Cultural Studies with *Gibberella Saubinetii* (Mont.) Sacc. which is parasitic on Rice-Plant.

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Introduction.

Two types of the Fusariose are so far known to us as attacking on the rice-plants (*Oryza sativa* L.) in Japan. One type is that due to the fungus *Lisea Fujikuroi* SAWADA (1920) and the other is caused through the attack of *Gibberella Saubinetii* (Mont.) Sacc. The phases we have to deal with here in this paper are concerned with the latter type only.

The conidial stage of *Gibberella Saubinetii* (Mont.) Sacc. has formerly been, and still sometimes is, identified by many observers, both abroad and at home, with *Fusarium roseum* Link. However, that the so called *Fusarium roseum* Autorum, is a collective name including several species of the genus, has been fully discussed by WOLLENWEBER (1910, 1914, 1917) and JACZEWSKI (1912). According to the former author (1917) *Fusarium graminearum* Schwabe is regarded to be the conidial form of *Gibberella Saubinetii* (Mont.) Sacc., and this view is accepted at present by many authors who have studied the wheat scab problems, such as ATANASOFF (1920), SHERBAKOFF (1922) and others.

In 1889, FELIX VON THÜMEN remarks in his monograph on the fungi of the rice-plant (1889, p. 3): "..... von *Fusarium roseum* Link überzogen sind, einem weissrötlichen Fadenpilz, welcher von vielen Autoren als in den Entwicklungskreis der *Gibberella Saubinetii* gehörig, angesehen wird. Obwohl der Verfasser den fraglichen Fadenpilz auch schon häufig gemeinsam mit der *Gibberella* gefunden hat, so kann er dieser Anschauung doch nicht beiphlichten und hält beide Pilzarten auseinander." He in this way holds doubts of the generic connection between *Fusarium graminearum* Schwabe (=THÜMEN's *F. roseum*) and *Gibberella Saubinetii* (Mont.) Sacc. And it is I. MIYAKE (1910) who for the first time found the perithecial stage of this fungus produced in open fields in Japan on the rice-plant. He writes literally: "In unserem

Lande wird seine Konidienform, *Fusarium roseum* Link., häufig auf verschiedenen Teilen vieler Pflanzen gefunden, aber ich habe noch nicht gehört, dass man seine Schlauchform beobachtet hätte. Nach meinen Untersuchungen befindet sich diese auf den abgestorbenen Pflanzenteilen, ..."

Epidemic invasions of this fungus have been comparatively often observed and reported in Japan on other cereals, such as wheat, barley, oats etc. Neverthless, all descriptions of the life cycle of this ascigenous fungus, in our country, have heretofore been based on field materials and not on pure cultures. It must be conceded that the taxonomy of *Fusaria* can be based on studies of pure cultures in a normal condition. It is through the efforts of WOLLENWEBER, ATANASOFF, NAUMOV and others that the perfect stage of *Gibberella Saubinetii* in many cereals has been demonstrated artificially on pure cultures. However, since we are not aware of any investigations made with regard to this fungus on the rice plant, it is thought that a few supplementary notes may be desirable.

The writer has been giving special attention since 1920 to the head-blight and node-rot of the rice-plant, collecting, from time to time as necessary, materials for the investigation, from the fields belonging to this Institute. Cultural works were undertaken, and the perfect stage of the fungus was obtained from cultures of sporodochial conidia; and conversely, sporodochial masses of conidia were yielded by cultures derived from ascospores. In view of these facts, the causal organism was identified with certainty as *Gibberella Saubinetii*, and the results of the cultures made are given in the following pages. Nothing will be touched upon in this place concerning the occurrence of the same fungus on other cereals, though this also is fairly wide spread in this country.

Common name and distribution of the fusariose.

The fusarial headblight of cereals is commonly known in this country under the name "Akakabi" (i. e. red mould). In the case of the rice-plant it is called "Ine-no-Akakabi," *Ine* being the Japanese name for *Oryza sativa* L. The origin of this name is not certain. So far as the writer is aware, in the second edition of IDETA's handbook which appeared as early as 1903 this common name is found though the author thinks *Fusarium heterosporum* Nees. to be the causal organism. For the node-rot of the rice-plant HARA (1910) gives a special name "Fushikuro" (i. e. node blackening) and S. ITO (1912) gives a special term, "Kokuten-byo" (i. e. black spotting disease), in the case of the head-blight of oats.

This disease of the rice-plant is distributed more or less commonly throughout this country. The reports on its occurrence are, however, com-

paratively few in number, the following being the only examples of them.

The late T. KAWAKAMI and R. SUSUKI (1908) reported on this disease from Formosa using the name of "Ine-no-Akakabi" which is mostprobably the first printed name of the disease though they regard *Fusarium heterosporum* Nees. as the causal fungus, according to the general belief that prevailed at that time. I. MIYAKE (1909, 1910) reports on materials of the diseased plant which he procured from Gunma and Tochigi, both being prefectures in the central part of this Island.

The occurrence of the same fungus on wheat, barley, oats etc, on the contrary, is reported often and from several localities, such as: Hokkaido, Chiba, Ibaraki, Tochigi, Saitama, Tokyo, Shizuoka, Gifu, Aichi, Osaka, Okayama, Ehime, and Formosa.

In Europe, Gibberella-disease of the rice-plant has been reported from Austria (VON THÜMEN, 1889), and Italy (NOVELLI, 1921). The fusariose of the same host is reported from Java (PALM, 1918) and also from Ceylon (GADD, 1921).

Modes of attack of the fungus.

Gibberella Saubinetii (Mont.) Sacc. attacks the rice-plant in at least three different ways, producing three distinct pathological conditions.

1. The first condition results from an attack on the germinating seeds in the irrigated seed-beds. DOYER's description (1921, p. 80) of the failure of germinating seeds of wheat is exactly applicable also to our case with the rice grains. When infected grains are kept moist before or after sowing, a considerable percentage of them are subjected to the attack of this fungus and the seedlings are destroyed.

2. The second condition results from an attack upon the heads causing so called scab or head-blight. On the surface of the husk of attacked grains many minute white mouldy spots appear at the beginning of the disease. Meanwhile the spots expand by and by, and turn pale yellow, then pale reddish and finally salmon or carmin, which is the color of the sporodochial masses of conidia, they being somewhat mucilaginous or viscid when moist. These masses are generally confined to only a limited area of the grain surface; but sometimes they cover the whole surface of the husk fully. However, as WOLLENWEBER (1914, p. 254) remarks, the formation of the conidial stage itself seems generally reduced in this fungus, and the production of perithecia on the grains comparatively rare in nature. Such diseased grains, mixed among other sound ones in the heads, often attract our attention while we walk through the fields where the host is in full ripeness and is waiting for harvest. The damage caused by this fungus is not so

serious as compared with the losses it causes in wheat, barley and oats. The attacked rice grains lack entirely or partially their normal contents. Such grains are light, shrunken, easily crushed, and fail without doubts to germinate.

3. The third condition results from a rotting of the stem-nodes. DOYER (1921, p. 80) well remarks on the node-rotting of wheat through *Gibberella Saubinetii* (Mont.) Sacc. and the description of this author applies equally to the case of the rice-plant. The perithecia of the fungus appear invariably on or near the node. As with the case of the grains, the diseased stems, sheaths, and leaves at first produce many minute spots of whitish mould. These spots starting chiefly on the node as the center, expand upwards and downwards near the node. The attacked part of nodes become blackish and disintegrated; finally the stems wilt, break down and fall to ground. Disease symptoms on the sheaths and leaves are nearly the same.

Cultural studies of the fungus.

Gibberella Saubinetii (Mont.) Sacc. has a vegetative stage and two spore stages. The conidial and perithecial development terminates the active vegetative period. The two spore stages of the fungus, are observed in the fields as it occurs on the rice-plant, as well as in artificial cultures; and by cultures we were able to establish generic connection between the two spore stages.

This species of ascigenous fungus produces, as DICKON and JOHNSON (1920) state, conidia at two different periods during its development. The first period of conidial production is in connection with the early mycelial growth, while the second occurs at the termination of the vigorous vegetative development. These later conidia are produced in definite sporodochia and are the only conidia generally described for this species.

It is generally believed that strains of this fungus producing abundant perithecia develope only few conidia. This was proved to be the case in our cultures also. ADAMS (1921) writes of one strain of *Gibberella Saubinetii* (Mont.) Sacc. which was isolated from corn, that it has shown the tendency to produce perithecia, but no culture from wheat has developed perithecia. While DICKSON and JOHNSON (1920, p. 236) inform us about the ability of the fungus to produce virulent spores in abundance in a short period of time during the early mycelial growth, in the majority of strains used in our cultures the conidial production was in general rather small.

WOLLENWEBER (1910, p. 133 and 1914, p. 278) obtained the perfect stage of this fungus on sterilized potato stem, *Vicia faba* stem, wheat straw and cotton stem. ATANASOFF (1920, p. 18) secured the perfect stages in the way originally described by APPEL and WOLLENWEBER and later extended by

WOLLENWEBER alone. ATANASOFF remarks: "It need only be emphasized that the perithecia will rarely be formed until the transfers and cultural work are begun from "normal" culture. Failure is bound to occur 95 times out of 100 before the culture which is to be used for development of the perfect stage is brought to this condition. Care must be taken that the cultures are kept uniformly moist until the perithecia are formed and the ascospores in them are ripe." Though special precautions for drying were not taken in our investigation, still fortunately we were able to get many perithecia on agar plates and on steamed potatoes as well.

HOPKINS (1921, Oct.) performed experiments on the relation of hydrogen-ion concentration to this fungus and has discovered the following facts:

Gibberella Saubinetii grown on both liquid and solid media in which the acidity is varied in different ways shows that a very wide range of hydrogen-ion concentration is tolerated. However, the growth of the acidity curve shows a decided minimum, which is about pH 5.0—6.0. The same author says in one of his hapers on the same problem (1921): "Growth increased with decreasing acidity from pH 2.5 to a maximum at pH 4.0—4.5. It then decreased to a minimum at pH 5.0—5.5 and rose again to a second maximum, the highest point of which was not determined." It is also proved by the same author that conidial production by this organism increases markedly as the hydrogen-ion concentration increases. No conidia are produced in the first few days on potato agar which is neutral (pH 7) while on potato agar which is made acid, the conidial production is greatly increased. The relation between hydrogen-ion concentration and conidial production may be expressed as a smooth curve. This smooth curve, when compared with the growth curve, shows that the conidial production varies inversely with the growth and directly with the hydrogen-ion concentration. Finally Hopkins says that the curve expressing the relation between acidity and spore germination shows a decided minimum, as found in the case of growth.

The following series of cultures are selected from a note of the writer's cultural work upon *Gibberella Saubinetii* (Mont.) Sacc., which, starting in October 1920, was performed at intervals until May, 1922.

(A) Cultures from conidia.

Culture 1. On Oct. 6, 1920, isolation work was started. Conidia taken directly from grenadine pink colored sporodochia produced in the field upon the rice grain were transferred to ten pieces of potato slants in the Roux tubes. The cultures were left at the room temperature which averaged 21°C. Mycelial growth progressed day by day and after 12 days a pinkish crimson coloration appeared chiefly in the margins of the substrata. The coloration became daily deeper and deeper. No sporodochia were rised.

Culture 2. On Oct. 12, 1920, another isolation culture was attempted. Conidia taken directly from rice grains in the field were introduced upon pieces of potato slants in the Roux tubes and the tubes were left at an ordinary room temperature which averaged 21°C. In the second day of culture white tufts of mycelial growth began to develope, and after 6 days pinkish crimson coloration appeared in the marginal part of the slants, the growth of mycelia being very vigorous. The mycelial growth extended upwards filling nearly the whole diameter of the tube. Conidial production was observed on the branches of mycelium which composed the mats on the surface of water in the bulb of the tube. However; definite sporodochial formation was never noticed.

Culture 3. On Oct. 13, 1920, Conidia from rice grains were placed on 5% glucose agar in the tubes at the room temperature of 20°C. Pale pinkish coloration appeared after 5 days. That part of the mycelial tufts which did not stain pinkish remained orange-yellow and deep penetration of such yellow-colored mycelium into the substrata was observed through the transparent medium in the tubes.

Culture 4. On Oct. 27, 1920, small bits of agar from Culture 3 with mycelial growth in or on them were transferred into the rice stem extract and left at the room temperature. In some of the tube-cultures, after a week, vigorous mycelial growth and pinkish coloration developed, while in other tube the growth was comparatively poor and the coloration did not appear.

Culture 5. Grenadine pinkish sporodochium of the fungus on grains of rice which had been kept moist developed a good deal of conidia. They were transferred into the rice stem extract on Nov. 6, 1920. Mycelial growth developed and flourished day after day upon the surface of the nutrient fluid and after a week a pinkish crimson coloration developed in the form of a ring along the inner wall of the tubes, where mycelia were in contact with it. The greater part of the colony was snow white. Sporodochial formation was not obtained.

Culture 6. Small bits of the mycelial growth from Culture 3 were transplanted, on Nov. 9, 1920, upon potato slants in the Roux tubes and were left in the room temperature. Very vigorous development of mycelial growth was noticed by the second day of culture, and after a week it extended upwards filling the tube full; the slant being completely covered with snow white mycelium. There appeared a shade of pinkish coloration on the surface of the water where it came into contact with mycelial growth in the bulb of the tubes. When observation was made on Dec. 15, 1920, sporodochial formations were noticed upon the surface of the slants or in the places of the contact of the mycelial growth with the inner-wall of the tubes.

Culture 7. On Oct. 21, 1921, sterilized cotton-plugged Roux tubes with a potato slant were prepared and conidia taken directly from sporodochia on the rice grain were transferred to the slants. These inoculated tubes were

all kept at a room temperature which ranged between 11° to 16°C. After four days mycelial tufts became denser and longer, but still no shade of coloration appeared. The mycelial tufts on the lower side of the potato slants extended downwards into the bulbpart of the tubes approaching to the surface of water contained there; and paler pinkish coloration was noticed in the mats of mycelium formed upon the surface of the water. This set of cultures was kept long and when observed on June 9, 1923 perithecial formations were noticed in three tubes of the set.

Culture 8. On Oct. 22, 1921, rice meal extract soft agar in the petri-dishes was inoculated with conidia taken from a sporodochium on rice grains. Cultures were attempted at the room temperature which varied from 11° to 16°C. Aerial mycelial development was extremely poor in this case and even after 5 days no kind of aerial growth was noticed. However, submerged mycelial growth was observed in comparatively large quantities and after 10 days a pinkish coloration was faintly visible.

Culture 9. On Oct. 26, 1921, rice meal extract medium agar (1.5%) in petri-dishes was introduced with small bits of the flourishing mycelium from Culture 7. All dishes were kept at ordinary room temperature. By the second day the bits of transplanted mycelium began to develope and some of them already had developed a pale pinkish coloration. After 5 days colonies developed in each petri-dish vigorously; and their marginal zone was composed of dense tufts of aerial mycelium, the inner part being colored pinkish. The cultures were carefully preserved and when observed on May 9, 1922, in all the dishes many perithecial formations were found.

Culture 10. On Oct. 26, 1921, boiled potato slices in the petri-dishes were inoculated with small bits of mycelium taken from Culture 7. Cultures were kept at ordinary room temperature. The rate of mycelial development was more vigorous than in the case of Culture 9 which was lead in parallel. Pale pinkish coloration appeared after 7 days, and potato slices became of salmon color. Such slices when cut into pieces were found completely covered with leather-like plectenchymatous stroma, formed by closely interwoven mycelia. These mycelia are composed of chains of swollen cells which have a thick membrane, and contain brown to red contents with many vacuoles. When these cultures were taken out and examined on May 10, 1922, a considerable number of perithecia was found to have been produced.

Culture 11. A small piece of growing mycelium was taken from Culture 7 and transplanted upon sterilized rice-halms in the Roux tubes. At room temperature the development of mycelium seemed retarded; and even after a week only poor, rough, scanty mycelial growth was obtained.

Culture 12. Mr. Y. NISIKADO of this institute attempted his isolation cultures on Sept. 8, 1921, taking ascospores from perithecia naturally produced upon rice-stems in the open field. After 4 days he was able to get sporodochia produced on rice-stem extract agar cultures. A culture was ob-

tained from him and on Nov. 4. 1921 sporodochial conidia were transplanted upon the same medium and the cultures were conducted under a constant temperature of 25°C in a thermostat. Flourishing white tufts of mycelium developed after 3 days, and after 7 days repeated crops of ochreous sporodochial masses of conidia were obtained in two tubes of the culture. In these tubes mycelial growth seemed to have cheased, it being not as vigorous as at the beginning of the culture period. At last after 10 days in all remainig tubes, smaller or larger sporodochia were found produced. This strain of the fungus had a tendency to produce conidia easily and in abundance.

(B) Cultures from the ascospores from open fields.

Culture 13. Ascospores from perithecia collected in the open field were taken as inocula, on May 27, 1921, upon wheat grain extract glucose agar. By the second day of culture period, cultures being grown at the constant temperature of 25°C, mycelial growth became macroscopically visible. When a small portion of the colony was placed under the microscope for examination, a vigorous germination of ascospores, sending out several promycelial strands from the different cells and a swelling of the ascospores themselves, were observed. In a majority of the tubes until the fourth day, the cottony growth of aerial mycelium was white or yellowish, while in one or two tubes only some pale pinkish shade of color began to be observed. On the fifth day carmine coloration of the plectenchyma manifested itself distinctly in nearly all tubes. The coloration became deeper and deeper and after 10 days beautiful crimson or pomegrant coloration was produced. Sporodochia, however, were not present.

Culture 14. Mucilaginous ascospore-masses, that oozed out of the ostiolum of a perithecium, were touched with a needle and ascospores that became attached thereto were, on June 10, 1921, applied as inocula upon wheat grain extract glucose agar in the tubes. The tube-cultures were kept during 4 days at the constant temperature of 30°C. Mycelial growth was evident on the second day, and after 3 days, a pinkish crimson coloration was produced in almost all tubes, a few remaining yellow. However by the fourth day the pomegranate coloration prevailed in all tubes, mycelial development being vigorous and covering nearly half the surface of the slants. And it was noticed that in the upper and lower parts of the colony, the orange-yellow color remained while the main part of the colony was of brilliant crimson.

Culture 15. On June 10, 1921, ascospore-suspension of water was prepared from masses ejected from perithecia. By means of a platinum wire-loop the suspension was carried upon sterilized wheat heads which were put in the tubes containing a small quantity of water. The tubes thus arranged were taken into a thermostat at 30°C. Cultures succeeded, mycelial growth

being very flourishing, and after 4 days the entire length of the wheat heads was nearly completely covered with mycelial growth. A paler or denser shade of pinkish color appeared. No sporodochial mass of conidia, however, was obtained.

Culture 16. Short pieces of wheat stem sterilized and kept in the tubes were used as the sustrata, upon which were introduced ascospores in the form of water suspension on June 10, 1921. The inoculated tubes were taken into a thermostat of 30°C. During first few days mycelial growth seemed show, but on the fourth day it increased in density and covered the entire length of the stem pieces, and a pale pinkish coloration appeared wherever mycelial growth was thicker. Mycelial growth was found thicker in the nodal part of the stems and there the coloration was deeper. After a week the said phenomenon became much more conspicuous.

Culture 17. On May 24, 1922, ascospores obtained from perithecia on the rice stem were isolated upon potato extract saccharose (5%) agar, which after isolation work was kept under a constant temperature at 25°C. After 6 days a beautiful pomegranate-purplish coloration developed, and was especially conspicuous in the side view of the medium slants. In the older part of the mycelial growth, however, the coloration was of orange-yellow, the younger part being snow white. Sporodochial formation was not reached in this set of cultures.

Culture 18. On May 29, 1922, ascospores taken from perithecia collected in the open field were placed upon rice straw extract agar. After 5 days, cultures being kept at a temperature of 25°C, a faint or more or less denser coloration of pomegranate purple was exhibited. The growth of aerial mycelium was in no way vigorous. In this case also, sporodochial formation of conidia was not obtained. The coloration is apt to be not fully developed, in the case of absence of sugars.

(C) Cultures from the ascospores produced in pure cultures.

Culture 19. On May 10, 1922, ascospores from perithecia produced on agar in Culture 9 were used as inocula upon potato extract glucose agar in the petri-dishes. When observed after 10 days culture at the room temperature which averaged 20°C., a very vigorous development of mycelium, and a brilliant pomegranate purplish color was noticed, conspicuously if the culture was observed from the bottom side of the dishes. The coloration leads us to think it to be very similar to the median cut surface of a peach, the flesh color of which is of pomegranate purple. No sporodochial formation was observed.

Culture 20. From perithecia produced in Culture 10, ascospores were taken and transferred upon potato slants sterilized in the common test-tubes.

The culture was started on May 13, 1922, and was left in a room, temperature of which averaged 20°C. Vigorous snow white mycelial tufts developed after a week and pomegranate purple exhibited itself to the fall extent in all the tubes. No trace of sporodochia was observed.

Culture 21. On May 13, 1922, ascospores taken from perithecia produced in Culture 10 were tranferred upon the heads of the naked barley sterilized in Roux's tubes, and all tubes were left at a room temperature which averaged 20°C.

Mycelial growth fully covered the heads after a week and pomegranate purple pigment appeared in the points where a part of the head came in contact with the inner wall of the tube. This coloration was also observed on the mycelial mats which covered the surface of water in the bulb of the tubes.

Culture 22. Ascospores procured from perithecia formed in Culture 9 were transferred to potato extract saccharose (5%) agar in the petri-dishes. After 8 days culture period in an electric thermostat of 25°C. white mycelial growth was found to be vigorously extending upwards or creeping along the inner wall of the upper cover of the dishes. And when observed from underneath of the dishes, a very brilliant pomegranate purple coloration was to be seen in all. Sporodochia were not observed.

Culture 23. On May 13, 1922, water suspension of ascospores from the perithecia developed in Culture 9 was applied to the heads of wheat and barley planted in the pots. The number of the heads was 17 in the case of the wheat; and 15 in that of barley; and all were just in the flowering season. All inoculated plants were subsequently covered with bell-jars to protect them from drying out. Fungus development progressed very soon after inoculation and positive infection resulted in all cases.

A few trials with the pigment produced by the fungus.

Generally the hyphae which lie upon or near the surface of substrata are colored in pomegranate purple or in yellow. Those somewhat apart from the surface remain always colorless. Even in one colored mycelium the colorless cells occur now and then. The coloring matter is formed within the cells of hyphae, and in the colored cells a few or many colored granules are to be seen. The pigment does not diffuse out into the substrata, which appear to be carmine color only owing to the fact that the colored hyphae are intermingled within them, such hyphae often forming the plectenchyma. It is a well known fact that this fungus produces carmine red in an alkaline substratum, while yellow in the presence of acid. On substrata rich in carbohydrates many fungi and bacteria produce alkaline substances; on those rich in pepton, acid substances. Each reaction is accompanied by special

colors, which change with the change of reactions of the substratum. And the relations of color and reaction of organisms, studied by MILBURN (1904) and BESSEY (1904) are said to be constant under constant conditions. HOPKINS says, in a private letter received by the writer on Jan. 31, 1922, that "the pigment produced by *Gibberella Saubinetii* (Mont.) Sacc. shows a rather sharp color change. In cultures with a reaction pH 4.0, and those more acid it is yellow, at pH 4.7 and in cultures more alkaline it is red or lavender while at pH 4.4 it shows an orange color. The pigment therefore acts like an indicator and its color change point may be placed approximately at pH 4.4."

A few trials performed by the writer with regard to the pigment of the fungus will be appended here in this place.

KLEIN (1892) working on a species which stands in a closer resemblance with *Fusarium graminearum* remarks that the part of mycelium which is as yet searcely reddish will become dark-red by warming in hot water. On Oct. 28, 1920, the present writer has selected out several culture-tubes within which the coloration was of buff-yellow, though in some tubes a very pale pinkish shade was also faintly observable. The tubes were placed in boiling water. The agar slant in the tubes melted down with the mycelial growth. The tubes which had pale pinkish shade changed directly into clear crimson, and almost all of those of buff-yellow changed also into the same color. However, a few which were yellow remained unchanged, which is most-probably due either to the reaction of the substrata or to the absence of easily volatiliging oxygen.

Potato slant cultures in Culture 1 were used as materials for the study. The slants, on which the pomegrante purple coloration was fully developed, were selected and they were directly dipped into the solvents. The subsequent dissolving out of the pigment was then studied at frequent intervals.

1. Ethylalcohol.—The pigment dissolves out very easily even while under observation, and gives the alcohol a color of safrano-pink or grenadine pink. (Rigdway)

2. Chloroform.—The pigment dissolves out sooner than in the case of ethylalcohol and stains the solvent a deeper pinkish, which corresponds to scarlet or scarlet red.

3. Benzene.—In comparison with both above noted solvents this solvent dissolves the pigment only slowly. After nearly 5 hours benzene became stained into shrimp pink or safaro pink.

4. Ether.—As in the case of benzene, this slowly dissolves out the pigment. Ether stained after nearly 10 hours faint yellow, corresponding to margnerite yellow.

5. Formic acid.—When a culture-slant was put into this acid, the white mycelium at once turned yellow and the crimson became gradually changed into orange. The acid itself soon became stained primuline yellow.

6. Acetic acid.—Gracile acetic acid was used, mycelium became yellow instantly and crimson coloration turned into orange gradually. The acid itself became mustard yellow, a little more faint than in the case of the formic acid.

7. Liquid paraffin.—This does not dissolve the pigment.

8. Sulfuric acid.—Dissolving out of pigment commences at once giving the acid yellowish brown coloration which afterward turns into dark slate purple or taupe brown.

9. Hydrochloric acid.—With diluted acid crimson fades away at once turning yellow. Dissolving action of this acid is slow and the acid itself becomes turbid, with a milky white color.

10. Aceton.—Pigment dissolves very easily into this chemical giving it a beautiful rose color. Dissolved pigment is apt to produce a sediment making the color of the lower part of the fluid denser. When observed, however, two days later the rosy color was found changed into mikado yellow.

11. Ammonia.—Pigment dissolves very slowly. Afterward, however, faint yellowish coloration was given to the fluid and after 6 hours it became denser yellow. And two days later it became pinkish and approached scarlet red when observed through the fluid and brazil red if the tube was placed upon a sheet of white paper.

12. Nitric acid.—Dissolving out of pigment instantly occurred and gave to the acid faint yellow which changed mustard yellow.

13. Turpentine.—Even after 2 days pigment did not dissolve.

Alkaline blue perithecia of *Gibberella Saubinetii* turn red or brown with the addition of acids, and this alternative change of color can be produced repeatedly.

Résumé.

1. There exists a species of *Fusarium* which causes seedling-blight, head-blight, and stem-rot in the rice plants. This species has heretofore been identified with *Fusarium roseum* Link, by many observers. The so called *F. roseum* Authorum, however, is a collective name including several species of the genus. With this question in mind, studies have been made upon this species of fungus.
2. *Gibberella Saubinetii* (Mont.) Sacc., which is common on the haulm of the diseased rice plants in open fields, has generally been described as the perfect stage of the said *Fusarium*—though it is in fact really the case—according to observation based upon field materials only and not on pure cultures.
3. Cultural studies have been undertaken to prove this generic connection.

Cultures started with conidia of the *Fusarium* taken from rice grains gave rise to the perithecial stage: *Gibberella Saubinetii* (Mont.) Sacc., and conversely also the conidia were produced in cultures derived from the ascospores of this ascigenous fungus. In view of these facts the life cycle of this species of *Fusarium* was determined and this organism was identified with certainty with *Gibberella Saubinetii* (Mont.) Sacc.; its conidial form being *Fusarium graminearum* Schwarbe.

4. Observed data with regard to 23 cultures of this fungus have been given; 12 series being those started from conidia, 6 series from the ascospores obtained from fields, and 5 series from the ascospores produced in our pure cultures.
5. Attempts have been made to investigate the dissolubility of a pomegranate pigment of this fungus and the results obtained thereby are briefly appended.

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