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Tulip veinal streak, a disorder probably caused by tobacco ringspot virus

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Abstract

A newly recognized disease of tulip called veinal streak is described. The disorder occurs more often in tulips grown under glass than in the field, but totally diseased stocks are found under both conditions. When sap from tulip leaves with veinal streak was manually inoculated to test species, no virus isolate was obtained whereas the sap clarified with a mixture of n-butanol and chloroform and concentrated by centrifugation consistently gave isolates of tobacco ringspot virus (TRSV). However, TRSV was also obtained from symptomless and diseased tulips infected with other viruses. Isolates were propagated in *Nicotiana tabacum* 'White Burley' and sap from systemically infected leaves was used for serological identification in microprecipitin tests. It is postulated that TRSV is the cause of tulip veinal streak. Generally the virus is latent in tulips. The development of symptoms is associated with particular environmental growing conditions (Asjes and Muller, 1972).

Introduction

Forced tulips of certain cultivars, especially 'Lustige Witwe' (syn.: 'Merry Widow'), 'William Copland' and sports, 'Paul Richter', 'Bing Crosby', etc., sometimes show severe brown necrotic, watery and sunken streaks along main veins. The disorder is also occasionally seen in field crops and all plants of a stock may be affected. I have named the disease tulip veinal streak.

Although the symptoms suggest a virus disease, it was not until February 1968 that a virus was isolated from tulips with veinal streak. Symptoms are different from those caused by *tulip breaking* (TBV; syn.: *tulip mosaic*), *cucumber mosaic* (CMV), *tobacco necrosis* (TNV), and *tobacco rattle* (TRV) viruses in tulips (van Slogteren and Asjes, 1970).

This paper reports the possible role of tobacco ringspot virus (TRSV; cryptogram: R/1:2.2/40:S/S/S/Ne; Nepovirus group) in its relation to the disorder tulip veinal streak. The induction of the development of the symptoms greatly depends on particular growing conditions, which will be discussed in a separate paper (Asjes and Muller, 1972).

Material and methods

Forcing of the tulips. Bulbs of many cultivars harvested in the field with circumferences of 10 cm or greater were subjected to temperature treatments in storage

rooms or planted in boxes in rooting chambers or outside in plunge beds. The bulbs were then 'forced' in glasshouses to produce flowers in winter.

Test plants. The test plants, *Chenopodium quinoa*, *Nicotiana rustica*, and *N. tabacum* 'White Burley', were grown in the glasshouse at about 20°C with additional illumination provided by hydrogen lamps (250 W) for 18h daily from November until March. These plants were more susceptible to infection by TRSV, than those grown under similar conditions in other months of the year. Carborundum (500/13) dusted onto leaves of test plants was used as an abrasive for manual inoculations.

Antisera. Antisera against TRSV were kindly supplied by Dr M. Hollings, Littlehampton, Great Britain; Dr H. A. Scott, Beltsville, USA, and Dr R. Stace-Smith, Vancouver, Canada. Virus isolates obtained from tulips were propagated in 'White Burley' tobacco plants, and the sap extracts from systemically infected leaves permitted the identification of TRSV in microprecipitin tests (van Slogteren, 1955).

Samples of tulips. Samples of different cultivars of tulips tested for TRSV were obtained from the collection of cultivars known to be infected with different viruses maintained at Lisse, and from various localities brought in by inspectors of the Plant Protection Service to be tested for viruses other than TRSV, e.g. TBV, TNV, TRV, CMV, AMV (*Arabis* mosaic virus), and TBRV (tomato black ring virus).

Symptoms

Forced tulips. Because the symptoms are more conspicuous on the upper surface of the leaves, the disease is most noticeable after the leaves unfold. Symptoms, although often present on all leaves and throughout the leaf area, are most pronounced on the basal part of the lower leaves. Consequently tulips which seem to be most severely diseased are these in which symptoms are readily visible on the upper leaves. Symptoms may also occur in the flowers. Usually tulip plants with veinal streak are somewhat stunted and twisted, but plants of normal height may also have symptoms in leaf and flower.

The main veins of the leaves show a translucent, yellowish-green discoloration, which may be restricted to elongated spots, or occurs as a vein-banding. In more severely diseased plants the centres of the spots or streaks may later turn brown necrotic and appear to be watery and sunken (Fig. 1), particularly near the leaf base. The main veins may also show dry-brownish discolorations bordered by chlorotic zones.

Petals either in the bud or in the open flower can also show watery, sunken, brown streaks along some veins. Green or greyish elongated spots may be present depending on the flower colour. The discoloration may occasionally be more extensive and the flowers may sometimes be severely deformed. Although the leaves may show no or mild symptoms, spots and streaks may occur in the flowers. In severely diseased plants the stems also show brown necrotic streaks.

In some cultivars the leaves only show a faint chlorosis, which may later turn into greyish-brown streaks. Under drier conditions in glasshouses brown watery necrotic streaks and spots may develop into 'dry necrotic' symptoms. These symptoms



Fig. 1. Watery brown necrotic streaks and lozenge-shaped spots along the main veins near the leaf base of the cultivar 'Lustige Witwe' (syn.: 'Merry Widow').

Fig. 1. Waterige bruin-necrotische strepen en langgerekte ovale vlekken langs de hoofdnerven nabij de basis van een blad van de cultivar 'Lustige Witwe' (syn.: 'Merry Widow').

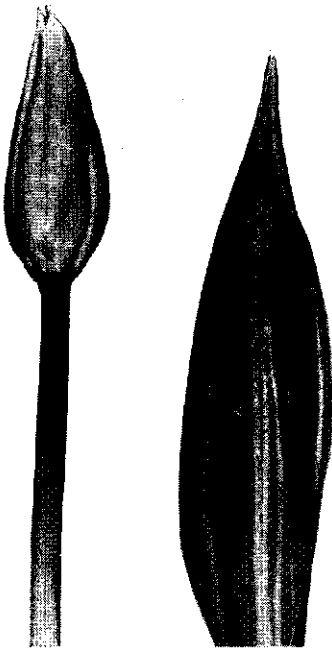


Fig. 2. Yellowish-green streaks on leaves of 'Bellona'.

Fig. 2. Geelgroene strepen op bladeren van 'Bellona'.

resemble those caused by tobacco necrosis virus (Asjes and Muller, 1972).

The development of symptoms on the cultivars 'Apeldoorn' and 'Bellona' parallels that in 'Lustige Witwe' except that the final stage of necrosis may not occur, and lighter green, chlorotic and greyish-brown streaks tend to prevail (Fig. 2). Although faint chlorotic streaks appear mild, these plants must be considered severely diseased, because the flowers are often affected.

Field-grown tulips. In these the symptoms usually remain milder than under glasshouse conditions. These symptoms consist of greenish discoloured or less pronounced chlorotic zones along the main veins in the early development of the plants. Symptoms may be more distinct in the form of necrosis on plants of susceptible cultivars having bright dark-green leaves if the growth is arrested by abnormally low temperatures. Symptoms in plants with a paler colour show narrower watery necrotic 'parallel' streaks all over the surface of the older leaves, in single or a few plants of a field crop.

In normal growth the milder symptoms disappear quite rapidly. No abnormalities have been found in the bulbs of severely diseased tulip plants.

Detection and identification of TRSV

No virus was obtained from plants with veinal streak by grinding leaf or flower material in 0.067M phosphate buffer (pH 7.2) and inoculating the homogenate to *C. quinoa*, *N. rustica* and *N. tabacum* 'White Burley'.

However, a virus was transmitted in February 1968 when clarified and concentrated extracts of diseased plants of 'Lustige Witwe' were used. The leaves were ground in phosphate buffer solution containing 0.1 % thioglycolic acid (w/w = 1/1)

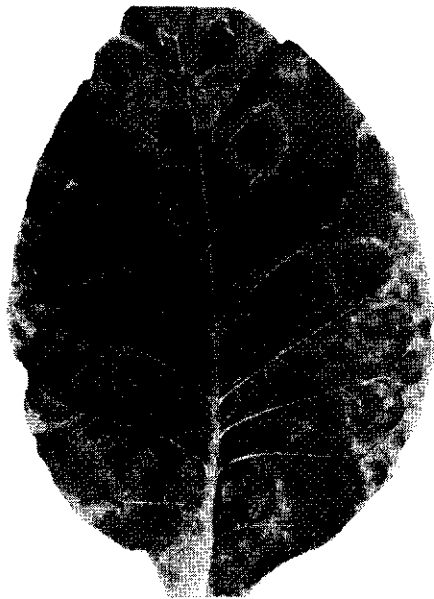


Fig. 3. Leaf of 'White Burley' tobacco plants with severe systemic ringspotting caused by tobacco ringspot virus.

Fig. 3. Blad van 'White Burley'-tabaksplanten met hevige systemische kringvlekvorming veroorzaakt door tabakskringvlekkenvirus.

with an Ultra-turrax (Janke and Kunkel KG – Stauffen). The sap was squeezed through cheese cloth and stored at -20°C for one night or sometimes even for several months. After thawing overnight at room temperature, n-butanol/chloroform (1/1) was added, and the mixture shaken periodically for 30 min.

The clarified sap (10 min at 1000g in an angle rotor of a 'Phywé P 15' centrifuge) was concentrated by centrifugation for 2 h at 92,000g in an angle rotor of a Spinco L 50 centrifuge. The pellet resuspended in phosphate buffer (1 ml resulting from 20–30g of leaves) was inoculated to test plants. In inoculated 'White Burley' plants a few and sometimes only one large lesion appeared after about two weeks. One and often two successive transfer inoculations were necessary before the tobacco plants produced systemic symptoms after three to four weeks (Fig. 3). Plants of *C. quinoa* became more readily systemically infected, and *N. rustica* initially gave watery lesions from which after a few subcultures a systemic reaction could be obtained.

Sap from systemically infected 'White Burley' tobacco plants extracted in phosphate buffer, untreated or clarified with butanol/chloroform gave positive reactions in micro-precipitin tests with several antisera to TRSV.

With preparations, purified by butanol/chloroform treatment of an isolate of TRSV from 'Lustige Witwe' cultured in *N. rustica* antisera of titres of 1/4096 were obtained by immunising rabbits with six to twelve intravenous injections given at three day intervals. However, similar preparations made from tulip leaves did not react against TRSV antisera nor did they produce antibodies against the virus when injected into rabbits.

Occurrence of TRSV in tulips

Two surveys were made to assess the occurrence of TRSV in stocks of tulip cultivars. In the first, cultivars more or less known to be susceptible to veinal streak were chosen at random, and samples of plants with and without the disease were taken. A sample consisted of the leaves of either one, or several single, or mixtures of two to four plants, and were homogenized in such a way. Samples of diseased plants were obtained from the following cultivars (number of stocks sampled given in brackets):

Mid-season: Mendel-tulips: 'Van der Eerden' (1)*; *Triumph-tulips:* Bing Crosby' (4), 'Lustige Witwe' (4), 'Mirjoran' (1)*, 'Olaf'(1)*, 'Snowstar' (1)*, 'Sulphur Glory' (2)*, 'Topscore'(1)*.

Late or May-flowering: Darwin tulips: 'Constellation' (1), 'Paul Richter'(5), 'Queen of Night'(1),* 'Rose Copland'(4); *Darwin hybrids:* 'Apeldoorn' (4), 'Diplomate'(2)*, 'General Eisenhower'(1)*.

Apparently healthy plants were taken from the cultivars: 'Lustige Witwe'(1), 'Paul Richter'(1) and 'Apeldoorn'(1).

From each sample a virus was obtained in the test species used. The reactions of the isolates in *N. tabacum* 'White Burley' in particular, together with a systemic reaction in plants of *C. quinoa* were indicative for TRSV to be detected. This was thought possible after sub-culturing several virus isolates in 'White Burley'-plants. The 16 isolates out of 48 tests which were checked serologically, all reacted with TRSV anti-

* Symptoms of veinal streak were only observed in a few plants of the sampled stock. The susceptibility is not indicated by mentioning the name of certain cultivars.

serum. The non-presence of other viruses infecting tulips in plants with veinal streak was assessed in order to exclude them as possible causes for the disease.

In a second survey 123 samples of either several single or mixtures of two to four plants from 90 stocks were investigated. The sampled plants showed either no symptoms at all, or viral symptoms to be attributed to different viruses detected, or non-descript symptom expressions of streaks and ringspot patterns which are not attributable to a described disease caused by the viruses mentioned under the next heading.

Totally 85 cultivars from 124 stocks were tested. A number of 61 were investigated by one sample of varying numbers of plants per stock whereas similarly the additional 24 were derived from 63 stocks of tulips. The results of the tests are grouped according to viral symptoms (viruses detected other than TRSV) and the classification of tulip cultivars (Anonymous, 1965). Seldomly the same cultivar was investigated in more than one group of viral symptoms, which is not separately mentioned. The indicative reactions for TRSV in 159 series of test plants were repeatedly additionally confirmed by 43 only positive serological reactions. It is separately mentioned in Table 1 that 24 cultivars were derived from 63 stocks.

In all but two of 161 tests the test species reacted with symptoms indicative for TRSV. The virus was detected in plants of cultivars of various groups of tulips. TRSV-infection predominantly is latent, because it was abundantly detected in plants which were apparently healthy or in complexes with TBV, and TRV. TRSV could be obtained from plants with CMV but not consistently (Asjes, unpublished). TRSV could not be detected in tulips already infected by TNV and AMV. These two viruses apparently were more readily transmitted to the test plants than TRSV. In tulip plants with symptoms of white streak or other streak and ringspot patterns the virus could be detected, but presumably some other non-identified virus may be involved in causing these symptoms.

Exclusion of other viruses infecting tulips as possible causes of tulip veinal streak

Tulip breaking virus (TBV): This virus causes a colour breaking and variably distinct mosaic patterns on the leaves (van Slogteren and Asjes, 1970). Symptoms of TBV are more distinct on the upper than on the lower leaves, which is not the case for veinal streak. TBV has thread-like particles detectable by negative staining using 2.5% phosphotungstic acid (pH 7.2) (Hitchborn and Hills, 1965). No thread-like particles of other viruses than TBV are thought to be present in tulips. In plants with veinal streak we never found thread-like particles. TRSV was obtained from TBV-plants.

Cucumber mosaic virus (CMV): This virus unlike veinal streak causes a necrotic reaction in bulbs of susceptible cultivars (van Slogteren and Asjes, 1970). CMV is readily transmissible mechanically to test plants. It was not detected in plants with veinal streak.

Tobacco necrosis virus (TNV): Symptoms of TNV may lead to necrosis in leaves and bulbs (van Slogteren and Asjes, 1970). Some of the leaf symptoms of veinal streak may be confused with those induced by TNV. Generally cultivars susceptible to TNV are not susceptible to veinal streak. TRSV could not be detected in plants with TNV by the procedure used because TNV more readily infected the test plants.

Table 1. Occurrence of tobacco ringspot virus (TRSV) in tulips, either apparently healthy, or with symptoms of known virus diseases, or symptoms resembling viral infections.

Viral symptoms/(viruses detected other than TRSV)/classification	Number of tests	Serological checks/indicative test plant reactions	Number of cultivars	Cultivars from more than one stock/stocks
<i>none (none)</i>				
early	1	1/1	1	
mid-season	4	2/4	4	
late or May-flowering	16	4/16	14	
species	1	0/1	1	
<i>tulip veinal streak¹ (none)</i>				
mid-season	16	8/16	10	3/9
late or May-flowering	22	8/22	8	5/15
<i>tulip breaking (TBV)</i>				
early	27	6/26	7	2/7
mid-season	28	6/28	10	5/12
late or May-flowering	15	1/15	9	3/6
species	1	0/1	1	
<i>tobacco rattle (TRV)</i>				
mid-season	4	2/4	4	
<i>'white streak' (none)</i>				
mid-season	8	2/8	4	2/6
late or May-flowering	1	1/1	1	
<i>non-descript streak and ringspot patterns (none)</i>				
mid-season	11	0/10	6	3/6
late or May-flowering	5	1/5	4	
species	2	1/2	1	1/2
total	161	43/159	85	24/63

¹ The results obtained for tulip veinal streak in the first survey are summarized. The stocks of cultivars from which apparently healthy material was taken, are counted twice in the third column.

Tabel 1. Het voorkomen van tabakskringvleckenvirus (TRSV) in tulpen met een gezond uiterlijk, met symptomen van een bekende virusziekte, of met symptomen die aan een virusziekte doen denken.

Tobacco rattle virus (TRV): Symptoms of TRV in tulips differ from those of veinal streak by less elongated, more pronounced diamond-shaped chlorotic or transparent flecks along the main veins of the leaves (van Slogteren and Asjes, 1970). A more conspicuous appearance in older than in newly formed leaves and a frequent occurrence in basal parts is common to both. TRV could be readily detected by electron microscopy in clarified preparations. It was also possible to separate TRV and TRSV by the use of test plants. In complex with TRV, TRSV seemed to infect 'White Burley'-plants more readily. Three to four weeks after inoculation TRSV could be distinguished from TRV because of its systemic reaction in *C. quinoa*.

Arabid mosaic virus (AMV): This virus was recently detected in field-grown tulips (Asjes, unpublished). AMV proved to infect the test plants more readily than TRSV. Mixtures of both viruses on test plants could not be detected. AMV caused a colour breaking in the flowers of the cultivars in which it was found.

Tobacco mosaic virus (TMV): This virus was recently detected in field-grown tulips (Asjes, unpublished). The infections in the plants were symptomless or there were symptoms noticeable not resembling those of veinal streak.

Tomato black ring virus (TBRV): Only once this virus was detected in tulips in the Netherlands. In the present investigations we could not find TBRV.

Latency of TRSV and appearance of veinal streak in tulips

The results in Table 1 indicate that TRSV is widespread and predominantly latent in tulips. It is postulated that tobacco ringspot virus is the cause of tulip veinal streak. The ubiquitous presence of TRSV in various groups of tulips suggests that the disorder may be occasionally observed too in other cultivars than those mentioned of the mid-season and late or May-flowering tulips. Once we observed a disease incidence up to 100% in a forced crop of the cultivar 'Bellona' (single early tulip).

The experience with the disorder for several years is that the appearance of symptoms of veinal streak seems to be dependent on particular growing conditions. Field-crops of a few stocks of 'Lustige Witwe' and 'Paul Richter' were severely diseased up to 100% in the spring of 1968. The plants of the progenies showed a very low percentage of only mild symptoms in the spring of 1969. The field crops of both cultivars in 1970 showed rather severe symptoms up to 50%. Similarly inspectors of the Plant Protection Service observed that the progeny of severely diseased stocks of tulips may look perfectly healthy in the field in the next season.

It was observed in forcing experiments with 'Lustige Witwe' and 'Paul Richter' that bulbs from severely diseased field crops only produced plants with very mild or no symptoms at all. On the other hand, the progeny of an apparently healthy field crop may give a disease incidence up to 100% under particular forcing conditions under glass. These will be discussed in a separate paper (Asjes and Muller, 1972).

Discussion

The testing of a diversity of cultivars from many stocks shows that the TRSV is widely spread in tulips. De Zeeuw (1965) and De Zeeuw and Hooker (1965) reported that some tulips grown in the field in Michigan (USA) were naturally infected by TRSV.

It is postulated that TRSV is the cause of tulip veinal streak. The virus is slightly readier transmitted to test plants from tulips with symptoms. The development of the symptoms is most conspicuous in the basal parts of the lower leaves of the tulips which is similar to that of other soil-borne viruses such as TRV and AMV. An air-borne virus such as TBV induces more conspicuous symptoms in the top parts of the upper leaves. The other viruses infecting tulips are excluded as possible causes. The symptoms are different. The disease incidences are very seldomly comparable with those of veinal streak, and may be more obviously correlated with the viruses detected.

The TRSV is presumedly spread up to 100% in certain cultivars and the disease incidences of veinal streak occasionally are up to 100%. However, the postulates of Koch were not applied.

The TRSV was obtained from plants showing symptoms of 'white streak' (Smith, 1957) and nondescript streak and ringspot patterns, and it might be suggested that TRSV would be a cause of these symptoms. However, a non-identified virus may play a role as well, because of the incidental occurrence in patches in the field of 'white streak' in particular. Moreover, it was not possible to induce these symptoms in forcing procedures as may be done for veinal streak (Asjes and Muller, 1972).

The ubiquitous occurrence of TRSV raises the question of how distribution occurs. Although in tulips seed transmission of TRSV has not yet been investigated, it is common in some of its hosts (for a survey of literature, Bennett, 1969). The overall infection of so many cultivars may be explained by seed transmission from the original breeding material if it was infected with TRSV. The original seedlings used to establish these cultivars may also have been infected at the places they were grown. In either case the ensuing vegetatively propagated cultivar would be overall infected.

TRSV is transmitted by *Xiphinema* spp. (for references see Harrison, 1964). Generally this virus is not present in sandy soils with a rather high water-level, which predominate in many of the bulb-growing regions in the Netherlands. In soils with a lower water-level *Xiphinema* spp. may occur (Van Hoof, 1966). We once isolated TRSV from 'White Burley' grown as a bait plant in soil sampled in an area with a lower water-level. This soil was not further investigated for the presence of the vector.

Transmission of TRSV has been reported for soybean by nymphs but not by adults of *Thrips tabaci* (Messieha, 1969). The virus was also transmitted by mites (*Tetranychus* sp.) in 3.14% of 605 attempts when known numbers and stages were picked from selected virus acquisition hosts (Thomwas, 1969). However, these arthropods do not seem to be of importance in the cultivation of tulips.

The recovery of virus of TRSV is not yet understood. Consistent transmission to test plants was obtained by the procedure described, but further concentration did not give a corresponding increase in infectivity. This could be due to an inhibitor in the plant extracts, but dilutions of sap did not improve infectivity. Moreover, inoculum prepared from lesions on newly inoculated test plants also were not very infective when transmitted to a new series of the same plants. It may be that little virus is present, or another factor not yet understood influences the transmissibility of the virus.

Samenvatting

Nerven- of strepenziekte in tulpen mogelijk veroorzaakt door tabakskringvlekkenvirus

Een nieuw herkende ziekte in tulpen, genoemd de nerven- of strepenziekte, wordt beschreven. De ziekte treedt meer op in tulpen in de kas dan te velde, doch totaal zieke partijen worden onder beide omstandigheden gevonden. Wanneer sap van tulpebladeren met de nervenziekte mechanisch geïnoculeerd werd op toetsplanten, kon geen virus-isolatie worden verkregen. Het sap gedeeltelijk gezuiverd met een mengsel van n-butanol en chloroform en door centrifugering geconcentreerd gaf steeds weer isolaties van het tabakskringvlekkenvirus (TRSV). Het virus werd echter ook verkregen

van symptoomloze planten en zieke tulpen die geïnfecteerd waren met andere virussen. Isolaties werden vermeerderd in *Nicotiana tabacum* 'White Burley' en sap van systemisch geïnfecteerde bladeren werd gebruikt voor serologische identificatie in microprecipitatietoetsen. Aangenomen wordt dat TRSV de oorzaak is van de nerven- of strepenziekte van tulpen. Gewoonlijk is het virus latent in tulpen aanwezig. De ontwikkeling van de symptomen gaat samen met bijzondere groeiomstandigheden (Asjes and Muller, 1972).

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