ISN 801018

Neth. J. Pl. Path. 79 (1973) 130–137

Two new disorders in freesias

H. J. M. VAN DORST

Glasshouse Crops Research and Experiment Station, Naaldwijk

Accepted 26 January 1973

Abstract

Two new disorders in freesia are described, viz. leaf necrosis (LN) and severe leaf necrosis (SLN). The agent causing LN could neither be identified nor transmitted to freesias or other plant species. It causes a mild necrosis on freesia leaves but no symptoms on the flowers. The agent could not be eliminated from the corms by heat treatment.

Severe leaf necrosis results from double infection by freesia mosaic virus (FMV) and the agent causing LN. The plant reacts with severe necrosis on the leaves, corms and cormels, and senesces and dies in most cases. Antiserum prepared against FMV reacts positively with sap from plants affected by SLN. Particles with a length of about 820 nm were found in dip preparations. Similar particles were observed in preparations made from plants infected by FMV only.

Introduction

Two new disorders were found in freesia plants 'Rose Marie' used during glasshouse experiments on freesia mosaic virus (FMV). One of them, called leaf necrosis (LN), was found on a small number of plants in 1963. The other disorder causing severe damage to the plant was noticed two years later. This disorder has tentativily been named severe leaf necrosis (SLN). Both diseases are now often found in freesia crops although LN is the most common. This paper describes the symptoms of both diseases and reports on some further studies on their causes.

Material and methods

Corms were grown in glasshouses, planted in October and harvested in June. In winter the temperature was kept at 10–15 °C. The soil was treated with methyl bromide before planting and the glasshouse kept free of aphids by soil treatment with Aldicarb granules and by fumigation with Sulfotep. Test plants were grown at about 20 °C.

The cv. 'Rose Marie' was used in all experiments. After harvesting and drying, the corms were prepared at a temperature of 31 °C for 13 weeks.

The serological precipitin tests for FMV were done by the Plant Protection Service at Wageningen, using crude sap from healthy and affected plants.

For electron microscopy dip preparations were stained with 2% phosphotungstic acid, pH 7.0.

Some more special techniques are described 'Experimental results' together with their results.

Fig. 1. Leaves of freesia 'Rose Marie' affected by leaf necrosis. Left: healthy leaf.

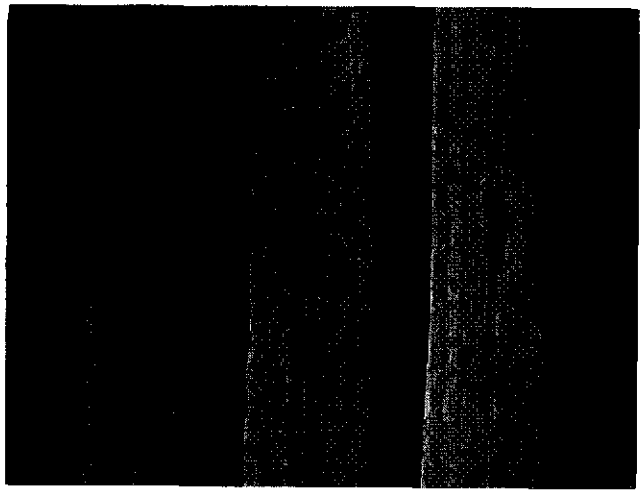

Fig. 1. Bladeren van freesia 'Rose Marie' aangetast door bladnecrose. Links: gezond blad.

Symptoms

Leaf necrosis. The first symptoms often appear on the fourth leaf of plants grown from corms and are seen 9 weeks after planting. On plants grown from cormels symptoms may begin on the second leaf.

Chlorotic spots and stripes start at the leaf tip, and eventually spread over the whole leaf. They later turn grey-brown and become necrotic (Fig. 1). In mildly diseased plants only the lower sheaths show some light green stripes. Flowers and corms look normal. The symptoms suggest a virus disease.

The symptoms of plants grown at low temperatures, in moist or wet soil and with fluctuating climatical conditions are usually more pronounced than of those cultivated at high temperatures and in dry soils. In conditions optimal for growing the disease sometimes is masked.

Severe leaf necrosis. Symptoms similar to those described for LN develop on affected plants. However, they appear on the first leaf of plants grown from cormels. In comparison to LN the disease progresses more rapid and symptoms are more severe (Fig. 2). Often the plants senesces and dies before flower formation. When flowers are formed, the petals severely discolour and a rachis sometimes has 2 or 3 small flowers. The petal tips are then greatly reduced in size.

Necrotic spots develop on the outer surface of nearly all corms and cormels of affected plants until the whole corm is affected, and then they rot (Fig. 3). Some corms rot before harvest, others develop the first necrotic spots during preplanting preparation or even after planting and rot then. Affected plants of 'Rose Marie' are completely valueless.

Fig. 2. Leaves of freesia 'Rose Marie' affected by severe leaf necrosis.

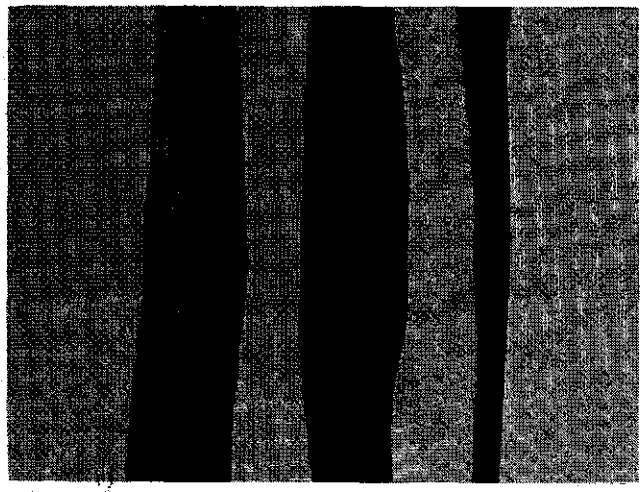

Fig. 2. Bladeren van freesia 'Rose Marie' aangetast door ernstige bladnecrose.

This disorder can easily be mistaken for that caused by *Fusarium oxysporum* but the vascular discoloration typical of *F. oxysporum* infection does not occur in plants with severe leaf necrosis.

Symptoms of both disorders are compared with those of freesia mosaic in Table 1.

Fig. 3. Corms of freesia 'Rose Marie' affected with severe leaf necrosis. Right: corm mummified during preparation. Left: healthy corm.

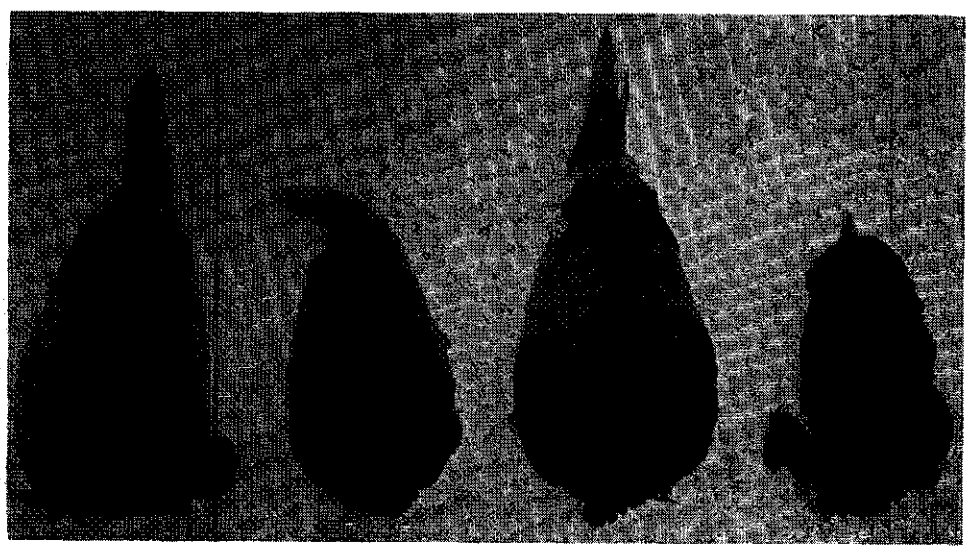

Fig. 3. Knollen van freesia 'Rose Marie' aangetast door ernstige bladnecrose. Rechts: knol gemummificeerd tijdens de preparatie. Links: gezonde knol.

Table 1. Symptoms of leaf necrosis, freesia mosaic, and severe leaf necrosis on plants of the freesia cultivar 'Rose Marie'[1].

Disease	Leaves	Flowers	Corms
leaf necrosis (LN)	necrosis (Fig. 1)	none	none
freesia mosaic (FM)	none	spots and streaks[2]	none
severe leaf necrosis (SLN)	severe necrosis (Fig. 2)	severely discolored and malformed when present	necrosis (Fig. 3)

[1] Many other new cultivars react similarly.
[2] Only in cultivars with red or blue flowers.

Tabel 1. Symptomen van bladnecrose (LN), freesiamozaïek (FM) en ernstige bladnecrose (SLN) op planten van de freesiacultivar 'Rose Marie'.

Experimental results

Perpetuation of the diseases through vegetative material. To study whether the agent causing LN could be maintained by vegetative propagation, all corms and cormels produced over a period of 4 year from 8 healthy and 16 affected plants, were indexed. The vegetative material obtained from the healthy plants remained healthy, and all grown from corms and cormels derived from affected plants, showed the disorder. Thus the disease is perpetuated by vegetative propagation.

The same is true for SLN. However, this could not be established experimentally over a period of several years as affected corms rarely produce new corms from which plants can be grown.

Manual inoculation from freesias with both disorders to freesia. In two experiments carborundum-dusted plants with three leaves were rubbed with sap expressed from leaves of LN-affected freesia plants and diluted 1:2 with distilled water. A reaction was neither observed on the 280 plants inoculated in the growing season nor on the plants grown in the next season from the corms and cormels.

In three other trials flower stems were inoculated. In one experiment the flower stem was cut across and a droplet of inoculum placed on top of the wound. In another experiment a rubber tube was placed over the excised stem tip, a drop of inoculum put into the tube and left there for a day. Finally, transmission of LN was attempted by cutting the flower stems of LN-affected plants and healthy ones in turn with a pair of scissors. None of the 270 plants inoculated and their corms indexed in the following season, reacted positively to any of these treatments.

To transmit SLN, healthy plants were inoculated on the flower stem as just described for LN. Out of the 459 plants used only one plant became infected by FMV, none showed symptoms of SLN. In an identical experiment with sap from FMV-infected freesia plants, 6 out of the 477 plants became infected. Sap from these 7 plants showing FMV symptoms, reacted positively with FMV antiserum. It was then tentatively concluded that SLN may be caused by two agents and FMV being one of them.

The possibility that SLN is caused by a mixed infection of FMV and the LN agent, was studied by inoculating plants affected by LN with FMV. A droplet of FMV sap was placed on top of the flower stem cut. During the growing season none of the 180 plants inoculated showed symptoms typical of SLN, but the next two seasons 1.7% and 15.0% of the plants, respectively did. Sap of these plants reacted positively with FMV antiserum. None of the healthy control plants or those affected with LN showed symptoms characteristic of FMV or SLN. These experiments show that SLN is the result of a mixed infection of FMV and the agent of LN. Here the incubation periods of FM and SLN were long. The rate of symptom development may depend on a number of factors including age of plants inoculated, site of inoculation, and cultivar sensitivity. Using plants of 'Ballerina' with two leaves, symptoms of SLN were seen 7 weeks after inoculation of LN-affected plants with FMV.

Manual inoculation of other plant species. Inoculum was prepared from leaves and roots of LN-affected plants of 'Rose Marie'. Sap obtained and diluted 1:1 with distilled water, was toxic to the plants tested. The toxicity was removed by dialyzing the inoculum against tap water for 24 h. In other experiments inoculum was prepared from roots using different buffers: a) Tris 0.01 M. pH 7.2; b) 0.01 M phosphate, 0.1 M KCl, 0.01 M NaCl, 0.001 M $MgCl_2$ and 0.001 M $CaCl_2$, pH 7.2 and c) 0.01 M phosphate pH 7.2. The plant species tested were *Beta vulgaris, Callistephus chinensis, Capsicum annuum, Chenopodium amaranticolor, C. quinoa, Cucumis sativus, Datura stramonium, Gomphrena globosa, Lycopersicum esculentum, Nicotiana clevelandii, N. glauca, N. glutinosa, N. rustica, N. tabacum* 'Samsun', 'White Burley' and 'Xanthi-nc', *Petunia hybrida, Phaseolus vulgaris, Physalis floridana, Pisum sativum, Plantago major, Raphanus sativus, Sonchus oleraceus, Spinacia oleracea, Torenia fourneri, Vinca rosea, Zea mays.* None reacted with any symptom. Back inoculations were not made.

Insect transmission. Attempts were made to transmit LN and SLN by aphids. In one experiment *Macrosiphum euphorbiae* was used and *Myzus persicae* in two other experiments. Both species were reared on healthy freesias.

M. euphorbiae was placed for one week on plants affected with LN and with FMV. After one week they were transferred to series of ten freesia plants having three leaves. Ten aphids were placed on each plant. The aphids which had fed on plants with LN were transferred to both healthy and FMV-infected plants; the aphids from FMV-infected plants were transferred to both healthy and LN-affected plants. After one week the aphids were killed. During the growing season no particular symptom was noticed. The corms were harvested and indexed the next season. Then, it appeared that LN was not transmitted, FMV was transmitted to 17% of the healthy plants and to 27% of the plants affected with LN. The latter showed symptoms characteristic for SLN.

In one experiment with *M. persicae*, 324 healthy freesias, 324 infected with FMV and two batches of 324 plants each with LN were placed in a closed compartment and while flowering infested with aphids. Six weeks later the aphids were killed and the corms harvested and indexed. FMV was transmitted to 11% of the healthy plants, but none of the healthy plants showed symptoms of LN. FMV was transmitted to 74% and 28% of the plants with LN as could be judged from SLN-symptoms on the progeny plants in the next growing season.

In another experiment transmission with *M. persicae* was attempted to *Beta vulgaris*, *Capsella bursa-pastoris*, *Capsicum annuum*, *Lactuca sativa*, *Nicotiana clevelandii*, *N. rustica*, *Physalis floridana*, *Pisum sativum*, *Plantago major*, *Raphanus sativus*, *Tetragonia expansa*, *Vicia faba*, and *Vinca rosea*. The aphids were placed on LN-affected plants for one week and then transferred to the test plants. Eight aphids were placed for two weeks on a test plant and 5–10 plants were used per species. The plants did not produce any symptom during the next 7 weeks. Back inoculations were not made with these plants.

Electron microscopy. No particles were found in dip preparations from plants with LN. Particles of about 820 nm in length were seen in preparations from plants infected with FMV or SLN. The particles found in both preparations did not differ morphologically. They occurred in low concentrations.

Heat treatment of corms and cormels with LN. To eliminate the agent causing LN corms and cormels which were in the 8th week of preparation were subjected either to a dry or to a wet heat treatment at 47 °C. Batches of corms and cormels were removed from incubator or waterbath at 1-day intervals and were further prepared at 31 °C, and indexed. Corms treated with dry air for more than 2 days did not survive. Of the corms treated in water 20% did not survive a treatment of 2 days. All surfiving corms produced plants with LN symptoms. From a batch of 25 cormels treated for 3 days in dry air, 4 survived, and produced healthy plants, but the corms harvested from these produced LN-affected plants in the next season.

In another experiment corms in the 4th week of their preparation, were treated for several periods between 1–24 hours at 48 °C, for 1–8 hours at 49 °C and for 1–5 hours at 50 °C. All 330 corms survived these treatments, and all produced plants with LN. None of the heat treatments reduced the incidence of LN in affected corms.

In a parallel experiment at 47 °C, heat treatment did not free cormels from FMV.

Effect of LN on production of flowers and cormels. In two experiments corms, 90 per m^2, were planted in November and measurements made from mid March to mid April. Then the number of stems, branches and flowers were counted, the length of the stems and branches measured and the corms and cormels counted and weighed. In these experiments measurements were made on 590 healthy and 490 cormels affected by LN.

No great difference was found between the data obtained with healthy plants and those affected with LN. The only significant difference ($P < 0{,}01$) could be found in the number of flowers per rachis, which was 7.5 for stems of healthy and 7 for those of the diseased plants. For the branches the numbers were 5.4 and 4.7, respectively. The flowers of plants with FMV showed discoloration, but no difference in number of flowers, stems and corms and other parameters when compared with healthy plants.

Discussion

Freesia mosaic virus and bean yellow mosaic virus have been described to cause diseases in freesia plants (Van Koot et al., 1954). The syndrome of leaf necrosis (LN) and its perpetuation in vegetative material over a period of several years suggest this disease

also to be caused by a virus. So far it has been impossible to transmit the disease to freesias or other hosts and to prove its infectious nature. However, it has been observed to spread in glasshouses and this excludes a genetic cause. Severe leaf necrosis (SLN) is the result of a combination of FMV and the agent causing LN. Hakkaart (1970, 1971) studying the same disorder arrived at the same conclusion.

Both described disorders were first found in an experiment with plants of a new cultivar ('Rose Marie'), but may have occurred for some time in the Netherlands. In a study on FMV, Van Koot et al. (1954), observed that symptoms on 'Snow Queen' differed from those on other freesia cultivars. The symptoms they described resemble those of SLN.

Leaf necrosis (LN) occurred also in a about 25-year-old batch of corms of 'Marion'. Plants grown from these corms were inoculated with FMV. Some of the plants developed symptoms characteristic for FMV, and a small number of the plants produced symptoms characteristic for SLN, indicating that some of the corms carried already a symptomless infection of LN. It is apparent from these and other results that a latent infection by the LN agent can be detected by inoculation of the plants with FMV.

The symptoms of freesia streak reported in England (Brunt, 1967) and ascribed to freesia streak virus resemble those of SLN. This disease may also occur in Germany (Caspar and Brunt, 1971). The identification of a special freesia streak virus (Brunt, 1967, 1968 and 1969) seems uncertain. The particles measuring 850 nm found by Brunt, may be those of FMV. I found such particles of about 820 nm in specimens prepared from FMV- and SLN-infected plants and could not confirm a particle length of 650 nm for FMV as given by Brandes (1964). Hakkaart (personal communication) also found particles with a length of 850 nm in preparations from plants infected with FMV and SLN.

Under experimental conditions LN had hardly any effect on growth and development of the affected plants. In my experiments growing conditions were optimal for freesias, but an effect on growth and development in suboptimal conditions cannot be excluded. Also, other cultivars may be more sensitive to this disease, than 'Rose Marie' which was used in my experiments.

Samenvatting

Twee nieuwe ziekten in freesia's

In dit artikel worden twee nieuwe ziekten in knolfreesia's beschreven. 'Bladnecrose' (LN, Fig. 1), valt uitsluitend op de bladeren waar te nemen. Bij 'ernstige bladnecrose' (SLN), komen behalve op de bladeren (Fig. 2) ook op de bloemen, voor zover aanwezig, en de knollen (Fig. 3) zeer duidelijke symptomen voor.

Voor de cultivar 'Rose Marie' is in Tabel 1 een overzicht gegeven van de symptomen bij aanwezigheid van LN, freesiamozaïek (FM) en de combinatie van beide (SLN). Deze tabel is op vele nieuwe cultivars van toepassing. De laatste ziekte is fataal voor 'Rose Marie', die in dit onderzoek voor alle experimenten werd gebruikt.

LN en SLN gaan over met het vegetatieve vermeerderingsmateriaal. Door middel van blad- en bloemstengelinoculaties en met de bladluizen *Macrosiphum euphorbiae* en *Myzus persicae* kon LN niet naar gezonde freesiaplanten worden overgebracht. Uit

planten met SLN werd freesiamozaïekvirus geïsoleerd. Indien FMV werd geïnoculeerd of met bladluizen werd overgebracht of freesiaplanten met LN dan ontstond SLN. In de elektronenmicroscoop werden alleen bij SLN deeltjes van FMV gevonden. Uit deze resultaten kan worden geconcludeerd, dat SLN wordt veroorzaakt door een combinatie van LN en FMV. De oorzaak van LN kon niet worden vastgesteld. Toetsplanten werden niet gevonden. Knollen en kralen met LN konden hiervan niet vrij gemaakt worden door droge en natte temperatuurbehandelingen tot 50 °C. In opbrengstvergelijkingsproeven tussen planten met en zonder LN waren de verschillen gering tot zeer gering.

Verondersteld wordt dat beide ziekteverschijnselen in Nederland reeds in 1950 of nog eerder in bepaalde cultivars, te weten 'Snow Queen' en 'Marion', aanwezig waren. LN is mogelijk latent in 'Marion'.

Het inoculeren van freesiaplanten met FMV biedt een mogelijkheid om het latent aanwezig zijn van LN aan te tonen.

De hevigheid waarin het ziektebeeld van LN zich openbaart kan van jaar tot jaar en van seizoen tot seizoen variëren. Dit wordt ongetwijfeld mede door de kwaliteit van het knolmateriaal en de cultuuromstandigheden bepaald. Onder gelijkmatige omstandigheden valt LN het minst op en is de nadelige invloed het geringst.

Acknowledgment

The author is greatly indebted to Dr D. Peters, Wageningen, for his help and advice, to Ir R. Zandvoort and Mr H. N. Cevat, Wageningen, for the large number of serological tests, to Miss W. Th. Runia, for her skilful assistance and to the N.V. Handelskwekerij M. C. van Staaveren, Aalsmeer, for supplying healthy freesia material in 1963. Finally I thank Dr J. Fletcher, Leeds, for his help in correcting the English text.

References

Brandes, J., 1964. Identifizierung von gestreckten pflanzenpathogenen Viren auf morphologischer Grundlage. Mitt. Biol. BundAnst. Ld- u. Forstw. 110: 1–130.

Brunt, A. A., 1967. Freesia streak virus (FSV). A. Rep. Glasshouse Crops Res. Inst.: 101.

Brunt, A. A., 1968. Freesia streak virus (FSV). A. Rep. Glasshouse Crops Res. Inst.: 104–105.

Brunt, A. A., 1969. Freesia. A. Rep. Glasshouse Crops Res. Inst.: 131–132.

Caspar, R. & Brunt, A. A., 1971. Das Freesia streak virus ein in Deutschland neues Freesienvirus. NachrBl.dt. PflSchutzdienst., Stuttg. 23: 89–90.

Hakkaart, F. A., 1970. Virusziekten in freesia's. Jversl. Inst. Plziektenk. Onderz.: 102–104.

Hakkaart, F. A., 1971. Virusziekten in freesia's. Jversl. Inst. Plziektenk. Onderz.: 104–105.

Koot, Y. van, Slogeteren, D. H. M. van, Cremer, M. C. & Camfferman, J., 1954. Virusverschijnselen in Freesia's. Tijdschr. PlZiekt. 60: 157–192.

Address

Proefstation voor de Groenten- en Fruitteelt onder Glas, Zuidweg 38, Naaldwijk, the Netherlands.