FURTHER STUDIES OF SUGAR-BEET SEED BALL EXTRACTS WITH SPECIAL REFERENCE TO THE TOXICITY OF HYDROLYZED AMMONIA
(Demonstration Paper)

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The presence of water soluble substances in sugar-beet seed balls which had a toxic effect on the germination of sugar-beet seed was reported in a paper presented at the 1938 meeting of the American Society of Sugar Beet Technologists.

The present paper is an evaluation of the effect of osmotic pressure and hydrolyzed free ammonia on the germination of sugar beet and certain other seeds. There was a marked differential tolerance of various seeds to osmotic pressure. It was evident, however, that even with seeds most sensitive to osmotic pressure this factor alone did not account for the major inhibiting effects of seed ball extracts or of solutions of urea or asparagin.

Free ammonia, in concentrations sufficient to account for the observed damage, was recovered from seed extracts and dilute solutions of urea and asparagin on which seeds had been placed to germinate. This ammonia was released by enzymatic hydrolysis of water soluble organic nitrogen compounds present in the extracts or solutions.

Increases up to four units in the pH of extracts were observed during germination tests (this is equivalent to a 10,000 fold decrease in hydrogen ion concentration). However, tests with dilute ammonia and slightly buffered Potassium Hydroxide solutions in which the pH of the liquid in contact with the seeds was maintained by a constant flow, indicated that free ammonia rather than hydroxyl ion was responsible for the injury. Inorganic ionized ammonium salt solutions which were slightly acid in reaction had little effect on germination other than that which could be attributed to osmotic pressure. This indicated that free ammonia rather than the ammonium ion caused the toxic effects observed.

RESISTANCE TO FUSARIUM YELLOWS IN SUGAR BEETS

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INTRODUCTION

In 1931, Stewart (1) reported the disease of sugar beets caused by Fusarium conglutinans var. betae. The symptoms on sugar beets were similar in many respects to those found in cabbage "yellows" and the casual organisms of the two diseases apparently were very closely related. The common name "yel-

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lows" was suggested for this disease. In plant pathology literature, this common name has been applied not only to such vascular diseases but also to a group of virus diseases. To avoid confusion, Nelson, Coons and Cochran (2) have suggested the designation Fusarium yellows be used for the celery disease caused by Fusarium apii. It seems desirable to introduce similar usage for the sugar beet disease.

The first collections of Fusarium yellows of sugar beets were made at Rocky Ford, Colo. in 1926 and 1927. Since that time, the disease has been found in many western sugar-beet districts. Stewart found it in several fields in northern Colorado; in 1936 and 1937, G. H. Coons collected specimens in western Nebraska, Wyoming, South Dakota, and Montana. In correspondence in 1937, M. M. Afanasiev reported observing the disease in many fields in Montana, but in rather small amounts.

SYMPTOMS OF THE DISEASE

The symptoms shown in this disease are those characteristic of a vascular mycosis and the general effects are yellowing, leaf blade stunting, and distortion. About the earliest foliage symptoms for positive diagnosis are the yellowing or blanching of one or more of the half- to fully-grown leaves. Only a portion of the foliage bouquet may be involved. These leaves commonly show paler areas between the main veins and the leaf blade has a mottled appearance. The leaves may also show slight inrolling at the margins. As the disease progresses, several whorls of leaves become involved, and the leaves may change from yellow to a red-brown color. Until the plant succumbs completely, the inner, partially-developed leaves remain erect like small furled flags. Affected plants stand out rather conspicuously not only because of their color but because of the upright habit. Older leaves die and dry up rather than wither. Complete wilting seldom occurs with older plants.

Stewart showed by greenhouse experiments that a high percentage of seedlings when exposed to the organism became in a 14-day period after emergence infected and died. Under field conditions at Rocky Ford, Colo., infected seedlings were found to die within a few days and the relatively high temperatures and low humidity caused them to dry and disappear rather quickly. The plants escaping infection in the seedling stage may become infected in later stages of growth. Infection of the roots apparently takes place through the tap root or some lateral.

Roots of plants in the early stages of the disease present almost no external indications of the disease; however, in the more advanced stages, the epidermis has a lifeless, grayish cast. When an affected root is cut transversely, it is found that the vascular bundles are gray or brown. In many cases, if the disease has not progressed too far, this discoloration may be limited to the sector of the root closest to the lateral root through which the pathogen made entry. Even after all the leaves of an affected plant have died, the root frequently remains firm until secondary organisms enter to complete the decomposition. F. conglutinans var. betae produces a slow, dry rot rather than a rapid, soft rot.

IMPORTANCE OF THE DISEASE

Because of its relationship to the group of Fusaria known to cause persistent soil infestation, the Fusarium attacking sugar beets has potentialities of becoming a serious disease which conceivably might render fields unfit for sugar-beet production. As yet, however, except for a comparatively few instances, the fungus apparently has not spread in fields at all comparable to the spread reported for the organisms causing cabbage yellows or Fursarium yellows of celery. In the long period of observation since 1926, the fields around Rocky Ford and those around Fort Collins, Colo., observed since 1932, in which affected plants were found continue to show diseased plants here and there, but no well-marked infested areas traceable to primary cases. A few fields, where sugar-beet crops have been rather frequently grown and where phosphate deficiency is becoming evident, show a comparatively high incidence of the disease. Thus, as high as 25 percent infection has been found in portions of fields near Rocky Ford and strong depression of the yields was clearly evident.

Some tests were made to determine the effects of the disease upon sucrose percentage and root weight. One hundred roots determined as affected with Fusarium yellows, and 100 roots determined as healthy were taken at random from piles of topped beets in a commercial field. The roots as picked up were grouped into 20-beet samples. The averages for the 5 samples of each class are given in Table 1.

These data show that Fusarium yellows may have effect upon yield, but significantly lowers quality of sugar beets, so much so in fact that a field with any high incidence would be seriously reduced in its acre-yield of sugar. Stewart (loc. cit.) has reported decisive weight reductions as well as significant depression of sucrose percentages. It is to be noted that the data given in Table 1 deal with roots of commercial size, large enough for field labor to top and pile and small, dwarfed beets as are produced by early attack were not included. Stewart has also called attention to the significance of the low sucrose percentages in relation to efficient fabrication of sugar.

As the incidence of the disease increases, the losses to the grower are not limited alone to smaller beets and lowered sucrose percentages. The effects that Fusarium yellows have on initial and early season stands also are important. Sugar beets can be infected by the organism from the seedling stage to maturity. One severely infested sugar-beet field was observed by the writer in October, 1937 in which the stand at that time was 50 percent of the initial, almost perfect, thinned stand as reported by the fieldman of the beet sugar company. Examination of plants from the field showed high prevalence of Fusarium yellows, and warranted considering this disease as the chief contributing factor to the stand loss.

Opportunity was afforded in a seriously infested field, turned over for experimental work by the American Crustal Sugar Company, to obtain information over a number of years as to the seasonal development of stand losses. This field was planted in 1933 to a commercial crop of sugar beets. Counts made on September 25 showed over 12 percent obviously diseased plants. Since many late infected plants fail to show definite foliage symptoms, it is probable that the incidence of the disease, which had developed under ordinary cultural practices involving frequent sugar-beet cropping, was at least twice as high. In 1934 and 1935, prior to planting sugar beets, barley grains which

Table 1.--Comparison of Fusarium yellows of sugar beets with non-affected control plants: sugar-beet roots of the two classes selected at random from topped sugar beets in a commercial field near Rocky Ford, Colo. (Results based on averages of five 20-beet samples.)

Class	Average root weight		Sucrose percentage		Apparent purity coefficient		Calculated indicated- available sugar per root	
	Actual	Comparative	By analysis	Comparative	As calculated	Comparative	As calculated	Comparative
	pounds	percent		percent		percent	pounds	percent
Diseased	1.469 ± .122	87	11.96 ± .788	71	77.3 ± 2.67	89	.1382 ± .019	55.6
Healthy	1.691 ± .033	100	16.82 ± .438	100	87.1 ± 1.83	100	.2484 ± .011	100.0

had been steamed and then inoculated with pure cultures of Fusarium conglutinans were drilled into soil at approximately the locations at which the sugar-beet seed was later to be planted. From the results of subsequent studies in the field, it seems safe to assume that extreme exposures of sugar-beet plants were thus given, the exposure approaching maximum in 1937 and 1938. The data given in Table 2 are based upon the counts made in four seasons on a highly susceptible sugar-beet variety which was randomly distributed throughout the field as a check variety. The table shows in each season the drastic drop in stand of thinned plants during the first month and a progressive decimation of stand throughout the season.

It is doubtful if the records for apparently free plants at harvest indicate significant differences in disease incidence for 1936, 1937, and 1938 but apparently the exposure in 1935 was not comparable in all sections of the field with that of later years. No inoculum was applied in 1937 or 1938 so that the data also may be interpreted as indicating strong persistence of the pathogen generally throughout the field.

Table 2.--Loss of stand assignable principally to Fusarium yellows in a highly susceptible variety grown in infested soil in the experimental field at Rocky Ford, Colo. Results given are sums of stand counts in check plots scattered throughout the field

Year	Total plants after thinning	Plants alive after approximately										Plants apparently free from infection at harvest[1]	
		1 month		2 months		3 months		4 months		5 months			
	No.	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
1935	7,845	3,835	49	2,922	37	-	-	2,690	34	-	-	1,640[2]	20.9[2]
1936	2,892	1,018	35	-	-	425	15	-	-	376	13	93	3.2
1937	17,158	6,889	40	4,362	15	-	-	-	-	3,362	20	913	5.3
1938	10,711	3,473	32	-	-	1,458	14	-	-	1,171	11	556	5.1

1/ The harvest record was based on root examinations as well as on foliage symptoms.

2/ Certain sections of the field in which the disease was not severe contributed very largely to these figures. Probably the bulk of the field had high infestation.

SELECTIONS FOR FUSARIUM-YELLOWS RESISTANCE

As stated previously, the history of spread and serious economic losses which occurred with the closely related diseases of cabbage and celery indicated potentialities for loss from the persistent, soil-inhabiting Fusarium concerned with Fusarium-yellows of sugar beet. It seemed advisable, even in advance of evidence that the disease was likely to be serious, to undertake selections to determine if Fusarium-yellows resistant could be secured as a result of mass selection or other breeding methods. Any varieties with Fusarium-yellows resistance, if otherwise satisfactory, would at least have a place in badly infested fields.

The investigations were begun in 1934 in the experimental field just discussed. During 1934 and 1935 a large number of European varieties as well as inbred strains and synthetic varieties developed in leaf-spot-breeding investigations were tested. Records were kept as to stands in the various plots after thinning, and at intervals throughout the summer.

Probably the major result from the 1934 tests was the finding that an inbred variety developed in the leaf-spot-breeding investigations, 20299-0, was extremely susceptible and that, judging from the persistence of plants in the plots, certain other varieties, especially certain strains from the leaf-spot-breeding work, offered possibilities for selection. Judging from plots, no European brand tested stood out from other brands, although desirable individual plants occurred here and there among them. The tests in 1935 were along the same line as those of 1934, except that 20299-0 as check on infestation occurred scattered throughout the field and was used to judge severity of exposure in making selections.

When the plots were harvested in November, interest was centered on those plots having the best record for stand maintenance or, in the case of certain varieties known to be otherwise satisfactory, the presence of a few outstanding plants led to their inclusion. All roots from plots thus chosen, which did not show foliage symptoms, were dug and the roots examined by cutting the ends of the tap roots or large lateral roots. As was to be expected, some beets were found which showed no positive symptoms on the leaves but did show typical brown vascular bundles. Others had apparently sound tap roots, but showed presence of the organism in one or more lateral roots. Positively infected, as well as suspected plants were discarded, irrespective of size of root and freedom from foliage symptoms.

The apparently healthy roots as selected were stored in crates or in a root cellar where the temperature was held at about 33-35° F. and the humidity was held as high as possible by frequent waterings. After 6 weeks of storage, a few of the roots were planted in 8-inch pots in a cool greenhouse for seed production, branches being bagged to secure selfing. Such of the remaining roots as were entirely sound in the spring were planted at various isolated locations in a number of groupings, dependent on the pedigrees, the following spring for seed production.

Reselections for Fusarium-yellows resistance have been made in the primary selections made in 1934 and 1935. As yet, the progress to be reported is not of such character that any one selection can be singled out as showing such resistance as to warrant introduction. The results obtained with certain selections in 1938 are given as strong evidence that highly resistant varieties may be obtainable by selection.

In the 1938 experiments, two 8 X 8 Latin squares were set up, each testing 6 selections and the check variety, 20299-0 which occurred twice. It seemed advisable to use this plan with the check variety in order to test more adequately uniformity of the infestation of the field.

The source material for the various breeders' strains consists of a commercial variety, Flat Foliage, produced by the American Crystal Sugar Company, a synthetic variety from the leaf-spot-resistance investigations of the Division of Sugar Plant Investigations, U. S. 215, and certain stocks arising from hybrids between sugar beet and Beta maritima. Since the results so far have not shown definite relations to the sources used, these details are omitted.

The selections shown in Table 3 represent stocks arising from primary selections made in 1934 which were reselected in 1936 for seed production in 1937. No significant differences in percentages of plants surviving for any of the counts made in the plots throughout the season were found, and the final record for percentages of plants apparently free from infection likewise showed no significant differences. The differences between the selected lots and the checks were highly significant.

In Table 4, two reselections for an original selection for Fusarium-yellows resistance also are compared with three first selections and three non-selected lots. In performance, the varieties appear to fall into three groups, with highly significant differences between the groups. Varieties without selection for Fusarium-yellows resistance averaged slightly over 3 per-cent in plants apparently free from infection; those having a single selection, about 20 percent apparently free from infection; and those with three selections, about 33 per cent.

These results are interpreted as extremely encouraging for the obtaining by mass selection varieties highly resistant to Fusarium yellows. The relatively small spread in percentages of plants apparently free between the primary selections and the reselections may be brought about by the inclusion of less resistant escapes when reselections of roots were made, which led to lowering of the general averages. With more drastic exposures, such as were afforded in the 1939 tests, a check on this assumption will be provided.

Table 3.— Performance of strains of sugar beets selected for Fusarium-yellows resistance when grown on infested soil at Rocky Ford, Colo. Results from first 8 X 8 Latin Square in 1938. (Data given as 8-plot averages.)

Variety	Description	Total thinned plots May 31	Percentage of plants alive on 1/			Percentage of plants apparently free from infection at harvest 1/
			July 7	Sept. 9	Nov. -	
7-201-0	Second Selection	1,012	65.1	36.6	33.4	26.8
7-202-0	do.	1,037	60.5	41.0	36.1	29.0
7-204-00	do.	1,090	57.6	40.0	37.4	28.3
7-205-0	do.	1,040	57.3	32.8	26.9	20.8
7-207-0	do.	971	57.4	35.6	30.9	20.8
7-208-0	do.	1,020	65.3	43.0	37.4	25.0
20299-0	Check	972	19.5	7.8	4.9	1.5
20299-0	do.	963	21.0	7.4	6.9	0.6
Difference required for significance 99:1		- - -	19.6	18.0	16.8	13.3

1/ Weighted averages.

Table 4.--Performance of strains of sugar beets selected for Fusarium-yellows Resistance when grown on infested soil at Rocky Ford, Colo. Result from second 8 X 8 Latin Square in 1938. (Data given as 8-plot averages.)

Variety	Description	Total thinned plants May 31	Percentage of plants alive on 1/ July 7	Sept. 9	Nov.-	Percentage of plants apparently free from infection at harvest 1/
7-206-0	Second Selection	1,014	72.6	51.1	44.5	35.4
7-209-0	do.	1,116	62.4	45.4	39.1	30.4
7-210-0	First Selection	997	71.8	40.4	31.3	21.9
7-211-00	do.	961	63.9	34.3	28.5	20.0
7-214-0	do.	1,048	58.0	31.9	26.5	17.4
7-216-00	No selection	988	65.3	27.3	15.4	4.1
20299-0	Check	960	21.3	9.0	7.6	2.8
20299-0	do.	985	29.3	11.3	9.5	2.6
Difference required for significance 99:1		- - -	14.7	13.0	11.1	9.3

1/ Weighted averages.

SUMMARY

Fusarium-yellows of sugar beet caused by Fusarium conglutinans var. betae has been found in Colorado, western Nebraska, South Dakota, Wyoming, and Montana.

Yellowing accompanied by mottling of the leaves in the early stages, which may change later to reddish-brown, is a characteristic foliage symptom. The distorted and curled leaves tend to remain erect until the plant is completely overcome. The root symptoms shown are gray to brown discolorations of the vascular bundles and a lifeless, grayish cast to the epidermis in the advanced stages. The pathogen produces a slow, dry rot rather than a rapid, soft rot.

The disease reduces the size and quality (sucrose percentage, and apparent purity coefficient) of sugar beets. Seedlings are especially susceptible to invasion by the pathogen, but the fungus attack continues with older plants throughout the growing season.

Definite progress in securing resistant varieties has been shown as a result of mass selections, the second selections being significantly improved over the primary selections.

References:

(1) Stewart, Dewey. Sugar-Beet Yellows Caused by Fusarium conglutinans var. betae. Phytopath. 21, No. 1, pp. 59-70. 1931.

(2) Nelson, Ray, Coons, G. H., and Cochran, L. C. The Fusarium Yellows Disease of Celery (Apium graveolens L. var. dulce D. C.). Michigan Tech. Bul. 155: 1-74. 1937.