

日本で発生したニンジンしみ腐病 (1)

発生,病徴および分離

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Brown-blotted Root Rot of Carrots in Japan

(1) Occurrence, Symptoms and Isolation

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Abstract

Blot-like lesions have occurred on taproots of carrots in summer-and winter-maturing crops in Chiba Prefecture for past 10 years, resulting in significant quality and yield losses. *Pythium sulcatum* (ref. (2) in the same title) was isolated from these diseased roots in various localities of Chiba Pref. The disease was severe in the fields with repeated cultivation of carrots, especially in the years with excessive rainfall prior to the harvest. The fungus was only pathogenic to the roots of carrots, but it did not cause damping-off nor leaf blight. In the summer-maturing crop, water-soaked brown circular or elliptical lesions, 3-5 mm in diam, were formed mainly on taproots at harvest, and often soft-rotted on the surface. In the winter-maturing crop, small brown slightly sunken spots, 2-3 mm in diam., accompanied occasionally with vertical cracks were formed on the taproots. In both cropping types, lateral roots were discolored to brown and decayed frequently and these roots got off from the taproots. Variations of the symptoms may be due to differences of cultivars, time of the disease occurrence and soil conditions.

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Key words : carrots, brown-blotted root rot, *Pythium sulcatum*, isolation.

Introduction

Chiba Prefecture has the second largest productive area (3,060 ha) of carrots (*Daucus carota* L.) in Japan, following Hokkaido. There are two types of carrot cropping in Chiba Pref., namely summer-and winter-maturing crops. In the former, carrot is seeded in January to February and harvested in May to June. In the latter, it is seeded in July to August and harvested in December to March. In these croppings, water-soaked brown spots, so-called blots occurred on the taproots of carrots at or near harvest since more than 10 years ago, resulting in significant quality and yield losses^{4,5)}.

In the summer crop of 1983, the blots on carrot roots occurred unusually heavily, probably due to frequent rainfall at harvest. During the etiological study of this carrot disease, some *Pythium* isolates were obtained from the lesions on the taproots. Although the root rots caused by *Rhizoctonia solani* Kühn⁵⁾ have been reported, the diseases caused by *Pythium* species have not yet been studied in Japan. Therefore, this study was conducted to clarify the causal organism of the so-called blots on the taproots of carrot

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occurred in Chiba Pref.^{1,6)}. This is one of two papers¹⁰⁾ with the same title, and it is mainly concerned with occurrence of the disease, symptoms, and experimental procedures for determining the causal organism.

Materials and Methods

Isolation. Diseased carrots with blot symptoms on the taproots were collected from the carrot fields or farmers' shipping markets in the main productive localities including Chiba and Funabashi City and Yachimata Town. The root tissues from the taproots with lesions were cut into small cubes, 1-2 mm long and washed under running tapwater for 1 h and rinsed with sterilized distilled water. Subsequently, they were placed on water agar medium in Petri dishes after removal of excess water and incubated for 1-2 days at 25 C. Then, single hyphal tips elongated from the tissue pieces were cut with agar blocks under a dissecting microscope and transferred onto potato sucrose agar (PSA) medium.

Inoculation *in vitro*. Isolates from the diseased plants were cultured on PSA in a 9 cm Petri dish for 4 days at 25 C. The mycelial agar discs, 4 mm in diam. were cut and removed from the edges of the colonies by a cork borer and used as inocula. Each inoculum was inoculated by placing on a taproot, a lateral root or a petiole of carrots in a humid large Petri dish, 18 cm in diam. with filter papers using 3-5 plants per treatment and incubated for 7 days or more at 25 C. The inoculation experiments were conducted usually using healthy fresh tissues of well-washed carrots (cvs. MS 4-sun and US Harumaki 5-sun). Artificially wounded tissues were also prepared by sticking 3-5 mm deep with a sterile needle. Young healthy seedlings of corn, bean, melon, cucumber, tomato, and peppers were also inoculated similarly *in vitro*. Disease severity was evaluated visually for each individual plant based on arbitrary index: 0=no disease, 1=mild, 2=moderate, and 3=severe.

Inoculation *in pots*. As all of 55 isolates were pathogenic to carrot taproots and judged as the same species on the basis of cultural and morphological characteristics together with mycelial growth rates, two isolates (A-1 and A-4) were selected and used for following works. These two isolates were cultured in potato sucrose broth in ten 500 ml flasks (300 ml broth per flask) for 3 weeks at 25 C, and the harvested mycelia were homogenized for 10 sec with a waring blender at low speed, and the suspension was further diluted with the same amount of distilled water. Nearly 2.5 kg of autoclaved volcanic ash soil in a 1/5000 a pot was infested with 40 ml of the diluted fungus suspension on the soil surface and 10-20 seeds each of the respective test plants were sown in each pot. Uninfested soils served as control. The plants tested were carrot (cv. MS 4-sun), corn (cv. Honney Bandum), bean (cv. Satsuki-Midori), melon (cv. Prince melon), cucumber (cv. Shin-Tokiwa), spinach (cv. Late Pionia) and radish (cv. Natsu Minowase No. 3).

For inoculation onto mature carrots, 115-day-old carrots grown in autoclaved soil in 1/2000 or 1/5000 a Wagner pots (10-15 plants per pot) were used. Inoculations were conducted by pouring 40 or 100 ml of the diluted mycelial suspension into 1/5000 or

1/2000 a potted soils, respectively. In addition, 4 mm agar discs used as inocula in *in vitro* inoculation study were also attached directly on the taproots in potted soil. The 1/5000 a pots were set in water bath at 20, 25 and 30 C together with the 1/2000 a pots in a greenhouse (15-25 C). The plants were watered rather excessively everyday. All inoculation experiments were repeated 3 times.

Results

Occurrence of brown-blotted root rot

The carrot disease occurred every year during harvest in summer crops (cvs. MS 4-sun, US Harumaki 5-sun and Yubeni) and also in winter crops (cvs. Kuroda 5-sun and Fuyubeni 5-sun).

Summer-maturing carrots with long cropping history cultivated in Chiba, Funabashi, Narashino and Yachiyo City have severely been damaged especially in the years with frequent rainfall at harvest, probably due to repeated cultivation for many years. On the other hand, winter-maturing carrots with rather recent cropping history cultivated in Yachimata and Matsuo Town in the northern upland areas of Chiba Pref. have also been damaged with small brown spots on the taproots increasingly every year, resulting in quality and yield losses.

Symptoms

The lesions of brown-blotted root rot of carrot are observed mainly on the taproots at or near harvest, and also on the lateral roots if the roots are removed from soil carefully, but not on the leaves nor petioles. These lesions in summer carrots are water-soaked and brown circles or ellipses, 3-5 mm in diam. (Fig. 1) and called "blots" locally, that occasionally develop into large irregular soft-rotted lesions, over 3 cm in diam. (Fig. 2). On the other hand, the lesions in winter carrots become small brown circular spots, 2-3 mm in diam. that are sunken slightly and occasionally cracked vertically in the center (Fig. 3). Two to 10, or more lesions are formed irregularly on the upper and middle parts of 12-18 cm long mature taproots, and fewer in the bottom. Although no pathogenic fungi are visible on the lesions by naked eyes, hyaline aseptate hyphae are observed microscopically.

Lateral roots are discolored to brown and rotted entirely or partially (Fig. 5). The rotted roots are easily detached from the taproots at harvest. Carrot plants infected early were poor in growth.

Symptoms on the taproots were classified into three types; A: water-soaked brown circular or elliptical lesion (Fig. 1). B: lenticel-like brown spot formed around the base of a lateral root (Fig. 4), C: small brown sunken spot with a vertical crack (Fig. 3).

Isolates

Pythium isolates were frequently obtained from summer carrots with the symptom of type A, and bacteria were isolated occasionally. All *Pythium* isolates tested formed water-soaked brown lesions on the taproots of carrots *in vitro*, whereas bacteria did not show any pathogenicity. From diseased carrots with the symptom of type B, various

Table 1. Occurrence of blot-like symptoms on the taproots of carrots in five carrot-growing localities of Chiba Prefecture in 1983 and 1984, types of symptoms, and pathogenicity^{a)} of *Pythium* isolates

Locality	Cropping type	Symptom type ^{b)}	Number of		
			Sample	<i>Pythium</i> isolate	Pathogenic isolate
Chiba	Summer-maturing	A	8	4	4
		B	5	0	—
		C	0	— ^{c)}	—
Funabashi	Summer-maturing	A	5	5	5
		B	0	—	—
		C	5	3	3
Narashino	Summer-maturing	A	1	1	1
		B	0	—	—
		C	6	6	6
Matsuo	Winter-maturing	A	1	1	1
		B	0	—	—
		C	2	2	2
Yachimata	Winter-maturing	A	3	2	2
		B	0	—	—
		C	13	13	13

a) Inoculated onto the taproots of carrots *in vitro*.

b) Classified into three types; A: water-soaked brown circular or elliptical lesion, B: lenticel-like brown spot formed around the base of a lateral root, C: small brown spot with a vertical crack.

c) Not tested.

organisms including *Rhizoctonia* sp., aseptate fungi and bacteria were isolated, but none of them were pathogenic to the taproots. From the type C on summer carrots, *Pythium* isolates were obtained frequently together with bacterium isolates. These *Pythium* isolates were also pathogenic to the taproots just like those in type A, but did not form any crack on the taproots *in vitro*. However, the seedlings planted in the artificially infested soil were frequently diseased with lesions with a crack after maturity. From the diseased winter carrots of type C, *Pythium* was predominantly isolated, and the fungus tested showed pathogenicity onto the taproots. All of these results were shown in Table 1.

A total of 55 isolates obtained in this study were all pathogenic to the taproots of carrots, and judged as the same species on the basis of cultural and morphological characteristics together with mycelial growth rates. This *Pythium* was later identified as *P. sulcatum* Pratt and Mitchell in our second paper¹⁰⁾.

Pathogenicity

When taproots and lateral roots of carrots (cvs. MS 4-sun, US Harumaki 5-sun and Fuyubeni 5-sun) were inoculated with mycelial agar discs of *Pythium* *in vitro*, water-soaked brown lesions were formed within 4 days after treatment around the inoculum irrespective of artificial wounding or not (Fig. 5). However, no clearcut symptoms were observed on leaves or petioles except slight discoloration occasionally occurred (Table

Table 2. Pathogenicity of *Pythium sulcatum* on to the roots and petioles of carrots in vitro

Isolate	Disease incidence rate (%) ^{a)} and severity ^{b)} of					
	Taproots		Lateral roots		Petioles	
	Rate	Severity	Rate	Severity	Rate	Severity
A-1	87	46	92	68	20	3
A-4	40	17	88	58	22	4
Uninoc.	0	0	10	2	0	0

a) A total of 15-20 samples tested in 2-3 separate experiments.

b) Severity = (Sum of disease Severity Index × no. of lesions/no. of samples inoculated × 3) × 100. Disease Severity Index was evaluated visually for each sample based on arbitrary index: 0 = no disease, 1 = mild, 2 = moderate, and 3 = severe.

Table 3. Pathogenicity of *Pythium sulcatum* (isolate A-4) to the young seedlings of several crops in the artificially-infested potted soils under three different soil temperature conditions

Temperature (C)	Crop	Inoculated			Non-inoculated		
		Seedlings ^{a)} tested (no.)	Damped-off ^{b)} (no.)	Blotted (no.)	Seedlings tested (no.)	Damped-off (no.)	Blotted (no.)
30	Cucumber	10	0	0	10	0	0
	Melon	7	0	0	7	0	0
	Corn	6	0	0	5	0	0
	Bean	9	0	0	5	0	0
	Tomato	14	0	0	10	0	0
	Pepper	13	0	0	10	0	0
	Turnip	14	0	0	9	0	0
	Carrot	6	0	3(+) ^{c)}	9	0	0
25	Cucumber	10	0	0	8	0	0
	Melon	8	0	0	8	0	0
	Corn	7	0	0	6	0	0
	Bean	9	0	0	7	0	0
	Tomato	17	0	0	20	0	0
	Pepper	15	0	0	20	0	0
	Turnip	18	0	0	18	0	0
	Carrot	4	0	4(+)	11	0	0
20	Cucumber	9	0	0	6	0	0
	Melon	6	0	0	2	0	0
	Corn	6	0	4(-)	9	0	3(-)
	Bean	9	0	0	10	0	0
	Tomato	15	0	0	18	0	0
	Pepper	13	0	0	13	0	0
	Turnip	19	0	0	18	0	0
	Carrot	5	0	1(+)	12	0	0

a) Ten seeds each of cucumber, melon, corn and bean, and 20 each of tomato, pepper, turnip and carrot were sown per pot.

b) The causes of failure of germination of seeds and emergence were not considered in this study.

c) +: *Pythium* was recovered, -: Not recovered.

Table 4. Incidence of brown-blotted root rot of carrots inoculated with *Pythium sulcatum* (isolate A-4) by two different methods under four soil temperature conditions

Temperature (C)	Treatment	Seedlings		
		No. tested	With taproots damaged(rate,%)	With lateral roots damaged(rate, %)
30	Mycelial agar ^{a)}	14	78	50
	Soil ^{b)}	23	11	22
	Uninoculated	21	4	11
25	Mycelial agar	13	85	62
	Soil	20	16	78
	Uninoculated	34	4	0
20	Mycelial agar	15	73	27
	Soil	24	12	21
	Uninoculated	33	3	0
13-20	Mycelial agar	20	75	5
	Soil	45	17	0
	Uninoculated	26	0	0

a) Inoculated directly with 4-mm mycelial agar discs onto the taproots.

b) The seedlings were grown in the artificially-infested soil with the mycelial suspension.

2, Fig. 6).

When carrot plants grown in pots were inoculated by pouring the mycelial suspension of *Pythium* near the roots, brown lesions were formed on both taproots and lateral roots of young seedlings, but no damping-off occurred (Table 3), whereas the symptoms developed on the taproots of mature plants similarly, but at the frequency of 20% or less even under excessively moistened soil conditions. However, more than 70% of the plants were diseased when the roots were directly inoculated with mycelial agar discs (Table 4).

No disease occurred on roots, basal stalks or leaves of tomato, pepper, cucumber, melon, kidney bean, turnip, spinach and corn by *in vitro* inoculation experiments. Furthermore, young seedlings of these crops grown in the artificially infested potted soil were free from the disease by this *Pythium* even under humid soil condition (Table 3).

Disease occurrence in relation to soil temperatures

When carrots were grown in potted soil in water baths with temperatures regulated at 20, 25, and 30 C and inoculated with the mycelial suspension of *Pythium*, infection and disease severity rates were highest at 25 C, although the disease occurred at 20 or 30 C. Infection rate of the taproots was lower than that of lateral roots when inoculated near maturity (Table 4).

Discussion

Carrot diseases caused by *Pythium* species have been reported in the USA and Canada

for the past 10 years^{2,3,8)}. The symptoms are various in the areas where the diseases occurred, and therefore, several disease names including brown rot^{3,7)}, rusty root^{8,9)}, rusty brown necrosis^{8,9)}, and *Pythium* root dieback²⁾ appeared in literature. These diseases appear similar with different *Pythium* pathogens. For example, root dieback is caused by *P. debaryanum* Hesse and rusty root by several *Pythium* species including *P. coloratum* Vaartaja, *P. irregulare* Buisman and *P. silyaticum* Campbell and Hendrix. However, brown rot is caused mainly by *P. sulcatum*⁷⁾. This disease is prevalent in Wisconsin and Florida, but the symptoms in both states are not always identical to each other. Brown rot reported by Pratt and Mitchell⁷⁾ is characterized by browning and decaying of taproots and lateral roots and forking of the taproots, whereas brown-blotted root rot in this study by brown spots on the taproots and browning and decaying of the lateral roots. These symptoms were more or less identical with the symptoms of brown rot in the United States except lack of forking.

Pythium sulcatum is presumed to be prevalent widely in carrot-growing areas in Chiba Pref. and probably in other prefectures and one of main pathogens of so-called blots on the taproots in various cropping types of carrots, because this fungus was isolated frequently from both water-soaked brown lesions of the summer-maturing carrots and small sunken brown lesions of the winter-maturing carrots irrespective of the differences of the cropping areas. In addition, the symptoms may be variable due to differences of the cultivars planted, cropping types, time of the disease occurrence and soil conditions including soil moisture and soil microorganisms. The symptom of type C in this study may be caused by *P. sulcatum* and a few unidentified soil microorganisms. Further work on this line is now in progress.

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和 文 摘 要

長井雄治・深見正信・村田明夫・渡辺恒雄：日本で発生したニンジンしみ腐病（1）発生，病徴および分離

千葉県で栽培されている夏どりニンジンと冬どりニンジンに近年しみ状斑点が発生し，品質低下と減収の原因となっていた。県内各産地のしみ状病斑から，いずれも *Pythium sulcatum* が分離された。本病は連作畑で発生し，特に収穫前に長雨に遭遇すると多発する。病原菌の寄生性はニンジン根部に限られ，葉枯れや立枯れは起こさず，ニンジン以外の作物には寄生しなかった。夏どりニンジンでは，主として収穫期の直根に径 3~5 mm の円形または長円形の褐色水浸状病斑を生じ，ひどい場合には病斑表面は軟化腐敗する。冬どりニンジンでは，直根上の病斑は径 2~3 mm の褐色斑点で，やや陥没し，しばしば病斑中央に縦の亀裂を生じる。いずれの作型でも，側根は褐変腐敗し，脱落しやすい。病徴は作型により異なり，その原因は品種，発生時期，土壌条件などの違いによるのではないかと推測された。

Explanation of plate

Plate I

- Fig. 1. Diseased taproots with water-soaked circular brown lesions, (Symptom type A) (left), and obscure symptoms in the early stage (right) of natural infection by *Pythium sulcatum* on summer-maturing carrots.
- Fig. 2. Diseased taproots of summer-maturing carrot with enlarged symptoms in natural infection by *P. sulcatum*.
- Fig. 3. Diseased taproots of winter-maturing carrot with small brown spots with a vertical crack (Symptom type C) in natural infection by *P. sulcatum*.
- Fig. 4. Diseased taproot of winter-maturing carrot with brown lenticel-like spot (Symptom type B). Note: No *Pythium* was recovered from the carrot with Symptom type B.
- Fig. 5. Formation of brown-blotted lesion on the taproot and browning of lateral roots of carrot by inoculation with 4-mm mycelial agar discs of *P. sulcatum* (isolate A-4) 5 days after inoculation.
- Fig. 6. Non-formation of symptoms on carrot petioles by inoculation with 4-mm mycelial agar discs of *P. sulcatum* (isolate A-4) 5 days after inoculation.

Plate I

