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Some factors influencing the infection of tulip sprouts by Botrytis tulipae

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Abstract

It is generally assumed that tulip bulbs with symptoms of infection by *Botrytis tulipae* will produce diseased sprouts, but experiments have shown this danger to be relatively small. Sprout infection is found to be influenced by several factors, e.g. the degree of infection of the bulb, the presence of the brown tunic around diseased bulbs, the length of the growth period between planting and flowering, and the soil temperature. The yield of salable flowers was found to depend on the location and intensity of sprout infection; no flowers are formed when sprouts are heavily infected.

Introduction

Tulip bulbs infected by *Botrytis tulipae* (Lib.) Lind may give rise to diseased plants. Spores formed on primaries (see under symptoms) may be dispersed by wind and rain, causing infections on leaves and flowers of neighbouring plants. In the field, spore infections may damage the foliage to such an extent that bulb production is reduced, and in the glasshouse this type of infection may render the flowers worthless for sale.

The effect of chemical control is satisfactory, either by disinfection of bulbs before planting to prevent development of primaries (e.g. Doornik and De Rooy, 1970) or by the use of fungicide sprays in the field (Hoogeterp, 1963) and smoke tablets (Daconil) in the glasshouse (De Rooy, 1968), which prevent spore infections.

However, information on factors favouring the development of disease on plants grown from diseased bulbs is scarce. The present paper discusses some factors found to influence the formation of primaries, including the speed of outgrowth of the sprout through the bulb neck and the rate of growth of the fungus from lesions present on the planted bulb.

Symptoms have been described in detail by several authors, such as Moore (1939) and Bergman and Doornik (1967), and can be summarized as follows.

On bulbs

The brown tunic may be cracked in several places; on the frayed dark rims along the cracks, small sclerotia may be present. The tunic may also remain free of symptoms, while circular or oval lesions (1 to 10 mm in diameter) are present on the underlying fleshy scale. These lesions show a sharp brown margin and a slightly depressed yellowish grey centre, where small sclerotia may be present. During storage of the bulbs

the size of lesions does not increase; it is only after planting that the symptoms are found to be spreading over the bulb surface and to the centre.

Primary infections on plants

A greyish brown oval patch may develop on the outer leaf enveloping the sprout during the early phases of development. When grown out, this leaf is malformed and does not stand erect but assumes a horizontal position close to the soil surface. The plant stem may bear a brown elongated patch under or slightly above the soil surface. When severely attacked in an early stage of development, the sprout may remain under the soil or may emerge as a withered stunted shaft; in the Netherlands these plants are called 'stekers' (stickers).

In the experiments discussed below, plants were considered diseased when they showed one or more of the symptoms mentioned, regardless the severity.

Materials and methods

In all trials except those performed to investigate the influence of the presence of the tunic on sprout infection, only bulbs were used which showed lesions caused by *B. tulipae* on the outer scale after removal of the tunic; symptom-free bulbs were discarded. Before planting, the bulbs were classified into 3 groups according to the number and size of the lesions present (see Fig. 1).

Unless otherwise stated, the bulbs used in experiments carried out in the glasshouse in the winter, had received a standard pre-planting temperature treatment: 20 °C until the end of August, followed by 17 °C for 5 weeks. Around the middle of October they were planted in flat wooden boxes which were ensiled outdoors till the end of

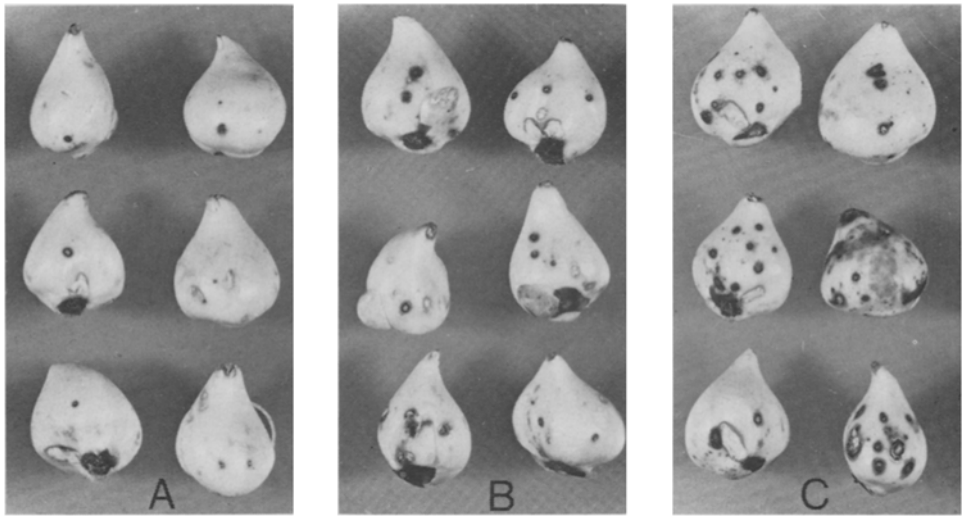

Fig. 1. Tulip bulbs, infected by *B. tulipae* arranged in 3 groups according to number and size of lesions shortly before planting; A = very light, B = light, C = moderate.
Fig. 1. Tulpebollen, geïnfecteerd door B. tulipae, geclassificeerd in 3 groepen naar het aantal en de afmetingen van de vlekken kort vóór het planten; A = zeer licht, B = licht, C = matig.

January, when the sprouts had a length of about 8 cm, then they were transferred to a glasshouse at about 15 °C soil temperature.

At several times after planting, samples of bulbs were lifted and examined for disease symptoms. Unless otherwise stated, the data in the tables represent results observed at the final examination at flowering time from 75 to 150 bulbs.

Results

Soon after planting of diseased bulbs, the infection may start to spread, both externally over the outer scale and internally to the bulb centre. The shoot may be attacked when it grows through the diseased tissue of the bulb neck (Bergman and Doornik, 1968; Price, 1970), but if the pathogen reaches the bulb centre rapidly, the sprout may be infected while still inside the bulb. However, sprouts often remain healthy, even when the disease has spread on the bulb surface and inwardly.

In a considerable proportion of our bulbs the disease did not spread further after planting, even after several months; it is not known whether the fungus remained dormant or had died. Plants grown from these bulbs showed no symptoms of primary attack.

The occurrence on infections on stems and leaves showed a close correlation with the rate of spread of the disease over and in the bulb, the percentages for both being higher when the disease symptoms on the bulb before planting had been more severe. However, the percentage of infected aerial parts was always low as compared with the percentage of bulbs showing spread of disease symptoms over the surface (Table 1).

The accordance between the percentages of diseased plants and the percentages of

Table 1. Percentages of bulbs showing spread of disease symptoms after planting and percentages diseased plants grown from these bulbs. The data were collected at flowering time in the glasshouse in the course of several experiments in various years. All bulbs showed symptoms at time of planting; number of bulbs 75–150.

Cultivar	Plants with disease symptoms on stem and/or leaves (%)			Bulbs with spread of symptoms (%)					
				over surface			to centre		
group[1]	A	B	C	A	B	C	A	B	C
'Ralph'	1	2	12	25	39	63	4	2	12
'Karel Doorman'	4	4	7	12	10	49	4	3	8
'Diplomate'	1	4	9	13	24	50	1	1	7
'Yellow Top'	3	4	16	21	30	67	2	4	11
'Bing Crosby'	4	6	12	48	69	85	3	5	17
'van der Eerden'	7	10	10	46	67	84	4	8	14

[1] Grading of infection according to symptoms on outer scale before planting (see Fig. 1; A = very light, B = light, C = moderate).

Tabel 1. Percentages bollen met uitbreiding van aantasting na het planten en percentages planten met symptomen, gegroeid uit deze bollen. Gegevens verzameld op het moment van bloei in de kas en samengevat uit verscheidene proeven in verschillende jaren. Classificatie van infectie op grond van symptomen op de buitenste rok vóór planten (zie Fig. 1; A = zeer licht, B = licht, C = matig).

bulbs showing spread of attack to the centre in Table 1 is only accidental, as was found in experiments not discussed here in detail. The sprout attack in this table was recorded at flowering time. However, when noted in an earlier stage of shoot development, it was found repeatedly that the sprout may be invaded before the fungus has reached the bulb centre, indicating that it has been attacked from the colonized bulb neck region. On the other hand many bulbs showing disease spread to the centre when examined at flowering time, proved to have produced a healthy plant.

The data in Table 1 suggest the existence of differences between cultivars in the susceptibility of sprouts and bulbs to infection by *B. tulipae*. The production of marketable flowers from diseased bulbs is commercially interesting. Heavily infected sprouts which have ceased to grow (i.e. the 'stickers') will not flower, but a high percentage of the diseased bulbs develop only slight symptoms or none at all on the stem base or the lowest leaf, without any loss of flower quality (Table 2).

Table 2. Percentage of marketable flowers grown from the diseased bulbs described in Table 1.

Cultivar	Group		
	A	B	C
'Ralph'	98	99	95
'Karel Doorman'	97	97	92
'Diplomate'	94	88	88
'Yellow Top'	93	94	86
'Bing Crosby'	94	93	91
'van der Eerden'	89	85	85

Tabel 2. Percentage verkoopbare bloemen afkomstig van de zieke bollen beschreven in Tabel 1.

Influence of the presence of the tunic on sprout infection

The preceding data were obtained in diseased bulbs planted after removal of the tunic. To investigate whether the presence of the tunic influences sprout infection, a selection of bulbs of various cultivars was made to form stocks having very high percentages of bulbs showing symptoms on the outer fleshy scale. Bulbs from these stocks were planted with and without the tunic under the same conditions. The results are shown in Table 3. In general, more plants showed disease symptoms and more bulbs showed severe rotting by *B. tulipae* when the tunics had not been removed.

Support for this tunic effect was provided by the following experiment. Pieces of tunic cut from bulbs showing lesions by *B. tulipae* in the underlying scales were attached to the scales of healthy bulbs, which were planted in an unheated glasshouse in October. When examined three months after planting, 35% of these bulbs showed disease symptoms, whereas the untreated bulbs were healthy.

Influence of the speed of sprout growth

Bulbs of three cultivars were given various pre-planting temperature treatments. One group was treated for glasshouse flowering in February (see under Materials and methods). A second lot was given the normal treatment for field planting (20 °C till the

Table 3. Influence of presence of the tunic around the planted bulb on infection of aerial parts and on the spread of the disease in and on the planted bulb. Data collected at flowering time in the glasshouse. Number of bulbs per experiment 75–150.

Cultivar	Bulbs with symptoms on outer scale before planting (%)	Infection on stem and leaves from bulbs (%)		Bulbs with spread of disease (%)			
				over the surface		to the centre	
		with tunic	without tunic	with tunic	without tunic	with tunic	without tunic
'Topscore'	93	30	21	79	66	17	25
'Red Champion'	85	22	14	48	46	19	11
'Goya'	90	38	19	78	64	34	20
'Ralph'	86	5	5	58	34	10	3
'Karel Doorman'	65	5	3	31	11	3	3
'Charles'	96	11	11	46	47	11	12
'Robinea'	75	13	16	42	51	17	23
'Oranje Nassau'	74	15	8	71	41	13	14

Tabel 3. Invloed van de aanwezigheid van de bolhuid om de geplante bol op de infectie van de bovengrondse delen en op de inwendige en uitwendige uitbreiding van de bolsymptomen. Gegevens tijdens de bloei in de kas verzameld. Aantal bollen 75–150 per groep.

end of August and 17 °C till planting in October). Of one cultivar ('Charles') a third lot was given a pre-planting treatment for flowering in December (20 °–17 °C till 25 August, followed by 5 °C for 9 weeks) and planted in a heated glasshouse. Thus, the length of the period between planting and flowering was varied from 10 to 29 weeks (Table 4). Some of the plants were examined at flowering time, but others were lifted and examined when the sprout length reached 8 cm. As can be seen from Table 4, the percentage of infected sprouts increased considerably with increasing length of the period between planting and flowering, i.e. with decreasing speed of sprout growth.

Although the sprout may become infected very soon after planting, comparison of the results for the two sampling dates (a and b) of the groups planted in the field (III) reveals that stems and leaves can become infected throughout the whole developmental period.

The data on disease spread on and inside the planted bulbs support the observations on infection of the aerial parts (Table 4).

In the preceding experiment, only the speed of shoot growth was taken into account. The fact that the pre-planting temperature of the various lots differed is left out of consideration. Also, soil temperatures after planting were not identical: group I was planted directly at 15 °C, group II was exposed to outdoor soil temperatures for 13 to 16 weeks, followed by 4 to 6 weeks at 15 °C after transfer to the glasshouse, and group III was exposed only to outdoor conditions.

It is known that *in vitro* the growth of *B. tulipae* is stimulated by higher temperature, 20 °C being about optimal (Valášková, 1963). But high soil temperature also stimulates shoot growth, provided the bulb has been exposed long enough to low temperature before or after planting, as was the case for our material. This makes it very difficult to study the influence of temperature on sprout and fungus growth separately in relation to sprout infection.

Table 4. Influence of speed of plant development (resulting from various pre-planting treatments and soil temperature) on percentage spread of infection on aerial parts and on bulbs. Number of bulbs 75–150.

Pre-planting treatment for:	Number of weeks		Infected plants (%)		Bulbs with spread of the disease (%)			
					over surface		to bulb centre	
	a[1]	b[2]	a[1]	b[2]	a[1]	b[2]	a[1]	b[2]
cv. 'Charles'								
I (flowering in December)	6	10	2	3	40	60	2	26
II (flowering in February)	15	19	12	19	62	74	11	35
III (flowering in the field)	19	29	21	42	74	87	14	49
cv. 'Robinea'								
II	14	20	16	16	41	51	16	23
III	19	29	15	32	44	84	12	30
cv. 'O. Nassau'								
II	16	19	15	8	45	41	12	14
III	23	29	17	25	46	52	12	22

[1]a = between planting and 8 cm sprout length, [2]b = between planting and flowering

Tabel 4. Invloed van de snelheid van uitgroei van de spruit tengevolge van verschillende voorbehandelingen en bodemtemperaturen na het planten op het percentage bovengronds geïnfecteerde planten en bollen met uitbreiding van de ziektesymptomen. a = tussen planten en spruitlengte van 8 cm; b = tussen planten en bloei.

The results of the following trials offer some additional information on this point. Diseased bulbs (cv. 'Kareol') were stored at 5 °C for 9 weeks prior to planting. After planting half of the lot was held at 15 °C, the other half at 5 °C. To obtain normal plants it was necessary to raise the soil temperature for the second lot to 15 °C during the last two weeks before flowering. Both lots were examined while in bloom (Table 5).

Although 15 °C is more favourable for fungal growth, the prolonged period of plant growth at 5 °C resulted in a more extensive growth of *B. tulipae* in the bulb and in a more frequent sprout infection at this temperature than at 15 °C.

In another experiment bulbs were kept (after the same pre-planting period as in the

Table 5. Influence of soil temperature on the external and internal spread of *B. tulipae* in planted bulbs (cv. 'Kareol') and the resulting percentage of infected sprouts. Number of bulbs 75–150.

Soil temperature	Number of weeks between planting and flowering	Diseased sprouts (%)	Bulbs with spread of disease (%)	
			over surface	to centre
15 °C	6	4	46	12
5 °C (+ 2 weeks 15 °C)	16 + 2	26	84	23

Tabel 5. Invloed van de bodemtemperatuur op de verspreiding van B. tulipae over en in de bol van geplante tulpen (cv. 'Kareol') en het percentage aangetaste spruiten.

Table 6. Influence of various periods of growth at 5° and 15°C soil temperature on the external and internal spread of *B. tulipae* in planted bulbs (cv. 'Kareol') and the resulting percentage of infected sprouts. Number of bulbs 75–150.

Length of period (weeks) between planting and flowering at soil temp.:		Diseased sprouts (%)	Bulbs with spread of disease (%)	
5°C	15°C		over surface	to centre
4	5	3	61	13
8	5	10	72	12
12	4	19	73	23
16	2	26	84	23

Tabel 6. Invloed van verschillende tijdsduur van groei bij 5° en 15°C bodemtemperatuur op de verspreiding van B. tulipae over en in de bol van geplante tulpen (cv. 'Kareol') en het percentage aangetaste spruiten als gevolg daarvan.

above experiment) for shorter periods at 5°C soil temperature, followed by 15°C to obtain flowering plants of normal habitus. The rate of spread in the bulb and the percentage of infected aerial parts decreased with shorter periods at 5°C, i.e. with more rapid shoot growth (Table 6). These differences are very marked, even though the period at 15°C prior to flowering (more favourable for fungal growth) was more than twice as long as in the preceding experiment.

Discussion

Most experiments described in this paper were done with bulbs which bore symptoms of attack by *B. tulipae* on the outer scale when planted, a situation hardly ever found in commercial stocks. In a considerable percentage of these bulbs the symptoms remained unchanged after planting, which suggests that the fungus remained dormant or was killed by causes unknown. Furthermore, far fewer bulbs produced diseased sprouts, especially when they had been forced into bloom in winter (Table 1).

The presence of the tunic may promote the infection of the aerial parts (Table 3), which may be caused by fungal inoculum present in the tunics, contributing to the spread of the disease in the scales. Of the plants recorded as showing symptoms nevertheless many produced flowers of a marketable quality (Tables 1 and 2).

It is not surprising that the severity of symptoms shown by planted bulbs is related to the frequency of sprout infection. There may be also an effect due to differences in susceptibility between cultivars. The speed of outgrowth of the sprout – as determined by the time needed after planting to reach a length of 8 cm or to reach flowering – has a pronounced influence in this respect, as can be seen from a comparison of bulbs planted in the field with bulbs of the same stocks used for forcing in the glasshouse (Table 4). The speed of sprout growth may be influenced by pre-planting temperature treatment and by soil temperature after planting. Persistent low soil temperature retards growth of the tulip plant, and during the prolonged period necessary to reach a given developmental stage (e.g. flowering), more plants are found to become infected by *B. tulipae* even when the temperature is far below the optimal values for fungal growth. It seems likely that the time factor is more important for the ultimate situation than a temperature more favourable for the fungus (Table 4). At present there

are indications that low temperature does not increase susceptibility of the bulb tissue to attack by *B. tulipae*.

Price (1970) has shown that higher temperatures indeed accelerate growth of the fungus *in vivo*, for he observed more extensive fungal outgrowth in inoculated bulbs planted at 10° and 15°5C as compared with 4°C, and this was accompanied by a more frequent incidence of diseased sprouts at the higher temperatures. However, in his experiments samples were examined at fixed times (41 and 82 days after planting), regardless the stage of sprout development. In our experiments one stage of development (flowering) was taken as a criterion, and this stage was reached after different lengths of time. Since Price used bulbs which had not been cooled before planting (personal communication), in this experiments shoots grew out slowly when soil temperature was relatively high (e.g. shoot length of about 15 cm reached at 15.5°C after 82 days). In our experiments plants reached the flowering stage in about 6 to 10 weeks at this temperature (Tables 4 and 5), because the bulbs had been pre-cooled. In view of this difference in conditions our results and those of Price, which seem contradictory, are not comparable. Moreover, Price used inoculated bulbs in which the fungus probably does not spread in the same way as the outgrowth from natural lesions caused by *B. tulipae*. This subject will be discussed in a later paper.

It will be interesting to collect data on sprout infection at higher soil temperatures, but under such conditions appreciable differences in speed of sprout growth can be reached only with some difficulty, by choosing widely different pre-planting treatments. This introduces a factor which in itself probably influences the rate of outgrowth of the fungus from lesions after planting. Price (1968) observed that the development of the fungus on inoculated bulbs increased with increasing delay of planting, i.e. after prolonged storage. In our opinion, in naturally infected bulbs, too, both the duration of the storage period and the conditions during storage may influence the spread of the fungus after planting; this point needs further investigation.

Samenvatting

Enkele faktoren, die de infectie van tulpespruiten door Botrytis tulipae beïnvloeden

Tot nu toe werd aangenomen dat spruiten, groeiende uit tulpebollen met symptomen van aantasting door *Botrytis tulipae*, bijna altijd door deze schimmel worden geïnfecteerd. Dit werd gebaseerd op het feit, dat de schimmel na het planten van de bol dikwijls in de richting van de bolneus groeit en/of tot in de binnenste rokken doordringt en zo gemakkelijk in contact kan komen met de groeiende spruit. Experimenteel bleek echter, dat een dergelijke spruitinfectie minder frequent voorkomt dan werd verondersteld.

Het ontstaan ervan is afhankelijk van een aantal faktoren. Behalve de mate van aantasting van de geplante bol, het al of niet aanwezig zijn van de bolhuid en de bodemtemperatuur blijkt ook de snelheid van uitgroeien van de spruit een belangrijke invloed te hebben. Hierdoor is het verklaarbaar dat aantasting van de bovengrondse delen veel vaker voorkomt in het veld (lengte van de periode tussen planten en bloei ongeveer 6 maanden), dan tijdens het forceren in de kas (lengte van deze periode 10–19 weken). Ondanks de aantasting bleek een deel van de spruiten in de kas nog verkoopbare bloemen te leveren.

De snelheid van uitgroeien wordt niet alleen beinvloed door de bodemtemperatuur maar ook door de temperatuurbehandeling tijdens de bewaring vóór het planten. Welke invloed deze laatste faktor op de spruitaantasting na het planten heeft, wordt nog onderzocht.

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