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Synergism of hydrogen fluoride and leaf necrosis on freesias

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Abstract

Continuous fumigation with HF at a concentration of 0.5 $\mu g/m^3$ and intermittent fumigation with 0.3 $\mu g/m^3$ aggravated leaf necrosis in freesia. In the cultivars Rose Marie and Royal Blue, symptoms of leaf necrosis develop earlier and increase in severety with these concentrations of HF. The agent of this disease has not yet been identified nor isolated. The normal symptoms are initially chlorosis and later necrosis but HF causes necrotic spots and stripes as the first foliar symptoms in the places where under uncontaminated conditions chlorotic spots would appear.

Introduction

During investigations in fumigation chambers in a greenhouse (Fig. 1) in 1970 and 1971, on the sensitivity of some freesia cultivars to very low concentrations of HF an anomalous rather serious injury developed (Wolting, 1973) as well as injury characteristic for HF. In some cultivars, most plants showed this symptom; in others, it occurred only in a few plants. In the unfumigated chamber, this injury was not observed. On closer examination, the symptoms resembled those of leaf necrosis, a disease whose cause is not yet known.

In 1971, 1972 and 1973, we studied this phenomenon more closely by fumigation experiments with the cultivars Rose Marie and Royal Blue.

Material and methods

In 1971, Mr Hakkaart (virologist of our Institute) provided two groups of corms of both cultivars, one free of leaf necrosis and other diseases, hereafter called healthy, and the other affected by leaf necrosis. In 1972, we tested with these same groups and a third group infected with freesia mosaic virus (FMV).

The fumigation experiments were in two plastic chambers with a floor area of 12 m^2 each and a volume of 30 m^3; one serving as control (air free from HF). The growth conditions for greenhouses where freesias are commercially grown were carefully simulated. The plants were grown directly in the soil. The temperature and relative humidity during the experiments were almost equal in the two chambers. For each group, 50 corms were planted in October; in April, the plants were lifted. For an injury rating, the surface of all leaves and the surface of the injured part of the leaf of 15 plants representative of each group were measured (half the width $\times$ height of

Fig. 1. Fumigation chambers for continuous fumigation of freesias.

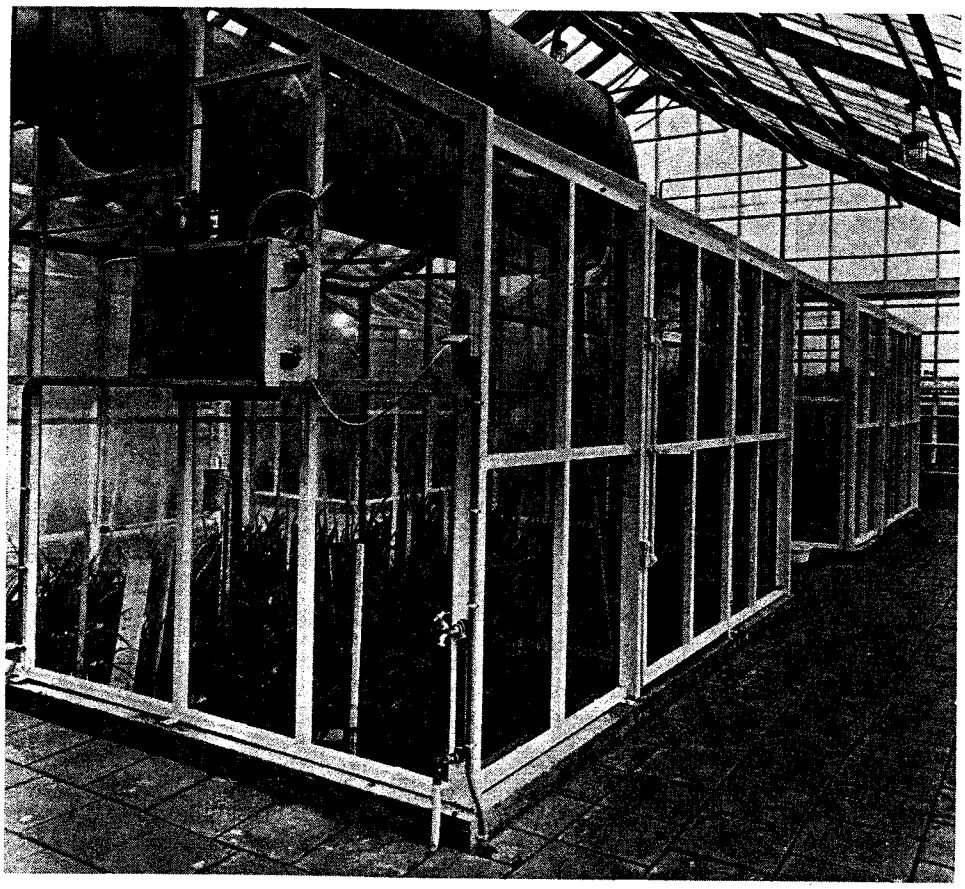

Fig. 1. Begassingskassen waarin langdurende begassingsproeven met fresia's werden uitgevoerd.

the lesion). The injury along the margins was estimated as leaftip injury.

An airstream from outside the chambers passed a fluoride-absorption filter which makes it practically free of hydrogen fluoride. Subsequently the air was led to the control chamber or was mixed with a known concentration of HF to the fumigation chamber.

In both chambers, the air was blown from a porous nylon tube, hanging in the ridge of the chamber, the air so being equally distributed. The air was sucked out through pipes ending just above the soil and equally distributed over the surface. Each chamber was ventilated at a rate of 90 m^3/min.

The HF concentration was measured once or twice during the day and once during the night. For this purpose an air sample from the chambers was led through a water bottle with demineralized water at the rate of 0.026 m^3/min. The concentration was determined by means of a spectrometer after adding lanthanium-alizarine complexan (Kooyman and Bergshoeff, 1966).

Results

In the first trial (1971/1972), the plants were fumigated with a concentration of HF of 0.5 $\mu g/m^3$. This concentration was repeatedly measured in industrial areas in and outside greenhouses. Fourteen days after planting, when the shoots of the plants appeared, fumigation started.

After almost continuous fumigation for six weeks, necrotic grey-brown spots and stripes between veins usually appeared in the second leaf, generally in the upper part of the leaf, in plants of both cultivars affected by leaf necrosis. The two groups of 'Royal Blue' plants showed the first well known ivory-coloured spots along the margins, typical of HF. The leaves of healthy 'Rose Marie' showed typical HF lesions when fumigated continuously for almost three months with the same concentration; 'Rose Marie' with leaf necrosis showed a severe necrosis between the veins too. 'Royal Blue' was considerably more sensitive to HF than 'Rose Marie'. The 'Royal Blue' plants affected with leaf necrosis were severely injured along the margins and between the veins, the healthy plants showed only the typical HF lesions.

During flowering, the plants were checked for the presence of freesia mosaic virus and bean yellow mosaic virus. In none of the plants were symptoms observed.

After flowering, when the plants had been continuously fumigated for about five months, the average leaf injury of the 'Rose Marie' plants, affected with leaf necrosis was about 40%. Healthy plants of 'Rose Marie' had only 16% injury, which was confined to the margin. Because of the great sensitivity of 'Royal Blue', differences in fluoride injury between healthy and affected plants had disappeared at the end of the trial. Both had about 60% injury.

In the unfumigated chamber, 'Rose Marie' plants affected by leaf necrosis showed many clear chlorotic spots, but only a few necrotic ones. In healthy plants growing in uncontaminated air, no injury was observed at all. 'Royal Blue' still had slight injury at the leaf margin; plants affected by leaf necrosis had chlorotic and some necrotic spots like 'Rose Marie'.

The aim of the second fumigation trial (1972/1973) was to investigate whether fumigation at a very low concentration, which causes little or no typical HF lesions to healthy plants, brings about a clearly visible injury to the plants with leaf necrosis. To simulate practical circumstances as far as possible, the plants were fumigated intermittently. The trial started five weeks after planting.

From the end of November until the beginning of January, a chamber was fumigated three times a week and subsequently until the beginning of April four times a week for six hours a day with an average HF concentration of 0.3 $\mu g/m^3$. In the control chamber, an average F^--concentration was measured of 0.2 $\mu g/m^3$. Most of this amount must be attributed to traces of fluoridecontaining dust, which would have no influence on plants. After fumigation for about four weeks, 'Royal Blue' plants with leaf necrosis already show the necrosis between the veins. They further exhibited watery dark-green spots along the margins, which later turned ivory.

'Rose Marie' plants with leaf necrosis first showed necrosis only some weeks later, when the plants had four leaves. At the end of flowering, after intermittent fumigation for four months, the chlorotic and necrotic injury in all plants had much increased. The symptoms, mainly appearing in the youngest leaves, extended over a length of

Fig. 2. Influence of leaf necrosis on sensitivity to hydrogen fluoride in the freesia 'Rose Marie'. a: Free from leaf necrosis ('healthy'), unfumigated. b: Free from leaf necrosis, after discontinuous fumigation with HF for 13 weeks with 0.3 $\mu g/m^3$. c: With leaf necrosis, unfumigated. d: With leaf necrosis, after discontinuous fumigation with HF for 13 weeks with 0.3 $\mu g/m^3$.

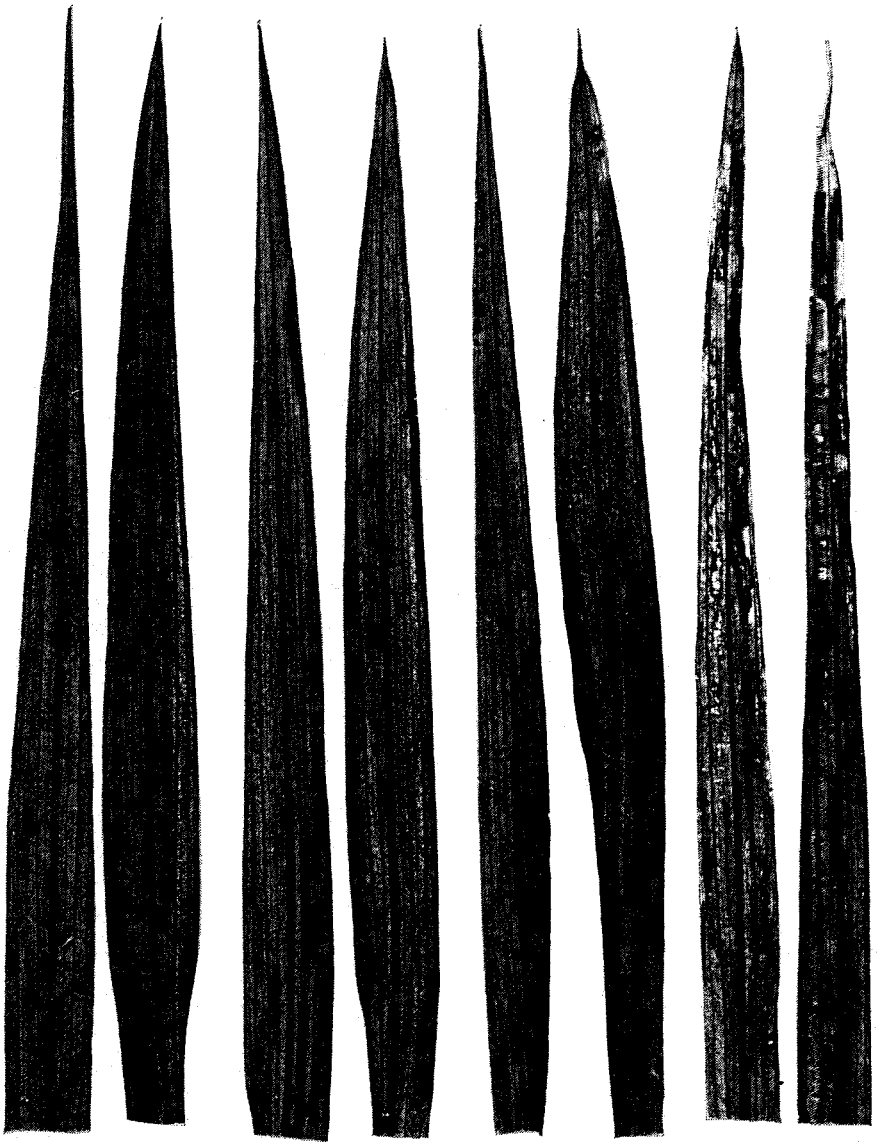

Fig. 2. De invloed van 'bladnecrose' op de gevoeligheid voor waterstoffluoride bij het fresiaras Rose Marie. a: vrij van 'bladnecrose', niet begast. b: vrij van 'bladnecrose', na 13 weken discontinue HF-begassing met 0,3 $\mu g/m^3$. c: besmet met 'bladnecrose', niet begast. d: besmet met 'bladnecrose', na 13 weken discontinue HF-begassing met 0,3 $\mu g/m^3$.

6 to 10 cm down from a few centimetres below the leaf tip (Fig. 2). A great deal of the healthy and mosaic-infected 'Rose Marie' plants showed very little necrosis at the leaf margins. Necrosis in the plants with leaf necrosis averaged 13% and ranged from 5 to 25%.

From the 'healthy' plants of 'Rose Marie', 40% were uninjured; the rest showed injuries in about 8% of the leaf surface. Of the 'Rose Marie' plants with FMV, 80% only showed lesions at the leaf margins (typical for HF); the average injury here was 6% of the leaf surface. The presence of FMV had no influence on sensitivity to HF.

As in the first trial, typical fluoride symptoms predominated in the three groups of 'Royal Blue' at the end of the trial. In all the fumigated groups, lesions covered about 30% of the leaf surface.

Most unfumigated plants of 'Rose Marie' with leaf necrosis showed chlorotic spots

Table 1. Effects of intermittent fumigation with hydrogen fluoride at 0.3 $\mu g/m^3$ on plants of the freesia cultivars Rose Marie and Royal Blue affected or not by leaf necrosis. Fumigation began five weeks after planting the corms.

Effects of fumigation after:	Fumigated		Unfumigated	
	with leaf necrosis	healthy	with leaf necrosis	healthy
		Rose Marie		
4 weeks	–	–	–	–
6 weeks	marginal necrosis; necrosis between veins	marginal necrosis	chlorosis	–
12 weeks	necrosis between veins; slight marginal necrosis	slight marginal necrosis	chlorosis; some necrosis between veins	–
18 weeks	Average proportion of leaf surface with lesions			
	13%	5%	ca 2%	< 1%
		Royal Blue		
4 weeks	necrosis between veins and at margin	necrosis at margin	chlorosis	–
6 weeks	number and size of lesions increase	number and size of lesions increase	chlorosis; necrosis at margin	necrosis at margin
12 weeks	marginal lesions predominate	severe marginal lesions	chlorosis; some necrosis between veins, slight marginal lesions	slight marginal lesions
18 weeks	Average proportion of leaf surface with lesions			
	30%	30%	ca 5%	ca 4%

Tabel 1. De invloed van een discontinue HF-begassing met 0,3 $\mu g/m^3$ op planten van de fresiacultivars Rose Marie en Royal Blue al dan niet besmet met 'bladnecrose'. Aanvang van de begassing vijf weken na het planten.

and only a few necrotic spots. Lesions extended over about 2% of the surface on average. The other unfumigated groups of 'Rose Marie' were practically uninjured. In this almost uncontaminated air, all the groups of 'Royal Blue' still had slight injury at the leaf margins. The plants with leaf necrosis had chlorotic spots and a few necrotic spots too. Results are summarized in Table 1.

Discussion

Studies on influence of air pollutants on diseases caused by viruses have concerned only tobacco mosaic virus (TMV) (Treshow et al., 1967). It was shown that fluoride predisposes 'Pinto' bean leaves to TMV infection. The number of TMV lesions per plant increased with increase in fluoride concentrations of leaves up to 500 μg/g, but when the leaves contained more the number decreased.

Leaf necrosis is often found in freesia crops and consists of chlorotic spots and stripes in the leaves. They turn grey-brown later and turn necrotic. The symptoms and their transmission in vegetative material suggest a virus as cause. The agent causing leaf necrosis could not be identified (Van Dorst, 1973; Hakaart, 1969). By studying this disorder, Van Dorst (1973) observed that under normal growth conditions, the first chlorotic symptoms are seen nine weeks after planting. In a continuous concentration of HF of 0.5 $\mu g/m^3$, necrotic spots appear in affected cultivars eight weeks after planting, and chlorosis is practically passed over. The places where the symptoms appear as well as the symptoms themselves agree with those of leaf necrosis under commercial conditions.

Intermittent fumigation with HF at a concentration of 0.3 $\mu g/m^3$ for 4 or 6 weeks, according to the sensitivity of the cultivar, causes necrotic spots and stripes as the first foliar symptoms in the places where chlorotic spots would appear with uncontaminated air.

In conclusion, a synergistic action between hydrogen fluoride and the agent of leaf necrosis has been demonstrated. There is strong evidence that freesias with leaf necrosis that are cultivated in areas with very low concentrations of HF will develop symptoms of leaf necrosis earlier and more severely.

Samenvatting

Synergistische werking van waterstoffluoride en 'bladnecrose' bij freesia's

Tijdens een begassingsonderzoek met freesia's werd als gevolg van de aanwezigheid van HF een vrij ernstige aantasting waargenomen, die afweek van de voor HF karakteristieke. Zowel de plaats van de aantasting als het ziekteverschijnsel zelf vertoonden veel overeenkomst met die van 'bladnecrose', een ziekte waarvan de oorzaak nog onbekend is. Aan de hand van begassingsproeven die in 1971/1972 en 1972/1973 in speciaal daartoe ingerichte kassen (Fig. 1) zijn uitgevoerd met de cultivars Rose Marie en Royal Blue kon worden vastgesteld dat reeds onder invloed van de aanwezighied van zeer lage HF-concentraties het verschijnsel 'bladnecrose' zich sneller manifesteert en in hevigheid toeneemt. Onder normale teeltomstandigheden verschijnen de eerste chlorotische vlekken aan de met 'bladnecrose' besmette planten ongeveer negen weken na het planten (Van Dorst, 1973). Bij een continue aanwezigheid van een waterstof-

fluoride concentratie van 0.5 $\mu g/m^3$ treden bij deze met 'bladnecrose' besmette cultivars meteen (acht weken na het planten) necrotische vlekken op en wordt de fase van chlorose overgeslagen. Nadat gedurende vier weken discontinu was begast met 0.3 $\mu g/m^3$ HF, ontstonden (negen weken na het planten) bij de met 'bladnecrose' besmette planten van het voor HF zeer gevoelige fresiaras Royal Blue de eerste necrosen op de plaats waar bij afwezigheid van HF eerst chlorotische vlekken zichtbaar worden. Bij de met 'bladnecrose' besmette planten van het minder voor HF gevoelige ras Rose Marie, traden bij deze intermitterende begassing pas enkele weken later de eerste necrotische verschijnselen op (Fig. 2).

Uit het voorgaande blijkt duidelijk dat er sprake is van een synergisme van waterstoffluoride en 'bladnecrose' bij fresia's. Op grond hiervan kan worden gesteld dat bij fresiarassen besmet met 'bladnecrose' – zowel gevoelige als minder gevoelige rassen – die geteeld worden in industriegebieden met een geringe mate van HF-luchtverontreiniging het ziektebeeld van 'bladnecrose' zich eerder en in ernstiger mate zal manifesteren.

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