THE ASSOCIATION OF CERTAIN CHEMICAL AND HISTOLOGICAL CHARACTERS WITH SUSCEPTIBILITY IN STRAWBERRY ROOTS TO BLACK ROOT ROT AS INFLUENCED BY SOIL TREATMENT

By

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Thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

1937

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ACKNOWLEDGEMENT

The writer wishes to express his most sincere gratitude to Director H. J. Patterson and Doctors C. O. Appleman and J. B. S. Norton for having made this study possible and for their kind interest and assistance. He also wishes to express his indebtedness to Doctor J. B. S. Norton for his able direction of the work and to Doctor R. P. Thomas for his invaluable advice and assistance with regard to soil treatment and analysis. Thanks are also due Doctor Mark Woods for his assistance in preparing the photographs.

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INTRODUCTION

Root rots of strawberry have been recognized as a serious disease problem in most areas where the plant is extensively cultivated. Humphrey (19) stated that in 1935 black root, the more generally distributed type of this disease, was reported from fifteen states causing losses ranging from a mere trace to fifty per cent. In Maryland it was the most serious strawberry disease, fifty per cent of the plants being killed in the most severe cases. A general survey of strawberry plantings in Maryland had been made by Dr. J. W. Heuberger in 1934 and a number of fungi had been isolated from diseased plants by Professor C. E. Temple. These isolates were in part provisionally identified by Dr. J. B. S. Norton previous to the time the writer was assigned to the problem. Of the identified isolates considered possible parasites species of Fusarium were predominant.

During the growing season of 1935 the writer made an intensive survey of areas on the Eastern Shore of Maryland which were reported to be infected with root rot. Diseased specimens and samples of soil from infected and non-infected areas were taken from thirty five locations between March 15 and July 1, 1935. Isolations from diseased specimens yielded sixty-five cultures. Of these only twenty-one produced spores or other characters which allowed classification to genus. Of these, Fusarium orthoceras appeared most frequently. This organism was selected for further studies and was

provisionally identified by C. D. Sherbakoff as Fusarium orthoceras App. et Wr. var. longius (Sherb.) Wr. It has been reported (39) in potato, onion, beet, cucurbits, and grasses in the United States and in Europe. The soil samples were submitted to Dr. Thomas, of the Department of Agronomy, for analyses. Results of these analyses indicated that the disease was more prevalent and serious among plants on soils low in available nutrients than on more fertile soils. As a result of these observations the work herein described was undertaken.

REVIEW OF LITERATURE

Heald (18) reported injury to strawberry roots by Rhizoctonia in 1921. Since that time numerous reports of organisms attacking and injuring strawberry roots have appeared. In England Akenhead, Harris, Berkeley and Massee (1) reviewed their own and other's work up to 1934 on degeneration of strawberries. In this review Berkeley gives descriptions of the two chief types of root rot and mentions eleven organisms which have been found associated with them. Berkeley and Lauder-Thompson (6) in Canada reported positive pathogenicity tests for five organisms including Fusarium orthoceras. Truscott (33) listed ten organisms which he found concerned in root rots of strawberry. Darrow (10) reviewed these and other works and pointed out the complexity of the problem. Strong and Strong (31) described the black root disease as a cortex destroying trouble and proved the pathogenicity of two organisms, (also found by Canadian and English workers), Hanesia lythri and Coniothyrium fuckelii. Anderson (2) described a stele destroying disease which he named "black stele". This disease was caused by an unidentified Phycomycete. This type, or one very similar to it, had also been mentioned by the English workers. It has been found in three localities in Maryland by the writer (20). The organism is apparently strictly parasitic and does not primarily cause blackening or lesions on the cortex, but destroys the stele, as shown in Plate 1.

Where present, it is more serious than the black root, or black lesion, type as shown in Plates 2 and 3, and as early lesions in Plates 4 and 5. Plakidas (28) described a root rot due to a Pythium sp. in Louisiana. Pythium spp. are also reported by the Canadian and British workers and were identified by Dr. J. B. S. Norton in isolates obtained from diseased roots in Maryland. Nolan (27) found a Diplodia sp. extremely pathogenic. Other organisms including Sclerotium rolfsii, Colletotrichum fragariae and several bacteria have been reported (10), but these appear to be incidental or concerned largely in troubles of plant parts other than the roots. White (37) described mycorrhiza on strawberry roots. Mycorrhizal fungi have been discussed also by the British workers mentioned above and apparently considered factors in the etiology of black root rot.

Ball and Mann (5) and Mann and Ball (23) studied the changes occurring in tops and roots of strawberry plants over a period of more than two years and found that during dormancy many fibrous roots were lost and that with age the cortex of all roots became darker in color and might be lost due to formation of secondary thickening. Such changes are gradual and not to be confused with the black and sunken lesions caused by the black root disease. The anatomical development of the strawberry as worked out by White (36) essentially confirms the developmental observations of Ball and Mann.

Much data, frequently conflicting, has been presented

on the problem of proper fertilizers for strawberry plants. In general, the findings of Chandler (7), 1913, have proven correct. This worker found that nitrate and potash fertilizers in the soil were sometimes detrimental to strawberries, whereas phosphates were distinctly beneficial. Davis, Hill and Johnson (11) found by sand culture trials that potassium deficiency was correlated with winter injury. These workers also found that potassium had a marked effect on carbohydrate storage. Withdrawal of this element gave a marked reduction in carbohydrate while the addition of phosphorus brought about a similar result. Commercial growers of strawberries avoid the use of potash and use little nitrate as compared with phosphates. Morris and Crist (26) found strawberry plant growth satisfactory in culture solutions having reactions from 5.0 to 7.0, but optimum at pH 5.7 to pH 6.0. Meyer (25) found pH 5.0 to 5.5 optimum during dry weather. Waltman (35) found growth to be best at pH 5.3 to 5.5 and that acidity at pH 4 was more harmful than alkalinity at pH 8. Clark (8) has more recently shown that optimum pH depends on type of fertilizer used since with the use of calcium nitrate pH 4.6 was optimum, while with the use of ammonium sulphate the optimum reaction was at pH 6.4.

Whitehouse (38) found from 3.26 to 25.98 per cent starch in strawberry roots. Greve (16) found only little or no starch in similar material. The writer has not made starch determinations, but by the use of iodine-potassium

iodide stains has observed starch deposits in the cortex, endodermis, and stelar parenchyma of roots from plants in all of the soil treatments used (shown in Plate 6).

There is considerable evidence in the literature to show that susceptibility of plants to disease may be modified by environmental factors, including mineral nutrition. Roberts (29) reports that black root of strawberries may be effectively reduced by mulching the beds in winter. Conant (9) was able to demonstrate that the rate of growth and ability to form cork were correlated with resistance in tobacco to Thielavia basicola. Thomas (32) found that tobacco was more severely injured by brown root rot on soils low in nitrate nitrogen and hypothesized that cellulose decomposing fungi attacked weakened plants as a source of nitrogen. Mathews (24) later demonstrated conclusively that the carbohydrate / nitrate-nitrogen ratio in soils was a highly significant factor in determining the severity of brown root rot (caused by Rhizoctonia bataticola) on tobacco. Dickson (12), Dickson, Link and Dickson (13), and Eckerson and Dickson (14) demonstrated a relationship between chemical composition of cereals and their pre-disposition to seedling blight. In their work sugars played an important role. Spinks (30) showed that nitrogen supply was an important factor in determining the susceptibility of wheat to powdery mildew. Forward (15) found that altered host metabolism influences the type of infection with Puccinia graminis. Valleau and co-workers (34) reported that the rate of con-

sumption of reserve foods by clovers during winter influenced their susceptibility to attack by Sclerotinia trifoliorum. Link and Wilcox (22) recently demonstrated a definite relationship between nitrogen-carbohydrate nutrition of Stayman apple trees and their susceptibility to fire blight.

MATERIALS AND METHODS

Plant Treatment

Six hundred recently rooted field grown strawberry plants of the Premier variety were obtained from the W. F. Allen Co., Salisbury, Maryland, in October, 1935. These plants were washed clean and carefully examined. Only plants with disease-free root systems were selected. Twenty plants were set in each of thirty 24 X 16 X 6 inch flats. The sand in which the plants were set was obtained from a barren field on the Experiment Station Farm. This sand gave negative tests for available nutrients as determined by spot plate tests made by the Department of Agronomy. That it was not entirely devoid of nutrients was shown by the fact that plants subsequently grew on it for several months without the addition of fertilizer. The thirty flats were then divided into ten sets, or plots, of three each. One set of three flats was filled with a rich loam soil. Each of the remaining nine sets was given a different treatment as in Table I.

The fertilizer mixtures used were made up of fish meal of known analysis, 20 percent commercial superphosphate, chemically pure sodium nitrate and potassium chloride to the desired N-P-K ratios. Where a 6-6-5 mixture was desired a commercial fish meal base ready mixed fertilizer was used. The lime used was a high calcium hydrated product and the sulphur was washed uninoculated flowers of sulphur.

TABLE I.

Plan of Plot Treatment

Plot No.	Treatment
I	Fertile loam soil
II	Sand without addition
III	Sand plus 6-6-5 fertilizer[1]
IV	Sand plus 6-6-5 fertilizer, heavily watered
V	Sand plus well-rotted manure[2]
VI	Sand plus 10-6-4 fertilizer
VII	Sand plus 2-12-4 fertilizer
VIII	Sand plus 2-6-10 fertilizer
IX	Sand plus 6-6-5 plus lime[3]
X	Sand plus 6-6-5 plus sulphur[4]

1. All fertilizer except manure used at rate of 1,000 lb. per A.
2. At rate of 20 tons per A.
3. Hydrated lime at rate of 1,000 lb. per A.
4. Flowers of sulphur at rate of 600 lb. per A.

The fertilizers, manure, lime and sulphur were added to the amount of sand necessary to fill three flats, thoroughly mixed in by repeated shoveling, and placed in the flats before the plants were set. The flats were set on a raised greenhouse

bench on gravel. The greenhouse temperature could not be accurately controlled. The mean temperature throughout the period of the tests for both years was approximately 60°F. as measured by thermographic record and by maximum-minimum thermometer records. Watering was done by means of a small sprinkling can. Four liters were applied per flat each week except during periods of very low humidity when more was required. Plot IV received double the amount of water given the others and was kept in a very wet condition throughout. Growth records were kept by means of leaf counts at periodic intervals. After fifty-four days two flats of each plot were inoculated, while the third was left uninoculated as a check. In 1935 one of the two flats per plot was inoculated with a culture of Fusarium orthoceras var. longius and the other with soil from an infected planting. In 1936 both inoculated flats per set were inoculated with the culture. Infected soil was not used in the tests of that year. The fungus had been grown in giant culture on autoclaved oats. Inoculation was effected by punching two holes under each plant and introducing into them about two and one half grams of the fungus-overgrown oats. In the case of inoculation with infected soil larger holes were made under the plants and were filled with the moist infected soil. The plants were then allowed to grow for another fifty days and harvested. The uninoculated check plants were examined and estimates of the amount of root injury which could be mistaken for root rot infection were made on three plants from each plot. The

inoculated plants were harvested and the diseased portions of roots separated from the healthy portions. Both diseased and healthy portions were dried to constant weight (±.1 gm.) and the percentage of diseased root tissue per plot thus calculated. The uninoculated flats are shown in Plate 7.

The second, or 1936, trial was carried out in as nearly as possible the same manner with the following exceptions. The plants were obtained and set thirty days earlier. The nitrogen content of Plot VI was reduced from 10-6-4 to 8-6-4 because of root injury and death of tops resulting from the high nitrate concentration of the first trial. Similarly the rate of sulphur addition to Plot X was reduced to four hundred pounds per acre. The time intervals between planting and inoculation and between inoculation and harvest were the same for both years. Sand and soil from the same place and fertilizer materials from the same lot were used for both years. The flats were placed on the greenhouse bench in the same order and frequently rotated in order to equalize light and temperature differences in the same manner each year. Hydrogen-ion concentration for the various plots after ninety days are shown below:

TABLE II.

Hydrogen-ion Concentration
of Sand and Soil Plots

	Year	I	II	III	IV	V	VI	VII	VIII	IX	X
pH	1935	6.1	6.1	6.3	6.1	6.0	6.0	5.9	6.0	6.6	4.4
	1936	6.4	6.6	5.9	5.9	5.7	5.5	5.0	5.2	5.9	4.7

Hydrogen-ion determinations were made by means of filtered soil solutions in a LaMotte comparator block in 1935 and by this means as well as by the use of an electrical pH meter using a glass electrode in 1936. Determination by these two methods agreed within 0.2 pH. The day length of the 1936 plots was extended to approximately fifteen hours throughout by the use of six one hundred watt electric lights with dull reflectors placed so that no plant was less than thirty six inches nor more than forty four inches away from a light.

Histological Method

Portions of roots of about equal size and from the same relative position with reference to root length were taken from twenty or more roots from each plot and fixed in formol-acetic-alcohol fluid. These were subsequently sectioned by the paraffin technique and mounted. Both cross and longitudinal sections were made the first year.

Cross sections were found more satisfactory and only these were made from the material of the second year. The sections were stained in safranin and light green, mounted in balsam and examined under oil immersion. Measurements of the thickness of the walls of the cells of the cortex in the second and fifth peripheral rows were made by the aid of a camera lucida at 2,400 diameters magnification. Sections from at least six individual plants per plot were examined. Sixty to one hundred twenty measurements per plot were recorded. Larger numbers were used at first but it was found that the calculated mean thickness from as many as six hundred measurements varied only in the second decimal from the calculated mean of thirty to sixty measurements. Because of the small size of this variation smaller numbers of measurements were later used. The mean, standard deviation and standard error for each plot was calculated.

Chemical Methods

Only roots and crowns from the uninoculated or check plants from each plot were used. These were cleared of all leaf material and sand, washed carefully, ground in a Nixtamal mill and thoroughly mixed. Samples of this ground material were taken for moisture, nitrogen and sugar determinations.

Moisture. One to four gram duplicate portions of the ground material were placed in tared moisture dishes

and dried to constant weight in a vacuum oven at 80°C.

Nitrogen. The dried residue from the moisture determination was used for the total nitrogen determination. This residue was moistened in the moisture dish, carefully broken up and transferred to Kjeldahl flasks. The determination was carried out according to the official method (3) with the following modifications recommended by Dr. Neil Stuart: Nitrates were included by the use of 3 grams of reduced iron powder and digestion over a low flame with 10cc. sulphuric acid diluted with an equal part of water. After a half hour the flasks were cooled, a few crystals of copper sulphate, 5 grams of potassium sulphate, and 20cc. concentrated low N sulphuric acid were added. Digestion was then completed over a high flame and continued one half hour after the liquid was clear. Distillation was carried out in the regular manner, the ammonia being caught in 10cc. standard acid to which had been added 2 drops of an indicator of the following composition: .3125 gm. methyl red, .2062 gm. methylene blue, 250 cc. 90% alcohol. After distillation the acid was titrated to a bright, pale green end point with standard alkali. Blank determinations were made for each ten determinations.

Soluble nitrogen was determined as alcohol soluble nitrogen, including nitrates. Extraction was carried out according to the method described by Appleman and Miller (4) on 20 gram samples put up in hot 95% alcohol so that the

final concentration of alcohol was 75%. Fifty cc. duplicate aliquots were then digested and determined according to the method given above for total nitrogen.

Sugars. Ten gram duplicate samples were put up in 75 cc. hot 95% alcohol. Determinations were carried out according to the Munson-Walker direct weighing method (3). Total sugars were determined by inverting 50 cc. aliquots of the cleared extract from the original samples with 5 cc. concentrated hydrochloric acid overnight. The aliquots were then neutralized with sodium carbonate and the sugar content determined by the direct weighing method as above. Both reducing and total sugar determinations were calculated as dextrose.

Sucrose was determined by calculating the total and reducing sugars as invert sugar, subtracting and multiplying by the factor .95.

RESULTS

Growth Rate of Tops.

The rate of growth of the plants was estimated on the basis of rate of leaf development. Only leaves in good condition, the leaflets of which were at least 15 mm. wide were counted. These records are expressed as percentage increase in leaf numbers in time, using the numbers counted 14 days after planting as the base.

TABLE III.

Percentage Increase of Number of Leaves.

Plot	Year	Percentage Increase in No. of Leaves After 26 days	After 54 days	After 90 days
I	1935	30	58	150
	1936	--	22.6	90.9
II	1935	0	0	-27
	1936	--	35.4	22.8
III	1935	66	158	210
	1936	--	42.6	111.5
IV	1935	118	250	283
	1936	--	44.1	138.9
V	1935	124	200	463
	1936	--	63.9	133.1
VI	1935[1]	53	60	10
	1936	--	65.7	187.9
VII	1935	94	157	225
	1936	--	39.3	156.3
VIII	1935	93	144	107
	1936	--	46.3	117.2
IX	1935	80	83	100
	1936	--	62.2	149.5
X	1935[1]	119	108	119
	1936	--	83.6	124.6

[1]. Plots later discarded because of fertilizer injury.

The results of the counts show that the plants on fertile soil, those on sand plus 6-6-5 fertilizer, on sand

plus manure, and on sand plus 2-12-4 made relatively uniform growth increases. Growth rates of plants on other treatments were slower in general after fifty-four days for the 1935 trials. Records for the 1936 trials are incomplete, but in general, though the increases were smaller the relative rates for these trials correspond fairly well with those of the previous year. It is noticeable that Plots IV, VI, VIII, IX, and X made greater relative increases in 1936 than in 1935. The fact that the plants used in 1936 were set 30 days earlier than in 1935 may have a bearing on the difference in rate of increase of leaf numbers for the two years. Comparison of the growth rate with the pH of the soil indicates that except for Plots I and II the soil reaction was probably more nearly optimum in 1936 than in 1935. Increased day length through the use of electric lights did not result in increased growth rate.

The plants of Plots VI and X of 1935 began to die after 90 days and were not used in other records. In 1936 these plots remained in good condition throughout, probably due to the use of smaller amounts of nitrates in Plot VI and less sulphur in Plot X.

There appears to be little correlation between growth rate of the tops and susceptibility of the roots to infection. In 1935 there was some degree of correlation between growth of tops and final total dry weights of roots. No such correlation appeared in 1936.

Degree of Infection

Estimates of injured root tissue that could be confused with infected tissue in the uninoculated plants were made on the basis of three plants arbitrarily chosen before harvest from the twenty plants of each plot which were not inoculated. These estimates ranged from 3.7% to 11.7% for the first year (1935) and from 5.34% to 8.36% for the second year (1936). Isolations from this material yielded cultures of Penicillium spp. and bacteria largely. Diseased, or infected, material in the inoculated flats ranged from 14.3% to 45.3% for the 1935 trials and from 13.03% to 28.33% for the 1936 trials. Fusarium orthoceras var. longius was recovered frequently, but by no means consistently, from this material. Since total dry weights of roots from inoculated plants and infection were calculated simultaneously and since the degree of infection per plot was probably the largest factor in determining the dry weight of the roots these data are presented together in Table IV.

TABLE IV.

Per Cent Infected Root Tissue and Total Dry Weight
Of Roots from Inoculated Plants per Plot

Plot No.	Year	% Decay in Checks	Per Cent Infected Tissue with Inoculum (a) Fusarium	(b) Soil[1]	Total Dry Wt. of Roots in Grams
I	1935	8.3	19.4	14.3	40.018
	1936	6.71	15.64		28.879
II	1935	7.9	30.8	37.03	26.901
	1936	7.02	15.15		31.166
III	1935	10.1	28.8	23.3	33.708
	1936	5.3	17.7		29.322
IV	1935	5.7	32.9	38.02	39.525
	1936	6.9	15.0		26.115
V	1935	6.2	41.7	29.4	37.27
	1936	5.5	14.68		27.678
VI	1935[2]				
	1936	8.19	22.48		22.185
VII	1935	5.8	33.7	33.0	35.545
	1936	6.63	13.03		24.562
VIII	1935	11.7	44.6	45.3	23.40
	1936	6.45	21.75		25.81
IX	1935	7.7	24.6	26.3	30.80
	1936	7.9	23.3		21.21
X	1935[2]				
	1936	8.3	28.3		20.02

1. Not used in the 1936 trials
2. Plots discarded because of fertilizer injury.

The per cent decayed root tissue in inoculated plants, here termed per cent infection, obtained by the use of the fungous culture and by the use of infected soil in the 1935 trials show some difference within a plot, but are comparable in general. For this reason and for the reason that infected soil from the same field could not be depended upon so far as similar soil flora was concerned in 1936, inoculation with soil was omitted and both flats were inoculated with fungus instead.

Degree of infection is reflected in the dry weights of the roots to some extent, especially in the 1935 trials. At least three factors are concerned in root weight per plot; first, weight at time of planting; second, influence of the growth rate of the entire plant; third the loss of tissue through decay. These factors can not easily be separated since it is difficult to accurately determine the weight of the roots at the time the plants are set, and there is little evidence to show that under different soil treatments the root to top growth ratios are similar. Furthermore, it is likely that a greater amount of root tissue is lost from plants having more infection than from those having less infection, and also, partially decayed tissue probably does not have the same weight per unit volume as that which is normal.

Throughout the plots the percentage of infected tissue was much higher in the 1935 trials than in those of 1936. This difference can be explained in part by the fact that the organism had been in artificial culture for nearly a year longer when used in the latter trials than in the former. When first used it had been in pure culture approximately one year. It is commonly accepted that many parasitic fungi decrease in pathogenicity when carried in artificial culture for a long period of time. The most striking differences occurred in Plot VIII (high potassium) and Plot V (manure). In the latter plot it is possible that the added organic matter in the soil combined with a more virulent organism

in the 1935 trials allowed a greater growth of the fungus which therefore was able to attack a larger portion of the roots, whereas in 1936 decreased virulence of the organism greatly limited its parasitism even though a greater growth took place. In the case of Plot VIII no definite explanation has been found for the great difference in infection for the two trials. In Plot X (sulphur) the degree of infection was highest of all the plots in 1936. This may be explained on the basis of optimum medium reaction for the growth of the fungus. It was found in laboratory trials with adjusted liquid medium that the organism enjoyed greatest growth at pH 3.5 and decreased in growth rapidly as the reaction of the medium approached the neutral point.

Cell Wall Thickness

As described under Materials and Methods, the thickness of the cell walls in the second and fifth peripheral rows of cells of the cortex was measured. Cross sections of roots showing as nearly as possible the same degree of development as estimated by the amount of xylem, phloem and pericycle tissue present were selected for these measurements. The results are recorded in Table V.

TABLE V.

Thickness of Walls of Cortical Cells

Plot	Thickness of Walls in Microns			
	Second Row		Fifth Row	
	1935	1936	1935	1936
I	1.61 ±.04	1.736 ±.017	1.32 ±.036	1.676 ±.027
II	1.38 ±.017	1.867 ±.025	1.19 ±.024	1.781 ±.042
III	1.4 ±.032	1.99 ±.039	1.18 ±.036	1.743 ±.042
IV	1.24 ±.025	1.803 ±.046	1.037 ±.026	1.61 ±.034
V	1.33 ±.022	2.087 ±.045	1.085 ±.04	1.73 ±.051
VI[1]		1.758 ±.03		1.638 ±.032
VII	1.103 ±.023	2.24 ±.049	.936 ±.026	1.683 ±.039
VIII	1.10 ±.035	1.82 ±.042	.933 ±.035	1.31 ±.034
IX	1.26 ±.031	1.67 ±.04	1.04 ±.035	1.278 ±.041
X[1]		1.524 ±.04		1.228 ±.03

1. Discarded in 1935 trials because of fertilizer injury.

Striking differences within a treatment between the two years trials is again evident. In general, wall thicknesses for the 1936 trial are greater than for the previous trial. Comparison of these data with those of Table IV show a negative correlation between cell wall thickness and degree of infection which holds true regardless of plot treatment or year, and seems to definitely indicate the association of increased cell wall thickness with decreased infection and vice-versa. The walls of the outer layers of

cells were in most cases rather heavily lignified and were thicker than those of the inner layers. It is to be assumed that increased thickness of cell walls offers increased resistance to fungous invasion. It might be equally as nearly correct to assume that the invasion of the fungus so disturbs the development of the host plant that normal thickening does not take place.

Nitrogen Content

Total and alcohol soluble nitrogen contents were found to be relatively consistent throughout the series of plots for each year with few exceptions. The results for the 1936 trials are higher than those for 1935. These figures compared with the percentage infected tissue per plot tend to indicate a relationship between nitrogen content and infection. However, this tendency is not borne out by comparisons of these figures among plots for a single year's trials. For the first year's trials the difference between total and alcohol soluble nitrogen for individual plots ranged from approximately .7 to 1.1 per cent. but seemed to have no bearing on the amount of infection. For the second year's trials this difference ranged from .51 to 1.44 per cent. with the greatest differences occurring in plots having the larger amounts of infected tissue. The results of nitrogen determinations are shown in Table VI.

The apparently conflicting evidence in these data seems not to justify conclusions as to the relation of nitro-

gen content to susceptibility of roots to infection. There appears to be a relationship between soil reaction and nitrogen content of root and crown tissue.

Sugar Content

The results of sugar determinations show considerable variation in the amounts and relative proportions of total, reducing, and sucrose sugar content of the plants of the different plots. There is consistent evidence that increased sugar content, particularly sucrose, is associated with an increased percentage of infected tissue both among treatments regardless of year and within treatments for the two year's trials, with the exception of Plot IX and possibly Plot III. There is also good evidence that increased sugar content, particularly sucrose, is associated with decreased thickness of cortical cell walls in all cases except Plot IX, which was limed, and possibly Plot VII. These results indicate that some factor in treatment or fungous activity limits the utilization of sugars for thickening of cell walls. The association of increased percentage infected tissue in plants with increased sugar content might be explained on the basis that increased sugar content of the plant tissue affords the fungus a better medium for growth once it has gained entrance. The results of the determinations of sugar content are shown in Table VII.

Increased total and sucrose sugar content also show some degree of association with decreased dry weight of

TABLE VI.

Nitrogen Content of Roots and Crowns

Determination	Year	Per Cent Nitrogen in Plot									
		I	II	III	IV	V	VI[1]	VII	VIII	IX	X[1]
Total	1935	1.70	1.28	1.58	1.71	1.85		1.39	1.58	1.92	
	1936	2.03	2.39	2.12	1.58	2.05	1.81	1.41	2.25	2.40	2.82
Alcohol Soluble	1935	.98	.57	.49	.80	.90		.60	.70	1.03	
	1936	1.06	1.24	1.03	.82	1.17	.85	.90	.93	1.20	1.38

1. Discarded in 1935 trials because of fertilizer injury.

roots. This would appear to confirm the opinion that factors operating to limit the utilization of sugar are concerned, however, the loss of material from heavily infected root systems as previously mentioned may nullify conclusions on this point.

Relationships among the factors discussed can best be seen in the summary of data. For convenient reference the plan of plot treatment is repeated.

TABLE VII.

Sugar Content of Roots and Crowns.

Determination	Year	Per Cent Sugar in Plants of Plots									
		I	II	III	IV	V	VI[1]	VII	VIII	IX[1]	X
Total Sugar	1935	6.96	7.45	6.56	7.07	6.90		6.63	9.06	6.78	
	1936	5.61	5.58	5.62	6.61	5.62	5.21	6.28	6.55	7.08	6.11
Reducing Sugar	1935	4.57	4.98	5.42	4.45	4.51		4.58	5.51	4.85	
	1936	4.09	4.42	3.79	4.48	4.24	4.32	4.07	4.25	3.97	3.71
Sucrose	1935	2.29	2.47	1.14	2.65	2.36		2.06	3.41	1.92	
	1936	1.63	1.26	1.86	2.13	1.43	1.11	2.35	2.06	3.16	2.42

[1]. Discarded in 1935 trials because of fertilizer injury.

SUMMARY OF DATA

TABLE VIII.

Plan of Plot Treatment

Plot No.	Treatment
I	Fertile loam soil
II	Sand without addition
III	Sand plus 6-6-5 fertilizer[1]
IV	Sand plus 6-6-5 fertilizer, heavily watered
V	Sand plus well-rotted manure[2]
VI	Sand plus 10-6-4 fertilizer
VII	Sand plus 2-12-4 fertilizer
VIII	Sand plus 2-6-10 fertilizer
IX	Sand plus 6-6-5 plus lime[3]
X	Sand plus 6-6-5 plus sulphur[4]

1. All fertilizer except manure used at rate of 1,000 lbs. per A.
2. At rate of 20 tons per A.
3. Hydrated lime at rate of 1,000 lbs. per A.
4. Flowers of sulphur at rate of 600 lbs. per A. in 1935, 400 lbs. per A. in 1936.

The tabulated data presented under each division of Results is combined in Table IX and shown in graphic form in Figure 1. Graphic representation of associations present in the data are also shown for each year's trial in Figures 2 and 3. In the summary wall thickness of cortical cells is given only as mean thickness of walls of the second row. Degree of infection is given in terms of

percentage infected root tissue on the basis of dry weight. Root weight is given in grams dry weight. Top growth is shown as percentage increase in numbers of leaves in time. Per cent infection is given only for plants inoculated with the culture of Fusarium orthoceras var. longius. Chemical data is given in per cent on the basis of dry weights of root and crown tissue. Top growth and root weights are not included in Figure 1.

TABLE IX.

Summary of Tabulated Data

Character	Year	Plot I	II	III	IV	V	VI[1]	VII	VIII	IX	X[1]
Per Cent Infection	1935	19.4	30.8	28.8	32.9	41.7		33.7	44.6	24.6	
	1936	15.64	15.15	17.7	15.01	14.68	22.48	13.03	21.75	23.38	28.33
Weight Roots In grams	1935	40.0	26.9	33.7	39.5	37.2		35.5	23.4	30.8	
	1936	28.87	31.16	29.32	26.1	27.67	22.18	24.56	25.81	21.2	20.0
Per Cent Increase In leaf no.	1935	150	-27	210	283	463	10	225	107	100	119
	1936	90.9	22.8	111.5	138.9	133	187.9	156.3	117.2	149.5	124.6
Cell Wall Thickness In microns	1935	1.61	1.31	1.39	1.03	1.32		1.10	1.10	1.26	
	1936	1.73	1.86	1.99	1.8	2.08	1.75	2.24	1.82	1.67	1.52
Per Cent Total N.	1935	1.70	1.28	1.58	1.71	1.85		1.31	1.58	1.92	
	1936	2.03	2.39	2.12	1.58	2.05	1.81	1.41	2.25	2.40	2.82
Per Cent Soluble N.	1935	.98	.57	.47	.79	.90		.60	.70	.10	
	1936	1.06	1.24	1.03	.82	1.17	.85	.90	.93	1.20	1.38
Per Cent Total Sugar	1935	6.96	7.45	6.56	7.07	6.9		6.63	9.06	6.78	
	1936	5.61	5.58	5.62	6.61	5.62	5.21	6.28	6.55	7.08	6.11
Per Cent Reducing Sugar	1935	4.57	4.98	5.42	4.45	4.51		4.58	5.51	4.85	
	1936	4.08	4.42	3.79	4.48	4.24	4.32	4.07	4.52	3.97	3.71
Per Cent Sucrose	1935	2.29	2.47	1.14	2.65	2.36		2.06	3.41	1.92	
	1936	1.63	1.26	1.86	2.13	1.43	1.11	2.35	2.06	3.16	2.42

1. Discarded in 1935 because of fertilizer injury.

DISCUSSION OF RESULTS

The results presented show a definite association of such characters as thickness of walls of cells of the outer portion of the strawberry root cortex and total sugar and sucrose content of roots and crowns with the amount of infected root tissue resulting from inoculation with a culture of Fusarium orthoceras App. et Wr. var. longius (Sherb) Wr. This association exists, with little exception, throughout the series of soil treatments used for both years the experiment was carried out. Increased thickness of cell wall was associated with a decreased amount of infection, while increased sugar content, particularly sucrose content, was associated with an increased amount of infection. In other words, increased sugar content was associated with decreased thickness of cortical cell wall and both of these conditions were associated with increased injury by the attack of the fungus. The data afford no definite evidence that plant growth as measured by rate of leaf development or total dry weight of roots was a factor in determining the degree of infection, or susceptibility of the roots to injury by the organism. Neither total, nor alcohol soluble nitrogen appeared to be associated with any other characters studied.

The decreased amount of infection obtained in the second year's trials might be explained as due to decreased virulence of the culture used resulting from long maintenance

on artificial media. The fact that, treatment for treatment, the cell wall thickness was greater and sugar content lower in the second year's trials than in the first can be less readily explained. The variable introduced by the use of supplementary light to increase the light period can probably be dismissed since additional light might be expected to increase top growth or sugar content over that of the trials which did not have supplementary light. Such responses did not occur. The numbers of leaves developed in the first year's trials were greater than in those of the second which may account for an increased sugar content of roots, but can hardly account for decreased cell wall thickness.

Association of a high degree of infection with increased sugar content of roots seems explainable on the basis that roots with a high sugar content furnish a more favorable medium for fungous development. Association of a high degree of infection with thin cell walls would indicate that such walls offer less resistance to fungous invasion. Since these associations appeared in both year's trials and since the degree of infection was higher, thickness of cell walls less, and sugar content greater throughout the first year's trials than in the second it would appear that plot treatment was not very effective in determining degree of infection except in Plots VI, VII, IX and X in which the concentration of nitrate, potash and H-ion respectively, approached the limits of tolerance of the host plant or as in X in which H-ion concentration was also more favorable for growth of

the fungus than in other plots.

The fact that an increased percentage infection is closely associated with thin cell walls and that this is particularly noticeable in comparing results for the two trials of the same treatment suggests that the fungous invasion so disturbs the host metabolism that normal cell wall thickening does not take place. This suggestion is to some extent supported by the work of Haymaker (17) and Lindford (21) which indicates that Fusarium species may be capable of producing substances toxic to the host plant.

SUMMARY

1. Fusarium orthoceras App. et Wr. var. longius (Sherb.) Wr. has been found associated with black root rot of strawberries in Maryland and has been shown to be parasitic.

2. A review of literature has shown that attack on plants by parasitic fungi may be modified by soil treatment.

3. An investigation of chemical and histological characters associated with susceptibility of strawberry roots to black root rot as influenced by soil treatment is described. The organism used was Fusarium orthoceras var. longius.

4. Growth rate of tops and dry weight of roots was not associated with degree of root rot infection.

5. Relatively thick walls in the cells of the outer cortex of roots and relatively low sugar content of roots and crowns were found associated with decreased amounts of infected root tissue.

6. Field observations have shown that strawberry plants grown on fertile soil were less injured by black root rot than those on soils low in nutritive elements. Experimental results indicate that treatments which increase the nitrate or potassium concentration or adjust the reaction of the soil to the vicinity of the limits of tolerance of the strawberry plant may increase its susceptibility to root injury by F. orthoceras var. longius.

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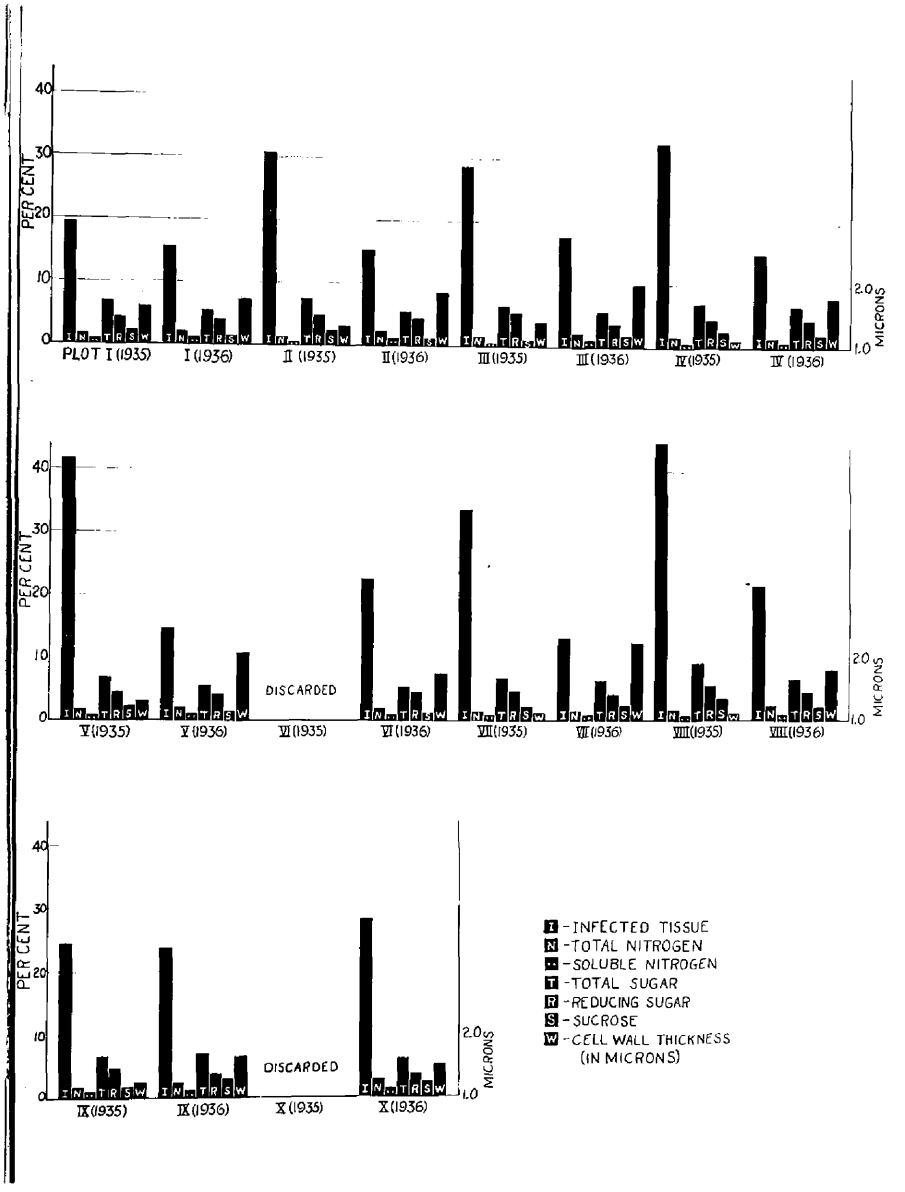

Figure 1. Diagrammatic representation of results of two year's trials.

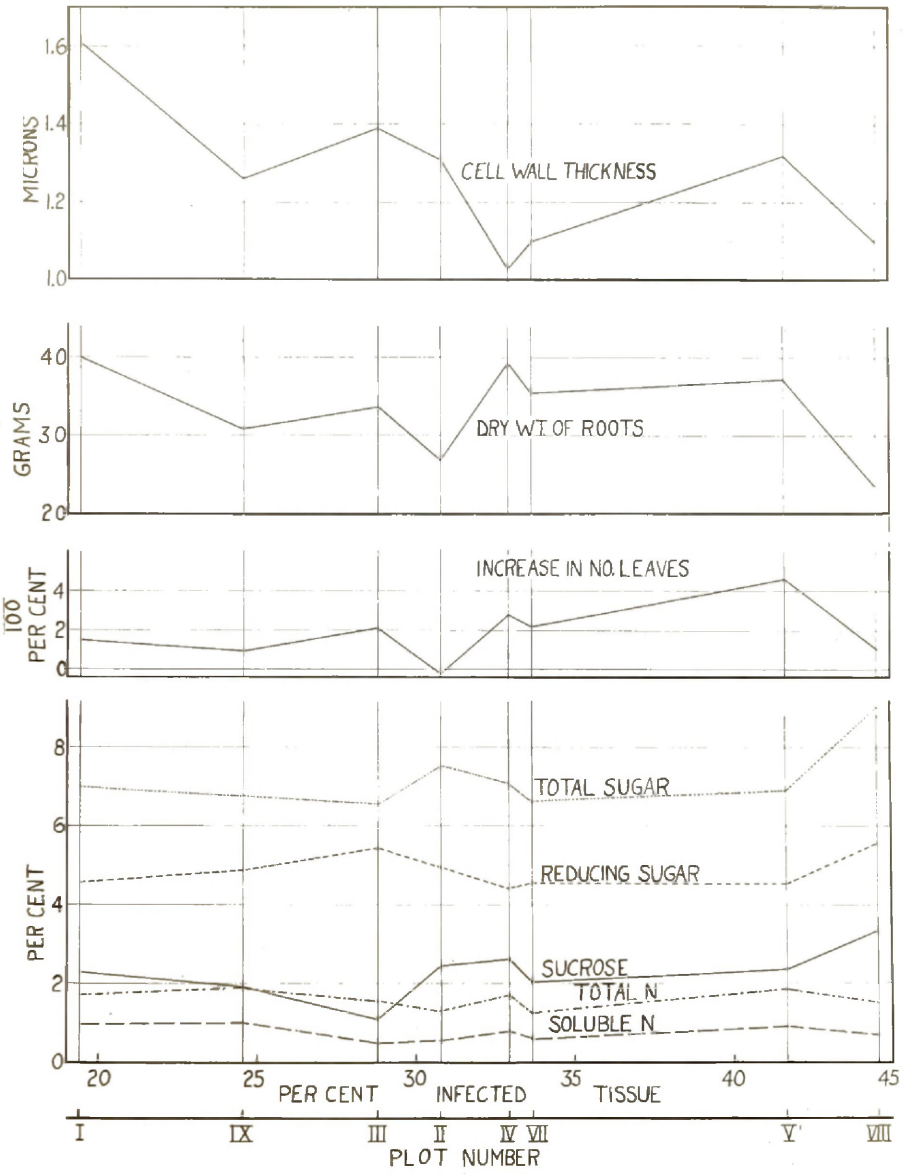

Figure 2. Graphic representation of associations in data from 1935-36 trials.

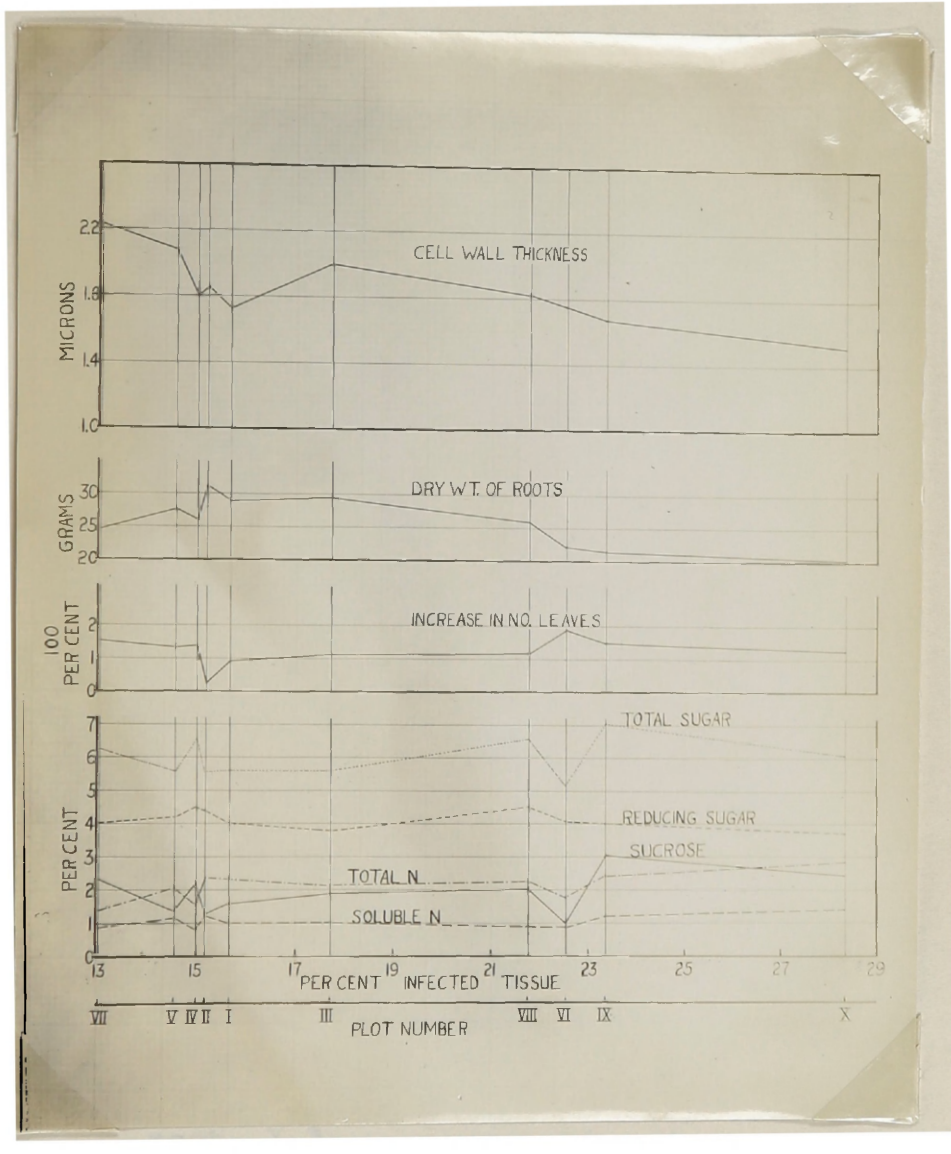

Figure 3. Graphic representation of associations in data from 1936-37 trials.

Plate 1. "Black stele" symptom in root, X 3.

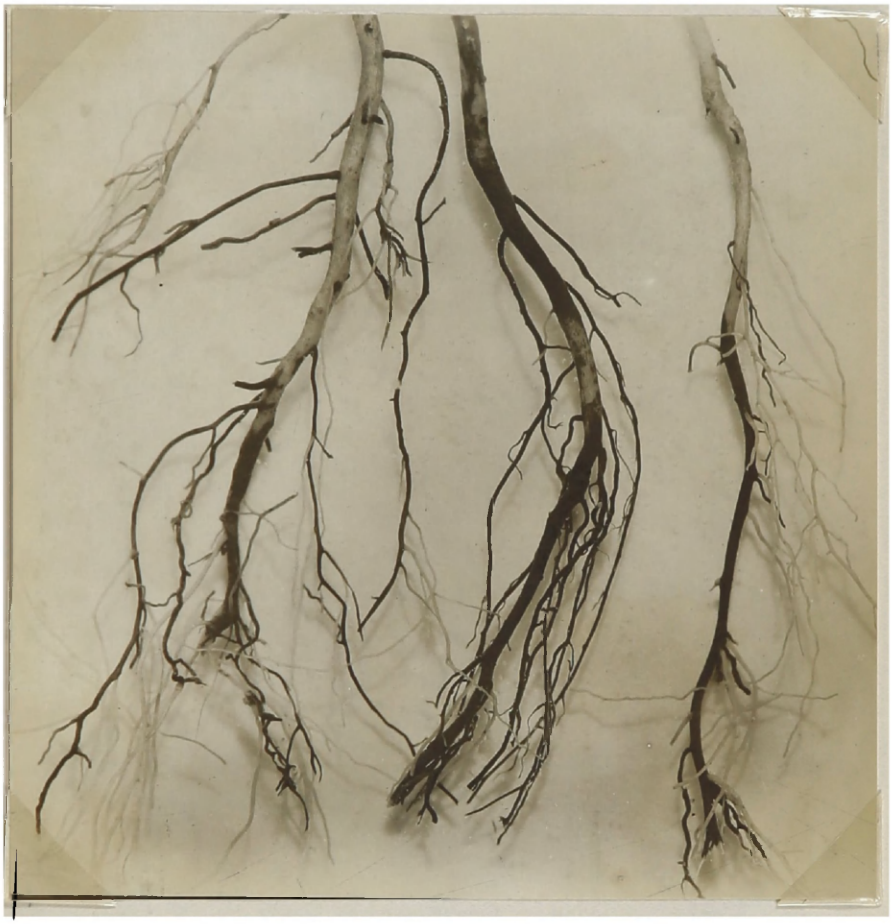

Plate 2. "Black root" symptom, X 1.7.

Plate 3. "Black root" symptom on roots compared with healthy root (right), X $\frac{1}{2}$.

Plate 4. Longitudinal section through an early "black root" lesion, X 250.

Plate 5. Longitudinal section through a sunken "black root" lesion, X 220.

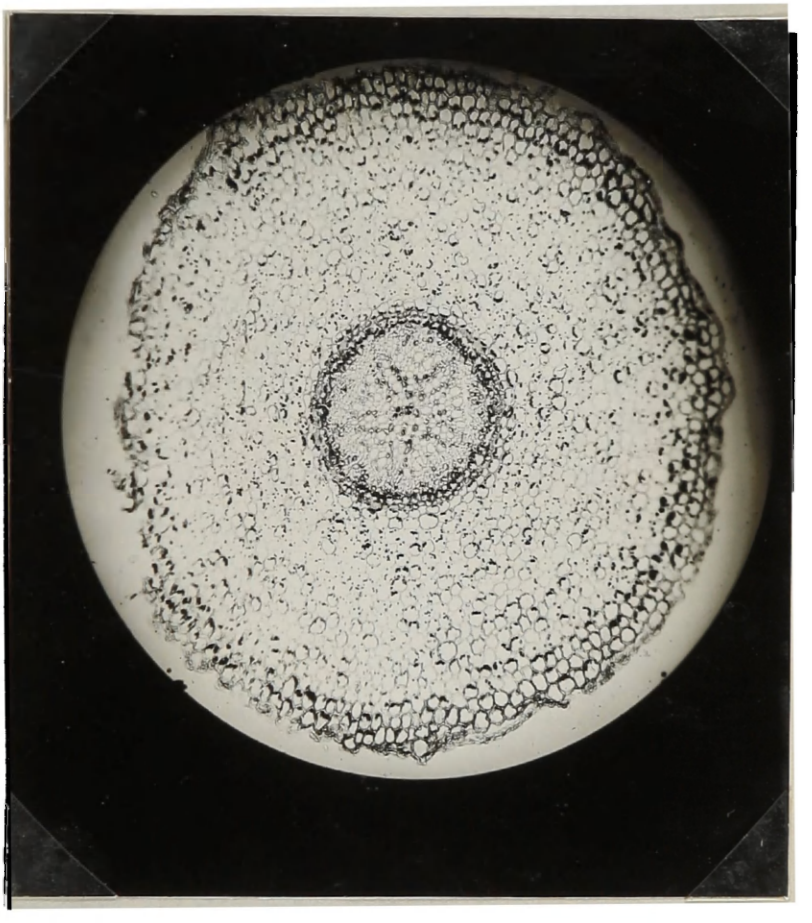

Plate 6. Cross section of a strawberry root showing starch deposits, X 90.

Plate 7. Uninoculated flats before harvest.