

ON THE RHIZOME ROT OF LOTUS, *NELUMBO NUCIFERA*
GAERTN., CAUSED BY A NEW FUSARIUM,
F. BULBIGENUM WR. *NELUMBICOLUM* NIS. et WAT.

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Introduction

Cultivation of lotus in Japan has been rather limited to certain areas due to its wide distribution of rhizome rot. Since 1907 the disease has been known to prevail in Chiba, Aichi, Osaka, Okayama, Hiroshima, Yamaguchi, Fukuoka, and Saga prefectures, and the cultivation of lotus has been greatly hampered in these areas. The cocurrence of the disease has been known for some time but has not been considered for extensive study. During the period between 1910 and 1925 some 110 to 120 million pounds were being produced annually ; but within the recent years the production has dropped to 40 or 50 million pounds, having been grown mainly in the prefectures mentioned above in latter years. This decrease in production is believed to be primarily due to the outbreaks of rhizome rot caused by a species of *Fusarium*, although lotus was replaced in part by staple food crops during and since the recent war.

Requests have been received from several growers to initiate studies on this disease. In this report the identification of the causal organism, its morphology, and its classification are covered. Studies were principally based on materials gathered in the prefectures of Okayama, Osaka, and those in Kyushu Island.

Concerning the rhizome rot of lotus, Hori (1907) reported a new species of bacterium, *Bacillus Nelumbii* Uyeda, as the cause of the rot on rhizomes growing in Shinobazu-no-ike in Tokyo. He stated that the accumulation of organic nitrogen draining into the lake from sewage lines was the cause of the rot ; and to prevent the disease, adjustment of fertilizer was required. However on this same disease, J. Okada (1922) at Yamaguchi prefecture found from experiments that the damage was not limited to the plot that had received an excess of nitrogen fertilizer, and there seemed to be other causes. Assuming Hori's *Bacillus Nelumbii* as the cause of the rot, Nisikado applied colloidal sulphur imported from the United States, to fields in Otaka district of Kurashiki, Okayama prefecture, where lotus was being grown intensively 20 years ago ; but its effect was not very definite. In the same test, the plot where lime was applied seemed to have had a better control.

Tests performed by Okayama Experiment Station also showed that the lime was better.

On a different disease of lotus rhizome, Nisikado (1944) reported a joint study made on a storage disease of the arrow head and lotus tubers grown in Fukuyama district of Hiroshima prefecture. This was a dry rot caused by *Cylindrocarpon radisicola* Wr.

Symptoms

Lotus is normally started from seed tubers which give rise to shoots with some 10 or more consecutive segments. Each of these segments at maturity will have a length of between 30 and 150 cm. Branch segments and petiole or foliage stalk also arise from the node. The edible rhizome is formed by the enlargement of the last three segments, whether it be the original shoot or the lateral branch. The enlargement begins in July or August, and the rhizome reaches its maturity in September. The rudimentary shoots on the enlarged healthy rhizome remain inactive throughout the remaining season, but those on the diseased rhizome begin to sprout in August and September and grow abnormal small foliage. The diameter of the normal leaves reach some 50 cm but those from the diseased rhizome will be only 10 to 20 cm having an appearance of mosaic, and the leaves stand shorter than the healthy leaves. (Fig. 9) Frequently the top of the petiole or stalk is crooked and gives a drooping appearance of the foliage. (Fig. 1 and 2) The color of the foliage is usually lighter and shows brownish discolored areas along the margin. The foliage finally dies as the discolored areas enlarge.

In the early stges of the disease the tuber shows only light brownish discoloration of the vascular tissues, but in the advanced stages this discoloration is enlarged. (Fig. 8) The discoloration of the vascular tissues extending from the diseased rhizome becomes gradually less pronounced toward the terminal. It may also extend to the petiole and the flower stalk.

There are two other distinguishig symptoms : one is the appearance of purplish color on the rhizome without accompanying any change in the shape ; the other is the appearance of longitudinal ridges on the bottoms side of the rhizome without the purplish color. (Fig. 6, 7 & 8) In either case the terminal portion of the diseased rhizome turn upward at an abrupt angle. (Fig. 7) The two above symptoms sometimes occur on the same rhizome.

Causal Organism

The organisms associated in this disease are a bacterium and a *Fusarium*. The bacterium was isolated in most cases from the rhizomes that were discolored purplish and from them that showed ridges and apparently in advanced stages of the disease. This bacterium seemed to be *Bacillus Nelumbii* Uyeda described by Hori, but possessed only a slight pathogenicity. For the infec-

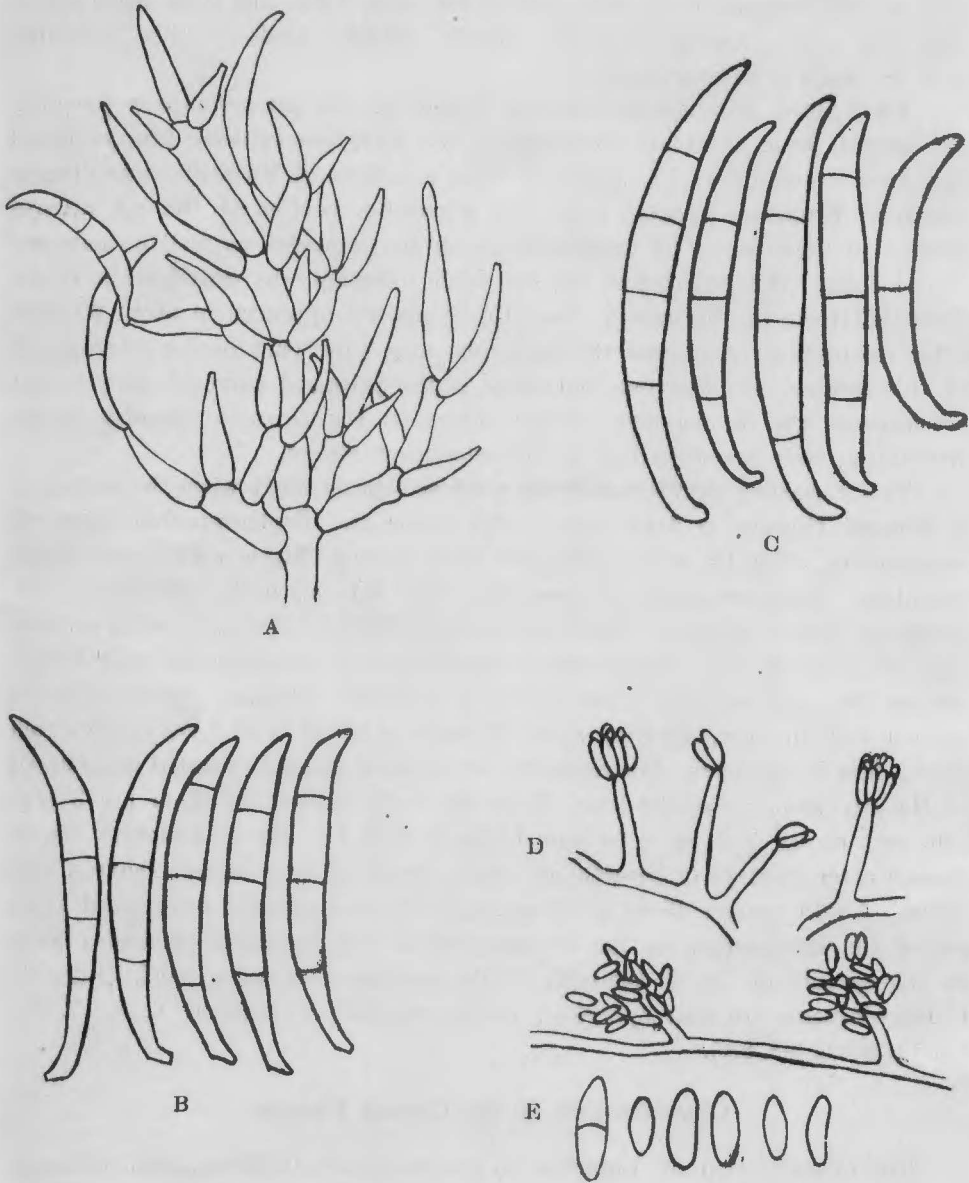
tion by this bacterium an open wound was required; and even when infection was noted, it progressed very slowly. Studies made on this bacterium will be dealt in a later report.

From those rhizomes that showed ridges but did not accompany purpling yet showed some signs of abnormality, the inner tissues were always found discolored brown (Fig. 7), and from them a species of *Fusarium* was always isolated. *Fusarium* isolated from the advancing portion of the rot always possessed a high degree of pathogenicity on the wounded as well as unwounded rhizome. The progress of the rot after infection was considerably faster than that from the bacterium, since the symptoms appeared in about 20 days after inoculation. Although the bacterium may take part in the development of this disease, the *Fusarium* definitely is the principal cause of the rot that accompanies the development of the ridges on the rhizome. Results on the inoculation tests are described in the subsequent section.

This *Fusarium* grows readily as a white cottony mold when the section of a diseased rhizome is kept moist, and forms the *Cephalosporium* type of microconidia. (Fig. D) Microconidia are short or long-elliptic with a very slight curvature. Some are pointed at one end (Fig. E), normally unicellular, but sometimes with a septum. Their sizes vary $5.5-12.5 \times 2.5-5.0\mu$ with an average of $8.59 \times 3.73\mu$. Macroconidia are formed as semispherical sporodochia and on the aerial mycelia when grown in a culture medium. Sporodochia are salmon pink in color, forming after 20 days at 25°C , and have a size from 1 to 2 mm in diameter. Macroconidia are crescent shaped, pointed and curved at the tip with a footlike base. From 90 to 95 percent of them are 3 septate and rarely 1, 2 or 4 septate. (Fig. A, B & C) These 3-septate spores formed after 20 days of growth on potato decoction agar medium have a size $38.06 \times 4.00\mu$ (range $25-46.3 \times 3.0-4.8\mu$). Chlamydospores are formed from one of the intermediate or the terminal cell of the mycelium, and also occur as enlargement of one of the cells of the macroconidia when aged. (Fig. F) Chlamydospores are near spherical, smooth walled and measure $6.95 \times 7.35\mu$ ($5.8-8.8 \times 6.3-8.8\mu$).

Classification of the Causal Fungus

The classification of *Fusarium* is comparatively difficult, and although there are differences in opinion, according to Wollenweber the fungus in question coincides in description to that of *Fusarium bulbigenum* Cke. et Mass, subgroup in *Constrictum*, a subsection to *Elegans* Group in Section XIV. This fungus resulted from combining several species. *F. Lycopersici* Brushi causing the wilt of tomatoes and *F. niveum* E. F. Smith causing the wilt of watermelon are its variations. The *Fusarium* causing the rhizome rot of lotus being distinct from the above species, a new variation name is hereby proposed: *Fusarium bulbigenum* Cke et Mass, var. *nelumbicolum* Nisikado et Watanabe.



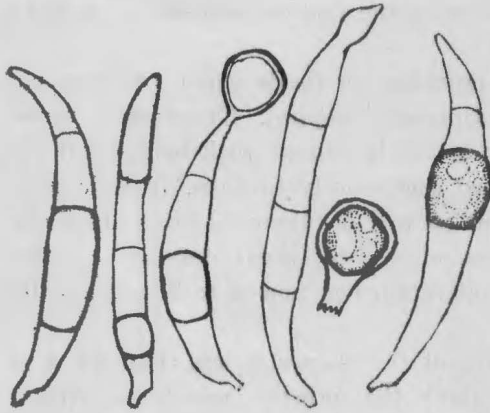
Fusarium bulbigenum Cke. et Mass. var. *nelumbicolum* Nisik. et Wat.

(A) Conidiophores $\times 800$. (B) Macroconidia produced on the sporodochia. $\times 1200$.

(C) Macroconidia produced on the initial colonies from the young germinated conidia. $\times 1200$

(D) Microconidium formation in Cephalosporium type. $\times 650$

(E) Microconidia, $\times 1500$,



F

Fusarium bulbigenum Cke. et Mass.
var. *nelumbicolum* Nisik. et Wat.
(F) Chlamydospores $\times 1500$.

Inoculation Experiments on Lotus Rhizome

1. For sterilizing the rhizome for inoculation, it was cut into full pieces at the node and sealed at the two ends with melted paraffin. It was then immersed in 1 : 1,000 solution of mercuric chloride for 15 minutes, and rinsed in several changes of sterile water. The enlarged internode portion was pricked with a sterilized needle to the air cavities, and inoculated at the bruised surface with an agar fragment of the fungus grown in pure culture. A white mycelial growth of the fungus was observed on the inoculated rhizome after 15 days at 24°C; when sections of the rhizome was made at the end of 20 days the inner tissues showed discoloration and growths of mycelia. The discolored area on the surface had a diameter of 1 cm and a depth of 15 cms. At the end of 25 days they were respectively 45 cms and 25 cms and the air cavities were filled with white masses of mycelial growths, here and there mixed with pinkish mycelia.

2. Spores formed in sporodochia taken up in sterile water were injected into air cavities of sterilized rhizomes. when the inoculated rhizomes were cut and observed after 15 days at 24°C showed numerous colonies of the fungus in the air cavities. After 20 days, these colonies became very distinct. At 25 days the cavities become filled with the fungus growths, accompanying a pinkish discoloration of the rhizome tissues. There were also seen fungus invasion of the air cavities of the adjacent internodes of the rhizome. when after 30 days there was a brownish discoloration of the outer surface of the rhizome and begins to show a distinct indication of a dry rot producing the typical longitudinal ridges. The terminal young shoot which is destined to become a rhizome showed an elongation of about 1 cm.

Conditions Affecting Disease Development

A knowledge on the factors affecting disease development is essential

in establishing control measures. Studies on the factors were made on fields of lotus.

1. Disease was found to be less prevalent in fields which the crop had not been grown, and more when continuously cropped. There were cases, however, where outbreak was greater in fields rotated with barley and rice for 2 to 3 years, than in fields cropped continuously in lotus. This is believed to be due to the drying of the soil as will be discussed later. In Kurashiki district 20 years ago there were considerable areas devoted to lotus, but at present, the center of lotus cultivation has moved to Tsurajima district some 5 miles away.

In the low wet fields the occurrence of the disease is less than the drier upland fields. In among the low wet fields the outbreak was found greater when supply of water was short, particularly when the surface of the soil begins to show large cracks from drying.

2. More outbreaks are seen in fields of light soil compared to heavy soil. Soils abundant in iron had less disease. Although it was two years test, an application of iron hydroxide to fields had some effect in reducing the outbreak.

3. Varieties of lotus that have a deep rooted habit of growth were less susceptible to the disease than the shallow rooted varieties.

Summary

1. This is the first of a series of reports on the rhizome rot of lotus in Japan.

2. The disease is found wherever lotus is cultivated. The damage received from this disease has been great, especially in recent years.

3. The disease starts from early July. It is noticed as a drooping of the foliage caused by crooking of the petiole near the top. Rhizomes show ridges over the surface or appear purplish. In cross sections, there will be discoloration of the inner tissues. Rudimentary shoots on the rhizome, which normally remain dormant till the following year, sprout in the fall and produce small leaves.

4. Species of *Fusarium* and bacterium were isolated from diseased rhizomes. The bacterium appeared to be that described by Hori, *Bacillus Nelumbii* Uyeda, but its pathogenicity was weak. The *Fusarium* possessed a high degree of pathogenicity.

5. From morphological studies, the *Fusarium* associated with this disease was established as *Fusarium bulbigenum* Cke. et Mass. var. *nelumbicolum* Nisikado et Watanabe.

6. The disease predominated in drier fields. There was a tendency for the disease to be associated with iron deficiency and light sandy soil.

PLATE I



Fig. 1



Fig. 2



Fig. 3

- Fig. 1.** Rhizome rot of lotus, *Nelumbo nucifera* Gaertn. Showing diseased drooping leaf stalks of lotus.
- Fig. 2.** Rhizome rot of lotus, *Nelumbo nucifera*. Showing somewhat advanced stage of the disease.
- Fig. 3.** Rhizome of lotus, showing the branching and the internodes. Plus sign shows the existence of hyphae of the causal fungus in that part, minus, the absence.

PETAL I



Fig. 4



Fig. 5



Fig. 8



Fig. 6



Fig. 7



Fig. 9

- Fig. 4. Healthy rhizome of lotus, showing the apical three swollen internodes, the edible part.
 Fig. 5. Lotus seedling inoculated with *Fusarium bulbigenum* var. *nelumbicolum*. Young seedlings were placed in soil, inoculated with the fungus conidia, and kept 4 days at 24°C. In right, the control is shown.
 Fig. 6. Rotted rhizome of lotus, showing longitudinal ridges on the surface of internodes.
 Fig. 7. Rotted rhizome of lotus, showing longitudinal ridges and abnormal apical sprouts turning acutely for the earth surface.
 Fig. 8. Transverse and oblique sections of lotus rhizomes, affected by the disease.
 Fig. 9. Abnormal small leaves, sprouted from affected rhizome in summer. A part of normal large leaf is shown for the sake of comparison.