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BY

W. H. TISDALE

Reprinted from **JOURNAL OF AGRICULTURAL RESEARCH**
Vol. XI, No. 11 : : : Washington, D. C., December 10, 1917

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION
OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON: GOVERNMENT PRINTING OFFICE: 1917

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FLAXWILT: A STUDY OF THE NATURE AND INHERITANCE OF WILT RESISTANCE

By W. H. TISDALE,
Wisconsin Agricultural Experiment Station

INTRODUCTION

The main object of these investigations has been to study the nature and inheritance of wilt resistance. Bolley (4-11)¹ has so thoroughly worked out the more practical phases of the problem that it has left a clear field for a study of this kind. It seems important, however, to give, by way of introduction, a few fundamental statements regarding the disease and the causal organism.

The flaxwilt problem has been of great importance in this country and is also a serious problem in some of the flax-growing countries of the East (5, 12, 19, p. 211-217). In America the flax industry has been forced rapidly westward, owing to the loss from wilt. The disease is typical of the wilt diseases which are produced by various species of *Fusarium*. Plants at any age from germination to maturity may be attacked and killed by the parasite. The disease is manifested by a sudden wilting of young seedlings and a yellowing of the foliage of older plants, followed by a wilting which may involve the entire plant or only one side of it, thus causing a bending or twisting of the plant toward the wilted side. The disease is highly destructive to common flax (*Linum usitatissimum*) when grown on thoroughly infected soil, and very often the entire crop is destroyed.

SOURCE OF MATERIAL

Flaxseed and "flax-sick soil" were kindly supplied for this work by Prof. H. L. Bolley,² of the North Dakota Agricultural Experiment Station. From plants grown from these seeds in the "sick soil" a species of *Fusarium* was isolated which agreed in cultural characteristics and pathogenicity with *Fusarium lini* Bolley (4). There was a wider range of spore size than Bolley gave in his original description, but this could be accounted for by the difference in environmental conditions under which the spores were produced, as environment was found to

¹ Reference is made by number (italic) to "Literature cited," p. 604-605.

² The writer wishes to express his sincere appreciation to Prof. H. L. Bolley for his hearty cooperation in offering invaluable suggestions and in supplying material for the work. He is also indebted to Prof. L. R. Jones, of the Plant Pathology Department, and to Prof. L. J. Cole, of the Experimental Breeding Department, of the University of Wisconsin, for their aid in defining the problems at the outset and for their respective supervision and kindly criticisms of the pathological and breeding phases as they progressed.

have a considerable influence on spore size in pure cultures. This organism, which is no doubt identical with *F. lini*, is the only pathogenic form of *Fusarium* isolated from flax during this work.

STRAINS OF FLAX USED

In undertaking a study of the inheritance of wilt resistance one of the most important considerations was to obtain for the crosses strains of flax which were highly resistant and strains which were highly susceptible to the disease. A number of both resistant and susceptible strains were obtained from Prof. Bolley. Several varieties of common flax were also obtained from various places in North Dakota and Minnesota. Before the crossing work was begun all of these strains, or varieties, were thoroughly tested as to resistant and susceptible qualities on "flax-sick soil" from North Dakota. Plants for these tests, as well as for all infection experiments, were grown in the greenhouse. Some of the strains proved satisfactory under greenhouse conditions, while others did not. All of the resistant strains except North Dakota Resistant 114 were discarded, that variety being used exclusively, as it proved to be superior in resistance to all other strains tested (Pl. 44, B, a). This strain is designated as No. 4 throughout this work. Prof. Bolley in sending the seed of No. 4 says:

This flax has been growing on the North Dakota State grounds for a number of years and ought to be highly resistant to wilt.

The most satisfactory strain of susceptible flax used is strain 3 (North Dakota Pure-Seed Laboratory No. 14654; Pl. 44, B, b). Bolley states that this strain died out completely on his seed plots at the North Dakota station and should be well suited for work of this kind. Two other strains of common flax have been used to some extent, but have not been so satisfactory as No. 3 in giving uniform results. One of these, No. 5—a white-flowered variety (Pl. 45, B, b)—was obtained from a linseed mill at Red Wing, Minn. This was the only white-flowered variety tested, and, although it was found to be entirely susceptible, it does not wilt so rapidly and uniformly as No. 3. The other, No. 6 (Pl. 45, A, d), is a strain of common flax obtained from Mr. M. S. Kirk, Devils Lake, N. Dak. It is similar to No. 3, but is slightly less susceptible.

DEFINITION OF THE PROBLEM

The first available remedy for controlling flaxwilt was introduced with Bolley's (4) discovery of the cause of the disease and his selection of flax plants for wilt resistance. The variation of such disease resistance of individual plants within a variety was the basis for Bolley's selection. This method of selection has likewise been successfully employed in obtaining disease-resistant strains of other cultivated plants (15, 18, 20, 21). In connection with this improvement by selection, there has

naturally arisen the question as to the nature of individual variation and the cause of disease resistance. Although there has been considerable theorizing concerning the possible cause, or nature of resistance, very little intensive research has been directed toward a positive solution of the problem. Biffen's (2, 3) hybridization experiments with wheat were the first to throw light on disease resistance as being due to inheritable factors which behave according to the laws of Mendel. It hardly seems possible, however, to explain the inheritance of such a character as resistance on so simple a basis as that stated by Biffen.

Having in mind these questions concerning the nature and inheritance of wilt resistance in flax, the writer undertook the present investigations with the Departments of Plant Pathology and Experimental Breeding at the University of Wisconsin in February, 1915, his objects being (1) to study the mode of penetration of flax plants by *F. lini*, (2) to make comparative studies on the penetration of cabbage seedlings by *F. conglutinans* (3) to determine whether or not *F. lini* enters the resistant flax plant, (4) to study the relation of the fungus to the tissues of susceptible and resistant flax plants, and (5) to study the inheritance of wilt resistance through hybridization. Flax was found to be a most suitable plant for the hybridization work for a number of reasons—namely, it has a short growing season, can be grown to maturity in the greenhouse, is easily cross-pollinated, and highly resistant and susceptible strains are available. Even with these advantages it was not expected that more than a clue as to the nature of the inheritance of wilt resistance might be obtained in the time allotted for the work, since breeding is a slow process and it was necessary to develop methods as the work progressed.

NATURE OF WILT RESISTANCE

MODE OF PENETRATION

Before making a detailed study of the relation of the fungus to the various host tissues it was considered of fundamental importance to know how it enters the host, and to know whether or not it penetrates the resistant plant. There seems to be very little definite information as to the exact mode of entrance of the parasitic soil fungi into their hosts. It was hoped that by using pure-culture methods penetrations of flax and cabbage seedlings by species of *Fusarium* might be obtained so that they could be detected by aid of the microscope. Bolley (4) states that *F. lini* penetrates the young flax plants at any point, through the seed, leaves, stem, or roots. His illustration shows very clearly that the fungus is able to penetrate the cell walls at any point, but he does not show conclusively the initial points of entrance. However, he was dealing with the subject mainly from a practical rather than from a standpoint of detailed microscopical study.

In order to make a careful study of penetration, special culture methods were necessary. Test tubes were prepared by placing in the bottom rolls of filter paper (Pl. 44, A) which were moistened and rendered suitable as a medium for fungus growth by pouring a small amount of melted potato agar over them. These tubes were autoclaved and then planted in equal numbers with seeds of both resistant and susceptible varieties of flax. These seeds had been previously treated for five minutes with a 1-to-1,000 solution of mercuric chlorid. At the time of planting, or in some cases just after the seeds had germinated, some of the tubes containing each strain were innoculated with *F. lini* from a pure culture, while others were left uninoculated to serve as controls, and in most cases these remained free from fungus growth (Pl. 44, A, b, c). Cabbage seedlings were also grown for penetration studies in tubes prepared as above and inoculated with *F. conglutinans* from a pure culture. The potato agar served as an excellent medium for the growth of the species of *Fusarium*, which had immediate access to the young seedlings growing in the tubes. As soon as any signs of wilting could be seen, the seedlings were carefully removed from the tubes, mounted on slides in a 20 per cent glycerin solution and examined carefully under the microscope. In this way the root hairs can be easily observed, and almost the entire young root system is so transparent that penetrating hyphæ can be detected. In some cases it was necessary to separate the cortical layer from the inner part of the root tissue in order to be able to examine it closely. This was done by carefully splitting the root on one side with a sharp scalpel and removing the cortical layer, which was then spread on the slide and mounted in glycerin, as mentioned above. Penetration studies were also made with young flax seedlings grown in very loose, infected soil. It is very difficult to obtain clean root hairs from plants grown in soil. The best results were secured by taking the plant up with a large lump of soil which was then dissolved away without destroying all of the root hairs by placing it in a vessel of still water and agitating gently. These roots were then mounted and examined in the same manner as those taken from the tubes. A small amount of eosin placed under the cover slip was often of considerable aid in observing root hairs and hyphæ.

A careful study of slides prepared as above described revealed root-hair (fig. 1, G, H, I), epidermal (fig. 2, 3), and stomatal (fig. 4), penetration of flax seedlings taken from tube cultures; root-hair (fig. 1) and epidermal (fig. 3), penetration of flax seedlings grown in infected soil; and root hair penetration (fig. 1, A, B) of cabbage seedlings taken from tube cultures. The cabbage seedlings produce a more abundant supply of root hairs, and are, for this reason, more desirable for the study of root hair penetration than are flax seedlings. By cross inoculation in tube culture it was found that *Fusarium conglutinans* could penetrate the root hairs of flax seedlings (fig. 1, K). Likewise, *F. lini*

FIG. 1.—A-F, *Fusarium conglutinans* penetrating root hairs of cabbage seedlings in pure culture in test tubes. G, H, and I, *F. lini* penetrating root hairs of flax seedlings grown in test tube cultures. J, *F. lini* penetrating root hair of flax plant grown in loose, infected soil. K, *F. conglutinans* penetrating root hair of flax seedling in pure culture in test tube. Camera-lucida drawings.

was evidently able to penetrate cabbage seedlings as they were killed by it in tube cultures. However, no penetration was observed in the latter as examination was limited. When flax was planted on "cabbage-sick soil" and the reverse, no wilting or yellowing occurred. The fungus, very likely, enters the root hairs to some extent, but probably is unable to invade the tissues of the plant for the same reason that *F. lini* is unable to invade the tissues of the resistant flax plant. It was shown by the

FIG. 2.—*Fusarium lini* penetrating epidermis of young flax root grown in loose, infected soil.

tube-culture method (Pl. 44, A, a, d) that *F. lini* can penetrate the young seedlings of the resistant strain of flax as readily as it can penetrate the seedlings of the susceptible strain under those conditions.

By what exact means the fungus is able to penetrate the cell walls of the host is not known. Perhaps the most feasible explanation is that given by Ward (26) that the fungus protoplasm overcomes the resistance

FIG. 3.—*Fusarium lini* penetrating epidermis of young flax seedling in test-tube culture.

of the cells of the host by means of enzymes or toxins. This might be interpreted as meaning that the fungus secretes an enzyme which has a solvent action on the cell wall, or that it may secrete a toxin which prevents any reaction on the part of the host-cell proto-

plasm by killing or weakening it, thus making possible the invasion of the cell. Perhaps both of these phenomena occur simultaneously. In the case of root-hair penetration there is a slight depression at the point of entrance of the fungus, and the diameter of the opening made by a hypha is somewhat less than the regular diameter of the hypha in question. Stomatal penetration was found on the stem of a young flax seedling near the point where the root began branching. Seedlings taken from the soil

showed stomata on the parts which were below the soil surface; therefore it is possible that stomatal penetration is a source of infection as well as root-hair penetration, and the penetration of the epidermis of young roots. The fungus is also capable of infecting through wounds, as was shown by artificial inoculations (Table I). This discovery of root-hair, epidermal, and stomatal penetration bears out Bolley's assumption that the fungus is able to penetrate the young plant at any point.

RELATION OF THE FUNGUS TO THE SUSCEPTIBLE PLANT

The relation of fungi to their host tissues is a very complicated one which varies greatly, according to the fungus and host under consideration. The object of this work was to study the relation of the fungus to the various tissues of the host and to seek any evidence that might be of value in explaining the possible cause for certain disease phenomena.

FIG. 4.—*Fusarium lini* entering stoma of young flax seedling in test tube culture.

After entering the susceptible plant, the fungus passes directly through the cell walls of the parenchyma tissues to the vascular system, which it invades to its limits. Bolley (4) states that sections through the stems and roots of wilted plants show that the parasite is able to penetrate the cell walls at any point and pass directly through any of the tissues, not excepting the woody parts. This statement holds true especially for plants in the later stages of wilt. Very few fungus hyphae can be found in the cortical tissues of the stems of plants which have just wilted, although these tissues become thoroughly ramified with hyphae as the plant begins to decay. The cortical parenchyma of the roots, however, is the first tissue to be invaded by the fungus. In the early stages of the disease the hyphae are confined largely to the woody tissues in the stem.

Eight newly wilted plants ranging from half-grown to the late-flowering stage were stripped of their foliage, treated for five minutes in a 1-to-1,000 solution of mercuric chlorid, washed in sterile water, cut in pieces with sterile instruments, and plated out on potato agar. Each piece was noted carefully, in order to be sure just what part of the plant it came from. Pure cultures of *F. lini* were obtained from all parts of

the stems of six of these plants up to the terminal bud, and, in one case, growth was obtained from the seed capsules. The two other plants showed growth up to within 1 inch of the terminal bud. Sections taken from near the top of the stem of a plant which had just wilted, and stained with "Pianeze IIIb stain" (24) showed the fungus hyphæ in the vascular system but not in the cortex. The method used in staining was varied somewhat from that described by Vaughan (24). The slides were passed from xylol through absolute and 95 per cent alcohol into the stain, where they were allowed to remain overnight. They were then washed rapidly in water to remove loose stain and detained in 95 per cent alcohol until the desired point was reached. After detaining they were passed through absolute alcohol and xylol and mounted in balsam

FIG. 5.—Longitudinal section of woody tissues of susceptible flax plant showing the invading hyphæ of *Fusarium lini*.

in the regular manner. Excellent results were obtained by this method of staining.

There seems to be very little reaction on the part of the protoplasm of the susceptible host toward checking the invasion by the fungus. The fungus grows rapidly within the tissues (fig. 5), and sometimes microconidia are produced in the vascular cells of the host plant (fig. 6). Some of the vessels may, in rare cases, become almost clogged with fungus hyphæ (fig. 5), but this is so rare that it would hardly seem possible that the wilting could be due to the cutting off of the water supply by this means (fig. 7). Gilman (14) believes that the yellowing of cabbage is due to the slow drain made by *Fusarium conglutinans* on the water supply combined with the high temperature, which causes an increased

growth of the fungus and which increases the transpiration of the plant. This seems reasonable, so far as it goes, but does not seem sufficient for a complete explanation. If it were a case of the water supply being cut off by the clogging of the vessels, we should not expect so much of the one-sided wilting of plants which is so common with flax. The leaves on one side of the stem may become yellow, while those on the other side remain perfectly normal. Stems of plants which are wilted on one side only become characteristically twisted or curved, owing perhaps to unequal growth and shrinkage of tissues. If this wilting were due to the mere cutting off of the water supply at some point, it would be reasonable to expect that when a normal plant has its stem cut half-through it would turn yellow and wilt on the cut side. Five flax plants were cut in this way, but none of them showed any yellowing or wilting from the wound. It is certain that by the time the foliage of the plant begins to wilt the root system has been invaded rather severely

FIG. 6.—Longitudinal section of the woody tissue of the susceptible flax plant showing the invading hyphae of *Fusarium lini*. Notice the microspores of the fungus in the host cells.

by the fungus, and the root hairs are largely destroyed. This is especially true in case of the flax, and would likely account to a considerable extent for any lack in the water supply, and for the general weakening of the plant. Furthermore, there must be a protoplasmic disturbance in cells of the invaded tissues, which helps to produce the local symptoms. Phenomena of this kind might be due to toxic substances produced by the fungus, which interferes with the normal functions of the host protoplasm. The fungus also consumes a part of the food and water supply of the plant. There are very likely, a number of factors which aid in the production of wilt symptoms due to the invasion of flax by *F. lini*—namely (1) Partial destruction of the root system which limits the food and water supply of the plant; (2) use of part of the food and water supply of the plant by the fungus; (3) an increase in transpiration and an increase in the growth of the fungus due to a rise in temperatures; and (4) the possible production of toxic substances by the fungus, which interferes with the normal functions of the host protoplasm.

RELATION OF THE FUNGUS TO THE RESISTANT PLANT

After finding that the fungus was able to penetrate seedlings of the resistant strain of flax in tube cultures, experiments were planned to determine whether or not the fungus was entering the resistant plants growing in infected soil, and, if so, why it was not able to invade the plant and cause wilt. It was found by inoculation experiments that the fungus was unable to produce wilt in resistant plants when introduced through wounds, although infection was obtained by inoculating plants of the susceptible strain. Inoculations were made by inserting bits of mycelium

FIG. 7.—Cross section of the vascular tissues of a susceptible flax plant, showing the invading hyphae of *Fusarium lini*. Camera-lucida drawing.

into needle wounds in the stems of plants. Some were inoculated just above and others just below the soil surface. Stems inoculated above ground were wrapped with moist cotton, while those inoculated below the surface of the ground were covered by replacing the soil. Table I gives the results of these inoculations. It will be seen from this table that the inoculations made in the field were less successful than those made in the greenhouse. The plants in the field were in the flowering stage, which is rather late for infection, and were growing under conditions where the moisture could not be satisfactorily controlled. In the greenhouse young plants were inoculated and kept under better controlled moisture conditions.

TABLE I.—Results of artificial inoculations of resistant and susceptible flax plants with *Fusarium lini*

Date.	Strain of flax.	Number of plants inoculated.	Number of field plants infected.	Number of greenhouse plants infected.
1915.				
August 7	Resistant	7	0	
Do	Susceptible	6	0	
August 17	Resistant	4		0
Do	Susceptible	2		2
Do	do.	3		3
August 26	Resistant	5	0	
Do	Susceptible	5	0	
Do	do.	5	0	
November 29	Resistant	11		0
Do	Susceptible	1		1
Do	do.	10		3
1916.				
November 26	Resistant	15		0
Do	Susceptible	15		13

Since no infection was obtained by inoculating plants of the resistant strain, while those of the susceptible strain did become infected, it seems that there must be some immediate reaction on the part of the protoplasm of the resistant plant to check invasion by the fungus.

Whatever the nature of resistance, it is not manifested to the highest degree unless the plant is kept under perfectly normal conditions. This fact was shown by the way in which seedlings of the resistant strain of flax were killed by the fungus in tube cultures (Pl. 44, A, d), and was further tested by planting disinfected seeds of both the resistant and susceptible strains in flasks of soil which had been sterilized and inoculated with *F. lini*. When these flasks were plugged with cotton and kept in the laboratory, the resistant strain of plants showed slightly more resistance at first, but later succumbed to the attack. However, when flasks were prepared as above and placed in the greenhouse without the cotton plugs, some of the plants of the resistant strain lived to the flowering stage, while plants of the susceptible strain immediately died of wilt. Even under greenhouse conditions, where the temperature runs above normal, some of the plants of the resistant strain wilt.

Careful examination of the root system of resistant plants grown in infected soil showed that some of the smaller roots were decaying and there were brownish spots on the larger roots. A number of these plants which showed no signs of wilt above ground were taken and the root system disinfected thoroughly on the surface with a 1-to-1,000 solution of mercuric chlorid for 2½ to 5 minutes. They were then washed thoroughly and plated out on potato agar. In a large percentage of cases pure cultures of *F. lini* were obtained from these roots. In some cases other

fungi appeared but not in so great an abundance as the parasitic form. Most of the root hairs and very small roots were left in the soil when these plants were removed; therefore the fungus obtained in these experiments came mainly from the larger roots, which were perhaps less likely to be penetrated than the smaller roots. Table II gives the results of these isolation experiments. It will be noticed that roots plated out on February 21, 1916, gave a much lower percentage of *F. lini* than did the others. This is probably due to the fact that these plants were grown in midwinter, when the soil was cooler and the fungus less vigorous. Some of the plants of the resistant strain succumb to the disease in the summer, when the temperatures are high; therefore we should be more likely to find the fungus in the roots under summer conditions, as infection is more abundant.

TABLE II.—Isolation of *Fusarium lini* from roots of resistant flax plants

Date.	Period of treatment with mercuric chlorid.	Number of plant roots plated out.	Number of roots showing growth of <i>F. lini</i> .
	Minutes.		
Oct. 4, 1915.....	2½	3	3
Oct. 7, 1915.....	2½	7	7
Feb. 21, 1916.....	5	16	4
Sept. 20, 1916.....	5	12	12
Sept. 26, 1916.....	5	28	23

This table shows that the fungus is at least able to penetrate deep enough beneath the surface of the resistant plant to protect it from the mercuric chlorid used in disinfecting the surface, but does not give any evidence as to the extent of invasion.

Parts of the roots of resistant flax plants which showed brown-spotting were fixed in Flemming's medium fixative, embedded, sectioned, and stained with "Pianeze IIb stain" (24) as previously described. This stain gives a pink color with the parenchyma cell walls of the host and with the fungus tissues. With lignified, cutinized, and suberized tissues it gives a light green. A careful study of these sections showed that the fungus entered the parenchyma tissues of the resistant plant (fig. 8), but seldom, if ever, penetrated so far as the xylem elements. This limited invasion by the fungus is accompanied by a number of cellular changes on the part of the host tissues which are in the immediate vicinity of the invading hyphæ.

(1) There is a slight breaking down of the invaded cells which, however, is not sufficient within itself to disconnect the hyphæ from surrounding cells. Marryat (17) and Ward (25) state that in the case of wheat which resists yellow-rust (caused by *Puccinia glumarum*) there is a sudden breaking down of the first cells to be invaded, thereby cutting

FIG. 8.—A, Longitudinal section of the cortical parenchyma of a resistant flax root showing the formation of cork walls around the point of invasion by *Fusarium lini*. B, Longitudinal section of the cortical parenchyma of a resistant flax root showing a cork layer formed between the point of invasion and the vascular system. Notice the increased cell division beneath the cork layer. C, Longitudinal section of the cortical parenchyma of a resistant flax root showing cell-wall penetration by *F. lini* and the formation of cork walls between the invading hypha and the vascular system of the root. The protoplasm in the cork cells was granular as indicated by stipling. D, Cross section of the cortical parenchyma of a resistant flax root showing the heavy cork walls formed around the point of invasion by *F. lini*. Camera-lucida drawings.

the fungus off from connection with other cells of the host. The fungus then dries up and dies or remains dormant in the dead host cells. The resistant flax plant behaves differently toward *F. lini* than does wheat toward *P. glumarum*. The rust fungus is an obligate parasite and is not capable of growing in dead tissues; *F. lini* may grow either as a parasite or as a saprophyte, and for this reason its development would not be checked by the death of the host cells. Furthermore, the breaking down of the cells in the flax plant is not so complete as that stated for wheat by Marryat and Ward, and would doubtless play very little part in decreasing further invasion by the fungus.

(2) In some cases the protoplasm of the host cells immediately surrounding the point of invasion becomes granular in appearance and stains green with the Planeze stain, whereas the protoplasm of the normal cell fails to take the green at all. The writer is unable to offer any definite explanation for this change other than to say that it is possibly a chemical change in the host protoplasm excited by the presence of the fungus. There is doubtless a certain amount of injury to the host protoplasm, which may account in part for the coarse granular condition. Furthermore, as will be mentioned later, there may be some substance produced which is injurious to the fungus.

(3) Surrounding the area in which the cells show the granular appearance, there is a stimulation to cell division. This cell division is more abundant toward the vascular system from the point of invasion. In some cases the dividing walls are formed more or less irregularly, while in other cases a typical cork cambium seems to be formed. The newly-formed cells are to all appearance cork cells.

(4) Accompanying this cell division and other phenomena is a thickening of cell walls, which is much more noticeable toward the vascular system from the invaded point. This thickening of walls may extend three or four cell layers beyond the point of invasion and is more pronounced with newly formed cells. However, the walls of cells which were formed previous to invasion may become thickened. The process of thickening seems to be a laying down of additional material which is produced by the protoplasm of the affected cells. The modification of old walls is more noticeable below and above the point of invasion and toward the epidermis, where cell division is not abundant. These thickened walls stain green with Planeze stain, which fact indicates that they are either lignified, cutinized, or suberized. In parenchyma tissues of this kind we should hardly expect to find lignin or cutin. When treated with concentrated potassium hydroxid, these walls gave the typical yellow reaction for suberin, which confirms the conclusion that they are of a corky nature.

Taking into consideration the above-mentioned phenomena with other possibilities, it seems that a combined explanation might be offered for the resistance of flax to extensive invasion by *F. lini*. In the first place

the protoplasm of the resistant plant may naturally contain a substance or substances injurious to the fungus. We know that the resistant plant differs from the susceptible plant in respect to its physiological nature. This difference might be due to some permanent chemical composition of the protoplasm as suggested above, or it might possibly be due to a hypersensitiveness of the protoplasm of the resistant plant which causes it to react much more readily than does the protoplasm of the susceptible plant in producing the phenomena which cause resistance. The fungus seems to be less vigorous in the invaded cells of the resistant plant than in the invaded cells of the susceptible plant; in other words, it is less abundant in the cells of the resistant plant. It seems possible, therefore, that some toxic or other chemical substance is produced by the protoplasm of the host which has a deleterious effect on the fungus. The coarse, granular appearance and staining reaction of the protoplasm of the invaded cells indicate that considerable change has taken place. Apparently this change is accompanied by an injury to both the host cells and the fungus hyphæ. Perhaps some substance is produced by the host protoplasm during the change which has an injurious effect on the fungus. When the hyphæ of the fungus come in contact with the modified or corky walls of the cells they fail to penetrate, and further invasion is prevented. Possibly these thickened walls would not be sufficient within themselves to prevent invasion, but they serve as a barrier to the fungus after it has been weakened by protoplasmic reaction on the part of the invaded host cells. These phenomena seem to indicate that resistance is due either directly or indirectly to the chemical nature of the host protoplasm. Appel (1) believes resistance in plants to be of a chemical nature and makes the following statement:

Efforts must be made to find the causes of immunity, and after solving this question to determine without infection the disease-resistant qualities in different varieties and individuals in order to be able to establish the desired resistance and at the same time eliminate undesirable qualities.

Such a theory might at first seem entirely feasible; but, when the multiplicity of constitutional and environmental factors influencing the production of the resistant character is considered, it appears more improbable that any such analysis will ever be satisfactorily made. Ward (25, 26) also speaks of the chemical nature of resistance. He (26, p. 21) says:

Infection, and resistance to infection, depend on the power of the Fungus-protoplasm to overcome the resistance of the cells of the host by means of enzymes or toxins; and, reciprocally, on that of the protoplasm of the cells of the host to form anti-bodies which destroy such enzymes or toxins, or to excrete chemotactic substances which repel or attack the Fungus-protoplasm.

This theory might be offered as a partial explanation for the resistance of *F. lini* by flax plants.

Finally, if we take into consideration the apparently weakened condition of the fungus in resistant host cells, the change in the nature of the protoplasm of the invaded cells, the new cell division, and the formation of cork walls around the point of invasion, all of which seem to play a part in the prevention of further invasion by the fungus, and all of which are due more or less to the chemical reaction of the host protoplasm, it seems safe to conclude that the resistance of flax to *F. lini* is essentially of a chemical nature.

INHERITANCE OF WILT RESISTANCE THROUGH HYBRIDIZATION

In undertaking a study of the inheritance of wilt resistance through hybridization it was very necessary that highly resistant and susceptible strains of flax be secured and thoroughly tested on infected soil before making crosses. The object of this chapter is to deal with methods of procedure in the work and to give such results as have been obtained from crosses up to date.

METHODS OF SOIL INOCULATION

Before the progeny from crosses could be tested it was highly important that the soil on which the plants were to be grown should be thoroughly infected with the wilt-producing organism, *F. lini*. The soil sent by Prof. Bolley from North Dakota was found by the preliminary tests to be satisfactory (Pl. 44, B), but the quantity was not sufficient. An attempt was therefore made to inoculate soil with pure cultures of the organism. In order to try this out, a small flat of greenhouse soil was sterilized in an autoclave one half being planted to flax No. 3 (susceptible), and the other half to flax No. 4 (resistant). After planting, about half a dozen tube cultures of *F. lini*, which were fruiting abundantly, were mixed thoroughly in a small pot of water and poured over the flat. Wilting of the susceptible plants did not begin until they were of considerable size, but they were completely killed in a short time after the disease started (Pl. 44, C). A large bench of soil was then inoculated. Water suspensions of the organism from pure culture were poured over the soil and worked into the surface. Seeds of the susceptible strain of flax were planted in abundance in the soil. As more fruiting cultures of the organism were obtained, the inoculation was repeated. When the plants from this seed showed considerable wilt they were turned under the soil and more seed planted. Only three or four plantings of this kind were necessary with the pure-culture inoculations to put the soil in suitable condition for the growth of hybrid plants (Pl. 46, A, B). It was also found that from 1½ to 2 inches of the North Dakota soil spread over Madison soil was sufficient to produce thorough wilting of susceptible plants (Pl. 46, C, b).

METHODS USED IN CROSSING AND SELFING PLANTS

After thoroughly testing the different strains of flax on "sick soil" and selecting the most suitable ones for the work, crosses were made between plants of the resistant and susceptible strains. The first crosses were made with plants grown in the greenhouse on soil free from *F. lini*. These crosses were fairly successful. In the summer of 1915, plants were grown in the field, and a large number of crosses were made between the different strains in a manner to be described later. These crosses were more successful than those made in the greenhouse. The best results were obtained from crosses made with the first few buds to appear on the plant. This is no doubt due to the fact that the plant is at its maximum sap content and highest state of activity at this stage and is more able to overcome any injury that might be done to the flower through the operation. After the plant becomes more mature, a lower percentage of the artificially pollinated flowers develops. The operation should be performed at about the time when the petals begin to show in the bud. At this stage the anthers are not fully mature and are not so easily broken open. The petals can be removed very easily by catching the tip of the bud with a small pair of forceps and pulling gently. Care should be taken not to catch too low on the bud, or the stigma may be broken or injured. Eyre and Smith (13) state that they removed the petals by a sudden jerk, removing the stamens at the same time. The writer was not able to do this with the varieties of flax used in this work, without injuring the stigma.

After removing the petals the sepals can be pushed aside and the anthers removed by carefully pinching off the filaments with forceps, which should be sterilized by dipping them in 50 per cent alcohol before and after each operation to avoid contamination. After this operation the flowers are ready for pollination. Flowers pollinated a day after emasculation gave no better results than those pollinated immediately after the process. Pollination is easily accomplished by taking a flower which has just opened from the plant to be used as the male parent and brushing the anthers directly over the stigma of the emasculated flower. It is very easy to tell when the anthers are open by the mealy appearance of the pollen on the surface. Before opening they are smooth and white. After the flower has been pollinated it should be covered to keep out insects and other agents of contamination. Small glazine bags and waxed paper rolled on a fountain pen and tied at the ends were found to be quite satisfactory for protecting the flowers. After the capsules begin to develop it is better to remove the covering, especially in the greenhouse, where there seems to be a smothering of parts covered in this way. In the field, where the rolled paper was used, the seed developed in good condition even though the paper was not removed. The bags, being tighter than the rolled paper, prevented aera-

tion to a greater extent, and for this reason it was more desirable to remove them as soon as possible after the seed began to set.

For selling plants the paper bags were used very little. The whole plant was covered with cloth. The most satisfactory method was to use wire cylinders about 3 inches in diameter and about 12 inches long, made of screening and covered with slips of finely woven white cloth made to fit. These cloth slips should be considerably longer than the cylinders, so that they can be tied at both ends. A piece of stout wire is pushed into the ground beside the plant, so that it extends a few inches above the top of the plant. The cylinder is then placed over the wire and the plant, and the cloth is brought together at the upper end and tied tightly around the large wire above the top of the plant. The lower end of the cloth is then tied around the wire and the plant above ground. The fruiting part of the plant is thus protected within the cylinder, where it produces seed in a fairly normal manner. These cylinders should be removed as soon as the flowering period is over and the fruit has set.

METHODS USED IN GROWING THE PROGENY FROM CROSSES

Plants of the first and second generations which were to be tested for resistance were grown in flats and on benches of North Dakota "flax-sick soil" and Madison soil which was inoculated as previously stated. These experiments were conducted in the greenhouse throughout the year. There is a slight variation in temperature in the greenhouse with change of seasons, a condition that can not be prevented. It was shown by temperature studies (23) that a difference of a few degrees might greatly influence the rate and amount of attack of flax by *F. lini*. By comparing the controls grown in winter and summer this difference will be observable. The high summer temperatures increased the severity of the wilt, even a few plants of the resistant strain wilting.

The soil was well pulverized and the seed planted about 1 inch apart in rows about 4 inches apart. This made it possible to grow a large number of plants in a comparatively small area. In every case rows of both parent strains were planted in every flat or bench in sufficient numbers to serve as controls, and in some experiments selfed seed from the parent plants of the crossed seed were planted as controls. The number of seeds planted was recorded in order to ascertain the percentage of germination. In the summer, when the greenhouse temperature ran high, the percentage of germination was low. In some cases practically none of the seeds germinated. This perhaps was not due to temperature alone but to the increased activity of other physical and biological agents in the soil. As soon as the seed germinated and the seedlings appeared above the ground, they were counted and recorded as plants. Any plant that made its appearance above the ground was counted, even though it died in this stage from wilt. The results are

given with this stage of the plant as a starting point. It would be impossible to determine in every case the exact reason for the failure of the seed to germinate; and as some seeds of both strains did not germinate, it was not thought wise to attribute any of the failure directly to *F. lini*, although it is quite likely that this organism was partly responsible. If there was any doubt as to the cause of the wilting of the seedling after it appeared above the ground, it was determined by isolation methods in the laboratory. After the number of plants was recorded they were kept under almost daily observation. Notes were taken every week where possible and the number of healthy, wilted, and dead plants recorded. Any plant which showed undoubted wilt symptoms was recorded as wilted. This method of note-taking made it possible to compare the rate of wilting of the hybrid plants with that of the susceptible strain. As time and space were very limited it was found to be undesirable to grow all plants to complete maturity. It was then necessary to select some stage in the development of the plant as the end point for observation and note-taking. At this stage all plants which were not to be kept for seed could be removed and other seed planted. The flowering stage was selected as being the most favorable to cease note-taking, for at this stage the plant has reached its maximum activity and very little noticeable infection takes place after this time.

Part of the hybrid seed was grown in clean soil in a different greenhouse where there was no chance for infection by *F. lini*. These plants were self-fertilized as previously described in order to obtain seed for the next generation. By this means seed was obtained from plants that might have been destroyed by wilt if they had been grown in infected soil.

RESULTS OBTAINED FROM THE CROSSES

The parent strains, as previously stated, were thoroughly tested on "flax-sick soil" before the crosses were made and were found to be uniformly resistant or susceptible, as the case might be. However, some of the plants of the resistant strain wilted in later experiments. Results obtained from the progeny of crosses show that there is a great difference in individuality among plants of any strain with respect to its resistance to wilt. This difference was shown very strongly in the two generations grown, the results being so widely different from individual crosses that it will be necessary to discuss the different crosses or groups of crosses separately. The first generation from certain crosses proved to be entirely, or almost entirely, resistant to wilt. Others were intermediate with respect to resistance, while still others were entirely susceptible. In cases where there was entire or partial susceptibility a difference in the time and rate of infection was noticed as compared with the time and rate of infection of the common, susceptible flax. In some experiments the common flax would be almost entirely

dead before the first-generation plants began wilting (Pl. 45, A, B). Later all of these F_1 plants would perhaps succumb to the disease. In some of the experiments there was segregation in the first-generation offspring, part of the plants wilting, while others were as resistant as the resistant parent. In this case there was an intermediate condition as to the time of infection of the F_1 plants also. This fact was apparently not due to excessive vigor of the plants, since vigor seems to play no part in the resistance to wilt by the flax plant. The plants of common flax seem to be even more vigorous than resistant plants in cases, but succumb very readily to the attacks of the fungus.

No such simple ratios were obtained from these flax crosses as Biffen (2, 3) reported from his wheat crosses. Biffen was studying the nature of the inheritance of resistance by wheat to yellow-rust. In his first set of experiments (2) he crossed Rivet wheat, which is resistant to yellow-rust, with Michigan Bronze, which he states is probably more susceptible to yellow-rust than any other wheat in existence. The first generation from this cross was entirely susceptible to attacks by the rust. No seed was obtained for a second generation, owing to the severity of the attack. Red King, a very susceptible variety, was crossed on Rivet, and the first generation was susceptible in this case also. A second generation from these plants gave practically a 1-to-3 ratio, which Biffen interpreted as indicating that resistance and susceptibility are unit characters, the latter being dominant to the former. Biffen fails to state just where he drew the line between resistance and susceptibility, a very important point. He says the plants which he placed in the resistant group were "relatively" or "almost" free from rust. He also states that the third generation gave results which confirmed those of the preceding generations, but, that statistics for this last generation were not altogether satisfactory, owing partly to the limited amount of grain harvested for the trial and partly to the unfavorable conditions at the time of sowing.

In 1907 Biffen (3) published on a piece of work which was much more thorough apparently than his first work. He reported in this case, as before, that the first generation of wheat plants from a cross between entirely immune and susceptible strains was entirely susceptible, and that the second generation gave approximately a ratio of one resistant to three susceptible plants. In these experiments every plant that showed symptoms of rust was considered as susceptible. He also states that there was a gradation between entirely resistant and entirely susceptible plants.

Stuckey (22), working with tomatoes at the Georgia Station, states that by crossing the Red Cherry, which is resistant to blossom endrot, with the Greater Baltimore, a large commercial type which is susceptible to the disease, both the first and second generations from the cross were resistant to blossom endrot and that there was no segregation in the second

generation according to Mendelian laws. Unless there is some possibility that these fruits were not subjected to uniformly favorable conditions for blossom endrot, this is apparently an unusual case of inheritance.

Orton (20, p. 463) says, in writing on the resistance of farm crops to disease:

When a disease-resistant variety is crossed with a nonresistant variety, the resulting offspring inherit resistance to a limited and varying extent.

The writer found that in crosses between resistant and susceptible flax plants there was considerable difference in the results obtained from the different progenies. The results seemed to depend largely on the individual plants crossed. One of the most promising crosses made was one between the resistant strain No. 4 and the most susceptible strain, No. 3. The resistant plant was used as female parent, and the progeny was designated as 4D20, "4" referring to the strain, "D" to the plot where the female parent was grown, and "20" to the number of the female plant. With these data on the female parent it was easy to refer to the original records of the cross for the strain of the male parent. This system of recording was used throughout the work. From crosses between the 2 plants mentioned above 5 capsules were obtained, and 26 first-generation plants were grown on soil thoroughly infected with *F. lini* (Pl. 45, D, b). These plants were distributed among three flats, one containing 8 and the two others containing 9 plants each. The plants in one flat were growing at a lower temperature than those in the two other flats, the temperature of the former ranging from 14° to 19° C., while that of the latter ranged from 18° to 21°. None of these F_1 plants, however, were infected, although the controls of susceptible flax No. 3 wilted completely (23). They were grown to maturity and seed was obtained for a second generation by selfing. There was a segregation in the second generation into resistant and nonresistant plants. Table III gives the results obtained from the first and second generations and their controls. In this case selfed seed from the parent strains were grown as controls. In order to show the comparison between hybrid plants and plants of the susceptible strain, the number of plants wilted at the end of three weeks and the number killed by wilt at the end of the experiment were recorded.

The second-generation plants, as indicated by the controls given in the table, were grown under somewhat more severe conditions than the first generation, as is shown by the fact that a number of the plants of the resistant parent strain were killed or infected by wilt. The F_1 plants were grown in pure North Dakota soil, while the plants of the second generation were grown in artificially infected soil. Bolley (9) says that a change in the type of soil may cause a weakening of the resistant character. There was also a difference of temperature under which these F_1 and F_2 plants were grown, which may have played some part. The F_2 plants were grown in the summer and autumn, while the F_1 plants

were grown in winter and early spring, when the temperature was considerably lower. A slight rise in temperature serves to accelerate the growth of the fungus, and the disease becomes more severe.

TABLE III.—Resistance to flaxwilt obtained from the progeny of a cross between resistant flax No. 4♀, and susceptible No. 3♂^a

Parent strain.	Date of planting.	Number of plants grown.	Ratio at end of three weeks.		Ratio at end of experiment.		Number of plants killed by wilt.
			Wilted.	Not wilted.	Wilted.	Resistant.	
1916.							
4D20(F ₁).....	Feb. 2.....	26	0	26	0	26	0
Resistant No. 4.....	do.....	39	0	39	0	39	0
Susceptible No. 3.....	do.....	52	34	18	52	0	52
F ₂ generation:							
4D20-1.....	July 22.....	68	37	31	45	23	40
4D20-2.....	Sept. 23.....	98	42	56	62	36	53
4D20-3.....	do.....	115	40	75	68	47	47
4D20-4.....	do.....	28	9	19	22	6	19
4D20-5.....	do.....	101	59	42	76	25	64
4D20-6.....	do.....	50	29	21	34	16	30
4D20-8.....	do.....	70	54	16	61	9	53
Total		530	270	260	368	162	306
Resistant No. 4.....	Sept. 23.....	56	6	50	10	46	6
Susceptible No. 3.....	do.....	82	76	6	82	0	82

^a ♀ = female; ♂ = male.

Since the first generation from this particular cross was entirely resistant, it was hoped that some reasonable explanation might be given for the results obtained in the second generation, although it was apparent at once that they could not be explained on a unit-factor basis. As the number of susceptible F₂ plants was very large, it was thought that they might be explained by Little's (16) hypothesis, which is an explanation of cases that appear to be a reversal of dominance. The individuals showing the character in question decrease in number in the F₂ generation, as there is an increase in factors which produce the particular character. The general principle is that with the addition of each factor involved the number of F₂ individuals possessing the character in question is multiplied by 3, while the total number of F₂ individuals is multiplied by 4. The difference between the number of individuals with the character and those lacking it grows progressively greater with each factor added. With the flax cross under consideration, the first generation was entirely resistant to wilt. In the second generation, from a total of 530 plants 162 were resistant and 368 susceptible. These figures approach very closely the expectation, if four factors are concerned in producing resistance. The actual expectation would be 81 resistant to 175 susceptible, which is a ratio of 1 to 2.16. The actual proportion obtained was 81 resistant to 184 susceptible, which is a ratio

of 1 to 2.27. This ratio is fairly close to the expectation. The number of susceptible plants ran rather high, although this was to be expected, since the F_2 plants were grown under slightly abnormal conditions, as we have already seen. Some of these plants showed only slight signs of infection and would no doubt have resisted entirely had they been under less severe conditions. In this case the discrepancy is in the direction expected, the number of susceptible plants running high. Since this is the only cross that gave definite ratios, too much emphasis should not be placed on these results until further experimental evidence is obtained. It is furthermore very desirable that experiments of this kind be conducted in environmental conditions which are kept fairly constant.

TABLE IV.—Resistance to flaxwilt obtained with reciprocal crosses of resistant flax No. 4 with susceptible No. 3 and their controls

Parent strain.	Date of planting.	Number of plants grown.	Ratio at end of three weeks.		Ratio at end of experiment.		Number of plants killed by wilt.
			Wilted.	Not wilted.	Wilted.	Resistant.	
1915.							
4E1 (F1).....	Oct. 15	9	2	7	9	0	9
6E1.....	do.	9	1	8	9	0	9
4E1 (self).....	do.	17	0	17	0	17	0
6E1 (self).....	do.	17	13	4	17	0	17
1916.							
F2 generation (6E1 ♀ and 4E1 ♂):	June 27						
6E1-1.....	do.	76	56	20	67	9	57
6E1-2.....	do.	133	100	33	128	5	115
6E1-3.....	do.	125	56	79	123	2	118
6E1-4.....	do.	104	49	55	101	3	97
6E1-5.....	do.	66	22	44	66	0	65
6E1-6.....	do.	34	15	19	34	0	33
Total.....		538	298	240	519	19	485
Controls, F2 generation:							
Resistant No. 4.....	do.	84	12	72	30	54	22
Susceptible No. 6.....	do.	8	8	0	8	0	8
Susceptible No. 3.....	do.	24	24	10	24	0	24
F2 generation (4E1 ♀, 6E1 ♂):							
4E1-1.....	do.	93	61	32	92	1	84
4E1-2.....	do.	74	52	22	73	1	68
4E1-3.....	do.	36	33	3	36	0	36
4E1-4.....	do.	86	63	23	86	0	84
4E1-5.....	do.	56	31	25	56	0	55
4E1-6.....	do.	84	55	19	80	4	78
Total.....		429	295	134	423	6	405

Another cross which was followed up with great care was a cross of resistant flax No. 4, with susceptible No. 6. Reciprocal crosses were made, and selfed seed was obtained from both parent plants. The resistant parent is designated as 4E1 and the susceptible parent as 6E1. The first generation (Pl. 45, A, b, c) proved to be entirely suscep-

ible, although it might be said that there was an intermediate condition, owing to the fact that there was considerable difference in the time of wilting of the first-generation plants as compared with plants grown from selfed seed from the susceptible parent. The second generation was almost entirely susceptible. These plants, however, were grown under more severe conditions than were the plants of the first generation. The severeness of these conditions can be seen from results of the resistant control given in Table IV.

In this case, as is shown by the table, the second-generation plants were grown under fairly severe conditions. Thirty of the eighty-four plants of the resistant control were infected. The second generation was grown on artificially infected soil. Considering the behavior of the first generation and the severe conditions under which the second generation was grown, together with the results obtained, it seems impossible at present to give any direct explanation on a Mendelian basis.

In order to see what the result would be if the seed from the crosses on a number of plants were mixed, seed from five different plants were thrown together and planted. In this case sight was lost of the individuality of parent plants, due to the mixing; consequently, no definite ratios could be expected as individuals behave very differently. Table V gives the results obtained from mixed progeny.

TABLE V.—Resistance to flaxwilt of the mixed progeny of crosses between resistant flax No. 4♂ and susceptible No. 3♀

Parent strain.	Date of planting.	Number of plants grown.	Ratio at end of three weeks.		Ratio at end of experiment.		Number of plants killed by wilt.
			Wilted.	Not wilted.	Wilted.	Resistant.	
3E-Mix. (F ₁).....	1915. Oct. 2	153	53	100	106	47	76
Resistant No. 4.....	do....	20	0	20	0	20	0
Susceptible No. 3.....	do....	46	43	3	46	0	46
F ₂ generation:	1916.						
3E-Mix-1.....	Apr. 22	89	41	48	49	40	46
3E-Mix-2.....	do....	81	63	18	78	3	72
3E-Mix-3.....	do....	88	55	33	84	4	67
Total.....		258	159	99	211	47	185
Resistant No. 4.....	Apr. 22	45	1	44	^a 3	42	0
Susceptible No. 3.....	do....	43	33	10	43	0	43

^a Slight.

As is shown by the controls in Table V, these plants were grown under very good conditions for the best test. The resistant parent strains stood up almost perfectly, while every plant of the susceptible parent strain died out completely. Since there are all gradations between an entirely resistant and an entirely susceptible first generation from individual crosses, we would expect the intermediate condition when seed

from a number of individuals are mixed. This was what actually happened. In the second generation there was great variation in the progeny from individuals of this first generation (Pl. 46, B, c, h). This is what might be expected, since the chances are that each group of second-generation plants came from a different cross. This method of growing the progeny was found to be very unsatisfactory as it gave no direct line on individual crosses which have been found to behave so differently.

TABLE VI.—Resistance to flaxwilt of the progeny from crosses between resistant and susceptible strains of flax in which about an equal number of resistant and susceptible plants occurred in the first generation

RESISTANT NO. 4 ♀ × SUSCEPTIBLE NO. 6 ♂

Parent strain.	Date of planting.	Number of plants grown.	Ratio at end of three weeks.		Ratio at end of experiment.		Number of plants killed by wilt.
			Wilted.	Not wilted.	Wilted.	Resistant.	
4E2 (F ₁).....	1916 Apr. 25	6	1	5	3	3	1
Resistant No. 4.....	do.....	6	0	6	0	6	0
Susceptible No. 6.....	do.....	9	9	0	9	0	9
F ₂ generation:							
4E2-1.....	Sept. 23	26	8	18	15	11	14
4E2-2.....	do.....	37	17	20	24	13	21
Total.....		63	25	38	39	24	35
Resistant No. 4.....	Sept. 23	26	4	22	7	19	5
Susceptible No. 3.....	do.....	24	14	10	24	0	24

RESISTANT NO. 4 ♀ × SUSCEPTIBLE NO. 3 ♂

4D3 (F ₁).....	1916 Feb. 2	18	0	18	10	8	2
Resistant No. 4.....	do.....	17	0	17	0	17	0
Susceptible No. 3.....	do.....	16	2	14	16	0	11
F ₂ generation:							
4D3-1.....	July 21	74	47	27	73	1	53
4D3-2.....	do.....	63	43	20	61	2	46
D3-3.....	do.....	105	69	36	103	2	82
Total.....		242	159	83	237	5	181
Resistant No. 4.....	July 21	37	6	31	14	23	8
Susceptible No. 3.....	do.....	26	25	1	26	0	26

RESISTANT NO. 4 ♂ AND SUSCEPTIBLE NO. 3 ♀

3E22 (F ₁).....	1916 July 21	9	1	8	4	5	3
Resistant No. 4.....	do.....	15	0	15	0	15	0
Susceptible No. 3.....	do.....	18	16	2	18	0	18
F ₂ generation:							
3E22-2.....	Sept. 23	24	20	4	23	1	22
Resistant No. 4.....	do.....	26	4	22	7	19	5
Susceptible No. 3.....	do.....	24	14	10	24	0	24

A number of cases were discovered in which the first generation split up into almost equal numbers of resistant and susceptible plants, but there seemed to be no definite order of segregation in the second generation in cases of this kind. The severeness of conditions under which these plants were grown can be judged from the controls in each case. Table VI gives results of crosses of this kind.

Another grouping of tables has been made to show the results of crosses where there was a larger number of resistant than susceptible plants in the first generation. There seems to be but little relation between the behavior of the first and second generations from these crosses. Table VII gives the results of crosses of this kind.

TABLE VII.—Resistance to flaxwilt of crosses between resistant and susceptible strains of flax in which a greater number of resistant than susceptible plants occurred in the first generation

RESISTANT NO. 4 ♀ × SUSCEPTIBLE NO. 3 ♂

Parent strain.	Date of planting.	Number of plants grown.	Ratio at end of three weeks.		Ratio at end of experiment.		Number of plants killed by wilt.
			Wilted.	Not wilted.	Wilted.	Resistant.	
	1916.						
4D1(F ₁).....	Mar. 29	21	4	17	4	17	2
Resistant No. 4.....	do.....	95	5	90	8	87	6
Susceptible No. 3.....	do.....	85	80	5	85	0	85
F ₂ generation:							
4D1-2.....	July 22	47	32	15	42	5	36
4D1-3.....	do.....	49	41	8	47	2	42
4D1-4.....	do.....	41	35	6	37	4	34
4D1-5.....	do.....	32	24	8	28	4	24
4D1-6.....	do.....	47	35	12	43	4	36
4D1-8.....	do.....	49	45	4	47	2	42
Total	do.....	265	212	53	244	21	214
Resistant No. 4.....	do.....	55	12	43	37	18	15
Susceptible No. 3.....	do.....	47	45	2	47	0	46

RESISTANT NO. 4 ♂ × SUSCEPTIBLE NO. 5 ♀

5E1(F) ₁	Feb. 2	8	0	8	2	6	0
Resistant No. 4.....	do.....	18	0	18	0	18	0
Susceptible No. 5.....	do.....	16	2	14	16	0	16
F ₂ generation:							
5E1-1.....	Sept. 23	78	56	22	67	11	51
5E1-2.....	do.....	8	2	6	4	4	4
5E1-3.....	do.....	18	8	10	18	0	15
5E1-4.....	do.....	24	14	10	21	3	18
5E1-5.....	do.....	20	7	13	13	7	10
Total	do.....	138	87	51	123	25	98
Resistant No. 4.....	do.....	26	4	22	7	19	5
Susceptible No. 5.....	do.....	2	2	0	2	0	2

TABLE VII.—Resistance to flaxwilt of crosses between resistant and susceptible strains of flax in which a greater number of resistant than susceptible plants occurred in the first generation—Continued

RESISTANT NO. 4 ♂ × SUSCEPTIBLE NO. 5 ♀

Parent strain.	Date of planting.	Number of plants grown.	Ratio at end of three weeks.		Ratio at end of experiment.		Number of plants killed by wilt.
			Wilted.	Not wilted.	Wilted.	Resistant.	
5E22(F ₁).....	1916. Apr. 26	14	0	14	5	9	1
Resistant No. 4.....	do.....	18	0	18	0	18	0
Susceptible No. 5.....	do.....	16	16	0	16	0	16
F ₂ generation:							
5E22-1.....	Sept. 23	53	21	32	40	13	32
Resistant No. 4.....	do.....	26	4	22	7	19	5
Susceptible No. 5.....	do.....	2	2	0	2	0	2

RESISTANT NO. 4 ♂ × SUSCEPTIBLE NO. 3 ♀

3E14(F ₁).....	Feb. 2	9	0	9	2	7	0
Resistant No. 4.....	do.....	14	0	14	0	14	0
Susceptible No. 3.....	do.....	21	7	14	21	0	21
F ₂ generation:							
3E14-1.....	July 21	80	62	18	79	1	67
Resistant No. 4.....	do.....	26	4	22	7	19	5
Susceptible No. 3.....	do.....	24	14	10	24	0	24

RESISTANT NO. 4 ♀ × SUSCEPTIBLE NO. 5 ♂

4E11(F ₁).....	Oct. 18	5	0	5	2	3	0
Resistant No. 4.....	do.....	79	3	76	6	73	3
Susceptible No. 5.....	do.....	51	36	15	51	0	50
F ₂ generation ^a :							
4E11-1.....	do.....	202	55	147	137	65	86
4E11-2.....	do.....	128	63	65	116	12	86
4E11-3.....	do.....	141	30	111	101	40	56
Total.....		471	148	323	354	117	228

RESISTANT NO. 4 ♀ × SUSCEPTIBLE NO. 5 ♂

4E7(F ₁).....	Oct. 18	5	0	5	2	3	0
Resistant No. 4.....	do.....	79	3	76	6	73	3
Susceptible No. 5.....	do.....	51	36	15	51	0	50
F ₂ generation ^a :							
4E7-1.....	do.....	44	10	34	18	26	10
4E7-2.....	do.....	52	7	45	16	36	11
Total.....		96	17	79	34	62	21

^aControls for F₂ same as for F₁ above.

Table VIII shows the results of crosses in which the first generation was entirely or almost entirely susceptible. The second generation in these cases behaved about as did the second generation from crosses which have been given above. There seems to be no common explanation for segregation in this case, which, of course, is to be expected when the first generation segregates irregularly, as was true with these crosses.

TABLE VIII.—Resistance to flax wilt of crosses between resistant and susceptible strains of flax in which the first generation plants were entirely or almost entirely susceptible

RESISTANT NO. 4 ♀ × SUSCEPTIBLE NO. 5 ♂

Parent strain.	Date of planting.	Number of plants grown.	Ratio at end of three weeks.		Ratio at end of experiment.		Number of plants killed by wilt.
			Wilted.	Not wilted.	Wilted.	Resistant.	
	1916.						
4E13 (F ₁).....	Feb. 2	14	4	10	12	2	3
Resistant No. 4.....	do....	18	0	18	0	18	0
Susceptible No. 5.....	do....	12	6	6	12	0	12
F ₂ generation:							
4E13-1.....	Sept. 23	127	82	45	126	1	125
Resistant No. 4.....	do....	26	4	22	7	19	5
Susceptible No. 5.....	do....	2	2	0	2	0	2

RESISTANT NO. 4 ♀ × SUSCEPTIBLE NO. 5 ♂

4E12 (F ₁).....	Oct. 18	11	5	6	11	0	8
Resistant No. 4.....	do....	79	3	76	6	73	3
Susceptible No. 5.....	do....	51	36	15	51	0	50
F ₂ generation: ^a 4E12-1.....	do....	123	62	61	106	17	83

^a Control same as for F₁.

A large number of first-generation plants have been grown from different crosses from which no second-generation plants have been grown. The first generations from these crosses vary from those which are entirely resistant to those which are entirely susceptible, as have been given in the previous tables. Table IX will show these results.

DISCUSSION OF RESULTS

The inspection of Tables III to IX reveals no common explanation for the results obtained. Except for one case (Table III), no definite ratios could be detected. However, there are a number of possible reasons for these results: (1) The plants were grown under abnormal greenhouse conditions. (2) The temperature was too high in some cases, as previously emphasized. (3) A different type of soil was used for some of the tests, which, according to Bolley (9), is likely to cause a breaking down of the resistant character. (4) There is a great variation among individual plants within a strain.

TABLE IX.—*Resistance to flax wilt of the first generation from individual crosses between resistant and susceptible strains of flax*

Parent strain.	Date of planting.	Number of plants grown.	Ratio at end of three weeks.		Ratio at end of experiment.		Number of plants killed by wilt.
			Wilted.	Not wilted.	Wilted.	Resistant.	
	1916.						
4D30 (F ₁).....	Feb. 2	18	0	18	0	18	0
4D8 (F ₁).....	do.	36	0	36	15	21	4
Resistant No. 4.....	do.	18	0	18	0	18	0
Controls:							
Susceptible No. 3.....	do.	18	5	13	17	1	13
4D21.....	Mar. 29	6	1	5	6	0	1
4D38.....	do.	22	6	16	7	15	3
4D37.....	do.	32	17	15	32	0	26
4D29.....	do.	21	16	5	21	0	20
4D26.....	do.	16	9	7	16	0	12
4D32.....	do.	17	9	8	17	0	17
4D-mix.....	do.	88	52	36	82	6	60
Resistant No. 4.....	do.	95	5	90	8	87	6
Susceptible No. 3.....	do.	85	80	5	85	0	85
4D36.....	May 13	16	6	10	6	10	6
4D31.....	do.	20	9	11	10	10	6
4D23.....	do.	20	15	5	15	5	11
4D-mix.....	do.	40	32	8	36	4	26
Resistant No. 4.....	do.	63	7	56	26	47	4
Susceptible No. 3.....	do.	75	74	1	75	0	75
6E2.....	Apr. 25	6	1	5	3	3	1
4E8.....	do.	8	3	5	4	4	2
5E3.....	do.	5	4	1	5	0	3
4E6.....	do.	10	7	3	9	1	4
Resistant No. 4.....	do.	10	0	10	1	9	0
Susceptible No. 5.....	do.	5	4	1	5	0	5

However, it seems that there is a possible explanation for the results obtained. The resistant strain of flax has been bred by selection to resist wilt under certain environmental conditions which we might call normal. As was pointed out in Table III, resistance is probably due to a number of factors. Then it might be assumed that under the normal environment certain factors, A, B, C, for example, are required to produce resistance. These factors, which may be of equal or unequal value in producing resistance, are possibly homozygous, as is evidenced by the fact that segregation is exceptional under what is termed a normal environment. If, however, the environment is made more severe, there is a certain amount of segregation among plants of the resistant strain, which was found to be true with plants growing in the greenhouse in the summer. Under these more severe conditions one or more additional factors, D, E, etc., might be supposed to be required to cause the plant to resist. Since the strain has not been selected to resist under these severe conditions of environment, no attempt has been made to make these factors homozygous; therefore they may be absent entirely, or may occur singly or in combination, and may be either homozygous or

heterozygous. Then, a certain amount of segregation would be expected when the plants are subjected to these conditions. Segregation would likewise be expected to take place in the first-generation offspring from crosses of these plants with plants of the susceptible strain, provided the plants are grown in the abnormal environment. Differences of the individual plants of the susceptible strain with regard to the resistant character might account for considerable variation in the amount of segregation in the first generation from such crosses. A difference of this kind has been the basis for the selection of resistant strains. It is possible that some of these plants have one or more of the factors for resistance, which may be either homozygous or heterozygous, but do not have a sufficient number of them to cause the plant to resist where the disease develops normally. In crossing susceptible plants of this kind with plants of the resistant strain various factor combinations would be obtained, some of which would produce resistance in the first generation under the severe conditions while others would not. Results obtained in these experiments could easily be correlated with a theory of this kind.

The perfecting of methods rather than the development of any definite proof of laws governing the inheritance of wilt resistance in flax has been the main accomplishment in this work. Therefore a few helpful suggestions might be offered by way of conclusion of this chapter.

(1) The resistant plant should be tested under severe disease conditions before making the cross. (2) Reciprocal crosses should be made in each case, and selfed seed should be obtained from both parent plants, this to be planted along with the crossed seed. (3) First-generation plants should be grown in disease-free soil to obtain seed for a second generation. (4) First and second-generation plants which are to be tested should be grown under similar conditions with selfed seed from the parents planted as controls. (5) The experiments should be conducted under uniform environmental conditions in order to obtain conclusive results.

CONCLUSIONS

(1) The flax plant is most suitable for a study of the nature and inheritance of wilt resistance, since it grows very well in the greenhouse, has a short growing season, is easily crossed, resistant and susceptible strains are available, and conditions for infection can be produced with certainty.

(2) *Fusarium lini* penetrates the flax plant through root hairs, young epidermal cells, stomata of seedlings, and perhaps through wounds.

(3) *Fusarium conglutinans* is able to penetrate the root hairs of young cabbage seedlings when the seedlings are grown in tube cultures.

(4) *F. lini* invades the various tissues of the susceptible flax plant causing the disease known as flaxwilt. No considerable clogging of

vessels can be seen. Wilting may be due to the combined action of several factors: (a) Destruction of the young active root system by the fungus, which cuts off a part of the food and water supply of the plant. (b) Use of the food and water supply of the plant by the fungus. (c) More vigorous growth of the fungus and increased transpiration of the host plant due to a rise in the temperature. (d) The possible production of toxins by the fungus which injure the host protoplasm.

(5) *F. lini* penetrates the resistant flax plant and stimulates division and cork wall formation in cells adjacent to those attacked, but is not able to invade the tissues to any considerable extent owing to a number of possible reasons: (a) The permanent chemical composition of the resistant plant may be of such nature as to be injurious to the fungus. (b) The protoplasm of the resistant plant may be more highly sensitive than that of the susceptible plant, thus reacting more readily in the production of those phenomena which cause wilt resistance. (3) The stimulation to new cell division and the laying down of cork walls which seem to serve as a barrier to further invasion by the already weakened hyphae.

(6) Wilt resistance in flax is an inheritable character which is apparently determined by multiple factors.

(7) There is a great difference in the individuality of plants of a strain with respect to the resistant character, as shown by their offspring. The first generation from some crosses is entirely resistant, from some intermediate, and from others entirely susceptible.

(8) The degree of resistance shown by a strain of flax depends to a considerable extent on the environmental conditions under which the plants are grown. A strain which was bred to resist under certain conditions may break down under a more severe environment. Plants of North Dakota Resistant No. 114, the best strain employed in this work, was not entirely resistant with the high summer temperatures in the greenhouse.

(9) All parent strains to be used in crossing should be thoroughly tested on infected soil under favorable disease conditions before making the crosses. The resistant parent to be used in the cross should be grown on infected soil.

(10) Hybridization experiments should be conducted under uniform environmental conditions in order to obtain conclusive results. The fact that such varied results were obtained in this work is probably due to the different environments under which the plants were grown.

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PLATE 44

A.—Flax seedlings as grown in the tube culture for penetration studies: *a*, Seedlings of susceptible flax in tubes inoculated with *Fusarium lini*. *b*, Susceptible flax seedlings growing in uninoculated tube. *c*, Resistant flax seedlings growing in uninoculated tube. *d*, Resistant flax seedlings in tubes inoculated with *F. lini*. These seedlings were killed as readily as susceptible seedlings under these conditions.

B.—Flax plants growing in North Dakota "flax-sick soil." *a*, Resistant flax No. 4. *b*, Susceptible No. 3.

C.—Flax plants grown on soil from Madison, Wis. This soil was sterilized and inoculated with pure cultures of *F. lini*. *a*, Susceptible No. 3. *b*, Resistant No. 4.

PLATE 45

A.—Flax plants growing on North Dakota "flax-sick soil." *a*, Resistant No. 4. *b*, *c*, Reciprocal crosses between resistant No. 4 and susceptible No. 6. All of these F_1 plants died later of wilt. *d*, Susceptible No. 6.

B.—Flax plants growing in North Dakota "flax-sick soil." *a*, Resistant No. 4. *b*, Cross between resistant No. 4 and susceptible No. 5. *c*, Susceptible No. 5.

C.—Flax plants growing in North Dakota "flax-sick soil." *a*, Resistant No. 4. *b*, *c*, Crosses between resistant No. 4 and susceptible No. 5. *d*, Susceptible No. 5.

D.—Flax plants growing in North Dakota "flax-sick soil." *a*, Resistant No. 4. *b*, Cross 4D20 between resistant No. 4 and susceptible No. 3. *c*, Cross 3E14 between plants of the same two strains as *b*. *d*, Susceptible No. 3 (all dead).

PLATE 46

A.—Flax plants growing in soil inoculated artificially with *Fusarium lini*. *a*, Resistant No. 4. *b*, Susceptible No. 3. *c-h*, First-generation plants from crosses between these two strains. Notice that some of the crosses were almost entirely resistant to the disease while others wilted almost as badly as plants of the susceptible strain.

B.—Second-generation flax plants growing on artificially infected soil. *a*, Susceptible No. 3. *b*, Resistant No. 4. *c-h*, Second-generation plants from crosses between these two strains. These plants show the difference in individual plants of the first generation. *c* and *d*, *e* and *f*, *g* and *h*, respectively, came from individual plants of the first generation.

C.—Second-generation flax plants growing in North Dakota "flax-sick soil." *b*, Susceptible No. 3. *c*, Resistant No. 4. *a*, *d-h*, Second-generation plants from the cross 4D20 in which the first generation were all resistant. Of the 530 plants of this F₂ generation 162 were resistant and 368 susceptible.

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