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Physiologic Specialization in Fusarium spp. Causing Headblight of Small Grains

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CONTENTS

PHYSIOLOGIC SPECIALIZATION IN FUSARIUM SPP. CAUSING HEADBLIGHT OF SMALL GRAINS [1]

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INTRODUCTION

The Fusarium headblight (scab) of wheat and other cereals, together with seedling blight caused by similar fungi, is an important factor in cereal growing in many countries of the world. In the United States, wheat scab causes heavy damage in the soft red winter wheat area and in the hard red spring wheat area. Altho the disease has been investigated extensively, no really satisfactory control measures have been devised. The development of resistant varieties apparently is the only adequate method of control. Very little is known, however, about the species of *Fusarium* which cause the disease and about the possible physiologic specialization within the different species. The present investigation was undertaken primarily to determine the relative virulence of the different species of *Fusarium* that cause headblight and to ascertain whether there are physiologic forms differing in pathogenicity and in their relation to environmental conditions. This knowledge is basic to a sound program of breeding disease-resistant varieties.

METHODS AND MATERIALS

Isolations were made primarily from blighted kernels of wheat and oats. The kernels were soaked in 70 per cent alcohol for two or three minutes and then burned over a free flame until slightly charred. The surface-sterilized kernel was then placed in a petri dish containing a suitable medium. Usually a colony of *Fusarium* grew sufficiently large in three or four days to be transferred to test tubes for stock cultures. Stock cultures were stored either in the ice box at 10° to 12° C. or at room temperature ranging from about 18° to 22° C. All the experiments were made with monosporous cultures. Sherbakoff's method of single-spore isolations was used exclusively (51, p. 104).

The sources of the different Fusaria used in the studies are listed in Table 1. Three cultures of *Fusarium graminearum* Schwabe (*Gibberella saubinetii* (Mont.) Sacc., three of *Fusarium culmorum* (W. G.

[1] Presented to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1929. The writer acknowledges his great indebtedness to Dr. E. C. Stakman, under whom the work has been done. He also wishes to thank Dr. C. D. Sherbakoff and Dr. H. W. Wollenweber for identifying and supplying cultures of *Fusarium*. He is also indebted to Dr. R. A. Gortner for furnishing laboratory facilities and other assistance in connection with the alcoholic fermentation studies. Dr. J. J. Christensen also has given valuable assistance.

Table I
Sources of Different Fusaria of Cereals Used in the Investigation

	Original host	Place of collection	Date of collection	Previous history
Fusarium graminearum Schwabe, form 1	wheat	Wisconsin	1924	Dr. L. R. Jones' culture furnished by Dr. Louise Dosdall
F. graminearum Schwabe, form 2[a]	wheat	Waseca, Minn.	Aug. 21, 1926	Material furnished by Dr. O. S. Aamodt
F. graminearum Schwabe, form 3[a]	oats	Purgatory Springs, Minn.	Aug. 10, 1926	Material furnished by Dr. J. J. Christensen
F. culmorum (W. G. Sm.) Sacc., form 1[b]	wheat	University Farm, St. Paul, Minn.	July 10, 1926	Material furnished by Dr. J. J. Christensen
F. culmorum (W. G. Sm.) Sacc., form 2[b]	wheat	University Farm, St. Paul, Minn.	Sept. 16, 1926	Material furnished by Dr. J. J. Christensen
F. culmorum (W. G. Sm.) Sacc., form 3[b]	wheat	Purgatory Springs, Minn.	Sept. 20, 1926	Material furnished by Dr. J. J. Christensen
F. avenaceum (Fr.) Sacc., form 1	wheat	Ithaca, N. Y.	Aug. 17, 1926	Material furnished by Dr. E. C. Stakman
F. avenaceum (Fr.) Sacc., form 2	......	....	....	Culture furnished by Dr. C. D. Sherbakoff
F. solani (Mart. p. Par.) Ap. et Wr.	......	....	....	Culture furnished by Dr. C. D. Sherbakoff
F. nivale (Fr.) Ces.	rye	Schlesien, Germany	Summer, 1925	Culture furnished by Dr. H. W. Wollenweber

[a] Cultures identified by Dr. C. D. Sherbakoff.
[b] Cultures identified by Dr. H. W. Wollenweber.

Sm.) Sacc., and two of *Fusarium avenaceum* (Fr.) Sacc. were found to comprise physiologic forms distinguishable on the basis of their pathogenicity. For convenience, these cultures are designated physiologic forms 1, 2, etc., as in the list. *Fusarium culmorum* form 1 mutated in the course of study and the resulting culture is designated as *F. culmorum* form 1 mutant. *F. solani* and *F. nivale* remained stable.

PATHOGENICITY AND PHYSIOLOGIC SPECIALIZATION

The fact that different cereal varieties differ in their degree of susceptibility to Fusarium headblight has long been recognized by investigators of the disease (5, 7, 8, 20, 21, 24, 40, 49, 50, 57).

As a result of extensive varietal tests carried on for eight years at University Farm, St. Paul, Minnesota, it was found that the amount of headblight in individual varieties of wheat varied from year to year and in different localities in the same year (20, 21). Christensen and Stakman (20) suggested that "there was also some circumstantial evidence that different parasitic strains may have been present in different years," in order to account for the different results obtained.

In studying the root rot of wheat, Henry (33, 34) found that different strains of *Fusarium moniliforme* Sheldon differed in virulence on wheat seedlings. Recently Greaney and Bailey (29) tested the pathogenicity of 28 cultures of unidentified Fusaria in the greenhouse and found that there were great differences in their degree of virulence on wheat seedlings.

In 1927 the writer made a comparative study of the pathogenicity of six cultures of *Fusarium:* three of *F. culmorum,* and three of *F. graminearum* (*Gibberella saubinetii*). The headblight phase of the disease was studied especially.

For all inoculation experiments, cultures were grown in Erlenmeyer flasks on a mixture of equal parts of sterilized wheat and oat seed. In most cases 25 gm. of seed and 50 cc. of water were placed in a 250-cc. flask and autoclaved for half an hour at 15 pounds pressure. In making head inoculations, a spore suspension was used, which was made by washing the culture with a definite amount of sterile water and then straining the washing through sterilized cheesecloth.

The seeds of cereals were obtained from A. C. Arny, Division of Agronomy and Plant Genetics, University of Minnesota. All the seeds used were pre-soaked in water from 8 to 12 hours and then treated with a 0.25 per cent solution of Semesan for 4 or 5 hours.

In the head-inoculation experiment in the field, seven varieties of wheat (Marquis, Minn. 1239; Preston, Minn. 188; Kota, C. I. 5878; Ceres, N. D. 1658; Glyndon Fife, Minn. 163; Pentad, C. I. 3322; Mindum, Minn. 470) and one variety of barley (Minsturdi, Minn. 439)

Table 2

Percentage of Headblight in One Variety of Barley and Seven Varieties of Wheat Grown in the Field at University Farm, St. Paul, Minnesota, in 1927 and Inoculated Artificially with Various Cultures of *Fusarium* spp.

Inoculum	Variety and percentage of head infection							
	Minsturdi barley	Marquis	Preston	Kota	Ceres	Glyndon	Pentad	Mindum
Fusarium culmorum, form 1	94.5±.92	5.5±.09	0.0±.00	0.5±.03	0.1±.02	0.0±.00	0.0±.00	1.0±.02
F. culmorum, form 2	61.4±.47	1.1±.02	0.0±.00	0.6±.02	3.9±.07	0.0±.00	3.6±.08	0.0±.00
F. culmorum, form 3	79.5±.61	14.3±.20	17.5±.11	16.2±.13	37.6±.21	4.8±.24	27.3±.23	3.0±.07
F. graminearum, form 1	78.6±.64	22.1±.34	23.4±.43	1.6±.05	44.3±.54	6.4±.37	34.9±.33	4.9±.09
F. graminearum, form 2	32.3±.27	0.2±.01	13.2±.09	0.3±.01	0.5±.07	0.0±.00	7.0±.15	1.9±.04
F. graminearum, form 3	18.7±.13	0.0±.00	0.2±.04	0.3±.04	0.2±.01	0.0±.00	1.3±.09	0.5±.02
Check	0.0±.00	0.0±.00	0.0±.00	0.0±.00	0.0±.00	0.0±.00	0.0±.00	0.0±.00

were used as differential hosts. The eight differential hosts were grown side by side in three 7-foot rows and replicated twice in each plot. The seven plots were systematically distributed in the field in order to reduce the danger of contamination. Each plot was sprayed with a spore suspension of a different culture. The first spraying was made at flowering time, and four subsequent sprayings were made at intervals of three or four days, while the check plot was sprayed with sterile water. Spraying was usually done in the evening, when the temperature was relatively low. The results are summarized in Table 2.

On the basis of the degree of infection produced by the different cultures, three physiologic forms of *F. culmorum* and three of *F. graminearum* were distinguished. It is interesting to note that headblight in Minsturdi barley ranged from 18.7 per cent, when *F. graminearum* form 3 was used as inoculum, to 94.5 per cent when *F. culmorum* form 1 was used. *Fusarium graminearum* form 1 and *F. culmorum* form 3, producing 78.6 and 79.5 per cent of headblight, respectively, ranked next in virulence on barley; while form 2 of *F. culmorum* and form 2 of *F. graminearum* both were still less virulent. The former produced 61.4 per cent infection, while the latter produced only 32.3 per cent. The percentage of infection was never so high on wheat as on barley.

On all varieties of wheat except Kota, *F. graminearum* form 1 produced consistently higher percentages of headblight than any of the other cultures. On Kota wheat, *F. culmorum* form 3 produced 16.2 per cent of headblight as compared with 1.6 per cent produced by *F. graminearum* form 1. *Fusarium culmorum* form 3 was the next most virulent form on wheat.

The rather low percentage of headblight on the wheat varieties inoculated may have been due to the hot, dry weather which followed the flowering period.

In 1928 a similar but more extensive head-inoculation experiment was made. In addition to the cultures used in the previous years, five more were tested, including *F. nivale* (*Calonectria graminicola*), *F. solani,* two physiologic forms of *F. avenaceum,* and *F. culmorum* form 1 mutant. As differential hosts, two varieties of barley (Minsturdi, Minn. 439; and Glabron, Minn. 445); seven varieties of wheat (Marquis, Minn. 1239; Preston, Minn. 188; Kota, C. I. 5878; Ceres, N. D. 1658; Haynes Bluestem, Minn. 169; Mindum, Minn. 470; and Akrona, C. I. 6891) and two varieties of oats (Victory, Minn. 564; and Anthony, Minn. 686) were used. The differential hosts were grown side by side in rod rows in each plot, and the plots were systematically replicated twice. The plants were inoculated in the same manner as those grown the previous year. The results are summarized in Table 3.

Table 3

Percentage of Headblight in Two Varieties of Barley, Seven Varieties of Wheat, and Two Varieties of Oats Grown in the Field at University Farm, St. Paul, Minnesota, in 1928, and Inoculated Artificially with Eleven Different Cultures of *Fusarium*

Inoculum	Variety and percentage of infection										
	Barley		Wheat							Oats	
	Minsturdi	Glabron	Marquis	Preston	Kota	Ceres	Haynes bluestem	Mindum	Akrona	Victory	Anthony
Fusarium culmorum form 1	87.4±.96	89.3±.99	45.5±.71	6.5±.13	42.6±.33	22.4±.35	21.1±.38	48.0±.45	62.3±.45	64.1±.71	62.1±.87
F. culmorum form 1—mutant	28.8±.33	56.1±.76	10.6±.33	14.2±.20	4.1±.09	7.6±.14	4.5±.12	18.5±.31	8.0±.25	46.2±.68	43.0±.48
F. culmorum form 2	42.4±.53	63.3±.71	29.5±.37	26.2±.21	14.0±.13	28.0±.11	16.6±.20	15.1±.20	27.5±.30	40.1±.81	39.2±.52
F. culmorum form 3	85.5±.40	74.8±.95	26.2±.28	41.5±.47	58.1±.27	47.6±.27	47.5±.44	63.5±.55	76.5±.98	70.1±.98	68.2±.79
F. avenaceum form 1	39.6±.47	47.8±.82	17.0±.20	3.1±.07	8.3±.13	7.6±.20	21.0±.53	27.2±.35	26.5±.45	37.0±.76	51.5±.81
F. avenaceum form 2	49.0±.67	60.0±.74	31.0±.41	18.2±.11	25.4±.26	31.0±.44	32.5±.21	15.4±.21	16.1±.47	47.2±.58	53.3±.49
F. solani	39.5±.43	61.6±.70	19.5±.34	10.3±.11	13.5±.16	14.1±.21	17.6±.20	12.2±.17	20.1±.63	27.3±.63	46.1±.32
F. graminearum form 1	84.4±.54	88.8±.99	82.1±.99	23.1±.20	46.0±.44	51.6±.61	24.5±.33	57.3±.27	57.5±.44	49.1±.37	58.2±.43
F. graminearum form 2	40.8±.47	50.7±.76	46.1±.76	6.5±.26	30.5±.57	21.3±.44	15.6±.45	28.5±.30	54.0±.47	32.4±.42	56.2±.73
F. graminearum form 3	32.6±.33	44.1±.50	15.6±.34	13.3±.23	9.1±.11	9.6±.15	8.5±.13	16.0±.33	27.5±.37	78.0±.77	80.2±.91
F. nivale	48.2±.44	62.4±.78	25.1±.44	18.1±.14	29.1±.13	15.1±.13	24.1±.12	9.0±.15	25.3±.33	42.1±.54	54.2±.37
Check	4.1±.15	5.6±.11	3.2±.11	2.1±.13	3.4±.09	3.0±.09	1.5±.08	2.9±.11	2.0±.15	3.2±.20	4.1±.17

The experiment of 1928 confirmed the results obtained in 1927. From Table 3 it is evident that the relative virulence of the physiologic forms of both *F. culmorum* and *F. graminearum* on barley and wheat was virtually the same at that in 1927, except that the percentages of infection on wheat were generally higher in 1928 than in 1927.

It seemed apparent that the relatively successful infection of wheat may have been due to the cloudy and rainy weather following the flowering period in 1928. The ability of different Fusaria to infect oats varied. *Fusarium graminearum* form 3 was the most virulent form on oats, producing 78.0 per cent on Victory and 80.2 per cent on Anthony; it was less virulent on barley and weak on most of the wheats. *Fusarium culmorum* forms 3 and 1 produced nearly as much infection, while *F. graminearum* form 2 was not so virulent on Victory, and *F. culmorum* form 2 least virulent on Anthony.

The two physiologic forms of *F. avenaceum* differed in their pathogenicity, altho they were weak in comparison with other virulent cultures. On barley and oats, and on wheat except the durums, form 2 was consistently more virulent than form 1. The form 1 produced 27.2 per cent infection on Mindum and 26.5 per cent on Akrona, while the form 2 produced only 15.4 and 16.1 per cent, respectively.

Fusarium solani and *F. nivale* were intermediate in their virulence.

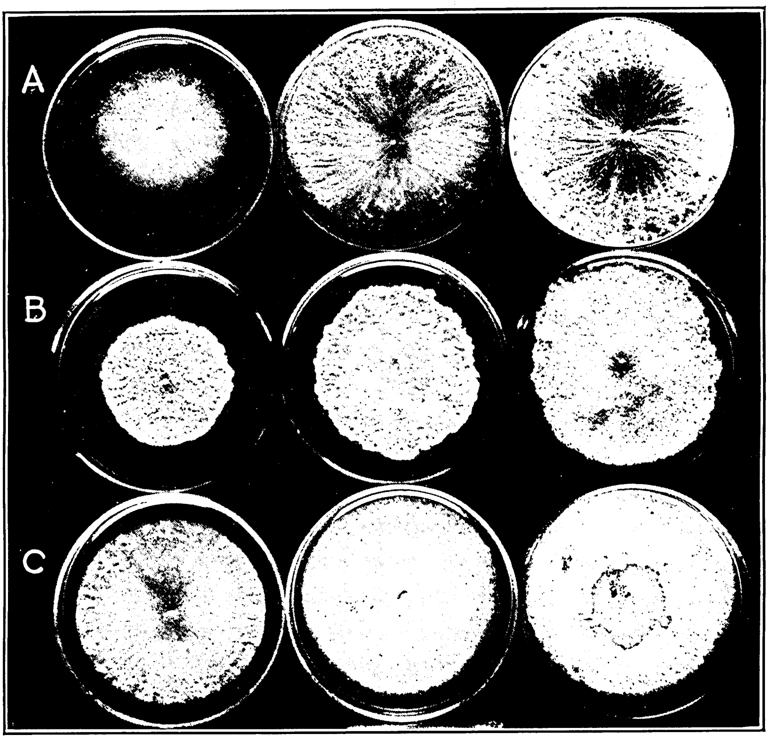
It seemed evident from these experiments that several species of *Fusarium* could cause headblight in small grains and that some of them comprised physiologic forms. As the development of headblight in the field is known to depend somewhat on temperature, it was decided to determine the effect of this factor on the development of different forms.

EFFECT OF TEMPERATURE ON GROWTH

The effect of temperature on the vegetative growth of species of *Fusarium* has been studied by various workers (12, 28, 36, 54, 55). In general, it was found that these organisms have a wide range of temperature adaptations and usually develop best at high temperatures. Wollenweber (56) also made the generalization that root-invading Fusaria are warm-soil organisms.

In studying the effect of temperature on the mycelial growth of *Gibberella saubinetii* (*F. graminearum*), MacInnes and Fogelman (40) found that the organism has the cardinal temperatures of 3°, 25-27°, and 33°C.; and Dickson (23) found that the cardinal temperatures of the organism are 3-4°, 24-28°, and 32-34° C.

However, different species of *Fusarium* attacking cereals may have different temperature requirements, as suspected by Atanasoff (8). Thus a comparative study of the effect of temperature on various Fusaria was undertaken by the author. In making the experiment, petri dishes of uniform size were poured with uniform amounts of Difco potato-dextrose agar, usually about 18 cc. Bits of mycelium of the cultures to be tested were transferred to the center of the plate. The plates were run in duplicate and were incubated at room temperature for 12 hours before being subjected to the various constant temperatures. For five days the diameter of each colony was measured daily. The averages of the three experiments for the fifth day are summarized in Table 4 and Figures 1, 2, and 3.



17° C. 22° C. 27° C.

Fig. 1. Effect of Temperature on the Growth of Two Physiologic Forms and the Mutant of *Fusarium culmorum* on Difco Potato-Dextrose Agar

A. *F. culmorum* form 3
B. *F. culmorum* form 1
C. Mutant of *F. culmorum* form 1.
Picture taken five days after inoculation.

It was found that some of the Fusaria had different temperature adaptations, altho most of them had a temperature similar to that of *F. graminearum*. Two interesting cases were *F. solani* and *F. nivale* (*Calonectria graminicola*). The optimum temperature of the former was about 32° C.; the latter had an optimum of about 22° C. and did not grow at all at 32° C. (see Fig. 2). It was also interesting to note

Table 4
Effect of Temperature on Growth of Various Fusaria

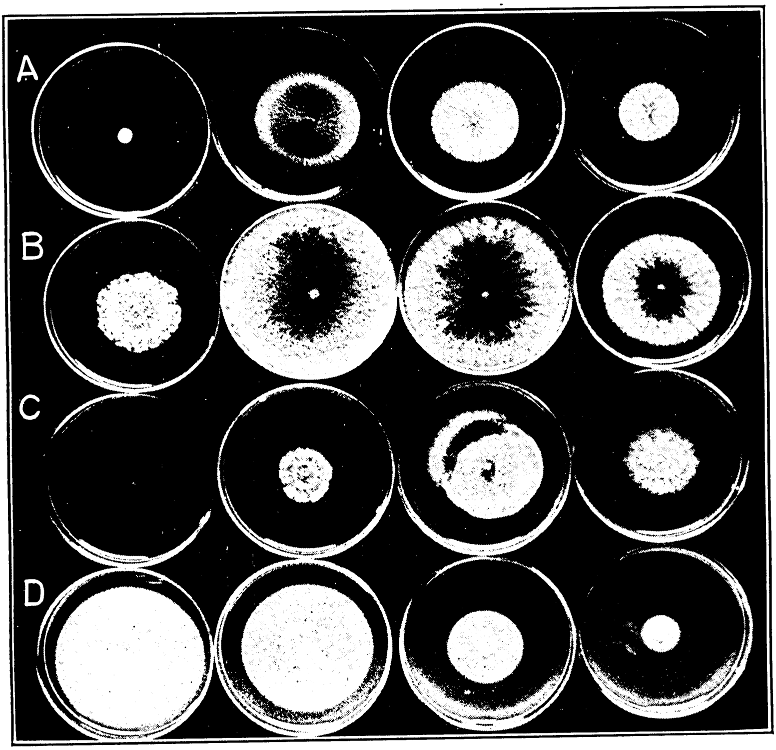
Fungus	Temperature in degrees C.						
	7	12	17	22	27	32	37
	Growth in millimeters 5 days after inoculation						
Fusarium culmorum, form 1	14.5±.07	35.0±.09	45.1±.21	57.3±.53	64.5±.47	34.0±.27	....
F. culmorum, form 1—mutant	11.0±.06	34.7±.14	47.2±.33	84.5±.61	86.5±.41	32.5±.33	....
F. culmorum, form 2	15.0±.13	42.1±.13	45.7±.30	59.4±.47	68.3±.53	32.5±.34	....
F. culmorum, form 3	14.2±.09	40.3±.13	59.5±.40	84.1±.54	89.9±.61	22.3±.20	....
F. avenaceum, form 1	13.2±.20	35.7±.11	45.6±.35	60.5±.37	42.3±.51	14.1±.11	....
F. avenaceum, form 2	12.0±.13	23.5±.09	28.7±.27	40.8±.27	49.9±.44	19.5±.09	....
F. solani	8.1±.09	10.6±.06	18.5±.20	34.2±.26	64.7±.32	71.8±.34	37.5±.54
F. graminearum, form 1	15.2±.11	37.0±.12	55.3±.35	83.1±.44	89.2±.62	41.5±.20	....
F. graminearum, form 2	15.4±.13	25.6±.08	42.3±.80	89.1±.47	98.9±.84	35.7±.19	....
F. graminearum, form 3	12.5±.21	35.2±.09	40.5±.37	52.8±.26	67.5±.56	23.1±.25	....
F. nivale	14.3±.06	31.4±.11	34.7±.13	48.5±.20	25.3±.34	....	....

that the two forms of *F. avenaceum* differed in their temperature requirements: form 1 grew best at 22° C. while form 2 grew best at 27° C., as was the case with most of the Fusaria studied.

It is evident that the different Fusaria might develop differently in seasons of different temperatures, and this easily could account for the marked differences in the amount of scab in different years.

ALCOHOLIC FERMENTATION

According to Guilliermond and Tanner (30), the idea that alcoholic fermentation is caused by living organisms originated with Linne. However, it was in 1859 that Pasteur first established experimentally that the alcoholic fermentation is correlated with the life of yeasts. Since then it has been shown by many workers that numerous fungi



32° C. 27° C. 22° C. 17° C.

Fig. 2. Effect of Temperature on the Growth of Four Species of *Fusarium* on Difco Potato-Dextrose Agar

A. *F. avenaceum* form 2
B. *F. graminearum* form 1
C. *F. nivale*
D. *Fusarium solani*
Picture taken five days after inoculation.

and bacteria have the power of fermenting various sugars, cellulose, and other starchy substances (26, 27, 31, 37, 41, 44, 45, 47).

In 1921 Harter and Weimer (32) found that among other sweet potato rotting organisms, *Fusarium acuminatum* can ferment glucose, with the production of alcohol and carbon dioxide as the two main end products.

In the study of the metabolism of *Fusarium lini* Bolley, Anderson (2) showed that ethyl alcohol and carbon dioxide were formed as the two main reaction products of the organism. He also found that the maximum percentage of alcohol produced, when a 10 per cent solution of glucose was used as a substrate, was 3.44 per cent by weight. Since this was practically the same percentage that is toxic to the fungus, Anderson suggested that the organism produced alcohol up to a point where it became toxic, then utilized the alcohol produced as food. Letcher and Willaman (39) corroborated Anderson's results and further showed that the virulence of the various physiologic forms of *F. lini* on flax plants was correlated with the amount of alcohol produced.

It seemed desirable, therefore, to study the metabolism of the various Fusaria used in the present investigation in order to ascertain whether there was a correlation between the fermentation capacity of Fusaria and their virulence. Accordingly, a comparative study was made of alcoholic production by three physiologic forms of *F. culmorum,* three of *F. graminearum,* two of *F. avenaceum,* and the mutant of *F. culmorum* form 1. To determine the difference in alcohol production among the nine cultures of *Fusarium,* a commercial brand (Aunt Dinah) of light New Orleans molasses was used as the substrate. This molasses was diluted with distilled water until the desired Brix (9.9-10.7) was obtained. One hundred cc. of the medium was used in 250-cc. Erlenmeyer flasks and autoclaved at 15 pounds pressure for 25 to 30 minutes. Ten flasks for each culture of *Fusarium* were prepared each time. A small bit of young, growing mycelium of the different Fusaria to be studied was used as inoculum. The flasks were then incubated from 10 to 50 days in a room at temperatures varying from 22 to 27° C. Every 10 days two flasks of each culture of *Fusarium* were analyzed for alcohol production.

The following method of ascertaining the percentage of alcohol was followed (39). The mycelium was filtered off from the medium by means of cheesecloth, and the mycelium was pressed as dry as possible to remove any adhering liquid. To the filtrate was added about 50 cc. of distilled water, and the whole mixture was then placed in a 500-cc. distillate flask connected to an ordinary straight-tube condenser. The first 100 cc. of the distillate was collected for alcohol determination.

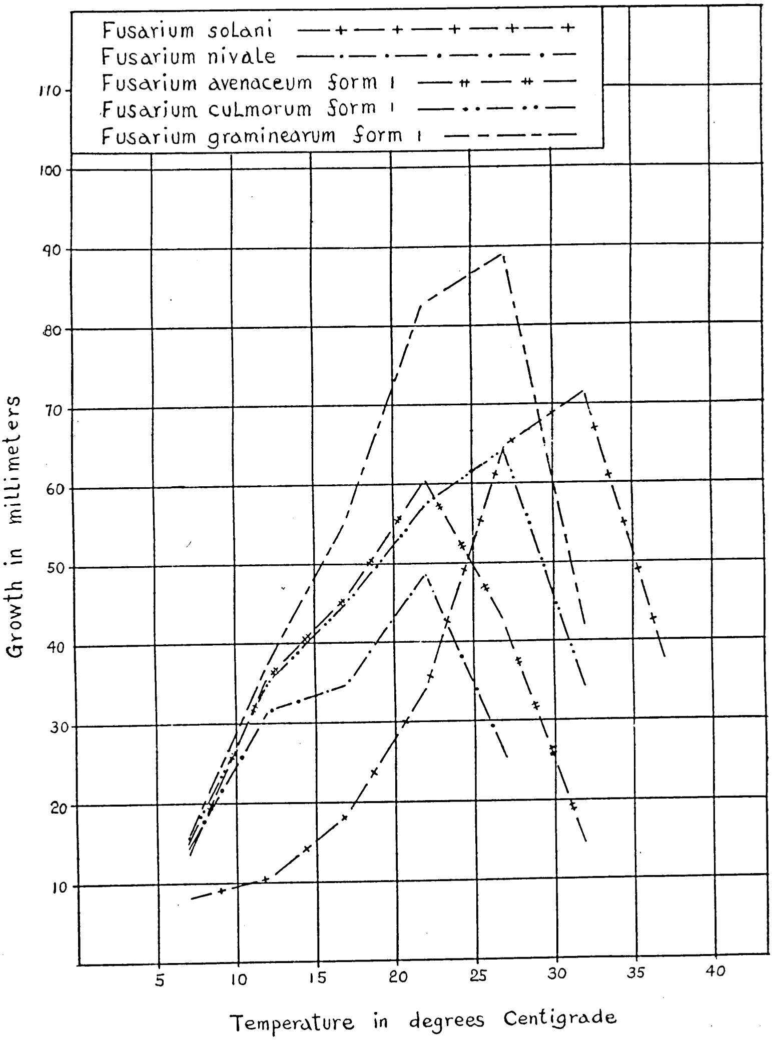


Fig. 3. Effect of Temperature on Growth of Five Species of *Fusarium* on Difco Potato-Dextrose Agar

The percentage of alcohol produced was obtained by the specific gravity method. The specific gravity of the distillate was determined by means of Chainomatic specific gravity balance. The plummet of the instrument was standardized for 20° C. and the balance was always adjusted to read 1.000 by using a sample of freshly distilled water. All readings were taken at 20° C.

For calculating the percentage of alcohol in the distillate, the specific gravity was converted by referring to standard tables (35). The results of four experiments are summarized in Table 5 and Figure 4.

From Table 5 it is apparent that the nine cultures of *Fusarium* studied differed in their ability to ferment molasses. The percentage of alcohol produced ranged from 1.8 in case of *F. graminearum* form 2 to 4.5 in *F. culmorum* form 1 mutant. The rate of fermentation was also different among the various cultures of *Fusarium*.

Fusarium culmorum forms 1, 2, and mutant reached the maximum production in about 30 days, while *F. culmorum* form 3, *F. graminearum* forms 1, 2, and 3, and *F. avenaceum* forms 1 and 2 required about 40 days for maximum alcohol production.

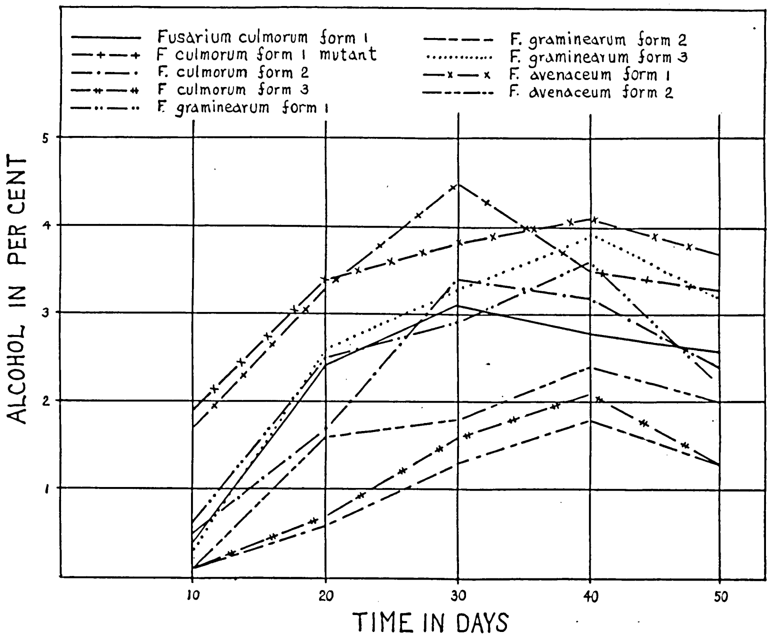


Fig. 4. Rate of Ethyl Alcohol Production of the Nine Cultures of *Fusarium* on Molasses

Table 5
Rate of Ethyl Alcohol Production of the Nine Cultures of *Fusarium* on Molasses

Fungus	Time in days				
	10	20	30	40	50
	Alcohol in per cent				
Fusarium culmorum, form 1........	0.4±.07	2.4±.13	3.1±.21	2.8±.09	2.6±.08
F. culmorum, form 1—mutant.......	1.7±.06	3.3±.14	4.5±.11	3.5±.05	3.3±.07
F. culmorum, form 2	0.5±.02	1.7±.07	3.4±.09	3.2±.05	2.4±.08
F. culmorum, form 3	0.1±.01	0.7±.05	1.6±.05	2.1±.02	1.3±.04
F. graminearum, form 1...........	0.6±.04	2.5±.05	2.9±.04	3.6±.03	2.2±.03
F. graminearum, form 2...........	0.1±.01	0.6±.04	1.3±.09	1.8±.07	1.3±.05
F. graminearum, form 3...........	0.3±.04	2.6±.12	3.3±.15	3.9±.08	3.2±.10
F. avenaceum, form 1..............	1.9±.09	3.4±.10	3.8±.09	4.1±.14	3.7±.12
F. avenaceum, form 2..............	0.1±.02	1.6±.11	1.8±.05	2.4±.09	2.0±.09

In order to determine whether there was a correlation between the amount of alcohol produced and the virulence of the various cultures of *Fusarium* on the varieties of small grains studied, a number of correlation coefficients were calculated. The percentage of headblight in the varieties of small grains in the summer of 1928 was used.

Table 6

Correlation Coefficients, Measuring the Relationship Between the Alcohol Production Capacity of the Nine Cultures of *Fusarium* and Their Ability to Cause Headblight of Two Varieties of Barley, Seven Varieties of Wheat, and Two Varieties of Oats Grown at University Farm, St. Paul, Minnesota, in 1928

Host	$r \pm E_r$	r / E_r
Minsturdi	−.2739±.1633	1.6773
Glabron	−.0014±.2112	0.0066
Marquis	−.1389±.1936	0.7174
Preston	−.0099±.2226	0.0445
Kota	−.0391±.2161	0.1809
Ceres	−.1791±.1848	0.9692
Haynes Bluestem	−.4133±.1319	3.1334
Akrona	−.6640±.0755	8.7947
Mindum	−.4454±.1247	3.5718
Victory	+.1825±.1838	0.9929
Anthony	−.1603±.1888	0.8490

All correlation coefficients were small and negative except one. There was probably only one significant negative correlation in light of its probable error. Thus we may conclude that there is no positive correlation between the alcohol production and percentage of headblight produced by the nine cultures of *Fusarium* studied.

MUTATION

Mutation is quite a common phenomenon in most of the fungi which have been studied thoroly in this respect (9, 10, 16, 53). In the genus *Fusarium,* mutation also occurs. Appel and Wollenweber (3) described certain results which indicated that mutation was taking place, altho the authors apparently did not recognize it as such. Later, Sherbakoff (51) probably obtained doubtful cases of mutation in his studies of Fusaria of potatoes. Recently Brown (14) found that saltation was quite a frequent phenomenon in various strains of *Fusarium fructigenum* Fr. on concentrated Richards solution agar.

The present writer found that in the course of temperature studies, a colony of *F. culmorum* form 1 produced a light, salmon-orange, wedge-shaped sector in the carmine red parent colony at the temperature of 27° C. (Fig. 5). In order to ascertain the constancy of the change, the apparent mutant culture was further studied, together with the parent.

Constancy of the Mutant

The mutant and the parent culture were grown side by side on Difco potato-dextrose agar for about 14 months under practically the same environmental conditions.

They were grown also at various constant temperatures ranging from 7° to 32° C., at 4-degree intervals, as indicated previously (Table 4 and Fig. 1). The mutant was consistently different from the parent. The cultural characteristics of *F. culmorum* form 1 and its mutant are tabulated in Table 7.

Fig. 5. The Mutant of *Fusarium culmorum* Form 1
Left, Top View; Right, Bottom View

From Table 7 it is evident that the mutant differed from its parent in type and rate of growth of the mycelium, in color production, and in type of fruiting when grown under practically the same environmental conditions. The parent and the mutant were also consistently different at various constant temperatures (Fig. 5). Furthermore, the mutant differed from the parent in alcoholic fermentation capacity on molasses (Table 5). The former produced 4.5 per cent in 30 days, while the latter produced only 3.1 per cent.

Table 7

Cultural Characteristics of *Fusarium culmorum* Form 1 and Its Mutant, Grown on Difco Potato-Dextrose Agar

Fungus	Type of mycelial growth	Amount of aerial mycelium	Growth in mm. in 5 days	Color of mycelium	Color of medium	Type of fruiting
Fusarium culmorum form 1	aerial	abundant	64.5±.47	carmine red at base; pale yellow at top	carmine red	sporodochia
Fusarium culmorum form 1 mutant	submerged	very scanty	86.5±.41	light salmon-orange	colorless	pseudo-pionnote

The mutant seemed to be culturally and physiologically different from its parent. The next problem was to ascertain whether the mutant differed from its parent in pathogenicity.

Pathogenicity of the Mutant

In addition to the morphologic and cultural differences, mutants have been shown to differ from their parents in pathogenicity, also. In the study of physiologic specialization and mutation in *Helminthosporium sativum,* Christensen (16, 17) found that some mutants differed from their parents not only in morphologic characters but also in pathogenicity. He stated that "some are more virulent than the parents, others are less so." Muller (43) found that, in general, mutants of *Gloeosporium fructigenum* were less virulent than their parents. Leonian (38) also observed that *Phytophthora omnivora* mutants were less virulent than their parents on pepper plants. In their work on physiologic specialization and mutation of *Ustilago zeae,* Christensen and Stakman (19) also concluded that "the mutants differ from their parents not only in general appearance on culture media but also in pathogenicity."

In order to determine the pathogenicity of *F. culmorum* form 1 mutant, its ability to cause headblight of cereals was studied in comparison with that of the parent culture in the summer of 1928, as mentioned previously. The results are summarized in Table 3. It was found that the mutant was decidedly less virulent on almost all the varieties of cereals studied. For instance, the parent produced 87.4 per cent of headblight on Minsturdi barley while only 28.8 per cent was produced by the mutant.

Since it was shown that, in addition to the cultural and physiological differences, the mutant differed decidedly from the parent in pathogenicity, it seemed important to study the influence of the various environmental factors upon the frequency of mutation of the various Fusaria used in the present investigation.

Effect of Environmental Factors on Mutation

Many mutations have been reported to be induced in cultures by various environmental factors, such as toxic substances and extremes of temperature. Arcichovskij (4) secured mutation in *Aspergillus niger* by the addition of 0.0001 N zinc sulphate to the culture solution. Schieman (48) also obtained mutation in *Aspergillus niger* by the addition to the culture medium of different concentrations of potassium dichromate. Mutations also were secured by subjecting the cultures to high temperatures, ranging from 40° to 48° C. According to Brierley (10), Waterman reported similar induced mutations in both *Aspergillus niger* and *Penicillium glaucum.* However, Brierley was unable to substantiate their results. Christensen (16, 17) showed that certain media were more favorable than others to mutation of certain physiologic forms of *Helminthosporium sativum.* Brown (13) found that saltation in *Fusarium fructigenum* Fr. occurred more frequently on concentrated Richards solution agar than on several other media. Recently Christensen (18) also observed that high temperatures (25° to 27° C.) favored mutations in *Helminthosporium sativum.*

A series of experiments was tried by the writer to determine the effect of various environmental factors upon the frequency of mutation of the various Fusaria in this study. In the study of the induced effect of toxic substances on frequency of mutation, zinc sulphate, potassium dichromate, copper sulphate, gentian violet, methyl blue, and eosin were added in various concentrations to the culture media. The media used were Difco potato-dextrose agar and Richards solution agar (13, 46). All the cultures were incubated at a temperature varying from 25°to 27° C. The toxic substances used and their concentrations are listed in Table 8.

In addition to the study of the action of toxic substances, the effect of temperature was studied. Again Difco potato-dextrose agar and Richards solution agar were used as the culture media. The cultures were incubated at constant temperatures of 12°, 27°, and 32° C., respectively.

Table 8

Toxic Substances and Their Concentrations Used in Mutation Studies

Toxic substances	Concentrations
Zinc sulphate	1-1,000
	1-2,000
Potassium dichromate	do
Copper sulphate	1- 500
	1-1,000
	1-1,500
Copper carbonate	do
Gentian violet	1-25,000
	1-30,000
	1-40,000
Methyl blue	do
Eosin	do

Besides Difco potato-dextrose agar and Richards solution agar used in the various studies as indicated above, Coons synthetic medium (22), oatmeal agar, cornmeal agar, and plain water agar also were tried.

Thus far, no mutation has been observed. Only retardation of growth, reduction in the amount of growth, and other temporary abnormal variations were observed. We may conclude that the cultures of *Fusarium* in the present investigation were rather stable and resistant to the stimuli of the various environmental factors.

More extensive studies must be made before final conclusions can be drawn.

MODE OF INFECTION

The mode of head infection is a question in dispute. Arthur (6) reported that scab in wheat heads "is due to a minute fungus that attacks the wheat heads at flowering time. The spores of the fungus blow through the air, lodge on the delicate parts inside the flower and soon penetrate the kernel and envelop it with a mesh of moldy filaments which sap the life of the kernel and forming new spores spread the disease to other flowers throughout the field." Adams (1), studying the pathologic histology of the Fusarium-blighted kernels, thought that infection took place first within the developing embryo and then spread to the storage or endosperm tissue. He also maintained that "a study of infected kernels from various parts of the state collected at the time of harvesting indicated that the prevalent type of infection occurred after the flowering stage." Dickson (24, 25) maintained that the scab organism usually first infected the extruded anthers of the flowers and that the percentage of scab depended upon the number of anthers which remained within the glumes. Promising results have been obtained by him by selecting individual plants from varieties having a marked freedom from "open" anthers. On the other hand, Atanasoff (8) observed that in a large number of cases the initial infection is by "contact" rather than through the anther, viz., "there where the first contact between host and pathogene is established is also the point of entrance for the fungus. This had been repeatedly observed on barley kernels on which the point of infection, a brownish spot, is always visible." The writer's observations seemed to agree with the view of Atanasoff. It was a common observation in the field that the initial point of infection on Minsturdi barley and Marquis wheat was always marked by a brownish, chlorotic spot on the glume.

In order to ascertain the method of infection, head inoculation experiments were made under controlled conditions in the greenhouse in the winter of 1927. Marquis and Mindum wheats and Minsturdi barley were used in the studies. The plants were grown usually in 6-inch, less frequently in 4-inch, pots. Shive's solution was added from time to time in order to insure a good growth of the plants (52). The head was inoculated with a spore suspension of *Fusarium graminearum* form 1. The inoculated head was then wrapped with moist cotton and covered with a glassine bag. The cover was left on for 4 to 5 days. The results of head inoculation are summarized in Table 9.

In the winter of 1928 the mode of infection was again studied. The method of inoculation was so modified that the development of infection could be observed readily. The outer glumes were inoculated with a small loopful of a spore suspension of *F. graminearum* form 1. The head was then covered by a large inverted test tube which was supported on a long glass rod. The head was loosely fastened in position to the rod. The opening of the test tube was plugged with sterile cotton. A small vial of sterile water was placed inside the tube to insure a uniformly saturated atmosphere. Marquis wheat and Minsturdi and Glabron barleys were the varieties studied. The results are summarized in Table 10.

Table 9

Effect of Inoculating Heads of Marquis and Mindum Wheats and Minsturdi Barley with *Fusarium graminearum* Form 1 in the Greenhouse in the Winter of 1927

Variety	Time of inoculation	Plant parts inoculated	No. of heads inoculated	No. of heads infected
Marquis	Head in sheath	sheath	17	16
Marquis	Head just out of sheath; before flowering	glume	5	5
Marquis	Kernels in milk stage; anther freed	glume	11	10
Marquis	Kernels in soft dough; anther freed	glume	4	3
Mindum	Head just out of sheath; before flowering	glume	5	5
Mindum	Kernels in milk stage; anther freed	glume	3	3
Mindum	Kernels in soft dough; anther freed	glume	9	8
Minsturdi	Head in sheath	sheath	7	7
Minsturdi	Head just out of sheath; before blossoming	glume	5	5
Minsturdi	Kernels in milk stage; anther freed	glume	13	12
Minsturdi	Kernels in soft dough; anther freed	glume	12	12

From Tables 9 and 10 it is apparent that heads become infected from the pre-flowering stage until the soft dough stage. It was also noticed that the initial point of infection was usually apparent as a brownish chlorotic spot on the glumes. When the inoculations were made before flowering time, the initial infection appeared before the anthers were extruded. Furthermore, in most cases no mycelium was found in the anther right after the appearance of the initial infection on the infected spikelet. In a few cases, however, the mycelium grew over the anthers a few days after the initial infection was noticed.

From the field observations and results of controlled experiments in the greenhouse, it may be concluded that the flower or the anther is not necessarily the court of initial infection. On the other hand, the glume seems to be a more common place for the entrance of the pathogene. The mycelium grows over the anthers in some cases, but that does not necessarily mean that initial infection takes place through the anther.

Table 10

Effect of Inoculating Heads of Marquis Wheat and Minsturdi and Glabron Barleys with *Fusarium graminearum* Form 1 in the Greenhouse in the Winter of 1928

Variety	Time of inoculation	Plant part inoculated	No. of heads inoculated	No. of heads infected	Time of first infection, days
Marquis	Head just out of sheath; before flowering	glume	17	16	6-8
Marquis	Kernels in milk stage; anther freed	glume	5	5	5-7
Marquis	Kernels in soft dough; anther freed	glume	5	4	6-7
Minsturdi	Heads just out of sheath; before flowering	glume	8	8	7-8
Glabron	Heads just out of sheath; before flowering	glume	5	5	5-8
Glabron	Kernels in soft dough; anther freed	glume	6	6	5-9
H 44	Head just out of sheath; before flowering	glume	6	1	10

In Marquis wheat the fungus seemed to travel down from the initial point of infection to other parts of the head, apparently by way of the rachis. Sometimes it traveled down to the neck, or peduncle, of the head, as far as 2 to 3 cm. in about two weeks.

However, detailed histological studies and more extensive field tests are highly desirable in order to clarify the question of mode of infection.

DISCUSSION AND CONCLUSIONS

As a result of the preliminary study of ten cultures of *Fusarium* causing headblight of cereals, experimental evidence proves that there are 3 physiologic forms of *Fusarium culmorum,* 3 of *F. graminearum,* and 2 of *F. avenaceum,* which are differentiated chiefly by their pathogenicity on different varieties of cereals. There also were differences in pathogenicity among the different species of *Fusarium* studied. Furthermore, it was shown that different Fusaria may have different optimum temperatures for growth. From the results thus far obtained it is quite possible that different physiologic forms, or even different species, of *Fusarium* might predominate in different years on account of differences in weather conditions. If such were the case, the conflicting results encountered in breeding work might be explained to some extent. The effect of environment on the development of the host plant and its resistance to Fusarium attack probably is important also.

The abilities of the different physiologic forms of the species of *Fusarium* to ferment sugar also were different. However, no positive correlation was found between the amount of alcohol production and the percentage of headblight caused by the different cultures of *Fusarium,* as Letcher and Willaman (39) found in case of the various physiologic forms of *Fusarium lini.*

The application of the term "mutation" in lower plants such as fungi and bacteria has caused considerable dispute. Brierley (11) objected to the use of the term in fungi and bacteria as, in his opinion, the criteria of purity that are used in the study of genetics of higher plants can not as yet be applied to these lower organisms. He considered that cases of so-called "mutation" are simply segregations from the parent. Morishima (42) thought that the term should be used only with higher plants and should not be introduced into bacteriology. He advised "to leave the term mutation to the botanist and for the present at least, to speak of atypical varieties of bacteria or simply variants." Stevens (53) used the term "saltation" with a new meaning to cover variations in non-sexual generations of fungi. Caldis and Coons (15) preferred to use the term "variant" to cover the "rather semi-permanent variations which are different from the parent form somatically rather than genetically," as they thought. On the other hand, Blakeslee (9) stated that "it became evident later that mutations could not be confined to cells associated with sexual reproduction, but as shown by the somatic mutations involved in bud sports in plants and in similar less common phenomena in animals they may occur in cells in which sexual processes are not involved. They have been found in lowly organized plants and animals in which non-sexual reproduction is the rule or in which sexual reproduction is not known to occur." Brown (13) also asserted that "it is obvious that this word (mutation) has been used by mycologists and bacteriologists to crystallize wheat is the salient feature of these changes occurring in culture, viz., that they are sudden changes which are neither the result of a process of gradual acclimatization or 'education,' nor of a mere sorting out of one strain from what was originally a mechanical mixture, used in this sense and this is the only sense in which the term mutation can be applied yet in microbiology."

In accordance with the general usage in mycology, the term "mutation" was retained in the present investigation in order to avoid further confusion.

Fusarium culmorum form I mutated on an artificial culture medium. The mutant was not only different from the parent in cultural characteristics but also in pathogenicity. Thus the occurrence of mutation may complicate the problem further, for it may alter the pathogenicity considerably. However, the phenomenon of mutation was rare in the various cultures of *Fusarium* used in the present investigation. Induced mutation with the aid of stimuli of various environmental factors, such as toxic substances and higher temperatures, was not observed.

The statement of Dickson (24) that the susceptibility of wheat plants to headblight depends upon the number of anthers which remain within the glumes, was not substantiated in this work. Field observations and controlled experimental evidence in the greenhouse both indicate that the initial infection is frequently through the glume. Furthermore, it is clearly shown that there are wide variations of susceptibility within the same variety of cereal to the different Fusaria under practically the same environmental conditions. It is probable, then, that Dickson's method of obtaining a resistant variety is not quite feasible.

SUMMARY

1. A preliminary, comparative study was made of ten different Fusaria attacking cereals.
2. Experimental evidence proves that there are three physiologic forms of *Fusarium graminearum* (*Gibberella saubinetii*), three of *F. culmorum*, and two of *F. avenaceum* that can be differentiated by their parasitism on varieties of cereals. The two physiologic forms of *F. avenaceum* also have different temperature requirements.
3. There also are differences in pathogenicity in the five species of *Fusarium* studied.
4. The optimum temperature for growth of *Fusarium avenaceum* form 2, three physiologic forms of *Fusarium graminearum* (*Gibberella saubinetii*), and three of *Fusarium culmorum* and its mutant on Difco potato-dextrose agar is about 27° C. *Fusarium avenaceum* form 1 and *Fusarium nivale* (*Calonectria graminicola*) have an optimum of about 22° C., and *Fusarium solani*, an optimum of about 32° C.
5. The physiologic forms of *F. graminearum*, *F. culmorum*, and *F. avenaceum* differ also in capacity for alcoholic fermentation.
6. *Fusarium culmorum* form 1 mutated on culture media. The mutant differed from the parent culturally and pathogenically.
7. Circumstantial and experimental evidence both indicate that initial head infection is probably by way of the glume rather than through the anther.

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