ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 16(1) 2017, 105–112
www.acta.media.pl ISSN 1644-0692

ORIGINAL PAPER

Accepted: 22.09.2016

A SURVEY OF VIRUSES' OCCURRENCE IN POLISH AND IMPORTED TULIP BULBS

Dariusz Sochacki[1]✉, Jadwiga Treder[2]

[1] Warsaw University of Life Sciences, Department of Ornamental Plants, 166 Nowoursynowska Street, 02-787 Warsaw, Poland
[2] Research Institute of Horticulture, 1/3 Konstytucji 3 Maja Street, 96-100 Skierniewice, Poland

ABSTRACT

Virus-infected tulip bulbs produce flowers with virus symptoms what makes cut flowers unmarketable. The aim of the work was to check frequency of virus presence in bulbs intended for forcing and originating both from the Polish farms and imported from The Netherlands. The tests were carried out on four tulip cultivars– 'Hermitage', 'Pretty Woman', 'Purple Prince' and 'Strong Gold' during four forcing seasons between 2010 and 2013. Bulb samples were tested by DAS-ELISA using antibodies specific for following viruses: *Tulip breaking*, TBV, *Tobacco necrosis*, TNV, *Lily symptomless*, LSV, *Cucumber mosaic*, CMV, *Tobacco rattle*, TRV (J and F strains) and *Tulip X*, TVX. The most frequently detected virus, both in Polish and Dutch bulbs, was TBV (138 positive results among 1,360 tested samples, during four seasons, what means approx. 10 percent). However, TBV was detected predominantly in bulbs originating from the Polish plantations. Other viruses were detected sporadically. Our results lead to the conclusion that the selection done during tulip vegetation on the examinated Polish farms and removal of diseased plants was not effective and chemical protection against aphids as TBV vectors may be insufficient. Consequently, many tulip bulb lots taken from the examined Polish plantations cannot meet the high quality level of Dutch bulbs, in particular in the case of TBV occurrence.

Key words: DAS-ELISA, forcing, TBV, TRV, TVX, virus diseases

INTRODUCTION

Twenty two viruses which can infect tulips [Mowat 1995] considerably affect yield and quality of bulbs and of forced flowers. Tulip plants are infected mainly by one virus, but sometimes mixed infections occurs [Ward et al. 2008, Sochacki 2013]. Commercial bulb production bases on vegetative propagation, what quickly leads to the accumulation of viruses. The most common in the world and the most dangerous are *Tulip breaking virus*, TBV; *Tobacco necrosis virus*, TNV; *Lily symptomless virus*, LSV; *Cucumber mosaic virus*, CMV and *Tobacco rattle virus*, TRV. Infected tulip bulbs produce flowers with virus symptoms like break, chlorosis and mosaic, what makes cut flowers unmarketable. In Poland cut tulips are very popular. Domestic production of cut tulips in Poland was estimated in 2003 as 110–120 million stems forced on area of 42 hectares [Jabłońska 2005]. Two years later the area increased to 46 hectares, what represented 8% of total area of cut flower production under covers [Jabłońska 2007] and the tendency is rising. Studies on the consumer behavior on the flower market in the capital of Poland, Warsaw showed that the greatest demand is for cut flowers, mainly roses (over 86%), tulips (74%), and flowering pot plants [Jabłońska et al. 2013]. That data shows economic importance of tulips as cut

✉ dariusz_sochacki@sggw.pl

flowers in Poland and importance of quality of bulbs intended for forcing. Tulip bulbs used for forcing in Poland are predominantly imported from The Netherlands. Polish bulbs are used marginally, often being forced at the same farm which produces the bulbs.

The aim of the work was to check the frequency of virus in bulbs intended for forcing originating from the Polish farms and imported from The Netherlands. The former evaluation of virus infections in four tulip plantations in Poland [Sochacki 2013] showed that the most common virus was TBV. Other viruses were detected sporadically, however, increase in LSV and TRV occurrence was noticed from year to year. *Tulip virus X*, TVX has become more important, both in The Netherlands [Beekwilder et al. 2008, Knippels 2011] and in Poland during last few years. In 2011 TVX was detected in few bulb samples of cultivar 'Strong Gold' from the Polish farms and it was the first case of detection of this virus in tulip in Poland [Sochacki and Komorowska 2012].

MATERIAL AND METHODS

The tests were done on four tulip (*Tulipa* L.) cultivars – 'Hermitage' (Triumph Group), 'Pretty Woman' (Lily-Flowered Group), 'Purple Prince' (Single Early Group) and 'Strong Gold' (Triumph), the most important among cultivars forced as cut flowers in Poland. Bulb for testing were taken from bulbs produced on different Polish plantations or from different lots of bulbs imported from the Netherlands and intended for forcing by the Polish growers, during four forcing seasons between 2010 and 2014.

For tests 20 bulbs, in each year, of each cultivar and from each plantation or imported lot were selected. Imported Dutch bulbs were taken in Poland, from bulb lots delivered to tulip growers. Number of sources of tested bulbs was different each season (from 6 and 10, and 6 and 14, for the Polish and Dutch lots, respectively). Totally, bulbs originating from 6 Polish plantations, 5 Dutch export companies and 1 Polish import company were evaluated. The samples were tested by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) [Clark and Adams 1977] using antibodies specific for TBV, TNV, LSV, CMV and TRV (TRV-J and TRV-F) and TVX. Kits of IgG and conjugates for detection of LSV, TBV, TNV and TRV-J and TRV-F originated from the Bulb Quality Support B.V., Lisse, The Netherlands, while that for detection of CMV – from the Research Institute of Horticulture, Skierniewice, Poland [Kamińska et al. 2005]. DAS-ELISA tests were performed according to the protocol of producers, with minor modifications. Bulb scales were grinded in phosphate buffer saline (PBS), pH 7.4 and all conjugates were diluted in PBS-T, containing 0.1% Tween 20 (w/v 1:5).Each sample was tested in 2–3 duplicates on each date. The absorbance was measured at 405 nm with a Ledetect 96 (Dynamica GmbH, Salzburg, Austria) reader. The samples were recorded as infected when the value of absorbance (A_{405}) was at least twice as high as the mean value A_{405} for the negative control [according to Clark et al. 1988]. The values of absorbance exceeding the negative control by 50 to 100% – usual recorded as "suspected" or "plus/minus" – were also recorded as infected.

RESULTS

In the first year of testing (2010) only TBV was detected in four single cases of 200 tested bulbs imported from The Netherlands. In the bulbs originating from the Polish plantations viruses were detected more often. TBV was detected in 1 and 2 bulbs of variety 'Hermitage' and 'Pretty Woman', respectively, and in 6 bulbs of 20 tested in one lot of 'Strong Gold'. LSV and CMV were detected in several individual cases in 'Pretty Woman' and 'Strong Gold' (tab. 1).

In the second year of indexing (2011) only LSV was detected in two single cases of 120 tested bulbs imported from The Netherlands. Both LSV-positive samples originated from variety 'Hermitage', but from two different bulb lots. No TBV nor any other virus were detected in Dutch bulbs in 2011. In Polish bulbs TBV was detected dramatically often, totally in 46 probes of 160 tested, what means almost 29 percent of infected bulbs. Thirty four of TBV-positive probes originated from 3 varieties ('Pretty Woman', 'Purple Prince' and 'Strong Gold') from the same plantation. LSV only in one probe and one of the two TRV-strains tested – TRV-F in two single probes were detected in Polish bulbs of 'Strong Gold' (tab. 2).

Table 1. Occurrence of viruses in Dutch and Polish tulip bulbs devoted for forcing, determined by DAS-ELISA in 2010 year

Tulip cultivar	Bulb lot Dutch (NL) or Polish (PL)	Detected viruses No. of positive probes among 20 tested						
		TBV	TNV	LSV	CMV	TRV-J	TRV-F	TVX
'Hermitage'	PL 01	1	0	0	0	0	0	0
	NL 01	1	0	0	0	0	0	0
	NL 02	1	0	0	0	0	0	0
'Pretty Woman'	PL 01	2	0	0	0	0	0	0
	PL 02	0	0	1	0	0	0	0
	NL 01	0	0	0	0	0	0	0
'Purple Prince'	PL 01	0	0	0	1	0	0	0
	NL 01	0	0	0	0	0	0	0
	NL 02	0	0	0	0	0	0	0
	NL 03	0	0	0	0	0	0	0
'Strong Gold'	PL 01	6	0	0	0	0	0	0
	PL 02	0	0	2	1	0	0	0
	NL 01	1	0	0	0	0	0	0
	NL 02	0	0	0	0	0	0	0
	NL 03	0	0	0	0	0	0	0
	NL 04	1	0	1	0	0	0	0
Total No. of positive probes among 320 tested (120 PL/200 NL)		13(9/4)	0	4(4/0)	2(2/0)	0	0	0

Table 2. Occurrence of viruses in Dutch and Polish tulip bulbs devoted for forcing, determined by DAS-ELISA in 2011 year

Tulip cultivar	Bulb lot Dutch (NL) or Polish (PL)	Detected viruses No. of positive probes among 20 tested						
		TBV	TNV	LSV	CMV	TRV-J	TRV-F	TVX
'Hermitage'	PL 01	4	0	0	0	0	0	0
	PL 02	1	0	0	0	0	0	0
	NL 01	0	0	1	0	0	0	0
	NL 02	0	0	1	0	0	0	0
'Pretty Woman'	PL 01	0	0	0	0	0	0	0
	PL 02	13*	0	0	0	0	0	0
	NL 01	0	0	0	0	0	0	0
'Purple Prince'	PL 01	3	0	0	0	0	1	0
	PL 02	10*	0	0	0	0	0	0
	NL 01	0	0	0	0	0	0	0
'Strong Gold'	PL 01	4	0	0	0	0	0	0
	PL 02	11*	0	1	0	0	1	0
	NL 01	0	0	0	0	0	0	0
	NL 02	0	0	0	0	0	0	0
Total No. of positive probes among 280 tested (160 PL/120 NL)		46(46/0)	0	3(1/2)	0	0	2(2/0)	0

* Results obtained by testing bulbs originating from the same farm

Table 3. Occurrence of viruses in Dutch and Polish tulip bulbs devoted for forcing, determined by DAS-ELISA in 2012 year

Tulip cultivar	Bulb lot Dutch (NL) or Polish (PL)	Detected viruses. No. of positive probes among 20 tested						
		TBV	TNV	LSV	CMV	TRV-J	TRV-F	TVX
'Hermitage'	NL 01	1	0	0	0	0	2	0
	NL 02	0	0	0	0	0	0	0
	NL 03	0	0	0	0	0	0	0
'Pretty Woman'	PL 01	0	0	0	0	0	0	0
	PL 02	9*	0	0	0	0	0	0
	NL 01	0	0	0	2	0	0	0
	NL 02	0	0	0	0	0	0	0
	NL 03	0	0	0	0	0	0	0
'Purple Prince'	PL 01	8*	0	0	0	0	0	1
	NL 01	0	0	0	0	1	1	0
	NL 02	1	1	1	2	0	0	0
	NL 03	0	0	0	0	0	0	0
'Strong Gold'	PL 01	1	0	2	0	0	0	0
	PL 02	9*	0	1	0	0	0	0
	PL 03	1	0	0	0	1	1	0
	NL 01	0	1	1	0	0	0	0
	NL 02	0	0	2	0	1	2	0
Total No. of positive probes among 340 tested (120 PL/220 NL)		30(28/2)	2(0/2)	6(3/3)	4(0/4)	3(1/2)	6(1/5)	1(1/0)

* Results obtained by testing bulbs originating from the same farm

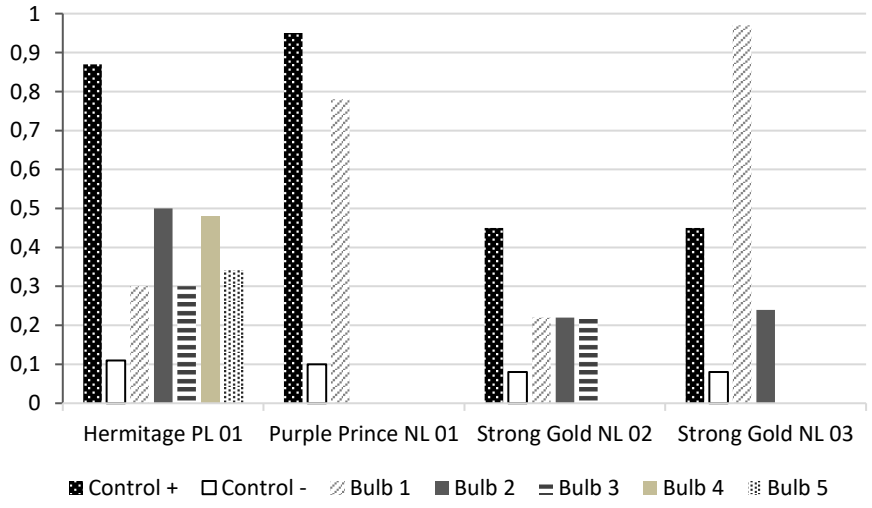

Fig. 1. Absorbance values (A_{405}) of ELISA against TVX – positive results for probes of tulip cultivars 'Hermitage' (lot PL 01), 'Purple Prince' (lot NL 01), 'Strong Gold' (lots NL 02 and NL 03) – bulbs devoted for forcing during the season 2013/14

Table 4. Occurrence of viruses in Dutch and Polish tulip bulbs devoted for forcing, determined by DAS-ELISA in 2013 year

Tulip cultivar	Bulb lot Dutch (NL) or Polish (PL)	Detected viruses No. of positive probes among 20 tested						
		TBV	TNV	LSV	CMV	TRV-J	TRV-F	TVX
'Hermitage'	PL 01	7	1	0	0	0	0	5
	NL 01	1	0	2	0	0	2	0
	NL 02	1	0	0	0	0	0	0
'Pretty Woman'	PL 01	2	0	0	1	0	0	1
	PL 02	9*	0	0	0	0	0	1
	NL 01	0	0	0	2	0	0	0
	NL 02	3	0	0	4	1	0	2
	NL 03	0	0	1	0	0	0	0
	NL 04	0	0	0	0	0	0	0
	NL 05	0	0	0	0	0	0	0
'Purple Prince'	PL 01	0	0	0	0	0	0	0
	PL 02	8*	0	0	0	0	0	1
	NL 01	0	0	0	0	0	0	1
	NL 02	0	0	0	1	0	0	0
'Strong Gold'	PL 01	0	0	0	2	0	0	1
	PL 02	6*	0	1	0	0	0	0
	NL 01	2	0	0	0	0	0	0
	NL 02	0	0	0	1	0	3	3
	NL 03	2	0	2	0	0	0	2
	NL 04	8	0	0	0	0	0	0
	NL 05	0	0	1	0	0	0	0
Total No. of positive probes among 420 tested (140 PL/280 NL)		49(32/17)	1(1/0)	7(1/6)	11(3/8)	1(0/1)	5(0/5)	17(9/8)

* Results obtained by testing bulbs originating from the same farm

In the third season of research TBV was still the most frequent virus detected both in Polish and Dutch bulbs. However, similarly as in previous years, TBV was more often detected in the bulbs from the Polish plantations (28 positive probes of 120 tested, i.e. 23%) in comparison with Dutch (2 positive probes of 220 tested, i.e. 0.9%). Eight – nine TBV-positive probes of 20 tested were noticed in 3 varieties ('Pretty Woman', 'Purple Prince' and 'Strong Gold') from the same plantation, similarly as in 2011. LSV only in two probes and TVX in one probe were detected in Polish bulbs. TNV, LSV, CMV and both strains of TRV were detected only in few cases of Dutch bulbs (tab. 3).

In the last year of testing (tab. 4) TBV, CMV and TVX were detected most frequently, both in Polish and imported lots of bulbs. TBV was detected in 32 probes (of 140 tested) of Polish bulbs and in 17 probes of 280 tested Dutch bulbs. TBV-positive samples were noticed in all cultivars coming from the Polish plantations while in Dutch bulbs TBV was found only in 'Hermitage' and 'Strong Gold'. CMV was detected in 11 samples. Total number of TVX-

positive probes (of Polish and Dutch lots) was 17 and was the highest during the all four seasons of indexing (tab. 4, fig. 1). LSV and TRV-F were found only in Dutch bulbs, totally 7 and 5 times, respectively. On the other hand, TNV was detected only in one sample of 'Hermitage' originating from a Polish plantation.

Quite often tulip bulbs were infected with more than only one virus. During the whole period of the indexing 18 samples were recorded as infected with two or three viruses. In most cases virus complex were consisted of TBV and TVX (5 times) or LSV and TRV-F (3 times).

DISCUSSION

The studies showed that the most common virus, both in Polish and in imported bulbs, is TBV (totally 138 positive results among 1,360 tested samples during four seasons, what means slightly over 10 percent). However, TBV was detected predominantly in bulbs originating from the Polish plantations (115 positive probes from Polish bulbs and 23 – from imported bulbs, what means 21.3 and 2.8 percent, respectively). Polder at al. [2014] reports a number of TBV-infected tulips in The Netherlands as 1.5 percent in 2011. Our results confirmed earlier research done on several Polish tulip plantations during the period 2006–2011 [Sochacki 2013], showing a high level of infection by the most common TBV, particularly in the yellow cultivar 'Strong Gold'. Other viruses detected in Polish and imported bulbs were: LSV (totally 25 positives), CMV, both strains of TRV (TRV-J and TRV-F) and TVX (17 positives for each virus), and TNV (only 3 positive probes of all tested). Except TBV, other viruses were detected rather in Dutch bulbs and less so in Polish bulbs. Such situation underlines TBV-infections as a predominant viral disease in tulip crops in Poland. Also in The Netherlands this virus is the most common, however, during the first three years no viruses were detected in imported bulbs or they were detected occasionally, usually in 1–2 samples out of 20 examined. In the final year of examinations an increase in the detectability of viruses was noticed in imported bulbs. What more, for the first time TVX was then detected in Dutch bulbs (in 7 samples coming from 3 different lots of bulbs). TVX in bulbs originating from Polish plantations was detected first time in the 3rd year of examinations. This fact confirms other report on TVX occurrence in Polish tulip crops in 2011 year [Sochacki and Komorowska 2012].

Mixed virus infections in tulip crops noticed during present investigations were reported also earlier by Ward et al. [2008] and Sochacki [2013], however, in different virus combinations.

The results showed, that bulbs imported from The Netherlands were more healthy than bulbs originating from Polish farms. It is commonly known, that negative selection of plants during the vegetation is of central importance for a healthy status of harvested tulip bulbs. During the inspection, all non-true-to--type or diseased plants should be removed (rogued out) from the field and destroyed [Okubo and Sochacki 2012]. In The Netherlands – world's leader in tulip bulbs production – quality inspection system for flower bulbs has been established many years ago, starting with foundation of The Dutch Flower Bulb Inspection Service (Bloembollenkeuringsdienst, BKD) in 1923 [Knippels 2005]. According to the existing inspection schemes and criteria of the BKD, all tulip fields and lots of flower bulbs are inspected and classified. In addition, for many tulip cultivars serological tests are obligatory. There are so called "ELISA-varieties", including as well white and yellow flowering cultivars such 'Yokohama', 'Monte Carlo' and 'Strong Gold' [Knippels 2005, Knippels 2011]. In Poland the area of tulip bulb production is limited to 200 hectares, which is rather small, especially in comparison to the Dutch area of 10.800 hectares [Buschman 2005]. The Polish State Plant Health and Seed Inspection Service perform regular inspections of registered producers offering to the market their bulbs or other plant products and objects, which may pose a phytosanitary risk of spreading quarantine organisms. However, the above control unit is not an inspection service dedicated to flower bulbs. Moreover, no virological tests are included in the inspection system. Virus-infection level of Polish tulip bulbs depends on effectiveness of roguing done by growers. It is noteworthy, that very

high level of TBV-positives in the lots of Polish bulbs in 2nd and 3rd year of investigations was associated only with one and the same farm. Thirty four and 26 TBV-positive probes of 46 and 28 TBV-positive probes of Polish bulbs respectively, were obtained from bulbs coming from the same farm. Similar situation occurred in the last year, when from 32 TBV-positive probes of Polish bulbs the virus was detected in 23 bulbs of 3 cultivars originating from one Polish plantation. Such high level of virus infections in bulbs originated from this one farm, suggest that plant protection and selection of plants with viruses symptoms were done unprofessionally.

Our results lead to the conclusion that the roguing done on certain of the examinated Polish farms is not effectiveand chemical protection against aphids as a vectors of TBV may be insufficient there. Consequently, many tulip bulb lots, originating from the examinated Polish plantations cannot meet high quality level of Dutch bulbs, in particular in the case of TBV.

CONCLUSIONS

The most frequently detected virus, both in Polish and Dutch bulbs, was TBV (138 positive results among 1,360 tested samples, during four seasons, what means approx. 10 percent). However, TBV was detected predominantly in bulbs originating from the Polish plantations (115 positive probes from the Polish bulbs while 23 from imported bulbs). Other viruses were detected sporadically. During the first three years in imported bulb lots no viruses were detected or they were detected occasionally, usually in 1–2 samples out of 20 examined per lot. In the final year of examinations the virus detectability in imported bulbs increased. As for TVX it was first detected in Dutch bulbs in the 4th season while in bulbs originating from the Polish plantations it was found in the 3rd year of trials. Our results lead to the conclusion that the negative selection done on some of the examinated Polish farms is not effective and chemical protection against aphids as TBV vectors may be insufficient. Consequently, many tulip bulb lots originating from the examinated Polish plantations cannot meet the high quality level of Dutch bulbs, in particular in the case of TBV.

ACKNOWLEDGEMENTS

We thank the tulip bulbs' producers and growers importing tulips bulbs who provided the plant material used during the research.

REFERENCES

Beekwilder, J., van Houwelingen, A., van Beckhoven, J., Speksnijder, A. (2008). Stable recombinant alpaca antibodies for detection of *Tulip virus X*. Eur. J. Plant Pathol., 121, 477–485.

Buschman, J.C.M. (2005). Globalisation – flower – flower bulbs – bulb flowers. Acta Hort., 673, 27–33.

Clark, M.F., Adams, A.N. (1977).Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 34, 475–483.

Clark, M.F., Lister, R.M., Bar-Joseph, M. (1988). ELISA Techniques. In: Methods for Plant Molecular Biology, Weissbaum, A., Weissbaum, H. (eds.). Acad. Press, 527.

Jabłońska, L. (2005). Rozwój polskiego kwiaciarstwa w minionym 15-leciu. Zesz. Probl. Post. Nauk Roln., 504, 21–31 (in Polish, with English abstract).

Jabłońska, L. (2007). Ekonomiczne aspekty rozwoju sektora kwiaciarskiego w Polsce. Wyd. SGGW, Warszawa (in Polish).

Jabłońska, L., Olewnicki, D., Ragan, M., (2013). Zachowania konsumenckie na warszawskim rynku kwiatów ciętych i doniczkowych. Zesz. Nauk. SGGW. Polit. Europ., Fin. Market., 9(58), 220–230 (in Polish, with English abstract).

Kamińska, M., Śliwa, H., Malinowski, T. (2005). Partial characterisation of cucumber mosaic virus isolate infecting *Loniceraca prifolium* L. plants. Acta Sci. Pol. Hortorum Cultus, 4(2), 3–10.

Knippels, P.J.M. (2005). The contribution of quality inspections to the improvement of the quality of the Dutch flower bulbs and access to export markets. Acta Hort., 673, 79–84.

Knippels, P.J.M. (2011). Recent developments in the inspection schemes of flower bulbs. Acta Hort., 886, 147–151.

Mowat, W.P. (1995). Tulip. In: Virus and virus-like diseases of bulb and flower crops, Loebenstein, G., Lawson, R.H., Brunt, A.A. (eds.). John Wiley & Sons, Chichester, New York, Brisbone, Toronto, Singapore, 352–383.

Okubo, H., Sochacki, D. (2012). Botanical and horticultural aspects of major ornamental geophytes. In: Ornamental geophytes. From basic science to sustainable production, Kamenetsky, R. Okubo, H. (eds.). CRC Press. Taylor and Francis Group, Boca Raton – London – New York, 77–121.

Polder, G., van der Heijden, G.W.A.M., van Doorn, J., Baltissen, T.A.H.M.C. (2014). Automatic detection of tulip breaking virus (TBV) in tulip fields using machine vision. Biosyst. Eng., 117, 35–42.

Sochacki, D. (2013). The occurrence of the viruses in tulip crops in Poland. J. Hort. Res., 21(1), 5–9.

Sochacki, D., Komorowska, B. (2012). First report of *Tulip virus X* on tulip in Poland. Plant Dis., 96, 4, 594–594.

Ward, L.I., Tang, J., Quinn, B.D., Martin, E.J., Clover, G.R.G. (2008). First report of *Tulip virus X* in New Zealand. New Dis. Rep., 17, 22.