STUDIES ON THE SOURCES OF *RHIZOCTONIA SOLANI* FOR PRIMARY INFECTION

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Abstract

Hyphae of *Rhizoctonia solani* showed stronger pathogenicity than sclerotia on kenaf seeds and seedlings. The saprophytic activity of *R. solani* in the soil was most prominent at the soil surface to a depth of 30 cm. Approximately 19.0–53.6% of the organic debris obtained at this depth carried inoculum of *R. solani*. The loofah root harbored less *R. solani* than that of 8 other previous crops in kenaf field. Isolates of *R. solani* differed in their ability to form sclerotia. Of the three isolates tested, K-1 and K-2 produced a slightly greater number of sclerotia in natural soil than sterilized soil, while the isolate S-1 formed more sclerotia in natural soil incorporated with sterilized soil. Therefore, the ability of sclerotia formation of *R. solani* seemed to be greatly depended upon the substrates as well as the isolates concerned.

Introduction

Rhizoctonia solani Kühn (= *Thanatephorus cucumeris* (Frank) Donk) is one of the most important pathogens involved in kenaf diseases (13). It has been regarded as one of the typical soil-inhabiting saprophytic fungi (4). Ferguson (3) mentioned that a saprophytic growth phase on the surface of the host plant prior to penetration of hyphae is a characteristic of *Rhizoctonia*, therefore, the presence of saprophytic hyphae of *R. solani* is believed to be an important source for primary infection. Formation of sclerotia is also one of the characters of this fungus (14). Sclerotia served not only as a long-lived resting stage for survival of the organism under unfavorable condition, but also as a source for re-establishing the parasitic phase of the fungus (4). However, further studies on the sources of primary infection of *Rhizoctonia* diseases are still necessitated.

This study was carried out to determine the source of *R. solani* for primary infection on kenaf, the density of saprophytic hyphae in natural soil, and the formation of sclerotia by three isolates of the fungus.

Materials and Methods

Three hyphal tip isolates of *R. solani*, K-1, K-2 and S-1, were used in this study.

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The first two isolates were obtained in 1968 from the mature plants and seedlings of kenaf, respectively. The last was isolated in the same year from the soil of a sugarcane field. These three isolates were maintained through serial transfers on potato-sucrose agar (PSA). Each hyphal cell of the isolates was found to be multinucleate.

To compare the pathogenicity of hyphal fragments and sclerotia of *R. solani*, the soil (sandy loam, pH 6.5) collected from field was first autoclaved at 20 lbs for 30 min., then mixed with either of the inocula at the rate of 0.2% by weight in a mixture apparatus (Fig. 1) for 10 min. The soil-inoculum mixtures were then filled in a 4-inch clay pot and 30 kenaf seeds were sown. Four replications were made for each inoculum-soil combination. All pots were kept in the laboratory where the temperature fluctuated around 26±2°C and provided with 9 hr. illumination. The total percentage of seed germination was counted after about 8 days when the first true leaves appeared. The diseased seedlings were recorded each day after seed germination.

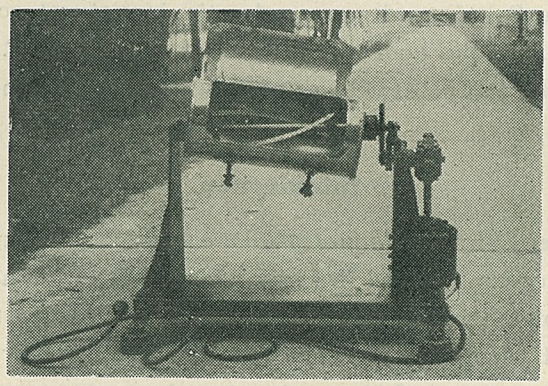


Figure 1. An apparatus for mixing soil and inoculum.

For preparing the hyphal fragments, *R. solani* was first grown in potato-sucrose broth for 4-6 days (no sclerotia formation was found at that time) at 28°C. The colony of *R. solani* was then filtered and macerated in a Waring blender with 20 cc distilled water for 30 seconds. The hyphal fragments were filtered again and air-dried on a sterile paper until the water content of the hyphal fragments was approximately 99.4%. The sclerotia used were obtained from the surface of PSA culture incubated at 28°C for 30 days.

The method described by Papavizas (9), with some modification, was followed to determine the density of saprophytic hyphae in the soil. Soil samples collected from different depths of 10 fields under different cultivation systems were passed through a 325-mesh sieve. The residues collected on the sieve were divided into 100 g aliquotes and each was suspended in 2 liters of tap water in a glass jar with a large stirring rod. The suspension was allowed to settle for 30 seconds and decanted onto a 60-mesh

sieve. The settled soil was again resuspended in 1 liter of tap water, allowed to settle for 30 seconds, and again decanted onto the 60-mesh sieve. The organic debris particles collected on the sieve were then washed thoroughly with a strong jet of tap water and spread on a paper to dry. 400 debris particles picked at random from each soil sample were transferred to 40 Petri plates (10 particles/plate) each containing 20 cc of water agar (pH 4.5). The particles were examined with a microscope after 24 and 48 hr. of incubation at 25°C, and the percentage of particles with *R. solani* was recorded.

Formation of sclerotia by isolates of *R. solani* in natural soil, autoclaved soil, autoclaved soil amended with 100 ppm sodium nitrate and the autoclaved soil recontaminated with 50% of natural soil was also studied. One part of soil-grown inoculum prepared in a manner described by Tu (15) was mixed with 9 parts of each soil tested. 900 g of the inoculum-soil mixture were then put into an 1 liter flask and covered with a glass cover. The water content of the soil was approximately 30% of its moisture holding capacity (m.h.c.). The soil-inoculum mixture was then incubated at 28°C for 30 days. To determine the number of sclerotia, the soil in 4 flasks of each test was mixed thoroughly in a pile; from it an 100 g sample was removed and the sclerotia sifted out and counted.

Formation of sclerotia by the isolates was again compared in three agar media. The fungi were cultured on Czapeck's agar (sucrose 30 g, $MgSO_4.7H_2O$ 0.5 g, $NaNO_3$ 2 g, $FeSO_4$ 0.01 g, KH_2PO_4 1 g, KCl 0.5 g, agar 20 g, and distilled water 1,000 cc), potato-sucrose agar, and water agar (agar 20 g, distilled water 1,000 cc) at 30°C for 30 days. The pH of the agar media was adjusted to 6.2 after sterilization. Each 8.5 cm Petri dish containing 20 cc of the agar media was considered a replicate, and 6 replicates were made for each combination of the isolates and culture media. The degree of formation of sclerotia was recorded according to the method described by Allington (1).

Results

Pathogenicity of hyphae and sclerotia of R. solani on kenaf seeds and seedlings: The hyphae of isolate K-1 showed stronger pathogenicity than sclerotia on kenaf seeds. The percentage of seed germination was 33.33 and 66.67 respectively. However, all kenaf seedlings were infected by the two infectional sources of isolate K-1 in 4 days after seed germination. The pathogenicity of both hyphae and sclerotia of isolate K-2 on kenaf seeds was almost the same as those of isolate K-1. Kenaf seedlings were also completely diseased in the soil infested with the hyphae of isolate K-2, but in the sclerotia-infested-soil there was still 25.70% of seedlings free from the disease 4 days after germination. The hyphae of isolate S-1 showed very weak pathogenicity on both kenaf seeds and seedlings; 84.44% of kenaf seed germination was observed and only 1.19% of seedlings were infected. No infected seeds and seedlings were observed

Table 1. A comparison of pathogenicity of hyphae and sclerotia of *Rhizoctonia solani* on kenaf seeds and seedlings

Isolate	Inoculum	Seed germination (%)	Diseased seedlings in 4 days after germiationn (%)
K-1	Hyphae	33.33	100
	Sclerotia	66.67	100
K-2	Hyphae	23.33	100
	Sclerotia	66.67	76.30
S-1	Hyphae	84.44	1.19
	Sclerotia	90.00	0
CK*		90.00	0

* Autoclaved soil

in the soil inoculaed with the sclerotia of S-1. The results are shown in Table 1.

Density of saprophytic hyphae in soil: The results summarized in Table 2 show that the saprophytic activity of *R. solani* in soil was much higher at the soil surface to a depth of 30 cm. The organic particles collected from this depth were 19.0-53.6% colonized by *R. solani*, while less than 4.9% of that were obtained from the soil at the depth of 30 to 40 cm. Approximately 21.7% of the particles which were distributed in the area of soil surface to the depth of 60 cm carried inocculum of *R. solani*.

Table 2. The percentage of *Rhizoctonia solani* in organic debris particles from different depths of soil

Depth of soil (cm)	Crops before planting of kenaf and locations of soil-sampling										Total
	Sugarcane		Bean	Cabbage	Sorghum	Sesame	Peanut	Sweetpotato	Sisal	Loofah	
	Luchu	Jente	Luchu	Luchu	Jente	Tainan	Heng-chun	Hengchun	Heng-chun	Yuan-chang	Ave.
0-5	73	50	63	53	73	40	67	47	60	10	53.6
5-10	45	30	47	60	27	53	53	45	47	5	41.2
10-20	50	17	47	52	27	13	20	43	33	0	30.2
20-30	40	10	20	7	13	27	15	43	15	0	19.0
30-40	10	7	3	10	4	0	5	5	5	0	4.9
40-50	5	0	2	3	0	7	0	0	2	0	1.9
50-60	4	0	0	1	1	3	0	1	1	0	1.1
Ave.	32.42	16.28	26.00	27.00	20.71	20.42	22.85	26.28	23.28	2.14	21.7

The saprophytic activity of *R. solani* in soil of kenaf field was greatly influenced by the previous crop. The highest saprophytic activity was obtained from the field where the preceding crop was sugarcane, bean, sorghum, peanut, or sisal. Much less

debris particles colonized by *R. solani* were collected from cabbage, sesame, sweetpotato, or especially loofah field.

Formation of sclerotia in the isolates: The isolates of *R. solani* differed in ability to form sclerotia in various soils and culture media. Isolate K-1 formed more sclerotia in natural than in other soils. Sterilized soil recontaminated with natural soil formed 57.92 sclerotia in 100 g samples of soil. No difference in formation of sclerotia was found in natural soil amended with sodium nitrate and in sterilized soil; both of them produced 49 sclerotia in 100 g samples. For isolate K-2, sclerotia were equally abundant in natural soil and in natural soil amended with sodium nitrate. The number of sclerotia in the sterilized soil was roughly 1/5-1/4 of that in the natural soil either amended with sodium nitrate or not. In striking contrast to the above two isolates, isolate S-1 only produced very few sclerotia in natural soil. Natural soil amended with sodium nitrate and sterilized soil recontaminated with natural soil seemed to be more suitable for the isolate to form sclerotia.

Czapeck's agar seemed to be the best culture medium for isolate K-1 and K-2 to form sclerotia. Potato sucrose agar was the next. Both isolates produced sclerotia sparingly or none on water agar. Isolate S-1 formed only a few sclerotia on potato sucrose agar, and none on both Czapeck's agar and water agar.

Discussion

Results obtained from this study indicate that both hyphae and sclerotia of *R. solani* are important sources of primary infection. From the comparison of pathogenicity of hyphae and sclerotia on kenaf seeds and seedlings, it can be assumed that the saprophytic hyphae in the soil cause more severe diseases in shorter time than the sclerotia. This is due to the fact that the saprophytic hyphae cause infection directly when suitable host plants are present, whereas the sclerotia need to germinate and produce hyphae first, and then infection follows.

Fungal population vary considerably with age and kinds of the crops grown (6). The effect of rhizosphere on *R. solani* is considered to be different from crop to crop (6,10). In addition, the vertical distribution of root debris particles in the soil is also different with rotation systems. A comparison of soil samples from 10 fields where different crops were grown before planting of kenaf showed that loofah root harbored less *R. solani* than those of any 8 other crops. The results of present work and those of others (2,8,9,12) indicate that hyphae and sclerotia of *R. solani* are well entrenched in soil organic debris. It was also found that the saprophytic activity of *R. solani* was greatest in a soil layer to a depth of 30 cm. Approximately 19.0-53.6% of the debris particles in this vertical area carried inoculum of *R. solani*. Martinson (7) demonstrated that damping-off incited by *R. solani* was primarily influenced by inoculum density. The severe occurrence of kenaf damping-off is definitely true

under such a high inoculum density of *R. solani* in the Southern Taiwan (Table 2).

The genotype of the fungus as an important factor of influencing formation of sclerotia is indicated by the capabilities of different isolates to produce sclerotia (Table 3 and 4). This is supported by Houston (5) and Sanford (11). The data in Table 3 also indicate that the isolate K-1 and K-2 produce more sclerotia in natural soil, whereas the isolate S-1 prefers natural soil incorporated with sterilized soil. Therefore, formation of sclerotia by *R. solani* seems to be closely related with the substrates and isolates concerned.

Table 3. Ability of sclerotia formation of the isolates of *Rhizoctonia solani* in various soils

Isolate	Number of solerotia in 100 g samples			
	Natural soil		Sterilized soil	Sterilized soil plus natural soil
	No $NaNO_3$	$NaNO_3$		
K-1	81.82	49.45	49.65	57.92
K-2	126.95	131.30	27.45	55.70
S-1	0.64	8.75	3.35	7.87

Table 4. Comparison on the ability of sclerotia formation among 3 isolates of *Rhizoctonia solani* on culture media

Culture media	Isolate		
	K-1	K-2	S-1
PSA	Medium	Medium	Slight
Czapeck's agar	Abundant	Abundant	None
Water agar	Very slight	None	None

* The degrees of sclerotia formation were classified into 5 classes: abundant, medium, slight, very slight, and none

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鐘麻腰折病病原菌（*Rhizoctonia solani*）初次侵染源之研究

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中 文 摘 要

土壤內 *Rhizoctonia solani* 之腐生菌絲及菌核均爲該病原菌之初次侵染源。惟兩者對蓖麻種子及幼苗之致病力菌絲較菌核強。*R. solani* 在土壤內之腐生活動大部份均集中在自土面以下 30 公分間的有機殘體上。在臺灣南部地區 9 種不同前作物之 10 處蓖麻田調查結果，此一深度範圍內之有機殘體平均 19.0—53.6% 帶有 *R. solani*，前作物絲瓜的蓖麻田含有 *R. solani* 之有機殘體則顯著減內產少。*R. solani* 產生菌核的能力因菌株而異。供試三菌株中之二菌株（K-1 及 K-2）在未消毒土壤生菌核數較消毒土壤內多，另一供試菌株（S-1）則在消毒土壤與未消毒土壤各一半均勻混合之土壤內最適合產生菌核。因此，判斷 *R. solani* 之菌核產生能力應同時考慮供應產生菌核之基質（土壤）種類及菌株類別。