– *A. casei* Gray (California)
– *A. pterocarpus* Watson (Nevada)
– *A. sclerocarpus* Gray (Washington)
– *A. tetrapterus* Gray (Nevada)
PREUSSII
+ *A. beathii* Porter (Arizona)
+ *A. pattersonii* Gray (Colorado)
+ *A. praelongus* Sheld. (New Mexico)
+ *A. preussii* Gray (Utah)
INFLATI
– *A. lentiginosus* var. *palans* Jones (Arizona)
SARCOCARPI
– *A. crassicarpus* Nutt. (Wyoming)
ULIGINOSI
– *A. canadensis* var. *carolinianus* (L.) Jones (Wyoming)

The group names in the list are those into which Jones[6] divided the genus on the basis of morphological characters. Using the criterion of physiological differentiation with reference to selenium, it is evident that the groups Galegiformes, Lonchocarpi and Podo-sclerocarpi need taxonomic revision, since each includes both indicator and non-indicator species.

The results of these germination tests are in agreement with those of field observations and growth experiments of longer duration.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

REARING GRASSHOPPERS UNDER LABORATORY CONDITIONS[1]

THE rearing of grasshoppers in the laboratory requires considerable care and attention.[2] The food must be grown and supplied daily to the insects, the cages must be cleaned at least once a week, dampness must be avoided, etc.

The present paper describes a simple method for rearing grasshoppers which is being used in the Division of Entomology and Economic Zoology of the University of Minnesota.

Two types of cages are used, a smaller, for the hatching of the eggs and as living quarters for the first instars, and a larger one for growth and reproduction.

Inside dimensions of the smaller cage are $6 \times 3\frac{1}{2} \times 3\frac{1}{2}$ inches. The sides and one of the ends are of wooden boards, the other end is left open for the attachment of a cheesecloth sleeve. The top and the bottom of the cage are made of screen wire cloth, 16 to an inch mesh. The bottom is elevated a quarter of an inch above the surface of the table.

The larger cage is of $12 \times 12 \times 12$ inches inside dimensions. The cage is made of wire cloth 12 to an inch mesh, nailed to the wooden framework. The bottom is elevated a half of an inch above the surface of the table. The lower third of one side is left open for a cheesecloth sleeve as in the smaller cage.

Food consists of a dry mixture of dried brewers' yeast, 1 part; skim milk powder, 2 parts; and dried alfalfa meal, 2 parts by weight. Water is given in shell vials plugged with cotton and laid on the bottom of the cage. Food can be supplied to the newly emerged insects in "Coca Cola" or similar caps from which cork has been removed. It is advisable to put 2 to 3 receptacles with food in the cage as well as 2 or 3 vials of water in order to avoid overcrowding and consequent undernourishment of some insects. One ounce ointment boxes are satisfactory for food containers in the bigger cage.

Grasshoppers are allowed to emerge from the eggs in the small cage. Water and food should already be present before hatching starts. Constant light is provided by bending an ordinary table lamp over the cage—about 3 to 4 inches from the top screen. The insects find the food and water without difficulty. It is important, however, to have the insects reared from eggs in the cage and not to introduce them from the outside after they will already have started feeding on their natural food. After all the insects enter their second instar, they may be transferred to a larger cage, the dimensions of which depend on the number of grasshoppers maintained for use in the laboratory.

The insects do not require any special attention, provided they always have food and water available. Feces which accumulate under the screen bottom may be removed from time to time.

In our experiment, hatching of overwintering eggs of *Melanoplus differentialis* started on May 27 and next molt occurred 5 days later. June 7, when all the grasshoppers had molted, they were transferred to the larger cage. There was no mortality. The time of appearance of the nymphs of the third instar was not noted, but June 9 the nymphs of the fourth instar began to appear, and succeeding molts occurred on June 13 and 20. The first adults appeared on June 24, the total developmental period being 28 days after hatching, during which 6 molts occurred. July 11, the last nymph molted, 31 days after hatching started. The insects were mostly segregated in a circle around the light where the temperature was about 34° C.

[1] Paper No. 1938, Scientific Journal Series. Minnesota Agricultural Experiment Station, St. Paul, Minn.

[2] E. E. Carothers, "Culture Methods for Grasshoppers. Culture Methods for Invertebrate Animals," pp. 287–291. Comstock Publishing Company, Ithaca, N. Y. 1938.

[6] M. E. Jones, "Revision of North American Species of *Astragalus*." 1923.

First death of adults occurred on July 12, 45 days after hatching, and 43 days later half of the adults were dead. The last adult, a male, died on October 26 at the approximate age of 152 days. Until July 31 the insects were kept in a basement laboratory having only artificial light available day and night. On that day the cage was taken to the greenhouse, where it remained till the end of the experiment. The insects behaved normally. They mated and the females oviposited in sod which had been placed on the floor of the cage.

The advantages of this method are obvious. The experimenter needs only to fill the dishes with food when necessary, provide water and occasionally remove the paper with feces from under the cages.

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VITAMIN SYNTHESIS BY A YEAST CONVERTED FROM A HETEROTROPHIC TO AN AUTOTROPHIC HABIT[1]

Saccharomyces cerevisiae is generally accepted to be heterotrophic with regard to a number of vitamins. However, the writers have succeeded in inducing ten strains of this yeast to grow without an exogenous supply of thiamin, pyridoxin, inositol and pantothenic acid; in addition, a rich growth without even biotin has been induced in the case of at least one strain. Thus has evolved a yeast that will readily grow in a synthetic medium containing no vitamins. This building-up process of autotrophic habit was accomplished by means of prolonged incubation, by the use of a large quantity of inoculum during the initial stages and by successive transfers to solutions from which one of the essential vitamins was omitted. Ordinarily from four to seven passages sufficed to induce the yeast to grow as well in the absence of a given vitamin as it did in its presence. Then a second vitamin was omitted from the medium, and the process was repeated until a complete, or nearly complete, autotrophic habit was established.

Yeast is a good source of vitamins, but since in its turn it is dependent upon an exogenous supply of growth factors, the question arises whether or not a conversion from heterotrophic to autotrophic habit might not affect vitamin synthesis and storage and thus leave the cell devoid of vitamins. In order to answer this, the writers grew their completely autotrophic strain of yeast in a synthetic medium prepared from vitamin-free chemicals. The cultures were incubated at 25° C for four days; the ensuing crop of cells was then harvested, washed, dried and tested for the various vitamins. This was done by weighing 0.2 gram of the cells for each 100 ml of the nutrient solution, boiling for 5 minutes to extract the soluble parts, filtering, sterilizing and inoculating with the test organisms.

The following organisms were used to detect the different vitamins: *Pythium ascophallon* for thiamin; *Ceratostomella ulmi* for pyridoxin; *Lactobacillus casei* for riboflavin; *Brucella suis* for nicotinamide; *Clostridium acetobutylicum* for para-aminobenzoic acid and strains of *Saccharomyces cerecisiae* for inositol, pantothenic acid and biotin.

Two lots of nutrient solution were prepared. The first lot, containing no vitamins, was divided into two portions; the first portion was used as the control, while the other contained, in addition, the water-soluble parts of the yeast. The second lot of the nutrient medium was divided into eight portions; each portion received all the vitamins mentioned in this paper except one. For instance, the first received all the vitamins except thiamin; the second received all except pyridoxin, and so on down the list. Then each one of the eight portions was divided into halves; the first was used as the control, while the water-soluble parts of the yeast were added to the second half to furnish the missing vitamin.

The bacterial cultures were incubated at 30° C, the yeasts and fungi at 25° C. Very slight or no growth was observed in all the controls, while a rich growth was made in all solutions containing the substances extracted from the yeast cells. This indicates that our strain of autotrophic yeast is capable of synthesizing in appreciable quantities all the vitamins mentioned in this paper.

In the building-up process of vitamin synthesis, the writers have developed many strains of yeasts from a single one. Some of these synthesize and store considerable quantities of vitamins; others produce smaller amounts, or almost none. This work will appear later.

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BOOKS RECEIVED

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