PHYSIOLOGY OF PARASITISM

2. Biochemical Changes in the Mung Bean Seedling Infected with *Rhizoctonia solani* Kuehn*

LUNG-CHI WU**

(Received July 28, 1967)

Introduction

Disease is a disturbance in a chain of metabolic events leading to the visible symptoms, thus, the biochemical alterations of the host plants induced by parasitic attack may be more characteristic than macroscopic symptoms (Kern, 1956; Kiraly and Farkas, 1959). The response of a plant to infection have recently been reviewed in terms of metabolic changes at molecular level (Allen, 1959; Farkas and Kiraly, 1958; Kiraly and Farkas, 1959; Uritani and Akazawa, 1959; Uritani, 1965). However, biochemical studies on the host plants infected with *Rhizoctonia solani* during the disease development appear to be very meager.

The mechanism behind the response of plant to Rhizoctonia infection is a problem on which many researchers are now engaged. Many ideas have been postulated as to the macerating enzymes produced by *R. solani*. Bateman (1963, 1964) and his associates (Van Etten et al., 1967) show that endopolygalacturonase (PG) is more important than cellulase during the early phase of pathogenesis. Lai et al. (1966) have attributed an increase of cell permeability in mung bean to PG activity during infection by this fungus. A high correlation is found between PG activity of various isolates of *R. solani* and their pathogenicity, though the pectin methylesterase (PME) activity is not correlated with pathogenicity as is the case with PG (Barker and Walker, 1962). A few workers emphasize that pectin lyase may have been at least partially responsible for tissue maceration (Ayers et al., 1966; Sherwood, 1964, 1966). Barker and Walker (1962) also indicate that there is no correlation between penetrability

* The research was partly supported by the National Council on Science Development. Given at International Symposium on "Plant Biochemical Regulation in Viral and Other Diseases or Injury." Tokyo, Japan, August, 1967.

** Professor, Department of Plant Pathology and Entomology, National Taiwan University. The author is concurrently a Research Fellow of the Institute of Botany, Academia Sinica.

and subsequent pathogenicity since they observe an isolate which does not penetrate bean, but has high PG activity, is highly pathogenic on cabbage.

Ideas have been put forth attempting to equate the action of other metabolites of the fungus with that of macerating enzymes. The basis for this supposition is the fact that a browning and discoloration of host cells in necrotic lesion is often in advance of the penetrating hyphae (Boosalis, 1947, 1950). The toxic substances, produced by the fungus, often reduce seed germination (Boosalis, 1947, 1950; Nishimura and Sasaki, 1963; Sherwood and Lindberg, 1962), inhibit development of secondary root (Boosalis, 1947, 1950; Sherwood and Lindberg, 1962; Wyllie, 1962) as well as growth of root (Aoki et al., 1963; Lai and Wu, 1963), and subsequently induce stunting of the host plants (Newton and Mayers, 1935; Lai and Wu, 1963; Wu, 1965). Necrosis formation is shown by the "Cellophane" bag technique (Kerr, 1956) and also with culture filtrate (Boosalis, 1947, 1950; Sherwood and Lindberg, 1962; Wyllie, 1962). Plant extracts obtained from infected seedlings show significant inhibitory effect on the growth of mung bean seedlings (Lai and Wu, 1963; Wu, 1965). The growth, pathogenicity, and toxin production of the fungus are also found to be directly correlated (Wu, 1965). These findings provide the further evidence of the important role of toxic metabolites in the disease incidence.

Recently, Sherwood and Lindberg (1962) show that the phytotoxic metabolite of the fungus is phenolic and glycosidic in nature. Nishimura and Sasaki (1963) isolate phenylacetic acid, *m*-hydroxy-phenylacetic acid, *p*-hydroxy-phenylacetic acid, and unidentified compound. They find these compounds toxic to Ladino clover. Of these, two are also found in both culture filtrates and infected roots of sugar beet by Aoki et al. (1963). From culture filtrate of this fungus, Nour el Dein and Sharkas (1965) also isolate a colorless needle shape crystalline compound characterized by absorption peaks in ethanol at 212, 270, and 436 mμ. However, the mode of action of these toxins is not understood in relation to disease development.

The present study is undertaken to determine the effect of infection by the fungus and of culture filtrate on the patterns of reducing sugars, amino acids, nucleic acids, and proteins in the mung bean during germination and growth. The objective of the present investigation is also to ascertain whether a metabolic change, accompanied by synthesis of the microbial toxin of the fungus, occurs in the nature when the host plant is inoculated with the fungus per se.

Materials and Methods

An isolate of *Rhizoctonia solani* (ReCS 5) highly pathogenic to mung bean seedling was used throughout the present studies. The mung bean (*Phaseolus*

aureus) used for this work included two groups of varieties, i. e. "Large grain" and "Small grain", which were different from that used for previous work (Wu, 1965).

Mung bean seeds were soaked in distilled water for 1 hour, and in an aqueous solution of 0.5% mercuric chloride for another 1 hour, and then rinsed in several changes of sterile distilled water. The disinfected seeds were placed in a specially made petri dish (12 cm in diameter and 10 cm high) containing 100 ml of 1% water agar, 150 seeds per dish, and allowed to germinate and grow at 26°C in the dark. Inoculation of the plants was made by pouring a fragmented mycelial suspension of *R. solani*, grown on potato-dextrose agar (PDA) for 2 days at 26°C, onto seeds at the time when they were seeded on water agar.

Culture fluid of the fungus was prepared by seeding 3 mycelial discs from 2-day old *R. solani* grown on PDA to 500-ml Erlenmeyer flask containing 100-ml of Czapek's sucrose nitrate solution. The cultures were incubated at 28°C for two weeks. Culture filtrates were obtained by straining through two layers of cheese cloth. An appropriate amount of culture fluid was distributed in a flask and heated in a boiling water for 10 minutes. The heated culture fluid was added to autoclaved melting agar to bring the concentration to 1% water agar impregnated with a given dilution of culture filtrate. The mung bean seeds were placed on the solidified agar plate as already described. Control plants were grown on water agar alone.

Seeds or seedlings of uniform size were harvested at intervals of 1 day, weighed, and stored in a freezer at -15°C. for later use. It was assumed that storage did not affect the components to be determined. Samples of 5 grams of seed or seedling were fractionated in the following manner. The frozen samples were blended with chilled 80% (v/v) ethanol solution (100 ml) for 1 minute to extract free amino acids and sugars. The extract combined with 20 ml washing from a blender jar was placed on a hot plate to boil for 10 minutes, cooled, and centrifuged at 10,000 X g. for 10 minutes. The supernatant was collected by decantation and the residue was reextracted with 20 ml portion of 80% ethanol. The supernatants were combined and diluted to 100 ml with 80% ethanol. Portion of the alcohol extract was saved for amino acid analysis and 50 ml of alcohol extract was placed in a beaker and evaporated on a hot plate to free the alcohol until the odor of alcohol disappeared. To the insoluble masses were added 25 ml distilled water and heated to break up. Having cooled to room temperature, the solution was transferred to a 100 ml volumetric flask and clarified by standard method using saturated neutral lead acetate and sodium oxalate (AOAC, 1960). The solution was analyzed for reducing sugar. Residues after the alcohol extraction were suspended in 35 ml distilled

water and 35 ml of 10% trichloroacetic acid (TCA). The mixture was heated for 15 minutes at 90°C with occasional stirring to extract and centrifuged. The residue was washed with 50 ml of 5% TCA and centrifuged. The combined supernatants were analyzed for nucleic acids. TCA insoluble residue was suspended in 100 ml of 0.2% sodium hydroxide, heated for 10 minutes at 90°C with occasional stirring, and centrifuged. The supernatant was saved for the analysis of protein. All the extracts were stored in a freezer until before use.

The photometric method of Somogyi (1952) and Nelson (1944) were adopted to analyze the reducing sugar and the values were expressed in mg glucose equivalents. Free amino acids were determined by the colorimetric method of Rosen (1957) with 3% ninhydrin solution. The yield of amino acids was calculated on the basis of glutamic acid. Nucleic acids were analyzed with orcinol as described by Schneider (1957). The Folin-Ciocalteu method (Lowry et al., 1951) was used for protein determination.

Results

The data of reducing sugar analysis indicated that the infection caused the drastic increase of simple sugar in the early stages of disease development (Fig. 1). In the ungerminated mung bean seeds, reducing sugar accounted for very low level and only slight increase was observed by day 2. The reducing sugar content began to increase between day 2 and day 3. By day 3 the reducing sugar accounted for 3.9% and 8.7% of the fresh weight of healthy and diseased seedlings, respectively. Apparently, the reducing sugar content in inoculated plants was more than twice that in healthy plants. The reducing sugar content started to decrease between day 4 and day 5 in both healthy and diseased plants. An extremely rapid decline in the reducing sugar may be attributed to the limited sources available for the reducing sugar. The visible symptoms, brown colored necrosis on hypocotyls and roots at agar level, appeared on day 4, thereafter the levels of reducing sugar in healthy and diseased seedlings decreased drastically. By day 8 the inoculated plants were almost colapsed.

The similar trend of reducing sugar pattern was obtained from mung bean seedlings grown on water agar impregnated with different dilutions of culture filtrates (0, 5, 10, 20%). As shown in Fig. 2, the trend is more obvious when the data expressed on the basis of fresh weight of seedlings were plotted against the time of seed germination and seedling growth. An abrupt increase in reducing sugar content might be ascribed to the sugars derived from culture filtrate since Czapek's solution contained 3% sucrose as a carbon source for growth of the fungus. Sucrose content was found to be very high in 6-day

old culture of the fungus grown in Czapek's solution containing 1.5% sucrose (Ogura et al., 1961). However, when the data were expressed on the basis of unit seedling, only those seedlings grown on water agar impregnated with 5% of culture filtrate showed the increase of reducing sugar about twice over that of control plants.

Amino acid content was also raised by the infection of the fungus in the early stages of disease development. The results are given in Fig. 3, in terms of μ moles of amino acid per gram fresh weight of seeds or seedlings and also of μ moles of amino acid per plant. The effect of infection on the free amino acid pattern was not as pronounced as that on the pattern of reducing sugar. In first 2 days after planting, there was not much difference in the amount of ninhydrin-positive materials between healthy and inoculated seedlings, i.e. from 25 μ moles to 53 μ moles in healthy plants and to 58 μ moles in inoculated plants. By day 3 the amino acid contents in inoculated plants reached a peak which was 1.4 times over that of control plants, thereafter free amino acid decreased steadily in the diseased plants throughout the disease development. This might be due to the accumulation of fungal metabolites which impaired the free amino acid pool. This was clearly illustrated by the data obtained from seedlings grown on different dilutions of culture filtrate of the fungus (Fig. 4).

Both nucleic acid and protein analyses indicated that total nucleic acid and protein were depleted rapidly during the fiirst three days of germination and growth. By day 3, the nucleic acids of healthy and inoculated seedlings accounted for only 25% and 18% of that in ungerminated seeds, respectively (Fig. 5); 33% and 26% of ungerminated seed protein were found in the healthy and inoculated seedlings, respectively (Fig. 7). Infection by the fungus seemed to cause a slight decrease in the nucleic acid and protein contents. When the seedlings were grown on the culture filtrates of different dilutions, the patterns of nucleic acid and protein were found to be very similar between the plants infected with the fungus and the plants grown on water agar impregnated with 5% culture filtrate (Fig. 6 and Fig. 8).

As shown in Fig. 6, when comparison was made between control plants and plants grown on water impregnated with 20% of culture filtrate, nucleic acid content was found to be higher in treated plants than in control plants. This may imply that a fungal toxin may stimulate the nucleic acid synthesis or inhibit the degradation of nucleic acid only at certain dilution of culture filtrate. Thus, the effect of more diluted culture filtrate could be masked by other toxic materials secreted by the fungus whereas higher concentration, say 50%, decreased nucleic acid of the treated seedlings (Table 1). The same phenomenon was also noted in the protein content of seedlings grown on water agar impregnated with 50% culture filtrate as shown in Table 1.

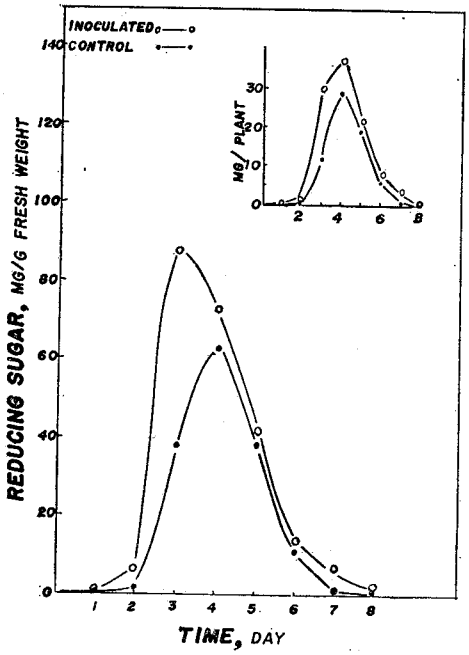

Fig. 1. Reducing sugars in healthy and diseased mung bean seedlings determined at various intervals following inoculation.

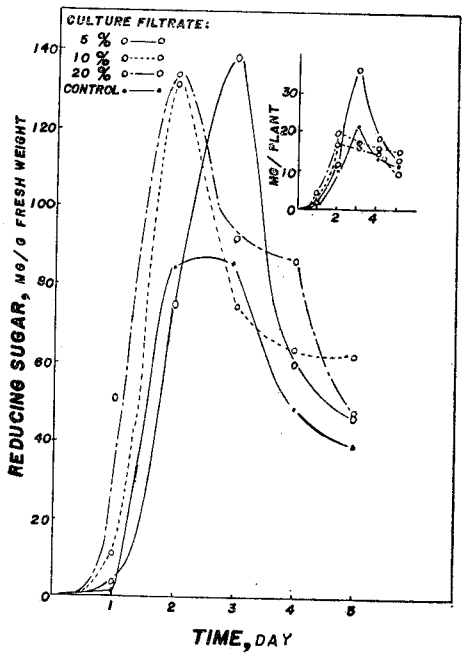

Fig. 2. Effect of culture filtrate on reducing sugar concentrations of mung bean seedling determined at various intervals following germination and growth.

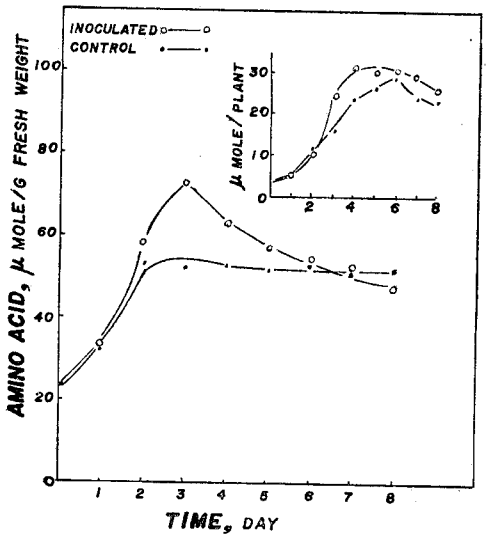

Fig. 3. Free amino acids in healthy and diseased mung bean seedlings determined at various intervals following inoculation.

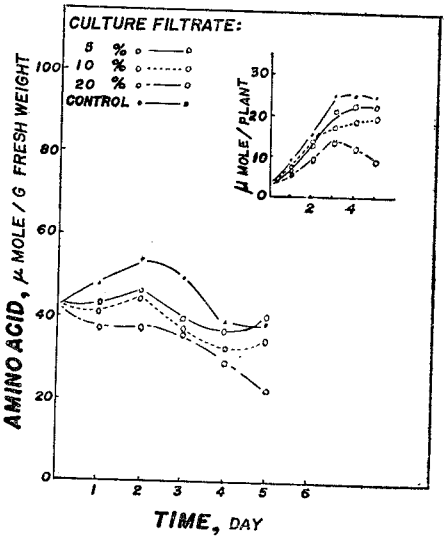

Fig. 4. Effect of culture filtrate on free amino acid concentrations of mung bean seedling determined at various intervals following germination and growth.

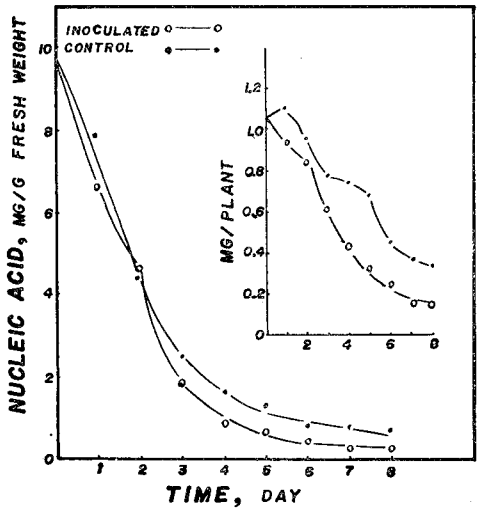

Fig. 5. Nucleic acids in healthy and diseased mung bean seedlings determined at various intervals following inoculation.

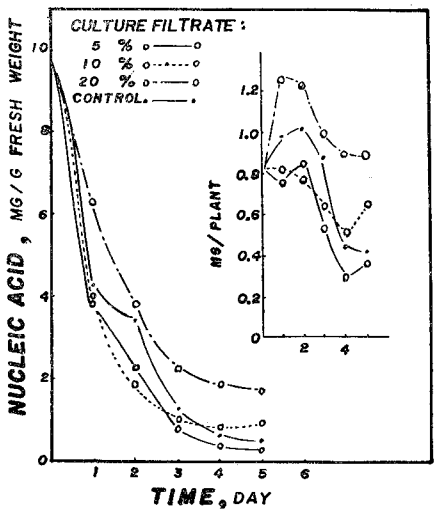

Fig. 6. Effect of culture filtrate on nucleic acid concentrations of mung bean seedling determined at various intervals following germination and growth.

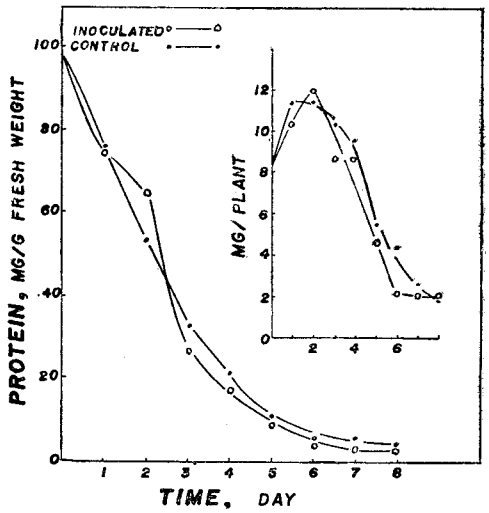

Fig. 7. Proteins in healthy and diseased mung bean seedlings determined at various intervals following inoculation.

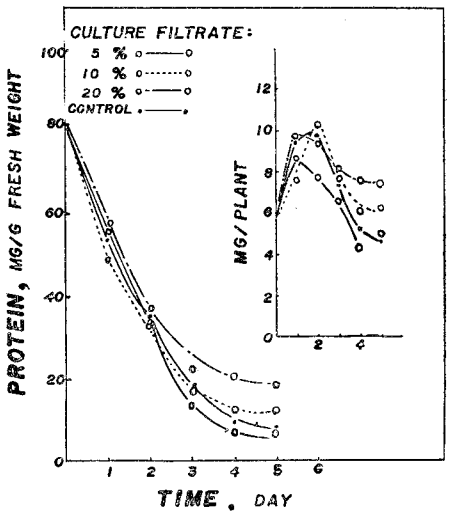

Fig. 8. Effect of culture filtrate on protein concentrations of mung bean seedling determined at various intervals following germination and growth.

Table 1. *Response of mung bean seedling to the culture filtrate of* ***Rhizoctonia solani****

Fraction	Age of seedling					
	1-day	2-day	3-day	4-day	5-day	6-day
Sugar	141	30	31	19	24	24
Amino acid	90	65	50	33	33	36
Nucleic acid	83	70	84	90	85	110
Protein	95	96	81	110	110	170

* Contents of reducing sugar, amino acid, nucleic acid, and protein in mung bean seedlings grown on water agar containing 50% culture filtrate of *R. solani* expressed as average percentage over the control plants grown on water agar.

Discussion

The primary objective of this study was to determine the biochemical changes in the inoculated plants and to compare the alteration in the chemical constituents of the plants grown on water agar containing different amounts of culture filtrates of *Rhizoctonia solani* thus to clarify the role of phytotoxic metabolites produced by the fungus in question.

Both reducing sugar and amino acid were found to rise in the early stages of disease development. On the fourth day after inoculation, the reducing sugar content of the inoculated plants became twice over that of healthy plants while the characteristic symptoms appeared one day later. Inman studied quantitative sugar changes in both obligate (1962) and facultative (1965) parasitism and indicated that the infection resulted in an increase in reducing sugars during early stages of infection and then declined as disease progressed. Although the effect of infection on free amino acid pool was not very great, ninhydrin-positive materials in inoculated plants rose to 1.4 times over that of control plants three days after inoculation. Similar trend was obtained by other workers (Burton and deZeeuw, 1961; Hrushovetz, 1954; McCombs and Winstead, 1961, 1964; Stretch and Cappellini, 1965). The critical period of pathogenesis is the time when the pathogen is becoming established. Changes in reducing sugars and free amino acids during the early period of disease development may be very meaningful, but the significance of such changes in pathogenesis is difficult to interpret. A careful studies on the metabolic turnover of the above mentioned biochemicals are necessary for an answer to the question.

Extremely rapid decline in the nucleic acid and protein in both healthy and inoculated plants was observed during the first three days of germination as calculated on the basis of fresh weight of tissues. However, with the graphs plotted with contents of nucleic acid and protein per plant slight

increase of both components were indicated within day 1 and day 2, and thereafter steady decrease throughout the growth was observed. The infection by the fungus seemed to reduce the nucleic acid and protein contents, particularly the nucleic acid content, whereas the RNA content of barley leaves was reported to increase immediately after the appearance of symptoms in obligate parasitism (Malca et al., 1964). Staples and Stahman (1964) also showed that small shift in protein synthesis was accompanied in the infection of a susceptible bean by a rust fungus.

Different dilutions of culture filtrate revealed different effects on the plants in their chemical patterns. When reducing sugar, amino acid, nucleic acid, and protein contents were calculated on unit plant, only reducing sugar content in the plants grown on water agar with 5% culture filtrate increased over control plants whereas an increase in nucleic acid and protein contents were observed in the plants grown on water agar containing 20% and 10% culture filtrates, respectively. However, the plants grown on water agar impregnated with 5% culture filtrate showed similar trend of reducing sugar, nucleic acid, and protein patterns to those infected with *R. solani*.

Although it can be mentioned with certainty from the foregoing results that culture filtrate involved multiple funtions in the biochemistry and physiology of the treated plants, it cannot, however, be stated with equal certainty that they are equally functional in the diseased plants infected with the fungus. Many biologically active substances produced by the fungus may be involved since many of them are found in the culture fluid of the fungus, e. g. a few derivatives of phenylacetic acid (Aoki et. al., 1963; Nishimura and Sasaki, 1963), gibberellin (Petersen et al., 1961, 1963), auxin (Dodman et al., 1966), and others (Van Etten et al., 1967). Protein concentration of pea is decreased in gibberellin A_3 treated plants whereas the carbohydrate fraction concentrations are increased reciprocally (Norcia et al., 1964). Accumulated evidence indicates that RNA synthesis and turnover is enhanced by plant hormones, other evidence suggests that the rate of RNA degradation may be reduced by indoleacetic acid (Davies, 1966).

From these findings mentioned above, one may speculate that a weak parasite with wide host range needs a complex of toxins, hormones, and enzymes to attack the host plants. A chain reaction of these biologically active substances may act at once or in sequence. This may be quite different from that of the host-specific plant toxins (Pringle and Scheffer, 1964) or pathotoxins (Wheeler and Luke, 1963) produced by the parasites with high specificity in their pathogenesis. The type of infection or infection mechanism may be determined by the nature of metabolites produced by the pathogen. The more specific the pathogenesis the narrower the host range may exist. It is

conceivable that the toxins (Ludwig, 1960) produced by a pathogen during the early development of infection may interfere the normal fluctuation of plant hormones so that the normal physiology and biochemistry of plant is disturbed. The plant hormones may regulate plant growth through nucleic acid and enzyme synthesis in sequence (Van Overbeek, 1966).

Summary

Infection of mung bean by *Rhizoctonia solani* caused an increase in both reducing sugar and amino acid fractions during the early stages of disease development. Three days after inoculation, reducing sugar content in inoculated plants was more than twice over that in healthy plants, whereas free amino acid rose to the level 1.4 times over that in the control plants followed by an appearance of characteristic symptoms on hypocotyls and roots one day later. Changes in reducing sugars and free amino acids during the early period of disease development may be very meaningful. The infection by the fungus reduced the nucleic acid and protein contents in diseased plants. Different dilutions of culture filtrate revealed different effect on the treated plants in their chemical changes. It seemed likely that the culture filtrates contained more than one biologically active substances. And the plants grown on water agar impregnated with 5% culture filtrate showed similar trend of reducing sugar, nucleic acid, and protein patterns to those mung bean seedlings infected with *Rhizoctonia solani* per se. Thus, the establishment of infection was thought to be determined by a complex of metabolites secreted by the fungus during the critical period of pathogenesis.

寄 生 生 理

2. 罹病綠豆苗之生化學研究

吳 龍 溪

綠豆受立枯病菌 *Rhizoctonia solani* 之感染後，還元醣與氨基酸在發病初期激烈增加，但核酸與蛋白質之含量却反而減少。接種後 3 日，其還元醣與氨基酸之含量增至對照區健全綠豆苗之 2 與 1.4 倍之多，然而接種後 4 日，典型病徵出現後，二者均遞減，其值漸次接近對照區健苗。核酸與蛋白質在綠豆發芽生長過程中，有遞減之傾向，尤以罹病綠豆苗特別顯著。接種後 3 日，核酸與蛋白質含量各已減至未發芽種子之18%與26%，比對照區健苗少 7%。以苗立枯病菌培養濾液處理綠豆時，綠豆發芽生長過程中，還元醣、氨基酸、核酸，以及蛋白質含量之變化，與受病原菌感染者頗相似，可見病原菌代謝物對本病害病態發展具有莫大之意義。而不同稀釋度病菌培養濾液對上述 4 成分之影響不一，各成分代謝對病菌代謝物敏感度固然不等，但病菌代謝物有多種，而其不同生理機能也可影響處理區綠豆苗化學組成一點，亦不可忽視。

Literature Cited

ALLEN, P. J. 1959. Physiology and biochemistry of defense. p. 435-467. In J. G. Horsfall and A. E. Dimond (ed.), Plant pathology Vol. 1. Academic Press, New York.

ALLEN, P. J. 1959. Metabolic considerations of obligate parasitism p. 119-129. In C. S. Holton, et al., (ed.), Plant pathology, Problems and progress, 1908-1958. Univ. Wisconsin Press, Madison.

AOAC. 1960. Official methods of Analysis, 9th Ed., Washington 4, D. C. 832 p.

AOKI, H., T. SASSA, and T. TAMURA. 1963. Phytotoxic metabolites of *Rhizoctonia solani*. Nature **200**: 575.

AYERS, W. A., G. C. PAPAVIZA, and A. F. DIEM. 1966. Polygalacturonate trans-eliminase and polygalacturonase prodcution by *Rhizoctonia solani*. Phytopathology 56: 1006-1011.

BARKER, K. R. and J. C. WALKER, 1962. Ralationship of pectolytic and cellulytic enzyame production by strains of *Pellicularia filamentosa* to their pathogenicity. Phytopathology **52**: 1119-1125.

BATEMAN, D. F. 1963. The "Macerating enzyme" of *Rhizoctonia solani*. Phytopathology **53**: 1178-1186.

BATEMAN, D. F. 1964. Cellulase and the Rhizoctonia disease of bean. Phytopathology **54**: 1372-1377.

BOCSALIS, M. G. 1947. Necrosis of soybean stem and root caused by *Rhizoctonia solani*. Phytopathology **37**: 3. (Abstr.).

BCOSALIS, M. G. 1950. Studies on the parasitism of *Rhizoctonia solani* Kuehn on soybeans. Phytopathology **40**: 820-831.

BURTON. C. L. and D. J. DEZEEUW. 1961. Free amino acid constitution of healthy and scab-infected cucumber foliage. Phytopathology **51**: 776-777.

DAVIES, D. D. 1966. The control of enzyme patterns in plant tissues. Biochem. J. 98: 37 p.

DODMAN, R. L., K. R. BARKER, and J. C. WALKER. 1966. Auxin production by *Rhizoctonia solani*. Phytopathology **56**: 875. (Abstr.).

FARKAS, G. L. and Z. KIRALY. 1958. Enzymological aspects of plant diseases. 1. Oxidative enzymes. Phytopathol. Z. **31**: 251-272.

HRUSHOVETZ, S. B. 1954. The effect of infection by *Helminthosporium sativum* on the amino content of wheat roots. Can. J. Bot. **32**: 571-575.

INMAN, R. E. 1962. Disease development, disease intensity, and carbohydrate levels in rusted bean plants. Phytopathology **52**: 1207-1211.

INMAN, R. E. 1965. Quantitative sugar changes in barley infected with a facultative parasite. Phytopathology **55**: 342-345.

KERN, H. 1956. Problems of incubation in plant diseases. Ann. Rev. Microbiol. **10**: 351-368.

KERR, A. 1956. Some interactions between plant roots and pathogenic soil fungi. Australian J. Biol. Sci. **9**: 45-52.

KIRALY, Z. and G. L. FARKAS. 1959. Biochemical trend in plant pathology. Phytopathol. Z. **34**: 341-364.

LAI, M.-T. and L.-C. WU. 1963. Some observations on the infection of mung bean seedling by *Rhizoctonia solani* Kuehn. Plant Prot. Bull. **5**: 386-397.

LAI, M.-T., A. R. WEINHOLD, and J. G. HANCOCK. 1966. Cell permeability increases in mung bean during infection by *Rhizoctonia solani*. Phytopathology **56**: 886. (Abstr.).

LOWRY, D. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265-275.

LUDWIG, R. A. 1960. Toxins. p. 315-357. In J. G. Horsfall and A. E. Dimand (ed.), Plant Pathology Vol. 2. Academic Press, New York.

MALCA, I., F. P. ZSCHEILE, and R. GULLI. 1964. Nucleatide composition of RNA in healthy and mildew-infected leaves of barley. Phytopathology **54**: 1112-1116.

MCCOMBS, C. L. and N. N. WINSTEAD. 1961. The amino acids and sugar compositions of healthy and anthracnose-infected watermelons. Phytopathology **51**: 644. (Abstr.).

MCCOMBS, C. L. and N. N. WINSTEAD. 1964. Changes in sugars and amino acids of cucumber fruits infected with *Pythium aphanidermatum*. Phytopathology **54**: 233-334.

NELSON, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. **153**: 375-380.

NEWTON, W. and N. MAYERS. 1935. The physiology of *Rhizoctonia solani* Kuehn. IV. The effect of a toxic substance produced by *Rhizoctonia solani* Kuehn when grown in liquid culture, on the growth of wheat, carrots, and turnips. Sci. Agr. 15: 399-401. (Abstr. in Rev. Appl. Mycol. **14**: 603).

NISHIMURA, S. and M. SASAKI. 1963. Isolation of the phytotoxic metabolites of *Pellicularia filamentosa*. Ann. Phytopathol. Soc. Japan **28**: 228-234.

NORCIA, L. N., J. D. EVANS, D. N. PAULKNER, and L. M. BOHRBANGH. 1964. Growth changes in pea plants induced by gibberellic acid and their effects on the concentration of tissue lipids. Biochem. J. **90**: 633-637.

NOUR EL DEIN, M. S. and M. S. SHARKAS. 1965. Isolation of a toxic substance from the culture filtrate of *Rhizoctonia solani*. Phytopathol. Z. **52**: 55-58. (Abstr. in Rev. Appl. Mycol. 44, 2614).

OGURA, H., S. AKAI, and T. SATO. 1961. Studies on *Pellicularia filamentosa* (Pat.) Rogers. II. On the formation of free amino acids and sugars in *P. filamentosa*. Ann. Phytopathol. Soc. Japan. **26**: 31-36.

PETERSEN, L. J., J. E. DEVAY, and B. R. HOUSTON. 1961. The effect of gibberellic acid on the parasitism of *Rhizoctonia solani* on bean cotton. Phytopathology 51: 67. (Abstr.).

PETERSEN, L. J., J. E. DEVAY, and B. R. HOUSTON. 1963. Effect of gibberellic acid on development of hypocotyl lesions caused by *Rhizoctonia solani* on red kidney bean. Phytopathology **53**: 630-633.

PRINGLE, R. B. and R. P. SCHEFFER. 1964. Host-specific plant toxins. Ann. Rev. Phytopathol. **2**: 133-156.

ROSEN, H. 1957. A modified ninhydrin colorimeter analysis for amino acids. Arch. Biochem. Biophys. **67**: 10-15.

SCHNEIDER, W. C. 1957. Determination of nucleic acids in tissues by pentose analysis p. 680-684. In S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology Vol. 3. Academic Press, New York.

SHERWOOD, R. T. 1964. Pectin trans-eliminase and polygalacturonase production by *Rhizoctonia solani* and other fungi. Phytopathology **54**: 907. (Abstr.).

SHERWOOD, R. T. 1966. Pectin lyase and polygalacturonase production by *Rhizoctonia solani* and other fungi. Phytopathology **56**: 279-286.

SHERWOOD, R. T. and C. G. LINDBERG. 1962. Production of a phytotoxin by *Rhizoctonia solani*. Phytopathology **52**: 586-587.

SOMOGYI, M. 1952. Notes on sugar determiantion. J. Biol. Chem. **195**: 19-23.

STAPLES, R. C. and M. A. STAHMANN. 1964. Changes in proteins and several enzymes in susceptible bean leaves after infection by the bean rust fungus. Phytopathology **54**: 760-764.

STRETCH, A. W. and R. A. CAPPELLINI. 1965. Changes in free amino acids and reducing sugars in Highbush blueberry fruit infected with *Glomerella cingulata*. Phytopathology **55**: 302-303.

URITANI, I. 1965. Molecular pathology in the plant field with special regard to defense action of the host. In Biochemische Probleme der kranken Pflanze. Vortaege eines Symposiums im Institut fuer Phytopathologie Aschersleben der Deutschen Akademic der Landwirtschaftswissenschaften. Tagungsberichte Nr. 74, Berlin.

URITANI, I. and T. AKAZAWA. 1959. Alteration of the respiratory pattern in infected plants. p. 349-390. In J. G. Horsfall and A. E. Dimond (ed.), Plant pathology Vol. 1. Academic Press, New York.

VAN ETTEN, H. D., D. P. MAXWELL, and D. F. BATEMAN. 1967. Lesion maturation, fungal development, and distribution of endopolygalacturonase cellulase in Rhizoctonia-infected bean hypocotyl tissues. Phytopathology **57**: 121-126.

VAN OVERBEEK, J. 1966. Plant hormones and regulators. Science 152: 721-731.

WHEELER, H. and H. H. LUKE. 1963. Microbial toxins in plant disease. Ann. Rev. Microbiol. **17**: 223-242.

WU. L.-C. 1965. Physiology of parasitism. 1. Growth, pathogenicity, and toxin production of *Rhizoctonia solani* Kuehn. Bot. Bull. Academia Sinica **6**: 144-152.

WYLLIE, T. D. 1962. Effect of metabolic by-products of *Rhizoctonia solani* on the roots of Chippewa soybean seedlings. Phytopathology **52**: 202-206.