Chromatography as a Method of Attack on the Problem of the Chemical Nature of Resistance of Sugar Beets to Curly Top

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Many studies have been made, in the past, in an attempt to correlate some property or the presence of some substance in sugar beet plants with resistance to curly top. In these investigations, comparative studies were made on the composition and the physical properties of juice expressed from different tissues of healthy and diseased plants of both resistant and susceptible varieties. The results of these comparative studies with plants grown in the greenhouse in sand culture, in heavily fertilized potting soil and with field-collected samples, often showed a greater variation among different plants of the same variety than between plants of resistant and susceptible varieties. When a number of plants were represented in the comparative studies, the differences found were not significant and frequently reversed.

It is known that under severe conditions of the disease a clear exudate often flows from the phloem of diseased leaves to the surface of the midrib and petiole through the intercellular spaces (3)[2]. Bennett (1, 2) has shown that this exudate collected over the bundles of diseased roots contains a high concentration of virus. A large amount of evidence has been accumulated also which indicates that the virus is more or less confined to the phloem and that multiplication may take place largely or only in the phloem. These findings have led to the conclusion that further studies should involve investigations of the chemistry of the phloem content. It is in this medium that one might expect to find the highest concentration of factors responsible for resistance.

It is known also that sugar beet roots will, under certain conditions which are not well understood, exude small amounts of liquid from the phloem tissue. Extensive tests of many collections made by the author (4) show this exudate to be strikingly different in composition and in physical properties from the expressed juice of the same roots and xylem exudate from similar roots. In view of the evidence that this exudate appears to be the phloem content in a relatively pure form, it should be of value for studies on the nature of resistance of sugar beets to curly top.

With the rapid advances made in the field of chromatography and by use of the newer ion exchange resins for the purpose of concentrating the samples, where necessary, the small volumes of phloem exudate which can be obtained from healthy and diseased sugar beet roots may be sufficient to find significant differences in the composition of phloem exudate from resistant and susceptible sugar beet plants. In view of such a promising method of attack on the problem, intensive studies have been undertaken using paper chromatography, to identify and to determine the relative

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[2] Numbers in parentheses refer to literature cited.

amounts of amino acids, organic acids and other compounds in the expressed juice and in the phloem exudate from diseased and healthy sugar beet plants of resistant and susceptible varieties. The present paper is a report on the investigations made thus far.

Methods and Results

Young beet plants of a variety susceptible to curly top were inoculated with a strain of virus which produced severe symptoms of the disease. After 21-28 days of growth in the greenhouse following inoculation, the leaves showing severe symptoms were collected and the petioles removed. Leaves of the same age were removed at the same time from healthy control plants. The juice was expressed from the frozen leaf samples and passed through a mat of celite which removed the chlorophyll. Thymol was added as a preservative and the juice stored at —10° F.

Four to 6 microliters of the undiluted juice were found to give the best unidirectional papergrams. These volumes of juice from healthy and diseased leaves were spotted on papers along with amino acid standards. One series of papers was developed in a phenol-water mixture while similar papers were developed in a butanol-acetic acid-water system by the ascending method. The temperature of the room was maintained at 23° C. with a relative humidity of 33 percent. The maximum variation in the room temperature was plus or minus one degree while the solvent and the air inside the jars was found to vary not more than 0.2° C.

When the solvent front reached approximately 25 cm. the papers were removed and dried, generally overnight, followed by two hours drying at 40° C. with air circulation. The papers were then sprayed with 0.5 percent ninhydrin in alcohol. Unidirectional papergrams, developed in phenol and a butanol-acetic acid-water system, using healthy and diseased juice of beet leaves and phloem exudate from diseased and healthy beet plants of resistant and susceptible varieties, are shown in Figure 1. Papergram A shows some of the striking differences found in the relative density of corresponding spots from healthy and diseased leaf juice.

It was found necessary to reduce the volume of juice from diseased leaves to one-tenth that of the healthy juice (the control) in order to make the density of the spots at Rf .50 approximately the same. Two directional papergrams revealed that the intensity of this spot in the diseased juice is due almost entirely to one amino acid. The amino acid has been tentatively identified as argenine. Aspartic acid, which is barely visible appeared to be present in both the healthy and diseased juice in approximately the same concentration while glutamic acid in all tests appeared to be considerably more concentrated in the healthy juice.

Papergram B (developed in butanol) shows three pairs of healthy and diseased juice. This papergram confirms the observations made from papergram A. With this solvent, as many as 10 distinct spots have been located on papergrams spotted with healthy and diseased juice. However, comparative spots were not found in all cases, indicating that certain amino acids may not be present in the juice of both healthy and diseased beet leaves.

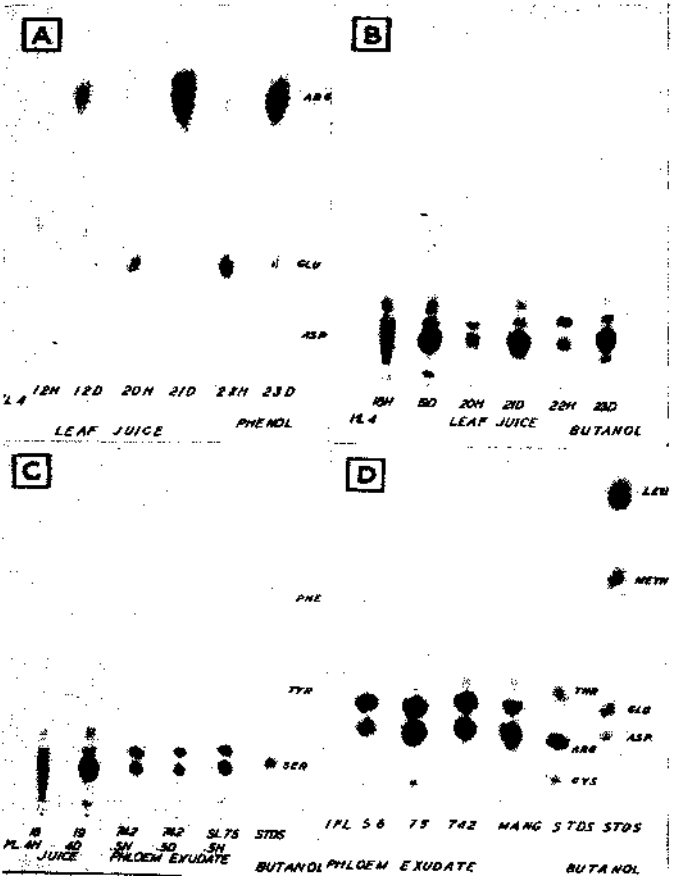

Figure 1. Papergrams showing the amino acids in juice from healthy and diseased beet leaves and phloem exudate from healthy and diseased roots of resistant and susceptible varieties.

The large extremely dense spot at Rf .12 in the diseased juice contains what has been tentatively identified as argenine. The papergram patterns and the differences in the relative density of corresponding spots were found to be uniform for the six pairs of healthy and diseased leaf juice studied.

The juice from healthy and diseased beet leaves was compared with phloem exudate collected from healthy and diseased roots of a susceptible variety (S. L. 742) and also with phloem exudate from roots of a variety resistant to curly top (U. S. 75). The results are shown in papergram C. In view of the fact that only 0.5 microliters of phloem exudate was sufficient to give a satisfactory papergram and at the same time produce comparative spots of approximately the same relative density as 4 microliters of leaf juice, it is evident that certain amino acids are present in the phloem exudate in concentrations several fold greater than in juice from expressed leaves. An amino acid, having an Rf value near that of the cysteine standard .08, is present in the healthy and diseased leaf juice and considerably more is present in the healthy phloem exudate of U. S. 75. A trace of this amino acid appears in the healthy susceptible phloem exudate but is apparently removed as a result of the disease. At Rf .10 an amino acid appears in the healthy and diseased leaf juice but is absent in the phloem exudate samples.

Unidirectional papergrams were prepared using phloem exudate from roots of two varieties resistant to curly top (U. S. 56 and U. S. 75), with exudate from a susceptible variety (S. L. 742) and with phloem exudate from mangels. The results are shown in papergram D. This papergram reveals differences mainly in concentration between amino acids in the phloem exudate from the resistant and susceptible roots. Distinct spots, at an Rf slightly below that of the standard cystein (Rf .07), appeared in the phloem exudate of the resistant varieties, whereas it is barely detectable in the susceptible variety S. L. 742 and is absent in mangels. At Rf .33, which is near the standard of tyrosine, spots are present in the resistant varieties but are absent in the phloem exudate of the susceptible variety and mangel.

Summary

By the use of paper chromatography, striking differences were found in the relative concentration of certain amino acids in the juice expressed from healthy and diseased beet leaves and in the phloem exudate collected from healthy and diseased sugar beet roots.

Distinct differences were found between the papergram patterns made from the phloem exudate collected from resistant and susceptible varieties of sugar beet roots and from mangels.

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