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Farming will never be a success unless the farmer had more voice in the disposal of his produce—P. Morrel.

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A WILT OF ZINNIA CAUSED BY SCLEROTIUM ROLFSII[1]

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Sclerotium rolfsii has a world-wide distribution and has been recorded from the United States of America, Australia and numerous islands of the Pacific and Atlantic Oceans, China, Ceylon, Bengal, Bombay and Madras in India and from Uganda in Africa. It was first reported by Rolfs in 1893 as being parasitic on tomatoes in the United States. Since then it has been known to cause diseases of several plants—140 varieties and species—including field crops and ornamental plants. As the cause of the rot of potatoes it has been studied by Taubenhas, Edson and Shapovalov. Under the name of *Rhizoctonia destruens* it was investigated by Shaw and Ajrekar in India and it was found to be parasitic on potato, piper-betel, groundnut, *Amorphophallus companulatus*, lucerne, Dianthus, delphenium and rice. In a later communication Ajrekar wrote that *R. destruens* was identical with *S. rolfsii*. Paintin who worked on the parasitology of the fungus found that in addition to these hosts the fungus is capable of infecting many more and has given a list of new hosts containing plants from widely different families. Higgins writes that *S. rolfsii* is a soil inhabiting fungus attacking a number of wild and cultivated plants in the field. It is also a common cause of decay in stored roots and vegetables and of cucurbita and other fruits that rest in the soil. In the nurseries too this fungus causes considerable damage.

During the year 1925 specimens of Zinnia plants were received from the Agri-Horticultural gardens, Madras with the report that the flower beds were affected by disease resulting in the wilting and drying up of grown-up plants. On the surface of the basal portions of the stem and sometimes extending over the roots a number of whitish mycelial strands were present. No

[1] Paper read before the Indian Science Congress, Madras Sessions, 1929.

fructifications or sclerotial bodies were noticed. Sections of the stem were found to be permeated by hyaline hyphae which were distributed both inter and intracellularly. The pith was hollow and in this the mycelium had accumulated to form flocculent cottony masses. From the mycelial growths in the pith region transfers were made on French bean agar. The fungus made a good whitish growth and by the end of a week dark brown mustard-seed-like sclerotia were formed in large numbers. Examination of the fungus revealed it to be *Sclerotium rolfsii*.

In order to establish the pathogenicity of the fungus on Zinnia (on which it has been found for the first time) inoculation experiments were conducted on a number of these plants both in the seedling and mature stages. In addition to Zinnia a few other hosts were also included in the experiments. The plants were grown in pots containing sterilised soil. Inoculations were made with cultures of the fungus 8—10 days old. Both the sclerotia and the mycelium of the fungus were used as inocula. After inoculation the plants were covered with bell jars and the inside of the bell jars were sprayed with water once a day to keep the air inside moist. Control plants were kept in all cases and these remained healthy throughout.

Results of the inoculation. (1) Zinnia: On young seedlings 15 days old the fungus caused a rotting of the collar resulting in the characteristic 'damping off' appearance in the course of 4—5 days. But with older plants it took a longer time for the effects of the infection to be externally visible. The plants wilted in the course of 8 days. There was a whitish felt of fungus mycelium formed at the base of the stem and on these a few sclerotia developed. At this place the stem was much shrunk. The parasitism of the fungus on Zinnia was thus confirmed.

(2) Groundnut: The plants were 1½ months old. The mycelium grew over the stem and on reaching the younger portions of the shoots caused them to rot. The leaves turned yellow and the branches exhibited a rotten water-soaked appearance and hung down bent at the rotten portions. The rotting spread to all the younger shoots and the mycelium which grew over the stem and even the surface of the pots produced a number of sclerotia.

(3) On cowpea, lucerne, Bengal gram and linseed (plants 2—3 weeks old) the fungus produced infection and grew over the stem forming a whitish felt of mycelium. The leaves were discoloured and dropped off. The basal portion of the stem was shrunk and the plants bent over at these portions. Sclerotia developed on the mycelium.

(4) Cosmos: The plants wilted and the fungus spread over the collar region as a white felt. Sclerotia developed on the mycelium.

(5) Seedlings of maize and potato easily succumbed to the fungus which produced a rotting of the collar.

(6) Cotton: Cambodia cotton seedlings 20 days old were easily affected by the fungus. But grown up plants (which had developed a thick stem) showed no signs of infection even after 15 days. The mycelium, however, grew on the outside of the stem without producing any signs of wilting of the plants. The fungus is able to infect this host only in the seedling stage.

Besides the specimens of diseased plants the Agri-Horticultural Society had sent a quantity of the soil from the affected plot. Two pots were filled with this soil. In another pair of pots sterilised garden soil was placed. Healthy Zinnia seedlings were planted in all the four pots. In the course of 20 days 7 out of the 8 seedlings planted in the soil received from the society

had wilted and sections of the diseased portions showed the presence of the fungus inside, while all the control plants were healthy. The disease is thus shown to be spread through the soil.

Growth of the fungus in culture media. The fungus grows readily on agar media producing a luxuriant white mycelial growth. After spreading throughout the length of the slant, sclerotial formation begins in about a week's time, varying to some extent with different media. The mycelial growth is mostly aerial and often several hyphae unite into strands. At the top of the slant and sometimes at the bottom more of mycelial accumulations are noticed. The nature of the growth on several media was as follows:

Medium	Nature of growth	Sclerotial formation
French bean agar ...	Luxuriant white growth of aerial mycelium, finally turning cream coloured at the top	Large number of sclerotia dark brown in colour formed at the end of 7 days singly or in groups.
Oat juice agar ...	A luxuriant white growth ...	"
Cornmeal and rice agars	A good growth of whitish mycelium not so luxuriant as on French bean ...	Large number of sclerotia formed.
Glucose agar ...	White luxuriant growth with plenty of aerial mycelium ...	Sclerotia rare or few in number.
Zinnia leaf-extract agar.	Poor growth with thin aerial mycelium	Sclerotia few in number smaller in size than in the above media and of a paler colour.
Zinnia stem-extract agar	Growth poor but better than in leaf extract agar ...	"
3 per cent. maltose and sucrose agars ...	Growth poor and more or less in the form of interlacing strands on the surface ...	Sclerotia rare or absent.
Potato slants ...	A very luxuriant white growth with plenty of mycelium spreading up the sides of the tubes in a fan like manner...	Numerous dark brown sclerotia formed.
Amorphophallus slants..	"	"
Stem of Zinnia ...	A white growth not luxuriant with a number of strands on the stem	Sclerotia numerous.

Of the different agar media French bean and Oat juice show the best growth, and are best suited for the rapid cultivation of the fungus. The plant tissues especially those of potato and Amorphophallus give a more luxuriant mycelial growth and a large number of sclerotia.

The effect of different concentrations of sugars such as sucrose, maltose and lactose on the growth of the fungus was investigated. For this purpose agar media containing these sugars in 1%, 2% and 3% concentrations were prepared. The cultures were grown in Petri dishes one sclerotium being planted in the centre of each plate. The diameter of the growth was measured at intervals. Duplicate cultures were grown and the average measurements are given below:

Date of inoculation	Date of observation	Diameter of the colony in m.m.								
		Maltose		Sucrose			Lactose			
		1%	3%	1%	2%	3%	1%	2%	3%	
21— 4—27	25— 4—27	40	50	33	36	42	14	13	13	
	26— 4—27	50	60	41	45	52	16	20	22	
	28— 4—27	62	68	60	64	67	27	31	33	

The dishes with 2% maltose were contaminated and as such no measurements were taken from them. The fungus grew much more quickly in maltose and sucrose than in lactose agar. Of the two former, there was an initial increase in the rate of growth in maltose but later no difference could be made out. In all the 3 kinds of sugars the rate of growth increased with the concentration, 3% showing the greatest growth.

The nature of the growth of fungus on media of different reactions was next studied. Oat-juice agar of different Fuller's scale varying from minus 10 to plus 20 was prepared and the rate of growth of the fungus in Petri dishes was observed at intervals. Duplicate cultures were kept and below are shown the average measurements and the number of sclerotia formed.

Diameter of the growth in mm. Sclerotia planted on August 13, 1928

No.	Reaction	15/8	16/8	17/8	18/8	19/8	Number of Sclerotia on 25/8
1	−10	25	49	73	88 (full)	...	50
2	− 5	23	50	75	88 ,,	...	60
3	N	27	55	78	90 ,,	...	42
4	+ 5	31	62	86	90 ,,	...	87
5	+10	27	54	75	90 ,,	...	80
6	+15	26	48	66	82	88 (full)	46
7	+20	18	41	56	74	88 ,,	43

An examination of the results shows that the growth is quickest in +5 with a gradual but slight falling off on either side. +20 shows the lowest growth. By the 18th the growth in the plates from −10 to +10 had completely filled the dishes. In +15 and +20 it was only on the 19th that the growths had extended completely over the dishes. Sclerotial formation had begun by the 20th in all the plates excepting +15 and +20 in which it was delayed by two days more. By the 25th the dishes of +5 and +10 reactions showed the largest number of sclerotia.

Change in the medium by the growth of fungus. Alkaline and neutral French bean agar are darker in colour than those of acidic reactions. But when S. rolfsii was allowed to grow over the former it was found that the media became paler by the growth of the fungus. The paler colour was noticed up to 3 mm. in advance of the aerial mycelial growth. This suggested that the fungus might have given rise to some acid during its growth.

The fungus was cultivated on congo-red agar and alkaline litmus agar. In the former the colour of the medium changed with the growth of the fungus from red to violet—an indication that acid is formed. The alkaline litmus agar developed a reddish tinge as the fungus extended over the medium.

To determine definitely the changes brought about in the acidity of the medium during the growth of the fungus it was grown in Richard's solution and the pH value of the medium determined periodically. Four flasks with 100 c.c. of the medium each were sterilised and the pH value adjusted to 5·4. Two of the flasks were inoculated with one sclerotium each on 19–9–27 and the other two were left as controls. On the 22nd the pH value of the medium in the inoculated flasks was 4·6 while in the control it was 5·4. On the 26th the medium in the inoculated flask showed 2·4 while the control had 5·4. On testing the media on the 29th again the inoculated one showed 2·4 and the control 5·4. The readings point out the increase in the acidity of the medium produced by the growth of the fungus. It developed very few sclerotia. Higgins who has worked on this problem describes a similar change in the pH value of the medium produced by the growth of *S. rolfsii*, and attributes the same to the formation of oxalic acid by the fungus.

Morphology of the fungus. The mycelium is hyaline much branched and spreads out in strands producing a fan-like appearance. The hyphae are septate and the branches generally arise from below a septum. Clamp connections and H connections are present. Often several hyphae become united to form thick white strands. In 3 to 4 days small whitish masses begin to form at the ends of the strands. These gradually increase in size and become rounded. The colour of these masses changes from white to cream and ultimately they develop into brown sclerotia. These occur singly or sometimes 3 or 4 of them fuse together to form irregular masses. On agar media sclerotia are formed in about 7 days. They are rounded or slightly flattened with one side—generally the side to which the mycelial strand is attached—slightly concave and with a number of minute pits on the surface. When mature they easily get separated from the mycelial strand. The individual sclerotia are usually 1·5 to 2 mm. in diameter but the massive ones formed by the fusion of 3 or 4 sclerotia often reach a length of ·8 cm. When placed in water or inside a moist chamber the sclerotium germinates by the production of long hyphal filaments from all over the surface. No kind of spore formation was observed in any of the cultures.

The size of the sclerotium varies to a certain extent with the medium on which it is formed. On some it is small while on others it is of a larger size. The averages of 50 sclerotia each from the following sources are as follows:

Source	Diameter of sclerotia in mm.		
	Maximum	Minimum	Average
Sterilised Zinnia stem ...	3	1	2·1
Oat agar	2·5	1	1·7
Zinnia leaf extract agar ...	1·25	·75	1
Zinnia stem extract agar ...	1·5	·7	1·36

The sclerotia formed on the Zinnia stem are of a bigger size while those from the Zinnia leaf—extract agar are very small. Taubenhas suggests that the acid in certain host tissues might be responsible for the bigger size of the sclerotia on them. In order to find out whether the same holds good on agar media also measurements were made of 50 sclerotia each from oat agar media of different reactions. The measurements were as follows:

Reaction	Diameter of sclerotia in mm.		
	Maximum	Minimum	Average
−10	2·5	1	1·5
− 5	2	1	1·7
N	2	1	1·5
+ 5	2·25	·9	1·6
+10	2	1	1·5
+15	2	1	1·8
+20	2·25	1	1·65

The acidity of the medium does not seem to exert any marked influence on the size of the sclerotia. The bigger size on some of the host tissues may probably be due to something other than acids contained in them.

Though at first round and big the sclerotium shrinks very much as the medium dries up. But when it is placed in water it again regains its original size.

Viability. Sclerotia retain their viability for long periods. But it depends on how they are preserved. A number of them removed from a culture on zinnia stem, 13 months old were alive and germinated readily. But others taken from a dried up French bean agar tube 8 months old did not show any signs of germination. Extreme drying up as that which occurs in old agar tubes (not sealed) is detrimental to the viability of the sclerotia.

Effect of heat on sclerotia. Sclerotia are more resistant to temperature variations than the ordinary mycelium and are produced to tide over unfavourable seasons. But above a maximum even the sclerotium is killed. The effect of exposure to dry heat at different temperatures for ½ hour and 1 hour on the germinating capacity of the sclerotia was determined. Mature sclerotia were kept on glass slides inside a hot water oven for the specified time at different temperatures. These were then removed and kept for germination in Petri dishes lined with sterilised and moistened filter papers. The Petri dishes were kept inside moist chambers. 25 sclerotia were kept in each case and the following are the results of germination.

Temperature	Time of exposure	Number germinated	Remarks
43°C	1 hour	25	Germinated on the next day, as good as control.
50°C	,,	25	Germinated on the next day, as good as control.
52°C	30 minutes	25	
52°C	1 hour	8	
53°C	30 minutes	25	Germination delayed by two days.
53°C	1 hour	7	
55°C	30 minutes	23	do
55°C	1 hour	Nil	
Control	...	25	

Sclerotia exposed to 55°C for 1 hour are killed.

The effect of chemicals on the Sclerotia. *Sclerotium rolfsii* is a soil inhabiting fungus. Various chemicals are used for sterilising the soil in gardens. The effect of some of the new fungicides which are being used for soil sterilisation in recent times and formalin and mercuric chloride on the sclerotia was tested. The sclerotia were steeped in the respective solutions for definite lengths of time, then washed in sterilised water and then kept for germination in Petri dishes with moistened filter papers. Twenty sclerotia were used on each occasion with the following results.

Fungicide	Strength of solution	Time of steeping	Date of steeping	Date of observation	Number germinated	Remarks
Germisan ...	0·5%	30 min.	24-2-26	1-3-26	4	
	0·5%	1 hour	,,	,,	...	
	1·0%	30 min.	,,	,,	...	
Uspulun ...	0·5%	30 min.	,,	,,	8	
	0·5%	1 hour	,,	,,	...	
	1·0%	30 min.	,,	,,	...	
Control ...	.....	.....	.....	.....	20	
Tillantin ...	0·5%	30 min.	25-2-26	3-3-26	18	
	0·5%	1 hour	,,	,,	12	
	0·5%	1½ hours	,,	,,	10	
	1·0%	30 min.	,,	,,	17	
	1·0%	1 hour	,,	,,	12	
	2·0%	30 min.	,,	,,	17	
	2·0%	1 hour	,,	,,	8	
Control ...	.....	.....	.....	.....	20	
Cheshunt compound.	Normal strength	30 min.	26-2-26	4-3-26	18	
	Do.	1 hour	,,	,,	20	
	1·0%	30 min.	,,	,,	20	
	1·0%	1 hour	,,	,,	18	
	1·0%	2 hours	,,	,,	15	
Control ...	.....	.....	.....	.....	20	
Formalin ...	2·0%	30 min.	15-10-27	25-10-27	6	Germination on the 4th day only.
	2·0%	1 hour	,,	,,	6	
	2·0%	1½ hours	,,	,,	...	
Control ...	.....	.....	.....	.....	20	
Mercuric chloride ...	0·1%	30 min.	21-9-27	30-9-27	14	
	0·1%	1 hour	,,	,,	12	
	0·2%	30 min.	,,	,,	6	
	0·2%	1 hour	,,	,,	...	
Control ...	.....	.....	.....	.....	20	

Of the fungicides 0.5 per cent solutions of germisan and uspulun and 0.2 per cent solution of mercuric chloride kill the sclerotia in one hour. Two per cent formalin kills in 1½ hours while Tillantin and Cheshunt compound are much less effective. Perhaps longer periods of immersion may be more effective. Shaw and Ajrekar mention that 0.1 per cent mercuric chloride kill the sclerotia in 2½ hours. Shorter periods with the same concentration are found to be less effective.

I am indebted to Mr. S. Sundararaman, Government Mycologist, Coimbatore for advice in carrying out this investigation and to Mr. P. D. Karunakar, Government Agricultural Bacteriologist for the determination of OH values of some media.

Summary

Sclerotium rolfsii was observed to cause a wilting of grown up zinnia plants. The fungus was isolated from diseased specimens and infection experiments proved its parasitism on zinnia resulting in the damping off of seedlings and wilting of grown up plants.

It grows luxuriantly on artificial media producing a whitish mucelium spreading out in a fanlike manner and forming in seven days numerous dark brown rounded sclerotia which measure on an average 1·5 to 1·7 m.m. in diameter. During its growth the fungus increases the acidity of the medium. The size of the sclerotium varies to a slight extent with the medium on which it is formed.

Sclerotia have been found to retain their viability for 13 months. They are killed when exposed to dry heat at 55° C for one hour. They die when immersed in ·5 per cent solutions of Germisan and Uspulun and ·2 per cent of mercuric chloride for one hour. In 2 per cent formalin they are killed in 1½ hours.

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USEFUL RECIPES

Lime sulphur sprays

Take 2 lbs. of quick lime in a kerosene oil tin and shake it with water. Add 2½ gallons of water, and boil. When the water has begun to boil add 4 lbs. of sulphur little by little, so that water and sulphur get well mixed up. Boil the mixture till the whole liquid gets a dark brown colour (this will be in about 1 hour.)

Caution. Never allow the mixture to become green. Remove the liquid, filter through a piece of gunny, and the solution is ready for use. Before spraying make it up to 20 gallons.

If the original mixture is to be preserved, it should be kept air-tight and if this should be effectively done pour some mineral oil (say kerosene) on the surface of the liquid.

(1 kerosene tin = 4 gallons = 24 ordinary bottles.)