Results of an Experiment in Colorado to Appraise Effects of Virus Yellows in Sugar Beet[1]

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Virus yellows may affect all or nearly all plants in the field and may occur so generally in a district that no healthy fields or portions of fields are available to serve as measuring sticks of what the crop might be without virus yellows. The period of disease onset whether early or late is extremely important with respect to crop damage, early infections being far more serious than those occurring in late season. As a further complication, other conditions may bring about a yellowing of foliage which superficially resembles virus yellows. For these reasons the assessment of losses caused by the disease presents some difficulties.

In Europe, a long period ensued after the first outbreaks of virus yellows were noted in sugar beets before the condition was recognized as an infectious plant disease. After that a considerable period elapsed before growers and processors became convinced that virus yellows may cause serious losses. Watson, Watson and Hull (5)[3] by a series of experiments begun in 1942 showed that plants which were infected in June or July lost half or more of their potential yield of sugar. The loss in sugar production varied directly with the length of infection period and amounted to 3 to 5 percent of the yield of healthy plants for each week of infection between the appearance of visible symptoms and harvest. Hull (3) states that numerous infection tests made in recent years have confirmed these findings. The experimental results were obtained from a series of replicated plots. The inoculation technique employed in the British experiments was to place five aphids *(Myzns persicae)* which had been fed on virus yellows plants upon each sugar beet plant to be infected. Plants not infested with aphids served as controls. After 24 hours the entire experimental area was fumigated with nicotine vapor.

On the basis of a number of experiments in the Netherlands, as reported by Hartsuijker (2) , 100 percent virus yellows infection at the end of June or beginning of July was estimated as causing 25-30 percent loss in sugar production. Bjorling (1) in his experiments in Sweden showed 61 percent decrease in sugar yield for parcels infected 100 percent on June 30, in comparison with parcels not infected. The difference fell to 34 percent for total infection July 20 versus non-infected.

Schloesser (4) *in* a replicated test at Buir, Germany, compared yields from plots subject to natural infestation of *Myzus persicae* carrying both virus yellows and beet mosaic with those in which insect infestation was largely prevented by spraying with a systemic insecticide (E605). The

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[3] Numbers in parentheses refer to literature cited.

spraying was reported to have held infection to a minimum. The period of infestation of the plots by the aphids was prevailingly at the end of June and the beginning of July, a date considered moderately early in the Rhineland for virus yellows infection. Two varieties were included in the test. The unsprayed plots of Kleinwanzleben E had an average root yield 23 percent below the yield of the plots treated with the systemic insecticide, a polariscope reading reduced by 0.68, and a 26 percent drop in sugar production. The corresponding figures for Kleinwanzleben CR, the other variety, were 18 percent reduction in root yield, 0.67 less in polariscope reading and 21 percent reduction in sugar production attributable to the insect-borne diseases.

In his tests Schloesser found that virus yellows combined with mosaic affected all important attributes which are concerned with returns from the beet crop, especially those related to quality. The soluble ash content increased in roots from diseased plots over that from the roots of sprayed plots and harmful nitrogen similarly was increased. The drop in polarization was less than has been reported for other experiments from early and severe virus yellows infections.

The severe losses caused by European virus strains do not necessarily mean that the strains of yellows virus in the United States will cause similar losses. Dr. Hull, on the basis of his observations of field symptoms in Colorado, Utah, California and Michigan, considered our strains to be of mild type, an opinion borne out by limited tests which he and Schloesser have been able to carry on with diseased plants sent from the United States.

It is therefore important to determine under our conditions, and with the strain or strains of yellows virus which occur here, the effects of the disease upon the sugar beet crop. An experiment was planned in which healthy plants and those affected with virus yellows were to be compared for yield and quality. Three dates of inoculation, namely, early, medium, and late season, were to be employed to give differential lengths of disease involvement. The yellows virus was to be the strain, or strains, found in the vicinity of Fort Collins, Colorado.

Viruliferous aphids were to be placed on all the sugar beet plants in the two center rows of the 8 X replicated plots at a given inoculation date, hence the onset of the disease would be at approximately the same time for these plots. For each inoculation date series, a similar number of adjacent plots not exposed to aphids was set up to serve as checks. The spread of aphids from the infested plots to the non-infested plots was to be prevented by frequent nicotine sulphate sprays which would also control, to some extent, aphids which might fly into the field from outside.

The design of the experiment called for the groups of plots for each of the three inoculation dates (early, medium, and late season) to occur at random in two blocks. Within the inoculation-date group of a block, the four infested and the four control plots were randomized. There were eight plots for each treatment at each inoculation date. Each plot consisted of four rows 25 feet long. The rows were 20 inches apart. The field chosen for the experiment had an excellent stand of the sugar beet variety GW 304.

On June 16 the first infestation with aphids was made. Only the two inner rows of the plot to be infested received the viruliferous aphids, about five to ten individuals, placed on each plant of these rows. The second inoculation with aphids as the vector of the virus was carried out July 16, following the same techniques; the late inoculation scheduled for August 10 was not carried out.

The colonies of *Myzus persicae* in this experiment were grown by the Entomology Section of the Colorado Agricultural Experiment Station. The aphids were hatched from eggs obtained from peach buds collected at Grand Junction, Colorado. Sugar beet roots known to be infected with virus yellows typical of that which occurred generally in the fields around Fort Collins were collected in October, 1952, from a field near Johnstown, Colorado, which was severely affected with virus yellows but apparently entirely free from beet mosaic. The roots were potted in early spring in the greenhouse and the new growth from each was tested by means of juice inoculations of healthy beet seedlings to make sure that beet mosaic was absent. No evidence of beet mosaic was found. They were put in cages and the virus-free *Myzus persicae* colonized on them.

By June 16 the leaves of the source plants for the yellows virus were heavily infested with aphids. The leaves were cut into small pieces with as little disturbance to the aphids as possible. The pieces were dropped into vials, each vial receiving not less than five aphids. Within a half hour, on the average, a crew of men in the field placed the small pieces of leaf and aphids on the young sugar beet plants, the contents of one vial being used for each plant. Conditions were excellent for aphid transfer at both infestation periods and the insects promptly moved to the young sugar beets.

Unfortunately, nicotine sulphate used as a spray to kill the aphids in the infested field after the June 16 inoculation was not entirely effective, so that viruliferous aphids moved into the control plots from the infested plots. These aphids plus any viruliferous aphids which may have come from adjacent fields led to a heavy incidence of virus yellows in the plots that should have been nearly yellows free. When it was found that the nicotine sulphate applications were not effective, a spray of tetraethyl pyrophosphate, TEPP, was substituted. TEPP gave a satisfactory kill.

The second inoculation date was July 16 and the same program for aphid transfer was followed. The spread of infectious insects from the inoculated plots to the checks was largely prevented by the TEPP spray, but because of the earlier movement of aphids from the infested plots occasioned by failure of nicotine sulphate to give an effective kill, together with movement of aphids from outside fields, the control plots in this series developed considerable virus yellows, the average for all control plots for this date of infestation being 32 percent. This high incidence, however, was in marked contrast to the control plots of the earlier infestation which showed, on the average, 64 percent virus yellows.

The results of the experiment, based on the entire plant population in the two inner rows of each plot, are summarized in Table 1.

Table 1.—Acre Yields of Roots and Sugar and the Sucrose Percentages from non-inoculated Plots and from Plots Inoculated with Virus Yellows by Means of *Myzus persicae:* Two Dates of Inoculation. All Plants in 40 Feet of Row Per Plot Harvested. Bay Farm, Fort Collins, Colorado, 1953. Results Given as 8-Plot Averages.

Treatment	Acre-yield		Sucrose	Plants per 100′ row	Incidence of virus yellows 9/29
	Gross sugar	Roots			
	Pounds	Tons	Percent	Number	Percent
A. June 16 inoculation:					
Not inoculated	5,350	16.43	16.27	105	64
Inoculated	5,248	16.14	16.28	106	93
Difference	102	0.29	0.01		
B. July 16 inoculation:					
Not inoculated	5,752	17.55	16.41	98	32
Inoculated	5,372	16.34	16.44	102	92
Difference	380[1]	1.19[1]	0.03		

[1] Differences marked are significant at 5 percent point.

No significant differences in acre yields of roots or sugar or in sucrose percentage were found between the early-inoculated plots and the non-inoculated plots set up as controls. In view of the high percentage of obvious virus yellows in the control plots, and since such readings may not fully reflect the incidence of the disease, little contrast between the inoculated plots and the control plots was to be expected. For the individual control plots, the readings of the number of plants obviously diseased with virus yellows up to September 29 were as follows: 76, 79, 64, 27, 73, 49, 59, and 84 percent; whereas, the inoculated plants gave readings of 98, 88, 93. 97, 91, 95, 84, and 95 percent virus yellows. Obviously there were not enough plants free from virus yellows in the control plots to bring about substantial yield differences.

The results from the series of plots inoculated with virus yellows July 16 show some significant differences. As shown in the table, the inoculated plots which averaged 92 percent virus yellows yielded significantly less than the control plots in spite of the fact that these averaged 32 percent virus yellows. The difference in root yield was 6.8 percent and the difference in sugar yield was 6.6 percent in favor of the controls. No appreciable difference in sucrose percentage between the inoculated plots and the controls was found. Clearly, virus yellows infection had a depressing effect on root growth.

In the July 16 series, as an average, 32 percent of the plants of the control plots had been staked by September 29 as showing virus yellows. This average, however, is misleading since the readings of virus yellows plants in the individual control plots ran 71, 27, 49, 56, 14, 3, 20, and 18 percent. The plots infested with viruliferous aphids read 93, 83, 93, 93, 88, 92, 95, and 95 percent virus yellows. Considering only the 5 control plots showing the least virus yellows and the 5 comparable infested plots, the latter averaged 11.4 percent less than the controls in yield of roots and 10.6 percent less in yield of gross sugar. The difference in sucrose percentage was negligible.

In considering the results from this test, it may be assumed that whatever natural virus yellows infection may have occurred before the inocula-

tions were made July 16 was balanced over the plots, that the virus yellows counts are low rather than high since only obviously diseased plants were noted, and that the high percentage of virus yellows in the control plots obscured to a very marked degree the contrasts between the inoculated and non-inoculated plots.

If the heavily infected plots when contrasted with those presumably only one-third infected show 6.8 percent reduction in weight of roots it is reasonable to assume that if the comparison could have been made between plots 100 percent diseased and 100 percent healthy then the difference would be more nearly on the order of 10 to 15 percent. The results obtained by selecting five of the least infected control plots for comparison with their neighboring infected plots support this assumption.

In Europe, as we have seen, differences in readings of sucrose percentage of 1 to 2 numerical percents may occur between early-infected and non-infected plants, although there is considerable variation with respect to this attribute. The data from this test do not show that virus yellows affected sucrose percentage.

This experiment represents the first attempt to appraise virus yellows effects in Colorado by means of controlled inoculations. Results over several years are needed. The techniques for infesting the plots with aphids, and for obtaining a complete kill to prevent the spread of viruliferous aphids to the control plots, have been explored. From the results, it is clear that infection from outside the experimental area also needs to be prevented. An experiment of this sort must be replicated many times to avoid bias which might come from soil heterogeneity and the entire area must be sampled. The sprayer as it is used for insect control must traverse the entire area to avoid differences in degree of soil compaction and of mechanical injury to the foliage; the insecticide used must not be such that its residues interfere with subsequent inoculations of plots in a late series by means of aphids. Next year's work should be done with greater effectiveness and some additional factors will be introduced to throw more light on the virus yellows problem.

Summary

Effects of virus yellows on sugar beet yield and quality were studied in a replicated test at Fort Collins, Colorado, in 1953 with the virus strain or strains prevalent there. Inoculations were made with viruliferous aphids.

Plots inoculated July 16 developed 92 percent obvious infection by September 29; the control plots did not remain free from virus yellows, but showed an average of 32 percent. The average root yield of the artifiicially infected plots was 6.8 percent less than that of the controls, a significant difference. Effect on sucrose percentage was negligible.

It is estimated that if the comparison could have been based on 100 percent diseased versus 100 percent healthy plants, virus yellows would have shown a repression of root growth of between 10 and 15 percent.

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