Neth. J. Pl. Path. 79 (1973) 243–248

Some factors influencing the outgrowth of Botrytis tulipae from lesions on tulip bulbs after planting

AMELITA W. DOORNIK and B. H. H. BERGMAN

Bulb Research Centre, Lisse

Accepted 7 May 1973

Abstract

Lesions of *Botrytis tulipae* on tulip bulbs do not give rise to new infections during storage but may do so after planting. Compared with storage at 20°C and a relative humidity (r.h.) of 40%, storage at 20°C and 95% r.h. reduces, and at 5°C and 95% r.h. tends to increase the rate of successful isolations from lesions during the storage period and the number of new infections after planting. The bulbs show more new infections during growth in soil at 9°C than at 18°C.

Introduction

Local lesions on tulip bulbs caused by *Botrytis tulipae* (Lib.) Lind remain unchanged during storage. After planting, fungus from some of these lesions grows out, causing the disease to spread over mother and daughter bulbs and occasionally over the outgrowing sprout (Doornik and Bergman, 1971).

Symptoms of infection on tulip bulbs after lifting have been described in detail by several authors, e.g. Moore (1939), Doornik and Bergman (1971). The first symptoms of new infection visible after planting are irregularly shaped brown patches, which may be located close to the old lesion but clearly separated from it by a white margin (Fig. 1), but may also be located at some distance, e.g. near the neck of the bulb. Sclerotia, which are rare on lesions before planting, may be formed on all scales soon after the development of new infections.

Microscopic observation of lesions at lifting showed that mycelium of *B. tulipae* generally does not penetrate the outer scale tissue deeper than about 10 cell layers (Doornik and De Rooy, 1971). It was therefore assumed that temperature and relative humidity during storage as well as the length of the storage period might influence the viability of the fungus.

Doornik and Bergman (1971) observed that a soil temperature of 5°C, and as a result a long interval between planting and flowering, led to a more frequent spread of the disease in planted bulbs than a soil temperature of 15°C and a shorter period until flowering.

In the present paper the influence of these factors is investigated.

Materials and methods

Unless otherwise stated, the brown tunics were removed before planting and diseased bulbs were classified into 3 groups, according to the size of the lesions, as previously

Fig. 1. Tulip bulb showing an irregularly shaped new infection by *B. tulipae* on outer scale close to the old circular local lesion (below).

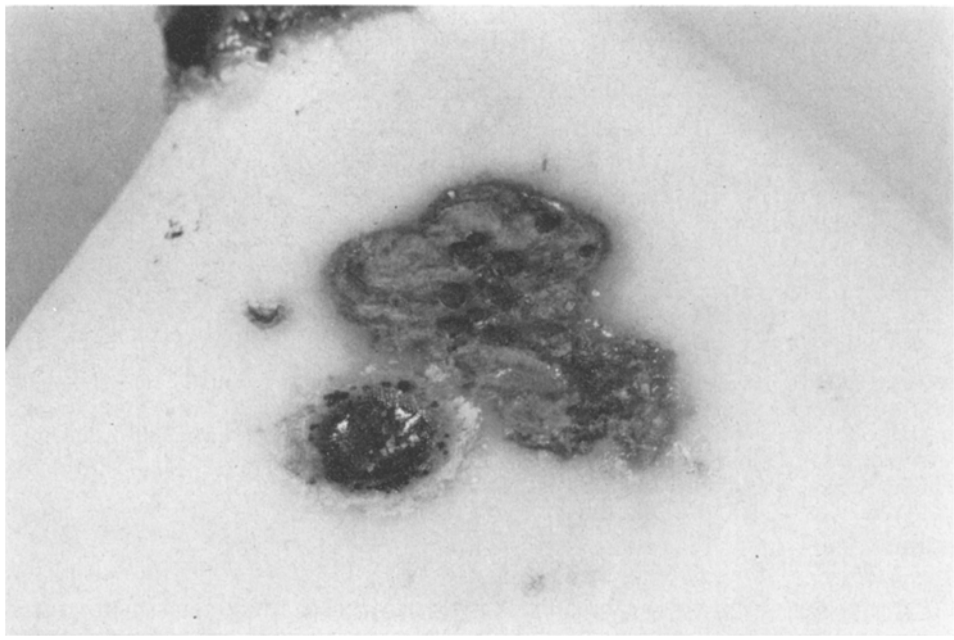

Fig. 1. Onregelmatig gevormde aantastingsplek tengevolge van een nieuwe infectie door B. tulipae vlak boven de oude, ronde lesie op de buitenste rok van een tulpebol.

described (Doornik and Bergman, 1971). In most of the trials a mixture of equal proportions of these groups was used; in general the experiments were carried out with several cultivars. For evaluation of the influence of the various factors on the spread of the disease over the bulb, bulbs were dug up for observation at flowering or at earlier moments, if desired.

Isolations of the fungus from local lesions were made on cherry agar (pH 4.5), and incubated at 20 °C under artificial light. Each lesion was plated separately, and *B. tulipae* was identified microscopically. Before plating, the lesions were assigned to 3 groups according to size.

Results

The first symptoms of new infections on the bulbs were found 6 to 8 weeks after planting. As shown for one of the cultivars used (Table 1), the percentage of bulbs with the disease spreading toward the bulb centre increased from then until flowering. Severity of infection on the bulb surface also tended to increase with time. Experiments with other cultivars showed the same trend (Doornik and Bergman, 1971), though often with different intensity.

The fungus could not be isolated from a number of lesions before planting (Table 2); this apparent inactivation may explain the observation that even at flowering a con-

Table 1. Percentages of tulip bulbs cv. 'Diplomate', showing new infections by *B. tulipae* on outer and inner scales after planting (15 October). Number of bulbs sampled at each date: 20–50. Moderate degree of infection before planting.

Date of sampling	14/12	17/1	16/2	14/3	17/4
scale 1*	50	46	56	83	48
scale 2	0	14	24	28	25
scale 3	0	0	16	22	14
scale 4	0	0	0	0	10

*Outer scale

Tabel 1. Percentages tulpebollen cv. 'Diplomate' met nieuwe aantastingen na het planten (15 oktober) door B. tulipae op de buitenste en overige rokken. Aantal bollen dat per datum werd nagezien: 20–50. Matige aantasting op de bollen vóór het planten.

Table 2. Percentages of successful isolations of *B. tulipae*. I: from lesions shortly before planting; II: from lesions which had not given rise to a new infection at flowering time. About 35 lesions were plated per object. Lesions classified as: s (small) = 1–3 mm; m (medium) = 4–7 mm; l (large) = > 7 mm diameter.

Cultivar	I			II		
	s	m	l	s	m	l
'Apeldoorn'	52	64	67	0	0	4
'Madame Lefeber'	14	27	47	4	5	2
'Van der Eerden'	39	34	53	14	0	10

Tabel 2. Percentage lesies, waaruit kort voor het planten B. tulipae kon worden geïsoleerd (I) en percentage lesies, die bij de bloei nog geen nieuwe infectie gevormd hadden, maar waaruit wel B. tulipae werd geïsoleerd (II). Per object werden ongeveer 35 lesies uitgelegd. Classificatie lesies: s = 1–3 mm, m = 4–7 mm, l = >7 mm diameter.

siderable number of bulbs did not show new symptoms of attack. On the other hand, however, it proved possible to isolate *B. tulipae* at flowering time from some lesions on bulbs without any spread of the disease (Table 2, II).

Temperature and relative humidity during storage and length of the storage period

Diseased bulbs with brown tunics present were stored at 5 °C and 95 % relative air humidity (r.h.), or at 20 °C and 40 or 95 % r.h. The influence of the various treatments on the proportion of successful isolations of the pathogen was checked at various intervals during storage by plating of lesions.

The percentages of lesions from which *B. tulipae* was recovered are given in Fig. 2. A temperature of 20 °C with 95 % r.h. significantly reduced ($P < 0.05$) the outgrowth of the fungus in the cultivars 'Apeldoorn' and 'Van der Eerden', and the data obtained with cv. 'Madame Lefeber' showed the same tendency. At 5 °C and 95 % r.h. storage, the percentages outgrowth of the fungus was promoted in 'Apeldoorn' and 'Madame Lefeber', but reduced in 'Van der Eerden'. Increasing duration of storage at 20 °C and 40 % r.h. did not give significant differences in the isolation rates.

For the investigation of the influence of storage conditions on disease development after planting, part of the same batches of bulbs were planted in autumn and dug when

Fig. 2. Influence of storage conditions on the percentages of lesions from which *B. tulipae* was isolated during storage. ——— cv. 'Apeldoorn'; . . . . . cv. 'Van der Eerden'; – – – – – cv. 'Madame Lefeber'.

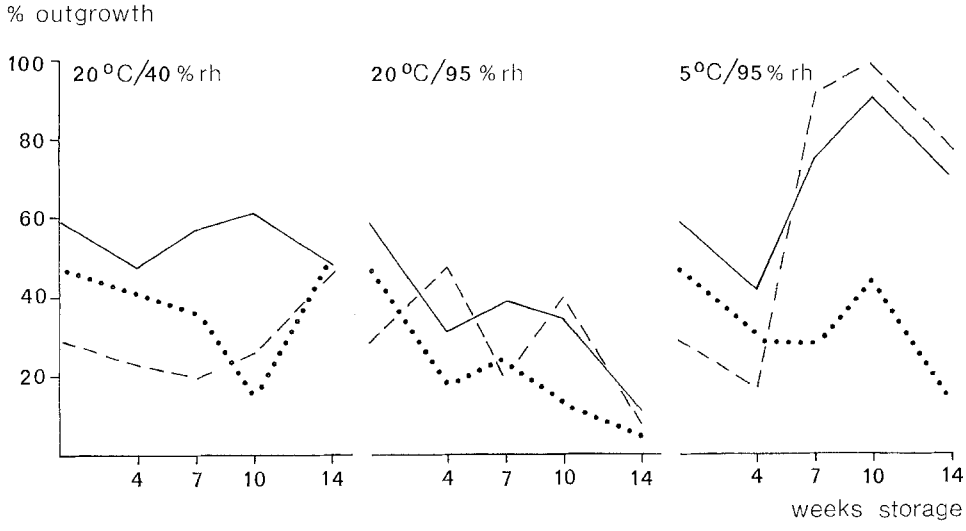

Fig. 2. Effect van de omstandigheden tijdens de bewaring op het percentage lesies, waaruit B. tulipae kon worden geïsoleerd gedurende die bewaring.

plants flowered. The development of new infections on these bulbs showed a similar trend as given in Fig. 2: bulbs with new symptoms were found more frequently after storage at 5 °C and 95% r.h. than after 20 °C and the same r.h. (Table 3). After storage at 20 °C and low r.h., however, the data of Table 3 confirm those of Fig. 2 only, when low and high humidity at 20 °C are compared: an increase in fungus viability during storage at 5 °C, as found in Fig. 2, cannot be observed in the data of Table 3. In trials with other cultivars, storage for various lengths of time at 20 °C and low but varying r.h. (40–70 %), i.e. the normal storage conditions for outside planting, did not significantly influence the frequency of spread of the disease in planted bulbs (Table 4).

Soil temperature

Soil temperature and the rate of outgrowth of the sprout, as determined from the

Table 3. Percentages of bulbs bearing lesions at planting, which show new infections at flowering time as influenced by storage conditions. Number of bulbs per object: 100–140.

Cultivar	Storage conditions		
	20 °C/40 % r.h.	20 °C/95 % r.h.	5 °C/95 % r.h.
'Apeldoorn'	85	66	82
'Mad. Lefeber'	67	31	87
'Van der Eerden'	84	35	59

Tabel 3. Effect van de behandeling tijdens de bewaring op het percentage aangetast-geplante bollen met nieuwe infecties rond de bloeitijd; 100–140 bollen per object.

Table 4. Influence of the duration of storage at 20 °C and 40–70 % r.h. on the percentages of bulbs showing new infections after planting. Number of bulbs used per object: 100–140.

Cultivar	Duration of storage period (in weeks)						
	6	9	11	13	14	17	19
'Kareol'	66	69		84		71	
'Parade'			70		68	58	65

Tabel 4. De invloed van de duur van de bewaring bij 20 °C en 40–70 % rv op het percentage bollen met nieuwe infecties rond de bloeitijd. Per object 100–140 aangetaste bollen geplant.

duration of the interval between planting and flowering, are known to influence the incidence of disease at flowering (Doornik and Bergman, 1971). Observation of the time limit rather than a certain stage of plant development might clarify the influence of soil temperature alone on disease development. To investigate this point, bulbs of cv. 'Van der Eerden' stored at 9 °C were planted at soil temperatures of 9° or 18 °C. At lifting 9 weeks later, 45 % of the bulbs grown at 9 °C had developed *B. tulipae* symptoms on the outer scale as against 10 % of those grown at 18 °C.

Discussion

At any time between planting and flowering, *B. tulipae* may start to grow out from local lesions on the outer scale. Even at flowering, the fungus can still be isolated from previous year's lesions on bulbs not showing new infections. It is not known whether an inhibiting factor present in or near the lesion is responsible for the apparent latency of the fungus in these cases; the effect of such a factor might differ in vitro and in vivo.

According to Válašková (1963), a high relative humidity and a temperature of 20 °C are optimal for the viability of conidia and sclerotia in vitro. In vivo, however, optimal conditions seem to be different, for a high r.h. (95 %) during storage of bulbs at 20 °C reduced the viability of the fungus in scale lesions, whereas 40 % r.h. at the same temperature seems to have no effect. On the other hand, a low temperature in combination with high r.h. probably favours fungal viability in the lesions. Nothing is known about the cause of these phenomena. No evidence is available about antagonists' activity in the soil or on the bulb surface during storage. Perhaps the hypothetical inhibitory factor is more effective at a high temperature, or the fungus is more rapidly killed by food depletion at this temperature and high r.h.

Bulbs planted at various soil temperatures reach a certain stage of plant development (e.g. flowering) at different times. An evaluation of symptom development at flowering led to the assumption that the prolonged period between planting and flowering prevailing at low soil temperatures was responsible for a more pronounced development of *B. tulipae* (Doornik and Bergman, 1971). Evaluation of disease development at a fixed time in bulbs grown at different soil temperatures showed, however, that a low soil temperature itself stimulates the formation and outgrowth of new infections.

Samenvatting

Enkele factoren, die het uitgroeien van Botrytis tulipae vanuit lesies op tulpebollen na het planten beïnvloeden

Lesies, veroorzaakt door *B. tulipae* op de buitenste rok van tulpebollen, veranderen niet van uiterlijk tijdens de bewaarperiode. Na het planten kan de schimmel tot aan het bloeitijdstip (waarna geen waarneming aan de moederbol meer mogelijk is) op elk moment, zelfs vlak voor het bloeitijdstip, vanuit de lesies uitgroeien en nieuwe infecties veroorzaken vlak naast de oude lesies of op enige afstand daarvan (Tabel 1). Het is niet bekend of bij deze late uitgroei een remmende factor in of rond de lesies een rol speelt. Bij een aantal lesies groeit de schimmel niet uit, hoewel hij uit enkele daarvan wel kon worden geïsoleerd, zelfs nog omstreeks de bloeitijd.

Hoewel in vitro een temperatuur van 20 °C en een hoge relatieve vochtigheid (rv) voor de schimmel optimale groeiomstandigheden vormen (Válašková, 1963), bleek dat na bewaring van de bollen bij 20 °C en een hoge rv (95 %), het uitgroeien van *B. tulipae* vanuit de lesies na het planten geringer was dan na bewaring bij 20 °C en 40 % rv, terwijl bewaring bij 5 °C de uitbreiding bevorderde (Tabel 3). Na het planten vond bij lage bodemtemperatuur (9 °C) meer uitbreiding van de lesies plaats dan bij hogere. De oorzaak van de verschillen tussen het uitgroeien van de schimmel in vitro en in vivo is niet bekend; misschien blijft de schimmel levenskrachtiger in het bolweefsel bij lage temperatuur of is bij hoge temperatuur een remmende factor meer actief.

References

Doornik, Amelita W. & Bergman, B. H. H., 1971. Some factors influencing the infection of tulip sprouts by *Botrytis tulipae*. Neth. J. Pl. Path. 77: 33–41.

Doornik, Amelita W. & Rooy, M. de, 1971. New developments in controlling *Botrytis tulipae* in tulips. First int. Symp. on Flowerbulbs, Noordwijk/Lisse, 30 March – 4 April 1970. Internat. Soc. hort. Science, Vol I: 194–198.

Moore, W. C., 1939. Diseases of bulbs. Bull. Minist. Agric. Fish. Fd, Lond. No. 117.

Válašková, E., 1963. Beitrag zum Studium der Biologie des Tulpenschimmels *Botrytis tulipae* (Lib.) Lind. Sb. čsl. Akad. zeměd. Věd. C. Rostlinná výroba 9: 671–680.

Address

Laboratorium voor Bloembollenonderzoek, Heereweg 345a, Lisse, the Netherlands.