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Soil-Borne Diseases and Ecology of Pathogens on Soybean Roots in Indonesia

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Abstract

Studies on root diseases and causal fungi on soybean in Indonesia were carried out. Missing plants due to damping-off of seedlings occurred frequently. In addition, the average infection rates of root rots amounted to 60% of all the surviving plants toward the pod filling stage. The more severe root rot was, the lower the yield of soybean seeds. The isolates from the seedlings showing damping-off belonged mainly to either *Pythium apanidermatum*, *Sclerotium rolfsii*, anastomosis groups (AG) 4 and 7 of *Rhizoctonia solani*, AG-E and other AGs of binucleate *Rhizoctonia* spp. As time went on, the severity of the root rot symptoms increased rapidly and *Fusarium* spp. and binucleate *Rhizoctonia* were predominantly isolated from rotten roots in the great majority of the fields, except for one field where root rot disease caused by *Cylindrocladium* sp. occurred. All the isolates of S. rolfsii, R. solani AG-4 and AG-7, binucleate Rhizoctonia AG-E, P. aphanidermatum, *P. myliotylum* and *Pythium* sp. caused damping-off as well as root rot on soybean, whereas the isolates of *F. solani* and *C. floridanum* from soybean were pathogenic to the plants in the middle or late stage of growth. A new root rot disease caused by *C. floridanum* and damping-off disease caused by AG-7 and AG-E of *Rhizoctonia* spp. were detected for the first time in soybean fields in Indonesia.

Discipline: Plant disease
Additional key words: Cylindrocladium, Fusarium, Pythium, Rhizoctonia, Sclerotium

Introduction

In Indonesia, soybean is the most important crop as a protein source for food among upland crops. Soybean is mainly grown in lowland fields at the end of the rainy season after harvesting of paddy rice, and in upland fields in the rainy season, with continuous cropping occasionally. The yield is currently low in many areas due to biological, chemical and physical constraints. Soil-borne diseases associated with several kinds of root-infecting fungi can also be a limiting factor for soybean seed production, as reported from other countries[1,3,4,7,10-12,15]. In Indonesia, root diseases of soybean have spread throughout the country especially in the rainy season. There is, however, little information about the yield loss due to soil-borne fungi in various stages of growth.

Therefore, field surveys on the occurrence of soil-borne diseases of soybean and identification of the causal fungi were carried out at Bogor Research Institute for Food Crops (BORIF) during the period April 1989 – March 1991. In this paper, the results obtained[5,6] are described.

The present paper was prepared on the basis of the results of the strengthening research for Palawija crops production project (ATA-378), which was jointly implemented by the Japan International Cooperation Agency, Japan, and the Bogor Research Institute for Food Crops, Indonesia, during the period 1989 to 1991.

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Present status of soil-borne diseases of soybean in Indonesia

Firstly, field surveys on the distribution and severity of soil-borne diseases of soybean were conducted in Java, Lampung and Aceh during the dry season of 1989 and rainy season in 1989/1990. Damping-off of the seedlings usually was not severe, except that in some fields the diseased plants accounted for 36% of all the plants. After the seedling stage, root rot disease with brown to dark brown lesions occurred widely throughout Java and Lampung at infection rates of more than 60% on an average (Table 1), while in North Aceh root rot was considerably less severe even in fields with soybean monoculture for 10 years, suggesting that the latter soil may be suppressive to the disease.

Secondly, based on the seasonal changes of root diseases on soybean in Citayam experimental farm, pre- and post-emergence damping-off disease occurred severely in the early stages of growth. As time went on, the severity of the root rot symptoms

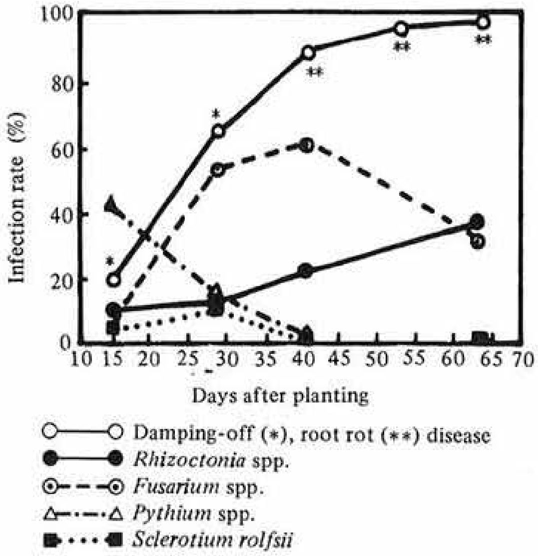


Fig. 1. Seasonal changes in damping-off, root rot disease and pathogens in soybean fields with continuous cropping at the Citayam experimental farm of BORIF
Date of planting: June 28, 1989.

Table 1. Outline of root rot diseases of soybean in Indonesia

Location	No. of fields	No. of plants	Diseased plants %	Diseased plants Severity[a]	No. of isolates	Pathogen isolated (%)[b] Rhiz.	Pyth.	Fus.	Scl.	Others
West Java										
Garut	8	–[c]	–	–	125	36.8	6.1	13.9	2.5	40.8
Cianjur	4	39	69.5	1.3	40	24.0	4.6	51.4	0.0	2.8
Tasikmalaya	2	7	100.0	1.6	23	34.3	16.7	23.1	0.0	26.0
Ciamis	1	18	100.0	1.8	14	21.3	7.1	50.0	0.0	21.4
Kuningan	2	49	87.8	1.5	28	67.9	0.0	17.8	0.0	10.7
Majalenka	2	31	86.1	1.2	18	4.2	8.4	33.4	0.0	37.5
Central Java										
Wonosari	5	111	85.6	1.3	71	79.0	0.6	20.4	0.0	6.3
East Java										
Ponorogo	2	70	44.9	0.5	50	56.8	1.2	42.5	0.0	0.0
Ngawi	2	39	56.9	0.7	15	64.3	0.0	35.9	0.0	0.0
Trenggalek	1	19	68.4	1.2	10	90.0	0.0	10.0	0.0	0.0
Mojosari	1	22	72.3	0.8	14	35.7	35.7	28.6	0.0	0.0
Malang	1	17	100.0	1.1	14	7.1	7.1	85.7	0.0	0.0
Sumatra										
North Aceh	2	30	27.8	0.3	–	–	–	–	–	–
East Aceh	3	40	55.2	0.6	–	–	–	–	–	–
Lampung	3	–	–	–	87	52.4	11.7	26.5	0.0	6.3

a): Disease severity; 0 (healthy) – 3 (severe infection).
b): Rhiz.; *Rhizoctonia*, Pyth.; *Pythium*, Fus.; *Fusarium*, Scl.; *Sclerotium*.
c): Not observed.

increased rapidly. Toward harvesting time, the diseased plants accounted for 72 to 92% of all the surviving plants (Fig. 1). *Sclerotium rolfsii* was predominantly isolated from diseased seedlings, followed by *Rhizoctonia solani* and binucleate *Rhizoctonia* spp. though most of the isolates from seedlings with preemergence damping-off consisted of *Pythium* spp. In the later stages of growth, a larger number of *Fusarium* spp. and binucleate *Rhizoctonia* spp. were recovered, whereas the number of *S. rolfsii* decreased. These results were similar to those obtained in farmers' fields in Java. Kinds and populations of causal fungi attacking roots seemed to vary with the growth stages of the plant.

Thirdly, experiments on yield loss of soybean due to root rot disease both in Citayam and Pacet experimental farms were conducted. The more severe the disease was, the lower the yield of soybean seeds. Light, moderate and severe infection resulted in 25, 50 and 75% yield losses, respectively, as compared with the absence of infection (Fig. 2). Since root rot disease causes infection rates of more than 60% on soybean in Java, countermeasures are important due to the large yield losses. Actually, soybean can grow without developing any root rot lesions, as shown in North Aceh.

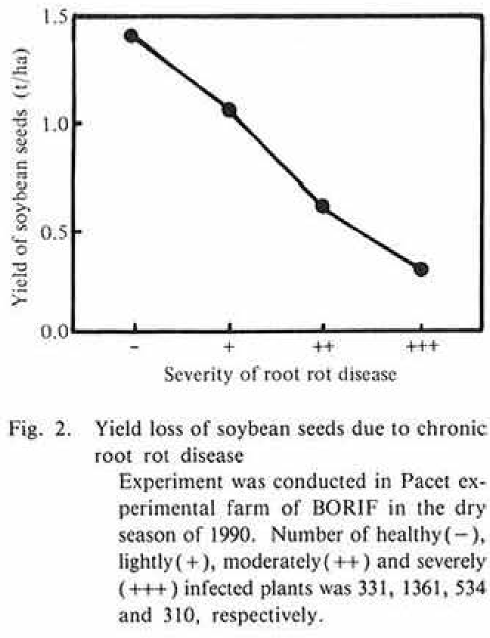


Fig. 2. Yield loss of soybean seeds due to chronic root rot disease
Experiment was conducted in Pacet experimental farm of BORIF in the dry season of 1990. Number of healthy(−), lightly(+), moderately(++) and severely (+++) infected plants was 331, 1361, 534 and 310, respectively.

Influence of cultural practices on the incidence of damping-off and root rot diseases of soybean was examined. Field experiments revealed that damping-off of soybean occurred severely in fields after harvesting of paddy rice as compared with fields after corn or mungbean cultivation. Earthing-up treatment at 2 weeks after planting promoted damping-off considerably. Lime treatment (3 t/ha) did not affect the disease severity. The application of barnyard manures (10 t/ha) reduced the incidence of damping-off slightly when they were mixed with soil at the time of plowing. However, these cultural practices did not affect the root rot severity 2 months after planting.

Pathogens and their ecology in relation to damping-off and root rot diseases of soybean

1) Anastomosis groups and pathogenicity of Rhizoctonia spp. isolated from soybean roots

The genus *Rhizoctonia* is complex. At present, *R. solani* and binucleate *Rhizoctonia* spp. are classified into 10 anastomosis groups from AG-1 through AG-10 and 12 groups from AG-A through AG-Q, respectively[12]. So far, AG-1, AG-2-2, AG-4 and AG-5 have been pathogenic to soybean[3,4,15].

Of the 283 isolates of *Rhizoctonia* spp. obtained from soybean plants with damping-off and root rot symptoms in Java and Lampung, 75% were

Table 2. Relationship between growth stage of soybean and types of anastomosis groups (AG) of isolates of *Rhizoctonia* spp.

Growth stage of soybean[a)]	No. of isolates	AG of binucleate *Rhizoctonia* spp. (%)		AG of *R. solani* (%)			*R. oryzae* (%)	*R. zeae* (%)
		AG-E	Others	AG-1	AG-4	AG-7		
Early	101	6.0	45.6	1.0	6.9	34.6	4.0	2.0
Middle	78	3.8	91.8	0.0	3.8	2.6	0.0	0.0
Late	98	3.1	86.7	0.0	3.1	6.1	0.0	0.0

a): Early; less than 1 month, Middle; 1 to 2 months, Late; more than 3 months after planting.

binucleate. The others were all multinucleate and assigned to either *R. solani* AG-1, AG-4, AG-7, *R. oryzae* or *R. zeae*. Isolation frequency of these groups varied with the growth stages of soybean and the kind of preceding crop (Table 2). In the early stages of growth, AG-4 was predominantly isolated from seedlings with pre-emergence damping-off while AG-7 and binucleate *Rhizoctonia* spp. were isolated mainly from seedlings with post-emergence damping-off. Most of the isolates of AG-7 originated from lowland soybean fields after harvesting of paddy rice. In the middle and later stages of growth, most isolates consisted of binucleate *Rhizoctonia* spp. whereas the incidence of *R. solani* declined rapidly. The isolation of AG-1, *R. oryzae* and *R. zeae* from seedlings throughout the growing season was infrequent.

Optimum growth temperature of AG-7 was nearly 30°C, higher than that of AG-4 and AG-E. At 37°C, AG-7 and AG-E grew well, while AG-4 growth was completely inhibited. In dual culture of isolates of AG-7 and AG-E, hyphal fusion occurred at a low frequency. Inoculation tests indicated that AG-4 was the most pathogenic to soybean, causing severe pre- and post-emergence damping-off, followed by AG-E and AG-7 (Table 3). These AGs caused root rot in the middle stage of growth. AG-1, known as a pathogen of foliar blight of soybean[15], did not attack the underground parts of the plants under natural field conditions though damping-off occurred severely in pots inoculated artificially. Many isolates of binucleate *Rhizoctonia* spp. excluding AG-E were weakly pathogenic to soybean in the middle stage of growth.

Table 3. Pathogenicity of *Rhizoctonia* spp. on soybean[a)]

Anastomosis group	Isolate tested	No. of seeds	Damping-off (%)		Disease severity[b)]
			Pre-emergence	Post-emergence	
R. solani AG-4	Rh- 1	50	100.0	–	–
	Rh- 45	50	100.0	–	–
	Rh-145	50	90.0	49.0	0.7
	Rh-166	50	100.0	–	–
R. solani AG-7	Rh- 19	50	8.0	97.8	1.0
	Rh- 51	50	4.0	100.0	1.0
	Rh-147	50	2.0	49.0	0.3
	Rh-234	50	4.0	98.0	0.4
	Rh-290	50	6.0	100.0	1.3
	Rh-292	50	0.0	88.0	0.7
	Rh-294	50	12.6	86.7	0.6
Binucleate *Rhizoctonia* AG-E	Rh- 20	50	12.0	88.4	0.9
	Rh- 24	50	16.0	97.6	1.0
	Rh- 71	50	28.0	97.2	1.5
	Rh- 72	50	40.0	96.7	1.1
	Rh- 74	50	26.0	97.3	0.9
	Rh-199	50	22.0	97.3	0.9
	Rh-202	50	60.0	100.0	0.8
Binucleate *Rhizoctonia* other group	Rh- 14	50	2.0	36.7	0.2
	Rh- 16	50	0.0	50.0	0.4
	Rh-104	50	0.0	34.0	0.2
	Rh-108	50	2.0	6.1	0.3
	Rh-122[c)]	50	0.0	4.0	0.3
	Rh-132	50	0.0	2.0	0.2
Not inoculated		50	0.0	8.0	0.1

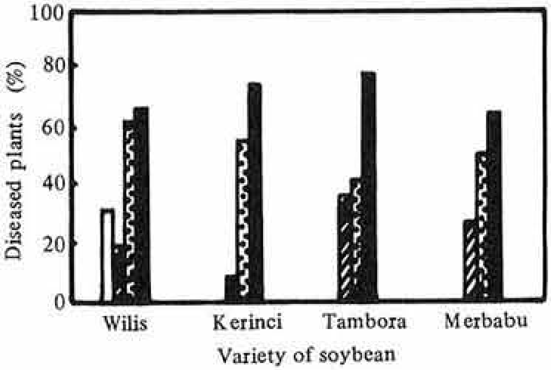
a): Inoculum; cultured on rice grains. Soil; autoclaved.
Five gram of inoculum cultured on rice grains was mixed with sterilized soil in a 12 cm pot, then 10 plants/pot were sown. Date of survey; 2 weeks after inoculation.

b): Disease severity 0; healthy, 1; light, 2; moderate, 3; severe infection.

c): Binucleate *Rhizoctonia* AG-G.

2) Characterization and pathogenicity of Cylindrocladium sp. isolated from soybean roots

We detected for the first time a root rot disease with foliar yellowing symptoms in the early pod stage of soybean in Pacet experimental farm of BORIF in March 1990. Though the symptoms were similar to red crown rot or black root rot caused by *C. crotalariae*, the isolates from these diseased roots were morphologically different from *C. crotalariae*[7]



(□) 30 days, (▩) 55 days, (▧) 75 days, and (■) 100 days after planting

Fig. 3. Occurrence of a new *Cylindrocladium* root rot of soybean in Pacet experimental farm of BORIF

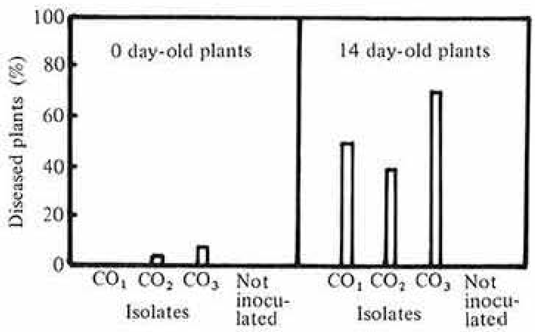


Fig. 4. Pathogenicity of isolates of *Cylindrocladium* sp. to soybean
The isolates, cultured on rice grains, were inoculated to 0 and 14 day-old plants. The plants, grown in pots containing sterilized soil, were inoculated with the isolates cultured on rice grains 0 and 14 days after planting in a glass house.
Data collected 1 month after inoculation.

and *C. clavatum*[1]. The disease occurred from one month after planting to harvesting time, and was more severe toward the pod filling stage (Fig. 3). The roots showed reddish to dark brown lesions of various sizes and shapes. The diseased roots often showed nematode infection. Inoculation tests revealed that *Cylindrocladium* sp. caused root rot disease on soybean and could be reisolated at a high percentage (Fig. 4). The fungus induced yield loss of soybean seeds.

The fungus showed whitish to reddish brown colonies on potato dextrose agar, producing reddish brown microsclerotia. Conidiospores arose laterally from a stipe that terminated in a hyaline, globose vesicle. Stipes were 12–100 μm long and 2–5 μm wide. Conidia developed on the apex of the phialide were hyaline, cylindrical, straight, uni-septate, rounded at both ends and 32–48 × 4–6 (41.5 × 4.9 on an average) μm in size. *Cylindrocladium* sp. from soybean was identical with *C. floridanum* Sobers et Seymour[8,13] morphologically. On the other hand, the spores of *C. crotalariae* and *C. clavatum* were 3- and uni-septate, respectively, and the vesicles of *C. crotalariae* and *C. clavatum* were globose and clavate, respectively[1,7]. Natural occurrence of root rot disease caused by *C. floridanum* was detected for the first time in Indonesia.

3) Characterization of Pythium spp. isolated from soybean root

Identification and inoculation tests of *Pythium* spp. obtained from soybean plants with damping-off and root rot symptoms were conducted in a glass house. Most of the isolates producing nematosporangia consisted of *P. aphanidermatum* and a few *P. myliotylum*. The other species which produced conidial-typed sporangia belonged to either unidentified *Pythium* species lacking a sex organ or *P. spinosum*-like species with projections on the oogonial wall. Maximum temperature for mycelial growth has been reported to be different among the *Pythium* species[14]. At a temperature of 37°C, most of the isolates from the highlands (1,150 m) did not grow, while those from the lowlands (150 m) grew well. The latter mostly belonged to *P. aphanidermatum* and a few *P. myliotylum*, both of which were seldom isolated from highland fields. Ecological niche of *Pythium* species in the highlands and lowlands seems to be determined by the maximum temperature for mycelial growth. Isolates of *P. aphanidermatum*, *P. myliotylum* and *Pythium* sp. were all pathogenic to soybean, causing pre- and post-emergence damping-off as well as root rot symptoms.

4) Pathogenicity of Fusarium spp. isolated from soybean roots

Inoculation tests showed that no isolate caused damping-off disease in the early growth stages of soybean, even when the seedlings were wounded with a sewing needle. However, some isolates caused root rot in the middle stage. Pathogenic isolates were identical with *F. solani* morphologically, especially based on the development of the conidiophore. At present, highly pathogenic strain (forma specialis) of *F. solani* causing sudden death syndrome as reported in the United States[10] has not been recorded yet in Indonesia.

5) Distribution of sclerotia of Sclerotium rolfsii in the field

Damping-off, stem or root rot disease caused by *S. rolfsii* occurred widely throughout the country, and was particularly severe in fields after paddy rice harvesting. Sclerotia of *S. rolfsii* are the most important source of infection[9]. Based on our data on the distribution of the fungus in fields, more sclerotia were formed on debris of rice stubbles after plowing; i.e. 10 sclerotia/g of dry debris. On the other hand, the density of sclerotia in soils of fields with paddy rice as preceding crop and soybean was less than 0.1 sclerotia/g of dry soil. These sclerotia usually germinated on nutrient agar medium, suggesting that they were viable.

Conclusion

As mentioned above, damping-off and root rot diseases are serious constraints on soybean production especially in the rainy season in Indonesia. Even though aerial parts of soybean appear healthy, many roots are infected with root rot fungi. The results of the pathogenicity tests and isolation frequency of the causal fungi indicated that root rot disease was mainly caused by AG-4 and AG-E of *Rhizoctonia* spp., *F. solani* or the complex though the kinds and populations of the causal fungi varied among the fields. Yield of soybean can increase to more than 25% in mosts areas in Java or Lampung, if soybean grows without any root rot lesion, for example in North Aceh where soybean roots are mostly healthy except for root or stem rot caused by *S. rolfsii* in fields after paddy rice harvesting. The reason why many soybean varieties can avoid root rot disease remains to be determined.

The presence of seedlings with damping-off led to missing plants. Mostly, *S. rolfsii*, *R. solani* and *P. aphanidermatum* were closely associated with such symptoms though some of the symptoms may be often attributable to the insect bean fly (*Ophiomya phaseoli*)[2]. Hence more attention should be paid to damping-off as well as root rot especially in the rainy season. Furthermore, analysis of the life cycle of the pathogen is important for developing integrated control methods combining cultural practices, biological control as well as selection of varieties.

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