土壌環境とホウレンソウ根腐病発生の関係(1):

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
著者	赤司, 和隆 前田, 要 生越, 明
巻/号	53巻3号
掲載ページ	p. 323-328
発行年月	1987年7月

日植病報 53：323-328 (1987)
Ann. Phytopath. Soc. Japan 53：323-328 (1987)

Soil Environment in Relation to the Incidence of Aphanomyces Root Rot of Spinach

(Ⅰ) Effect of Nitrate Nitrogen on Disease Outbreak*

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Abstract

It was recognized from surveys in 30 fields around Sapporo city that soil pH values were neutral (6.5-7.5) and nitrate nitrogen was deficient (less than 50 ppm) in fields where Aphanomyces root rot of spinach was severe.

The effects of applied nitrate nitrogen and decreased pH on the root rot severity were examined. Disease severity was remarkably decreased at more than ca. 200 ppm of nitrate nitrogen in soil solution. The effect of soil pH on the severity was not significant at high inoculum potential. It was elucidated from these results that nitrate nitrogen is more closely related to the outbreak than soil pH, and the severe outbreak occurs when nitrate nitrogen is deficient.

(Received January 16, 1987)

Key words: Aphanomyces root rot, spinach, nitrate nitrogen, soil pH.

Introduction

Spinach is well known as a host of *Aphanomyces cochlioides* Drechsler[13]. In Japan, the outbreak of spinach root rot caused by *A. cochlioides* was first observed in Tochigi Prefecture[11]. The occurrence of this root disease in Hokkaido Prefecture has been discussed in the previous report[1]. The summary is as follows: Outbreaks of Aphanomyces root rot are frequently observed in summer cropping (July-August). The hypocotyl of diseased plant in cotyledonary stage is water-soaked and browned.

This symptom is extremely difficult to distinguish from that produced by *Pythium* spp.. In middle growing stage, the tap root may be broken off from the part at soil surface due to high wind. This phenomenon is therefore called "Kamairazu" in cropping area which can be literally translated as "able to harvest without a sickle". There are many reports about the effect of soil environment on the outbreak of root rot caused by *A. euteiches*[2,9,14,15], however, there are few reports about *A. cochlioides*, especially in Japan. In this paper, we intend to clarify the chemical characteristics of soil in fields where Aphanomyces root rot is severe.

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* A preliminary report of this study was presented in the Annual Meeting of the Hokkaido Division of the Phytopathological Society of Japan (November, 1985).
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Materials and Methods

Surveys. Research on disease incidence, pathogen and chemical characteristics of soil has been carried out in 30 fields in the Ariake area around Sapporo city during the period 1984-1985. This area of brown lowland soil, a sandy loam, is the largest spinach growing area in Hokkaido Prefecture. Forty and twenty samples of diseased plants and soil, respectively, were collected from the same 20 points of each field.

Root segments, 3-5 mm in length, were placed in a 9 cm Petri plate containing 10 ml of sterilized water at 25 C for one day.

After microscopic observation of primary zoospore cysts, some of the segments were put on Petri plates of corn meal agar (Difco's CMA) with a Van Tieghem cell. The mycelia which appeared after incubation at 25 C, were immediately transferred. Soil samples were analyzed for nitrate nitrogen, available phosphorus and exchangeable bases, and were measured for pH and EC (electric conductivity).

Treatment of infested soil. Pot tests were undertaken in greenhouse at 25 C for 10 days in order to clarify effects of soil pH and nitrate nitrogen on disease severity.

The infested soil used for tests was collected from a field where Aphanomyces root rot was most severe (A-field). The chemical analyses were as follows: pH; 7.3 (in water), EC; 0.009 mS/cm (1 : 5 water), nitrate nitrogen; 19 ppm, available phosphorus (Truog-P_2O_5); 721 ppm, exchangeable bases, K; 1.6 me, Ca; 11.3 me, Mg; 2.3 me/100 g. The pH values of pH-series without nitrogen application were adjusted with sulfuric acid (0.3 N and 0.4 N) to levels of 6.0 and 5.5. In N-series without the decrease in pH, nitrogen, as $Ca(NO_3)_2 \cdot 4H_2O$, was applied in the form of liquid fertilizer at three levels; 0, 15 and 30 mg per 100 g soil.

Ten seeds of spinach (cv. Sun light) were sown to pots packed with 500 g air-dry soil treated as mentioned above. Three pots were used for each treatment.

Inoculation tests. Pot tests were undertaken to determine the effects of soil pH and nitrate on disease severity. Two series of greenhouse tests were conducted at 25 C for 10-30 days. The soil used for N-series was brown lowland soil with clay loam texture from Hokkaido Prefectural Central Agricultural Experiment Station. The results of chemical analyses were as follows: pH; 6.2 (in water), nitrate nitrogen; 16 ppm, exchangeable bases, K; 0.7 me, Ca; 9.3 me, Mg; 5.4 me/100 g.

Nitrogen, as $Ca(NO_3)_2 \cdot 4H_2O$, was applied in the form of liquid fertilizer to each pot containing 500 g of sterilized, air-dry soil at four levels: 0 (deficient), 200 (standard), 400 (upper limit), 800 (excess) ppm. The change of soil pH based on this treatment was scarcely recognizable. Before inoculation, the concentration of nitrate nitrogen in soil solution at maximum water capacity was analyzed by the centrifuge method.

The soil used for pH-series was the same as for the N-series, and was adjusted with calcium carbonate to pH levels of 6.2, 6.5, 7.0 and 7.5 two years before. The soil used for 5.5 level was from a field on the experiment station where chinese cabbage had been cultivated four times without lime application. The results of analyses were as follows: pH; 5.5, nitrate nitrogen; 5 ppm, exchangeable bases, K; 0.6 me, Ca; 6.5 me, Mg; 5.4 me/100 g. Nitrogen was not applied in this series.

The isolate of *A. cochlioides* used for tests was A-K-1 as described in previous paper[1]. Three methods were adopted for inoculations, i. e., zoospores (10 or 10^3/g soil), oospores (20 or 200) and mycelia (per pot) grown at 25 C for 7 days on CMA in a 9 cm Petri plate. Zoospores were produced from mycelia grown in a 0.3% peptone solution according to Herr's method[8]. Oospores were obtained from colonies containing oospores grown for 3 weeks on Difco's CMA by drying mycelia at room temperature for 10 hours on a clean bench. The colonies were obtained by scraping up from the surface of the culture. Inoculations were made to cotyledons of 15 plants per pot. Four pots were used for each treatment.

Results and Discussion

Chemical characteristics of soil from diseased fields

Most of *Aphanomyces* isolates were identified as *A. cochlioides* based on the morphological observation and pot tests for determining host range[1]. It was observed from surveys that detection frequencies of *A. cochlioides* from diseased spinach were correlated with soil pH (Fig. 1). The frequency was high (20-60%) at fields of which pH values were neutral (6.5-7.5). Soil pH values depended on the amount of nitrate nitrogen rather than that of exchangeable calcium or calcium saturation percentage. The higher the pH values, the less the amount of nitrate nitrogen (Fig. 2). This phenomenon was mainly caused by both nitritification and nitrification of ammonium nitrogen[7]; $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$ + energy, because nitrogen fertilizer containing ammonium

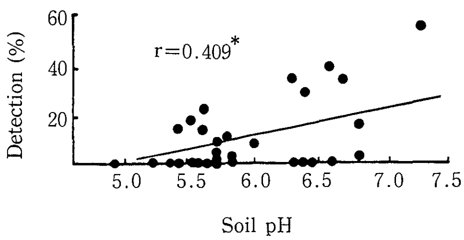

Fig. 1. Correlation between soil pH and detection frequency of *Aphanomyces cochlioides* from spinach seedlings. Detection (%) =No. of *A. cochlioides* detected/No. of root segments tested. The pH is average value of 20 samples per field.
* : significant at P=0.05.
●: the results from 30 fields investigated.

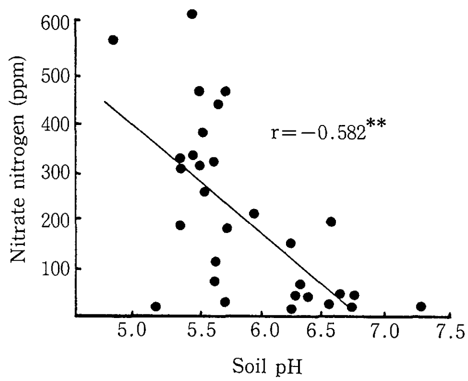

Fig. 2. Correlation between soil pH and the amount of nitrate nitrogen in soil.
** : significant at P=0.01.
●: the results from 30 fields investigated.

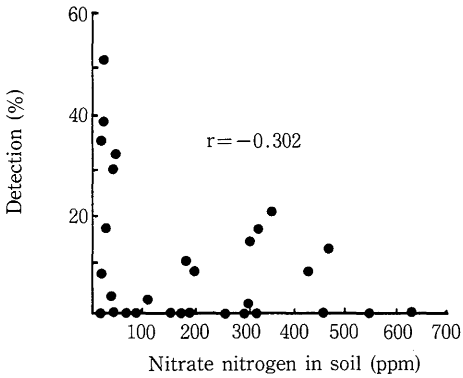

Fig. 3. Correlation between nitrate nitrogen and detection frequency of *A. cochlioides* from spinach seedlings.
●: the results from 30 fields investigated.

nitrogen is often applied in spinach fields. Hashimoto[6] reported that ammonium nitrogen (0.56 g) applied to soil (4 kg) rich in calcium reduced soil pH value by ca. 1.0 due to these oxidations.

It proved accordingly that *A. cochlioides* was frequently detected from fields at deficient level of nitrate nitrogen (Fig. 3, less than 50 ppm) and at neutral level of soil pH. Besides, the EC values which are correlated with the amount of nitrate nitrogen, were low in fields with deficient levels of nitrate nitrogen.

Effects of soil pH and nitrate on disease severity

To elucidate whether pH or nitrate nitrogen is a principal factor in the root rot severity, the effect of the nitrate application and decrease in pH on disease outbreak in the infested soil (A-field) was investigated. As shown in Table 1, severity was more remarkably decreased by nitrate application than by the decrease in pH.

Severity was most remarkably decreased at nitrogen level of 30 mg/100 g than in any other treatments (Table 1). A similar phenomenon was recognized in inoculation tests. In all inoculations, disease outbreak was remarkably reduced at more than ca. 200 ppm of nitrate nitrogen in soil solution (Table 2).

Symptoms produced by inoculations started to appear within ca. 10 and 30 days for zoospores and oospores, respectively. As shown in Table 3, the effect of soil pH on severity was most significant at a level of 5.5 in inoculations of zoospores (10 zoospores /g of soil) and mycelia, while a significant difference was not recognized in high inoculum potential (10^3 zoospores/g of soil). These results obtained from pot tests suggest

Table 1. Effect of nitrate application and soil acidification on Aphanomyces root rot severity of spinach in infested soil[a)]

Soil amendments	pH (in water)	NO_3-N (ppm)	Root rot (%)
Infested soil (check)	7.3	19	84.2
Nitrate application (N : 15mg/100g)	7.1	169[b)]	38.3
Nitrate application (N : 30mg/100g)	7.2	319[b)]	12.5
Decrease in pH (6.0)	5.8	19	73.0
Decrease in pH (5.5)	5.5	19	55.1

a) The soil from A-field.
b) The calculated value : the applied nitrogen+the amount of nitrogen of check.

Table 2. Effect of nitrate in soil on Aphanomyces root rot severity of spinach

NO_3-N level		% of diseased plants						Plant height of check[d)] (cm)
mg/100g[a)]	ppm[b)]	Zoospores[c)] (no./g) 10	Zoospores[c)] (no./g) 10^3	Mycelia[c)] (CMA)	Oospores[d)] (no./g) 20	Oospores[d)] (no./g) 200	Check[d)]	
0	14	36.7[a e)]	86.7[a]	31.7[a]	73.3[a]	98.3[a]	0	4.4[a]
20	178	5.0[b]	48.3[b]	5.0[b]	0[b]	5.0[b]	0	8.2[b]
40	236	trace[b]	20.0[c]	trace[b]	0[b]	0[b]	0	7.1[c]
80	650	trace[b]	5.0[d]	trace[b]	0[b]	0[b]	0	5.9[d]

a) The amount of nitrate nitrogen applied.
b) The concentration of nitrate nitrogen in soil solution.
c) Observed 10 days after inoculation.
d) Observed 30 days after inoculation.
e) In each row, means followed by a common letter are not significantly different by the Duncan's new multiple range test ($P<0.01$).

Table 3. Effect of soil pH on Aphanomyces root rot severity of spinach

pH level	% of diseased plants[a)]			
	Zoospores (no./g) 10	Zoospores (no./g) 10^3	Mycelia (CMA)	Check
5.5	16.7[a b)]	73.3[a]	8.3[a]	0
6.2	45.0[b c]	85.0[a]	26.7[b]	0
6.5	46.7[b c]	83.3[a]	33.7[b c]	0
7.0	60.0[b]	83.3[a]	45.0[c]	0
7.5	36.7[c]	76.7[a]	38.3[b c]	0

a) Observed 10 days after inoculation.
b) See legend e) of Table 2.

that nitrate nitrogen is more closely related to disease outbreak than soil pH, and severe outbreak occurs at deficient nitrate nitrogen levels.

Walker and Musbach[17)] reported that nitrogen is more important in reducing pea root rot than phosphorus and potassium, which supports these results. It is also known that the use of various fertilizer to soil with low salt content reduces Aphanomyces root rot of peas[3-5,12,16)] and sugar beet[10)]. However, it is unlikely in spinach fields that phosphorus and potassium deficiency is responsible for the increase in root rot, because excess accumulation of these elements from continuous cropping is a serious problem in spinach fields nowadays. Nitrate deficiency seems to be a principal cause of severe outbreak, because nitrate nitrogen is apt to become the most limiting of all minerals by leaching (sandy soil) or dilution (heavy clay soil), due to rainfall and excess watering.

Apparent growth inhibition was also observed in check (non-inoculation) at a nitrogen level of 0 (Table 2). It is accordingly conceivable that decreasing in resistance caused by deficiency of nitrate nitrogen is responsible for severe disease outbreak. However, it may not be the only explanation, because disease outbreak was remarkably reduced at a nitrogen level of 800 ppm in which growth inhibition based on excess injury was observed (Table 2). Further research is needed to clarify the outbreak mechanisms from aspects of the pathogen behavior, antagonists and host susceptibility.

We would like to thank Mr. E. N. Bassett, Department of plant pathology, Washington State University for reading the manuscript.

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和 文 摘 要

赤司和隆・前田　要・生越　明：土壌環境とホウレンソウ根腐病発生の関係（第1報）発生に及ぼす硝酸態窒素の影響

札幌市近郊のホウレンソウ畑30圃場における実態調査の結果，*Aphanomyces cochlioides* が多く検出される根腐病多発生圃場では，土壌 pH が中性域（6.5-7.5）でかつ硝酸態窒素含量は欠乏域（50 ppm 以下）にあることが認められた。次に，根腐病発生に及ぼす土壌硝酸態窒素並びに土壌 pH の影響をポット試験で検討した。その結果，高い感染ポテンシャル下では pH の影響は判然としなかったのに対し，硝酸態窒素の施用はいずれの接種方法においても発病株率を低下させ，とくに土壌溶液中の硝酸態窒素含量約 200 ppm 以上で著しかった。以上のことから，根腐病の発生には土壌 pH に比べ硝酸態窒素含量がより密接に関与しており，欠乏域で多発生することが判明した。