Pyrenochaeta sp. as One of the Causal Agents of Upland Rice Soil Sickness

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The soil sickness of upland rice that occurs when the plant is continuously cropped on the same field year after year is a serious problem in the intensive agriculture. Even when water, nutrients, weeds, pests and soil pH are adequately controlled, maintaining the production of continuously cropped upland rice at the highest level is very difficult due to the soil sickness. Symptoms of upland rice soil sickness are reduction of growth and yield, and browning of roots. In most cases in Japan, where the plant is cultivated once a year from May to October, the yield of rice after 3 or 4 successive crops, decreases to as low as 50–70% of that of the control plant, not continuously cropped, although no further reduction in yield is observed by a further continuation of successive cropping. Many investigators have studied the cause of upland rice soil sickness and agreed that it is not caused by a single agent, but by multiple agents such as phytotoxins excreted by the plant, pathogenic nematodes and fungi as reviewed recently by the authors[8].

Change of inhibitory action of root residues

Since upland rice contains toxic substances in roots and excretes some of them into a surrounding medium, a possibility has been proposed that they may take a direct part in the appearance of the soil sickness. To elucidate this possibility the authors conducted the following experiments: roots, which are the origin of phytotoxins in the soil, were left as stubbles in the field after harvest and a water-extract of the root residues was prepared so as to test aseptically its inhibitory action on the growth of germinating seedlings of upland rice. As Fig. 1 shows, the inhibitory action of the membrane-filtered water-extract

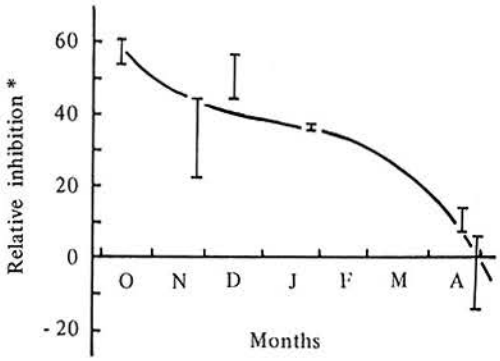

Fig. 1. Inhibition of root-elongation of germinating seedlings of upland rice by the water-extract of the root residues left in the field[5]

A water-extract of root residues was prepared by homogenizing 2.0 g fresh weight of root residues with 15 ml of distilled water and then membrane-filtered.

* As expressed by $\frac{w-a}{w} \times 100$

where w = root length of seedlings germinated in distilled water, and a = that in the extract

Pyrenochaeta sp. was identified, after the completion of this manuscript, as *Phoma terrestris* Hansen, commonly known as *Pyrenochaeta terrestris* (Hansen) Gorenz et al. by Dr.. G. H Boerema, Plantenziektenkundige Dienst, Wageningen, Netherlands, to whom the authors wish to express their sincere thanks.

from root residues stayed relatively high from autumn to winter, when the soil temperature was very low, but diminished in spring just before the next sowing. This result suggests that the root residues left in the field until spring may not contain an appreciable amount of phytotoxins.

Therefore phytotoxins do not seem to take a direct part in the appearance of upland rice soil sickness. However, there is a possibility that they may take an indirect part, that is, they may weaken root activity at the site where root residue comes into contact with living root, and stimulate the infection of pathogenic fungi and nematodes on and in roots.

Implication of aerobic organisms in the soil sickness

Many studies indicate that animate factors are the main causal agents, because the appearance of the soil sickness is almost overcome by the sterilization of soil. Of animate factors, aerobic orgainisms are suspected as the causal agents. Table 1 shows the effect of homogenate of root residues of upland rice left in the field up to the following spring on the growth of seedlings of the plant. The unsterilized homogenate of the root residues had high inhibitory action. This inhibitory action may not be due to phytotoxins in the root residues, as already mentioned, but due to detrimental aerobic organisms, because anaerobic preincubation of the homogenate for 6 days lost its inhibitory action. The speculation that aerobic organisms may be the main causal agents of the soil sickness agrees with the fact that lowland rice grown in anaerobic soil condition is immune to the soil sickness.

Enrichment of *Pyrenochaeta* sp. on and in roots of upland rice

A great number of aerobic organisms are living on and in roots of upland rice and some of them are thought to give a detrimental effect on the growth of the plant. The authors examined the fungal genera living there. The root samples were washed 15 times with sterile water by vigorous shaking. An aliquot of the washed roots was cut aseptically into 2 mm segments for the detection of fungi on the root surfaces, and another aliquot was surface-sterilized by immersing them into 0.1% mercuric chloride solution for 2 min and then cut into segments for the detection of fungi in the root tissues. Root segments were planted in Czapek-Dox agar supplemented with rosebengal and streptomycin, and incubated at 17–25°C for 7–10 days. The frequency of occurrence of each fungal genus was expressed as the fungal activity, which means the percentage of root segments colonized by the genus.

The dominant genera on the root surfaces of upland rice were found to be *Penicillium* on the tip region and *Fusarium* and *Pyreno-*

Table 1. Fffect of homogenate of root residues of upland rice on the growth of seedlings of the plant[5)]

	Expt. I		Expt. II	
	No. of plants tested	Mean* height (cm)	No. of plants tested	Mean* height (cm)
1. Sterile water	10	10.9±1.1	14	9.8±2.2
2. Unsterilized homogenate	15	8.4±0.9	15	4.7±2.2
3. Membrane-filtrate of homogenate	15	10.4±0.9		
4. Anaerobically preincubated homogenate			15	11.4±2.2

Sterile seedlings were inoculated with either non-treated or treated homogenate and were cultivated in the light preventing further microbial contamination.

*Mean height with confidence limit at P=0.05.

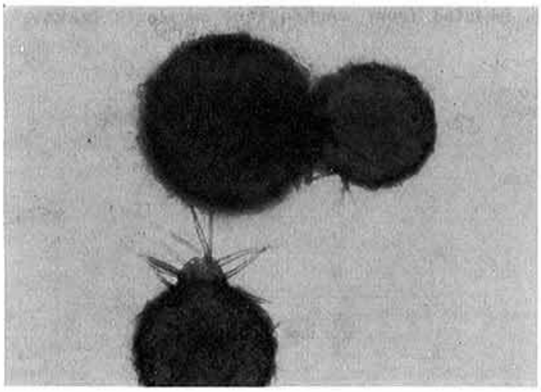

Plate 1. Pycnidia of *Pyrenochaeta* sp.

Table 2. Frequency of occurrence of *Pyrenochaeta* sp. isolated from root segments taken from continuously cropped upland rice[2].
(expressed as the fungal activity)

		Days after germination						
		3	17	31	51	84	109	138
Washed roots								
Plot	1	10	20	5	20	8	6	18
	2	33	40	40	35	13	42	56
	3	—	33	—	10	15	25	41
Surface sterilized roots								
Plot	1	—	—	—	0	5	0	15
	2	—	—	—	10	8	12	20

Plot 1: control plot (Norinmochi No. 1)
Plot 2: continuously cropped plot (Norinmochi No. 1)
Plot 3: continuously cropped plot (Hatasangoku)

chaeta on the crown region[2]. Of these only *Pyrenochaeta* sp. was noticed to enrich on and in roots of upland rice through its continuous cropping. This fungus had not been recognized before as an inhabitant on and in roots of continuously cropped upland rice. Plate 1 shows pycnidia of *Pyrenochaeta* sp. Table 2 shows that *Prenochaeta* sp. occurred 1.6–8.0 times more frequently on the root surfaces taken from the continuously cropped plants at every plant age, and that this fungus also penetrated more frequently into the root tissues of the continuously cropped plant. The enrichment of the fungus on and in roots through continuous cropping was reconfirmed also in the next year using 9 plots. On the root residues left in the field after harvest, the fungus survived during winter until the following spring[2].

Thus, the following life cycle of *Pyrenochaeta* sp. in the field was speculated: this fungus colonizes the root surfaces soon after the germination, multiplies there with root growth, then penetrates into the root tissues, thereafter can survive during winter on infected root residues left in the field, and in the next spring colonizes the roots of new seedlings; therefore the fungus accumulates on and in roots through the continuous cropping.

Enrichment of *Pyrenochaeta* sp. on roots of upland rice through its continuous cropping was confirmed not only in our experimental field (Kitamoto, Saitama Pref.) but also in the other fields (Konosu, Saitama Pref. and Mito, Ibaraki Pref.)[1].

Inhibition of the growth of upland rice by *Pyrenochaeta* sp.

Pyrenochaeta sp. was indicated to have an ability to inhibit the growth of upland rice by the following results. 1) It inhibited the growth of the sterile seedlings when inoculated in a pure form[3]. 2) While partial sterilization of the continuously cropped soil by autoclaving or chloropicrin eliminated the fungus on roots and enhanced the seedling growth, the fungus inoculated into the partially sterilized soil colonized on roots and reduced the plant growth[3]. 3) The higher the frequency of occurrence of the fungus on the root surfaces, the lower the top weights of the plant grown in the field[2].

Comparison of frequency of occurrence of *Pyrenochaeta* sp. on roots among several different kinds of plants continuously cropped

Pyrenochaeta sp. was noticed to have a considerable and specific affinity to roots of up-

Table 3. Frequency of occurrence of *Pyrenochaeta* sp. isolated from washed root segments taken from continuously cropped plants[2,4)]

	Continuously cropped for	Fungal activity	Sampled at
Upland rice	9 yrs	15	1 Oct. 1971
Peanut	9	9	
Taro	9	2	
Sweet potato	9	6	
Upland rice	9	38	1 Oct. 1971
Peanut	9	2	
Upland rice	9	71	18 July 1974
	12	40	
Lowland rice in upland	9	44	
Chinese cabbage	0	2	18 Oct. 1974
	4	0	
Barley following upland rice	9	37	8 Apr. 1975
following taro	4	9	
following peanut	4	17	

land rice when compared with roots of several other kinds of plants continuously cropped. As Table 3 shows, the fungus occurred most frequently on roots of upland rice and lowland rice growing in upland field, followed by roots of barley, and rarely on roots of peanut, taro, sweet potato and chinese cabbage. The fungus could not be detected at all on roots of lowland rice in paddy field[1)].

A substance of selective action on plant growth produced by *Pyrenochaeta* sp.

Why does *Pyrenochaeta* sp. have a particular affinity for roots of upland rice? The water-extract of roots inoculated with the fungus in a pure form was found to contain a substance which inhibited the growth of germinating seedlings of upland rice but not that of chinese cabbage or radish[3)]. The authors attempted to isolate this substance and obtained the partially purified pink substance from mycelia of *Pyrenochaeta* sp. cultured in liquid medium as well as from roots inoculated with the fungus in a pure form. Its ethanol solution had absorption spectrum at the visible region as shown in Fig. 2.

This partially purified substance had a selective action on plant growth. It inhibited the growth of germinating seedlings of five monocotyledonous plants (upland rice, sorghum, wheat, barley and oat) at a concentration higher than 10 ppm, while it stimulated those of five dicotyledonous plants (chinese cabbage, cucumber, radish, turnip and burdock) at 10–100 ppm in the light (Fig. 3). Even when sprayed on shoots of seedlings which had been germinated in pots, it inhibited the growth of upland rich but stimulated that of chinese cabbage at ca. 70 μg/plant of the partially purified pink substance[7)].

Discussion and conclusion

These results show that plants susceptible to the pink substance tend to have a higher frequency of occurrence of *Pyrenochaeta* sp. on their roots. Therefore, it is concluded that this fungus may have an affinity to roots of upland rice by producing the pink substance

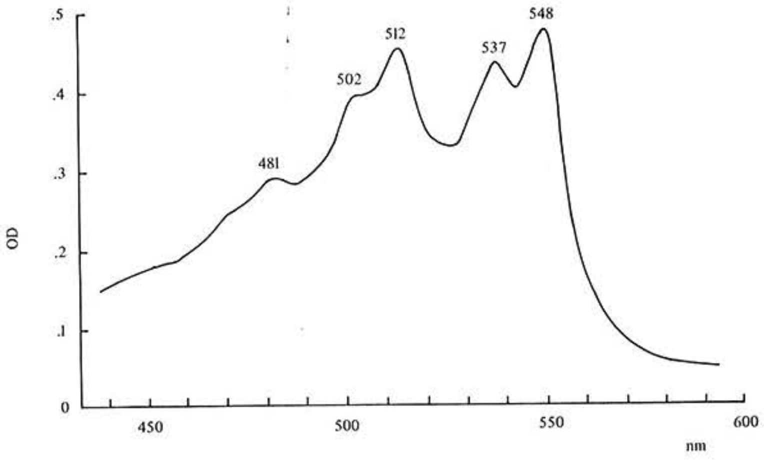

Fig. 2. Spectrum of ethanol solution of the pink substance produced by *Pyrenochaeta* sp.[6]

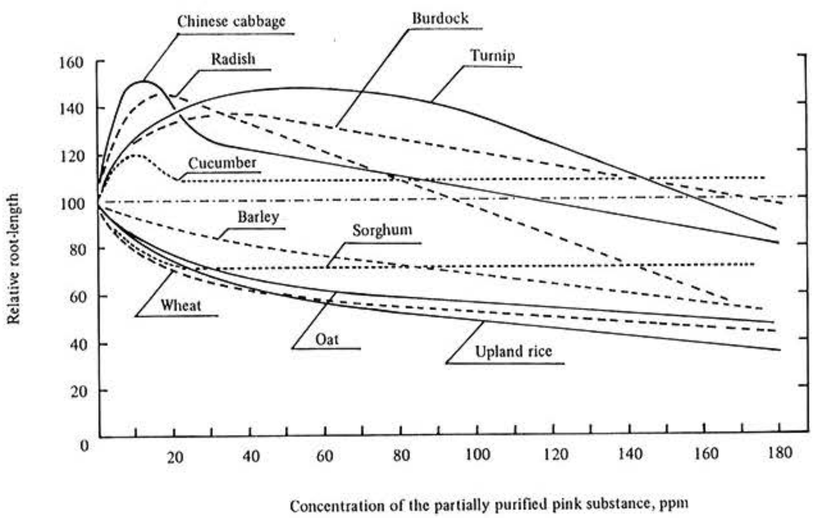

Fig. 3. Effect of the pink substance on root-elongation of germinating seedlings in the light (summarized)[7]

on and in roots of the plant. The following would be the process of infection: the pink substance produced by *Pyrenochaeta* sp. lowers the activity of roots, and as a result, it promotes infection to the host plant. Thus, *Pyrenochaeta* sp. may be one of the causal agents of upland rice soil sickness. But before coming to the final conclusion further efforts are necessary for the detection of the pink substance in roots of upland rice in the continuously cropped field, and for the purification and identification of the pink substance. Since the main monocotyledonous summer crops common in Japanese upland farming are limited to upland rice, lowland rice in upland field and maize, the pink substance is regardable in practice as an inhibitor specific to the growth of upland rice.

Pyrenochaeta sp. is not the sole organism causing upland rice soil sickness. Other investigators showed enrichments of particular pathogenic nematodes and other fungi in the field through the continuous cropping of upland rice[8]. *Pyrenochaeta* sp. and other organisms are thought to cause the soil sickness in co-operation with each other, probably with the indirect aid of phytotoxins remained in a small amount in root residues. In the Philippines where twice or more continuous cropping of upland rice in a year is possible by irrigation, upland rice soil sickness appeared more severely than in Japan, as shown by Ventura and Watanabe[9]. They suggested a possibility that the cause of the soil sickness in the Philippines may be different from that in Japan. It is an interesting further problem to compare the symptoms and causes of upland rice soil sickness in the countries of different climate and agricultural systems.

References

1) Matsumoto, M., Nishio, M. & Kusano, S.: Fungi on roots of upland rice continuously cropped in the different regions of Japan. *J. Sci. Soil Manure, Japan,* [In Japanese with English summary] (In press).
2) Nishio, M. & Kusano, S.: Fungi associated with roots of continuously cropped upland rice. *Soil Sci. Plant Nutr.,* 19, 205–217 (1973).
3) Nishio, M. & Kusano, S.: Effect of fungi associated with roots on the growth of continuously cropped upland rice. *Soil Sci. Plant Nutr.,* 21, 161–171 (1975)
4) Nishio, M. & Kusano, S.: Fungi on roots of continuously cropped upland rice and their effect on the plant growth. *Soils & Microorganisms,* 17, 29–36 (1975).
5) Nishio, M. & Kusano, S.: Effect of root residues on the growth of upland rice. *Soil Sci. Plant Nutr.,* 21, 391–395 (1975).
6) Nishio, M. & Kusano, S.: A growth inhibitor specific to upland rice produced by *Pyrenochaeta* sp., *Soil Sci. Plant Nutr.,* 22, 277–286 (1976).
7) Nishio, M. & Kusano, S.: A fungal substance of selective action on plant growth produced by *Pyrenochaeta* sp., *Soil Sci. Plant Nutr.,* 22, 467–472 (1976).
8) Nishio, M. & Kusano, S.: Problems in upland rice soil sickness, *In* Proceedings of the International Seminar on Soil Environment and Fertility Management in Intensive Agriculture, Tokyo, 744–749 (1977).
9) Ventura, W. & Watanabe, I.: Growth inhibition due to continuous cropping of dryland rice and other crops. *Soil Sci. Plant Nutr.,* (In press).