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Viruses and virus diseases in Dutch bulbous irises (Iris hollandica) in the Netherlands

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Abstract

The occurrence in Dutch bulbous irises *(Iris hollandica)* of two viruses – iris mild mosaic virus (IMMV) and iris severe mosaic virus (ISMV) – in association with two diseases – mosaic (mozaïek) and grey (grijs) – was reported so far. In the Netherlands, three virus diseases have been distinguished: mild mosaic (mozaïek), mild yellow mosaic (bont), and severe mosaic (grijs). These diseases were associated with IMMV (750 nm), IMMV plus iris mild yellow mosaic virus (IMYMV, a newly recognized virus; 660 nm), and IMMV plus ISMV (750 nm), respectively. The viruses are antigenically distinct and their presence could be established serologically. Tobacco mosaic virus (TMV), tobacco rattle virus (TRV), and tobacco ringspot virus (TRSV) were also detected in irises, but not in association with particular symptoms.

Generally, the symptoms of the diseases can be distinguished early in the growing season, particularly in March. Later on, the distinctive symptoms mostly disappear on plants showing mild symptoms but not on severely affected plants. Growing and forcing conditions influence the symptoms. The IMYMV and the ISMV transmitted in May and early in June by *Macrosiphum euphorbiae* cause more severe symptoms than those induced by transmissions late in June and in July. Problems related to disease control in irises are discussed.

Additional keywords: iris mild mosaic virus, iris mild yellow mosaic virus, iris severe mosaic virus, *Macrosiphum euphorbiae*, tobacco mosaic virus, tobacco rattle virus, tobacco ringspot virus.

Introduction

In the Netherlands the acreage of Dutch bulbous iris *(Iris hollandica)* is about 2000. The irises are grown on loamy and sandy soils. They are planted in the field in October/November and harvested in July/August of the following year. Vegetative propagation facilitates and includes virus transmission to progeny bulbs. Virus infection may decrease commercial value of the bulbs of many cultivars when these are used for forcing into flowering during various periods throughout the year (Durieux and De Pagter, 1967).

Till now symptomatology and etiology of virus diseases of Dutch bulbous irises have been confusing due to lack of information on the viruses known to be involved so far. Iris mild mosaic virus (IMMV) and iris severe mosaic virus (ISMV) have long been known to occur in Dutch bulbous irises in the Netherlands (Van Slogteren, 1958; 1963). IMMV occurs in all cultivars grown commercially (Van Slogteren, 1958). In my own investigations of the crop, I have come across a number of other viruses, as

tentatively reported: iris mild yellow mosaic virus (IMYMV; Dutch name: irisbontvirus; Asjes, 1974a), tobacco mosaic virus (TMV), tobacco ringspot virus (TRSV; Asjes, 1969) and tobacco rattle virus (TRV; Asjes, 1974b). The present paper reports in more detail on the occurrence of these viruses in Dutch bulbous irises, their symptoms, particularly in view of field diagnosis, transmission by aphids and control measures applicable in irises in the Netherlands.

Materials and methods

Virus disease survey. Samples of several iris cultivars showing virus syndromes were available for investigation. They were sent by growers and by inspectors, or collected in the field by myself.

Virus symptom expression. In order to describe symptoms and to study their variations more reliably, stocks of 'Dominator', 'Ideal', 'Professor Blaauw', and 'Wedgwood', which upon testing had been found naturally infected by IMMV, IMYMV, and occasionally ISMV, were grown in the field on light sandy, peaty bog, and heavy loam soils. The bulbs were replanted in sandy soil and re-examined for symptom expression in the following growing season.

In addition, similar stocks of 'Dominator', 'Professor Blaauw', 'Wedgwood' planted in sandy, peaty bog, and heavy loam soils were given the usual temperature treatments and forced into flowering in February/March at 13–15 °C under glass. The severity of symptoms of IMMV and IMYMV on plants of 'Professor Blaauw' in sandy soil was evaluated in sequentially forced samples throughout the year (Durieux and De Pagter, 1967).

Electron microscopy. Leaf epidermal strips of 'Dominator', 'Professor Blaauw', and 'Wedgwood' were macerated in phosphate buffer (0.067M; pH 7.2) and the extracts treated with 2% phosphotungstic acid (pH 7.2). TMV was added as an internal standard (Bos, 1975) or as an external standard when photographs on separate grids were taken intermittently. The virus particles photographed at a magnification of 10000 in a Philips 201S electron microscope were enlarged by projection to approximately 200000, the images drawn on paper, and measured.

Purification of viruses. The leaves of plants of various cultivars containing IMMV, plus ISMV, or IMYMV, or TMV, or TRV, or TRSV, were homogenized with an Ultraturrax in NaK-phosphate buffer (0.067M; pH 7.2; w/v = 1/1) containing 0.1% thioglycollic acid, or in 0.2M boric acid plus 0.05M sodium borate (pH 7.2; w/v = 1/1). The extract, which was used directly or after freezing at –20 °C for at least a day, was treated with n-butanol/chloroform (w/v = 1/1; 1/2h) and the virus in the aqueous phase differentially centrifuged (Asjes, 1972). The viruscontaining pellet was resuspended in the phosphate or borate buffer.

Antisera. IMMV antiserum had earlier been prepared with partially purified virus preparations from apparently healthy iris plants 'Albino' (Van Slogteren, 1955), and ISMV antiserum from mosaic showing material of crocus 'King of the Blues' (Van Slogteren, 1958). Antisera against TRSV, and similarly against TRV, were prepared by

injecting rabbits with virus suspensions from infected *Nicotiana tabacum* 'White Burley', as described elsewhere (Asjes, 1972).

An antiserum to IMYMV (titre 1/320 in microprecipitin tests under paraffin oil) was prepared by injecting rabbits six times intraveneously with partially purified virus preparations of mild yellow mosaic-infected material of 'Dominator'. Prior to injection, IMMV and ISMV were absorbed from the suspensions.

Test plants. TRSV, TRV and TMV were detected on *Chenopodium quinoa* and *N. tabacum* 'White Burley' after manual inoculation. The virus preparations were partially purified in order to enhance transmission percentages of the viruses, in particular of TRSV, onto the test plants. The test plants were grown at ca. 20 °C under hydrogen lamps (250 W; 18h/day).

Transmission of IMYMV and ISMV by aphids. Plants of 'Professor Blaauw' with mild mosaic containing only IMMV and plants of 'Wedgwood' with severe mosaic or mild yellow mosaic containing ISMV and/or IMYMV in addition to IMMV, were exposed in field cages to large numbers of *Macrosiphum euphorbiae* for 21-day periods starting on May 11, June 1 and 22, and July 13. The symptoms induced by transmitting ISMV and/or IMYMV in this way were investigated in progeny plants after replanting the bulbs of 'Professor Blaauw' in the field or under glass.

In another experiment IMMV-infected bulbs of 'Professor Blaauw' and 'Wedgwood' were planted in cages together with IMYMV-infected bulbs of 'Professor Blaauw' and 'Dominator', also containing IMMV. The plants were exposed to large numbers of *M. euphorbiae* from June 1 onwards. The symptoms and the viruses to be detected were studied on plants of replanted bulbs in the following growing season.

Under storage conditions, samples of bulbs of 'Wedgwood' only infected with IMMV and samples of bulbs of 'Dominator' containing ISMV or IMYMV in addition to IMMV were exposed for 14-day periods to colonies of *M. euphorbiae* in October, November and December. The bulbs of 'Wedgwood' were replanted in the field so that any development of symptoms could be followed.

Results

Symptoms of mild mosaic. Until about two months after emergence the plants do not show symptoms of mild mosaic, but then a rather distinct fine yellowish-green mosaic-like streaking (Fig. 1) or a faint chlorotic mosaic develops in the newly developing leaves. The mosaic pattern is regular. As the plants mature, the symptoms become more prominent on both old and new leaves. Late in the growing season, a mosaic pattern also showing grey necrotic areas develops along leaf margins of plants of some cultivars, e.g. 'Professor Blaauw'. Conspicuous mosaic symptoms may develop on the flower stalk and spathe. On flowers, one to several dark spots sometimes develop at the tip of the fall in blue- and white-flowered cultivars. The flowers are normal in size and shape. The market value and the vigour of the plants do not seem to be markedly reduced.

The severity of symptoms depends on the sensitivity of the cultivar and the growing conditions. In 'Professor Blaauw' a fairly mild mosaic pattern develops, whereas in 'Ideal' the mosaic can be very conspicuous. The mild mosaic visible on field plants

Fig. 1. Left: Faint yellow-green streaking due to mild mosaic on leaves of a plant of 'Professor Blaauw', two to three months after emergence. Right: Mild yellow mosaic manifested mainly along leaf margins on a plant of 'Professor Blaauw' photographed two to three months after emergence.

Fig. 1. Links: Milde, fijne, geelgroene streping (mozaïek) op bladeren van een plant van 'Professor Blaauw' ongeveer twee tot drie maanden na opkomst. Rechts: Geelgroen mozaïek (bont) voornamelijk langs de bladranden bij 'Professor Blaauw' ongeveer twee tot drie maanden na opkomst.

grown in sandy soil can be more conspicuous when the bulbs were previously grown in heavy loam or peat soil than after the growth in sandy soil. The mild mosaic on forced plants can be more prominent on plants in heavy loam soil than in sandy soil.

Symptoms of mild yellow mosaic. An intense yellowish-green mosaic pattern developing mainly along all leaf margins (Fig. 1 and 2) is visible on emerging plants. Symptoms visible near or sometimes just below soil level do not tend to develop rapidly in growing plants. The mild yellow mosaic pattern is irregular with fairly conspicuous

Fig. 2. Left: Apparently healthy plant of 'Professor Blaauw'. Middle: Mild yellow mosaic, mainly along leaf margins and on a flower bud sheath of a plant of 'Professor Blaauw'. Right: Severe mosaic on basal areas of all leaves and on a flower bud sheath of a plant of 'Professor Blaauw'.

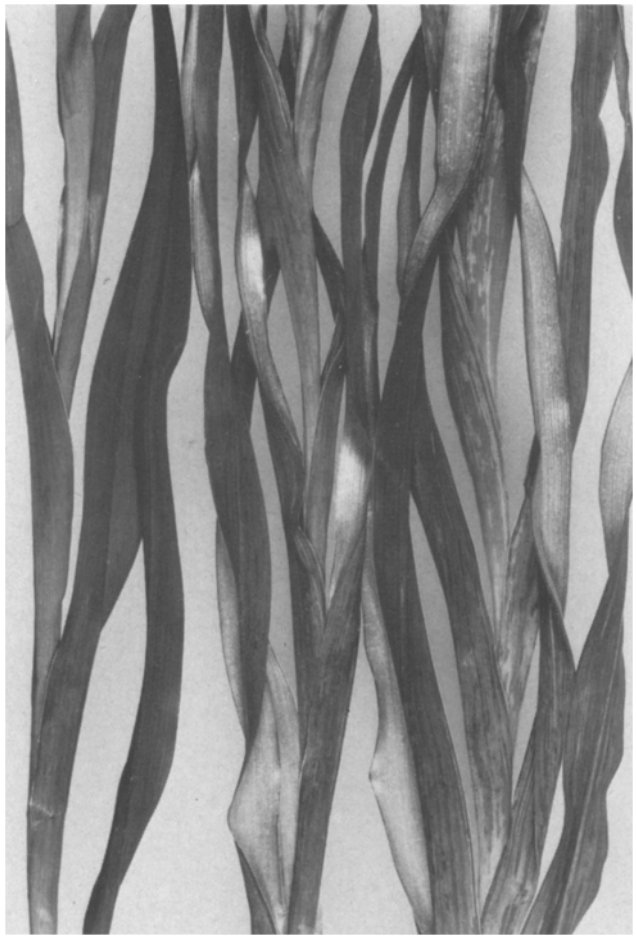

Fig. 2. Links: Ogenschijnlijk gezonde plant van 'Professor Blaauw'. Midden: Geelgroen mozaïek (bont) voornamelijk langs de bladranden en op een bloemschedeblad van een plant van 'Professor Blaauw'. Rechts: Ernstig mozaïek (grijs) in de vorm van geelgroene strepen op de basale bladeren en op een bloemschedeblad van een plant van 'Professor Blaauw'.

shades of yellowish-green. The plants are lighter yellowish-green than those with mild mosaic. The characteristic symptoms of mild yellow mosaic gradually disappear when mild mosaic symptoms become fairly conspicuous later on.

Symptoms on plants on the verge of flowering consist of yellowish-green or sometimes bluish-green stripy mosaic patterns, especially on the sheaths of the flower buds. The flowers show a more irregular colour pattern than when mild mosaic is present only. Affected plants die a few days prematurely. The market value of the flowers is slightly lower than that of flowers with mild mosaic.

The severity of the symptoms of mild yellow mosaic is intermediate between mild mosaic and severe mosaic.

Field plants in sandy soil show more severe symptoms when the bulbs were previously grown in heavy loam or peat soil in stead of continuously in sandy soil. Mild yellow mosaic may develop more conspicuously on plants forced in sandy soil than on plants in heavy loam or peat soil. Plants of 'Professor Blaauw' forced in successive groups throughout the year develop symptoms in winter, spring, and autumn, but not in summer.

Symptoms of severe mosaic. On emerging plants, mild symptoms of severe mosaic consist of yellowish-green stripes in restricted areas on the outer leaves or on developing middle leaves. Severe symptoms consist of pale-green and yellowish-green stripes and wider bands (Fig. 2) in irregular patterns extending upward from below soil level. The stripes and wider bands against a dark-green to bluish-green background on leaves above the soil level turn to light-green and bluish shades on the plants. The plants may be stunted and distorted. Initially, the symptoms remain most conspicuously visible on leaves close to and below the soil level, but later, around flowering time, they also become very prominent on the top parts of the plants. Severely affected plants die prematurely.

Fig. 3. Histograms of lengths of virus particles associated with syndromes in irises: A. mild mosaic in 'Wedgwood'; B. severe mosaic in 'Professor Blaauw'; C. mild yellow mosaic in 'Dominator'; and D. a severe form of mild yellow mosaic in 'Wedgwood'.

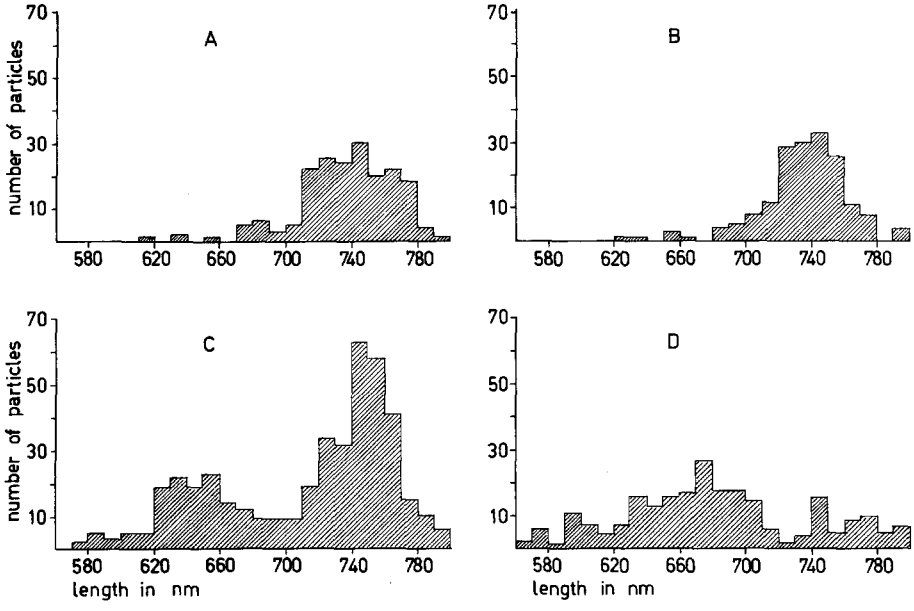

Fig. 3. Histogrammen van de lengtes van virusdeeltjes als gevolg van het optreden van ziektebeelden in irissen: A. mozaïek in 'Wedgwood'; B. grijs in 'Professor Blaauw'; C. bont in 'Dominator'; en D. ernstig bont in 'Wedgwood'.

Flowers on severely affected plants of blue, yellow, or white cultivars show mild to severe colour-breaking in petals. Teardrop markings may appear in either the standards or falls or in both. Flowers may be reduced in size and are often twisted to one side. The cut blooms do not last as long as uninfected flowers.

On plants produced by very late forcing (September/November), severe mosaic symptoms can be extremely pronounced. The plants may become yellow, stunted and therefore worthless. The symptoms may be more severe on plants forced in sandy soil than on plants forced in heavy loam and peaty soil.

After emergence of the plants, mild expression of severe mosaic can easily be mistaken for mild yellow mosaic, and the reverse can alco occur. When the development of symptoms of mild yellow mosaic is retarded during the rapid growth of the plants, the differential diagnosis more strongly indicates iris severe mosaic virus infection and the differential symptoms can be more easily evaluated visually than at emergence. Around flowering time, mild mosaic can be mistaken for mild yellow mosaic by evaluating symptomatology, and mild yellow mosaic can similarly be interpreted as a mild expression of severe mosaic.

Symptoms due to TMV, TRV, and TRSV. So far, no particular symptoms were associated with the occurrence of these viruses in irises.

Electron microscopy. Virus particles from about 20 plants showing distinct symptoms of mild mosaic, mild yellow mosaic or severe mosaic were measured. For mild mosaic and for severe mosaic a peak length of 750 nm (Fig. 3 A, B) was found. Virus from plants with mild yellow mosaic showed two peaks of 660 nm (680 nm) and 750 nm (Fig. 3 C, D).

Serology. The viruses in iris sap extracts from fresh or frozen plant material were partially purified or not when tested in microprecipitation tests under paraffin oil. On the whole, all test results were comparable.

In extracts of all plants showing mild mosaic symptoms, which were obtained from various cultivars and/or stocks, IMMV was invariably present. The sap dilution 1/4–1/8 generally resulted in weak precipitation reactions. Plants showing mild yellow mosaic distinctly contained IMMV and IMYMV. Sap dilutions of 1/2 generally performed weak positive reactions. IMYMV was sometimes difficult to detect serologically in plants showing less distinct mild yellow mosaic.

Tests with plants showing severe mosaic indicated the presence of IMMV plus ISMV (sap dilution 1/4–1/8 generally performed weakly positive) or IMMV plus IMYMV and ISMV.

On the whole, the serological results were relatively distinct when severe and easily recognizable syndromes of mild mosaic, mild yellow mosaic or severe mosaic were shown on irises.

Occurrence of viruses. All cultivars grown commercially in the Netherlands were invariably found to be infected with IMMV as reported by Van Slogteren, 1958. Similar findings were reported elsewhere (Loebenstein et al., 1961, cited by Barnett, 1972; Brunt, 1973; 1975).

Infection of IMYMV was particularly obvious in sensitive cultivars, e.g. 'Domi-

nator'. The detrimental effect of IMYMV, which was clearly recognized for several years already, led to the elimination of too seriously infected stocks.

In the majority of commercial cultivars, the incidence of infection of ISMV was found to be relatively low. However, occasionally a high incidence was observed in stocks. Similar findings were made elsewhere (Lawson, 1967; Brunt, 1975).

TMV was occasionally detected on test plants. Once, its occurrence was confirmed during the measurement of virus particles from a plant of 'Professor Blaauw' affected by severe mosaic.

TRV was only detected in two samples of plants of 'Professor Blaauw'.

Using test plants TRSV was occasionally detected in plants of 'Dominator', 'Ideal', 'Professor Blaauw', 'Wedgwood', and 'Yellow Queen'. In a few test series in winter months, TRSV was detected in as much as 70% of the material, whereas in comparable trials in summer it was hardly detectable. In general, TRSV was obtained more readily from severely diseased than from mildly affected iris plants. The incidental occurrence of TRSV in irises in Britain was also reported by Brunt (1975).

Transmission of IMYMV and ISMV. The plants of 'Professor Blaauw' exposed to aphids for 21 days starting on May 11 or June 1 were judged to be infected (100%) on the basis of severe yellowish-green symptoms of IMYMV and/or ISMV on the progeny plants. About 50% of the forced plants showed ISMV symptoms. The progeny of plants exposed to aphids from June 22 and July 13 onward showed a low incidence (15%) of IMYMV and/or ISMV symptoms. Symptoms of IMYMV were observed in the progeny of bulbs of 'Professor Blaauw' and 'Wedgwood' exposed to aphids transmitting IMYMV from plants of 'Dominator'.

Under the experimental conditions, IMYMV and ISMV were not transmitted during storage.

Disease control. The occurrence of symptoms in virus-infected irises is dependent on the type of virus, the sensitivity of the cultivar, the growing conditions in the field, and the forcing conditions under glass. In the field, for instance, IMYMV and ISMV symptoms can be temporarily masked under certain growing conditions, especially after spells of warm weather in spring. The symptoms on forced plants can be more pronounced in winter, spring, and autumn, or when bulbs are planted in sandy soil or are severely infected by pathogenic fungi. This can lead to differences between disease incidence in the field and under glass. However, other factors pertaining to disease control in the field must also be taken into consideration.

Disease control by rogueing diseased plants should preferably be carried out by people working on their knees, which permits better inspection of the plant parts close to the soil. Unfortunately, the early spring control usually has to be done in cold weather, which may be indirectly responsable for a higher percentage of overlooked plants, even above the 50% known for other crops, e.g. tulips (Bulsink, 1974). Furthermore, symptoms are more easily overlooked on plants grown from small, densely planted bulbs than on those of larger, more widely spaced bulbs, and the difference will be reflected in the infection rates of the stocks. Disease control is more difficult when the recognition of symptoms is complicated by regular spraying of fungicides which tends to change the green colour of the leaves into grey shades. In spring, too, incidental night frosts occasionally cause early death of the leaf tips, giving

yellowish discolorations that can be confused with virus symptoms on leaves.

Disease control should be carried out as early as possible, preferably in March and April, at least before the appearance of distinct symptoms on leaf tips and flowers later on, because around this time aphids capable of transmitting viruses start flying, albeit in low numbers. Moreover, disease control is then hampered by the masking of IMYMV and ISMV symptoms when the IMMV syndrome develops simultaneously.

Other methods besides the rogueing of plants must be developed to improve the health of stocks. Mineral-oil sprays diminish the spread of virus in irises (Deutsch and Loebenstein, 1967). Our results with this method will be reported in due course. The ultimate use of virus-free material has also to be considered (Stone and Hollings, 1965; Baruch and Quak, 1966; Paludan, 1971) if rapid propagation by tissue culture (Fujino et al., 1972; 1973; Fujino, 1974; 1976) can be successfully developed for large-scale production. So far, the only approach to grow irises economically is to rogue as many as possible plants showing ISMV- and IMYMV-symptoms out of field stocks in order to attain reasonable health standards. However, at least IMMV will still be invariably present in irises. Highly qualified stocks of certain cultivars, e.g. 'Professor Blaauw', infected only with IMMV are certified in the Netherlands. This approach to the improvement of the state of health of stocks is similar to that used in Israel, where maintenance of virus-tested stocks is applied (Loebenstein, 1972).

As the production of virus-free cultivars is not feasible economically, so far, serious attention must still be given to the breeding of commercial cultivars tolerant to IMMV in the future.

Discussion

The description of virus symptoms on irises as reported in literature (Barnett, 1972; Brierley and McWhorter, 1936; Brunt, 1968; 1975; Loebenstein and Alper, 1963; Lawson, 1967) were of help in this study in the further differentiation of virus syndromes under variable conditions in the field and under glass in the Netherlands.

Since virus names often refer to specific symptoms, presumably more names have been introduced than the number of filamentous viruses occurring in irises. Probably the terms iris latent mosaic virus (Loebenstein and Alper, 1963) and iris mosaic virus (Brierley and McWhorter, 1936; Van Slogteren, 1955; Brunt, 1968) refer to the same virus as IMMV (Lawson, 1967; Brunt, 1973; 1975). Iris grijs (= grey) virus (Van Slogteren, 1958; 1963) and iris yellow mosaic virus (Loebenstein and Alper, 1963) probably refer to ISMV (Lawson, 1967; Brunt, 1975).

Besides these two viruses, a third filamentous virus, iris mild yellow mosaic virus (= iris bontvirus; Asjes, 1974a) was detected. This virus proved to be associated with a conspicuous syndrome earlier called 'bont' in Dutch (De Vos, 1967). However, it still remains to be determined whether, according to Koch's postulates, IMYMV or ISMV can cause the mild yellow mosaic and severe mosaic diseases, respectively, without IMMV being present.

Samenvatting

Virussen en virusziekten in Hollandse irissen (Iris hollandica) in Nederland

Het virusonderzoek bij Hollandse irissen *(Iris hollandica)* in Nederland leidde tot het onderscheiden van drie ziekten, namelijk: het mozaïek ('mild mosaic'), het bont ('mild yellow mosaic') en het grijs ('severe mosaic'). Het voorkomen van iris-mozaïekvirus (deeltjeslengte 750 nm), iris-mozaïekvirus plus iris-bontvirus (660 nm) en iris-mozaïekvirus plus iris-grijsvirus (750 nm), welke virussen serologisch zijn te onderscheiden, werd in verband gebracht met respectievelijk het mozaïek, het bont en het grijs. Geen verband werd gevonden tussen het voorkomen van tabaksmozaïekvirus, tabaksratelvirus en tabakskringvlekkenvirus en de genoemde ziekten.

Op basis van de symptomen zijn de ziekten te velde vroeg in het voorjaar meestal wel te onderscheiden. Later in het groeiseizoen verdwijnt dit onderscheid veelal bij planten met milde symptomen, maar niet bij planten met ernstige symptomen. De symptomen van het mozaïek worden pas een paar maanden na de opkomst van de planten zichtbaar. De licht- en donkergroene mozaïeksymptomen doen zich duidelijk voor omstreeks de bloei. Het bont is bij opkomst te herkennen aan het geelgroene mozaïek, dat voornamelijk aan de bladranden voorkomt (Fig. 1). Na de lengtegroei van de planten worden de symptomen op de bloemscheden en op de brede bladgedeelten zichtbaar (Fig. 2). Het grijs (Fig. 2) uit zich met brede geel- en donkergroene strepen op de bladeren, die tot onder het grondniveau doorlopen en bij opkomst duidelijk zichtbaar zijn. Het geelgroene, soms streepvormige mozaïek blijft bij de lengtegroei van de planten duidelijk zichtbaar op de bladbases. Ernstige grijs-symptomen zijn bloembreking, gedraaide stand van smallere bladeren en dwerggroei van de planten. De duidelijkheid van de ziektebeelden, zowel van het mozaïek als van het bont en het grijs, is afhankelijk van de cultivar en van de teeltomstandigheden te velde en in de kas.

Het iris-bontvirus en het iris-grijsvirus geven bij overdracht door de bladluis (*Macrosiphum euphorbiae*) in mei en in de eerste helft van juni in het volgende groeiseizoen ernstiger aangetaste planten dan bij latere overdracht.

De mogelijkheden van de herkenning en de bestrijding van virusaantastingen in irissen worden beschreven.

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