TAXONOMIC AND CULTURAL CHARACTERIZATION OF *RHIZOCTONIA SOLANI* KUHN ISOLATE FROM SOYBEAN (*GLYCINE MAX* MERRIL)[1]

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ABSTRACT

Unsuccessful attempts were made to produce the perfect basidial stage of *R. solani* isolate designated as R. 5, from Beltsville – Maryland USA, soybean fields. The isolate was characterized according to its ability to sucessfully anastomose with tester isolates belonging to anastomosis group 4 (AG–4). Growth responses of the isolate were studied at four different temperatures the isolate growth was relatively insensitive to temperatures between 24 – 32ºC.

(Fitopatologia Brasileira 3: 241-247, 1978)

RESUMO

Caracterização taxonômica e cultural de um isolado de *Rhizoctonia solani* Kuhn obtido a partir de soja (*Glycine max* Merril).

Revelaram-se infrutíferas as tentativas de Produção do estágio perfeito de um isolado de *R. solani*, designado como R–5, obtido a partir de soja em Beltsville – Maryland (USA). O isolado em apreço foi caracterizado de acordo com sua habilidade de anastomosar com isolados testes pertencentes ao grupo 4 de anastomose (AG–4). O crescimento do isolado foi estudado em quatro diferentes temperaturas, tendo crescido indiferentemente na faixa de 24 – 32ºC.

(Fitopatologia Brasileira 3: 241-247, 1978)

(1) This work is a portion of the senior author's M.S. thesis at University of Wisconsin Madison.

INTRODUCTION

Because of the wide variation in morphology, pathogenicity, and physiology, the taxonomy and momenclature of *R. solani* has been the subject of much controversy for many years.

In 1891, Prillieux and Delacroix cited by Kotila, (1947) described the basidial stage of *R. solani*, Since then, the perfect stage has been used as a defined taxonomic characteristic. However, the relative infrequency of the basidial stage in nature, and the lack of a reliable method of inducing fruiting in many isolates are still to be overcome. Thus, a technique to induce sporulation may have to be developed for particular isolates.

Schultz in 1937 and Richter & Schneider in 1953, found another important phenomenon could be used for taxonomic and nomenclatural purposes. They grouped *R. solani* isolates according to the capacity to anastomose. The phenomenon has been demonstrated very extensively (Schultz, 1937; Richter & Schneider, 1953; Papavizas *et al.*, 1965; Parmeter *et al.*, 1969; Ogoshi, 1972a; 1972b), among isolates of fungus.

Studies on growth of *R. solani* isolates showed that the isolates frequently differ in their response to temperature requirements (Mildenhall, 1971).

In these studies attempts were made to: 1) induce the perfect stage of a *R. solani* isolate in culture; 2) place this isolate in one of the known anastomosis groups; and 3) determine the effect of temperature on the growth in liquid still culture.

MATERIAL AND METHODS

The *R. solani* isolate used, was designated as R–5 isolate (Lewis Personal communication), was obtained from Beltsville – Maryland (USA) soybean fields.

Three of the most commonly used methods were used to attemps the induction of sporulation of the R–5 isolate:

i) Agar culture tecnique (Hawn & Vanterpool, 1953; Flentje, 1956): This method has been used sucessfully for a large number of isolates. It is based on an application of the principles of transferring well nourished, actively growing vegetative cells into a less nutritive substratum Sherwood, 1970.

The R–5 isolate was cultured for seven days in potato dextrose agar amended with 5/liter of yeast extract (PYDA), followed by transferring a portion of the mycelium to 2% water agar and incubating at 26°C at a high relative humidity (above 80%), and in complete darkness. After 10 days, the cultures were examined for hymenial formations.

ii) **Water surface method** (Sims, 1956; Stretton & Flentje, 1964; Tuite, 1969): This method has been commonly used, basically because of its simplicity and relative efficiency. Like the first one, the isolate was cultured for seven days on PYDA, then the plates were covered with distilled water and incubated at 26°C in complete darkness. Similarly, the cultures were examined after 10 days.

iii) **Soil-oven-culture method** (Tu, 1970): This method is the most laborious and may be the most efficient. It is based on very recent studies on nutritional and physical requirements for vegetative growth and sporulation of *R. solani*. To accomplish this method, the isolate was grown on PYDA in 9-cm diamenter petri dishes at 28°C, and in the dark. When the mycelium covered the agar surface (approximately 3 days), the mycelium was covered with 90g of an oven-dried clay loam soil and adjusted to pH 8.5 with $CaCO_3$. The woil was maintained at 30% moisture (by weigth) and incubated under 220 foot candles light intensity in a 12-hour regime and at 24–26°C of temperature. Three days later the cultures were examined for basidium formation.

The anastomosis tester isolates used were supplied by Dr. N.A. Anderson (University of Minnesota).

The anastomosis tests were performed

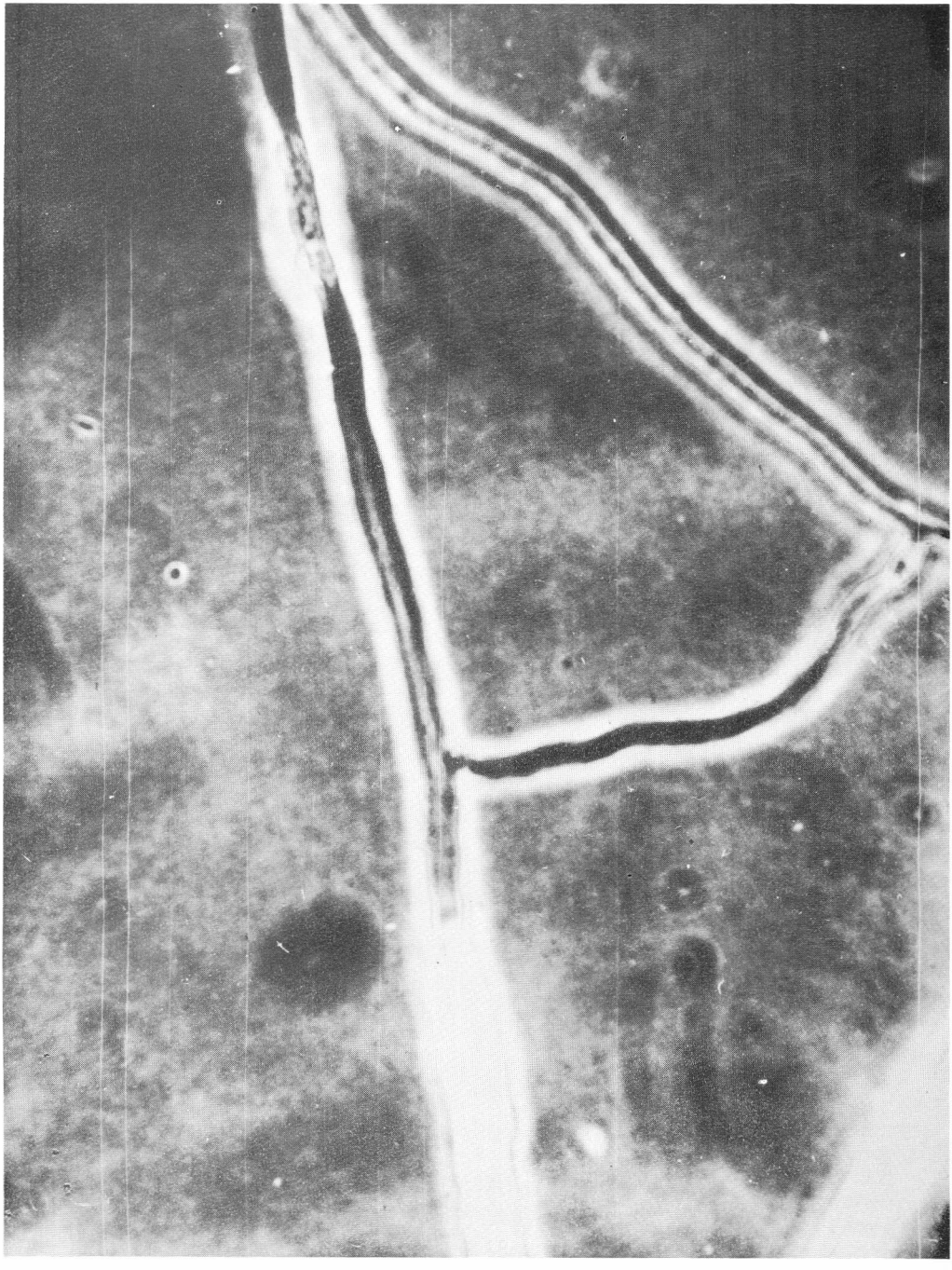

Fig. 1 Hyphal anastomosis between the R-5 isolare and AG-4 tester isolate of *R. solani. (phase contrast)*

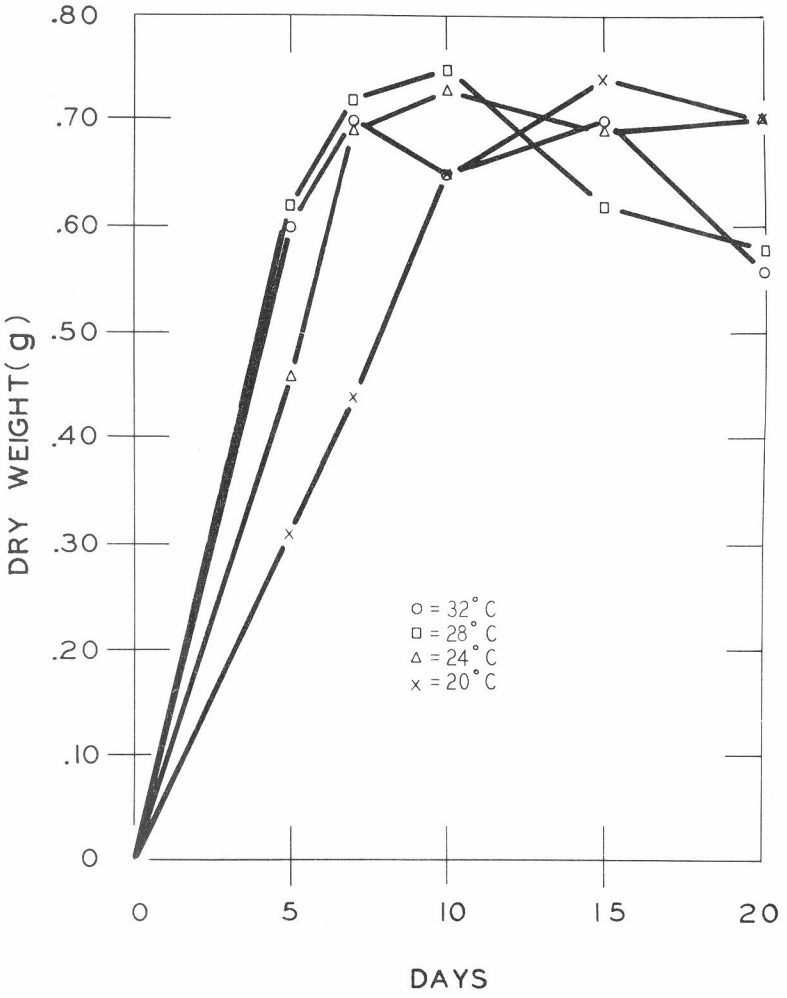

Fig. 2 Mean dry weight of *R. solani* (isolate R-5) grown at four different temperatures.

by a method described by Parmeter et al. (1969). The R–5 isolate was opponed to the tester isolates on 2% water in petri dishes. One pair of isolates was tested per petri dish. The culture was incubated at 24°C and in dark-ness until the growing hypha made contact (approximately 24 hours). A small portion (1 cm^2) of the area of contact between the two isolates was mounted on a microscope slide, stained with 0.001% cotton blue in diluted lactophenol (1 lactophenol: 9 water) and scanned microcopically for hyphal anastomosis.

The *R. solani* R–5 isolate from a four-day old culture on potato dextrose agar (PDA) was transferred to 300-ml flasks with 50ml of potato dextrose broth amended with 1 gram/liter of yeast extract, pH 6.3. The temperatures were 20°, 24°, 28°, and 32°C, and the experiment was conducted under complete darkness.

After 5, 7, 10, 15, and 20 days the oven-dried weight was determined.

The experiment was repeated three times and the mean was calculated.

RESULTS AND DISCUSSION

None of methods here tested induced any hymenial formation in any culture of the R–5 isolate. The experiments were repeated for three consecutive trials and no fruiting could be seen in any of those trials.

There may be several reasons for the lack of positive results.

Stretton *et al.* (1964) found that pathogenic isolates failed to fruit on agar. They also suggested that there were many different conditions required for fruiting among the different isolates. As the precise conditions are not known for many isolates, the lack of fruiting under artificial conditions is not surprising.

Isolates of *R. solani* vary as far as required conditions for sporulation, and the lack of knowledge of these factors could be a possible explanation for the unsuccessful attempts here presented. Only three methods were used and one single isolate out of hundreads of known in most of the reports.

Some authors also have reported negative results. Pitt (1964) did not succeed using water agar, soil and distilled water in inducing isolates of *R. solani* from eyespot of wheat to sporulate. Papavizas (1965) applied the method of transferring from rich to poor substrates with eight different rich media and five poor media, and out for 69 isolates, only eight fruited in only one combination of media (yeast extract – PDA to soil-extract agar).

Isolate R–5 is evidently a strain that is difficult to induce to form the perfect state. Studies on the influence of the environment on fruiting of this fungus have to be done in order to make use of this very important state, not only for taxonomic and nomenclatural use but also for genetic and cytological studies.

The R–5 isolate of *R. solani* successfully anastomosed with tester isolates of AG–4 (fig. 1), but failed to anastomose with other AG tester isolates. Following the suggestion of Parmeter *et al.* (1969), the successful anastomosis was confirmed by tracing back individual hyphae to insure that anastomosis was between paired isolate and not between branches of the same isolate.

The results confirm some suggestions as to host range of some groups. Ogoshi (1972a) pointed out that the majority of isolates of AG–4 were from leguminosae and sugar beets. Also, Flentje *et al.* (1970) reported that no successful anastomosis occurs between isolates that differ in their host range. Papavizas *et al* (1975) working with isolates from a soybean field in Maryland found the majority of isolates from soil and debris in this field belonged to AG–4 and all highly pathogenic isolates were in AG–4, a characteristic of the R–5 isolate.

The average growth on a dry weight basis of the fungus is presented in fig. 2. The R–5 isolates attained maximum growth

around 7 days at temperatures above 25°C. At 20°C there was a delay of three to four days until maximum growth occurred.

The data here presented indicated that the growth of the isolate was not limited by temperatures of 24°C or above until 32°C. It also confirms the findings of Gottlieb (1971) who pointed out the limited growth of fungi, including *R. solani*.

Lewis and Papaviazas (unpublished data) stated that the R–5 isolate was able to grow increasingly from 16° to 32°C, and cause preemergence damping-off at considerably higher temperatures.

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