SOME INTERACTIONS BETWEEN PLANT ROOTS AND PATHOGENIC SOIL FUNGI

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Summary

A "Cellophane" bag technique is described which enables some chemical interactions between organisms to be studied *in situ*.

Both *Pellicularia filamentosa* and *Sclerotinia homeocarpa* affect a wide range of plants. *P. filamentosa* causes typical damping-off of seedlings whereas *S. homeocarpa* causes a severe stunting without penetrating the plant tissue. It was shown by the "Cellophane" bag technique that these fungi excrete substances toxic to the plants, and also that the roots of lettuce and radish seedlings stimulate the growth of *P. filamentosa* but the roots of tomato seedlings do not. Preliminary investigations on the relation of the root excretions to infection and susceptibility of seedlings are described.

I. INTRODUCTION

The interactions between plant hosts and fungal pathogens have been studied intensively by workers in many countries, and much is now known about the post-penetration stages of infection; the pre-penetration stage of infection, however, has been relatively neglected although it is well established that the host plant can influence the pathogen before penetration takes place. W. Brown (1922) showed that the germination of spores of *Botrytis cinerea* was stimulated by diffusions from damaged plant tissues, and R. Brown (1946) has reviewed the literature on the influence of root excretions on the germination of spores of pathogenic fungi. The reverse effect of pathogen on host has also been noted by some workers. Müller (1924) observed that necrotic spots appeared on potato roots prior to penetration by hyphae of *Pellicularia filamentosa*, and Lebeau and Dickson (1953) have shown that an unidentified snow-mould pathogen liberates hydrogen cyanide in toxic quantities.

In the course of work on two lawn diseases, brown patch and dollar spot, caused by *P. filamentosa* (Pat) Rogers and *Sclerotinia homeocarpa* Bennett respectively, some interesting results were obtained on the mutual influence of host and pathogen prior to penetration. The present paper reports these results and discusses their significance.

II. EXPERIMENTAL

(a) Pathogenicity of P. filamentosa and S. homeocarpa to Several Plants

At least 230 plant species belonging to 66 families have been recorded as hosts of *P. filamentosa* (Braun 1930). Storey (1941) showed that the fungus exists as several strains, some of which have a wide host range while others are more specialized and may be restricted to hosts of a single family. The literature on physiological specialization of *P. filamentosa* has been reviewed by Kernkamp *et al.* (1952).

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S. homeocarpa has been recorded as causing disease only in grass lawns, but Montieth and Dahl (1932) state that the fungus may attack a wide range of weeds in lawns. In the present work, the pathogenicity of *P. filamentosa* and *S. homeocarpa* to several plants of economic importance was tested. *P. filamentosa* (Waite Institute strain St 3) was isolated from a lawn infected with brown patch, and *S. homeocarpa* from a golf green infected with dollar spot.

The plants tested were: *Raphanus sativus* L. (radish), *Lactuca sativa* L. (lettuce), *Beta vulgaris* L. (red beet), *Lycopersicon esculentum* Mill. (tomato), *Trifolium subterraneum* L. (subterranean clover), *Triticum vulgare* Vill. (wheat), *Pisum sativum* L. (pea), and *Phaseolus vulgaris* L. (bean).

TABLE 1

THE PATHOGENICITY OF P. FILAMENTOSA AND S. HOMEOCARPA TO SEVERAL

PLANTS

Percentage of healthy, emerged seedlings from four jars

Plant	Control	P. filamentosa	S. homeocarpa	No. of Seeds Sown
Radish	81	1	0	80
Lettuce	83	4	4	80
Beet	130	1	1	80
Tomato	91	19	16	80
Subterranean clover	80	8	3	80
Pea	83	42	0	40
Bean	90	95	0	20
Wheat	93	83	75	40

Pathogenicity was measured by sowing seeds of the various test plants in inoculated and in uninoculated soil and counting the subsequent emergence of seedlings. To inoculate soil, the fungi were grown separately on sand-maize-meal for 10 days and then mixed thoroughly with unsterilized soil at the rate of 2 g inoculum to 100 g soil. Cylindrical glass jars, 9 cm deep and 9 cm wide were used as soil containers. Unless otherwise stated this method of soil inoculation was used throughout. There were four replicates of each treatment and the experiment was run in a constant temperature growth room at 22°C. The results were recorded after 10 days (Table 1).

The results indicate that *P. filamentosa* (strain St 3) has a wide host range. Most of the susceptible seedlings were attacked in the pre-emergence stage, while the few seedlings that did emerge were often diseased. Of the small-seeded, non-graminaceous plants tested, tomato appeared to be more resistant than the others. The emergence of beans was not affected by the fungus, which produced limited, dark brown lesions on the hypocotyls near soil level. The effect on peas was similar but they were more susceptible than beans, and the lesions sometimes completely encircled the hypocotyls causing the seedlings to collapse. Symptoms produced on wheat closely resembled the disease "sharp eye spot" described by Glynne and Ritchie (1943). There was very little damage to the roots of any of the plants tested.

Sclerotinia homeocarpa also appeared to have a wide host range but the effect of this fungus on the host was quite different to that caused by *P. filamentosa*. The seedlings which emerged were severely stunted (Plate 1) but when they were dug up, washed, and examined, there was no sign of penetration of plant tissue by the fungus. The non-emerged seedlings were then recovered from the soil and examined. Tomato, beet, radish, lettuce, subterranean clover, and wheat seedlings were all severely stunted, but showed no sign of fungal infection. Half of the bean seeds sown had not germinated while the others had produced radicles about $\frac{1}{2}$ in. long. The tips of the radicles were brown and necrotic, but no mycelium could be found within the root tissue. Necrotic roots were washed thoroughly in sterile water and planted on tap water-agar; the presence of *S. homeocarpa* could not be demonstrated. Peas were even more severely affected than beans and germination had been arrested at a very early stage. The pea and bean seeds were soft, and microscopic examination showed that the cotyledons were heavily infected with bacteria and fungal hyphae. *S. homeocarpa* was readily isolated from these seeds.

(b) *Chemical Interactions between Hosts and Pathogens*

The previous experiment showed that the pathogenic activities of *P. filamentosa* (strain St 3) and *S. homeocarpa* were very different: *P. filamentosa* penetrated the tissues of many plants and caused pre-emergence or post-emergence damping-off of seedlings; *S. homeocarpa* also damaged a wide range of plant seedlings, but the effect of the fungus on all hosts except beans and peas was not direct parasitic attack, but appeared due to the production of a toxin which prevented or retarded growth.

Further studies of the interactions of hosts and fungi have been undertaken using a "Cellophane" bag technique to prevent physical contact between host and fungus and yet allow diffusion of chemicals from one to the other. Dobbs and Hinson (1953) have used "Cellophane" bags to study the behaviour of fungal spores in soil, and by this method were able to demonstrate a widespread fungistasis in soil that prevented the germination of spores of many soil fungi. In the present studies, seeds of various hosts were placed in "Cellophane" bags which were then part buried in soil inoculated with the appropriate fungus. "Cellophane" sheets, 25 μ thick, were boiled in water, as recommended by Dobbs and Hinson, to remove any coating material, and then made into bags 2 by 2·5 in. using adhesive "Cellophane" to seal them. Eight seeds of lettuce or radish were placed in the lower half of a "Cellophane" bag, and two bags of each plant were then partly buried in 400 g unsterilized soil in a glass jar. The soil had been inoculated previously with sand-maize-meal cultures of *P. filamentosa* or *S. homeocarpa* or left uninoculated. The soil containers were covered with clear plastic to prevent evaporation, and the experiment was carried out in quadruplicate in a constant temperature growth room at 22°C. After 3 days incubation, the bags were taken carefully from the soil and washed gently in water to remove adhering soil particles.

The seedlings from the bags buried in soil inoculated with *S. homeocarpa* were markedly stunted compared with the controls (Plate 2, Fig. 1). Some of the lettuce roots were necrotic but in no case had the fungus penetrated the "Cellophane". Stunted and necrotic seedlings were planted on tap water-agar and potato-dextrose-

agar but *S. homeocarpa* did not grow from them. This confirms that *S. homeocarpa* produces a substance toxic to plants, and that the plants may be affected without the fungus penetrating them. The substance is soluble in water and is capable of diffusion through "Cellophane".

The "Cellophane" bags buried in soil inoculated with *P. filamentosa* were examined, and it was found that there was marked aggregation of hyphae on the outside of the "Cellophane" directly opposed to some of the roots of both radish and lettuce seedlings. Where aggregation had occurred the radish roots were blackened and the lettuce roots severely necrotic. The aggregated hyphae outside the bags were removed from the "Cellophane" by means of a needle and forceps, planted on tap water-agar, and were shown to be *P. filamentosa*.

Further experiments showed that the aggregation of *P. filamentosa* hyphae could be demonstrated more clearly if sterile, germinating seedlings (four per bag) were used instead of seed, and if the soil was left for 1 week after inoculation before the "Cellophane" bags were buried. If the bags were buried in the soil immediately after inoculation, there was dense hyphal growth over the "Cellophane" in the soil and stimulation of the fungus by the roots was sometimes obscured. Staining the bags with cotton blue in lactophenol, after removal from the soil, facilitated examination of the fungal hyphae.

Using this modified technique, tomato, lettuce, and radish seedlings were tested in unsterilized soil inoculated with *P. filamentosa* and in uninoculated soil, and four bags from each treatment were examined after 3, 5, and 7 days incubation. After 3 days incubation there was a marked aggregation of hyphae opposite the roots of lettuce and radish seedlings in inoculated soil (Plate 2, Fig. 2). There was no aggregation of fungal hyphae opposite tomato roots in inoculated soil or opposite the roots of the three test species in control soils. It was again observed that where aggregation had occurred, the roots of the plants were damaged and the seedlings were markedly stunted (Plate 3). The bags were opened and the "Cellophane" carefully examined under a microscope for hyphal penetration but none were found. Hyphae of *P. filamentosa* did not grow from affected seedlings which were planted on tap water-agar and potato-dextrose-agar. After 5 days incubation, aggregation of hyphae was more intensive and damage to seedlings more severe. Cultural studies showed that the fungus had penetrated four of the "Cellophane" bags, two containing radish and two containing lettuce seedlings, and that the seedlings in the bags were infected. After 7 days incubation, all bags containing radish or lettuce seedlings in inoculated soil were severely rotted and it was difficult to remove them from the soil without damage; the bags tore only where there was intensive hyphal aggregation. Those bags containing tomato seedlings in inoculated soil and those containing radish, lettuce, and tomato seedlings in uninoculated soil were intact. There was no aggregation opposite the hypocotyls of any seedlings of the three test plants.

The aggregation must have resulted from the diffusion of a substance, or substances, from the plant roots through the "Cellophane" to the soil, and this substance stimulated the growth of *P. filamentosa*. The damage to the plant roots prior to penetration of the "Cellophane" by the fungus, must have been caused by the

diffusion of a substance from the aggregated hyphae through the "Cellophane" to the plant roots.

In inoculated soil the weakening of the bags containing radish and lettuce seedlings could have been caused by the physical penetration of the "Cellophane" by the fungal hyphae or by the cellulolytic activity of *P. filamentosa* or associated soil organisms. *P. filamentosa* has been classified by Garrett (1951) as a "sugar fungus", a term first used by Burges (1939) to denote those soil fungi that are unable to decompose either cellulose or lignin. Blair (1943) has demonstrated, however, that *P. filamentosa* is able to decompose cellulose, although much more slowly than typical cellulose decomposers, and Siu (1951) lists *P. filamentosa* as a cellulolytic fungus.

(c) The Relation of Root Stimulation to Infection and Susceptibility of Seedlings

Experiments with the "Cellophane" bags had indicated that growth of *P. filamentosa* over the "Cellophane" was denser when the bags were placed in the soil immediately after inoculation than when they were added 1 week later (see Section II (b)). It is unlikely that this difference was the result of a rapid decrease in the fungal population in the soil; a simpler explanation would be that shortly after inoculation

TABLE 2

THE INFLUENCE OF TIME OF SOWING AFTER SOIL INOCULATION WITH P. FILAMENTOSA
ON THE EMERGENCE OF SEEDLINGS

Percentage of healthy, emerged seedlings from four pots each sown with 20 seeds

Time of Sowing after Inoculation	Lettuce	Radish	Tomato
Not inoculated	78	71	83
Two weeks	0	1	51
Immediately	0	0	13

the fungus was actively colonizing the soil, whereas after 1 week the process of colonization was largely completed, resulting in a decrease in the number of growing tips of the fungus. If infection of seedlings is dependent on a stimulation of the fungus before penetration, the artificial stimulus following soil inoculation might influence the number of seedlings infected. To test this, radish, lettuce, and tomato seeds were sown in soil (not in "Cellophane" bags) immediately after inoculation and 2 weeks after inoculation, and also in uninoculated soil.

The results were recorded after 10 days incubation at 22°C. Table 2 shows that there was a much higher number of healthy tomato seedlings in the soil sown 2 weeks after inoculation than in the soil sown immediately after inoculation, but that the number of healthy radish and lettuce seedlings in the two soils was not significantly different. These results show that an increase in the activity of the fungus in the soil leads to an increase in the number of tomato seedlings infected; they do not indicate, however, whether the difference in emergence between tomato seedlings on the one hand and lettuce and radish seedlings on the other, is due to the latter being more

susceptible, or to the stimulation of the fungus by their root excretions. If the second alternative is true, it is possible that by growing tomato plants in close association with radish and lettuce plants, stimulation of the fungus by the latter could influence the number of tomato seedlings infected. This was tested by sowing tomato seed in contact with radish and lettuce seed in soil inoculated 2 weeks previously, and in uninoculated soil. Seeds of the three plants were also sown separately. The emergence of tomato seedlings from inoculated soil was much higher when sown alone than when grown with radish and lettuce seedlings (Table 3), suggesting that stimulation of the pathogen by root excretions from the latter plants caused an increase in the number of infected tomato seedlings. Stimulation of the fungus resulting from infection of radish and lettuce seedlings may be an alternative explanation.

TABLE 3

THE EFFECT OF SOWING LETTUCE, RADISH, AND TOMATO SEEDS ALONE AND ALL TOGETHER IN SOIL INOCULATED WITH P. FILAMENTOSA

Percentage of healthy, emerged seedlings from four pots

	Method of Sowing	Lettuce	Radish	Tomato
Control	Alone	81	66	81
	All together	78	66	82
P. filamentosa	Alone	3	2	61
	All together	1	0	20

From these experiments a marked difference in the susceptibility of tomato seedlings and radish and lettuce seedlings was not demonstrated conclusively. The difficulty has been to distinguish between the effect of fungal activity as influenced by root excretions and true host susceptibility. The "Cellophane" bag experiments, however, had failed to demonstrate a stimulation of the fungus by the hypocotyls of any seedlings, and in an attempt to determine the true susceptibility of radish, lettuce, and tomato to *P. filamentosa* (strain St 3), a different method of inoculation was used. Seeds of the three plants were sown separately in uninoculated soil in eight glass jars, 20 seeds being sown in each. Immediately after seedling emergence, discs (6 mm in diameter) of potato-dextrose-agar inoculum were placed on the soil surface at the centre of half the jars, the remainder being left uninoculated, and all were covered with clear plastic. The fungus grew out radially from the inoculum over the surface of the soil. Although some hyphae probably grew through the soil, the effect of root stimulation must have been considerably reduced. 100, 96·7, and 39·6 per cent. of lettuce, radish, and tomato seedlings respectively, were diseased. This indicates that tomato seedlings are more resistant to infection by *P. filamentosa* than are lettuce and radish seedlings, although the possibility of a chemical stimulation by the hypocotyls of the susceptible seedlings cannot be entirely ruled out. There was a marked difference, however, between the appearance of the affected plants; the hypocotyls and cotyledons of infected lettuce seedlings became completely per-

meated by the fungus mycelium, whereas damage to the tomato seedlings was largely confined to the base of the hypocotyls at soil level. The appearance of radish seedlings was intermediate to that of lettuce and tomato seedlings; frequently the cotyledons and the upper part of the hypocotyls were infected. These observations support the belief that tomato seedlings are more resistant to infection by *P. filamentosa* than are lettuce and radish seedlings.

III. DISCUSSION

Both *P. filamentosa* and *S. homeocarpa* would be classified by Gäumann (1950) as "necrobionts"; they "never feed directly on living plant substance because the toxic secretions of their hyphae kill the host cells in advance of them." When *S. homeocarpa* is inoculated into soil it grows very vigorously for a limited period, and in the process secretes a toxic substance in sufficient concentration to depress the growth of seedlings of several plant species which are not penetrated by the fungus as far as I have been able to discover. Most seedlings are not markedly damaged by the toxin, the exceptions being pea and bean seedlings, whose roots are severely damaged by the toxin. An effect similar to this has been demonstrated by Newton and Mayers (1935) for a strain of *P. filamentosa* on turnips and carrots. "Turnips are markedly stunted when grown on infected soil but exhibit no lesions or other symptoms." *P. filamentosa* (strain St 3) also excretes a toxin which may cause a necrosis of roots before the plant tissues are penetrated by the fungus. The production of toxins by both fungi was demonstrated conclusively by the "Cellophane" bag technique. This technique also showed that *P. filamentosa* (strain St 3) is stimulated by a substance excreted by the roots of radish and lettuce seedlings. This stimulation caused the hyphae of *P. filamentosa* to aggregate on the outside of the "Cellophane" bags directly opposed to the roots. Both stimulatory and toxic substances must be soluble in water and be capable of diffusion through thin "Cellophane".

Evidence was presented to show that the excretion of stimulatory substances by roots makes seedlings more liable to infection, and also that tomatoes which did not stimulate the fungus were less susceptible than radish and lettuce which did. This aspect of the work is being investigated further.

In the "Cellophane" bag experiments, fungal growth was not stimulated by the hypocotyls of any of the test plants, and yet when seedlings are not enclosed in "Cellophane" bags, *P. filamentosa* (strain St 3) attacks the hypocotyls and not the roots. A root does not have an impervious covering of cuticle and so presumably substances can diffuse into the soil much more readily from a root than from a hypocotyl. Either the hypocotyls did not excrete a stimulatory substance, or the substance did not diffuse through the "Cellophane" in sufficient quantity to stimulate growth. The contact between hypocotyl and "Cellophane" was never as close as between root and "Cellophane", as the cotyledons in their upward growth pushed apart the two sides of the bag, and this may have prevented the diffusion of substances from the hypocotyls to the surrounding soil. It is possible that when host and pathogen are in direct contact there is sufficient diffusion from the hypocotyls to influence the growth and behaviour of the fungus. If this is the case, the stimulation of the fungus by the hypocotyls might be much more important than stimulation by roots, and root

excretions may be largely fortuitous due to the "leaky" character of the root, although they may influence the ability of a pathogenic fungus to "find" a susceptible host.

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PLANT-PATHOGEN INTERACTIONS
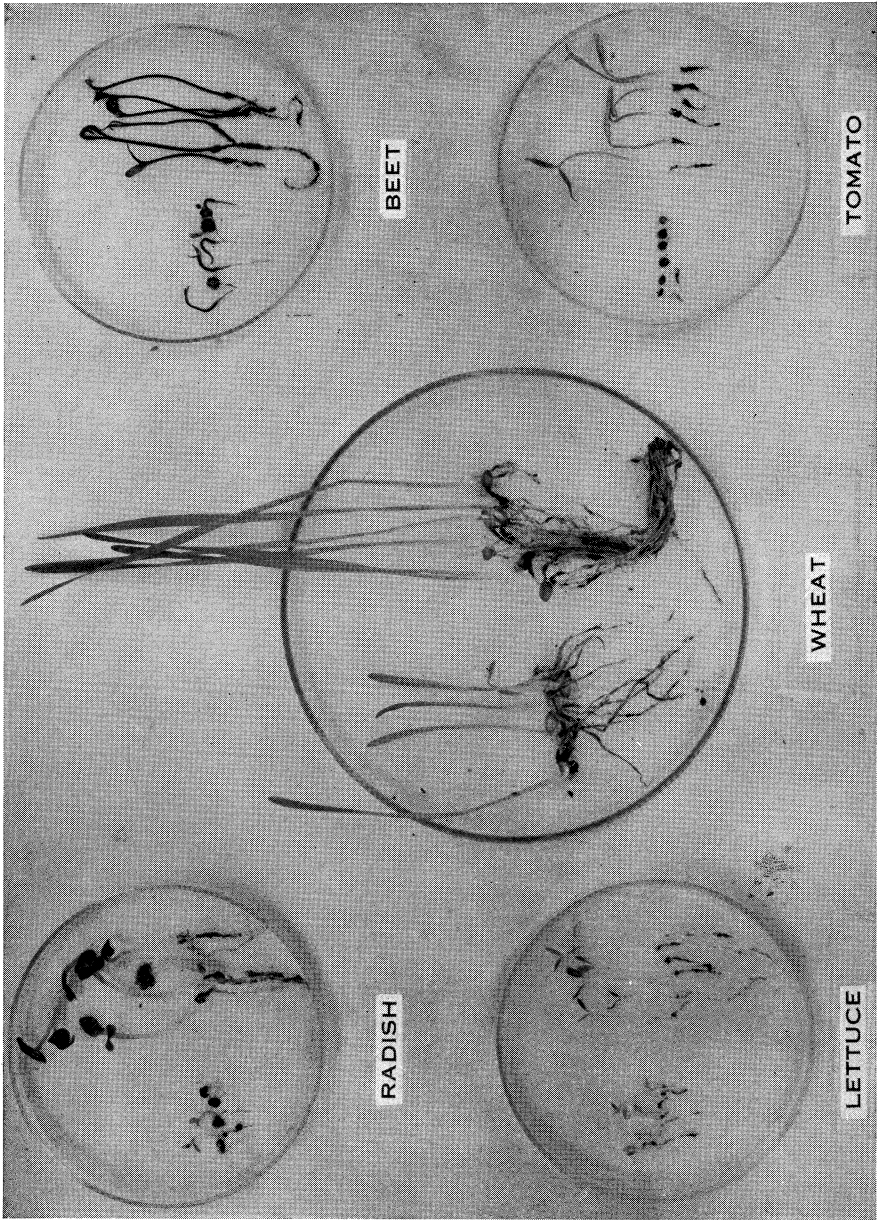
The pathogenic effects of *Sclerotinia homeocarpa* on five hosts. Left, seedlings from inoculated soil. Right, seedlings from uninoculated soil.
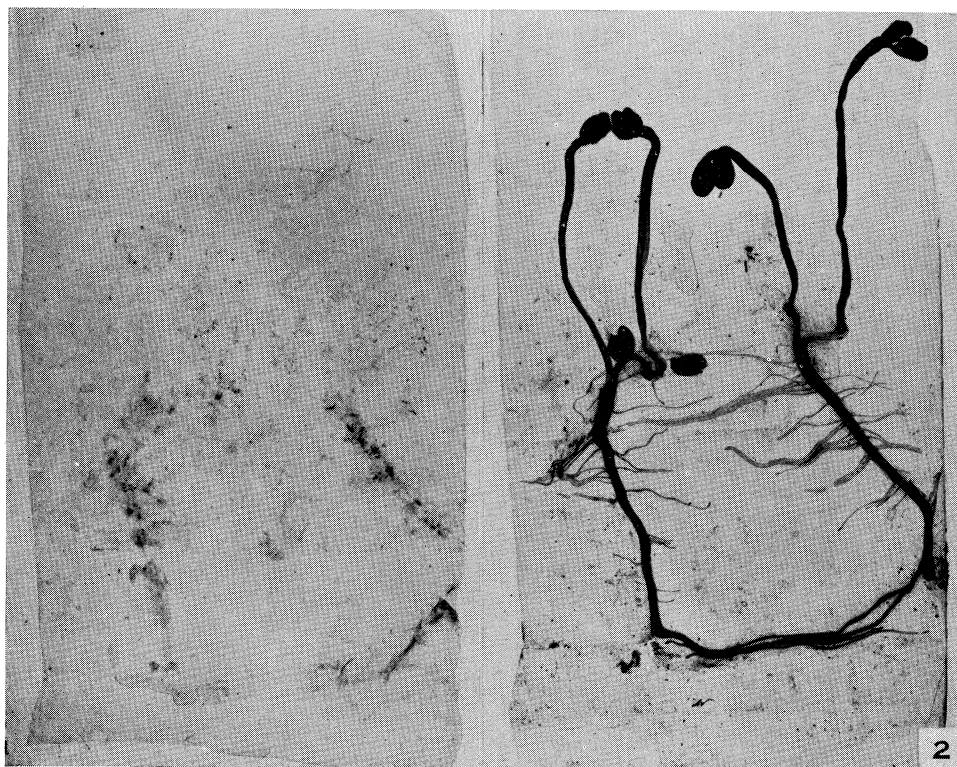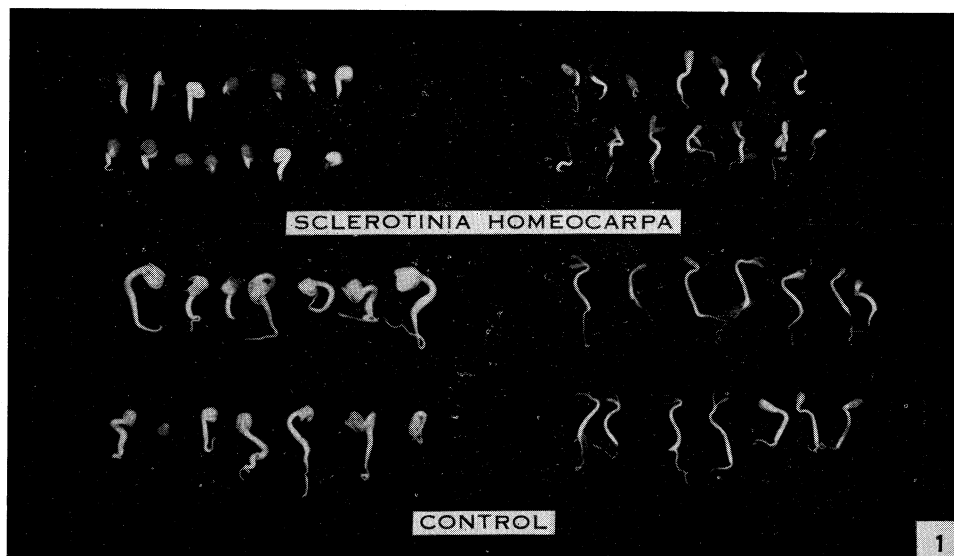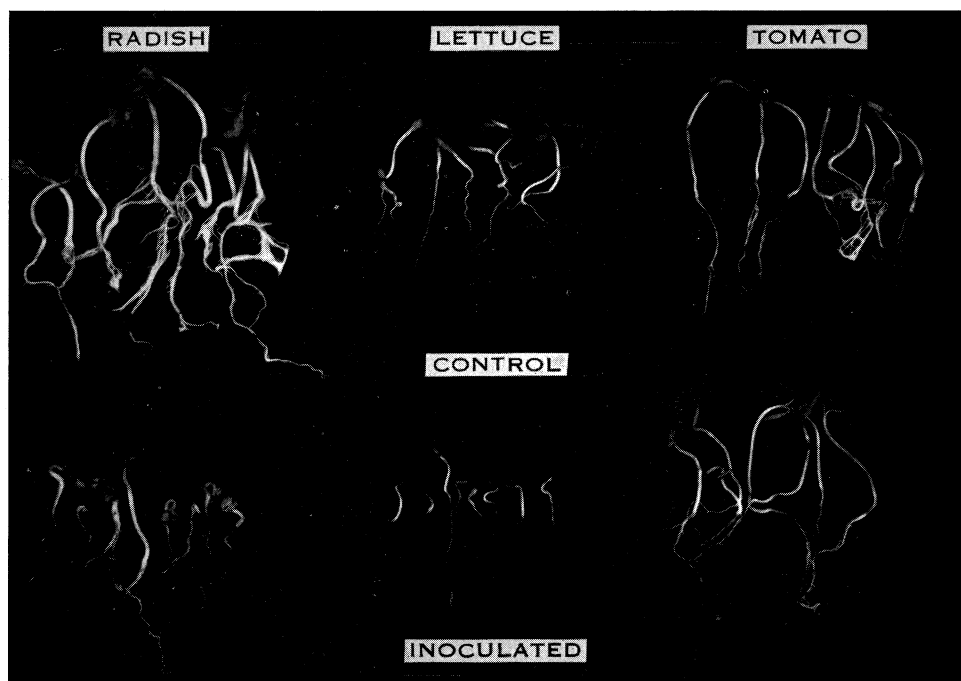
PLANT-PATHOGEN INTERACTIONS

Fig. 1.—The growth of radish (left) and lettuce (right) seedlings inside "Cellophane" bags buried in uninoculated soil, and in soil inoculated with *Sclerotinia homeocarpa*.

Fig. 2.—The aggregation of *Pellicularia filamentosa* hyphae on the outside of a "Cellophane" bag directly opposite the roots of radish seedlings.

PLANT-PATHOGEN INTERACTIONS

The growth of radish, lettuce, and tomato seedlings inside "Cellophane" bags buried in uninoculated soil, and in soil inoculated with *Pellicularia filamentosa*.