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Detection of tobacco rattle virus by ELISA and test plants in main sprouts of tulip bulbs during storage at different temperatures

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Abstract

The detectability of tobacco rattle virus (TRV) in the main sprouts of primarily and secondarily infected tulip bulbs of cv. Apeldoorn stored at different temperatures from the lifting in July up to February is described. Detection by ELISA was not affected by the size of the main sprout, nor by the size of the bulbs. The rates of TRV-infected bulbs found by ELISA were highest during storage at 13, 9 or 5 °C continuously, and when temperatures were lowered from 20 or 17 °C to 5 °C in October. The percentages detected via test plants, but undetectable by ELISA were also lowest at these temperatures. The unfavourable effect of continuous storage at 20, 17, or 2 °C as expressed in low ELISA absorbances not significantly different from the mean value of healthy bulbs, was largely overcome during long storage by the change of temperature down to 5 °C from 20 and 17 °C or upwards from 2 °C. The reverse from 5 °C upwards to 17 and 20 °C affected the detectability by ELISA unfavourably. The rate of detection via test plants in the main sprout and in the small sprouts from different positions in bulbs was only possible at low percentages.

The effect of some factors, like different temperatures during storage, detectability of different TRV serotypes, interference of irregular occurrence of TRV in the removed scale and basal-plate tissue with the main sprout, and variable recurrence of TRV in progeny bulbs, is discussed in view of its impact on routine testing of bulbs during storage.

Introduction

The aim to monitor bulbs of ornamental crops in the Netherlands for the absence of viruses requires the development of suitable test procedures on bulbs in the laboratory as an addition to visual detection of infected plants in the field. The routine testing of samples of tulip bulbs during storage can be of help to predict the virus rates in the lots replanted in the field. This has been attained on a routine basis for tulip breaking virus in tulip (TBV; Van Schadewijk and Eggink, 1984), and TBV and lily symptomless virus in lily (LSV; Van Schadewijk, 1986). The development of test procedures on bulbs was reported for iris mild mosaic virus in iris (IMMV; Van Schadewijk et al., 1988) and tobacco rattle virus in tulip (TRV; Van der Vlugt et al., 1988). The detection of TRV in tulip bulbs was complicated by the non-uniform occurrence of detectable virus in parts of the same scale and in the different scales of bulbs. However, in a single test the detection of TRV in the main sprouts of bulbs after storage for seven months

at 5 °C seemed fairly accurate (Van der Vlugt et al., 1988). In this paper we describe the detection of TRV in the main sprouts of bulbs stored at different temperatures for several periods in order to test extensively the possible use of sprouts in routine monitoring.

Material and methods

Plant material. Bulbs primarily infected by TRV as visually determined were obtained from a lot of cv. Apeldoorn grown at Julianadorp. Secondarily infected bulbs from another lot of cv. Apeldoorn were maintained at the Bulb Research Centre. Healthy bulbs were obtained from a TRV-free lot grown in soil disinfested with methyl bromide. The primarily infected bulbs were lifted at the end of June; other lots were lifted on July 3. After being dried and cleaned from tunic and roots, the bulbs were put into storage on July 8, at different temperatures as listed in the tables.

In most experiments bulbs with a circumference larger than 11 cm were used. Small-sized bulbs (7/8 cm) were also used in the quantitative testing for TRV. Sap extracts were made of the main sprouts removed from the bulbs with a cork borer and subsequently made free from scale tissue by hand. The length of the sprouts was measured. Testing started at the end of October when sprouts had some length to be used properly.

In an additional experiment on November 12, the main and the other sprouts from ten bulbs were tested. The sprouts were coded as 'main' and a through e, as positioned from the inner to the outer scales of a bulb. The outermost sprouts were very small. Therefore these sprouts were homogenized with surrounding scale tissue to obtain the extracts for inoculation.

Test plants. The extracts with ELISA absorbances lower than the mean value of healthy bulbs were kept at −20 °C for some weeks. They were tested later by the mechanical inoculation of test plants of *Nicotiana edwardsonii*, *N. debneyi* and *N. tabacum* 'White Burley' grown under glass at c. 20 °C.

The series of ten healthy bulbs tested gave ELISA absorbances larger than 0.200 in 20%, values between 0.100-0.200 in 25% and smaller than 0.100 in 55% of the tests. Arbitrarily the criterium of a $A_{405} < 0.200$ was applied to facilitate the choice of suspensions to be inoculated on test plants.

ELISA procedure. In quantitative tests 2 g of sprout were homogenized in 8 ml PBS extraction buffer in a glass tube with an Ultra Turrax for about ten seconds at high speed. In the qualitative tests the sprouts were squeezed through a ribbed Pollähne rollerpress with simultaneous additon of buffer with a hand dispenser at a rate of c. 4 ml per g. In both tests the debris was left to sediment at room temperature for c. 1 h. The supernatant was incubated overnight in microtitre plates at 6 °C. The ELISA was applied as described by Van der Vlugt et al. (1988). The anti-TRV-conjugate (type PV) was incubated at 37 °C for 2 h. The conjugate buffer contained 0.3% Tween-20 and 0.4% normal horse serum. Absorbance values at 405 nm were determined with a Titertek Multiskan Spectrophotometer. Mean absorbance values are presented in the tables after subtraction of the high absorbance occasionally obtained from healthy bulbs. This means that low ELISA absorbances may not always be significantly

different from the values of healthy bulbs. In random tests during storage the possible presence of other different serotypes was tested with antisera coded TJ, TY, and UM. These antisera were prepared from different TRV isolates obtained from tulip and narcissus.

Results

Most serotypes of TRV were detectable in ELISA with the PV-antiserum. The antisera coded TJ, TY, and UM detected only one or two or no serotypes in the random tests done during the testing period. The length of the main sprout did not affect the detectability of TRV. This was not affected by the size of the bulb either.

Quantitative detection. Mean ELISA values of series of main sprouts of individual bulbs are shown in Table 1. The absorbances obtained in December were lower than those in November, January and February, whereas in October high values were found. The extinction values of primarily and secondarily infected bulbs were largely similar. The storage temperatures did not affect these values.

The percentages of detectable TRV in primarily and secondarily infected bulbs determined by ELISA and in test plants are shown in Table 2. Higher rates of secondarily infected bulbs were found by ELISA than of those primarily infected. The percentages of infected bulbs stored at 5 °C were only a little lower than of those stored at 9 °C. The additional percentages of infected bulbs obtained via test plants were fairly considerable. No virus was detected in 7-14% of the bulbs.

Qualitative detection. The length of the sprouts increased during storage at the same temperature, or when temperatures were lowered near the end of storage. The average lengths decreased when the temperatures were raised. The ELISA values were not affected by the lengths of the sprouts. The data from qualitative ELISA on the primarily and secondarily infected bulbs together stored at different temperatures

Table 1. Quantitative ELISA tests on tobacco rattle virus in the main sprouts of primarily and secondarily infected bulbs (n=20) of cv. Apeldoorn during storage at 5 and 9 °C.

Date	Mean ELISA absorbance[1] (bulbs with TRV)				Mean length of sprout in mm
	primary infection		secondary infection		
	5 °C	9 °C	5 °C	9 °C	(*n* = 80)
October 20	99 (9)	133 (9)	56 (15)	90 (18)	27
November 12	56 (14)	52 (13)	39 (17)	49 (19)	35
December 1	33 (10)	25 (10)	28 (10)	45 (13)	43
December 23	39 (16)	39 (17)	45 (19)	38 (17)	51
January 14	53 (9)	55 (8)	46 (16)	55 (18)	61
February 3	54 (15)	54 (18)	110 (19)	64 (20)	70
February 24	52 (14)	53 (14)	41 (13)	46 (16)	57

[1] A_{405} × 100 minus occasional high mean value of healthy bulbs, i.e., 0.20.

Table 2. Percentages of tobacco rattle virus infection of the main sprouts determined by quantitative ELISA and test plants of primarily and secondarily infected bulbs of cv. Apeldoorn (n=100) tested in December up to February after storage at 5 and 9 °C since July 8.

Type of infection	% ELISA positive $A_{405} > 0.20$	% additionally positive on test plants		% positive by ELISA and test plants
		after ELISA $A_{405} = 0.10-0.20$	after ELISA $A_{405} < 0.10$	
Primary				
storage at 5 °C	64	11	15	90
storage at 9 °C	67	6	13	86
Secondary				
storage at 5 °C	77	8	8	93
storage at 9 °C	84	2	5	91

Table 3. Qualitative ELISA on tobacco rattle virus in the main sprouts of bulbs (n = 20) of cv. Apeldoorn stored at different temperatures for variable periods since July 8.

Storage temperature[1] °C	Mean ELISA absorbance [2] (bulbs positive TRV)				
	29 October	16 November	9 December	6 January	9 February
25	51 (6)	–[3]	(0)	–	–
20	(0)	–	8 (10)	6 (4)	(0)
20-5	–	9 (3)	49 (20)	55 (16)	64 (14)
5-20	–	38 (8)x[4]	65 (18)	54 (8)	76 (7)
17	(0)	–	10 (3)	9 (11)	7 (4)
17-5	–	16 (1)x	26 (13)	56 (6)	65 (11)
5-17	–	56 (6)x	35 (17)	100 (10)	33 (8)
13	72 (12)	–	56 (19)	81 (15)	66 (10)
13-5	–	29 (8)x	36 (16)	57 (15)	50 (18)
5-13	–	36 (6)x	60 (20)	86 (18)	54 (13)
9	–	–	94 (10)	84 (16)	56 (16)
9-5	–	50 (10)x	59 (17)	47 (14)	57 (18)
5-9	–	50 (7)x	68 (20)	97 (18)	59 (15)
5	–	–	72 (11)	81 (13)	62 (12)
2	42 (8)	–	11 (3)	42 (5)	48 (9)
2-5	–	–	15 (17)	41 (11)	46 (10)

[1] Temperatures were lowered at October 2 and raised at October 17.
[2] $A_{405} \times 100$ minus occasional high mean value of healthy bulbs, i.e.,0.20.
[3] – = no datum.
[4] x: n = 10.

continuously or changed during storage are shown in Table 3. The 13, 9 and 5 °C temperature regimes were best in all treatments. Low absorbance values and percentages of TRV detection were obtained after continuous storage at 25, 20, 17, and 2 °C.

Table 4. Percentages of tobacco rattle virus infection determined by qualitative ELISA and additionally by test plants, of the main sprouts of bulbs of cv. Apeldoorn stored at different temperatures from July 8 till February 9.

Storage temperature[1] (°C)	Number of bulbs	% positive by ELISA	Additional % positive in test plants	Total % positive
25	40	60	28	88
20	80	24	56	80
20-5	80	66	7	73
5-20	70	58	17	75
17	80	23	55	78
17-5	70	44	22	66
5-17	70	58	17	75
13	80	70	6	76
13-5	70	81	12	93
5-13	70	81	0	81
9	50	84	5	89
9-5	70	84	11	95
5-9	70	86	6	92
5	70	60	11	71
2	70	36	21	57
2-5	60	63	24	87

[1] Temperatures were lowered at October 2 or raised at October 17.

TRV was increasingly detectable when the temperatures of 20 and 17 °C were lowered to 5 °C for two months. The change of 2 °C to 5 °C was similarly effective.

The data of the qualitative detection of TRV by ELISA and the additional testing in plants of the bulbs referred to in Table 3 are shown in Table 4. The ELISA gave high percentages of bulbs with positive values for TRV when storage between 5 and 13 °C was applied continuously or after a change up from 2 °C or downwards from 20 and 17 °C in October. The highest rates of TRV detection by ELISA and test plants were obtained in the 9 and 13 °C series. High rates of TRV detected via test plants only were found in the bulbs submitted to regimes of 25, 20, 17, and 2 °C.

Detection in different sprouts of bulbs. Results on the TRV detection in sprouts from different positions in bulbs are shown in Table 5. TRV was mainly detected in the main sprouts. Low percentages of virus detection were obtained from the other sprouts. All sprouts of a bulb did not contain detectable virus. If no virus was obtained from the outer sprouts, the main sprout did not contain detectable virus either.

Discussion

The detection of TRV by ELISA in the main sprouts of tulip bulbs was affected by different temperatures during storage. The continuous regimes of 5, 9, or 13 °C induced the best results in view of high ELISA absorbances and high percentages of detectable virus-infected bulbs. The difference in level of absorbances obtained in

Table 5. Detection of tobacco rattle virus via *Nicotiana* species determined at November 12 in the sprouts of secondarily infected bulbs of cv. Apeldoorn stored at 5 °C since July 8.

Bulb	Circum-ference	Length in mm position of the sprout[1]						Test plant reaction[2]					
	(cm)	main	a	b	c	d	e	main	a	b	c	d	e
1	12/up	40	8	20	4	10	10	+	–	–	–	–	–
2	12/up	50	20	25	1	5	10	+	–	–	–	–	–
3	12/up	35	5	5	5	5	5	–	–	–	–	–	–
4	12/up	45	10	3	2	3	8	–	–	–	–	–	–
5	12/up	25	5	7	10	7	7	+	–	+	–	–	
6	11/12	35	14	5	4	3	5	–	–	–	–	–	–
7	11/12	35	5	2	1	2	2	–	–	–	–	–	–
8	10/11	35	7	5	2	1	+	–	–	–	–	–	
9	9/10	35	5	4	3	3	5	+	(+)	–	(+)	–	–
10	9/10	40	5	2	2	2	4	±	(±)	–	–	(±)	–

[1] Main through e means that the position of the sprout was at increasing distance from the centre of the bulb.
[2] + = positive; – = negative; (+) = weak positive reaction.

quantitative and qualitative tests in consecutive months of storage can not be explained (Tables 1 and 3). The 9 °C regime either applied continuously, or changed in October, scored the highest rates after ELISA. The unfavourable effect of continuous storage at 2, 17, 20 or 25 °C was evident (Table 3) in view of low ELISA absorbances which mostly were most likely insignificantly different from ELISA values of healthy bulbs, which consequently affect the low percentages of detectable virus-infected bulbs additionally. The low percentages of infected bulbs detected by ELISA and additionally by test plants of the bulbs stored at 20, 17, and 2 °C were only partially corrected by a decrease to 5 °C, if the total rates of the 9 °C regimes were considered to be maximal (Table 4). These percentages may be the highest as variable small percentages of TRV-infected bulbs will not give rise to infected plants after planting in the field. This percentage of diseased plants becoming healthy after replanting may also differ in consecutive years (Asjes, 1989). The lower total rates of TRV detected in the 17 and 20 °C series might also be due to the loss of the TRV infection in the bulbs, which, however, is contrary to experience obtained in experiments several years ago.

The TRV undetected by ELISA but traced by test plants, might represent different serotypes which were not traceable with the PV-antiserum. However, only occasionally one or two bulbs or none at all contained the TJ, TY, and UM-coded serotypes as randomly tested during storage. This very low incidence was due to the known source of the infected bulbs used. Presumably this would not have been true, if tulip bulbs of other locations had also been tested in these series of experiments. The main sprout had to be tested as the other sprouts gave variable results (Table 5).

The main sprouts were fairly easy to use from October onwards considering their lengths (Table 1). The inconvenient removal of the main sprout from the bulbs, especi-

ally in the early-dated tests, was not completely overcome in December and later on, when the sprouts protruded outside the bulbs during the storage at 5, 9, and 13 °C. The scale and basal-plate tissue around the sprouts may interfere with the level of detectability considering the irregular occurrence of TRV in bulbs (Van der Vlugt et al., 1988).

As discussed the detection rate of TRV by ELISA in main sprouts of tulip bulbs varies with a. temperature regime during storage; b. identity of TRV serotype and available antisera; c. interference of the irregular occurrence of TRV in the adjacent bulb scale and basal-plate tissue; and d. variable recurrence of TRV in progeny bulbs (Asjes, 1989). Therefore, the routine testing for TRV of bulbs during storage is not as obvious as has been exemplified for other viruses, e.g., TBV in tulip, and TBV and LSV in lily (Van Schadewijk and Eggink, 1984; Van Schadewijk, 1986).

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