STUDIES ON THE WHEAT SCAB, CAUSED BY *GIBBERELLA ZEAE* (SCHW.) PETCH, AND ITS CONTROL

By

Yosikazu NISIKADO

INTRODUCTION

The present disease seems to have been known since long before, not only in Japan, but also in almost all over the world. As the rainy season, so called "Tsuyu", in Japan sets usually in the ripening period of wheat and other cereals, the damage of this disease might be sometimes especially severe, although it had been attributed to the rain damage. The disease was caused by a parasitic fungus, which has a very wide range of host plants of Gramineae and other families. Among these, the damage on wheat, known as head blight, scab or seedling blight, was especially prominent. The disease was studied by many plant pathologists of all the world, and great many numbers of reports had been published. But no suitable method to control this disease had been shown.

In 1913 the writer began to investigate the scab or head blight disease of wheat fundamentally, hoping to ascertain some reliable method for the protection. After then the project was continuously supported by the Ministry of Agriculture and Forestry. The results of the writers' investigations have already been published in progressive annual reports to the Ministry of Agriculture and Forestry and in other papers. Although further investigations must be done in many points, the writer thought it well to compile all the available data of his works, as circumstances compelled him to do so. Lately they were published in a paper in Japanese "Komugi no Akakabi-byo ni kansuru Kenkyu", or Studies on the wheat scab caused by *Gibberella zeae* (SCHW.) Petch and its control, published in the "Nôgaku Kenkyû" or Report of the Ohara Institute for Agricultural Biology, Okayama University, Vol. **45** pp 59—86, 141—158, 159—220, Vol. **46**, pp 1—47, 1958, and also in the "Nôgyô Kairyô Gizyutsu Shiryô" or Publications for the Improving Agricultural Technique of the Ministry of Agriculture and Forestry, No. **97**, pp. i —x, 1—162, References: pp. 144—153, English summary: pp. 155—162, Plate I—XVIII, March 1958. The contents of the original paper in Japanese, above mentioned, and the summary and some of the Plates are given in this paper.

During the course of the investigation the writer has been much obliged to Messrs. Hideo Nishigori, Director of the Research Department; Shinjirô Akimoto and Jiro Takeuchi, Former Heads of the Agricultural Production Section; Masaakira Hori, Head of the Plant Protection Section; Umenojô Bokura, Kazuo Gotô, Project Leader of the Plant Protection; Yoshihisa Iizuka, Chief of the Forecast of Disease Outbreak, and others of the Ministry

of Agriculture and Forestry; Takao Nakayama, and many other former and pressent members of the Plant Pathology Laboratory of the Ohara Institute for Agricultural Biology, Okayama University. To these gentlemen the writer wishes to express his sincere thanks. Thanks are also due to Dr. Shunsuke Kusano, Professor emeritus of Tokyô and other professors and researchers in the plant pathology laboratories of many National Universities and National or Prefectural Agricultural Experiment Stations for their kind suggestion and supply of research materials.

Contents of the Original Paper in Japanese

SUMMARY

I. NAME AND DISTRIBUTION OF THE DISEASE

1. Name of the Disease: A blight disease of head and seedling of wheat was first called in England, Wheat scab. In America it was called variouly as Head blight of wheat, Fusarium blight, Fusarial head blight, Ear blight, and on seedlings as Seedling blight. In Germany it is called Weizenschorf, and in Italy it is Golpe bianca del frumento. In Japan such names as Akakabi-byô (Red mould disease) or Kokuten-byô (Black spot disease) have been used widely.

2. In years past, the disease was believed to be the direct damage caused by the rain. In Japan the disease was first recorded in 1902, while in 1914 it made an extensive outbreak and attracted attention as one of the most serious diseases of small grains. The disease is now widely distributed throughout the world, but in the United States, its damage has been great and there are numerous reports published on this disease.

II. SYMPTOMS

3. The blight on the wheat normally occurs on the head when there is rain during the heading period.

4. During the early period of infection, a part or entire spikelet becomes discolored brown. Then normally there appears a salmon colored fungus growth between the layers of glumes, which are the masses of conidia. When dry days follow after the infection, further spread of the disease is checked, but the portion of a head above the infected spikelet normally dies. When the diseased head is exposed to more rain, the disease progresses rapidly and causes the entire head to rot. As the infected head ages, there appears on the surface minute black bodies which are the perithecia that bear ascospores.

5. Infection occurs also on the tender root and colyoptile of the young plant, which appears as brown, reddish brown or dark brown spots. Sometimes blotches of spots are produced on the blades of the weakened plant.

III. CAUSAL FUNGUS

6. The blight is caused by a fungus belonging to the genus Gibberella. As to its name, the authors have adopted *G. zeae* (Schw.) Petch (1936), with *G. saubinetii* (Mont.) Sacc. as its synonym. In the asexual or conidial stage of this fungus it is called *Fusarium graminearum* Schwabe.

7. The causal fungus produces conidia, ascospores, and to a limited extent chlamydospores. Conidia are hyaline, cresent shaped, normally 6-celled, pointed at one end and with a distinct base or foot at the other end. Size averages $49.5 \times 6.5 \mu$. Conidiophores are either simple or branched, and bear one conidium at the apex.

Perithecia are blue-black to black in color, near spherical and each provided with a beak having an opening or ostiole. Size of the perithecia averages $272 \times 218.1\,\mu$.

Asci are hyaline, club shaped, and each contains 8 ascospores. Their size averages $99.8 \times 11\,\mu$. Ascospores are hyaline, spindle-shaped, and consist of 4 cells. The size averages $21.4 \times 4.1\,\mu$.

Chlamydospores may be formed in the cells of the mycelium or the conidium. They are usually imperfectly formed.

8. Variation in the morphology of the mycelia and conidia may be influenced by the portion of the fungus body removed for the isolation of the fungus. The fungus isolates those originated from ascospores normally exhibited vigor and reproduced readily conidia. Those originating from conidia or mycelia may yield perithecia.

9. The optimum temperature for the growth of mycelia is 27°C. Maximum and minimum temperatures are, respectively, 32° to 35° and 5°C. Optimum pH range for the growth of fungus is 5.0 to 6.0. The fungus will grow at a wide range of between pH 2.7 and 11.7. For the formation of conidia, temperature higher than 15°C and a relative humidity greater than 98% are required.

The optimum temperature for the germination of conidia is in the neighborhood of 25°C. For an optimum germination a relative humidity of 100% is required. There will be no germination at 93% or less. Aerial mycelium is produced most abundantly at 98% relative humidity.

10. Formation of conidia is gradually increased after each cycle consisting of several days. Light is required for the formation of conida and the ultra-violet light influences the most. An extremely intense light is harmful to the formation of conidia. Germination of conidia is best when they are fresh and the density is low. Addition of small amounts of glucose or magnesium sulfate in the medium stimulates germination. Removing the adhesive coating of the conidia will not cause reduction in the germination.

11. For the formation of perithecia a minimum relative humidity of 98%, a temperature of above 15°C, diffused light, and a certain amount of air circulation are required. A high nutrient level is not essential for the formation of perithecia.

12. From results of mix culturing of the fungus isolates from single ascospore, the fungus has been determined homothallic in its sexual behavior.

IV. INVASION OF HOST PLANT BY THE CAUSAL FUNGUS

13. When invading the wheat head the fungus first infected the anthers, then progressed into the outer and the inner glumes. It was rare that the fungus penentrated the epidermal cells of the outer glume. The most common mode of entrance was through the stomata. It appeared that the invasion of the inner glume was more common than the outer glume. The fungus reach-

ed endosperm and embryo of the developing kernel from the glumes by progressing through vascular bundles. Invasion into the kernel was made more difficult as the tissues of the kernel develop and become thickened. Infection of the kernel was therefore usually limited to the milk stage.

14. The causal fungus also attacked germinating seeds, young roots, sheath and hypocotyl. Invation of the young roots took place by either directly penetrating the epidermal cells or entering between the epidermal cells and the root hairs. Mycelial mass was formed on the sheath by which infection occured through the seam of the cell or through the stomata. Infection of the hypocotyl also took place by means of mycelial mass through stomata.

V. PATHOGENICITY AND VARIATION IN RESISTANCE TO FUNGICIDES

15. In the 124 isolates of the fungus studied, the pathogenicity varied widely from very weak to very strong.

16. In the six variants that were separated from the mother isolates as having distinct morphological differences, the pathogenicity was found to have been changed. The direction of the change was in both directions—some increased the pathogenicity while the others decreased. Variation in the pathogenicity among the conidia and ascospores was found to be very little. Pathogenicity decreased by repeated transplanting. Isolates made from wheat having varying degrees of susceptibility to wheat scab, showed no distinct tendency in their degree of pathogenicity. In a study of reaction of different isolates to copper fungicides, it was found that variation existed among the isolates in the resistance to copper fungicides.

VI. SUSCEPTIBILITY OF WHEAT VARIETIES TO THE WHEAT SCAB

17. From the result of tests conducted on some 70 varieties of wheat, it was found that the susceptibility type of the wheat head to scab can be classified in the following 3 large groups, in relation to the period of blooming and that of infection.

Group 1. Maximum infection occurs during the period of blooming.

Group 2. Maximum infection occurs during the period following the blooming. Infection declines rapidly after this period.

- a. Uniform rate of infection occurs during the period of one weeks after blooming.
- b. Uniform rate of infection occurs during the period of two weeks after blooming.

Group 3. Rate of infection rises for a short time after blooming, which is followed by a gradual decline.

- a. Rate of infection reaches a maximum on 3 to 5 days

after blooming, then it gradually declines.

b. Rate of infection reaches a maximum on 7 to 9 days after blooming, the it gradually declines.

c. Rate of infection reaches a maximum on 11 to 13 days after blooming, then it gradually declines.

18. Most varieties of wheat showed higher rates of infection when inoculated and incubated at 27°C or 30°C than at 20°C.

19. There were no significant correlation between the susceptibility of wheat varieties and the length, width, thickness of the head, length of awn, angle of the opened glumes, hairyness, as well as pigmentation of the head.

20. Correlation was high on the susceptibility of wheat between the stages of seed germination and plant maturity, and $r = 0.472 \pm 0.085$ ($P < 0.01$). Also a certain degree of correlation was observed between the susceptibilities of the varieties in the seedling and matured stages, and between seed germination and seedling infection.

21. From a 7 year study of wheat varieties on the susceptibility on the wheat scab, it was found that there were no varieties that showed consistent resistance to the wheat scab. Although the reaction varied somewhat from year to year, such varieties as Sôshû, Wase-Komugi, Homan, Homan No. 1, Iwate-Sôshû, Kinai No. 9, Sanshû-Kotake showed comparatively less infected heads, whereas, Saikai No. 45, Kitakanto No. 14, Pusa No. 12, Mihara, Mubô-chiko, Shirage-Nankin, Norin No. 5, Yamaguchi-Komugi and Bei No. 1 had more infected heads from year to year, and were thus considered susceptible varieties.

VII. SHAPE AND GERMINATION OF DISEASED KERNEL

22. Healthy kernels passed mostly through sieves having openings between 2.5 and 2.7 mm ; whereas in the diseased kernels they were 2.2 and 2.5 mm. The diseased kernels also showed a general relationship of width being less than thickness. The diseased kernels are thus more slenderer than the healthy kernels and their weight of 1000 grains was less than that of healthy kernels.

23. Germination of wheat seed was best at temperatures between 15° and 17°C. In the inoculated seeds the germination was best in the neighborhood of 10°C. At 10°C damage from the disease was at a minimum. Germination of inoculated seeds decreased with the reduction in soil moisture up to 45.5 percent (percent of moisture in air dry soil).

24. Germination of diseased grain was better on larger grain than the smaller grain. The diseased grains germinated better when they were only lightly covered with soil. Those diseased grains that had a specific gravity of more than 1.24 showed least effect on the germination. Lighter grains had poorer germination. Less fungus mycelium was present in kernels with greater specific gravity.

25. When samples of diseased grains were blown by a current of air, a majority of the heavier grains had the specific gravity of greater than 1.20. In the healthy grains the percentage of germination had but little relationship with the specific gravity.

VIII. PRIMARY AND SECONDARY INFECTIONS

26. The fungus survived on rice straw agar cultur medium as follows: 36 months or more at 5°C, 33 to 36 months at 10° to 15°C, 14 months at 20°C, 10 to 15 months at 25°C, 4 to 7 months at 30°C, and 2 to 4 months at 35°C.

27. Formation of perithecia was affected by the environmental temperature, but in pure culture, perithecium was produced at any time of period between late March and November. In nature, perithecia were produced on rice straw that were piled in the open and on rice stubbles remaining in the field after the middle of April. These perithecia mature in May. They were also produced and thriving in abundance on other monocotyledonous plants.

28. Flight of ascospores was affected by environment. Ascospores occurred mostly during the period soon after a prolonged rain. The spores were shot out into the air when the humidity of the air was saturated or near saturated. They may fly to a height of 1 to 3 cm. It was found that the time between absorption of moisture by the perithecia and the flight of spores was 4 to 9 hours.

29. On the wheat head, ascospores germinated best at 24° to 25°C. Between 20°C and 30°C, the latter temperature was more favorable for the germination. Ascospores germinated better on the head than in plain water. Germination of ascopores took place within 3 hours, when the temperature might be between 20° and 30°C.

30. Observations made on the infection of wheat head by the ascospores produced in nature in the open showed that the wheat close to the source of infection became diseased as much as 96 percent. This percentage decreased in proportion to the distance from the source of inoculum. Plants in the leeward direction showed greater percentage of infected heads. These observations indicated that the ascospores possess high degree of pathogenicity to the wheat ear.

31. The time requiring for the infection of wheat head by the fungus spores under natural condition was studied, by exposing the wheat head to the source of infection at specified periods. It was shown that the head, becoming infected within one week after blooming, received the greatest damage from the disease. The infection was more severe, when there was rain or when the ascospores were in flight.

32. Studies were made on the conditions under which conidia are pro duced on the diseased wheat head. It was found that the conidia are not

produced in abundance when there is a prolonged period of wetness or dryness; but rather that, improvement in the weather conditions takes place, after the fungus has made sufficient growth, by rainy weather.

33. The percentage of germination of conidia was greatly increased by using the water adhering to the surface of the plant. The germination tube was also observed to elongate rapidly in this water.

34. Conidia produced on the diseased head were carried by the wind much more, when there was rain and high humidity.

35. So far as observations were made, an infected head produced at least 16×10^6 conidia, which upon contact with water might yield suspension of the conidia in short period of time. As the conidial suspension flowed down the side of the wheat head, the conidia adhered to the new surface. In this manner considerable number of conidia were left on the surface of the wheat head as a source of inoculum.

IX. EPIDEMIOLOGY OF THE SCAB DISEASE IN RELATION TO ENVIRONMENTAL FACTORS.

36. Occurrence of rain prior to infection stage had little effect on the susceptibility of wheat head to the scab.

37. Wheat plants growing in soil containing 60% soil moisture, had the greatest number of scab development; and either an increase or decrease in soil moisture from this content, caused reduction in the disease development.

38. Increased use and late application of nitrogen fertilizers caused increased scab development. Phosphorus and potassium fertilizers showed but little effect on the development of the scab, but appeared to have an opposite effect to that of the nitrogen fertilizers.

X. TOXICITY OF GRAINS AFFECTED WITH SCAB

39. Toxicity of the scabbed grain, as tested by feeding mice and rabbits, disclosed that these animals were not affected by the diseased grains. Further feeding tests using young chickens also did not show significant effect on the weight, body temperature, fecal mater, and development of the birds.

40. Toxicity of the scabbed grain was observed in Okayama prefecture on two calves fed with a ration containing diseased barley grain. As a result of consuming the grains, the calves exhibited such abnormal behavior as reduced appetite, difficulty in walking, also difficulty in the rising and sluggish in action, loss in firmness of the feces, and loss in body weight. The mothers of calves, however, did not show any effect from consuming diseased barley grains. Analyses of the feed showed that it contained, in large proportion, grains infected with *Gibberella zeae*, *Alternaria* sp., *Peni-*

cillium sp. and species of bacteria. Since *Gibberlla zeae* was particularly prevalent in the mixture it was believed that the toxicity effect, shown by the calves, was caused the scabbed grains.

XI. CONTROL OF THE DISEASE

41. For preventing the seedling blight a seed sterilization by a 5 minute soaking in hot water held at 50° to 52°C, or in 1/2000 dilution of mercuric chloride; and a 3 hours soaking in 1/400 dilution of formalin or uspulun were effective. Fumigation with 1/2500 formalin reduced the viability of the seed; whereas chloropicrin at the same rate did not reduce the viability, when the treatment was made after allowing 2 weeks of after-ripening period.

42. Tests using various fungicide compounds on slides showed that the germination of conidia was checked by fermate and bordeaux dusts. On agar medium arasan and fermate at a concentration of 0.01% effectively checked the germination of conidia. In volatile materials, growth was checked most by spergon and phygon.

43. On the effectiveness of various fungicides on the wheat head in preventing the scab, arasan and dust and water dispersible fermate were found highly effective. A certain degree of effectiveness was obtained from phygon and PMF (1/2000). The effectiveness was increased when dusting was made while the dew still remained on the surface of the plant, as it increased adherance of the chemicals to the plant.

44. Using cover glasses to test the effect of elapse of time on the effectiveness of fungicides, it was found that fermate retains effectiveness for at least 15 days which was equivalent to that of bordeaux mixture of 0.5%. Using the wheat heads, farmate was still effective at 6 days after the application.

45. Application of fungicides on the wheat head was most effective when it was made within one week after blooming. Effective control was noted when fungicides were applied 2 days after inoculating the wheat head, which was followed by favorable temperature and humidity conditions for infection by the fungus. Spraying after 3 day inoculation was still moderately effective. Elapse of 6 days or more had but little effect in reducing the damage on the head. The effectiveness of the fungicides was increased, if a period of dryness follows after application of fungicides to the head.

46. Effectiveness of the deposit of fungicides was reduced by a 20 minute rain; however, this reduction was comparatively less on such fungicides as arasan, fermate dust and phygon. Application of dust compounds for wheat scab was more effective, when made at the time dew still remained on the surface of the plant.

EXPLANATION OF PLATES

Plate I Symptoms of the scab disease of wheat caused by *Gibberella zeae* (Schw.) Petch.

(1) Wheat heads affected by the scab. At the left showing a seriously scabbed head, at the right a healthy head, and between the two various grades of the scab.
(2) Leaf blotch of wheat caused by artificial inoculation with *Gibberella zeae* (Schw.) Petch showing large irregular shaped blotches.

Plate II. Hyphae of *Gibberella zeae* (Schw.) Petch.

(1), (2) Showing thick-walled segments of the comparatively aged hyphae grown in culture.
(3) Showing ends of young hyphae grown on potato dextrose agar medium.
(4) Showing some conidium producing types of hyphae grown on potato dextrose agar.
(5) Fully grown hyphae.
(6) Vigorously growing young hyphae.

Plate III. Conidia and conidiophores of *Gibberella zeae* (Schw.) Petch.

(1) Showing conidia produced on a scabbed head wheat. (× 800)
(2) Showing conidiophores produced on a scabbed wheat head. (× 450)
(3) Showing conidia produced on a leaf blotch caused by artificial inoculation. (×900)
(4) Showing conidiophores produced on malt extract agar. (× 450)

Plate IV. Conidia, asci and ascospores of *Gibberella zeae* (Schw.) Petch.

(1) Conidia produced on scabbed wheat heads. (× 900)
(2) Conidia produced on potato-dextrose agar. (× 840)
(3) Asci and ascospores produced on rice straw. (Asci × 800, ascospores × 900)

Plate V. Formation, shape and contents of perithecia and conidium germination of *Gibberella zeae* (Schw.) Petch.

(1) Germination of conidia produced on a leaf blotch caused by artificial inoculation. (×450)
(2) Perithecia profusely produced on a scabbed wheat heads
(3) Perithecia broduced on germinating rice grains, artificially inoculated.
(4) Section of a perithecium produced on rice straw. (×120)
(5) Asci and ascospores of a perithecium produced on rice straw. (×540)

Plate VI. Perithecium, asci and ascospores of *Gibberella zeae* (Schw.) Petch.

(1) A perithecium produced on boiled rice straw medium, showing asci and ascospores. (×270)
(2) Asci and ascospores. (×240)
(3) An ascus. (×700)
(4) Ascospores. (×1000)

Plate VII. Infection of *Gibberella zeae* (Schw.) Petch to anther and glume tissue of wheat.

(1) Longitudinal section of a glume inoculated with the scab fungus, showing fungus hyphae and pollen.
(2) Transverse section of anthers. Showing hyphal attack to pollen. 24 hours after inoculation.
(3) Transverse section of a glume, showing maceration and collapse of leaf tissue near the stomata affected by the fungus hyphae.
(4) The same as above, enlarged.

Plate VIII. Infection of *Gibberella zeae* (Schw.) Petch to wheat tissue.

(1) Transverse section of an outer glume, showing the fungus hyphae in the host cells. (×600)
(2) Transverse section of outer glume, showing the hyphal penetration through stomata.
(3) Infection of hyphae to the ovary. (×500)
(4) Infection of hyphae to leaf tissues. (×200)

Plate IX. Infection of *Gibberella zeae* (Schw.) Petch to wheat tissues.

(1), (2) Hyphal infection to the tip of tender root. through epidermal cells.
(3) Hyphal infection to tender root, through epidermal cells.
(4), (5) Hyphal infection to tender young root, through the space between epidermal cell and root hairs.
(6), (7) Hyphal tips, somewhat swelling on the epidermis of the host.
(8), (9) Penetration of infection hyphae from am appressoria-like swelling tip of the hyphae into the host tissue.

Plate X. Infection of *Gibberella zeae* (Schw.) Petch to wheat tissues.

(1), (2) Penetration of hyphal mass into the epidermal cells.
(3)—(5) Penetration of hyphae through the stomata of cotyledon. Surface view of the stomata, 3—4 days after inoculation.
(6), (7) Transverse section of the stomata of cotyledon, showing the hyphal penetration and macerated chloroplasts.

Plate XI. Perithecium formation of *Gibberella zeae* (Schw.) Petch.

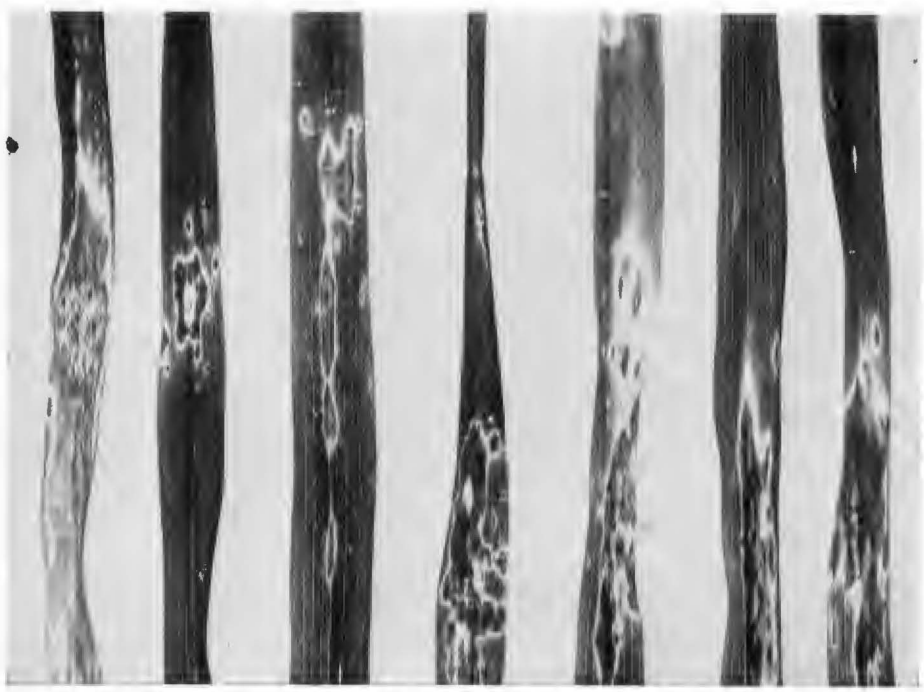
(1) Tall beaker experiment for the perithecium formation of *Gibberella zeae* (Schw.) Petch. Pieces of rice straw sterilized in reagent glasses, were inoculated with the scab fungus. They were then kept in tall beakers under diffused sun light.
(2) Some of rice stubbs affected by the scab fungus in fields. Showing abundant formation of perithecia.
(3) Scabbed wheat grains infected by *Gibberella zeae* (Schw.) Petch. Scabbed grains are thinner and more wrinkled than the healthy ones.

Plate XII. Inoculation experiments of *Gibberella zeae* (Schw.) Petch.

(1) Wheat plants in fields, showing inoculation experiment with *Gibberella zeae* (Schw.) Petch, wheat heads beeing sacked with cellophane to protect the heads from the natural infection of the scab before the experiments.
(2) Wheat plants in pots in a greenhouse, used for the inoculation experiment with *Gibberella zeae* (Schw.) Petch. Each head was labelled to show the date of shooting and flowering.

Plate I

(1)



(2)

Plate II

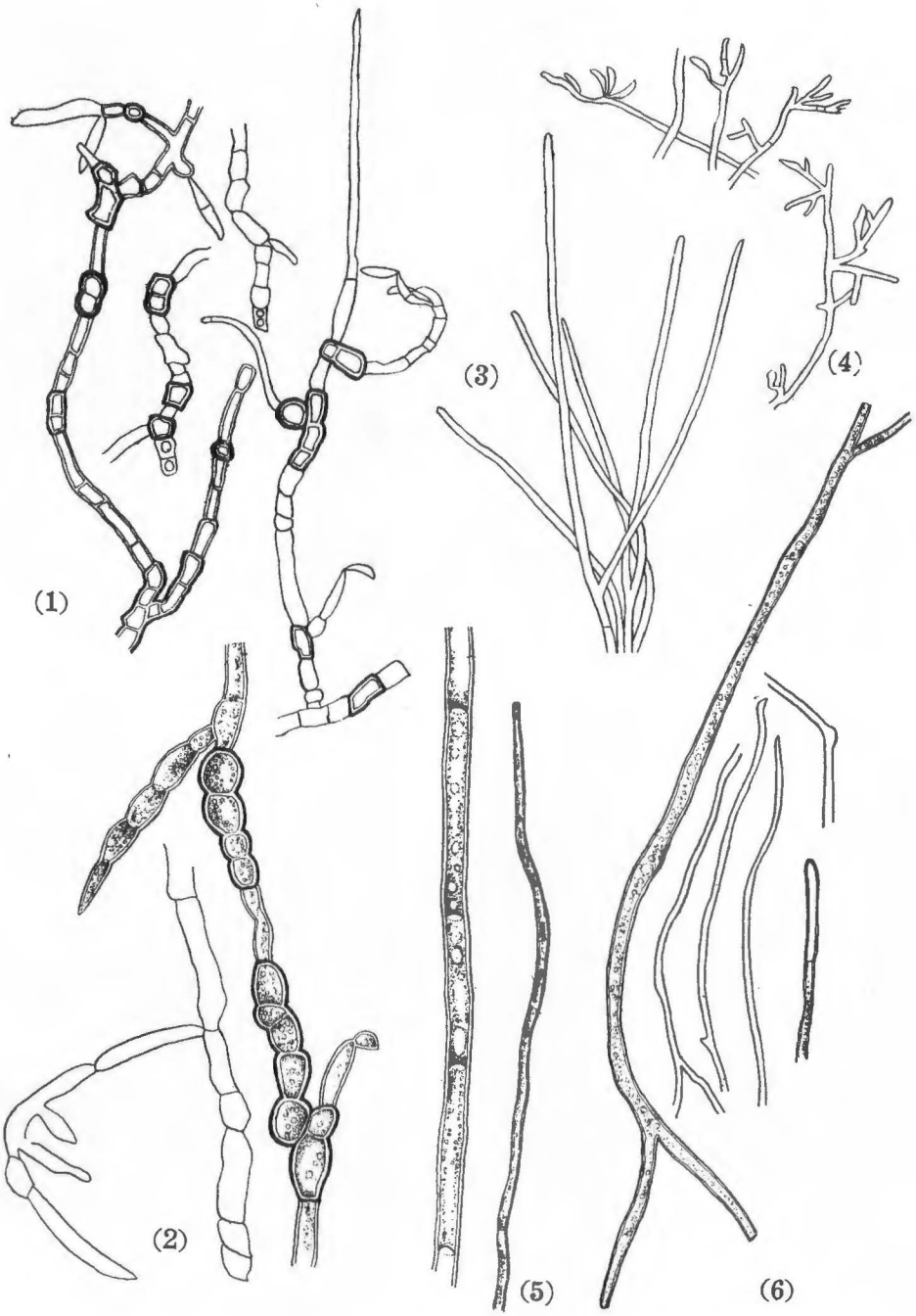
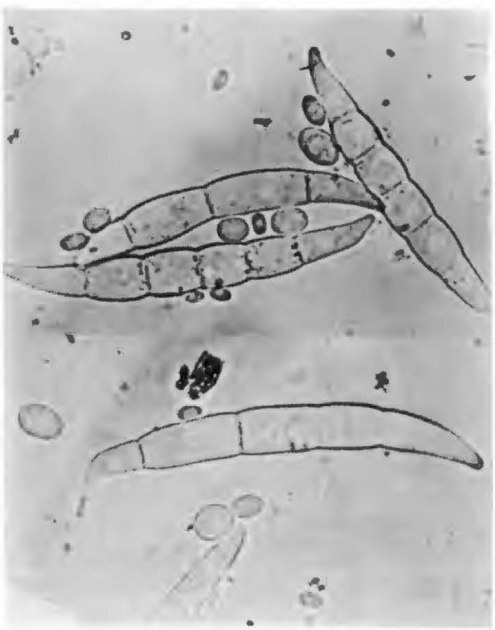


Plate III

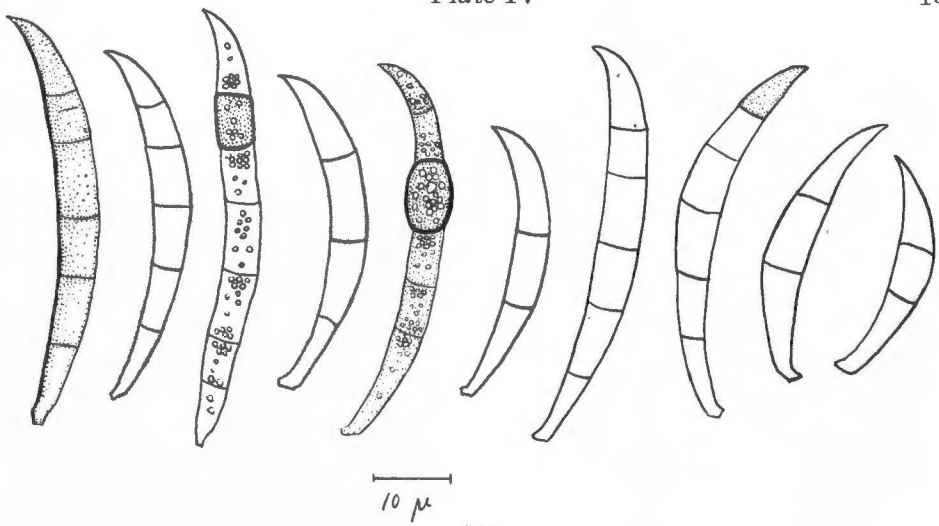
(1)

(2)

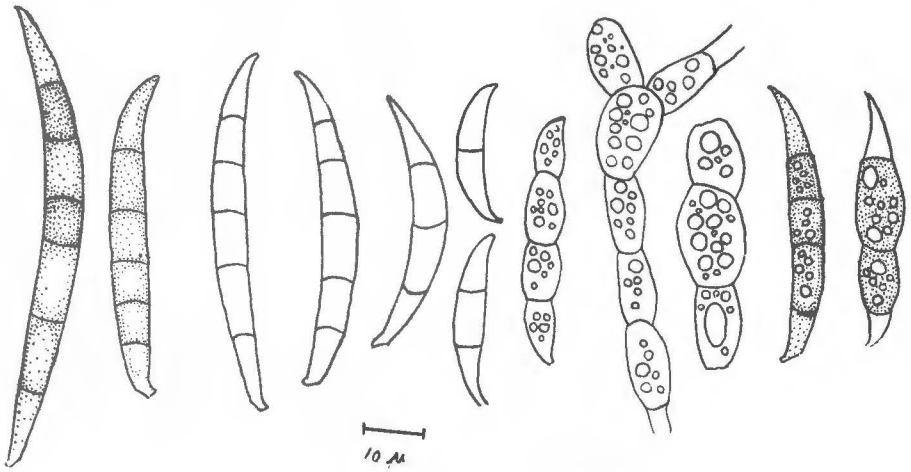


(3)

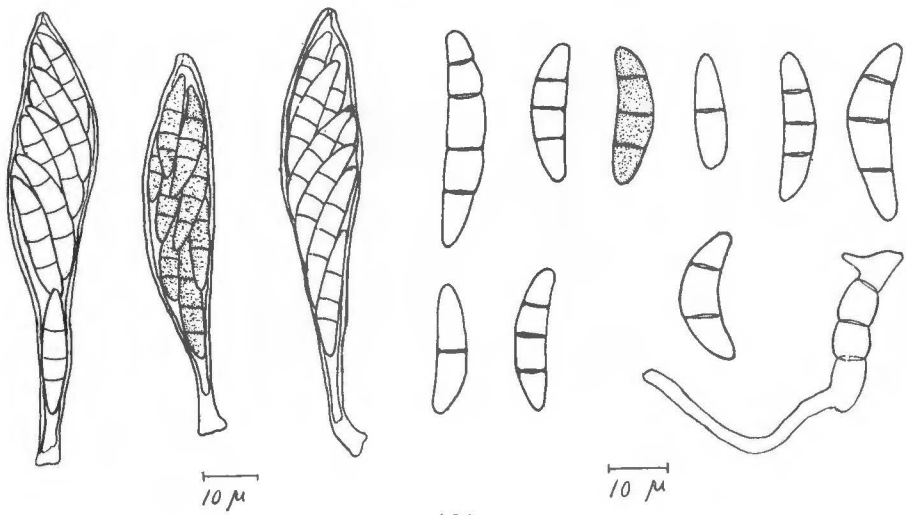
(4)



(1)



(2)



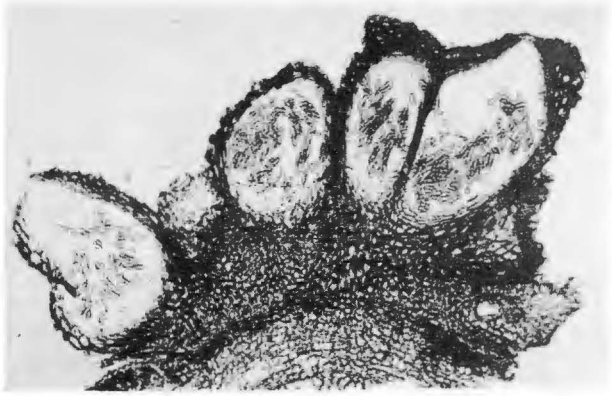
(3)

Plate V

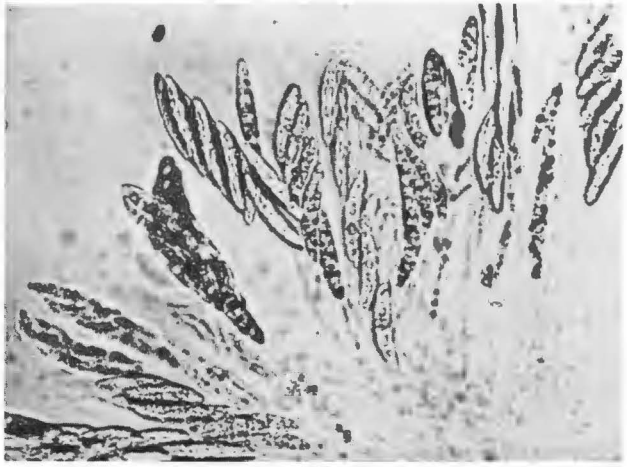
(1)

(2)

(3)



(4)



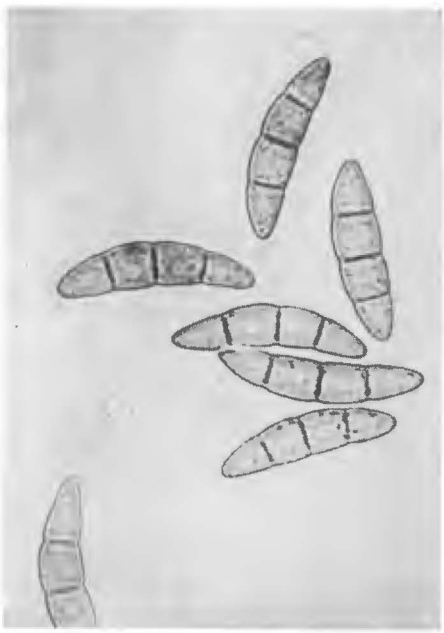
(5)

Plate VI

(1)

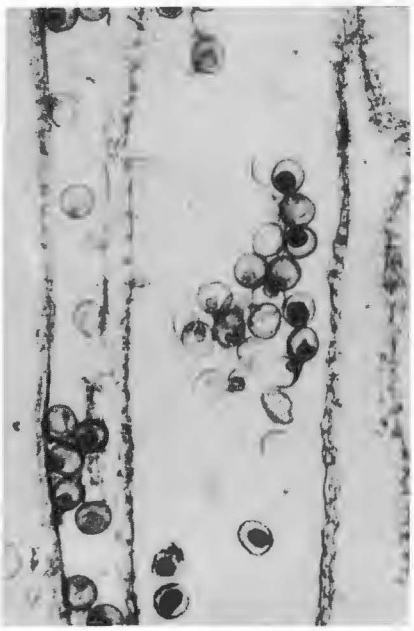
(2)

(3)



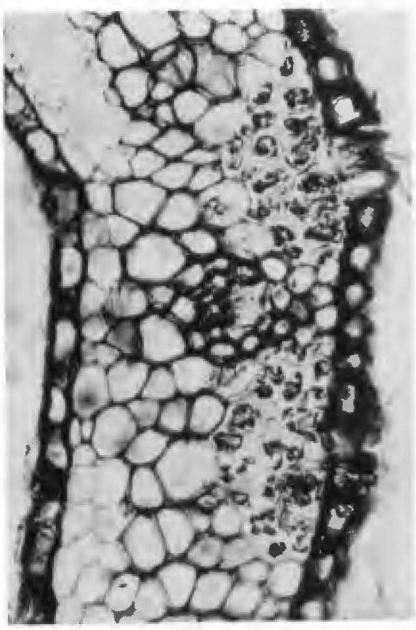
(4)

Plate VII

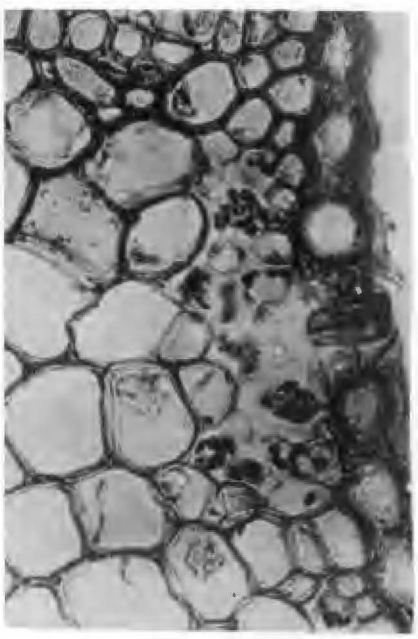


(1)

(2)

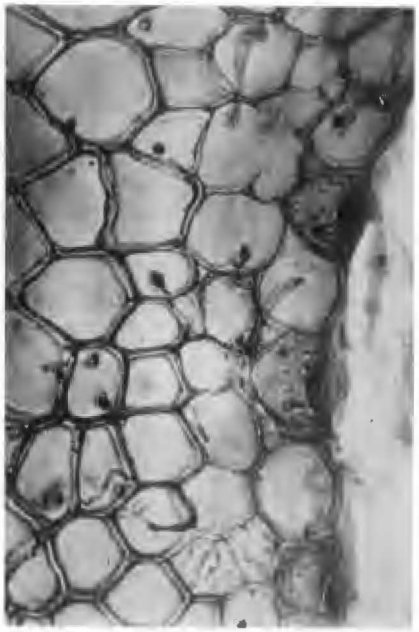


(3)



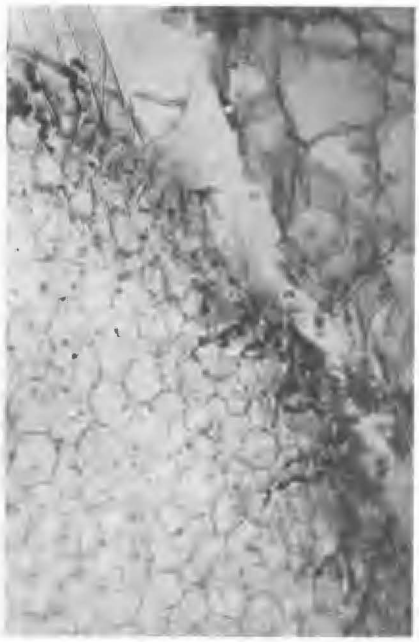
(4)

Plate VIII



(1)

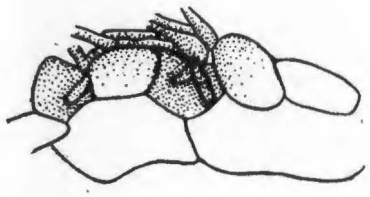
(2)



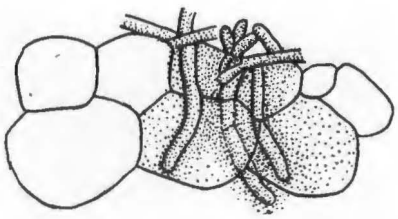
(3)

(4)

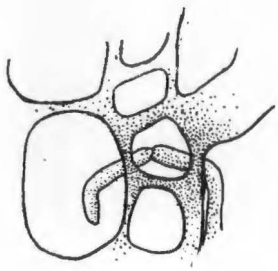
Plate IX



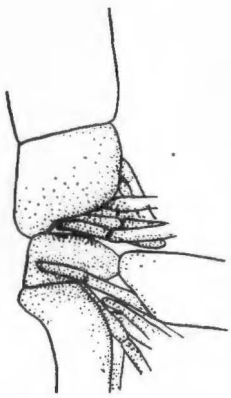
(1)



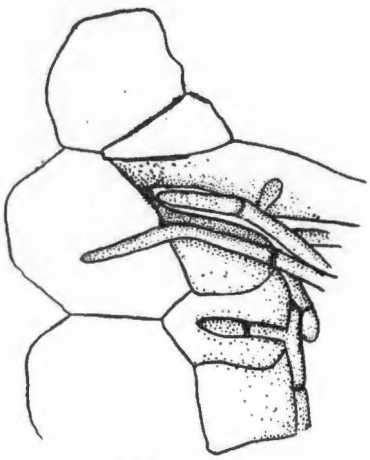
(2)



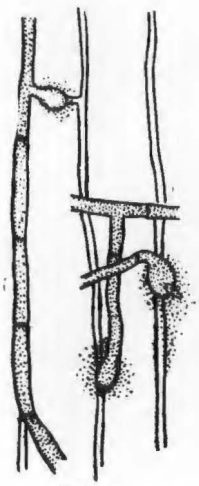
(3)



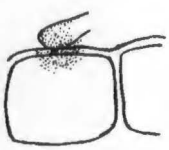
(4)



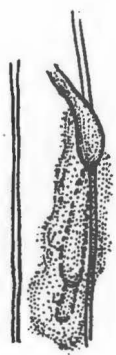
(5)



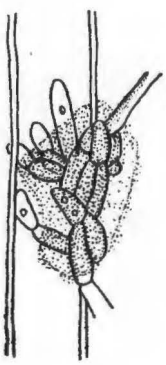
(6)



(7)

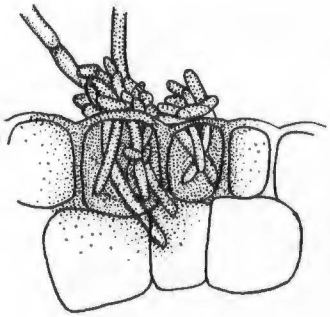


(8)

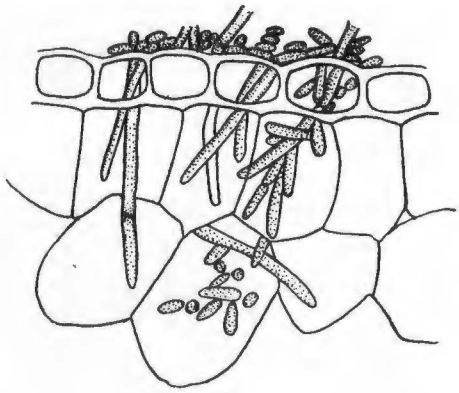


(9)

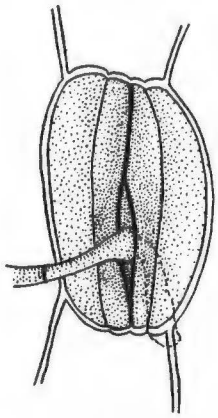
Plate X



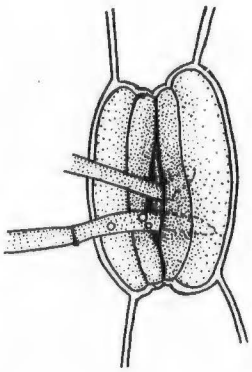
(1)



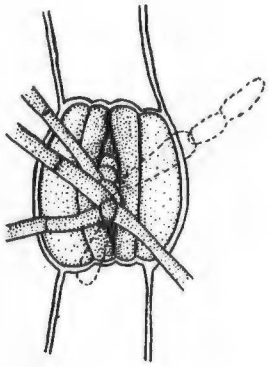
(2)



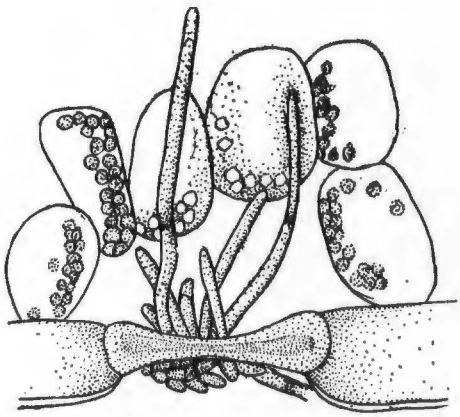
(3)



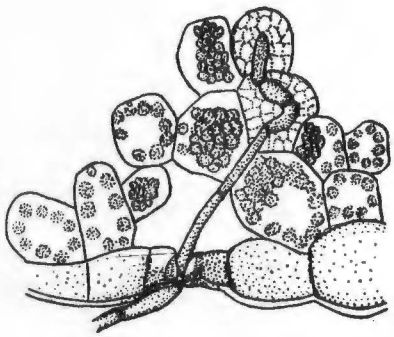
(4)



(5)



(6)



(7)

Plate XI

(1)

(2)

(3)

Plate XII

(1)

(2)