Emergence, Growth, and Development of Dry Bean Seedlings in Response to Temperature, Soil Moisture, and *Rhizoctonia solani*

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ABSTRACT

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The rates of emergence and initial seedling growth of red kidney beans were determined in response to various conditions of temperature and soil moisture in the presence and absence of *Rhizoctonia solani*. In incubators, the optimal temperature and soil moisture for seedling emergence were around 27 C and 20% (−0.2 bar). *R. solani* delayed emergence, reduced the growth rate, and increased the shoot:root ratio, particularly at temperatures above 18 C and at low soil moisture levels. The proportion of plants infected was dependent only on temperature (optimum at 24–30 C). Lesion size was determined primarily by soil moisture (largest at 10% or −9.5 bar).

In a field study of the effects of *Rhizoctonia solani* Kühn on growth and yield of dry beans (*Phaseolus vulgaris* L.), we observed that this pathogen delayed emergence and further development and reduced final stand (23). It did not reduce overall yield but delayed maturation so that uneven ripening resulted. The delayed maturation was directly related to the delay in emergence, and *R. solani* appeared to have little further effect on plant growth and development after the unifoliate stage. Thus, further research was focused on emergence and initial development of bean seedlings.

Under optimal soil moisture conditions, heat sums can be successfully used to predict emergence (25), but under varying soil moisture and in the presence of soilborne pathogens those predictions would be less accurate. Under these circumstances, a computer simulation model (taking effects of temperature, soil moisture, and soilborne pathogens into account) would be more suitable for predicting emergence.

A range of optimum temperatures for infection of different crops by *R. solani* has been reported (1,18,22). The optimum temperature for pathogenicity of *R. solani* is considered to be a characteristic of the fungus isolate and is not influenced by temperature requirements of the host (1,9,18). The effect of soil moisture on infection of beans by *R. solani* has not been studied intensively. On crops other than beans, infection slowly increased with increasing soil moisture from about 30 to 70–80% moisture-holding capacity and decreased at higher moisture levels (2,4,13,19). In only a few cases were the effects of temperature and moisture investigated simultaneously. Bateman (3) demonstrated several interactions between soil moisture, temperature, and *R. solani* in their effects on poinsettia root rot.

In this paper, we report on the effects of temperature and soil moisture on emergence, growth, and development of dry bean seedlings in uninfested soil or soil infested with *R. solani*. The objectives were to collect input data for a simulation model on the rates of emergence and initial growth of dry beans, as affected by soil moisture, temperature, and *R. solani*. We also wanted to determine at which stage and under which conditions *R. solani* infected the roots and hypocotyls.

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MATERIALS AND METHODS

Soil. A gravelly silt loam (Darien) was used for all experiments; its properties were described previously (24). Air-dried soil was sieved through a 1-cm-mesh screen and mixed with 5 g of agricultural hydrated lime per liter of soil (fineness: 85% passing 200 mesh) in a concrete mixer, raising the pH in water from 5.8 to 7.2.

Soil infestation. One isolate of *R. solani*, R-2, obtained from G. S. Abawi (New York State Agricultural Experiment Station, Geneva) was used. This isolate was originally isolated from beet roots but was highly pathogenic to beans (11). It did not anastomose with any of the tester isolates that were available (AG1-4) (11). Sclerotia (300- to 710-μm diameter) were produced on autoclaved green beans (defrosted frozen cut beans) as described previously (24). Their viability was checked by sprinkling sclerotia onto the surface of acidified water agar (pH 4.8) in three or four petri plates and counting germination after 24 hr at 27 C. Percent germination ranged from 78 to 92%. Half of the soil to be used in each experiment was infested with 500 sclerotia per liter of air-dried soil, and half was left uninfested for the control treatments. This inoculum density had resulted in the highest infection level in field experiments (23).

Soil moisture. To measure the response of seedling emergence to soil moisture and temperature, the soil moisture was adjusted to seven levels ranging from 10 to 25%. Tap water was added to air-dried soil in plastic bags to obtain the desired soil moisture content. The plastic bags were shaken vigorously, and the clumps were broken. Water and soil were allowed to equilibrate for 1–2 hr. Plastic trays with soil (see below) were kept in plastic bags to maintain the moisture levels. The soil moisture content was determined by oven drying two or three samples per batch for about 30 hr at 105 C, before and after each experiment. The measured initial and final moisture contents ranged from 12.1 to 24.0 and from 8.8 to 21.5%, respectively. A soil moisture retention curve was obtained with the use of a pressure-plate apparatus at a temperature of 25 ± 3 C (Fig. 1).

Seed treatment and planting. Since the response surfaces were intended to be used for construction of a simulation model, cultural practices were similar to those used by growers. Dry bean seeds, cultivar Redkloud, were treated with captan 50 WP at a rate of 1.2 g active ingredient per kilogram of seeds, because this practice was recommended to growers (20). Twelve seeds were planted in trays (15 × 20 × 7 cm, containing 0.5–0.6 L of soil) in three rows at a depth of 2 cm. All trays were placed in plastic zip-lock bags, which were opened daily for air exchange and observations on emergence.

Environmental conditions. For the construction of statistical response surfaces (to temperature and soil moisture), the effects of a wide range of temperature and soil moisture levels had to be measured simultaneously. Since as many as eight growth chambers were not available simultaneously, the experiments were executed in incubators in the dark; it was assumed that light would not have a major influence at these early stages of seedling development. The temperatures ranged from 12 to 33 C with intervals of 3 C.

Observations. Daily observations were made on emergence. When the hypocotyl loop became visible, the seedling was counted as emerged. Preemergence damping-off was not evaluated in this study, because it could not be distinguished from nonemergence.

The seedlings in a tray were "harvested" when the primary leaves of four plants protruded from between the cotyledons. Fresh weights of shoots and roots were determined after washing soil from the roots with tap water and blotting the roots dry with paper towels. Net growth rates per plant per day were calculated by subtracting the seed weight from the total weight and dividing this by the number of plants and the number of days from planting to "harvest." Shoot:root ratios were calculated on a fresh weight basis.

The numbers of plants with hypocotyl and/or root lesions were counted. The length and width of each lesion (or infected area, when lesions had coalesced) were measured in millimeters and multiplied to calculate lesion areas, which were assumed to have a rectangular shape.

Experimental design and analysis. The experiments were factorial and had a split-plot design with temperature as the main plot and soil moisture and infestation with *R. solani* as subplots. Each tray planted with 12 seeds was considered to be an experimental unit. There were three replications over time, considered to be blocks in the statistical analysis. The numbers of seedlings emerged were transformed into percents of the maximum number at "harvest" time, and then into probits. The probits were regressed on number of days after planting. When development was fast, it was necessary to include 0 or 100% emergence in the regression. In that case, 0 and 100% were considered as 0.1 and 99.9% with probits of 1.91 and 8.09, respectively. The mean period for the population to emerge (in days) was calculated as $m = (5 - a)/b$, in which a is the intercept and b is the slope of the regression line. This mean period was considered a dependent variable in the analysis to test effects of temperature, soil moisture, and infestation with *R. solani*. The data were analyzed by using multiple regression analysis with the Statistical Analysis System (SAS Institute, Carey, NC). Initially all possible interactions and polynomial terms of temperature and moisture were included in the statistical models. The statistical models were then simplified based on sequential sums of squares. For infection data only infested units were included in the analysis, since plants in uninfested trays were virtually free of symptoms.

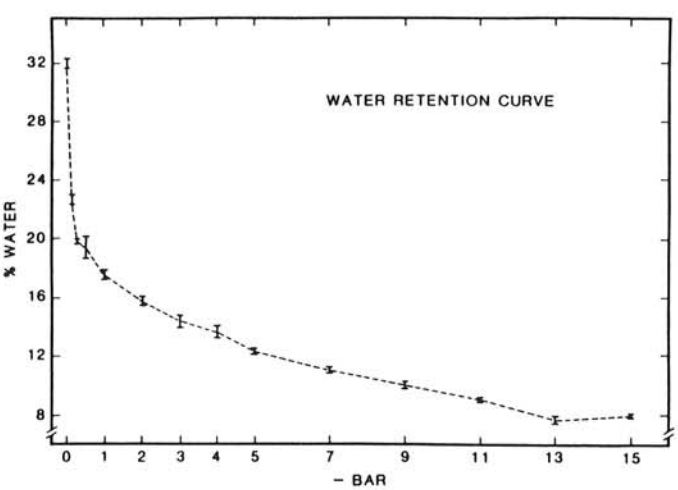

Fig. 1. Relationship between gravimetric soil moisture content (%) and matric water potential (−bar) for Darien gravelly silt loam, as determined with a pressure-plate apparatus. Vertical bars indicate standard deviations (five replications).

RESULTS

Infection by *R. solani*. The percentage of plants infected (disease incidence) depended only on temperature (Table 1), with an optimum temperature range from 21 to 27 C (Fig. 2A). The total lesion area per hypocotyl (determined when the primary leaves became visible) was influenced more by moisture than temperature, and there was a significant interaction between these two factors (Table 1). The largest lesions developed between 21 and 30 C in combination with low soil moisture (Fig. 2B).

Emergence. Regression of the mean period from sowing to emergence on temperature, moisture, and *R. solani* indicated that the temperature and moisture effects and their interaction were highly significant (Table 2). The effect of *R. solani* also was significant, but interactions of *R. solani* with temperature or moisture were not. Response surfaces of the period from sowing to emergence on temperature and moisture was located at a higher level after infestation with *R. solani* (Fig. 3A and B). At 18 C and 17% soil moisture, seedling emergence in infested soil was delayed by 24% compared to that in control soil. The minimal period was at about 27 C and 20% moisture in both cases.

Growth rate. The growth rate based on fresh weight was significantly affected by temperature, soil moisture, and their interaction (Table 3). *R. solani* reduced the growth rate of the plants in infested soil, especially at 18–30 C and at low soil moisture (Fig. 4).

Shoot:root ratio. The shoot:root ratio based on fresh weight was affected more by soil moisture than by temperature (Table 3). It was significantly larger in the presence of *R. solani* than in the controls, especially from 21 to 27 C combined with low moisture levels, and at temperatures above 27 C combined with high moisture levels (Fig. 5).

DISCUSSION

Disease incidence was dependent on temperature, and not on soil moisture. The optimum temperature ranged from 21 to 27 C, the same optimum range as for the linear growth rate of the same isolate of *R. solani* on potato-dextrose agar (23). Disease severity decreased steadily at increasing soil moisture levels, when evaluated at the same stage of seedling development, viz., when primary leaves became visible. However, in this way, the time period allowed for lesion expansion was longer at lower moisture levels, so that is is not clear whether rate of lesion expansion would also decrease with increasing soil moisture content. Nevertheless, a decrease in disease severity by *R. solani* at increasing moisture levels has been observed for crops other than beans (2,13,19). Several

TABLE 1. ANOVA table for regression of percent bean plants (in primary leaf stage) infected by *Rhizoctonia solani* (root or hypocotyl lesions), and lesion area per hypocotyl on temperature and percent soil moisture

	Plants infected (%)			Lesion area per hypocotyl		
Source	*df*	*SS*[a]	*F*	*df*	*SS*[a]	*F*
Experiment	2	1,836	6.0 *[b]	2	24,047	1.3 n.s.[b]
Temperature[c]	7	46,429	43.3 **	7	159,220	2.4 n.s.
Error *a*	10	1,531		10	93,524	
Moisture[d]	5	2,887	2.3 n.s.	5	65,049	2.7 *
Temp. × moisture	...	...			27,092	5.7 *
Error *b*	39	9,603		32	153,129	
Corrected total	63	62,286		57	522,062	
R^2(%)		84.6			72.8	
C.V. (%)		21.1			107.7	
Mean		74.2			62.8	

[a] Sequential sums of squares.
[b] Asterisks ** and * indicate F significant at $\alpha = 0.01$ and $\alpha = 0.05$, respectively; n.s. = not significant.
[c] Linear and quadratic terms significant.
[d] Linear term significant for lesion area.

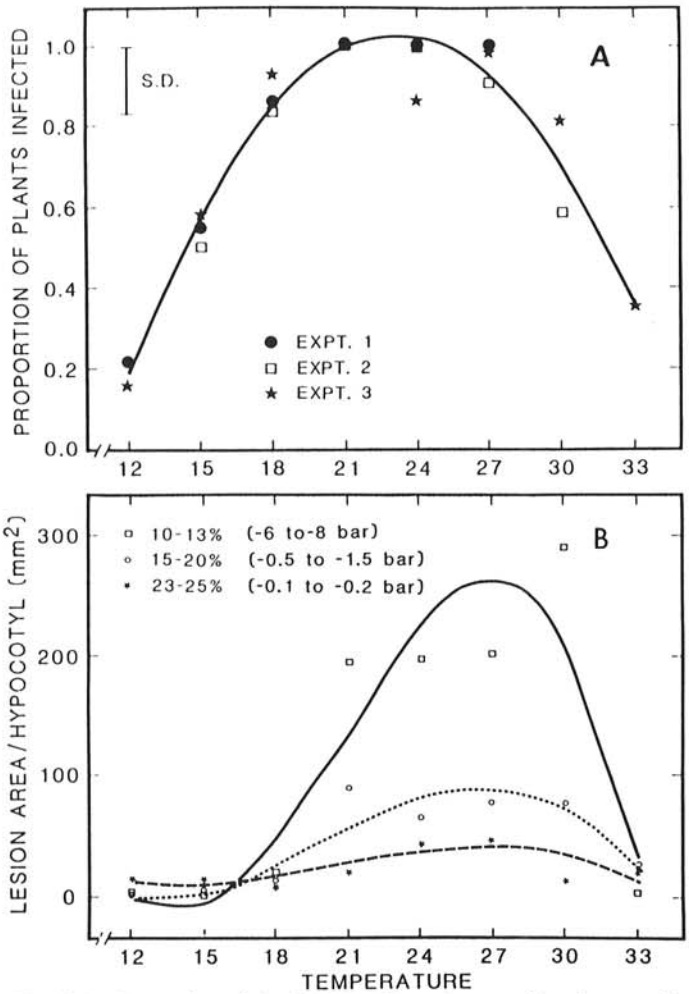

Fig. 2. A, Proportion of dry bean seedlings (roots and/or hypocotyls) infected by *Rhizoctonia solani* and **B,** lesion area per hypocotyl at different soil moisture levels (%), as related to temperature (C). Note: Despite a significant effect of experiment on proportion of plants infected, the prediction lines were too close to each other to be drawn separately.

authors have reported a sudden drop in infection at soil moisture contents above 70–80% (2,4) of the moisture-holding capacity. The moisture-holding capacity of the soil used here was about 17% (25% at field capacity and 8% at the permanent wilting point). Seventy to 80% of the moisture holding capacity would be 19.4–21.5%, corresponding to −0.5 to −0.3 bar. These values are close to the critical value of −0.49 bar for emergence, below which the time needed for emergence increases rapidly (10). We observed similar critical values for seedling development between −0.25 and −1.0 bar. The sudden drop in infection above about 70% moisture-holding capacity could be ascribed to insufficient aeration for growth of *R. solani* in soil (2) but may also be connected with the sudden rise in development rate of seedlings at a similar moisture content. However, soil moisture content or seedling growth rate by themselves may not be directly responsible for the levels of infection. Leach (15) proposed that disease incidence was inversely related to the coefficient of velocity of seedling emergence and the growth rate of a pathogen on agar, but this view was disputed by later authors (1,9).

TABLE 2. ANOVA table for regression of period from sowing to emergence (days) on temperature, percent soil moisture, and *R. solani*

Source	*df*	*SS*[a]	*F*
Experiment	2	188	13 **[b]
Temperature[c]	7	1,598	33 **
Error *a*	10	70	
Moisture[d]	5	677	73 **
Temp. × moisture	33	197	3 **
R. solani	1	10	6 *
Temp. × *R. solani*	...	...	n.s.[e]
Moisture × *R. solani*	...	...	n.s.
Error *b*	76	141	
Corrected total	134	2,881	
R^2(%)		95.1	
C.V. (%)		21.1	
Mean		6.4	

[a] Sequential sums of squares.
[b] Asterisks ** and * indicate *F* significant at $\alpha = 0.01$ and $\alpha = 0.05$, respectively; n.s. = not significant.
[c] Linear and quadratic terms significant.
[d] Linear, quadratic, and cubic terms significant.
[e] Tested in a full model.

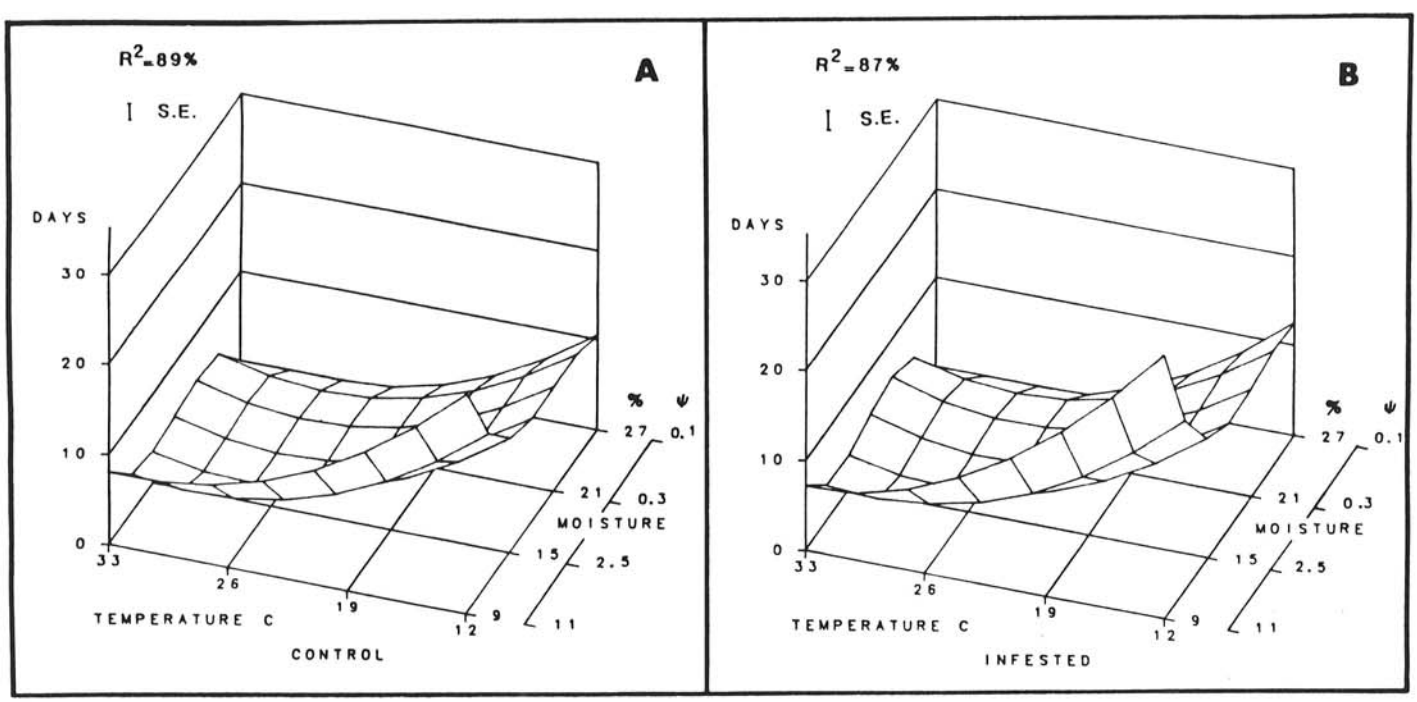

Fig 3. Response surfaces from multiple regression of the period from sowing to emergence of dry beans on temperature and soil moisture (%) **A,** in the absence or **B,** in the presence of *Rhizoctonia solani*.

The optimum temperature range for seedling development was 27–30 C, which was a little lower than that of a snap bean cultivar (30–35 C) given by Harrington (12). At the temperature extremes (>30 C and <18 C) development slowed drastically, which also was reported by Harrington (12). In temperate climates, the lower temperature range is more crucial in connection with the planting date (20). Although the minimum temperature for germination has been determined to be about 8 C (6), temperatures below 15 C generally slow germination drastically (14).

R. solani delayed emergence in these experiments as it did in field experiments (23), and the delay was independent of temperature and soil moisture levels. In field experiments at an inoculum density of 500 sclerotia per kilogram of soil, emergence was delayed by 2.5 days, or 24% of the emergence period in uninfested soil (23). At temperature and soil moisture levels similar to those encountered in the field (18 C and 17% moisture content), *R. solani* also delayed emergence in incubators by 24%. However, in both infested and uninfested trays, emergence was much faster in the experiments described here than under field conditions, probably due to the high relative humidity in the plastic bags. McIntyre and Boyer (16) ascribed the effect of relative humidity on seedling development to an increase in water potential and cell turgor in the growing region due to a decrease in transpiration.

The relationship between growth rate and temperature and soil

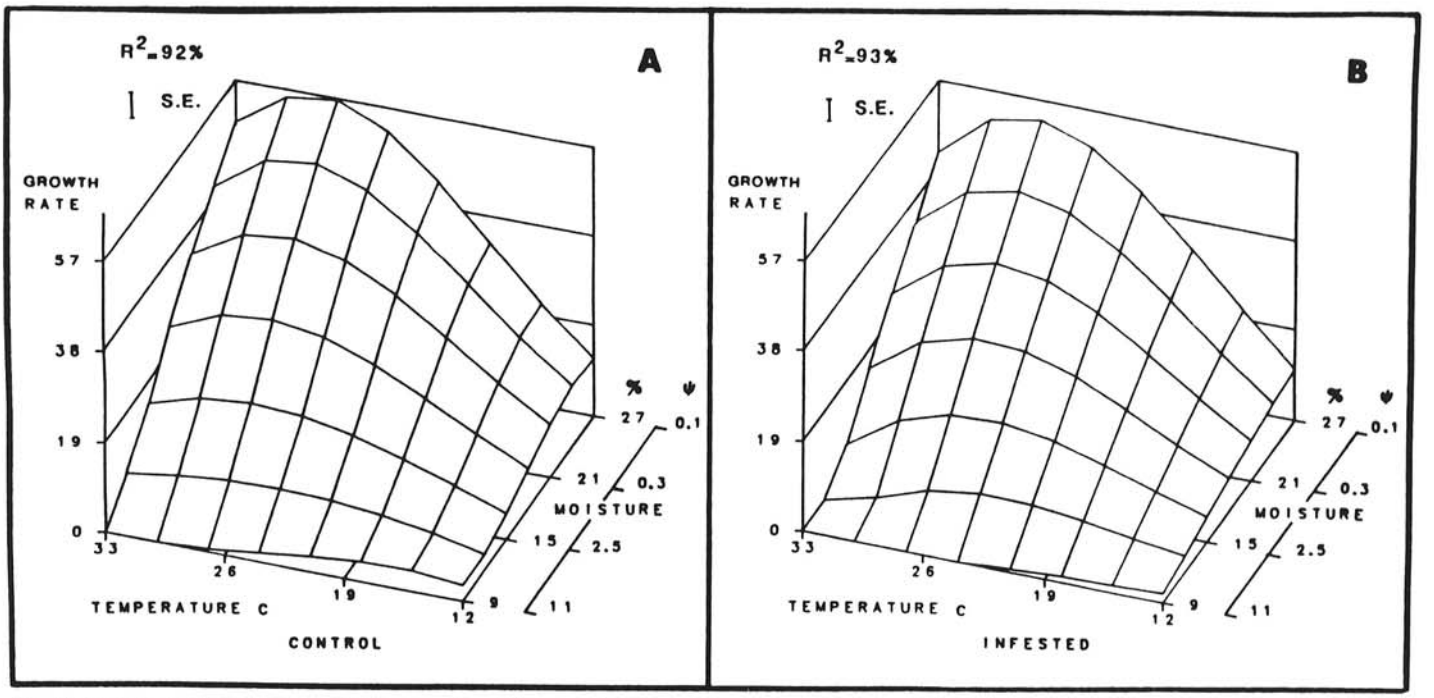

Fig. 4. Response surfaces from multiple regression of the growth rates (milligrams per plant per day) of dry bean seedlings based on fresh weights on temperature and soil moisture **A,** in the absence or **B,** in the presence of *Rhizoctonia solani.*

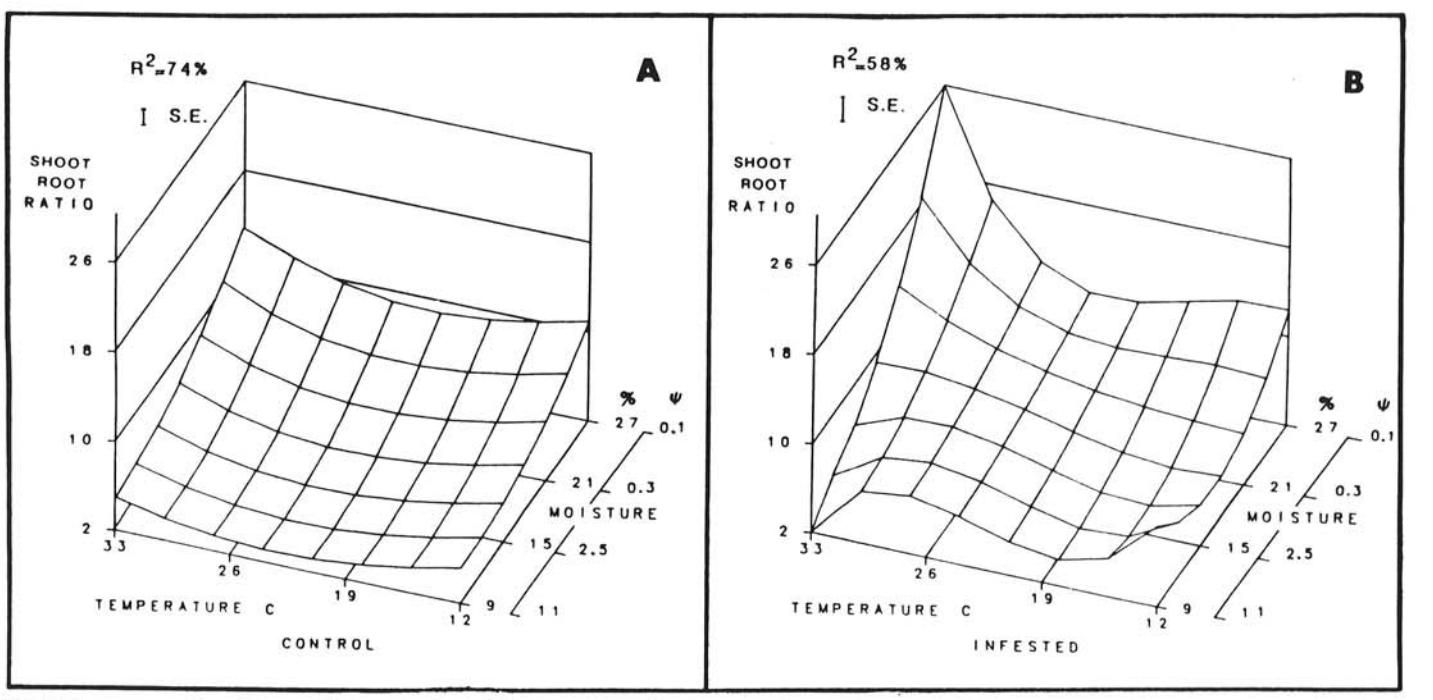

Fig. 5. Response surfaces from multiple regression of the shoot:root ratios (based on fresh weights) of dry bean seedlings on temperature and percent soil moisture **A,** in uninfested soil or **B,** in soil infested with *Rhizoctonia solani.*

TABLE 3. ANOVA table for regression of growth rate based on fresh weight (10 mg/plant/day) and of the shoot:root ratio on temperature, soil moisture, and *R. solani*

Source	Growth rate			Shoot:root		
	df	*SS*[a]	*F*	*df*	*SS*[a]	*F*
Experiment	2	213	10 **[b]	2	12	2 n.s.[b]
Temperature[c]	7	8,330	116 **	7	71	3 *
Error *a*	10	103		10	33	
Moisture[d]	5	15,564	330 **	5	232	54 **
Temp. × moisture	33	3,131	10 **	33	132	5 **
R. solani	1	155	16 **	1	47	55 **
Temp. × *R. solani*	7	270	4 **	7	23	4 *
Moisture × *R. solani*	6	359	6 **	6	17	3 *
Error *b*	64	604		64	55	
Corrected total	135	28,728		135	622	
R^2(%)		97.9			91.1	
C.V. (%)		14.7			17.1	
Mean		20.9			5.4	

[a]Sequential sums of squares.
[b]Asterisks ** and * indicate *F* significant at $\alpha = 0.01$ and $\alpha = 0.05$, respectively; n.s. = not significant.
[c]Linear, quadratic, and cubic terms are significant for growth rate, and only the quadratic term is significant for shoot:root ratio.
[d]Linear, quadratic, and cubic terms significant.

moisture was similar to that between development rate and temperature and soil moisture. *R. solani* decreased the growth rate especially at high temperature and low moisture levels. This is consistent with the higher disease severity at high temperature and low moisture levels.

The shoot:root ratios increased at the temperature extremes and at higher water potential. This is in agreement with Brouwer's (7,8) observations on bean seedlings in nutrient solution. *R. solani* increased shoot:root ratios, again at relatively high temperatures and low moisture levels.

In another series of experiments (23), there were no significant interactions between light and temperature or soil moisture in their effect on emergence and seedling development. Yet, the emergence data obtained from these experiments can only cautiously be used as input data for a simulation model intended to simulate field conditions, because the environmental conditions were quite different from those in the field. Moreover, only one inoculum density of one isolate of *R. solani* was used. Other isolates are likely to have different optimum curves for growth and infection (1), and the relationship between emergence and temperature could depend on inoculum density of the pathogen (5). Besides, seedlings and the pathogen may react differently to alternating temperatures compared to constant temperatures. At extremely low temperatures, preconditioning is not an uncommon feature during imbibition and early seedling growth. For example, during the period from about 6 to 48 hr after imbibition of bean seeds, the embryos are very sensitive to chilling (4 C) and may become irreversibly damaged (17); such seedlings become very susceptible to *R. solani* when they are returned to high temperatures (21). Therefore, additional experiments would be needed on the effect of alternating chilling temperatures and higher temperatures and of different levels of relative humidity and light intensity on infection by *R. solani* and seedling development, in order to be able to predict emergence under the wide range of environmental conditions encountered in the field.

LITERATURE CITED

1. Barker, K. R. 1961. Factors affecting the pathogenicity of *Pellicularia filamentosa*. Diss. Abstr. 22:699.
2. Bateman, D. F. 1961. The effect of soil moisture upon development of poinsettia root rots. Phytopathology 51:445-451.
3. Bateman, D. F. 1963. Factorial analysis of environment and pathogens in relation to development of the poinsettia root rot complex. Phytopathology 53:509-516.
4. Beach, W. S. 1949. The effects of excess solutes, temperatures, and moisture upon damping-off. Pa. Agric. Exp. Stn. Bull. 509:1-29.
5. Benson, D. M., and Baker, R. 1974. Epidemiology of *Rhizoctonia solani* preemergence damping-off of radish: Inoculum potential and disease potential interaction. Phytopathology 64:957-962.
6. Bierhuizen, J. F., and Wagenvoort, J. F. 1974. Some aspects of seed germination in vegetables. 1. The determination and application of heat sums and minimum temperature for germination. Sci. Hortic. 2:213-219.
7. Brouwer, R. 1963. Some physiological aspects of the influence of growth factors in the root medium on growth and dry matter production. Meded. Inst. Biol. Scheik. Onderz. Landbouwgewassen, Wageningen 212:11-30.
8. Brouwer, R. 1964. Responses of bean plants to root temperatures. I. Root temperatures and growth in the vegetative stage. Jaarboek Inst. Biol. Scheik. Onderz. Landbouwgewassen 235:11-22.
9. Doornik, A. W. 1981. Temperature dependence of the pathogenicity of several isolates of *Rhizoctonia solani* in some bulb crops as an intrinsic property of the isolate. Neth. J. Plant Pathol. 87:139-147.
10. Feddes, R. A. 1971. Water, heat and crop growth. Meded. Landbouwhogesch. Wageningen 71-12:1-184.
11. Galindo, J. J., Abawi, G. S., and Thurston, H. D. 1982. Variability among isolates of *Rhizoctonia solani* associated with snap bean hypocotyls and soils in New York. Plant Dis. 66:390-394.
12. Harrington, J. F. 1963. The effect of temperature on the germination of several kinds of vegetable seeds. Pages 435-441 in: Proc. Int. Hortic. Congr. 16th, 1962. Vol. II.
13. Hartill, W. F. T. 1968. The influence of soil conditions on development of *Rhizoctonia solani* Kühn on tobacco. Rhodesia J. Agric. Res. 6:77-79.
14. Kooistra, E. 1971. Germinability of beans (*Phaseolus vulgaris* L.) at low temperatures. Euphytica 20:208-213.
15. Leach, L. D. 1947. Growth rates of host and pathogen as factors determining the severity of preemergence damping-off. J. Agric. Res. 75:161-179.
16. McIntyre, G. I., and Boyer, J. S. 1984. The effect of humidity, root excision, and potassium supply on hypocotyl elongation in dark-grown seedlings of *Helianthus annuus*. Can. J. Bot. 62:420-428.
17. Reuther, G. 1970. Wachstumsphysiologische Untersuchungen temperatursensibler Stadien keimender Samen. Ber. Dtsch. Bot. Ges. 83:151-160.
18. Richards, B. L. 1923. Soil temperature as a factor affecting the pathogenicity of *Corticium vagum* on the pea and the bean. J. Agric. Res. 25:431-449.
19. Roth, L. F., and Riker, A. J. 1943. Influence of temperature, moisture, and soil reaction on the damping-off of red pine seedlings by *Pythium* and *Rhizoctonia*. J. Agric. Res. 67:273-293.
20. Sandsted, R. F., Ellerbrock, L. A., Halseth, D. E., Kelly, W. C., Kline, R. A., Minottie, P. L., Muka, A. A., Siecka, J. B., Sweet, R. D., Tingey, W., Topoleski, L., Warholic, D. T., Wien, H. C., and Zitter, T. A. 1983. Cornell recommendations for commercial vegetable production. NY State College of Agriculture and Life Sciences, Cornell University. 68 pp.
21. Schulz, F. A., and Bateman, D. F. 1969. Temperature response of seeds during the early phases of germination and its relation to injury by *Rhizoctonia solani*. Phytopathology 59:352-355.
22. Townsend, G. B. 1934. Bottom rot of lettuce. Mem. N.Y. Agric. Exp. Stn. (Ithaca) 158. 46 pp.
23. van Bruggen, A. H. C. 1985. The effects of *Rhizoctonia solani* Kühn on emergence, growth and yield of dry beans. Ph.D. thesis. Cornell University, Ithaca, NY. 296 pp.
24. van Bruggen, A. H. C., and Arneson, P. A. 1985. A quantifiable type of inoculum of *Rhizoctonia solani*. Plant Dis. 69:966-969.
25. Wagenvoort, W. A., and Bierhuizen, J. F. 1977. Some aspects of seed germination in vegetables. II. The effects of temperature fluctuations, depth of sowing, seed size and cultivar on heat sum and minimum temperature for germination. Sci. Hortic. 6:259-270.