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Cercospora Needle Blight of Pines in Japan

By

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Summary: The needle blight of pines caused by *Cercospora pini-densiflorae* HORI et NAMBU was first reported from Japan in 1917. In the second decade of this century, the disease had affected seedlings of *Pinus densiflora* and *P. thunbergii* in several forestry nurseries in the southern parts of Kyushu, Japan. Since then, no information had been available for a long time, and it was considered to be a sporadic disease occurring in limited parts of Kyushu. However, since the Second World War the causal fungus has been found to be distributed in the central and western parts of Honshu, Japan's main island, and Shikoku Island as well as in Kyushu. The fungus severely attacks seedlings of both native and exotic species of the genus *Pinus*, and often brings about death of the diseased seedlings. The fungus has recently been reported by foreign pathologists to be distributed in Africa, India and Malaysia. This paper deals with an outline of researches on the disease made by Japanese workers as follows: Morphology, geographic distribution and sporulation of the causal fungus; inoculations with the fungus to several kinds of conifers; fertilizers relating to the disease incidence; relative susceptibility of pine species to the disease; and fungicidal control of the disease.

Introduction

In 1913 a serious foliage disease, later known as Cercospora needle blight, was observed on seedlings of *Pinus pinaster* and *P. thunbergii* in several forestry nurseries in Kumamoto Prefecture, Kyushu, the southern part of Japan, and the disease heavily affected seedlings of *P. densiflora* and *P. thunbergii* in other nurseries in Kumamoto and Kagoshima Prefectures during the period 1913～1920 (HIDAKA 1932)[11].

As the causal fungus of the needle blight, NAMBU (1917)[29] described newly *Cercospora pini-densiflorae* HORI et NAMBU from material on *Pinus densiflora* seedlings collected at Magome, Kagoshima.

Since that time, no information had been available concerning the out-break of the disease, and it had been generally believed for a long time to be a minor disease of pines found sporadically in limited parts of Kyushu.

After the Second World War a destructive disease of pine seedlings occurred in epidemic form in Kumamoto, Miyazaki, and Kagoshima Prefectures, in southern Kyushu. Growers in all sections of these districts lost almost their entire crops, and, as a result, some growers

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abandoned cultivation of pine seedlings. Dr. O. CHIBA, of the Government Forest Experiment Station, identified the causal organism of the disease as *Cercospora pini-densiflorae* HORI et NAMBU. Here, the pathologists' and growers' attention was again called to the fungus as an important causal agency of pine seedling diseases (ITO 1954)[15].

Since the Second World War, further surveys made by many workers showed a more extensive distribution of *Cercospora pini-densiflorae* as follows: Shikoku (YOSHII & SOGAWA 1955)[43], Honshu, Japan's main island, Mie (Anon. 1954[1]), ITO 1962[18]), Shizuoka (ITO 1961)[17]), Shimane (SUTO 1967[32]), 1968[33]), and Okinawa (Ryukyu Islands) (TOKUSHIGE 1971)[41] Prefectures. Accordingly, now, the needle blight caused by the fungus is a major obstacle to the satisfactory production of pine seedlings throughout the southwestern districts of Japan.

Cercospora pini-densiflorae was known in Taiwan (Formosa) as early as 1923, and it has been reported to attack seedlings of *Pinus massoniana* and *P. luchuensis* (SAWADA 1928[31]), T. ITO 1935[21]). According to foreign researchers its occurrence in Africa (Tanganyika, Tanzania, Zambia and Rhodesia) has been recorded by GILL (1963)[5], HODGES (1964)[12], GIBSON (1964[5], 1970[6], 1971[7]), ETHERIDGE (1965)[3] and GRIFFIN (1968)[9]; in Southeastern Asia (India, Malaysia) by GIBSON (1971)[7]. Dr. P. D. GADGIL, of the Forest Research Institute, New Zealand, has recently written to the author that "we are very much interested in this fungus [*Cercospora pini-densiflorae*] because the macroscopic symptoms are very similar to those of an unidentified disease we have noticed on *Pinus radiata*……".

The Cercospora needle blight of pines has been considered to be one of the most important forest-tree diseases dangerous internationally (IMAZEKI & ITO 1961[13], 1963[14]). It is the purpose of the present article to give a review of the studies on the disease reported by many workers and to note the author's unpublished data.

The author takes pleasure in making acknowledgement to Prof. Dr. Y. TOKUSHIGE, of Kagoshima University, for helpful suggestions during the progress of the work, and to Dr. I. A. S. GIBSON, of the Commonwealth Mycological Institute, England, for literature references and kind advice. He is also indebted to Mr. Y. MAMIYA and Mr. Y. ZINNO, of the Government Forest Experiment Station, and Mr. K. NAKANO, exresearcher of Shizuoka Prefectural Forest Experiment Station, for assistance in the preparation of the photographs and the illustrations.

Symptoms and damage of the disease

Lesions, pale green at first, 5~10 mm in length, appear on the needle, then turn yellowish brown, and finally become grayish brown. Infection gradually progresses upward from the lowest needles. In the later stage of the disease, a number of sooty spots, fruit-bodies of the causal fungus, are produced on lesions. Discolored gray band with dark minute fruit-bodies of the fungus and brown band appear reciprocally on the diseased needle. Blighted needles finally become dried and shrunken. Severely infected seedlings are completely killed by the disease (Plates 1, 3, 4, 5).

The needle blight is essentially a serious disease of seedlings in many species of *Pinus*. In an epidemic condition, approximately 100 per cent of the seedlings are infected and 50 to 80 per cent killed (Plate 2).

The damage of the needle blight is usually limited to 1- and 2-year-old seedlings in the following species: *Pinus densiflora*, *P. thunbergii*, *P. taeda*, and *P. caribaea*. However, on the

contrary, *Pinus pinaster*, *P. radiata*, and *P. halepensis* are commonly attacked by the disease not only in nurseries but also in young plantations (Hidaka 1932[11], Kiyohara & Tokushige 1969[27]).

Morphology of *Cercospora pini-densiflorae*

Cercospora pini-densiflorae Hori et Nambu
in Nambu, N. (1917). Jour. Plant Protection (Tokyo)
4, 353～354. semi nomen nudum

Nambu's (1917)[29] original description written in Japanese is translated into English as follows: "Conidiophores in fascicle arising from the stomatal openings, dark brown, 44×4.4 μ. Conidia filiform or long-clavate, slightly curved or straight, light yellow, 4～6 septate, 41.49～50.7×1.23～4.6 μ. Habitat: On 2-year-old seedlings of *Pinus densiflora*. Locality: Magome*, Kagoshima. Date of collection: September 20, 1915."

In Taiwan (Formosa), Sawada (1928)[31] noted the morphological characters of the fungus on *Pinus massoniana* and *P. luchuensis*. Chupp (1953)[2], in his monograph of the genus

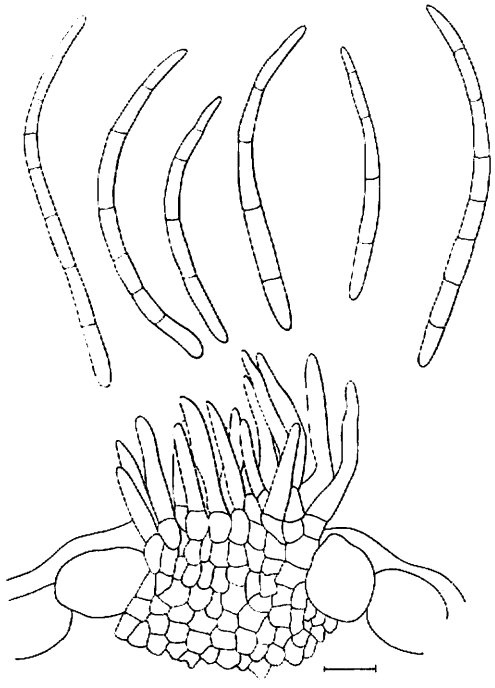

Fig. 1 *Cercospora pini-densiflorae* on *Pinus densiflora* collected in Hamakita, Shizuoka. (⊢—⊣ = 10 μ)

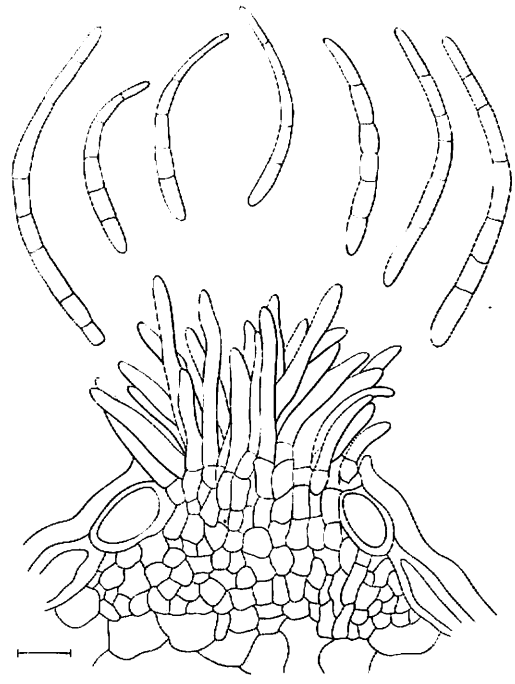

Fig. 2 *Cercospora pini-densiflorae* on *Pinus strobus* collected in Hamakita, Shizuoka. (⊢—⊣ = 10 μ)

* "Makago" noted erroneously by Tanaka (1918)[37] and Katsuki (1965)[23] is correct to be spelled as "Magome".

Cercospora, gave a description of the fungus on *Pinus massoniana* collected in Taiwan, and said that "I have seen only the Formosan collection". Recently, KATSUKI (1965)[23], in his monographic study of Japanese Cercosporae, reported in detail the morphology of the fungus on material of *Pinus densiflora*, *P. thunbergii* and *P. pentaphylla* collected in Kyushu, Japan.

Dimensions of the fungus measured by the earlier workers and the present author are summarized in Table 1.

Table 1. Dimensions of *Cercospora pini-densiflorae*

Author	Locality	Host	Stroma in diameter	Conidiophore	Conidium	Number of septum in a conidium
			μ	μ	μ	
NAMBU (1917)	Kagoshima, Japan	*P. densiflora*	—	44×4.4	41～51×1.2～4.6	4～6
SAWADA (1928)	Taiwan	*P. massoniana* *P. luchuensis*	—	16～22×3	34～65×2.5～3.5	1～7 (mostly 3～5)
CHUPP (1953)	Taiwan	*P. massoniana*	60	10～45×2.5～5	20～60×3～6	3～7
KATSUKI (1965)	Kumamoto, Fukuoka, Japan	*P. densiflora* *P. thunbergii* *P. pentaphylla*	63～70	12～15×2.5～3	35～63×2.5～3	—
ITO	Kumamoto, Japan	*P. densiflora*	60～96	13～20×2.5～3	23～42×2.2～3	1～3
ITO	Shizuoka, Japan	*P. densiflora*	—	13～28×3～4	30～64×2.7	3～7 (mostly 4～5)
ITO	*do.*	*P. thunbergii*	—	11～36×3～4	28～57×2.7	3～7 (mostly 4～5)
ITO	*do.*	*P. strobus*	—	13～28×3～4	30～64×2.7	3～7 (mostly 4～5)

The author's description of the fungus is as follows: Stromata dark brown, tuberculated, filled the stomatal openings, 60～96 μ in diameter. Conidiophores dense fascicle, straight or slightly curved, olivaceous brown, rarely septate, not branched, usually attenuated toward the apex, 11～36×2.5～4 μ. Conidia obclavate to obclavate-cylindric, straight or curved, rounded to obconically truncate base, obtuse tip, 3～7 septate (mostly 4～5 septate), light olivaceous, 28～64×2.2～2.7 μ (Figs. 1, 2).

Geographic distributiin of *Cercospora pini-densiflorae* in Japan

The distribution of the fungus had been believed to be restricted to Kyushu, the southern part of Japan. Since the Second World War numerous reports and collections of the fungus have indicated its more wide distribution in the southwestern districts of the country.

The accompanying map (Fig. 3), which has been drawn up on data collected from all the sources available and reports made by earlier workers (NAMBU 1917[29], HIDAKA 1932[10], Anon. 1954[1], T. ITO 1954[22], NUKUMIZU 1956[30], ITO 1961[17], 1962[18], KATSUKI 1965[23], SUTO 1967[32], 1968[33], TOKUSHIGE 1971[41]) shows the present known distribution of the fungus.

Besides Japan, the fungus has been recovered from Taiwan (SAWADA 1928[31], T. ITO 1935[21]), Africa (GILL 1963[5], HODGES 1964[12], GIBSON 1964[5], 1970[6], 1971[7], ETHERIDGE 1965[3]), India (GIBSON 1971[7]), Malaysia (GIBSON 1971[7]), and probably New Zealand. Accordingly, now, it may be one

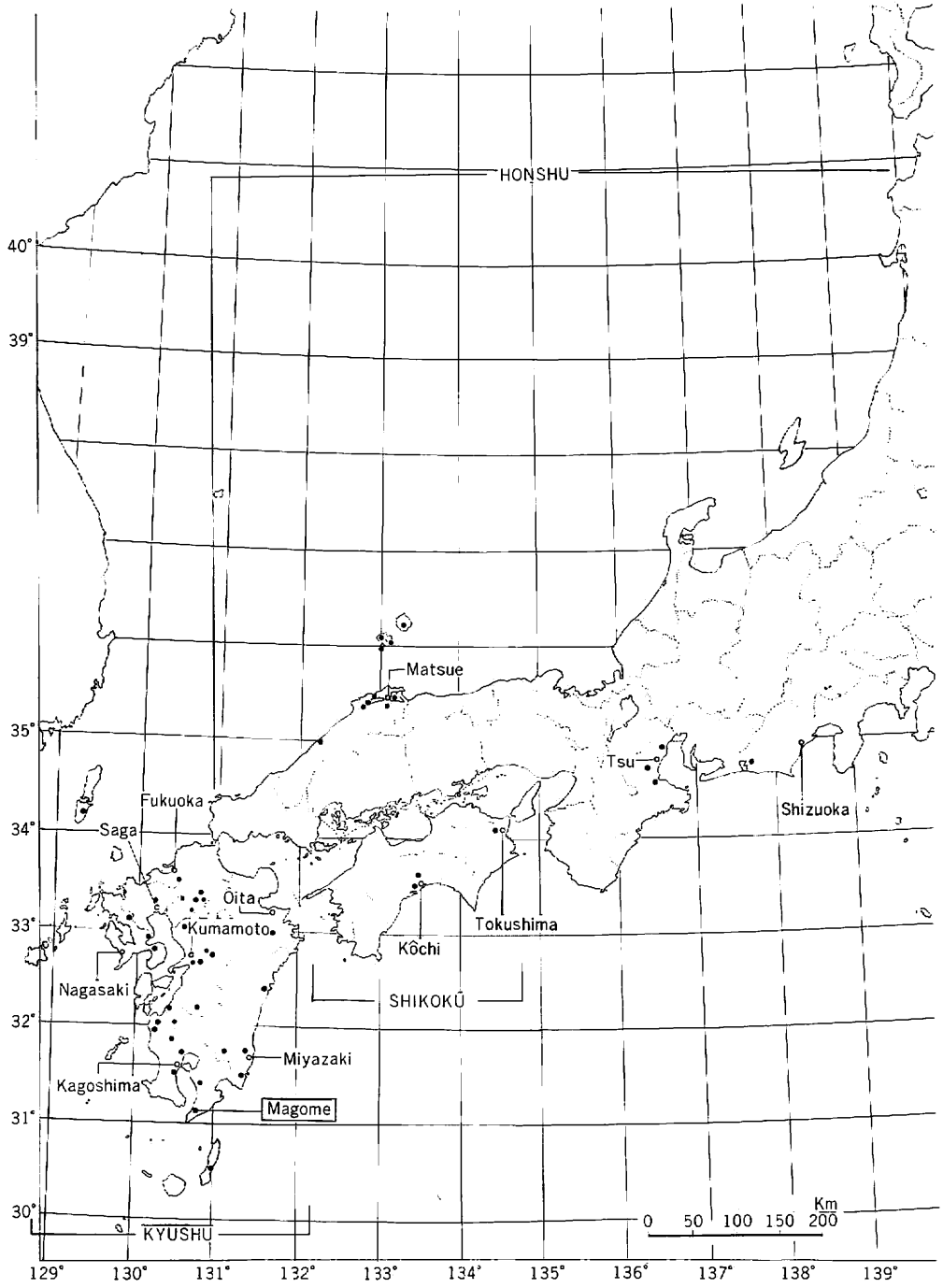

Fig. 3 Map of distribution of *Cercospora pini-densiflorae* in Japan.

□ ……Type locality

of the world-wide distributed fungi.

Inoculations with *Cercospora pini-densiflorae* to various conifers

Fresh conidia collected from the diseased needles germinated readily (Fig. 4). Mono-conidial isolates were cultured on potato-dextrose agar.

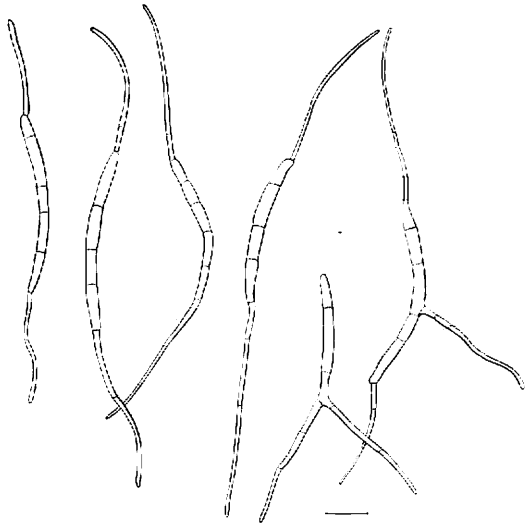

Fig. 4 Germinating conidia of *Cercospora pini-densiflorae*. (⊢——⊣ =10 μ)

Colonies of the fungus on potato-dextrose agar are compact, elevated, almost hemispherical especially at first, and the surface of colonies is flat. Aerial mycelium is at first pale olive gray in color, and then becomes smoky gray. Mycelia in the inner part of colony are dark gray.

Inoculation experiments with the fungus were made to the healthy potted seedlings of the following tree species: *Pinus densiflora*, *Pinus thunbergii*, *Larix kaempferi*, *Cryptomeria japonica*, and *Chamaecyparis obtusa*.

The fungus colonies on potato-dextrose agar were broken in sterile distilled water, then filtered through double sheets of cotton cloth.

In early September, the needles of potted seedlings were inoculated by atomizing with the fungus suspension in the greenhouse, then being covered with bell-jars keeping in moist condition for two days. The check plants were sprayed with sterile water instead of the fungus suspension.

On the inoculated needles of *Pinus densiflora* and *P. thunbergii* typical symptoms of the disease began to appear about 4 weeks after the inoculation, while on those of the other species no symptom appeared.

Cercospora pini-densiflorae is confined as a pathogen almost entirely to pine foliages, but infection of *Pseudotsuga menziesii* is known (Kiyohara & Tokushige 1969)[27].

Sporulation of *Cercospora pini-densiflorae* on culture media

It has been well known that the Cercosporae as a group have been found to produce few typical conidia in pure culture. Various workers have reported many results of their studies on the conidial production of Cercosporae in pure culture, because considerable difficulties were encountered in obtaining and maintaining sporulating conditions in artificial media in many species of *Cercospora*.

Cercospora pini-densiflorae has been considered to be one of the fungi producing very few

conidia on culture media. Recently, KIYOHARA and TOKUSHIGE (1969)[28] and SUTO (1971)[36] have reported, respectively, methods for artificial sporulation of the fungus.

KIYOHARA and TOKUSHIGE's (1969) procedures for sporulation were as follows: (1) The fungus was cultured on potato-dextrose agar in test tube for 15 days at 25°C., (2) the mycelial colony on agar slant was pulled out from the tube, agar medium beneath the colony was removed, and then the colony was placed on filter paper in a desiccator containing $SiO_2 \cdot nH_2O$ for about a week, (3) the mycelial colony was cut into small pieces (4～6 milligrams a piece), (4) small pieces of the mycelial colony absorbed water were kept at about 28°C and at 55% or 88% in relative humidity, and then (5) a great number of conidia were produced on the under surface of the mycelial colony. KIYOHARA and TOKUSHIGE (1969)[28] have explained that environmental factors such as drying and moistening, and mechanical actions such as peeling and cutting, upon the mycelial colony may stimulate sporulation of the fungus.

SUTO (1968[35], 1971[36]), in several experimental series on sporulation of the fungus, obtained abundant conidia on culture media by the following method: (1) As the production of conidia was stimulated by fragmentation of the young colony, successive transplantation of the suspension of the fungus consisting of conidia and fragmental hyphae was employed, (2) among the several kinds of agar media used, pine-needle decoction plus V-8 juice agar proved to be suitable for the sporulation of the fungus. He noted that, in this method, abundant conidia were obtained continuously, but, among 10 isolates tested, only one isolate possessed ability to produce conidia on culture media.

Effect of fertilizers on incidence of the disease

The effect of the mineral nutrition of pine seedlings upon the development of Cercospora needle blight was determined by estimating the degree of infection on blighted needles of plants grown in solution complete in all nutrients, or deficient in nitrogen (N), phosphorus (P), or potassium (K).

Two-year-old seedlings of *Pinus thunbergii* were grown in glazed pots, 5 pots of each of the four treatments. The seedlings were sprayed with a suspension of mycelia of *Cercospora pini-densiflorae* cultured on potato-dextrose agar and placed in a moist chamber for 48 hours. Then, the seedlings inoculated were kept in the greenhouse.

Results obtained at the end of 4 weeks' experiment showed that the influence of each of nitrogen (N), phosphorus (P), and potassium (K) of pine seedlings was not remarkable on the incidence of Cercospora needle blight, while deficiency in all of the three elements (N, P, and K) of fertilizers slightly increased disease incidence.

In field-plot tests, KAWABATA et *al.* (1962)[25] reported that nitrogen, phosphorus, and potassium in relation to the disease of *Pinus thunbergii* seedlings were not evident, respectively, and seedlings in unfertilized plot were slightly more susceptible to the disease than those in fertilized plots.

Susceptibility of pine species to the disease

According to the results obtained by field observations and artificial inoculations made in Japan (T. Ito 1954[22], Nukumizu 1956[30], Ito 1961[17], Kiyohara & Tokushige 1969[27], Kawabata 1971[26]) the relative susceptibility in many species of the genus *Pinus* to the disease is as follows:

Highly susceptible……*P. radiata, P. lambertiana, P. jeffreyi, P. pinaster, P. sylvestris, P. nigra, P. pinea, P. halepensis, P. canariensis, P. muricata, P. murrayana, P. ponderosa, P. contorta, P. resinosa, P. flexilis, P. montana, P. strobus, P. aristata, P. attenuata*

Susceptible……*P. densiflora, P. thunbergii, P. luchuensis, P. massoniana, P. echinata*

Resistant……*P. rigida, P. taeda, P. caribaea, P. griffithii, P. torreyana, P. patula.*

Generally speaking, several exotic pine species such as *Pinus radiata, P. pinaster, P. sylvestris, P. pinea, P. halepensis, P. canariensis* and *P. muricata* are very highly susceptible, whereas the indigenous species, *P. densiflora, P. thunbergii* and *P. luchuensis*, are intermediate, although the other exotic species, *P. taeda, P. caribaea, P. rigida, P. griffithii, P. patula*, are resistant (Plate 2).

In tests carried out in Japan (Kiyohara & Tokushige 1969)[27], *Pinus patula* was mildly attacked by the fungus, but, in Tanzania, Africa, the species was reported to be completely immune to the diseases (Gibson 1970)[6].

Fungicidal control of the disease in nurseries

About 40 years ago, Hidaka (1932)[11] had reported that Bordeaux mixture spray from April to October, except August, at the interval of 2 weeks, was very effective for control of the disease in Kyushu.

After the Second World War, Tokushige and Kiyohara (1962)[35] made control experiments for the disease of *Pinus thunbergii* seedlings in Kyushu with the following fungicides: Bordeaux mixture*, other copper containing fungicides, Uspulun, Ceresan, and Dithane. They noted that good results were obtained from spraying with Bordeaux mixture with Uspulun** from May to October, at the interval of 2 weeks.

Results of the field-plot tests made by Kawabata (1960[24], 1971[26]), in Kyushu, showed also that Bordeaux mixture* spraying from May to November proved to be the best from the standpoint of effectiveness and injuriousness.

Suto (1968)[31], in the field-plot tests at Oki Islands, Shimane, observed that mercury Bordeaux (wettable and powder) appeared to be effective.

The causal fungus commonly overwinters as mycelial masses or immature stromata in the tissues of diseased needles, and produced new conidia as early as in the middle of April in Kyushu (Tokushige & Kiyohara 1965[39], Tokushige et *al.* 1965[40]). These newly formed conidia cause the first infection of the disease, and it is very reasonable that fungicide spraying

* It was prepared at the rate of 4 grams of copper sulfate and 4 grams of hydrate lime in 1 liter of water. To this, spreader was added.

** Bordeaux mixture was added with Uspulun at the rate of 1 gram to 1 liter of spray-solution.

should be begun in late April to early May in Kyushu. The most important period for the spraying may be in early June to mid-July, the moist season in the southwestern parts of Japan (Tokushige & Kiyohara 1962)[38].

It is very noteworthy that the no-sprayed 1-year-old seedlings in the seedbed are severely affected by the disease after being transplanted to the next nursery bed, while, on the contrary, the well controlled ones are healthy. To keep the 2-year-old seedlings entirely free from the disease, the spraying should be begun soon after germination and continued at regular intervals (Tokushige & Kiyohara 1962[38], Suto 1968[34], Kawabata 1971[26]).

From the forgoing experimental data, the following control measures are generally carried out in forestry nurseries where Cercospora needle blight of pines is distributed: (1) All diseased seedlings should be removed and burned early in the season when infection occurs, (2) Copper fungicides should be sprayed from May to October at the interval of two weeks, and (3) The spraying should be begun soon after germination of the seedlings in seedbeds and continued to the end of growing seasons. Transplanted 2-year-old seedlings also should be sprayed with the fungicide periodically.

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Explanations of Plates

Plate 1.

A seedling of *Pinus thunbergii* attacked by *Cercospora pini-densiflorae*. Collected in Hamakita, Shizuoka.

a. Diseased seedling　　b. Affected needles

Plate 2.

A. One-year-old seedlings of *Pinus thunbergii* attacked by *Cercospora pini-densiflorae* in Hamakita, Shizuoka. Photo. by Mr. K. Nakano

B. Two-year-old seedlings of *Pinus thunbergii* attacked by *Cercospora pini-densiflorae* in Yatsushiro, Kumamoto. Photo. by Dr. Y. Tokushige

C. Two-year-old seedlings of *Pinus strobus* attacked by *Cercospora pini-densiflorae* in Hamakita, Shizuoka. Photo. by Mr. K. Nakano

D. Seedlings of *Pinus radiata* and *P. halepensis* attacked by *Cercospora pini-densiflorae* in Kumamoto.

Left row: *Pinus radiata*　　Right row: *P. halepensis*

Photo. by Dr. Y. Tokushige

E. Seedlings of *Pinus radiata* heavily attacked by *Cercospora pini-densiflorae* and healthy ones of *P. taeda* in Kumamoto.

Central row: *Pinus radiata*　Left and right rows: *P. taeda*

Photo. by Dr. Y. Tokushige

Plate 3.

A. A 2-year-old seedling of *Pinus densiflora* attacked by *Cercospora pini-densiflorae*. Collected in Mie, Oita. ×0.8

B. A 1-year-old seedling of *Pinus thunbergii* attacked by *Cercospora pini-densiflorae*. Collected in Hamakita, Shizuoka. ×1

C. A 2-year-old seedling of *Pinus thunbergii* attacked by *Cercospora pini-densiflorae*. Collected in Hamakita, Shizuoka. ×0.8

D. A 2-year-old seedling of *Pinus strobus* attacked by *Cercospora pini-densiflorae*. Collected in Hamakita, Shizuoka. ×0.8

Plate 4.

A. Seedlings of *Pinus pinaster* attacked by *Cercospora pini-densiflorae* in Kumamoto.

B. Needles of *Pinus densiflora* attacked by *Cercospora pini-densiflorae*. ×1.2

C. *Ditto*. ×3

Plate 5.

A. Needles of *Pinus thunbergii* attacked by *Cercospora pini-densiflorae*. ×1.2

B. *Ditto*. ×2.2

C. *Ditto*. ×3

D. Needles of *Pinus strobus* attacked by *Cercospora pini-densiflorae*. ×1.2

E. *Ditto*. ×3

日本におけるマツ葉枯病の研究

伊　藤　一　雄[1]

摘　要

マツ葉枯病菌 *Cercospora pini-densiflorae* Hori et Nambu は鹿児島県産資料によって1917年（大正6年）にわが国で命名・記載されたものである。

大正年代に本菌によるアカマツおよびクロマツ苗の葉枯病が熊本，鹿児島両県に発生して少なからぬ被害を与えたと報じられていたが，その後は絶えて久しく本病に関する記録はなく，これは南部九州の一部に散発的に発生する風土病的なものと考えられてきた。

第二次世界大戦後やはり熊本，鹿児島および宮崎の各県でマツ苗に激烈な疾病が発生，これによってマツ苗養成苗畑は壊滅的打撃をこうむっていることが明らかになり、病因調査の結果，それは本菌による葉枯病にほかならぬことが知られ，再びこれはおおいに注目されるようになった。

その後の調査により，本病病原菌は九州一円はもとより，四国，静岡県，三重県および島根県にも分布してマツ苗にはなはだしい被害を与え，その病状はスギ赤枯病にも匹敵することが明らかにされ，日本西南部における最も重要な苗病の一つに数えられるようになっている。

本菌は台湾においてタイワンアカマツおよびリュウキュウマツに被害を及ぼすことはすでに1928年（昭和3年）に報告されているのであるが，なお琉球本島にも存在することが最近明らかにされている。

本菌はアカマツ，クロマツ，リュウキュウマツなど本邦産マツ類のほか，多くの外国産マツを侵すことが知られており，なお外来種のなかには邦産マツ類よりもいっそうはなはだしく感受性のものが少なからず所在することも報じられている。そして，強感受性マツ類は山出し後も造林地で病状が進展し，この点被害が苗木時代にほとんど限定されるアカマツおよびクロマツとはおおいに趣を異にする。

近年本病病原菌はアフリカ（タンガニカ，タンザニア，ザンビア，ローデシア），インドおよびマレーシアにも分布することが明らかにされ，なお外国種マツ類に対する病原性が強いことから，海外においてもこれに対して大きな関心が払われつつある。

このように，本病はひとり本邦のみならず国際的にも重要樹病の一つと認められていることから，病徴，被害状況，病原菌の形態，培地上の胞子形成，地理的分布，病原性，被害に及ぼす肥料成分，内外マツ類の本病に対する耐病性および薬剤防除法などについて，これまでわが国で公表された試験研究の概要を紹介し，あわせて著者の未発表の成績をもとりまとめて，海外における諸研究者の参考に供する。

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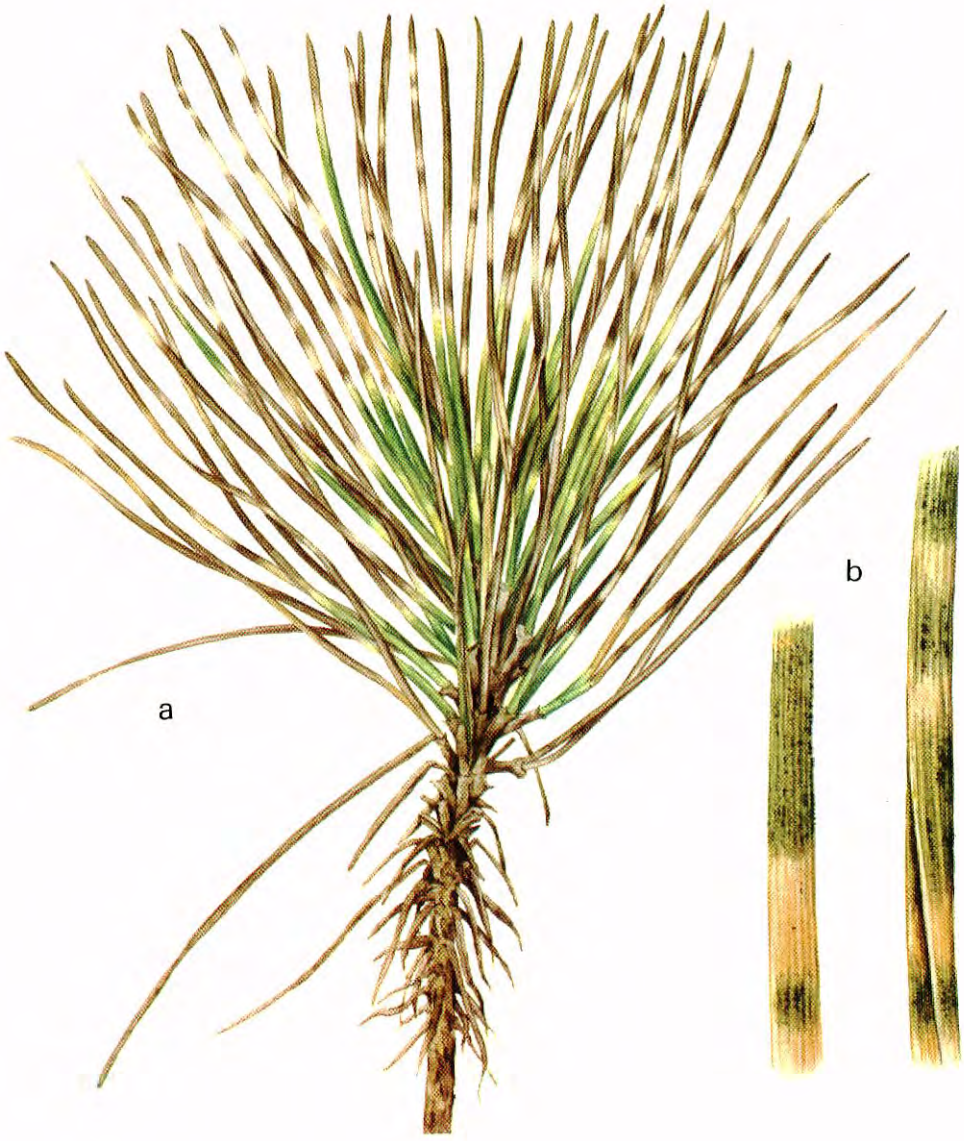
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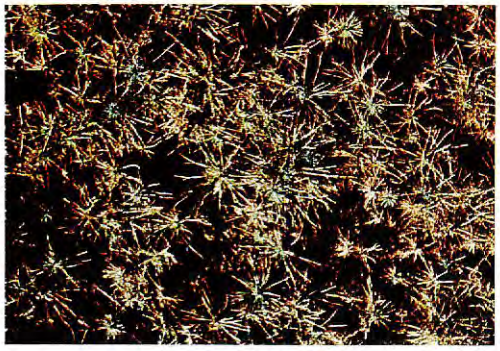

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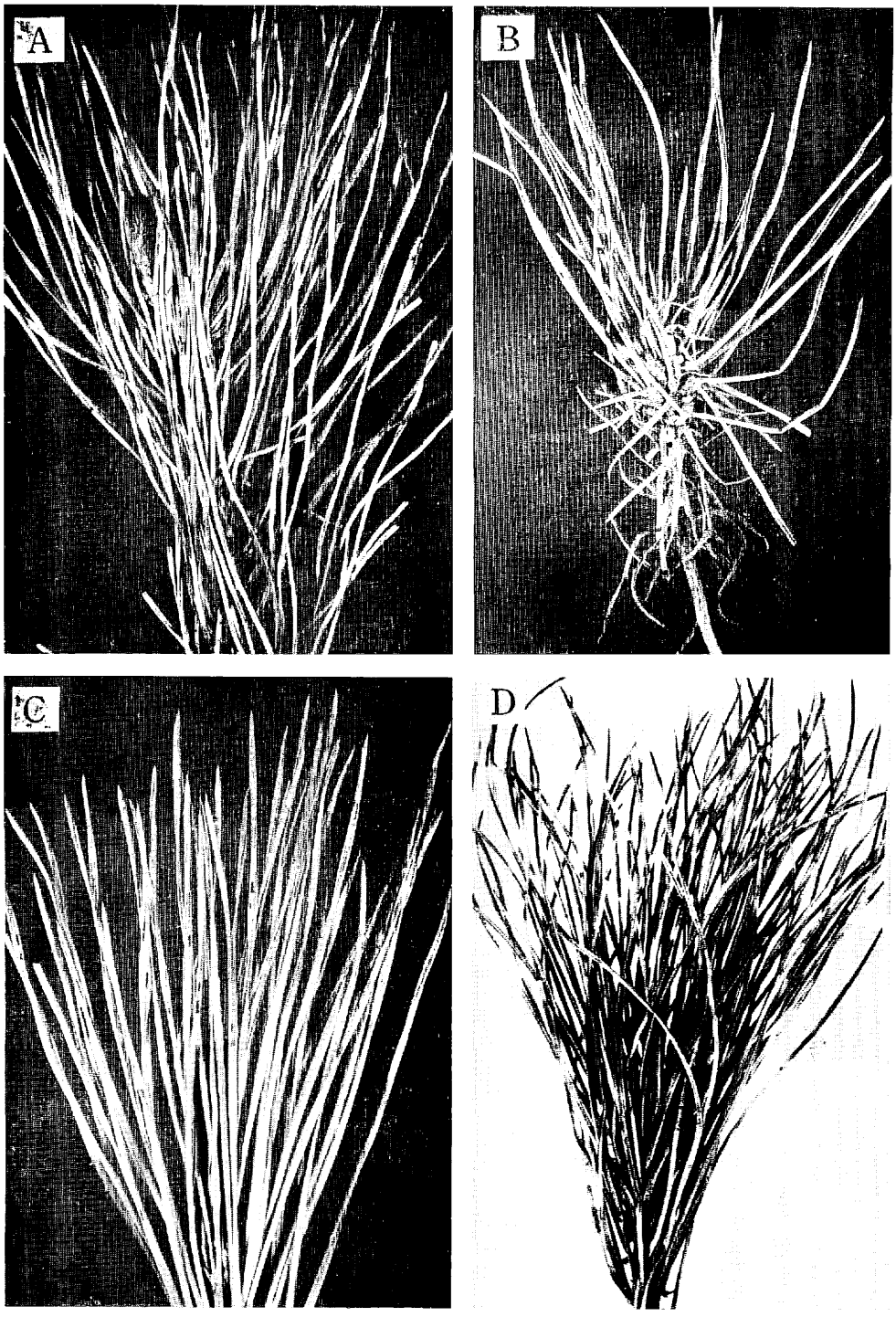
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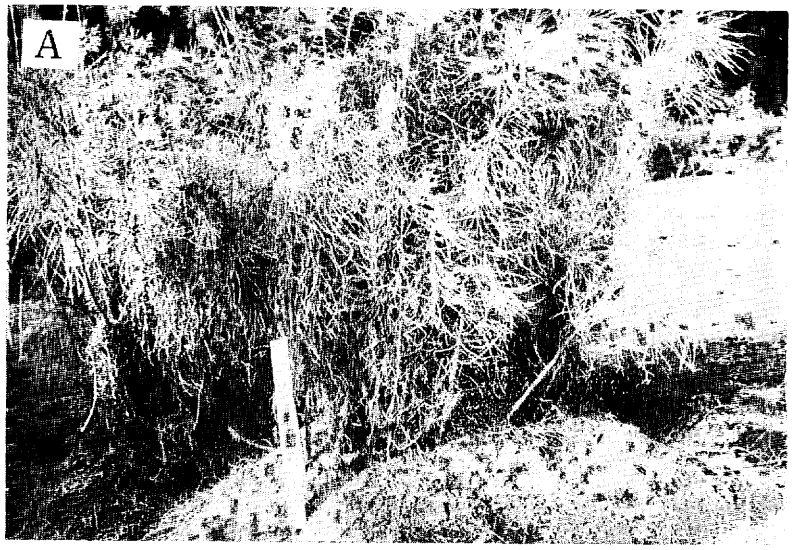
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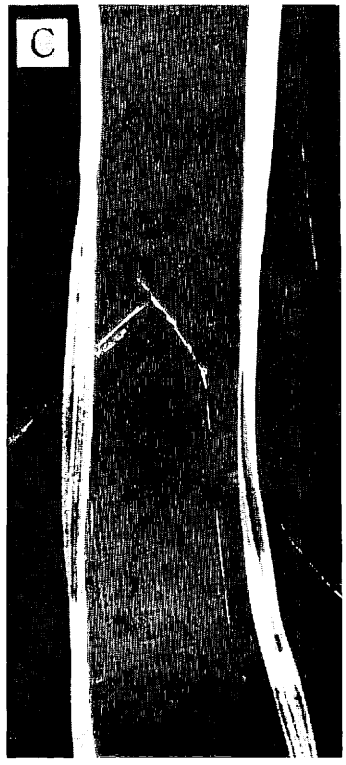
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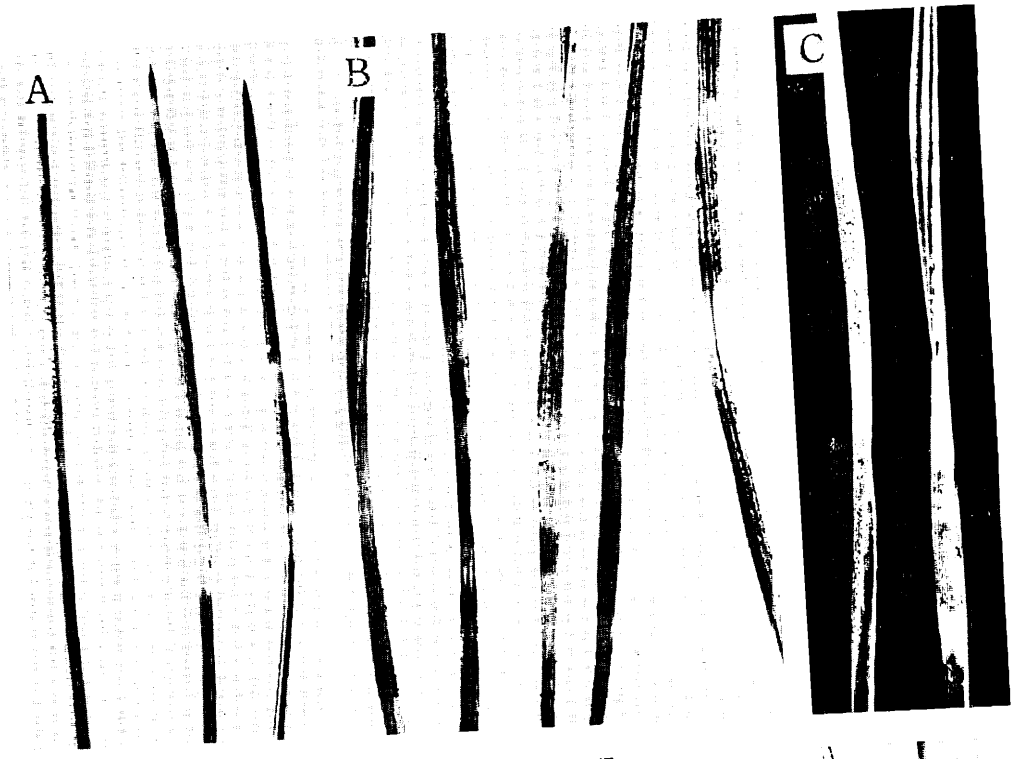
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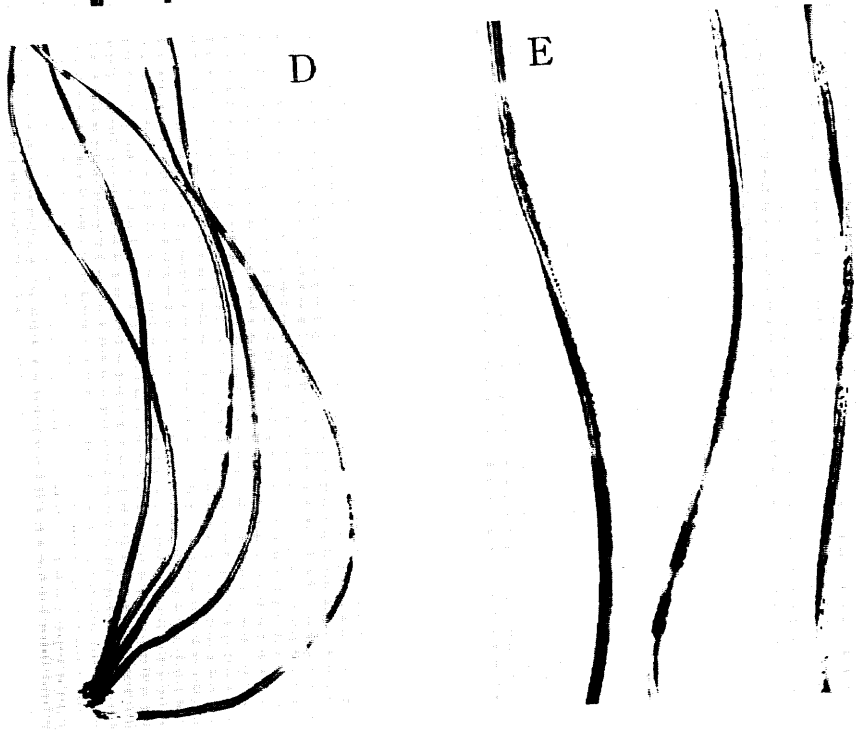
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