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Influence of soil temperature on field infection of tulip bulbs by Fusarium oxysporum

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Abstract

Soil temperature is an important factor in field infection of tulip bulbs by *F. oxysporum* in both autumn and late spring. Three types of infection and the associated symptoms are discussed in relation to temperature.

(1) The infection through the browning tunic at any place in the outer scale, which occurs during the last few weeks before harvest, being most common in the Netherlands.

(2) A basal rot is thought to be favoured by a relatively high temperature after planting in combination with a warm spring, conditions which sometimes also cause premature death of plants; these symptoms are attributed to infection of the root plate immediately after planting.

(3) After a warm spring period several weeks before normal lifting time brown spots may be present in the otherwise white tunic, frequently accompanied by an infection of the scale below the spot.

It is suggested that a high inoculum level built up in the decaying scales of the planted bulb under warm conditions may weaken the tunic barrier by local reduction of the tuliposid concentration in the white tunic, which compound is a precursor of the fungitoxic tulipalin. A semi-quantitative assay for estimation of the number of fungus propagules in the old scales is described.

Introduction

Losses due to *Fusarium oxysporum* Schlecht f. sp. *tulipae* Apt are known to be most severe in the Netherlands in years with warm wheather conditions in spring and early summer. The effect of soil temperature has been mentioned in several papers (Bergman, 1965, 1966), but experimental data have not yet been published in detail. Infection of daughter bulbs usually occurs in the Netherlands during the last few weeks before harvest (Bergman, 1965); roots and plant stems generally show little damage. The absence of apparent host damage after prolonged contact with the parasite (autumn to June) has been suggested to be due to the presence of a tuliposid precursor of the fungitoxic lactone tulipalin A (Beijersbergen and Lemmers, 1972b) which is found in extracts of roots, stems, and white tunics of young bulbs (Bergman and Beijersbergen, 1968). Daughter bulbs usually become infected only during the last weeks of growth, when the tuliposid content of the tunics is decreasing.

In some years, however, some plants may die off suddenly with leaves turning bluish or reddish during late May or early June. Although this premature death may also have other causes (e.g. infection by *Botrytis tulipae*), often a greyish area of decay in the root plate and in the lower part of the stem is found, which is specific for attack by *F. oxysporum*. Daughter bulbs of such plants are usually attacked at the base.

Stocks dug early in June of 1970 and 1971 included bulbs showing sharply defined brown spots on the otherwise white and fleshy tunics. The bulb scale underneath such spots frequently showed symptoms of infection by *F. oxysporum*, demonstrating that the fungus had been able to pierce the tunic in which the chemical barrier was then still present.

In this paper the ways in which soil temperature can influence the development of each of the symptoms is discussed. Moreover, it was attempted to estimate the influence of soil temperature on the amount of *F. oxysporum* present in the gradually decaying scales of the planted bulbs.

Materials and methods

Bulbs of cv. 'Enterprise' were planted in metal containers (14 × 26 × 30 cm) filled with sand contaminated with coarsely ground fungus cultured on rice grains. After the containers had been held for several months at 5 °C to promote root and shoot outgrowth, the soil temperature was raised gradually, according to a fixed scheme, to various constant maximum levels. Because of the effect of soil temperature on plant growth, the intervals between temperature changes were chosen such that the desired maxima were reached three weeks before the harvest time. The bulbs were lifted when the plants had died and the tunics had turned brown, after which they were dried rapidly and stored at 20 °C for several weeks before being examined for symptoms.

In another experiment with cv. 'Apeldoorn', soil temperatures were varied during the first 3 weeks after planting in combination with various temperatures in spring. The containers were held at 11 ° or 17 °C for 3 weeks prior to cooling and late in spring half of each group was exposed to a high (14–16–18 °C) or a low (10–12–14 °C) soil temperature range.

A similar set-up was used to study the colonization by *F. oxysporum* of the scales of spore-contaminated bulbs (cv. 'Rose Copland', pre-cooled at 5 °C) after maintenance at constant soil temperatures of 13 ° or 18 °C. At various sampling dates the excised scales of each of 4 bulbs were ground separately with some sterile water in an Ultra-Turrax grinder for 1 minute, after which the volume was made up to 200 ml with water and the suspension was ground a second time in a Servall Omni-Mixer for 2 minutes at about 10,000 rpm. This finely ground material was diluted 1:10 to 1:10,000, 1 ml aliquots were pipetted into petri dishes and mixed with 9 ml of a warm (45 °C) agar. The selective agar medium after Kerr (1962) was modified as follows: a higher dose of streptomycin sulphate (150 ppm) and Rose Bengal (125 ppm) was used and 150 ppm oxytetracyclin hydrochloride was added. The dishes were stored at 27 °C for 3 days, after which the colonies of *F. oxysporum* were counted. From these numbers of the various dilutions, the mean numbers of *Fusarium* propagules per bulb and per 4-bulb sample were calculated.

Similar experiments and field plantings with various cultivars – some on artificially heated soil – served for additional observations of symptoms or disease counts.

Results

In two experiments with cv. 'Enterprise', temperatures were raised gradually to maxima of 10 ° to 24 °C, starting on April 1st. After the harvest of the first experiment,

Table 1. Percentages of bulbs infected by *F. oxysporum* after growth under various soil temperature schedules; maximum temperatures during the last 3 weeks before harvest. Cv. 'Enterprise', healthy bulbs in contaminated soil; about 100 bulbs per treatment.

Maximum temperature during last 3 weeks (°C)	% diseased bulbs during storage	
	1st exp.	2nd exp.
10	–	4
12	0	–
13	–	3
14	0	–
16	1	13
18	2	–
20	8	42
22	12	–
24	–	80

Tabel 1. Percentages door F. oxysporum geïnfecteerde bollen in de oogst na teelt bij verschillende bodemtemperaturen; maxima gedurende de laatste 3 weken voor de oogst. Cv. 'Enterprise', gezonde bollen in besmette grond; ongeveer 100 bollen per object.

no diseased bulbs were found in the material grown at 12° or 14°C, and the numbers of diseased bulbs increased with higher temperatures. In the second experiment more inoculum had been added to the soil, and much higher disease percentages were found (Table 1).

When the effect of various soil temperatures immediately after planting was studied with cv .'Apeldoorn' in combination with high or low temperatures in spring, the results were as shown in Table 2. Although warm conditions during the last part of the growth period were predominant, high temperatures in the autumn had a distinct effect on the ultimate disease percentages. When the temperature was high during both periods, its cumulative effect was frequently expressed by a sudden blueing and

Table 2. Effect of soil temperature in autumn and late spring on percentages of *Fusarium*-infected bulbs after harvest; maximum temperatures during 3 weeks prior to harvest; cv. 'Apeldoorn', healthy bulbs in contaminated soil; about 160 bulbs per treatment; values between parentheses are percentages with symptoms beginning at the bulb base.

Temperature (°C)		% diseased bulbs during storage
October (3 weeks)	May/June	
11	10–12–14	0
11	14–16–18	16 (2)
17	10–12–14	5 (1)
17	14–16–18	42 (22)

Tabel 2. Invloed van de bodemtemperatuur in de herfst en het voorjaar op de percentages door F. oxysporum geïnfecteerde bollen na de oogst (maxima in het voorjaar gedurende 3 weken voor de oogst); cv. 'Apeldoorn', gezonde bollen in besmette grond; ongeveer 160 bollen per object; () = percentages bollen met symptomen beginnend bij de bolbasis.

early death of the plants, the daughter bulbs of which being always diseased at the base.

The same symptoms were found in field plantings in June after a warm spring in crops which had been planted in early October when soil temperature was relatively high.

Plants dying prematurely were counted in an experiment with cv. 'Topscore', planted on 9 October in contaminated soil provided with an electrical heating system. One part of this soil was heated to about 2 °C above the temperatures actually prevailing from 10 October to 20 November (which were 1–2 °C above normal in 1970). A second part was heated to 2–4 °C above normal beginning on 11 May, and a third part remained unheated. Numbers of prematurely dying plants are given in Table 3. These high numbers are not common in tulip cultivation in the Netherlands, and must be ascribed to the combined effect of artificial soil contamination and high temperatures which were also distinctly above normal in the unheated parts of the soil both in October 1970 and spring 1971. When the bulbs were harvested at the end of June, many were so severely rotted that it was impossible to determine whether infection had started at the base as could be expected, or elsewhere.

In several tulip stocks (including the highly susceptible cvs 'Paul Richter' and 'Prominence') planted in non-heated contaminated soil in the same field six weeks later (20 November), no 'blue plants' were found in late spring and the percentages of stored bulbs with symptoms beginning at the bulb base were not unusually high.

Table 3. Premature death of tulip plants (cv. 'Topscore') grown in field soil contaminated with *F. oxysporum* and heated to about 2 °C above naturally prevailing temperatures in either autumn or spring; total number of plants: 300.

Period of temperature raise	Number of blueing and dying plants on:			
	12 May	18 May	27 May	3 June
11 Oct. – 20 Nov. 1970	1	3	36	all
11 May – 10 June 1971	0	0	28[1]	nearly all[1]
soil not heated	0	1	10[2]	67[1]

[1] Mild symptoms
[2] Inconspicuous symptoms (leaf tips only)

Tabel 3. Aantallen voortijdig afstervende planten (cv. 'Topscore') gegroeid in met F. oxysporum besmette grond, die of in het najaar of in het voorjaar ongeveer 2 °C werd verwarmd boven de heersende bodemtemperatuur; 300 planten per object.

After a prolonged warm period in spring, the third type of symptom was found in bulbs lifted early in June: sharply bordered brown spots on the otherwise white and fleshy tunics, frequently with disease symptoms in the underlying scales (Fig. 1). This symptom was found, for instance, in early-planted cv. 'Topscore', and in late-planted cvs 'Apeldoorn' and 'Prominence'. After 20 minutes of disinfection in calcium hypochlorite the pathogen was readily isolated from brown-spot tissue, but not from the white parts of the tunics. In extracts of brown-spot tissue (Beijersbergen, 1972) no tulipalin A was found, whereas extracts of the white parts of the same tunics contained high concentrations.

Fig. 1. Left: white tunic of a young bulb with sharply bordered brown spots caused by *F. oxysporum*. Right: similar bulb after some weeks' storage (tunic turned light brown), showing symptom in the underlying scale.

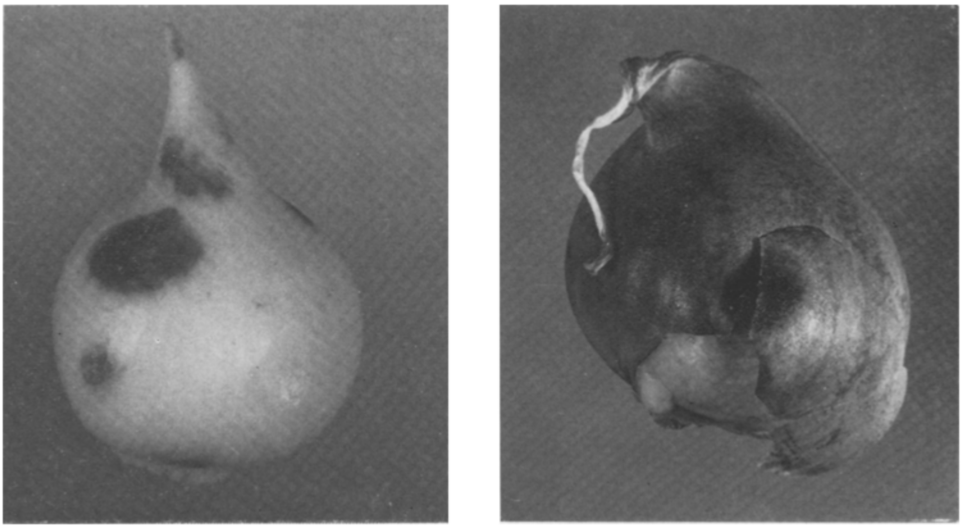

Fig. 1. Links: jonge bol met nog witte huid, waarop scherp-begrensde bruine vlekken veroorzaakt door F. oxysporum. Rechts: soortgelijke bol na enkele weken bewaring (huid is lichtbruin verkleurd) met symptoom van aantasting in de rok onder de bruine vlek.

It was supposed that the tulipalin barrier in the tunics is broken by a local build-up of a high inoculum density in the scales of the mother bulb. For this reason, an attempt was made to isolate *F. oxysporum* semi-quantitatively from these scales, and to determine whether soil temperature influences the numbers of propagules of the pathogen. To this end, samples of cv. 'Rose Copland', spore-contaminated before planting, were taken 2 weeks after planting and then every week or – later on – every 3 or 4 days. Bulbs were used regardless of whether symptoms of *F. oxysporum* infection were visible or not. The average numbers of colonies calculated per 4 bulbs are given in Fig. 2. The lower dilutions of each sample yielded relatively fewer colonies than the more diluted samples. Since all were used for calculating the ultimate numbers of propagules, the values in Fig. 2 represent the lower limits of the propagule numbers actually present. Counts made on different sampling dates varied considerably. This may be partially due to the fact that the samples consisted of only 4 bulbs, but it proved impossible to handle larger numbers in this strictly standardized procedure. At all sampling dates except one (4 April), however, counts were distinctly higher for bulbs grown at 18 °C than for those grown in cool soil.

Discussion

Soil temperature must be regarded as a very important factor in the infection of tulips by *F. oxysporum* in the field. This explains why losses are severe only in some years in the Netherlands, but frequent in countries with a milder climate in autumn and/or spring (Schenk, 1967).

Fig. 2. Numbers of propagules of *F. oxysporum* as calculated for 4 bulbs per sampling date from colony counts on a selective agar medium mixed with a suspension of ground scale tissue of bulbs grown at 18° or 13 °C soil temperature.

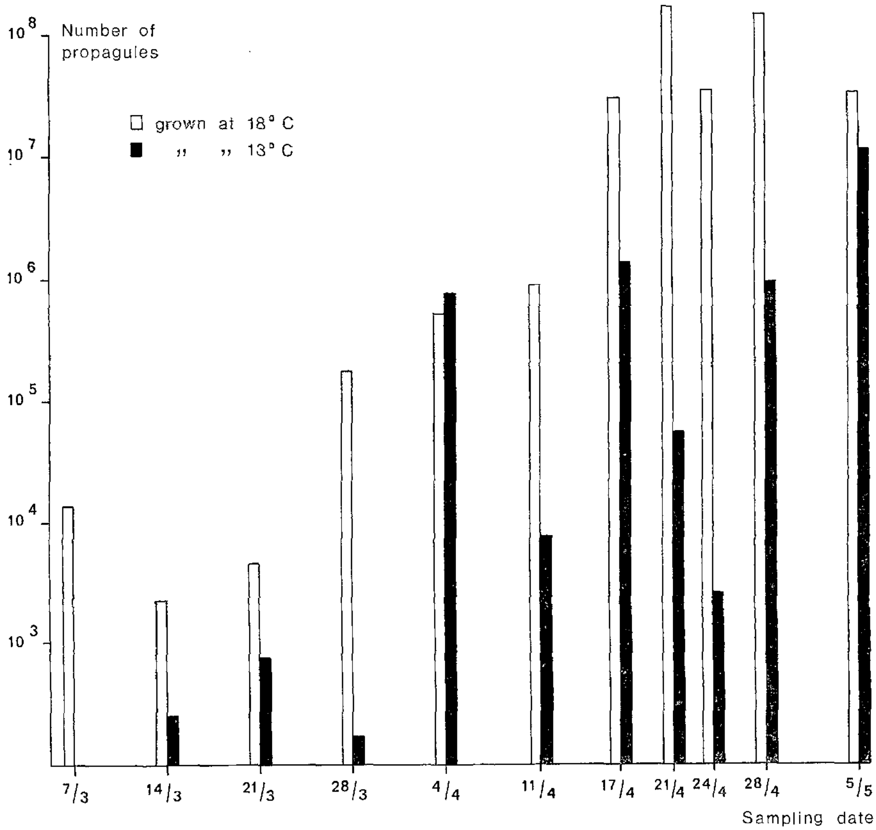

Fig. 2. Aantal kiemkrachtige eenheden van F. oxysporum berekend voor 4 bollen per monster uit tellingen van kolonies op een selectieve voedingsbodem, waaraan suspensies werden toegevoegd van gemalen rokken van bollen, die geteeld werden in grond met 18° of 13°C bodemtemperatuur.

Bulb growers tend to advance the planting date, mainly to suit the mechanisation of planting. As a result, bulbs are often planted in relatively warm soil, which has undoubtedly contributed to the high losses in some years. The effect of high temperatures after planting is not surprising: it is known that in tulips pre-cooled at 5 °C and planted in a heated glasshouse for forcing, the basal plate may be invaded rapidly by *F. oxysporum*, which often results in the death of the plants before flowering (Schenk and Bergman, 1968). In field plantings such invasions are arrested by the low winter temperatures and the plants emerge and flower normally. In mild springs decay of the bulb base may progress so rapidly that the plants die prematurely early in June. The concentration of the precursor of tulipalin A present in the basal plate tissues is apparently insufficient to prevent invasion by the fungus, which grows vigorously under such conditions. After early planting (10 October 1970) in non-heated contaminated soil, a considerable number of plants of cv. 'Topscore' died prematurely, whereas crops of several cultivars planted in the same field 6 weeks later showed no blue plants. This indicates that infection of the planted bulb in the autumn – favoured by relatively high temperatures – is mainly responsible for this field symptom. In crops containing numerous 'blue plants' in late spring, a high proportion of the harvested bulbs show symptoms emanating from the base (Table 2). In this case daughter bulbs are usually infected through the basal plate of the planted bulb.

In Dutch bulb production symptoms are usually found somewhere on the side of the bulbs, which indicates that the fungus has passed directly through the tunic. In general, this infection route is only accessible during the last few weeks of the growth period, when the concentration of the tulipalin precursor decreases in the dying tunic (Bergman, 1965, 1966; Bergman and Beijersbergen, 1968). The tunic is considered to function as a barrier against infection except during the last few weeks of the growth period.

In vitro, the fungus can become adapted to a high concentration of tulipalin A (Beijersbergen and Lemmers, 1972a). This has been ascribed to an irreversible binding of the compound, which leads to the death of part of the mycelium, allowing surviving parts to grow out. It was suggested that the compactness of fungus growth plays the essential role in this process of tulipalin inactivation.

The scales of the planted bulb surrounding the daughter bulbs may contain a considerable amount of *F. oxysporum* propagules, and the amount proved to increase with both time and increasing soil temperature (Fig. 2). This suggests that after a prolonged warm period in spring the fungus may reach a sufficiently high inoculum level locally to overcome the tunic barrier, thus leading to the brown-spot symptom and early lateral infection.

Samenvatting

De invloed van de bodemtemperatuur op de aantasting van tulpebollen te velde door F. oxysporum

De mate waarin en de wijze waarop tulpebollen door *Fusarium oxysporum* f. sp. *tulipae* worden aangetast, wordt sterk beïnvloed door de bodemtemperatuur na het planten en in het voorjaar. Drie typen van infectie konden worden onderscheiden en in verband gebracht met een bepaald temperatuurverloop tijdens de groei.

(1) Het in Nederland het meest voorkomende infectietype is de aantasting gedurende de laatste weken voor de oogst, wanneer de bolhuid bruin wordt en zijn beschermende functie verliest. Beginsymptomen kunnen dan op elke plaats op de zijkant of bij de bolbodem worden gevonden. De invloed van de grondtemperatuur gedurende de laatste weken van het groeiseizoen is duidelijk waarneembaar (Tabel 1).

(2) Indien de schimmel de bolschijf van de geplante bol binnendringt en van daaruit de schijf van de dochterbollen bereikt, is steeds sprake van 'bodemzuur' ('basal rot' in o.a. Japanse en Amerikaanse literatuur). Dit infectietype wordt veelvuldig gevonden, indien de geplante bol reeds in het najaar via de wortelkrans wordt aangetast. Men vindt dit vooral bij partijen die vroeg in het najaar worden geplant bij relatief hoge bodemtemperatuur (Tabel 2). Indien het daaropvolgende voorjaar warm is, kan de schimmel dermate snel door de bolbodem tot in de stengelbasis groeien, dat de bovengrondse delen reeds eind mei of begin juni plotseling afsterven, waarbij de bladeren aanvankelijk blauw of roodachtig verkleuren (Tabel 3). De bollen afkomstig

van dergelijke 'blauwe planten' vertonen dan ook vrijwel steeds symptomen van 'bodemzuur'.

(3) Een derde type van aantasting ontstaat na een langdurige warme periode in het voorjaar, waarbij geen bovengrondse symptomen worden gevonden, maar reeds in mei of begin juni op de nog witte huid van de jonge bol scherpbegrensde bruine vlekken ontstaan. In een aantal gevallen blijkt de bolrok onder de vlek geïnfecteerd te zijn (Fig. 1). In extracten gemaakt van het weefsel van bruine vlekken werd geen fungitoxisch tulipaline A gevonden, dat in extracten van de nog witte delen van dezelfde huiden nog in hoge concentratie werd aangetroffen. Uit het weefsel van de bruine vlekken kan de schimmel gemakkelijk worden geïsoleerd.

Voor de isolatie van *F. oxysporum* uit de verterende rokken van de geplante bol werd een gestandaardiseerde methode ontwikkeld, waarbij het weefsel gemalen werd en na verdunning van de suspensie werd uitgeplaat op een selectieve voedingsbodem. De aanwijzing werd gevonden, dat de aantallen kiemkrachtige eenheden van de schimmel sterk kunnen toenemen, zowel in de tijd als onder invloed van de bodemtemperatuur (Fig. 2). Op grond daarvan werd verondersteld, dat de biochemische barrière in de witte huid lokaal kan worden gebroken indien de dichtheid van de schimmelgroei in de oude bolrokken onder invloed van de bodemtemperatuur sterk toeneemt.

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