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FIELD INFECTION OF TULIP BULBS BY *FUSARIUM OXYSPORUM*[1]

Veldinfectie van tulpen door Fusarium oxysporum

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Fusarium oxysporum in tulips is able to penetrate through the roots into the tissue of the planted bulb and from there it can grow into the basal plate of the young bulb. However, evidence is given that under natural conditions infection more often takes place directly into the fleshy outer scale of the new bulb, often during the last weeks before the bulb skin turns brown. This fungus seems to be not a vascular parasite and behaves in a way completely different from other strains of *F. oxysporum*. Often disease symptoms are not visible on freshly harvested bulbs from infested stocks, while after storage for several weeks a number of bulbs prove to be infected. This is also true when storage conditions make infection during this period very improbable.

INTRODUCTION

One of the most important diseases of tulip bulbs is caused by a strain of *Fusarium oxysporum* Schlecht., specific for this host plant. Many forms of this species are known to be vascular parasites, invading their hosts through the roots. SLOOTWEG (1955) and GERLACH (1959) observed that the acid-smelling rot typical of *Fusarium* infection of tulip bulbs generally starts at the basal plate, which suggests that in this host plant also, the fungus usually invades the bulb through the roots. The pictures of IWAKIRI *et al.* (1961) showed only bulbs with disease symptoms at the base. However, in the writer's experience similar disease spots can be found more frequently on the side or the top of the bulb, without any visible connection with the basal plate (Fig. 1). It was therefore important to collect more information about the mode of infection of the tulip bulb during the growth period. Conditions favouring infection during storage of the bulbs and the way of infection under these circumstances are not discussed in this paper.

INFECTION THROUGH THE ROOTS

Fourty tulip bulbs of the susceptible cultivar 'Preludium' were planted in sterilized moist dune sand to induce root formation. When the roots had a length of about 2–3 cm, 30 of the bulbs were transferred to glass vessels, with a narrowed neck, which were partly filled with soil inoculated with *F. oxysporum* isolated from a diseased tulip and grown on boiled rice grains. The inoculation level was 200 ml of the rice culture in 10 liters of soil. Ten bulbs were planted in the same way in non-inoculated soil. The roots of the bulbs were gently pressed into the soil surface while the bulb itself remained about 2 cm above the soil,

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resting on the narrow neck of the glass vessel. In this way the roots were growing in contaminated soil while the bulb had no contact with the inoculum (Fig. 2). In order to stimulate root growth the vessels were kept at 9 °C for five weeks, by which time most roots had penetrated several centimeters into the soil. The glass vessels were then buried in wet peat-dust in metal containers suspended in Wisconsin temperature tanks at 10° and 20 °C. Fifteen plants with their roots in infested soil were placed at each temperature, while the plants in non-inoculated soil were all placed at 20 °C. The root systems could be examined through the glass walls, while for closer observation the soil in the glasses was washed out by a gentle flow of water without damaging the roots.

After five weeks five plants grown in inoculated soil at 20 °C were examined. All root systems were showing a yellowish-brown discoloration. In two plants most of the roots were heavily damaged and the basal plate of the bulb had started decaying, while the leaf tips were yellowing. Microscopical investigation showed that the mycelium was growing abundantly in the root cortex, destroying the cortical parenchyma completely, but it was only very rarely found in the xylem vessels. *Fusarium* was re-isolated both from the basal plates and from the roots.

After eight weeks all root systems of the remaining ten plants grown in infested soil at 20 °C were more or less decaying. In four plants the leaves were yellow and the flower bud shrivelled, while the bulb showed the typical disease symptoms of *Fusarium*, extending from the basal plate.

The roots of plants grown in inoculated soil at 10 °C showed very slight discolorations at that time and the bulbs and foliage of these plants looked healthy. The plants grown in uninoculated soil at 20 °C had a completely healthy appearance (Fig. 3).

Three plants grown in infested soil at 20 °C and four of those grown at 10 °C yielded a small new bulb under these unnatural growth conditions. After some weeks' storage two respectively one bulb in each group proved to be diseased at the base. All new bulbs from plants in uninfested soil were not affected.

These observations prove that *F. oxysporum* is able to penetrate through the roots of tulips into the basal plate of the planted bulb and from there reach the base of the newly formed bulb of the next generation. Under the conditions of the experiment, this type of attack sometimes caused complete destruction of the root system and death of the plant during the flowering period, giving no opportunity for the formation of a new bulb. It must be emphasized that such a severe attack in an early stage of development of the plant is not found under field conditions in Holland. The severe disease symptoms in our experiment may have been due not only to the very high inoculum potential of the soil, but also to the high soil temperature, a temperature very uncommon in this country during this early stage of development of the plant.

INFECTION DIRECTLY INTO THE SCALES

The mode of infection described does not explain the occurrence of diseased spots on the side or the top of the outer bulb scale, apparently without any connection with the basal parts.

To study this phenomenon bulbs were used from diseased stocks of the cultivars 'Enterprise', 'Red Giant' and 'Mantilla', which are all very susceptible to

Fusarium. The bulbs were cleaned directly after lifting and the brown skins were removed. They were then placed in cardboard egg-trays in the laboratory. Storage in this way prevented contact infection completely, while infection by conidia of *Fusarium* formed on diseased bulbs was very improbable under the existing conditions of low air humidity (own observations and IWAKIRI *et al.*, 1961). The exposed outer fleshy scales of the bulbs were examined weekly for *Fusarium* symptoms, beginning directly after cleaning.

It is very important to note that on the day of lifting only a small number of bulbs showed disease symptoms, the number increasing considerably at every weekly inspection, as is shown in Table 1. In several cases bulbs were found for the first time to be diseased only after more than four weeks of storage.

TABLE 1. [illegible]
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Tijdstip van zichtbaar worden van
ring na de oogst, waarbij contactinfectie of infectie door conidia werd voorkomen. natuurlijk besmette partijen.

Cultivar	Number of bulbs examined *Aantal onderzochte bollen*	Number of days after lifting *Aantal dagen na rooien*							
		0	7	14	21	28	35	42	49
		Number of bulbs with symptoms *Aantal bollen met symptomen*							
'Enterprise'	998	13	26	33	38	40	40	41	42
'Red Giant'	870	13	42	62	64	66	67	67	67
'Mantilla'	336	4	12	15	19	20	21	22	22

In the cultivar 'Enterprise' less than one third of the ultimate number of diseased bulbs showed symptoms at the time of lifting, while in 'Red Giant' and 'Mantilla' the proportion was less than one fifth. Apparently many infections were so small at that time, that they could not be recognized, even during careful examination after removal of the bulb skin.

During the weekly inspections, the position of the initial disease symptoms was recorded, after which the diseased bulbs were discarded. The data obtained are summarized in Table 2.

TABLE 2. Position of Fusarium lesions on the outer fleshy scale of tulip bulbs after lifting; same stocks as in Table 1.
Plaats van beginsymptomen van Fusarium-*aantasting in de buitenste rok van tulpebollen na de oogst; dezelfde partijen als in tabel 1.*

Cultivar	Number of bulbs with initial symptoms on the following areas: *Aantal bollen met beginsymptomen op de volgende plaatsen:*			
	Top *Neus*	Side *Zijkant*	Base *Bodem*	Uncertain *Onzeker*
'Enterprise'	3	24	11	4
'Red Giant'	10	41	15	1
'Mantilla'	10	8	4	0

These data show clearly that more bulbs developed the initial symptoms of *Fusarium* attack somewhere on the side or the top of the bulb than on the base or in the fleshy scale bordering the basal plate. This conclusion was confirmed in other tulip stocks examined in other years.

The question now arose whether the initial symptoms really show on the place where the fungus invades the host. It seemed possible that infection in the basal plate might occasionally give no symptoms and the fungus might grow upward into the scale to burst out somewhere on the side of the bulb.

To investigate this possibility bulbs from stocks known to be diseased were lifted some days before normal harvesting time and cleaned. The outside of the bulbs was disinfected by dipping in a 4% formalin solution for five minutes. After rinsing in sterile water the bulbs were cut in slices about 3 mm thick parallel to the basal plate. The knife was sterilized after each cut. The rings obtained by these cuttings from the first, second etc. scales were separated and plated on potato dextrose agar to which 100 ppm streptomycin and terramycin had been added to suppress bacterial growth. Both antibiotics do not influence *Fusarium* growth at this concentration. This procedure was applied to bulbs both with and without *Fusarium* symptoms.

Though outside disinfection as described greatly reduces contamination of the surface, it is difficult to obtain complete sterility (SAALTINK, 1959). It is therefore not absolutely certain that fungus growth on the plates originated from an infection and not from a contamination of the surface. In most cases of *Fusarium* growth on the plates the fungus grew from both the outer and the inner side of the scale, suggesting that the fungus was present within the scale tissue and did not originate from outside contamination. Moreover, growth of other fungi from these slices was not observed.

In Table 3 data are summarized obtained from 55 bulbs treated in this way.

TABLE 3. Number of bulbs showing *Fusarium* growth from the outer scale after cutting in slices 3 mm thick and plating on potato dextrose agar.
Aantal bollen, waaruit na snijden in plakken van 3 mm dikte en uitleggen op aardappel-glucose-agar Fusarium *uit de buitenste rok groeide.*

Growth of *Fusarium* from bulbs with symptoms on the outer scale *Bollen met symptomen*			Growth of *Fusarium* from bulbs without symptoms on the outer scale *Bollen zonder symptomen*			
Base *Bodem*	Side *Zijkant*	Base + side separated (type II, Fig. 4) *Bodem + zijkant gescheiden (type II, fig. 4)*	Base *Bodem*	Side *Zijkant*	Base + side separated *Bodem + zijkant gescheiden*	No *Fusarium* *Geen* Fusarium
3	14	2	8	9	0	19

The places where *Fusarium* grew from the slices were marked in situation sketches of which Fig. 4 gives some representative examples. As can be seen from this figure, the size of the visible symptoms on the outer scale was usually smaller than the spot from which the fungus could be isolated (Nos I and II). The fungus was also often isolated from the second and even from the third

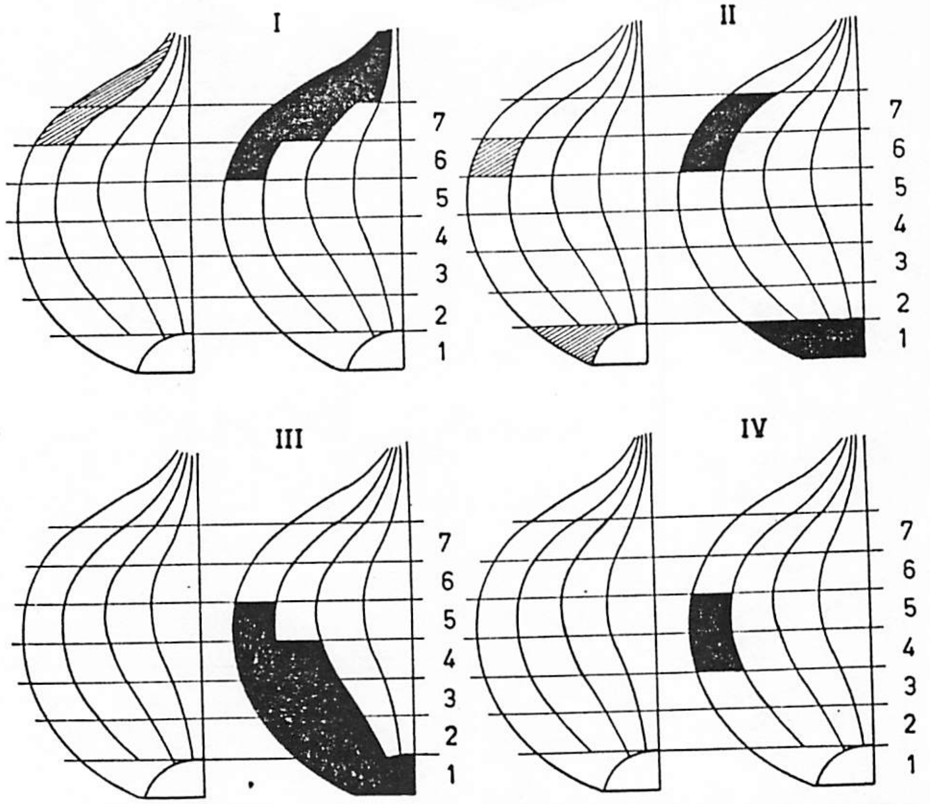

FIG. 4. Presence of *Fusarium* symptoms on the outside of tulip bulbs (striped areas in left-hand-figure of each diagram) and growth of *Fusarium* on potato dextrose agar (black areas in right-hand-figures); data from slices about 3 mm thick cut parallel to the basal plate from the same bulbs.
Aanwezigheid van Fusarium-*symptomen op de buitenzijde van tulpebollen (gearceerde gedeelte links in ieder diagram) en groei van de schimmel op aardappel-glucose-agar (zwart gekleurde gedeelte in de rechter helft van elk diagram) vanuit plakken van ongeveer 3 mm dikte, gesneden van dezelfde bollen, evenwijdig aan de bodem.*

scale, when symptoms were present on the first scale only. Probably the fungus had already penetrated into the inner scales without causing symptoms, but the possibility of contamination of the inner scales during cutting of the heavily infested outer one could not be excluded.

Often *Fusarium* could be isolated when no symptoms were visible on the outside of the bulb (Nos III and IV) and occasionally the infected area was surprisingly large (No III).

Diagrams I, II and IV show clearly that in a number of bulbs no connection could be demonstrated between the base of the bulb and the diseased spots on the side. This supports strongly the idea already mentioned, that *Fusarium* in tulips is able to penetrate directly into the tissue of the outer fleshy scale of the bulb without needing the root tissue as an approach.

DISCUSSION

Under natural conditions in the Netherlands infection by *Fusarium* directly into the scales of tulip bulbs occurs more frequently than infection through the roots into the base, for in naturally infected stocks more bulbs are found with diseased spots on the side of the bulb than at the basal plate. Only in bulbs grown in artificially heavily infested soil has there been a larger proportion showing disease symptoms emanating from the basal plate. Probably under these circumstances infection through the roots is favoured.

In roots, mycelium was found in the cortex in great quantity, causing quick decay of this tissue. In the vessels it was found only in a few instances. In the fleshy scales the fungus grows both inter- and intracellularly in the disorganized tissue of the storage parenchyma and no preference for vascular bundles was observed. Therefore it is concluded that *F. oxysporum* in tulips behaves in a way completely different from other strains of this fungus species, in which, as far as is known, growth is almost always restricted to the xylem vessels (DIMOND, 1955).

When tulip bulbs with slight infections or contaminated with microconidia are planted, usually the plants grow normally, but a large proportion of the progeny of these bulbs is often found to be diseased after the following harvest. Apparently the fungus present in or on the planted bulb usually does not destroy the root system, causing premature death of the plant. It probably more often invades either the young bulb directly or attacks the roots only in the latest part of the growth period. This may explain why field symptoms typical for *Fusarium* disease in tulips do not exist. Whether the fungus grows saprophytically in the gradually decaying tissue of the planted bulb or remains dormant (e.g. as chlamydospores) is not known.

Freshly harvested bulbs often seem to be healthy but start showing disease symptoms after some time of storage, even when precautions have been taken to prevent infection during storage. Apparently these infections have already taken place in the field, but they are so small at the time of harvesting that they cannot be recognized. It is obvious that either these infections have occurred during the last weeks before harvest or the development of the parasite in the host plant tissue is very slow under these circumstances. The first supposition is supported by the fact that both in the growers' experience and in experimental diggings not reported here in detail it has been found that an early harvest can reduce the incidence of *Fusarium* attack considerably. The extent to which this occurs may be related to climatic conditions, e.g. soil temperature, which is usually rising during the first weeks of July when most tulips are harvested. However, it may also be influenced by other factors as yet poorly understood, such for instance, as a change in susceptibility of the bulb tissue to fungus attack.

SAMENVATTING

Fusarium oxysporum, de veroorzaker van het „zuur" in tulpen, is in staat om via de wortels in het weefsel van de moederbol binnen te dringen en van daaruit de bodem van de nieuwgevormde bol te bereiken (fig. 1 boven, fig. 3). Infectie van de jonge bol geschiedt onder natuurlijke omstandigheden echter vaker rechtstreeks in de buitenste bolrok, waarbij dikwijls geen schimmel kan worden aangetoond in het bolweefsel tussen de aangetaste plek en de bolbodem (tabel 2

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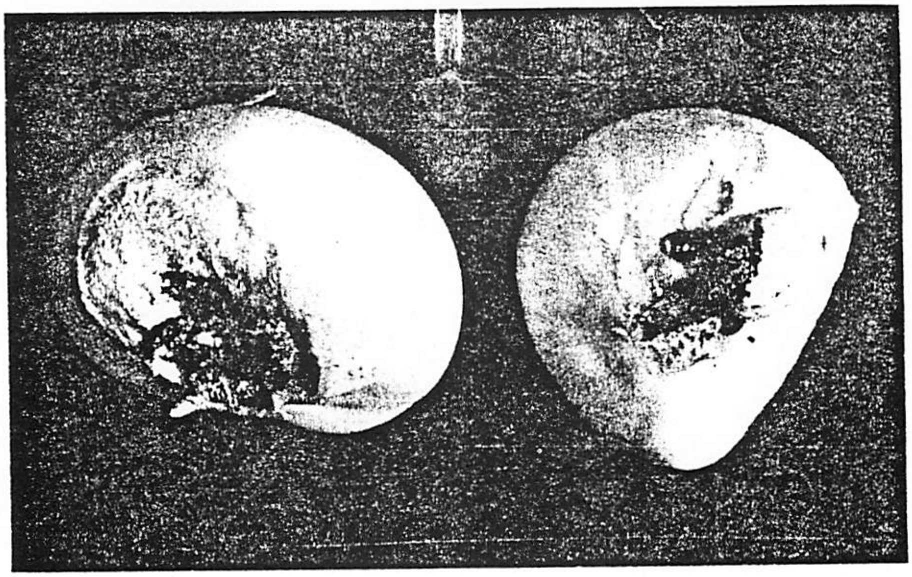

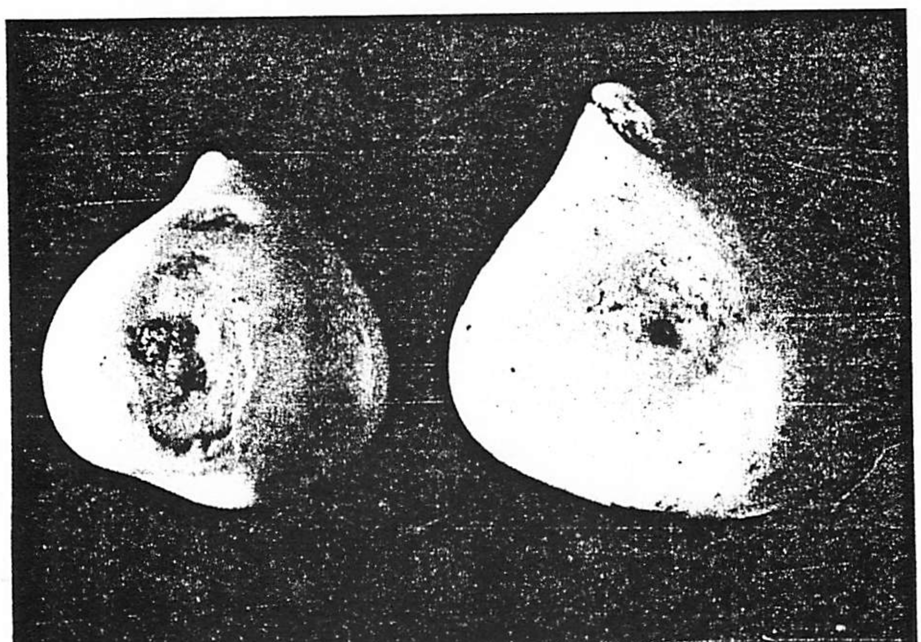

FIG. 1. *Fusarium* symptoms on tulip bulbs a few weeks after harvesting.
Above: diseased spots emanating from the basal plate.
Below: diseased spots on the side and the top of the bulb, without connection with the basal plate.
Fusarium-*symptomen op tulpebollen kort na het rooien.*
Boven: aantasting van de rok, uitgaande van de bodem.
Onder: aantasting op de zijkant en neus zonder verbinding met de bodem.

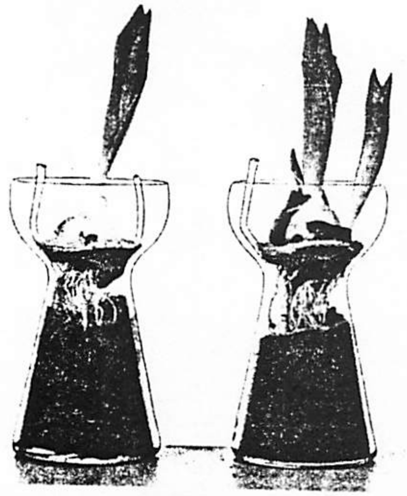

FIG. 2. Tulips in glass vessels, the roots growing in inoculated soil, while the bulb remained free from contact with the inoculum.
Tulpen, gekweekt in hyacinte-glazen, waarbij de wortels groeiden in geïnoculeerde grond, waarmee de bol niet in contact kwam.

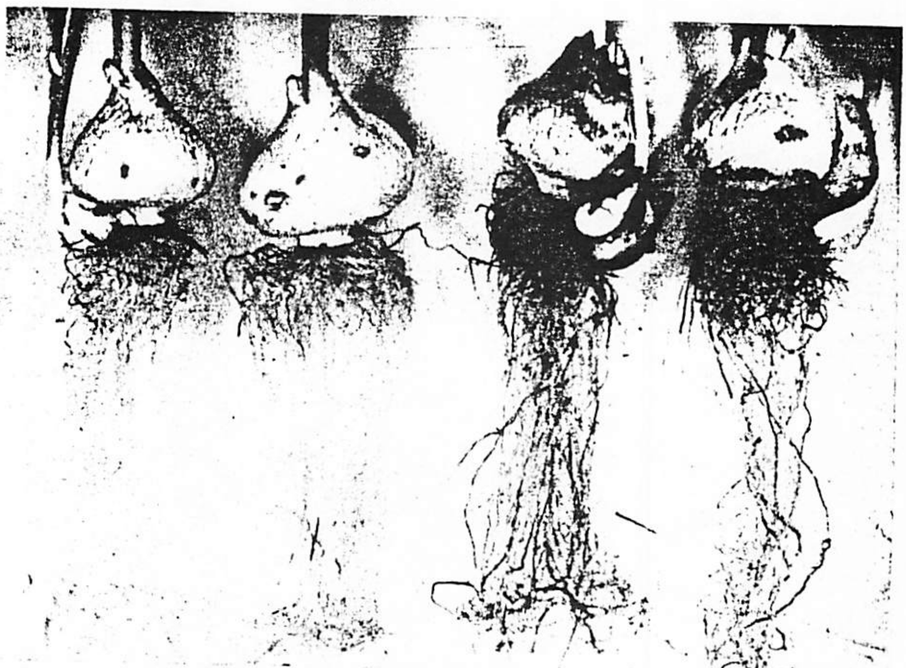

FIG. 3. Roots of tulips grown in glass vessels of Fig. 2, after eight weeks at 10° or 20° C soil temperature.
From left to right: in inoculated soil at 10°C, roots without discoloration; in non-inoculated soil at 20°C, roots healthy; two plants in inoculated soil at 20 C, roots heavily damaged and partly decaying; base of bulb at extreme right affected by *Fusarium*.
Wortels van tulpen gekweekt in hyacinte-glazen van fig. 2 na acht weken bij bodemtemperaturen van 10° en 20°C.
Van links naar rechts: in besmette grond bij 10°C, wortels zonder verkleuring; in onbesmette grond bij 20°C, wortels normaal; twee planten in besmette grond bij 20 C, wortels zwaar beschadigd en deels rottend; bodem van meest rechtse bol aangetast door Fusarium.

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en 3, fig. 1 beneden en 4). Slechts zelden werden hyfen van *Fusarium* in de houtvaten, doch wel in grote massa's in het schorsweefsel van de wortels van de waardplant gevonden. De groei van *Fusarium oxysporum* bij de tulp is dus geheel afwijkend van die van andere stammen van dezelfde soort bij andere waardplanten.

Veelal zijn symptomen van aantasting van de bol ten tijde van de oogst zelfs bij nauwkeurig waarnemen niet zichtbaar; deze verschijnen vaak pas verscheidene dagen of weken na het rooien. Dit is ook het geval wanneer de omstandigheden tijdens de bewaring in de schuur zodanig zijn, dat infectie tijdens deze periode zeer onwaarschijnlijk mag worden geacht (tabel 1).

Argumenten worden besproken, welke pleiten voor de opvatting dat infectie te velde dikwijls pas zeer laat in het groeiseizoen plaatsvindt.

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