STUDIES ON THE VARIABILITY OF PATHOGENICITY AND CULTURAL CHARACTERS OF GIBBERELLA SAUBINETII [1]

By ARNOLD J. ULLSTRUP [2]

Research associate in plant pathology, Wisconsin Agricultural Experiment Station, and formerly agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Variability in pathogenicity and cultural characters in the fungus *Gibberella saubinetii* (Mont.) Sacc. was brought to the attention of the writer during the course of an investigation on the influence of soil temperature upon the development of seedling blight in barley (*Hordeum vulgare* L.) caused by this organism. As a result of these observations the present investigation was directed toward determining the nature, frequency, and magnitude of the apparent variability in *G. saubinetii*, particularly with respect to pathogenicity and cultural characters.

MATERIAL AND METHODS

Perithecia of *Gibberella saubinetii* collected by the writer in the summer of 1933 from barley fields in Illinois, Iowa, and Minnesota furnished the material on which this study was based. All cultures used were grown from ascospores isolated in sets of 8, each set comprising the 8 ascospores from a single ascus. Each subsequent subculture, however, was derived from a single conidium unless otherwise indicated. Ascospores, rather than conidia, were selected as starting points because, theoretically, the ascospores should be homocaryotic, whereas the conidia may or may not be in that condition. Granting the possibility of the existence of nuclei of different genetic constitution, a random assortment of nuclei by virtue of the frequently occurring phenomenon of anastomosis would not preclude the possibility of conidia of different nuclear make-up.

The procedure employed in the isolation of the ascospores was to strip off a bit of the epidermis of a cornstalk bearing the perithecia and wash it in several changes of sterile distilled water. Then it was placed in a sterile Petri dish and the excess water allowed to evaporate. The dish with cover removed was placed under a dissecting microscope and an individual perithecium was picked off with a small flattened needle and placed in a drop of distilled water on a flamed microscope slide. The perithecium was then crushed between the slide and a flamed cover slip. The asci and spores thus removed

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from the confines of the fruiting body were observed under the microscope to determine their stage of maturity. Experience has shown that apparently there is a rather definite stage in the development of the asci and ascospores before or after which successful isolation of a set of eight spores from a single ascus is impossible. If the spores are immature, although they may be well defined within the ascus, they are difficult to remove. Furthermore, germination of the spores either does not take place or is at best very weak. If, on the other hand, the contents of the perithecium have advanced beyond the desirable stage of development, the spores are liberated from the ascus while the latter is still within the perithecium, thus making it impossible to isolate the spores with regard to the ascus in which they were borne.

Spores and asci that had attained the proper stage of maturity, as determined by observation, were picked up with a fine wire loop along with the water in which they were suspended, and the suspension was streaked over the surface of hard 4-percent water agar contained in Petri dishes. After about 5 hours' incubation at 24° C., the spores within the asci showed germ tubes, approximately one-half the length of the spore, protruding through the ascus wall. With the aid of the low power of the microscope the locations of asci bearing eight germinating ascospores were marked by scarifying the surface of the agar adjacent to each ascus. When a sufficient number of asci had thus been located, the agar plate was placed under a dissecting microscope, and each ascus was moved across the surface of the agar to an area free from spores and fragments of the perithecial wall. With the asci thus removed from extraneous material and separated at sufficient distances from each other, the spores were "teased" out of each ascus and separated from one another at convenient distances within the field of vision. All manipulations, including the separation of the asci from spores and other material and the removal of spores from the asci, were done by hand with the aid of a sterilized fine glass wire 2μ to 3μ in diameter and bent to form a right angle or semiloop. After the spores were removed from the asci and separated from one another they were examined under the microscope and then allowed to continue germination for a period of about 3 hours. By means of a small loop cutter 0.5 mm in diameter, a cylinder of agar was cut around each spore and the entire piece of agar with the spore on its surface was lifted out and placed in the center of a Petri dish containing hard potato-dextrose agar. Each plate was placed under a miscrosope and again examined to make certain that only a single ascospore had been transferred.

Hyphal-tip cultures were obtained by the same general method as that just described for single ascosporic cultures. After the eight spores from a single ascus had been separated from one another, they were allowed to continue germination until the germ tubes were 5 to 6 times the length of the spore. By means of a fine sterilized glass needle a light scratch was made across the tip of the germ tube, back of a septum, severing it from the spore proper. The free hyphal tip was picked up and placed in the center of a Petri dish containing hard potato-dextrose agar. Two hyphal tips were thus cut off from each spore and grown in separate Petri dishes.

A system of numbering was devised whereby the cultures and culture sets just described could be designated. In this system a number was given to each culture that would indicate the State, the location in the State where the collection was made, the perithecium, the ascus from which the eight spores were isolated, and finally the single ascospores or hyphal tip from which the culture was grown. In table 1 are given the culture sets used, together with the location where the original perithecial material was collected and the date on which the isolation was made.

TABLE 1.—*Cultures used in the investigations reported in this paper, together with the origin of the perithecial material and the date of isolation*

Culture no.	Origin of perithecial material	Date of isolation	Culture no.	Origin of perithecial material	Date of isolation
		1933			*1933*
Ill. 111-1 to 7	Mineral, Ill.	July 14	Minn. 111-1 to 8	Olivia, Minn.	July 17
Ill. 121-H. T[1]	do	Do.	Minn. 112-H. T	do	Do.
Ill. 132-H. T	do	Oct. 15	Minn. 211-1 to 8	Alpha, Minn.	Nov. 2
Iowa 111-1 to 8	Calumet, Iowa	July 14	Minn. 212-1 to 8	do	Do.
Iowa 211-1 to 8	West Liberty, Iowa	July 28			
Iowa 221-1 to 8	do	Oct. 15			

[1] Hyphal tip.

CULTURAL STUDIES

In order to obtain some information as to the range, type, and factors influencing variability in cultural characters, four different experiments were set up. In these experiments potato-dextrose agar and a modification of Brown's agar were used. Each subculture studied was grown from a single conidium unless otherwise indicated. Isolations of single conidia were made by streaking a spore suspension over the surface of hard agar and allowing the spores to germinate. After germination had commenced the spores were picked up singly and placed on the particular agar medium on which they were to be grown. The quantity of agar used in the Petri dishes in which the single-spore colonies were grown was approximately 20 cc. The colonies were allowed to develop in the dark at room temperature, which varied between 21° and 23° C. At the end of 5 days, except in case of first isolation, descriptive notes were taken on each culture with respect to color, type of growth, diameter of colony, type of margin, and the expression of zonation and radiation. Although it is admitted that descriptive notes do not adequately convey a picture of the real differences and changes that occurred in the cultures, nevertheless, such notes, together with photographs, are of value in recording the major variations in cultural behavior.

The following is a brief description of the original colonies grown at room temperature for a period of 4 days:

Color of colony, pomegranate purple[3] concentrated in a central area 2 to 3 cm in diameter and gradually fading to white toward the edge of the colony. Aerial mycelium abundant, extending over the entire colony, and of a cottony texture. Margin of colony, generally regular and entire. No pronounced zonation or radiation detectable. Colony diameter, 8 cm.

[3] RIDGWAY, R. COLOR STANDARDS AND NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912

The foregoing description applies to all of the original isolates, whether grown from ascospores or hyphal tips (figs. 1 and 2) and regardless of their geographical origin. These isolates constitute a

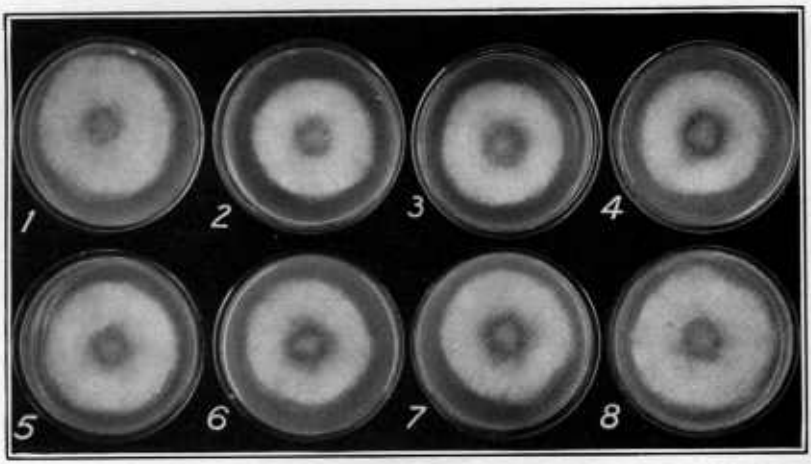

FIGURE 1.—Eight single ascosporic colonies at 4 days of age, grown from the eight ascospores of a single ascus.

type always encountered on first isolation and referred to in this paper as "type A."

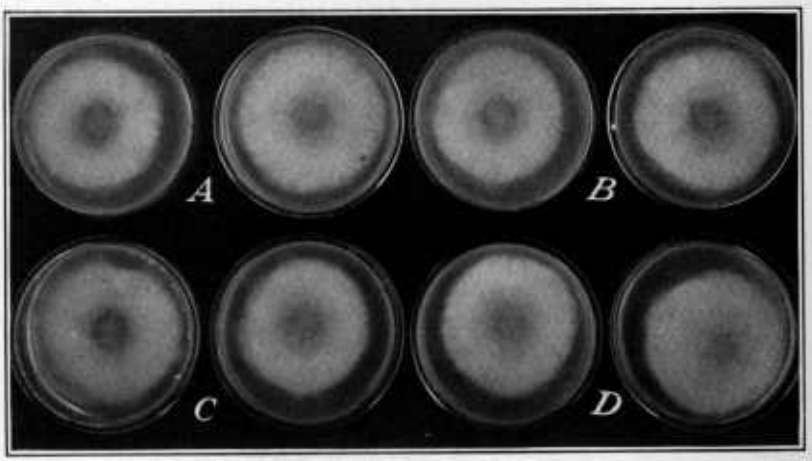

FIGURE 2.—Four pairs (*A–D*) of hyphal-tip colonies at 4 days of age. The two members of a pair were grown, respectively, from hyphal tips arising from opposite ends of a germinating ascospore.

EXPERIMENT 1

Experiment 1 was designed to study the effect of the medium and the cultural technic on the changes in cultural characters. Eight single ascosporic cultures from an individual ascus were employed in the experiment. The eight original colonies were strikingly similar in appearance (fig. 1). Duplicate mass transfers were made from each

of the colonies, one transfer to a slant of potato-dextrose agar and the other to a slant of modified Brown's agar. Three days later, spore suspensions were made in these culture tubes and single conidial isolations were made in triplicate from each culture. Part of the spore suspension was used to seed fresh slants of agar from which single conidial isolations were again made 3 days later. This procedure of subculturing from slant cultures that had been transferred every 3 days was repeated 28 times.

During the course of the experiment several of the cultures showed marked changes in cultural behavior from that of the original ascosporic culture. In the cultures propagated on potato-dextrose agar, nos. 1, 2, 4, 6, and 7 were strikingly different by the end of the experiment, while nos. 3, 5, and 8 remained constant (fig. 3). Of the cultures grown on modified Brown's agar, only no. 7 maintained its

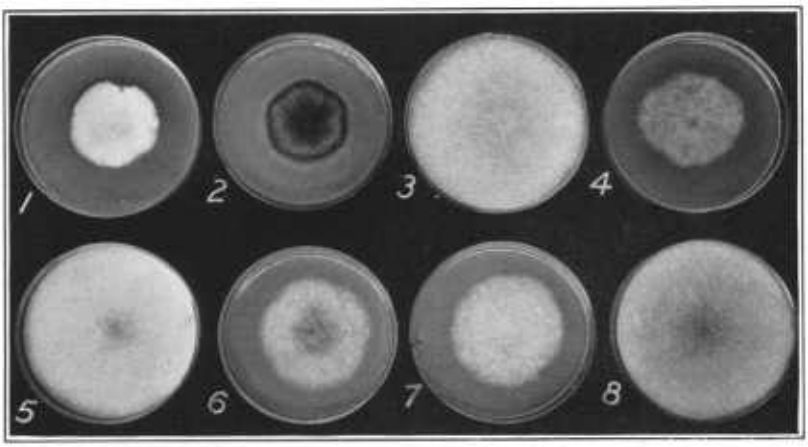

FIGURE 3.—Eight monoconidial colonies after having been subcultured 28 times on potato-dextrose agar. Originally, these cultures were all similar in appearance and were derived from the eight ascospores of a single ascus. Nos. 1, 2, 4, 6, and 7 were strikingly different at end of experiment. Nos. 3, 5, and 8 remained constant.

original appearance, the others having changed decidedly in cultural behavior (fig. 4).

It is apparent that the medium on which the cultures were grown had no influence in determining the extent and magnitude of variation that occurred in cultural characters. Duplicate cultures, each on a separate medium, did not behave in a manner that would indicate their relationship. If the capacity or tendency of a culture to vary in its behavior while growing under artificial conditions is associated with its genetic constitution, then it is not unreasonable to expect cultures arising from the same original ascosporic colony to behave in a similar manner unless irregularities of one sort or another have occurred. Under the conditions of the experiment, in which considerable care was taken to maintain the cultures at a constant environment, there seemed to be no uniformity in the changes that the cultures underwent, regardless of their origin or the media used. The variant cultural types that appeared were generally characterized by a slower radial growth rate and a decrease in abundance of aerial mycelium.

EXPERIMENT 2

The primary object of experiment 2 was to determine what influence the frequency of transfer might have on the cultural behavior of a set of eight single ascosporic isolates from the same ascus.

Duplicate transfers were made from the eight original colonies, which were essentially alike in appearance, to slants on modified Brown's agar. One of these sets of slant cultures was subsequently transferred every 4 days, the other set every 8 days. Each time the cultures were transferred single conidial isolations were made in triplicate from each isolate.

During the course of the experiment the majority of isolates changed in cultural appearance. There was apparently no difference in behavior between the isolates of the group transferred every 4 days and those transferred every 8 days. The time elapsing before the changes became evident and the extent and the magnitude of these

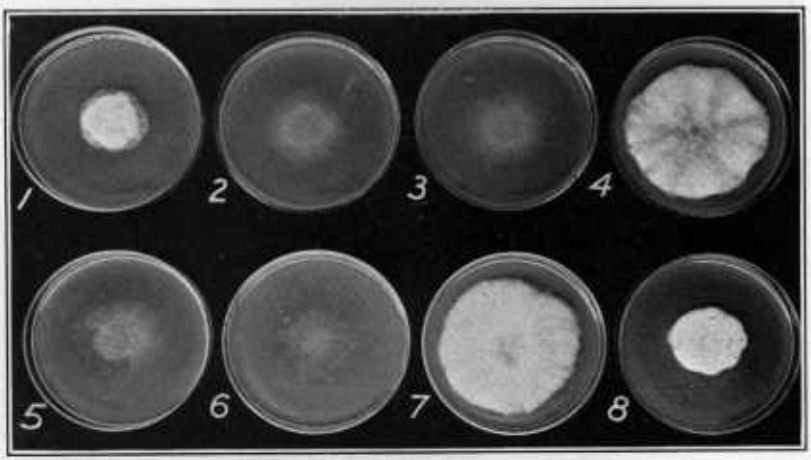

FIGURE 4.—Duplicates of colonies shown in figure 3 and originally derived from the same ascus. They were subcultured 28 times on modified Brown's agar. Nos. 1 to 6 and 8 changed in cultural behavior. No. 7 remained constant.

changes in cultural type were essentially the same in both groups. The results from this experiment, like those from experiment 1, seemed to indicate that changes in cultural characters were not of a systematic, genetic nature.

EXPERIMENT 3

Studies were made to determine the effect of continued subculturing on the stability of a set of eight single ascosporic isolates from an individual ascus. In experiment 3 the isolates were propagated only in Petri dishes instead of in culture tubes, as in experiments 1 and 2. As in the other experiments, the original ascosporic colonies were alike in cultural appearance. When the original ascosporic colonies were 6 days old a spore suspension was made in each dish containing a colony, and from each, single conidial isolations were made in triplicate. Although this procedure was continued for 10 successive conidial generations, no changes in cultural behavior became evident. Each of the 8 cultures was now transferred to a slant of modified Brown's

agar and subsequent transfers were made every 6 days. At each time of transfer single conidial isolations were made in triplicate from each of the cultures. After the second transfer on agar slants, changes in cultural characters began to appear in two of the isolates. The variant cultural types were characterized by a reduced growth rate and a decrease in aerial mycelium.

EXPERIMENT 4

The object of experiment 4 was to determine what effect continued subculturing might have on single cultures. Only 2 cultures were

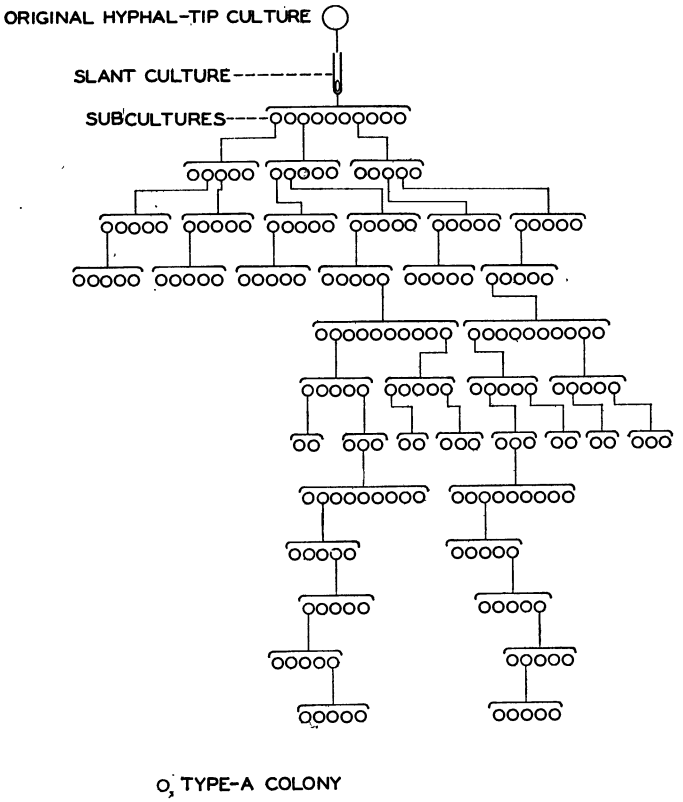

FIGURE 5.—Diagram of procedure followed in experiment 4 in the use of culture Ill. 132–1A. Each circle represents a colony of monoconidial origin isolated from a colony of the previous conidial generation.

studied in detail, namely, Ill. 132–1A and Ill. 132–1B. These were hyphal-tip cultures from a single ascospore and, on original isolation, were exactly alike in cultural behavior. From each of these 2 original cultures, 10 single conidial isolations were made and plated out on hard potato-dextrose agar. After these 20 cultures were 6 days old, single conidial isolations were again made from some colonies of the previous conidial generation. This procedure was continued for more than 16 conidial generations in the culture Ill. 132–1B and 12 conidial generations in the culture Ill. 132–1A (figs. 5, 6).

In the first conidial generation of culture Ill. 132–1B all of the 10 colonies were alike and similar to the original parent colony, that

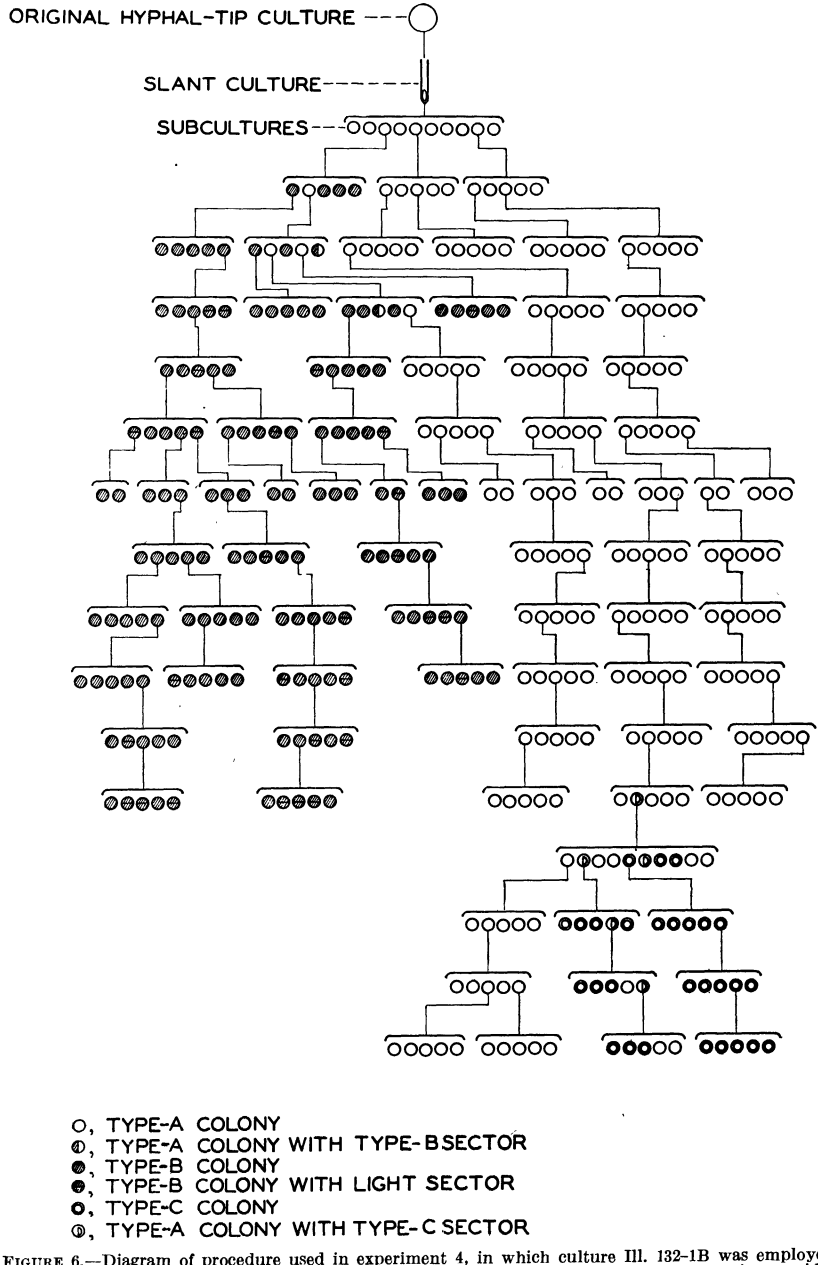

FIGURE 6.—Diagram of procedure used in experiment 4, in which culture Ill. 132-1B was employed. Each circle represents a colony of monoconidial origin isolated from a colony of the previous conidial generation.

is, type A. In the second conidial generation 5 spores selected from one of these colonies produced 5 colonies, 3 of which were alike but

differed distinctly from the parent colony in color, rate of growth, and type of growth. This cultural type will be referred to as type B. The type-B colony, grown at room temperature for a period of 5 days, may be described as follows:

Color of colony, pomegranate purple [4] in central area 0.5 cm in diameter, surrounded by yellow olivaceous zone that fades to pale yellow at edge of colony. Aerial mycelium scant and extending to 1 cm from edge of colony. Margin regular and entire. Color zones as described, radiations in yellow olivaceous zone. Diameter of colony, 5 cm.

The fourth colony was like the parent colony, and the fifth, although resembling the parent colony in the main, had a small sector resembling the type-B colony (fig. 7). Subsequent isolations from type-A colonies of this line produced type A, type B, and type A with type-B sectors. Further isolations from type-B colonies always

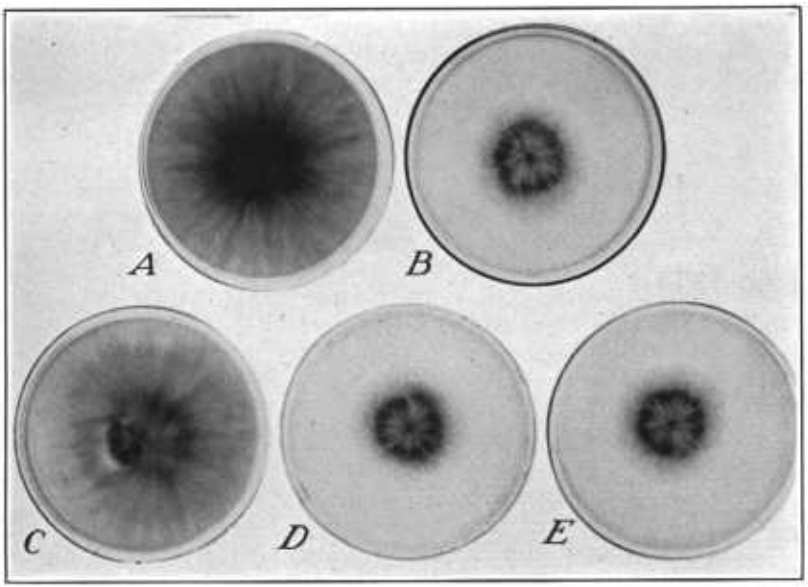

FIGURE 7.—Five single conidial colonies at 5 days of age, all of which were isolated from a type-A colony. *A*, Colony of type A; *B*, *D*, *E*, three colonies of type B; *C*, colony of type A with a type-B sector.

gave rise to colonies resembling the type-B parent, although frequently, after the colonies were 5 days old, light-colored sectors appeared in some of these type-B colonies.

The type-A colonies from the other lines remained constant until the twelfth conidial generation, when one colony produced a type-C sector. Isolations from this colony gave rise to colonies of type C, type A, and type A with type-C sectors. Subsequent type-A colonies remained constant, as did the type-C colonies. The following is a description of the type-C colony:

Color of colony, spinel pink [4] in central area surrounded by narrow zone of pomegranate purple,[4] which, in turn, was surrounded by a zone of spinel pink, which faded gradually to white at edge of colony. Aerial mycelium abundant, extensive, and of a dense cottony texture. Margin regular and entire. Color zonation as indicated. Radiations in spinel-pink zone. Diameter of colony, 6.5 cm.

[4] RIDGWAY, R. See footnote 3.

The culture Ill. 132–1A was studied parallel with Ill. 132–1B. During the course of 12 conidial generations no deviations from the original cultural type were found, that is, all colonies were of type A throughout the experiment.

In this experiment, as well as in the experiments reported earlier in this paper, the results appear to lend themselves to 1 of 2 possible interpretations: (1) Variations in cultural behavior may have been the result of a reassortment and somatic segregation of nuclei of different potentialities that arose through atypical division, or (2) they may have resulted from a fortuitous change in the arrangement and organization of heritable material. The latter interpretation appears to be the more tenable, and until a detailed and comparative cytological study of the variant and normal type cultures is made the possibility of atypical nuclear divisions and subsequent reassortment of these nuclei to account for variation can be advanced only as a hypothesis.

It is not surprising that greater variation in cultural behavior was found in cultures propagated on agar slants than in those carried in Petri dishes. On the limited surface of the agar slant these variations may take place without being observed; in subsequent mass transfers to fresh agar slants a large number of spores and mycelial fragments are carried over, some of which may be of one or more variant cultural types. Continued transferring may well eliminate one or another type until a practically pure culture of a particular and distinct appearance is obtained. Where cultures are carried in Petri dishes and only relatively few spores are isolated from a given colony, the chances of obtaining a variant type culture are considerably reduced even though the variant type may be present but unobservable in the parent colony. If a larger number of conidia had been isolated from colonies carried in Petri dishes, then perhaps greater variation would have been observed.

PATHOGENICITY STUDIES

The pathogenicity studies were undertaken not only with the object of determining the relative virulence of the various cultures, but also with a view to finding a possible correlation between colony type and degree of virulence.

These studies were conducted in benches in the greenhouse where the soil temperature was maintained between 15° and 18° C. The relatively low soil temperature was selected in order to produce a sufficient amount of infection to obtain a differential in virulence between the cultures. Dickson (*9*)[5] has already pointed out that seedling blight of corn, caused by *Gibberella saubinetii*, is favored by soil temperatures ranging from 8° to 20°, whereas at temperatures above 24° no blighting occurs. No attempt was made to control moisture other than to maintain the soil at a moisture content that would permit normal growth and development of the seedlings.

Two lines of corn, obtained through the department of plant pathology of the University of Wisconsin and produced by J. R. Holbert at Bloomington, Ill., were used in the experiments. One line, designated as R4, has proved for a period of years very susceptible to

[5] Reference is made by number (italic) to Literature Cited, p. 161.

seedling blight; while under comparable conditions, the other line, Br10, has been relatively resistant. The line R4 was used in all series of the pathogenicity studies, whereas Br10 was used only in series 6 and 7. In all, 45 single isolates, the cultural behavior of which had not been followed so much in detail as had those isolates used in the section called "Cultural Studies", were employed in the first five series of the experiment. Most of these were single ascosporic cultures from individual asci; a few of the cultures, however, were grown from isolated hyphal tips of germinating ascospores. Originally all of these isolates were alike in their cultural behavior; after being propagated for a comparatively short time on potato-dextrose agar, however, certain variations in their appearance became evident. In order to study these variations in greater detail single conidial isolations were made of each isolate where this was possible. In certain variant types spores were no longer produced and hyphal tips were isolated from the mycelium. Descriptive notes and photographs of these cultures later proved useful in making correlations between colony type and virulence.

Inoculations were made by immersing 50-kernel samples of corn in a suspension of spores and mycelial fragments from an individual culture. Since an extreme variation existed in the sporulation of the cultures, ranging from those in which conidia were very abundant to those in which none were produced, standardization of the inoculum was impossible. Notes on the relative abundance of sporulation of each culture were taken to determine whether a correlation existed between the abundance of conidia and virulence. After the corn kernels had been soaked in a suspension of a single culture for 3 to 5 minutes they were planted in the soil in the bench at a depth of 1 inch. Check plantings were immersed in sterile distilled water for the same period of time and planted in the same bench.

When the control seedlings, grown from the uninoculated seed, had reached the third-leaf stage, which required about 30 days at this soil temperature, both the inoculated and uninoculated lots were removed from the soil and classified according to the severity of infection. The classes in which the seedlings were placed were assigned arbitrary values ranging from 10 for healthy seedlings to 0 for those that failed to emerge because of disease. The number of seedlings in each class was multiplied by the given value of that class and the sum of these values for each class was divided by the number of kernels planted. If the quotient is subtracted from 10, the resulting value represents the disease index. Thus, a disease index of 10 indicates that all seedlings had been killed before emergence, whereas a disease index of 0 indicates that all seedlings emerged and were healthy.

The disease indices of the cultures for each of the five series, together with an average disease index for each culture for all series, are given in table 2. It will be seen at once that there were wide differences in pathogenicity in the cultures. In the set of cultures Iowa 211–1 to 8, originally grown from the eight spores, respectively, of a single ascus, such differences are strikingly evident; the average disease indices range from 3.17 to 9.75, showing an extreme difference of 6.58.

TABLE 2.—*Disease indices of cultures of Gibberella saubinetii used in inoculation studies on an inbred strain of corn, R4*

Culture no.	Disease indices for—					Average for—	
	Series 1	Series 2	Series 3	Series 4	Series 5	Individual cultures	Set of cultures
Ill. 111–1	7.76	5.54	4.08	8.20	8.40	6.79	
–2	3.46	2.88	2.68	6.44	5.60	4.21	
–3	8.66	7.92	6.68	9.12	8.52	8.18	
–4	4.00	2.78	1.12	4.92	2.96	3.15	4.66
–5	5.38	4.02	.86	2.58	2.12	2.99	
–6	2.82	1.20	.88	2.44	2.54	1.97	
–7	6.10	4.30	2.84	6.84	6.48	5.31	
Ill. 121–1A	9.60	9.32	8.66	10.00	9.72	9.46	
–1B	6.16	6.50	3.56	7.74	6.40	6.07	
–3A	6.90	7.00	4.44	8.44	7.10	6.77	
–3B	9.62	9.84	8.98	9.96	9.66	9.61	
–5A	5.16	3.48	2.48	6.24	5.68	4.61	6.55
–5B	7.34	6.24	4.18	9.24	8.24	7.05	
–6A	1.40	1.82	.64	1.24	2.96	1.61	
–6B	7.12	5.66	6.58	9.28	7.48	7.22	
Iowa 111–1	.86	1.20	.48	.80	1.24	.91	
–2	6.68	4.98	3.00	6.76	5.36	5.35	
–3	7.40	5.34	3.82	8.88	6.76	6.44	
–4	5.12	3.72	2.72	5.92	5.60	4.61	
–5	2.26	1.40	.72	1.94	1.40	1.54	4.20
–6	.80	.66	.32	1.84	1.00	.92	
–7	8.00	6.34	4.48	8.34	7.72	6.97	
–8	7.10	7.78	3.92	8.64	7.60	7.00	
Iowa 211–1	9.06	9.10	8.60	9.96	8.54	9.05	
–2	6.42	5.06	4.56	7.66	7.08	6.15	
–3	4.88	1.90	2.34	1.86	4.86	3.17	
–4	10.00	9.88	9.24	9.92	9.72	9.75	
–5	5.56	5.22	.66	2.74	2.22	3.30	7.47
–6	9.42	9.62	9.36	9.96	9.74	9.62	
–7	10.00	9.72	9.06	9.90	9.54	9.64	
–8	9.86	8.84	8.30	9.48	8.90	9.07	
Minn. 111–1	4.30	2.36	2.12	5.78	4.52	3.81	
–2	1.00	1.32	.40	1.70	1.12	1.11	
–3	2.64	1.64	.60	2.36	2.60	1.97	
–4	1.04	.88	.32	1.01	1.04	.86	
–5	8.74	8.00	6.48	9.84	9.64	8.54	3.55
–6	1.16	.72	.52	1.40	1.00	.96	
–7	3.76	3.86	1.28	5.82	4.50	3.84	
–8	7.42	6.76	5.94	7.88	8.58	7.31	
Minn. 112–4A	5.86	2.52	.68	3.92	1.52	2.90	
–4B	2.14	.94	.20	2.40	.64	1.26	
–6A	1.78	1.24	.44	1.46	1.00	1.18	
–6B	.86	1.68	.54	1.04	1.06	1.03	1.36
–7A	.80	.68	.92	1.54	1.62	1.11	
–7B	.80	.80	.48	.76	.44	.65	
Average indices for all cultures in each series	5.29	4.50	3.36	5.65	5.12	----------	----------
Average for control plantings	.79	.65	.62	1.09	1.37	----------	----------

In every case type-A colonies showing rapid radial growth along with an abundance of aerial mycelium were highly pathogenic. No colonies showing this type of growth were weakly parasitic. A few colonies, however, that did not exhibit such cultural characters were rather virulent. Nevertheless, the indications are that rapid mycelial growth and abundant aerial mycelium are directly correlated with a high degree of pathogenicity in the majority of cases. There may, however, be still other factors responsible for pathogenicity. Similar observations on species of *Fusarium* have been made by other workers. Brown (*3*), working on certain fruit-rotting species of *Fusaria*, has pointed out that the mycelial type of culture is the most pathogenic. He also states that this type of growth is the form found on first isolations from diseased tissue. Harvey (*11*), in a study of the parasitic

abilities of cultures of *F. fructigenum* Fr., found that high virulence was correlated with vigorous mycelial growth.

The data obtained on the relative sporulating ability of the cultures indicate that no positive correlation exists between sporulation and pathogenicity. Some of the most virulent cultures, as well as those that show very weak parasitic tendencies, produce conidia in abundance. Likewise, cultures that produce very few spores may or may not be pathogenic. The sporulating ability of the cultures used in the pathogenicity studies was determined by observing the relative number of spores in a drop of inoculum. No actual counts were made.

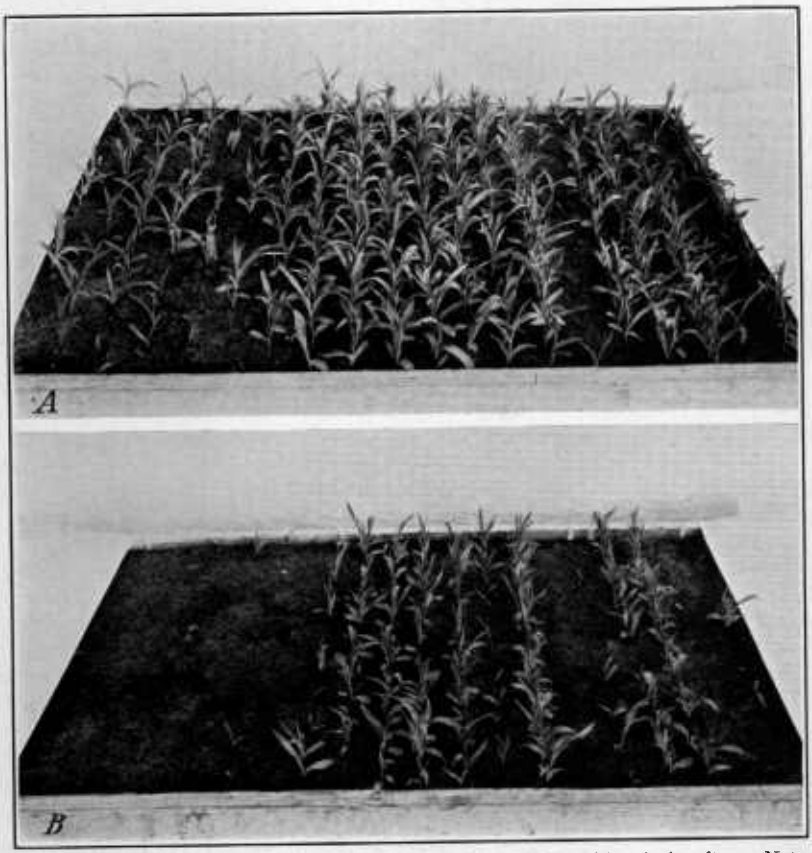

FIGURE 8.—*A*, Corn seedlings of resistant line, Br10, each row inoculated with a single culture. Note the difference in virulence between the cultures used. *B*, Corn seedlings of susceptible line, R4, inoculated with the same cultures as in *A*. Note the relative susceptibility of R4.

In two additional inoculation series (series 6 and 7) both inbred lines of corn, R4 and Br10, were used. In these series an entirely different group of cultures was employed. The same general results were obtained as in the first five series, that is, high virulence was directly correlated with rapid mycelial growth and vigorous aerial mycelium. No indication was found of selective pathogenicity of cultures between two lines of corn, all cultures being more virulent on the susceptible line, R4, than on the resistant line, Br10 (fig. 8 and table 3).

TABLE 3.—*Disease indices of cultures of Gibberella saubinetii used in inoculation studies on 2 inbred lines of corn, Br10 and R4*

Culture no.	Disease indices for indicated culture series on—					
	Inbred Br10			Inbred R4		
	Series 6	Series 7	Average	Series 6	Series 7	Average
Minn. 211-1	8.54	7.62	8.08	9.91	9.72	9.81
-2	8.23	7.80	8.01	9.85	10.00	9 92
-3	8.00	7.58	7.79	9.94	10.00	9.97
-4	7.39	6.58	6.98	9.84	9.68	9.76
-5	8.36	7.94	8.15	9.74	9.86	9.80
-6	8.63	7.66	8.14	9.76	9.20	9.48
-7	7.74	7.90	7.82	9.79	9.84	9.81
-8	4.01	2.94	3.47	8.13	7.80	7.96
Iowa 221-1 (A)	2.09	2.56	2.32	6.57	5.52	6.04
-2	2.53	1.56	2.04	5.57	5.24	5.40
-3	1.95	1.12	1.53	5.40	4.28	4.84
-4	2.19	1.24	1.71	3.99	1.60	2.79
-5	3.73	6.12	4.92	7.35	8.36	7.85
-6	1.18	.64	.91	1.14	.52	.83
-7	8.38	7.12	7.75	9.96	10.00	9.98
-8	5.42	5.28	5.35	9.13	9.32	9.22
Ill. 132-1B-(B)	2.01	1.56	1.78	6.34	5.46	5.90
1B-(B)-S	2.43	1.44	1.93	5.35	3.74	4.54
1B-(A)	8.72	9.20	8.96	9.86	9.76	9.81
1B-(A)-S	4.42	5.46	4.94	8.70	8.88	8.79
Checks (averages)	1.02	.58	.80	1.35	1.19	1.27

EFFECT OF PASSING CULTURES THROUGH THE HOST

Experiments of a preliminary nature were conducted to determine what effect the parasitic habit might have on the cultural behavior of the fungus.

Fifteen lots of corn seedlings, each of which had been inoculated at planting with a different isolate of known cultural behavior, were removed from the greenhouse bench, surface-disinfected, and the diseased tissue plated on hard potato-dextrose agar. After 3 days the fungus had grown out of the diseased portion and could be readily identified as *Gibberella saubinettii*. Mass transfers of apparently pure cultures were made to plates of potato-dextrose agar and 5 days later cultural characters were studied and recorded.

In all cases the reisolated cultures were the same in their cultural behavior as at the time of inoculation, indicating that the sojourn within the host tissue, which was about 1 month, had no perceptible influence on the cultural characters of the fungus. These results are not in accord with the findings of Burkholder (*4*) in a species of *Fusarium*. He reported morphological and physiological changes attending *F. martii phaseoli* Burk. when kept in culture for a long period of time, but after inoculation and reisolation the fungus assumed its original cultural aspects. He also noted that loss of virulence was restored after two inoculations and reisolations. Coons and Larmer (*7*) found that aberrant cultures of *Cercospora beticola* Sacc. have a tendency to revert to the original form after inoculation and reisolation from the host.

DISCUSSION

Throughout the present investigations it has been definitely shown that considerable variation exists among single ascosporic cultures and hyphal-tip cultures of the fungus *Gibberella saubinetii* while growing

under an artificial environment. This variability has been expressed both in cultural behavior and pathogenicity.

An extensive volume of literature has been devoted to the subject of variability of fungi in pure culture. Most of the authors, however, simply report the occurrence of the variations as differences in cultural behavior, morphology, physiology, or pathogenicity, without attempting to analyze their true nature.

Several different views regarding the nature of variations in fungi have been put forward by different groups of workers. Stakman (*18*), Christensen (*6*), and Stevens (*19*) believe that most of the variability in fungi probably is due to true mutation or saltation similar to bud mutation in the higher plants. Brown (*2*) and Mohendra (*17*) also are of the opinion that variants arise as true mutants. La Rue (*14*) suggests that loss of virulence in pathogenic fungi is brought about by the appearance of saprophytic strains, which arise as mutants that are able to thrive better under artificial culture and thus outgrow the parasitic strains.

Caldis and Coons (*5*) have demonstrated that certain variations in the fungi were of a more or less permanent nature and probably induced by nutritional disturbances or a poisoning of the protoplasm. These writers regard such variations as similar to the "Dauermodification" described by Jollos (*13*) for certain Protista.

Holton (*12*), substantiating some of Dickinson's work (*8*), has suggested that in some of the smuts, and in other fungi where true sexuality exists, delayed segregation of certain heritable factors may account for the appearance of variants.

Leonian (*15*, *16*) has explained variation in the fungi as a natural phenomenon whereby the culture traces the variability of the species. Attempts by that author to induce mixochimaera in *Fusarium moniliforme* Sheld. were unsuccessful. After two distinct isolates had been grown in combinations for a period, reisolation revealed both parent strains and in addition a new type. The latter was believed to be a dissociant of one of the parent strains and not a heterotype resulting from anastomosis and intimate association of the protoplasm of the two parents.

In opposition to this opinion, Brierley (*1*) holds that the appearance of variants in many instances may be accounted for by a heterocaryotic condition of the original isolate, and that through subsequent culturing a reassortment of nuclei may take place that gives rise to cultures phenotypically divergent from the parent. Brierley further states that true mutation, defined as "a fundamental change in one or more of the hereditary units, and carried from one generation to another", has not been adequately shown in the fungi. He concedes the possibility of aberrant nuclear divisions by virtue of which new forms might arise. Hanson and Smith (*10*) have brought out experimental evidence with *Botrytis* in support of Brierley's contention that a reassortment of the nuclei of a heterocaryotic isolate may give rise to strains differing from the original.

In regard to the pathogenicity of different cultures, Tanja (*20*), working with three isolations of *Gibberella saubinetii* procured in culture from different sources, found that they differed from one another in virulence. Tu (*21*), in an investigation of species of *Fusarium* causing head blight of small grains, has shown that three isolates of *F. graminearum* Schwabe could be separated on the basis of their viru-

lence on several varieties of wheat and on their growth rates. Some information concerning the changes that went on in the cultures employed by these workers before they were tested for pathogenicity would be of interest.

The nature of the variability observed in the investigations reported here does not appear to be directly due to a condition of heterocaryosis in the original isolates. Theoretically, the four nuclei in an ascospore are of the same genetic constitution, and if segregation occurs it takes place in the ascus before the spores are formed. Likewise, all nuclei in the hypha of a germinating ascospore have their origin in the mother nucleus of the cell from which the hypha was produced, and should be genetically alike. Under these assumptions, barring any aberrant nuclear divisions, duplicate ascosporic cultures from a common isolate and hyphal-tip cultures from the same ascospore should behave in the same manner.

Whether aberrant nuclear divisions occur would be difficult to determine cytologically with the material used in these studies. If atypical divisions of the nuclei take place, it is conceivable that only a few of such aberrances could, through the reassortment and segregation of the nuclear complex of a single culture, give rise to forms differing in several respects from the original cultural type. There is also the possibility, and this is perhaps the most tenable explanation, that variations in cultural behavior and pathogenicity in this organism are brought about by gene changes or chromosomal aberrations. That the variant types have appeared rather suddenly and have in some cases passed unaltered through the ascigerous stage strengthens this supposition.

In the experience of the writer, as well as of some other workers, abnormal cultural types were never found under natural conditions. That these abnormal or variant forms are weak pathogenes may preclude their isolation from diseased tissue. In addition, it has been observed that few of these forms pass through the ascigerous stage, which may account for their absence from the isolations made from perithecial material. If these forms do exist in nature they probably do so as saprophytes and are constantly being eliminated through competition and natural selection of the more vigorous forms.

SUMMARY

Single ascosporic and hyphal-tip cultures of *Gibberella saubinetii* (Mont.) Sacc. were studied in relation to cultural behavior and pathogenicity.

The method employed in isolating the eight ascospores from a single ascus is described in detail.

All original ascosporic and hyphal-tip isolates were strikingly similar in cultural behavior regardless of the locality from which the perithecial material was collected. During the course of the studies considerable variability was observed in cultural behavior. The variation was more or less haphazard and did not appear as a result of an orderly segregation within the ascus.

Wide differences were found among the isolates with respect to their ability to cause seedling blight on corn. Some isolates were highly virulent while others were practically nonpathogenic.

A direct correlation was found between colony type and virulence. Those cultures that showed a rapid radial growth and an abundance

of aerial mycelium were always highly pathogenic, whereas those having a relatively slow growth rate and a pionnotes type of growth were generally poor pathogenes. No correlation could be established between abundant conidial production and degree of pathogenicity.

Passage through the host apparently had no influence on the cultural characters of the isolates.

It is suggested that the variability observed in the present investigations may be due to 1 of 2 possible causes: (1) Abnormal nuclear divisions with subsequent reassortment and segregation of a new nuclear complex, or (2) the existence of true mutants.

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