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Leaf-yellowing in combination with corm necrosis in freesia caused by bean yellow mosaic virus: factors involved in syndrome development

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Abstract

In freesia cv. Aurora grown in the field for cutflower production, a disease occurred with symptoms of leaf-yellowing in combination with corm necrosis (LYCN). It is shown that this disease is caused by bean yellow mosaic virus (BYMV).

No differences in symptoms of LYCN were observed between the freesia cultivars Aurora, Imperial and Rose Marie. Most BYMV isolates gave rise to LYCN; the isolates from crocus and *Ixia* sp. did not. LYCN was stimulated by a high BYMV concentration in the inoculum, a temperature above 20 °C, inoculation soon after emergence of the freesias, and by the absence of freesia mosaic virus. Freesias with mosaic symptoms and infected with a cross-protecting BYMV strain, did not show symptoms of leaf-yellowing and/or corm necrosis after inoculation with BYMV-Cm. The presence of the unknown agent causing leaf necrosis in freesias did not have an influence on symptom development after infection with BYMV.

Additional keywords: *Crocosmia* sp., crocus, gladiolus, iris, *Ixia* sp., leaf necrosis, freesia mosaic virus.

Introduction

Occasionally, a serious disease occurred in *Freesia refracta,* mainly in cv. Aurora, when corms are planted in May in the field for cutflower production. The characteristic symptoms, viz. of leaf-yellowing in combination with corm necrosis (LYCN; Derks et al., 1980), were observed for instance in 1974 and 1979. LYCN can be distinguished visually from other syndromes that involve corm rot or necrosis, such as severe leaf necrosis (SLN; Van Dorst, 1973), and the one caused by *Fusarium oxysporum* (McClellan, 1945). SLN develops in freesias after infection with freesia mosaic virus (FMV) and an unknown agent causing leaf necrosis (LN; Van Dorst, 1973).

Bean yellow mosaic virus (BYMV) has been isolated from freesias of cv. Aurora with LYCN (Derks et al., 1980). As freesia seedlings of cv. Imperial show similar symptoms after mechanical inoculation with BYMV (Derks et al., 1980), we investigated whether LYCN found in cv. Aurora is also caused by BYMV.

Because it is known that infection with BYMV may give rise to a mosaic pattern in leaves of various freesia cultivars (Van Koot et al., 1954), we examined some factors that determine the type of syndrome which will develop in freesias after BYMV infection.

Materials and methods

Plant material and experimental conditions. Corms of cv. Aurora originating from a stock in which FMV and BYMV were not detected with their respective antisera in ELISA, were planted in the field in gauze cages (40 corms per treatment) at the end of April. Plants were inoculated with a BYMV isolate (BYMV-Fr, BYMV-Cm or BYMV-Ih) either mechanically or by aphids at 4, 6 and 10 weeks after planting. Leaves and corms, after careful removal of the soil, were observed periodically. In the first week of November the remaining corms were lifted.

Freesia seedlings of cv. Imperial were grown at ca 20 °C in an aphid-proof glasshouse. From November till March plants were given supplementary light (Philips HPL 250 W; 5 h/day). Unless stated otherwise, the seedlings were inoculated mechanically nine weeks after emergence. After inoculation with BYMV, the plants remained in the aphid-proof glasshouses at ca 20 °C. The effects of temperature (15, 20 or 25 °C) and light intensity (3.8 or 7.1 W m^{-2}; TL 33; 16 h photoperiod) on symptom development were investigated on freesia seedlings in growth-chambers. The results were analysed with a Chi-square test.

Corms of cv. Rose Marie free from BYMV, FMV and the LN agent or infected with the LN agent or infected with FMV, were planted in glasshouses at ca 20 or ca 25 °C. In each treatment at least 44 corms were used. Four weeks after planting, the leaves were inoculated mechanically with BYMV.

Inoculation methods and BYMV isolates. In case of mechanical inoculations, the outer 2-3 leaves were inoculated with sap after dusting the plants with carborundum (500 mesh). The sap was prepared by grinding BYMV-infected leaves of *Vicia faba* in 0.07 M phosphate buffer (pH 7.0) containing 80 mM sodium sulphite (w/v = 1 : 2). Unless stated otherwise, these homogenates were used undiluted.

Inoculations by aphids *(Macrosiphum euphorbiae)* were performed by placing pots with virus-free tulips colonized by aphids in the gauze cages. At the same time, in each cage two *Vicia faba* plants infected with BYMV were planted among the freesias. After two weeks the aphids were killed with 0.2% nicotine.

The BYMV isolates from freesia (BYMV-Fr), *Crocosmia masonorum* (BYMV-Cm), iris (BYMV-Ih), and *Vicia faba* (BYMV-Vf) have been described previously (Derks et al., 1980). The other isolates were obtained from *Crocus flavus* with a faint mosaic pattern in the leaf bases (BYMV-Cf), from *Ixia* hybrid cv. Venus with a very faint mosaic pattern in the leaves and a dark colour-breaking in the flowers (BYMV-I), and from *Gladiolus* × *hortulans* cv. Hopmans Glorie with a pronounced lightgreen or yellow mosaic pattern in the leaves (BYMV-Gh). All isolates were maintained in *Vicia faba* cv. Witkiem.

Virus detection. Samples of either randomly chosen or all plants were checked for the presence of BYMV and other viruses, with the electron microscope, the microprecipitin test, ELISA (Clark and Adams, 1977) or by test plants (Derks et al., 1980). The test plants for diagnosis and propagation of the BYMV isolates were grown from seed under the same conditions as described for the freesia seedlings.

Results

Experimental evocation of LYCN

Four weeks after planting virus-free plants of cv. Aurora were inoculated with BYMV-Fr or BYMV-Cm either in sap or with viruliferous aphids. In all treatments ca 80% of 40 inoculated plants became infected. Symptoms started 3-5 weeks after inoculation with a yellowing of the inner leaves and small reddish-brown necrotic spots on the leaf veins. The vein necrosis extended rapidly in the direction of the new corms resulting in necrotic areas in the corms at the sites of insertion of the leaves (Fig. 1). Most infected new corms necrotized completely during the growing season starting two months after inoculation, the other ones after lifting within a week of storage at 30 °C. The old corms on which the new corms are formed, did not show necrosis during the growing season.

Factors influencing symptom development

Inoculated virus. The influence of the virus concentration in the inoculum on the type of syndrome in cv. Imperial was determined with dilution series of BYMV-Vf (Table 1) and of BYMV-Fr (results not shown). LYCN was observed with dilutions up to 1/5 - 1/9, and mosaic with dilution up to 1/25 - 1/81. Virus particles were only observed in leaf dip preparations of plants with either LYCN or mosaic.

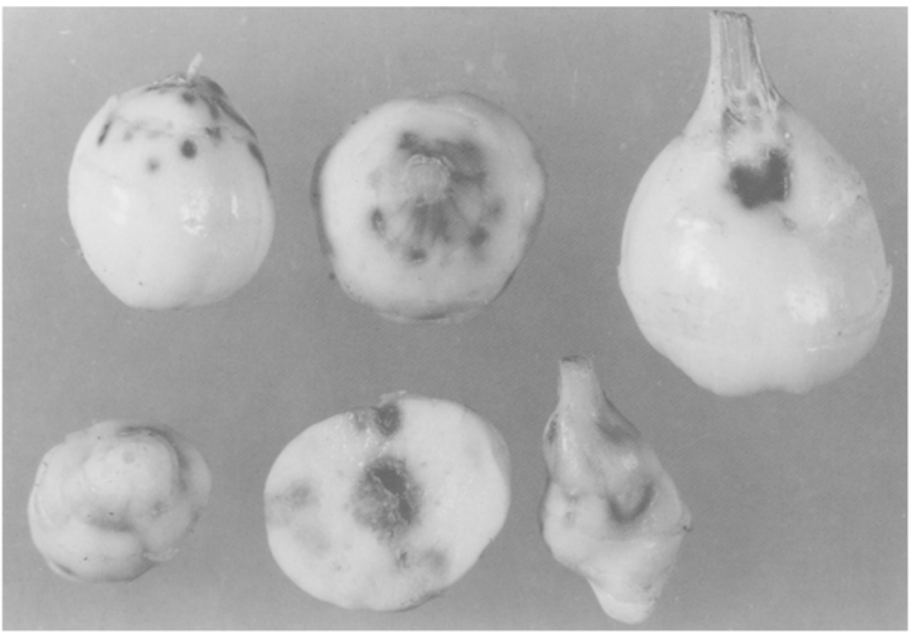

Fig. 1. Corm necrosis in freesia cv. Aurora infected with BYMV-Cm. Necrotic areas in the corms at the sites of insertion of the leaves (corm in the middle of top row), extending to the central part (corm in cross-section in the middle of bottom row).

Table 1. Effect of dilution of the leaf homogenate containing BYMV-Vf on the numbers of plants with the various syndromes in freesia cv. Imperial (n = 10).

Dilution	Numbers of plants with		
	LYCN	mosaic	none
undiluted	3	6	1
1/3	3	4	3
1/9	3	4	3
1/27	0	1	9
1/81	0	4	6
1/243	0	0	10

Table 2. Effect of BYMV isolates in undiluted leaf homogenates on the type of syndrome in freesia cv. Imperial (n = 6). The isolates were tested 2-4 times.

BYMV isolate	Numbers of plants with		
	LYCN	mosaic	no or local symptoms[1]
BYMV-Cm	3.3 ± 0.3[2]	1.7 ± 0.3	1.0 ± 0.6
BYMV-Ih	3.3 ± 0.8	1.5 ± 0.9	1.3 ± 0.3
BYMV-Gh	2.5 ± 0.5	3.5 ± 0.5	0
BYMV-Fr	1.8 ± 0.4	3.2 ± 0.5	1.0 ± 0.5
BYMV-Vf	1.5 ± 0.5	4.0 ± 1.0	0.5 ± 0.5
BYMV-I	0	4.5 ± 0.5	1.5 ± 0.5
BYMV-Cf	0	4.5 ± 1.5	1.5 ± 1.5

[1] Local symptoms: small, green or brown necrotic spots in inoculated leaves only. The virus did not spread from these spots to other leaves or to the corms.
[2] Mean and standard error of the mean.

The effect of different isolates on symptom expression in cultivar Imperial was tested with undiluted homogenates. According to their efficiency in causing LYCN, we distinguished three groups of BYMV isolates (Table 2): 1) mainly LYCN, 2) more mosaic than LYCN, 3) mosaic only and no LYCN. Simultaneous inoculation of *Chenopodium quinoa* with the various homogenates resulted in a similar number (30-60) of local lesions, indicating comparable virus concentrations. The presence of BYMV in the infected freesia plants has been established by the microprecipitin test or in ELISA.

Host plant. No differences in LYCN symptoms were observed between the cultivars Aurora, Imperial and Rose Marie with each isolate used.

Most plants of cv. Aurora (75-85%) developed LYCN when inoculated 4 or 6 weeks after planting with either BYMV-Fr, or BYMV-Cm or BYMV-Ih. Plants inoculated ten weeks after planting showed a mosaic pattern (5-10%) or escaped infection.

When freesias were inoculated 6 weeks after planting, about a third of the planted

Fig. 2. (Left) Freesia cv. Imperial 10 weeks after inoculation with BYMV-Ih: New healthy shoots (on the left side from both diseased plants) developing next to shoots with LYCN.

Fig. 3. (Right) Freesia cv. Imperial 25 days after inoculation with BYMV-Cm and grown at a light intensity of 7.1 W m^{-2}. Influence of temperature on symptom development. From left to right:
15 °C: green spots in the inoculated leaves, beginning of yellowing of central leaf, small white corm;
20 °C: necrotic spots in the inoculated leaves, advanced yellowing of central leaves, corm necrosis;
25 °C: plants with yellow and brown-grey necrotic leaves and corm necrosis.

corms produced new shoots without symptoms and without virus particles, next to those with LYCN (recovery growth). At lifting time a healthy and a necrotized corm were found on the shrivelled old corms. With cv. Imperial similar symptoms (Fig. 2 and 3) and similar percentages of plants with the various syndromes were obtained after inoculation with BYMV-Ih.

External factors. The factors temperature and light intensity were studied in several experiments. No significant interaction could be established between these two factors. At 25 °C the freesias showed significantly more LYCN than at 15 and 20 °C (Table 3). At the lower temperatures the symptoms developed more slowly (Fig. 3). At a light intensity of 7.1 W m^{-2} significantly more LYCN occurred than at 3.8 W m^{-2} (Table 4).

Table 3. Effect of temperature on the type of syndrome in freesia cv. Imperial after inoculation with BYMV-Cm (n = 12).

Temperature (°C)	Numbers of plants with		
	LYCN	mosaic	no or local symptoms[1]
15	4	3	5
20	5	2	5
25	10	1	1

[1] See subscript to Table 2.

Table 4. Effect of light intensity on the type of syndrome in freesia cv. Imperial after inoculation with BYMV-Cm (n = 18).

Light intensity ($W\ m^{-2}$)	Numbers of plants with		
	LYCN	mosaic	no or local symptoms[1]
3.8	7	3	8
7.1	12	3	3

[1] See subscript to Table 2.

Viruses present in host plant. When 11 freesia plants of cv. Imperial, infected with BYMV-Fr and showing mosaic symptoms, were challenged with BYMV-Cm, none of the plants showed additional leaf-yellowing and/or corm necrosis, indicating a complete premunition.

In freesia cv. Rose Marie the presence of the LN agent did not influence the incidence of LYCN after inoculation with BYMV-Cm. However, the presence of FMV resulted in a lower percentage of plants with LYCN; after inoculation with BYMV-Cm, ca 15% of the FMV-infected plants showed LYCN against ca 50% of the virus-free plants.

Discussion

As the LN agent is sometimes masked in freesias (Van Dorst, 1973), LYCN in cv. Aurora may have been caused by BYMV in combination with this agent, as is described for SLN, a disease in which FMV and the LN agent together are responsible for corm necrosis (Van Dorst, 1973). The experiments with virus-free cv. Rose Marie (this article) and those with freesia seedlings cv. Imperial (Derks et al., 1980) exclude this combined effect. Therefore, we may conclude that LYCN is caused by BYMV.

In our experiments no differences were found between the cultivars Aurora and Imperial in LYCN symptoms and in infection percentages with BYMV. Therefore, we chose cv. Imperial for studying most of the factors involved, as this cultivar was grown from seed and seed transmission of BYMV and FMV (Van Koot et al., 1954) and of the LN agent (H. van Dorst, pers. comm.) has never been established in freesias.

Analysing the problems in practice, LYCN is found in freesias grown from certified stocks (thus free from BYMV and FMV) in the neighbourhood of gladioli, *Crocosmia* sp. or old stocks of freesia, i.e., crops infected with BYMV. Freesias are planted in the field at the end of May for cutflower production, so they are frequently exposed to day temperatures of 20 °C or more from June on. In June these plants are in the stage of 2-3 leaves. Moreover, aphid species flying in May and June, like *Acyrthosiphon pisum* and *Aphis fabae* (Prinsen, 1984), are very effective in spreading BYMV (Bos, 1970). All these facts fit in very well with the observations made in our experiments. That the disease has been observed in some years only, like 1974 and 1979, is probably due to an accidental combination of these factors together with an excessive spread of the virus. This is supported by the finding of an excessive spread in June 1979 of another non-persistently transmitted potyvirus, viz. FMV, in freesias in the Netherlands (Buijs, 1979).

The recovery growth in some of our experiments has been observed in commercially grown stocks too. It is possible that this phenomenon masks the occurrence of LYCN in years with a slight spread of BYMV.

We have to point out the absence of LYCN in older freesia stocks of cv. Aurora infected with FMV and/or BYMV. Our suggestion of a premunitive effect of BYMV (Derks et al., 1980) has been confirmed, but an additional disease-reducing factor was found in the presence of FMV.

In freesias grown under glass LYCN has not been observed. This may be explained as follows: mostly, these freesias are planted from September until March at soil temperatures below 17 °C; in this period there is little or no aphid influx in the glasshouses; an effective aphid control is possible; and BYMV-infected crops are grown neither in the same glasshouses nor outdoors at that time of the year.

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Samenvatting

Bladvergeling in combinatie met knolnecrose in freesia veroorzaakt door bonescherpmozaïekvirus: factoren betrokken bij de ontwikkeling van het ziektebeeld

In freesia's cv. Aurora die buiten geteeld werden voor produktie van snijbloemen, is een ziekte waargenomen die gepaard gaat met vergeling van de bladeren en knolnecrose (LYCN). Bonescherpmozaïekvirus (BYMV) blijkt verantwoordelijk te zijn voor deze ziekte.

Tussen de freesia-cultivars Aurora, Imperial en Rose Marie werden geen verschillen geconstateerd in LYCN-symptomen. De meeste BYMV-isolaten veroorzaakten LYCN in meer of mindere mate; alleen de isolaten van crocus en *Ixia* sp. deden dit niet. LYCN

werd bevorderd door een hoge BYMV-concentratie in het inoculum, een temperatuur boven de 20 °C, inoculatie kort na opkomst van de freesia's en de afwezigheid van freesiamozaïekvirus. Bij freesia's met mozaïeksymptomen en geïnfecteerd met BYMV-Fr werden na inoculatie met een tweede BYMV-isolaat, BYMV-Cm, geen symptomen als bladvergeling en/of knolnecrose waargenomen. De aanwezigheid van de veroorzaker van bladnecrose in freesia had geen invloed op de symptoomontwikkeling na infectie met BYMV.

References

Bos, L., 1970. Bean yellow mosaic virus. Commonwealth Mycological Institute/Association of Applied Biologists, Descriptions of Plant Viruses No. 40.

Buijs, G., 1979. Virusexplosie in freesia in Nederland. Vakblad voor de Bloemisterij 35: 71.

Clark, M.F. & Adams, A.N., 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology 34: 475-483.

Derks, A.F.L.M., Vink-van den Abeele, J.L. & Muller, P.J., 1980. Bean yellow mosaic virus in some iridaceous plants. Acta Horticulturae 110: 31-37.

Dorst, H.J.M. van, 1973. Two new disorders in freesias. Netherlands Journal of Plant Pathology 79: 130-137.

Koot, IJ. van, Slogteren, D.H.M. van, Cremer, M.C. & Camfferman, J., 1954. Virusverschijnselen in freesia's. Tijdschrift over Plantenziekten 60: 157-192.

McClellan, W.D., 1945. Pathogenicity of the vascular *Fusarium* of gladiolus to some additional iridaceous plants. Phytopathology 35: 921-930.

Prinsen, J.D., 1984. Waarnemen en interpreteren van bladluisvluchten in verband met de epidemiologie van schadelijke plantevirussen. Jaarverslag Instituut voor Plantenziektenkundig Onderzoek, Wageningen 1983: 106-110.