# On *Neocosmospora vasinfecta* Smith, a Causal Fungus of Seedling-Wilt of Silk-Tree, *Albizzia Julibrissin* Durraz.

By

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## I. Introduction.

In Japan sand dunes lie in many sea side districts, especially arong the coast of the Japan Sea. Planting the sand dunes, to prevent the movement of sand drifted by wind onto the crop fields, is one of important problems in such districts. For this purpose so-called black-pine tree, *Pinus Thunbergii* Parl., was commonly planted, but the growth was not good. To promote the growth of the pine tree, it was planted mixed with any one or two of *Albizzia Julibrissin* Durraz., *Lespedeza bicolor* Turcz., *Alnus firma* Sieb. et Zucc. var. *multinerva* Regel., *Robinia pseudoacacia* L., etc. Especially *Albizzia Julibrissin*, or silk-tree, was proved to be good and the seedlings were raised by the authorities of the districts. Recently, however, a serious wilt disease of the seedlings outbroke in the nurseries of silk-tree.

In September 1934, the writers had received such diseased seedlings of the silk-tree from the Kanazawa Local Forestry Office. Since then they set up an investigation on the wilt disease of silk-tree seedlings.

A damping off of young silk-tree was observed in the Government Railway's nurseries of North Eastern Honsyû. As to the cause of the seedling death, M. KASAI (1935) ascribed it to a species of *Fusarium*, which was provisionally identified as *Fusarium pseudoacaciae* RAPAICS (1910). The present writers have also found *Fusarium* sp. resembling to the KASAI's fungus on the diseased part of the silk-tree seedling grown in the nurseries at Nenbutu near Kanazawa. Their inoculation experiment showed that the *Fusarium* was able to infect the seedling of silk-tree, etc. As to this *Fusarium*, a detailed report will be published in future.

Meanwhile the wilt disease of the seedlings was caused at least partly by the attack of *Neocosmospora vasinfecta* SMITH. This fungus was first described by E. F. SMITH (1899) in North America as the causal fungus of the wilt disease of cotton watermelon and cowpea (DUGGAR, 1909). Since then WOLF ascribed the fungus as the cause of red rot of pea nut. The fungus was reported as the cause of so-called collar-rot of cotton in Egypt (SHEARER, 1924), in Central Asia (ZAPROMETOFF, 1925, 1926), in Congo (GHESQUIERE, 1928), etc. It has also been reported on *Sesame* in Central Asia (ZAPROMETOFF, 1926), on coconut in Morocco (SERGENT and BUQUET, 1921), and on *Crotalaria* in Tonking (VINCENS, 1921).

Prior to these, BUTLER (1910) had studied the wilt of pigeon pea, *Cajunus indicus*, and concluded from his inoculation experiment that *Neocosmospora vasinfecta* was not the true cause of the wilt of pigeon pea, cotton and indigo. He ascribed the cause of the wilt to such *Fusarium* as *F. vasinfectum* ATK., etc. This opinion has been cited by many authors.

Recently, however, MITRA (1934) studied the wilt disease of *Crotalaria juncea* LINN. and noted *Neocosmospora vasinfecta* as one of the causal agents. The present writers' experiment showed that the fungus under consideration was able to infect the seedlings of silk-tree, etc.

The present paper deals with the morphological characteristics of *Neocosmospora vasinfecta* SMITH found in Japan.

The writers are obliges to Messrs. S. YOSIKAWA, S. TAKITA, Y. INUKAI, H. ÔGAMI and other staffs of Osaka Local Forestry Bureau for their help during the investigation. Thanks are also due to Prof. J. WESTERDIJK, Baarn, Holland, for her kind supply of pure cultures.

## II. Occurrence and Symptoms of the Wilt.

According to the observation of the Kanazawa Local Forestry Office, the present disease outbreaks seriously in the nurseries set at the fields where the silk-tree has been repeatedly grown. While the damage is usually slight in the newly cleared land.

Young seedlings, germinated in May, show commonly no signs of the wilt until the middle of June, but then they are attacked by the disease, if it rains for a long time. The attacked seedlings wilt suddenly and shed the leaves. Then they are not able to form new leaves, and killed. In 1935, 99 percent. of the seedlings of silk-tree grown in the nurseries at Nenbutu near Kanazawa were killed by the disease and only 2—3,000 out of about 200,000 seedlings survibed. (cf. Plate XXVII, Fig. 1 and 2.)

Roots of diseased seedling turn to brown color. If the diseased parts of the seedlings are kept in a moist chamber for a few days, they are covered with white hyphae. Among the hyphae, sometimes, many small reddish bodies are also found, which are the fruit-bodies of the fungus under consideration.

## III. Material Studied.

The material studied was sent from the Kanazawa Local Forestry Office. On September 8, 1934, the senior writer had received the diseased specimens of silk-trees. From this material, pure cultures were secured, the isolations being repeated for several times. The physiological characteristics of the fungus strains, thus isolated, were studied. For the identification of the fungus, comparisons were made not only by the description but also by the culture sent by Prof. J. WESTERDIJK.

## IV. Morphological Characteristics.

*(1) Mycelium.* Young hyphae are colorless, but later become light yellow to dark brown and guttulate. They are from 2 to 9 $\mu$ and commonly 4—6 $\mu$, and septated at intervals of 15—36 $\mu$, and slightly constricted at septa.

*(2) Conidium.* The conidia are formed at the apices of short branches of aerial hyphae or conidiophores in the shape of *Cephalosporium*. They are generally continuous, colorless, elliptical of long elliptical or oval shape. (cf. Plate XXIX, Fig. 6.) They are 6—14×2—5 $\mu$, commonly 8—9×3 $\mu$. Results of measurement of the non-septated conidia produced on 3% malt-extract agar or potato agar at 27°C. after 7 days' culture are given in Table 1 (I) on next page.

Among these non-septated ones, 1-3-septated, comparatively large-sized conidia are also observed, which are resembling to those of *Fusarium* spp., but sausage, club, or sickle-shaped. The results of the measurements of the septated conidia, although small in number, are given in Table 1 (II).

*(3) Chlamydospores.* The chlamydospores of this fungus are not found in young cultures. While in old cultures, such as two or three months old, they are produced apically or intercalary. They are colorless, thick-walled, generally granular or elliptical, 5—12.5×5—12 $\mu$ in limits and 8.75×7.50 $\mu$ in average, the contents being granular. (cf. Plate XXIX, Fig. 7.)

Table 1.

Size of Conidia of *Neocosmospora vasinfecta* Smith.

(I) Non-septate Conidia.

Grown after 7 days' culture at 27°C.

| | Culture media | Number measured | Range | Mean | Standard deviation |
|---|---|---|---|---|---|
| Length | (A) Malt-extract agar | 100 | 6 – 13 μ | 8.47±0.16 μ | 1.47 μ |
| | (B) Potato agar · · | „ | 6 – 14 | 9.84±0.17 | 1.64 |
| Width | (A) Malt-extract agar | „ | 2 – 4 | 2.88±0.03 | 0.26 |
| | (B) Potato agar · · | „ | 2 – 5 | 3.22±0.03 | 0.28 |

(II) Septate Conidia.

Grown on potato agar after 7 days' culture at 27°C.

| Number of septa | Number measured | Length | | Width | |
|---|---|---|---|---|---|
| | | Range | Mean | Range | Mean |
| 1-septate conidia | 50 | 11 – 26 μ | 19.96 μ | 3 – 5 μ | 3.97 μ |
| 2-septate „ | 10 | 21 – 37 | 30.17 | 3 – 5 | 3.92 |

*(4) Perithecium.* The perithecia are formed superficially on the affected parts of the host above the ground or sometimes in the earth near the roots. (cf. Plate XXVIII, Fig. 3.) They are almost globose or ovate, and provided with short neck to the ostiolum. They are variable in color, yellowish when young, but later orange to red. The size of the perithecia is also variable and 150—340×120—270 μ in average, 248.9×213.9 μ as shown in Table 2.

*(5) Ascus and Ascospore.* The asci are numerous and colorless, cylindric to clavate, stipitate, the base being somewhat narrowly constricted and almost equal to the length of the spore-bearing part. They contain 8 ascospores, commonly in one row, and 78—130×10—15 μ in limits and 96.8×10.65 μ in average, as shown in Table 2. The ascospores are continuous, globose, short elliptical, ovate, sometimes irregular in shape and rather thick-walled. They are colorless when young, but then becoming brown with age and dark brown when matured. The contents are hyaline and one to several guttulate. The size of those formed on the host is 10—18×7—13 μ and in average 14.11×9.30 μ as shown in Table 2, and those formed in culture 11—16×9—11 μ. (cf. Plate XXVIII, Fig. 4.) They germinate readily in water and in nutrient solutions and produced septated germ-tubes as shown in Plate XXIX, Fig. 5.

Table 2.

Size of Perithecia, Asci and Ascospores of *Neocosmospora vasinfecta* Smith.

| | | | Number measured | Range | Mean | Standard deviation |
|---|---|---|---|---|---|---|
| Peritecia | (1) On host | Height | 100 | 150 – 340 μ | 248.9 ± 4.03 μ | 40.30 μ |
| | | Diameter | „ | 120 – 270 | 213.9 ± 3.92 | 39.20 |
| Asci | (1) On host | Length | 100 | 75 – 130 | 96.80 ± 3.83 | 38.30 |
| | | Width | „ | 10 – 15 | 10.65 ± 0.37 | 3.67 |
| Ascospores | (1) On host | Length | 100 | 10 – 18 | 14.11 ± 0.15 | 1.48 |
| | (2) In culture | „ | „ | 11 – 16 | 14.05 ± 0.09 | 0.85 |
| | (1) On host | Width | „ | 7 – 13 | 9.30 ± 0.15 | 1.53 |
| | (2) In culture | „ | „ | 9 – 11 | 10.03 ± 0.04 | 0.43 |

Remarks: (1) shows the perithecia, asci and ascospores produced on the host plants in nature; (2) those produced on 3% malt-extract agar after 40 days culture at 27°C.

*(6) Paraphyses.* The presence of paraphyses is not distinct as they are colorless. They grow mixed with asci, and are composed of 4—8 cells of angular shape, which are thin-walled, 15—40 × 10—25 μ and contain some granules. The total length of the paraphyses is somewhat larger than that of the asci and measured 75—200 μ.

## V. Toxonomical Consideration.

The above given morphological characteristics show that the present fungus must be of the genus *Neocosmospora* of *Hypocreaceae*. The genus was first established by E. F. SMITH in 1899 and the single species of his new genus given was *Neocosmospora vasinfecta* SMITH. The important characteristics of the SMITH's fungus and the writers' one are compared in Table 3 on next page.

The comparison given in Table 3 shows that the more important characteristics of the writer's fungus agree to those of the SMITH's fungus, and the both fungi must be the same.

In 1910, BUTLER gave a description on the fungus found on pigeon pea and cotton, the chief morphological characteristics being the same with those given by E. F. SMITH. Moreover the characteristics given by WOLLENWEBER (1926) in his Fusaria autographice delineata do not differ from those of the present fungus. To make the identification sure, the senior writer asked Prof. WESTERDIJK to donate

Table 3.

Comparison in Morphological Characteristics of *Neocosmospora vasinfecta* Smith, and of the Present Fungus.

| | *Neocosmospora vasinfecta* described by SMITH | The present fungus |
|---|---|---|
| Perithecia | Height: 210–400 μ, mostly 250–350 μ | 150–340 μ, mean 240 μ |
| | Diameter: 150–328 μ, mostly 200–300 μ | 120–270 μ, mean 214 μ |
| | Peridium: 20 μ thick, coral red to vermilion red | Ditto. |
| | Neck: 30–40 μ, sometimes 80 μ long, never hairly or fimbriate at the apex | Ditto. |
| Asci | 8-spored, cylindric, stipitate | Ditto. |
| | 70–100 × 11–14 (12) μ | 75 – 130 × 10 – 15 μ |
| Ascospores | Globose to short elliptical, continuous rather thick-walled, colorless, then light brown, with thick-wrikled exospore | Ditto. |
| | 10–12 μ in diam. or 8–12 × 11–14 μ | 10 – 18 × 7 – 13 μ |
| Parahyses | Present, 2–3 times of the diam. of asci | Ditto. |
| Conidia (Cephalosporium stage) | Colorless, oval to narrowly elliptical, non-septate, 4–25 × 2–6 μ | Mostly non-sept., 6–14 × 2–5 μ |
| | Frequently 1-septate | Freq. 1-sept., 11–26 × 3–5 μ |
| | Rarely 2-septate | Rarely 2-sept., 21–37 × 3–5 μ |
| Chlamydospores | Globose, thin-walled, smooth, terminal or intercalary | Ditto. |
| | 10–12 μ, mostly 7–5 μ | 5–12.5 × 5–12 μ, mostly 8.8 × 7.5 μ |

the type culture of *Neocosmospora vasinfecta* and received a strain of the fungus isolated by WOLF. And the cultural characteristics of this strain were similar to those of the writers' fungus. As the WOLF's strain, however, did not produced the perithecia, the writers were not able to compare the most important characteristics of the both strains. As to the perithecium production, MOHENDRA (1928) reported the formation of saltant which lost the ability to form perithecia in his culture studies on this fungus.

The above statements show that the present fungus must be called with the name *Neocosmospora vasinfecta* SMITH.

## VI. Pathogenicity.

The pathogenicity of *Neocosmospora vasinfecta* SMITH to watermelon, cotton, etc., has been generally denied since the report of BUTLER (1910). The result of the writers' experiment, however, showed that it was able to infect the seedlings

of silk-tree, watermelon and cotton, at least under suitable condition. Therefore it is not able to suppose the *N. vasinfecta* SMITH to be a mere saprophyte to the above given hosts, like heretofore. The detailed results of the writers' experiments on the pathogenicity of this fungus are given in another paper.

## VII. Summary.

1) The present paper deals with the occurrence of *Neocosmospora vasinfecta* SMITH in Japan and its morphological characteristics.

2) The fungus may cause the seedling-wilt of silk-tree, *Albizzia Julibrissin* DURRAZ., under suitable conditions.

3) The seedling-wilt of silk-tree is prevalent in north-eastern districts of Japan and the coast of the Japan Sea. Sometimes the damage was very serious in the nurseries, where the seedlings were grown repeatly on the same land.

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## Explanation of Plates.

**Fig. 1.** Diseased seedlings of silk-tree, *Albizzia Julibrissin* DURRAZ., attacked partly by *Neocosmospora vasinfecta* SMITH. Pictured by Mr. S. KATÔ on Aug. 7, 1933 in a nursery of silk-tree at Nenbutu near Kanazawa.

**Fig. 2.** Healthy seedlings of silk-tree, pictured by Mr. S. KATÔ at the same time with Fig. 1 for the sake of comparisons.

**Fig. 3.** Perithecia of *N. vasinfecta* SMITH, produced on the diseased root of silk-tree. (Magnified ca. ×200.)

**Fig. 4.** Asci, paraphyses (ca. ×400) and ascospores (ca. ×800) of *N. vasinfecta* SMITH.

**Fig. 5.** Germination of the ascospores of *N. vasinfecta* SMITH, after 24 hours in 3% malt-extract agar at 24°C.

**Fig. 6.** Conidia and conidiophores of *N. vasinfecta* SMITH (Strain No. 1027), produced on 3% malt-extract agar. (ca. ×800.)

**Fig. 7.** Chlamydospores of *N. vasinfecta* SMITH (Strain No. 1027), produced on 3% malt-extract agar. (ca. ×800.)

# PLATE XXVII

Fig. 1.

Fig. 2.

# PLATE XXVIII.

Fig. 3.

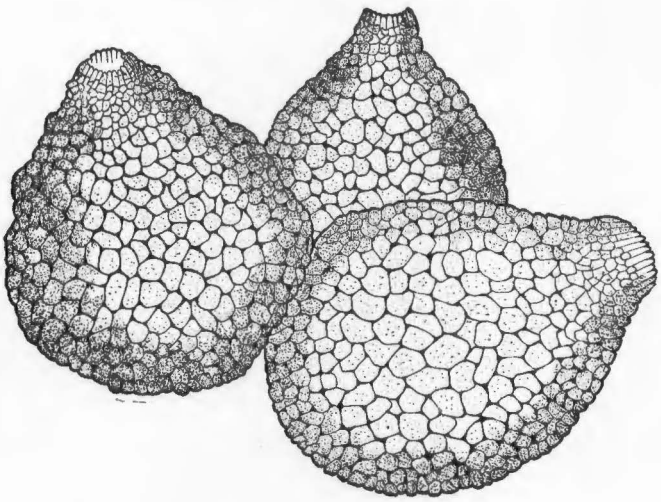
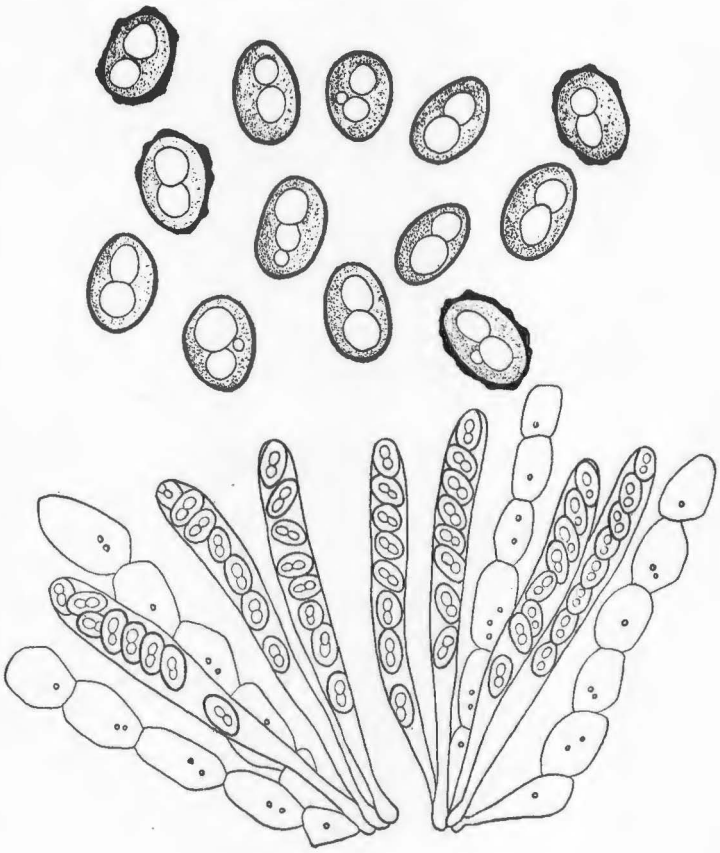


Fig. 4.



# PLATE XXIX.

Fig. 5.

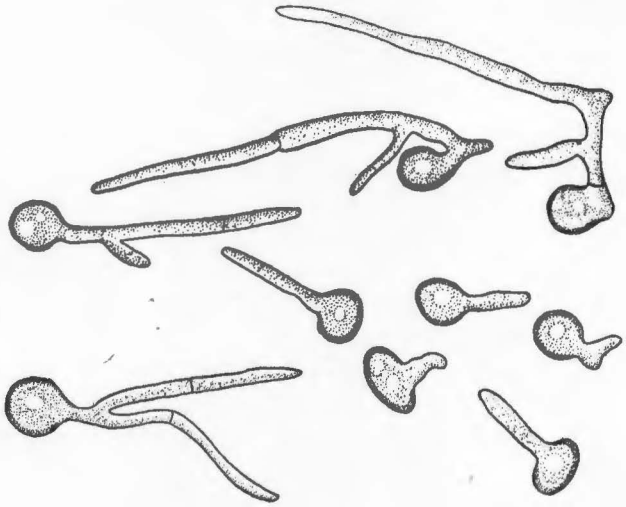


Fig. 6.

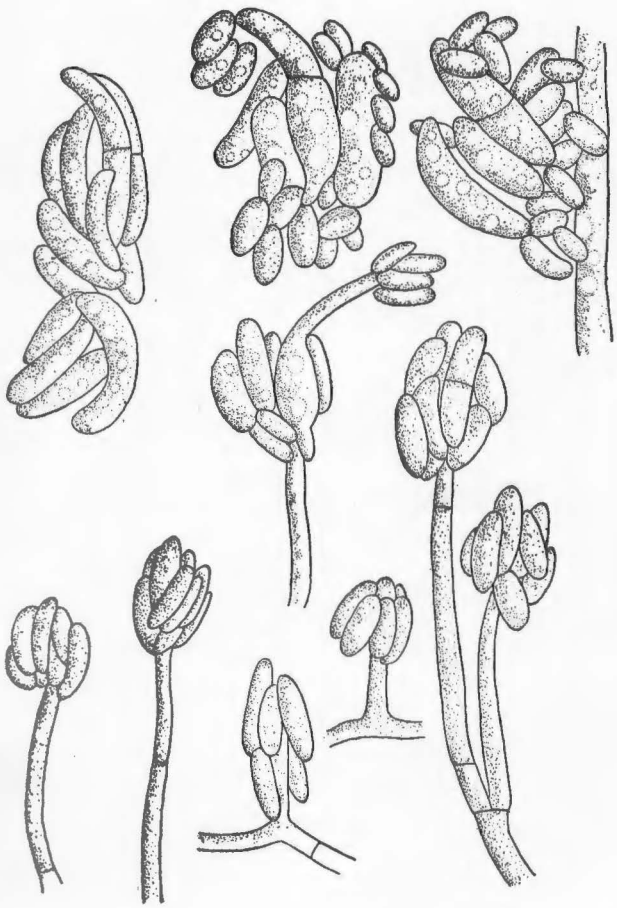


Fig. 7.