RELATIONSHIPS OF CONCENTRATION AND AGE OF INOCULUM OF *RHIZOCTONIA SOLANI* KUHN ON PATHOGENICITY TO GERMINATING SOYBEAN SEEDS.

J. EMILSON CARDOSO[1], A.C. HILDEBRANDT[2] AND C.R. GRAU[2]

[1]Centro de Pesquisas Agropecuária do Trópico Úmido CPATU/EMBRAPA
Caixa Postal 48 – 66.000 Belém – Pará;
[2]Department of Plant Pathology – University of Wisconsin Madison Wi 53706 U.S.A.
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ABSTRACT

The relationship of inoculum concentration, determined by partially dried mycelial weight, to pathogenicity on germinating soybean seeds demonstrated that concentrations between 4 and 10g of mycelium per 2 liter of vermiculite were statistically equal and superior to concentrations lower than 4g.

Studies on the effect of inoculum age upon pathogenicity on soybean showed that as the fungus aged, the pathogenicity decreased.

(Fitopatologia Brasileira 3: 193–204, 1978)

RESUMO

Relações entre concentração e idade do inoculum de *Rhizoctonia solani* Kuhn e a patogenicidade em sementes de soja durante a germinação.

As relações entre a idade do inóculo de Rhizoctonia solani Kuhn, bem como de sua concentração, determinada através do peso seco do micélio parcialmente desidratado, e a patogenicidade sobre a germinação de sementes de soja demonstraram que concentrações entre 4 e 10g de micélio por 2 litros de vermiculite não diferenciaram estatisticamente entre si, mas foram superiores às concentrações abaixo de 4g micélio.

Estudo sobre o efeito da idade da micélio em relação a patogenicidade em soja mostraram que o fungo diminui sua patogenicidade à medida que o micélio envelhece.

(Fitopatologia Brasileira 3: 193–204, 1978)

INTRODUCTION

Rhizoctonia solani Kunh (Thanatephorus cucumeris (Frank) Donk) induces severe epidemics and results in consequent lower stands in soybean fields in Southern Brazil. The disease has caused stand losses up to 40% in soybean fields. (Lehman *et al.*, 1974). No control measure other than crop rotation is known, a fact which may be due to the lack of studies on the various aspects of the disease.

Since *R. solani* does not produce conidia and basidiospore production is either rare, difficult to obtain in culture, or lacking, as in the case of the R–5 isolate here used, mycelia and/or sclerotia are used as inoculum for pathogenicity tests.

Many techniques have been used to test pathogenicity of *R. solani* in the soil (Lehman, 1940; Leclerg, 1941; Smith, 1943; Staten & Cole, 1948; Houston, 1949; Bruehl, 1951; Sinclair, 1958; Ashworth & Amin, 1964; Owen & Gray, 1964) the technique of choice will depend upon several factors such as the host, ii) the type of disease, iii) the praticability of the technique and iv) the purpose of the work.

The R–5 isolate obtained from Maryland USA soybean fields and belonging to anastomosis group four primarily induce damping-off of germinating soybean seeds but it is also capable of attacking seedling roots and hypocotyls and forming lesions of variable sizes (Cardoso *et al.*, unpublished data) The damping-off effect of R–5 was the main concern of this research. However, the effects ou roots were not disregarded.

It was the purpose of these experiments i) to examine the effectiveness of a method of testing pathogenicity of *R. solani* R--5 isolate to soybean seeds and seedlings; ii) to correlate the amount of inoculum to damping-off and root and hypocotyl lesion inicidence in soybean and finnally iii) to establish a relationship between inoculum age and pathogenicity of *R. solani* isolate.

MATERIAL AND METHODS

A slight modification of the methods developed by Sinclair (1958) and Sims (1960) was used for testing pathogenicity of *R. solani*.

The *R. solani* R–5 isolate was grown for 10 days in 250ml flasks containing 50ml potato dextrose broth amended with 1g/liter of yeast extract (PYDB), pH 6.3, at 28°C, and in darkness. The broth mycelial mat was decanted and the mat was washed at least three times with distilled water and kept at approximately 30°C and low relative humidity for half an hour. The fresh mycelia were weighed. The inoculum concentrations were 1, 2, 4, 6, 8, and 10g. They were blendend in 200ml of distilled water for 20 seconds and incorporated to 2000cc of vermiculite. The infested vermiculite was placed into a 30 x 24 x 6cm pan and seeded with 5 rows of 10 seeds each. The soybean cultivar Chippewa 64 was used throughout the experiment. The pans were placed at 28°C temperature at 12--hour photoperiod, and uniformly watered until emergence occurred.

After 10 days the seedlings were harvested, counted, and the root lesions were scored. Also the dry weight and hypocotyl height were measured. A completely ramdomized design was used with five replications and the experiment was repeated three times.

A disease severity index (DSI) used consisted of a numerical index from 0 (zero) to 4; 0 = healthy non-infested plants; 1 plants with slight root or hypocotyl lesions; 2 = plants with larger root root or hypocotyl lesions byt without any apparent on growth; 3 = plants with large lesions girdling and showing visible efects on growth; and 4 = plants with roots or hypocotyl completely rotted, stunted and dying. (Fig. 1).

To teste the influence of inoculum age on the pathogenic ability of *R. solani*, the fungus was grown for 5, 7, 11 and 7 days on

the same liquid media: Four grams of mycelium was used for each different age.

RESULTS

The inoculum concentration of 1 and 2 gram of mycelium did not show any statistical difference as to damping-off and root/ hypocotyl lesions from the non-infested control. Howeves concentrations above 4 gram were similar, data which suggest that the inoculum threshold would be between 2 and 4 gram/ 2 liter of vermiculite concentrations (Fig. 2,3) (Table 1).

The effect of age inoculum in culture on pre-emergence damping-off and root/ hypocotyl lesion and dry weight of soybean cultivar Chippewa 64 is presented (Figs 4, 5) (Table 3, 4).

The data show a sharp decrease in pathogenicity as the fungal culture gets old. In fact after 17 days in culture the fungus no longer differed statistically from the infested control as to damping-off and did so as to disease severity.

No post-emergence damping-off was reported from the presente experiments.

DISCUSSION

According to many reports (Vi & Tachinai, 1955; Rich & Miller, 1962; Sneh *et al.*, 1966) it would be expected that as the inoculum concentrations increased there would be an increased in severity of disease. Since R. solani in soil is unlike the classical foliar pathogens where there is a need for multiple infection and yet show differences in severity. Other reports (Sanford, 1941; Blair, 1942; Das & Western, 1959) however suggested that high concentrations of inoculum reduced the pathogeniaty due to accumulation of staling products in the concentrated inoculum in can be concluded that at certain concentrations of inoculum there is an increased in disease incidence and, severity for an increase of inoculum density but when the density gets high it reverses the correlation.

The data here presented show a lack of positive correlation between inoculum density and pathogenicity above 4 gram/ 2 liter of vermiculite concentration. The absence of inough substrate for mycelial growth in two liters of vermiculite must account for these results.

The loss of pathogenic capabilities of the R–5 isolate of *R. solani* with time in culture is shown clearly on the presented data (Figs 4.5).

Gottlieb's (1971) concept of stopping growth of fungi after a certain period of time independently of the nutritional level of the substrate and the accumulation of exotoxin was somewhat confirmed by the senior authour (unpublished data). He found that the maximumum growth of this fungus (R–5 isolate) occurred around 7 days. The results showed here suggestedtaht after 7 days the fungus started drastically to lose its ability to cause damping-off.

Physiological and chemical changes must account for this los of pathogenicity since no morphological and number of living and dead cells differed (Ashworth & Amin, 1964; van Etten & Molitonis, 1966; Gottlieb, 1966; 1971).

From the present data it can be conclused that there is a correlation between cessation of growth and loss of pathogenic ability.

The fungus ability to attack roots and hypocotyl is also going to decreased with age in culture media.

The lack of interference of root and hypocotyl lesion on the growth of soybean seedlings and the absence of post-emergence damping-off confirmed the statment that the R–5 isolate of *R. solani* is primarily a pre-emergence damping-off inciting isolate.

Table 1. Disease severity index (DSI) of soybean seedlings (cv. Chippewa 64) grown in *Rhizoctonia solani* infested vermiculite.[1]

Inoculum concentration (g)	Mean DSI [2]	Range
0	0.00a	0
1	2.82b	0–4
2	3.06bc	1–3
4	3.48d	3–4
6	3.42cd	2–4
8	3.86d	2–4
10	3.72d	3–4

(1) Completely randomized design. Means not having similar letters were significantly different (P = 0.05)
Duncan's test.
(2) Mean of all germinated plants.

Table 2. Disease severity index of soybean (cv. Chippewa 64) grown at 28°C on *R. solani* infested and non-infested vermiculite. [1]

Age of inoculum (days)	DSI
Non-infested	0.0a
17	1.5b
11	1.8b
7	4.6c
5	5.0c[2]

[1] Completely design with five replications.
Numbers not having similar letters were significantly different (P = 0.05) as determined by Duncan's test.

[2] Non-germinated seeds not included in other germinated treatments.

Table 3. Dry weigth of soybean seedling (cv. Chippewa 64) grown at 28°C on *R. solani* infested and non-infested vermiculite.

Age of culture (days)	Mean seedling dry weigth mg/seedling
Non-infested	200a
17	160a
11	170a
7	120a
5	000b

Table 4. Dry weight of soybean seedlings (cv. Chippewa 64) planted in R. solani infested vermiculite at different inoculum concentrations.

Inoculum concentration (g)	Seedling dry weight[1] (mg/seedling)
0	210a
1	179b
2	178b
4	150bc
6	148c
8	128c
10	150bc

[1] All germinated plants of 3 experiments and 5 replications each. Means not having simmilar letters were significantly different (P = 0.05) by Duncan's test.

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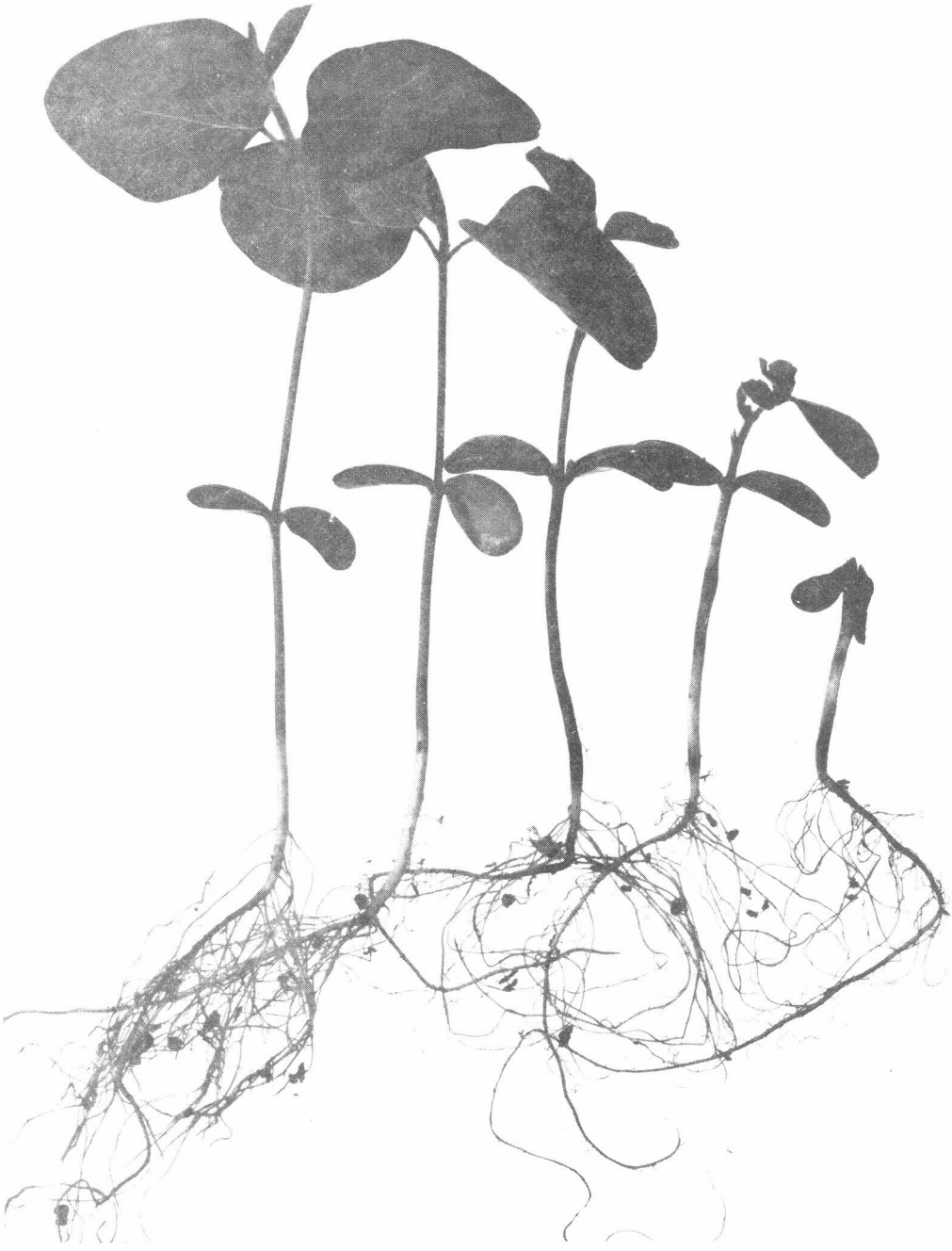

Figura 1. Soybean seedlings illustrating the classes of the disease severity index (DSI) used. From left to right classes 0, 1, 2, 3, and 4, respectively.

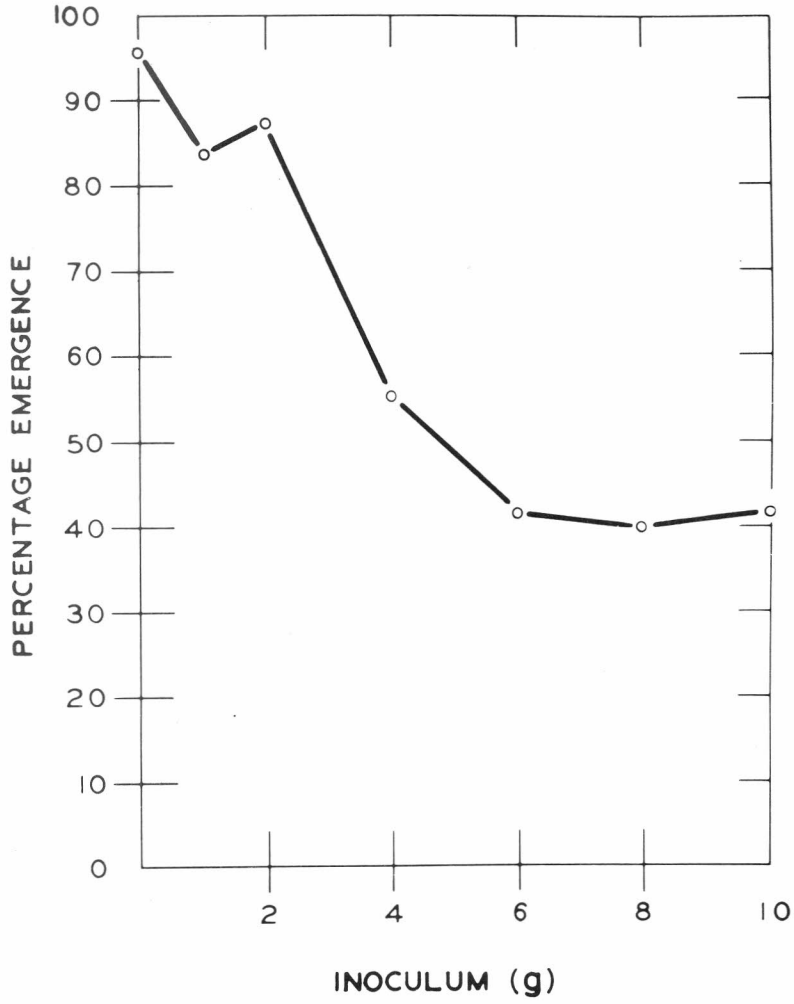

Figura 2. Percentage emergence of soybean seedlings (cv. Chippewa 64) grown at 28°C in noninfested vermiculite and in vermiculite infested with *R. solani* at six inoculum densities.

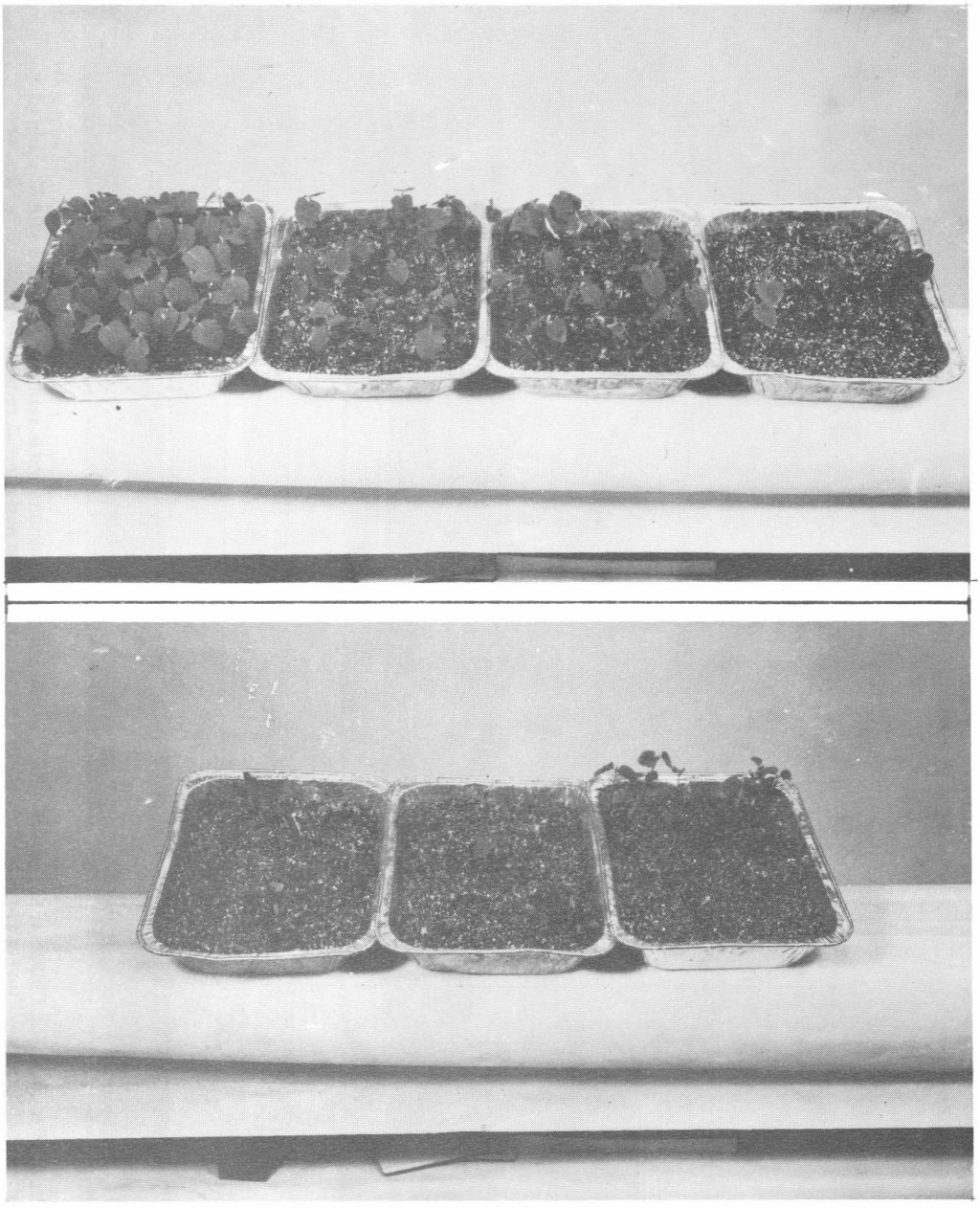

Figura 3. Germination of soybean seedlings (cv. Chippewa 64) growm at 28°C in non-infested vermiculite and in vermiculite infested with *R. solani* at six inoculum densities. (Above) From left to right: non-infested, 1g, 2g and 4g of fresh weight mycelium, veight mycelium, respectively (Below) 6g, 8g, and 10g of fresh weight mycelium, respectively.

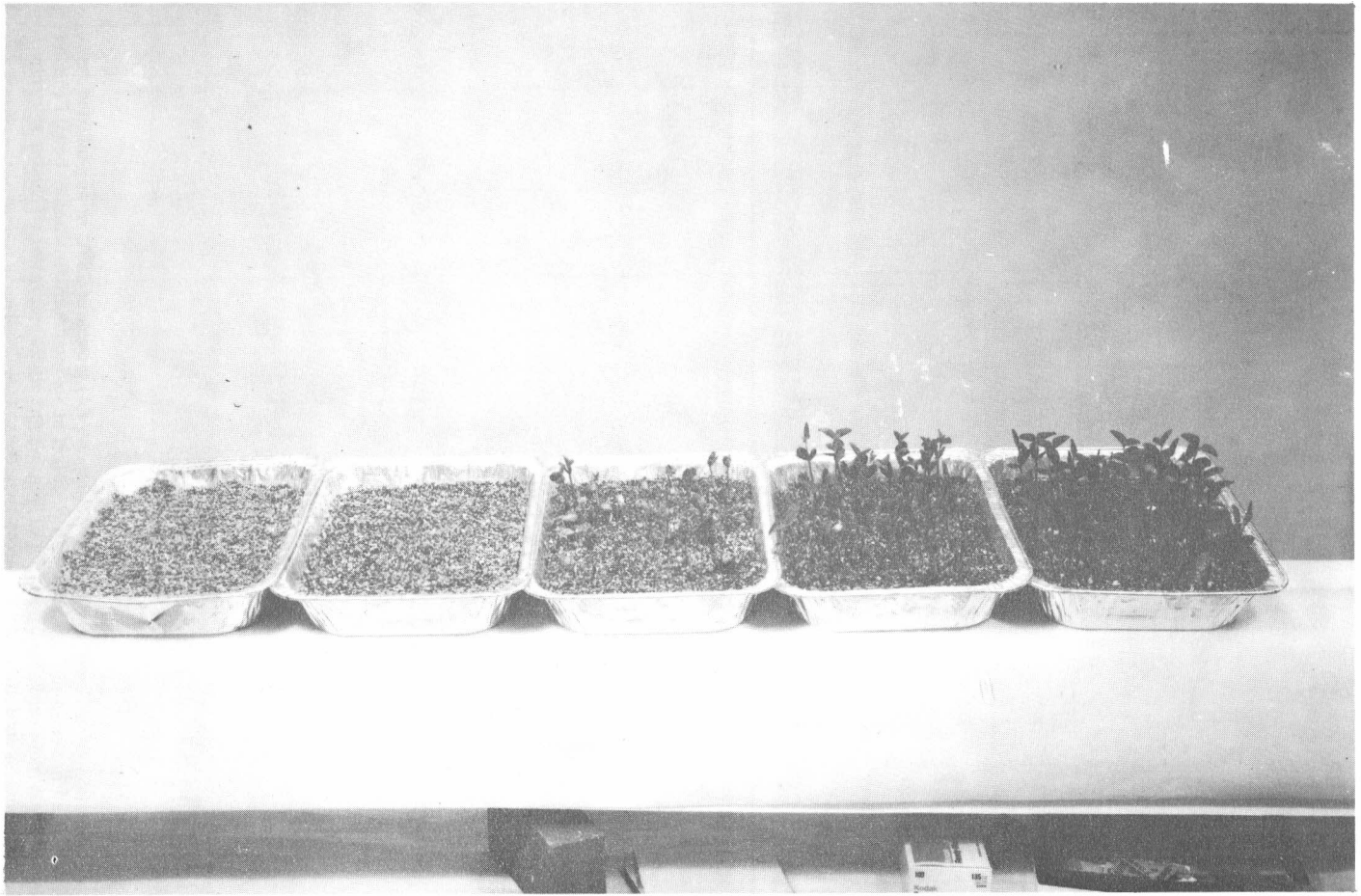

Figura 4. Emergence of soybean (cv. Chippewa 64) grown at 28°C in non-infested vermiculite and in vermiculite infested with *R. solani* of four different culture ages. From left to right: 5, 7, 11, 17 days old cultures and non-infested, respectively.

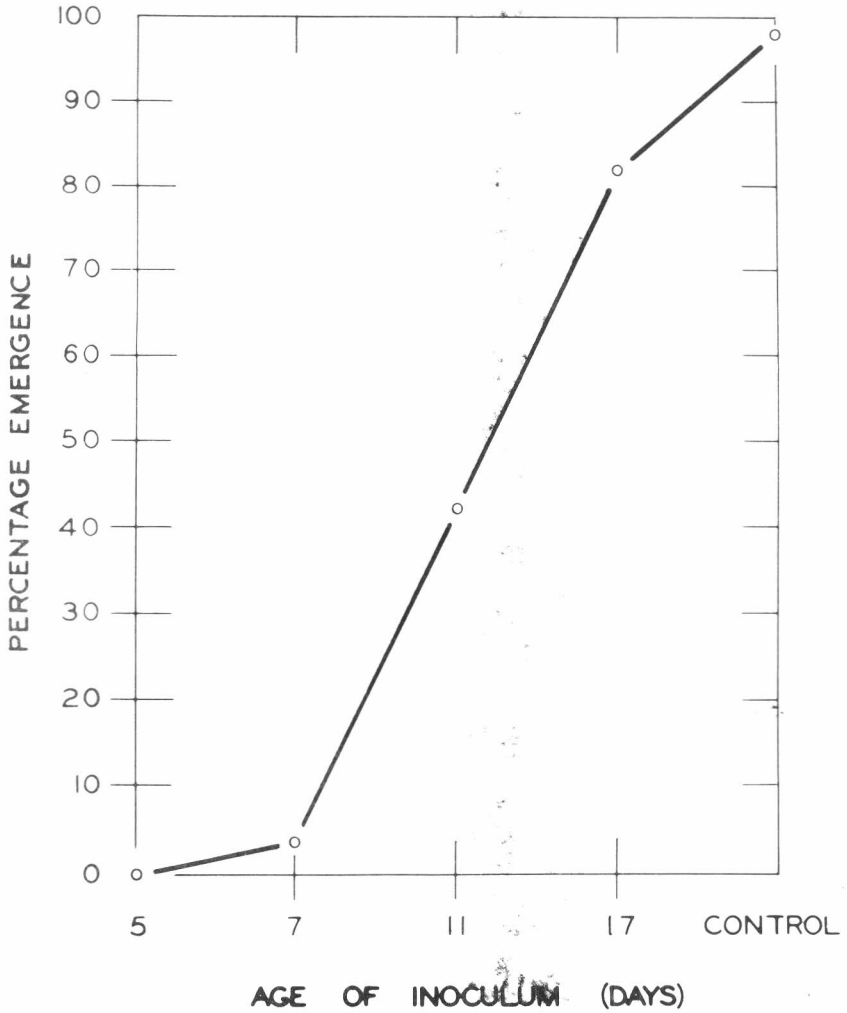

Figura 5. Percentage emergence of soybean seedling (cv. Chippewa 64) gr-wn at 28°C in non-infested vermiculite and vermiculite infested with *R. solani* of four different culture ages.

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