RELATION OF TEMPERATURE AND MOISTURE TO NEAR-WILT OF PEA [1]

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INTRODUCTION

Practically complete control of the wilt disease (*Fusarium orthoceras* App. and Wr. var. *pisi* Linford), which was formerly very destructive to the pea crop of Wisconsin, eastern Washington, and parts of Maryland, has now been accomplished by the development and general adoption of suitable wilt-resistant varieties. The description of this disease and the causal organism has been given in detail by Linford.[2] More recently another disease of pea, known as near-wilt, has been distinguished in the same areas and even over a wider geographical range. This malady and the causal pathogen (*F. oxysporum* Schlect. f. 8 Snyder) have been described by Snyder and Walker.[3] While the near-wilt disease appears to be much more widespread in the United States than wilt, it has never become so acutely destructive in the regions in which it has become established. In fact it is usually to be found on occasional plants, widely scattered in the field, and only uncommonly does it destroy a majority of the pea plants within a given area. Whether this is due to the relatively poor capacity of the near-wilt organism to establish itself as abundantly and rapidly in favorable soils as does the wilt fungus remains to be determined, but it does appear from field observations that the former organism occurs over a wider range of soil types than the latter.

Symptoms of the two diseases resemble one another in many respects. The foliage becomes yellow with both and the leaflets and stipules of the plants may curve downward and inward, while at high temperatures they may quickly wither from the base of the plant upward. Stunting and rapid loss of turgidity of the wilt disease are less common with near-wilt. On the other hand, unilateral development of symptoms is more common with the latter disease. The pathogen, traveling rather rapidly up the stem, in one or a few vascular bundles, affects leaves and stipules in its path from base to tip. This localized effect commonly extends to one stipule in a pair, to leaflets on one side of the petiole, and even to the portion of the leaf lamina on one side of the midrib. In general the affected vascular system is orange to deep red in color in contrast to the lighter orange characteristic of bundles affected by wilt. This color difference, together with the fact that the near-wilt organism and the discoloration following it travel up the stem often to the growing tip while in wilt they seldom advance above the fifth node, is a very useful means of distinguishing the two diseases.

[1] Received for publication May 22, 1939.
[2] LINFORD, MAURICE B. A FUSARIUM WILT OF PEAS IN WISCONSIN. Wis. Agr. Expt. Sta. Res. Bul. 85 44 pp., illus. 1928.
[3] SNYDER, W. C., and WALKER, J. C. FUSARIUM NEAR-WILT OF PEA. Zentbl. f. Bakt. [etc.] (II) 91: 355-378, illus. 1935. See p. 373.

The purpose of the investigation reported in this paper was to study the effect of certain environal factors, especially temperature and moisture, upon the expression of near-wilt symptoms. This seemed to the writers to be particularly important in view of the attention being given to the possibility of developing near-wilt-resistant varieties suitable for commercial production.

EXPERIMENTAL RESULTS

TEMPERATURE RELATIONS OF THE NEAR-WILT ORGANISM

The relation of temperature to the growth of *Fusarium oxysporum* f. 8 in pure culture was studied in plate cultures of potato-dextrose agar which had been adjusted to pH 6.2. The isolate was from typically diseased plants in the field and its identity was confirmed by

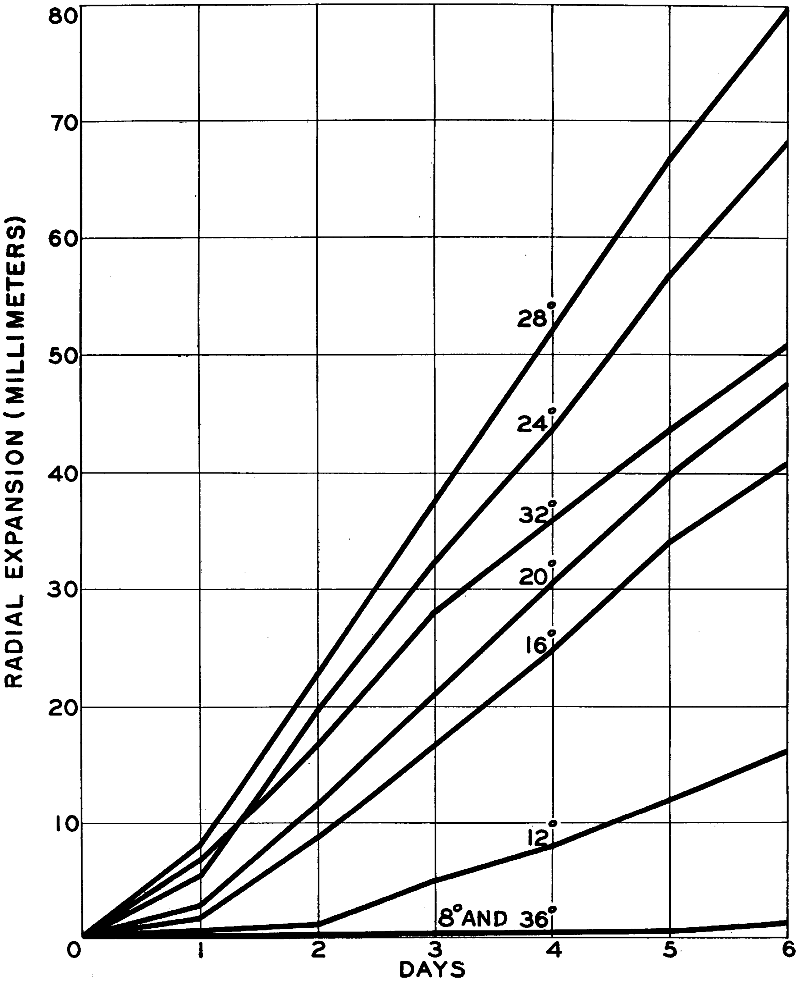

FIGURE 1.—Daily radial expansion of *Fusarium oxysporum* f. 8 on potato-dextrose agar plates incubated at various temperatures.

W. C. Snyder of the University of California. Five inoculated plates were placed in controlled incubators at each of the following temperatures: 4°, 8°, 12°, 16°, 20°, 24°, 28°, 32°, 36° C. After colonies had become established the radial growth of each plate was recorded every 24 hours until the thallus covered the entire plate. No measurable growth occurred at 4°. The average daily radial growth is plotted in a graph for each of the other eight temperatures (fig. 1). Since each of these graphs is practically a straight line it may be assumed that little or no staling material which retarded growth was produced by the fungus. Rates of growth at 8° and 36° were approximately the same. Most rapid radial expansion occurred at 28°. These results agree in general with those reported for other forms of *F. oxysporum*.[4] The maximum, minimum, and optimum temperature for growth as measured by this method came relatively close to coinciding with those of the wilt fungus.[5] It may be pointed out here, however, that whereas the optimum temperature for wilt development is much lower than that for its growth on agar, the data to be presented later in this paper show that the optima are closer in the case of near-wilt.

RELATION OF VARIETY TO DISEASE EXPRESSION

During the course of this investigation a study of varieties of peas (*Pisum sativum* L.) in relation to near-wilt was carried out in both field and greenhouse. It was noted that, generally speaking, the symptoms of near-wilt were slower to appear than those of wilt. Furthermore their development in the case of near-wilt varied with the variety when a number of varieties were grown in the same location. In most cases there was a distinct coincidence under field conditions in southern Wisconsin and in the greenhouse at Madison, Wis., between appearance of near-wilt and the beginning of the blossoming period. Thus early-blossoming varieties showed the disease early while in late-blossoming varieties the appearance of symptoms was delayed. A common example of this is the contrast between the Alaska, a variety which blooms and matures early, and Alderman, a late variety. Under optimum conditions for the disease the plants of Alaska are commonly dead from near-wilt before any signs of disease are shown by the Alderman plants, although the latter eventually succumb. In certain varieties which are very susceptible the plants may wilt before blossoming has occurred. The differential effect of varieties on the progress of the disease has been taken into account, therefore, in the present study of the effect of external environment upon near-wilt.

TEMPERATURE RELATIONS OF THE DISEASE

Soil-temperature experiments were carried out in the greenhouse in Wisconsin tanks. The pathogen was increased on a medium of corn meal and sand which was incorporated with soil that had been collected from an uncultivated wood lot and autoclaved for 5 hours at 15 pounds pressure. Since the volume of inoculum used was very small in relation to that of the soil, a period of 2 months was allowed to elapse during which the mixture was stirred and watered occasionally in order

[4] GOSS, R. W. RELATION OF ENVIRONMENT AND OTHER FACTORS TO POTATO WILT CAUSED BY FUSARIUM OXYSPORUM. Nebr. Agr. Expt. Sta. Res. Bul. 23, 84 pp., illus. 1923.
JOHNSON, JAMES. FUSARIUM WILT OF TOBACCO. Jour. Agr. Res. 20: 515–535, illus. 1921.
[5] See footnote 2.

to permit the organism to become uniformly established. It was then placed in galvanized iron cans which were set into the respective tanks. A quantity of sterilized, uninoculated soil from the same source was placed in other cans to serve as controls. For comparative purposes soil naturally infested with the wilt organism was placed in still other cans.

Five tanks were adjusted to 16°, 20°, 24°, 28°, and 30° C., respectively. In each tank were placed four cans of near-wilt-infested soil, two cans of wilt-infested soil, and 2 cans of uninoculated soil. The moisture content of the soils was approximately 60 percent of their water-holding capacity and the moisture was kept fairly constant by frequent weighings of the cans and replacement of the water lost by evaporation and transpiration.

In each tank two cans of near-wilt soil and one can of uninoculated soil were planted with seeds of Early Kay, a wilt-resistant and somewhat near-wilt-resistant variety of pea, and a similar set was planted with Wilt Resistant Perfection, a wilt-resistant, near-wilt-susceptible variety. The wilt soil (two cans in each tank) was planted with wilt-susceptible Perfection. Ten plants were grown in each can.

Plants were removed and counted as soon as they were permanently wilted and the identity of the disease was confirmed by recovering the respective fungus from each plant. A wilt index or near-wilt index was calculated for each can or duplicate cans by adding the number of days from sowing to wilting for each plant and dividing by the total number of plants, the index being the average number of days for the plants to reach the wilt stage. Thus the more rapid the development of the disease the lower the index. The data from this experiment are given in table 1.

Since the plants in the uninoculated soil all remained healthy they are omitted from the table. The most rapid development of wilt was at 20° C. which is in accord with the report of Linford [6] that the optimum was between 21° and 22°. It is to be seen, however, that the disease development was nearly as rapid at 24°, 28°, and 30° as at 20° while a definite retardation occurred at 16°. In the case of Wilt Resistant Perfection growing on near-wilt soil the most rapid disease development was at 24°, although there was little difference at this temperature from 20° and 28°. Distinct retardation occurred at 16° and 30°. It is evident that the disease was limited more definitely at 30° than was wilt, while at 16° they were both distinctly retarded.

TABLE 1.—*Indices of wilt and near-wilt in Wilt Resistant Perfection (wilt-resistant, near-wilt-susceptible), Perfection (wilt-susceptible), and Early Kay (wilt-resistant, near-wilt-resistant) pea varieties at various soil temperatures*

Variety	Wilt index (days) at soil temperature of—					Near-wilt index (days) at soil temperature of—				
	16° C.	20° C.	24° C.	28° C.	30° C.	16° C.	20° C.	24° C.	28° C.	30° C.
Perfection	[1] 39	25	27	28	[2] 27					
Wilt Resistant Perfection						[3] 64	46	44	46	[4] 55
Early Kay						(5)	(6)	62	63	68

[1] 4 plants alive at the end of the experiment.
[2] 1 plant alive at the end of the experiment.
[3] 2 plants alive at the end of the experiment.
[4] 4 plants dead at the end of the experiment.
[5] No plants dead at the end of the experiment.
[6] 16 plants living, 4 dead at the end of the experiment.

[6] See footnote 2.

In addition to being somewhat more restricted in its optimum soil-temperature range, near-wilt was definitely slower in appearing. Thus in the two strains of Perfection it required, on an average, nearly twice as long for all plants to succumb to near-wilt at 20° to 28° C. as was the case with wilt at the same temperatures. The

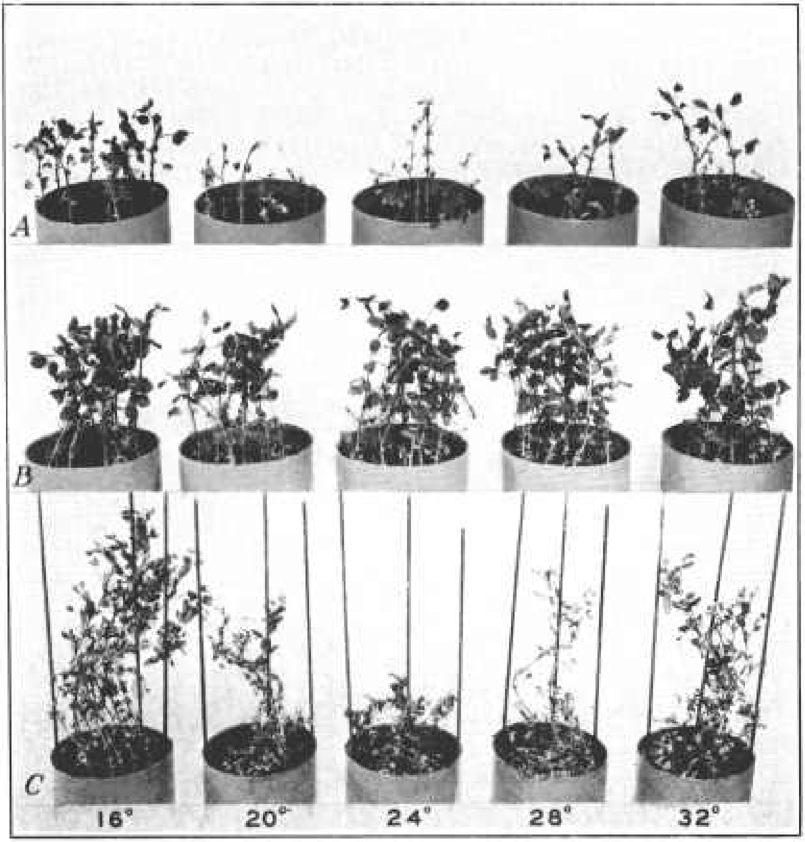

FIGURE 2.—Relation of soil temperature to the development of wilt and near-wilt. *A*, Wilt-susceptible Perfection peas grown in wilt-infested soil for 30 days at the respective constant soil temperatures indicated at the bottom of the figure. *B*, Near-wilt-susceptible Wilt Resistant Perfection plants grown for 30 days in near-wilt-infested soil in parallel series with those in *A*. Note that at this interval the wilt-susceptible plants are all dead or severely diseased at 20°, 24°, and 28° C., while no signs of disease have appeared in the plants on near-wilt soil. *C*, The same series as *B* which has been continued at the respective temperatures for 22 days longer. Note that near-wilt has now developed to a severe degree at 20°, 24°, and 28°, showing that it requires much longer to develop than does wilt, even in optimum environment.

data from Early Kay showed the effect of plant resistance on lengthening the time required for plants to succumb. They showed also that at the higher temperatures the disease eventually affected all plants, even of a variety which shows marked resistance under field conditions. Certain of the plants in this experiment are illustrated in figure 2.

The same test was applied with the inoculated near-wilt soil in a second experiment. Only the most favorable temperatures, 20°, 24°, and 28° C., were included. The two varieties used were Alaska and Horal. The latter, being a late-blooming variety, had shown a longer incubation period in the field and definite evidence of resistance. In a third test Alaska and Wilt Resistant Perfection were run at 16°, 20°, and 24° in naturally-infested near-wilt soil from a field near Winneconne, Wis. The results of these trials are given in table 2. The near-wilt index tended to be lower in the naturally-infested than in the artificially inoculated soil. This is found to be the case when the two Alaska tests in table 2 and the Wilt Resistant Perfection tests in tables 1 and 2 are compared. It may be due to a heavier infection or to the supplementary effect of other organisms which are pathogenic on pea roots, in the naturally infested soil. The same inoculated soil was used several months later for the next experiment (table 3), in which it will be noted that the near-wilt index is about the same for Wilt Resistant Perfection as that in the naturally infested soil of the last experiment. Whether this change is due to the increase in the amount of near-wilt inoculum or to the influence of other organisms with which contamination had occurred cannot be stated.

TABLE 2.—*Near-wilt indices in certain pea varieties in artificially inoculated and in naturally infested soil*

Type of soil infestation	Variety of pea	Near-wilt index [1] at soil temperature of—			
		16° C.	20° C.	24° C.	28° C.
Artificially inoculated	Horal	------	82	80	74
	Alaska	------	46	42	49
Naturally infested	Alaska	(2)	39	36	------
	Wilt Resistant Perfection	(3)	46	38	------

[1] The near-wilt index in each case is based on 10 plants.
[2] At 60 days only 1 plant had wilted.
[3] At 60 days only 2 plants had wilted.

It is to be noted that, in accord with what has been pointed out in the previous section, the index was higher in Wilt Resistant Perfection, a late-blooming variety, than in Alaska, an early-blooming variety. This difference was only slight, however, at the optimum. Alaska and Wilt Resistant Perfection are regarded as very susceptible to near-wilt. The comparison between Alaska and Horal brings out again the striking difference in index between an early susceptible and a late resistant variety. Horal is about the same in season as Wilt Resistant Perfection but the near-wilt index of the former is nearly twice that of the latter, discounting the slight discrepancy to be attributed to the difference between inoculated and naturally infested soil. Thus the variety usually highly resistant in the field shows considerable resistance in the greenhouse, but eventually succumbs.

The same artificially inoculated soil used in the above experiments was employed in a study of the relation of air temperature to the development of near-wilt. Three soil temperatures were used—16°, 22°, and 28° C.—at each of three air temperatures—16°, 20 to 22°, and 28°. Wilt Resistant Perfection was planted in three cans at each soil temperature in each air temperature, the total number of plants in each three-can group averaging about 35. The near-wilt indices secured in this experiment are recorded in table 3. The optimum soil temperature was 28° regardless of the air temperature to which the tops were exposed. At any given soil temperature the differences in rate of disease development between the three air temperatures was not great although the smallest index in each case was at 20°–22°.

TABLE 3.—*Near-wilt indices in Wilt Resistant Perfection peas grown in artificially inoculated soil at 3 soil temperatures in each of 3 air temperatures for 57 days*

Air temperature (° C.)	Near-wilt index at soil temperature of—		
	16° C.	22° C.	28° C.
16°	[1] 51	38	28
20°–22°	[2] 47	34	25
28°	[3] 52	35	27

[1] Based on 8 diseased plants from a total of 37.
[2] Based on 25 diseased plants from a total of 48.
[3] Based on 13 diseased plants from a total of 42.

It may be concluded from these experiments that the soil temperature is much more influential upon near-wilt than is air temperature. While an exact soil-temperature optimum cannot be defined it is evident that constant soil temperatures of 24° to 28° C. usually result in the most rapid development of the disease. Thus the optimum for near-wilt may be regarded as about 5° higher than that for wilt. Temperatures around 16° greatly retard near-wilt regardless of the air temperature. On the other hand, an air temperature of 16° does not appreciably affect the progress of the disease provided the soil temperature is maintained near the optimum. These statements apply particularly to the near-wilt susceptible varieties such as Alaska and Wilt Resistant Perfection. In the second experiment (table 2) there is an indication that the resistant variety, Horal, has a higher optimum than the susceptible variety, Alaska. These studies should be continued with resistant varieties.

RELATION OF SOIL MOISTURE TO THE DISEASE

Soil-moisture studies were conducted in Wisconsin soil-temperature tanks where the temperature variable could be eliminated by running all tanks at 24° C. in a common air temperature of 21° to 22°. Three soil types were used—two infested with near-wilt, one not infested. The infested soils were: (1) A sandy loam from a naturally infested field at Winneconne, already mentioned in connection with the soil-temperature experiments; and (2) a black silt loam which had been artificially inoculated. The noninfested soil was also a silt loam, but was lower in organic matter than the inoculated soil. It was sterilized before use. These soils were each made up into three groups designated as dry, medium-moist, and wet. The water-holding capacity of each soil and the actual moisture content of each group were determined. The percentage of water-holding capacity for each group is given in table 4.

The data from two series, including two susceptible (Alaska and Wilt Resistant Perfection) and two resistant (Kay and Early Kay) varieties are given in table 5. Inasmuch as no disease developed in the noninfested soil no data therefrom are included. The best growth in this soil occurred in the medium-moist lot. There was distinct retardation in growth in the dry soil. In the wet soil, yellowing of the lower leaves was pronounced.

TABLE 4.—*The water-holding capacity of the lots of soil used in the study of the relation of soil moisture to near-wilt*

Description of soil	Moisture as water-holding capacity in—		
	Dry group	Medium-moist group	Wet group
	Percent	*Percent*	*Percent*
Naturally infested sandy loam	25	47	67
Artificially inoculated silt loam	32	69	84
Sterilized uninoculated silt loam	30	55	75

TABLE 5.—*The relation of soil moisture to the development of near-wilt in pea*

Description of soil	Variety of pea	Series No.	Dry soil: Plants used [1]	Dry soil: Near-wilt index	Medium-moist soil: Plants used [1]	Medium-moist soil: Near-wilt index	Wet soil: Plants used [1]	Wet soil: Near-wilt index
			Number		*Number*		*Number*	
Inoculated silt loam	Wilt Resistant Perfection.	1	19	64	13	68	27	
		2	12	54	17	50	17	
	Alaska	1	41	51	51	47	54	
		2	20	51	20	47	20	
	Early Kay	2	19/6	55	19/8	61	20/4	
	Kay	2	19/8	67	19/0	--------	20/14	
Naturally infested sandy loam.	Wilt Resistant Perfection.	1	31	54	37	51	14	
		2	17	44	18	46	19	
	Alaska	1	31	41	56	41	42	
		2	17	41	20	40	17	
	Early Kay	2	16	82	14	39	19	
	Kay	2	20	80	17	54	14/12	

[1] In those cases in which all plants did not wilt permanently before the close of the experiment a fraction is given of which the numerator is the total number of plants and the denominator is the number of plants that did wilt permanently; the index is based on the data from the latter group only.

It will be seen from the data that in the case of Wilt Resistant Perfection and Alaska there was little difference between the indices in dry and medium-moist soil, while those in the wet soil were consistently the lowest. In the naturally infested soil disease development was generally more rapid, but the same relation prevailed between the dry, medium, and wet levels. Whether the greater rate of disease advance in the naturally infested soil is due to the supplementary effect of other organisms is not known, but it is recalled that the same relation occurred between artificially inoculated and naturally infested soil in the soil-temperature studies.

When the resistant varieties, Kay and Early Kay, are considered the soil-moisture effects are not so clear. The fact that disease development was so slow that usually only a small portion of the plants entered into the index may account in part for the lack of conformity. In the naturally infested soil, where practically all of the plants wilted, the index of both resistant varieties was distinctly lower in the medium-moist soil than in either the dry or the wet soil.

DISCUSSION

Although the wilt and near-wilt diseases have closely related causal organisms and certain symptoms in common, they nevertheless are distinct in several respects. Perhaps the greatest important difference is that pea plants and varieties fall into two discontinuous groups insofar as resistance and susceptibility to wilt are concerned, while in the case of near-wilt, the distinction between susceptible and resistant types is not so clear. Under very favorable conditions for near-wilt all plants of resistant forms slowly but eventually succumb. The effect of the host plant itself upon the development of the disease is more striking in near-wilt. This is shown in the correlation between rate of disease development and the rate of blooming of the host. It is again shown in the increase in incubation period in proportion to host resistance. These varietal differences usually held at various soil temperatures and moistures.

The relative slowness of near-wilt development as compared with wilt development, regardless of variety, is outstanding. It is probably as important a factor as any in determining the fact that although the near-wilt organism is more widespread it is generally less destructive than wilt.

The fact that the optimum soil temperature for near-wilt is somewhat higher than that for wilt is not likely to be very important since near-wilt is nearly as destructive at 20° and 28° C. as at 24° and is limited at 16° to about the same degree as wilt. While susceptible varieties succumb most rapidly in wet soils the progress in moderately moist and dry soils is sufficiently rapid to indicate relatively little retardation in dry seasons.

Although the effects of temperature and moisture were quite consistent and clear-cut when susceptible varieties were used, it is important to note that they did not always coincide with those secured with resistant forms. These discrepancies warrant further study on these and other resistant varieties and need to be considered in the evaluation of plants in improvement of the pea for resistance to near-wilt.

SUMMARY

The investigations comprise a study of the temperature and soil-moisture relations of the near-wilt fungus (*Fusarium oxysporum* Schlect. f. 8 Snyder) in relation to the pea plant.

On potato-dextrose agar the most rapid radial expansion of the organism occurred at 28° C. and the upper and lower limits for growth were somewhat above 36° and below 8°.

The near-wilt disease develops more slowly in a favorable environment than does wilt (*F. orthoceras* App. and Wr. var. *pisi* Linford).

Varieties differ in the rate at which near-wilt develops, the disease appearing more slowly as a rule in late-blossoming forms than in early-blossoming ones.

The optimum soil temperature for near-wilt is about 24° to 28° C. and it is thus about 5° higher than that for wilt. The disease develops readily, however, at temperatures as low as 20°; at 16° it is distinctly retarded. Air temperature has relatively little influence upon the disease.

In near-wilt-susceptible varieties there is little difference in the rate of wilting in dry and medium-moist soil, but it is consistently more rapid in moist soil. In the resistant varieties used the wilting is most rapid in medium-moist soil. The rate in all soils is sufficiently rapid, however, to indicate little retardation of the disease in dry seasons.