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FACTORS AFFECTING GROWTH, SPORULATION, PATHOGENICITY, AND SURVIVAL OF ASCOCHYTA RABIEI [1]

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SUMMARY

Isolates of *Ascochyta rabiei* from diseased chickpea (*Cicer arietinum*) from India, Iran, Turkey, and West Pakistan varied greatly in growth rate, sporulation, and colony appearance. Maximum spore production on various nutrient media occurred with 8% chickpea seed meal agar (CSMA), whereas mycelial growth was usually greatest on CSMA or oatmeal agar. The optimum temperature for growth and sporulation was 15–20 C and the upper limit for growth was 30 C. Exposure of uniform colonies to continuous light on potato-dextrose agar increased mycelial growth rates and conidial production, whereas zonation occurred in most isolates exposed to alternating light and dark periods. Wavelengths of light in the near-ultraviolet and blue regions of the electromagnetic spectrum were responsible for increased growth and sporulation. In inoculation studies, *A. rabiei* infected chickpea, *Phaseolus vulgaris*, and *Vigna sinensis*, but was highly pathogenic only to chickpea. Of numerous chickpea lines tested, only one black-seeded type was highly resistant to most isolates of the pathogen. Pycnidia containing viable spores formed on dried chickpea stem pieces at temperatures of 10–30 C, but developed most quickly at 20 C. Mature pycnidia formed at 20 C in 46 hr in continuous light, 50 hr in alternating light and dark, and 68 hr in continuous dark. The fungus survived over 2 yr in naturally-infected chickpea tissue at temperatures of 10–35 C, relative humidities of 0–30% and on the soil surface. *Ascochyta* lost its viability rapidly at 65–100% relative humidity and at soil depths of 10–40 cm.

Chickpeas (*Cicer arietinum* L.) are the most important food legume grown in many pulse producing and consuming countries of the Middle East and Southeast Asia. In India alone this crop is cultivated on more than 8,000,000 hectares (2) and is an extremely important protein source for a large segment of the population.

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Numerous diseases affect *Cicer* in this region of the world (**6, 7, 8, 17**). In areas where rainfall and/or high humidity occur during part of the growing season *Ascochyta* blight, caused by the foliar pathogen *A. rabiei* (Pass.) Lab., is a disease of major importance (**4, 6, 9, 10, 12, 13, 14, 15, 19**).

This study was undertaken to determine the effects of various nutritional and environmental factors on growth, sporulation, pathogenicity and survival of isolates of *A. rabiei* from India, Iran, Turkey, and West Pakistan.

MATERIALS AND METHODS

Disease surveys were made from 1966 to 1971 in chickpea producing areas of India, Iran, Turkey, and West Pakistan where *Ascochyta* blight commonly occurs. Isolations were made by plating surface-sterilized (0.5% sodium hypochlorite for 5 min) pieces of diseased tissue on water agar (WA), potato-dextrose agar (PDA) or acidified potato-dextrose agar (APDA). Cultures were maintained on slants of PDA or 8% chickpea seed meal agar (CSMA).

Nutrient media used in these studies, except PDA and 8% CSMA, were from Difco Laboratories and were prepared according to the directions on the labels. CSMA was prepared by incorporating 2 g of freshly crushed chickpea seed of a large white-seeded Iranian variety in 25 cc of 2% WA in pyrex tubes, 20 × 200 mm, and autoclaving for 15–20 min at 15 psi. PDA was prepared by steeping 250 g of freshly peeled potatoes at 60 C for 1 hr, filtering the liquid through four layers of gauze, incorporating 20 g dextrose and 20 g agar, and adding water to make a final volume of 1,000 cc. Tubes of media were autoclaved for 15–20 min at 15 psi.

Isolates of *A. rabiei* used in these studies were from India (Punjab 1); Iran (Dezful 2, Ghazvin 3, Gorgan 4, and Saqez 5); Turkey (Ankara 6 and Eskisehir 7); and West Pakistan (Lyallpur 8).

Pathogenicity tests were conducted with different isolates of *A. rabiei*. Test plants utilized in these studies were bean (*Phaseolus vulgaris* L.), mungbean (*P. aureus* Roxb.), broadbean (*Vicia faba* L.), chickpea, cowpea [*Vigna sinensis* (L.) Savi ex Hassk.], lentil (*Lens esculenta* Moench), pea (*Pisum sativum* L.), cucumber (*Cucumis sativus* L.), *Chenopodium amaranticolor* Coste & Reyn., *Gomphrena globosa* L., and *Nicotiana glutinosa* L. Spores were washed from agar slants and strained through gauze before being sprayed on plants of differing ages with an aerosol spray kit. Inoculum density in the various inoculation tests varied from 10–5,000 × 10^6 spores/cc. Control plants were sprayed

with sterile water. Immediately after inoculation, plants were placed in a moist chamber for 3–5 days where the relative humidity was maintained at 100% with a humidifier. Plants were returned to the greenhouse after removal from the moist chamber. The temperatures in the chamber and greenhouses varied from 12 to 28 C.

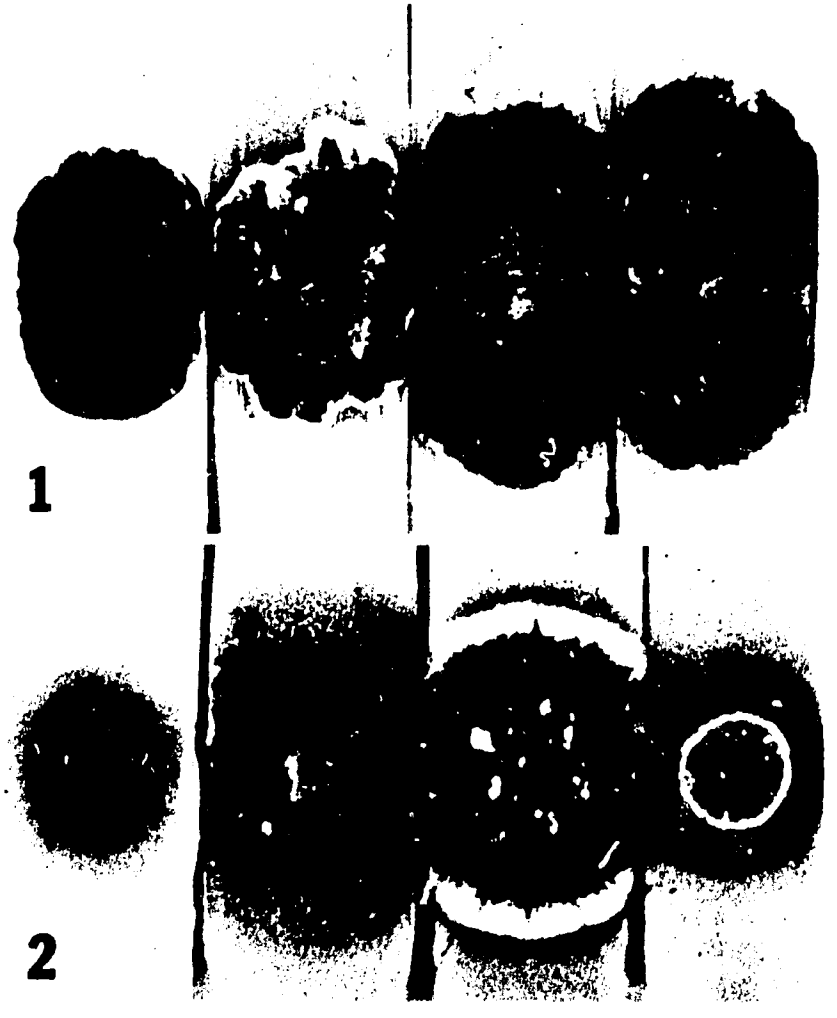
Survival of *A. rabiei* in naturally-infected chickpea stems and pods from Dezful, Iran, was studied at different temperatures, relative humidities, and in soil. Diseased tissue was incubated at 10–35 C in temperature cabinets and outdoors in a weather station shelter. Relative humidities of 0, 15, 30, 65, 85, 95, and 100% were maintained in desiccators containing different salt solutions (**18**). Diseased stems and pods were placed between two pieces of nylon screen (36 squares/cm^2) and buried at depths of 0–40 cm in a clay loam soil at Karaj, Iran. The buried samples were located in a field sown to chickpeas from May to August, 1970.

Viability of the fungus was periodically checked by macerating diseased tissue in a drop of sterile water between frosted ends of sterilized microscope slides. The pulverized tissue was washed onto the surface of WA and incubated at 25 C for 1–5 days. Germinated spores were transferred to PDA and APDA plates where fungous identifications were made.

Light experiments were carried out in a plant growth chamber which contained a bank of 8 Sylvania F48T12-CW-HO Lifetime fluorescent lamps. The temperature in the cabinet was set at 20 ± 1 C and was monitored with a recording thermograph. In experiments testing the effects of continuous light or alternating light and dark periods, the isolates were exposed to a light intensity of 10,750 lux (1 lux = 0.0929 ft-c) for 16 days. Corning glass filters were used to study the effects of different wavelengths of light on growth and sporulation of isolates of *A. rabiei*. The light intensity beneath each filter was adjusted to 550–750 lux with a lux meter by interposing the top of a Petri dish cover between the light source and filter and the lux meter. No additional adjustments were made to correct for differences in sensitivity of the lux meter to various wavelengths of light. Fungi used in the filter studies were cultured in Petri dishes (16 × 52 mm) with the lids in place. Dark-grown cultures which were kept in the same cabinet were wrapped in aluminum foil or were placed in light-proof boxes.

In all experiments, except those with colored filters, fungous isolates were cultured in pyrex tubes (20 × 200 mm) containing 25 cc of medium and measurements were made at periodic intervals of linear mycelial growth. Sporulation was determined by cutting out an entire

colony and placing it in a measured volume of water in an Erlenmeyer flask. The flasks were shaken vigorously for 5 min, and spore counts were made with a hemacytometer. There were generally 4–6 replications per treatment and data were usually the average of two or more experiments.



FIGS. 1–2. *Ascochyta rabiei*. 1. Variation in single-spore isolates from Iran after 18 days on potato-dextrose agar at 20 C. 2. Effect of temperatures of 15, 20, 25, and 28 C (from left) on growth and colony appearance of an Iranian isolate grown for 13 days on chickpea seed meal agar.

RESULTS

Effects of different nutrient media on growth and sporulation.—Different isolates of *Ascochyta rabiei* (FIG. 1) varied greatly in growth rate, sporulation, and colony appearance on different nutrient media (TABLE I). Maximum spore production occurred on CSMA, whereas maximum mycelial growth usually occurred on CSMA or oatmeal agar (OMA). There was an abundant formation of orange spore masses on CSMA and OMA with little formation of aerial mycelia or black pycnidia. When the concentration of CSMA was reduced to 1%, conidial production was decreased by 67–93%. Formation of dark masses of pycnidia was common on some media, e.g., PDA, malt agar, and mycological agar. Spore production was most abundant on CSMA with the Iranian isolate and least abundant with the West Pakistani isolate (TABLE I). Growth rate and conidial production of all isolates were poor on several agar media tested, especially nutrient, Czapek solution, and yeast extract. In comparison with these media, spore production on CSMA was increased by 23–97 times.

TABLE I

EFFECTS OF DIFFERENT NUTRIENT MEDIA ON GROWTH AND SPORULATION OF FOUR ISOLATES OF ASCOCHYTA RABIEI FOR 16 DAYS AT 20 C IN CONTINUOUS DARKNESS

Nutrient media	% Concentration	pH	*Ascochyta* isolates							
			India 1		Iran 3		Turkey 7		West Pakistan 8	
			MG[a]	Sp[b]	MG	Sp	MG	Sp	MG	Sp
Chickpea seed meal	8	5.8	50[d]	315	52	472	51	462	43	97
Chickpea seed meal	1	6.0	39	54	36	32	51	32	36	32
Oatmeal	7.2	6.0	49	275	62	406	54	113	42	37
Malt extract	2.5	5.5	35	186	39	81	45	54	28	50
Lima bean	2.3	5.6	40	122	37	98	37	60	43	62
Mycological	3.5	7.0	34	111	40	52	42	34	42	23
Potato-dextrose	....[c]	6.2	40	115	39	27	48	69	27	5
Bean pod	2.2	5.6	29	103	34	224	30	59	31	62
Peptone	2.5	7.0	29	96	30	49	26	12	38	29
Czapek solution	4.9	7.3	26	62	24	23	28	8	20	1
Corn meal	1.7	6.0	32	38	33	15	42	24	20	19
Yeast extract	1.0	6.6	28	17	36	60	24	16	33	14
Nutrient	2.3	6.8	26	14	25	7	28	9	25	3

[a] MG = Linear mycelial growth (mm) in tubes (20 × 200 mm).
[b] Sp = Spores/cc × 10^6.
[c] All nutrient media, except chickpea seed meal agar and potoato-dextrose agar, were from Difco Laboratories.
[d] Means of 3–5 experiments.

TABLE II

EFFECT OF TEMPERATURE ON GROWTH AND SPORULATION OF FOUR ISOLATES OF ASCOCHYTA RABIEI ON TWO NUTRIENT MEDIA FOR 16 DAYS IN CONTINUOUS DARKNESS

Temperature C	Potato-dextrose agar								Chickpea seed meal agar, 8%							
	Ascochyta isolate								*Ascochyta* isolate							
	India 1		Iran 3		Turkey 7		West Pakistan 8		India 1		Iran 3		Turkey 7		West Pakistan 8	
	MG[a]	Sp[b]	MG	Sp	MG	Sp	MG	Sp	MG	Sp	MG	Sp	MG	Sp	MG	Sp
10	14[c]	18	20	11	16	15	20	8	21	23	26	78	23	54	23	23
15	25	58	27	15	41	47	25	23	43	144	35	242	44	196	45	137
20	40	115	39	27	48	69	27	5	50	315	52	472	51	462	43	97
25	25	2	35	3	23	1	25	1	33	71	47	127	32	88	36	44
28	9	0	20	tr[d]	21	tr	13	tr	7	1	18	2	18	3	15	1
30	1	0	5	0	10	0	4	0	2	0	4	0	3	tr	1	0
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] MG = Linear mycelial growth (mm) in tubes (20 × 200 mm).
[b] Sp = Spores/cc × 10^6.
[c] Means of 2–4 experiments.
[d] tr = less than 0.5 × 10^6 spores/cc.

Effects of temperature on growth and sporulation.—The optimum temperature for mycelial growth and conidial production on PDA varied among the four *Ascochyta* isolates from various countries, but was always 20 C or less (TABLE II). As temperatures increased above 25 C, spore production and mycelial growth decreased rapidly and ceased at 30 and 32 C, respectively. On CSMA the optimum temperature for mycelial growth and conidial formation in all isolates was 20 C. At 10, 15, and 20 C there was an abundant formation of orange spore masses (FIG. 2). However, at 25 and 28 C the color of the colonies became black and spore production dropped off rapidly (TABLE II; FIG. 2). At 30 C conidia were detected only with the Turkish isolate. No growth occurred at 32 C, even when cultures were transferred to temperatures favorable for growth after 16 days.

Response to light.—Light had striking effects on growth and sporulation of various isolates of *A. rabiei*. On PDA and CSMA mycelial growth was always greater in cultures exposed to continuous light than continuous dark (TABLE III). On PDA exposure to light increased spore production in all isolates by 2–11 times and stimulated mycelial growth (FIG. 3). The effects of light on conidial production were greatest with the Iranian isolate and least with the West Pakistani isolate. On CSMA continuous light increased spore formation in two of four isolates studied, but the differences were less than those with PDA (TABLE III).

TABLE III

GROWTH AND SPORULATION OF FOUR ISOLATES OF ASCOCHYTA RABIEI ON TWO NUTRIENT MEDIA IN CONTINUOUS LIGHT OR DARK FOR 16 DAYS AT 20 C

Ascochyta isolate	Continuous light[a]				Continuous dark			
	PDA[b]		CSMA[b]		PDA		CSMA	
	Mycelial growth[c] (mm)	Spores/cc × 10^6	Mycelial growth (mm)	Spores/cc × 10^6	Mycelial growth (mm)	Spores/cc × 10^6	Mycelial growth (mm)	Spores cc × 10^6
India 1	55	270	55	258	40	115	50	315
Iran 3	70	297	65	440	39	27	52	472
Turkey 6	50	87	47	239	35	8	26	36
West Pakistan 8	45	33	52	129	27	5	43	97

[a] Light intensity of 10,750 lux.
[b] PDA = potato-dextrose agar; CSMA = 8% chickpea seed meal agar.
[c] Measurements of linear mycelial growth were made in pyrex tubes (20 × 200 mm) and are the means of 2–4 experiments.

TABLE IV

EFFECT OF CONTINUOUS AND ALTERNATING DARK AND LIGHT PERIODS ON SPORULATION OF SEVEN ISOLATES OF ASCOCHYTA RABIEI ON POTATO-DEXTROSE AGAR FOR 16 DAYS AT 30 C

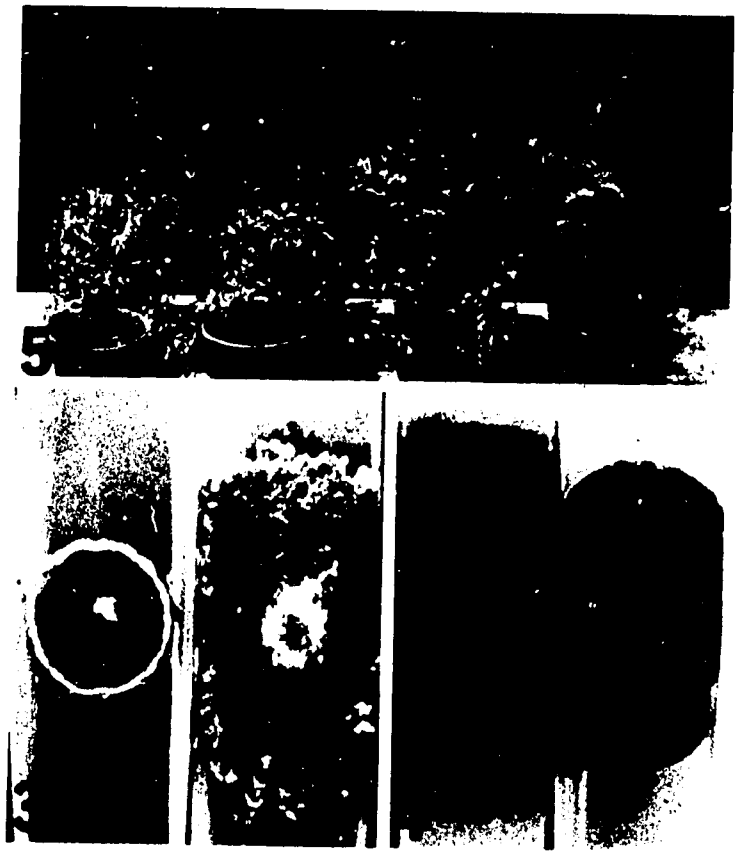
Ascochyta isolate	Sporulation[a]		
	Continuous light[b]	Alternating light-dark[c]	Continuous dark
India 1	207	194[d]	115
Iran 2	82	53	8
Iran 3	297	149	27
Iran 4	310	344	119
Iran 5	44	70	12
Turkey 7	99	177	69
West Pakistan 8	33	110	5

[a] Spores/cc × 10^5.
[b] Light intensity of 10,750 lux.
[c] 12-hr light–12-hr dark.
[d] Zonation occurred in all isolates, except Iran 2.

Effect of alternating light and dark periods on zonation.—When *A. rabiei* was grown on an alternating schedule of 12-hr dark–12-hr light at 10,750 lux on PDA at 20 C, zonation occurred in all isolates tested, except one. Zonation was more evident in some isolates, especially Iranian isolate 4 from Gorgan (FIG. 4). Zonation also occurred on CSMA in alternating light and dark periods, but was less pronounced than on PDA. Zonation was absent in cultures grown in continuous light or continuous dark. Spore production in diurnal cycles of light/dark was always greater than in continuous dark, but was often less than in cultures grown in continuous light (TABLE IV).

Effect of different wavelengths of light on growth and sporulation.—Exposure of cultures to wavelengths in the near-ultraviolet and blue regions of the electromagnetic spectrum increased the rate of mycelial growth, pycnidial formation, and spore production of all isolates of *A. rabiei* (TABLE V). The largest increase in conidial production (11.3 times) at 550–750 lux occurred with the Turkish isolate, and the smallest (2.1 times) with the Indian isolate (TABLE V). With some isolates, mycelial growth and spore production were greater in cultures exposed to continuous white light at an intensity of 10,750 lux than at lower light intensities.

Pathogenicity studies.—Various test plants, including all food legumes cultivated in Iran, were inoculated with heavy spore suspensions (10–5,000 × 10^6 spores/cc) of four Iranian isolates of *A. rabiei*. Cowpea



FIGS. 3–5. *Ascochyta rabiei*. 3. Growth of an isolate from West Pakistan on potato-dextrose agar at 20 C for 12 days in continuous dark (left) and continuous light (right). 4. Effect of alternating 12-hr light–12-hr dark (left) and continuous dark (right) on growth of an Iranian isolate after 18 days at 20 C on potato-dextrose agar. Zonation occurred only in alternating light and dark. 5. Reaction of different chickpea lines inoculated at the same time. Plants varied in their reaction from very susceptible (all plants dead) (extreme left) to highly resistant (extreme right).

and bean were the only plants besides chickpea that became infected. Small reddish brown spots developed on the stems, petioles, and leaves of cowpea and on leaves of bean, but the lesions did not increase in size. Numerous lesions developed within 5–8 days of inoculation on all foliar parts of inoculated chickpeas. Pycnidia developed abundantly in lesions on stems, petioles, leaves, and pods. Lesions increased in size and eventually girdled the stems resulting in death of all inoculated plants. No symptoms developed on noninoculated plants.

TABLE V

EFFECTS OF RADIATION OF DIFFERENT WAVELENGTHS ON MYCELIAL GROWTH AND SPORULATION OF FOUR ISOLATES OF ASCOCHYTA RABIEI ON POTATO-DEXTROSE AGAR FOR 12 DAYS AT 20 C

Filter color[a]	Filter No.	Wavelength range[b] nm	Mycelial growth (mm)[c]				Sporulation[d]			
			Ascochyta isolates				*Ascochyta* isolates			
			India 1	Iran 3	Turkey 6	West Pakistan 8	India 1	Iran 3	Turkey 6	West Pakistan 8
No filter[e]			44	50	40	44	413	318	89	30
Clear	3850	>360	32	50	38	30	144	138	93	44
Blue	9782	350–600	36	50	40	42	156	134	113	37
Green	4010	460–580	28	42	36	25	67	83	43	19
Orange	3482	>540	31	35	18	18	61	35	25	16
Red	2408	>610	31	26	21	18	105	32	10	9
Dark			28	28	25	20	75	36	10	10

[a] The light intensity beneath each Corning glass filter was 550–750 lux.
[b] Transmission characteristics of Corning glass filters.
[c] Fungi were cultured in Petri dishes (15 × 52 mm).
[d] Spores/cc × 10^6.
[e] Light intensity of 10,750 lux.

Inoculations were conducted with several isolates of *A. rabiei* to determine the resistance of numerous lines of *Cicer arietinum* to the pathogen, as well as to observe whether any differences in pathogenicity occurred between isolates of the fungus. There did not appear to be significant differences in pathogenicity of isolates to white-seeded lines which were generally more susceptible to infection by *Ascochyta*. However, one black-seeded chickpea line from Israel was highly resistant to most isolates of *A. rabiei* (FIG. 5), although this line was less resistant to some isolates from West Pakistan, indicating that there may be different strains of the pathogen.

Effect of temperature on pycnidial formation.—Propylene-oxide sterilized chickpea stem pieces were infected with a heavy spore suspension of an Iranian isolate of *A. rabiei* and plated on WA at different temperatures. In continuous dark, mature pycnidia formed in 68 hr at 20 C, 78 hr at 25 C, 96 hr at 15 C, and 120 hr at 10 and 28 C. Pycnidia occasionally developed after 10 days at 30 C. There was no mycelial growth or pycnidial formation at 32 C. Mature pycnidia formed in 46 hr at 20 C when incubated in continuous light (5,375 lux) and within 50 hr at a 12-hr alternating light and dark sequence.

Survival of the fungus.—The effect of temperature, relative humidity, and soil on survival of *A. rabiei* in naturally-infected stem and pod tissue

from Iran was observed over a 2-yr period. At temperatures of 20 and 35 C the pathogen could no longer be recovered from infected tissue after 115 weeks. The fungus was still viable at 136 weeks at 10, 15, and 30 C, and outside in a weather station shelter (FIG. 6). At lower relative humidities of 0–30% the fungus was viable at 128 weeks, but at higher relative humidities of 65, 85, 95, and 100% the fungus survived only 18, 8, 5, and 2 weeks, respectively. *Ascochyta* lost its via-

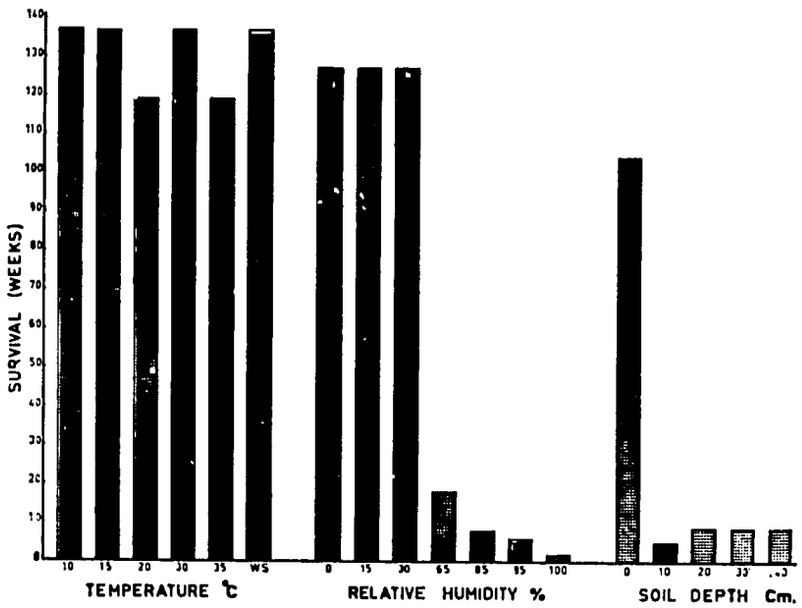


FIG. 6. *Ascochyta rabiei.* Survival of the fungus in naturally infected chickpea tissue at different temperatures, relative humidities and in soil.

bility within 8 weeks at soil depths of 10–40 cm, but remained alive for more than 100 weeks on the soil surface.

DISCUSSION

Isolates of *Ascochyta rabiei* from India, Iran, Turkey, and West Pakistan sporulated most abundantly on WA which contained 5–8% crushed chickpea seed. Heavy sporulation occurred when WA was supplemented with chickpea seed from different lines, even resistant types. In a chickpea breeding program where numerous lines are screened for resistance to *A. rabiei* large quantities of inoculum are needed in greenhouse and field inoculation tests. The use of CSMA is

recommended because it is easily prepared from various chickpea seed sources and supports abundant sporulation of numerous isolates of the pathogen.

Leach (11) reported that sporulation was initiated or increased in isolates of *Ascochyta pinodella* Jones, *A. pisi* Lib. and the *Ascochyta* state of *Mycosphaerella pinodes* (Berk. and Blox.) Vest. under continuous exposure to near-ultraviolet light. Sporulation was also increased in all isolates of *A. rabiei* tested, including Iranian isolates of *A. pisi* and *M. pinodes* (*Ascochyta* state) from *Vicia faba* (unpublished data), when exposed to near-ultraviolet and blue light. Zonation which is common in some *Ascochyta* spp. (11) occurred in most isolates of *A. rabiei* when exposed to alternating light and dark periods at 20 C. Zonation, however, was more pronounced in some isolates of the pathogen. These results differ from those of Hafiz (3) who found that zonation did not occur in three isolates of *A. rabiei* (two from Lyallpur, West Pakistan) on potato agar that were exposed to alternating light and darkness at constant temperatures. In his experiments, zonation occurred only when there was a fluctuation in temperature.

The host range of *A. rabiei* was reported to be restricted to *Cicer* (9), although some Iranian isolates of the pathogen produced small, necrotic lesions on bean and cowpea which did not increase in size. In India chickpea varieties that had been resistant to *Ascochyta* blight, subsequently succumbed to the disease (1), indicating the presence of different strains of the pathogen. Evidence was provided by Aujla (1) who identified 11 races of the fungus using 15 differential chickpea varieties. In Iran, strains of *A. rabiei* may occur, although the author has insufficient evidence to support this from pathogenicity tests with different isolates. In inoculation studies there appeared to be differences in pathogenicity to differential chickpea lines of isolates of the pathogen from India, Iran, Turkey, and West Pakistan.

In Bulgaria (10) and Greece (19), perithecia of *Mycosphaerella rabiei* Kovačevski, the perfect state of *A. rabiei*, were observed on naturally-infected chickpea debris that had overwintered in the field. Perithecia of *M. rabiei* were not observed on diseased chickpea tissue collected from various parts of India, Iran, Turkey, and West Pakistan, or when isolates of the fungus were cultured on various agar media. In Iran the perfect state did not form on infected chickpea tissue left on the soil surface for 2 years, nor did they develop on chickpea plants which were inoculated singly or in combination with various isolates of the pathogen. The absence of the perithecial state in isolates of *A. rabiei* from these countries may indicate that certain environmental

and/or nutritional factors essential for induction of the perfect state were lacking or that the proper mating types of the fungus were not present if the fungus was heterothallic.

In Iran, *A. rabiei* survived over 2 years in naturally-infected chickpea tissue at various temperatures up to 35 C, relative humidities of 30% or less, and on the soil surface. Cultures of the pathogen isolated from these tissues after 2 years were still highly pathogenic to susceptible chickpea lines. Various investigators have indicated the importance of chickpea debris in survival of *A. rabiei* and as a source of infection to subsequent chickpea plantings (6, 9, 10, 12, 13, 14, 15, 19). However, in Iran the fungus was killed rapidly when infested debris was buried in the soil, an observation that was also made by Luthra et al. (13) in India. Some *Ascochyta* spp. produce chlamydospores which are resistant structures important in survival (16). Isolates of *A. rabiei* from various countries did not form chlamydospores in culture or infected tissue. In Iran, *A. rabiei* survived in diseased chickpea tissue and seeds as mycelium and/or pycnidia.

The information obtained from survival studies, especially in soil, illustrate an important principle which may be highly effective in controlling the pathogen after harvest of *Ascochyta*-infected plantings. Incorporation of *Ascochyta*-infected chickpea debris in soil by plowing will result in rapid inactivation of the pathogen and reduce greatly the likelihood of reinfection of subsequent chickpea plantings. However, great care must be taken to avoid introduction of *A. rabiei* into chickpea plantings on contaminated seed. Seed transmission is an important factor in spread and survival of the fungus (5, 6, 12, 13, 14, 15, 19). In Iran, *Ascochyta* survived long periods in diseased chickpea seed, even under adverse environmental conditions (6).

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