

resulted partly from inanition. All the chicks in both high and low pantothenic acid-supplemented groups were forced-fed the same amounts of food each day. This was unsuccessful as growth ceased entirely in the chicks on the low pantothenic acid level, and nearly all of them died after two weeks.

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IDENTIFICATION OF SELENIUM INDICATOR SPECIES OF *ASTRAGALUS* BY GERMINATION TESTS

INDICATOR plants belonging to the genus *Astragalus* are of great aid in locating seleniferous areas that may be capable of producing toxic forage and grain.¹ From chemical analyses of plants collected in the field, 28 species of *Astragalus* are known to be selenium accumulators and indicators, whereas 54 other species of *Astragalus* absorb only small amounts of selenium and are not limited to seleniferous soils.² It is important that many of the remaining 218 species in North America and 1,200 species in the rest of the world be examined for their ability to absorb selenium.

The present paper describes a simple test with germinating seedlings that enables one to determine whether or not a given species of *Astragalus* is capable of accumulating selenium. The test is based on an earlier observation that a selenium indicator species tolerates and is even stimulated by a high concentration of sodium selenite, whereas a non-indicator species is severely poisoned by a low concentration of this salt.³ No other case has been reported in which closely related species differ so markedly in their response to a mineral poison.

It has been found that the addition of 20 parts per million of selenium (as sodium selenite) to the culture solution has no observable effect upon the early seedling growth of an indicator species of *Astragalus*, whereas it completely inhibits root development of a non-indicator species.

The culture technique is an adaptation of a method that has been used for many years.⁴ Seeds of most of the species included in these tests were collected during the summer of 1941. To soften their coats, the seeds were treated for from 30 to 60 minutes with concentrated sulphuric acid. They were then soaked

in tap water for three hours and subsequently allowed to germinate on wet filter paper in a glass moist chamber. After about 24 hours, when the seedlings were approximately 10 mm in length, they were transferred to culture vessels. A piece of paraffined bobbinet (1/16-inch mesh) was stretched over the top of a 300-cc beaker and fastened by a ligature of paraffined linen thread. This beaker was placed in a 600-cc beaker, and both the inner beaker and the space around it were filled to the level of the netting with culture solution. Twenty-five seedlings were placed upon the netting, with their roots dipping into the solution. Two cultures of each species of *Astragalus* were prepared, one supplied with the usual mineral salts⁵ and the other receiving in addition 20 parts per million of selenium (as sodium selenite). The cultures were kept in darkness at 28°–29° C., and a moist chamber was provided for each during the first day by covering the larger beaker with an inverted watch glass. At the end of three days, the seedlings were put in chrom-acetic fixing solution, and the lengths of roots and hypocotyls were later recorded. I am indebted to Dr. Sydney S. Greenfield for assistance in making these tests.

The results are summarized in the following tabulation, in which a *plus* sign signifies a selenium indicator species and a *minus* sign denotes a non-indicator species. The roots of the indicator species made the same amount of growth (25–30 mm) in the absence of selenium and in the presence of 20 parts per million. Root growth of the non-indicator species was completely inhibited in the solution containing 20 parts per million of selenium but amounted to 25–30 mm in the selenium-free solution. The hypocotyls of the non-indicator species in the selenium solution grew only about 5 mm, while those of all the other cultures grew 20–30 mm.

BISULCATI

- + *Astragalus bisulcatus* (Hook.) Gray (Wyoming)
- + *A. haydenianus* Gray (Colorado)
- + *A. oocalycis* Jones (Colorado)

GALEGIFORMES

- + *A. racemosus* Pursh (South Dakota)
- *A. drummondii* Hooker (Wyoming)

LONCHOCARPI

- + *A. osterhoutii* Jones (Colorado)
- *A. lonchocarpus* Torr. (New Mexico)

OCREATI

- + *A. confertiflorus* Gray (Utah)
- + *A. flavus* Nutt. (Wyoming)

PODO-SCLEROCARPI

- + *A. grayi* Parry (Wyoming)
- + *A. pectinatus* Dougl. (Wyoming)
- + *A. rafaelsensis* Jones (Utah)
- + *A. toanus* Jones (Nevada)
- *A. canonis* Jones (California)

⁵ See footnote 3.

¹ For a general review of the selenium problem, see: S. F. Trelease, *The Scientific Monthly*, 54: 12, January, 1942.

² O. A. Beath, C. S. Gilbert and H. F. Eppson, *Am. Jour. Bot.*, 28: 887, 1941.

³ S. F. Trelease and H. M. Trelease, *Am. Jour. Bot.*, 26: 530, 1939.

⁴ S. F. Trelease and H. M. Trelease, *Bot. Gaz.*, 80: 74, 1925.

- *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. crassicaipus* Nutt. (Wyoming)
- ULIGINOSI
- *A. canadensis* var. *carolinianus* (L.) Jones (Wyoming)

The group names in the list are those into which Jones⁶ 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 CONDITIONS¹

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.

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

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.