STUDIES IN THE INHERITANCE AND ENZYMATIC DETECTION OF RATOON

STUNTING DISEASE OF SUGARCANE

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By

Mohamed Awadalla Yassin

Thesis Committee:

Ukio Urata, Chairman
James A. Silva
Don J. Heinz

We certify that we have read this thesis and
that in our opinion it is satisfactory in scope and
quality as a thesis for the degree of Master of
Science in Agronomy.

THESIS COMMITTEE

_____
Chairman

_____

_____

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# INTRODUCTION

Ratoon stunting disease (R.S.D.) of sugarcane (Saccharum sp.) is
a suspected virus disease (hereafter referred to as virus disease), and
was first reported (1944-1945) in Queensland, Australia. It is character-
ized by general stunting of the affected cane resulting in reduced
tonnage, particularly in ratoon crops. Primary infection is caused by
planting diseased material and secondary infection takes place through
cane knives and blades of cutter planters. No insect vector is known
but the disease can be transmitted mechanically. Loss in yield due to
the disease may be as high as 10 percent in plant cane and 20 percent
in ratoon crops.

Unlike other plant diseases, R.S.D. does not produce any external
diagnostic symptoms, thereby making identification of diseased plants
difficult, especially in young vigorously growing canes. Symptoms
associated with the disease (stunted growth and low yield) may be
caused by a number of other plant diseases and by unfavorable growth
conditions, e.g. unfavorable soil and weather. Good growing conditions
also tend to mask the symptoms. Visually, diseased plants are recog-
nized by the presence of an orange-red discoloration of the vascular
bundles at the mature basal (internodal) regions. The efficiency of
this test depends on the variety and the environmental conditions. A
number of susceptible varieties do not show these symptoms and are
referred to as symptomless carriers.

Since a number of susceptible sugarcane clones do not respond to
the visual test (symptomless carriers), it was one of the objectives
of this research to determine if the differences in isozyme patterns

and increase in peroxidase activity reported to occur in other virus-host combinations could be made use of in identifying diseased sugarcane plants.

The second objective was to attempt to determine the mode of inheritance of resistance of sugarcane varieties to R.S.D.

The importance of the disease arises from the loss in profits due to reduced cane yields, expense of control, and the part it is suspected to have played in the yield decline of sugarcane varieties. A knowledge of the mode of inheritance of the disease would aid in the development of resistant varieties.

LITERATURE REVIEW

History and Nature of the Disease:

Ratoon stunting disease, a suspected virus disease affecting

sugarcane, was recognized in the "Mackay" district in Queensland,

Australia, when a number of fields and portions of fields of the new

and very promising variety Q.28 produced very low ratoon crops compared

with adjacent ratoons of the same variety. Steindl (1950) described

the symptoms as a marked reduction in growth and yield, particularly

in ratoons. Diseased plants had fewer stalks per stool than normal

ones and were the first to show symptoms of stress, e.g. water or

nutrient shortages. The disease did not occur in patches; either

fields or parts of fields showed the abnormality corresponding to the

various sources of the planting material.

The fact that the external symptoms associated with the disease

can be caused and/or enhanced by unfavorable environmental conditions

such as poor soil and drought, and may be masked by good growing condi-

tions, led to the search for better diagnostic symptoms. Steindl and

Hughes (1953) reported that the abnormality was associated with an

orange-red discoloration of the vascular bundles of the mature basal

nodes of diseased stalks. Microscopic examination through the dis-

colored vascular bundles showed that the xylem elements were clogged

with a gummy material which interfered with the free flow of sap in

the plant. The absence of any bacterial or fungal infection in the

diseased plants together with the presence of gummy masses in xylem

vessels led to the belief that the causal organism is a virus. They

also mentioned that trials to isolate the virus failed and the electron

microscope also failed to demonstrate any differences in saps of diseased and healthy plants. However, they reported, when diseased sap was injected into rabbits evidence of formation of antibodies was observed, giving further support to the belief that the causal organism was a virus.

Hughes and Steindl (1956) reported that germination, early growth and ultimate length of stalk were poorer than in healthy canes but no difference in thickness of stalk or number of stalks per stool was observed. A survey of all the varieties and localities in Queensland in 1953-54 revealed that the effects of the disease were more pronounced in drier years and that different varieties react differently to infection. Losses in yield of Q.28, Pindar and P.O.J. 2878 were particularly high while Badila and Comus did not show consistent losses, indicating some degree of tolerance. They also reported that the virus was found to occur in the leaves, stems and roots in approximately equal concentrations and remained infective at dilutions of 1:10,000. A high percentage of infection was obtained by cutting roots with infected knives or cutting roots in infected juice. They mentioned that no disease symptoms were observed when sorghum, sudan grass and maize were inoculated with infected sap but the disease symptoms were observed on Q.28 when it was inoculated with sap from these plants.

The source of primary infection was established to be the planting of diseased material. Secondary infection occurs through cane knives and blades of cutter planters. The disease may be mechanically transmitted into healthy plants by artificial inoculation.

The discovery of the disease and the recognition of the losses it can produce instigated research work in many of the sugarcane growing countries to determine the presence of the disease and the extent of loss from it, and to develop control measures which could be taken to eliminate it.

The Experiment Station, Hawaiian Sugar Planters' Association (HSPA) Anon. (1953) reported acute symptoms of growth failure in varieties H47-4991 and H47-112. These symptoms which appeared in many other varieties, were found to be identical to those produced by R.S.D.

Weihe (1954) reported the presence of the disease particularly in the super humid region of Mauritius. The disease was severest on varieties D-109, Co.419, and Co.421. Weihe mentioned that the disease was the major factor in the declining yields of the variety M.134/32. King (1956) reported that the disease might have been the main reason for the deterioration of N.Co.310, a variety that occupied about 50 percent of the sugarcane growing areas in Natal, South Africa. Sheffield (1956) claimed that, because of the disease, Co.281 in Natal deteriorated from a yield of 23 tons per acre in 1924 to 14 tons per acre in 1952.

Surveys made in 1954-55 and 1955-56 showed an R.S.D. infection of 57.6 and 72.4 percent, respectively, in N.Co.310 in Taiwan sugarcane fields, Taiwan (Chu and Liu, 1956).

Veiga (1956) reported the presence of the disease in the variety H32-8560. Sap from this plant could infect and cause yield losses in a number of local varieties. Wehlberg reported the presence of the

disease in Cuba in 1956. The disease was reported to occur in Louisiana by Schexnayder in 1956. Barnes (1957) reported the presence of the internodal symptoms associated with R.S.D. in a number of local varieties and in ratoons of recently introduced varieties in Nigerian sugarcane growing areas. In 1959, Navarrete reported the disease in San Cristobal, Mexico.

King and Steindl (1953) reported that the virus could be inactivated in vitro by soaking infected tissue or sap with 2.5 percent lysol, 1 percent phenol, 5 percent alcohol or 10 percent formalin for 10 minutes and by heat, 55° C. for 10 minutes. The juice remained infective for 5 days when stored at room temperature.

Artschwager (1959) reported that the discolored strands in the nodal region showed pathological changes in both xylem and phloem. The large metaxylem vessels became plugged with a red-staining gummy substance when stained with phloroglucin and HCl, while the changes in the phloem consisted of lignification in the cell wall and contents of the sieve tubes.

Forbes and Ling (1960), using the electron microscope, reported the presence of two types of particles in the juice extracted from leaves of diseased plants not found in the leaves of healthy plants of the same variety. The smaller type of particles was more or less spheroidal in shape and uniform in size averaging 31.9 mu and the larger type had a verticulate appearance averaging 73.3 mu in diameter. The smaller particles simulating other plant virus particles were considered to be the R.S.D. virus particles and the larger ones were by-products of the virus.

Besides mechanical transmission by artificial inoculation or by cane knives and blades of cutter planters, Wehlberg (1956) reported that the virus could also be transmitted by rats in sugarcane fields. Rats fed on diseased stalks caused 40 percent infection in healthy seed pieces which were introduced into the rat cages directly after the rats were fed on diseased stalks. However, this method of transmission has not been duplicated elsewhere.

Steindl (1957) reported that the virus could infect <u>Brachiaria</u> <u>miliiformis</u> (Presl) A. Chase, <u>Brachiaria</u> <u>mutica</u> (Forsk.) Stapf, <u>Chloris</u> <u>gayana</u> Kunth; <u>Cynodon</u> <u>dactylon</u> (L.) Pers., <u>Echinocloa</u> <u>colonum</u> (L.) Link. and <u>Panicum</u> <u>maximum</u> Jacq. Juice from these plants infected and produced symptoms on R.S.D. susceptible sugarcane plants.

### Symptoms and Methods of Identification

Hughes and Steindl (1956) divided the disease symptoms that can be of diagnostic value into:

1. A pinkish cast appearing in the immature nodes of diseased cane.

2. The occurrence of a reddish-orange discoloration of the internodal regions of the more mature stalks of diseased cane.

As this discoloration was not readily shown by a number of varieties, attempts were made to develop some sort of chemical test to recognize diseased plants. Farr (1957) mentioned that longitudinal sections cut from the periphery of mature basal nodes of healthy cane gave a blue green color in the parenchymatous tissue around the fibro-

vascular bundles at the leaf scar level when treated with 3 percent $H_2O_2$ and conc. HCl. No such reaction was observed in diseased cane. Antoine (1959) claimed that longitudinal sections from the mature basal nodes of 8-9 month-old diseased cane gave a deeper red coloration when treated with 2,3,5 triphenyl tetrazolium chloride than sections taken from nodes of healthy cane. However, these two tests were said to be inconsistent and cannot be considered reliable. Schexnayder (1959) developed the use of an indicator plant which was a sugarcane variety that showed the internodal symptoms clearly when inoculated with juice from an infected plant. In this test the indicator plant is inoculated with juice from a plant suspected to be diseased and 8-10 weeks later the indicator plant is examined for the discoloration in the internodal regions. Fife and Stokes (1959) using paper chromatography could detect higher concentrations of certain amino acids in the leaves of diseased plants.

Changes in enzyme levels or isozyme patterns have been reported to occur in a number of host-parasite combinations. Lobenstein and Linsey (1961), studying peroxidase activity in the leaves and roots of sweet potatoes infected with vein-clearing virus, reported that infected plants showed a higher level of peroxidase activity starting at the time of symptom appearance and increasing with the age of the organ and the age of the infection. The increase seemed to be associated with the concentration of the virus in the tissue per se. The same study revealed that respiration rates in infected plants were significantly higher than those in healthy ones. Respiration rate values were dependent on leaf age, time of measurement and interval

after infection. They attributed the increase in peroxidase activity
to either enzyme protein synthesis triggered by the parasitic attack
or to the activation of inactive enzymes. Lobenstein and Linsey (1963)
observed vein clearing, mosaic, and necrosis with stunting when they
infected tobacco plants with potato virus Y (PVY), potato virus X (PVX),
and PVX + tobacco mosaic virus (TMV), respectively. The more severe
the symptoms, the greater the increase in peroxidase activity (P.A.).
P.A. was two to three times higher when the infection induced local
lesions instead of systemic mottling or mosaic. P.A. varied in dif-
ferent regions of the same leaf and was highest where symptoms were
severest.

Farkas and Stahmann (1966), reported that infection of Pinto bean
leaves (Phaseolus vulgaris) with southern bean mosaic virus resulted
in an increase in peroxidase activity and changes in peroxidase iso-
zymes or multiple components. Using polyacrylamide gel electro-
phoresis, they separated peroxidases of healthy Pinto bean leaves
into two components (Peroxidases I and II). Associated with the virus
lesion development, two new peroxidases (III and IV) appeared at the
site of lesion formation. Changes in peorxidase activity were
attributed to the parasitically induced acceleration of senescence
and the mechanical damage which accompanied lesion development.
Solymosy, Szirmai Beczner and Farkas (1967), showed that extracts from
leaf tissues of Phaseolus vulgaris and Nicotiana glutinosa infected
with alfalfa mosaic virus and T.M.V., respectively, showed different
peroxidase isozyme patterns. The virus infection resulting in local
lesions led to the formation of new peroxidase components. The type
of change was determined by the host tissue rather than by the virus.

Menke and Walker (1963), using a modified colorimetric technique, detected an increase in the respiratory rates, virus concentrations and peroxidase activity of the first, second and third leaves from resistant and susceptible cucumber varieties (Cucumis sativas L.) inoculated with cucumber mosaic virus (C.M.V.). Peroxidase activity increased in all leaves of resistant plants, but was consistently higher in comparable leaves of susceptible plants.

## Losses and Control Measures:

Steindl and Hughes (1953), reported that the losses in yield of a susceptible variety like Q.28 could be as high as 60 percent in ratoons and 12-37 percent in plant canes. They estimated an average of 10-20 percent loss in yield of the sugarcane varieties tested in Queensland, Australia. Among a number of hot water treatments they found that treating planting material with 50° C. hot water for 2 hours gave effective control with the least damage to germination. A short hot water treatment, 52° C. for 1½ hours, had also been recommended. Steib and Chilton (1959), recommended use of hot air at temperature 54° C. for 8 hours. The arguments they presented against hot water treatment were:

1. Greater danger of damaging the eyes than with hot air.

2. Hot water softened the eyes and made them more susceptible to damage in handling and rotting by microorganisms.

3. Hot water treatment could not be provided in farms without an available source of steam. With hot air treatment none of the above mentioned disadvantages were encountered.

It was reported in the Annual Report of the Exp. Sta., H.S.P.A. (Anon., 1956) that knife transmission of the disease could be prevented by the following chemicals: Lysol, 5 percent solution; bionol, 5 percent; isopropyl alcohol, 50 percent; mescodyne, 5 percent; and phenyl mercuric acetate (P.M.A.), 5 percent. Contaminated knives were used to cut healthy stalks of H45-2120, immediately and at intervals of 2, 6, 24 and 48 hours to study the longevity of virus infectivity on percentage transmission. The percentage transmission immediately, after 2 hours and after 6 hours following knife contamination was found to be 81.3, 8.3, and 4.6 percent, respectively. R.S.D. was not transmitted by contaminated knives after 24 hours.

Steib and Chilton (1957), using the hot water treatment in a number of trials involving the varieties CP44-101 and CP36-105, found an increase in yield of 9 tons cane per acre in the plant cane and 17 tons per acre in the first ratoon over diseased cane for CP44-101 and 9.4 tons and 15.9 tons per acre for CP36-105. There was no significant difference in sucrose content in plant cane or stubble of diseased and healthy canes. The spread of the disease from diseased to healthy canes by cane knives was found to be very rapid. Plots observed to be 16 percent and 50 percent infected in the plant cane were found to be 47 percent and 80-90 percent infected in the stubble, respectively. Because of this, it was recommended that cane knives and blades of cutter-planters be sterilized when moving from infected to clean fields.

Chu and Ling (1958) reported an increase in yield over diseased N.Co.310 of 19.5 percent when using diseased untreated cane and 33.8 percent when using hot water treated cane. The use of hot water

treatment, 50° C. for 2 hours in P.O.J.2883 resulted in a yield increase
of 45.7 percent over that of diseased material. Wismer (1964 and 1965)
reported that R.S.D. in the variety H50-7209 produced a loss of 20.2,
and 21.5 percent in tons sugar per acre of plant cane, and first
ratoons, respectively.

## Importance and Implications of the Disease:

The disease is held to be of major economic importance since
losses in susceptible canes can be very high, particularly in ratoons.
According to King (1954), the worldwide distribution of the disease
implied that it was not recent in origin and might have significantly
contributed to yield decline of sugarcane varieties. Moreover, King
argued that the hot water treatment has saved a number of varieties
which were on the verge of being discarded. The same line of argument
was followed by Abbot (1959) who claimed that the restoration of former
yields of a number of commercial varieties such as C.P.28-19 furnished
a clue that the disease was involved in the yield decline of these
varieties. Elimination of the disease, he mentioned, besides increas-
ing yields isgnificantly, has a stabilizing effect on production. King
(1954), cited the absence of external diagnostic symptoms, the clear-cut
low yields of infected plants as compared with healthy plants and the
progressive spread of the disease over the life of the variety as
further evidence supporting the hypothesis that the disease has been
one of the major factors in yield decline of sugarcane varieties.

MATERIALS AND METHODS

Materials:

Resistant and susceptible sugarcane varieties were intercrossed by personnel of the Genetics Department, Experiment Station, Hawaiian Sugar Planters' Association (Exp. Sta., H.S.P.A.) to obtain the following combinations:

> Susceptible x susceptible
>
> Resistant x susceptible
>
> Resistant x resistant

Progenies were studied from the following crosses:

|   | Cross No. | Parentage | Type of Cross |
|---|-----------|-----------|---------------|
| 1 | 333 A | H53-263 x H53-1447 | S x S |
| 2 | 481 A | H60-6909 x H49-3533 | R x R |
| 3 | 481 B | H49-3533 x H60-6906 | R x R |
| 4 | 482 B | H39-7028 x H60-6909 | R x R |
| 5 | 483 | H50-723 x H60-6909 | R x R |
| 6 | 484 A | H50-7209 x H60-6909 | S x R |
| 7 | 484 B | H60-6909 x H50-7209 | R x S |
| 8 | 485 A | H53-263 x H60-6909 | S x R |
| 9 | 485 B | H60-6909 x H53-263 | R x S |

> R = Resistant
> S = Susceptible
> B = Refers to the Reciprocal cross of A

Diseased and healthy plants of the indicator variety H45-2120 were also utilized in the study.

Methods of Planting and Testing:

Crosses were made according to the method described by Mangelsdorf (1953), at the Kailua substation of the Exp. Sta., H.S.P.A., during the crossing season of 1966-67.

Individual seedlings were space-planted in the field in April 1967 at the Kunia Research substation of the Exp. Sta., H.S.P.A.

Four months later, about 300 three-eyed seed pieces (one seed piece from each separate seedling) from each of the crosses H39-7028 x H60-6909, H50-7209 x H60-6909 and H53-263 x H53-1447 were pressure inoculated and planted in 5 x 3 foot plots at Kunia Research sub-station. The pressure inoculator (Bell, 1935), recommended by Dr. Wismer (Pathologist, Exp. Sta., H.S.P.A.) consisted of a cast iron cylinder within which there were small flanged, sharp-edged stainless steel cups (Figure 1). Inoculum was obtained from diseased plants as judged by the presence of internodal discoloration. A Cuban-A mill was used to express the sap. The extracted juice was diluted 1:1 with water, poured into the inoculator until the level was slightly above the cutting edge of the cup. The freshly-cut seed piece was then placed centrally and vertically above the cutting edge of the cup and forced downwards until stopped by the flange. The increasing outward pressure forced the inoculum into the vascular system of the seed piece. Before planting, the seed pieces were dipped into 10 percent phenyl mercuric acetate (P.M.A.) diluted 1:800 with water. The inoculum was changed daily and used for about 3 hours a day.

For peroxidase isozyme comparisons one seed piece from each of the 80 clones studied was inoculated with R.S.D. virus, then planted, and another seed piece was planted without inoculation.

FIGURE 1. THE PRESSURE INOCULATOR.

To determine the success of the inoculation technique, 30 seed pieces of the indicator variety H45-2120 were inoculated, treated with P.M.A. and planted.

In January 1968, when there was enough millable cane, the plants were checked visually for the presence of internodal discoloration (Figure 2). The procedure followed was to cut a longitudinal section through the mature basal nodes of two stalks per seedling. The plants were classified into five categories.

0 = No symptoms

1 = No definite symptoms

2-5 = Definite symptoms (depending on intensity of symptoms)

Since certain clones could be symptomless carriers, the test-plant method suggested by Schexnayder (1959) was used to detect the presence of the virus in the plants of the first and second categories (0 and 1). The juice from each plant was extracted by crushing the stalk in a nut-cracker (Figure 3) and used to inoculate a three-eyed seed piece of the indicator variety H45-2120 by dipping freshly-cut ends of the seed piece into the extracted juice. The nut-cracker was sterilized in 2 percent lysol after each application and the inoculated seed pieces were sprayed with 10 percent P.M.A. diluted 1:800 with water nefore planting. Due to the cold, cloudy weather, over-aged planting material (H45-2120) and hard soil, only 50 percent germination occurred. Replanting was done a month later. In late June 1968, these plants were checked again for the presence of the internodal discoloration.
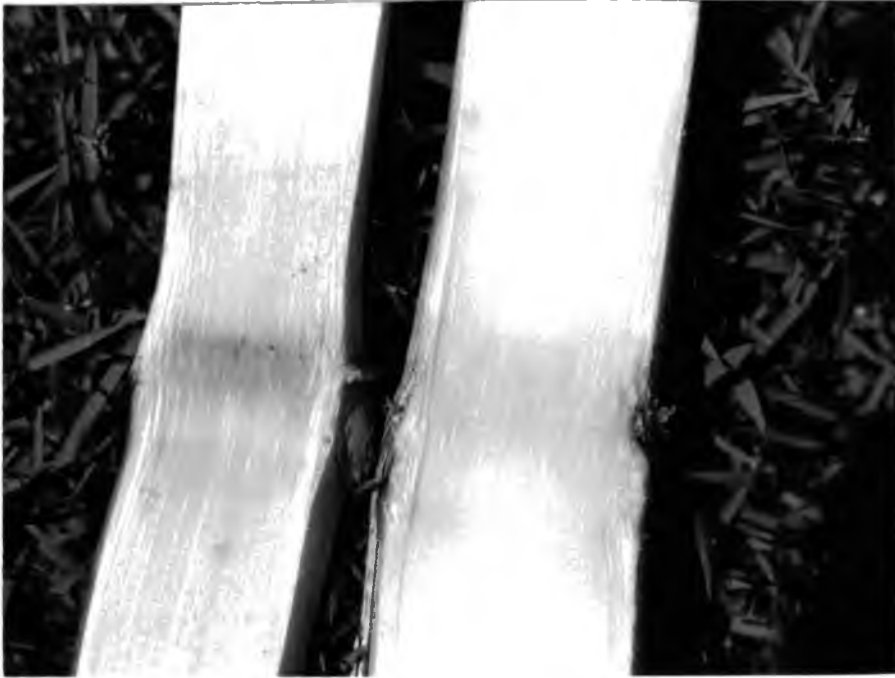
Diseased                Healthy

FIGURE 2.   R.S.D.  INTERNODAL SYMPTOMS.

FIGURE 3.   THE NUT-CRACKER.

Peroxidase Studies:

Sample preparation: The sixth leaf, counting from the spindle leaf of the plant, was used in this study. For efficient juice extraction, leaf samples were frozen till analyzed. The midribs were then stripped off and one gram of the remaining leaf blade was crushed in a mortar in six drops of distilled water.

Starch-gel preparation: The procedure outlined below follows that reported by Ashton (1967). Forty-eight grams of hydrolyzed starch were added to 400 ml. Ashton's gel electrolyte (40 ml. Ashton A + 360 ml. Ashton B). The mixture was heated until the starch was dissolved, and then evacuated by suction to remove all air mixed with the solutions. The starch was then poured into the holders. These consisted of plastic trays with troughs 0.3 cm. deep, 4 cm. wide and 26 cm. long. The gels were levelled by passing a stretched wire over the top of the holder. The excess gel was discarded. A slit was then cut across the starch channels near the anode (negative pole), and small rectangular pieces of Whatman No. 17 filter paper (4.6 cm.) saturated in the leaf extract were inserted in the slit.

Electrophoresis: Ashton A solution was put into the two tanks which were connected with two cellulose wicks. The inner holders were connected to the gel holder with similar wicks. The starch-gel holder was mounted on the two tanks and covered with the two wicks connecting the tanks. A current of 25 MA at 100 V was passed through the gel. The filter papers containing the leaf extracts were removed after 30 minutes of electrophoresis. During electrophoresis, the starch gels were covered with thin polyvinyl film to prevent evaporation.

Electrophoresis was performed "at room temperature (75° F.)" for about 10 hours.

The buffers used for gel preparation and electrophoresis were:

Ashton A:               2.61 g. LiOH

                        45.18 g. boric acid

                        3.8 liters water


Ashton B:               6.08 g. citric acid

                        23.56 g. trizma base

                        3.8 liters water

After the electrophoretic run, the starch gels were removed from the holder and stained for peroxidases.

Staining:  The staining procedure of Siegel (1967) which employs a mixture of equal volumes of benzidine solution and 3 percent $H_2O_2$ was used.  When the peroxidase bands were clear, 100 ml. of tris maleate B were added to stop the reaction.  The starch-gels were then destained and washed with wash solution.  The solution constituents are given below:

            Benzidine solution:     1 g. benzidine heated to dissolve

                                    in 9 g. acetic acid + 40 ml. dis-

                                    tilled water.


            Tris maleate B    :     25.4 g. NaOH dissolved in 3.8

                                    liters distilled water.

Wash solution:              10 parts methyl alcohol

                            10 parts distilled water

                             2 parts glacial acetic acid

                             1 part ethyl alcohol.

RESULTS AND DISCUSSION

Enzymatic Detection of the Disease:

Since the ratoon stunting disease of sugarcane does not show any
external diagnostic symptoms and since the chemical tests so far de-
scribed cannot be considered reliable, it was the purpose of the
present investigation to determine whether the changes in peroxidase
isozymes reported to occur in other virus-host plant systems (Farkas
and Stahmann, 1966) could be made use of in identifying sugarcane
plants infected with ratoon stunting disease.

To study this, leaf extracts from 26 Hawaiian sugarcane varie-
ties and 280 plants representing 9 different crosses were analyzed by
starch-gel electrophoresis for peroxidase. Schematic diagrams of the
electrophoretic patterns obtained are shown in Figures 4-8. Each
starch strip or trough contained two different leaf extract samples,
one from an inoculated seed piece from a variety or seedling and the
other from a control or an uninoculated seed piece of the same variety
or seedling. It is clear from the diagrams that an additional one or
two peroxidase isozymes appeared in the leaf extracts from inoculated
plants or seedlings. Leaf tissues of sugarcane seem to contain be-
tween three and five original peroxidase components, numbered either
1-3 or 1-5, according to their electrophoretic mobility.

The differences in numbers of the original peroxidase components
in leaves of different plants may be accounted for by the complex
genetic nature of sugarcane varieties, which are high polyploids and/or
aneuploids. In such systems differences in isozyme numbers would be

1, 2, 3 and 4    Original components
5 and 6          Due to virus infection



FIGURE 4.  A SCHEMATIC DIAGRAM OF PEROXIDASES OF SEEDLING 113.

1, 2, 3, 4 and 5    Original components
6 and 7             Due to virus infection
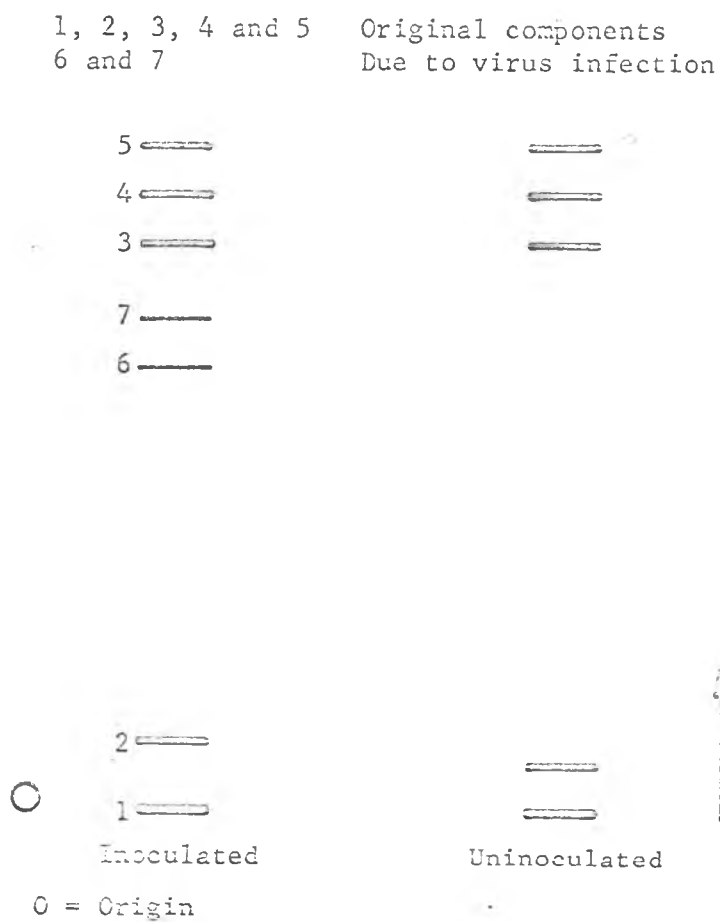


FIGURE 5.  A SCHEMATIC DIAGRAM OF PEROXIDASES OF SEEDLING 125.

1, 2, 3, and 4    Original components
5                 Due to virus infection

4 ⸺⸺

3 ⸺⸺

2 ⸺⸺
5 ⸺⸺

O      1 ⸺⸺                    ⸺⸺         ⊖
       Inoculated                Uninoculated

0 = Origin

FIGURE 6.  A SCHEMATIC DIAGRAM OF PEROXIDASES OF SEEDLING 5.

1, 2 and 3    Original components
4 and 5       Due to virus infection
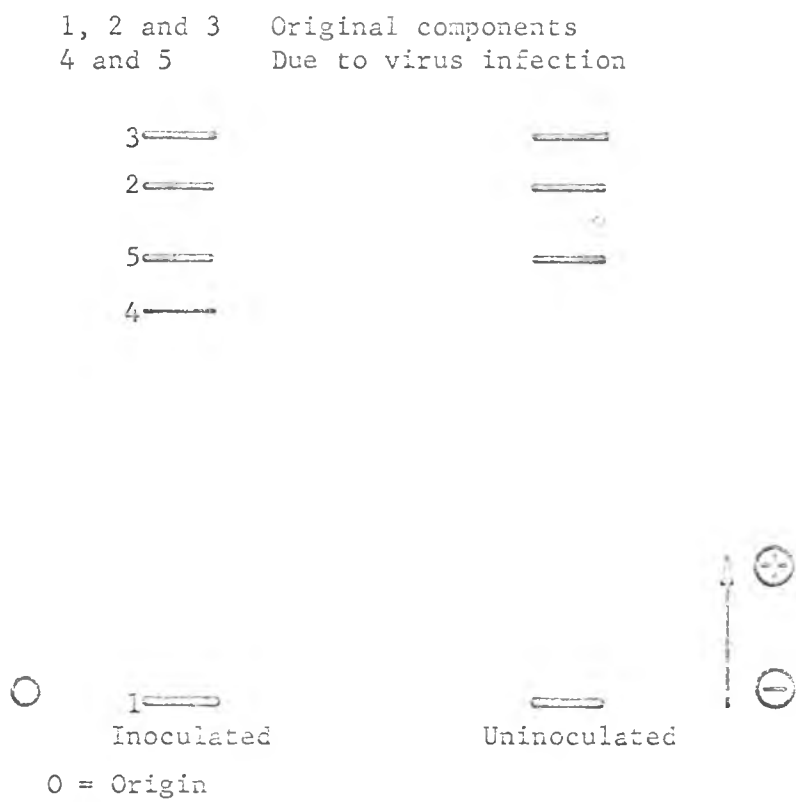


FIGURE 7.   A SCHEMATIC DIAGRAM OF PEROXIDASES OF H45-2120.

1, 2 and 3    Original components
4             Due to virus infection

3 ━━━━                           ━━━━

2 ━━━━                           ━━━━
4 ━━━━

○    1 ━━━                    ━━━         ⊕
      Inoculated              Uninoculated        ⊖
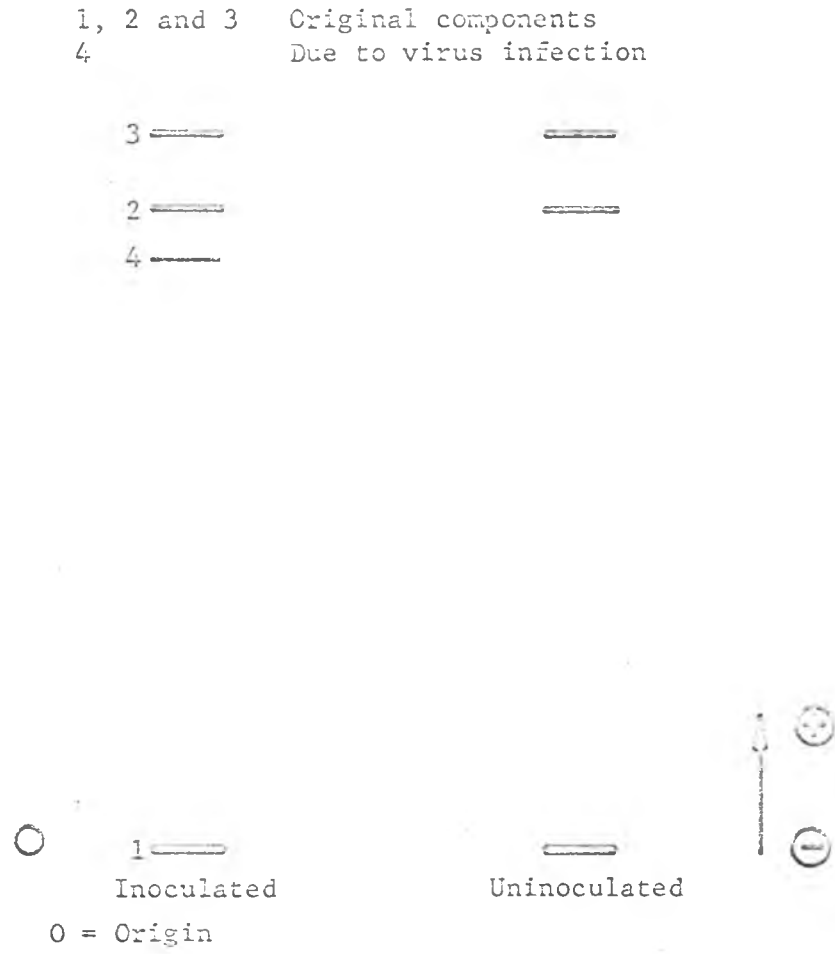
0 = Origin

FIGURE 8.  A SCHEMATIC DIAGRAM OF PEROXIDASES OF H58-8622.

expected as each isozyme is controlled by a different gene (Markert and Moller, 1959).

Due to the virus infection an additional one or two components seem to develop. These are numbered 5 and 6 (Figure 4), 6 and 7 (Figure 5), 5 (Figure 6), 4 and 5 (Figure 7), and 4 (Figure 8). This seems to be in agreement with the findings of other research workers in this field (Farkas and Stahmann, 1966; Lobenstein and Linsey, 1961, 1963).

As to the physiological role of peroxidases in plant life, Lobenstein and Linsey (1961) reported that peroxidases are involved in the oxidation of many mono- and di-phenols, and aromatic amines to quinones in the presence of $H_2O_2$. Lobenstein and Linsey (1963) suggested a possible change from the glycolytic to the pentose pathway in virus-infected tissues. Also, they reported that increase in peroxidase activity were invariably associated with increased respiration rates. Farkas and Stahmann (1966) reported that the appearance of additional peroxidases was found to be associated with an overall increase in peroxidase activity.

According to them, new peroxidases are formed by the triggering of enzyme protein synthesis in the host, as the formation of new peroxidases in their study could be inhibited by three different inhibitors of protein synthesis, actinomycin D, P-fluorophenyl-alanine and puromycin. They attributed the triggering of enzyme protein synthesis to the accelerated aging of the virus-infected leaves and the necrobiosis associated with symptoms development.

This study, which is qualitative in nature, revealed that virus infection resulted in increased peroxidase activity demonstrated by the

development of one or two additional peroxidase components. This could be used to identify diseased plants. The study was conducted when the seedlings were 5 months old and the varieties were 8 months old.

Many sugarcane varieties and clones are symptomless carriers of the ratoon stunting disease virus. To investigate whether these symptomless carriers show increases in peroxidase activity and whether this increase can be used to identify them, a Chi square test was conducted to compare the efficiency of the visual test and the peroxidase test.

Table I shows the reaction of the different Hawaiian varieties to ratoon stunting disease infection when examined visually, by the peroxidase, and by the indicator plant methods. An insignificant Chi square (Table II) was obtained when comparing the visual test plant counts of the Hawaiian varieties and those of peroxidase counts, indicating that there is no significant difference between the two tests. However, since only the plants which do not show the visual symptoms are tested by the use of the indicator plant, there would not be any basis for comparing the visual test and the indicator plant test. The data in Table I show that the visual test followed by the indicator plant test of the plants that did not show the visual symptoms is more exact in identifying all diseased plants in a progeny.

Table III shows the reaction of the progeny of the nine crosses to R.S.D. infection when examined visually, by the peroxidase and the indicator plant methods. An insignificant Chi square was obtained when comparing the visual and peroxidase methods of testing (Table IV), indicating that there is no statistically significant difference

TABLE I.  HAWAIIAN SUGARCANE VARIETIES TESTED FOR
R.S.D. VISUALLY, BY THE USE OF TEST PLANT
AND BY PEROXIDASE ISOZYMES METHODS

| No. | Variety | Method | | |
|-----|---------|--------|---|---|
|     |         | Visual | Peroxidase | Test Plant |
| 1   | H45-2120 | +[a] | + | -- |
| 2   | H60-6909 | 0    | 0 | 0  |
| 3   | H61-2757 | PS   | 0 | +  |
| 4   | H60-6744 | 0    | + | +  |
| 5   | H60-6314 | +    | + | -- |
| 6   | H59-3932 | PS   | 0 | +  |
| 7   | H59-6699 | 0    | 0 | +  |
| 8   | H60-3081 | PS   | + | +  |
| 9   | H58-9236 | +    | 0 | -- |
| 10  | H59-7444 | +    | + | -- |
| 11  | H59-3749 | PS   | + | +  |
| 12  | H59-3775 | 0    | 0 | +  |
| 13  | H57-1472 | 0    | + | +  |
| 14  | H58-3820 | 0    | + | +  |
| 15  | H59-9084 | +    | + | -- |
| 16  | H61-2757 | PS   | + | +  |
| 17  | H58-6247 | PS   | 0 | +  |
| 18  | H58-6273 | 0    | 0 | +  |
| 19  | H58-5087 | PS   | + | +  |

[a]PS = Poor Symptoms
 +  = Symptoms
 0  = No symptoms
 -- = Not tested

TABLE II.  TEST OF THE HYPOTHESIS OF INDEPENDENCE
OF THE VISUAL AND PEROXIDASE TESTS
FOR THE HAWAIIAN VARIETIES

| Test | Diseased | Healthy | Total |
|------|----------|---------|-------|
| Visual | 12 | 7 | 19 |
| Peroxidase | 11 | 8 | 19 |
| Total | 23 | 15 | 38 |

$$X^2 = 0.1100 \text{ n.s.}$$

There is no significant difference between the two
methods of testing, therefore, the hypothesis of independence
is accepted.

TABLE III.   PROGENY OF THE CROSSES TESTED BY THE VISUAL, THE
PEROXIDASE AND THE INDICATOR PLANT METHODS.   THE
VALUES UNDER EACH METHOD REPRESENT THE
NUMBERS OF DISEASED PROGENY

|  | Cross | Total No. Tested | Method | | |
|---|---|---|---|---|---|
|  |  |  | Visual | Peroxidase | Indicator Plant |
| 1 | 333 A | 31 | 7 | 8 | 17 |
| 2 | 481 A | 34 | 19 | 20 | 11 |
| 3 | 481 B | 32 | 3 | 5 | 1 |
| 4 | 482 B | 56 | 3 | 4 | 7 |
| 5 | 483 | 24 | 1 | 2 | 6 |
| 6 | 484 A | 27 | 2 | 4 | 4 |
| 7 | 484 B | 33 | 19 | 21 | 13 |
| 8 | 485 A | 22 | 3 | 3 | 6 |
| 9 | 485 B | 21 | 5 | 7 | 11 |
| Total |  | 280 | 62 | 74 | 76 |

TABLE IV.  TEST OF THE HYPOTHESIS OF INDEPENDENCE OF THE
VISUAL AND PEROXIDASE TESTS FOR THE PROGENY
OF THE CROSSES INVESTIGATED

| Test | Healthy | Diseased | Total |
|------|---------|----------|-------|
| Visual | 218 | 62 | 280 |
| Peroxidase | 206 | 74 | 280 |
| Total | 424 | 136 | 560 |

$$x^2 = 1.3984 \text{ n.s.}$$

There is no significant difference between the two methods
of testing, therefore, the hypothesis of independence is accepted.

bewtwen the two methods of testing. This implies that the increase in peroxidase activity and development of additional peroxidase components is closely associated with the sensitivity of the variety to virus infection and the development of the internodal discoloration. This seems to be in agreement with the findings of Solymosy, Szirmai, Beczner and Farkas (1967), who reported that increase of peroxidase activity is closely associated with symptom development. Lobenstein and Linsey (1961) also mentioned that the increase in peroxidase activity follows symptom development.

The analysis for peroxidase isozymes was carried out again when the progeny was 8 months old. Only the plants which showed increases in peroxidase activity at 5 months showed it at 8 months.

At this stage, it may be worthwhile to note that differences in peroxidase isozymes observable in plants that show the internodal discoloration are of no value in identifying symptomless carrier sugarcane plants. The test plant procedure remains the sole known method of identifying the symptomless carriers.

Inheritance of Resistance to R.S.D.:

Indications of existence of sugarcane clones that are tolerant to ratoon stunting disease trace back to the time when the disease was described. Steindl and Hughes (1953) described the varieties Badila and Comus as showing some degree of tolerance. Todd (1956) claimed that inherent resistance to ratoon stunting disease existed in some sugarcane clones in Louisiana. However, no further support to his conclusions was reported.

Sugarcane is a high polyploid and heterozygous, making it difficult to determine the genetic nature of its characters, including disease resistance. The purpose of this investigation was to determine if some of the clones involved in these crosses exhibit some degree of resistance which could be used in breeding.

As indicated previously, the selection of the parents of the progeny under investigation was based on their reaction to ratoon stunting disease following a series of inoculations with infected juice and observations at the Exp. Sta., HSPA.

The method of inoculation utilized in the study proved to be 100 percent successful. Table V, consisting of limited populations of each progeny tested visually and by the test plant method to determine the symptomless carriers, suggests that these progenies reacted differently to infection. This is indicated by the highly significant Chi square (Table VI) when testing the hypothesis of independence.

Table VII consisting of larger populations of the three major categories (resistant x resistant, resistant x susceptible and susceptible x susceptible parents), further supports the hypothesis that reaction to infection was dependent on the type of cross since the differences were highly significant. Also, the 100 percent infection obtained in H45-2120 which was raised under the same environmental conditions and cultural treatments implies that the differences in reaction to the disease shown by the different progeny might have been due to genetic variation.

The histogram (Figure 9) shows that except for the progeny of the cross "H60-6909 x H49-3533", all other progenies of which one of the

TABLE V.  PERCENTAGE OF RESISTANT PLANTS IN THE
PROGENIES OF THE CROSSES STUDIED

| | Cross | No. of Plants Tested | Diseased | Symptom-less Carriers | Total Diseased | Percent Resistance |
|---|---|---|---|---|---|---|
| 1 | H53-263 x H53-1447 | 31 | 7 | 17 | 24 | 22.58 |
| 2 | H60-6909 x H49-3533 | 34 | 19 | 11 | 30 | 11.76 |
| 3 | H49-3533 x H60-6909 | 32 | 3 | 1 | 4 | 87.50 |
| 4 | H39-7028 x H60-6909 | 56 | 3 | 7 | 10 | 82.14 |
| 5 | H50-723 x H60-6909 | 24 | 1 | 6 | 7 | 72.00 |
| 6 | H50-7209 x H60-6909 | 27 | 2 | 4 | 6 | 77.77 |
| 7 | H60-6909 x H50-7209 | 33 | 19 | 13 | 32 | 96.97 |
| 8 | H53-263 x H60-6909 | 22 | 3 | 6 | 9 | 59.09 |
| 9 | H60-6909 x H53-263 | 21 | 5 | 11 | 16 | 23.81 |

TABLE VI.  SUMMARY OF THE REACTIONS OF THE
VARIOUS PROGENIES TO INFECTION

|   | Cross | Healthy | Diseased | Total |
|---|---|---|---|---|
| 1 | H53-263 x H53-1447 | 7 | 24 | 31 |
| 2 | H60-6909 x H49-3533 | 4 | 30 | 34 |
| 3 | H49-3533 x H60-6909 | 24 | 4 | 32 |
| 4 | H39-7028 x H60-6909 | 46 | 10 | 56 |
| 5 | H50-723 x H60-6909 | 17 | 7 | 24 |
| 6 | H50-7209 x H60-6909 | 21 | 6 | 27 |
| 7 | H60-6909 x H50-7209 | 1 | 32 | 33 |
| 8 | H53-263 x H60-6909 | 13 | 9 | 22 |
| 9 | H60-6909 x H53-263 | 5 | 16 | 21 |
| Total | | 142 | 138 | 280 |

$$X^2 = \frac{(f - F)^2}{F} \quad \text{(Snedecor, p. 226)}$$

Where f  = Observed values

$$F = \frac{(\text{row total}) (\text{column total})}{n}$$

n  = Grand total

$$X^2 = 118.425**$$

The highly significant Chi square shows that the reaction to infection is dependent on the type of cross.

TABLE VII.  EFFECT OF INOCULATION ON THE POPULATIONS
OF R x R, R x S AND S x S CROSSES

| Cross | Type | No Symptoms | Symptoms | Symptomless Carriers | Total | Percent Resistance |
|-------|------|-------------|----------|----------------------|-------|--------------------|
| H39-7028 x H60-6909 | R x R | 100 | 41 | 98 | 239 | 41.84 |
| H50-7209 x H60-6909 | S x R | 74 | 109 | 79 | 262 | 28.24 |
| H53-263 x H53-1447 | S x S | 82 | 14 | 118 | 214 | 38.31 |
| Total | | 256 | 164 | 295 | 715 | |

$$x^2 = 93.1836**$$

The highly significant Chi square indicates the dependence of response to R.S.D. infection on the type of cross.
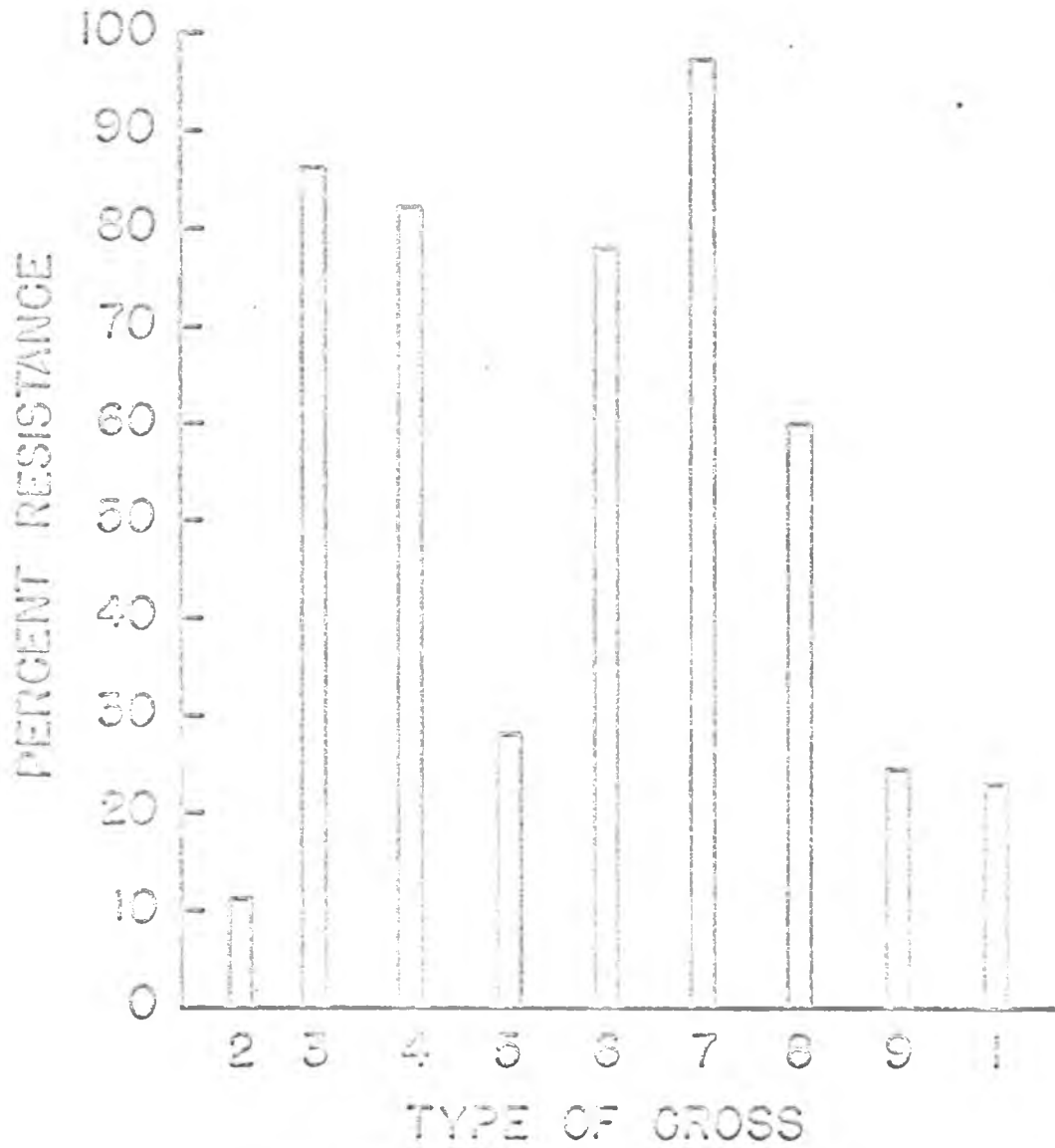
2 = H60-6909 x H49-3533
3 = H49-355 x H60-6909
4 = H60-6909 x H49-3533
5 = H50-723 x H60-6909
6 = H50-7209 x H60-6909

7 = H60-6909 x H50-7209
8 = H53-263 x H60-6909
9 = H60-6909 x H53-263
1 = H53-263 x H53-1447

FIGURE 9.   REACTION OF THE VARIOUS PROGENIES TO INFECTION.

parents was resistant to the disease gave larger numbers of resistant plants than progeny of the cross H53-263 x H53-1447 in which both parents were susceptible.

The data (Table V) imply that resistance to R.S.D. was not inherited as a simple Mendelian character or a typical polygene system but rather by a few major genes, since we get a high percentage (38.31) from the cross H53-263 x H53-1447 in which both parents were susceptible. However, the existence of modifier genes influencing the expression of resistance cannot be ruled out, especially when dealing with such a genetically complex crop.

Such conclusions were also reported by other authors investigating inheritance to resistance of sugarcane diseases. Azab and Chilton (1952) reported that resistance of sugarcane varieties to red rot disease is governed by one or a few major genes for resistance from S. spontaneum together with a dominant inhibitor gene from S. officinarum. The inhibitor gene from S. officinarum masked the effects of genes for resistance from S. spontaneum. They also suggested that a group of modifier factors influenced the degree of reaction of the plants to the disease. They also mentioned that resistance of sugarcane varieties to mosaic disease is governed by complementary factors with resistance being dominant. Modifier factors were also said to exist.

The cross H49-3533 x H60-6909 gave almost eight times more resistant progeny than its reciprocal, while the cross H60-6909 x H50-7209 gave about one and one-fourth times more resistant progeny than its reciprocal. This implies a certain degree of cytoplasmic inheritance which might have been modified by genic interaction. The

susceptible progeny comprises the plants which responded positively to the visual test plus the symptomless carriers.

Table VIII shows that a significant Chi square was obtained when comparing the data resulting from the two crosses (H60-6909 x H49-3533 and H49-3533 x H60-6909) indicating significant differences in the progeny of the two crosses with regard to their reaction to the disease.

Although the crosses H60-6909 x H49-3533 and H49-3533 x H60-6909 resulted from parents considered resistant to the disease, the variety H60-6909 was found to behave poorly as a female parent in transmission of disease resistance to its progeny, thus giving less resistant progeny when used as a female parent. This behavior of H60-6909 will be further clarified later in this discussion.

The progeny of the crosses H50-7209 x H60-6909 and H60-6909 x H50-7209 resulting from susceptible x resistant and resistant x susceptible parents, respectively, shows that more resistant progeny were obtained when the resistant parent was used as the female than vice versa. This is indicated by the highly significant Chi square (Table IX).

This implies that more resistant progeny should be expected from the cross H60-6909 x H53-263 (R x S) rather than from its reciprocal cross H53-263 x H60-6909 (S x R); but again this might have been due to the poor performance of H60-6909 as a female parent as is the case with the cross H60-6909 x H53-263. Table X shows a significant Chi square value was obtained which indicates that the progeny of the two crosses reacted differently to the disease.

The phenomenon of cytoplasmic inheritance, referring to a higher degree of expression of the maternal characters in the offspring due

TABLE VIII.  EFFECT OF INOCULATION ON THE CROSS
H60-6909 x H49-3533 AND ITS RECIPROCAL
H49-3533 x H60-6909

| Cross | Healthy | Diseased | Total |
|---|---|---|---|
| H60-6909 x H49-3533 | 4 | 30 | 34 |
| H49-3533 x H60-6909 | 28 | 4 | 32 |
| Total | 32 | 34 | 66 |

$$X^2 = 37.8564**$$

The reaction to infection depends on the type of cross.

TABLE IX.  EFFECT OF INOCULATION ON THE CROSS
H50-7209 x H60-6909 AND ITS RECIPROCAL
H60-6909 x H50-7209

| Cross | Healthy | Diseased | Total |
|---|---|---|---|
| H50-7209 x H60-6909 | 21 | 6 | 27 |
| H60-6909 x H50-7209 | 1 | 32 | 33 |
| Total | 22 | 38 | 60 |

$$X^2 = 35.7284**$$

The reaction to infection depends on the type of cross.

TABLE X.  EFFECT OF INOCULATION ON THE CROSS
H53-263 x H60-6909 AND ITS RECIPROCAL
H60-6909 x H53-263

| Cross | Healthy | Diseased | Total |
|-------|---------|----------|-------|
| H53-263 x H60-6909 | 13 | 9 | 22 |
| H60-6909 x H53-263 | 5 | 16 | 21 |
| Total | 22 | 38 | 60 |

$$X^2 = 5.4950*$$

The reaction to infection depends on the type of cross.

to the contribution of the largest amount of cytoplasm by the female germ cell in the egg, has support from a number of authors studying inheritance of characters in sugarcane. Natarajan, Krishnamurthy and Thuljaram (1967) reported the occurrence of higher maternal influence in progeny performance of certain sugarcane crosses with regard to millable canes at harvest, height of stalk and brix percent in juice irrespective of the effects of the pollen parent. Raghavan (1964) interpreted the results of the cross between P 8831 (higher brix) and Co.605 (higher weight), made reciprocally, on the basis of cytoplasmic inheritance. More seedlings with higher brix were obtained when P 8831 was used as the female parent while more seedlings with higher weight were obtained when Co.605 was used. Raghavan (1964) argued that a much wider range of variation would be expected instead of so much similarity to the pistillate parent with regard to these characters were the inheritance purely genic.

To investigate the behavior of the variety H60-6909 as a female and a male parent, the data obtained from the crosses H60-6909 x H49-3533, H60-6909 x H50-7209 and H60-6909 x H53-263 in which H60-6909 is the female parent and the data obtained from the crosses H49-3533 x H60-6909, H50-723 x H60-6909, H50-7209 x H60-6909, H53-263 x H60-6909 and H39-7028 x H60-6909 in which H60-6909 is the male parent were analyzed.

Table XI shows that in these three crosses less resistant progenies were obtained when H60-6909 was used as the female and, as indicated by the insignificant Chi square, H60-6909 behaved similarly in both crosses. The discrepancy in the data was due to chance variation.

TABLE XI.  COMPARISON OF THE BEHAVIOR OF H60-6909 AS THE FEMALE
PARENT IN CROSSES H60-6909 x H49-3533, H60-6909 x
H50-7209 AND H60-6909 x H53-236

| | Cross | Healthy | Diseased | Total |
|---|---|---|---|---|
| 1 | H60-6909 x H49-3533 | 4 | 30 | 34 |
| 2 | H60-6909 x H50-7209 | 1 | 32 | 33 |
| 3 | H60-6909 x H53-236 | 5 | 16 | 21 |
| | Total | 10 | 78 | 88 |

$$X^2 = 5.5101 \text{ n.s.}$$

The reaction to infection does not depend on the type of cross.
H60-6909 behaved similarly in the three crosses.

In the other five crosses, where H60-6909 was the male parent, more resistant progenies were obtained (Table XII), and the variety also behaved similarly in both crosses as indicated by the non-significant Chi square value. Since the data obtained were found to be homogeneous, a comparison of the behavior of H60-6909 as a female and a male parent could be made.

A highly significant Chi square value was obtained when comparing the data (Table XIII) which represented the totals obtained from the two crosses utilizing H60-6909 as a female parent on one hand as a a male parent on the other. This implies that H60-6909 behaved differently when used as a male and a female parent. It was more able to transmit resistance to R.S.D. when used as a male parent. This also supports the view that cytoplasmic genic inheritance might have been involved in transmission of resistance to ratoon stunting disease. Michaelis (1952) mentioned that such behavior can be attributed to either the male cytoplasm of the pollen tube entering the egg cell or to the selective power of genes on multiplication or elimination of plasmic units.

TABLE XII. COMPARISON OF THE BEHAVIOR OF H60-6909 AS THE
MALE PARENT IN CROSSES H49-3533 x H60-6909, H50-723 x
H60-6909, H50-7209 x H60-6909, H53-263 x H60-6909
x H39-7028 x H60-6909

| | Cross | Healthy | Diseased | Total |
|---|---|---|---|---|
| 1 | H49-3533 x H60-6909 | 28 | 4 | 32 |
| 2 | H50-723 x H60-6909 | 17 | 7 | 24 |
| 3 | H50-7209 x H60-6909 | 21 | 6 | 27 |
| 4 | H53-263 x H60-6909 | 13 | 9 | 22 |
| 5 | H39-7028 x H60-6909 | 46 | 10 | 56 |
| | Total | 125 | 36 | 161 |

$$X^2 = 7.446 \text{ n.s.}$$

H60-6909 behaved similarly in the five crosses.

TABLE XIII.  COMPARISON OF THE BEHAVIOR OF H60-6909
AS THE FEMALE OR MALE PARENT UTILIZING THE
DATA FROM TABLES XI AND XII

|                                   | Healthy | Diseased | Total |
|-----------------------------------|---------|----------|-------|
| Total for H60-6909 as female      | 10      | 78       | 88    |
| Total for H60-6909 as male        | 125     | 36       | 161   |
| Total                             | 135     | 114      | 249   |

$$X^2 = 100.6892**$$

The reaction to infection depends on the type of cross.  There
is significant difference between the behavior of H60-6909 as male
and female.

## SUMMARY AND CONCLUSIONS

The objectives of this investigation were:

1. To determine if there were differences in peroxidases acti-
   vity in healthy and ratoon stunting disease infected
   sugarcane plants and if these differences could be made
   use of in identifying diseased plants.

2. To determine the mode of inheritance of resistance to ratoon
   stunting disease of sugarcane.

Nine different crosses were made to give the following combina-
tions:

> resistant x resistant
>
> resistant x susceptible
>
> susceptible x susceptible

Starch-gel electrophoresis was used to determine the differences
in peroxidase isozymes in healthy and diseased sugarcane plants.
Besides the progeny of the above listed crosses, the differences in
peroxidase isozymes of 26 Hawaiian sugarcane varieties were also
investigated. Sap from leaves of diseased and healthy plants was
extracted and analyzed for peroxidase isozymes.

Peroxidase activity was found to be higher in leaves of plants
which showed the internodal discoloration. The increase in peroxidase
activity was indicated by the appearance of one or two additional
peroxidase components in the leaves of diseased plants.

Although this might be beneficial in identifying diseased
sugarcane plants, its use would be limited to those plants which could

be identified more rapidly and easily by the visual test, since the
method was inefficient in determining the symptomless carriers.

To determine the mode of inheritance of ratoon stunting disease
of sugarcane, the progeny resulting from the various crosses was
pressure-inoculated with sap from ratoon stunting diseased sugarcane
plants. On examining these for the disease they were rated as fol-
lows:

0 = No symptoms

1 = No definite symptoms

2-5 = Definite symptoms

Juice from the plants falling in the first two categories (0 and
1) was used to inoculate seed pieces from the indicator variety
H45-2120. On re-testing, the plants which induced the internodal
discoloration of the test variety were designated as symptomless
carriers.

The data implied that inheritance of ratoon stunting disease was
governed by a few major genes for resistance, the influence of which
might have been modified by various modifying factors.

Evidence of the occurrence of cytoplasmic inheritance modified
by genic interaction was also observed on examining progeny of the re-
ciprocal crosses. Also, the resistant variety H60-6909 was found to
transmit resistance to ratoon stunting disease better when it was used
as the male parent instead of the female parent. This breeding behavior
of H60-6909 is anomalous, since it indicates that the variety lacks the
cytoplasmic component for R.S.D. resistance, and yet strongly displays
the characteristic of R.S.D. resistance. Further work is needed to
elucidate this point.

# LITERATURE CITED

Anon. 1953. Ann. Rept., Exp. Sta., HSPA. P. 20-22.

Anon. 1956. Ann. Rept., Exp. Sta., HSPA. P. 25.

Antoine, R. 1959. Note on the Tetrafolium test for detecting R.S.D. in sugar cane. I.S.S.C.T. 10th Cong. P. 1042-1043.

Artschwager, E. 1959. Sieve tube lignification in sugar cane and its significance in relation to the ratoon stunting disease. I.S.S.C.T. 10th Cong. P. 1077-1083.

Ashton, G. C. 1967. Private communication.

Azab, Y. E. and Chilton, J. P. 1952. Studies on inheritance of resistance to red rot disease of sugar cane. Phytopath. 42: 282. (Abstr.).

Barnes, A. C. 1957. Sugar cane growing in Nigeria. South African Sug. J. 41: 191-195.

Bell, A. F. 1935. Two inoculation methods. Proc. I.S.S.C.T. 5th Cong. P. 199-201.

Chu, H. T. and Liu, H. P. 1956. Investigations on the stunting disease in NCO. 310. Rep. Taiwan Expt. Sta. No. 14. P. 83-92.

Chu, H. T. and Ling, K. C. 1958. Recent progress in stunting disease control in Taiwan. Taiwan Sug. J. 1958: 26-31.

Farkas, G. L. and Stahmann, M. A. 1966. On the nature of changes in peroxidase isozymes in bean leaves infected by Southern bean mosaic virus. Phytopath. 56: 669-677.

Farrar, L. L. 1957. A chemical test for ratoon stunting disease of sugarcane. Phytopath 47: 10. (Abstr.).

Fife, J. M. and Stokes, I. E. 1959. Amino acid changes identify ratoon stunting disease. Sugar Y Azucar. December. P. 27-28.

Forbes, I. L. and Ling, K. C. 1960. Particles associated with the ratoon stunting disease of sugar cane. Sug. J. 23(2): 15.

Hughes, C. G. and Steindl, D. R. L. 1956. Some further developments in the study of R.S.D. in Queensland. Proc. I.S.S.C.T. 9th Cong. P. 1012-1022.

King, N. J. and Steindl, D. R. L. 1953. The relationship between varietal yield deterioration and R.S.D. Proc. I.S.S.C.T. 8th Cong. P. 851-862.

King, N. J. 1954. The implications of R.S.D. Int. Sug. J. May. P. 125-127.

King, N. C. 1956. R.S.D. in Natal. South Afr. Sug. J. 40: 107-119.

King, N. J., Mungomery, R. W. and Hughes, C. G. 1965. Manual of Cane Growing. Elsevier Pub. Co. P. 270-274.

Lobenstein, G. and Linsey, N. 1961. Peroxidase activity in virus infected sweet potatoes. Phytopath. 51: 533-537.

Lobenstein, G. and Linsey, N. 1963. Effects of virus infection on peroxidase activity and C6/C ratios. Phytopath. 53: 350. (Abstr.).

Mangelsdorf, A. J. 1953. Sugarcane breeding in Hawaii. Part II. 1921-1952. The Hawaiian Planters Record 54: 101-137.

Markert, C. L. and Moller, F. 1959. Multiple forms of enzymes tissue, ontogenetic and species, specific patterns. Proc. Nat'l. Acad. Sci. U.S. 45: 753-763.

Michaelis, P. 1952. Interactions between genes and cytoplasm in Epilbium. Cold Spring Harb. Symp. on Quant. Biol. 16: 121-129.

Menke, J. H. and Walker, J. C. 1963. Metabolism of resistant and susceptible cucumber varieties infected with cucumber mosaic virus. Phytopath. 53: 1349-1355.

Natarajan, B. V., Krishnamurthy, T. N. and Thuljaram, J. Rao. 1967. Relative effects of parents on some economic characters in sugarcane. Euphytica 16: 104-108.

Navarrete, F. S. 1959. R.S.D. at San Cristobal. Proc. I.S.S.C.T. 10th Cong. P. 1083-1091.

Raghavan, T. S. 1964. Cytoplasmic inheritance in Saccharum. Indian J. Sug. Cane Res. Development 8: 134-139.

Schexnayder, C. A. 1956. The ratoon stunting disease in Louisiana with notes on its control. Proc. I.S.S.C.T. 9th Cong. P. 1058-1065.

Schexnayder, C. A. 1959. The use of a sugarcane "test plant" as a means of detecting the presence of ratoon stunting diseases in sugarcane. Proc. I.S.S.C.T. 10th Cong. P. 1068-1072.

Sheffield, F. M. L. 1956. R.S.D. A degeneration disease of sugar cane. East Afr. Agric. J. 22: 70-72.

Snedecor, G. W.  1962.  Statistical Methods.  5th Ed.  The Iowa State University Press, Ames, Iowa, U.S.A.  P. 219.

Solymosy, F., Szirmai, L. Beczner, L. and Farkas, G. L.  1967. Peroxidase isozyme patterns induced by virus infection.  Virology. 32: 117-121.

Siegel, B. Z.  1967.  Private communication.

Steib, R. J., Forbes, I. L. and Chilton, S. J. P.  1957.  A report on further studies on ratoon stunting disease of sugar cane in Louisiana.  Sug. J. 19(10): 35-36.  (Abstr.).

Steib, R. J. and Chilton, S. J. P.  1959.  Control and rate of increase of ratoon stunting disease in hot air treated sugar cane in Louisiana.  Proc. I.S.S.C.T.  10th Cong.  P. 1061-1068.

Steindl, D. R. L.  1950.  R.S.D.  Proc. I.S.S.C.T.  7th Cong.  P. 457-465.

Steindl, D. R. L. and Hughes, C. G.  1953.  R.S.D.  Cane Grower's Quarterly.  Queensland.  16: 79-95.

Steindl, D. R. L.  1957.  Host range of the sugar cane ratoon stunting disease virus.  J. Aust. Inst. Agr. Sci. 23: 238.

Todd, E. H.  1956.  Apparent resistance to R.S.D.  Proc. I.S.S.C.T. 9th Cong.  P. 1169-1173.

Veiga, F. M.  1956.  R.S.D. in Brazil.  Cane Grower's Quarterly Bull. Queensland.  20(1): 2-6.

Wehlberg, C.  1956.  R.S.D. in Cuba.  Sugar 51(3): 27-29.

Weihe, P. O.  1954.  R.S.D.  Annual Report.  Mauritius Sug. Inds. Res. Instit.  P. 47-48.

Wismer, C. A.  1964.  Ann. Rept. Exp. Sta., HSPA.  P. 30.

Wismer, C. A.  1965.  Ann. Rept. Exp. Sta., HSPA.  P. 32.