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Latent infection in tulip bulbs by Fusarium oxysporum

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Abstract

Latent infections by *Fusarium oxysporum* Schlecht. f. sp. *tulipae* can easily be induced in tulip bulbs in the laboratory. They may also occur in commercial stocks and their presence in planting stocks of susceptible cultivars may affect the health of the daughter bulbs harvested in the following year, even when the planted bulbs have been disinfected. Latent infections do probably not reduce the flowering ability of bulbs during forcing in winter. By histopathological techniques the infective hyphae were shown to penetrate the host tissue superficially in latent infections. The pathogen remains viable during dry storage of the bulbs for several months and can become reactivated after planting.

Additional keywords
Fusarium oxysporum f. sp. *tulipae*, histopathology, transfer of disease to progeny.

Introduction

Latent or dormant infections caused by *Colletotrichum* (*Gloeosporium*) species have been described in a number of tropical fruits, e.g. banana, mango, papaw, avocado, and citrus. They have also been demonstrated in apples (by *Cryptosporiopsis* spp.), apricots (by *Sclerotinia fructicola*), and strawberry and tomato fruits (by *Botrytis cinerea*). A review of the literature has been given by Verhoeff (1974).

In ornamental bulbs, the existence of latent infections in gladiolus corms by *Fusarium oxysporum* Schlecht. f. sp. *gladioli* (Massey) Snijder & Hansen has been suggested by Littrell (1964), and evidence of it has been provided by, among others, Magie (1971) and Henis and Zilberstein (1973). Langerak and Haanstra-Verbeek (1977) demonstrated with histological methods latent infections by *F. oxysporum* f. sp. *narcissi* (Cooke & Massee) Snijder & Hansen in the root plate of narcissus bulbs and proved their importance in the epidemiology of the disease. The first indications of latent infections caused in tulip bulbs by *F. oxysporum* f. sp. *tulipae* Apt were obtained by Bergman (1965), who isolated the pathogen from the scales of seemingly unaffected bulbs.

Since it proved difficult to study this phenomenon in more detail in naturally infected stocks, a method was developed to induce latent infections in bulb scales artificially. This method is described here together with histopathological observations. The consequences of latent infections for the transmission of the disease to the next generation of bulbs are discussed.

Materials and methods

Bulbs were usually lifted early, before the tunics had turned brown, in order to avoid natural infection in the field (Bergman and Beijrsbergen, 1971). Remnants of the scales of the mother bulb, roots and shoot were removed. Bulbs with closed tunics were selected, washed and the tunics were removed. The undamaged outer scale was inoculated with an agar disk (4 mm diameter) punched from the edge of a colony of *F. oxysporum* f. sp. *tulipae* (isolate B 1 mono) grown on Czapek Dox agar (Oxoid) enriched with 2% glucose and 1.5% yeast extract (Difco). The inoculation site was marked with dots of Indian ink. Bulbs were incubated in a constant water-saturated air stream at 25 °C for 44–48 h (Bergman, 1975), after which the inoculum was removed and the bulbs were stored in trays in a well-ventilated room at 25 °C and 60–80% relative humidity. During the following weeks of dry storage all bulbs developing typical symptoms of *Fusarium* rot were discarded.

When inoculation sites were to be plated, the surface was disinfected by rubbing with a cottonwool plug dipped in a 6 or 10% solution of formaldehyde or by submersion of the bulbs in 10% formaldehyde for 5 minutes, followed by rinsing in tap water. Scale parts of the inoculation sites were plated on potato-dextrose agar (P.D.A.) containing terramycin hydrochloride and streptomycin sulphate (0.01% each) or on the selective medium described by Papavizas (1967).

Results

Under the conditions described, 40–60% of the bulbs of susceptible cultivars showed symptoms of *Fusarium* infection within about six weeks after inoculation. These losses of bulbs can be influenced by the size of the inoculum, the chemical composition of the culture medium, and the temperature and duration of incubation. This high percentage of lost bulbs was accepted, because otherwise too many of the symptom-free specimens might have escaped infection.

Atypical symptoms of damage developed at the inoculation sites of many of the seemingly non-infected bulbs during dry storage: yellowish-brown and slightly sunken pinpoint specks or small spots, scattered over the surface (Fig. 1). These phenomena were not seen on non-inoculated bulbs treated in the same way or on inoculated bulbs beyond the inoculation site. Once these discolourations had arisen, they did not enlarge or increase in number.

At the end of the storage period of 3 months or more, the inoculated tissue was classified as 'spots', 'specks', or 'symptomless', disinfected externally, and plated, as shown for two representative experiments in Table 1 and Fig. 2 (right).

Scales without any discolouration sometimes yielded the pathogen to a considerable percentage, although the percentages outgrowth of *Fusarium* from scales showing 'speck' of 'spot' symptoms were usually much higher. Even after much longer periods of dry storage (up to 6 months) of a more drastic external disinfection (dipping in 10% formaldehyde for 15 min), the fungus was recovered frequently (e.g. 84 and 21% resp.).

To evaluate the effectiveness of external disinfection with formaldehyde, bulbs were inoculated and incubated for several days. The extent of hyphal growth over the surface of the scales was observed microscopically and the location of the hyphal tips was marked with Indian ink. The surface was then disinfected with formaldehyde of

Fig. 1. 'Pinpoint' and 'speck' symptoms on the scale surface of a tulip bulb, caused by latent infections by *F. oxysporum* f. sp. *tulipae*: photographed after 3 months dry storage, about 3 × enlarged. Black ink dots mark the site of inoculation.

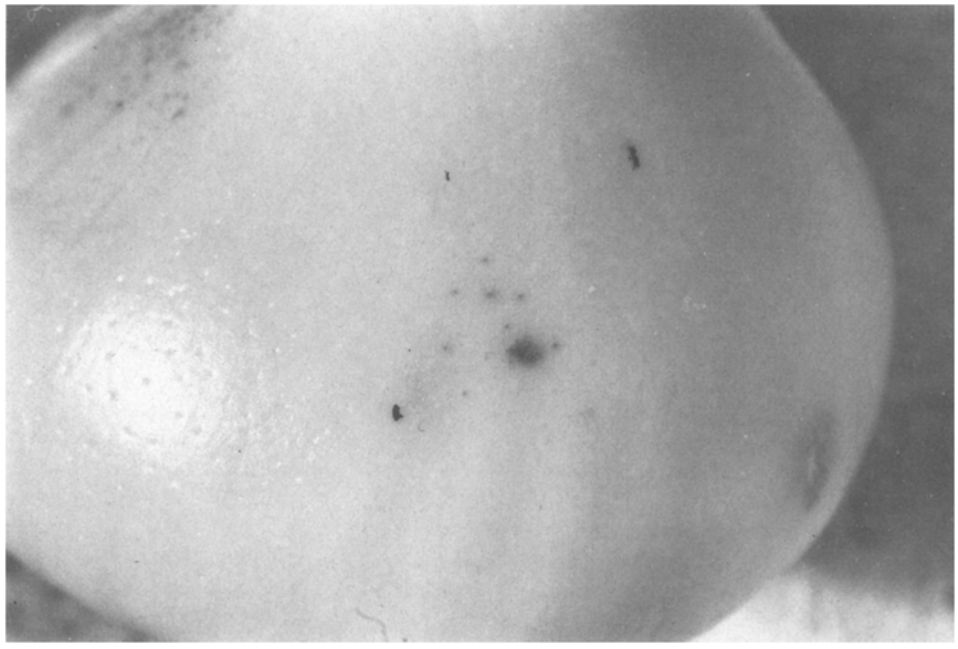

Fig. 1. 'Punt'- en 'vlek'-symptomen in de buitenste bolrok van een tulp, veroorzaakt door latent gebleven infecties van F. oxysporum f. sp. tulipae; gefotografeerd na 3 maanden droge bewaring, vergroting ong. 3 ×. Zwarte stippen markeren de inoculatieplaats.

various concentrations (4–10%) for 5 min, and in one treatment additionally carefully rubbed with cottonwool wetted with 10% formaldehyde. Pieces measuring about 1 × 3 mm were cut from the edges of the marked area for plating, this tissue being considered the least likely to be invaded by the hyphae.

While the non-disinfected scale pieces yielded Fusarium outgrowth upon plating for more than 70%, this percentage was reduced to 17% or less in the disinfected samples,

Table 1. Ratio between numbers of platings of inoculated scales yielding *F. oxysporum* f. sp. *tulipae*, and total numbers of platings; cv. 'Apeldoorn'; inoculation sites classified according to aspecific symptoms of damage; disinfection with 10% formaldehyde.

	Spots	Specks	Without symptoms	Total % outgrowth	Non-inoculated controls
Exp. I	9/11	42/44	4/10	84	0/20
Exp. II	25/27	31/39	0/9	75	0/20

Tabel 1. Verhouding tussen het aantal geïnoculeerde rokstukjes waaruit bij uitplaten F. oxysporum f. sp. tulipae groeide en het totale aantal rokstukjes; cv. 'Apeldoorn'; inoculatieplaatsen geclassificeerd naar de a-specifieke symptomen van beschadiging; ontsmetting in 10% formaldehyde.

Fig. 2. Schematic representation of an experiment in which latent infections by *F. oxysporum* f. sp. *tulipae* are induced in the outer scale of tulip bulbs by a short incubation after inoculation, followed by dry storage for 13 weeks (right side), or dry storage interrupted by storage for 1 week in a moist atmosphere (left). Cv. 'Apeldoorn', 360 bulbs.

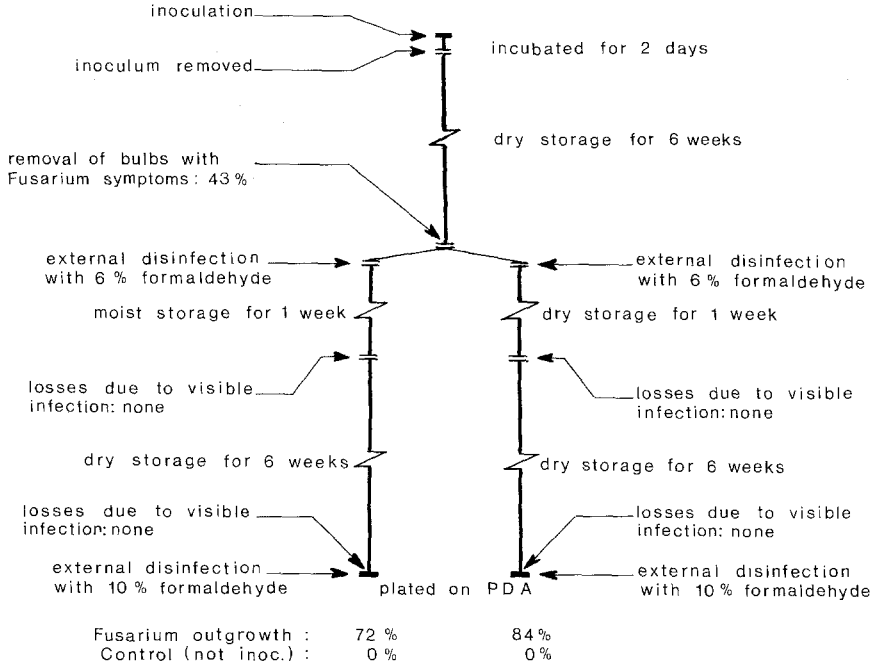

Fig. 2. Schema van het opwekken van latente infecties door F. oxysporum f. sp. tulipae in de buitenste rok van tulpebollen door een korte incubatie gevolgd door 13 weken droge bewaring (rechts) of droge bewaring onderbroken door 1 week bewaring in een vochtige atmosfeer (links). Cv. 'Apeldoorn', 360 bollen.

but outgrowth was never entirely suppressed. This may indicate that none of the disinfections completely killed the fungus growing over the scale surface, or that occasionally it had already penetrated the scale tissue, even at the edge of the area overgrown by the mycelium. The histopathological observations (see below) make the latter explanation more likely.

When after 6 weeks dry storage after inoculation, the bulbs were exposed to high humidity (100% r.h. at 25 °C) for one week, no *Fusarium* disease symptoms developed from the latent infections. The fungus apparently was not reactivated by a moist atmosphere. Neither did such a moisture treatment affect the viability of the fungus in the latent infections (Fig. 2).

When bulbs were not dried quickly after the incubation period, but kept in a moist (100% r.h. at 25 °C) atmosphere for one further week after removal of the inoculum, the percentage of bulbs developing visible symptoms increased considerably (e.g. 74% of the bulbs developed typical symptoms after moist storage as compared with 31% after quick drying), and the percentage of seemingly unaffected bulbs in which latent

infections could be demonstrated later on decreased considerably, e.g. in the same experiment from 34% to 11% upon plating after a 2 months' storage. In this experiment in fewer bulbs (65%) infection (visible + latent) could be demonstrated after quick drying than after another moist week (85%). In other experiments this difference was less pronounced, though always present.

These results suggest that the fungus may be forced into a latent phase and sometimes even may be killed by drought during the initial infection period but, once latency is reached, it is not readily affected by air humidity.

The effect of latent infections in planting stock on disease incidence in the offspring bulbs. As the parasite present in latent infections proved to remain viable during several months of dry storage, the health of the offspring from bulbs with latent infections was investigated in the following year. The transmission of the pathogen seemed probable, because Bergman and Noordermeer-Luyk (1973) had shown that, even in competition with other organisms, the pathogen can grow readily in the decaying scales of the planted bulb in spring.

Bulbs of three cultivars ('Red Giant', susceptible; 'Lustige Witwe', fairly susceptible; 'Black Parrot', resistant) which had been inoculated and stored for about 4 months without developing disease symptoms, were disinfected with 10% formaldehyde just before planting in steam-sterilized soil. Non-inoculated bulbs from the same stocks which had undergone the same procedure served as controls. The daughter bulbs were harvested when the tunics had turned brown, dried, and examined in September (Table 2).

The data show that the disease can be easily transmitted from a seemingly healthy stock of a susceptible cultivar containing bulbs with latent infections, also after a careful disinfection of the bulbs to be planted. In a resistant cultivar, in which latency can also occur, this danger is much less or even absent.

The same holds for naturally infested stocks, as shown in the following example. From stocks of the susceptible cvs 'Enterprise' and 'Red Giant' which has suffered

Table 2. Attack by *F. oxysporum* f. sp. *tulipae* in bulbs of 3 cultivars ('Red Giant': susceptible; 'Lustige Witwe': fairly susceptible; 'Black Parrot': resistant) grown from inoculated mother bulbs with latent infections, after disinfection in 10% formaldehyde prior to planting in steam-sterilized soil.

	Number of bulbs harvested	% diseased	% diseased in controls (non-inoculated)
Red Giant	114	30	1
Lustige Witwe	775	8	0
Black Parrot	677	0	0

Tabel 2. Voorkomen van aantasting door F. oxysporum f. sp. tulipae bij bollen van 3 cultivars ('Red Giant': vatbaar; 'Lustige Witwe': tamelijk vatbaar; 'Black Parrot': resistent), geteeld van bollen welke na inoculatie geen ziektesymptomen hadden ontwikkeld. Vóór het planten in gestoomde grond ontsmet in 10% formaldehyde.

Table 3. Outgrowth of *F. oxysporum* f. sp. *tulipae* from scales of bulbs with latent infections, planted in sterilized soil after a benomyl treatment and kept at 5°C for 15 weeks, followed by 18°C for 5 weeks. Numbers of platings yielding *Fusarium* per total numbers of plated scale fragments.

Disinfection before planting	Recovery of *Fusarium* from:		
	1st scale	2nd scale	3rd scale*
Benlate 0.5%, 15 min.	21/21	12/21	8/16
No disinfection	17/22	10/22	3/8

*Some bulbs did not have a 3rd scale.

Tabel 3. Uitgroei van F. oxysporum f. sp. tulipae uit rokstukjes van bollen met latente infecties, die na ontsmetting in benomyl werden geplant in gestoomde grond by 5°C gedurende 15 weken, gevolgd door 5 weken bij 18°C. Verhouding tussen de aantallen stukjes waaruit Fusarium groeide en de totale aantallen uitgelegde rokstukjes.

severely from attack by *F. oxysporum* f. sp. *tulipae*, bulbs showing no symptoms of pathogenic attack or mechanical damage after removal of the tunics were selected and planted in clean soil after external disinfection. After harvest, 8 and 13%, respectively, of the offspring was found to be affected by *Fusarium*. This indicates that in naturally infested stocks latent infections may have important consequences for the health of the bulbs harvested in the following year.

The transition of latent infections into an active phase. The high incidence of *Fusarium*-diseased offspring from bulbs bearing latent infections before planting suggests that latency shifts to activity some time after planting, thus enabling the pathogen to colonize the scales of the mother bulb (Bergman and Noordermeer-Luyk, 1973) and from there to invade the newly formed bulbs.

To investigate this aspect further, bulbs of cv. 'Paul Richter' with induced latent infections were treated with benomyl and planted in sterilized soil in a glasshouse at about 5°C to meet the cold requirement of the bulbs. The temperature was raised to about 18°C after 15 weeks. When the plants flowered 5 weeks later, the bulbs were dug up and disinfected externally in 10% formaldehyde. The marked inoculation site and underlying parts of the 2nd and 3rd scales were plated on Papavizas' medium (Table 3).

In both treatments the pathogen was recovered frequently from both the 2nd and the 3rd scales, indicating a vigorous outgrowth of the fungus from the latent infections in the outer scale, which probably occurred mainly during the few weeks of favourable temperature.

The fungus can reach the inner scales in two ways: either by growing inward through the outer scale, or by invading the basal plate and penetrating from there upwardly into the inner scales. Since no symptoms (i.e. discolouration of the basal plate, rotting of basal parts of the scales) indicated the latter pathway (Schenk and Bergman, 1969), it must be concluded that the pathogen isolated from the 2nd and 3rd scales followed the former pathway.

Since the benomyl treatment had no effect in this experiment, the concentration of this compound inside the scale tissue apparently has been too low to be efficient. This may be due to the relatively low sensitivity of *F. oxysporum* f. sp. *tulipae* to this compound (Duineveld and Beijersbergen, 1975).

Histopathology. Pieces of inoculated tissue were fixed in glutaraldehyde in vacuo, dehydrated in a range of water/ethanol/tertiary butanol mixtures, and embedded in diglycolstearate; the sections were stained with toluidin blue.

Penetration of the fungus usually – and in undamaged tissue probably always – occurs via the stomata, which are numerous in the outer epidermis of the scale. In actively expanding infections, some mycelium is found in the stomatal cavity and intercellularly between the adjacent cells, but initially mainly between the cuticle and the epidermal cell wall, forming a thick strand of hyphae. The cuticle soon bursts and a protruding fluffy mycelium forms microconidia. At this stage, hyphae have usually also penetrated deeper into the parenchyma, at first between and later into the cells. The cell contents are disorganized before the hyphae reach them, the middle lamellae are dissolved, and the host tissue becomes pulpy. The spread is often so rapid that the tissue is ruptured within a few days, when external symptoms have barely become visible as greyish, sometimes slightly sunken spots.

When scale tissue showing atypical symptoms (Fig. 1) is examined some months after inoculation, i.e. when only latent infections may be expected, mycelium – if present – can only be found in the stomatal cavity and the porus or intercellulary between some cells directly under the cavity, but not at a greater depth or under the cuticle. Hyphae may be branched and hyphal tips are sometimes swollen. Usually, the contents of the guard cells and some adjacent cells are disorganized, and cell walls have turned yellowish brown and may have collapsed or dissolved, causing the scale surface to sink slightly where 'pinpoint' of 'speck' symptoms are present. Chlamydospores were not observed.

Discussion

Gäumann (as cited by Verhoeff, 1974) defined a latent or dormant infection as the situation in which 'the actual infection has taken place, though macroscopically not yet visible, but further growth of the infection hypha is delayed'. This definition does not apply strictly to the tulip, where symptoms are often visible: 'pin-points' or 'specks'. However, these are non-specific as to the pathogen (e.g. *Penicillium* spp. may cause similar symptoms), and are completely different from those caused by an active infection.

Some differences between fruits with latent infections of anthracnose fungi and tulip bulbs infected by *F. oxysporum* f. sp. *tulipae* may be mentioned here. In fruits, all early infections seem to remain dormant in the peel until the fruit ripens, whereas in the tulip many scale infections remain active and cause a manifest decay shortly after infection, while others become latent. Secondly, latent infections in fruits are normally activated during ripening of the fruits. In the tulip they remain dormant throughout dry storage and are not easily affected by storage conditions (Fig. 2). Transition into the active phase apparently occurs only after planting, when food supplies are mobilized for root and shoot outgrowth, which may cause changes in the chemical composition of the host tissue as drastic as those occurring in the ripening fruit peel.

It is not known in what proportion of the latent infections the fungus resumes activity after planting. The data in Tables 2 and 3 indicate that this may be considerable and that latent infections may strongly affect the health of offspring bulbs. It is unlikely, however, that their presence is of importance when bulbs are used for flower forcing. Cool storage (5°C) of the dry bulbs will not favour the transition into the active phase, and the period between planting in the glasshouse and blooming (about 6 weeks) will generally not permit the parasite to affect flowering adversely. Some preliminary observations support this supposition.

Likewise the proportion of the actual infections that may become dormant in bulb farm stocks is unknown. Some observations indicate that this percentage of bulbs with latent infections may be considerable.

Once the latent phase has been established, humidity conditions during storage do not seem to affect the viability of the fungus appreciably. However, when bulbs are dried quickly directly after harvest, the development of visible symptoms is reduced considerably because many infections are forced to become latent and sometimes the fungus is even killed. This means that losses due to rotting of bulbs during storage are reduced, but the number of bulbs with latent infections may increase considerably in the planting stock.

The existence of latent infections may explain why, despite the appreciable beneficial effect, a disinfection of planting stocks never eradicates the disease. Even with highly toxic compounds in concentrations far above the lethal level (e.g. the organic mercury compounds used until recently), or compounds with a prolonged fungistatic activity, such as benomyl (Table 3), this goal has never been reached.

The fact that the disease was found in the offspring grown from bulbs with latent infections of two susceptible cultivars, but not in those of the resistant cultivar 'Black Parrot' (Table 2) does not indicate that latent infections do not occur in the latter, as will be shown in a next paper. Probably in this cultivar resistance factors prevent the outgrowth of the fungus in the scales of the planted bulb and the invasion of the young bulbs to such a degree that the offspring bulbs are not threatened.

It is tempting to suppose that the rapid synthesis of the glycosidic precursor of the fungitoxic tulipalin in the scale tissue during the first days after lifting (Bergman and Beijersbergen, 1971) plays an important role in the arrest of the infection hypha, thus causing the infection to become latent, but conclusive proof of this is not available. There are indications that after planting either the tuliposid precursor concentration decreases considerably, or the splitting of the non-toxic precursor into the active tulipalin is less readily accomplished (Beijersbergen, pers. comm.). However, supportive evidence for the hypothesis that these factors play a role in the reactivation of latent infections after planting is also lacking.

Samenvatting

Latent blijvende infecties in tulpebollen door Fusarium oxysporum

Het is mogelijk latent blijvende infecties veroorzaakt door *Fusarium oxysporum* Schlecht. f. sp. *tulipae* kunstmatig op te wekken (Fig. 2), waarbij geen of voor de ziekte aspecifieke, onopvallende symptomen ontstaan (Fig. 1). Ook in partijen, waarin de ziekte uitval heeft veroorzaakt, kunnen in bollen die op het oog gezond lijken, latente

infecties voorkomen. Indien dergelijke bollen worden geplant, is de kans aanwezig dat de ziekte in een onverwacht grote mate voorkomt in de oogst van het volgende jaar (Tabel 2). Dit geldt eveneens indien het plantgoed is ontsmet en geplant in onbesmette grond. Waarschijnlijk heeft de aanwezigheid van latente infecties in bollen bestemd voor de bloemproduktie in de winter geen invloed op de slaging van de bloei, omdat de periode waarin de temperatuur een voor de parasiet gunstige waarde heeft, te kort is. De schimmel blijft inactief tijdens de droge bewaring van de bollen en wordt weinig beïnvloed door de bewaar-omstandigheden. In een aantal gevallen groeit hij na het planten uit in de rokken van de moederbol (Tabel 3), van waaruit hij in het late voorjaar de jonge bollen kan infecteren. Uit histologisch onderzoek bleek, dat het pathogeen meestal of uitsluitend via de huidmondjes in de onbeschadigde bolrok binnendringt, van waaruit hij inter- en later intracellulair groeiend in korte tijd het omringende weefsel vernietigt. Indien de infectie door nog onbekende oorzaak in de latente fase overgaat, kan het mycelium slechts spaarzaam in de ademholte of intercellulair tussen de deze omringende cellen worden gevonden.

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