STUDIES ON SCLEROTIUM-FORMING FUNGI

I. *Sclerotium cepivorum* Berk and *S. tuliparum* Klebahn

Part 2. Symptoms, Mode of Infection and Host Range

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Introduction

Bulb-rot of tulips and white-rot of onions were first reported by Wakker[22] in Holland and Berkeley[3] in Great Britain respectively. Bulb-rot, caused by *Sclerotium tuliparum* Klebahn, was subsequently recorded in Germany by Klebahn,[13] in Switzerland by Muller-Thurgau[18] and Lendner,[14] in United States by Whetzel and Arthur[24] and Buddin,[7] in England by Brooks[6] and Dowson,[10] in Holland by Van Beyma Theo Kingma[20] and in Japan by Kawamure.[12] Similarly white-rot, caused by *Sclerotium cepivorum* Berk, was later reported in Italy by Voglino[21], in Spain by Caballero,[8] in Europe and America by Walker,[23] in Egypt and Cyprus by Nattrass,[19] in South Africa by Du Plessis,[11] in Russia by Matzulevitch,[16] in Argentina by Marchionatto,[15] in Germany by Bremer,[5] in Brazil by Brandão[4] and in various parts of Australia (Anon,[1–2] 1938, 1943).

Symptoms and Mode of Infection

The earliest signs of attack of *S. cepivorum* on spring-sown seedling onions usually become noticeable about the end of May or beginning of June. The older leaves first turn yellow, starting from the tips downwards, and then fall over. The inner leaves collapse later on. Affected plants can readily be pulled from the soil, because the stem base is more or less completely rotted. Around the base of affected bulbs a white, fluffy mycelial growth is frequently seen. Later on sclerotia are formed on or inside the scales as the mycelium penetrates into the interior and gradually destroys the base and the scales so that the bulb becomes rotten and worthless (Plate V, Figs. 1–3). The fungus causes a semi-water decay of the scales. The sclerotia formed are minute, black and hard. Attack is not limited to young seedlings, but one frequently finds large bulbs in the autumn showing early stages of attack.

Hyphæ of the fungus can readily be demonstrated ramifying through all the disintegrated tissue, roots, stem and leaf bases.

When onion seed is sown in ground artificially contaminated by the fungus, *e.g.*, by placing the seed on pieces of the fungal mycelium in the ground, more drastic attack may be shown. In such a case a large percentage of the seedlings may fail to come above ground.

The fungus may be seed-borne, as reported by Moore,[17] but is usually transmitted by infected seedlings, and distributed locally by cultivation. Cotton and Owen[9] suggest that in all cases the roots are attacked before the bulbs, but in the writer's opinion this is not the case. Field observation shows from time to time bulbs with the appearance illustrated in Plate V, Fig. 4. In such a case the base of the bulb is entirely rotted though a large percentage of the roots are still not invaded. Unless special percautions are taken such a bulb when pulled will tear apart at the stem base, part of the latter being still anchored to the ground by the healthy roots.

Inoculation experiments carried out in the laboratory support the view that the stem base is the part of the bulb most susceptible to invasion. Healthy bulbs, with the leaves cut off were suspended, after careful washing and surface sterilisation, in moist glass jars and inoculated either—(*a*) on the uninjured surface of scale leaves; (*b*) on the injured surface of scale leaves; (*c*) on the uninjured stem base at the point of emergence of roots; (*d*) on the uninjured root surfaces. Ten bulbs were tested in each lot. The results are indicated in Table I.

TABLE I

Rot on the uninjured surface of scale leaves	Rot on the injured surface of scale leaves	Rot on the uninjured stem base at the pint of emergence of roots	Rot on the uninjured root surface
Nil	+ +	+ +	+

While the fungus appears to be able to penetrate the surface of uninjured roots, it does so relatively slowly and uncertainly whereas it freely enters at the stem base. The port of entry has been found to be the natural wound caused by the emerging root. It is to be noted that no attack takes place through the surface of the intact outer scales.

The symptomatology of "gray-bulb rot" of tulips due to *S. tuliparum* is somewhat different from that of the "white-rot" of onions by *S. cepivorum*. In the field the disease is first indicated in the spring by the failure of bulbs in certain patches to appear above ground. Bulbs which are less severely attacked may send up some distorted leaves but as a rule no flower is formed. When such plants are dug up, one finds that the rot is at the

base of the leaves, *i.e.*, at the nose of the bulb and that the leaves are only attached to the bulb by a thin brown connection of rotted tissues. In the cases where bulbs fail to come up, one is struck by the fact that soil clings to the exterior of the rotted part. The roots of a badly infected bulb may be perfectly sound. The rot is of a dry type and the healthy white tissue turns to a reddish gray colour and becomes brittle. Sometimes brownish-black sclerotia are formed inside the bulb scales but more usually they occur in the soil adhering to the bulbs. In advanced stages of infection the mycelium forms a felty layer between the scales of the bulbs.

The fact that tulips when planted in infected grounds at the normal depth (about 5 inches) are attacked at different stages probably indicates that the base of the shoot is infectible over a considerable period of time. If however the bulbs are planted very shallow, so that their noses are just below ground level, the base of the shoot passes through the susceptible stage rather quickly. This is illustrated in Table II which gives the results of an experiment with potted plants.

TABLE II

(20 bulbs were planted and inoculated in each case)

Description of inoculation	No. of plants growing above soil level	No. of plants flowering
1. Control (uninoculated) ..	20	19
2. Bulbs inoculated at nose when shoots are 1–1½ inches long ..	5	Nil
3. Bulbs inoculated at nose when shoots are 2½–3 inches long	17	15

Under such conditions the base of the shoot presumably becomes harder with thicker cuticle than when the bulb is deeply planted.

Attempts in the laboratory to infect portions of the flowering stem above ground or the leaf-blades, wounded or unwounded, were uniformly negative.

Pot experiments indicated that the roots are not directly attacked by the fungus. Thus in one case 20 bulbs were planted over the fungal mycelium, while 20 others had the mycelium placed on the nose. Of the latter 5 only grew and 2 produced flowers, of the former 16 flowered. In all cases it was the shoot bases and not the roots which were attacked.

The experiments on influence of soil moisture and temperature on infection were mostly confined to the attack of *S. cepivorum* on white spring onion (Lisbon variety).

The soil, a mixture of medium loam, sand and leaf-mould, was autoclaved and dried down. The water-holding capacity of such a soil was found to be 46 per cent. of the dry weight. Six batches of this soil were then adjusted to moisture contents of 100, 80, 60, 40, 20 and 10 per cent. of water-holding capacity. These were placed in varnished earthen pots 6″ in diameter and with a depth of soil of about two inches. The pots were placed under bell-jars and sufficient water added daily to each to keep up a constant weight. The results of two sets of such experiments are given in Table III when the seedlings had grown for eight weeks.

TABLE III

Percentage of soil moisture	Experiment I		Experiment II	
	No. of plants growing in uninoculated soil	No. of plants growing in soil inoculated with *S. cepivorum*	No. of plants growing in uninoculated soil	No. of plants growing in soil inoculated with *S. cepivorum*
100	65	40	70	36
80	67	25	66	30
60	50	12	47	15
40	36	2	32	5
20	12	10	18	12
10	Nil	Nil	Nil	Nil

These results indicate that *S. cepivorum* is able to attack onion seedlings over the whole range at which ready germination takes place. There is some suggestion also that the greatest development of the disease is near about 40 to 60 per cent. soil moisture, higher or lower percentages reducing the disease.

The effect after six weeks of varied soil temperature is shown in Table IV. The experimental pots were placed in a range of green-houses. The temperatures were not under very strict control.

TABLE IV

(Number of seeds used was 90 in each case)

Temperature in Centigrade	Number of healthy plants growing		
	Control	*S. tuliparum*	*S. cepivorum*
25°–30°	21	18	15
13°–18°	74	72	24
8°–13°	65	66	32
2°—8°	55	53	46

The figures indicate an optimum of attack somewhere in the neighbourhood of 13°–18° C. At the higher temperatures, germination was very poor even in the controls, but there was relatively little attack. At the lower temperatures germination was good, and there was little attack.

The good agreement shown between the series which was uninoculated and the one which was inoculated with *S. tuliparum* indicates, as before, that this fungus causes no attack.

Host Range

An experiment was set up in a green-house in which 20 tulip bulbs were planted early in December in pots, five per pot, (*a*) without addition of fungus, (*b*) with mycelium of *S. cepivorum* at top and bottom of bulbs, and (*c*) with mycelium of *S. tuliparum* placed as in (*b*). Observation five months later gave the results shown in Table V.

Table V

(20 tulip bulbs were planted in each case)

Fungus	No. of plants grown	No. of plants flowering
Soil uninoculated ..	20	18
S. cepivorum ..	20	18
S. tuliparum ..	6	5

There was thus no evidence that *S. cepivorum* had produced any effect.

This experiment was repeated on a larger scale in the following year in the open ground. 12′ × 12′ area was divided into 12 rows, and in each row 20 Prince of Austria tulip bulbs were planted at a distance of six inches apart. The first row was uninoculated, the second inoculated by *S. cepivorum* at the top and at the base of the bulbs and the third similarly by *S. tuliparum*. This scheme was replicated four times so that altogether 80 bulbs were subjected to each treatment. All the bulbs were planted and inoculated in November. The number of plants which had come above ground were counted in March and the number of plants flowering were recorded after another two months. The data are given in Table VI.

It is thus clear that under conditions which were sufficiently favourable to enable *S. tuliparum* to produce nearly 100 per cent. infection, *S. cepivorum* had no ascertainable effect whatsoever. None of the bulbs inoculated with *S. cepivorum* showed any trace of the latter fungus, either on the scales or on the roots, at the time of lifting.

TABLE VI

(80 bulbs were planted in each case)

Fungus	No. of plants appearing above ground	No. of plants flowering
Soil uninoculated ..	75	73
S. cepivorum ..	78	78
S. tuliparum ..	4	1

From laboratory experiments, it did not appear that *S. cepivorum* could attack tulip tissue even when the epidermis was removed. Some growth of the fungus took place but there was no obvious effect on the bulb tissue.

The behaviour of the two fungi towards onion plants was tested in a series of pot experiments, the results of which are set out in Tables VII and VIII. 80 seeds were sown in drills, with or without inoculation, in each case in the unautoclaved soil series while 108 in the autoclaved one. More extended tests which gave substantially the same conclusions were carried out on a field plot scale and the results are given in Table IX.

TABLE VII

(Ten autumn-sown sets were planted in each case)

Treatment	Number of plants grown	Number of plants healthy
Uninoculated soil ..	10	10
Inoculated by *S. cepivorum* ..	6	4
Inoculated by *S. tuliparum* ..	9	9

TABLE VIII

Percentage of growth

Treatment	Unautoclaved soil	Autoclaved soil
Uninoculated soil ..	72·0	82
Inoculated by *S. cepivorum* ..	42·7	30
Inoculated by *S. tuliparum* ..	67·0	76

On account of a certain amount of variation in the percentage germination of the seeds, the results are not so clear cut as in the converse case of tulip bulbs described above. The two tables however show definitely that *S. tuliparum* produces no attack of onion sets or seedlings under conditions

where *S. cepivorum* caused the loss of approximately 50 per cent. of the plants.

A comparison of the results of the Table VIII suggests that the capacity of *S. cepivorum* to attack is somewhat greater in autoclaved than in ordinary soil.

TABLE IX

(400 onion seeds were used in each case)

Treatment	Percentage of healthy plants
Control (uninoculated) ..	43·0
S. tuliparum ..	48·0
S. cepivorum ..	25·0

It appears therefore from these experiments that neither *S. cepivorum* nor *S. tuliparum* is able to attack the host of the other.

A series of inoculations was carried out with *S. tuliparum* and *S. cepivorum* on a miscellaneous assortment of plants possessing bulbs, corms, etc. Inoculations were made or wounded or unwounded materials either in moist chambers or in the soil of pots. The following is a summary of the results obtained.

(*a*) *S. tuliparum* caused 80–100 per cent. infection of Single Early tulip (Artus), Single Tulip (Prince of Austria), *Scilla sibirica*, Hyacinth (Crimson), *Chionodoxa luciliæ*, *Iris hispanica* (King of Whites).

(*b*) *S. tuliparum* caused infection in 40–60 per cent. of Gladiolus (peach Blossom), Narcissus (*Poeticus ornatus*), Daffodil (Princeps), Crocus (Light Blue), Snowdrop (Single). Rhizomes of winter Aconite was less frequently attacked, only 20 per cent.

(*c*) *S. tuliparum* produced no attack on English and Spanish mature onion bulbs, Spanish and English grown autumn sown sets, seedlings of white Lisbon and Red onion, Shallots, Leek (Musselburgh).

(*b*) *S. cepivorum* was not seen under any conditions to attack any of the plants listed under (*a*) or (*b*), whereas it vigorously attacked most of the onion types given under (*c*). Red onions, leeks and shallots were attacked to an extent of 20–25 per cent. only, *i.e.*, less than the other onion types.

It was noticed with both fungi that moist atmospheric conditions and autoclaved soil increased the pathogenicity on almost all the hosts.

The symptoms and mode of infection of both fungi on the above hosts are almost the same as described above for the natural hosts, *i.e.*, *S. cepivorum*

attacks the base of the bulbs and *S. tuliparum* the top and young growing hoots.

Summary

1. Symptoms on the natural hosts and the modes of infection of *S. cepivorum* and *S. tuliparum* are described. While *S. cepivorum* appears to be able to penetrate the surface of uninjured roots, it does so relatively slowly and uncertainly whereas it freely enters at the stem base. The port of entry has been found the natural wound caused by the emerging root. In case of *S. tuliparum* it was the shoot bases and not the roots which were attacked.

2. *S. cepivorum* is able to attack onion seedlings over the whole range of soil moisture at which ready germination takes place—the greatest development of the disease being near about 40 to 60 per cent. soil moisture. As regards temperature effect, the optimum attack is somewhere in the neighbourhood of 13° to 18° C.

3. Under conditions which were sufficiently favourable to enable *S. tuliparum* to produce nearly 100 per cent. infection on tulips, *S. cepivorum* had no ascertainable effect whatsoever.

4. Neither *S. cepivorum* nor *S. tuliparum* is able to attack the host of the other.

5. *S. tuliparum* caused 80–100 per cent. infection of Tulips, *Scilla sibirica*, Hyacinth, *Chionodoxa luciliæ*, *Iris hispanica*; 40–60 per cent. of Gladiolus, Narcissus, Daffodil, Crocus, Snowdrop; 20 per cent. of rhizomes of winter Aconite; and produced no attack on onions, shallots and leek. *S. cepivorum* on the other hand attacked vigorously most of the onion types, leeks, shallots and red-onions only up to an extent of 20–25 per cent., while it could not attack the hosts of *S. tuliparum*.

6. It was noticed with both fungi that moist atmospheric conditions and autoclaved soil increased the pathogenicity on almost all the hosts.

References

1. Anon .. *Agric. Gaz. N.S.W.*, 1938, **49**, 423–27.
2. ———— .. *J. Dep. Agric. Vict.*, 1943, **41**, 312.
3. Berkeley, M. J. .. *Ann. and Mag. Nat. Hist.*, 1841, **6**, 355–65.
4. Brändao, J. S. .. *Bol. Minist. Agric. Riode. J.*, 1942, June 6.
5. Bremer, H. .. *Nachrichtenbl. Deutsch. Pflenzenschutzdienst.*, 1934, **14**, 37-38.
6. Brooks, F. T. .. *Gard. Chron.*, 1926, **79**, 271–72.
7. Buddin, W. .. *J. Minist. Agric.*, 1937, **44**, 54–59.
8. Caballero, A. .. *Bol. de la R. Soc. Esp. de Hist. Nat.*, 1922, **22**, 210–12.
9. Cotton, A. D. & Owen, M. N. *Jour. Board of Agric. of Great Britain*, 1919–20, **26**, 1093-99,
10. Dowson, W. J. .. *Jour. Royal Horti. Soc.*, 1928, **53**, 45–54,

11. Du Plessis, S. J. .. *Farming in South Africa*, 1932, **7**, 112–14.
12. Kawamura, T. .. *Ann. Phtopath. Soc. Japan*, 1936, **6**, 1–14.
13. Klebahn, H. .. *Ztschr. Pflanzenkrank*, 1904, **14**, 18–36.
14. Lendner, A. .. *Jour. Hort. etc. vit. suisse.*, 1911, **7**, 263–67.
15. Marchionatto, J. B. .. *Physis (Rev. Soc. Argentina Cien. Nat.)*, 1933, **XI**, **39**, 301-05.
16. Matzulevitch, B. P. .. *Leningrad Publ.*, 1932, **10**, 24.
17. Moore, W. C. .. *Report of Fungus, Bacterial and other Diseascs of Crops in England and Wales for the years* 1933–42, London, H.M. Stationary Office, 1944.
18. Muller-Thurgau, H. .. *Landw. Jahrb. Schweiz*, 1908, **22**, 743–45, Abstr. in *Ztschr. Pflanzen-krank.*, 1910, **20**, 50.
19. Nattrass, R. M. .. *Cyprus Agri. Journ.*, 1933, **28**, 98–100.
20. Van Beyma Theo Kingma, F. H. .. *Phytopath.* Lab. "Willie Commelin Scholten" Baarn (Holland), 1928, **12**, 28–30.
21. Voglino, P. .. *Staz. Sper. Agr. Ital.*, 1903, **36**, 89–106.
22. Wakker, J. H. .. *Algem. Vereening. Bloembotten—culture Haarlem. Verslag*, 1884, 1885, 22–26.
23, Walker, J. C. .. *Phytopath*, 1926, **16**, 697–710.
24. Whetzel, H. H., and Arthur, J. .. *Cornell Univ. Agri. Expt. Sta. Memoir*, 1924, **89**, 3–18.

Explanation of Plate

Fig. 1. Two healthy spring onion bulbs with roots.

Fig. 2. Two diseased spring onion plants. The black covering all round the base in the mycelial mat and sclerotia of the pathogen.

Fig. 3. An advanced case of attack of *S. cepivorum*. The photo shows the whole plant of a spring onion, the leaves have all dried and fallen off.

Fig. 4. An early stage of infection by *S. cepivorum* on onion bulb. Though the base of the bulb is rotted yet only a few roots have gone. A high percentage of healthy roots is shown here.

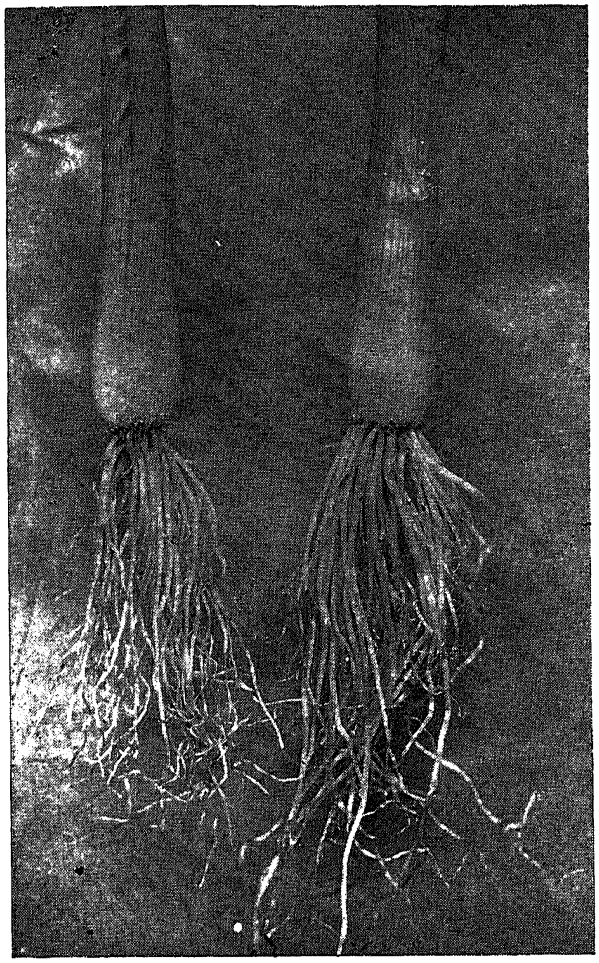

FIG. 1

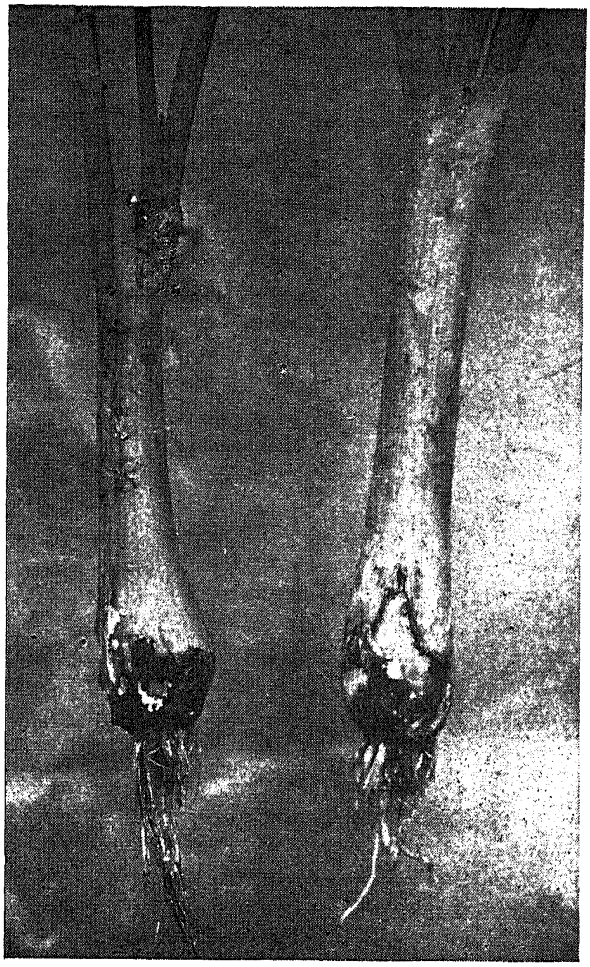

FIG. 2

FIG. 3

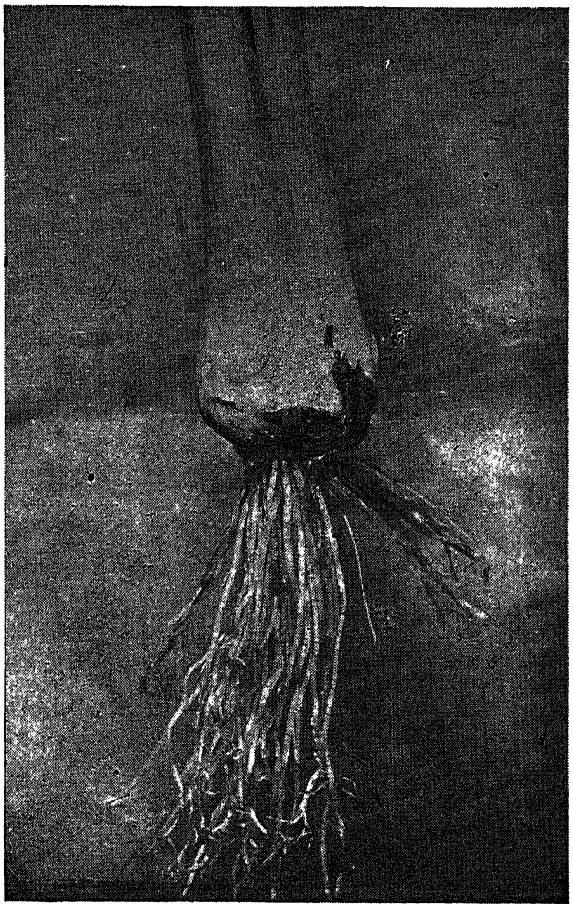

FIG. 4