Sugar Beet Virus Yellows Situation in the United States

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Since the first reports of the occurrence of sugar beet yellows in Europe, and especially after the investigations by Quanjer (7)[2], Roland (8), Watson (11), and others definitely established its virus nature, numerous attempts have been made to determine whether the disease occurs in the United States. The earlier European accounts were somewhat conflicting and the symptoms of the disease were not clearly described. The black and white photographs gave little idea of the effects on individual plants, or of the field aspect of the disease. It had been shown that plants exposed to virus-bearing aphids became the foci of virus infection when taken to the field. In many cases, the success of inoculations was determined by the capacity of inoculated plants set out in the field to become infection centers.

Much of this early evidence seemed at variance with American experience with virus infections, so that considerable skepticism concerning virus yellows existed among plant pathologists here. Any such confusion has now been entirely corrected by the critical research in the Netherlands, France, England, Belgium, Germany and elsewhere, so that now virus yellows is recognized as a distinct disease complex concerning which there is definite concordance of views among experts.

The beet sugar industry of Europe was extremely slow to believe that the yellowing of fields, which took place in early August or which in some years was deferred, was assignable to disease and not the result of early ripening, drought, excessive moisture, nutritional deficiencies or soil conditions. Many of these conditions do cause plants to take on a yellow appearance, but these may be definitely separated from the virus yellows syndrome.

It now seems clear that virus yellows is not a new disease in Europe. Authentic reports in the Netherlands of a yellowing of fields of the same character as those which occur nowadays date from 1912[3]. In Denmark, E. Gram (3) believed there were acceptable records from that country as far back as 1914. In Belgium, the reports indicate that in 1925, 1927, 1928 and, above all, in 1929, virus yellows was generally present and destructive. In England (11) the disease was identified in 1940, although it had been suggested in 1935 that the disease might be present. Apparently the outbreak of virus yellows in Germany is relatively recent—1937 (9) .

Beginning in 1934 and continuing over many seasons, sugar beets showing symptoms which appeared to correspond to European descriptions of virus yellows were collected from commercial fields in Michigan, Ohio and Colorado, and taken to the greenhouse where aphids were colonized upon them. The aphids were transferred to healthy plants to determine whether a virus was present. Attempts at mechanical inoculation were also made.

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[2] Numbers in parentheses refer to literature cited.

[3] Verbal communication—Henk Rietberg, Director, Instituut voor Rationele Suikerproductie, Bergen-op-Zoom, The Netherlands.

Consistent failures from all attempts had almost lead to a fixed opinion that virus yellows was not present in United States and that we should look to other factors to explain the types of yellowing which occurred with us.

On September 26, 1940, the writer noted in the field at the Colorado Agricultural Experiment Station, Fort Collins, Colorado, where sugar beet agronomic experiments were being conducted, that the edges of the field and certain spots 30 to 50 feet in diameter were conspicuous because of sugar beet plants whose outer leaves were yellow, especially at the tips of the leaves. The condition was designated at the time as "brittle yellows." Similar observations were made in September, 1947. About the same time, A. C. Maxson and H. E. Brewbaker reported to the writer that they had observed for several seasons a yellowing of foliage which began to develop about mid-season in a few sugar beets growing near the Great Western Experiment Station at Longmont, Colorado, and that this yellowing before the end of the season would apparently involve almost all the sugar beets growing in the plot in the immediate proximity of the Station.

In the fall of 1949, many yellowed plants having symptoms which apparently conformed to those of virus yellows in Europe were collected in Michigan and Colorado. Because of the high incidence of sugar beet mosaic in the plants, they were grown in the sugarcane greenhouse at Plant Industry Station at Beltsville, Maryland, in order to avoid chance of mosaic spreading to other sugar beet cultures at the Station.

Dr. Rietberg[3] in February, 1950, brought anti-yellows serum (1) as prepared by the Laboratorium voor Bloembollenonderzoek at Lisse, Netherlands, to Beltsville, Maryland, together with apparatus and other check sera as necessary for testing for presence of virus yellows in suspected plants.[4] He trained the late Dr. J. E. Kotila and the writer in the technique but was not able, during his brief period at Beltsville, to demonstrate the presence of virus in suspected plants.

No positive results were obtained in later work during that spring. We now know that the spontaneous flocculation which made it impossible to read the preparations was due to warm conditions in the greenhouse in which the plants were grown and the very warm room conditions when the juices were being extracted.

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[3] In the technique commonly followed at the Lisse Laboratory, the anti-yellows serum is obtained from a rabbit which has been repeatedly injected, at about two-week intervals, with juice from sugar beets showing typical virus yellows symptoms. When the rabbit has reached a high state of immunization to the yellows virus, it is bled and the serum separated. Such serum will keep for an indefinite period if frozen. Prior to making a test of suspected plants, it is necessary to saturate the serum with juice from healthy sugar beet leaves to remove any antibodies which might react with the protein of the normal sugar beet plant. Usually 0.1 cc. of serum is added to 1.0 cc. of juice from healthy leaves. After incubation at 37° C. for two hours, the juice—plus anti-serum—is kept for 24 hours at slightly above the freezing point. The solution is then centrifuged at high speed to obtain a crystal-clear solution for the tests. To test a suspected plant, mature or nearly mature leaves are pressed to obtain a small quantity of juice. This is diluted 50:50 with 0.9 percent NaCl solution. Small drops of approximately equal size of the anti-yellows serum, diluted as indicated, and the diluted plant juice are placed side by side on a cover glass. The drops are then mixed with a small glass rod. The test drop on the cover glass is then inverted and placed over a glass ring as a hanging drop. Vaseline is used to seal the cover glass to the van Tieghem ring.

After periods of one hour and two hours, respectively, at 37° C., the drop is examined under the microscope, a low-power objective and a darkfield condenser being used. Positive reactions are manifested by clouds forming in the drop and by flocculation of the minute particles into discrete clumps. With a negative reaction, the field remains free from clouds and the small particles are scattered. As checks, the juice from the suspected plant is tested against a serum prepared by injecting the rabbit with juice from healthy plants, and against normal rabbit serum. These are saturated with healthy juice as described for the anti-yellows serum. For a test to be acceptable, there must be no clouding or flocculation with the check sera.

In November, 1950, a new shipment of anti-yellows serum was sent by Dr. Rietberg, and in February, 1951, he again visited the United States. In tests with the anti-yellows serum from the Netherlands, Plant X-100—collected at Cone, Michigan, in September, 1950—was selected by Dr. Rietberg for the first tests because it showed a mild netting of the veins of older leaves. This plant gave positive reactions against anti-yellows serum entirely comparable with the reactions obtained with juice from virus yellows plants of the Netherlands known to have been affected with virus yellows which Dr. Rietberg had brought to the United States (2). Since that time, Michigan Plant X-100 and a companion plant from the same field, X-105, have been tested repeatedly and always with positive reactions. Frozen juice of Plant X-100, taken to Holland in June, 1951, gave positive readings in the serological tests conducted by Dr. J. A. Hijner.

During late winter and spring of 1951, many serological tests were made by the writer on suspected plants which had been collected in Michigan, Ohio, Colorado and with clones of Utah plants that had been maintained in the Beltsville greenhouse. Out of seven plants collected in Michigan and Ohio, positive tests were obtained from two plants as already mentioned. These plants giving positive tests gave such reactions consistently and the plants which were negative likewise remained negative. Out of twenty-six plants picked up in Colorado because of yellowed foliage, nine were positive, two probable and fifteen negatives. Two monogerm plants received originally from Salt Lake City in 1950, which had been maintained by cloning, gave positive reactions.

In the spring of 1951 aphids were colonized on plants which had given positive reactions, but all tests failed because of death of the aphids from parathion residues on the greenhouse plants.

Kassanis (5) reported that mechanical inoculation of sugar beets with virus yellows was possible, about ten percent of the plants becoming systemically infected. When plants were kept four days in the dark, three times as many became systemically infected. Juice inoculations were tried in two experiments in which sap from the leaves of Michigan Plant X-100 was rubbed on the leaves of healthy sugar beet plants following a light dusting of the leaf surface with carborundum powder.

In the experiment performed March 17, 1951, fourteen plants of the variety SP 503036-01 in about the six-leaf stage were so inoculated and one plant, No. 11 of the series, showed a yellow spot about four millimeters in diameter on March 22. Yellow spots or blotches continued to appear on the new leaves which developed on this plant. The spots were sometimes circular, but usually more of a lemon-yellow blotch. One week later juice was pressed from the leaf, which had been kept in the icebox, and the juice tested as antigen against anti-yellows serum. It gave a positive test. Attempts to colonize aphids on Plant No. 11 failed because of death of the aphids.

On April 19, 1951, nine plants of the variety 46108-01, which were about seven months old, were similarly inoculated with sap of X-100. These plants and three companion plants had previously been placed for six days in the dark in a refrigerated cold-storage room where mother beets are kept. In the April 19 inoculations, two or three leaves per plant were

dusted with carborundum and then rubbed lightly with juice obtained by grinding a leaf of Plant X-100 in a mortar. Nine plants were so inoculated with juice from X-100; three other plants were rubbed with water as check. Untreated plants of the same planting stood nearby. On May 14, 1951, all plants including the checks and the untreated plants, except Plant No. 5, were normal. Plant No. 5 was noted as having a suspicious mottling not unlike beet mosaic but occurring everywhere throughout the leaves. Some excrescences had formed at the scratches. The juice of a leaf of this plant was squeezed out on June 8, frozen, and taken in the frozen condition to Netherlands by air where it was tested along with many other frozen juices by Dr. Hijner. The juice of Plant No. 5 of the April 19 inoculation gave a positive reading, tests being made in the blind by Dr. Hijner, Dr. Rietberg and the writer. Juice from a check plant (Plant No. 12) similarly treated gave negative results.

In August and September, 1951, Dr. Raymond Hull of the Rothamsted Experiment Station, who is stationed at Dunholme, England, and is a specialist on virus yellows (4) , traveled in Colorado, Utah, Oregon, California and Michigan. In his inspection of sugar beet fields, he found plants with typical virus yellows aspect in all states visited. In Colorado and Utah, he found such plants only *in* close proximity to greenhouses or seed plots where mother beets could have carried over the virus. Yellowed plants of this type were not found in commercial fields. In Oregon he found red garden beets and volunteer sugar beets growing in the stubble of a recently harvested sugar beet seed crop which he suspected of being diseased with virus yellows.

Both red garden beets and sugar beets collected in Oregon and brought to Beltsville have given positive serological tests. Plants taken from Colorado fields in which Dr. Hull had noted plants with a typical virus yellows aspect have also been shipped to Beltsville. These have given positive serological reactions. Plants from the greenhouse of the U. S. Sugar Plant Field Laboratory at Salt Lake City, Utah, where Dr. Hull suspected virus yellows to be prevalent, have also given positive reactions whereas seedling plants collected from a field at a distance from the greenhouse have given negative reactions.

At Salinas, California, Dr. Hull investigated a condition of sugar beets which had been noted for several years and was spoken of as "Salinas yellows." By mid-August the fields had become almost totally yellow. One field (Salinas Vegetable Growers' Exchange field) was noted in which the yellowing occurred only as a few limited areas, resembling the early stages of the disease in European sugar beet fields. The symptomatology shown by the yellowed plants in California was entirely comparable to that of European plants known to be affected with virus yellows. This was so stated by Dr. Hull.

During October 22-25, C. W. Bennett, E. Carsner and the writer inspected sugar beets fields near Salinas, Clarksburg and Linden, California. The extreme yellow phase was being succeeded by a green growth of inner leaves, but the fields still had a yellow cast and the outer leaves were yellow and brittle, resembling virus yellows as seen by the writer in Europe. Curly-top and severe red spider injury which had obscured the disease symptoms

in other years were almost absent in 1951. The field of the Salinas Valley Vegetable Growers' Exchange was now totally infected.

Plants had been collected from the fields visited by Dr. Hull by Dr. J. S. McFarlane. Additional collections were made at Salinas, Spreckels, Clarksburg and Linden. These were shipped to Beltsville, Maryland. Juice also was expressed from fresh leaves, frozen and shipped to Beltsville. Representative material from the California collections has given positive reaction with the anti-yellows serum supplied by Dr. Rietberg.

Dr. Pierre Limasset of the Station Centrale Pathologie Vegetale, Versailles, France, in January, 1952, tested juice from suspected plants with an anti-yellows serum which he had produced at the Versailles station. With the technique in use at Versailles (6) , leaves from plants sent from Spreckels and elsewhere were tested serologically with positive reactions. Dr. Limasset stated that with a reaction so specific as that of virus yellows protein against an anti-yellows serum that he had no doubt of the presence of virus yellows in California and in Utah, as a result of the tests he had conducted.

Aphid transmissions of virus yellows have been accomplished, using both Oregon and California plants as virus sources. Plants collected in Oregon by Dr. Hull, and plants from the Salinas, California, fields that he visited, had been growing under quarantine at Plant Industry Station, Beltsville, Maryland. Several transmission tests started in November and December, 1951, gave positive results. One of these is cited since an Oregon plant was used as a virus source.

A test was made using a sugar beet (No. 2) collected by Dr. Hull in Oregon as showing virus yellows symptoms. Aphids *(Myzus persicae)* had fed on this plant for a few weeks. On November 14, 1951, small pieces of two yellow outer leaves, each piece with about ten aphids, were placed on healthy sugar beet seedlings in the four-leaf stage. The aphids promptly moved to the test plants. The next day, aphids were noted as feeding. Three days later the aphids were killed by nicotine spray and the plants were placed in the quarantine greenhouse. On December 12, 1951, and January 29, 1952, four out of nine inoculated plants were found to show yellow, brittle leaves typical of virus yellows. Five check plants *in* this experiment not exposed to aphids remained healthy. Three of the plants diagnosed as positive were tested with anti-yellows serum and gave positive reactions. One check plant was tested serologically and was found to be negative.

The aphid transmission test started December 13, 1951, is reported in detail. Aphids [5] had been colonized for nearly a month on a plant from the Jensen field (Salinas 1) and on a plant from the Jacobs field (Salinas 2) . A piece of leaf carrying approximately ten aphids was placed alongside a healthy plant in the two-leaf stage and the aphids allowed to crawl to the young plant. Observation was made the next day and, in all cases but one, feeding was recorded as positive, and this one was questioned.

Repeated examinations of these plants showed absence of veinlet clearing. The signs of disease were very obscure or absent until January 31 when

[5] Obtained from the stock cultures of Dr. Floyd F. Smith, Bureau of Entomology and Plant Quarantine.

Table I.—Results from Inoculation Test in Which Plants from Jensen Field (Lot 1) and Jacobs Field (Lot 2) Were Sources of Inoculum. Ten Apterous Aphids *(Myzus Persicae)* Were Placed on Healthy Two-leaf Stage Sugar Beet Plants, Decmber 13, 1951. (Read Jan. 31, 1952, and Feb. 1, 1952. No Veinlet Clearing)

One Plant—Salinas Lot 1						One Plant—Salinas Lot 2						Check					
		Readings 1/31/52 and 2/1/52						Readings 1/31/52 and 2/1/52						Readings 1/31/52 and 2/1/52			
Plt. No.	Aphids	Feeding 12/14	Color Oldest Leaf	Texture	Serum Test	Plt. No.	Aphids	Feeding 12/14	Color Oldest Leaf	Texture	Serum Test	Plt. No.	Aphids	Feeding 12/14	Color Oldest Leaf	Texture	Serum Test
1	10+	+	Yel.	Br.	+	11	10+	+	Yel.	Not Br.	—	20	0	—	pl. yel.	nor.	—
2	10+	+	Yel.	Wil.	—	12	8	+	pl. yel.	Br.	—	21	0	—	pl. yel.	nor.	—
3	10+	+	Yel.	Br.	+	13	10	+	yel. bl.	Br.	+	22	0	—	pl. yel.	nor.	—
4	10+	+	Yel.	Br. th.	—	14	10	+	pl. yel.	Sl. br.	+	23	0	—	pl. yel.	wil.	—
5	10+	+	Pale	Sl. Br.	—	15	10	+	pl. yel.	Sl. br.	±	24	0	—	gr.	nor.	—
6	10+	+	Yel-Gr.	Br. th.	±	16	10	+	pl. yel.	Br.	+	25	0	—	pale	nor.	—
7	10+	+	Gr.	Br.	+	17	10	+	pl. yel.	Sl. br.	+	26	0	—	gr.	nor.	—
8	10+	+	Gr.	Br. th.	+	18	10	+	pl. yel.	Sl. br.	—	27	0	—	pl. yel.	nor.	—
9	10+	+	[1]Gr-Yel.	Br.	+	19	5	±	pl. yel.	Sl. br.	—	28	0	—	gr.	nor.	—
10	10+	+	[1]Yel.	Not Br.	—							29	0	—	blan.	nor.	—
												30	0	—	gr.	nor.	—

[1] Lowest leaf dead; next one read.

Abbreviations key: Yel. = yellow; Gr. = green; Br. = brittle; Wil. = wilted; Th. = thick; Pl. = pale; Sl. = slight; Blan. = blanched; Bl. = blotched; Nor. = normal

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Table 2.—Results from Inoculation Test in Which a Plant from Salinas Valley Vegetable Growers' Exchange Field was the Source of Inoculum. Aphids Were Colonized One Month on this Plant, Then Transferred on Jan. 8 to Test Plants, About 25 Aphids Per Plant. Test Plants Were in Two- to Four-leaf Stage. Feeding Period was Two Days. (When Read on Jan. 29 and Mar. 21, no Veinlet Clearing was Noted.)

Check					On Plt. From Salinas Valley Veg. Gr. Exch. Field as Inoculum Source					
Test Plt. No.	Aphids	Condition of Lower Leaves 1/29/52	3/21/52	Results of Serological Tests[1]	Test Plt. No.	No. of Aphids Appl'd 1/8/52	Aphids Feeding 1/9/52	Condition of Lower Leaves 1/29/52	3/21/52	Decision as to Virus Yellows	Results of Serological Tests[1]
1	0	normal	normal	3/21 lost	13	25+	many	normal	normal	±	3/21 —; +
2	0	normal	normal	3/21 lost	14	25+	many	normal	brittle	+	3/21 +
3	0	normal	normal	3/21 lost	15	25+	many	normal	normal	—	3/21 ±
4	0	normal	normal	3/21 —	16	25+	many	normal	brittle	+	3/21 +
5	0	normal	normal	4/1 —	17	25+	many	normal	brit; th.	+	3/21 +
6	0	normal	normal	4/1 —	18	25+	many	normal	normal	—	1/29 —; 4/1 —
7	0	normal	normal	4/1 lost	19	25+	many	normal	brittle	+	4/1 +++
				4/2 lost	20	25+	many	normal	brittle	+	4/1 +
8	0	normal	normal	4/2 —	21	25+	many	normal	brittle	+	4/1 +
9	0	normal	normal	4/2 —	22	25+	many	normal	normal	—	4/2 —
10	0	normal	normal	4/1 —	23	25+	many	normal	brittle	+	4/2 ++++
11	0	normal	normal	4/3 —	24	25+	many	normal	normal	—	4/2 —
12	0	normal	normal	4/3 —	25	25+	many	normal	normal	—	4/2 —; —
					26	25+	many	normal	yel., brit.	+	4/3 +++
					27	25+	many	normal	normal	—	4/3 + (weak)
					28	25+	5 or 6	normal	brittle	+	4/3 +++
					29	25+	many	yel., brit.	brittle	+	1/29 +; 4/3 +++
					30	25+	many	yel., brit.	brittle	+	1/29 +; 4/3 +++
					31	25+	many	normal	brittle	+	4/3 +++
					32	25+	many	normal	normal	—	4/3 —
					33	25+	many	normal	brittle	+	4/3 lost

[1] Serological readings of — mean no flocculation with anti-yellows serum from Bergen-op-Zoom; ± means slight cloud, not clear positive; readings of +, ++, +++, and ++++ represent definite flocculation, heaviest virus concentrations giving the +++ and ++++ reactions.

samples were taken of the oldest leaves for serological tests. Then it was found that in very high percent of plants the first and second leaves, i.e. the oldest, of inoculated plants were greenish-yellow, thickened and brittle whereas leaves *on* the check plants usually were green or, if yellow, were a clear, bright yellow. Leaf texture of check plants was normal, tending to be more flaccid than brittle. As the juice was being squeezed for the serological tests, the brittleness of the leaves of many of the inoculated plants was very evident in contrast to the soft, yielding condition of the leaves of the check plants. Results of the tests are given in Table 1.

Failure to obtain pronounced symptoms of virus yellows in greenhouse culture in winter is characteristic of the disease (10) . Attention is also called to the fact that the virus strain from the Salinas plants did not cause veinlet clearing. The strains of virus yellows recently investigated in Netherlands and England, in contrast to those studied earlier, show veinlet clearing of young leaves, a sign which assists in determination of positive reacting plants.

January 8, 1952, aphids *(Myzus persicae)* which had been colonized for a month on a plant from the Salinas Valley Vegetable Growers' Exchange field were placed on healthy sugar beet plants in the two- to four-leaf stage, about twenty-five aphids being used per plant. The aphids were confined by a glass cylinder placed over each plant. The next day, it was found that in all cases the aphids were feeding. Next day the aphids were killed with nicotine and the plants put in the quarantine greenhouse. After three weeks three plants which showed signs of brittleness of the first true leaf were tested serologically. Two of these plants gave positive reactions. One large plant in the check series which showed a bright yellow lower leaf was tested—it was negative.

The details of this test and the subsequent readings in which a high percentage of the inoculated plants became affected with virus yellows are reported in Table 2 along with results of the serological tests. These results are of particular importance because Dr. Hull had made most positive statements concerning this field, as showing typical virus yellows aspect.

The concordance of disease symptoms of European and American plants, as stated by experts who are familiar with virus yellows in Europe, and the experimental work to date with anti-yellows sera and the inoculation tests, including the last reported tests of aphid transmission, lead the writer to a definite conclusion—that virus yellows exists in United States and that the condition known as "Salinas yellows," together with the similar yellowing effects noted near Clarksburg and near Linden, are indeed manifestations of virus yellows. The presence of virus yellows in Oregon also has been demonstrated. *On* the basis of symptomatology and serum tests, it can be stated that virus yellows also occurs in Utah, Colorado and Michigan. The Michigan type of virus yellows is being investigated further.

Summary

Virus yellows of sugar beet, known as **a** widespread **and** serious disease of **sugar and** forage beets **in Europe,** has **now** been positively **determined as occurring in Michigan, Colorado, Utah, Oregon and California- European specialists on virus yellows have confirmed the similarity of symptoms and have supervised serological tests of suspected plants. Utilizing and-**

yellows sera obtained from Lisse, Netherlands, and Versailles, France, positive tests have been obtained from plants collected in the states mentioned. Transmission tests with juice (Michigan plants) and with aphids, *Myzus persicae*, (California and Oregon plants) have given positive results. The disease known in California as "Salinas yellows," on the basis of aphid transmissions and serological tests, has been shown to be virus yellows. The virus causes yellowing and thickening of outer leaves and brittleness; veinlet clearing has not been noted.

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