Gibberella Saubinetii (Mont.) Sacc. as a Causal Fungus of the Wilt-disease of Horse-bean.

with Plate XIV—XV.

By

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1. Introduction.

It was in June 1919 that a wilt-disease in the Horse-bean (*Vicia Faba* L. var. *equina* Pers.) first came under the notice of the author, at a time when the crop was just ready for harvest. In the previous year, in Kurashiki, this disease had caused considerable damage to this plant, reducing the production of bean by a large percentage. Since that date the writer has been paying special attention to this matter, in order to ascertain the exact cause of the disease; and the following kinds of fungus were found to be associated with it.

1) *Phoma* sp.
2) *Macrophoma* sp.
3) *Rhizoctonia* sp.
4) *Penicillium* sp.
5) *Botrytis* sp.
6) *Sclerotinia* sp.
7) *Sclerotium* sp. (2 kinds)
8) *Fusarium* sp.
9) *Gibberella Saubinetii* (Mont.) Sacc.

Among these fungi *Gibberella Saubinetii* (Mont.) Sacc. seems to the writer, to play the most important rôle in producing the disease. Through the attack of this fungus, the Horse-beans, when they are full grown and ready to be picked, gradually wilt, or lose their leaves. The ripenning of the pods is checked, and it happens that the whole plant completely withers away with serious results to the bean crop.

So far as the writer is aware, there exists no extended experiment on the Gibberella-disease of the Horse-bean; hence it is thought worth while to put this paper into publication.

F. L. Stevens[1] reports from America that the conidial stage of *G. Saubinetii* causes diseases in clover and alfalfa, both hosts belonging to the

Leguminosae. The Horse-bean is also a member of the same family. Through the cultural and infection experiments made by him, it was demonstrated that the fungus which attacks clover is identical with that found on wheat, barley, rye, spelt, emmer and oats.

O. KIRCHNER[3] makes very short mention of *G. Saubinetii* in his handbook, saying that it attacks *Vicia Faba* L. It appears to the present writer, that this statement of KIRCHNER is the only statement upon the Gibberella disease of the Horse-bean that has so far been made in other countries.

2. Symptoms of the disease.

This disease occurs, in late spring, upon Horse-beans which have been planted in fields where rice was planted under irrigation in the previous year. The writer has never observed the host plant, when planted in dry soil, to suffer from this disease.

The symptoms are various, but generally speaking the diseased plants develop slender, the pods produced are always few in number and smaller than usual. When the plant is almost at the ripe stage, in the middle of May, if the weather is moist, this disease makes its appearance in such a way as to attract our attention. The fungus appears in the basal part of the stem, from ground surface upward to a limit of 2 or 3 inches; and in the affected part, the reddish growth of fungus mycelium is always to be noticed. By this time whole plant tends to wilt; the leaves droop and hang down; the majority of the pods wither and some blackish specks appear upon them; while the beans themselves are covered with brownish spots.

The rootlets, being attacked in the early period of the disease, are completely destroyed sooner or later, and it is very easy to pull out the diseased plants as only the main root is left. The lower affected parts of the stem are blackish in color, and the cortex of that portion is entirely destroyed. In this portion minute black perithecia, sometimes singly but generally in groups, are easily found with the unaided eye.

3. Predisposition.

Surrounding conditions seem to have an important bearing on the occurrence of the disease. This disease often appears after a long spell of wet weather, when the water-content of the soil is high, without regard to other soil conditions. In Kurashiki and its neighbourhood the underground water

is rather high and precipitation is often abundant. The following figures are shown to indicate the precipitation amount during from March till June, since 1918 till 1923.

	Frequency	Amount	Remarks
		m.m.	
1918	54	496.3	Disease very heavy.
1919	34	400.9	„ rare.
1920	44	406.8	„ comparatively heavy.
1921	47	642.3	„ very heavy.
1922	30	305.5	„ rare.
1923	44	688.6	„ very heavy.

4. Morphology of the causal fungus.

A) *Conidial stage.*

Fusarium graminearum SCHWARBE is the name given to the conidial stage of this fungus. The ascospores develop very easily on many artificial or natural substrata. After they have been cultured for a few days, many tufts of aerial mycelium are produced. Aerial mycelium is septate, branched, and hyaline, but becomes staind afterwards to a pinkish, brownish, or yellowish tint. Conidia are borne on conidiophores which branch out of mycelium. Conidia are sickle shaped, hyaline in color, curved to one side, and so forming a dorsi-ventral distinction, broadest in the part immediately above the middle, then gradually tapering towards both ends. At the basal end of conidia a rudimentary pedicel is visible. Dimensions of conidia are 26—51 $\times$ 3.7—5 μ, the average being 34.7 $\times$ 4.5 μ. The number of septum is 3—5, in majority of cases being 3, and in rare cases 6.

B) *Perithecial stage.*

Many minute blackish perithecia are produced, rarely singly but commonly in aggregations, on the basal stem part of the diseased plant. As viewed with the naked eye the surface is seen to be rough and when observed under the microscope it appears jagged and irregular. The cells composing outer part of the perithecium are thick walled and have a beatiful bluish or inky-black color. The shape of perithecia is spherical or egg-form, and provided with an ostiolum on the upper part, and measure 60—280 $\times$ 50—250 μ, the average being 198 $\times$ 172 μ. Those produced in cultures on rice extract agar measured 95—330 $\times$ 95—310 μ, the average being 228 $\times$ 195.5 μ. Ascus is hyaline, (but if gathered together is of a pale brownish color,) club-shaped, a little swollen at the attaching basal part, perishes easily, containing

eight ascospores arranged in two oblique rows, 55—100 × 10—15 μ large, averaging 79.8 × 12 μ.

Ascospores are hyaline, curved, fusiform, obtuse at both ends, 3-septate, constricted at septum, and smaller or larger oil globules are found while young; dimensions are 17—28 × 3.8—5.6 μ, the average of 600 spores which were obtained from the natural host *Vicia Faba*, being 22.4 × 4.5 μ; while those from perithecia, produced on rice extract agar, have somewhat larger dimensions being 21.5—36 × 4—6.2 μ; the average of 100 spores being 25.9 × 4.9 μ.

Ascospores germinate easily in distilled water at the room temperature in middle of June, the germ-tube reached 15—30 μ within 6 hours. In general, germination takes place from one of the terminal cells of the spore, and when a germ-tube is thus elongated and branched, the other cell sends out another. Paraphyses of rather peculiar shape and inconspicuous are found standing among the asci. They are very flexible; broken bits are often observed, while perfect ones are very rarely found. Paraphyses are composed of several ovoid cells, and are very deeply constricted at the septum. These cells are various, the larger ones measuring 35 × 24 μ, while the smaller measure 10 × 11 μ. Both larger and smaller cells, are arranged straight irregularly, the basal cell being smaller than the others and club-shaped. The length of paraphyses is difficult to measure as they are easily broken as mentioned above, however there are many which are a little longer than the ascus.

Under higher magnification of the microscope the paraphyses are difficult to see, whereas on the contrary under 300—400 magnification they are easily observed.

5. Cultural characters.

The materials used for this study were those collected on June 20, 1919 from a Horse-bean field in Ōtakamura, a village near Kurashiki. A single ascospore was used in the starting culture. Mycelial growth from this isolation culture was taken, and transplanted upon the following substrata, and the results obtained thereby will be briefly given.

1) *Rice extract agar.* Test-tube cultures in a thermostat of 30°C.
(Rice-stem 200 gr, agar 18 gr, distiled water 1000 cc.).

Growth was vigorous, provided with long white aerial mycelium. Conidia were produced after 5 days, and after 2 weeks white knots of mycelium were found in or near the surface of the substratum. These knots become larger and numerous after 3 weeks. They are stroma for the perithecia. The diameter of the colony at the end of 5 days averaged 62—65 mm, and the

coloration at the same period of growth was rosy-red. The coloration varied in density and time of appearance.

2) *Potato-extract agar.* Tube-cultures in a thermostat of 30°C.
(Raw leaves of potato 200 gr, agar 20 gr, water 1000 cc.)

A flourishing development of aerial mycelium was observed, the growing tip of mycelium was round and smooth. Conidial production was also normal. The growth after one week measured 51.1 mm, being a little inferior when compared with the 64.5 mm. of experiment No. 1. Many white knots were found, just as in the first experiment.

3) *Potato-slice culture.* In Petri-dishes at room temperature.

After 2 days dense mycelial growth developed. Rosy coloration appeared, and diameter of the growth reached 40 mm. in 3 days, and after 5 days, the central part of the colonies became grayish, the other parts being Cameo Pink, and when observed from outer bottom side of the dish-cultures, these parts appeared Pomegranate purple. No conidial formation was noticed.

4) *Steamed potato stem.* In the test-tubes in a thermostat of 25—28°C.

Growth was vigorous in the second day, and after 3 days diameter of the growth reached 23—30 mm. White aerial mycelium grew conspicuously, but no conidia were found. The whole surface of the substratum was completely covered with mycelium after 4 days. The characteristic Crimson color appeared after 8 days, and in 2 weeks white knots were observed. By this period of time, conidial production occurred normally, yet there was no trace of the production of perithecia.

5) *Horse-bean stem.* In the test-tubes in a thermostat of 25—28°C.

After 3 days development took place, and growth reached to 90 mm. in diameter in 2 weeks. However, development was found to be not vigorous. White knots were produced after 3 weeks and rosy color began to be denser by this period of time. A number of perithecia was produced.

6) *Steamed rice grain.* In Petri-dishes at room temperature in the end of September.

Growth diameter was 60 mm. after 3 days, and the central portien of the colony was yellowish, while white aerial mycelium bordering the margin.

By the 4th-day the mycelium had completely covered the whole surface of the substratum in the dishes, and rosy coloration appeared. On the fifth day few conidia were found and the intensity of the rosy coloration became deeper and deeper. Conidia were produced in small numbers only and no perithecia have developed.

7) *Glucose (5%) agar.* Test-tube cultures at room temperature in August.

Upon this substratum the fungus behaved almost in the same way as in the case of the rice-extract agar, the only difference lying in the greater depth of rosy color in favour of this case.

The color, by reflected light corresponded to Victoria lake, and Carmine or Ox-blood Red by transmitted light. A large number of perithecia was produced, upon this substratum.

8) *Steamed Horse-bean.* In the test-tubes at the constant temperature of 25—28°C.

Vigorous growth was obtained, the beans being completely covered by aerial mycelium which was found fully occupying the inner portion of the seed coat; and it was also well developed on the surface of the water which remained at the bottom of the tubes.

The characteristic deep carmine coloration developed in the part of mycelial growth that lay over the water, and conidial production was observed. Still no perithecia were formed.

As has been described above, the formation of conidia and perithecia was rather rare, or even lacking, in several kinds of the cultural substrata used. Even when both conidia and perithecia were formed they were few in number. Perithecial formation was not obtained at all on steamed rice grains, potato slices and potato-stems in our experiments.

6. Development in relation to temperature.

After few trials having been attempted to ascertain this relation, it was found that the dimensions of the colonies, after 5 days, averaged 44.5 mm at 30°C; 27.9 mm at 15°C; and 6.29 mm at 5°C. The average maximum air temperature of every March in the 6 years since 1918 till 1923 is 12—16°C; that of April 18—19°C, that of May 21—25°C; and that of June 25—30°C. From these facts it is thought the optimum temperature for the growth of this fungus lies between 25—30°C. And as it makes daily development of 1.26 mm at 5°C, a still lower temperature seems not to check the growth. The respective minimum temperatures during the four months, from March to June, of these 6 years are 1.34—3.8°C in March; 6.2—7.4°C in April; 10.5—14.1°C in May; and 17.6—18.2°C in June. From these data it is obvious that the lower air temperature in the middle or end of March does not hinder the development of this fungus in any way, if the other conditions are favorable.

7. Summary.

1) The wilt-disease of Horse-bean (*Vicia Faba* L. var. *equina* Pers.), in Okayama, a district of western Japan, is found to be caused by a number of fungi such as *Phoma*, *Rhizoctonia*, *Penicillium*, *Botrytis*, *Sclerotinia*, *Sclerotium*, and *Gibberella Saubinetii* (Mont.) Sacc. Among these the last named species [*Gibberella Saubinetii* (Mont.) Sacc.] is the most prevalent cause of the disease.

2) The general morphological characters of *Gibberella Saubinetii* (Mont.) Sacc. as observed upon the host plant, and its cultural results have been described.

3) The optimum temperature for the growth of this fungus in cultures seems to lie near 30°C, but even at 5°C a growth of 1.26 mm per day does occur.

By compering this optimum with the atmospheric temperature of this district of Japan, the air temperature of June seems to be adequatly fitted for the development of this fungus.

4) An unusually heavy precipitation and higher underground water furnish the two important predisposing factors in the appearance of this disease.

Acknowledgment.

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3) KIRCHNER, O., Krankheiten und Beschadigungen unserer Landwirschaftlichen Kulturpflanzen. p. 63 und p. 128, 1890.

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Explanation of the figures.

Plate XIV. Perithecial stage of *Gibberella Saubinetii* (Mont.) Sacc.

Fig. 1. Longitudinal section of a perithecium produced on the host plant. (*Vicia Faba* L. var. *equina*. Pers.)

Fig. 2. A group of asci and paraphyses.

Fig. 3. Matured and young asci.

Fig. 4. Ascospores.

Fig. 5. Ascospores germinated in distilled water.

Fig. 6. Cross-section through aggregated perithecia upon the stroma on the host plant.

Plate XV. Conidial stage of *Gibberella Saubinetii* (Mont.) Sacc.

Fig. 1. Mycelium and conidia obtained in a pure culture on steamed potato stem.

Fig. 2. Mycelium with very large conidia found in cultures on steamed stem of the host plant.

Fig. 3. Mycelium and conidia developed in an Oryza decoct agar culture.

PLATE XIV.

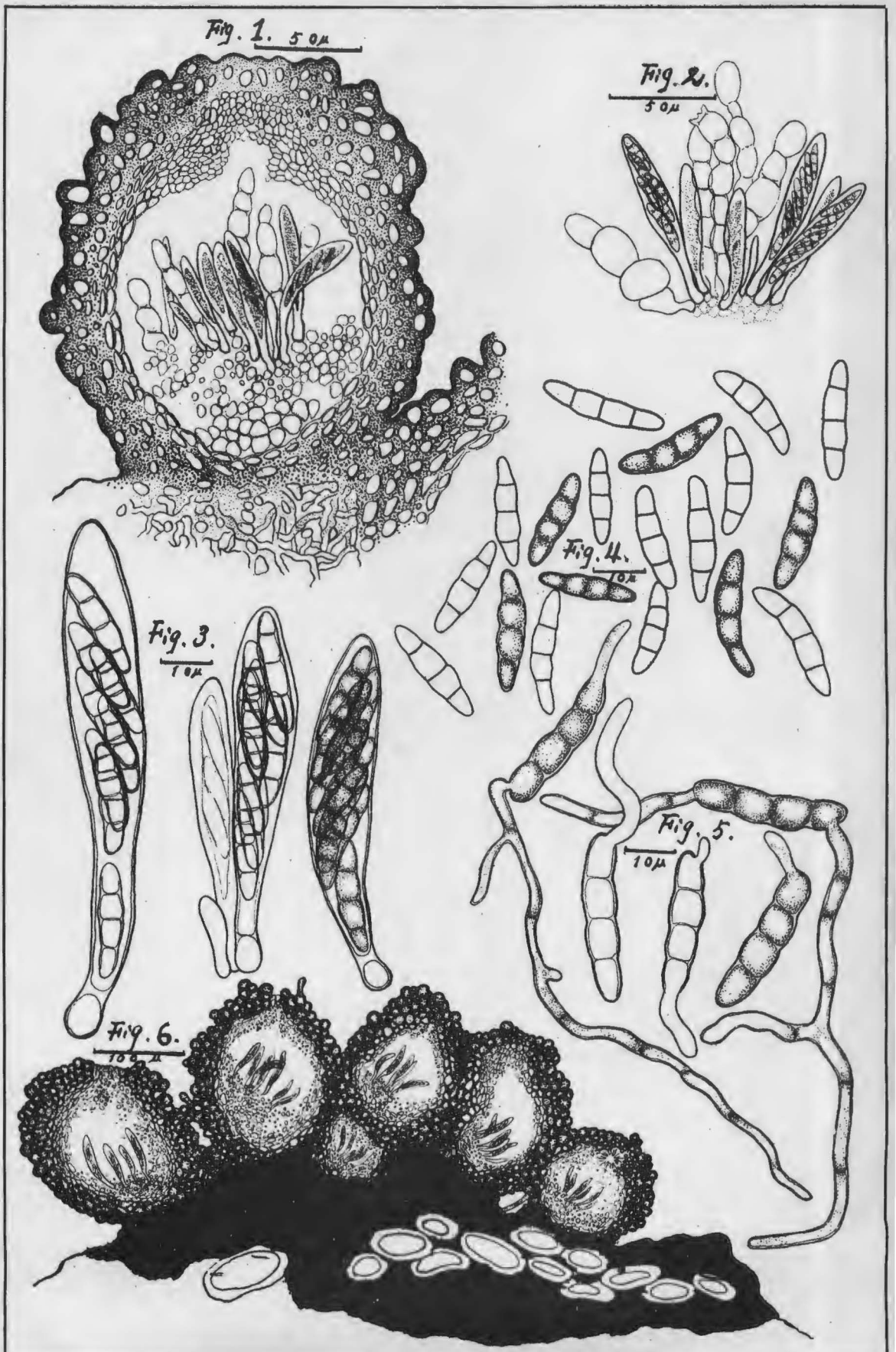

PLATE XV.