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Bud necrosis, a storage disease of tulips. II. Analysis of disease-promoting storage conditions

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Abstract

The syndrome of bud necrosis, a storage disease of tulips, is analysed and relationships between several phenomena are described:

1. In 'White Sail', bud necrosis occurred in cardboard boxes only when bulbs infected with *Fusarium oxysporum f. tulipae* were present.
2. Buds with symptoms of bud necrosis were open at the tip.
3. Whereas in 'White Sail', open buds occurred in boxes only when *Fusarium*-infected bulbs were present, in 'Red Champion', open buds occurred also in well-ventilated open trays with wire-gauze bottoms stored at higher temperatures but without *Fusarium*-infected bulbs. In 'Rose Copland', open buds were only found exceptionally.
4. In buds with symptoms of bud necrosis, mites were almost always present.
5. In boxes in which *Fusarium*-infected bulbs were present, ethylene concentrations up to 23 ppm were found.

On the basis of these findings it seems highly probable that bud necrosis develops only if mites penetrate into the buds and reach the stamens. A prerequisite for penetration is the presence of a pore in the bud tip. Ethylene, emanated by *Fusarium*-infected bulbs or from other sources and accumulating under conditions of poor ventilation, is probably the direct cause of open buds.

In 'Red Champion', however, this aberration can also be caused by higher storage temperatures even with good ventilation. In a subsequent paper proof of this hypothesis will be presented.

Introduction

The symptoms of bud necrosis in tulips have been described (De Munk and Beijer, 1971). The occurrence of the disorder was found to be promoted by storage under conditions of poor ventilation and temperatures between 17 and 23 °C. The wide variation in the disease percentages both in the experiments of different years and in replicates in the same year, in spite of comparable storage conditions (temperature and ventilation), suggests that the disease may be caused by the combined action of several factors (Kamerbeek and De Munk, 1966).

Preliminary experiments had demonstrated a relationship between the disorder and inhibition of bud growth (Kamerbeek and De Munk, 1968). Furthermore, mites were often found in decaying buds. This raised several questions: whether growth inhibition and/or mites are involved in the genesis of bud necrosis or play a secondary role; whether a growth-inhibiting substance, viz ethylene, accumulates under poor ventilation conditions; what the source of the growth-inhibiting substance might be.

A closer analysis of the syndrome and the composition of the air surrounding the bulbs under disease-promoting conditions was necessary to answer these questions and to determine the sequence of events leading to bud necrosis.

Materials and methods

In the period from 1964 to 1967, bulbs of the bud necrosis-sensitive cultivars 'White Sail' and 'Red Champion' and of the bud necrosis-insensitive cultivar 'Rose Copland' after being dug up in July, were packed in cardboard boxes (20 bulbs of 10/11 cm circumference in a box of $10 \times 10 \times 15$ cm). The boxes were sealed and stored at temperatures of 13, 17, 20 and 23 °C. During the storage period, which lasted till the middle of November, some of the boxes were opened at regular intervals and the bulbs out and dissected for measurement of the length of the buds (i.e. length of the first leaf) and the stamens, and to determine the degree of bud necrosis according to the description of De Munk and Beijer (1971). A comparison was made between bulbs stored in boxes and those in open trays with wire-gauze bottoms, the latter being denoted as 'open storage'.

Before the boxes were opened, air samples were drawn from them by means of gas-tight syringes. Oxygen and carbon dioxide concentrations were determined volumetrically according to Haldane with the Lloyd Gas analyser G.C. 400 (Lloyd, 1958). The presence of ethylene was detected by the triple response bio-assay with etiolated pea seedlings (Neljubow, 1911; Knight and Crocker, 1913). Analyses were then carried out by gas chromatography with a Varian Aerograph, Model 1522*B*, with 5-ft-long columns of stainless steel 3 mm in diameter, filled with Porapak Q 80–100 mesh or Alumina-Alcoa type F_1, 60–80 mesh, temperature 40 °C; carrier gas N_2, flame ionization detection (Meigh, 1959). Statistical tests for calculations of independence in two by two tables were performed according to Wijvekate (1960).

Results

Besides the effect of temperature, the effect of limited ventilation differed widely among the cultivars used. In 'White Sail' the open-storage bulbs showed hardly any bud necrosis. In bulbs kept in closed boxes a relatively low percentage showed bud necrosis: in 46 out of 216 boxes in the 1964/1965 season and in 34 out of 127 boxes in the 1967/1968 season. In 'Red Champion', however, the same proportion of necrosis occurred in open-storage bulbs as in those stored in cardboard boxes, i.e. in 24 out of 71 boxes. In 'Rose Copland' almost no bud necrosis was observed in either open-storage bulbs or in bulbs stored in boxes.

Because of these differences, the results of the experiments will be described separately for each cultivar.

Experiments with 'White Sail'

For the calculations, the data of all samples from season 67/68 were pooled, because no significant differences were found between the phenomena in the samples pertaining to different storage temperatures. The four most important relationships are:

1. *Relationship between bud necrosis and the presence of Fusarium-infected bulbs.* The samples in the boxes can be divided into two groups, one without and the other with bud necrosis. The samples can also be divided into two groups on the basis of the presence or absence of *Fusarium oxysporum f. tulipae*-infected bulbs in the boxes. When these two distinctions are combined, a correlation is found between the presence of

Table 1. Numbers of storage boxes with bulbs of 'White Sail', 'Red Champion', and 'Rose Copland' in which bud necrosis did or did not coincide with the presence of *Fusarium*-infected bulbs.

	Fusarium-infected bulbs present	*Fusarium*-infected bulbs absent	P-value
'White Sail'			
bud necrosis observed	34	0	
no bud necrosis observed	56	37	0,00
'Red Champion'			
bud necrosis observed	16	8	
no bud necrosis observed	21	26	0.08
'Rose Copland'			
bud necrosis observed	0	3	
no bud necrosis observed	0	99	—

Tabel 1. Aantallen doosjes met bollen van 'White Sail', 'Red Champion' en 'Rose Copland' waarin het verschijnsel kernrot al dan niet samenvalt met de aanwezigheid van zure bollen.

Fusarium-infected bulbs and the occurrence of bud necrosis (Table 1), indicating that bud necrosis occurs only when *Fusarium*-infected bulbs are present in closed boxes. Conversely, *Fusarium*-infected bulbs do not always coincide with bud necrosis.

2. *Relationship between open buds caused by growth inhibition and the presence of Fusarium-infected bulbs.* As could be expected, the growth of the buds in open-stored bulbs was very regular. The growth of the buds in box-stored bulbs does not always correspond with the normal growth of open-storage bulbs. The length of buds from boxes without *Fusarium*-infected bulbs showed good agreement with the normal growth pattern whereas in the presence of *Fusarium*-infected bulbs the buds were mostly shorter (Fig. 1).

The growth inhibition affected both the foliar and the flower primordia, but the former more than the latter. This is illustrated by Fig. 2, which shows the ratio between the lengths of the stamens and buds. This unequal inhibition leads to an abnormal and premature opening of the buds and as a consequence the stamens are no longer en-

Table 2. Numbers of storage boxes with bulbs of 'White Sail', 'Red Champion', and 'Rose Copland' in which open and closed buds did or did not coincide with the presence of *Fusarium*-infected bulbs.

	Fusarium-infected bulbs present	*Fusarium*-infected bulbs absent	P-value
'White Sail'			
open buds	70	0	
closed buds	20	37	0.00
'Red Champion'			
open buds	32	21	
closed buds	5	13	0.02
'Rose Copland'			
open buds	0	7	
closed buds	0	95	–

Tabel 2. Aantallen doosjes met bollen van 'White Sail', 'Red Champion' en 'Rose Copland' waarin open of gesloten knoppen al of niet samengaan met door F. oxysporum aangetaste bollen.

closed by the foliar primordia. This morphological aberration is termed 'open bud'. In severe cases the stamens may even project through the tip of the bud.

A significant correlation exists between these open buds and the presence of *Fusarium*-infected bulbs (Table 2), but the presence of *Fusarium*-infected bulbs does not always coincide with open buds.

3. *Relationship between open buds and bud necrosis.* Bud necrosis almost always coincides with the occurrence of open buds (Table 3). On the other hand, bud necrosis was observed in only 37.8% of the bulbs with open buds. Bud necrosis in normal closed buds was observed in only 2 out of 774 bulbs.

Fig. 1. Lengths of buds (○ and ●) and stamens (□ and ■) from bulbs ('White Sail') stored in cardboard boxes during different periods of time.
○ and □ lengths in boxes without Fusarium-infected bulbs; ● and ■ lengths in boxes with Fusarium-infected bulbs.

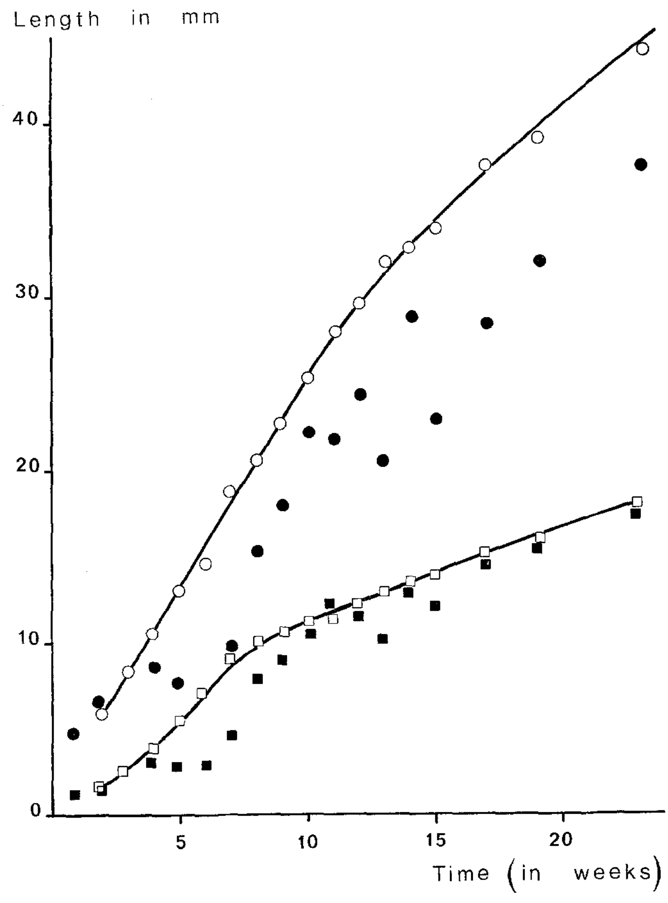

Fig. 1. Lengte van knoppen (○ en ●) en meeldraden (□ en ■) van bollen ('White Sail') welke gedurende verschillende perioden waren bewaard in kartonnen doosjes.
○ en □ lengten in doosjes zonder 'zure bollen' ● en ■ lengten in doosjes met 'zure bollen'.

Fig. 2. Ratio between lengths of stamens and buds of bulbs ('White Sail') stored during different periods of time in cardboard boxes with (•) and without (○) Fusarium-infected bulbs present.

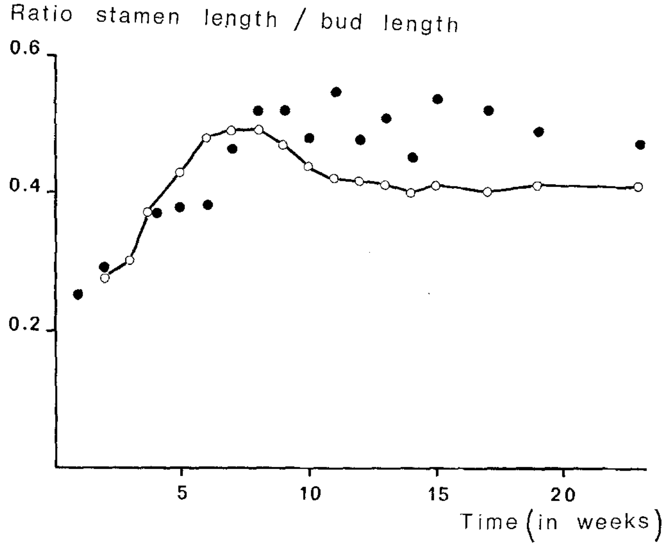

Fig. 2. Verhouding tussen de lengten van meeldraden en knoppen van bollen ('White Sail') welke gedurende verschillende perioden waren bewaard in kartonnen doosjes waarin wel (•) of geen (○) 'zure bollen' aanwezig waren.

4. *Relationship between bud necrosis and the presence of mites.* Bud necrosis is also correlated similarly with the presence of mites (*Rhizoglyphus echinopus* Fumouze & Robin and *Tyrophagus* spp.) in and on the buds (Table 4): when bud necrosis was observed, mites were almost always present, although mites are also found in buds without disease symptoms. When mites were present on closed buds (in 105 out of 774 bulbs), this coincided with bud necrosis only 2 times.

To summarize the foregoing it can be said that out of 1,240 bulbs from boxes in which *Fusarium*-infected bulbs were present, 466 showed open buds, 363 mites and 178 bud necrosis. Almost all of the necrotic changes were found in bulbs with open buds in which mites were present. This is shown graphically in Fig. 3 (the 2 and 3 cases in Tables 3 and 4, respectively, in which the phenomena did not coincide have been left out). These findings were used to formulate a hypothesis to be discussed below.

Table 3. Numbers of bulbs of 'White Sail', 'Red Champion' and 'Rose Copland' in which bud necrosis occurred in combination with open or closed buds.

	Open buds	Closed buds
'White Sail'		
bud necrosis observed	176	2
no bud necrosis observed	290	772
'Red Champion'		
bud necrosis observed	173	12
no bud necrosis observed	203	323
'Rose Copland'		
bud necrosis observed	7	2
no bud necrosis observed	0	427

Tabel 3. Aantallen bollen van 'White Sail', 'Red Champion' en 'Rose Copland' waarin het al of niet voorkomen van kernrot gecombineerd is met open of gesloten knoppen.

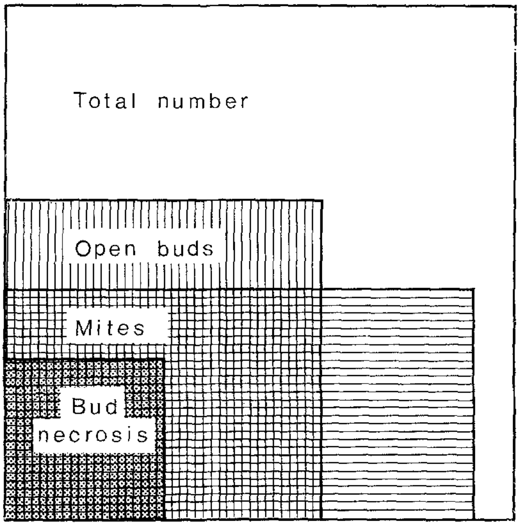

Fig. 3. Distribution in 'White Sail' of open buds (vertical lines); mites (horizontal lines), and bud necrosis (dotted area) over the total number of examined bulbs from boxes in which *Fusarium*-infected bulbs were present.

Fig. 3. Verdeling in 'White Sail' van open knoppen (verticaal gearceerd), mijten (horizontaal gearceerd) en kernrot (gestippeld) over het totale aantal onderzochte bollen uit doosjes waarin zure bollen voorkwamen.

Experiments with 'Red Champion'

The relationships found for 'White Sail' hold only partially for 'Red Champion'. Relationships 1 and 2, i.e. concerning the presence of *Fusarium*-infected bulbs and bud necrosis and open buds, respectively, are not significant at a one-side tail-probability of 5% and 1%, respectively, for this latter cultivar, although the same phenomena were observed. Bud necrosis and open buds in the absence of *Fusarium*-infected bulbs occurred in 8 and 21 boxes, respectively, out of 71 (Table 1 and 2).

Relationships 3 and 4, i.e. between bud necrosis and open buds (Table 3) and bud necrosis and the presence of mites (Table 4) did occur, however. In contrast to the findings in 'White Sail', where bud necrosis and open buds occurred only in boxes with *Fusarium*-infected bulbs, in 'Red Champion' both phenomena were also found in open-storage bulbs (Table 5.) This cultivar, unlike 'White Sail', showed the effect of the storage temperature very strongly. At a low temperature (13 °C) far fewer open buds were observed than at a higher temperature (23 °C), viz 10% as against 90%.

Table 4. Numbers of bulbs of 'White Sail', 'Red Champion' and 'Rose Copland' in which bud necrosis did or did not coincide with the presence of mites.

	Mites present	Mites absent
'White Sail'		
bud necrosis observed	175	3
no bud necrosis observed	188	874
'Red Champion'		
bud necrosis observed	153	32
no bud necrosis observed	47	479
'Rose Copland'		
bud necrosis observed	7	2
no bud necrosis observed	1	426

Tabel 4. Aantallen bollen van 'White Sail', 'Red Champion' en 'Rose Copland' waarin het voorkomen van kernrot al of niet samengaat met de aan- of afwezigheid van mijten.

Table 5. Numbers of bulbs of 'Red Champion' in open storage at 20 °C and without *Fusarium* infection, in which bud necrosis occurred in combination with open or closed buds.

	Open buds	Closed buds
Bud necrosis observed	12	0
No bud necrosis observed	129	147

Tabel 5. Aantallen bollen van 'Red Champion' welke open bewaard zijn bij 20 °C zonder 'zure bollen', waarin het voorkomen van kernrot gekombineerd is met open of gesloten knoppen.

Experiments with 'Rose Copland'

Fusarium-infected bulbs were not found in 'Rose Copland' and bud necrosis was rare (Table 1). The phenomenon of open buds was exceptional, having occurred only 7 times in 436 bulbs. The buds that where open showed bud necrosis (Table 2 and 3). Seven of 9 bulbs with bud necrosis also had mites (Table 4).

Although the scores for the various phenomena are very low, the relationships between bud necrosis and open buds and the presence of mites still exist.

Analysis of air samples from boxes

The lowest oxygen concentration measured was 20.0 %, the highest concentration of carbon dioxide 0.85 %. Although these values differ from the normal concentrations (20.9 and 0.04 %, respectively) in the air surrounding the boxes, no relationships between these gas concentrations were found either with bulbs affected by bud necrosis, with those infected with *Fusarium oxysporum*, or with otherwise aberrant symptoms.

Gas-chromatographic analyses of air samples from all boxes revealed the presence of ethylene. This component was identified by means of the triple response bio-assay (Table 6) and by comparison with known amounts of several hydrocarbons injected into columns of the gas chromatograph.

The concentrations of ethylene varied from 0 to 23 ppm. Concentrations higher than 0.06 ppm were found only when one or more of the bulbs in the boxes showed infection with *Fusarium oxysporum*. Furthermore, the mean concentrations of ethylene in those boxes were higher with increasing storage temperature of the bulbs (Fig. 4). At the end of August, after four weeks of storage, the mean concentrations were 0.3 ppm at 13 °C; 1.0 ppm at 17 °C; 2.4 ppm at 20 °C and 4.7 at 23 °C.

Table 6. Mean increase in length of 12 etiolated pea seedlings ('Alaska') in a 4-day period under the influence of the presence of *Fusarium*-infected bulbs in a cultivation room (cm).

Length of pea seedlings	Without bulbs	20 healthy bulbs present	1 *Fusarium*-infected bulb present
at the beginning of the experiment	0.7	0.8	0.7
after 4 days of incubation	11.2	11.2	0.9
Mean increase	10.5	10.4	0.2

Tabel 6. Gemiddelde lengtetoename van 12 erwtenkiemplantjes 'Alaska' in 4 dagen onder invloed van 'zure bollen' in de groeiruimte.

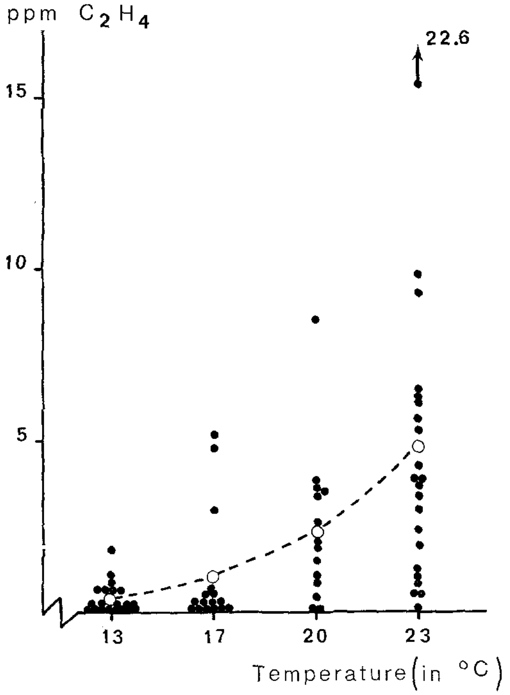

Fig. 4. Concentration of (●) ethylene determined in air samples from cardboard boxes containing 'White Sail' in which one or more *Fusarium*-infected bulbs were present and which were stored at 13, 17, 20 or 23 °C (○ = mean concentration).

Fig. 4. Gemeten ethyleenconcentraties (●) in luchtmonsters uit kartonnen doosjes waarin zure bollen voorkwamen en welke werden bewaard bij 13, 17, 20 of 23°C (○ = gemiddelde concentratie).

Discussion

On the basis of the above mentioned relationships between the phenomena, the hypothesis can be formulated, that bud necrosis is the result of a process of decay caused by the activity of mites in the buds. This activity can only occur if the buds are open and the mites can therefore reach the stamens. In this conception bud necrosis is a multifactorial disorder depending on at least two conditions, i.e. the existence of open buds and the activity of mites in the buds (Fig. 5).

Open buds develop in 'White Sail' when the bulbs are stored at poorly ventilated places (cardboard boxes) and *Fusarium*-infected bulbs are present. It is likely that the development of these anomalies is promoted by accumulated ethylene, because open buds are a result of growth inhibition, a known effect of ethylene (Hitchcock *et al.*, 1932; Elmer, 1936; Burg, 1962; De Munk and De Rooy, 1971).

Though the mean concentrations of ethylene increased with increasing storage temperature in boxes with 'White Sail' with *Fusarium*-infected bulbs, there was no rela-

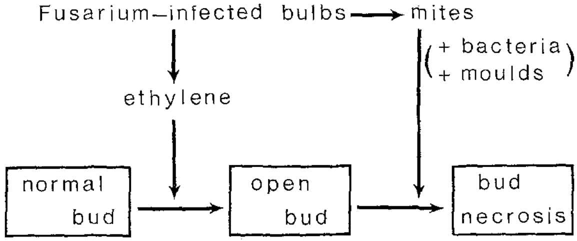

Fig. 5. Schematic representation of the hypothesis concerning the ontogeny of bud necrosis.

Fig. 5. Schematische voorstelling van hypothese voor het ontstaan van kernrot.

tionship between the storage temperature and the percentage of bulbs with bud necrosis. This can indicate that a certain threshold concentration of ethylene was already exceeded at the temperature of 13 °C. This threshold must be somewhere below 0.3 ppm.

In 'Red Champion' open buds can be caused by ethylene from *Fusarium*-infected bulbs but also by higher storage temperatures. In 'Rose Copland' even higher storage temperatures generally did not cause open buds. Furthermore the lower frequency of *Fusarium* infections in this cultivar might explain the lower frequency of bud necrosis.

The activity of mites can include feeding on stamens and transmission of fungi and bacteria. We observed fretting on stamens, and colonies of fungi and bacteria developed in traces made by mites on sterile agar plates.

The main source of mites in our experiments were decaying *Fusarium*-infected bulbs, which offer an excellent substrate from which these animals (Seemüller, 1968) swarm out during storage. As stated as early as 1928 by Hodson, the common bulb mite is not harmful to tulips as long as bulbs are stored well and not damaged. Our hypothesis indicates the storage conditions under which these animals can be harmful.

The hypothesis covers all our results and explains the wide variations in the disease percentages in preliminary experiments (De Munk and Beijer 1971). In years and replicates with less bud necrosis the frequency of *Fusarium* infection and consequently the concentration of ethylene may have been too low to cause open buds. It also remains possible that the bulbs used in those experiments were free of mites at the beginning of the experiments and that the enclosure of the bulbs in boxes prevented invasion.

Because the hypothesis is a post facto explanation, its validity must be checked experimentally. If it holds, it should be possible, for instance, to obtain bud necrosis in 'White Sail' after artifical administration of ethylene and application of mites. Bud necrosis may not occur after treatment of mite-free bulbs with ethylene or after exposure of ethylene-free stored bulbs to mites. For 'Red Champion' the same prediction can be made except that ethylene administration would have to be replaced by storage at high temperatures (20–23 °C). The results of such experiments will be described in a subsequent paper.

Samenvatting

Kernrot, een bewaarziekte in tulpen. II. Analyse van bewaaromstandigheden die de afwijking bevorderen

In een voorgaande publikatie werd gesuggereerd dat kernrot ontstaat door een combinatie van faktoren. De aard ervan was tot nu toe onbekend.

Om deze faktoren te identificeren zijn het ziektesyndroom en de bewaaromstandigheden verder geanalyseerd. Enkele relaties konden worden vastgesteld, die het opstellen van een verklaringshypothese mogelijk maken.

Deze relaties zijn: 1. Bij 'White Sail' komt kernrot voor als de bollen worden bewaard in doosjes waarin ook bollen voorkomen die aangetast zijn door *Fusarium oxysporum f. tulipae* ('zure bollen'); 2. Knoppen met symptomen van kernrot zijn aan de toppen open. Dit komt zowel bij 'White Sail' en 'Red Champion' voor als bij 'Rose

Copland'. Als deze afwijking in sterke mate optreedt groeien de meeldraden door de opening aan de top van de knop; 3. Open knoppen komen bij 'White Sail' alleen voor in doosjes waarin ook 'zure bollen' voorkomen. Bij 'Red Champion' kan dit verschijnsel behalve door bewaring in doosjes samen met 'zure bollen', ook ontstaan tengevolge van hogere bewaartemperaturen (20–23 °C). Bij 'Rose Copland' komen open knoppen slechts bij uitzondering voor; 4. In knoppen met symptomen van kernrot komen bijna steeds mijten voor; 5. In doosjes waarin 'zure bollen' voorkomen, is ethyleen aangetoond in concentraties variërend van 0 tot 23 dpm.

Op grond van bovenstaande relaties is de hypothese opgesteld dat kernrot alleen dan ontstaat, wanneer mijten tot in de bloemknoppen kunnen doordringen en de meeldraden kunnen bereiken. Voorwaarde hiervoor is de aanwezigheid van een opening in de top van de knop.

Ethyleen uit met *Fusarium* besmette bollen is waarschijnlijk het werkzame agens bij het ontstaan van open knoppen in 'White Sail' en 'Red Champion', onder omstandigheden van slechte ventilatie. Bij 'Red Champion' kan het verschijnsel ook bij goede ventilatie en dan tengevolge van hoge bewaartemperaturen ontstaan.

Resultaten van verder onderzoek die de hypothese bevestigen, zullen in een volgende publikatie worden beschreven.

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