土壌病菌量Pythiumの生態および分類 第XI報 ジエチルジチオカルバミンド酸ナトリウム処理をしたカボチャ胚軸部に現われる水浸状病斑

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
著者名	一谷,多喜郎 米谷,冨男 高橋,実
発行元	日本植物病理學會
巻/号	38巻4号
掲載ページ	p. 313-322
発行年月	1972年9月

日植病報 38: 313-322 (1972)
Ann. Phytopath. Soc. Japan 38: 313-322 (1972)

Ecologic and Taxonomic Studies on *Pythium* as Pathogenic Soil Fungi. XI.

Water-soaked and Spreading Lesions on Squash Hypocotyls treated with Sodium diethyldithiocarbamate

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一谷多喜郎*・米谷富男*・高橋　実*: 土壌病菌 *Pythium* の生態および分類
第 XI 報　ジエチルジチオカルバミド酸ナトリウム処理をした
カボチャ胚軸部に現われる水浸状病斑

Abstract

Amakuri squash seedlings were cultivated in Hoagland solution in plant growth chamber, and water-soaked spots and spreading lesions were described on *Pythium*-infected hypocotyls when the seedlings were pre-treated with sodium diethyldithiocarbamate (NaDEDC) in relation to increase in host susceptibility. A slight abnormal elongation of the growth of the seedlings was found both in plant growth chamber and in water culture. Browning spots were formed in both soil and solution-grown seedlings after inoculation. The nutrient solution did not give any effect on the mycelial growth of the pathogen. The cell sap expressed from hypocotyls treated with NaDEDC did neither stimulate nor inhibit the fungal growth. Water-soaked lesions were found without any phytotoxicity when pre-treated with ether for 20-120 seconds. Only NaDEDC and sodium fluoride were considered good among the metabolic inhibitors used which induced water-soaked lesions because of the absence of phytotoxicity and stimulative effects on the fungal growth. Cuttings of the seedling which were supplied with the NaDEDC through their cut ends did not exhibit browning spots but rather formed water-soaked lesions. The water culture was more desirable method with NaDEDC than the injection in obtaining plant materials with water-soaked lesions.

(Received December 2, 1971)

Introduction

There are numerous species of *Pythium* causing soil-borne diseases. *Pythium* causes storage rots of fruits and tubers, and damping-off of different kinds of seedlings[8,9,15,16,18,19]. This fungus has been reported to be generally highly pathogenic[8,9] but the pathogenicity of the fungus varies considerably with the species or the isolate[5,19]. Likewise, susceptibility of the hosts also varies with the species or the isolate of *Pythium*[5,19]. These differences in susceptibility may be explained either as the relative degree of pathogenicity of the parasites or a defense reaction of the hosts[4,5].

Pythium ultimum Trow is known to be pathogenic to family *Cucurbitaceae*[20]. Regarding the host susceptibility to this fungus, cucumber, tomato and egg-plant are classified as highly susceptible, whereas "Amakuri" and "Delicious" squash are resistant, and rice plant is immune[16]. Resistant squash Amakuri shows a hypersensitive reaction against the invasion of *P. ultimum* by producing

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a heat-labile antifungal substance (s) in the tissues adjacent to the invaded part, and the diseased tissues forming brown spots on the hypocotyl. On the contrary, highly susceptible cucumber seedlings neither produce the substance (s) in the adjacent part nor show brown spots on the hypocotyl but rather become water-soaked and damped off[18]. Thus, water-soaking and spreading of the lesions have considered as an indication of increasing host susceptibility[17,18].

The brown water-soaked spots and enlarged lesions were observed on *Pythium*-infected hypocotyls of Amakuri squash seedlings grown in pots or in nutrient solution when the seedlings were pre-treated with ether, alcohol, chloroform and different metabolic inhibitors[3,12,17]. These phenomena have also been reported in other diseases[1,7,10,11]. All these experiments were, however, done under ordinary glasshouse although a characteristic of disease symptoms may vary with experimental conditions.

In the present paper, Amakuri squash seedlings were cultivated in nutrient solution in plant growth chamber, and water-soaked spots and spreading lesions on the hypocotyls were described in relation to increase in host susceptibility after treating the seedlings with sodium diethyldithiocarbamate (NaDEDC).

Materials and methods

Host and pathogen used The seedlings of squash Amakuri (*Cucurbita maxima* Duch. cv. Uchiki Akagawa Amakuri) were used as the test plant. The seeds were obtained from Uchinada-mura, Ishikawa prefecture, and stored in desiccator at 10°C throughout the year. *Pythium ultimum* Trow (Isolate No. 77) was used as pathogen. This fungus was originally isolated from *Hibiscus manihot* and is known to be pathogenic to the *Cucurbitaceae* and Amakuri squash[16,20]. The fungus has been maintained as described in previous paper[4].

Cultivation of host seedlings Medium sized seeds, with a lustrous seed coat, were carefully selected and disinfested with 0.1% $HgCl_2$ solution for 20 minutes. They were washed in sterilized water several times and finally soaked in it for 2 hours. Germination of about 50 seeds was hasten by putting them between two moist filter papers in a dark colored glassware (30 cm in diam.) at 26°C. Several black polypots (7 cm in diam.) with sterilized sandy soil and each planted to seven germinated seeds were used. After covering the seeds with sterilized sand, the pots were kept in plant growth chamber at 25±1.8°C and exposed to light for 14 hours daily (10,000 lux, Mitsubishi fluorescent lamp "Delux White"). The relative humidity of the air and the velocity of the wind inside the chamber were 60–80% and less than 0.1 m a second, respectively. When cotyledons have been fully developed but the leaves were not yet formed, the seedlings were used for the experiments.

For the water culture, the germinated seeds were pre-cultured in glass-distilled water (pH 6.0) in 65 m*l* capacity black bottle for a day and grown on a half-strength Hoagland nutrient solution* (pH 6.0) for another days in plant growth chamber. Changing of the nutrient solution was done every day and thus aeration of the solution was facilitated. Seedlings with a height of 4–5 cm top length were uniformly selected and used in the experiments.

Fungitoxicity of nutrient solution used The effect of the nutrient solution on the mycelial growth of the pathogen was determined by modifying Ryan *et al.* method[14]. Five millimeter-diameter agar discs of the colony grown on 2% water agar at 26°C for 5 days were cut with a cork borer. A colony disc was placed in every tube (12 mm in diam. and 120 mm in length) containing different

* One liter of undiluted solution contained, 115.0 mg $NH_4H_2PO_4$, 606.6 mg KNO_3, 944.6 mg $Ca(NO_3)_2 \cdot 4H_2O$, 493.0 mg $MgSO_4 \cdot 7H_2O$. One mole of each component of the solution was prepared in advance with glass-distilled water (pH 6.0). The solution was stored at 4°C and used within 3 weeks.

dilutions of 10 m*l* sterilized Hoagland solution. It was made sure that the discs of inoculum sunk at the bottom of the tube. The tubes were placed upright and incubated at a temperature of 26°C. The distance from the distal edge of the growing mycelium to the point of seeding (height of mycelium) was measured after 14–84 hours of incubation. The growth intensity of the mycelium was also observed. Similarly, the effect of Hoagland solution on the mycelial growth was also determined on agar plate. Furthermore, the effect was determined on liquid and agar plate culture media with corn-meal decoction. The radii of colonies in plate culture were measured after 12–48 hours of incubation.

Kinds of chemicals used Chemicals used were as follows: L-ascorbic acid, cupric sulfate, 2, 4-dinitrophenol, α, α'-dipyridyl, ethyl ether, malonic acid, NaDEDC, phenylthiourea, potassium cyanide, sodium bisulfate, sodium fluoride, sodium monofluoroacetate, thiourea, and urethane. All these chemicals were analytical grades and obtained from Wako Pure Chemical Industries, Ltd., Osaka.

Fungitoxicity and phytotoxicity of chemicals used The fungitoxicity of metabolic inhibitors was determined by using substantially the same method which has been described elsewhere. Ten m*l* sterilized Czapek's solution was poured into several test tubes (18 mm in diam. and 180 mm in length). Each tube was seeded with one 5-mm agar disc of mycelium which has been previously incubated at 26°C for 5 days on 2% Czapek's agar medium. The height of mycelium was measured after 3–5 days of incubation at 26°C.

The fungitoxicity of NaDEDC absorbed by squash seedlings was determined by getting expressed cell sap of the hypocotyls treated with NaDEDC after maceration and centrifugation at 15,000 g for 30 minutes. The supernatant liquid was sterilized either by millipore filtration (MF-Millipore Filter GSWP 04700, Nihon Millipore Ltd., Tokyo) or by autoclaving (1.2 kg/cm^2, 40 min.) and then seeded with 5-mm agar disc. The height of mycelium was measured after 24–72 hours of incubation at 26°C.

Phytotoxicity was judged both from a visible discoloration produced on hypocotyls and from the reduction in the growth of the seedlings.

Ethyl ether or metabolic inhibitor treatments Ether treatment was conducted with a slight modification of the method described in a previous paper[17]. Stripe of absorbent cotton (5 mm in width and 15 mm in length) were prepared in advance. The hypocotyls were covered with the cotton and about 0.5 m*l* of ether was dropped onto it. After treatment, the cotton was removed and the seedlings were left for 1 hour in a well ventilated room (about 26°C) before inoculation.

Treatments with metabolic inhibitors were done by replacing the Hoagland solution with inhibitor solution. The treatment was also done by injecting 0.05 m*l* inhibitor solution into a pith where the cotyledons jointed. Metabolic inhibitor was first dissolved in glass-distilled water of pH 7.0 or 6.0 immediately before using for either water culture or injection. However, NaDEDC was always dissolved in it at pH 7.0, since this compound decomposes at lower pH values[13]. After an uptake period of 24 hours, the plants were rinsed in distilled water and put back to the Hoagland solution before inoculation.

Inoculation *P. ultimum* was subcultured as described in the previous paper[4]. Bacterial contamination was determined by using nutrient broth. Eight m*l* of corn-meal agar (1% agar) was poured into a petri dish (8.5 cm in diam.). It was seeded at the center with *P. ultimum* and incubated for 4 days at 26°C. Mycelial mat together with the agar (0.8 cm in width and 1.5 cm in length) was cut out and placed on paraffin paper. An inoculum was wound around the hypocotyl near the soil line and covered with adhesive tape. Agar blocks served as checks. Both inoculated and uninoculated plants were put in moist chamber for 36 hours inside the plant growth chamber. Afterwards, the plants were kept in plant growth chamber for another 12 hours.

Description of external symptoms External symptom development was generally observed 36 hours after inoculation, according to six different common types of symptoms which will be described later.

Results and discussion

Inoculation tests on solution-grown squash seedlings in plant growth chamber The growth of solution-grown seedlings in plant growth chamber was first compared with that in ordinary glasshouse (21–30°C with a maximum light intensity of 60,000 lux) (Table 1). Results indicate that a slight abnormal elongation of growth of the seedlings was found both in plant growth chamber and in water culture. Under ordinary glasshouse, cucurbitaceous plants grown in pots are generally known to be highly susceptible to *Pythium*, but Amakuri squash seedlings are resistant or slightly susceptible, exhibiting browning spots[16,18]. To determine whether solution-grown Amakuri squash seedlings are also capable to exhibit browning spots in plant growth chamber, the inoculation tests were done and the results showed that browning spots were found in both soil and solution-grown seedlings in this chamber (Table 2). The cuttings of squash Amakuri were first thought to be resistant to *Pythium*, exhibiting browning spots, but the results of the experiment showed that it was not the case.

From the above-mentioned results, solution-grown seedlings as well as those grown in soil in plant growth chamber are good materials for the study of disease resistance.

Table 1. Growth of Amakuri squash seedlings in ordinary glasshouse and in plant growth chamber[a)]

Exp.	Avg. top length (mm)	Avg. dry weight (mg) per seedling
Ordinary glasshouse (21–30°C)		
Soil	40	101.2
Solution	77	104.0
Plant growth chamber (25±1.8°C)		
Soil	67	98.4
Solution	90	100.6

a) Sampled 15 seedlings at the cotyledonous stage for each trial immediately before inoculation.

Table 2. Symptoms in resistant squash seedlings grown in ordinary glasshouse and in plant growth chamber

Exp.	No. of seedlings exhibiting symptoms[a)]					
	1	2	3	4	5	6
Ordinary glasshouse (21–30°C)						
Soil	0	3	27	5	0	0
Solution	0	0	7	9	0	0
Plant growth chamber (25±1.8°C)						
Soil	0	1	33	6	0	0
Solution	0	3	14	0	0	0

a) Numbers (1–6) denote 6 common types of symptoms of NaDEDC treated, inoculated Amakuri squash hypocotyls as shown in Fig. 1.

Effect of nutrient solution on mycelial growth of the pathogen It was first considered that the nutrient solution itself may result in producing water-soaked lesions by promoting mycelial growth of the pathogen on the surface of hypocotyl. Results showed, however, that the solution did not give any effect expected and that the appearance of water-soaked lesions on solution-grown seedlings was not due to the stimulatory effects of the solution on mycelial growth of the pathogen.

Effect of metabolic inhibitors on mycelial growth of the pathogen It was also thought that metabolic inhibitors themselves absorbed by seedlings may induce water-soaked lesions by promoting mycelial growth of the pathogen. The growth of the pathogen in Czapek's solution in the presence of each metabolic inhibitor was first compared. Out of 13 inhibitors used, cupric sulfate and 2, 4-dinitrophenol were fungitoxic even at the concentration of 10^{-6} M. No metabolic inhibitors stimulate mycelial growth, but they were generally fungitoxic to some extent at the ranges of 10^{-3} -10^{-5} M. The effect of different concentrations of NaDEDC on mycelial growth of the pathogen in Czapek's solution was again determined in detail and the concentrations used were not remarkably stimulating but rather inhibiting (Table 3). Then, the growth of the pathogen in cell sap expressed from hypocotyls treated with NaDEDC was examined and the cell sap did neither significantly stimulate nor inhibit the fungal growth (Table 4). These results indicate that no effect of NaDEDC was found on

Table 3. Effect of NaDEDC dissolved in Czapek's liquid medium on mycelial growth of *P. ultimum* (Isolate No. 77)

NaDEDC (M)	Avg. height (mm) of mycelium incubated for:				Mycelial growth intensity[a)]
	1 day	2 days	3 days	4 days	
10^{-3}	0	0	0	0	−
10^{-4}	0	0	6	16	++
10^{-5}	10	19	24	31	+++
10^{-6}	12	21	27	31	+++
10^{-7}	9	16	23	26	+
10^{-8}	8	18	25	29	++
Untreated	13	19	26	31	++

a) Incubated for 5 days; −, no mycelium; a rating of (+++) denotes mycelial mat was opaque; a rating of (+) denotes mycelial mat was transparent; a rating of (++) denotes an intermediate degree of transparency of mycelial mat.

Table 4. Effect of cell sap from squash hypocotyls pre-treated with NaDEDC on mycelial growth of the pathogen

Concentration (M) pre-treated	Avg. height (mm) of mycelium in autoclaved cell sap incubated for:			Avg. height (mm) of mycelium in filtered cell sap incubated for:		
	24 hr	48 hr	72 hr	36 hr	48 hr	60 hr
10^{-3}	0.6	1.8	2.5 (++)[a)]	1.7	2.0	2.4 (+)[b)]
10^{-4}	0.8	1.7	2.5 (++)	1.6	2.0	2.5 (+)
10^{-5}	0.7	1.7	2.5 (++)	1.4	1.8	2.3 (+)
10^{-6}	1.0	2.0	2.5 (++)	1.4	1.9	2.4 (+)
Untreated	0.8	1.9	2.5 (+)	1.6	1.8	2.2 (+)

a) and b) were mycelial growth intensity at 96 hr- and 60 hr-incubations, respectively. A rating of (+) denotes mycelial mat was more transparent than a rating of (++).

the mycelial growth of the pathogen in the plant, suggesting no direct stimulatory effect on mycelial growth *in vivo*.

Phytotoxic effects of ether or metabolic inhibitors Ether treatment for inducing water-soaked lesions should be done in the absence of any significant levels of phytotoxicity. Ether-treated hypocotyls showed amorphous water-soaked phytotoxic symptoms with a pale yellow color. But hypoctoyl normal color returned within 6 hours even when treated for 2 minutes only under the laboratory condition (23–26°C). No effects of ether on the growth of the seedling were observed. The length of the treatment was, therefore, determined within 2 minutes, since no fungal penetration into the hypocotyl is recognized up to 8 hours after inoculation[18]. One hour was sufficient to eliminate the remaining ether from the hypocotyls by evaporation in a well ventilated room (23–26°C).

Metabolic inhibitor treatments for inducing water-soaked lesions should also be done in the absence of any siginificant levels of phytotoxicity. Phytotoxicity induced by NaDEDC was as follows: depressed top and root extension growth of the seedlings, slight chlorosis of the cotyledons, reduction in lateral root size and slight yellowed or soft rotted tip. The degree of phytotoxicity were graded as shown in Table 5. The phytotoxic symptoms produced by different metabolic inhibitors were essentially the same as those produced by NaDEDC.

Water-soaked and spreading lesions on hypocotyls pre-treated with ether or metabolic inhibitors Five different common types of symptom which were expressing the degrees of water-soaking

Table 5. Indices for the degree of phytotoxicity by various metabolic inhibitors

Index	Phytotoxic symptoms
###	Root etiolated, rotted and stunted; lateral roots considerably poor; top wilted.
##	Root etiolated and rotted; lateral roots poor; top water-soaked or oil immersed, stunted.
#	Roots scanty; lateral roots stunted; top slightly stunted.
+	Lateral roots stunted or root tip slightly enlarged.
−	No visible phytotoxic symptoms

Table 6. Symptoms in resistant solution-grown squash seedlings treated with ethyl ether

Duration (sec.) of treatment	No. of hypocotyls used	No. of seedlings exhibiting symptoms[a]					
		1	2	3	4	5	6
0	3	0	0	3	0	0	0
20	3	0	0	0	3	0	0
40	3	0	0	0	3	0	0
60	3	0	0	0	2	1	0
80	3	0	0	0	2	1	0
100	3	0	0	0	1	2	0
120	3	0	0	0	2	1	0
140	3	0	0	0	2	1	0
160	3	0	0	0	3	0	0
180	3	0	0	0	2	1	0

[a] Numbers (1–6) denote 6 common types of symptoms as shown in Fig. 1.

Table 7. Symptoms in solution-grown resistant squash Amakuri seedlings treated with various metabolic inhibitors

Inhibitor	Conc (M)	No. of seedlings exhibiting symptoms[a]						Phyto-toxicity[b]
		1	2	3	4	5	6	
L-Ascorbic acid	10^{-3}	0	0	0	1	3	0	+
	10^{-4}	0	0	2	0	2	0	−
	10^{-5}	0	0	1	1	2	0	−
Cupric sulfate	10^{-3}	3	0	1	0	0	0	++
	10^{-4}	3	0	1	0	0	0	++
	10^{-5}	0	0	1	0	2	1	+
2, 4-Dinitrophenol	10^{-4}	0	0	0	2	2	0	+++
	10^{-5}	0	0	1	3	0	0	+
	10^{-6}	0	0	3	1	0	0	−
α, α'-Dipyridyl	10^{-3}	0	0	0	0	0	4	+++
	10^{-4}	0	0	0	0	3	1	++
	10^{-5}	0	0	2	1	1	0	−
Ethyl ether	0[c]	0	0	3	0	0	0	−
	20	0	0	0	3	0	0	−
	40	0	0	0	3	0	0	−
	60	0	0	0	2	1	0	−
Malonic acid	10^{-3}	0	0	0	2	2	0	++
	10^{-4}	0	0	1	2	1	0	+
	10^{-5}	0	0	3	1	0	0	−
Phenylthiourea	10^{-3}	0	0	1	1	1	1	+
	10^{-4}	0	0	1	1	2	0	−
	10^{-5}	0	0	1	1	2	0	−
Potassium cyanide	10^{-3}	0	0	0	2	0	2	+++
	10^{-4}	0	0	2	1	0	0	+
	10^{-5}	0	1	2	1	0	0	−
Sodium bisulfate	10^{-3}	0	0	0	1	2	1	++
	10^{-4}	0	0	1	1	2	0	+
	10^{-5}	0	0	1	1	2	0	−
Sodium diethyl dithiocarbamate	10^{-3}	0	0	0	0	2	2	++
	10^{-4}	0	0	0	2	2	0	−
	10^{-5}	0	0	1	3	0	0	−
Sodium fluoride	10^{-3}	0	0	0	1	3	0	+
	10^{-4}	0	0	0	1	2	1	−
	10^{-5}	0	0	1	2	1	0	−
Sodium monofluoro-acetate	10^{-3}	0	0	0	3	1	0	++
	10^{-4}	0	0	2	1	1	0	+
	10^{-5}	0	1	1	2	0	0	−
Thiourea	10^{-3}	0	0	0	2	2	0	−
	10^{-4}	0	0	1	1	2	0	−
	10^{-5}	0	0	2	0	2	0	−
Urethane	10^{-3}	0	0	2	1	1	0	+
	10^{-4}	0	0	1	1	2	0	−
	10^{-5}	0	0	1	0	3	0	−
Untreated		0	0	3	1	0	0	−

[a] and [b] were determined the same as those described in Fig. 1 and Table 5, respectively.
[c] duration (sec.) of treatment.

and spreading appeared in fresh hypocotyls when treated with ether or any of the metabolic inhibitors used (Fig. 1). Chlorotic fleck consisted of a few pale yellow fleck (less than 1 mm in diam.) in the invaded areas seems to be the first symptom. Browning spot was scattered more or less at random, or usually a chain-like reddish brown discolorations. Browning spot with water-soaking has a yellowish green border. Spreading water-soaked lesion has spreading amorphous greenish symptom enlarged on both sides of the browning spot. Damping-off was seen on the hypocotyls which were much thinner and softer than the upper uninvaded portion.

When the seedlings were treated with ether for 20–120 seconds, water-soaked lesions appeared without any phytotoxicity (Table 6). Taking NaDEDC as one of the metabolic inhibitors, the length of treatment as well as the concentrations required for the appearance of water-soaked lesions were studied. Twenty-four hours treatment with 10^{-4} M NaDEDC was enough to obtain water-soaked lesions. Seedlings were, then, treated equally for 24 hours with 13 metabolic inhibitors at the concentrations from 10^{-3} to 10^{-6} M and the appearance of water-soaked lesions was compared (Table 7). Water-soaked lesions were induced by L-ascorbic acid, malonic acid, potassium cyanide and sodium bisulfate at lesser concentration of 10^{-3} M. Sodium fluoride, 2, 4-dinitrophenol, α, α'-dipyridyl and NaDEDC induced water-soaked lesions at the concentrations from 10^{-3} to 10^{-5} M. Among the metabolic inhibitors which induced water-soaked lesions, only NaDEDC and sodium fluoride were considered good because of the absence of phytotoxicity and stimulative effects on the fungal growth. Besides, the fate of NaDEDC in plants has been studied intensively[2] although its biological activity is not yet elucidated[13].

Since the cuttings were unable to be used in the present experiment, the injection of NaDEDC to the hypocotyls was, then, performed at different times before and after inoculation. The water-soaked lesions appeared when the seedlings were injected 5–27 hours before or immediately after inoculation. With this method, a little more pronounced water-soaking of the symptoms in the seedlings was observed. The results obtained, however, were inconsistent to some extent. Thus, the water-culture is more desirable treatment method than the injection in obtaining plant materials with increased host susceptibility.

Attempts to compare the formation of a heat-liable antifungal substance (s) both in the tissues adjacent to water-soaked lesions and in those adjacent to browning spots by the method described in previous paper[4] were unsuccessful. This should, however, be analyzed, together with the specific role of pathogenic factors of the fungus in water-soaked lesions, before water-soaked lesions in relation to increase in host susceptibility could be properly evaluated.

Acknowledgement

The authors are indebted to Professor R. V. Alicbusan of the Department of Plant Pathology, University of the Philippines, for his helpful suggestions and critical reading of the manuscript. Technical assistance of M. Ikegami is also gratefully acknowledged.

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和文摘要

土壌病菌 *Pythium* の生態および分類

第XI報　ジエチルジチオカルバミド酸ナトリウム処理をした カボチャ胚軸部に現われる水浸状病斑

一谷多喜郎・米谷冨男・高橋　実

人工気象室において甘栗カボチャ幼苗を Hoagland 液で水耕栽培し，根部にジエチルジチオカルバミド酸ナトリウム (NaDEDC) 処理を行ない，*Pythium ultimum* (77 号菌) を胚軸に接種して出現する病徴を記載した。

幼苗は若干徒長したが，接種によりガラス室のポット栽培と類似の褐色病斑を胚軸上に形成した。幼苗の cutting は接種後ほとんどすべて水浸状病斑を形成した。幼苗の胚軸の上端部から髄腔に NaDEDC の注入処理を行なうと，水浸状拡大病斑の顕著な形成がみられたが，結果は不規則であった。Hoagland 液および処理時間内に根から胚軸に吸収された NaDEDC は，ともに供試菌の菌糸発育にまったく影響しなかった。10^{-4} M NaDEDC の根部処理は幼苗に薬害を与えることなく，胚軸上に一様な水浸状拡大病斑の形成を促進した。上記および既報の結果から，NaDEDC の根部処理による水浸状拡大病斑の形成は寄主感受性の増大にもとづくものと考えられる。

Fig. 1. Common types of symptoms of NaDEDC treated, inoculated Amakuri squash hypocotyls.

1. Symptomless
2. Chlorotic fleck (arrows)
3. Browning spot
4. Browning spot with water-soaking
5. Spreading water-soaked lesion
6. Damping-off

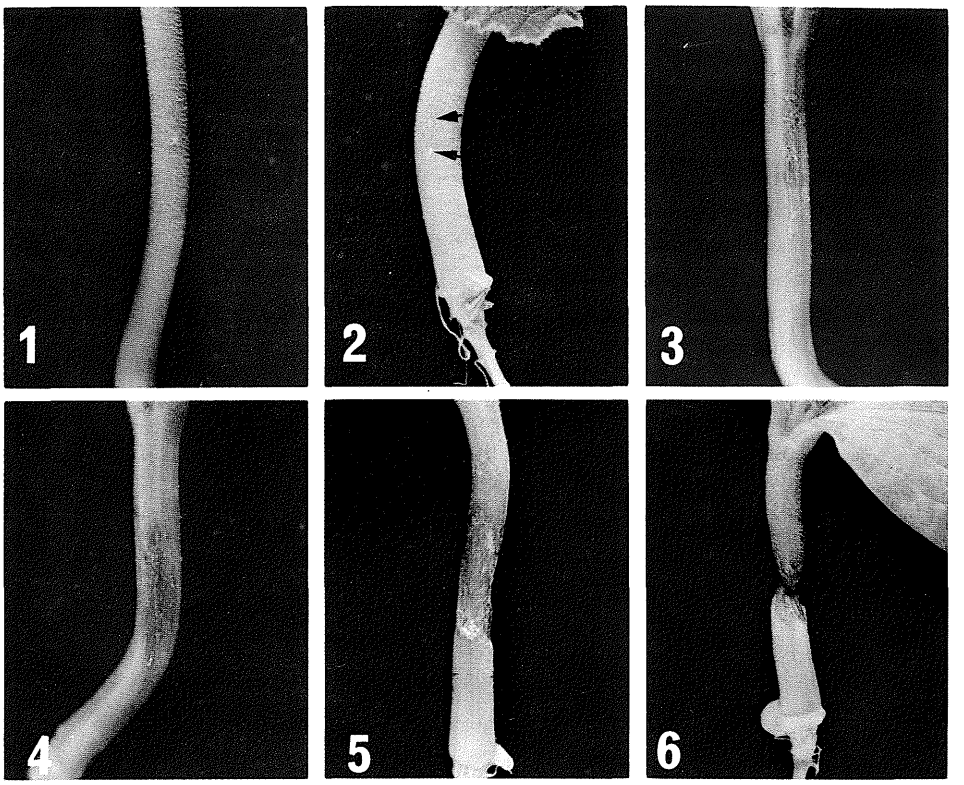

Fig. 1.