PHYSIOLOGY OF PARASITISM

1. Growth, pathogenicity, and toxin production of *Rhizoctonia solani* Kuehn[1]

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Introduction

Microbial toxins which play causal roles in the disease development may be useful to clarify the nature and course of diseases since they permit the exclusion of the pathogens and simplify the elucidation of host-parasite interactions (Wheeler and Luke, 1963). This model probably does not hold true for all plant diseases, however, it seems very promising.

Newton and Mayers (1935) showed that autoclaved filtrates from culture of *Rhizoctonia solani* Kuehn caused stunting of carrot and turnip. Their findings were confirmed by Boosalis (1947, 1950) who demonstrated the role of toxin secretion by *R. solani* in relation to the formation of necrosis of soybean stem and root. It was also shown by the "cellophane" bag technique to illustrate the excretion of phytotoxic substances from this fungus (Kerr, 1956). Attention was also directed to the extracts of the Rhizoctonia-infected plants. The extracts from diseased plants showed significant inhibitory effect on the growth of mung bean seedlings (Lai and Wu, 1963). Recently, Sherwood and Lindberg (1962) reported that phytotoxic materials had phenolic and glycosidic properties. Nishimura and Sasaki (1963) detected 8 acidic substances in the course of isolation and purification of phytotoxic metabolites produced by this fungus. A marked toxicity to Ladino clover was caused by phenylacetic acid, *m*-hydroxy-phenylacetic acid, *p*-hydroxy-phenylacetic acid, and an unidentified compound isolated.

It has been shown that the pathogenicity of *Helminthosporium victoriae* Meehan and Murphy is closely correlated with its ability to produce victorin

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(Luke and Wheeler, 1955). And differences in toxin production are directly related to growth rates of the isolates tested. The present report will deal with the relation between pathogenicity and growth as well as toxin production of *R. solani* incubated at various temperatures, a possibility that appears not to have been investigated before. Reversal of Rhizoctonia toxin will be also concerned which may add to knowledge of the mode of action in disease development.

Materials and Methods

An isolate of *Rhizoctonia solani* Kuehn (ReCS5) highly pathogenic to mung bean seedling was used through-out the present studies. Mung bean (*Phaseolus aureus*) seeds were soaked in distilled water for 1 hour followed by treatment with an aqueous solution of 0.5 per cent mercuric chloride for another 1 hour. The treated seeds were rinsed in several changes of sterile distilled water. Two-day old cultures of *R. solani* grown on potato-dextrose agar (PDA) at 28°C were served as the sources of inocula (Lai and Wu, 1963).

Bioassay of the toxin was carried out by soaking the treated seeds in the various lots of culture filtrates or plant extracts examined. Usually, 20 seeds were placed in a Petri-dish containing 20 ml of the material to be assayed. These were incubated in a 28°C incubater for 2 days to allow the germination and growth of mung bean. The lengths of the seedlings were measured at the end of the experiments. Unless otherwise stated, culture filtrates or plant extracts to be assayed for phytotoxicity were strained through several layers of cheesecloth. They were then heated in a boiling water bath for 10 minutes. The supernatants thus obtained were diluted 2-fold with sterile water.

Culture fluids of the fungus was prepared by transferring several mycelial agar blocks from PDA slants to 500-ml Erlenmeyer flask containing 100-ml of Czapek's sucrose nitrate solution. These cultures were incubated at 28°C for two weeks as already mentioned in previous report (Lai and Wu, 1963). Plant extracts were made by grinding 10 g of chilled mung bean in a mortar with 40 ml of chilled sterile water. They were then treated in the manner stated prior to bioassay.

Gibberellic acid of Eastman Kodak products indicating 93% purity was used for the study on the reversal of Rhizoctonia toxin.

Results

Growth and pathogenicity.—Linear growth of *R. solani* on PDA and Czapek's agar showed that optimum temperatures were within the range of 25° and 31°C (Fig. 1). Colonies appeared to be lighter in color when they were grown on

Czapek's agar. At favorable temperatures, the mycelia of the fungus reached the edge of the plate of both agar media in 2 to 3 days.

As to the optimum tomperature for the elongation of mung bean seedling, it was found to be between 25° to 34°C though the dry weight of seedlings tended to be lower at the temperatures above 22°C when the seedlings were grown on water agar for 6 days (Fig. 1). The same results were obtained from the mung bean grown on Hoagland solution (Hoagland and Arnon, 1950) plus 1% agar and 1 or 4% glucose. The lowering of the dry weight at these temperatures might be attributed to the fact that all the plants were grown in dark since under light condition the growth of mung bean based on dry weight was shown to be higher between 28° and 30°C (Reddick, 1917).

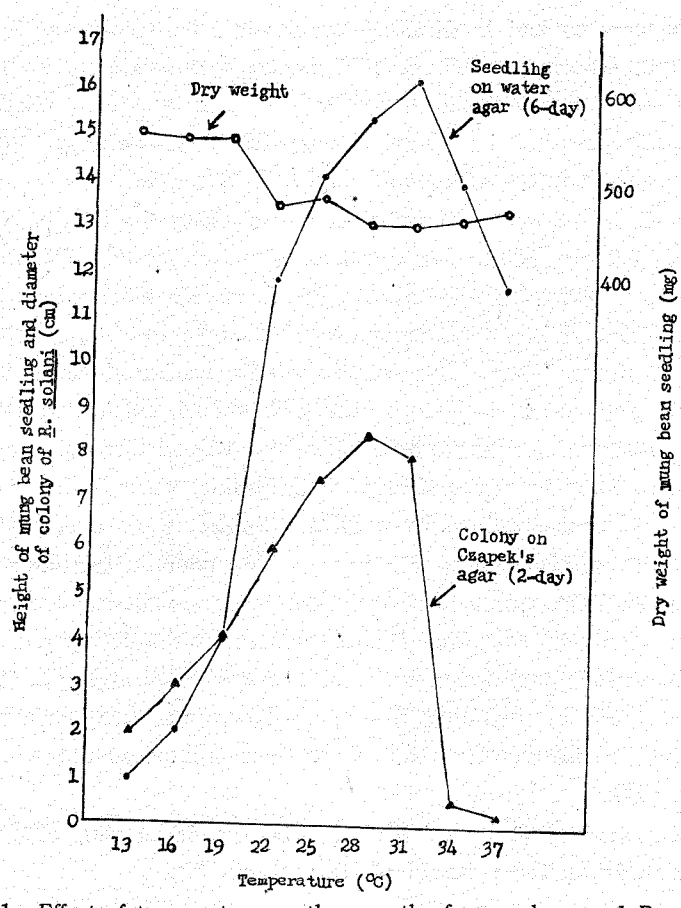

Fig. 1. Effect of temperature on the growth of mung bean and *R. solani*.

In order to study the relation between temperature and the pathogenicity of *R. solani*, U tubes of regular test-tube size were applied. Fifteen ml of Czapek's solution with 2% agar were pipetted into each tube, slanted to make a slope in the shorter arm of U tube, and kept at room temperature overnight.

Next day, 10 ml of 2% water agar were pipetted into the longer arm of U tube. All the procedures were carried out under aseptical conditions. Inoculation of the cultures was made by transferring a mycelial agar block on the agar slant of the shorter arm. These cultures were incubated at the temperatures of 13°, 16°, 19°, 22°, 25°, 28°, 31°, 34°, and 37°C for 3 days allowing the excreted substances to diffuse through agar. Five pregerminated mung bean seeds were then seeded on the surface of water agar in the longer arm of the tube to observe the growth of mung bean seedlings at 22°C. Tubes without inoculation of *R. solani* were served as control.

Table 1. *Effect of temperature on the growth and pathogenicity of R. solani.**

Pre-incubation Temperature (°C)	Growth of *R. solani***	Elongation of mung bean (cm)	
		Inoculated	Control
13	±	8-10	9-12
16	+	6-10	9-10
19	++	5-7	9-10
22	++	2-3	9-11
25	+++	3-8	9-11
28	++++	2-3	8-10
31	++++	2-3	8-11
34	+++	6-11	9-11
37	+	6-9	8-11

* *R. solani* was grown on the Czapek's agar slant in one arm of U tube for 3 days at a given temperature prior to seeding 5 pregerminated mung bean seeds on the water agar in another arm. The growth was measured 4 days after post-incubation at 22°C.

** Growth indicated in the order of increasing number of "+" was recorded 3 days after inoculation.

The data in Table 1 illustrated the results obtained. Elongation of mung bean seedlings which were grown in the tubes preincubated at temperatures of 22°, 25°, 28°, and 31° showed inhibitory effect of agar diffusible metabolites excreted by cultures of *R. solani* though the seedlings were infected by *R. solani* per se by submerged mycelia in agar at the end of experiment, particularly at these temperatures. The experiments were repeated several times with the same results. Apparently, 3-day old cultures of *R. solani* grown at these temperatures exerted the phytotoxic activity on the elongation of mung bean seedlings, however, the inhibitory effect caused by the fungus infection was not entirely ruled out by this technique. It should be emphasized that the data in Fig. 1, obtained from both host plant and the parasitic fungus under experimental conditions, are comparable to those in Table 1 where the inhibition

of seedling elongation was found at the temperatures favorable for the growths of the fungus and mung bean seedling.

Toxin production.—Culture filtrates, obtained from the cultures of *R. solani* incubated at temperatures of 13°, 16°, 19°, 22°, 25°, 28°, 31°, and 34°C, were bioassayed by taking 5 ml of culture fluid from a 500-ml flask each time at 2 days intervals. In this series of experiments, the growth of test plants grown in Petri-dish containing culture filtrate and a piece of filter paper was better than those grown in Petri-dish with culture filtrate alone. The active materials seemed to be absorbed by filter paper. For this reason, filter paper was no longer used in the later experiments. The data from one of the tests are shown in Table 2.

Table 2. *Effect of temperature on the growth and toxin production of R. solani.*

Temperature (°C)	13	16	19	22	25	28	31	34
Growth of *R. solani**	+	++	++	++++	++++	++++	++++	++
Toxin formed after incubation for (day)**	—	—	—	14	10	10	10	—

* Growth was recorded by visual inspection, number of "+" indicated the extent of mycelial growth on liquid media.

** "—" showed the failure in detecting toxin 16 days after incubation of the cultures of *R. solani*.

The resultant data (Table 2) showed that the time required for the toxin production was shorter in the cultures incubated at the temperatures of 25° to 31°C where the favorable growth of *R. solani* was observed.

Toxin obtained from Rhizoctonia-infected seedling—Water extracts of inoculated and uninoculated mung bean seedling grown on 1% water agar incubated at various temperatures were assayed for the phytotoxic materials. At the same time, water extracts obtained from water agar on which mung bean seedling had grown were also tested. The extracts of water agar were made by adding 40 ml of sterilized distilled water to each flask in which the seedling was previously removed. Extraction was made by shaking at 3–5°C for 2 to 3 hours and collecting the water extracts by decantation. They were then treated in the manner already mentioned prior to assay. Here, the inoculated plant materials were prepared by transferring mycelial suspension of *R. solani* to 500-ml Erlenmeyer flask in which mung bean seeds were germinated and grown for 5 days at 25°C prior to inoculation. They were then incubated at 16°, 19°, 22°, 25°, 28°, 31°, and 34°C for 10 days to secure the heavily infection of mung seedlings. Before extraction, they were kept in a cold room of 2°C for a week to chill them before grinding and extraction. The data in Table 3 illustrated

the results obtained in one of the experiments in this series.

Attempts to determine the phytotoxic substances in water extracts obtained from mung bean seedlings inoculated and uninoculated with *R. solani* were complicated by random fluctuations which occurred among replicates when the measurements were made by the present assay method. Although the inhibitory effect of both inoculated and control plants fluctuated considerably, the similar patterns of phytotoxic effect were obtained from inoculated plant and water agar on which inoculated plants were grown at the temperatures of 28° and 31°C.

Table 3. *Phytotoxicity of water extracts obtained from mung bean seedling incubated at various temperatures.**

Temperature (°C)	16	19	22	25	28	31	34
D-A**	2.9*** (0.4-9.3)	2.1 (0.2-7.1)	3.2 (0.4-8.4)	1.2 (0.2-8.0)	0.4 (0.1-4.5)	1.1 (0.2-4.1)	1.1 (0.2-4.6)
H-A	1.9 (0.2-6.8)	3.5 (0.2-7.6)	4.3 (0.2-7.5)	2.7 (0.4-8.5)	3.1 (0.3-8.7)	2.1 (0.2-7.5)	3.3 (0.2-8.5)
D-P	1.0 (0.1-7.5)	1.4 (0.2-7.1)	1.1 (0.2-7.2)	2.4 (0.2-10.1)	0.6 (0.2-3.0)	1.0 (0.1-5.6)	0.8 (0.1-5.5)
H-P	2.8 (0.4-7.9)	1.5 (0.1-7.2)	3.5 (0.2-10.1)	2.3 (0.1-9.1)	2.3 (0.1-6.8)	2.0 (0.1-5.9)	1.3 (0.2-6.4)

* Seedlings grown in sterile water were 0.3 to 10.2 cm in length with average of 5.7 cm.

** D-A indicated the water agar on which inoculated plants, D-P, were grown; H-A, the water agar on which uninoculated plants, H-P, were grown.

*** Average figures and the ranges of figures in parentheses were the lengths of mung bean seedlings (cm).

Reversal of Rhizoctonia-toxin by gibberellic acid—an attemp was made to search for a possible growth regulator to encounter the effect of Rhizoctonia-toxin by using different concentrations of gibberellic acid with 2-fold diluted culture filtrate. Repeated experiments in this series failed to eliminate the inhibitory effect of Rhizoctonia-toxin on elongation of mung bean seedlings by gibberellic acid when culture filtrate was mixed with gibberellic acid at different concentrations, *i.e.* 5, 10, 15, 20, and 25 p. p. m. In contrast, the inhibitory effect of Rhizoctonia-toxin was reversed when seeds presoaked in culture filtrate for 2 hours were successively treated with gibberellic acid for another 2 hours prior to germinating and growing in sterile water. The results shown in Table 4 illustrated a possible reversal of Rhizoctonia-toxin by gibberellic acid at the concentrations of 10 and 20 p. p. m.

Table 4. *Reversal of Rhizoctonia-toxin by different concentrations of gibberellic acid (GA).*

Concentration of GA (p. p. m.)	Treated mung* bean seeds	Control**
0	2.7	2.2
5	3.2	2.8
10	3.9	1.6
15	2.1	2.6
20	3.8	2.8
25	3.1	2.5

* Mung bean seeds were successively presoaked in 2-fold diluted culture filtrate and a given concentration of GA for 2 hours before germinating and growing in sterile water at 28°C.

** Control was carried out exactly the same except that sterile water was used instead of culture filtrate for presoaking.

Discussion

Differences in pathogenicity among the isolates of *H. victoriae* were found to be directly related to differences in the quantity of toxin produced and the time required for toxin production which in turn was correlated to the growth rates of the 3 cultures (Luke and Wheeler, 1955). Contrarily, Wyllie (1962) found that growth rate and pathogenicity failed to meet a direct correlation among the isolates of *R. solani*. The difference in observed host reaction was assumed to be a quantitative difference in toxin production by the isolates of the same fungus. Studies of the effect of temperature on the infection of mung bean seedling by an isolate of *R. solani* showed that the pattern of the temperature effect on infection was more or less similar to that on the growth of the fungus (Lai, and Wu, 1963), Evidence of the correlation among the growth, pathogenicity, and toxin production of an isolate of *R. solani* was demonstrated in the present experiments. This seemed to eliminate the possibility that host resistance was the only factor for the failure of infection of mung bean by this fungus at extreme temperatures, *i. e.* 13°, 34°, and 37°C. Water extracts obtained from Rhizoctonia-infected seedling and water agar from which infected seedlings were removed provided the further evidence of the important role of Rhizoctonia-toxin in disease development.

Germinations of soybean (Boosalis, 1947, 1950), alfalfa (Sherwood and Lindberg, 1962), and clover (Nishimura and Sasaki, 1963) were greatly impaired by Rhizoctonia-toxin. Stunting (Newton and Mayers, 1935) and necrosis (Boosalis 1947, 1950; Kerr, 1956; Wyllie, 1962; Sherwood and Lindberg, 1962) were also known to be the phytotoxic effect of this toxin. Secondary root

inhibition (Boosalis, 1947, 1950; Wyllie, 1962; Sherwood and Lindberg, 1962) were also observed. Yet, the mode of action of this toxin is not known.

Wooley (1954) used a growth factor, strepogenin structurally similar to lycomarasmin produced by *F. lycopersici*, to overcome the toxic action of lycomarasmin in tomato leaves. Recently, Wheeler and Luke (1963) cited Farkas and Levrekoich's work that the decrease in soluble protein as well as chlorosis in tobacco leaves induced by culture filtrate of *P. tabaci* could be suppressed by spraying the leaves with kinetin. Petersen et al. (1963) reported that severity of the disease caused by *R. solani* was enhanced by treatment of gibberel lic acid. In view of the phytotoxic effect of Rhizoctonia-toxin, an attempt to search for a possible antagonist of this toxin seemed to be plausible for the elucidation of the mode of action of this toxin. The data obtained showed that gibberellic acid seemed to reverse the inhibitory effect of mung bean seedling by Rhizoctonia-toxin.

Summary

In the present studies, the growth, pathogenicity, and toxin production of an isolate of *R. solani* were found to be directly correlated when the cultures of this fungus were incubated at different temperatures. Plant extracts obtained from infected seedlings provided the further evidence of important role of Rhizoctonia-toxin in the disease incidence. A possible reversal of Rhizoctonia-toxin by gibberellic acid was illustrated.

寄 生 生 理

1. *Rhizoctonia solani* Kuehn 之生長，病原性，及毒素生產之關係

吳 龍 溪

本試驗發現綠豆苗立枯病病原菌 (*Rhizoctonia solani*) 所分泌之毒素與該病菌病原性具有密切關係。病原菌培養液在攝氏 25°、28°、以及 31°C 培養十日後毒素之含量可達抑制綠豆苗生長之程度。上述溫度亦係該病菌及綠豆生長之適溫，由此可知苗立枯病之爲害程度與病菌分泌毒素有關。以在上述溫度生長之病植物抽出液測定毒素之存在，更可瞭解病菌所分泌之毒素對于病害產生之重要性。植物生長素之一，Gibberellic acid，能抵消病菌培養液抑制綠豆苗生長之作用。

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