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Infection of offspring tulip bulbs by Botrytis tulipae during the growth period and after lifting

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Abstract

During the growth season young bulbs may become infected by *Botrytis tulipae* via the infections present on scales of the planted bulb and from plant stems or by conidia washed down from leaf spots. During storage, infection can only occur after wound inoculation or when bulbs are stored at 100% relative humidity for some days immediately after lifting.

There are no perceptible variations in susceptibility or sensitivity of the bulb tunic during growth, whereas the sensitivity of the outer scale is more pronounced during the first weeks after flowering. During storage, the sensitivity of the scale tissue tends to increase in October and November.

Introduction

After planting, the tulip bulb may be infected by *Botrytis tulipae* (Lib.) Lind from various sources, viz. conidia present on the bulb surface (Doornik and Bergman, 1974), mycelium present in the brown tunic or in local lesions in the outer scale (Price, 1970; Doornik and Bergman, 1971), or inoculum in the soil (Coley-Smith and Javed, 1972; Doornik and Bergman, 1974). However, there is no information in the literature on the mode and source of infection of the offspring bulbs during the growth period and during storage after harvest. Symptoms on young bulbs suggested three modes of attack:

1. by conidia originating from leaf infections and washed down along the plant stem;
2. by mycelium growing downward superficially in the stem; or
3. by inoculum present in the gradually decaying scales of the mother bulb.

After lifting, infections may be caused by the same sources of inoculum as in the soil.

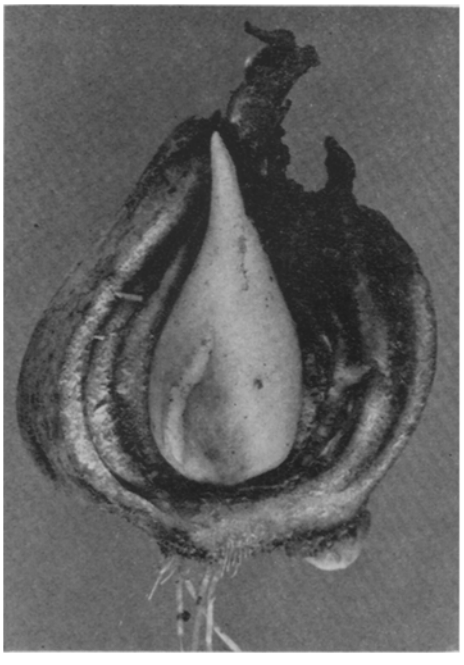
The relative importance of each of these inoculum sources was studied with respect to the transmission of the disease to the young bulbs before and after harvest, as well as the conditions favouring infection. Some information on changes in sensitivity of scale tissue for infection was also obtained at various moments.

Materials and methods

Conidial suspensions were made from 7 to 10-day-old cultures grown on cherry agar (pH 4.5) at 20 °C under Philips HPL lamps. When required, dry conidia were brushed off from similar cultures. Mycelial inoculum consisted of 4 mm disks taken from a 7-day-old culture on cherry agar grown in darkness to prevent formation of conidia. Inoculum for stem inoculations was derived from a 2-month-old culture on rice grains

Fig. 1. Young bulb showing tunic infection, originating from *Botrytis*-infested scales of the old bulb.

Fig. 2. Young bulb with infected, cracked tunics with sclerotia and underlying scale with lesions.



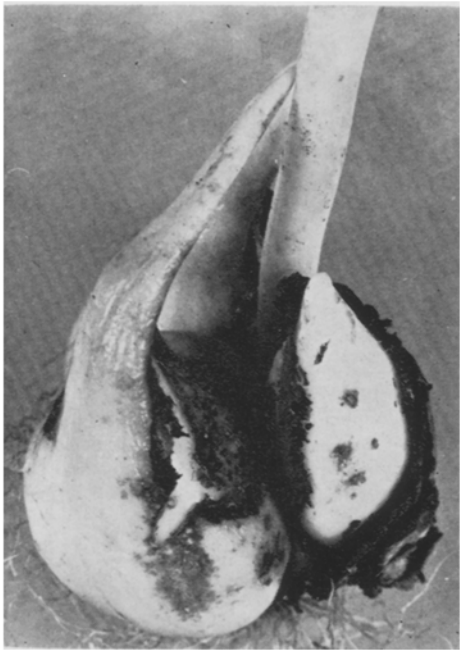


Fig. 1. Huidaantasting van de jonge bol, afkomstig uit de door Botrytis doorwoekerde rokken van de oude bol.

Fig. 2. Jonge bollen waar op aangetaste, gescheurde huiden sclerotiën zijn gevormd en lesies op de onderliggende buitenste rok.

prepared by autoclaving 50 ml rice in 20 ml water for 25 min at 120 °C.

Various air humidities were achieved in conditioned-climate rooms. When storage at 100 % relative humidity (r.h.) and subsequent drying was to be simulated, the air humidity was lowered to 95, 90, 80 and 70 % r.h. for 1 day each, after which bulbs were stored in a well-ventilated room at 20 °C with the air humidity varying from 40 to 70 %.

For the inoculation of offspring bulbs growing in the field, an agar disk with mycelium was stuck to the tunic. For inoculation directly on the scale, a large segment of the tunic was removed, taking care not to damage the scale. These bulbs were lifted for examination two weeks after inoculation.

Inoculations with conidia were performed directly after lifting by dipping bulbs – without removal of remnants of the old bulb – in a suspension of 5×10^5 conidia/ml. During storage, dry conidia were brushed on wounds inflicted with carborundum. Inoculation by conidia washed down from the plants by rain was imitated by spraying 500 ml of a conidial suspension (8×10^6 conidia/ml) per 100 plants, followed by 2.5 l water. Plant stems were inoculated 2 cm below soil surface; the infection site remained under the soil and no formation of conidia was observed.

Results

Symptoms on young bulbs

The fungus may attack the white tunic of young bulbs anywhere, causing an expanding area of brown discoloration (Fig. 1). If the tunic is attacked at about flowering time, the tissue may tear and sclerotia may form on the frayed borders (Fig. 2). When infection of the tunic is severe at this time, the symptoms shown by the underlying scale may consist of irregularly shaped blackish-brown patches, and the infection may penetrate even to the second scale. When the infection occurs later or is less severe, the scale symptoms are restricted to pinpoint specks or 2–10 mm circular or oval and slightly sunken spots, initially pinkish-grey and later yellowish of colour and surrounded by a sharp brown border (Fig. 3). Once this border is formed, the spots do not enlarge. These symptoms resemble the local lesions already described (Doornik and Bergman, 1971).

When inoculation is done in the field on the undamaged bulb scale after removal of the tunic, the symptoms are similar to those seen after severe tunic infection at flowering time (Fig. 4), regardless of the time of inoculation. Under these conditions, the fungus is apparently able to spread freely in the scales of the growing bulb.

Fig. 3. Young bulbs with infection symptoms on outer scales. The bulb on the right shows very young, pinkish-grey, local lesions still without the sharp brown margin. The bulb in the middle shows older local lesions and pin-point specks.

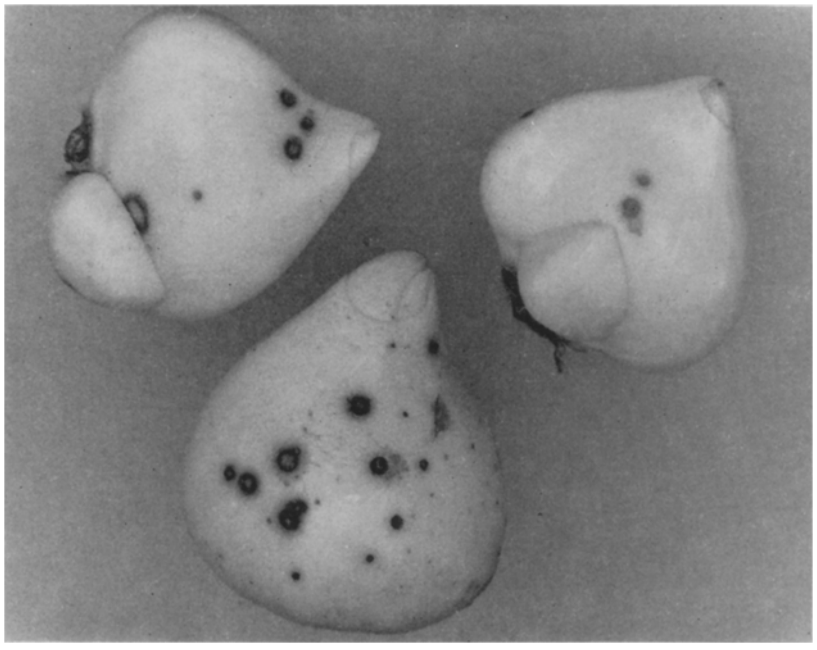


Fig. 3. Jonge bollen met infectie-symptomen op buitenste rok. De rechtse bol toont heel jonge, rose-achtig grijze lesies, nog zonder scherpe begrenzing door een donkerbruine ring. De middelste bol toont naast oudere lesies ook de 'speldeknopsymptomen'.

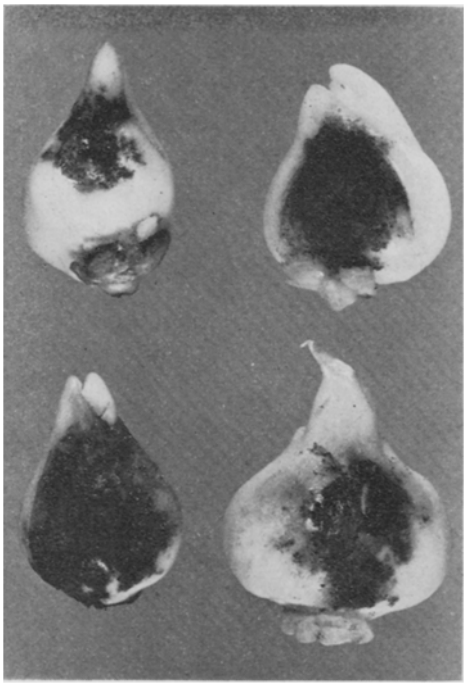


Fig. 4. Infection symptoms after inoculation made on the scale in the field.

Fig. 4. Infectie-symptomen na inoculatie op de buitenste rok, tijdens de groei op het veld.

Sources and modes of infection during the growing period

a. Conidia from aerial plant parts. Symptoms of infection are often localized in the top region of the bulbs, which suggests that conidia originating from sporulating leaf spots and washed down along the plant stem may have been the source of the infection. To verify this point, plants of several bulb lots were sprayed with a conidial suspension on May 15th, and conidia were washed down with an excess of water. As compared with the untreated plots, no appreciable increase in disease incidence was found after harvest of the bulbs. When 3 ml of a very dense suspension was pipetted directly around the plant stems in another experiment, however, 65% of the offspring bulbs showed infection, as against 9% in the untreated bulbs. Symptoms were found almost exclusively in the area close to the bulb tops.

b. Fungal growth in the plant stem. Symptoms on the scales are often localized on the flat side of the bulbs, turned towards the plant stem. Here, fungus growing downward through the stem from above-ground infections might be involved. Subsoil stem inoculations resulted in an increase in disease incidence in the offspring bulbs in only one out of three experiments. In this case almost all of the infections were situated on the flat sides of the bulbs.

c. Fungus present in the scales of the planted bulb. Observations made at 2–4 weeks intervals in the autumn showed that growth of the pathogen in the outer scale starts soon after planting. If the fungus, which can originate from various sources (Doornik and

Bergman, 1971, 1973, 1974; Coley-Smith and Javed, 1972), spreads strongly through the scales after planting, the first symptoms on the tunics of offspring bulbs may be found as early as several weeks before the plant flowers. Usually, however, these symptoms are seen after flowering, when the young bulbs start to grow vigorously. First symptoms on the scales appear 2–4 weeks later, after penetration of the tunic. The number of bulbs with tunic and scale symptoms may increase considerably with time (Table 1).

Table 1. Percentages of offspring bulbs with symptoms of *B. tulipae* on tunics and outer scales caused by the fungus present in lesions on outer scales at planting; all plants flowered at the end of April.

Cultivar	Number of bulbs per sample	Symptoms on	Sampling date				
			2/5	15/5	11/6	17/6	27/6
Diplomat	40–100	tunic	0	2	–	46	46
		scale	0	0	–	17	35
Chinese Bandit	80–120	tunic	–	4	17	17	22
		scale	–	2	9	11	12
Ornament	100–200	tunic	13	13	26	–	*
		scale	0	11	11	–	17

* Observation impossible because of natural browning.

Tabel 1. Percentage jonge bollen met symptomen van B. tulipae op huid en buitenste rok, veroorzaakt door mycelium aanwezig in lesies op de buitenste rok bij het planten; alle cultivars bloeiden rond eind april.

The importance of the presence of the fungus in or on the bulbs of planting stock for the transmission to the offspring bulbs is illustrated by the effect of a bulb disinfection with tetrachloronitrobenzene (TCNB) before planting (Table 2). The same holds for the protection of planted bulbs against attack by inoculum present in the soil by means of a soil treatment with the same chemical.

Infection after harvest

Sources of infection of harvested bulbs may be:

Table 2. Influence of disinfection of diseased planting stock and contaminated soil on the infection percentages of offspring bulbs during the growth season. Cv. Ornament; number of bulbs per sample 100–200.

	% of planted bulbs with new symptoms on 2 May	% of offspring bulbs with symptoms on outer scale on:				
		2/5	15/5	11/6	27/6	4/7
1. diseased bulbs in clean soil	83	0	11	11	17	21
2. as 1, bulbs treated with TCNB	9	0	0	1	1	1
3. healthy bulbs in contaminated soil	49	0	0	2	5	10
4. as 3, soil treated with TCNB	4	0	0	0.3	0.3	1

Tabel 2. Invloed van ontsmetting van ziek plantmateriaal en besmette grond op de aantastingspercentages van jonge bollen gedurende het groeiseizoen. Cv. Ornament; 100–200 bollen per beoordeling.

Table 3. Percentages of bulbs with scale symptoms after inoculation by dipping in a conidial suspension immediately after harvest; cv. Ornament.

1. Inoculated and stored at 100% r.h. at 20 °C for 1 week	27
2. Non-inoculated, stored as 1	5
3. Inoculated and dried immediately after inoculation	4
4. Non-inoculated and dried as 3	5

Tabel 3. Percentages bollen met aantasting op de buitenste rok na dompeling in een sporensuspensie, onmiddellijk na het rooien; cv. Ornament.

1. Conidia deposited on the bulbs during harvest and cleaning, mainly originating from diseased leaves and stems;
2. Fungus which has already penetrated into the tunic but not yet into the scales; and
3. Inoculum present in the remnants of the old bulbs.

As shown earlier (Doornik and Bergman, 1971), fungus present in local scale lesions is inactive during the storage period. Inoculation of undamaged bulbs with conidia was only successful when performed directly after lifting and when the bulbs were kept moist for about a week. When the bulbs were dried immediately after inoculation, disease incidence did not increase (Table 3). Once the bulbs had been dried for a few weeks, inoculations with conidia did not cause infections on undamaged bulbs, even if they were subsequently stored in air with a high relative humidity for a week. Inoculations on carborundum-inflicted wounds resulted in infections at any time during storage if the bulbs were kept moist for a few days, but were without effect under dry conditions. Twenty percent of the bulbs of cv. Ornament showed symptoms on the outer scale upon cleaning and removal of the tunic directly after harvest, and this percentage did not increase upon storage under moist conditions. If the tunics of bulbs from the same lot were not removed and the bulbs were kept under high-humidity conditions for 6 days, 31% were diseased. When remnants of the old bulb were also left undisturbed, the disease percentage rose to 35 after moist storage for the same period. These results suggest that inoculum present in the old bulb scales and on or in the tunic may contribute to the disease incidence if the bulbs are not dried quickly after lifting.

The influence of the storage conditions during the first week was also illustrated in a stock of cv. Solanus, 28% of which showed scale symptoms when harvested. This percentage did not increase when uncleaned bulbs were stored immediately at 40–70% r.h., but rose to 48% when they were stored without drying for 6 days.

Sensitivity and susceptibility of tunic and scale

The terms sensitivity (tendency of an organism attacked by a pathogen to give more or less strong symptoms) and susceptibility (non-immunity to a given pathogen) are defined here as given by Ainsworth (1971).

Under natural conditions, the percentages of bulbs with symptoms on the tunic and outer scale increased with time (Table 1 and 2), suggesting an increase in susceptibility. Field inoculations made on the tunics of growing bulbs of several cultivars did not demonstrate perceptible variations in susceptibility, since the percentages of successful inoculations nearly always reached 100%. Judging from the severity of the symptoms, the sensitivity of the tunic also persisted on a very high level from early

Fig. 5. Sensitivity of the outer bulb scale of 3 tulip cultivars to *B. tulipae* infection upon inoculation in the field at various intervals after flowering.

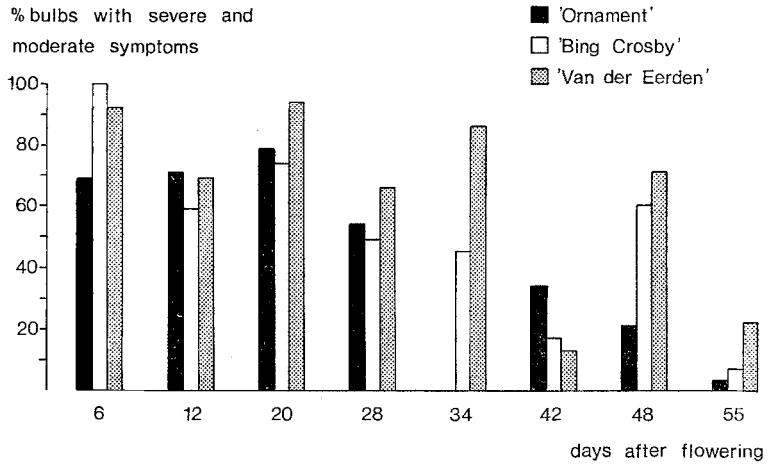


Fig. 5. De gevoeligheid van de buitenste bolrok van 3 tulpe-cultivars voor B. tulipae-infectie bij inoculatie op het veld op verschillende tijdstippen na de bloei.

May (beginning of vigorous growth of bulblets) till the middle of June (when natural browning of the tunics begins to obscure the symptoms).

Field inoculations made directly on the scale after removal of the tunic at various times up to the 55th day after flowering and examination of results two weeks later revealed a decrease in severity of symptoms, i.e. in the sensitivity of the scale tissue during the later part of the growth period (Fig. 5). Analysis of the data by the test on the trend of random samples (De Jonge, 1963) showed this decrease to be highly significant for cv. Ornament ($P < 0.01$) and significant for the other cultivars ($P < 0.05$). Field inoculations on the tunics led to a similar and significant decrease in the sensitivity of the underlying scale tissue in two out of three cultivars.

During storage, inoculations on the scales are only successful during the first few days after harvest. Later on, the dry bulbs have to be wounded and incubation at a high relative humidity is necessary. Under these extreme conditions, inoculations performed every two weeks gave no evidence of variations in susceptibility during the storage period, but sensitivity tended to increase in October and November, as judged from the size and depth of the areas with symptoms.

Discussion

Although the experimental results indicate that the infection of offspring bulbs during the growth period may be caused by conidia washed down from infected plant parts or by mycelium growing downward through the stem, the main source of the inoculum responsible for transmission of the disease to the new bulbs is probably fungus present in the decaying scales of the planted bulb. This inoculum can originate from contamination of the soil, but the persistence of *B. tulipae* in soil is limited (Doornik and Bergman, 1974).

It may therefore be assumed that inoculum present on the bulbs, e.g. conidia on the tunic (Doornik and Bergman, 1971), or mycelium in the local lesions in the scales

(Doornik and Bergman, 1973), is mainly responsible for the transmission of the disease to the next generation of bulbs. This assumption is supported by the observation that stocks which have suffered severely from 'fire' (heavily sporulating leaf damage, frequently accompanied by growth of the fungus in the stem), often appeared to have only a few diseased bulbs after lifting. On the other hand, the use of diseased planting stock with local scale lesions often results in a high percentage of diseased offspring.

In general, infections begin to occur after flowering. The white tunic proved to be very sensitive to attack throughout the growth period and therefore does not adequately protect the underlying scale against this pathogen. Inoculations made on the browning tunic during the last few weeks before harvest and immediately after lifting resulted in symptoms in the underlying scale as well, thus demonstrating the ability of the fungus to penetrate this dying tissue.

The situation in the tunic changes after planting, because now the tunic acts as a protective layer against infection (Doornik and Bergman, 1974), though on the other hand, the tunic invaded in the previous growth season may be a source of infection (Doornik and Bergman, 1971).

The first symptoms on the outer scale are found slightly later than those on the tunics. The increase of disease incidence on the scales at the end of the spring season seems to contradict the demonstrated decrease in sensitivity of the scale tissue, but may be due to an increasing fungus mass in the scales of the mother bulb and the tunic during late spring. Early inoculations on tunics resulted in an unrestricted spread of the symptoms in the underlying scales, similar to those caused by inoculations made directly on the scales. Later in the growth period, however, tunic inoculations gradually gave more localized spots in the scale, resembling the 'local lesions' found in commercial production (Doornik and Bergman, 1971). Therefore it seems likely that in nature many infections occur at a late stage of bulb development, as also is illustrated by the data in Table 1 and 2.

When the bulbs are harvested, infection can occur only during the first days after lifting, when the bulbs are still wet.

Once they have been dried, infection is only possible after superficial wounding and moist incubation. This low sensitivity and susceptibility of the scale tissue is consistent with Doornik and De Rooy's (1971) hypothesis that during storage the scale tissue has some kind of inhibitory capacity, because fungus present in local lesions is not able to develop further. The proven decrease in the sensitivity of scale tissue during the last part of the growth period, as described in this paper, provides solid support for this hypothesis.

It is evident that the situation in the scale tissue changes drastically after planting, since *B. tulipae* can then grow out from the local lesions (Doornik and Bergman, 1973), and the scales are easily invaded by inoculum present in the soil or on the bulb surface (Doornik and Bergman, 1974).

Samenvatting

Infectie van jonge tulpebollen door Botrytis tulipae tijdens de groei en na het rooien

Tijdens de groei kunnen de jonge tulpebollen worden aangetast door *B. tulipae* afkomstig 1) uit de rokken van de oude bol, 2) uit de geïnfecteerde bloemsteel, en 3) van conidia die van bladvlekken naar beneden worden gespoeld.

Van deze drie oorzaken speelt de eerstgenoemde de belangrijkste rol. Aantasting van de jonge bol kan reeds enige weken voor de bloei plaatsvinden. Gewoonlijk verschijnen de eerste symptomen op de nog witte huid kort na de bloei, dus in de periode waarin de jonge bollen goed beginnen te groeien. Op de buitenste rok kunnen de eerste symptomen 2–4 weken later worden gevonden. Infecties op huid en rok nemen toe met de tijd (Tabel 1).

Oorzaken van infectie na het rooien kunnen zijn: 1) conidia, afkomstig van bladvlekken, die tijdens het rooien op de bollen terechtkomen, 2) reeds in de huid aanwezig inoculum dat de rok nog niet is binnengedrongen, en 3) in de overblijfselen van de oude bol aanwezig inoculum.

Na het rooien vinden infecties alleen plaats, wanneer de bollen direct gedurende meerdere dagen bij 100% r.v. worden bewaard (Tabel 3). Zijn de bollen na het rooien gedroogd, dan worden onverwonde bollen niet meer aangetast, ook niet als deze na het drogen geïnoculeerd werden en gedurende 1 week bij 100% r.v. en 20°C werden bewaard. Een verwonding van de buitenste rok met carborundumpoeder is voldoende om een inoculatie met conidiën op elk moment tijdens de bewaring te doen slagen, wanneer de bollen na inoculatie 1 week bij hoge r.v. worden bewaard. Er is geen verschil in vatbaarheid of gevoeligheid van de huid waargenomen bij inoculaties op verschillende tijdstippen tijdens de groei op het veld. De gevoeligheid van de buitenste rok is echter in de eerste weken na de bloei groter dan later tijdens de groei. Deze afname in gevoeligheid is significant in de inoculatieproeven. Dit lijkt in tegenspraak met de gevonden toename van de aantastingspercentages ten gevolge van natuurlijke infectie. Deze toename kan echter het gevolg zijn van een groter wordende myceliummassa in de rokken van de oude bol en in de huid van de jonge bol later tijdens de groei.

Tijdens de bewaring is geen verandering in de vatbaarheid van verwonde bollen geconstateerd. Afgaande op de omvang van de aangetaste plekken na sporen-inoculatie, lijkt de gevoeligheid van de buitenste rok in de maanden oktober en november echter toe te nemen.

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