agent has been transferred from mouse to culture, back through mice and to culture again. The same procedure was carried out with a culture in the tenth generation.

Furthermore, fluid from the culture of the seventh generation was inoculated intranasally into a susceptible ferret. The ferret developed marked fever and nasal symptoms, and mild pulmonary involvement was observed at autopsy. The disease picture produced was that of experimental influenza.

That the infectious agent in the cultures is the influenza virus is shown by the fact that the capacity of the culture fluid to produce lung lesions in mice is completely inhibited by the serum of ferrets that have recovered from infection with the P. R. 8, the Philadelphia or the Alaska[5] strains of ferret passage influenza virus which had never been passed through mice, while normal ferret serum does not exert this effect. Tests of this nature were performed with the fifth, eighth and fifteenth transfer cultures. Control cultures of the uninoculated medium have been entirely innocuous for mice.

The virus has been carried through 20 serial transfers in an artificial medium. Furthermore, the infectious titer of the culture virus after these repeated subcultures, while varying somewhat, remains essentially the same as that of the mouse passage virus from which the culture virus was derived. These facts indicate clearly that the virus has multiplied *in vitro*. The question of mere survival or of the carrying over of surviving virus in serial transfers appears to be excluded by the dilution factor.

Dochez, Mills and Kneeland[6,7] have reported observations concerning the cultivation *in vitro* of a filterable agent obtained from the throat washings of patients suffering from an upper respiratory disease resembling influenza. The inoculation of their culture into the nasal passages of human volunteers provoked upper respiratory disorders. The presence of the filterable agent in cultures was demonstrated only by the inoculation of human volunteers.

In the present experiments the culture virus has been transmitted readily to mice and ferrets, and it has been found that its capacity to infect experimental animals is inhibited by specific anti-influenza immune serum. Nevertheless, certain alterations in the immunological characteristics of the virus have been noted after repeated cultivation *in vitro*. Whether these differences in the behavior of the mouse passage virus and of the culture virus are merely quantitative or represent a definite change in the biological character of the agent is at present the subject of further investigation.

THOMAS FRANCIS, JR.
THOMAS P. MAGILL

[5] T. Francis, Jr. Unpublished experiments.

[6] A. R. Dochez, K. C. Mills and Y. Kneeland, Jr., *Proc. Soc. Exp. Biol. and Med.*, 30: 1017, 1933.

[7] *Idem.: ibid.*, 32: 406, 1934.

RELATIVE EFFICIENCY OF ROOTS AND TOPS OF PLANTS IN PROTECTING THE SOIL FROM EROSION

PLANT cover, soil and topography are the major factors concerned in the problem of erosion. During the earlier studies, conducted largely by engineers, chief emphasis was placed upon topography. Following extensive work by pedologists, it is now generally conceded that type of soil is an equally important factor. Intensive studies on the effect of plant cover reveal the extremely important rôle played by plants in holding the soil against the forces of erosion. In this preliminary account of the relative effectiveness of roots as compared to tops in holding the soil in place, the results are based on extensive experiments with cultivated crops and native vegetation.

Samples of soil 1 by 0.5 meter in area were obtained from fields, pastures and prairies by placing a wooden frame tightly about the sample, cutting away the core of soil and attaching a tight bottom to the frame at 10 cm depth without disturbing either soil structure or plant cover. The samples, taken in pairs, were transported to a rack which sloped at an angle of 10°, and the relative time required to wash away the soil was determined with plant cover intact and the cover clipped to the soil surface and removed. A stream of 13 gallons of water per minute from an open hose was played uniformly over the sample at a height of 2.5 feet and under a total force of approximately one pound per square inch.

Results with winter wheat are shown in Table 1.

TABLE 1
TURKEY RED WINTER WHEAT (TRITICUM AESTIVUM L.) GROWN IN WABASH SILT LOAM SOIL

Date samples taken	Height wheat inches	Stage of development	Erosion time, min.		Dry wt. gm.	
			Without tops	With tops	Underground parts	Tops
April 12 ...	3	"Rosettes" spread 6.5 inches	7	11	22	23
April 29 ...	7	Erect, heavily tillered	11	19	38	88
May 17 ...	20	Early "boot" stage	13	40	70	247
June 5 ...	36	Flowering	17	59	75	417
June 19 ...	45	Early dough	16	113	74	520
July 2 ...	45	Nearly ripe	15	99	65	521

Table 1 shows that with the growth of the roots the erosion time was somewhat more than doubled, but with the corresponding development of tops it increased 9 to 10 times. Decrease in efficiency in the ripening stage was due to the shriveling and drying

of the leaves as a result of an epidemic of rust. The protection afforded by the monocotyledonous "grass type" of leaf was in every case found to be far more efficient than that of the dicotyledonous type. This is illustrated by the results with alfalfa.

Samples of common alfalfa (*Medicago sativa* L.) were taken from a 5-year-old stand on alluvial soil on April 20. The tops were 5 inches high and completely concealed the soil. The clipped sample was entirely eroded in 11.5 minutes; that with tops intact in 32 minutes. The soil of another set of samples on May 16, when the plants were 16 inches tall and better developed, was completely washed away in 14 and 28 minutes, respectively. These last samples were secured after heavy rains which delayed cutting and resulted in the loss of most of the leaves to a height of 18 inches. Hence, samples on June 5 were entirely eroded in 11.5 and 30.5 minutes, respectively. The second growth, when 28 inches tall, prolonged the time of erosion to 43 minutes; with tops removed to 21 minutes. Thus well established stands of alfalfa protect the soil from erosion to a much smaller degree than does a crop of maturing wheat. Young alfalfa is much less efficient.

Root systems of native grasses are far more efficient than are those of most crops. By May 15, 1934, Kentucky bluegrass (*Poa pratensis* L.) was killed in most pastures in eastern Nebraska by the great drought. Samples with tops removed were eroded in Carrington silt loam soil in the spring of 1934 in 2 hours with the open hose plus 2 hours with a stream of water $\frac{3}{8}$ inch in diameter delivering 3.5 gallons of water per minute with a force of 1.4 pounds on .11 square inch of surface. By the next spring similar samples were eroded in 80 minutes with the open hose, and in late July, when the rhizomes and most of the roots were decayed, in only one fourth this time.

A thick, fully grown stand of wheat grass (*Agropyron Smithii* Rydb.) held the soil for 46 minutes when the tops were removed. A period of 3 hours and 31 minutes (with open hose) was required for similar results when the tops were left intact. Big bluestem (*Andropogon furcatus* Muhl.) is even more efficient. The period for erosion with tops removed was, in addition to two hours with the open hose, 2 hours and 40 minutes. A period of 13 hours was required when the maturing tops were intact. The soil beneath a fully grown, thick stand of slough grass (*Spartina Michauxiana* Hitchc.) can scarcely be eroded, although the underground parts are only moderately efficient. Roots and rhizomes alone held the soil against erosion for about 2 hours (open hose). Application of the water stream under the greater pressure during a period of 13 hours failed to remove any perceptible amount of soil when the cover was intact.

A complete report on the efficiency of important field and garden crops, weeds and native grasses is in preparation.

J. E. WEAVER
JOSEPH KRAMER

UNIVERSITY OF NEBRASKA

SCIENTIFIC APPARATUS AND LABORATORY METHODS

RIO-HORTEGA'S DOUBLE SILVER IMPREGNATION TECHNIQUE ADAPTED TO THE STAINING OF TISSUE CULTURES[1, 2]

WE have had equivocal results in employing the various techniques described in the literature for silver staining of tissue cultures *in situ* in the plasma clot. As a result we strove to find a simple, dependable technique which could be carried out quickly. Such a one follows in detail:

(1) Fix for 24 hours in equal amounts of 10 per cent. neutral formalin and normal salt solution after removing all paraffin and vaseline from the cover slip.

(2) Wash in 30 cc of distilled water to which has been added 6 drops of ammonium hydroxide—5 minutes for thin clots on cover slips and up to 15 minutes for thicker clots in Carrel flasks. (Petri dishes used throughout. It is necessary that the water employed be doubly distilled.)

(3) Wash in distilled water.

(4) Place into the following mixture: 30 cc of 2 per cent. silver nitrate (reagent), 50 drops of 95 per cent. alcohol, 25 drops of pyridine, 5 drops of ammonium hydroxide. This is heated slowly up to 40° C. until the characteristic yellow color develops (about 12 minutes).

(5) Wash in distilled water.

(6) Place in the following mixture: 30 cc of silver carbonate, 25 drops of 95 per cent. alcohol, 15 drops of pyridine, and heat slowly up to 40° C. until the fragments take on a brown color (about 9 minutes). The silver carbonate is made by adding to 5 cc of a 10 per cent. silver nitrate (reagent) 20 cc of a 5 per cent. sodium carbonate. A white precipitate is formed which is dissolved by the addition of ammonium hydroxide drop by drop, being careful not to add an excess. It is then made up to 75 cc with distilled water.

(7) Wash in distilled water.

[1] From the Montreal Neurological Institute, McGill University.

[2] Read at the meeting of the International Association of Medical Museums, New York, April 17, 1935.