

and Illinois A with an inbred line of Luces Favorite. Individual tetraploid plants induced in this three-way hybrid by the heat treatment technique of Randolph² were mass pollinated during two subsequent generations and from this tetraploid strain samples of grain from 10 ears selected at random were taken for analysis. Diploid sister plants of the original tetraploids were intercrossed in a similar manner for two generations to provide a comparable diploid strain for comparison with the tetraploid.

For the fractionation and determination of the carotinoids the procedure of Kuhn and Brockmann³ was adopted with certain modifications. The pigments were extracted directly from the corn meal with anhydrous methyl alcohol, saponified and fractionated

with petroleum ether. For determining concentration we used a photoelectric colorimeter equipped with Corning glass filters 428 and 585 and calibrated against standard solutions of crystalline beta carotin.

We attribute the observed differences between diploid and tetraploid yellow corn to quantitative rather than qualitative gene differences, since the comparison was made between strains having a common origin and an essentially identical genetic constitution. There is a possibility that tetraploid corn will be of practical importance due to its increased vitamin A activity.

L. F. RANDOLPH
DAVID B. HAND

CORNELL UNIVERSITY

SCIENTIFIC APPARATUS AND LABORATORY METHODS

SIMPLIFIED SCHAEFFER SPORE STAIN

BEGINNING students of bacteriology frequently find it difficult to follow the original Schaeffer¹ technique, which calls for heating a flooded slide over an open flame, without breaking the slide. Consequently, the following technique was first worked out for beginning students in bacteriology at the Agricultural and Mechanical College of Texas, and since then, over a period of time and with a variety of cultures, it has given better results than the technique originally described. In addition, the method of drying and the time of staining have been modified.

A simple, inexpensive steam bath, perhaps a tin can or beaker of proper diameter or a metal tray about three inches deep and two inches wide, on an asbestos centered wire gauze, is used to heat the slides.

(1) Smears, prepared from spore suspensions, are dried for staining by laying the slide on the table top near the base of the burner used for heating the steam bath.

(2) Dried slides are placed across the steam bath until definite droplets of water collect on the bottom of the slide.

(3) The slides are then flooded with 5 per cent. aqueous malachite green and left on the steam bath for one minute.

(4) Stained slides are removed from the steam bath with the thumb and index finger of one hand and dropped into cool water. This is done by spanning the length of the slide. The overhanging ends of the slides are cool enough to do this without danger of burns.

(5) The slides are thoroughly rinsed and, while still

wet, are counterstained with 0.5 per cent. aqueous safranin for thirty seconds and again rinsed in cool water.

(6) Rinsed and dried slides are easily examined under the microscope. As in the original Schaeffer staining technique, the spores stain green and the vegetative cells stain red.

GERALD K. ASHBY

MEAD JOHNSON & Co.,
EVANSVILLE, IND.

A METHOD FOR FIXING AND STAINING EARTHWORMS

NOTHING furnishes more satisfactory material for classroom work than sections of earthworms properly fixed and stained. In almost every course in general biology or zoology some time is devoted to the histological study of the common earthworm. It is unfortunate that the material used is often poorly fixed and the sections do not show up well. I have found the method given below simple, quick and one which gives excellent results.

Collect several earthworms and rinse off the dirt. Place these in a covered dish and sprinkle a small amount of well-sifted corn meal and powdered agar mixed in equal proportions on the bottom of the dish. Some finely chopped lettuce may also be added. Cover the worms with a moist paper and leave in a cool dark place. Transfer the worms to clean dishes and change the food each day for three days. By this time their alimentary tract should be free of all dirt and grit.

The specimens are fixed by cutting them into pieces about three fourths of an inch long and dropped directly into warm (about 50° C.) Allen's B-15. Fix for twelve hours and then rinse in water and run through 35, 50, 70, 80, 95 and two changes of 100 per cent. alcohol. Leave in each alcohol one hour. Now run

² L. F. Randolph, *Proc. Nat. Acad. Sci.*, 18: 222-229, 1932.

³ R. Kuhn and H. Brockmann, *Zeit. Physiol. Chemie.*, 206: 41, 1932.

¹ Alice B. Schaeffer and McDonald Fulton, *SCIENCE*, 77: 194, 1933.

through an alcohol-chloroform series as follows: One part chloroform to three parts of 100 per cent. alcohol, one hour; one part chloroform to one part alcohol, one hour; three parts chloroform to one part alcohol, one hour; pure chloroform, one hour. Saturate the chloroform with paraffin and leave twelve hours, add more paraffin and put in an oven at 58° C. for six hours. Pour off the liquid and add melted paraffin and leave in the oven for forty-eight hours, then embed and section at 12 microns.

Delafield's haematoxylin is a very satisfactory stain, since it shows up the tissues well and has the added advantage of being permanent. The sections should be stained from 10 to 30 minutes and then washed in water and destained in 50 per cent. acid alcohol (3 drops of HCl to 100 cc of alcohol) until the sections are a deep pink color, then wash in ammonia alcohol (5 drops of ammonia to 100 cc of 50 per cent. alcohol) until they are a light blue color. Run up and mount in dammar or balsam.

ELTON C. COCKE

UNIVERSITY OF VIRGINIA

GRASS VOLUME TABLES FOR DETERMINING RANGE UTILIZATION

FORESTERS have used tree volume tables for decades, but the authors know of no previous effort to develop volume tables for range grasses. There is an urgent need for a mechanical means of determining the degree of grazing of forage plants on national forests and other ranges in the West. The use of such tables offers a very promising aid in range research and in practical range inspection.

More than seven hundred samples of sixteen important range grasses have been collected, mainly from Montana national forests. The plants were cut and weighed to show the weight per inch of height, and tables have been prepared for each species. There appears to be very satisfactory correlation between weight and volume. A much greater concentration of weight exists in the lower portions than is generally known among range ecologists. In *Festuca idahoensis* approximately three fourths of the weight is in the lowest one fourth of height, and other species show similar distribution. The basic method was devised in 1927 by the senior author. Under his direction further development was made in 1934-35 by Chandler Jensen and Kenneth E. Chriswell, and the present form was completed in 1937-38 by Chandler Jensen and Adolph Hecht.

For determining utilization on grazed ranges a table was devised to convert inches of stubble remaining into percentage of height removed. The latter was then aligned with a percentage of volume utilization scale for the species concerned. Edward C. Crafts, of the

Southwestern Forest and Range Experiment Station at Tucson, Ariz., replaced the conversion table with a modified slide rule scale. In this region, we have now developed a partial three-cycle, semi-circular logarithmic scale which converts inches of stubble remaining directly into percentage volume utilization. A full account of this work will be published later.

TOM LOMMASSON
CHANDLER JENSEN

U. S. FOREST SERVICE,
MISSOULA, MONT.

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