

ハクサイのVerticillium albo-atrumによる新病害とその発病に及ぼす二三の要因

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A New Disease of Chinese Cabbage Caused by *Verticillium albo-atrum* and Some Factors Related to the Incidence of the Disease

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渡辺恒雄*・尾沢 賢**・酒井隆太郎*: ハクサイの *Verticillium albo-atrum* による新病害とその発病に及ぼす二三の要因

Abstract

A new disease of chinese cabbage has been observed since 1966, and the occurrence area has been gradually increasing every year.

The leaves of the diseased plants turn blight yellow in color, and the plants are a little stunted. These plants do not form compact central heads even near a harvest time. Their roots and stems become dark brown or blackened in the vascular tissues.

Hyphae were always observed in the vascular tissues of the diseased plants. *Fusarium oxysporum*, the suspected causal fungus, was never isolated from the freshly dissected stem or root tissues of the diseased plants. *Cephalosporium* sp. was one of the most frequently isolated fungi, but its pathogenicity onto chinese cabbages was not positive. *Verticillium albo-atrum* is another frequently isolated fungus. It showed pathogenicity onto 3 varieties of chinese cabbages tested under a greenhouse condition at 20C. The symptoms of the plants grown in artificially infested soils were more or less identical with those in naturally infested soils. In addition, *V. albo-atrum* was recovered from the diseased plants. Vascular discoloration of the roots or the stems were generally observed in some 40 days after seeding in the artificially infested soil at 20C.

As *Verticillium* disease of chinese cabbage in nature appears not to have been previously reported, this plant may be included as a new host of this fungus. "Yellows" (Japanese name, Ohkabyo) may be coined to this new disease.

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A new disease of chinese cabbage (*Brassica pekinensis* Rupr.) has become serious since 1966 in the chinese cabbage fields located in high lands of Nagano prefecture. Some 10 ha out of 140 of the fields were damaged due to this disease in 1969, and the occurrence area has increased gradually every year³⁾.

The symptoms of this disease become obvious near a mature stage. The leaves of the diseased plants turn blight yellow in color, and the plants are a little stunted. These plants do not form compact central heads even near a harvest time. Their tap

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roots and stems become dark brown or blackened in the vascular tissues.

The cause of the disease has been suspected to be due to *Fusarium oxysporum*, but this fungus has not been definitely proved to be the pathogen.

In the present investigation, the cause of the disease, and some factors related to the incidence of this disease were studied.

Materials and methods

Hyphae were always observed in the tissues of the roots and stems of the diseased chinese cabbages examined (Plate I, 1-3). Therefore, isolates of the fungi were only focused in this investigation.

Isolation. Fungi were isolated from small pieces of root or stem vascular tissues incubated on water agar (WA), or potato dextrose agar (PDA) containing 50 ppm streptomycin sulfate by single-hyphal tipplings. Freshly diseased tissues were always used for the isolation with or without treatment of 50 ppm streptomycin sulfate solution for 30 min. A total of 8 diseased chinese cabbages were sampled from 2 different areas in 3 different occasions, and used for the fungal isolation.

Inoculation method. Three different inoculation methods were used for testing the pathogenicity of the isolated fungi. In the first method, an aqueous suspension of minced PDA slant culture was poured near each seedling grown in pasteurized soil at the rate of 1 tube culture for infestation of soil (0.6 kg of air-dried soil) filled in a 12 cm clay pot. In the second, a fresh PD broth culture (0.12 g in air-drying) used as an inoculum was placed at the equal position of seeding without direct contact of each other, and pasteurized soil was further added over the inoculum or seeds about 2 cm deep. Two seeds were generally seeded per pot, but young seedlings were occasionally used instead of seeds. In the third, PD broth cultures containing hyphae, resting mycelium, and spores were minced by a homogenizer at the high speed for 2 min, and used as an inoculum. Then it was artificially mixed with pasteurized soil at the rate of 1 petri dish culture per 1 potted soil. In the second, or the third, the fungus was generally grown in PD broth stationary in a 9 cm petri dish for at least 10 days at 23C, and used for further experiments.

Plants tested. Varieties of chinese cabbages used for the experiments are Chirimen, Nagaoka-hybrid Yokozuna no. 1, or Nozaki no. 2. These commercial seeds used for the experiments were previously confirmed not to carry *Verticillium* and other pathogens in examining a total of 1,000 seeds of 3 varieties by culturing. However, the seeds were always surface-sterilized with Nichinomeru (major content: ethylmercuric sulfate) before use.

Pathogenicity of 2 isolates onto 5 other plants, by the first, or the second inoculation method, was also tested using 4-6 plants each of the following plants, and the experiment was repeated twice. The plants tested are tomato (variety: Sekai no. 1), egg plant (early-matured Minjyu), bell pepper (Mie-midori), radish (Minowase, and Kuroba-minowase), or cabbage (Shiki ehsu).

Disease incidence in relation to inoculum quantity. Artificially infested soils were prepared by mixing pasteurized soils with 0.1, 0.2, or 0.4 g of minced air-dried hyphae and resting mycelium fragment per kg of air-dried soil. Incidence rate in relation to inoculum quantity was determined on the basis of the number of plants with vascular discoloration, and that of infected plants per a total of plants tested. When the fungus was reisolated from any plant tested by culturing, the plant was tentatively determined to be infected by the fungus. The plants grown in the non-infested soil served as a control.

Infection rate in elapse of time. Artificially infested soils were prepared by the third inoculation method at the rate of 0.2 g of the inoculum per kg of air-dried soil. All pathogenicity experiments were conducted under a greenhouse condition at 20C. Young seedlings grown in the artificially

infested soils were removed 10, 20, or 30 days after seeding. And then they were assayed for the fungal infection by culturing.

Results

The fungi isolated. A total of 143 isolates of the fungi were obtained, and among them 93 isolates (65% of the total) were *Cephalosporium*, followed by 37 of *Verticillium* (26%). The former genus was isolated from any plant tested, whereas the latter from 5 out of 8 plants tested. Other genera isolated were *Mortierella*, *Rhizoctonia*, *Penicillium*, and *Fusarium*. Any of these fungi was isolated from only 1 or 2 plants tested. One isolate of *Fusarium*, *F. roseum* was obtained, but *F. oxysporum*, the suspected causal fungus was never isolated.

Characteristics and identity of *Verticillium* isolated. The *Verticillium* isolates formed hyaline verticillate conidiophores, but no dark foot cells of conidiophores were observed by culturing these isolates on natural, or artificial media tested. The conidia are hyaline in color, and 5.3-8.8 (average 6.8) μm long, and 2.5-4.5 (3.3) μm wide in size by measurement of 50 spores formed on PD broth culture. The fungus also formed characteristically dark thick-walled resting mycelium. Each thick-walled cell is 8.8-17.5 (11.5) μm long, and 7.5-13.8 (9.1) μm wide in size (Plate I, 4-6).

Growth responses of 2 isolates of *Verticillium* at the 4 temperature levels of 20, 23, 26, or 28C were compared both on Czapek, or PDA in 4 separate experiments. In both media, the optimum growth was observed at 20C, or 23C days after seeding (Fig. 1, Plate-I, 7).

Most of the morphological characteristics and growth responses were closely corresponded with the description of *Verticillium albo-atrum* Reinke and Berth. by previous workers^{5),7)~12)}, and our isolates were identified as *V. albo-atrum*.

In the pathogenicity experiment of this fungus onto other plants, it was found that bell pepper become diseased, showing a stunting, but other plants tested did not show any visible symptom within 60 days after seeding although some of these plants were found to be infected.

Pathogenicity of *V. albo-atrum* in naturally or artificially infested soil. When 21 days old greenhouse grown seedlings of chinese cabbages were transplanted onto naturally infested soils, the plants became stunted, and turned pale yellowish within 40 days after transplanting; and withering occurred in the lower leaves. All of 3 chinese cabbage varieties tested were infected and blackened in the vascular tissues. *V. albo-atrum* was always reisolated from any test plants by culturing.

In the artificially infested soils, the symptoms were not severe probably due to young ages of plants however the diseased plants showed a slight stunting, yellowing of the leaves, or withering from the lower leaves. The symptoms are more or less identical with those of the diseased plants when grown in naturally infested soil. In addition, infected plants were easily diagnosed on the basis of vascular discoloration by examining the inside of the roots or stems of the plants (Plate I, 8, 9). Some visibly healthy plants were also found to be infected with this fungus. Two isolates

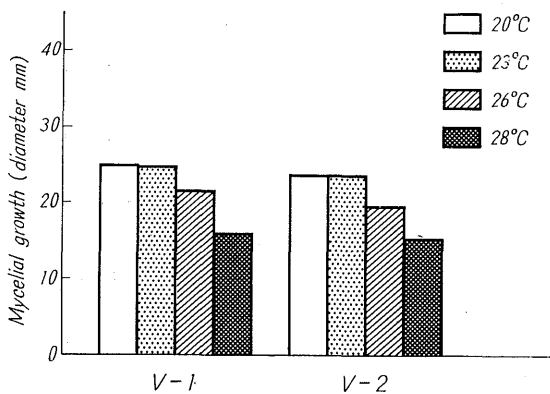


Fig. 1. Influence of temperatures on the mycelial growth (diameter, mm) of 2 isolates of *Verticillium albo-atrum* on PDA 10 days after seeding.

of *V. albo-atrum* tested caused vascular discoloration of 3 varieties of chinese cabbages whatever inoculation method might be used. For example, 17% of 123 plants of Chirimen variety were blackened in the vascular tissues with the isolate, V_1 , and 30% of them were infected as shown in a summarized data of 8 separate experiments. Other varieties were also diseased in the soil artificially infested with this fungus. On the contrary, chinese cabbages were never diseased in the soils treated with *Cephalosporium* sp., or non-treated control (Table 1).

Table 1. Pathogenicity of *Verticillium albo-atrum*, or *Cephalosporium* sp. to chinese cabbages

Variety	Age		Infestation method ^{a)}	Rate of disease incidence			Control
	Initial	Final		<i>Verticillium</i> V_1	V_2	<i>Cephalosporium</i>	
Chirimen	21	60	1st	1/2 ^{b)} (1) ^{c)}	1/2 (1)	0/2 (2)	0/2 (0)
	0-14	53-70 ^{d)}	2nd	4/13 (7)	4/13 (7)	0/9 (9)	0/22 (0)
	0	50-64 ^{d)}	3rd	16/108 (29)	6/35 (15)	0/32 (30)	0/78 (0)
	Total			21/123 (37)	11/50 (23)	0/43 (41)	0/102 (0)
Yokozuna No. 1	21	60	1st	2/2 (2)	0/2 (0)	0/2 (1)	0/2 (0)
	14	53	2nd	1/6 (1)	2/6 (4)	0/6 (6)	0/6 (0)
	Total			3/8 (3)	2/8 (4)	0/8 (7)	0/8 (0)
Nozaki No. 2	21	60	1st	2/2 (1)	1/2 (2)	0/2 (1)	0/2 (0)
	14	53	2nd	0/6 (1)	4/6 (2)	0/6 (6)	0/6 (0)
	Total			2/8 (2)	5/8 (4)	0/8 (7)	0/8 (0)

a) Inocula were poured near each seedling, placed near seeds or seedlings, or mixed with soil for the 1st, the 2nd, or the 3rd infestation method, respectively.

b) Numerator indicates number of plants with vascular discoloration; denominator number of plants tested.

c) Figure in parenthesis is number of infected plants from which the respective fungi were reisolated.

d) Summarized data of 2-5 separate experiments.

Disease incidence in relation to inoculum quantity. Twenty or 22% of 20 or 18 plants became blackened in the vascular tissues at the inoculum quantity of 0.2, or 0.4 g per kg of soil, respectively.

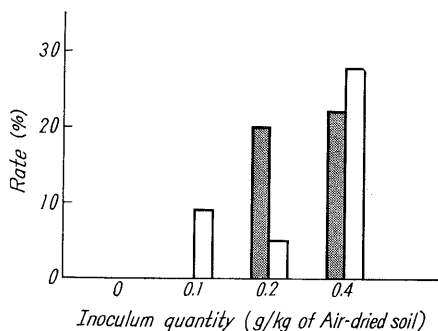


Fig. 2. Rates of vascular discoloration (black bar), and infection (blank bar) of Chinese cabbage in relation to inoculum quantity in the artificially infested soils.

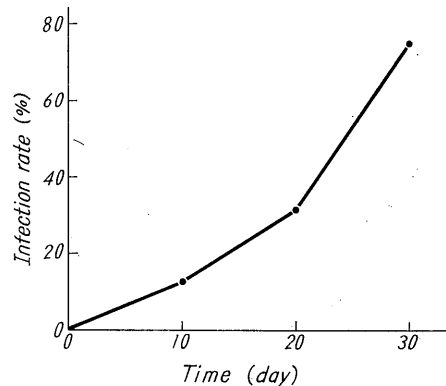


Fig. 3. Infection rates of Chinese cabbage with *Verticillium albo-atrum* in relation to time after seeding in the artificially infested soils.

However, no vascular discoloration occurred in any test plant at 0.1 g, or control. Infection rates in the inoculum level of 0.1, 0.2, or 0.4 g were 9.1, 5.0, or 27.8%, respectively (Fig. 2).

Infection rate in elapse of time. An average of 12.7% of 102 plants tested were infected in 10 days after seeding at 20C, and in 20 or 30 days, 31.3 or 75.0% of the plants tested were infected, respectively (Fig. 3).

Discussion

The cause of a new disease of chinese cabbage in Nagano prefecture had been suspected to be due to *Fusarium oxysporum*. Therefore, inoculation experiments with *F. oxysporum* isolates from chinese cabbage were tried, but the results were not satisfactory³⁾. *F. oxysporum* f. *conglutinans* isolated from cabbage yellows was, however, shown to be pathogenic, causing yellowing of leaves, twisting leaf veins, and vascular discoloration of roots and stems even in the young seedling stage (Unpublished data). This fungus was also listed as a pathogen of chinese cabbage⁴⁾. Therefore, this fungus, if present, may be possible to be the cause of a new disease of chinese cabbage found in Nagano prefecture.

However, *F. oxysporum* was never isolated from freshly dissected root or stem tissues of the diseased chinese cabbages in our experiment, but *V. albo-atrum* was frequently isolated. In addition, *V. albo-atrum* isolates from the chinese cabbage was proved to be infective and pathogenic to chinese cabbages under green house conditions, although the symptoms observed were generally mild. The symptoms of the plants observed were more or less identical with those in nature, and this is especially true when symptoms were compared between the plants grown in naturally-infested and artificially-infested soil. The vascular blackening was always observed generally 40 days after seeding in both cases.

The fungus appears to infect or to be intimately associated with the seedlings grown in the artificially infested soils as early as 10 days after seeding. The development of the symptoms, however, may be influenced by the plant age, varieties to be tested, nutritional or enviromental conditions. Furthermore, unknown factors may be involved in nature for predisposing plants more susceptible to this pathogen, and intensifying the development of the symptoms.

As *Verticillium* disease of chinese cabbage found in nature appears not to have been previously reported in Japan²⁾, or other countries^{1),5),6),11)}, this plant may be included as a new host of *V. albo-atrum*. "Yellows" (Japanese name, Ohkabyo) may be coined to this new disease of chinese cabbage.

Literature Cited

- 1) Anonymous. (1960). Index of plant diseases in the United States. U.S. Dept. Agr. Agr. Handbook 165: 1-531.
- 2) Anonymous. (1965). Common names of economic plant diseases in Japan. The Phytopath. Soc. Japan 2: 1-329.
- 3) Anonymous. (1970). Nagano Pref. Hort. Exp. Stn. Rept. 28: 69-83.
- 4) Armstrong, G. M. and Armstrong, Joanne K. (1966). Phytopathology 56: 525-530.
- 5) Devaux, A. L. and Sackston, W. E. (1966). Can. J. Bot. 44: 803-811.
- 6) Engelhard, A. W. (1957). Plant Dis. Repr. Suppl. 244: 23-49.
- 7) Isaac, I. (1949). Trans. Brit. mycol. Soc. 32: 135-157.
- 8) Isaac, I. (1953). *Ibid.* 36: 180-195.
- 9) Isaac, I. (1967). Ann. Rev. Phytopathology 5: 201-222.
- 10) Ludbrook, K. V. (1933). Phytopathology 23: 117-154.
- 11) Smith, H. C. (1965). N. Z. J. agric. Res. 8: 450-478.
- 12) Wilhelm, S. and Taylor, J. B. (1965). Phytopathology 55: 310-316.

和文摘要

ハクサイの *Verticillium albo-atrum* による新病害と
その発病に及ぼす二三の要因

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長野県でハクサイの新病害が1966年頃から見出され年々その発生面積を増しつつある。罹病植物は葉が黄化し、やや発育が衰え収穫期が近づいても結球しない。それらの根、茎部の導管部組織は黒変し、菌糸が観察された。それらの罹病組織からは病原菌として疑われた *Fusarium oxysporum* はまったく分離されなかった。*Cephalosporium* sp. は最も高い頻度で分離されたがハクサイには病原性を示さなかった。*Verticillium albo-atrum* はその次によく分離され、ハクサイに対して、20°Cの温室条件下で病原性を示した。人為的な病土と自然病土にハクサイを播種し罹病植物を比較すると両者の病徴はほぼ一致し、*V. albo-atrum* が再分離された。根、茎部の導管変色は人為的な病土では播種後約40日頃から観察された。ハクサイの *V. albo-atrum* による自然発病はこれまでのところ我国をはじめ諸外国でも報告されていないので、ここにハクサイを同菌の新しい寄主植物の一つとして報告し、同病害を黄化病と命名したい。

Explanation of plate

- Fig. 1. Cross section of a diseased chinese cabbage naturally infected with *Verticillium albo-atrum* showing vascular discoloration of the root and stem tissue.
- Fig. 2-3. Longitudinal (Fig. 2), or cross (Fig. 3) section of the root of the diseased chinese cabbage in nature showing hyphae in the vascular tissues.
- Fig. 4. Verticillate conidiophore of *V. albo-atrum*.
- Fig. 5. Conidia of *V. albo-atrum*.
- Fig. 6. Dark resting mycelium of *V. albo-atrum* formed in PD broth 10 days after seeding.
- Fig. 7. Colonies of *V. albo-atrum* on PDA 10 days after seeding.
- Fig. 8-9. Cross (Fig. 8), or longitudinal (Fig. 9) sections of the roots of chinese cabbages grown in the soil artificially infested with *V. albo-atrum*, or non-infested soil showing the diseased (left 2 plants in Fig. 8, or left 2 pieces in Fig. 9), or the healthy (right 1 plant in Fig. 8, or right 2 pieces in Fig. 9) plants 50 days after seeding. Note the vascular discoloration of the diseased plants.

Plate I

