STUDIES ON VARIATION IN GIBBERELLA SAUBINETII (MONT.) SACC. (FUSARIUM GRAMINEARUM SCHWABE)[1]

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Introduction

In recent years much attention has been given to variation in fungi, chiefly as a step in pathogenicity studies in combating diseases of economic plants. The exact nature of these variations has been, and still remains, very controversial. In artificial culture, variations may be in the form of sectors or islands in apparently homogenous cultures, or the whole culture may vary perceptibly from the parent organism. The variations may be only temporary, reverting to the parental type in the next cultural generation; they may persist through several generations and then revert to the parental type; or remain as permanent variants; or, they may in turn form still other variants.

Brierley ('31) summarizes the theoretical modes of origin of new forms as follows: "(1) by adaptation of an existing form, (2) by hybridization of two existing forms, or by some other mode of genetic fusion and segregation, and (3) mutation." He adds that what is apparently a new form may possibly be only the re-emergence and stabilization of a suppressed or latent character, or grouping of characters, or of a particular cyclogenic phase in a polyphasic organism.

The objects of the experiments described in the first part of the paper were: (1) to determine whether variation can be

[1] An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

induced by altering environmental conditions, and to compare the variant with the parental type in order to obtain evidence of possible genetic change in constitution; (2) to determine whether there is a definite cycle of growth stages, such as mycelial, sporulating, and pionnotal, which can be influenced by environmental conditions; and (3) to observe whether the pionnotal stage reverts to the stage with aerial mycelium.

The experiments described in the latter part of this paper were devised to show whether, when contrasting monosporous strains are grown together on culture media, the hyphal anastomoses result in heterocaryosis, thus producing new strains.

HISTORICAL REVIEW

Variations in fungi have been referred to as mutations, saltations, discontinuous variations, dissociations, and semi-permanent variations. De Vries ('06) used the term "mutation" for a means of change which lies in the sudden and spontaneous production of new forms from old stock. Muller ('22) refers to mutation as a variation in the individual gene. "Saltation" has been used more or less synonymously with mutation. However, it was probably used first by Stevens ('22) to designate heritable variations for which the sexual stages are not known, and in which the cytological conditions have not been thoroughly investigated. Das Gupta ('34) states that saltation includes only those variations which are of the order of mutation in higher plants. Brierley ('31) prefers to use the non-committal descriptive term "discontinuous variation," which "has no genetic implications." "Dissociation," as defined by Leonian ('32), the originator of this view, "is that phenomenon whereby a given organism traces the sphere of variability of the species." He states that no two members of a given species are identical, and that if tests could be sufficiently refined, differences between any two isolants of the same variety would probably be detected. Dissociations serve to bring forth such differences and to enlarge our species concept. Caldis and Coons ('26) state that the variants which they studied rep-

resent semi-permanent variations which differ from the parent form somatically rather than genetically. They suggest that the variations may be due to a nutritional disturbance which may be overcome when the necessary conditions are supplied.

Studies on variation in fungi under cultural conditions were recorded at least as early as 1908 by Edgerton, who considered one of his variant forms of *Glomerella* as a mutation. In a later publication ('14) he remarked that this mutation was no doubt the minus strain of this fungus. Crabill ('14) observed a mutation in a pure Petri-dish culture of *Phyllosticta,* and later ('15) he reported the sudden development of a minus strain from a plus strain in *Coniothyrium pirinum.* This phenomenon was observed four times in single spore cultures, and his explanation was that the minus strain was a "sport or mutant arising from the plus strain at irregular and unprognosticable intervals." Blakeslee ('20) observed two mutations in non-sexually propagated races of *Mucor genevensis.* Other recorded mutations are those of Burger ('21) in *Colletotrichum gloeosporioides;* Chodat ('26) in *Aspergillus ochraceus* and *Phoma alternariacearum;* Christensen ('26), Christensen and Davies ('37), in *Helminthosporium sativum;* Christensen and Stakman ('26), Stakman, Christensen, Eide, and Peturson ('29), and Stakman, Christensen, and Hanna ('29), in *Ustilago Zeae,* in which they reported numerous mutations in monosporous cultures; Newton and Johnson ('27) in *Puccinia graminis Tritici;* Sellsschop ('29) in *Gloeosporium;* Rodenhiser ('30) in *Phlyctaena linicola;* Blochwitz ('31) in *Citromyces luteus,* which was described at first as *Penicillium javanicum;* and Eide ('35) in *Gibberella Saubinetii,* in which he attributed the variations to true mutations or at least resulting from genotypic changes. Burkholder ('23) isolated the gamma strain of *Colletotrichum Lindemuthianum* from beans in a field where only the alpha and beta strains had been known. Since it was nearer to the beta strain in its range of susceptible hosts, he concluded that it was a mutation from that strain.

Following the studies of Stevens ('22) on *Helminthosporium,* Mitter ('29), in his work on saltations in the genus *Fu-*

sarium, found a greater difference between parent and variant than between species and species.

Horne and Das Gupta ('29) reported an "ever-saltating" strain in *Diaporthe perniciosa.* It was impossible to prevent saltation from occurring in every cultural generation. The resulting variant or strain was always the same. The ability of the strain to saltate was independent of the medium and was inherent in the strain itself. In 1934, Das Gupta, after further work on these strains, reported saltation to be a conversion phenomenon. He concluded that properties of both strains were included in a single culture of DH_C and even in a single hypha but that the properties might be spatially separated in the hypha. One strain of DH_F had no visible expression in the presence of the other strain DH_C. However, if small segments were cut from a young hypha of DH_C mycelium, the majority would develop into DH_F, and the remainder into DH_C. Also the young DH_C mycelium was able to convert a DH_F culture into DH_C.

Matsuura ('30, '30A, '32) described four types of saltations in *Ophiobolus, Brachysporium, Alternaria,* and an Ascomycete from pears. Some of these saltations partially or totally reverted.

Leonian ('26) reported a reversible mutation in *Phytophthora.* Strain I gave rise at times to strain IV or a mixture of I and IV, and strain IV by mutation resulted at times in strain I.

Chaudhuri ('24) described some saltations which were permanent on Coons' agar and on oatmeal agar, but which reverted to the original when transferred to potato-mush agar. Transfers made to Coons' or oatmeal agar from the potato-mush agar did not produce the variant again.

The preponderance of cases of saltations or mutations have been of a varietal or specific nature. However, Wiltshire ('29, '32) reported a reversible saltation, where a *Stemphylium* culture in the presence of a bacterial colony gave rise to an *Alternaria* colony which in turn produced the *Stemphylium* colony again. Brett ('31) found a cyclic saltation in *Stemphylium.*

Within a colony of *Stemphylium,* dark spore heads were produced. Spores from these gave rise to *Alternaria* colonies in which the spores were produced in chains. The *Alternaria* spores gave rise to *Stemphylium* again, completing the cycle. Das Gupta ('30) working with *Cytosporina,* obtained not only the characteristic bent filiform spores, but also oval spores, characteristic of the genus *Phomopsis.* Christensen ('32) observed that sectors formed in monospore cultures of *Pestalozzia funerea* produced colonies conforming to those described for *Monochaetia.* Cultures of the *Monochaetia* type were also obtained from spores produced in pustules of *Pestalozzia funerea* on long-leaf pine. Saccardo (1884) distinguished the genus *Pestalozzia* from the genus *Monochaetia* on the number of setae, the former having two to six and the latter only one. Christensen believed that our previous conception of the genus *Pestalozzia* should be modified to include this form with one seta.

Orthogenetic or unidirectional saltations have been reported by Crabill ('15) in *Coniothyrium,* referred to above; and Das Gupta ('30) in *Cytosporina.* A particular strain may be reached in one saltation, or two or three saltations may take place before it has been developed. For example, Brown ('26) and Mohandra ('28) found that sometimes strain I gave rise to strain III, and at other times it produced strain II. The latter did not remain stable but gave rise to strain III. Das Gupta ('30) observed a particular strain in *Cytosporina* to be reached by one, or by a series of three saltations.

Burkholder ('25), working with *Fusarium mortiiphaseoli* grown in culture for a period of five years, discovered that it varied both in morphological characters and in virulence but would revert when inoculated into a bean plant and re-isolated. He thought that this might explain the great number of species and varieties in *Fusarium.* Chaudhuri ('31) has found the same to be true with some fungi with which he worked. Palmiter ('34) suggested, from his observations of cultures of *Venturia inaequalis,* that this species was not homogeneous but composed of many strains differing physiologically and morphologically.

La Rue ('22), from his work on *Pestalozzia Guepini,* stated that he found no evidence that distinct lines could be established by selection. However, Curzi ('30) reported that by selecting transfers of sectors of a monoconidial culture of *Fusarium Moronei,* he obtained two non-reversible strains. Strain alpha was the result of selection, through several generations, of the sector having the most profuse aerial mycelium; likewise, strain gamma was secured by selecting the sector having the scantiest aerial mycelium.

Greene ('33) found two types of variants in *Aspergillus Fischeri.* In the first type, the ascospores produced cultures practically identical with the original stock culture, while the conidia continued to give rise to the variant form. In the second type both ascospores and conidia produced the variant form.

Experimental Work

A. Sources of Cultures.—Cultures of two strains of *Gibberella Saubinetti* (Mont.) Sacc. were obtained in the summer of 1937 from Dr. Carl J. Eide, of the Division of Plant Pathology and Botany at the University of Minnesota. He collected the original perithecial material on old corn stubble in grain fields in Minnesota in 1932-1933.[1] These strains were designated by him as A36–1–V and A43–4–I–I. The first was obtained from a single ascospore of the original perithecial material but did not in turn produce perithecia in culture. The second culture was from an ascospore of a perithecium which formed on an old piece of inoculum after it had been transferred to fresh agar in a flask. It had been noted that the original ascospore culture, that from the ascus of a perithecium on corn stubble, had not formed fertile asci in culture on synthetic media. The same is true of this variant or strain.

B. Materials and Methods.—For the greater part of the culture work the substratum used was potato-dextrose agar. The special media used were Brown's "synthetic potato-dextrose" agar, Coons' synthetic medium, Leonian's agar, and

[1] These strains, along with some others, were used in investigations for his doctoral thesis (Eide, '35).

Richards' agar. Malt extract agar medium was used for some of the stock cultures.[2]

Single conidia were isolated with the Zeiss, and Bausch and Lomb micro-manipulators, using a glass needle in the manner described by Dickinson ('33). The conidia were immediately transferred to fresh drops of agar on cover slips which were then inverted on Van Tieghem cells in Petri dishes lined with moist filter-paper. After germination of the conidia, the agar drops were transferred to culture media in Erlenmeyer flasks, Petri dishes, or test-tubes. Where cultures produced no conidia, hyphal tips were cut off and used. This was done by transferring a bit of the mycelium to "water agar"[3] in Petri dishes. The scanty growth on this substratum made possible the isolation of single hyphae. In one instance the cutting tool consisted of a small piece of safety razor blade soldered to the end of a sewing needle, as described by Eide ('35). At another time, fine dissecting scissors were used. The tips of the hyphae were cut off under a dissecting microscope and, with a small portion of the agar, were transferred by means of a sterile instrument to fresh agar drops on cover slips. After the mycelia had developed slightly, the drops were transferred to media in test-tubes, Petri dishes, or Erlenmeyer flasks, as in the case of the conidia.

For cytological study, perithecia were dissected from the agar culture, killed and fixed in Hermann's fluid, embedded in paraffin, and serial sections cut at 7 microns. The stain used was Haidenhain's iron-alum haematoxylin, with phloxine as a counter-stain. The results were fairly good.

[2] The *potato-dextrose* agar consisted of: peeled potatoes, 400 gms.; dextrose, 10 gms.; agar, 17 gms.; and distilled water, 1 liter. *Brown's "synthetic potato-dextrose" agar:* glucose, 2 gms.; asparagin, 2 gms.; K_3PO_4, 1.25 gms.; $MgSO_4$, 0.75 gms.; agar, 17 gms.; and distilled water, 1 liter. *Coons' synthetic medium:* sucrose, 7.20 gms.; dextrose, 3.60 gms.; $MgSO_4$, 1.23 gms.; KH_2PO_4, 2.72 gms.; KNO_3, 2.02 gms.; agar, 17 gms.; and distilled water, 1 liter. *Leonian's agar:* peptone, 5 gms.; KH_2PO_4, 1 gm.; $MgSO_4$, 1 gm.; dextrose, 20 gms.; agar, 17 gms.; and distilled water, 1 liter. *Richards' agar:* cane sugar, 50 gms.; KNO_3, 10 gms.; KH_2PO_4, 5 gms.; $MgSO_4$, 2.5 gms.; $FeSO_4$, a trace; agar, 17 gms.; and distilled water, 1 liter. *Malt extract agar:* malt extract, 33.5 gms.; agar, 20 gms.; and distilled water, 1 liter.

[3] "Water agar" consisted of: agar, 1.5 gms.; and distilled water, 100 cc.

Small blocks of the mycelial culture of variants on agar were killed and fixed in Hermann's fluid, and embedded in celloidin. Serial sections were cut at 7½ microns (Foster, '26) and stained in Haidenhain's iron-alum haematoxylin and phloxine. The nuclei and cell walls were well differentiated, but individual hyphae could not be easily traced because of the compacted condition of the mycelium.

For the study of hyphal anastomoses, the following procedure was used: Conidia from two contrasting strains were allowed to germinate on a thin agar drop on a cover slip suspended over a Van Tieghem cell on a glass slide. Anastomoses of the hyphae were observed under the high power of the microscope, and camera-lucida drawings were made. For the stained preparations of hyphal anastomoses, the same procedure was used except that the agar drop was placed on the slide instead of the cover slip. The same killing fluid and stains were used as for perithecia. Differentiation of the septa was difficult because the greater part of the stain was removed from them in order to destain the agar drop.

C. Types of Strains at the Beginning of the Experiments.—A43–4–I–I, when grown on potato-dextrose agar in Petri plates, produced only a scant amount of white to pale pink aerial mycelium. The 2¼–2½-cm. salmon buff center of the upper surface was sometimes surrounded by two narrow bands, a wide band, and a narrow border. The first of these was purplish gray, the second vinaceous purple, the third purplish gray, and the border of submerged hyphae was vinaceous purple.[4] In other cases, the two narrow bands were absent (pl. 9, fig. 1). The center of the reverse was the same pattern as the upper surface, with the following colors, beginning at the center: apricot buff, dull violet black, dark vinaceous purple, dull violet black, and the border of dark vinaceous purple. There were numerous rudimentary perithecia on the upper surface. The dark vinaceous purple color was due to very numer-

[4] Colors given are those of Ridgway's "Color Standards and Color Nomenclature." 1912.

ous short thick-walled dark blue cells embedded in the agar. Conidia were abundant.

A36–1–V, when grown on potato-dextrose agar in flasks or Petri plates, produced an abundance of pale pink with some clay-colored aerial mycelium, cottony at first, then compacted with age (pl. 9, fig. 2). The bottom of the culture was dahlia purple to blackish red. The upper surface was level. Empty perithecia were numerous over the entire surface of the agar and many were embedded in it. Conidia were moderately numerous.

For convenience in this paper, the former strain will be referred to as A, and the latter as B.

D. Preliminary Cultural Work.—In order to determine the relative stability or variability in each strain, a number of conidia were isolated and grown under ordinary laboratory conditions on potato-dextrose agar. Forty-six conidia were isolated from strain B and transferred to agar drops in the manner described under "Materials and Methods." After germination of the conidia, the agar drops were transferred to potato-dextrose agar slants in test-tubes where they were allowed to grow until the surface of the medium was covered. No variation appeared in the test-tube cultures. Transfers were made in triplicate from each of the 46 test-tubes to potato-dextrose agar in Petri plates. After 25 days, the cultures appeared uniformly constant. About as much variation appeared between the plates from one conidium as between the sets of triplicates. Two and one-half months later, transfers were again made in triplicate to potato-dextrose agar in Erlenmeyer flasks. This time two types of variations appeared in six of the sets of triplicates, in from one to three flasks of each set (table 1). The remaining 126 flasks were of the "normal"[5] type for B.

Transfers of these variant cultures were made to Leonian's agar in Erlenmeyer flasks, along with transfers of the pa-

[5] "Normal" as used in this paper refers to the original B or original A when grown on potato-dextrose agar.

TABLE I

VARIATION IN SIX SETS OF TRIPLICATES OF SERIES B GROWN ON POTATO-DEXTROSE AGAR IN FLASKS AT ROOM TEMPERATURE

Triplicates	Form of variant	Color		Amount of aerial mycelium	Rudiments of perithecia
		Upper surface	Reverse		
B 5 3 flasks B35 2 flasks B44 2 flasks	Islands	"Normal" except for white islands	Normal	Much. Islands extended slightly above remainder of colony	Very few
B26 2 flasks B32 1 flask B45 2 flasks	Whole colony	Hay's maroon to acajou red	Normal	Little	Very numerous on surface and embedded in agar

rental type. B 26, B 32, and B 45[6] produced cultures identical with the parental type, while transfers from the white islands of B 5, B 35, and B 44 all produced cultures which were about 4 cm. tall and very cottony, in contrast to the parental type which was about 1 cm. tall, with the aerial hyphae more yellowish brown. Perithecia were not so abundant in the variant type and were embedded in the agar.

Cultures B 5–1, B 35–1, and B 44–1, from Leonian's agar, were grown again on potato-dextrose agar in flasks at the same time as B 5 of the parental type. The resulting cultures showed that B 35–1 and B 44–1 had reverted to the original "normal" B type identical with B 5, while B 5–1 remained a white cottony variant.

[6] The system of nomenclature used in this series of cultural experiments is as follows: A or B represents the original cultures with which this work was begun. The arabic numeral following is the number of the conidium isolated at random from that culture. Another arabic numeral following a dash represents the variant (island or sector) from this type. For example, A17–1 represents the culture from the first sector or island from the seventeenth conidial isolate of series A. If two sectors are formed in the same culture of A17, they are then designated as A17–1 and A17–2. If A17–1 again forms a sector, it will be designated as A17–1–1. This system was suggested by Dr. E. C. Stakman as a graphic way of recording the genealogy of each variant.

B 5 and B 5–1 were included in the experiment on "Attempts to Induce Variation in Strains," to be described later in this paper. Up to the present time B 5–1 has remained stable.

Of 46 single conidial cultures, 40 have remained stable through five cultural generations on potato-dextrose agar extending over a period of ten months; 6 formed variants in the fourth cultural generation. Three variants produced the parental type when transferred to Leonian's medium. Two of the remaining three reverted to the parental type when transferred from Leonian's medium to potato-dextrose agar again. One variant has remained stable through five cultural generations and on various media.

Fifty-two single conidial isolates were made from culture A in the same manner as described for culture B. No variations appeared in the test-tubes, but when transfers were made in

TABLE II

VARIATION IN FOUR SETS OF TRIPLICATES OF SERIES A GROWN ON POTATO-DEXTROSE AGAR IN PETRI PLATES AT ROOM TEMPERATURE

Triplicates	Form of variant	Color		Amount of aerial mycelium	Rudiments of perithecia
		Upper surface	Reverse		
A4 1 plate	Whole culture	Salmon orange with Eugenia red bands	Cinnamon rufous with Eugenia red bands	Some in center, border submerged	None
A17 1 plate	Sector	Same as above	Same as above	Same as above	None
A24 1 plate	Two sectors	Same as A17 with addition of dark purple zone toward center	Same as upper surface	Same as above	None
A43 1 plate	Whole culture	Salmon orange center, wide zone of dark greenish black, and a narrow zone of Eugenia red	Same as upper surface	Only scant amount on the dark zone	None

TABLE III

VARIATION IN FOURTEEN SETS OF TRIPLICATES OF SERIES A GROWN ON POTATO-DEXTROSE AGAR IN FLASKS AT ROOM TEMPERATURE

Triplicates	Form of variant	Color		Amount of aerial mycelium	Rudiments of perithecia
		Upper surface	Reverse		
A 5 1 flask A 7 2 flasks A 9 1 flask A15 1 flask A25 2 flasks A37 1 flask A45 1 flask	Whole culture	Light salmon orange center, wide dark purple band, and light salmon border	Same as upper surface	Very slight growth	Abundant on surface
A 8 2 flasks A12 1 flask A20 1 flask A21 3 flasks A23 2 flasks A46 1 flask	Whole culture	Same as A5 but with Eugenia red border	Same as A5 but with Eugenia red border	Very slight growth	Abundant on surface
A35 1 flask	Three sectors	Light salmon orange with 4 concentric narrow bands of light Eugenia red	Same as upper surface	None	None

triplicate to potato-dextrose agar in Petri plates, variations appeared in the four sets of triplicates which are recorded in table II. All except A17–1 produced the parental type in the next cultural generation, on potato-dextrose agar. About three months later, transfers were again made in triplicate to potato-dextrose agar in Erlenmeyer flasks, and the 14 variants which appeared this time were recorded in table III. All these possible variations, A5, A7, A8, A9, A12, A15, A20, A21, A23, A25, A35 (three sectors), A37, A45, and A46 were replated on potato-dextrose agar, and, with the exception of A35–1 (sector 1 of A35), all the plates showed the characters of the parental type.

E. Attempts to Induce Variation in Strains.—Since strains A and B were found to be fairly stable in the preliminary experiments when grown on potato-dextrose agar at room temperature, the cultures were grown on different substrata and

TABLE IV

VARIATION IN MACROSCOPIC CHARACTERS OF A17 GROWN ON VARIOUS MEDIA AT DIFFERENT TEMPERATURES

A 17	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		18° C.		
Color upper surface	Flesh-color mottled with Indian lake, dark purple border	Dark purple with wide rose-pink border	Seashell pink center, carmine border, remainder dark purple with slight mottling of seashell pink	Salmon buff center, remainder dark purple with slight mixture of salmon buff
Color reverse	Salmon with dahlia-purple mottling and dark purple fringed border	Dark purple with Vandyke red border	Same as upper but apricot buff instead of seashell pink	Same as upper
Type of growth	Uniform medium-heavy growth of aerial hypae	Uniform medium-heavy growth of aerial hyphae	Center and spots of medium-heavy growth of aerial hyphae, remainder light growth	Uniform light growth of aerial hyphae
Topography	Deeply wrinkled	Concentric furrow, few short radial furrows, lobed margin	Very shallow concentric furrow, and large shallow wrinkles	Short radial furrows, very uneven margin
Rudiments of perithecia	Few, small	Very numerous	Very numerous	Very few

at different temperatures in order to learn whether variations could be induced. Consequently, A17, A17–1, A4, A24, A43, A35, A35–1, B5, and B5–1 were grown in duplicate on Brown's "synthetic potato-dextrose" agar, Coons' synthetic medium, Leonian's agar, potato-dextrose agar, and Richards' agar at 18°, 20°, 25°, and 30°C. and the macroscopic characters recorded after 25 to 30 days. With the exception of A17–1, which produced no conidia and in which hyphal tips were substituted, single conidial isolates were used.

On Brown's medium (pH 5.6), A17 showed the appressed type of growth, or with very little aerial hyphae in the center. The color of both upper and reverse surfaces was seashell pink at temperatures 18°, 20°, and 25° C. At 30° C. the color was

TABLE IV (Continued)

A 17	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		20° C.		
Color upper surface	Salmon with very light mottling of Indian lake with fringe of dark purple	Center and wide border rose pink, wide uneven band of dark purple around center	Seashell pink center, carmine border, remainder dark purple with very slight mottling of seashell pink	Salmon buff center, remainder dark purple
Color reverse	Same as upper	Same as upper	Same as upper but carmine instead of rose pink	Same as upper but apricot buff instead of seashell pink
Type of growth	Uniform light growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Uniform light growth of aerial hyphae except center of very light aerial growth	Uniform light growth of aerial hyphae
Topography	Entire surface deeply wrinkled	Very shallow concentric furrow, slight radial furrowing, very uneven margin	Very shallow concentric furrow, hint of radial furrows around center	Level, no furrows
Rudiments of perithecia	Numerous	Numerous	Very numerous	Very few

pale salmon. The surface was smooth and no rudiments of perithecia were formed. Variations on other media are shown in table IV.

Growth of A17–1 on Brown's medium was appressed at 18° C., and appressed with few aerial hyphae at all other temperatures. The color was seashell pink; the surface was level, with no wrinkles or furrows; and no rudiments of perithecia were formed. Variations on other media are shown in table V.

A4, on Brown's medium, was mostly of the appressed type with very few aerial hyphae in the center. At 20° C., the growth did not exceed 1 cm. in diameter and developed no color. At 18° and 25° C., it was seashell pink, and at 30°, pale salmon color with orange pink reverse. The surface was

TABLE IV (Continued)

A 17	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		25° C.		
Color upper surface	Salmon	Center and border flesh pink, remainder dark purple mottled with flesh pink	Orange pink with radiate splashes of dark purple	Salmon buff with wide border of dark purple
Color reverse	Salmon	Same as upper but carmine instead of flesh pink	Cinnamon rufous with radiate splashes of dark purple	Same as upper but salmon instead of salmon buff
Type of growth	Mostly appressed, only very slight uniform growth of aerial hyphae	Felt-like growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Uniform light growth of aerial hyphae
Topography	Entire surface deeply wrinkled	Very shallow concentric furrow	Very shallow concentric furrow, hint of radial furrows or large wrinkles	Level except for very shallow concentric furrows near center
Rudiments of perithecia	None	Numerous	None	None
		30° C.		
Color upper surface	Light apricot orange	Chatenay pink	Apricot buff	Light salmon orange
Color reverse	Apricot buff	Apricot buff, very deeply mottled with carmine	Apricot buff	Light salmon orange
Type of growth	Appressed mostly; very slight growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Appressed except light growth of aerial hyphae in center and furrows	Appressed
Topography	Entire surface deeply wrinkled	Deep concentric furrow or none near center; 18–20 radial furrows	Level except 18–20 shallow radial furrows	Level except few shallow wrinkles in center
Rudiments of perithecia	None	None	None	None

TABLE V

VARIATION IN MACROSCOPIC CHARACTERS OF A17-1 GROWN ON VARIOUS MEDIA AT DIFFERENT TEMPERATURES

A 17–1	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		18° C.		
Color upper surface	Flesh pink	Alizarine pink; sector 1—rose red; sector 2—alizarine pink with rose red border	Orange pink with slight mottling of dark purple	Orange pink with light fringe of dark purple
Color reverse	Deep seashell pink	Acajou red with apricot buff margin; sector 1—Vandyke red; sector 2—acajou red	Salmon with dark purple mottling	Light salmon orange with light fringe of dark purple
Type of growth	Uniform medium-heavy growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Uniform light growth of aerial hyphae
Topography	Level—no furrows	Level except furrows between sectors and parent, and few radial wrinkles near center	Level except hint of radial furrowing	Level—no furrows
Rudiments of perithecia	None	None	Few, very small	None

smooth with no rudiments of perithecia produced. Variations on other media are shown in table VI.

A24, on Brown's medium, showed no variation except at 30° C. where there was no growth. Growth was of the appressed type with very little aerial hyphae in the center, the color was seashell pink, and rudiments of perithecia were lacking. Variations on other media are shown in table VII.

A43, on Brown's medium, was identical with A17 described above, except that there was very slight growth at 20° C. Variations on other media are shown in table VIII.

TABLE V (Continued)

A 17–1	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		20° C.		
Color upper surface	Orange pink	Alizarine pink with narrow circle of dark purple around center	White to pale pink	Orange with light fringe of dark purple
Color reverse	Orange pink	Carmine mottled with apricot buff	White to pale pink	Light salmon orange with dark purple border
Type of growth	Uniform light growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Extremely slight growth, appressed	Uniform light growth of aerial hyphae
Topography	Deep concentric furrow, large wrinkles, very uneven border	Shallow concentric furrow; 8–10 short radial furrows near center	Level	Level—no furrows
Rudiments of perithecia	None	None	Very few	Very few
		25° C.		
Color upper surface	Salmon color	Alizarine pink with salmon center	Onion-skin pink	La France pink, some dark purple in border
Color reverse	Apricot buff	Carmine red slightly mottled with apricot buff	Buff pink	Same as above
Type of growth	Uniform light growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Appressed except light growth of aerial hyphae in center, border, and furrows	Appressed except few aerial hyphae in center and border
Topography	Shallow concentric furrow, 12–14 radial furrows	Shallow concentric furrow near center; 6–12 short radial furrows	Shallow concentric furrow or none; 5–10 short to long radial furrows	Level, no furrows
Rudiments of perithecia	None	None	None	None

TABLE V (Continued)

A 17–1	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		30° C.		
Color upper surface	Salmon color	Flesh pink	Orange pink	Orange pink; sector—La France pink with traces of deep blue in dark purple border
Color reverse	Apricot buff	Light salmon orange with slight carmine mottling	Apricot buff	Orange pink; sector—same with slight traces of dahlia purple and dark blue
Type of growth	Light growth of aerial hyphae, lighter in center	Uniform medium-heavy growth of aerial hyphae	Uniform light growth of aerial hyphae	Appressed except few aerial hyphae in center and covering sector
Topography	Level except numerous short radial furrows in the lobed margin	Level—no furrows	Level—no furrows	Level—no furrows
Rudiments of perithecia	None	None	None	None

A35, on Brown's medium, varied only in color at the different temperatures. At 18° and 20° C., the color was seashell pink, at 25° it was salmon-buff, with the reverse seashell pink, and at 30° orange pink with safrano pink reverse. The growth type was appressed and no rudiments of perithecia were formed. Variations on other media are shown in table IX.

A35–1 produced only the appressed type of growth on Brown's medium, except at 20° C., where there was no growth. The color varied from seashell pink at 18°, through salmon buff with seashell buff reverse at 25°, to light salmon orange with orange pink reverse at 30°. The surface was smooth and no rudiments of perithecia were formed. Variations on other media are recorded in table X. B 5 and B 5–1 will be discussed later in this paper.

TABLE VI

VARIATION IN MACROSCOPIC CHARACTERS OF A4 GROWN ON VARIOUS MEDIA AT DIFFERENT TEMPERATURES

A 4	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		18° C.		
Color upper surface	Flesh color mottled with Indian lake and having very wide border of dark purple	Dark purple with wide rose pink border; sector—dusky auricula purple	Seashell pink center, carmine border, remainder dark purple with slight mottling of seashell pink	Salmon pink center, remainder dark purple with slight mixture of salmon buff
Color reverse	Salmon with dahlia purple mottling, very wide border of dark purple	Same as above	Same as upper, but apricot buff instead of seashell pink	Same as above
Type of growth	Uniform medium-heavy growth of aerial hyphae	Medium-heavy growth of aerial hyphae; sector—light growth of aerial hyphae	Center and spots of medium-heavy growth of aerial hyphae, remainder light growth	Uniform light growth of aerial hyphae
Topography	Level—except few shallow wrinkles near center	Very shallow concentric furrow; uneven margin	Very shallow concentric furrow, and large shallow wrinkles	Some short radial furrows, very uneven margin
Rudiments of perithecia	Very few	Very numerous	Very numerous	Very few
		20° C.		
Color upper surface	Flesh color slightly mottled with Indian lake and having dark purple fringe	Center and wide border rose pink; between, wide uneven band of dark purple	Salmon pink center, carmine border, remainder dark purple with very slight mottling of seashell pink	Salmon buff center and dark purple border
Color reverse	Salmon with dahlia purple mottling and dark purple fringe	Same as above but carmine instead of rose pink	Same as upper but apricot buff instead of seashell pink	Same as above
Type of growth	Uniform light growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Light growth of aerial hyphae with ring of very light growth around center	Uniform light growth of aerial hyphae
Topography	Entire surface deeply wrinkled	Shallow concentric furrows; 8–10 short radial furrows near center	Very shallow concentric furrow, very shallow large wrinkles	Level—no furrows
Rudiments of perithecia	Numerous	Numerous	Very numerous	Very few

TABLE VI (Continued)

A 4	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		25° C.		
Color upper surface	Salmon	Center and border flesh pink; between, dark purple mottled with flesh pink	One plate orange-pink with radiate splashes of dark purple; one plate orange-pink, dark purple mottled; secant —⅓ size of plate, apricot buff	Salmon-buff with wide border of dark purple
Color reverse	Salmon	Same as upper, but carmine instead of flesh pink	Apricot buff with dark purple mottling	Salmon color with dark purple border
Type of growth	Only very light growth of aerial hyphae; mostly appressed	Felt-like growth of aerial hyphae	Uniform light growth of aerial hyphae; secant—very light growth of aerial hyphae	Uniform light growth of aerial hyphae
Topography	Entire surface deeply wrinkled	Shallow concentric furrow; some large wrinkles	Very shallow concentric furrow; hint of radial furrows or large wrinkles; no wrinkles in secant	Level—hint of concentric furrow
Rudiments of perithecia	None	Numerous	None	None

In general with strain A, media seemed to be responsible for greater variation in a given culture than temperature. Growth was greatest on Leonian's agar, decreasing in amount on potato-dextrose agar, Richards', Coons', to Brown's medium, where the development was least. Brown ('26) found the optimum pH for growth in his *Fusarium* species to lie toward the acid end. This is in agreement with the work here on *Gibberella Saubinetii.*

All cultures tended to produce the appressed type of growth, or with few aerial hyphae in the center on Brown's medium. Through the series the amount of aerial hyphae increased with

TABLE VI (Continued)

A 4	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		30° C.		
Color upper surface	Apricot buff; sectors (2), daphne pink	Chatenay pink	Orange pink	Orange pink with light fringe of dark purple
Color reverse	Apricot buff; sectors (2), dahlia purple	Light salmon orange with slight carmine mottling	Cinnamon rufous	Orange pink
Type of growth	Appressed mostly; very slight growth of aerial hyphae; sectors—light growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Very light growth of aerial hyphae
Topography	Entire surface deeply wrinkled	20 or more shallow radial furrows	Shallow concentric circle near center; 25–30 shallow to deep radial furrows	Level except for very slight wrinkling in center
Rudiments of perithecia	None	None	None	None

the amount of growth, being greatest on Leonian's agar as is shown in pl. 10, fig. 4.

Rudiments of perithecia were not produced on Brown's medium at any temperature, but developed on all other media at 18° and 20° C., and on Leonian's at 25° C. also. Lower temperatures seemed to favor development of more numerous and larger perithecial rudiments. Tschudy ('37) observed that species of *Chaetomium* do not develop normal perithecia on peptone media. Abundant primordia would form on the surface of the agar but would develop no further. The fact that mature perithecia developed on agar alone showed the peptone to be an inhibiting factor. The addition of 2 per cent alcohol to the sterilized nutrient agar had the same inhibiting effect on the development of perithecia as the peptone. In this investigation on *Gibberella Saubinetii,* larger and more numerous

TABLE VII

VARIATION IN MACROSCOPIC CHARACTERS OF A 24 GROWN ON VARIOUS MEDIA AT DIFFERENT TEMPERATURES

A 24	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		18° C.		
Color upper surface	Flesh color mottled with Indian lake; very wide border of dark purple	Dark purple with wide rose pink border	Seashell pink center, border carmine, remainder dark purple with slight mottling of seashell pink	Salmon buff center, remainder dark purple with slight mixture of salmon buff
Color reverse	Salmon with dahlia purple mottling and dark purple border	Dark purple with Vandyke red border	Same as upper except apricot buff instead of seashell pink	Same as above
Type of growth	Uniform medium-heavy growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Center and spots of medium-heavy growth of aerial hyphae; remainder light growth	Uniform light growth of aerial hyphae
Topography	Few shallow wrinkles near center	Concentric furrow, few short radial furrows, lobed margin	Very shallow concentric furrow, and large shallow wrinkling	Some short radial furrows, very uneven margin
Rudiments of perithecia	Very few	Very numerous	Very numerous	Very few
		20° C.		
Color upper surface	Flesh color mottled with Indian lake; dark purple margin	Center and wide border rose pink; wide uneven band of dark purple between	Seashell pink center, carmine border, remainder dark purple with very slight mottling of seashell pink	No growth
Color reverse	Salmon with dahlia purple mottling and dark purple border	Same as upper but carmine instead of rose pink	Same as upper but apricot buff instead of seashell pink	No growth
Type of growth	Uniform light growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Uniform light growth of aerial hyphae except ring of very light aerial hyphae around center	No growth
Topography	Entire surface deeply wrinkled	Level—no furrows, uneven margin	Very shallow concentric furrow, hint of radial furrows near center	No growth
Rudiments of perithecia	Numerous	Numerous	Very numerous	None

TABLE VII (Continued)

A 24	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		25° C.		
Color upper surface	Salmon	Center and border flesh pink, remainder dark purple mottled with flesh pink	Seashell pink center; remainder dark purple mottled with seashell pink; dahlia purple traces in border	Salmon buff, wide border of dark purple
Color reverse	Salmon	Same as upper but carmine instead of flesh pink	Same as upper but cinnamon rufous instead of seashell pink	Salmon color with dark purple border
Type of growth	Mostly appressed; very slight uniform growth of aerial hyphae	Felt-like growth of aerial hyphae	Uniform light growth of aerial hyphae	Uniform light growth of aerial hyphae
Topography	Entire surface deeply wrinkled	Very shallow concentric furrow; 10 very shallow radial furrows; uneven margin	Very shallow concentric furrow, hint of radial furrows or large wrinkles	Level, except one very shallow concentric furrow around center
Rudiments of perithecia	None	Numerous	None	None
		30° C.		
Color upper surface	Apricot buff	No growth	White	Light salmon orange
Color reverse	Apricot buff	No growth	White	Light salmon orange
Type of growth	Appressed, mostly very slight growth of aerial hyphae	No growth	Appressed extremely light growth	Appressed
Topography	Entire surface deeply wrinkled	No growth	Level	Level except few shallow wrinkles in center
Rudiments of perithecia	None	No growth	None	None

TABLE VIII

VARIATION IN MACROSCOPIC CHARACTERS OF A43 GROWN ON VARIOUS MEDIA AT DIFFERENT TEMPERATURES

A 43	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		18° C.		
Color upper surface	Flesh color mottled with Indian lake, very wide border of dark purple	Dark purple with wide rose pink border; sectors (2), old rose with claret brown apices	Seashell pink center, carmine border, remainder dark purple with slight mottling of seashell pink	Salmon buff center, remainder dark purple with slight mixture of salmon buff
Color reverse	Salmon with dahlia purple mottling, wide border of dark purple	Dark purple with Vandyke red border; sectors (2), same as for upper surface	Same as upper but apricot buff instead of seashell pink	Same as above
Type of growth	Uniform medium-heavy growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae; sectors, light growth of aerial hyphae	Center and spots of medium-heavy growth of aerial hyphae, remainder light growth	Uniform light growth of aerial hyphae
Topography	Level, no furrows	Concentric furrow, few short radial furrows, much-lobed margin	Very shallow concentric furrow, large shallow wrinkling	Some short radial furrows, very uneven margin
Rudiments of perithecia	Very few	Very numerous	Very numerous	Very few

perithecial rudiments were produced on Leonian's medium, a peptone agar, than on other media employed. It has been stated previously that no fertile perithecia have been observed in strains A and B.

The color and color patterns varied widely with the different media used, and to a less extent with the temperature. With the decrease in temperature the colors became uniformly darker, usually red and dark purple. A deeper pigmentation was developed at 18° and 20° C. (see pl. 11). This agrees with the work of Crabill ('15) on production of pigmentation in *Coniothyrium,* but accords only in part with the statement of

TABLE VIII (Continued)

A 43	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		20° C.		
Color upper surface	Flesh color slightly mottled with Indian lake, dark purple fringed border	Center and wide border rose pink, remainder wide uneven band of dark purple	Seashell pink center, light carmine border, remainder dark purple with slight mottling of seashell pink	Salmon buff center, dark purple border
Color reverse	Salmon with dahlia purple mottling and dark purple fringed border	Same as upper but carmine instead of rose pink	Same as upper but apricot buff instead of seashell pink	Same as above
Type of growth	Uniform light growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Uniform light growth of aerial hyphae except ring of very light aerial hyphae around center	Uniform light growth of aerial hyphae
Topography	Deeply wrinkled near center, more shallow toward margin	Shallow concentric furrow, uneven margin	Very shallow concentric furrow, very large shallow wrinkles	Level, no furrows
Rudiments of perithecia	Numerous	Numerous	Very numerous	Very few

Ashley, Hobbs, and Roistrick ('37) that the optimum temperature for development and pigmentation in *Gibberella Saubinetii* is 24° C. Horne and Mitter ('27) found the intensity of coloring in some *Fusarium* species to be associated with a high C:N ratio. Snyder ('33) observed that the pH value, as well as the high carbohydrate content of the medium, may influence pigmentation. "The color of the mycelium" as stated in Gäumann-Dodge ('28, p. 233) "is largely dependent on the nutrition, especially on the reaction of the substrate.—The red mycelium of *Gibberella Saubinetii* on alkaline media becomes yellow on acid." In the present investigation, strain A became yellow on Richards' medium (pH 4.5) but red and purple on Leonian's (pH 4.8) and potato-dextrose agar (pH 5.8). On

TABLE VIII (Continued)

A 43	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		25° C.		
Color upper surface	Salmon	Center and border flesh pink, remainder dark purple mottled with flesh pink	Orange pink with radiate splashes of dark purple	Salmon buff, wide border of dark purple
Color reverse	Salmon	Same as upper but carmine instead of flesh pink	Cinnamon rufous with radiate splashes of dark purple	Salmon with wide border of dark purple
Type of growth	Mostly appressed, only very slight uniform growth of aerial hyphae	Felt-like growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Uniform light growth of aerial hyphae
Topography	Entire surface deeply wrinkled	Level except radial furrows in the lobed margin	Very shallow concentric furrow, hint of radial furrows or large wrinkles	Level, no furrows
Rudiments of perithecia	None	Numerous	None	None
		30° C.		
Color upper surface	Apricot buff	Chatenay pink	Apricot buff	Light salmon orange
Color reverse	Apricot buff	Apricot buff very deeply mottled with carmine	Apricot buff	Apricot buff
Type of growth	Mostly appressed, very slight growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Appressed except light growth of aerial hyphae in center and furrows	Extremely light growth of aerial hyphae
Topography	Entire surface deeply wrinkled	Shallow concentric circle near center, 10–12 shallow radial furrows	Level except 18–20 shallow radial furrows	Level except very shallow wrinkling in center
Rudiments of perithecia	None	None	None	None

TABLE IX

VARIATION IN MACROSCOPIC CHARACTERS OF A 35 GROWN ON VARIOUS MEDIA AT DIFFERENT TEMPERATURES

A 35	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		18° C.		
Color upper surface	Flesh color mottled with Indian lake, dark purple border	Dark purple with wide rose pink border	Seashell pink center, carmine border, remainder dark purple slightly mottled with seashell pink	Salmon buff center, remainder dark purple with slight mixture of salmon buff
Color reverse	Salmon with dahlia purple mottling and dark purple fringed border	Dark purple with Vandyke red border	Same as upper but apricot buff instead of seashell pink	Same as above
Type of growth	Uniform medium-heavy growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Center and spots medium-heavy growth of aerial hyphae, remainder light growth	Uniform light growth of aerial hyphae
Topography	Level, no furrows	Concentric furrow, few short radial furrows, lobed margin	Very shallow concentric furrow, and wide shallow wrinkles	Same short radial furrows, very uneven margin
Rudiments of perithecia	Few, small	Very numerous	Very numerous	Very few
		20° C.		
Color upper surface	Flesh color mottled with Indian lake, dark purple fringed border	Center and wide border rose pink, wide uneven band of dark purple between	Whitish to very pale pink	Salmon buff center, remainder dark purple
Color reverse	Salmon with dahlia purple mottling, dark purple fringed border	Same as upper but carmine instead of rose pink	Same as above	Same as above
Type of growth	Uniform light growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Very slight development, appressed except for few aerial hyphae	Uniform light growth of aerial hyphae
Topography	Surface deeply wrinkled in center, more shallow toward margin	Very shallow concentric furrow, hint of radial furrows, uneven margin	Level, no furrows	Level, no furrows
Rudiments of perithecia	Numerous	Numerous	None	Very few

TABLE IX (Continued)

A 35	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		25° C.		
Color upper surface	Salmon slightly mottled with deep slate violet	Center and border flesh pink, remainder dark purple mottled with flesh pink	Orange pink with radiate splashes of dark purple	Salmon buff with wide border of dark purple
Color reverse	Salmon slightly mottled with dull dusky purple	Same as upper but carmine instead of flesh pink	Cinnamon rufous with radiate splashes of dark purple	Salmon with dark purple border
Type of growth	Uniform light growth of aerial hyphae	Felt-like growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Uniform light growth of aerial hyphae
Topography	Entire surface deeply wrinkled	Shallow concentric furrow, large wrinkles somewhat radial, uneven margin	Very shallow concentric furrow, hint of radial furrows or large wrinkles	Very shallow concentric furrow near center, or none
Rudiments of perithecia	None	Numerous	None	None
		30° C.		
Color upper surface	Apricot buff	Chatenay pink	Flesh color	Grenadine
Color reverse	Apricot buff	Apricot buff very deeply mottled with carmine	Apricot buff	Grenadine pink
Type of growth	Mostly appressed, very slight growth of aerial hyphae	Uniform medium-heavy growth of aerial hyphae	Heavy growth of aerial hyphae	Light growth of aerial hyphae
Topography	Entire surface deeply wrinkled	8–12 very shallow radial furrows	1 deep concentric furrow near margin, about 12 radial furrows	1 concentric furrow, remainder wrinkled and somewhat warty
Rudiments of perithecia	None	None	None	None

TABLE X

VARIATION IN MACROSCOPIC CHARACTERS OF A 35-1 GROWN ON VARIOUS MEDIA AT DIFFERENT TEMPERATURES

A 35-1	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		18° C.		
Color upper surface	Bittersweet pink	Bordeaux with few apricot buff aerial hyphae in center	Chestnut with radiating broken lines of ox-blood red	Orange pink
Color reverse	Orient pink	Bordeaux	Burnt sienna	Orange pink
Type of growth	Appressed	Appressed	Appressed	Appressed
Topography	Shallow wrinkles over entire surface	Level, no furrows	Level, no furrows	Some short radial furrows, very uneven margin
Rudiments of perithecia	None	None	None	None
		20° C.		
Color upper surface	Light salmon orange	Bordeaux with few apricot buff aerial hyphae in center	Light buff	Orange pink
Color reverse	Orange pink	Bordeaux	Light buff	Orange pink
Type of growth	Appressed	Appressed	Appressed, very light growth	Appressed
Topography	Shallow wrinkles toward center	Very shallow concentric furrow	Level, no furrows	Level, no furrows
Rudiments of perithecia	None	None	None	None

Coons' (pH 6.5) and Brown's (pH 5.6) the strain was very pale pink to colorless. The hydrogen-ion concentration of the media was taken only at the beginning of the experiment before inoculations had been made, and it is quite probable that substances produced by the fungus during growth on certain media tend to neutralize some of the acid, thus producing the red color. A correlation between rate of growth and pigmentation

TABLE X (Continued)

A 35-1	Richards' pH 4.5	Leonian's pH 4.8	Potato-dextrose pH 5.8	Coons' pH 6.5
		25° C.		
Color upper surface	Salmon orange	Bordeaux	Cinnamon rufous with broken radiate lines of dahlia purple	Salmon buff
Color reverse	Apricot buff	Bordeaux	Same as above	Pale flesh color
Type of growth	Appressed	Appressed	Appressed	Appressed
Topography	Very shallow concentric furrow	Very shallow concentric furrow	Very shallow concentric furrow	Level, no furrows
Rudiments of perithecia	None	None	None	None
		30° C.		
Color upper surface	Orange chrome	Brick red	Flesh color	No growth
Color reverse	Medium salmon orange	Hay's russet	Apricot buff	No growth
Type of growth	Appressed	Appressed	Light growth of aerial hyphae	No growth
Topography	Level, no furrows	Level, no furrows	About 12–13 shallow radial furrows	No growth
Rudiments of perithecia	None	None	None	No growth

in *Alternaria Solani* was pointed out by Bonde ('29). The maximum for both was at 25–30° C.

Variations as referred to in the previous paragraphs are not of a permanent nature, as was shown in subsequent transfers to other media. They are the non-heritable changes due to environment, the "eco-variants" of Dickinson ('32).

A total of nine sectors was formed in six of the 360 plates used in this investigation, five being on Leonian's agar at 18° C., one on potato-dextrose agar at 25° C., one on Coons' medium at 30° C., and two on Richards' medium at 30° C. Distribution of sectors according to cultures, media, and temperature are shown in table XI.

TABLE XI

DISTRIBUTION OF SECTORS ACCORDING TO CULTURES, MEDIA, AND TEMPERATURES

Cultures	Number of sectors	Medium	Temperature
A4	1	Leonian's	18° C.
A4	1	potato-dextrose	25° C.
A4	2	Richards'	30° C.
A17–1	2	Leonian's	18° C.
A17–1	1	Coons'	30° C.
A43	2	Leonian's	18° C.

In an attempt to isolate these possible saltants, inocula were taken from the apex, center, and outer edge of each sector and transferred to fresh media. A transfer of the part not sectoring was made at the same time, for comparison. Only the two sectors from A17–1 on Leonian's agar at 18° C. proved to be of a different form from the parent culture. These variants, designated as A17–1–1 and A17–1–2, were identical in appearance. They will be discussed further under the next topic.

B 5 and B 5–1 produced no sectors, islands, or other visible modifications when grown on the different media at different temperatures. B 5–1, on all media and at all temperatures, was lighter in color than the corresponding culture of B 5. The colors ranged from ox-blood red to Eugenia red except on Brown's medium, where it was light buff or pale pinkish buff to white. Depth of color increased with decrease in temperature.

The B series, like the A series previously mentioned, did not grow well on Brown's medium, the growth ranging from none (B 5 at 20° C.) through 1 cm. (B 5–1 at 20° C.) to covering the surface of the medium at higher temperatures. No rudiments[7] of perithecia were formed on this medium.

On all other media and at all temperatures used, the aerial growth filled the Petri plate. No rudiments of perithecia were

[7] The term "perithecial rudiments" has been used, because up to this time no mature ascospores had been found. One mature perithecium of B 5 on Leonian's agar at 18° C. was found when serial sections were cut.

produced at 30° C. Distribution at all other temperatures is shown in table xii.

TABLE XII

DISTRIBUTION OF PERITHECIAL RUDIMENTS ACCORDING TO MEDIA AND TEMPERATURE

Media	Strain or variant	25° C.	20° C.	18° C.
Coons'	B 5	Numerous	Very numerous	Very numerous
	B 5–1	None	Very few	Very few
Richards'	B 5	None	Numerous	Numerous
	B 5–1	None	None	None
Potato-dextrose	B 5	Numerous	Very numerous	Very numerous
	B 5–1	None	None	Numerous
Leonian's	B 5	Numerous	Very numerous	Very numerous
	B 5–1	None	Numerous	Numerous

Vasudeva ('30) found that certain strains of *Fusarium* when grown on shallow plates of an acid phosphate medium readily gave rise to strangely diverging sectors. Many of these sectors did not prove to be variants and were therefore termed "false sectors." Matsuura ('32) observed that temperature and composition of the nutrient media influence the frequency of mutation, being greater on potato decoction agar. Stakman, Christensen, Eide, and Peturson ('29) have shown there were more numerous variations in *Ustilago Zeae* on some media than on others and that they occur at comparatively high temperatures. However, Tu ('30) was unable to induce permanent variations in species of *Fusarium* by subjecting them to different media and incubating them at various temperatures. Brown ('26, '28) reported that saltations in *Fusarium* were more frequent on concentrated Richards' solution agar than on many other media. This was confirmed by Chaudhuri ('31). Christensen ('26) observed more numerous mutations in strains of *Helminthosporium* on one media than on another under similar conditions. In 1937 he and Davies demonstrated the frequency of mutation of *Helminthosporium* on a bacteria-staled medium.

One race cultured for seventeen years produced eighty-one varieties from seventeen colonies on the bacteria-staled medium, while on the same number of colonies on potato-dextrose agar none was produced. Paxton ('33) was able to secure consistent mutations in *Helminthosporium sativum* on Czapek's medium, with the $NaNo_3$ omitted. An average of five sectors in each plate was observed. On Czapek's medium which was used as a check, very few sectors were formed. Chaudhuri ('31) found that the greater number of variants would revert to the original form when grown on some medium or when returned to the original host. He considered saltation in fungi to be purely a nutritive phenomenon, unless it be a rare case of true mutation. Coons and Larmer ('30) obtained variants of *Cercospora beticola* in cultures on artificial media. They regarded them as modified forms with nutritional disturbances playing a role in their development. Caldis and Coons ('26) expressed the same opinion for variants in *Colletotrichum* and *Cladosporium*. This disturbance might have been due to the connections with the substratum being severed by the drying of the mycelium, or the variant might have arisen from cells the protoplasm of which had been poisoned or had been affected by some other unknown factor.

Chodat ('26) favored the premutation theory of de Vries as an explanation for the appearance of variants in the various media. His belief was that the media did not produce the variants but only made visible the pre-existing mutations. Shear and Wood ('13) found it impossible to trace any causal relation or connection between most of the phenomena of variation observed in *Glomerella* and the conditions of environment to which the cultures were subjected.

Induced variation by heating the ascospores of *Eurotium* was reported by Barnes ('28, '31). Some of these variants had remained stable over a period of about four years. He suggested that, apart from the probable nuclear changes, a general derangement of the physiological balance of the cell may be responsible for variation. Dickson ('32) and Goldring ('36) were unable to obtain any variants by this method. Chris-

tensen ('29), and later Mitra ('31), observed that certain lines of *Helminthosporium* species mutate only at the higher temperatures for growth. Christensen found the optimum temperature for mutations to be 25–27° C., while Mitra noted that it was 30° C. In the present investigation on *Gibberella Saubinetii,* more variations appeared at 18° C. than at the three higher temperatures, as is shown in table XI.

F. Constancy of Mycelial and Conidial Forms.—In the present investigations, also in *Fusarium* studies carried on by Brown ('28), sectors were formed which were mycelial in character and produced few or no conidia. At other times in mycelial types, sectors have been formed with very numerous conidia and very little aerial mycelium. On A17–1, a sector of A17, no conidia developed when transferred to fresh potato-dextrose agar and grown at room temperature as described earlier in this paper. A17, although mycelial in character, produced many conidia under the same conditions.

A17 and A17–1 were grown on five different media and at four different temperatures as described in the preceding experiment, and the macroscopic characters are represented in tables IV and V. They were examined microscopically to determine the constancy of these forms under various cultural conditions. These results are given in table XIII.

Inocula from the two sectors of A17–1 were transferred to fresh potato-dextrose agar in Petri plates. A transfer of inoculum of A17–1 was made at the same time for comparison. A17–1–1 and A17–1–2 remained the typical sector color, or perhaps the color was slightly deeper. The upper surface was Eugenia red to Vandyke red and the reverse was apricot buff to acajou red. The mycelium was mostly appressed, with very little aerial hyphae, and was somewhat wet in appearance. The surface was smooth except for one (rarely two) concentric furrows and numerous short shallow radial furrows. These cultures were definitely of the conidial type. A17–1 remained a mycelial type and produced no condia on potato-dextrose agar at room temperature.

TABLE XIII

PRODUCTION OF CONIDIA IN LINES A17 AND A17–1 UNDER VARIED ENVIRONMENTAL CONDITIONS.

Temperature	Richards'	Potato-dextrose	Leonian's	Coons'	Brown's
A 17 (mycelial form producing numerous conidia):					
18° C.	Many	Few	Few	Many	Few
20° C.	Many	Very few	Very few	Few	None
25° C.	Very many	Few	Few	Few	Very few
30° C.	Very many	Few	Very few	Very few	Very, very few
A 17–1 (mycelial form producing no conidia):					
18° C.	Very few	None	None, except on 2 sectors where numerous	None	None
20° C.	Very few	Very few	None	None	None
25° C.	Few	Very few	None	1 plate—many 1 plate—very few	None
30° C.	Very few	Very few	None	None	Very few

Inocula from A17–1–1 and A17–1–2 were transferred to potato-dextrose agar in test-tubes. Instead of the appressed conidial form again, an aerial form was produced. It was pale pinkish and powdery in appearance, and microscopic examination showed numerous conidia.

Attempts to Induce the Pionnotal Stage to Revert to the Aerial Mycelial Stage

In cultural work with species of *Fusarium,* variants have developed which were in the form of pionnotes.[8] However, so far the writer has been able to find no instance reported of a culture completely reverting to the aerial mycelial phase after it once had gone into the pionnotal phase. In this work A35–1

[8] Pionnotes is merely a biological term for an effuse conidial stage, with a maximum of conidia and a minimum of aerial mycelium, which, as a rule, is slimy when young and resin-like or powdery-dry in old age. (Wollenweber, '13.)

developed as an appressed form with very numerous conidia. When grown on various media at a rather wide range of temperatures, as described under "Attempts to Induce Variation," the growth was always appressed except on potato-dextrose agar at 30° C. where a light growth of aerial hyphae developed. At 20° C. on Leonian's agar the color of this culture was Bordeaux. When transfers were made to malt extract agar in tubes the color reverted to the light salmon orange characteristic of this culture on this medium, but the growth had a slimy appearance with no aerial mycelium. When examined microscopically, the growth was found to consist of a maximum of conidia, the pionnotal stage. A transfer to Leonian's agar made at the same time produced the Bordeaux color again, but the mycelium, instead of being completely appressed, was largely aerial. Conidia were very numerous and were 3- to 7- (mostly 5-) septate.

A subsequent transfer to potato-dextrose agar in a Petri plate produced a moderately heavy growth of aerial hyphae which were pink with a yellowish tint. This growth soon collapsed, and the color of the upper surface changed to Eugenia red and the reverse to acajou red to Vandyke red.

To learn whether the pionnotal phase would revert to the aerial mycelial phase, the series of transfers were made as shown in fig. 1. In each case, a small portion of mycelium was transferred with a sterile needle to the fresh media. The Roman numbers in fig. 1 represent the stages in the development of the aerial mycelial phase from the pionnotal phase, and are as follows:

I. Pionnotal (described previously in text).
II. Appressed type, salmon orange with very faint zoning of Eugenia red. Very numerous conidia.
III. Appressed type, salmon orange with Eugenia red center. Very numerous conidia.
IV. Wide zone of aerial hyphae about 1 cm. from the center, remainder appressed. Salmon orange except for a wide zone of irregular radially striped Eugenia red coinciding with the zone of aerial hyphae. Very numerous conidia.

V. Aerial mycelial stage. Aerial hyphae cottony, white to pale pink, reverse surface acajou red. Conidia fairly numerous.

VI. Dense aerial growth. Aerial hyphae cottony, white to slightly mottled with Eugenia red, substratum Eugenia red. Few or no conidia.

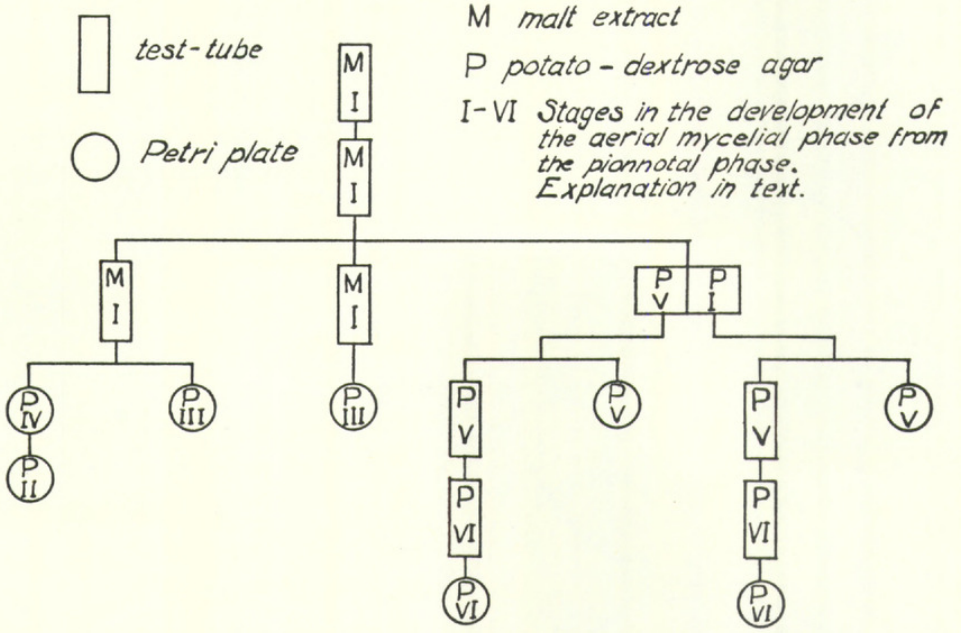

Fig. 1. Graphic representation of the development of the aerial mycelial phase from the pionnotal stage. The culture at the right in the third generation was of type I at first but later developed an area of type V.

In this series of cultures there seemed to be a transition from the pionnotal phase with no aerial hyphae (I) and the appressed type (II, III), through the aerial and appressed forms (IV), to the aerial mycelial forms (V, VI); from the orange color (I, II), through the orange and red (III, IV), to the red substratum (V, VI); from forms with a maximum or very numerous conidia (I, II, III, IV), through forms with fairly numerous conidia (V) to forms with few or no conidia (VI) (see pl. 12).

Observations on Sector Formation

Figure 2 shows the form and comparative distance from the inoculum of all variant sectors formed in an attempt to induce

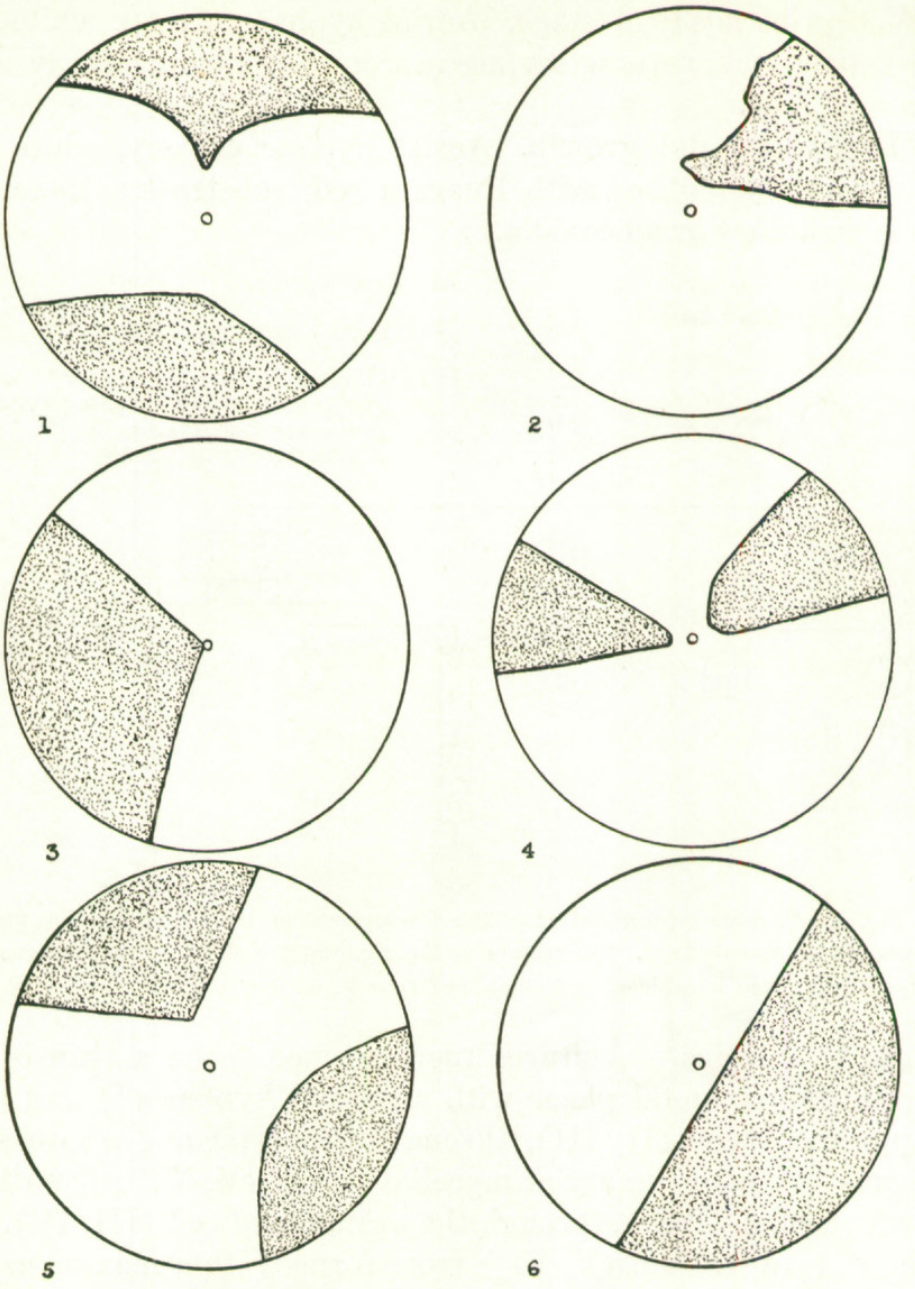

Fig. 2. Sectors formed in cultures grown at various temperatures on different media: *1*, A 17–1, on Leonian's agar at 18° C. One sector 15 mm. from inoculum, nearer one 8 mm. from inoculum; *2*, A 17–1, on Coons' agar at 30° C. Sector 4 mm. from inoculum; *3*, A 4, on Leonian's agar at 18° C. Sector starts at inoculum; *4*, A 4, on Richards' agar at 30° C. Both sectors start 3 mm. from inoculum; *5*, A 43, on Leonian's agar at 18° C. One sector 16 mm. from inoculum, nearer one 8 mm. from inoculum; *6*, A 4 on potato-dextrose agar at 25° C. Sector (secant) 3 mm. from inoculum.

variation by varying the temperature and medium. It can readily be seen that sectoring began at varying distances from the center or from the piece of inoculum. One sector (A 4 on Leonian's agar at 18° C.) started at the piece of inoculum and another (A 43 on Leonian's agar at 18° C.) started 8 and 16 mm. from the inoculum. In one, both sectors on the plate started at the same time, but in the other two cases one sector started at approximately twice the distance of the first sector from the inoculum. Christensen ('26) found that more sectors started near the edge of the colony than near the center in *Helminthosporium*. Brown ('26) found that more saltations took place near the center or in the older mycelia and that they occurred in irregular patches.

In a total of 360 Petri plates, six plates formed sectors. Of these, three plates formed two each, and three formed one each, making a total of nine sectors. The chance of getting a plate with one sector then is 9/360 or 1/40, and the chance of getting a plate with two sectors would be $1/40 \times 1/40$ or 1/1600. Yet in this experiment there were three plates with two sectors each, or 3/360 or 1/120.

The question then arises as to why there were so many cases of double sectors. In two sectors the growth type was observed to be the same in both; or if the sectors were differentiated on the basis of deeper or lighter pigmentation than the remainder of the culture, both showed this character; or if it were ability to produce conidia, both exhibited this character. It seems then that whatever factor or factors were operating to produce one sector tends to produce two. It acts in a qualitative as well as in a quantitative way.

Attempts to Produce Intermediate Strains by Crossing Two Morphologically Different Strains

Brierley ('29) suggested that certain recorded variations in fungi might be explained on the basis of the "mixochimaera" hypothesis. This term was used first by Burgeff ('14) for a mycelium in which nuclei and cytoplasm of distinct types were associated as the result of hyphal fusion.

Hyphal anastomoses are common in fungi. Ward ('88), Beauverie and Guillermond ('03), and Brierley ('22) have demonstrated anastomoses in *Botrytis*. Ezekiel ('24) observed fusions between hyphae of two varieties of *Sclerotinia americana* but no further growth. Matsumoto ('21) noted in *Rhizoctonia Solani* fusions between hyphae of the same or closely related strains; or strains which have recently originated from the same ancestral type. He was of the opinion that it was not a sexual process. Stevens ('22) reported that hyphal fusions were common in *Helminthosporium*. Dosdall ('23) and Christensen ('26) observed numerous fusions of germ tubes in *Helminthosporium*, Christensen noting as many as seven instances of lateral fusion in one series. Ocfemia ('24), in his work on *Helminthosporium*, showed figures of anastomoses very similar to those of the present author on *Gibberella Saubinetii* as shown in pl. 14. Drechsler ('23) noted in *Helminthosporium Bromi* the same type of fusion of germ tubes as did Christensen, but the former observed also that some of the hyphal fusions would swell into subglobose bodies and proliferate short irregular processes of inflated segments, the whole resulting in dark brown, knotty masses of mycelium. Some of these continued to increase in size, developing into subspherical sclerotia readily visible to the naked eye. He did not cultivate these further but was of the opinion that they represented immature perithecia.

Aside from these observations on hyphal anastomoses, there are the investigations of those who have studied hyphal fusions as such, or who have attempted to synthesize new types from pre-existing strains or even species. Burgeff ('14, '15) produced a neutral strain by mechanically mixing the cell contents of a plus and a minus hypha of *Phycomyces nitens*. This product, as previously mentioned, he termed "mixochimaera." Leonian ('30) attempted to induce mixochimaera in *Fusarium moniliforme* by growing two morphologically different types together in nutrient media, but no new strain was produced. However, Hansen and Smith ('32, '34, '35) reported that heterogenic types of *Botrytis cinerea* resulted from mixing

together two homogenic strains of the fungus in the same culture. Mixing of cell contents by anastomosis resulting in "heterocaryosis" was suggested as the mechanism of this phenomenon. Interspecific combinations were made in the same way by using two distinct species. The authors suggested that the production of these aberrant homotypes was due to gene changes brought about in some way by interspecific anastomosis.

Davidson, Dowding, and Buller ('32) used hyphal anastomosis as a character for differentiation of species of *Microsporum*. They observed fusions between mycelia of the same species but never between different species. Dickinson ('32) isolated fusion cells which were the product of the anastomosis of two contrasting strains of *Fusarium fructigenum*. On isolation from the subsequent growth of such fusion cells, the cultural characters of the two parent strains were found unchanged. Das Gupta's observations ('34) of hyphal anastomoses in *Diaporthe perniciosa* led him to suggest that the fusion of DH_C with DH_F mycelium exerted an influence on the latter and brought about the conversion of DH_F into DH_C mycelium.

The present work was undertaken, first, to find if hyphal anastomosis took place when conidia[9] from two contrasting strains of *Gibberella Saubinetii* were allowed to germinate on the same drop of nutrient agar (this procedure has been described under "Materials and Methods"); and second, to determine whether new and different strains were produced as a result of this fusion. The following combinations were made: A35–1 × B5–1; A35 × A35–1; and B5 × B5–1. Camera-lucida drawings of hyphal anastomoses are shown in pl. 14, figs. 1–8. Conidia of A35–1 and B5–1 were germinated separately on nutrient agar drops, and hyphal anastomoses occurred also in single spore colonies (pl. 14, figs. 9–11).

The agar drops with the germinated conidia were transferred to Petri plates of nutrient agar. In the first two combi-

[9] During several months in culture, A35 lost its capacity for production of conidia. Hyphal tips were substituted.

nations, there were sharp lines of demarcation between the two strains showing that each had inhibited the growth of the other. In the third combination the two colonies intermixed for the most part. Conidia were isolated along the line separating the two colonies, or along the surface where the two colonies overlapped. Of the total of 56 conidia isolated from the A35–1 × B5–1 combination, 31 produced the A35–1 type and 25 the B5–1 type. In the second combination, A35 × A35–1, 44 conidia were isolated and all exhibited the A35–1 type. Of the 55 conidia isolated from the third combination, B5 × B5–1, 28 were the B5 type and 27 the B5–1 type. No intermediate or new strain resulted from mixing the mycelia.

As can be seen from the data recorded, there was approximately a 1:1 ratio of the original types which appeared in the isolates from the mixed cultures of A35–1 × B5–1, and B5 ×B5–1. The occurrence of only one original type in the isolates from the "A35 × A35–1" cross may be explained by the supposition that A35, which was not producing conidia when the cross was made, still did not produce conidia. This evidence obtained from these crosses and re-isolations indicates that although hyphal anastomoses were common between the different pairs of variants, the strains remained separate.

CYTOLOGICAL STUDY

No intensive study of the whole cytological phenomenon in variants of *Gibberella Saubinetii* was attempted. However, observations were made of structures which proved to be perithecial rudiments; also of the nuclear condition in hyphal anastomoses.

An examination of the perithecial rudiments showed that the vast majority had not developed to maturity. One perithecium, nevertheless, which had been produced in a culture of B 5 on Leonian's agar at 18° C. was found to contain mature ascospores (pl. 13). The asci had probably already disintegrated, for the ascospores appeared to be loose in the perithecial cavity. The ascospores were 4-septate. Eide ('35) found them to be 3-septate in the strains which he examined.

Four-septate ascospores are exceptional in this species (Wollenweber and Reinking, '35).

In the study of hyphal anastomoses, the nuclear condition was found to be more complex than the data on isolation of single-spore colonies from the mixed cultures would lead one to suspect. Dickinson ('32) found the fusion cells in hyphae of *Fusarium fructigenum* to be binucleate, although both nuclei were sometimes found in one half of the cell. The hyphal cells are generally uninucleate except in the fusion cells, or sometimes in cells adjacent or very near to the fusion cells.

In this study, lateral processes were observed to push out from adjacent filaments and come together and fuse. The wall between them disintegrates, thus forming a connection between cells of the two filaments. Plates 15 and 16 show various nuclear conditions, many of which cannot be explained until some additional information has been gained. Figures 2 and 3 of pl. 15, and figs. 2a, 5a, 6a, and 7a of pl. 16 show binucleate fusion cells either before or after one nucleus has migrated into the other half of the cell, conditions described by Dickinson ('32, fig. 6) for *Fusarium fructigenum*. In other fusion cells one or both nuclei appear to have divided once or more, and in a few cases some of the daughter nuclei are seen migrating through the anastomosing tube (pl. 15, figs. 4, 5 and 7, pl. 16, figs. 1, 2b, 4, 5, 6b). D'Oliveira ('36, pls. 4 and 7) reported a similar phenomenon in *Fusicladium*. His figures showed anastomoses between two hyphae, and between two conidia. In the former, nuclei are seen which have recently divided, and in the latter some recently divided nuclei are seen passing into the anastomosing tube. No instance of caryogamy in these anastomoses was observed. All fusion cells were two- to several-nucleate.

Discussion and Conclusions

Gibberella Saubinetii appears to be a very variable species exhibiting a number of forms or phases differing from each other in type and rate of growth, color, and amount of conidial production. These characters may be influenced by media and

temperature. Brown ('26) remarks, with regard to *Fusarium fructigenum,* that "the tendency of these *Fusarium* strains to saltate is a function of the cultural medium." That the majority of variations recorded in this paper were due to reaction to the environment is evidenced by the return of the variants to the original form when grown under the original conditions of culture. Others, however, did not revert to the original form but continued to produce the same type of variation or to form still other variant types.

One culture, originating as a sector from A35, went through a cycle of growth phases. It was mentioned under "Sources of Cultures" that the original cultures used in this study were obtained from Dr. Eide, who had isolated them from corn stubble in 1932 and 1933. He described his original ascospore isolate A43–4–I, from which this A43–4–I–I or A line developed, as follows: " 'Normal' type; characterized by abundant, cottony, aerial mycelium, red, often with a tinge of yellow. The bottoms of the cultures were pink to deep Eugenia red." (Eide '32, p. 12, table 4, and p. 13.) A43–4–I–I or A of this paper is described in detail on page 106, and is illustrated in pl. 9, fig. 1. Briefly, it was an orange pink form at first with scant aerial mycelium but with age produced abundant thick-walled deep purple cells in the substratum. Upon further culture, A35–1 was produced as a sector in one of the conidial isolates (table III). It differed from A35 in having no aerial mycelium and developed no deep purple thick-walled hyphae. It was of the conidial or sporulating type. On five different media and at four different temperatures it produced this appressed type of growth with very numerous conidia, but on potato-dextrose agar at 30° C. a light aerial growth resulted. When transferred from Leonian's agar at 20° C. to potato-dextrose agar and malt agar, the culture assumed the pionnotal phase. In subsequent transfers to potato-dextrose agar, as is shown in fig. 1, the pionnotal phase changed to the aerial mycelial phase with few or no conidia. This phase seems comparable to the description of Eide's original ascospore isolate, although a stock culture of the original could not be obtained for purposes of comparison.

Whether this culture will again pass from the aerial mycelial phase, through the appressed or conidial phase and the pionnotal phase, to the aerial mycelial phase again is still being investigated. Upon the basis of data obtained thus far it seems a more logical conclusion that there is a definite cycle of growth phases through which this fungus passes, than to conclude that all these variations represent separate strains within the species.

It has been definitely shown that the pionnotal phase reverts to the aerial mycelial phase. This, it seems, is a very significant contribution.

Summary

1. A number of conidial isolates of two strains of *Gibberella Saubinetii* were allowed to grow for several generations on potato-dextrose agar to determine the relative stability of the strains. Only one permanent variant was formed in one strain and two in the other strain.

2. Nine conidial isolates, including the three variants, were grown on Brown's, Coons', Richards', Leonian's, and potato-dextrose agar at 18°, 20°, 25°, and 30° C., to induce variations. Ecovariants, or temporary variants due to environment, as well as some permanent variations, were produced. More variations, temporary and permanent, occurred at the lower temperatures and on Leonian's agar. Growth also was best on Leonian's agar. The optimum temperature for growth was 25° C.

3. Mycelial and conidial forms were only fairly constant when subjected to variations in temperature and media. Numerous conidia appeared in sectors of the mycelial types which had previously formed no conidia; and conidial types have changed to aerial forms producing only very few or no conidia.

4. The pionnotal stage in one strain reverted to the aerial mycelial stage which seemed to answer the description of the original ascospore isolate from corn stubble. It completed a cycle of growth by passing from the aerial mycelial phase through the conidial and appressed phase, through the pionnotal phase back to the aerial mycelial phase.

5. No new or intermediate strains resulted from crossing two morphologically different strains. While nuclear migration occurred between hyphal anastomoses, there was no evidence that caryogamy took place in the hyphal anastomoses.

6. The two facts: (1) production of two sectors at about the same time, and (2) identical mutant sectors, favor the hypothesis that the saltation is somatic rather than germinal. The soma, rather than the germ-plasm, might more readily be expected to saltate more or less simultaneously at diverse points under the proper stimulus. Germinal changes are usually somewhat at random and even if two occurred in the same culture it might confidently be expected that they would be different mutants.

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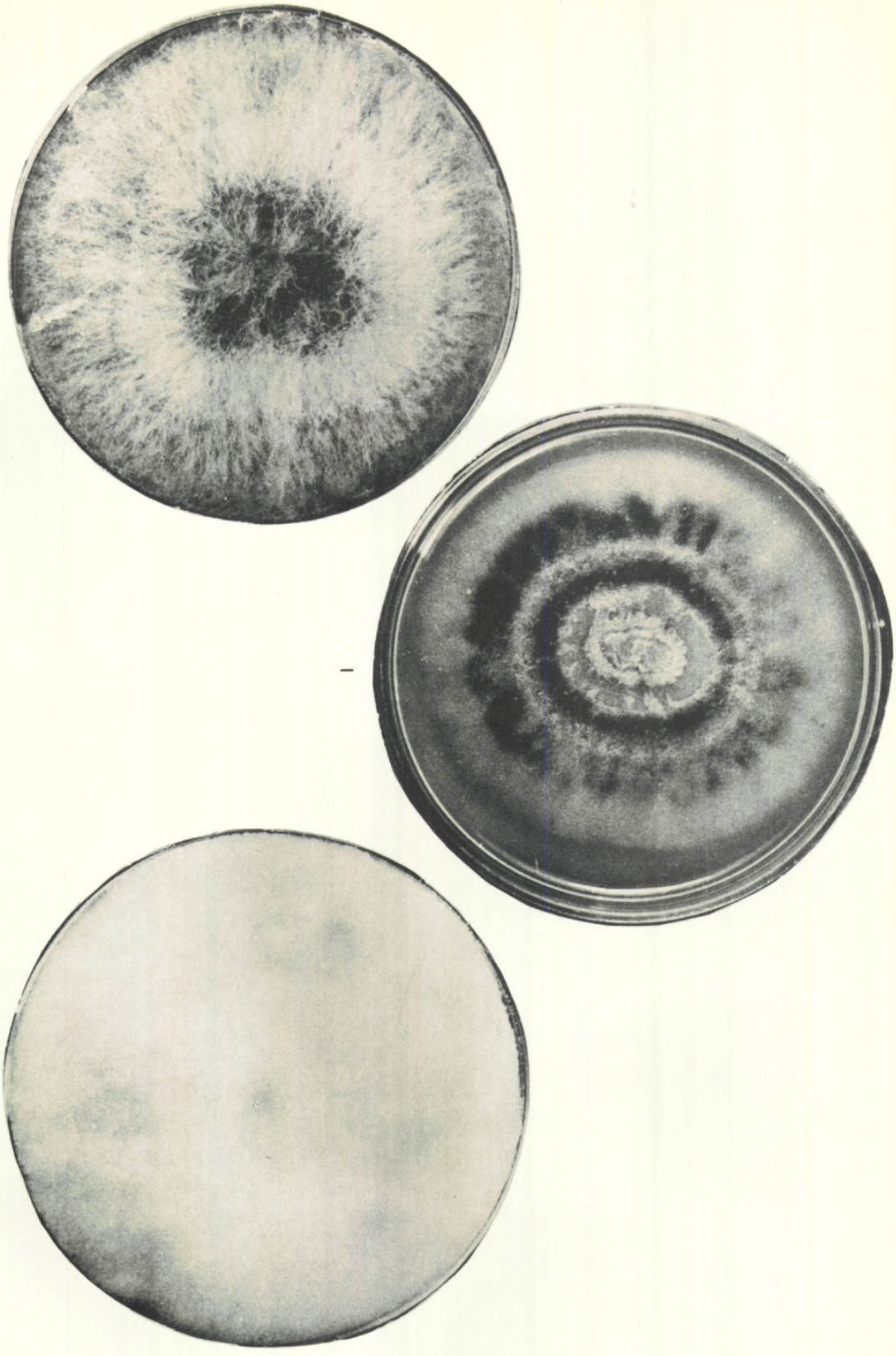
Explanation of Plate

PLATE 9

Cultures grown on potato-dextrose agar at room temperature.

Fig. 1. Petri plate culture of strain A.
Fig. 2. Petri plate culture of strain B.
Fig. 3. Petri plate culture of B 5–1, a variant from strain B (fig. 2).



2 1 3

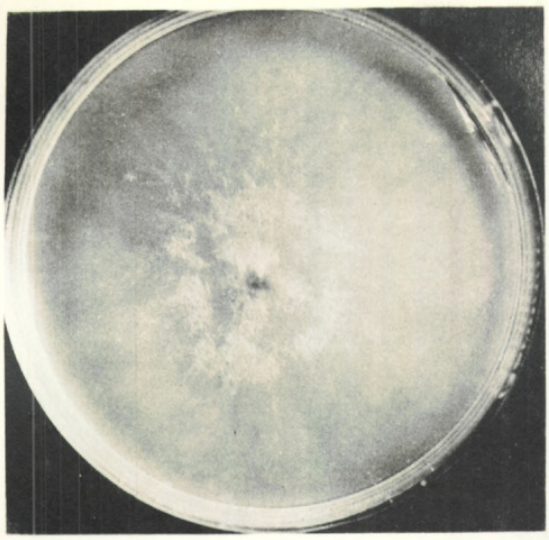
GODDARD — VARIATION IN GIBBERELLA SAUBINETII

Explanation of Plate

PLATE 10

Variations in a single culture due to differences in the composition of the medium. Temperature, 25° C.

Fig. 1. A 17 on Brown's agar.
Fig. 2. A 17 on Coons' agar.
Fig. 3. A 17 on Richards' agar.
Fig. 4. A 17 on Leonian's agar.
Fig. 5. A 17 on potato-dextrose agar.



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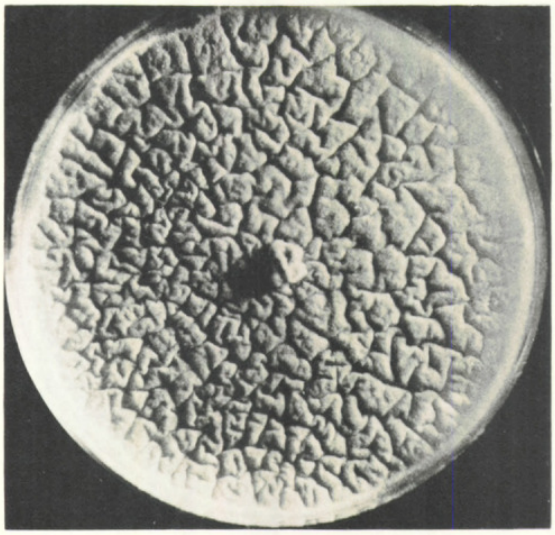

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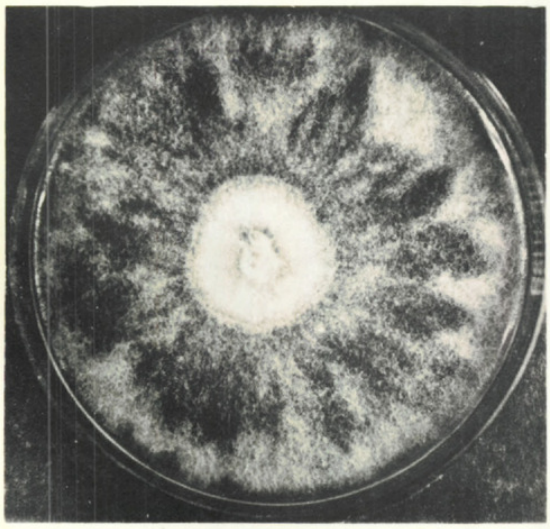

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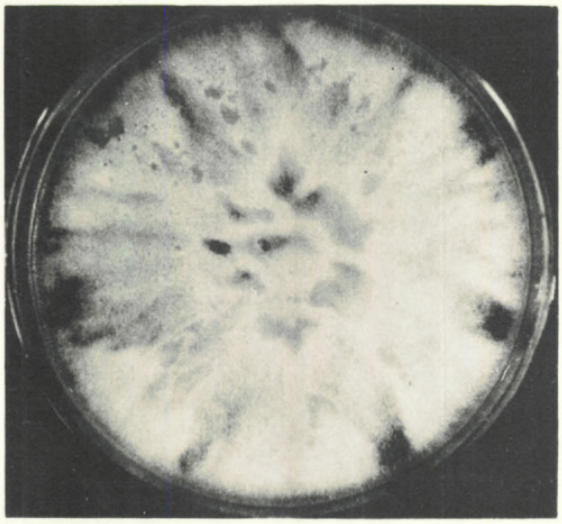


5

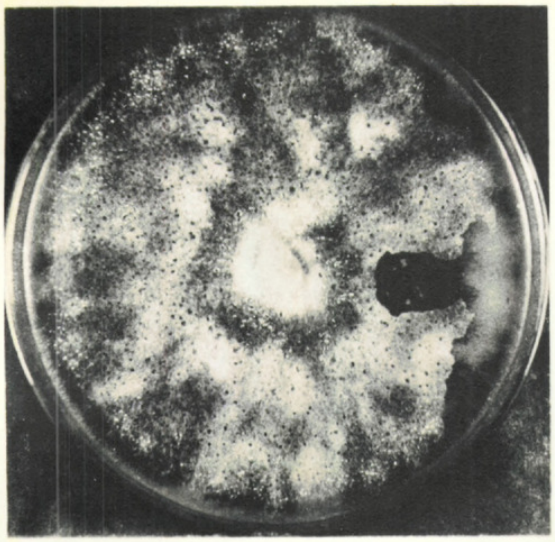
GODDARD — VARIATION IN GIBBERELLA SAUBINETII

EXPLANATION OF PLATE

PLATE 11

Variations in a single culture due to differences in temperature. Grown on potato-dextrose agar.

Fig. 1. A 17 at 18° C.
Fig. 2. A 17 at 20° C.
Fig. 3. A 17 at 25° C.
Fig. 4. A 17 at 30° C.



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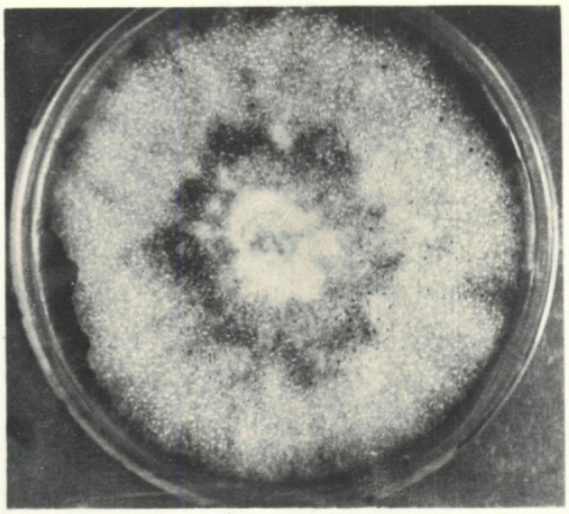

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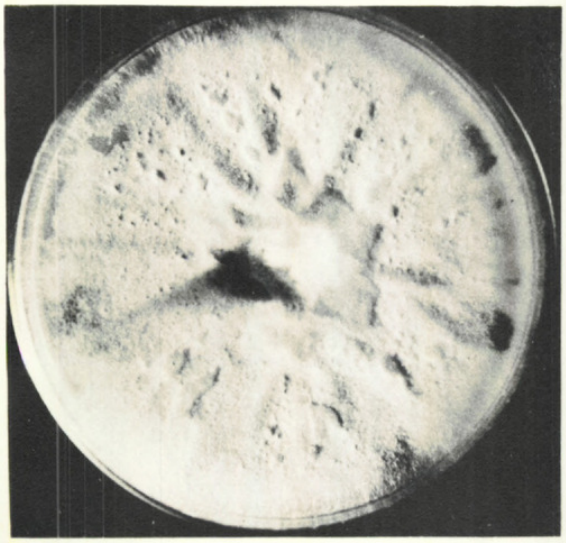

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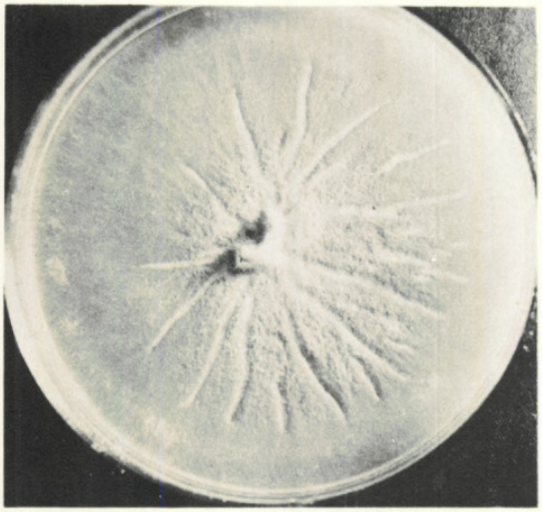


4

GODDARD — VARIATION IN GIBBERELLA SAUBINETII

Explanation of Plate

PLATE 12

Culture A 35–1 on potato-dextrose agar, showing some stages in the development of the aerial mycelial phase from the pionnotal phase. Stage I, the pionnotal phase, was grown in test-tube cultures.

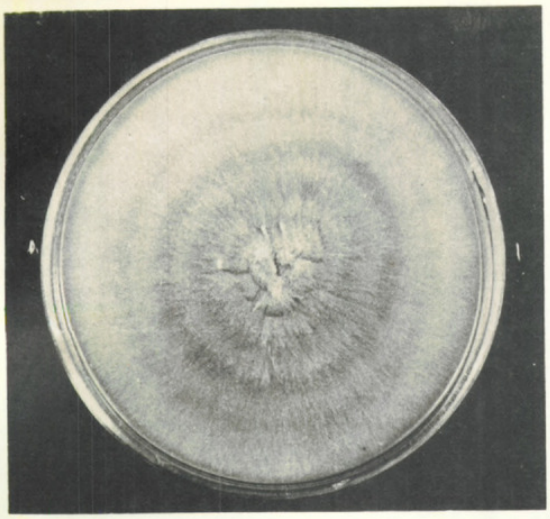
Fig. 1. Stage II. Appressed type, salmon orange with Eugenia red center; very numerous conidia.

Fig. 2. Stage III. Appressed type, salmon orange with Eugenia red center; very numerous conidia.

Fig. 3. Stage IV. Wide zone of aerial hyphae about 1 cm. from the center, remainder appressed; salmon orange except for a wide zone of irregular radially striped Eugenia red coinciding with the zone of aerial hyphae; very numerous conidia. This stage is intermediate between the appressed type and the aerial mycelial type. It was also intermediate in color between salmon orange and Eugenia red.

Stage V. was identical in appearance with Stage VI. Conidia were fairly numerous.

Figs. 4 and 5. Stage VI. Dense aerial mycelium; aerial hyphae cottony, white to slightly mottled with Eugenia red; few or no conidia (see fig. 1 in text). Fig. 5 is a culture developed from the pionnotal region of the test-tube culture, and fig. 4 from the aerial mycelium of the same culture. The two resultant cultures are identical.



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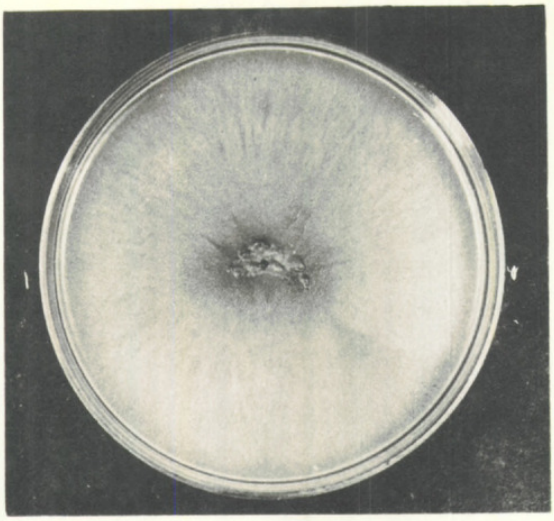

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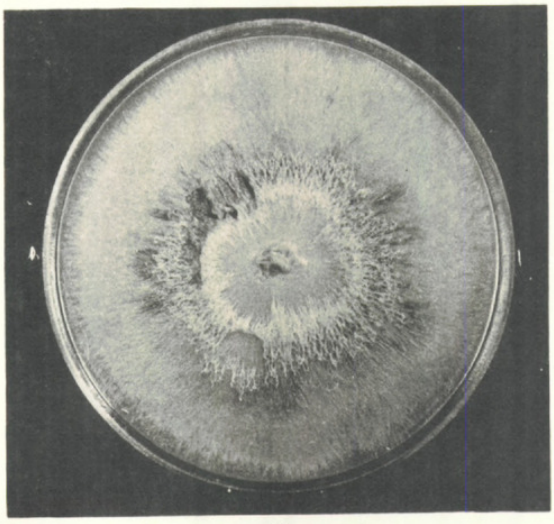

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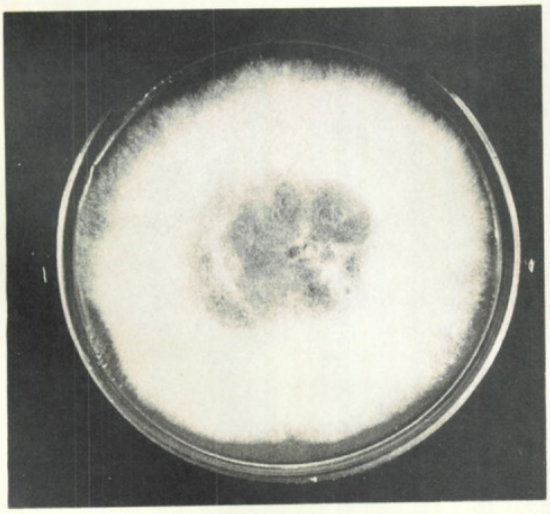

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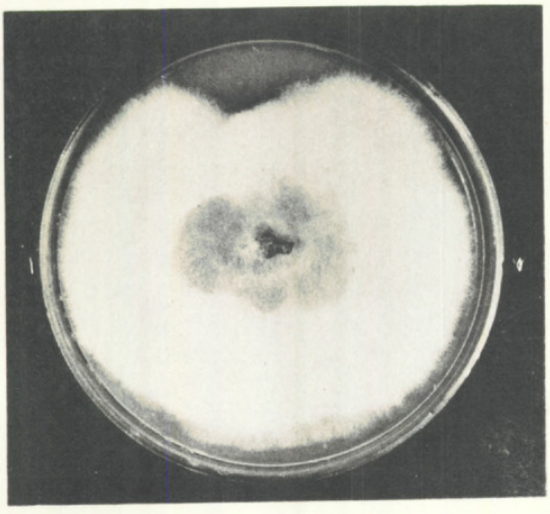

5

GODDARD — VARIATION IN GIBBERELLA SAUBINETII

EXPLANATION OF PLATE

PLATE 13

The microscopic structure of a fertile perithecium.

Figs. 1–3. Serial sections of a fertile perithecium from a Petri-dish culture of B 5 grown on Leonian's agar at 18° C; × 350. In fig. 3, a median section, ascospores are plainly visible.

Fig. 4. The ascospores of fig. 3 shown at a higher magnification; × 865. Note that these spores are 4-septate (3-septate ascospores are usually found in this species).

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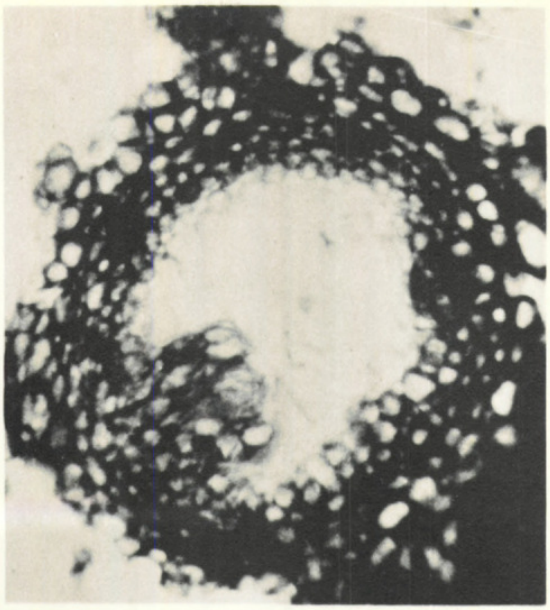

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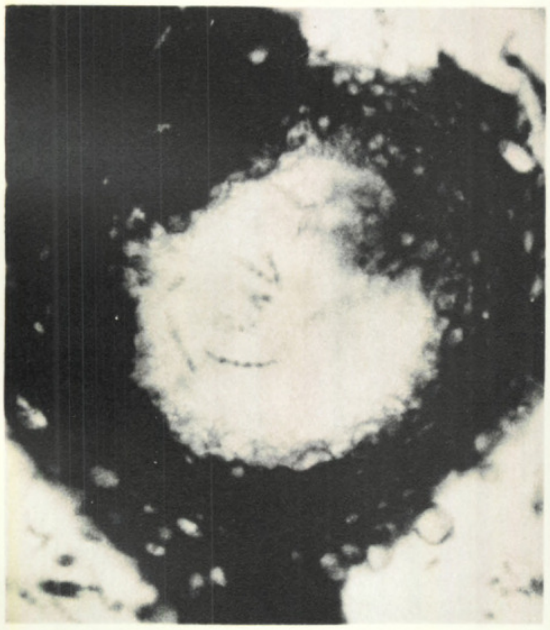

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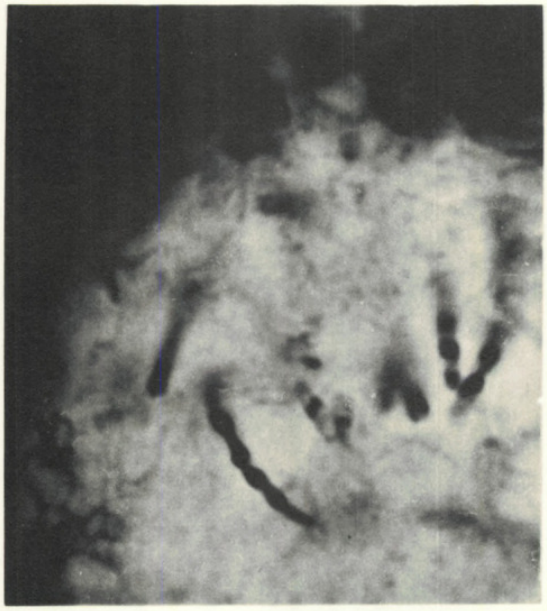


4

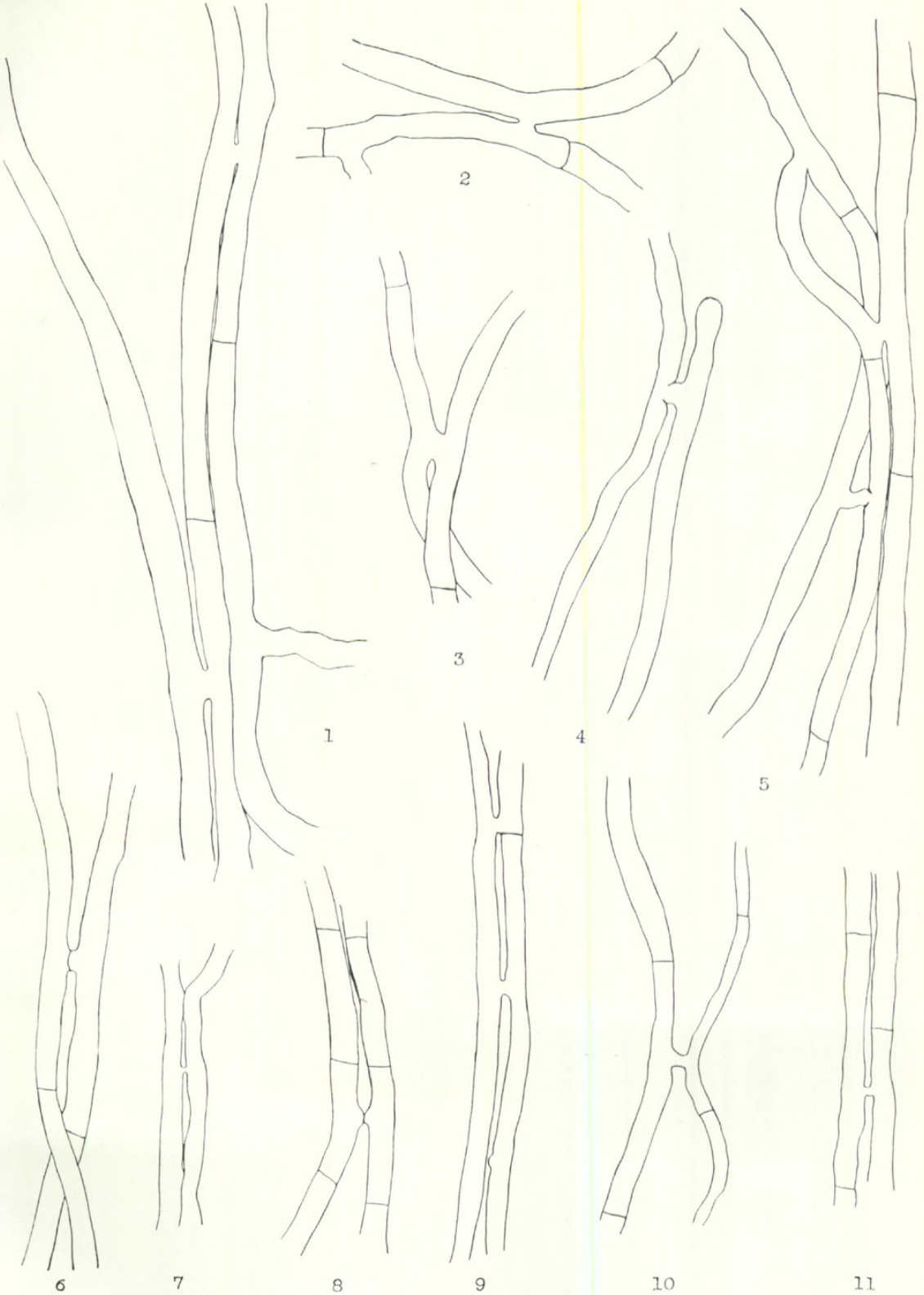
GODDARD — VARIATION IN GIBBERELLA SAUBINETII

Explanation of Plate

PLATE 14

Hyphal anastomoses drawn with the aid of a camera lucida from living material; × 625.

Figs. 1 and 2. B 5–1 × A 35–1.
Figs. 3 and 4. A 35 × A 35–1.
Figs. 5, 6, 7, and 8. B 5 × B 5–1.
Figs. 9 and 10. B 5–1.
Fig. 11. A 35–1.



GODDARD—VARIATION IN GIBBERELLA SAUBINETII

Explanation of Plate

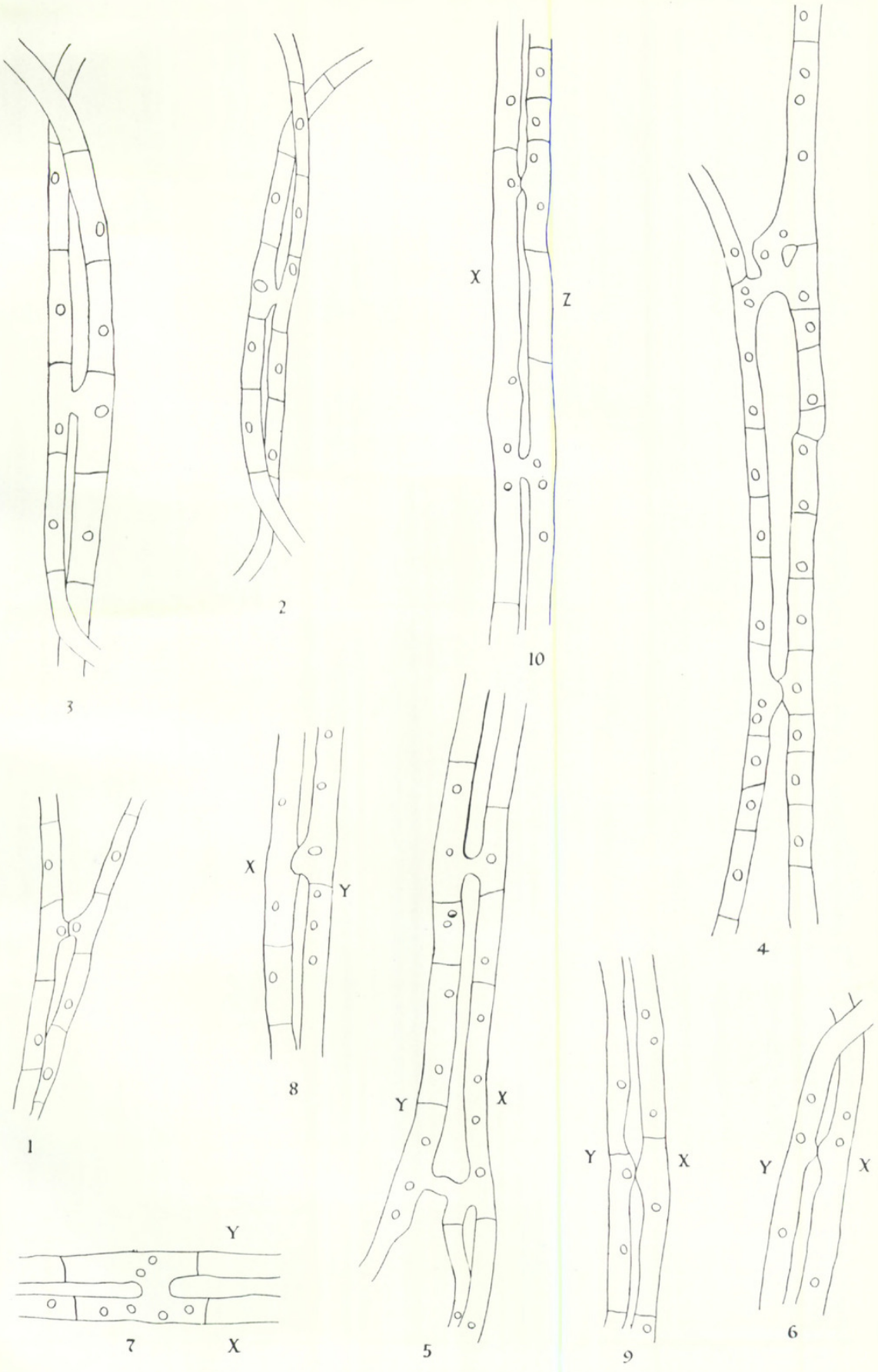
PLATE 15

Hyphal anastomoses drawn with the aid of a camera lucida from stained material; × 450.

Figs. 1, 2, and 3. A 35 × A 35–1; the remainder, B 5 × B 5–1.

Figs. 5–9 inclusive show 6 fusions or beginnings of fusion of hypha X with hypha Y.

Fig. 10 shows hypha X fusing with hypha Z.



GODDARD—VARIATION IN GIBBERELLA SAUBINETII

EXPLANATION OF PLATE

PLATE 16

Hyphal anastomoses drawn with the aid of a camera lucida from stained material.

Figs. 1–8. B 5 × B 5–1; × 450.

Fig. 9. A single hypha showing type of branching; × 225.