THE LARGE LEAF-SPOT OF CHESTNUT

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Introduction

Chestnut culture in recent years has been endangered by the appearance of "Kuri-Tama-Bati" (*Dryocosmos* sp.) as reported by Ii (1951). The authors have made some studies on leaf diseases of chestnut associated with this insect. In this report morphology of the fungus causing the 'large leaf-spot, is given. Materials used were disease samples from Utogawa, Yoshii-cho, Shitsuki-gun, Okayama prefecture, gathered through the courtesy of Mr. Kameichi Kodani.

The disease in Japan was first reported from Shizuoka prefecture by Tsuruta (1917), later Kitajima (1925) isolated the causal fungus in pure culture. There has not been any further studies reported on this disease.

This disease, although occurs on the leaves, makes its appearance rather late in the season; for which reason by the time the disease has spread and caused damage, the tree has already completed a greater part of its current growth, which accounts for the damage to be rather light in most cases. When seedlings are affected by this disease, it usually results in considerable damage due to the checking of growth. In the United States this disease has been reported to have resulted in considerable damage from wide distribution, particularly in the southern Appalachian districts.

Symptoms

This is a foliage disease that begins its infection in September and reaches its peak of damage during October and November. At first, pale whitish, circular spot about 1 cm in diameter appears on the upper surface. It enlarges and begins to show slight corrugation at the margin. The spot may coalesce with others and becomes irregular in outline. The margin of the spot is either dark brown or dark green. The center of the spot is grayish. With the enlargement of the spot, there will be concentric rings, which are very distinct when viewed against the light. On these concentric rings are formed small dark bodies which are the ascervuli. (Fig. 1 and 2) The disease spots are usually brittle and readily fall from the foliage, leaving large holes. One to several spots may occur on a leaf, but when there is a heavy infection leaves fall prematurely.

Morphology of the Fungus

Ascervulus normally forms on the upper side of the leaves in a concentric pattern over the spot, black in color and 100 to 380 μ in diameter. It first forms just below the leaf epidermis, but later breaks this and becomes exposed. (Fig 3) Conidiophores are pointed at the tip, hyaline and $10.5-18.5\times1.5-2.5\mu$. Conidia are spindle shaped, straight, rarely curved, and normally 5 celled; the middle 3 cells are larger, deep olive in color; the lateral cells are hyaline, somewhat constricted at the septum, measuring $20.5-29.5\times7.0-9.5\mu$, average $23.0\times7.5\mu$; spine appendage at the terminal is straight or curved, hyaline, $6-11\times0.7-1.0\mu$. (Fig. 4)

Conidia begin germinating from any one of the colored cells. These cells may separate from one another upon germination. Germinated hyphae are hyaline, rarely light-olive in color, $3-4\mu$ in width, septate at irregular distances, and germinate at an optimum temperature of 25°C.

Fungus Nomenclature

The causal fungus was first discovered on the leaves of oak (*Quercus* spp.) in France by Desmazières (1849) who named it *Pestalozzia monochaeta* Desm. Later Saccardo (Syll. 3 : 397, 1884) created within *Pestalozzia* a subgenus *Monochaetia* for those that have an appendage at one end of the conidium. Allescher (1903) raised this to a genus, and named the fungus *Monochaetia monochaeta* (Desm.) Allesch. Later Saccardo listed this as *Monochaetia Desmazierii* Sacc. (Syll. 18 : 485, 1906).

Graves (1912) studied this disease on oak and applied 'large leaf-spot' from its large sized spot distinguishing 'small leaf-spot' caused by *Marssonia ochroleuca* B. et C. Farlow identified the causal fungus as *Monochaetia Desmazierii* Sacc. Hedgcock (1929) reported its hosts, chestnut (*Castanea dentata*) and 18 other species covering 6 genuses. Most of these occur in southern Appalachian districts according to Boyce (1938).

In a book written by Stevens and Hall (1910), among fungi attacking chestnut and oak, listsed *Monochaetia pachyspora* Bubák. Hall (1912) stressed the use of *Mon. pachyspora* against Graves' *Mon. Desmazierii* (1912) after studying and deciding that both fungi are the same.

In Japan, Tsuruta (1917), Nanbu (1920) and Kitajima (1925) used *Mon. pachyspora*. Like in the case between Graves and Hall, we assume the fungi to be identical, and propose to use in this report *Mon. Desmazierii* being the older name of the two, as reported by Stevens (1923). Table 1 shows comparative sizes of conidium and appendage of the fungi described under both names by different authors.

Physiological Characteristics

Kitajima cultured the fungus on various culture media. He found that

TABLE 1. *Conidium and appendage of Monochaetia desmazierii and Mon. pachyspora.*

Author	Name	Number of Septa	Size of Conidia in μ	Size of Appendage in μ
Desmazières (1849)	*Pestalozzia monochaeta*	4	10×4	5-6
Wollenweber (1936)	*Monochaetia Desmazierii*	5	18-30×4.5-7 (23.5×6.0)	6-20×0.5-1.0
Bubák (1904)	*Mon. pachyspora*	5	20-26×7.9	20-40×1.5
Kitajima (1925)	*Mon. pachyspora*	5	19.2-28.0×8.0-9.6	16.0-19.2×1.6
Nisikado& Watanabe (1950)	On chestnut	5	20.5-29.5×7.0-9.5 (av. 23.0×7.5)	6-11×0.7-1.0 (av. 10×0.9)
Nisikado & Watanabe (1950)	On potato decoction agar	5	17.5-32.5×4.5-7.5 (av. 25.9×5.6)	35-50×0.7-1.8 (av. 43.7×1.3)

soy sauce agar yielded a best growth, having been able to cause the fungus to sporulate in 12 days at 25 C. There was no sporulation on potato decoction agar or glucose agar medium.

The authors studied growth as influenced by types of culture media and environment, particularly by the temperature.

1. *Relation of temperature to growth.*

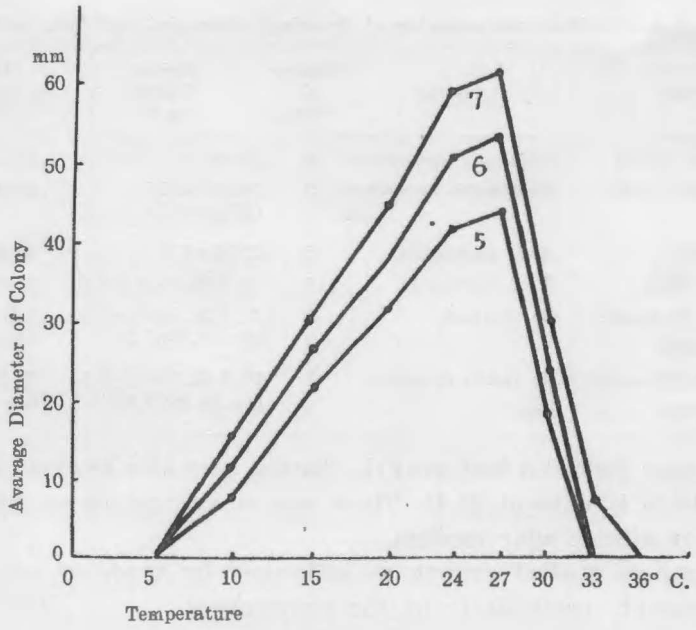
The test fungus was isolated from diseased chestnut leaf collected at Utogawa, Yoshii-cho, Shitsuki-gun, Okayama prefecture. It was cultured on potato decoction agar medium poured in petri dishes. The agar medium was prepared from the followings: potato 200 grams, glucose 20 grams, agar 20 grams, water 1,000 ml. Culturing petri dishes were held at temperatures between 5 and 36°C divided into nine different classes. Each class consisted of five petri dishes. The amount of growth was measured daily from the fourth day after inoculation. Table 2 and Graph 1 show average diameter of mycelial growth at respective days of measurement.

TABLE 2. *Mycelial growth of Monochaetia desmazierii Sacc. on potato decoction agar medium at various temperatures. Measurements are in millimeters of dizmeter of colony.*

Incubation	5°	10°	15°	20°	24°	27°	30°	33°	36°C
4 days	0	5.9	16.0	24.0	31.7	33.2	0	0	0
5 days	0	7.8	21.0	31.0	40.2	42.8	18.5	0	0
6 days	0	11.3	27.0	38.3	49.8	53.8	23.0	0	0
7 days	0	14.7	29.8	45.0	58.0	60.8	28.5	7.5	0

Table 2 shows the fungus' optimum temperature for growth at about 27°C, minimum between 5 and 10°C, and maximum between 33 and 36°C. On the agar medium, the mycelium shows a concentric pattern of growth, particularly when cultured at temperatures near the optimum. It was pale cinnamon in color.

On the effect of temperature upon conidia formation, it was found on



GRAPH 1. Effect of Temperature on the Mycelial Growth of *Monochaetia Desmarzierii*. Sacc. Showing Diameter of Colonies on Potato Decoction Agar Medium after 5, 6 and 7 Days' Culture, Respectively.

potato decoction agar medium that they are formed at temperatures between 10 and 30°C, having most formation at 24°C. Conidia formed at different temperature did not vary in their morphology. Spore mass was moist, sticky, black, and either round, rhomboid or club shaped, scatteredly formed in radiating pattern over the colony.

When the conidia germinate on a culture medium low in nutrient, the germ tube becomes a conidiophore and bears one to several secondary conidia. The secondary conidia are of various sizes, but normally they are smaller than the mother conidia, and there is littte coloring in the normally colored cells (Fig. 5). The size of secondary conidia are 17-32.5 × 6.0μ, average 25.0 × 4.8μ. The appendage is very short.

2. *Growth of mycelium and spore formation in relatation to pH of the culture medium*

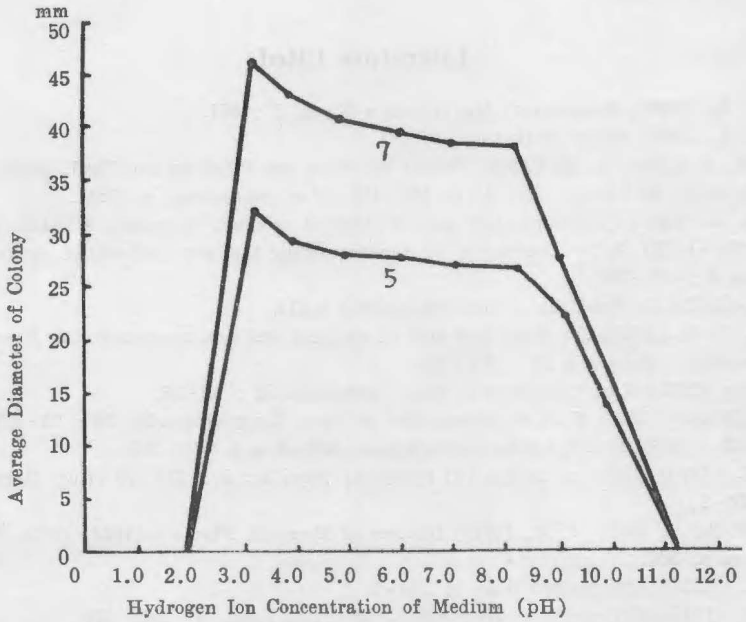
A. Growth of mycelium : In preparing culture medium adjusted to various pH, an appropriate amount of N/5 HCl or N/5 NaOH was poured into a petri dishes, over which melted potato decoction agar medium was added. The two well mixed prior to allowing the agar to solidify. The pH readings were made by comparing with the standard color solutions prepared according to Clark & Lubs method. An agar fragment of the fungus growth was inoculated at the center of the petri dishes. There were 8 petri dishes replicated for each class of pH, all incubated at 24°C. Results obtained were

averaged and are shown in Table 3 and Graph 2.

Reviewing Table 3, a pH of 3.2 had the greatest mycelial growth, but others up to pH 8.2 had but slight difference. At pH 9.0 and 9.8 the growth declined. There was no growth at pH 2.0 and 11.2.

TABLE 3. *Mycelial growth of Monocahetia desmazierii Sacc. on patato decoction agar medium at various hydrogen ion concentration.*

pH	1.2	2.0	3.2	3.9	4.9	6.0	7.0	8.2	9.0	9.8	11.2	11.8
No. of drops added	62	35	6	2	1	1	3	4	5	28	37	46
After												
5 days	0	0	31.0	29.3	28.0	27.7	27.0	26.8	22.5	13.5	0	0
7 days	0	0	46.3	43.0	40.5	39.3	38.3	38.0	27.0	17.0	0	0



GRAPH 2. Effect of Hydrogen Ion Concentration of Medium on the Mycelial Growth of *Monochaelia Desmazierii* Sacc.
Showing Diameter of Colonies on Potato Decoction Agar Medium after 5, 6 and 7 days Culture, Respectively.

B. Spore formation: Spores were formed at the end 12 days at pH between 3.2 and 9.0, most abundantly, at pH 3.9 and 4.9, and none at 9.8. In contrast to mycelial growth, spore formation at pH 3.2 was about same as at pH 8.2 and 9.0. There was no distinguishing difference in the morphology of the conidia formed at different pH.

Summary

1. This is a report on 'large leaf-spot, fungus attacking chestnut leaves.

2. *Monochaetia Desmazierii* Sacc. was used as the name of the causal fungus in this report.

3. Symptoms of the disease and the morphology of the fungus were described in detail.

4. The fungus has the following growth temperatures: minimum 5-10°C, optimum 27°C, and maximum 33°C.

5. Spores were formed best at temperature of 24°C.

6. On the culture medium, formation of secondary conidia was observed.

7. The fungus mycelium showed following reaction to pH: minimum less than 3.2, optimum 3.2, and maximum 9.8.

8. Spores were formed at pH between 3.2 and 9.0 having the optimum at 3.9 to 4.9.

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Fig. 1. Large leaf-spot disease of chestnut. Black bodies represent ascervuli. ×0.7.

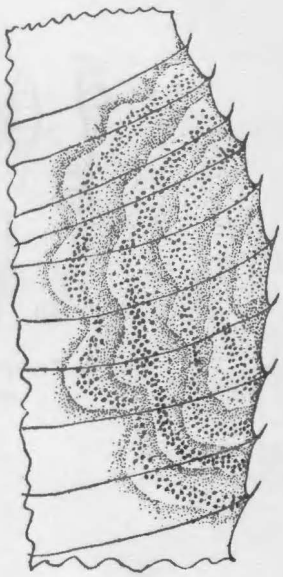

Fig. 2. Same enlarged. ×1.3.

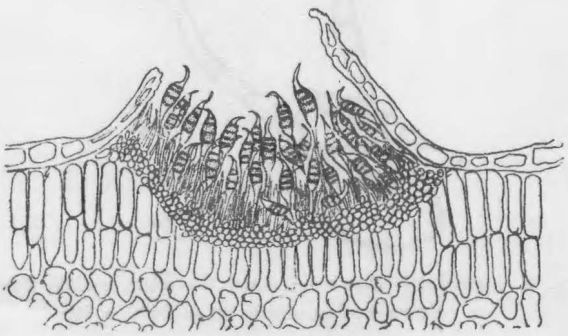

Fig. 3. *Monochaetia Desmazierii* Sacc. Section of a ascervulus. ×200.

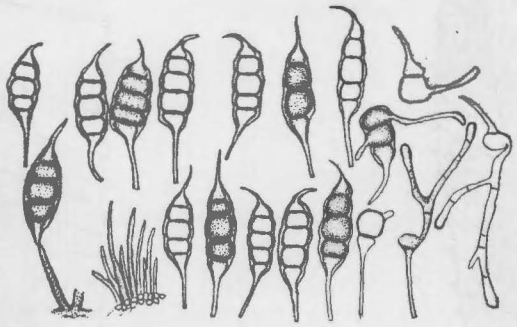

Fig. 4. *Monochaetia Desmazierii* Sacc. Conidia, conidiophores, and germination of conidia × 400.

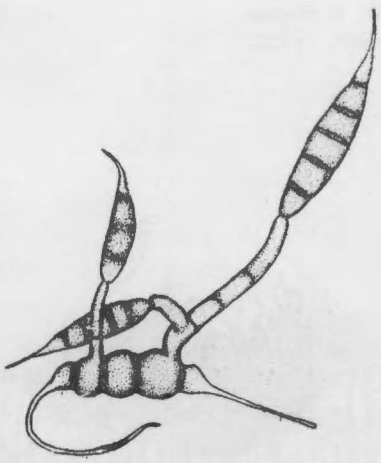

Fig. 5. *Monochaetia Desmazierii* Sacc. Secondary conidia formed on culture medium incubated at 24°C. × 740.