflet	Color of bloom Standard deviation
Utah Common	19.7	18.5	22.4	11.2	12.2	18.0	2.93
"	22.2	18.8	18.0	18.4	15.1	25.7	3.03
"	19.9	26.0	21.7	17.1	13.4	26.8	3.35
"	16.6	24.4	12.0	15.3	17.6	23.7	3.59
"	21.8	26.1	17.0	18.0	13.2	21.8	2.65
Grimm	22.9	26.9	15.0	18.8	17.2	23.6	10.80
Grimm Sask. No. 666	26.9	31.4	25.3	19.2	17.6	24.8	6.66
Hardigan	22.0	24.2	14.2	19.3	14.9	25.7	5.87
Dak. Common	21.9	25.8	7.6	16.2	12.1	21.4	2.37
Ont. Variegated	25.0	25.3	20.0	20.8	17.8	21.3	6.66

The result of one generation of inbreeding is summarized in Table 2 where the variability is reported for progenies. Comparing plant height and width in the two tables, it is clear that some of the progenies are similar in variability to the parent varieties while others are distinctly more variable and others distinctly less. This is true of each character studied, even for blossom color. There is every evidence of segregation of distinct strains of alfalfa at the end of the first inbred generation. This indication is so definite that high hopes are held for producing improved sorts after several generations of inbreeding. The second inbred generation shows rather marked tendencies to the further purification of some strains, but since these are not yet available they can be only noted here.

TABLE 2

VARIABILITY OF PROGENY ROWS OF ALFALFA, EACH GROWN FROM SELF-FERTILIZED SEED OF A SINGLE PLANT. THREE PLANTS EACH OF THE HIGHEST, OF INTERMEDIATE, AND OF THE LOWEST VARIABILITY OF EACH CHARACTER

Variability group	Plant height	Plant width	Angle of erectness	Diameter of stem	Length of leaflet	Width of leaflet	Color of bloom
Highest	38.2	45.6	50.7	25.7	27.5	23.7	12.19
"	36.7	43.8	46.2	22.0	26.8	29.6	10.64
"	30.8	39.4	44.6	21.1	18.9	28.8	10.15
Intermediate	20.8	22.9	21.1	14.2	12.6	16.7	4.35
"	19.9	20.8	23.1	13.8	11.9	16.7	3.83
"	18.4	21.2	19.5	13.3	11.2	15.5	3.33
Lowest	9.3	11.0	8.6	8.5	7.1	11.2	1.22
"	9.6	11.2	7.8	6.1	6.6	10.3	1.10
"	7.9	7.5	7.0	4.5	4.8	9.0	0.0

Inbreeding which tends to purify the various subvarieties, or strains which make up a variety, allows for the appearance of any abnormalities which in open pollination (such as occurs in the field) are obscured by being in a crossed condition with normal types. In the first inbred generation, three rather distinct sorts of abnormal plants have appeared.

Each of two progenies, both from the same parent strain, showed several plants on which the leaflets had tiny brown markings not characteristic of any recognized disease when examined by a plant pathologist. The leaves, partly folded and partly crinkled, suggested that small sections of the leaf structure had been lost or had died after development had started. Sufficient study has not yet been made to enable more to be said at the present time.

In three other progenies there occurred some distinctly shorter plants extraordinarily leafy but which would produce only about half as much hay as the parent variety. One strain of nearly normal size showed in a large degree this same condition of extreme leafiness.

In a single progeny almost half the plants had the blossom replaced by a freak vegetative development that resembles somewhat the appearance of the flower buds on a plant of seed onion. This condition occurs naturally in the field at rare intervals. Here a progeny was found in the first inbred generation in which the character was thousands of times more frequent than is found in the commercial varieties or strains.

Some strains are becoming pure for erectness, while others are pure for a nearly flat position; others are showing a strong tendency to coarseness and others to a suggestion of "vininess" in the upper branches. This last character suggests a faint resemblance to twining branches such as occur on vetches and some beans.

There is also considerable evidence that seeding ability in certain strains is governed in part by the genetic constitution of the plants.

While an immense amount of careful scientific work would be required to develop and maintain economic strains, the promise is such as to warrant its being given a thorough trial.

GEORGE STEWART[4]

NON-ACID-FAST TUBERCLE BACILLI

NON-ACID-FAST organisms long have been known to exist as a phase in the life cycle of the acid-fast group. Until recently no pure cultures of the non-acid-fast varieties have been obtained. It has been thought that the occasional non-acid-fast rod and the forms described as Much's granules[1] were involution forms which had lost their acid-fast property. From Kahn's recent work,[2] it would appear that these granules were the precursors of numerous young organisms. Recently Dreyer and Vollum[3] reported the cultivation of a non-acid-fast strain of human tubercle bacilli which showed optimum growth at the bottom of veal peptone bouillon medium. These organisms were fully virulent.

In this communication we describe a non-acid-fast, almost a virulent type of organism which has much in common with the acid-fast strain from which it was isolated.

A two-months-old culture of H-37 strain of human tubercle bacilli developed a group of yellow-orange, chromogenic colonies in the midst of other normal-appearing colonies. These chromogenic colonies appeared to have the same morphological characteristics as the known acid-fast ones surrounding them. They were made up of numerous non-acid-fast rods and granules and were Gram-positive. There were also present a few acid-fast rods and granules, and some of the non-acid-fast rods contained from one to three acid-fast granules.

Three subcultures were made at different times from the original colonies. The cultures grew rap-

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[1] H. Much, *Beitr. z. Klin. d. Tuberc.*, 8, 85, 1907.

[2] M. Kahn, *Amer. Rev. Tuberc.*, 20, 150, 1929.

[3] G. Dreyer and R. L. Vollum, *Lancet*, 220, 1015, 1931.