

# THE INFLUENCE OF WATER ACTIVITY ON THE GROWTH OF *RHIZOCTONIA SOLANI*

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## Summary

The growth of *R. solani* on agar decreased from 7.5 to 0 mm/day with reduction in water activity from 0.995 to 0.960. Growth differed slightly when sucrose was used as a solute, as compared with a salt mixture, to produce a number of water activities. The effects of decreasing water activity were similar with a number of isolates of *R. solani*.

## I. INTRODUCTION

A strain of *Rhizoctonia solani* Kühn, which causes "bare patch" disease of cereals in South Australia, oversummers in soil as dormant brown hyphae (De Beer 1965). Little is known about the relationship between soil moisture and the activity of this strain of *R. solani* in soil. The present paper attempts to determine the effect of different moisture tensions on hyphal growth and the minimum moisture tension for growth. The latter point will define the lower limit for growth and the upper limit for dormant survival.

Experimentation on soil moisture and its effect on growth of fungi is complicated by many factors, e.g. aeration, soil texture, bulk density, solute diffusion, and microbial antagonism. As pointed out by Griffin (1969) it is necessary to set up a model system, where the factor under consideration can be investigated unambiguously. The model system used in these experiments is a fungus growing on agar of a defined water activity ( $a_w$ ), which is in equilibrium with the water vapour pressure of the atmosphere above it. If in this system it is assumed that, at a given water activity, the amount of energy required by the fungus to remove water molecules that are bound to solute molecules is similar to the amount of energy required by the fungus to remove water molecules from soil at an equivalent relative humidity, or matric suction (e.g.  $a_w 0.99 \equiv$  relative humidity  $0.99 \equiv pF 4.16$ ), then the results would give an indication of the likely behaviour of the fungus in soil. A full explanation of water activity is given by Scott (1956). Water activity is the water vapour pressure of a solution ( $p$ ) expressed as a fraction of the vapour pressure of pure water ( $p_0$ ) at the same temperature (i.e.  $a_w = p/p_0$ ), and is numerically equal to relative humidity expressed as a fraction (i.e.  $a_w =$  relative humidity/100).

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The use of solutes to produce a medium with a defined water activity was suggested by Scott (1953) and depends upon hydration of solute molecules or ions. The force with which water molecules are bound to a solute molecule or ion decreases with each successive hydration shell. In dilute solutions (high water activity) the distance between solute molecules or ions is relatively large, e.g. in an 0.01 molal NaCl solution the distance between ions is approximately 14 water molecules (Robinson and Stokes 1959). However, in a concentrated solution (low water activity) the distance between solute molecules or ions is relatively small, e.g. in a 1.0 molal NaCl solution the distance between ions is approximately 3 water molecules. The solutions used in the present paper can be considered concentrated (0.2–1.9 molal). When a fungus grows in these solutions it competes directly with solute molecules or ions for the water it requires for growth. The addition of solutes to produce a medium with a defined water activity was used in preference to solutions controlling water vapour because it eliminates lengthy equilibration of substrates, it is accurate, and preparation is simple and rapid.

## II. GENERAL METHOD

Cultures of *R. solani* used for inoculum were grown at 25°C on Neutral Dox Yeast agar at one-fifth normal strength (NDY/5) (i.e. NaNO<sub>3</sub> 0.4 g, KH<sub>2</sub>PO<sub>4</sub> 0.2 g, KCl 0.1 g, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.1 g, yeast extract 0.1 g, sucrose 6 g, agar 15 g, in 1 litre of distilled water); this medium has a water activity of approximately 0.999. Plugs of agar cultures or fungus-covered Cellophane disks were cut or removed from the edge of a culture of *R. solani*. These disks or plugs, each 0.6 cm diameter, were then transferred to agars of defined water activity and subsequent growth measured. The assessment of growth in all experiments was colony diameter, which was measured with sliding vernier callipers.

Care was taken in these experiments to prevent water loss by covering solutions while autoclaving, pouring plates only with cooled agar, and sealing plates with tape immediately after pouring.

All media used for assessing the effects of water activity had a nutrient base of KH<sub>2</sub>PO<sub>4</sub> 0.68 g, K<sub>2</sub>HPO<sub>4</sub> 1.4 g, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.12 g, sucrose 4.0 g, NaNO<sub>3</sub> 0.68 g, added to 1 litre of distilled water and containing 1% agar (Difco Bacto). Scott (1953) demonstrated that this concentration of agar had a negligible effect in reducing water activity.

The isolate used except where otherwise stated was isolate 108, a root-rotting strain of *R. solani*, isolated from Port Vincent, S.A.

## III. EXPERIMENTAL DETAILS AND RESULTS

### (a) Comparison of Solutes

The solutions required to produce water activities of 0.990 or less are concentrated (e.g.  $a_w$  0.990 is produced by 0.53 molal sucrose solution). It is therefore necessary to compare the growth of a fungus on a variety of solutes over the same range of water activities to detect aberrant results produced by solute toxicity. Similar growth curves, i.e. similar optimal and minimal water activities, would provide evidence that the effects observed are a result of decreased water activity, and independent of other solute effects.

Two different types of solutes were compared, a disaccharide (sucrose) (Robinson and Stokes 1959), and a salt mixture (NaCl, KCl, Na<sub>2</sub>SO<sub>4</sub> in the ratio 5 : 3 : 2 moles) (Scott 1953).

Agar plugs were used as inoculum for this comparison and growth from these was assessed after 5 days. Colony diameter was measured five times on each colony, and each treatment was replicated three times. The relation between fungal growth and water activity produced by different solutes is shown by the regressions in Figure 1. There is a marked reduction in the amount of growth with both solutes when the

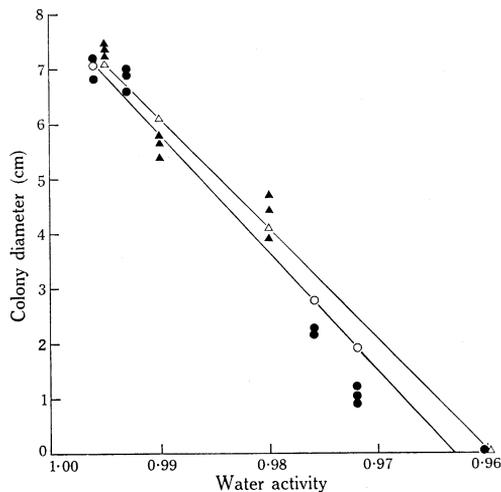


Fig. 1.—Relation between growth of *R. solani* and water activity.  $\Delta$ ,  $\blacktriangle$  Salt mixture.  $\circ$ ,  $\bullet$  Sucrose. Solid symbols indicate replicate means.

water activity is decreased from 0.995 to 0.960. With salts as solutes the colony diameters decrease from 7.1 cm at a water activity of 0.995 to no measurable growth at a water activity of 0.960. The regression, with sucrose, shows no measurable growth at a water activity of 0.963; however, it must be remembered that this is a fitted line. Actual results show that at a water activity of 0.960 there was no measurable growth.

Inoculum plugs from a water activity of 0.960 (produced by sucrose or the salt mixture), all of which showed no growth over the experimental period, were transferred to NDY/5. All plugs produced normal colonies, illustrating that the hyphae had been dormant and viable.

Straight-line regressions have been fitted to these data, because they are a good fit; however, the best fit would have been obtained with a higher-order polynomial regression. But, because the method of statistical comparison is difficult to interpret, straight-line regressions have been used. A *t*-test, used to compare the regression slopes of the two solutes, shows that the probability of the slopes being similar is less than 1 in 20 ( $t_{153} = 2.1$ ). The sensitivity of the *t*-test is dependent upon the total number of degrees of freedom in the comparison. In this comparison there were 153 degrees of freedom, and the test was therefore capable of detecting small differences. The differences detected may be due to the type of solute or to other factors. In Figure 1 the slope of the sucrose regression was increased by the values obtained at water activities of 0.976 and 0.973. However, the difference in slope is small, resulting in a maximum difference between the two solutes of 0.5 cm growth in 5 days, compared with the effect of water activity which reduced the amount of

growth obtained in 5 days from approximately 7.1 cm to zero with a change in water activity from 0.998 to 0.960.

(b) *Comparison of Temperature Control*

The water activity of a given solution varies only slightly within the range of temperatures permitting microbial growth (Scott 1956). However, the relative humidity of the atmosphere above the solution or agar is not independent of temperature; a decrease of 0.5 degC at 25°C will change the relative humidity by 2.9%, whereas a decrease of 0.01 degC will alter the relative humidity by only 0.6%. *R. solani* has some aerial hyphae, and at water activities of 0.971 and greater, a decrease of 0.5 degC could produce condensation on these hyphae, and confound the effects of water activity by allowing the fungus to take up pure water which has a water activity of 1.00. For this reason it was decided to test the importance of temperature control and its effect on water activity and subsequent growth of the fungus. A constant-temperature room kept at  $25 \pm 0.5^\circ\text{C}$  and a water-bath, in which the temperature was controlled at  $25 \pm 0.01^\circ\text{C}$ , provided the two types of temperature

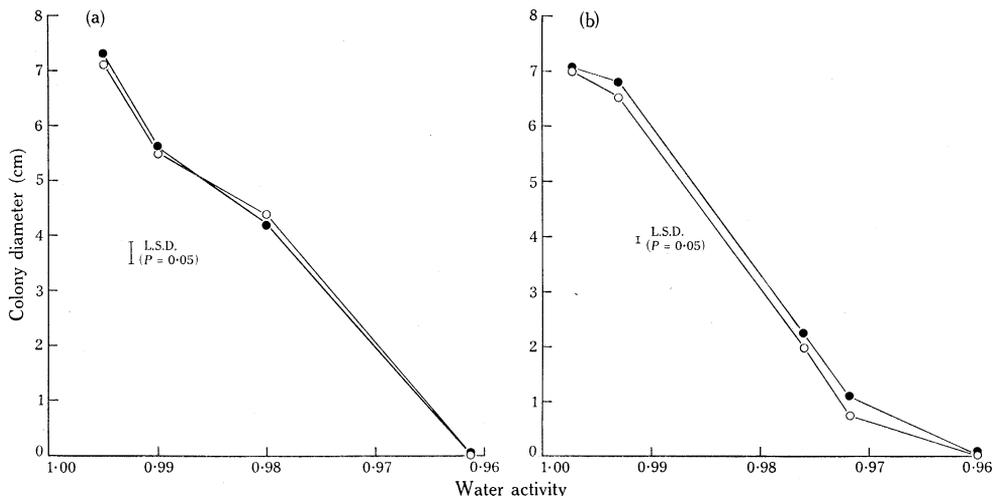


Fig. 2.—Relation between temperature control and growth of *R. solani* on agar containing salt mixture (a) or sucrose (b) of differing water activities. In (a) the salt mixture comprised NaCl, KCl, and  $\text{Na}_2\text{SO}_4$  in the molal ratio of 5 : 3 : 2). ●  $25 \pm 0.01^\circ\text{C}$ . ○  $25 \pm 0.5^\circ\text{C}$ .

control. Two types of solute, sucrose and a salt mixture, were used to produce a number of water activities. Agar plugs were used as inoculum for this comparison, and growth from these was assessed after 5 days. Colony diameter was estimated five times on each colony, and each treatment was replicated four times in the salt mixture experiment, and three times in the sucrose experiment. The experiments were not done at the same time; the salt mixture experiment was completed first, and it was found that the same information could be obtained with three replicates. For this reason the sucrose experiment had only three replicates.

Analysis of variance from the salt mixture experiment shows that there is no significant difference ( $P < 0.05$ ) in the amount of growth of *R. solani* at a number of water activities with different types of temperature control [Fig. 2(a)]. No inter-

action was detected between the type of temperature control and water activity. Analysis of variance of data from the sucrose experiment, however, shows there is a significant difference ( $P > 0.05$ ) in the amount of fungal growth between the types of temperature control. It can be assumed therefore that there is a difference in results between the types of temperature control [Fig. 2(b)]. No interaction was detected between the type of temperature control and water activity. Because the growth curves with both solutes over the same range of water activities are similar, and because there is no interaction between water activity and type of temperature control, it can be assumed that the difference in colony diameter is due to a small but consistent difference in temperature throughout the experiment.

(c) *Comparison of Types of Inoculum*

In the previous experiment agar plugs which have a water activity of approximately 0.999 were used as inoculum. Fungus-covered Cellophane disks were used as inoculum source to determine whether the water in agar plugs is sufficient to confound the effects of decreasing water activity. A salt mixture was used to produce the same range of water activities as in Section III(a). Inoculated plates were incubated at  $25 \pm 0.5^\circ\text{C}$ , and fungal growth assessed after 6 days. For comparison of agar plugs and Cellophane disks the straight-line regression of this experiment is compared with that of the pooled salt mixture data of Section III(b) (Fig. 3). Although the Cello-

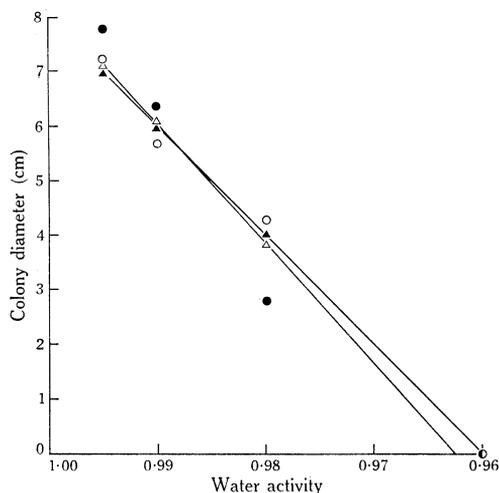


Fig. 3.—Relation between water activity and growth of *R. solani* from different inocula. Growth medium contained salt mixture as for Figure 2(a).  $\triangle$  Cellophane disks ( $\bullet$  mean values).  $\blacktriangle$  Agar plugs ( $\circ$  mean values).

phane-disk regression shows no growth at approximately 0.963, actual results show no growth at a water activity of 0.960. Statistical comparison of the regression slopes shows they are significantly different ( $P > 0.05$ ). However, the *t*-test was based on 286 degrees of freedom, and therefore was capable of detecting small differences. The regressions show that the amount of growth from Cellophane disks is similar to that from agar plugs, and that the differences detected are slight over the range of water activities studied. It can be concluded that the water supplied by the agar plugs has little effect on the subsequent growth of the fungus on agars of known water activity.

*(d) Comparison of Rates of Growth*

This experiment was designed to determine whether lowered water activity was decreasing the rate of growth, or increasing the lag period without alteration of growth rate.

Two solute types, sucrose and a salt mixture, were used to produce a range of water activities. The agars of known water activity were inoculated with fungus-covered Cellophane disks, plates were incubated at  $25 \pm 0.5^\circ\text{C}$  and growth assessed after 2, 4, and 5 days. The diameter of each colony was measured three times on each plate, and each treatment replicated five times.

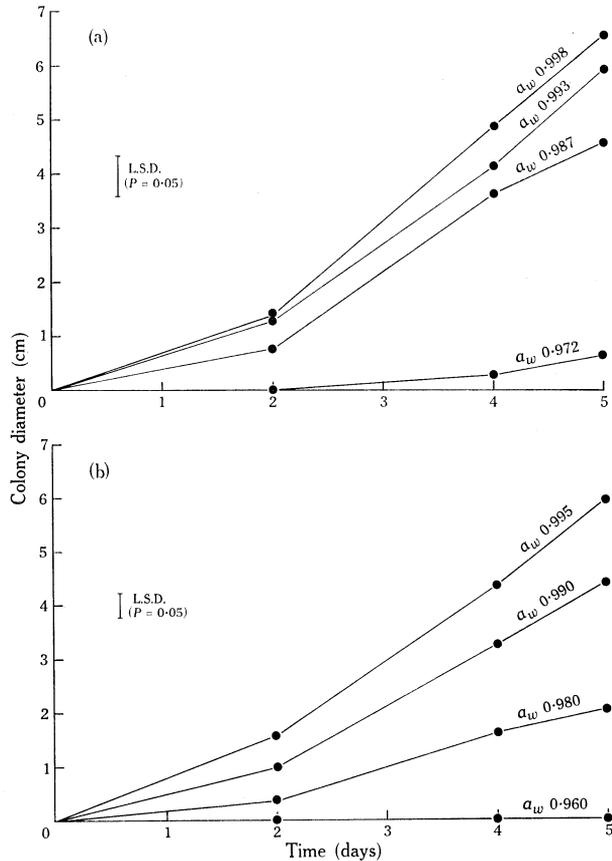


Fig. 4.—Relation between rate of growth of *R. solani* and water activity. (a) Solute = sucrose; (b) solute = salt mixture as for Figure 2(a).

The sucrose experiment and salt mixture experiment each show that a decrease in water activity decreases the rate of growth [Figs. 4(a) and 4(b)]. Tomkins (1929) achieved a similar result with the growth of *Alternaria citri* on substrates equilibrated with solutions controlling water vapour.

## (e) Comparison of Isolates

The effects of water activity on the growth of isolate 108 had now been defined, but it was not known whether isolates from different climates, soil types, or with different pathogenicity behaved in a similar way. Isolates of *R. solani* from different soils, hosts, and geographical areas were compared over a range of water activities from 0.998 to 0.963. The isolates are listed in Table 1.

TABLE 1

PERFECT STATE, ORIGIN, SOIL TYPE, AND PATHOGENIC SPECIFICITY OF ISOLATES OF *R. SOLANI* USED IN THE COMPARISON OF GROWTH AT A NUMBER OF WATER ACTIVITIES

Isolate No.	Perfect State	Origin	Soil Type	pH	Pathogenic Specificity
42	<i>Thanatephorus praticola</i>	Slough, U.K.	Medium red-brown alluvial loam	5.3	Stems; not specialized
48	<i>Thanatephorus cucumeris</i>	Waite Institute, S.A.	Heavy red-brown silt loam	6.5	Crucifer stems
69	<i>Thanatephorus cucumeris</i>	Clare, S.A.	Heavy red-brown silt loam	—	Crucifer stems
75	Unknown	Ceduna, S.A.	Brown calcareous sandy loam	8.0	Stems and roots; not specialized
99	<i>Thanatephorus cucumeris</i>	Moonta, S.A.	Brown calcareous sand	8.5	Roots; not specialized
108	Unknown	Port Vincent, S.A.	Brown calcareous sand	8.0	Roots; not specialized

Petri dishes containing agars of known water activity produced by sucrose solutions were inoculated with fungus-covered Cellophane disks, and incubated for 3 days at  $25 \pm 0.5^\circ\text{C}$ .

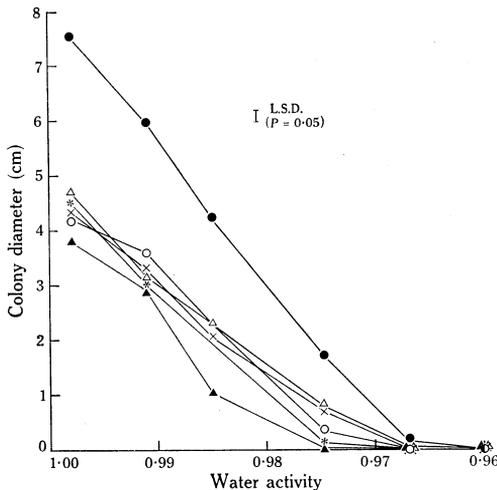


Fig. 5.—Relation between growth of isolates 42 (●), 108 (\*), 75 (×), 48 (▲), 69 (○), and 99 (△) of *R. solani* and water activity.

Colony diameter was measured five times and each treatment was replicated three times. Isolate 42 (*Thanatephorus praticola*) grew most rapidly at all water activities from 0.998 to 0.976 (Fig. 5). Isolates 69, 75, 99, and 108 were similar to

each other in their response to water activity, but they produced much less growth than isolate 42. Isolate 48 (*T. cucumeris*) grew least at all water activities from 0.998 to 0.976. From this comparison it can be concluded that all isolates tested grow over the same range of water activities. The differences exhibited in the amount of growth between isolates at equivalent water activities can be accounted for by their inherent rates of growth, e.g. a feature commonly used to distinguish *T. praticola* from *T. cucumeris* is rate of growth.

#### IV. DISCUSSION

If the earlier assumption is made, viz. energy required by the fungus to remove water bound to solute molecules is similar to that required to remove water from soil at equivalent relative humidity, then water activity values can be related to soil moisture tensions. A decrease in water activity from 0.998 to 0.990 would be equivalent to a change in  $pF$  from 3.2 to 4.2; this results in a reduction of 14–18% in the growth of the fungus. A decrease in water activity from 0.990 to 0.963, which is equivalent to a change in  $pF$  from 4.2 to 4.7, results in a reduction of 80–85% in the growth of *R. solani*. The fungus does not grow at a water activity of 0.963. The results show therefore that several isolates of *R. solani* from different soil types and geographical locations are capable of growth below the permanent wilting point of plants ( $pF$  4.2), but fail to grow at or below  $pF$  4.7.

These results are similar to those of Schneider (1954) who demonstrated a marked reduction in the growth of *R. solani* and *Gäumanomyces graminis* v. Arx & Olivier below the wilting point of plants ( $pF$  4.2), these fungi having minimum water activities for growth of 0.965 ( $pF$  4.7) and 0.955 ( $pF$  4.8) respectively. Cook and Papendick (1970) showed that the optimum soil water tension for germination of the spores of *Fusarium roseum* (Lk. ex Fr.) emend. Snyder & Hans. f. sp. *cerealis* (Cke.) Snyder & Hans. "culmorum" was  $pF$  4.2, and the minimum soil water tension for germination was  $pF$  4.9. Thus if moisture tensions for germination and growth are similar then cereal root-rotting pathogens are capable of growth and may attack plant roots and stems well below the wilting point of plants. Chen and Griffin (1966) showed, however, that *Aspergilli* and *Penicillia* grew at soil moisture tensions from  $pF$  5.2 to  $pF$  5.6.

These differences in minimum water activity for growth of different fungi may be important in influencing populations of individual fungi in soil because of their effect on general substrate colonization, including, specifically, attack of both plants and other fungi around these minimum water activity levels.

#### V. ACKNOWLEDGMENTS

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