Ecologic and Taxonomic Studies on Pythium as Pathogenic Soil Fungi : IX. Effect of Inorganic Nitrogen on Vertical Distribution and Pathogenicity of Several Soil-borne Plant Pathogens

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

IX. Effect of Inorganic Nitrogen on Vertical Distribution and Pathogenicity of Several Soil-borne Plant Pathogens

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Introduction

The survival and pathogenicity of the pathogens causing soil-borne diseases are influenced by vertical distirbution of the pathogens or by plant residues, nitrogen and the carbon : nitrogen (C : N) ratio of the soil (1, 2, 3, 5, 6, 7, 8, 9). DURBIN (4) observed that the vertical distribution of *Rhizoctonia solani* on the host plant varies with their clones and that the subterranean-type clones have a higher CO_2 tolerance than the aerial and surface-type clones. He also pointed out that there is correlationship between the habitat of clones and aeration. Nitrate and nitrite have generally been used as substrates in anaerobic respiration of *Escherichia coli*(14), and it is presumed that nitrate might be related to CO_2 tolerance and anaerobic respiration. Since the vertical distribution of *R. solani* in the soil is relatively high(12) nitrate and nitrite supplied may influence considerably its vertical distribution and pathogenicity.

The present paper deals with a comparative study on the effects of forms and amounts of nitrogen on vertical distribution and pathogenicity of soil-borne disease fungi such as *R. solani*, *Pythium aphanidermatum* and *Sclerotium rolfsii*.

Materials and Methods

Along with *R. solani*, *P. aphanidermatum* and *S. rolfsii* which were used in previous paper(12), different isolates of *R. solani* which causes damping-off of pine, carnation, radish, cauliflower, chrysanthemum, and sugar beet, potato scab, and *Pellicularia sasakii* which causes sheath blight of rice were used in this experiment.

The vertical distribution and pathogenicity were determined by the agar coated contact slide technique and indicator plant method(12). A sterilized soil was placed in a Wagner pot and mixed thoroughly after adding 100 g of soil infested with the pathogens and 10 g of nitrogen. The agar coated slide was buried vertically and the remaining 10 g of nitrogen dissolved in a liter of water was poured. To remove entrapped air from the soil 2 liters of water was added in each pot. The excess water was allowed to flow before the drainage hole was plugged. The buried slide was removed after 6 days and mycelial growth intensity was determined by examining 100 microscopic fields with 150 total magnification(12). The effects of different concentrations of nitrate and nitrite nitrogens on mycelial growth in the soil were determined by mixing the entire quantity of nitrogen source and infested soil with sterilized soil. The resulting soil was placed in a pot. After giving 3 liters of tap water, the experiment was followed by the above-mentioned procedures. The effects of nitrogen forms on pathogenicity was determined by introducing 20 g of infested soil into a depth of 5 cm

from the soil surface in the Wagner pot then covered with sterilized soil. In the case of *S. rolfsii* 30 g of infested soil was introduced on the surface of soil. After giving 2 liters of tap water, 10 g of nitrogen dissolved in a liter of tap water was poured. The excess water was removed and the pot drainage was plugged. Thirty-five cucumber seeds were sown on each pot and pathogenicity was determined by taking the number of healthy seedlings. Soy bean instead of cucumber was used for *S. rolfsii*. Mycelial growth intensity at different depths was determined by the use of agar coated buried slide technique examined after 5 days.

Results

1. Effects of nitrogen on vertical distribution of *R. solani*

It has been shown that *R. solani* utilizes specifically nitrite in vitro as compared with *P. aphanidermatum* and *S. rolfsii*. Nitrate and nitrite nitrogen are utilized by the bacteria and fungi as substrates of reductive enzymes in anaerobic respiration, fermentation and assimulation(14). Since it has been considered that the presence or absence of nitrogen source and the forms of nitrogen affect the survival of *R. solani* in the soil, the effect of $(NH_4)_2SO_4$, KNO_3 and KNO_2 on the vertical distribution of *R. solani* was determined by means of the buried slide technique (Fig. 1).

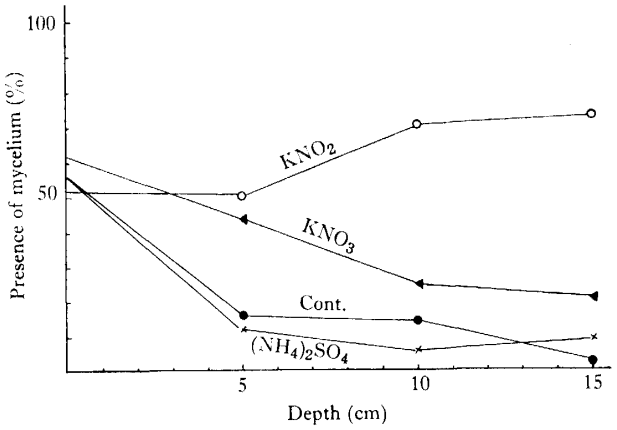

Fig. 1. Effect on the vertical distribution of *R. solani* of inorganic nitrogen added in the soil.

The mycelial growth intensity and vertical distribution of *R. solani* in the soil were generally favored by the addition of nitrate and nitrite, and no effect was observed with ammonium nitrogen. It may be considered that the vertical distribution of *R. solani* is largely affected by oxidized nitrogen and its growth is dependent on anaerobic respiration.

2. Effects of amounts of KNO_3 and KNO_2 on vertical distribution of *R. solani*

At the rate of 5, 10 20, and 40 grams of KNO_3 or KNO_2 added to the soil mycelial growth intensity of *R. solani* was determined.

Similar favorable increase in mycelial growth intensity and vertical distribution was observed at different depths of the soil when 5 to 40 g of KNO_3 was added (Fig. 2). With KNO_2 at the rate ranging from 5 to 20 g the same vertical distribution was observed but at the rate of 40 g the growth was suppressed at different depths indicating the inhibition of the mycelial growth by the excess supply of the chemical (Fig. 3). From the results obtained it showed that mycelial intensity and vertical distribution of *R. solani* were affected by the forms and amounts of nitrogen added.

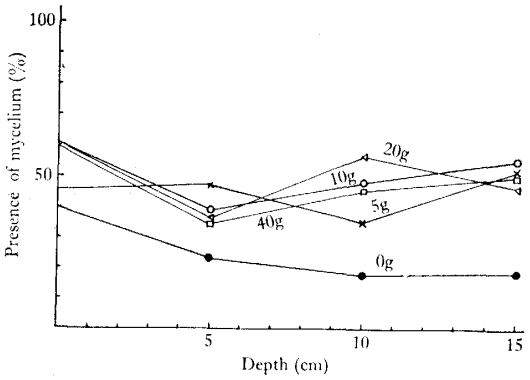

Fig. 2. Effect on the vertical distribution of *R. solani* of the amounts of potassium nitrate added in the soil.

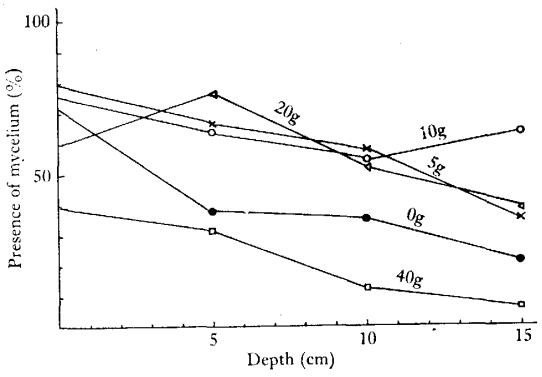

Fig. 3. Effect on the vertical distribution of *R. solani* of the amounts of potassium nitrite added in the soil.

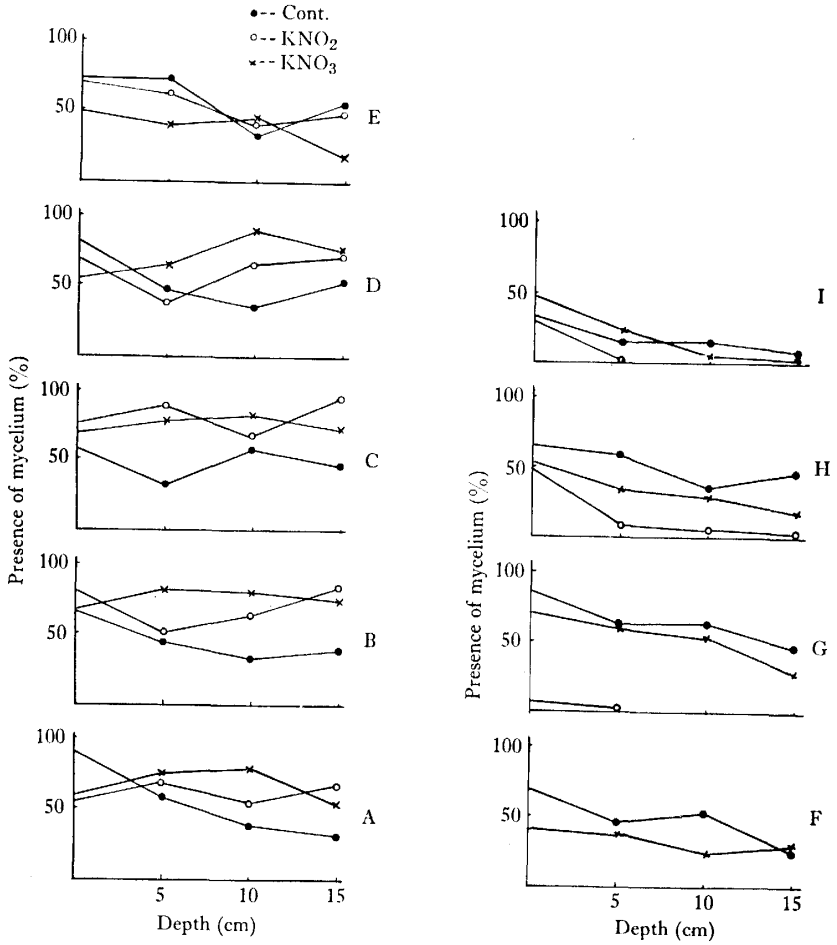

Fig. 4. Effect on the vertical distribution of different isolates of *R. solani* of inorganic nitrogen added in the soil.
Isolates from (A) broad bean, (B) pine seedling,
(C) carnation, (D) radish, (E) rice plant,
(F) cauliflower, (G) chrysanthemum,
(H) potato, and (I) sugar beet.

3. Vertical distribution of different isolates of *R. solani*

Since the vertical distribution of *R. solani* is influenced by the supply of nitrate or nitrite the effect was determined using eight isolates of *R. solani* together with *P. sasakii* (Fig. 4).

Twenty grams of nitrogen was added. The supply of KNO_3 and KNO_2 increased mycelial growth intensity and vertical distribution of the isolates from broad bean, pine, carnation and radish seedlings. The supply of KNO_2 inhibited remarkably or completely the mycelial growth of the isolates from cauliflower, chrysanthemum, potato and sugar beet, and the supply of KNO_3 decreased very significantly their mycelial intensity. The mycelial growth of *P. sasakii* occurring in aerial and surface environment was not affected but inhibited in deeper layer of the soil. Thus, the vertical distribution of *R. solani* in the soil may be affected by the presence or absence of KNO_3 or KNO_2 and the amounts added.

4. Relation of nitrogen forms to mycelial extension and pathogenicity in the soil

The vertical distribution as well as mycelial growth intensity of *R. solani* in the soil was promoted by supplying nitrogen. The effects of nitrogen on mycelial extension and pathogenicity in the soil was determined using *R. solani, P. aphanidermatum* and *S. rolfsii.*

Five different kinds of nitrogen such as KNO_3, KNO_2, $(NH_4)_2SO_4$, NH_4NO_3, NH_2-$CONH_2$ were used. Pathogenicity was determined by the percentage of healthy indicator plant seedlings and mycelial extension was determined by the agar coated contact slide technique (Table 1, Figs. 5, 6, 7).

Table 1. The effect of inorganic nitrogen on the pathogenicity of 3 pathogenic fungi

Fungi	Days after inoculation	KNO_3	KNO_2	$(NH_4)_2SO_4$	NH_4NO_3	$\begin{matrix}NH_2\\CO\\NH_2\end{matrix}$	Non-Nitrogen
R. solani	3	56	44	17	80	64	92
	5	16	12	0	10	2	14
	7	0	0	0	0	0	0
P. aphanidermatum	7	92	47[a]	24	72	5[a]	52
	9	72	55	1	26	6	48
	14	52	8[b]	0	2	0	48
S. rolfsii	7	0	0[a]	0	0	0	0

Data show adjusted ratio of healthy plants.
a : Germination and growth of test plant were inhibited by the addition of nitrite.
b: Number decreased as plants were attacked by higher growth intensity of the mycelium.

As shown in Table 1, the disease incidence incited by *R. solani* was considerably increased when nitrogen was added. Three days after inoculation the degree of infection was observed to be five times more serious than the control when $(NH_4)_2SO_4$ was supplied and all seedlings were infected 5 days after inoculation. All other seedlings in the experimental blocks supplied with 5 different kinds of nitrogen got infected after 7 days.

The disease incidence caused by *P. aphanidermatum* was significantly increased by the addition of $(NH_4)_2SO_4$ and also influenced by NH_4NO_3. Nitrate nitrogen only retarded the disease development because the same degree of severity was observed in the non-nitrogen control treatment 14 days after inoculation. Nitrite and urea nitrogen reduced the germinability of the seeds retarded the growth of the test plants. The few seedlings got infected 14 days after inoculation. Thus, the effect of ammonium and nitrate nitrogen on the disease incidence caused by *P. aphanidermatum* were similar to that caused by *R. solani.* Nitrite and

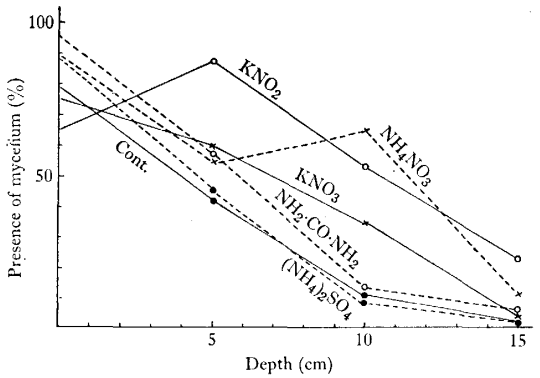

Fig. 5. Effect on the vertical distribution of *R. solani* of inorganic nitrogen added in the soil which was planted with cucumber seeds.

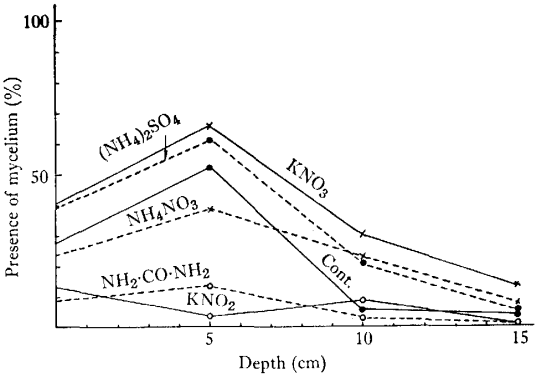

Fig. 6. Effect on the vertical distribution of *P. aphanidermatum* of inorganic nitrogen added in the soil which was planted with cucumber seeds.

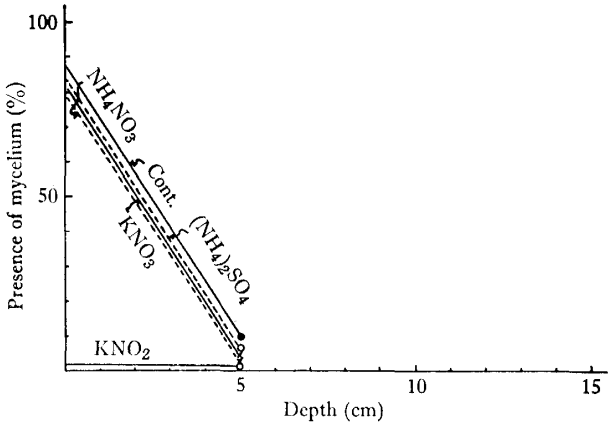

Fig. 7. Effect on the vertical distribution of *S. rolfsii* of inorganic nitrogen added in the soil which was planted with cucumber seeds.

urea nitrogen, however, favored the outbreak of the disease caused by *R. solani*.

Both mycelial extension of *S. rolfsii* and germination of soy bean were inhibited by the application of nitrite. No difference was observed among the different forms of nitrogen sources on the disease incidence since there was a uniform severity in all treatments.

Mycelial extension of *R. solani* was evident as shown in Fig. 5 especially higher in the upper layer of soil when ammonium and urea nitrogen were added. The fact that mycelium is abundant towards the upper layer of the soil, therefore, indicates a good correlation between mycelial extension and pathogenicity.

When nitrate and nitrite nitrogen such as KNO_3, KNO_2 and NH_4NO_3 were supplied, mycelial growth intensity of *R. solani* was significantly higher even at a depth of 10 cm and downward mycelial extension in the soil layer was promoted indicating broad vertical distribution of the fungus.

All nitrogen except KNO_2 and NH_2CONH_2 increased mycelial extension of *P. aphanidermatum* at a depth of 5 cm and did not promote it at the upper layer of the soil unlike *R. solani* (Fig. 6). Potassium nitrate and ammonium sulphate stimulated mycelial growth at a depth of 5 cm similar to non-nitrogen control. The fact that KNO_3, $(NH_4)_2SO_4$ and NH_4NO_3 stimulated mycelial growth at a depth of 10 cm indicates slight promotion of mycelial extension towards lower layer of the soil. Mycelial growth was suppressed by KNO_2

and NH_2CONH_2 at all levels. *P. aphnidermatum* differs from *R. solani* by the fact that the mycelial growth of the former was suppressed by nitrite nitrogen whereas the latter was stimulated.

The mycelial growth of *S. rolfsii* was equally unaffected by all nitrogen except that it was completely suppressed by KNO_2. Abundant mycelium was observed only on soil surface and scarcely observed in the lower layer of the soil (Fig. 7).

Discussion

There are many reports regarding changes of saprophytic activity and pathogenicity of soil pathogenic fungi by an application of inorganic nitrogenous fertilizers and organic matter such as plant residues, barnyard and green manures. PAPAVIZAS *et al.* (8) claimed that enrichment of the substrate with nitrogen increased saprophytic activity of *R. solani*. Disease severity of *Rhizoctonia* brown patch in turfgrasses was much greater at high nitrogen level than at normal or low levels and low nitrogen gave significantly less disease than a normal level(2). Increase in soil nitrogen enhanced pathogenicity of *R. solani* to kidney bean(1). On the other hand, SANFORD(9) pointed out that an increase in nitrogen promotes fungal saprophytic growth and reduces pathogenicity. This is similar to the present result because KNO_3 and KNO_2 enhances mycelial growth with lesser pathogenicity. It was observed that there was a decreased growth as well as low microbial population when inorganic fertilizers such as NH_4NO_3 or $(NH_4)_2SO_4$ was added. When organic matter such as bean cake or manure was added the growth was inhibited but microbial population increased(7). The growth of *R. solani* in the soil was suppressed with the addition of mature organic amendments such as soybeans and corn. The supplementary nitrogen added appears to favor the increase in numbers of fungi, streptomycetes and bacteria in the soil, partially to favor the C:N ratio of the amendment(3). UI(15) emphasizes organic matter utilization of *R. solani* in the soil and claims that although generally considered as soil inhabitant it also requires plant roots or plant residues. There are considerable works conducted to determine the growth and pathogenicity of *Rhizoctonia* in the soil but limited experiments were done on its vertical distribution in the soil. The present experiment showed that the different forms of nitrogen influenced not only the growth in the soil and its vertical distribution but also pathogenicity.

A fungus basically needs organic matter and the *Phycomycetes* such as *Pythium* which can utilize low molecular sugar will be the first group to dominate as the organic residues started to decompose followed by the more saprophytic groups like, in the order of occurrence, the *Fungi Imperfecti*, the *Ascomycetes*, the *Basidiomycetes*(11). This was proved partially by the fact that when the crops were planted just after green manure was applied the damping-off disease on barley or kidney bean will occur since *Pythium* will start first to grow saprophytically(10).

Regarding the diseases caused by *S. rolfsii*, the writers have been found that organic matter is important in the incidence of the disease. The disease can develope in the soil mixed with the inoculum containing immature organic matter such as wheat-bran or barley grains, but not in the soil which contained completely decomposed organic matter(13). WATANABE(16) also pointed out that a fungus needs organic matter for its pathogenic activity. There was, however, no study regarding the effect of inorganic nitrogen on vertical distribution and pathogenicity of the fungus in the soil.

IWASE(5) reported that the incidence of *Sclerotinia* disease of rape varies depending on the time of applying fertilizers. The present paper shows that the incubation period in damping-off diseases caused by *R. solani* or *P. aphanidermatum* was also shortened when nitrogen especially $(NH_4)_2SO_4$ was applied. The supply of nitrogen did not only induced

the severity of the disease but also increased the pathogenicity. Thus, manuring for each crop could be performed provided that the forms of nitrogen needed by the crop and the pathogens should be given due consideration in order to control damping-off and root rot. Ammonium nitrogen enhances the incidence of damping-off on soil surface. Nitrate and nitrite nitrogen promote the mycelial growth of *R. solani* towards the deeper layer of the soil and nitrate nitrogen gave the same effect on the growth of *P. aphanidermatum.* Thus, the possibilities to inducing root rot disease cannot be overlooked. The forms of fertilizers should also be given due in consideration in view of inactivation by adsorption which influences their mobility in the soil.

Summary

1. The present paper deals with the comparative study on the effects of inorganic nitrogen on vertical distribution and pathogenicity of soil-borne disease fungi such as *Pythium aphanidermatum, Rhizoctonia solani* and *Sclerotium rolfsii,* and on the effects of nitrate and nitrite on the vertical distribution in the soil of different isolates of *R. solani.*

2. The mycelial growth intensity of *R. solani* at different depths of soil was enhanced by addition of KNO_3 and KNO_2 unlike when $(NH_4)_2SO_4$ was added.

Similar enhancement of mycelial intensity and vertical distribution at different depths of the soil when 5 to 40 g of KNO_3 was added. Both mycelial growth intensity and vertical distribution have shown similar curves to those obtained with KNO_3 when 5 to 20 g of KNO_2 was added. When 40 g of KNO_2 was added the growth of the fungus was suppressed probably due to the excess supply of KNO_2.

3. The addition of KNO_3 and KNO_2 increased mycelial growth intensity of subterranean-type of *Rhizoctonia* in the deeper layer of the soil but had no effect on *P. sasakii* occurring in aerial and surface environment.

4. The early stage of disease development caused by *R. solani* was significantly promoted and acute symptom of damping-off appeared when any form of nitrogen was supplied. Disease development was the most severe when ammonium sulphate was added. Ammonium and urea nitrogen stimulated mycelial extension towards the upper layer of the soil while the KNO_3 and KNO_2 promoted the downward growth in the deeper layer.

5. The disease incidence caused by *P. aphanidermatum* was enhanced by the application of ammonium or nitrate nitrogen. When KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$ were added, mycelial intensity was remarkably increased in the deeper layer of the soil but it was inhibited by KNO_2 and NH_2CONH_2.

6. The mycelial growth and pathogenicity of *S. rolfsii* were equally unaffected by all nitrogen forms except KNO_2. Abundant mycelium was observed only on soil surface and scarcely observed in the lower layer of the soil.

Acknowledgement

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