

mechanically from plant to plant have been made, using various methods of inoculation, both with untreated juice from infected plants and also after the addition of materials to the juice to alter the osmotic pressure, pH, and oxidation-reduction potential with the aim of stabilizing the virus. Juice has been rubbed onto the leaves and injected into the stems and crowns of plants by different techniques. Several species of susceptible plants have been used in these experiments, but an infection has never been obtained by any of these methods. The inoculation of plants with juices from infective insects also failed to produce infection, even though such juice was proved to have infective virus when tested by the successful technique described here. Leafhoppers fed on juices from diseased plants, from viruliferous insects, and on concentrates of these juices prepared by high speed centrifugation did not become infective. It is possible, of course, that more extensive trials with these procedures or variations of them might yield positive results.

Storey (5) was the first to transmit a plant virus from insect to insect by inoculating virus-free vector leafhoppers, *Cicadulina mbila* Naude, with juice from insects carrying maize-streak virus, *Fractilinea maidis* (Holmes) McKinney (3). Using fine glass capillaries, Black (1) transmitted the aster-yellows virus (*Chlorogenus callistephi* H. var. *vulgaris* H.) by injecting juice from viruliferous into nonviruliferous leafhoppers (*Macrostelus divinus* Uhler). In view of these earlier successes, it seemed logical to attempt transmission of the virus by injecting healthy vector leafhoppers with extracts from insects carrying wound-tumor virus.

Three hundred and fifty nonviruliferous leafhoppers (*Agallia constricta* Van Duzee) were caged on crimson clover plants (*Trifolium incarnatum* L.) showing pronounced symptoms of wound-tumor disease and were kept there for four weeks at about 25° C. Maramorosch had previously (4) obtained good transmission of the virus at 25° C with this vector, after a minimum incubation period in the insect of two weeks. The insects were then collected and ground at 0° C with an equal weight of 0.25 M NaCl. The suspension was centrifuged at 3500 rpm for 5 min, and the supernatant used without further dilution for the injection of 28 virus-free nymphs. The solution was drawn up into a fine glass capillary, and a small amount forced into the insect through a puncture made in the abdomen with a glass capillary. Twelve of the 16 insects which survived the injection proved to be infective when tested on crimson clover plants. Although no controls were included in this experiment, the results are considered significant because there were no accidental infections with this virus in the greenhouse during the course of this experiment or the two previous years.

A second experiment was carried out to confirm these results as well as to obtain a first approximation of the dilution end point. An extract of viruliferous insects was prepared as in the first experiment, and 0, 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴ dilutions were made with 0.25 M NaCl. Groups of 20-30 insects were inoculated with each dilution, and an equal number of uninoculated insects were

kept as controls. All insects were tested individually on two sets of crimson clover plants over a period of two months, and the plants were then observed in the greenhouse for an additional period of two months. None of the control insects became infective, but some insects inoculated with each dilution of viruliferous insect extract up to and including 10⁻³ did. There was a long incubation period before inoculated insects were able to infect healthy plants with wound-tumor virus.

These experiments show that wound-tumor virus can be detected in extracts from insects, and the experiments also make possible the determination of some of the physical and chemical properties of the virus. The fact that three leafhopper-transmitted plant viruses have proved transmissible by this method of insect inoculation suggests that the method may succeed in certain instances where other techniques have failed.

References

1. BLACK, L. M. *Phytopathology*, 1941, **31**, 120.
2. ———. *Amer. J. Bot.*, 1945, **32**, 408.
3. HOLMES, F. O. The filterable viruses. Suppl. 2. *Bergey's Manual of determinative bacteriology* (6th Ed.). 1948. Baltimore: Williams and Wilkins. Pp. 1127-1286.
4. MARAMOROSCH, K. *Phytopathology*, 1949, **39**, 14.
5. STOREY, H. H. *Proc. roy. Soc. Lond.*, Ser. B., 1933, **113**, 463.

The Chromatographic Estimation of Lysine and Some Applications of the Method¹

Anthony A. Albanese² and Marilyn Lein

Department of Pediatrics,
New York University College of Medicine

In exploring the various published chromatographic techniques, it occurred to us that paper chromatography of the copper salts of amino acids might provide a simple means of estimating some of the amino acids. The salts were prepared as described in our modification of the Pope and Stevens procedure for determination of the amino acid N (1) and 0.02-cc aliquots of the resulting solution partitioned on paper strips in an aqueous phenol atmosphere for 6 hr by the capillary ascent principle described by Williams and Kirby (2). After drying in air the strips were developed by painting with a freshly prepared 10% solution of aqueous iron ferrocyanide. The characteristic pink color of copper ferrocyanide, which appears distinctly on drying, indicates the position and relative amounts of various amino acids on the strip. Tests with 60 γ of purified specimens showed glutamic and aspartic acid to have overlapping R_f values in the area 0.08-0.10. Lysine occurred in the region 0.42-0.50. Arginine and histidine overlapped in the 0.55-0.62 R_f zone. The other amino acids, glycine, serine, threonine, valine, leucine, isoleucine, tyrosine, proline, hydroxypro-

¹ Supported in part by funds from the Office of Naval Research.

² Present address; Nutritional Research Laboratory, St. Luke's Hospital, New York.

line were to be found in the zone 0.7–1.0. Tryptophane, methionine, cystine, and phenylalanine could not be identified by this technique, since they are oxidized during the partitioning process.

For our purposes the lysine band, because of its relative consistency, proved most useful. The band spread of the copper lysine salt is roughly proportional to the concentration in the range 30–60 γ of lysine. With protein hydrolyzates containing initially 0.5 mg N/cc it was found that zein gave negative tests; wheat gluten gave faintly positive tests; and casein, gelatin, lactalbumin, human hemoglobin, and fibrin gave bands which approximated roughly the lysine content of the preparations.

Fivefold concentrates of urines of adult or infants on normal diets showed the lysine concentration to be greater for the infant than for the adult. When a wheat gluten diet supplemented to contain 4% L-lysine was fed to infants, the lysine output was similar to that of an evaporated milk diet. When the infants were fed wheat gluten diet without the lysine supplement, the urine lysine level fell below the sensitivity of the test. Supplementation of this diet with 6% D-lysine caused a tremendous increase in the lysine output, which became normal on changing to 6% L-lysine. On the basis of this observation and the poor N-retention and weight changes manifested by infants maintained on the D-lysine-supplemented wheat gluten diet it would appear that D-lysine is not utilized for growth by the infant.

Blood filtrates could not be manipulated satisfactorily to give positive tests by this procedure.

Within limitations this method is a useful one, but it cannot be expected to yield better than semiquantitative results. Other metal salts of the amino acids—namely, nickel, silver, chromium, mercury, lead, and barium—are being studied to determine their chromatographic characteristics.

References

1. ALBANESE, A. A. and IRBY, V. *J. Biol. Chem.*, 1944, **153**, 583.
2. WILLIAMS, R. J. and KIRBY, H. *Science*, 1948, **107**, 481.

Quantitative Study of Root Systems in Different Soil Types

J. E. Weaver and R. W. Darland

Department of Botany, University of Nebraska, Lincoln

Study of soil-root relations of various crop plants and range grasses has lagged far behind the pressing need for an understanding of these relationships. Little progress in devising new methods for such study, at least methods that have been widely used in the field, has been made since the extensive researches by the direct method, using trench and hand pick, employed by Weaver (1, 2) during the period 1919 to 1926. This lack of a quantitative approach to comparative root studies in various soil types has undoubtedly been a chief reason for the dearth of more definite information on this important subject.

A new method has recently been devised by which a complete sample of an entire root system from soil sur-

face to maximum depth of penetration may be taken, separated from the soil without injury to the root or displacement of individual roots from their natural position, and examined in the laboratory in relation to the various horizons of the soil profile. The method, applied to range grasses, consists in obtaining monoliths of soil 12 in. wide and 3 in. thick to a depth, varying with root extent, of 3–6 ft.

A trench about 3 ft wide and 4–5 ft long is dug in a site where there is normal development of vegetation. The depth is usually 4–6 ft. Beneath the particular sample of grass, previously selected and left undisturbed in the side wall, the wall of the trench is made smooth and vertical, as shown by a plumb line. A long shallow wooden box, 12 in. wide and 3 in. deep (inside dimensions), without a top and lacking one end, is employed. It is placed on end, with the closed end downward. The open top is placed against the vertical trench wall, the upper end of the box just reaching the soil surface. An impression of the sides and lower end of the box is made on the vertical wall of the trench by tapping the bottom of the box vigorously with a 4-lb sledge. The box is then removed and the soil column marked out with butchers' knives having rigid blades. The soil on the sides and below these marks is removed by means of knives and spades until the monolith protrudes from the trench wall, its sides and bottom extending outward at least 3 in. The box is then fitted tightly over the monolith and the bottom and lower end of the box are braced to hold the soil column in place. Finally, the soil on the inner, attached face of the monolith is cut away by working inward with knives and spades from each side. The soil is not cut close to the top of the box, but a V-shaped ridge of soil is left protruding throughout its length. This is a part of the sample when the braces are removed and the monolith is lifted out of the trench. The entire monolith is transported to the laboratory, where a description of the profile as regards soil texture, structure, consistence, pH, etc. is made. Only then is the monolith reduced to exactly 3 in. in thickness.

The soil is removed from the box by a process of repeated soaking, often for several days, and gentle washing, mostly under water, even when it is extremely compact or contains a claypan. A flaring rose nozzle attached to a garden hose is employed. During this process one may study the intimate relations of soil and roots. Roots are unharmed and in their natural position in the water after the soil has been washed away.

The root system is transferred to a large smooth painted board. The board is kept wet and tilted while the roots are finally washed free of any remaining soil. Excess water is then removed by large blotters and the damp root system is transferred to a mounting board covered with cloth of black felt. This is done by placing the mounting board, face downward, over the root system, holding the two boards tightly together, and inverting them. The painted board is then removed. The root system is lighted for photographing by electroflash units. It may then be dried and preserved indefinitely or cut into sections according to depth in feet or according to