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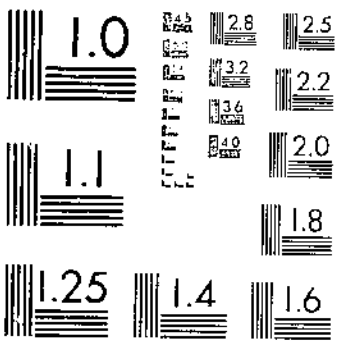

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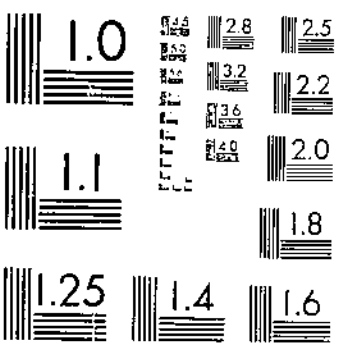

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HISTOLOGICAL CHARACTERS OF FLAX ROOTS IN RELATION TO RESISTANCE TO WILT AND ROOT ROT

By LYTTON W. BOYLE[1]

Assistant pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry

The Bureau of Plant Industry in cooperation with the North Dakota Agricultural Experiment Station and the University of Wisconsin

CONTENTS

INTRODUCTION

In field plantings of flax (*Linum usitatissimum* L.) varieties, many degrees of resistance to the diseases due to soil-infesting fungi are apparent. The purpose of the study here reported was to determine whether there was a correlation between histological characters of the roots of certain flax varieties and their relative resistance to fungi that infest the soil of flax fields at Fargo, N.Dak.

REVIEW OF LITERATURE

Difficulty in producing successive flax crops on account of a condition that develops in the soil was noted by Pliny (*4*) [2] in the first

[1] The writer wishes to thank J. G. Dickson, of the University of Wisconsin, for advice in the pursuit of these studies; also to acknowledge indebtedness to E. H. Herrling, of the plant pathology department, University of Wisconsin, for assistance in obtaining photomicrographs; to H. H. Flor, of the Division of Cereal Crops and Diseases, for growing and collecting field specimens during the season of 1931; to Spas Ivanoff, of the plant pathology department, University of Wisconsin, for assistance in preparation of the material illustrated in plate 1; and to A. G. Johnson, of the Division of Cereal Crops and Diseases, for criticism of the manuscript.

[2] *Italic numbers in parentheses refer to* Literature Cited, p. 17.

century. He wrote that flax "has the property of scorching (exhausting) the ground where it is grown and of deteriorating the quality of the very soil itself." Similar ideas prevailed until the latter part of the nineteenth century. In 1890 Lugger (*17*) reported that failures of flax crops were due to the flax straw itself when in the soil, and not to depletion of the soil. A few years later Snyder (*25*) showed by chemical analyses of both soils and crops that in comparison with small-grain crops flax did "not remove an excessive amount of fertility from the soil."

During the period from 1892 to 1901 it was discovered that the difficulty in the production of successive flax crops on the same land was due to parasitic fungi in the soil. It is reported that in 1892 "Miyabe first found a species of *Fusarium* concerned" in the failures of successive flax crops in Japan (*27*). His findings were confirmed by Hiratsuka (*15*). In 1893 Broekema (*7*) in the Netherlands studied "Vlasbrand", a soil-borne disease of flax, which doubtless was wilt. He suggested the parasitic nature of the disease and pointed out the possibilities of selecting seed from surviving plants for developing resistant strains. In 1901 Bolley in North Dakota described flax wilt as due to a parasitic soil-inhabiting fungus, which he named *Fusarium lini*, sp. nov. (*2*, *3*). Since these early discoveries much study has been made of the diseases of flax due to soil-inhabiting fungi. A list of fungi reported on flax, with short descriptions of the diseases they cause, has been compiled by Schilling (*23*).

Specific diseases of flax have been described, but the symptoms may not be distinctive. *Asterocystis radicis* De Wild., *Thielavia basicola* Zopf, and *Pythium megalanthum* DBy. have been noted as organisms that may occur together and not show distinctive symptoms (*23*). In the United States, plants infected with *Rhizoctonia* have been described by Brentzel (*5*) as "taking on the general appearance of wilt." The possibility of "wilt" due to soil and environmental conditions has been reported by Boerger (*1*). Streets[3], in a study of species of *Fusarium* that may cause flax wilt, described *F. martii* App. and Wr. var. *viride* Sherb. as very virulent and *F. zonatum* (Sherb.) Wr. as less virulent. The first species is known to discolor the fibrovascular bundles of potatoes and the latter to cause dry rot of potato tubers. It is a question whether Streets made a distinction of virulence within wilt, a vascular disease, or between wilt and root rot.

Resistance of flax to wilt has been attributed to different characters of the plants. Tisdale (*26*) described resistance of the flax root to infection as due to the development of a corky barrier in the cortical tissues ahead of the fungus. Burnham (*8*) has reported a strain of flax from which "it was possible to isolate the organism from the stem tissues of plants which were completely healthy." He points out that resistance cannot be due wholly to the formation of a corky barrier in such a strain of flax. Burnham also states that "Crosses between certain resistant strains of different origin showed a high percentage of wilt, indicating that they may carry different factors for resistance."

[3] STREETS, R. B. STUDIES OF THE WILT DISEASE OF FLAX. (Unpublished thesis. Library, University of Wisconsin.) 1924.

Resistance of flax to *Fusarium lini* has been attributed by Reynolds (*19*, *20*, *21*) to the presence of toxic principles in the flax plant. He found that these toxic principles occurred in different amounts in flax and suggested that environmental conditions may inhibit the expression of the hereditary factor in this case. Nelson and Dvorak (*18*) correlate resistance of flax to wilt with specific globulin fractions which they obtained from the seeds of resistant varieties.

A contrast is apparent between the various parasitic fungi that infest the soil and attack flax plants. Some of the fungi are described as invading the vascular tissues of the roots and causing wilt, others as invading the cortical tissues of the roots and causing root rot. However, as previously noted, this contrast may not always be apparent from macroscopic symptoms, and confusion results.

VARIETIES OF FLAX STUDIED

Five varieties of flax that differ distinctly in their resistance to the complex of parasitic fungi that occur in soil continuously cropped to flax were studied. These are as follows:

Bison (C.I. 3893).[4]—A very resistant variety of seed flax originally selected by H. L. Bolley, of the North Dakota Agricultural Experiment Station, and distributed to farmers in 1927.

Morye (C.I. 112) and *Pehanjo* (C.I. 160).—These are of Argentine linseed type and very resistant.

Ottawa White Flower (C.I. 24).—This was chosen as typical of a group of varieties in which symptoms of root disease do not usually become apparent until after midseason. A high percentage of the plants survive to mature some seeds, but a low percentage mature as healthy plants.

Common.—A very susceptible selection from commercial seed flax, none of which survived when sown in badly infested soil.

All varieties were pure-line selections. All varieties except Bison were selected and selfed for five generations by C. R. Burnham at the department of genetics, University of Wisconsin. His original seed was furnished by the Division of Cereal Crops and Diseases from the crop of 1925 grown in badly infested soil at Mandan, N.Dak.

The five varieties were chosen for this study after tests had been made of a large number of varieties by growing them in very badly infested soil. The tests were made on "plot 30" at the North Dakota Agricultural Experiment Station (*2*), where flax had been grown almost continuously for more than 30 years. The data on the behavior of these five varieties are given in table 1. Three types are apparent in the comparative resistance of these varieties to diseases due to root infections: (1) A type in which a high percentage of plants survive the season and remain healthy until mature; (2) a type in which a high percentage of the plants appear as healthy until about midseason and survive to mature some seeds, but a low percentage of the population mature as healthy plants; and (3) a type in which no plants survive later than midseason.

[4] C.I. refers to accession number of the Division of Cereal Crops and Diseases.

TABLE 1.—*Comparative resistance of certain flax varieties to diseases due to root infections, when grown in a badly infested field at Fargo, N. Dak., 1929 and 1930*

Group and variety	Year	Plants emerged	Plants healthy				Plants survived at harvest [1]		Condition of survived plants at harvest	
			June 5	June 14	July 26	At harvest			Healthy	Diseased
		Number	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*
Resistant:										
Bison	1929	150	100	93.3		77.3	116	78.7	98.2	1.8
	1930	338				71.8	258	76.3	94.1	5.9
Morye	1929	158	100	95.6	70.3	72.8	135	85.4	85.2	14.8
	1930	70				92.9	70	100.0	92.9	7.1
Pehanjo	1929	154	100	97.4	74.0	61.7	129	83.8	73.6	26.4
	1930	85				82.2	75	88.2	100.0	.0
Average			100	95.4	72.2	76.5		85.4	90.7	9.3
Partially resistant:										
Ottawa White Flower	1929	153	100	100.0	43.2	39.3	142	92.8	42.3	57.7
	1930	73				38.3	42	57.5	66.6	33.4
Average						38.8		75.2	54.5	45.5
Susceptible: Common	1929	111	82	25.2	.0	.0		.0		
	1930	42				.0		.0		

[1] Under "Plants survived at harvest" are included all plants that resisted the attack of soil-borne parasites sufficiently to mature any seed.

METHODS

PRODUCTION AND PREPARATION OF SPECIMENS

Plants for histological studies were grown in the field at Fargo, N.Dak., and in the greenhouse at Madison, Wis. The field plants were grown in soil that was only moderately infested with the fungi that attack flax. The soil temperatures in the field were recorded for use in comparison with temperatures in the greenhouse. The seed was sown in the field May 21, 1930, and specimens of roots were collected at intervals of 6 to 9 days during the following 65 days. On account of an unusual deficiency in rainfall and the resulting low moisture content of the surface soil, emergence of the plants was irregular. In collecting the specimens care was taken to obtain plants of the same relative development.

In the greenhouse, specimens were grown under controlled environments in chambers described by Dickson (*10*). The plants were grown in pots in three chambers held at 16°, 20°, and 24° C. respectively. The soil was maintained at approximately 60 percent of its water-holding capacity. Comparable plantings were made in sterilized soil and in badly infested soil obtained from plot 30 at Fargo, where the field tests of resistance were conducted. Supplementary illumination, by means of electric lights, was given during the winter months. Temperature, which is a significant factor in the development of flax wilt (*16*), was the only factor that was varied in the series of controlled environments in the greenhouse.

Root specimens were obtained by digging the plants. The roots were washed carefully to remove the soil. When the roots appeared clean they were washed further with a camel's-hair brush while

immersed in the water. This method was fairly satisfactory for removing the finer soil particles lodged on the root surfaces. The roots were stored for a short time in a refrigerator and then sectioned by hand. The sections were kept in 95-percent alcohol for subsequent examination. With few exceptions the roots were sectioned within 48 hours after being dug. All sections were cut from the upper 2 inches of the root systems, and the development of xylem tissues that had occurred was used as a basis in the selection of comparable root sections when microscopic observations were made. Conclusions were not based entirely upon observation of specimens that had been killed or preserved in solutions other than alcohol, and embedded or fixed to slides before examination. Some specimens were killed in formol-acetic-alcohol, embedded in paraffin, cut, and fixed to slides with a very dilute formalin-gelatin solution. This faster method was satisfactory to confirm observations made on specimens prepared by the slower freehand method. Sections fixed to glass slides were not entirely satisfactory, since, in spite of precautions, there was some interference with the free action of certain reagents such as acids.

HISTOLOGICAL COMPARISON OF SPECIMENS

The present study was planned to find histological characters of the flax root that might be significant in determining resistance or susceptibility to soil-borne fungus parasites. Sections were studied to determine (1) the occurrence and amounts of suberin and lignin in the root tissues, (2) the comparative resistance of the cortical cell walls to the action of certain acids, and (3) the resistance of the middle lamellae to a pectin solvent.

The following sequence for studying the cell walls and cementing substances was first carried through with specimens from each environment and of different ages:

(1) Sections were stored in 95-percent alcohol.

(2) Sections were stained with ruthenium red (1:10,000 aqueous solution) in the dark. This stains most pentosan materials, lignin, and hydrocellulose.

(3) Sections were kept in 0.5-percent ammonium oxalate solution, at 85° to 95° C., for 3 hours. By this treatment the protopectin is hydrolyzed and becomes soluble. A few sections were stained with ruthenium red (as described above) and examined. The ammonium oxalate solution was replaced by a fresh solution of the same, and the sections were boiled gently for 30 minutes. A sand bath and a small Erlenmeyer flask with a reflux condenser were used for this purpose. This treatment dissolves pectins, pectates, and certain pentosans. Sections were then washed and stained in ruthenium red, Sudan III, safranine, or phloroglucin (1 percent in alcohol) with hydrochloric acid.

(4) Sections were heated in 1- or 2-percent sulphuric acid, at 85° to 95° C. for 3 to 6 hours, to remove xylan, araban, and some hemicelluloses, and were then stained as in no. 3.

(5) A few sections were treated in 2- to 5-percent sodium hydroxide at 85° to 95° C., to saponify certain suberin lamellae and fat compounds in the walls, and were stained with Sudan III.

(6) Sections were stained with Sudan III for 12 hours and mounted in a potassium iodide-iodine solution.[5] The excess solution was removed from the mount and the sections were treated with 60- to 75-percent solution of sulphuric acid while being observed under the microscope. The cell walls are stained with the potassium iodide-iodine solution as their cellulose becomes hydrolyzed and dissolves, leaving the lignin and suberin of the walls.

[5] Iodine 0.3 g and potassium iodide 1.5 g, dissolved in 100 cc of distilled water.

Results from the foregoing procedure suggested a shorter method, allowing examination of a greater number of specimens and including only those parts of the above-described technic that indicated where differences in resistant and susceptible specimens seemed to lie. Sections were macerated by the use of ammonium oxalate and examined as stated, stained with Sudan III or IV, and treated with a potassium iodide-iodine solution and phloroglucin and examined under the microscope as sulphuric acid or hydrochloric acid was applied. Various concentrations of the acids were used. A mount was prepared, consisting of two specimens to be compared. Successive additions of sulphuric acid, in increasing concentrations, were added at one side of the cover slip and withdrawn from the opposite side with absorbent paper, the specimens being watched at the same time under the microscope to determine the strength of acid that would allow time for comparing the relative rate of hydrolysis in the two specimens. Further comparisons between sections from the same specimens could then be made more accurately by immediate addition of acid of suitable concentration. Note was made (1) of the rate at which the cellulose compounds were hydrolyzed by the acid, (2) of the amount of residue left after the action of the acid, and (3) to what extent the residue remained intact as an outline of the original cellular structure of the tissues.

HISTOLOGY OF HEALTHY AND DISEASED ROOTS

A number of specimens of healthy and diseased flax roots from young plants grown under field conditions were studied to locate the fungi in them.

The flax root is diarch in arrangement of protoxylem. Quickly following the differentiation of the few cells at each protoxylem point, the metaxylem develops to form a compact cylinder of lignified tissue between them. At this stage in the root development some suberization occurs in the radial and inner tangential walls of the endodermal cells in an arc opposite the two protophloem points. As the root develops, suberization of the cell walls of the endodermis continues toward completion of the circumference formed by this layer of cells. The rate and extent of this suberization differed according to the environment in which the specimens were grown.

The increase in diameter of the root results chiefly from the addition of secondary xylem elements. These develop to form a large central cylinder of compact lignified tissue. Increase in the cortex is mainly in circumference to compensate for the enlarging core of xylem tissue. No secondary meristem was observed to develop in the cortex either naturally or because of stimulus such as might result from wound or infection.

The cortex of the root is variable both in its duration and in the character of its cell walls, as will be discussed later.

It was not always clear from the material studied whether the exposed suberized surface of older roots was endodermal or pericyclic in origin. In some cases the continuity of the suberized layer formed by the cell walls was unbroken and Casparian strips were evident, indicating endodermis.

No fungus hyphae were observed in specimens of roots of susceptible flax plants gathered 15 days after seeding (May 16) in

infested soil at Fargo. Five days later, however, the vascular tissues or the whole of the steles of the young roots of comparable plants were well filled with fungus hyphae (pl. 1, *A*). Hyphae also had emerged through walls of the xylem elements between the spiral or annular thickenings. In some cases in which the stele was well filled with mycelium, hyphae extended into the cortex, which was still intact, from the part of the stele near the protoxylem points (pl. 1, *B*). In such specimens only the pericycle and endodermis, which latter had not become suberized, separated the protoxylem from the cortical parenchyma of the root.

It was not observed how the fungus gained entrance into the xylem of the root, but indications were that it entered at or near the root tip, under the natural conditions in the field. No hyphae were observed in the vascular tissues of roots of the resistant flax grown under the same conditions. On the surface of roots from comparable resistant plants, mats of hyphae were noted. An extreme case of this is shown in plate 1, *C*. Other specimens showed evidence that such a condition may have existed in the soil but that the hyphal mats had been lost in the washing process. No penetration was observed in such cases, and it is a matter of speculation whether such fungi would penetrate the cortex of a young susceptible root and readily rot it so that it would not be noticed in the collection of specimens. In comparable specimens of roots of resistant flax, large nonseptate hyphae of an unidentified fungus were found in and between the cells in the cortex (pl. 1, *D*). Some of the cortical cells were greatly enlarged and contained a striking deposit, which presumably had developed in response to the hyphal invasion in the adjacent tissues (pl. 1, *D*, *d*). Very young collapsed roots were frequently noted in specimens from comparable susceptible plants. The cortical cell walls of such roots stained with safranine in contrast to those of young healthy roots, which stained with fast green. The cause of the collapsed condition of the roots was not apparent in the specimens observed, but it is suspected that parasitic fungi were responsible for it. The cellulose, which in the healthy roots was stained by fast green, had been so modified that it was stained by safranine, which shows a possible relation to the ligninlike compounds found in wounded tissues as described later.

The observations in this study were (1) that in the roots of susceptible flax plants the vascular-invading fungus grew well in the vascular tissue and in young roots might spread from the stele to the cortex, and (2) that in comparable roots from highly resistant plants no development of fungi occurred in the vascular elements under field conditions. Further study is required of the mats of hyphae found on the surface of the roots of resistant plants, the unidentified fungus with nonseptate hyphae found in the cortex of resistant plants, and the frequent collapse and modification of the cell walls of very young roots of susceptible plants.

COMPARATIVE HISTOLOGY AND PATHOLOGICAL RESPONSES OF ROOTS

SPECIMENS FROM GREENHOUSE PLANTINGS

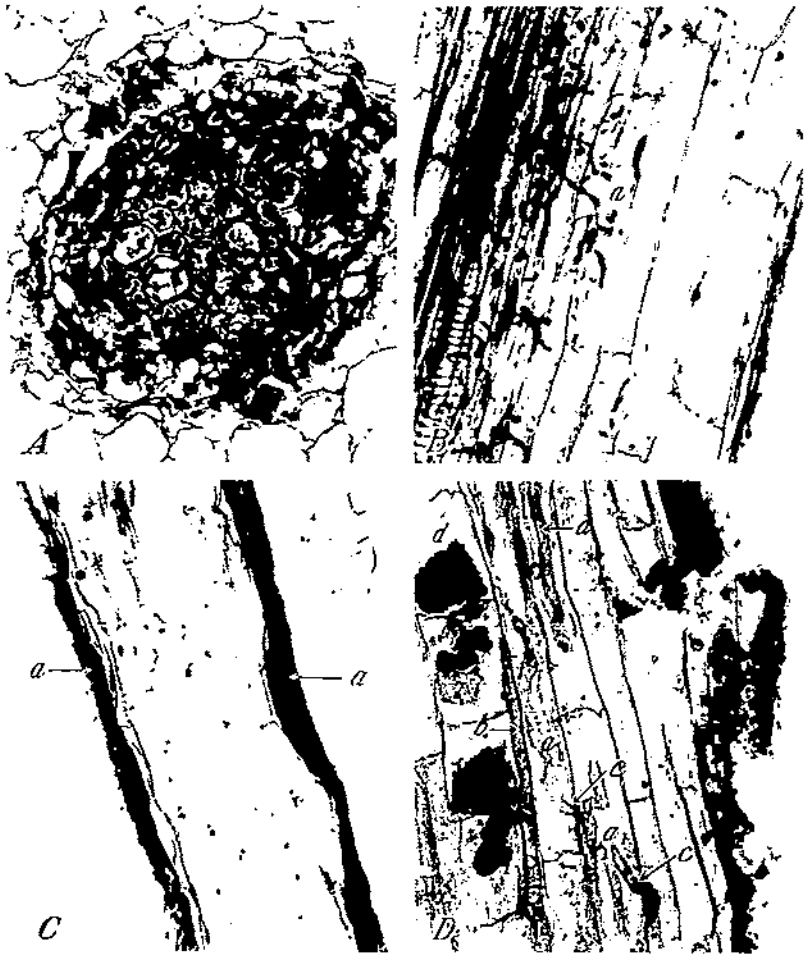
Studies were made to determine differences in the cell walls of the root cortex (1) by the use of stains, (2) by comparison of the relative

amounts of hydrolyzable and nonhydrolyzable materials, and (3) by comparison of the rate at which the cellulose compounds became hydrolyzed by sulphuric acid as previously described. No significant differences were observed between comparable plants grown under greenhouse conditions at 15° to 16° and at 20° to 22° C., respectively.

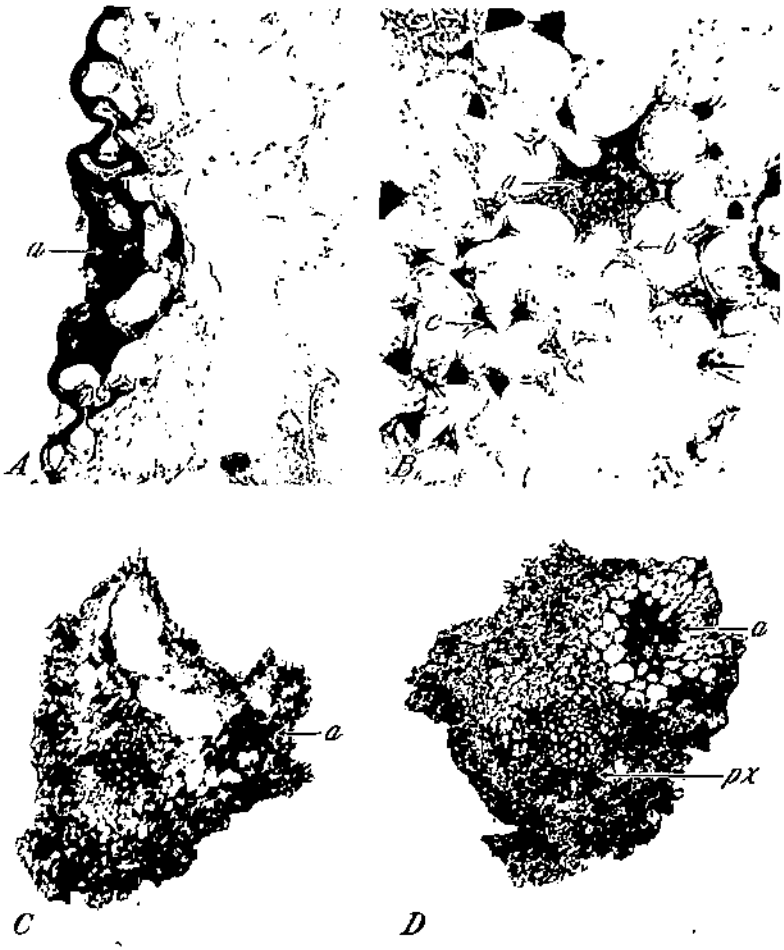
Differences were not apparent in the character of the middle lamellae in the roots of flax growing at 20° to 22° and at 24° when examined at intervals from 7 to 28 days after planting. Later collections of root specimens from plants growing at 20° to 22°, made at intervals during a period of 107 days, showed that a change occurred in the chemical constitution of the cell-cementing substances in the cortical parenchyma. As the plants developed, this tissue gradually became more resistant to maceration by ammonium oxalate solution, as previously described. This increase in resistance to maceration was most marked in the peripheral region of the cortex and gradually decreased toward the inner part of the cortex. This gradient was more uniform in the resistant variety Pehanjo. In the susceptible variety Common the resistance to maceration was greatest at the epidermis but patchy rather than uniform through the cortex. In the older roots of the resistant variety this increased resistance to maceration appeared also just outside of the endodermis. The more uniform resistance to maceration that developed in the cortical tissues of the roots from resistant strains when grown in the greenhouse corresponds with the greater amount of cortical tissues that were found intact on the roots of these strains when collected from field plots.

The root specimens from flax grown in the greenhouse at 15° to 16° C. for 40 days showed only the beginning of change in the chemical constitution of the cell-cementing substance in the peripheral region of the cortical parenchyma. In the case of the susceptible Common flax, the root sections were too completely macerated to permit examination after boiling in the ammonium oxalate solution. Examinations of specimens collected from the same plantings at 58, 68, and 107 days showed that the same progressive change in resistance to maceration had occurred as described above for plants growing at 20° to 22°. Comparisons between the varieties showed the susceptible variety lagging behind the resistant varieties and more patchy in its resistance to maceration. Differences of a constitutional nature in the middle lamellae of root tissues were not found either between the plants grown at 15° to 16° as compared with those grown at 20° to 22° or between different strains of flax grown under comparable conditions.

In view of the role that suberization of tissues in the flax root might play in resistance to invasion by root parasites (*26, 9, 11, 24*), comparisons were made of the development of the endodermis in the roots of a number of strains of flax grown at different temperatures. Differences were not found that were characteristic of any variety or of plants grown at temperatures of 15° to 16° or 20° to 22° C. Plants grown at these temperatures showed suberization only in the very distinct Casparian strips in the oldest portion of the roots at 11 days after sowing. After 55 days, the endodermis had developed so that the walls of the endodermal cells in arcs opposite the protophloem were suberized, and heavy Casparian strips were evident in



A, Central portion of cross section of root of susceptible flax plant (Common, 20 days old, showing abundant fungus hyphae in the xylem elements. ×270. *B*, Longitudinal section of root of susceptible flax plant (Common, 20 days old, showing the fungus hyphae extended from the stele near the protoxylem into the cortical tissues *a*. ×267. *C*, Longitudinal section of root of resistant flax plant (Pehanjo, 20 days old, growing in badly infested soil, showing a mat of hyphae *a* on surface of root. ×143. *D*, Portion of longitudinal section of cortex of root from resistant flax plant (Pehanjo, 20 days old, showing nonseptate hyphae within *a* and between *b* cells of cortex. Cell wall penetration is shown at *c*. Enlarged cortical cells with strikingly abnormal content are shown at *d*. ×270.



A, Cross section of portion of cortex from root of susceptible flax plant (Common) 11 days old, grown in the greenhouse at 20° to 22° C., showing deposit of lignitlike materials in outer cells *a*. Stained with phloroglucin and sulphuric acid. ×256. *B*, Cross section of portion of cortex of root from susceptible flax plant (Common) 11 days old, grown in the greenhouse at 20° to 22°, showing deposit of lignitlike materials in the cell *a*, on the cell walls *b*, and in the intercellular spaces *c*. Stained with phloroglucin and sulphuric acid. ×256. *C*, Cross section of root from susceptible flax plant (Common) 11 days old, grown in the greenhouse at 20° to 22° in badly infested soil from the field plats, showing a lignitlike deposit *a* in a small group of cortical cells. Stained with phloroglucin and sulphuric acid. ×68. *D*, Cross section of root shown in *C*, showing pathological response in a small group of cells *a* in cortex, when remainder of cortex is a mass of partially disorganized tissue and mycelium. Stained with Sudan IV, but color reaction very faint. Dark color in photograph due to density of lignitlike deposit; note protoxylem *px* for comparison. ×68.

the walls of the cells in arcs opposite the protoxylem. After 107 days at the same temperatures, at least the inner walls of all the endodermal cells were suberized.

Pathological responses also were studied in roots of flax plants grown under the controlled environments in the greenhouse. The root specimens for this study were obtained either from plants grown in uninfested soil and mechanically injured or from plants grown in badly infested soil and thus subjected to the action of a natural complex of parasites. The infested soil was obtained from "plot 30" of the North Dakota station, as previously noted.

The general nature of the response found in the cells of the root cortex was the same from mechanical injury as from injury which general evidence indicated as due to parasites. No response was found in the stele of the roots observed. In the cells of the cortex there was a deposition of tan-colored substances on the cell walls, in the intercellular spaces, and rarely in the cell lumina. These substances were resistant to the action of a 62-percent solution of sulphuric acid and stained red with phloroglucin as either hydrochloric or sulphuric acid was applied (pl. 2, *A*–*C*). Tests for suberin with Sudan III or IV showed a faint, almost questionable reaction. The reaction to this stain was not so distinct as the illustration would suggest (pl. 2, *D*). The dark color in the photograph for the most part is due to the density of the deposit. Test [6] for tannins with ferric salts gave negative results. The resistance of these deposited substances to the action of acids and their red color reaction when phloroglucin with either hydrochloric or sulphuric acid is applied indicates a relationship with the ligninlike materials that are found in plant tissues (*13*). Such a reaction in response to injury occurred in all the strains of flax used in this study and was not limited to plants grown in the greenhouse chambers either at 15° to 16° or at 20° to 22° C. Specimens grown at 24°, because they readily became diseased and rotted, did not withstand the handling necessary in preparing them for observation.

The cause of such a pathological response was not always apparent in the specimens examined, nor indicated by the location or organization of the pathological tissue that resulted. In cases where the plants had been purposely wounded, the response occurred in the cell walls of tissue following along or just beneath the surface exposed by the wound (pl. 3, *A*) and in a few isolated spots within the cortical parenchyma, where cells no doubt had been injured by crushing when the wounds were made. Thus mechanical injury produced the same type of response in the wounded tissue as is found in regions of fungus invasion. In cases where the roots had not been wounded by mechanical means, much variation was found in the extent of response which had resulted from causes not apparent in the specimens as observed. In an otherwise normal part of the root, deposition of substances might be limited to the wall on one side of a single epidermal cell as it appeared in cross-section, or it might occur on the whole of the cell wall and parts of the walls of the adjacent cells. Again, several cells might be involved so as to form a small pocket on the root surface (pl. 2, *A*). At times deposition

[6] ECKERSON, S. H. MICROCHEMISTRY. (Mimeographed outlines.)

of substances on the walls of the exposed cells of the cortex would cover large enough areas to be evident to the naked eye as tan or light-brown spots on the roots as they were dug from the soil. Such tan spots were due to the reaction in the outer cells of the cortex and when examined under the microscope appeared clean as compared with those where the reaction had occurred in a layer of cells beneath the surface on which some dead tissue and bits of soil persisted. Such specimens were found more frequently in roots taken from plants grown in infested soil at temperatures favorable for the development of disease than in roots grown at lower temperatures or in uninfested soil at the same temperature. Also, in these cases the amount of modified tissue was much greater in the susceptible than in the resistant strain. This suggested that the injuries were due to the action of soil parasites.

Tisdale (*26*), in a discussion of the relation of *Fusarium lini* to the resistant flax plant, mentioned brown spots on the roots and described them as occurring where the outer cells of the cortical parenchyma had been invaded by hyphae and a modification had occurred in the cells of the cortex just beneath. This seemed a very plausible explanation of the location of the change in the tissues in such specimens and of their resistance to the parasite. However, in some specimens studied by the writer the response was centered in a cortex filled with fungus hyphae (pl. 2, *D*). In such cases, the relation between such localized responses in the tissue and invasion of fungus hyphae is not apparent. The fungus penetrated the modified cells of the cortex, but a great deal less mycelium developed in them than in those where no response by deposition of substances on the cell walls had occurred. The walls of the cortical cells in which the fungus hyphae were abundant hydrolyzed with acid more readily than those of corresponding cells in the modified tissue, where hyphae were scarce, or those in comparable healthy specimens. It was evident, therefore, that the fungus had destroyed some of the stability normally present in the walls of the cortex.

SPECIMENS FROM FIELD PLANTINGS

Comparisons of root tissues in field-grown specimens were made between the same strains of flax and in the same manner as described above for specimens from greenhouse plantings. These comparisons were made to determine whether differences in the root tissues of these strains of flax could be correlated with their differences in resistance to disease caused by soil-borne parasites. Material for this study was collected from a planting made May 21, 1930, at Fargo, N.Dak., on land where flax had grown previously but where the soil was not sufficiently infested with parasites to interfere with the growth of a very susceptible strain. Record was kept of the soil temperatures at a depth of 2 to 3 inches in this plot, in order to compare the root specimens with those produced under controlled temperatures in the greenhouse.

For comparison of the population of the respective varieties and strains, a comparable number of root sections from each of 10 or more plants were taken as a composite sample about every 10 days. Since comparisons between such samples were made by examination of only root sections of comparable development as indicated by the

xylem tissues, their classification is taken as an index to the classification of the populations in the respective plantings. The relative resistance of the cell walls of the root cortices to hydrolysis by acids and the relative amounts of nonhydrolyzable substances they contained were used as a basis of classification and comparison. The specimens were divided into four groups, as follows: Group 1 contained roots in which hydrolysis of compounds in the cortical cell walls was readily accomplished and in which the cell walls contained only insignificant amounts of nonhydrolyzable materials, if any. Groups 2, 3, and 4 included plant roots in which relatively greater degrees of stability of materials in the cortical cell walls were developed, respectively, as indicated by their resistance to the action of acids and by the amounts of nonhydrolyzable substances present (pl. 3, *B*, *C*). Root specimens from the different varieties of flax, collected 20 days after seeding, showed the following distribution of plants in the different groups (table 2).

The differences in the amounts of nonhydrolyzable substances in these roots were chiefly in the cell walls in the peripheral region of the cortices, as but very little was left of the midcortical parenchyma, in any case, after the action of the acid was completed, and as the inner cortex was variable.

TABLE 2.—*Distribution of flax plants in groups based on relative stability of cell walls of root cortices*

Type of variety	Percentage of population in group—			
	1	2	3	4
Resistant	0	50	40	10
Susceptible	50	40	10	0
Partially resistant	50	40	10	0

Specimens from a comparable position in the upper portion of the roots of plants, collected 28 and 36 days, respectively, after sowing, showed that a gradual change had occurred in the relative differences between roots of resistant and susceptible strains. They were alike in time necessary for complete hydrolysis of cortical cell-wall substances. However, the differences in the amount of residual wall materials left after the cellulose materials had been digested were still noticeable but were less marked. The cortices of root specimens collected at later dates could not be compared, since only fragmentary parts or none of the cortices were found to persist on the older roots of susceptible plants. An example of the persistence of the root cortex of a resistant strain, although infested, is illustrated in plate 3, *D*, which shows cortical root rot but no infection in the xylem and therefore not typical wilt.

A comparison between the anatomy of the resistant and susceptible strains did not show differences other than those just described. Qualitative differences in the cell-cementing substances were not apparent when ruthenium red was used as a stain after treatment to remove the pectic compounds from the middle lamellae. No differences were found in the endodermis or stele at any stage studied.

Specimens of infected roots were found rather infrequently in the collections from the field, because they were grown in soil comparatively free from infestation. The response to injury of the root tissues (pl. 4, *A*, *B*) was similar to that described in the roots of plants that had grown in the greenhouse except that suberization was more distinct in the field specimens. At the point of emergence of a branch root, a very distinct response of this type occurs in the naturally injured and more or less exposed cortical tissue (pl. 4, *C*). Such responses were noted both in resistant and in susceptible strains of flax.

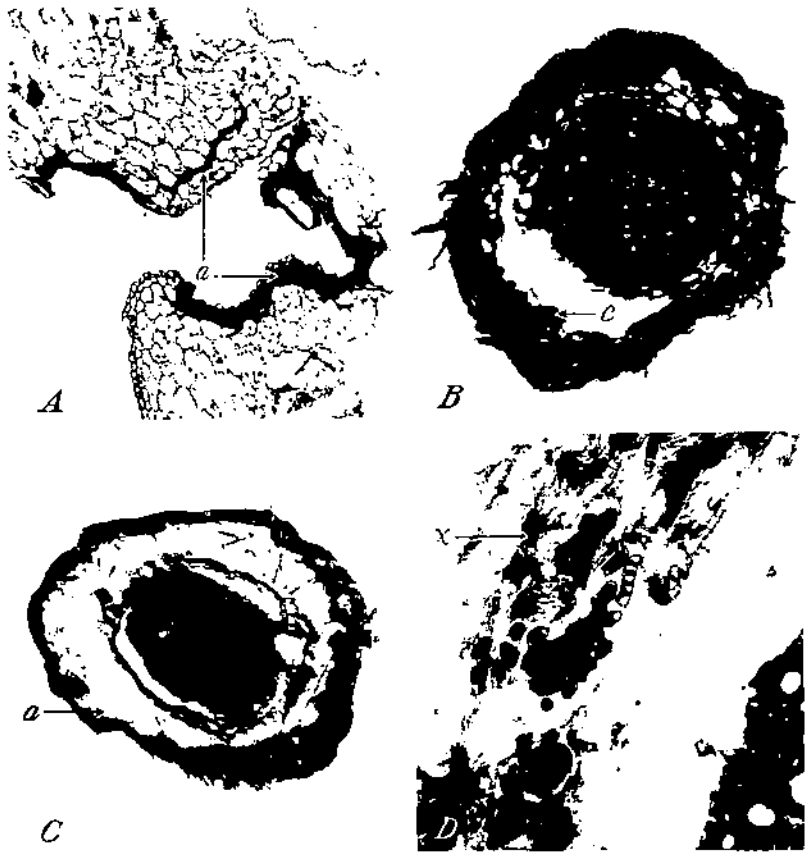
COMPARISON OF SPECIMENS FROM FIELD AND GREENHOUSE PLANTINGS

A flax root that has grown under field conditions during the normal growing season is very different from one that has grown during the winter in a controlled environment in a greenhouse, where the light is much decreased and of shorter duration than that in the field during the spring and summer. The rate of development of the flax root is much slower under the greenhouse conditions. Comparison of comparable sections of the primary roots from plants grown in the greenhouse and field, respectively, showed that the root of the plant grown in the field (pl. 4, *C*) had developed much more after 20 days from the date of seeding than the root of the plant grown in the greenhouse (pl. 4, *D*) had developed after 31 days. The diameter of the root grown in the field was about twice that of the root from the greenhouse. The endodermis in the latter showed only a trace of suberization as compared to the well-developed, heavily suberized endodermis in the root from the field. There was also a difference in the composition of the cell walls of the cortical parenchyma of the roots. Sulphuric acid of a concentration that readily dissolved most of the cell walls in the cortical parenchyma of the roots grown in the greenhouse had very little apparent effect upon comparable cell walls in the roots grown in the field.

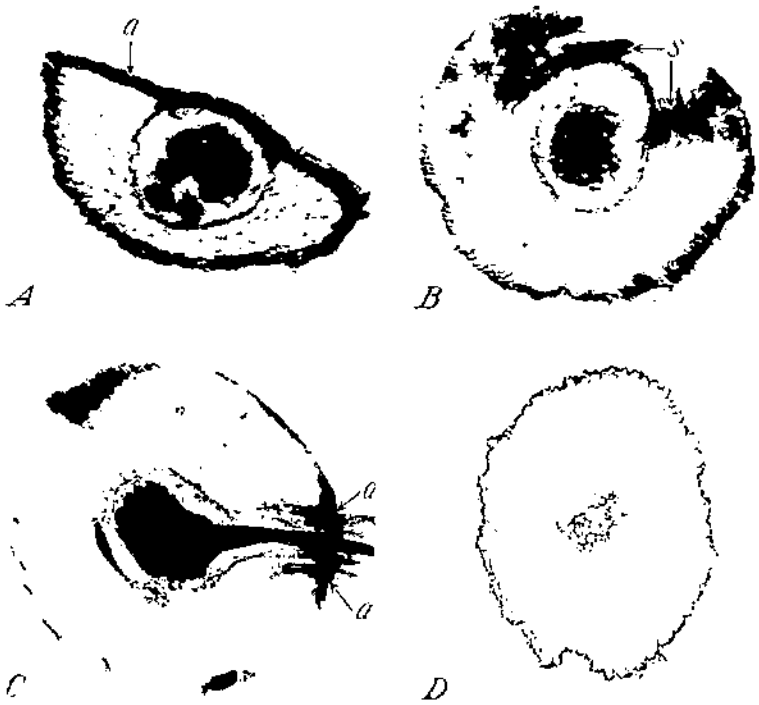
DISCUSSION

NATURE AND SIGNIFICANCE OF THE PATHOLOGICAL RESPONSES

The principal pathological response in the cortical cell walls of flax roots is the deposition of a ligninlike substance on the cell wall, with possibly some intussusception in the wall (pl. 2, *A*, *B*). The term ligninlike deposit has been used, since it is apparent from the work of Ritter (*22*) that, without qualification, such a term as lignification should not be applied to the microchemical nature of the cell walls. Neither was it within the scope of this study with a small amount of pathological material to attempt further classification in view of the many open questions in the details of chemistry and technic in the study of the nature of plant-cell walls. The significant points, insofar as this study is concerned, were that this material was ligninlike in its reaction to stains and resistant to hydrolysis by acids and that only when plants were grown under field conditions was suberization distinctly evident in response to injury of the tissues. Tisdale (*26*), after examination of field-grown specimens of infected roots, described the modification of the cell



A, Cross sections of parts of root cortices of susceptible flax plants (Common) grown in uninfested soil in the greenhouse at 20° to 22° C., showing response to wounds made by mechanical means. Stained with phloroglucin and sulphuric acid to show location of lignin-like deposits *a*. ×135. *B* and *C*, Cross sections of roots of susceptible flax plants (Common) collected from field plantings 28 days after seeding, showing extremes of a wide range in the stability of the cortical cell walls as measured by their resistance to the action of sulphuric acid. In *B* the cellular structure persisted and tore *c* when the mount was handled, while in *C* the cellular structure of the cortex *a* was completely dissolved by the acid. ×40. *D*, Cross section of a part of the root cortex of a resistant flax plant collected from a field planting in badly infested soil 79 days after seeding, showing *Thielavia* sp. in the resistant cortical parenchyma. Stained with phloroglucin and sulphuric acid. ×135



A, Cross section of a root from resistant flax plant (Bison) collected from a field planting, showing a beadlike deposit *a* on exposed surface. Stained with phloroglucin and sulphuric acid. ×43. *B*, Cross section of root from susceptible flax plant (Common) grown in the field, showing suberization *s* on the exposed surface of the wound. ×43. *C*, Cross section of root from susceptible flax plant (Common) collected from a field planting when 20 days old, showing deposit of suberized point *a* where branch root emerges. Stained with Sudan III. ×43. (Compare with *B*.) *D*, Cross section of root from susceptible flax plant (Common) collected from a greenhouse planting when 31 days old, showing development of root as compared with that shown in *C*. Stained with Sudan III. ×73.

walls in the cortical tissues, resulting from injury by fungi, as "corky." His analysis was incomplete, however, in that he used "Pianeze III b" stain, which colors both suberin and lignin alike. No stimulation of cell division after injury of the cortex by fungi, as described by him, was observed by the writer.

The modification of the cell walls by deposition of substances in response to injury as described above is not a primary character of resistance to fungi such as commonly infest soils in the region of Fargo and make such soils unfit for flax production, as this response was noted both in resistant and in susceptible strains of flax. Penetration of such modified walls by hyphae was observed to occur, but growth of hyphae in such tissue was sparse as compared with growth in parts of the cortex where no response was evident. In earlier studies, Tisdale (*26*) observed the resistance offered to the fungus by modified cell walls in resistant flax roots. As he did not observe the response to injury by fungi in roots of a susceptible strain of flax growing on infested soil, he interpreted the phenomenon as a characteristic of the resistant plant instead of its being a normal response to injury regardless of resistance to disease. He stated, however, that "Possibly these thickened walls would not be sufficient within themselves to prevent invasion", and this seems to be true.

The rupture of the cortex by emerging secondary roots stimulated the same modification in the adjacent cortical tissue as that resulting from wounds inflicted by other means (pl. 4, *A–C*). This type of response seems equal in both resistant and susceptible strains.

CORRELATION BETWEEN HISTOLOGICAL AND FIELD DATA

Stability due to substances that were either more resistant to hydrolysis by sulphuric acid or nonhydrolyzable developed more readily and to a greater degree in the cortical cell walls of the roots of resistant flax plants than in those of corresponding roots of partially resistant or susceptible plants. If this stability of the cortical cell walls had been the only character concerned in the resistance of flax strains to soil-infesting parasites, only the 10 percent of the population of the resistant strains that were found to have greater stability in their cortical cell walls than any of the susceptible plants would have survived the season, since 100 percent of the susceptible strains were killed by disease due to such parasites. Also, after comparing the root specimens from plants grown in the field with those grown under controlled environments of the greenhouse, one would expect that all plantings in infested soil in the greenhouse would be killed very readily by disease. However, marked differences in the resistance of the respective varieties of flax to disease were apparent in the greenhouse material except that grown at 24° C. At 20° some plants of the resistant strains did not wilt even though they did not at any age develop a stability of their cortical cell wall that approached that found in 20-day-old susceptible plants grown in the field.

The fact that some of the plants persisted when growing in infested soil under conditions that did not allow the development of stability in their root cortical cell walls suggests possibilities that are illustrated by the following diseases caused by wilt-producing

or vascular-invading fungi. Haymaker (*14*) found that resistance of tomatoes to wilt (*Fusarium lycopersici* Sacc.) is due to the ability of the host to tolerate certain toxic products excreted by the fungus. Smith and Walker (*24*) have shown by cytological comparison between cabbage plants that are resistant and susceptible to yellows (*Fusarium conglutinans* Wr.) that infection of the root takes place "through the growing point and zone of elongation, and infrequently through root hairs"; and as they found neither "morphological differences between resistant and susceptible hosts nor visible reactions produced by the resistant host to the presence of the parasite", they suggest that resistance may be due to the cell contents of the resistant host. They also note that the heavily suberized cells on the inner cortex of the root form a barrier which prevents entrance of the parasite to the stele. As previously mentioned, Reynolds (*19*, *20*, *21*) has attributed the resistance of flax to wilt (*Fusarium lini*) to toxic substances in flax extracts that inhibit the growth of the parasite. Fahmy (*12*) has described the fungus (*Fusarium vasinfectum* (Atk.) var. *aegyptiacum* T. Fahmy) that causes wilt of cotton as entering the plant principally through the region just back of the root cap. *Rhizoctonia*, he points out, enters the young plant through the hypocotyl and disorganizes the root, thus causing death; while in wilt, death of the plant may not occur. He also notes that the parasite that causes wilt does not cause disorganization of the root as compared to *Rhizoctonia* and that it prefers the vascular system of the host, where it develops rapidly. He also describes growth of the organism on inorganic media such as might be available to it in fluids of the xylem vessels. Fahmy, in his conclusion, emphasizes the importance of the rotting of the extremities of the roots in the development of disease and points out how the disease may be less severe if the plant is able to develop a sufficient root system below the levels in the soil where the parasite is most prevalent and active.

That fungi of the root-rotting type, or cortical invaders, as well as the wilt-producing type, or vascular invaders, may infest the soil and make it unfit for production of flax has been mentioned. *Rhizoctonia*, which is noted in the above comparison with wilt of cotton, may be frequently encountered on flax roots growing in infested soil. A fungus tentatively identified as *Thielavia basicola* has been isolated from the roots of diseased flax plants collected in South Dakota and was observed in specimens collected at Fargo, N.Dak. (pl. 3, *D*). As species of *Fusarium* are difficult to identify, it is possible that some of the fusaria found in flax roots may be of the root-rotting type. Species of *Fusarium* that were noted by Streets [7] as least virulent on flax are not of the vascular-invading type on other hosts. That the relative stability of materials in the cell walls may be a significant character of resistance to cortical invasion and development of disease caused by a parasite of this type has been demonstrated in corn and wheat by Dickson and Holbert (*11*) and in tobacco by Conant (*9*).

In young flax plants collected from field plantings, it does not seem a mere chance that the cortical cell walls in the roots of the resistant strains should be so consistently equal to or better than those of the susceptible strain in stability as determined by comparative

[7] See footnote 3.

treatment with acid. Further, when such a wide range in degree of stability exists in the cell walls (pl. 3, *B*, *C*) within pure lines of plants and when cortical invaders may frequently attack flax roots, it seems that in some instances correlation may exist between the stability of the cell walls in the cortical tissues of the roots and the degree of resistance shown by plants growing on infested soil. For example, a plant completely resistant to a vascular parasite (such as *Fusarium lini*) might mistakenly be interpreted as only relatively resistant to this parasite if the possibility of its susceptibility to less virulent cortical invaders, or root-rotting fungi, is not considered. This possibility is supported by the data gained from field tests and from microscopic examination of root specimens from field-grown plants, in the following manner:

(1) Plants growing in infested soil are subject to two types of disease, namely, wilt and root rot.

(2) In view of previously reported studies of resistance to disease in flax and other crops (*8*, *9*, *11*, *14*, *18*, *19*, *20*, *21*, *24*, *26*, *28*), it is assumed that resistance to each of these two types of disease is due to characters of the plant that are distinct in nature.

(3) The average loss of plants, 14.6 percent (table 1), in the most resistant varieties is due either to incomplete genetic resistance to wilt or to some cause against which the plants have no resistance. Invasion by fungi (pl. 1, *D*) possibly may account for a part of this loss. Also, it has been reported (*6*) that resistance to strains of *Fusarium lini* may be specific in some cases. In either case it must be considered, in keeping with the hypothesis above, that this susceptibility is independent and uniform through the populations of the respective varieties. Compensation must then be made in the previously described classification of plants according to the stability of the cortical cell walls or cortical resistance of their roots, since this is taken as a measure of their resistance to infection and disorganization by other fungus parasites. In other words, 14.6 percent of the populations of the respective strains were eliminated by some independent undetermined cause and consideration is given only the remaining 85.4 percent of the populations in their classification according to their relative resistance to root rot as determined by the relative resistance of the cell walls of the root cortices to hydrolysis by acids and the amounts of nonhydrolyzable substances they contained. The classification described earlier (table 1) would then be as shown in table 3.

TABLE 3.—*Distribution of flax plants based on 85.4 percent of populations as shown in table 2*

Type of variety	Percentage of population in group—				
	1	2	3	4	Total
Resistant	0.0	42.7	34.2	8.5	85.4
Susceptible	42.7	34.2	8.5	.0	85.4
Partially resistant	42.7	34.2	8.5	.0	85.4

(4) If a pure-line strain equal to the most resistant strains in its resistance to wilt and equal to the most susceptible strain in its resistance to cortical rot were grown in infested soil, it would be

expected that 42.7 percent (34.2+8.5, groups 2 and 3, table 3), or that part of its population which equals the resistant strains in the above classification, would be equally as healthy. Thus, when 90.7 percent of the surviving plants of resistant strains are healthy (table 1), we would expect 90.7 percent of the 42.7 percent, or 38.7 percent of such a partially resistant strain to be healthy. Resistance to wilt without resistance to root rot seems to characterize the partially resistant variety Ottawa White Flower. Microscopic examination of root specimens showed that this strain was comparable to the most susceptible strain in stability of the cell walls and therefore in probable susceptibility to root rot. It is considered resistant to wilt (1) because the average of 38.8 percent of its population which survived as healthy plants (table 1) checks closely with the 38.7 percent of healthy plants expected as above; and (2) because a high percentage of its population remained healthy until late in the season, after wilt apparently had taken its toll (table 1).

The diseased individuals of Ottawa White Flower did not show symptoms entirely distinct from wilt. There was a difference in the fact that plants of this strain appeared to grow well and to be healthy until after the susceptible Common flax had been killed by wilt. It was during the later part of the season that the growth of the plants of Ottawa White Flower was checked and the plants ripened prematurely. If this was due to a difference in resistance to wilt, the stunting effect should be more apparent throughout the season. Moreover, such differences in the time when symptoms of the diseases occurred varied with the seasons. This suggests a possible difference in the cause of the symptoms. The observations also show that in plantings of flax in infested soil, such as were used in this study, a distinction should be made between reaction to two or more diseases of different virulence and reaction to one disease.

SUMMARY

Three types of flax were selected according to their reaction to diseases due to root infections by fungi infesting the soil where successive flax crops had been grown at frequent intervals for more than 30 years. In their reactions these three types were respectively resistant, partially resistant, and susceptible.

Histological studies of the relation of disease resistance and anatomical characters showed that roots from plants grown in the greenhouse were not comparable to roots from plants grown in the field.

Pathological responses, such as the deposition of suberin and lignin-like materials on or in the cortical cell walls of the plant roots, were not correlated with resistance to diseases due to infections of the root by soil-inhabiting fungi.

Differences were found between resistant and partially resistant or susceptible strains of flax in the stability of the cortical cell walls of the roots as measured by their resistance to hydrolysis by sulphuric acid and the amounts of nonhydrolyzable materials they contained. These differences were not constant during the life of the plants but were most marked in field-grown plants when 20 days old.

Evidence gained in this study and taken from reports of previous investigations shows (1) that two types of disease, namely, wilt and

root rot, may occur when flax is grown in soil that has produced this crop at frequent intervals for many years, and (2) that resistance to each of these two types of disease is due to distinctly different characters of the flax plants.

Histological data and data from field plantings in badly infested soil may be correlated to show that resistance to root rot is due to the quicker and greater development of stability in the cortical cell walls of the roots in the resistant varieties than in the susceptible varieties.

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