ABSTRACT

The Effect of Latent Virus Diseases on the Growth, Yield and Quality of Sebago Potatoes

By Sung Man Lim

The effects of various virus diseases upon the symptomatology, vigor, yield and quality of Sebago variety, Solanum tuberosum L. were observed over a two year period. Serological techniques tested by bioassay methods were applied to greenhouse grown plants to determine the absence or presence of the viruses. The plants were classified as healthy or infected with virus S, X or the combination of viruses S and X. For field plantings young potato plants were separated from the mother tubers with a sterilized knife and transplanted into the flats according to the serological classifications.

All plants classified as virus infected were inoculated with specific viruses to insure infection with single viruses or combinations of the viruses. Since virus Y had not been found in the tested plants, healthy plants were inoculated with virus Y, virus S or virus X to give all combinations of the virus.

Three field experiments were planted at the Lake City Experiment Station. Ten replicated hills, each comprised of four plants, were space planted five feet apart in a randomized complete block design. The number of plants infected with each virus changed from the original numbers due to the absence of virus Y and its combinations with the other viruses-infected plants. Many of these subsequently

became infected with virus X and the combination of viruses S and X.

The number of plants was, therefore, regrouped and data were analyzed on the basis of the results of the agglutination tests at different dates by combining the three field experiments.

In this experiment, both viruses S and X were mild viruses and the symptoms were not severe. A somewhat lighter green with slight mottling was observed on the leaves of virus S-infected plants, but these symptoms were not consistently present or clearly visible. A mottling, light yellowish areas and a number of small necrotic lesions on the leaves of virus X and the combination of viruses S and X-infected plants were observed. The combination of viruses S and X did not appear to be a new complex symptom although the small necrotic lesions seemed to be more pronounced on the leaves of the plants infected with both viruses S and X.

The healthy plants exhibited a more luxuriant growth and were larger throughout the growing season than all virus-infected plants except those infected with virus S. There was no difference in plant growth between healthy and virus S-infected plants in the early growing season. However, the size of the healthy plants exceeded that of the virus S infected plants later in the season.

The reductions in tuber yield varied with the various potato viruses. The virus infections affected the large tubers, those above 5 cm in diameter, for both number and weight. Virus S did not affect the number of tubers but it seemed to affect tuber weight.

Both virus X and the combination of viruses S and X reduced both the number and weight of tubers. The percentage reduction in tuber yield was 17.5% for virus X, 15.5% for the combination of viruses S and X, and 6% for virus S respectively.

Apparently virus infection in this experiment did not affect the specific gravity and was not associated with the color of the potato chips.

Foliage weight and tuber yield were positively correlated at a highly significant level. The greatest tuber yield and foliage weight were obtained from the healthy plants. Correlation of dry matter of foliage and tuber yield was negative but there were no significant differences. The dry matter of the potato foliage of the healthy plants was not greater than that of the virus X or virus S-infected plants. The combination of viruses S and X infected plants reduced the dry matter of the foliage, but differences were very small. In this experiment, the virus-infected plants could have had a reduced rate of photosynthesis and disturbed carbohydrate metabolism causing interference with the translocating of reserves from diseased leaves to the tubers. It is concluded that the effect of virus X appeared early in growing stage. The effect of virus S appeared after the period of tuber set and resulted in a decreased number of large tubers.

Finally, it has been shown that the agglutination test is a technique applicable to the identification of mild infections of viruses, and that plants in which the virus was not detected produced the greatest yields of marketable tubers. It can be utilized in a seed program to eliminate many plants which are a potential source of infection.

THE EFFECT OF LATENT VIRUS DISEASES ON THE GROWTH, YIELD AND QUALITY OF SEBAGO POTATOES

By

Sung Man Lim

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INTRODUCTION

In modern potato production a high yield of marketable potatoes is synonomous with efficiency and profit. To attain high yields, disease free seed is essential. Some diseases may be readily identified by symptomatology and are easily removed from seed stocks. Others are latent and difficult to diagnose. Their effects are not immediately obvious and their importance has been questioned.

Although there has been some work on problems concerning the effect of viruses upon the growth and yield of potatoes, the results are somewhat inconsistent so cannot be applied universally. For instance, the reduction in yield from virus-infected potatoes reported by several investigators varies from a very small amount to as high as 30 percent.

Roguing or early lifting of visibly diseased plants is general practice in seed programs. However, the viruses that are practically symptomless and difficult to diagnose remain. Only since the development of serological methods, whereby antisera are mass produced, has a tool been available to attempt to remove the latent viruses.

The present study was undertaken to obtain information on the growth, yield and quality of potatoes infected with virus diseases. Serological and bioassay techniques were used to determine the absence or presence of the viruses.

REVIEW OF LITERATURE

Since Schultz and Folsom (37) in the United States and Quanjer (34) in Holland, described a number of virus diseases of the potato Solanum tuberosum L. in 1923, a great amount of research has been conducted and many papers published.

The reduction in yield from virus X-infected potatoes reported by several investigators varies from a very small amount to as high as 30%. The reduction varies with the potato variety and the strain of virus X (29, 32, 38, 39, 47). Information regarding the effect of virus S on potato yield is scarce and no reports were found regarding the effect of viruses S and X- in combination on potato yields.

In Maine, yield reductions of approximately 12 to 22% were obtained by Schultz et. al. (38) over a four-year period with potato virus X-infected stocks of the Chippewa and Katahdin varieties when compared with potato virus X-free stocks of the same varieties. Although a very few potato virus X-free stocks have been found among some of the old varieties by the above authors, they were not able to obtain a single tuber free from virus X when 1000 Green Mountain tubers from several potato regions were tested. They found it practically impossible to diagnose latent mosaic in the field.

Seed stocks of the varieties Chippewa, Sebago and Teton, carrying a mild strain of potato virus X, were compared with virus X-free stock and commercial stock for each variety by Lombard (29). Both

mild X and commercial seed produced reduced yields when compared to yields from virus X-free stock. Percentage reductions in yield for mild X were: Chippewa 6%, Sebago 4%, and Teton 9%. Percentage reductions in yield for commercial stock were: Chippewa 6.8%, Sebago 9.8% and Teton 5.1%.

Yield reduction in Chippewa and Katahdin was 6% with virus X-infected plants in New York and reduced stands were observed in virus X-infected Sebago stock according to Wilkinson et. al. (47). In the Sebago variety, there was a marked effect on the stands in addition to an effect on yields.

In England, yield reduction in Majestic and Arran Banner ranged from 5 to 22% depending upon the severity of the virus X present, according to Bawden et. al. (5).

Norris (32) found that potato virus X (latent mosaic) in the President and Up-to-Date varieties reduced the yield about 30% and that it was one of the chief causes of the reduction in yield of potatoes in Australia. Its effects were evenly spread over the entire crop.

Scott (39) reported that in Scotland potato virus X (latent mosaic) was responsible for yield reductions of 16 to 25% and that similar yield losses resulted from potato virus A.

Bald (4) has shown that the effect of a masked strain of virus X upon the yield of the Up-to-Date variety is due to the inability of diseased plants to transport hydrolyzable reserve leaf proteins to the tubers for late-season expansion. There was no difference between the

yields of healthy and X-infected plants which were harvested 14 to 15 weeks after emergence of the plants, but at maturity, diseased plants yielded about 11 percent less than healthy ones. He suggested that final rapid tuber expansion at maturity was due to the translocation of reserve proteins from aging leaves in normal plants, and that virus infection interfered with the emptying of such reserves from diseased leaves.

Transport and distribution of products of photosynthesis in potato plants were studied using ^{14}C and a starch test by Kosmakova (25). During the flowering phase, products of photosynthesis were assimilated chiefly into the tubers, with a small amount entering the roots and infloresences. After flowering ^{14}C was found only in the roots and tubers, but mostly in the latter. Later in the season, just before harvest, the products from the leaves was translocated only to the tubers. A starch test showed that in plants with mottling, leaf curl, and wrinkled mosaic, carbohydrate metabolism in the leaves was disturbed with less starch accumulating in them than in the leaves of healthy plants.

Daniel et. al. (15) reported that infection by a masked strain of virus X caused decreased photosynthesis in leaf tissue. Photosynthetic rates were decreased in symptomless Y-infected leaves, and double infections resulted in the greatest decrease in rates of photosynthesis. Systemic infections by viruses which invade leaf parenchyma apparently result in a decrease in the rate of photosynthesis, regardless of the symptoms produced. The reductions in yield caused by

masked strains of virus X can be attributed, at least in part, to reduced photosynthesis in diseased plants. The effects upon photosynthesis of the several chronic infections closely parallel those upon yields.

Yield reduction of virus S-infected Bintje has been observed in the Netherlands (36). In general the yield of virus S-infected clones was 10-15% below the healthy clones. Virus S-infected clones produced a higher percentage of a smaller tuber and a smaller percentage of the larger tubers. The average reduction of the size larger than 50 mm in diameter was 15-20%.

A similar reduction in yield was found in Switzerland, reported by Rozendaal (36).

Clark (12) counted the number of tubers per plant of the Rural New Yorker No. 2 variety, beginning at weekly intervals soon after the period of tuber formation and up to the time the plants were killed by frost. The range of growth period was from 64 to 127 days. He found very little increase in tuber set after the blossom stage and concluded that small tubers at harvesttime are the result of an uneven growth rate rather than a late set. It indicates that most small potatoes are of the same chronological age as the large ones.

Neither soil moisture nor loss of vines affected chip color at harvest (26). However, when potatoes were held at 60°F., differences between treatments developed. The tubers from saturated soil produced darker chips than tubers from dry soil. Wet soil conditions produced an intermediate effect on chip color. Loss of vines caused consistently darker chips at all moisture levels after 2 weeks storage, but the effect

of vine removal on chip color was less than the effect of soil moisture.

The problem of black discoloration after cooking was studied by Tottingham et. al. (43). One of their first concerns in approaching this problem was the possibility that the abnormality had a pathological origin. However, their trials in two seasons on field plots at several locations failed to prove the transmissibility of a pathological condition to the succeeding crop. They concluded that the blackening condition was due to physiological factors in the growth of the plants.

Akeley et. al. (1) observed that relatively large differences in density of tubers of the same variety occurred in the same field. The differences are still greater when the same variety is grown in different locations. Katahdin grown in 7 locations showed wide extremes in mean tuber-density class values from 3.1 to 8.5, a highly significant difference. He concluded that environmental conditions affect tuber density to a high degree, a variety grown in one location differing very greatly from the same variety grown in another place.

Heinze et. al. (20) has shown that the average specific gravity differs from year to year for the lots obtained from the same general location. Deviations indicate that these same lots of potato varieties were much more variable in some years than in others. The weighted average deviations for location show that potatoes tend to be more variable in specific gravity in some locations.

Transmission of one or more viruses, mottle virus, from apparently healthy potatoes of common varieties was reported by Johnson in 1925 (21). In Europe, this disease which shows a mild type of mottling in some varieties and has been described as simple mosaic or crinkle (31).

It is generally believed that Schultz and Folsom's rugose mosaic (37) is the same as the crinkle of Murphy and McKay (31) and the common mosaic as described by Quanjer (34).

In 1931 Smith (40) designated the virus causing this disease as X, which had been known in Europe as simple mosaic, and in the United States as latent mosaic or the healthy potato disease.

Different strains of virus X have been found in some of the European and American potato varieties. The intensity of symptoms of such complexes causing crinkle and mild mosaic varied depending upon the strain of X present in the complex (18).

A mosaic of the Chippewa variety characterized by irregular chlorotic-mottle on upper leaves and small scattered necrotic-flecks on older leaves was observed in the field by Larson (27). The external and internal root, stem, and petiole tissues of affected plants were normal. The tubers were slightly smaller than normal, but showed no pathologic symptoms. The question of insect transmission of potato virus X has been investigated by many workers. Clinch (13) stated that neither aphids nor leafhoppers need be considered as possible vectors of virus X. Thrips, flea beetles and various sucking insects have all been tested by Smith with negative results (41). However, he observed that in carefully controlled experiments odd infections do turn up from time to time. He commented that these infections were due to casual spread of the virus by grasshoppers or to a mechanical transport of the virus on the boots or implements of workers.

Walters (46) demonstrated that grasshoppers transmitted the virus from tobacco to tobacco in 6 percent of greenhouse trials.

Experiments on the spread of virus X in the field have been carried out by several workers. It is generally known that virus X is one of the few plant viruses which spreads in the field by contact between healthy and virus-infected plants (31, 40).

Virus X spreads through contact of sprouts in storage or foliage in the field (13, 35). It is also spread by the cutting knife and there is evidence that it is spread by contact between roots (35).

Observations were made by Roberts (35) on the spread of virus X in 40 plots containing from 8 to 99 healthy potato plants and a single infector plant in the central area of each plot during a period of five years. From the 40 infected sources 34 transmissions were recorded as the possible result of direct contact between healthy and infected plants. In 15 plots there was no spread of virus X within the season; in 17 plots only a single plant was infected; in 7 plots two plants adjacent to the infective source were infected; and in 1 plot infection spread to 3 adjacent plants.

Studies on the spread of potato virus X were carried out at 10 different locations by Hansen (19). The average of three years results showed that 18% of the original virus free Bintje plants were infected with potato virus X. During each of the three seasons the infection was 19, 20 and 14% respectively. The percentage for an individual location varied from 0 to 60%. The experiments comprised altogether 335 primary infected plants. The progency of these, about 3000,plants was tested

individually. About 40% of the tubers from these plants were virus X-infected. Only 10% of the primary infected plants gave completely infected progeny.

Cockerham (14) reported 19.7% infection in a stock of the Majestic variety grown adjacent to virus X-infected stocks, 54.2% in a stock which had been grown between infected Arran Banner, and 82.8% in a stock which had been grown throughout virus X-infected surroundings.

Beemster (8) studied the translocation of potato viruses in the potato plant from the inoculated leaf to the tubers. The results showed that very few tubers were infected with virus X when the inoculation was made on mature plants. On the other hand, a higher percentage of infected tubers was found when the tubers were harvested five weeks after inoculation than when harvested three weeks after inoculation. From these results he concluded that the translocation of virus X from the leaf to the tubers continued from the third to the fifth week even when the plants had been inoculated in a later stage.

A survey conducted by Todd (42) indicated that: 1) the spread of potato virus X is not normally rapid, 2) usually, infected plants could not be found until the second growing season but the content of infected plants would increase roughly at the rate of doubling every year, 3) extensive and rapid infection occurred in the stocks comprising a mixture of potato virus X and virus X-free potatoes and in plots which were surrounded by a great mass of infective materials, 4) infection did not depend on the stock being next to a virus-infected stock and some grown in fields by themselves become infected, 5) virus X is rapidly transmissible on the person, on clothing and on animals.

Bawden et. al. (6) reported that when certain potato varieties, already infected with virus X, were inoculated with virus Y, symptoms of current-season infections were no more severe than those caused by virus Y alone.

Daniel et. al. (15) reported that chronic infections by both virus X and Y invariably resulted in diagnostic symptoms of rugose mosaic and leaf-drop streak in Placid potatoes. Symptoms were easily recognizable at all times during the season. Masked virus X in single infections caused no symptoms. Symptoms caused by virus Y alone were detected only during a short period about 6 weeks after planting, when diseased plants were smaller and lighter green than healthy ones. Plants infected by virus Y recovered rapidly, and were undistinguishable from healthy or X-infected ones by midseason. However, mixed infections by virus X and Y in the variety Placid can be readily diagnosed in the field, even though each virus may be carried symptomlessly in single infections.

Potato virus S was detected first by serological techniques at the Laboratory for Flower Bulb Research at Lisse, The Netherlands under the direction of E. van Slogteren (16, 36, 44). An attempt was made to prepare an antiserum against potato virus A by infecting rabbits with extracts from virus A-infected potato leaves. The antiserum he prepared did not react against virus A but, against a quite different antigen which had been obtained from Bintje as well from Light and Dark Industrie. Transmissibility of this virus antigen was proved (16, 36), by tuber plug

graftings and sap inoculations. It was named as virus S from the first letter of E. van Slogteren's family name. Bagnall stated (2) that potato virus S was very wide spread in North American potatoes. Symptoms incited on potatoes usually were so slight that they passed for normal effects of maturity. However, virus S could be distinguished from other potato viruses by serological tests and by the reactions of a number of differential test plants; Chenopodium album, Nicotiana debneyi etc.

Rozendaal (36) stated that the virus S infected plants are generally characterized by a lighter green color, a deepening of the veins (rugosity) a slight bending downward of the tip of the leaves, a more open plant growth and by more or less drooping of the haulm of older plants. The symptoms frequently are inconspicuous and in many transition forms. He found the virus to be easily transmitted by stem grafting or sap inoculation. A contaminated knife or needle infected 25% or more plants of susceptible varieties when the tubers were cut or when the eyes or young sprouts were picked. These results did not agree with Levieil's who reported that sap transmission of virus S is comparatively difficult.

Kassanis (22) was unable to transmit virus S by Myzus persicae from infected to virus free plants of the King Edward variety. This is in agreement with Rozendaal (36). However, both workers stated that if aphids play a part in the transmission of virus S it can only be a very minor one.

According to Rozendaal (36) the combination of virus S with other viruses does not give rise to a new complex disease, but only to more pronounced symptoms. In contrast, the combination of viruses X and A

in many varieties causes a complex disease with symptoms different from those of the separate viruses. The variety Light Red Star, when infected with stipple streak virus is much more mottled and rugose when virus S is also present.

The first serological investigations in connection with plant viruses were carried out in 1927 by Dvorak (17). She showed evidence that mosaic disease altered the serologic specificity of the globulin reaction of the cell sap and cytoplasm of the potato plant. Beale (8) also showed that tomato and tobacco plants infected with mosaic contained an antigenic substance which was associated with the virus. Several investigators have confirmed and extended these findings.

The results of serological reactions, specific for a virus, may be arbitrarily summarized as follows: 1). virus-infected plants contain an antigenic substance which is not present in healthy plants (17)., 2). The serologic titre for the antigenic substance is correlated with the concentration of virus ((7), 3). Virus-containing plant juice stimulates the production of antibodies which are specific for the virus ((11), 4). Purified preparation of several viruses give positive reactions only with homologous virus antigens ((10), 5). Antisera prepared against a plant virus can be absorbed with a healthy plant antigen. The absorbed serum contained reactive substances (10). From these studies the first antisera, for routine analysis, was mass produced and used in the potato industry.

Van Slogteren (45) stated that the serological diagnosis is: 1) independent of the symptoms, 2) quick and objective, 3) makes possible the identification and classification of many viruses, 4) can help to

determine the concentration of a virus together with its localization and transport in the plant.

He stressed the fact that its simplicity made it possible to test many plants to identify virus-free potatoes. Furthermore, the serological diagnosis can be helpful for 1) speeding up the breeding of immune varieties, 2) the disentangling of complex virus diseases, 3) the discovery of unknown viruses.

As a routine testing method, the agglutination test was applied to identify potato virus X by Kristensen of Denmark (24). Potato plants selected in the field were serologically tested twice during the growing season. These two serological tests showed corresponding results in 94.5% of the tests. The same potato plants were tested by inoculation to Gomphrena globosa and when these tests were compared with the serological test the results corresponded in 92.2% of the cases. He stated that partial infection occurred frequently in the field and caused some variation in the different tests on the same individual plant. In most cases when the tested plant was virus-infected the agglutination reaction occurred within a few seconds and was performed much more quickly than by any other method.

MATERIALS AND METHODS

Variety: The Sebago potato variety was used in this experiment. The initial seed was obtained from a Michigan Foundation seed grower. The perfection of serological techniques as a tool to detect the presence of viruses permitted the classification of adequate numbers of plants for agronomic studies.

Serological tests: As a modification of the agglutination test, flat-bottomed petri dishes about 10 cm. in diameter were coated with a thin film of polyvinyl formal. A 1% solution of "formvar" in chloroform was poured into the dry petri dishes and poured out again immediately. A hydrophobic film forms on drying. Small drops of antisera were placed in rows on the petri dish with one end of a toothpick and mixed with a drop of the antigen (plant juice) with the other end. The toothpick was then discarded. Drops were covered with mineral oil to prevent evaporation and spontaneous flocculation at their edges. Later, flat-bottomed plastic dishes about 10 cm. square were used instead of petri dishes. It was found that drops of antiserum can be mixed with drops of antigen on the plastic dishes without the coating of polyvinyl. The antisera used in this test were normal serum, anti-S-serum, anti-M-serum, anti-Y-serum and anti-X-serum. These were obtained from the Laboratorium Voor Bloembollenanderzoek, Lisse, The Netherlands. A press was designed and built to extract plant juice from the leaf samples of test potato plants. Each leaf sample was placed between two 5 cm. square metal plates and pressed. A few drops of extracted juice from the press were collected. The metal plates were

washed and sterilized between each leaf sample.

Bioassay Method: Bioassay was carried out in the greenhouse where the temperature was usually held at 20-25°C to verify the use of serological techniques as a tool for screening large numbers of plants in a seed program. The indicator plants to be inoculated were sprayed uniformly with carborundum on the upper surface of the leaves. The plant leaves were inoculated by rubbing the extracted crude-sap on the upper surfaces of the leaves with a tongue depressor. A new tongue depressor was used for each individual inoculation. The leaves were washed with water after the inoculation was completed.

Gomphrena globosa plants were used as the indicator plant of potato virus X. The half-leaf method was applied on Gomphrena globosa plants. Local necrotic lesions surrounded by reddish borders developed within 5 days on the inoculated leaves when test potato plants were carrying virus X.

Nicotiana debeneyi plants were used as the indicator plant for potato virus S. The whole plant method was used. N. debeneyi plants were inoculated in the young 2 or 3-leaf stage. Potato virus S incited no local symptoms on the inoculated leaves but about 25-30 days after inoculation, a vein-clearing appeared on the first leaf above the base of the inoculated leaf when test potato plants were carrying virus S.

Greenhouse experiment: In the winter of 1963, 200 tubers from individual clones of the Sebago variety were planted in pots 10 x 13 cm and placed on benches in the greenhouse.

Three leaflets were collected from each stem of the 200 plants when they were 25-30 cm high. Extracted plant juice from these leaf samples

was then used in the agglutination tests in order to detect virus diseases which might be present. Three weeks later the plants were tested again. All tested plants were then classified into different groups according to their reactions in the agglutination tests. Viruses S, X and the combination of S and X were detected. No positive reactions were obtained with antisera of virus M and virus Y. The number of virus diseased Sebago clones determined by the agglutination test in the winter of 1963 are presented in Table 1.

Table 1. Number of clones infected with various virus diseases determined by the agglutination test.

Viruses	Virus free	Virus S	Virus X	Virus S plus Virus X
First test	163	8	17	12
Second test	157	11	18	14

Since there was a slight variation between the two agglutination tests, only clones which reacted similarily in both tests were saved: Virus S, 7; Virus X, 17; Virus S plus Virus X, 11 and Virus free, 157. Tubers from these clones were space-planted 5 feet apart in a randomized complete block design with 10 replications in 1964. Washing, caused by excessive rainfall, created great variations in emergence and stand. It was not possible to evaluate the effect of virus diseases on growth and yield. The experiment was repeated in 1965.

The procedures of 1963 were used to identify the potato virus diseases in the 1964 plantings. Bioassay was also carried out on each plant which showed a positive reaction in the agglutination test. During the winter of 1964-65 a tuber from each hill grown in the field in 1964

was grown in the greenhouse and tested by both methods for the presence of virus. Tubers, free of virus and with specific viruses were planted in sterilized sand. The agglutination test provided a technique for positive identification of plants infected with a virus.

Ten plants for each of the potato viruses; S, X, the combination of S and X, and virus free plants were grown in the greenhouse for observation on their growth and for the expression of virus symptoms.

Plants for the field experiments of 1965 were started in the greenhouse to be transplanted to the field in order to obtain a perfect stand and an equal number of stems in each potato hill. Tubers, free of virus and with specific viruses were planted in sterilized sand. When the young potato plants were about 7-10 cm. high, they were separated from the mother tubers with a sterilized knife and transplanted into flats according to their classification. Approximately 150 plants for each category, i.e., infected with virus S, X, or the combination of viruses S and X and virus free plants, were started in flats. An additional 150 virus free plants were started to be inoculated with virus Y since it had not been found in the original 200 tubers. Virus M was not detected in any tubers tested so was not included in field experiments.

The transplanted plants were inoculated when they were about 15 cm high to insure that they were infected with the virus diseases. Also, the combination of viruses S and Y, viruses S and X, viruses Y and X and viruses S, Y and X were inoculated on Sebago plants by individual inoculum of virus S, virus Y and virus X. The inoculum for virus S was prepared from virus S - infected N. debeneyi; the inoculum for the virus X

from virus X - infected Sebago plants and inoculum for virus Y from the virus Y - infected N. tabacco.

Twenty-five grams of 600 size grit of carborundum mixed with one hundred cc. of prepared inoculum for each of the virus groups was diluted with distilled water in the ratio of 1 to 50. The diluted inoculum was sprayed on the leaves of the transplanted potato plants with 65 pounds of air pressure. The atomizer was sterilized each time inocula were changed.

Field experiment: Three field experiments were planted at the Lake City Experiment Station. Each experiment consisted of virus free plants or plants carrying and inoculated with either virus S, virus Y or virus X to provide plants infected with a single virus, or combinations of viruses S and Y, viruses S and X, viruses Y and X and viruses S, Y and X. Four plants were transplanted together to make each hill on June 15, 1965. These hills were space-planted 5 feet apart in a randomized complete block experiment with 10 replications. Each replication consisted of 9 hills, 7 diseased hills and 2 virus-free hills. Two weeks after transplanting, any missing plants in each hill were replaced.

Since the agglutination tests showed that the viruses had spread during the growing season, all the data were grouped and analysed according to the results of agglutination tests at different dates by combining analysis of variance for the three experiments. Duncan's multiple-range test was used to compare each mean with every other mean obtained in the results of this experiment.

Normal cultural practices were followed during the growing season. The field was irrigated and sprayed with fungicides plus insecticides

throughout the growing season. Weeds were controlled by hand hoeing. Later, a small hand cultivator was used instead of a hoe.

The agglutination test was carried out at three different times; August 19, September 18 and September 17.

The following observations were recorded:

1) Growth: a) observations were made weekly throughout the growing season to record the expression of any disease symptoms on the potato plants in each individual hill. b) the plant height was measured three times. c) flowering and maturity were also recorded. d) vines of 45 hills from each of three experiments were harvested and the fresh weight and dry weight were obtained.

2) Number of tubers: At harvesttime the tubers from each hill were divided into two groups and counted on the basis of size; larger or smaller than 5 cm in diameter.

3) Weight of tubers: Weights of tubers per hill were obtained for each of the two size groups.

4) Potato chips: Three slices approximately 2 mm thick were cut from the center of 2 or 3 tubers from each individual potato hill. Slices were rinsed in water to remove excess starch, blotted with paper towels, and cooked in a commercial grade of homogenized vegetable shortening at a temperature $\pm$ 345°F. They were removed from the cooker when bubbling stopped. All the chips were placed in cellophane bags and kept at room temperature, 20-25°C.

Three readers evaluated the chip color according to the color reference standard of the Proctor and Gamble Company. There are ten classes ranging from a very light, almost white, to very dark brown in the color reference standard. The scores were averaged.

RESULTS AND DISCUSSION

1. Observations on the spread of potato viruses.

Inoculations with potato virus Y and the various combinations of virus Y with the other viruses were not successful. Consequently, no data on virus Y could be included in this experiment. The trends in virus infections are illustrated in Table 2.

Table 2. The number of potato hills either free of virus or infected with virus on three different dates.

Date of Agglutination Tests		Virus free	Virus S	Virus X	Virus S + Virus X	Total
1st test August 17	Exp. 1	24	10	33	23	90
	Exp. 2	21	10	32	27	90
	Exp. 3	27	8	34	21	90
Total		72	28	99	71	270
2nd test September 8	Exp. 1	21	8	24	37	90
	Exp. 2	22	7	22	39	90
	Exp. 3	21	9	27	33	90
Total		64	24	73	109	270
3rd test September 17	Exp. 1	24	3	13	50	90
	Exp. 2	19	9	16	46	90
	Exp. 3	14	14	21	41	90
Total		57	26	50	137	270

The result of the third and final agglutination test showed a decrease in the number of virus free potato plants and plants infected with either virus S or virus X and an increase in the number of hills infected with the combination of virus S and virus X. The greater than anticipated number of potato plants infected with virus X and the combination of viruses S and X in the first agglutination test

was due to the absence of virus Y-infected plants which subsequently become infected with virus X or the combination of viruses S and X. The reduced number of virus X-infected plants in the final test may be largely due to the appearance of virus S, which had previously existed in the virus X-infected plants but could not be detected on the first test.

The total number of virus S-infected hills was fairly constant throughout all the agglutination tests. There was a slight decrease in the number of infected plants in the second test. However, at the time of the third test there was a marked decrease in Experiment 1 and a sizeable increase in Experiment 3. Some of these plants accounted for the increase in the number of plants infected with the combination of virus S and virus X.

It may be that virus S required a long time to become established and the virus concentration was not high enough to show a positive serologic reaction in the first agglutination test.

This is in agreement with Rozendaal et. al. (36) who was unable to observe primary symptoms on potato leaves inoculated with virus S. In his work the infection was detected by the agglutination test, but occasionally it was detected only by the micro-precipitation test. Frequently both tests were negative, yet the progeny proved to be infected. Potato virus S has been shown to be very widespread in many potato varieties since the application of the serological test.

II. Influence of virus infections upon plant growth and symptomatology.

All the potato plants in the field were slow to recover from

transplanting. Growth during the early growing season, i.e., in late June was slow. The average temperature in June was 16.5°C., the weather often cloudy. After frequent irrigation and an application of ammonium nitrate fertilizer, the plants gradually recovered from transplanting.

By early July, the plants had fully recovered and the color of the leaves was changing from light yellowish green to dark green. The average temperature in July was 18.0°C. and total rainfall was quite low, 23.6 mm. The departure from normal was -56.6 mm. A light yellowish mottling and small necrotic lesions were observed on the leaves of virus X and the combination of viruses S and X-infected plants. All these symptoms were mild and no severe symptoms were observed during this period. In late July, all plants were growing vigorously and were fairly uniform.

In August, the average temperature was 18.5°C. and the total rainfall was 129.8 mm. Very little irrigation was required during this period. By this time, the plants were blossoming profusely. The mild mottling, the light yellowish areas and the number of small necrotic lesions were more evident on the leaves of virus X and the combination of virus S and virus X-infected plants. The small necrotic lesions seemed to be more pronounced on the leaves of plants infected with the combination of viruses S and virus X. The combination of virus S and X did not appear to be a new complex symptom.

A somewhat lighter green with slight mottling was observed on the leaves of virus S infected plants. But these symptoms were not consistently present and were not clearly distinguishable.

In September, the average temperature dropped down to 14.0°C. from the 18.5°C. in August. The total rainfall was 161.3 mm and the departure from normal was +82.0 mm. Rainfall was frequent. The plant foliage was killed by a heavy frost on September 23.

The symptoms of various viruses are shown in Figure 1 and 2.

Rozendaal et. al. (36) pointed out that the combination of virus S with other viruses, i.e. virus A, virus X and a strain of virus Y, does not give rise to a new complex disease, but only to more pronounced symptoms, depending upon the potato variety. The symptoms of virus S are very mild and variable depending upon the strain of the virus, the weather conditions and the soil. In addition to a deepening of the veins causing rugosity of the leaves, they observed a distinct mottle on the leaves of virus S-infected some potato varieties, i.e. Gloria and Benelander, and small necrotic spots in the Profijt. In general symptoms of virus S appear later than those of virus X and virus A.

Figure 1-a. Leaflet of a healthy Sebago. (3-88)

Figure 1-b. Leaflet of virus S-infected Sebago showing a somewhat lighter green with slight mottling and vein-clearing. (2-86)

3-88

2-86

Figure 1-c. Leaflet of virus X-infected Sebago showing mild mottling and small necrotic lesions. (2-33)

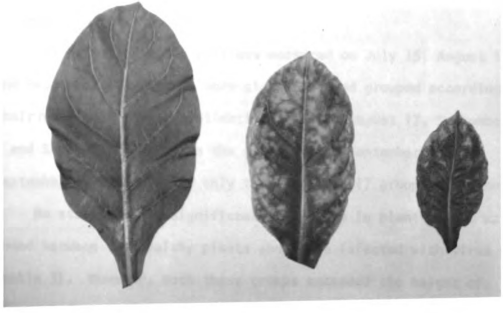
Figure 1-d. Leaflet of the combination of viruses S and X-infected Sebago showing similar symptoms of virus X. (2-2)

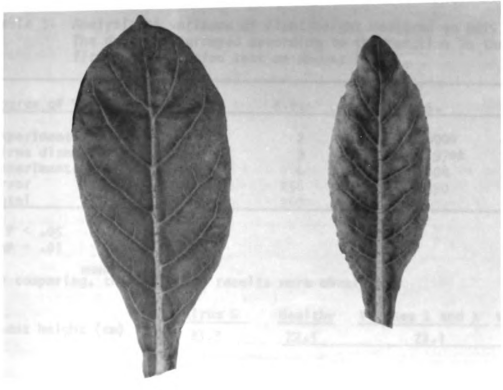
2-33

2-2

Figure 2-a. Leaves from a single plant of Nicotiana debneyi inoculated with virus S. Inoculated basal leaf (left) is symptomless while middle and upper leaves show vein-clearing.

Figure 2-b. A vein-clearing and somewhat mottling show on the leaves of Nicotiana debneyi inoculated with the combination of viruses S and X.





Plant height

The height of all plants was measured on July 15, August 10 and September 12. Plants were classified and grouped according to their reaction to the agglutination tests on August 17, September 8 and September 17. Since the groupings on September 8 and September 17 were similar only the September 17 grouping is shown.

No statistically significant difference in plant height was found between the healthy plants and those infected with virus S (table 3). However, both these groups exceeded the height of plants infected with virus X and the combination of viruses X and S.

Table 3. Analysis of variance of plant height measured on July 15. The data were grouped according to the reaction in the ffrst agglutination test on August 17.

Source of Variance	d.f.	M.S.
Experiments	2	90.400*
Virus diseases	3	434.197**
Experiments x virus diseases	6	36.604
Error	258	25.350
Total	269	

* P < .05
** P < .01

By comparing means, the following results were obtained:

	Virus S	Healthy	Viruses S and X	Virus X
plant height (cm)	23.3	22.3	21.1	20.7

Any two means not underscored by the same line are significantly different. When the data were regrouped by the reactions on September 17, there were no significant differences in plant height between plants infected with viruses and healthy plants.

The plant height measured on August 10 is presented in Table 4-a and 4-b.

Table 4-a. Analysis of variance of plant height measured on August 10. The data were grouped according to the reaction in the first agglutination test on August 17.

Source of Variance		
Experiments	2	804.915**
Virus diseases	3	226.485*
Experiments x virus diseases	6	135.592
Error	258	63.713
Total	269	

*P < .05
**P < .01

By comparing means, the following results were obtained.

	Healthy	Virus S	Viruses S and X	Virus X
Plant height (cm)	55.6	54.6	52.6	51.8

Any two means not underscored by the same line are significantly different.

Table 4-b. Analysis of variance of plant height measured on August 10. The data were grouped according to the reaction in the third and final agglutination test on September 17.

Source of Variance	d.f.	M.S.
Experiments	2	803.579**
Virus diseases	3	163.282*
Experiments x virus diseases	6	138.429
Error	258	61.797
Total	269	

*P < .05
**P < .01

By comparing means, the following results were obtained:

	Healthy	Virus S	Viruses S and X	Virus X
Plant height (cm)	55.4	55.1	53.7	52.2

Any two means not underscored by the same line are significantly different.

The healthy plants were significantly taller than the plants infected with virus X and the combination of viruses S and X. There was again no significant difference between virus free and virus S-infected plants (Table 4-a).

In Table 4-b, the healthy and virus S-infected plants were significantly taller than the plants infected with virus X.

Results of the final measurement of the plant height were presented in Table 5-a and 5-b.

Table 5-a. Analysis of variance of plant height measured on September 12. The data were grouped according to the reaction in the first agglutination test on August 17.

Source of Variance	d.f.	M.S.
Experiments	2	80.772
Virus diseases	3	493.818**
Experiments x virus diseases	6	393.624**
Error	258	65.306
Total	269	

*P < .05
**P < .01

By comparing means, the following results were obtained.

	Healthy	Viruses S and X	Virus S	Virus X
Plant height (cm)	73.4	69.8	68.8	67.6

Any two means not underscored by the same line were significantly different.

Table 5-b. Analysis of variance of plant height measured on September 12. The data were grouped according to the reaction in the third and final agglutination test on September 17.

Source of Variance	d.f.	M.S.
Experiments	2	80.051
Virus diseases	3	383.562**
Experiments x virus diseases	6	229.613**
Error	258	69.099
Total	269	

*P < .05
**P < .01

By comparing means, the following results were obtained.

	Healthy	Virus X	Viruses S and X	Virus S
Plant height (cm)	73.8	69.2	68.7	68.1

Any two means not underscored by the same line were significantly different.

The measurements of September 12 showed that the height of healthy plants exceeded that of the virus-infected plants, when grouped according to reactions of both August and September agglutination tests.

The influence of virus S on plant height did not appear in the early part of the growing season but had a pronounced effect late in the growth cycle. On the contrary virus X reduced the plant growth early in the season and this retardation remained throughout.

It should be pointed out that the number of virus free plants and plants infected with viruses varied between the first and final agglutination tests. The number of plants infected with the combination of viruses S and X detected in the final test was increased by the appearance of virus S into virus X-infected plants

or by a current season infection of both viruses S and X. The late infection apparently did not influence the plant height whereas the plants infected with both viruses at the time of the first agglutination test were affected.

III) Influence of virus infections upon tuber yield.

a) Number of Potato Tubers

The number of tubers above and below 5 cm in diameter was determined at harvesttime. The average number of sized tubers per hill for virus free and virus-infected tubers is presented in Table 6.

The virus free plants produced the largest total number of tubers in all cases when the plants were grouped according to their virus reactions on different test dates.

There was a gradual decline in the total number of tubers between virus free and virus S, virus S and the combination of viruses S and X and between the combination and virus X alone, (Table 7-a and 7-b). Virus S did not affect the number of large tubers per hill but the hills infected with virus X and the combination of viruses X and S produced fewer large tubers. The agglutination test of September 17 detected current season infection and showed that viruses had spread. Some healthy plants became infected either with a single virus or the combination of viruses S and X. Regrouped according to the findings of the third test portrays the same trends but a slightly different picture. Plants that were virus free for the first period of growth had acquired a virus and

Table 6. Average number of potato tubers from virus-free and virus-infected plants grouped according to the reaction in the agglutination tests on three dates.

Date of agglutination tests	Infected viruses and tuber size	Virus free			Virus S			Virus X			Viruses S and X		
		Large	Small	Total	Large	Small	Total	Large	Small	Total	Large	Small	Total
	Exp. I	6.4	2.6	9.0	6.2	2.5	8.7	5.1	2.2	7.3	5.0	1.9	6.9
1st test	II	7.4	2.3	9.7	6.5	3.1	9.6	5.7	1.9	7.6	7.1	2.3	9.4
August 17	III	7.5	2.5	10.0	7.0	1.0	8.0	6.4	1.8	8.2	6.1	1.6	7.7
	Exp. I	6.2	2.7	8.9	6.9	2.0	8.9	4.9	2.0	6.9	5.1	2.1	7.2
2nd test	II	7.4	2.2	9.6	6.3	3.7	10.0	4.9	1.7	7.6	6.6	2.3	8.9
September 8	III	7.3	2.6	9.9	7.8	1.4	9.2	6.2	1.7	7.9	6.4	1.5	7.9
	Exp. I	6.3	2.6	8.9	5.7	2.3	8.0	4.5	1.3	5.8	5.3	2.4	7.7
3rd test	II	7.2	2.3	9.5	7.1	2.9	10.0	6.8	1.7	8.5	6.2	2.3	8.5
September 17	III	8.1	2.1	10.2	6.5	1.9	8.4	6.3	1.7	8.0	6.5	1.8	8.3

consequently were placed in another category.

Table 7-a. Analysis of variance on the total number of potato tubers per potato hill classified according to the reaction in the first agglutination test on August 17.

Source of Variance	d.f.	M.S.
Experiments	2	29.581*
Virus diseases	3	55.170**
Experiments x virus diseases	6	10.767
Error	258	6.335
Total	269	

*P < .05
**P < .01

By comparing means, the following results were obtained:

	Healthy	Virus S	Viruses S and X	Virus X
Total number of tubers	9.6	8.8	8.1	7.6

Any two means not underscored by the same line were significantly different.

Table 7-b. Analysis of variance on the number of large potato tubers (larger than 5 cm.) per potato hill classified according to the reaction in the first agglutination test on August 17.

Source of Variance	d.f.	M.S.
Experiments	2	37.315**
Virus diseases	3	25.016**
Experiments x virus diseases	6	5.842
Error	258	3.916
Total	269	

*P < .05
**P < .01

By comparing means, the following results were obtained.

	Healthy	Virus S	Viruses S and X	Virus X
Number of large tubers	7.1	6.5	6.1	5.7

Any two means not underscored by the same line were significantly different.

There were no significant differences in the number of small potato tubers. The means of small tubers for healthy and viruses infected plants were as follows:

	Healthy	Virus S	Viruses S and X	Virus X
Number of small tubers	2.5	2.3	2.0	1.9

Table 8-a. Analysis of variance on the total number of potato tubers per potato hill classified according to the reaction in the second agglutination test on September 8.

Source of Variance	d.f.	M.S.
Experiments	2	33.848**
Virus diseases	3	48.615**
Experiments x virus diseases	6	5.662
Error	258	6.832
Total	269	

*P < .05
**P < .01

By comparing means, the following results were obtained:

	Healthy	Virus S	Viruses S and X	Virus X
Total number of tubers	9.5	9.3	8.0	7.5

Any two means not underscored by the same line were significantly different.

Table 8-b. Analysis of variance on the number of large potato tubers per potato hill classified according to the reaction in the second agglutination test on September 8.

Source of Variance	d.f.	M.S.
Experiments	2	42.681**
Virus diseases	3	26.202**
Experiments x virus diseases	6	2.473
Error	258	3.764
Total	269	

*P .05
**P .01

By comparing means, the following results were obtained:

	Healthy	Virus S	Viruses S and X	Virus X
Number of large tubers	7.1	7.0	6.0	5.7

Any two means not underscored by the same line was significantly different.

No significant differences were found in the number of small potato tubers.

	Healthy	Virus S	Viruses S and X	Virus X
Number of small tubers	2.4	2.3	2.0	1.8

The number of tubers obtained when the data were grouped according to the reaction in the second agglutination test show the same trend as the results in Table 7-a except the virus S infected plants which are significantly different from the plants infected with the combination of viruses S and X for both the total number and the large size tubers (Table 8-a and 8-b).

The analysed data on the number of potato tubers according to the reactions in the third and final agglutination test are presented in Table 9-a and 9-b.

Table 9-a. Analysis of variance on the total number of potato tubers per potato hill classified according to the reactions in the third and final agglutination test on September 17.

Source of Variance	d.f.	M.S.
Experiments	2	33.061**
Virus diseases	3	37.707**
Experiments x virus diseases	6	7.681
Error	258	6.700
Total	269	

*P < .05
**P < .01

By comparing means, the following results were obtained:

	Healthy	Virus S	Viruses S and X	Virus X
Total number of tubers	9.4	8.9	8.1	7.6

Any two means not underscored by the same line were significantly different.

Table 9-b. Analysis of variance on the number of large potato tubers per potato hill classified according to the reactions in the third and final agglutination test on September 17.

Source of Variance	d.f.	M.S.
Experiments	2	42.233**
Virus diseases	3	17.887**
Experiment x virus diseases	6	4.241
Error	258	3.813
Total	269	

*P <.05
**P <.01

By comparing means, the following results were obtained:

	Healthy	Virus S	Viruses S and X	Virus X
Number of large tubers	7.0	6.6	6.0	5.9

Any two means not underscored by the same line were significantly different.

No significant differences were found in the number of small potato tubers.

	Healthy	Virus S	Viruses S and X	Virus X
Number of small tubers	2.4	2.3	2.1	1.6

Results similar to Table 7-a are shown in Table 9-a except that the virus S-infected plants were not significantly different from the other viruses-infected plants for the number of large tubers (Table 9-b).

Results of statistical analysis would indicate that virus S did not affect the number of tubers set during the growing season. Since there is evidence of very little increase in the number of tubers set after the blossom stage (12), most small potatoes are of the same chronological age as the large ones. The effect of virus S did not appear until the blossom stage of the potato plants. This was also true in plant height where the effect of virus S did not appear until August 10. However, the height of virus S-infected plants was less than that of the virus free plants in the final measurement of September 12.

There was a great decrease in the total number of potato tubers due to virus X infection of the plants when compared to virus free plants and virus S-infected plants.

It would indicate that the effect of virus X appeared in the earlier growing stage.

The plants infected with the combination of viruses S and X set a lesser number of tubers than the virus free plants. However, there was

no difference between the combination of viruses S and X, and virus X-infected plants.

It would indicate that the combination of viruses S with X did not give rise to a new complex disease and their effect on the number of tuber set is somewhat between the effect of virus S and virus X. This is in agreement with previous works (22, 36). The percentage reduction in the number of tubers set, due to infection with specific viruses is summarized in Figure 3.

Percentage reduction in the total number of tubers of virus X-infected plants and of the plants infected with the combination of viruses S and X was fairly constant, 20% and 15% respectively. On the other hand, the number of tubers from virus S-infected plants depended upon the groupings of the reactions in the different agglutination tests; 8.3% in the first, 2.1% in the second and 5.3% in the third groups respectively. This variation resulted from the changing of a plant from one classification to another. Alternatively, there might be more variation within virus S-infected plants since fewer plants were available in this category.

The trends of percentage reduction in the number of large tubers were very similar to the number of total tubers.

Although there were no significant differences among means of the small tubers of the plants infected with various viruses, there was a difference among them when expressed as a percent of the healthy plants. There was a little, about 5.5%, reduction in the number of

small tubers of virus S-infected plants when compared to virus free plants. But a great reduction in the number of small tubers occurred in virus X and the combination of viruses S and X-infected plants: 17.4% and 27.4% respectively.

Although Brust (36) stated that the average reduction in the size 50 mm and larger was between 15 or 20% in the variety Bintje, he also observed very great differences between plant families. For the variety Bintje, it has been shown that the virus S-infected families produced a higher percentage of small tubers.

There is a great variation among the different varieties since very great differences in the tuber size per family have been observed in previous works (36).

In this experiment, virus S-infected plants produced more tubers both large and small size than the other virus-infected plants. There was no statistical difference between the number of tubers from virus S-infected plants and the virus free plants.

b) Weight of Potato Tubers

Data of the tuber weight were grouped and analyzed by the procedures applied to tuber number.

Means of the tuber weight from individual experiments are presented in Table 10.

In Table 11-a total weights of potato tubers for the healthy and virus S-infected plants were highly significantly different from the weights of both virus X and the combination of viruses S and X-infected plants. The results of large size tubers were similar to the total

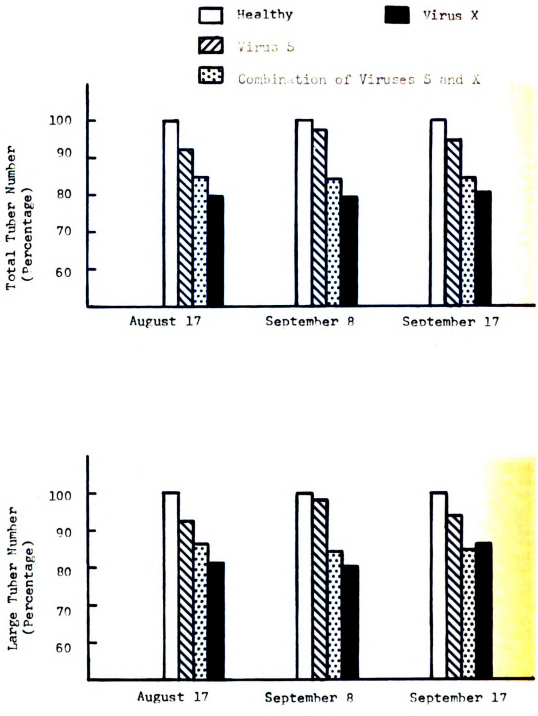


Figure 3. Number of tubers from virus infected potato hills expressed as percent of the weight of tubers from healthy hills.

Table 10. Average weight of large and small potato tubers from healthy and virus infected potato hills grouped by the serological reactions on three test dates.

(expressed in grams)

Agglutination test		Virus free		Virus S		Virus X		Viruses S and X	
		Large	Small	Large	Small	Large	Small	Large	Small
	Exp. I	1,237.5	78.7	1,21.00	73.0	951.9	54.1	1,013.7	63.7
1st test	II	1,411.5	70.7	1,224.5	113.5	1,104.1	81.4	859.2	88.9
August 17	III	1,428.1	62.6	1,608.1	26.21	1,272.6	64.4	1,173.1	58.1
	Exp. I	1,225.9	80.2	1,412.5	58.1	915.9	58.3	1,013.4	62.70
2nd test	II	1,410.1	72.7	1,170.0	127.1	1,183.6	83.4	1,118.5	77.4
September 8	III	1,490.0	55.7	1,346.7	41.1	1,232.2	60.4	1,292.4	64.8
	Exp. I	1,232.9	76.2	1,485.0	80.0	868.2	41.5	1,024.1	65.2
3rd test	II	1,408.8	73.2	1,303.3	93.9	1,287.8	61.6	1,221.2	94.6
September 17	III	1,632.5	52.9	1.163.2	49.3	1,222.6	46.2	1,329.6	70.7

weight of potato tubers. Analysis of variance is presented in Table 11-b. The comparison of the means are presented.

Table 11-a. Analysis of variance of the total weight of potato tubers grouped according to the reaction in the first agglutination test on August 17.

Source of Variance	d.f.	M.S.
Experiments	2	1,472,864.5**
Virus diseases	3	1,102,838.7**
Experiments x virus diseases	6	141,075.5
Error	258	169,102.9
Total	269	

*P <.05
**P <.01

By comparing means, the following results were obtained:

	Healthy	Virus S	Virus S and X	Virus X
Weight of total tubers (grams)	1,430.1	1,403.03	1,223.5	1,177.7

Any two means not underscored by the same line are significantly different.

Table 11-b. Analysis of variance of the weight of large potato tubers grouped according to the reaction in the first agglutination test on August 17.

Source of Variance	d.f.	M.S.
Experiments	2	1,488,297.6**
Virus diseases	3	1,059,755.4**
Experiments x virus diseases	6	159,492.8
Error	258	165,986.5
Total	269	

*P <.05
**P <.01

By comparing means, the following results were obtained:

	Healthy	Virus S	Viruses S and X	Virus X
Weight of large tubers (grams)	1,359.75	1,328.93	1,111.23	1,156.4

Any two means not underscored by the same line are significantly different.

No significant differences were found for the weight of small tubers.

	Healthy	Virus S	Viruses S and X	Virus X
Weight of small tubers (grams)	70.25	74.10	112.27	21.30

Table 12-a. Analysis of variance of the total weight of potato tubers grouped according to the reaction in the second agglutination test on September 8.

Source of Variance	d.f.	M.S.
Experiments	2	1,473.720.50**
Virus diseases	3	1,024,961.67**
Experiments x virus diseases	6	130,338.83
Error	258	170,283.91
Total	269	

*P <.05
**P <.01

By comparing means, the following results were obtained:

	Healthy	Virus S	Virus S and X	Virus X
Weight of total tubers (grams)	1,445.5	1,388.96	1,226.65	1,180.22

Any two means not underscored by the same line are significantly different.

Table 12-b. Analysis of variance of the weight of large potato tubers grouped according to the reaction in the second agglutination test on September 8.

Source of Variance	d.f.	M.S.
Experiments	2	11,449,512.5**
Virus diseases	3	2,870,508.0**
Experiments x virus diseases	6	158,897.2
Error	258	134,111.15
Total	269	

*P < .05
**P < .01

By comparing means, the following results were obtained:

	Healthy	Virus S	Viruses S and X	Virus X
Weight of large tubers (grams)	1,375.89	1,317.08	1,158.03	1,113.57

Any two means not underscored by the same line are significant different.

No significant differences were found for the weight of small tubers.

	Healthy	Virus S	Viruses S and X	Virus X
Weight of small tubers (grams)	69.6	71.9	68.6	66.6

Table 13-a shows that the tuber weight of virus S-infected plants was not significantly different from the other viruses infected plants. There was also no difference between yields of the healthy and the virus S-infected plants. A similar trend was found for the large size potato tubers (Table 13-b).

Table 13-a. Analysis of variance of the total weight of potato tubers grouped according to the reaction in the third agglutination test on September 17.

Source of Variance	d.f.	M.S.
Experiments	2	1,470,394.2**
Virus diseases	3	848,679.2**
Experiments x virus diseases	6	408,739.0*
Error	258	165,894
Total	269	

*P< .05
**P< .01

By comparing means, the following results were obtained:

	Virus Free	Virus S	Virus S and X	Virus X
Weight of total tubers (grams)	1,459.20	1,317.10	1,228.0	1,201.20

Any two means not underscored by the same line are significantly different.

Table 13-b. Analysis of variance of the weight of large potato tubers grouped according to the reaction in the third and final agglutination test on September 17.

Source of Variance	d.f.	M.S.
Experiments	2	1,470,394.2**
Virus diseases	3	848,679.2**
Experiments x virus diseases	6	408,739.0*
Error	258	165,894.0
Total	269	

*P < .05
**P < .01

By comparing means the following results were obtained:

	Healthy	Virus S	Viruses S and X	Virus X
Weight of large tubers (grams)	1,389.68	1,248.8	1,152.9	1,151.3

Any two means not underscored by the same line are significantly different.

No significant differences were found for the weight of small tubers.

	Healthy	Virus S	Viruses S and X	Virus X
Weight of small tubers (grams)	69.5	68.2	75.1	49.9

The yield depressions resulting from the infections of various viruses was due primarily to the effect on the large size tubers, above 5 cm. diameter. The viruses did not affect the weight of small size tubers, those less than 5 cm. diameter.

The effects of virus X and the combination of viruses S and X on yields were similar to the results of tuber number.

For virus S-infected plants, it has been pointed out that the number of virus S-infected plants varied within an experiment for the agglutination tests of the different dates. Some virus S-infected plants became infected with both virus S and virus X or, a very few of the infected plants appeared healthy in the final agglutination test. Such variations in the number of virus S-infected plants could have been due to a low or variable concentration of the virus within the infected plants.

Most of the plants added to the virus S-infected group in the final agglutination test had been classed as healthy plants in the previous tests. The tuber weight of virus S-infected plants which gave a positive reaction in all three agglutination tests was compared to the tuber weight of the virus S-infected plants detected in the final agglutination test. There was no difference between

them. Virus S did not affect the tuber weight in the statistically analysed data. However, there was an indication that virus S did influence yield since there were no significant differences between virus S and the other viruses-infected plants. It should be pointed out that the weight of tubers from healthy plants was greater than from virus S-infected plants. This was not true in the number of potato tubers in which the difference between healthy and virus S-infected plants was negligible.

The percentage reduction in total yield due to the infection of specific viruses is presented in Figure 4.

The percentage reduction in yield of virus X-infected plants was consistent, approximately 17.5% throughout all the plant groups based on their reaction in the agglutination tests of different date.

Similarly the yield of the plants infected with the combination of viruses S and X, was reduced 15.5%. This result would indicate as in the other evaluations that the combination of viruses S and X did not create a new complex disease.

A greater percentage reduction in yields of the virus S-infected plants was observed between the groupings on the first and the final agglutination tests. There was a 2% reduction in yield of the plants on the first test and a 10% reduction on the final agglutination tests. These results indicate that virus S affected the total yields but did not effect the number of potato tubers. On the other hand, virus X and the combination of viruses S and X affected

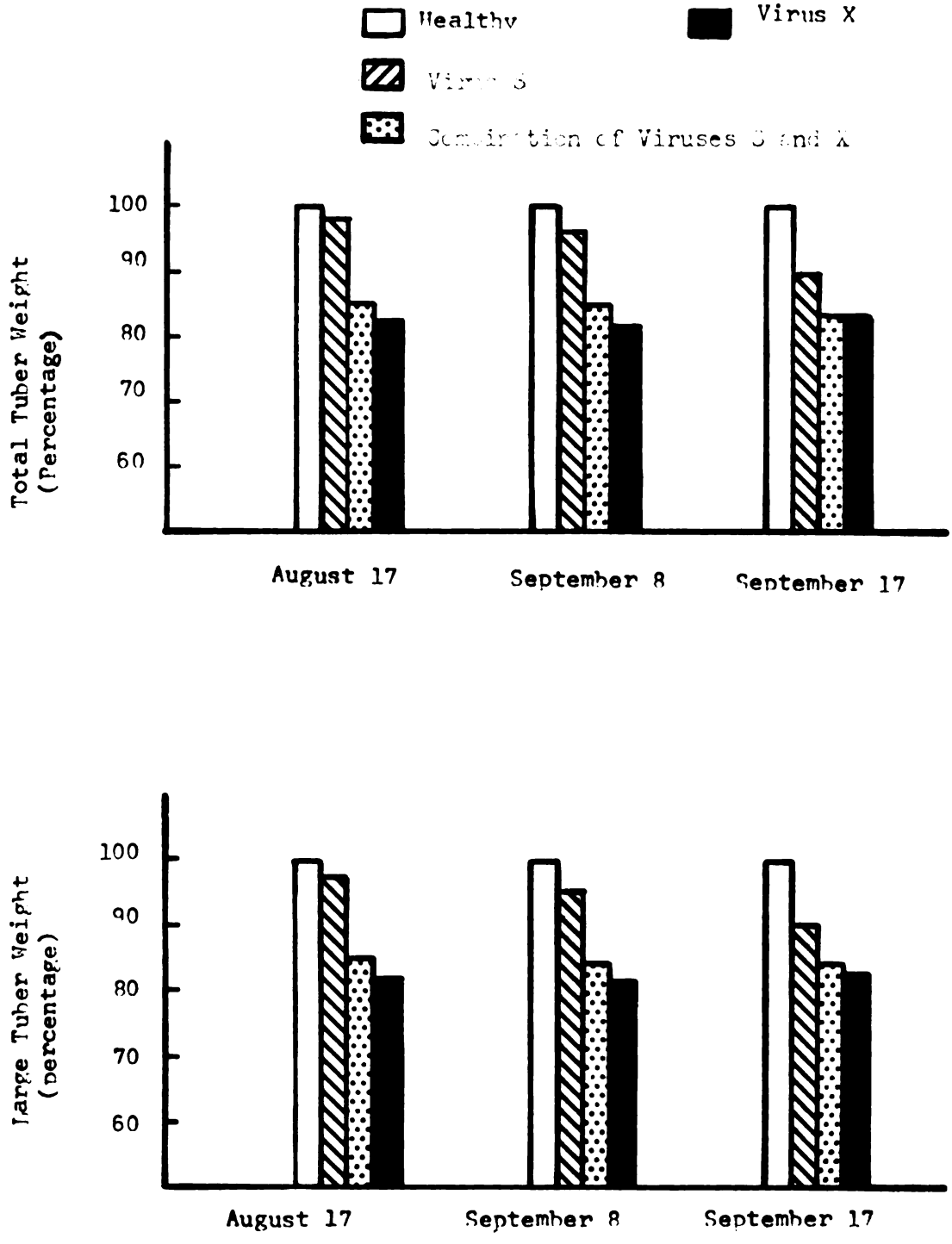


Figure 4. Weight of tubers from virus infected potato hills expressed as percent of the weight of tubers from healthy hills.

both the number and the weight of potato tubers.

IV) Influence of virus infections upon the specific gravity of potatoes.

The average specific gravity of potato tubers from each of the different virus-infected plants is given in Table 14.

Table 14. The average specific gravity of Sebago potato tubers from various viruses-infected plants grouped by serological reactions on three test dates.

Date of agglutination tests	Viruses	Healthy	Virus S	Virus X	Viruses S and X
1st test	Exp. I	1.068	1.075	1.071	1.071
August 17	II	1.074	1.073	1.073	1.069
	III	1.071	1.077	1.074	1.076
Grand Mean		1.071	1.075	1.073	1.072
2nd test	Exp. I	1.068	1.069	1.073	1.071
September 8	II	1.074	1.072	1.075	1.070
	III	1.070	1.073	1.075	1.076
Grand Mean		1.070	1.071	1.074	1.072
3rd test	Exp. I	1.068	1.072	1.070	1.072
September 17	II	1.074	1.072	1.079	1.069
	III	1.070	1.073	1.075	1.075
Grand Mean		1.070	1.073	1.075	1.072

The data show that the specific gravity varied among the experiments as well as among the groupings for the different dates of the agglutination tests. The differences in specific gravity of all the virus-infected potatoes were not greater than those variations (Table 15).

There were statistically no significant differences among means of the specific gravity of potato tubers from the specific virus-infected plants. Therefore, the infection with virus, did not effect the specific gravity of the Sebago potatoes.

Table 15. Analysis of variance of specific gravity data of Sebago tubers grouped according to the reaction in the first agglutination test on August 17.

Source of Variance	d.f.	M.S.
Experiments	2	.000.222.5
Treatments	3	.000.198.7
Experiments x treatments	6	.000.210.2
Error	258	.000.175.0
Total	269	

Considerable variation in specific gravity of the individual tubers occurs within a variety. These variations are caused by such factors as location, seasonal variation, fertilizer, irrigation, chemicals, etc. (20). The accumulative effect of these factors could account for the existing variations since no trend in specific gravity could be associated with the virus infections.

V). Influence of virus infections upon the color of potato chips.

The average scores of the color of potato chips made from potatoes infected with specific viruses are presented in Table 16. No statistically significant differences were found in potato chip color (Table 17). The color of the potato samples of chips in all tests was barely acceptable or below. There was some variation in chip color among the experiments, but the differences in chip color

due to the infection of virus were not greater than these variation. The data indicated that the infection of various viruses was not associated with discoloration.

The plant foliage was killed by a heavy frost on September 23, but the tubers could not be harvested until October 8. During this period, the temperature was quite low and the soil was wet due to frequent rainfall.. Potatoes not harvested until after the frost of September 23 had been chilled in the ground and consequently did not make potato chips of an acceptable color. The condition prevailed in all potatoes on the station. Kushman et. al. (26) has reported that neither soil moisture nor loss of vines affected chip color at harvest. However, when the potatoes were held at 60°F., differences between treatments developed. Tubers from wet soil produced darker chips than tubers from dry soil. Loss of vines (9 days before harvest) caused consistently darker chips at all soil moisture levels after 2 weeks storage, but the effects of vine removal on chip color was less than the effect of soil moisture. In this experiment, the potatoes were held 2 weeks before chipping so that these two factors could have contributed to the overall poor color rating. Since the Sebago does not recondition no attempt was made to evaluate chip color during the storage periods.

The blackening of cooked potatoes does not appear to be associated with a transmissible, pathological condition (43).

Table 16. Influence of virus infections upon chip color of potatoes from healthy and virus infected potatoes grouped according to serological reactions on three test dates.

Date of Agglutination tests		Healthy	Virus S	Virus X	Viruses S and X
1st test August 17	Exp. I	6.69	6.21	6.73	6.46
	II	6.92	6.76	6.31	6.99
	III	6.82	6.34	6.38	6.83
Grand Mean		6.81	6.44	6.48	6.74
2nd test September 8	Exp. I	6.70	6.89	6.51	6.74
	II	6.79	7.21	6.27	6.76
	III	6.90	6.36	6.33	6.73
Grand Mean		6.80	6.78	6.37	6.67
3rd test September 17	Exp. I	6.81	6.90	7.04	6.38
	II	7.00	6.60	6.21	6.73
	III	6.95	6.38	6.24	6.78
Grand Mean		6.91	6.51	6.44	6.62

Table 17. Analysis of variance of potato chip data of Sebago variety grouped according to the reaction in the first agglutination test on August 17.

Source of Variance	d.f.	M.S.
Experiments	2	.185
Treatments	3	2.147
Experiments x treatments	6	2.561
Error	258	1.142
Total	269	

VI) Influence of virus infections upon the potato foliage

a. Fresh weight of the potato foliage

Data of the fresh weight of potato foliage was classified and grouped according to their reaction to the agglutination tests of

different dates (Table 18).

Table 18. Fresh weight of the potato foliage per hill (grams).

Date of Agglutination tests		Healthy	Virus S	Virus X	Viruses S and X
1st test August 17	Exp. I	1,118.88	982.80	946.51	873.17
	II	1,254.95	945.02	942.09	1,015.56
	III	846.73	916.28	745.61	740.05
Grand Mean		1,057.0	949.9	872.1	918.3
2nd test September 8	Exp. I	1,116.56	975.25	933.65	907.19
	II	1,224.72	988.86	997.93	1,025.99
	III	982.81	710.65	738.73	756.95
Grand Mean		1,109.13	858.4	875.7	908.8
3rd test September 17	Exp. I	1,046.78	1,179.40	890.2	960.4
	II	1,231.2	1,065.9	1,030.3	1,010.6
	III	1,141.57	680.37	719.29	759.79
Grand Mean		1,118.3	867.9	880.4	911.0

Analysis of variance of grouped data based on the first and third agglutination tests are shown in Table 19-a and 19-b. The groupings on the second test are not shown since their results were similar to the third test.

Table 19-a. Analysis of variance of fresh weight of the potato foliage grouped according to the reaction in the first agglutination test on August 17.

Source of Variance	d.f.	M.S.
Experiments	2	835,872.10**
Treatments	3	225,131.07**
Experiments x treatments	6	82,271.11*
Error	123	33,899.28
Total	124	

*P < .05
**P < .01

By comparing means, the following results were obtained:

	Healthy	Virus S	Virus S and X	Virus X
Fresh weight of potato foliage (grams)	1,057.0	949.9	918.3	872.1

Any two means not underscored by the same line are significant different.

Table 19-b. Analysis of variance of fresh weight of the potato foliage grouped according to the reaction in the third and final agglutination test on September 17.

Source of Variance	d.f.	M.S.
Experiments	2	835,842.1**
Treatments	3	347,714.1**
Experiments x treatments	6	48,965.0
Error	123	33,248.4
Total	134	

*P <.05
**P <.01

By comparing means, the following results were obtained?

	Healthy	Virus S	Virus S and X	Virus X
Fresh weight of potato foliage (grams)	1,118.3	910.9	880.4	867.9

Any two means not underscored by the same line are significantly different.

The healthy plants exhibited a more luxuriant growth, were bigger and produced more foliage than the virus-infected plants. The foliage weight of healthy plants was significantly greater than the weight of the virus-infected plants. There were no significant differences among the virus infected plants.

A correlation coefficient was calculated from the data to test the relation between tuber yield (number and weight) and foliage weight. The correlations of foliage weight with the number of tubers and with the weight of tubers are shown in Figure 5 and 6. A highly significant positive correlation was found between the foliage weight and tuber weight.

The foliage weight of virus S-infected plants was not consistent when grouped according to the reactions on the different test dates. However, a mean foliage weight of 949.9 grams per potato hill was obtained from the data of the first agglutination test: 858.4 grams from the date of the second and 867.9 grams from the date of the third test, respectively.

For the healthy plants or the plants infected with the other viruses, the foliage weight was fairly constant among the data grouped by the results of agglutination tests of different dates. The weights of both foliage and tubers appear to have fluctuated as the spread or evidence of virus was detected, causing the plants to be placed in another group.

In both cases, virus S-infected plants were not significantly different from the other virus-infected plants in the data of the third agglutination test.

Figure 5. Relationship between fresh weight of potato foliage and number of tubers grouped according to disease reading on each of three agglutination tests.

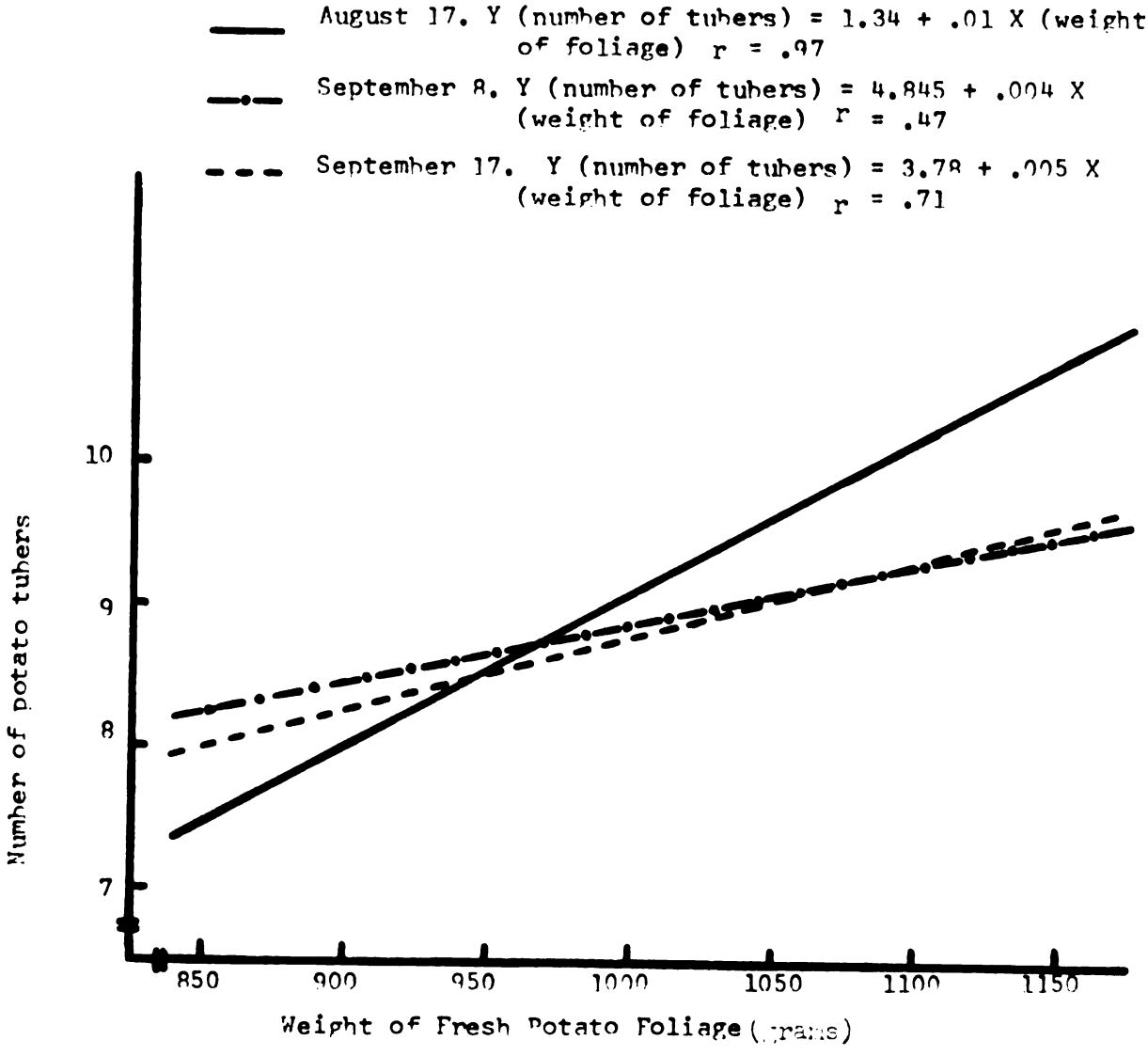
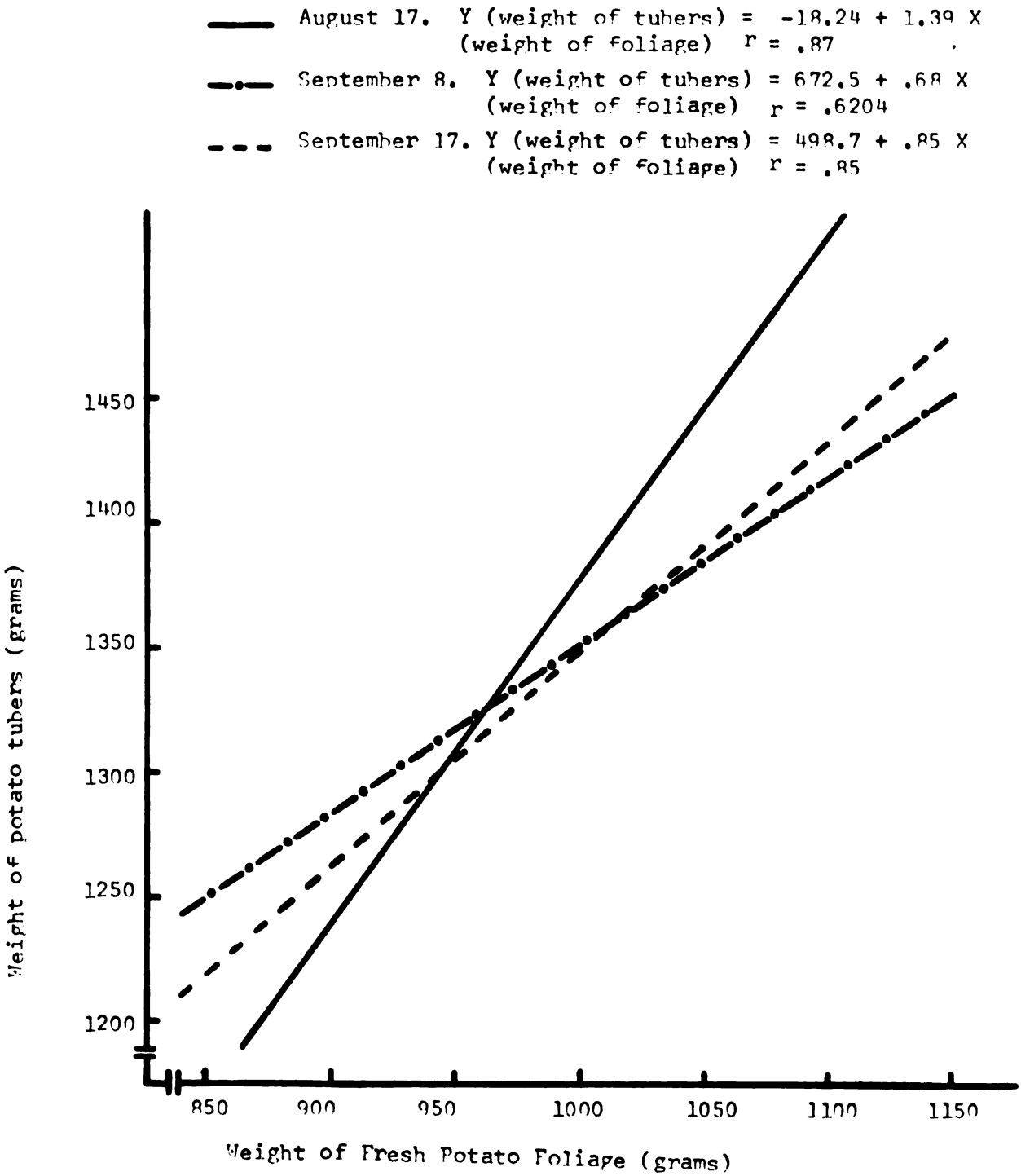


Figure 6. Relationship between fresh weight of potato foliage and weight of tubers grouped according to disease readings on each of three agglutination tests.



b. Percentage dry matter of potato foliage

Table 20. Means of the percentage dry matter of the potato foliage of healthy and virus-infected plants grouped by serological reactions on three test dates.

Date of Agglutination Tests		Healthy	Virus S	Virus X	Viruses S and X
1st test August 17	Exp. I	27.01	28.31	27.42	27.03
	II	30.23	27.57	28.70	28.68
	III	26.71	26.94	28.99	25.24
Grand Mean		27.78	27.64	28.39	27.19
2nd test September 8	Exp. I	26.95	25.69	26.67	27.59
	II	28.97	29.72	28.83	28.65
	III	27.84	24.38	28.80	26.37
Grand Mean		27.80	26.64	28.10	27.64
3rd test September 17	Exp. I	26.73	38.46	27.12	27.25
	II	30.49	28.00	29.23	28.53
	III	30.37	23.73	29.50	26.86
Grand Mean		28.58	26.42	28.55	27.55

The method of angular transformation was applied to the data for analysis of variance.

A significant difference was found only in the data grouped by reactions on the first agglutination test (Table 21).

Table 21. Analysis of variance of dry matter data of the potato foliage grouped according to the reactions in the first agglutination test on August 17.

Source of Variance	d.f.	M.S.
Experiments	2	23.6188**
Virus diseases	3	217.2798**
Experiments x virus diseases	6	111.1173**
Error	123	3.1862
Total	134	

*P <.05
**P <.01

When the means were compared, the following results were obtained.

	Virus X	Healthy	Virus S	Virus S and X
Mean of dry matter %	23.3954	27.7827	27.6453	27.1927

Any two means not underscored by the same line are significantly different.

The percent dry matter of virus X-infected plants was significantly greater than the percent dry matter of all the other plants. Healthy and virus S-infected plants were also significantly different from the combination of viruses S and X-infected plants. There was no significant difference between healthy and virus S-infected plants.

Attempts were made to relate tuber yield to dry matter of the potato foliage. The correlation of dry matter with tuber yield was negative but not significantly different.

The dry matter of the potato foliage of virus X-infected plants was higher than that of the other virus infected or healthy plants. Therefore, it can be stated that the greater fresh foliage weight of healthy plants must have been due to the water content of the foliage.

There have been several reports of a decreased rate of photosynthesis in virus infected leaves (3, 15, 25). Bald (4) suggested that final rapid tuber expansion at maturity was due to the translocation of reserve proteins from senescent leaves in normal plants, and that virus infection interfered with the translocating of such reserves from diseased leaves.

The systemic infections of viruses which invade the leaf parenchyma cells apparently resulted in a decrease in the rate of photosynthesis, regardless of the symptoms produced. It would appear that the decreased photosynthesis in diseased plants limited growth and tuber formation and probably made less carbohydrate available to tubers during the phase of their most rapid expansion. In this experiment, all the virus-infected plants could have had a reduced rate of photosynthesis and a disturbed carbohydrate metabolism which interfered with the translocation of such reserves from diseased leaves to the tubers.

SUMMARY AND CONCLUSION

This study was conducted to obtain information on the effect of some virus diseases upon the symptom expression, plant vigor, yield and quality of Sebago variety, Solanum tuberosum, L.. Serological and bioassay methods were used to determine the absence or presence of the viruses.

In the winter of 1963, 200 tubers of the Sebago variety from individual clones were planted in the greenhouse. The agglutination test was applied on each of 200 plants and all the tested plants were classified into different groups according to their reaction; healthy, virus S, X and the combination of viruses S and X respectively. Tubers from these grouped clones were space-planted five feet apart in the field of 1964. Washing caused by excessive rainfall created great variations in emergence and stand. It was not possible to evaluate the effect of virus diseases on growth and yield. The experiment was repeated in 1965. The procedures of 1963 were used to identify potato virus diseases in the 1964 plantings. Bioassay was carried out on each plant which showed a positive reaction in the agglutination test.

Approximately 150 plants for each of the potato viruses; S, X, and the combination of viruses S and X, and healthy plants were grown in the greenhouse. When the young potato plants were about 7-10 cm high, they were separated from the mother tubers with a

sterilized knife and transplanted into flats according to their classification. An additional 150 healthy plants were started to be inoculated with virus Y since it has not been found in all the tested plants. All the transplanted plants were inoculated to insure that they were infected with the virus diseases. Also, virus Y, the combination of viruses S and Y, viruses X and Y, and viruses S, Y and X were inoculated on healthy and already infected plant with an individual virus.

Three field experiments were planted at the Lake City Experiment Station. Four plants for each hill were space-transplanted 5 feet apart in a randomized complete block experiment with 10 replications. Each replication consisted of 9 hills; 7 diseased hills and 2 healthy plant hills. The agglutination test was carried out three times during the growing season.

Inoculations with virus Y and the various combinations of virus Y with the other viruses were not successful. The number of plants for each virus changed from the original numbers due to the absence of virus Y and the combination with the other viruses-infected plants, which subsequently become virus X and the combination of viruses S and X-infected, or to the current season infection.

The number of plants were therefore regrouped and analyzed based on the results of the agglutination test in different date by combining analysis of variance for three field experiments.

In this experiment, both of viruses S and X were mild viruses and the symptoms were not severe. A somewhat lighter green with slight mottling was observed on the leaves of virus S-infected plants. But

these symptoms were not consistently present and were not clearly visible. The mottling, the light yellowish areas and the number of small necrotic lesions were observed on the leaves of virus X and the combination of viruses S and X-infected plants. The combination of viruses S and X did not appear to be a new complex symptom. There was no visible difference in the expression of symptoms between them. However, the smaller necrotic lesions seemed to be more pronounced on the leaves of the plants infected with both virus S and X.

The healthy plants were significantly taller than the plants infected with virus X and the combination of viruses S and X throughout all the growing season. In early growing stage, there was no significant difference between healthy and virus S-infected plants. However, the healthy plants exceeded that of virus S-infected plants in later season.

It has been shown that the reductions in yield varies with the different viruses. The total number and weight of tubers of healthy plants were significantly greater than virus X and the combination of viruses S and X-infected plants. However, there was no significant difference between healthy and virus S-infected plants on tuber yield. Significant difference at the 5% level was found between virus S and virus X-infected plants in the number of tubers. Also, the virus S-infected plants were significantly difference at the 5% level compared to virus X and the combination of viruses S and X-infected plants in the data grouped based on the serological results obtained August 17. However, the weight of tubers of virus S-infected plants was not significantly

difference from the other viruses-infected plants.

Since there were no statistically significant differences in the small tubers of all the virus-infected and healthy plants, the effect of viruses on tuber yield was mainly due to the reduction in the yield of large tubers above 5 cm size.

Percentage reduction in yield was 17.5 with virus X, 15.5 with the combination of viruses S and X, and 6% with virus S respectively. From this experiment, it is concluded that the effect of virus X appeared in earlier growing stage. The influence of virus S appeared after the period of tuber sets. It produced more small tubers than the other viruses. The combination of viruses S and X did not give rise to a new complex disease. Reduction in tuber yield was not less than virus X.

Apparently, infection of viruses, in this experiment, did not effect the specific gravity of the Sebago potatoes. Infection of viruses was not associated with the color of potato chips. A highly significant positive correlation was found between fresh foliage weight and tuber yield. Both the greatest tuber yield and foliage weight was obtained from the healthy plants. The correlation of dry matter of foliage with tuber yield was negative but there was no significant differences.

Since the dry matter of potato foliage from the healthy plants was not higher than virus X-infected plants, it might be stated that the greater fresh foliage weight of healthy plants was mainly due to the sufficient water content of the foliage. In this experiment, it

was interpreted that all the virus infected plants could have had a reduced rate of photosynthesis and disturbed carbohydrate metabolism causing interference with the translocating of such reserves from diseased leaves to the tubers.

Finally, it has been shown that the agglutination test is a technique applicable to the identification of mild infections of viruses, and that plants in which the virus was not detected produced the greatest yields of marketable tubers. It can be utilized in a seed program to eliminate many plants which are a potential source of infection.

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