CONTROL REVIEW OF AIR-BORNE TULIP BREAKING VIRUS AND LILY SYMPTOMLESS VIRUS IN LILIUM IN THE NETHERLANDS.

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Abstract

In this study on the air-borne viruses in *Lilium*, mainly lily symptomless virus (LSV) and tulip breaking virus (TBV), various factors involved in the efficiency of control are dealt with. The incidence of LSV was reduced very substantially from the old 100% rate. TBV occurs at fairly low rates. Lily virus X (LVX) and cucumber mosaic virus (CMV) occurred at low level in the last decade. The symptoms of TBV to induce the roguing of diseased plants became less important, if the ELISA-testing of bulbs before bulk propagation by the scaling of bulbs and by tissue culture procedures was effectively applied. The reduction in bulb yield and the relative reduced quality of cut flowers indicate the need for virus control. The virus spread as affected by the elimination of virus source plants strongly became dependent on the ELISA-testing. The speed of spread was generally experienced to be differentially rapid over the season, while a range of c. 25 aphid species were able to transmit LSV and TBV. The chemical control of spread is efficiently done by the routine spraying of mineral oil plus pyrethroid. Terpenoids were not effective. Some effect was observed from a pheromone, plant oil, and β-pinene polymer emulsion. Deltamethrin and zetamethrin sprays were most effective. The non-chemical control by the polymer web coverage of crops proved impractical under field conditions. The factors in the chain of control which have a weak impact, e.g., delayed elimination of TBV-diseased plants in the field if the ELISA-bulb testing prior to planting is routinely unapplicable in varieties of *L. longiflorium* and Oriental hybrids, or strong impact, e.g., testing of bulbs by ELISA of a large part of the assortiment of cultivars, chemical control of virus spread, and rapid propagation procedures, on the efficiency to improve the health situation in the lily culture were indicated.

Introduction

The lily acreage increased from tens of hectares in the early 1960s up to c. 3500 ha in 1995 for various reasons. One was the growers' routine spraying of mineral oil and synthetic pyrethroids to effectively curtail the spread of non-persistently transmitted viruses, e.g., lily symptomless virus (LSV; ahpid-borne; carlavirus), tulip breaking virus (TBV; aphid-borne; potyvirus) and cucumber mosaic virus (CMV; aphid-borne; cucumovirus), and the persistently transmissible lily virus X (LVX; potexvirus with an unknown vector; Asjes, 1976; 1981; 1984; 1985; 1989; 1990; 1991; Asjes and Blom-Barnhoorn, 1994). However, other factors also determine the control of the virus situation, e.g., incidence of viruses, symptoms, yield-reduction aspects, detection by visual and serological means, virus spread as affected by the elimination of virus sources, speed of spread and chemical control, and the methods of propagation to restart from the best possible health state to

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build up large lots of bulbs. These factors are reviewed on the basis of results obtained in past decades, and additional data on transmission by aphid species and on chemical and on non-chemical control of virus spread will be presented.

Material and methods

Plant material

Control of TBV-infection in cut-flower production. The bulbs of *Lillium* cv. Star Gazer (12-14 cm in circumference; 100% LSV, less than 1% TBV) were interplanted with *L. longiflorum* cv. Gelria (100% LSV, c. 30% TBV) in the open field. Iin 1993 bulbs (100/plot; in duplicate) were planted on April 15 and June 1, and in 1992 on April 22.

Control of virus infection in bulb production. The bulbs (9-10 cm) to produce stalk-forming plants, and 5-6 cm in circumference for non-stalk-forming lilies (c. 98%)) of cv. Enchantment at rates of 0-2% LSV, and c. 2% TBV were planted in early April. Flower buds were picked in early July. Initial virus rates were subtracted from the infection data.

Experimental lay-out

Cut-flower production. The plots were planted with 100 bulbs *L.* cv. Star Gazer in ten rows in 1m-wide beds with 18 cm interrow distance. *L. longiflorum* cv. Gelria was interplanted at row 3, 7, and 11.

Bulb production. The plots in triplicate were planted in two pairs of five rows (18 cm between the rows) in 1-m wide beds. One row of *L.* cv. Enchantment (100% LSV and TBV) was planted between the two pairs.

In both types of experiments the distance between plots was 0.75 m in the beds, and 2.0 m between beds by flanking paths of 0.5 m and a 1-m wide bed with dahlias in 1993 and grass in 1994. The treatments were arranged in randomized blocks.

Virus transmission. In 1990 lily cvs. Montreux and Enchantment infected with LSV and TBV at very high rate were grown, in which migrating aphids were caught on a whitish gauze screen. In 1991 and 1992 *L.* cv. Enchantment was used. The lily bulbs cv. Enchantment (9-10 cm in circumference) to be inoculated contained less than 1% LSV or TBV, which bulbs were discarded beforehand by the additional testing with ELISA of bulbs and leaf material. The bulbs were planted in pots at different dates to produce susceptible plants throughout the season. The lilies were grown in an aphid-proof gauze house before and after the transmission procedure. Flying aphids caught on whitish gauze screens were handled for different trial options. The aphids were either directly put on virus-tested lilies, or given intermediate access to LSV + TBV-infected leaves of cvs. Enchantment and Montreux, or put on virus-tested lilies with an additional virus-infected piece of leaf of cv. Enchantment. The aphids were individually given access and kept on the leaf in small circular cages (3.5 cm in diameter, 1 cm in height, and via sieve holes connected with open air). Contrary to 1990 and 1992 the aphids were starved (1 hr) before transfer to plant tissue. The direct catch of aphids from the air and immediate transfer to plants was done in 1990 and not in 1991 and 1992. The intermediate access was for 1-2 hours, while the inoculation period on the plants to be infected was lengthened to some hours. The individual aphids were kept in 70% ethanol until identification. In 1990 all caught aphids were identified, whereas in 1991 and 1992 this was done for the transmitting species only. The bulbs were tested with ELISA in January of the following year.

Fleece cover of crops

A propylene fiber fleece (polymer web; 17 g/m^2; Lutrasil) effective to prevent completely potyvirus spread in potato experiments (Harrewijn et al., 1991) and a fleece (30 g/m^2)

were tested. In the cut-flower trials plants were covered at emergence, and in the other experiments from late April-1st week of May.

Chemicals
The natural monoterpenoid myrcene (Harrewijn et al., 1994) was tested as 1. 0.5% myrcene (Aldrich), 0.5% ionol (Bayer) and 0.5% Tween-20 (Sigma); 2. 0.5% myrcene, 0.5% (E)-ß-farnesene, 1% ionol and 1.0% Tween-20; and 3. 0.5% ionol, and 0.5% Tween-20. The pheromone (E)-ß-farnesene was in a liquid formulation and in a dust carrier (Denka International, Barneveld). The insecticide imidacloprid in Admire (70% a.i.; Bayer BV, Arnhem) was applied to immerse bulbs (0.04% solution) before planting and to spray on plants in the field (20 g ha^{-1}). The pyrethroids were deltamethrin in Decis (2.5% a.i.; 10 g ha^{-1}) and zetamethrin in Fury (ew; 10% a.i.; 20 g ha^{-1}; Luxan BV, Elst). The plant oils consisted of rapeseed oil at high and low erucic acid rate. β-pinene polymer on Wilt Pruf (1:6 dilution of product; Wilt-Pruf Products, Connecticut) was tested. The mineral-oil/pyrethroid spray means the use of Luxan oil H (93% oil; 6.25 l ha^{-1}) plus deltamethrin. All chemicals were sprayed in volumes equivalent to 400 l ha^{-1}.

Applications of sprays
Emulsions prepared shortly before use were sprayed with a propane knapsack sprayer (Birchmeijer Helico nozzles 1.2) at 400 kPa. The weekly spraying in May, June and July started in the first week of May. In August-early September fortnightly sprays were applied. The cut-flower trials were sprayed weekly.

Assessment of virus infection and bulb weight
Virus infection of cut flowers. In some Oriental cultivars, e.g., cv. Star Gazer, in plants of which the bulbs were preparatively stored at -1°C severe TBV-symptoms may develop within 3-4 weeks or more, which consist of a mosaic in the upper leaves and yellowing and browning of lower leaves, which results in the early death of plants (Derks and Muller, 1993).
Bulb production experiments. The bulbs were harvested in mid-October and stored at 0-1°C till January. Then the bulbs were cleaned from soil debris. The mean weight ratios were calculated as (weight of n-treated bulbs): (weight of n-control bulbs) x 100. The presence of TBV, LSV, and occasionally LVX in scales of individual bulbs was assessed by DAS-ELISA (Van Schadewijk, 1986). The virus rates were calculated on the basis of single infections per plant of the two viruses LSV and TBV.

Past and present results
Virus incidence
The symptomless field appearance of LSV precludes the effective roguing of infected plants. The overall incidence reduced most substantially by the use of virus-ELISA-tested material. The incidence of TBV either detected by visible symptoms or the ELISA-testing of bulbs in cultivars except those of *L. longiflorum* and Oriental hybrids was strongly reduced. The types of TBV differently infectious to various lily groups were found to differ in host range of lilies and herbaceous hosts, but these were not distinguishable serologically (Derks and Lemmers, 1991; Derks et al., 1994). The reintroduction of the name Lily mottle virus instead of TBV is pursued (Dekker et al., 1993; Derks et al., 1994). CMV occurs at low rate. The incidence of LVX was strongly reduced as a result of the ELISA-testing of bulbs and the control by insecticide sprays (Asjes, 1991).

Symptoms and detection

LSV in field plants is apparently symptomless. In cut flowers the vase life is shortened by an earlier yellowing of the lower leaves (Boontjes, 1978). LVX occurs symptomlessly (Derks and Hendriks, 1990). TBV is expressed in mosaic in leaves, breaking of the flower colour, and occasionally ring patterns in bulbs, which is different in cultivars due to their sensitivity (Derks, 1988). TBV symptoms helpful in the roguing of plants are still important in some *L. longiflorum* and Oriental cultivars. The laborious roguing of field plants can be replaced in many cultivars by the use of ELISA to build up well-qualified stocks.

Bulb yield

The qualitative impact of TBV in cut flowers is noted above, and LSV shortens the vase life (Boontjes, 1978). The reduction in bulb yield of TBV- and LSV-infected lilies (Boontjes, 1987) encourages growers to grow virus-tested lilies.

Virus spread

Elimination of virus sources. Initial virus infection rates in bulb lots considerably affects virus spread, which implies the necessity of their reduction. The impact of roguing plants with visible symptoms and/or the testing of bulbs by ELISA to build up qualitatively best-improved lots became evident, while the stocks with high infection rates could be disapproved for further propagation. The Flowerbulb Inspection Service supervises and implemented the ELISA-testing service most accurately (Asjes, 1990).

Speed of spread. In very susceptible cultivars the viruses spread very rapidly if 5-10% source plants are present in the lots (Asjes 1985; 1989; 1991; Asjes and Blom-Barnhoorn, 1994). The susceptibility of plants before flowering is at high level, whereas circumstantial evidence indicates the development of mature plant resistance after flowering (Asjes and Blom-Barnhoorn, 1994).

The results on LSV- and TBV transmission by aphid species trapped on a gauze screen in 1990-1992 are shown in Table 1. The transmission rate of the aphids is indicated by the number of transmitting aphids: total of tested aphids. The percentages of virus transmission were in 1990: 35 : 593 = 5.9%; 1991: 20 : 416 = 4,8%, and 1992: 15 : 498 = 3.0%. The rate of virus transmission by aphids caught and directly put on virus-tested plants in 1990 was substantial: 3.3% versus the total of 5.9%. LVX was not transmitted in any transfer.

LSV was transmitted by *Aphis sp., Brevicoryne brassicae, Myzus persicae*, and *Uroleucon sp.*. TBV was transmitted by *Acyrthosiphum pisum*: 2/7 (virus transmission/aphids caught), *Anoecia corni*: 1/53, *Aphis species*: 6/106, *Brevicoryne brassicae*: 1/10, *Cavariella theobaldi:* 1/7, *Capitophorus sp.*: 2/7, *Dysaphis sp.*: 2/4, *Hyalopterus pruni*: 1/17, *Hyperomyzus lactucae*: 2/28, *H. pallidus*: 3/14, *Kallistaphis basalis*: 1/1, *Macrospiphum rosae:* 1/1, *Myzus cerasi*: 1/2, *M. persicae*: 2/13, *Nasonovia ribisnigri*: 1/4, *Rhopalosiphum padi*: 2/69, *Tetraneura ulmi*: 1/3, and *Uroleucon sp.*: 1/24. The 18 species for which transmission was shown accounted for 62% of aphids trapped in 1990. Overall 76 species were caught. Some species caught in relative large numbers, e.g., *C.aegopodii* (51), *Metoplolophium dirhodum* (13), and *Sitobion avenae* (17) were not found to transmit virus in 1990, however, the latter two did in 1991-1992. TBV was more effectively transmitted than LSV, and almost all as single infection except three.

In 1991 and 1992 LSV was transmitted by 9 species: *A. pisum, Aphis sp., Liosomaphis berberidis, Metopolophium dirhodum, M. persicae, R. insertum*, and *R. padi*. TBV was transmitted by *A. pisum, A. corni, Aphis sp., B. persicaecola, C. hippophaes, Liaphis erysimi, Macrosiphoniella sejuncta, M. dirhodum, R. padi, R. pilipes, S. avenae*, and

Uroleucon sp. In 1990-1992 LSV was transmitted by 11 aphid species and TBV by 24.

Chemical control

Mineral-oil sprays have been applied since the mid-1960s (Asjes, 1976), and differences in efficacy of oil brands became evident (Asjes, 1984). The synthetic pyrethroid insecticides effective both on the acquisition and inoculation in the virus transmission were introduced in early 1980s (Asjes, 1981). The decrease in volume of mineral oil compensated by added pyrethroid was applied from early 1990 (Asjes, 1989; 1991). The spray frequency was adapted to rapid spread in May-July, i.e., weekly, and fairly slow spread in August-September, i.e., fortnightly sprays (Asjes and Blom-Barnhoorn, 1994). The use of plant oils and pheromone was not applicable as yet (Asjes and Blom-Barnhoorn, 1994). The search for substitutes is still pursued. The results on compounds tested in 1994 are shown in Table 2. The data indicate that sprays of terpenoid, pheromone, imidacloprid, plant oil, and an anti-transpirant were moderately till not effective. Deltamethrin was much more effective than observed before (Asjes, 1985; 1991; Asjes and Blom-Barnhoorn, 1994). The mineral-oil/pyrethroid spray was most effective.

Non-chemical control

Polymer webs to cover and protect crops from virus spread were compared with the effect of mineral-oil/pyrethroid sprays in experiments for cut-flower and bulb production.
Cut flower production. The results on the protection from primary TBV-infection in cut flowers of *L.* cv. Star Gazer are shown in Table 3. The data indicate that a cover for 12 weeks protected completely from infection in the April 15-planting, which did not occur in the June 1-crop. The mineral-oil/pyrethroid sprays more effectively protected plants from infection in the first than in the second series. The distortion of buds started within eight weeks after emergence. In 1994 the sprayed plots rated 0-0.5% TBV, whereas the fleece-covered plots were infected at 2.0% (4 wks), 12.5% (8 wks), and 9.0% (12 wks). The bud distortion by the sprays was 33% (6 wks) and 52% (8 wks).
Bulb production. In 1993 the TBV + LSV infection of *L.* cv. Enchantment rated 75.7% in the untreated plots, whereas the covered plots were infected at 21.1% (17 g/m^2-fleece) and 18.4% (30 g/m^2-fleece).

Propagation

The scaling of bulbs to achieve substantially increased propagation per bulb enhances the aim to build up bulky virus-tested foundation stocks grown in a gauze house. The latter will be the more true by the well-established application of tissue culture procedures by which hundreds of bulblets can be harvested from one bulb (Van Aartrijk and Van der Linde, 1986).

Discussion

Since the 1960s the health state in the lily culture improved most substantially. The reduction of bulb yields and the qualitative impact of virus infection induced the developments of improvement.
The strong items in the chain of control factors are 1. the ELISA-testing of bulbs of the larger part of the assortment of cultivars, by which growers know about the health state of their lots at an early stage of propagation; 2. the rapid propagation by the scaling of bulbs and tissue culture is most important to build up stocks of best quality; 3. the mineral-oil/pyrethroid sprays efficiently curtail the air-borne virus spread. A fairly weak link in the chain is the ELISA-testing of bulbs not applicable to all cultivars. The diversity of TBVs

is not really a weak item as the spread as potyvirus does not change.
The difference in efficiency of virus(es) transmission due to some aphid species only was not found. Some tree species even tranmitted LSV and TBV. This might be a characteristic of the virus group (carla, poty). Until now it was not shown that a potyvirus could be transmitted to potato plants by tree aphids (De Bokx and Piron, 1990). Single virus infections were mostly obtained, while the incidence of multiple infection in one transfer should be confirmed by further tests. LVX is assumed to be air-borne as insecticides curtail its spread (Asjes, 1991), but could not be associated with any of the tested species.
The effect of myrcene by which aphids refuse to penetrate into plants (Harrewijn et al., 1994) was not reflected in the control of virus spread, which may be due to the rapid decrease of the terpenoids, partly by evaporation, partly by decomposition, under field conditions. The short chain polymer product of β-pinene lost its effect fairly quickly under field conditions.
The non-chemical control by polymer-web-crop cover proved inappropriate due to rupture of the tissue too easily on stalk-forming lilies, by straw remains left on soil, and by the effect of strong winds. The weed growth is less easily controllable, and aphid intrusion may occasionally worsen the situation by which virus spread accelerates.
The need to produce lilies with the lowest virus(es) rates to avoid occasional undesirable current-season infection in cut-flower production is a matter of constant concern. The complexity of control still has some weak links in the chain, which need further investigation.

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Table 1: Virus infection of *Lilium* cv. Enchantment by alate aphid species trapped on a whitish gauze screen, by transmission either directly from air, or with an intermediate virus source, or with an added source in 1990-1992.

Virus	Number of infected plants after aphid transfer									total
	directly from air			intermediate virus source			added virus source			
	1990	1991	1992	1990	1991	1992	1990	1991	1992	
Lily symptomless virus	1	-	-	2	4	3	1	4	0	15
Tulip breaking virus	18	-	-	1	4	8	9	8	4	52
Lily virus X	0	-	-	0	0	0	0	0	0	0
Lily symptomless + tulip breaking virus	1	-	-	0	0	0	2	0	0	3
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Total number		20	-	-	3	8	11	12	2	4
70										
% transmission	3.3	-	-	0.5	1.9	2.2	2.0	2.9	0.8	4.6

Table 2: Effect of terpenoids, pheromone, insecticides, plant oils, anti-transpirant on the spread of lily symptomless virus and tulip breaking virus in *Lilium* cv. Enchantment in 1994.

Treatment	Type of material	% LSV + % spread TBS	reduction	Weight ratio
Untreated	-	59.3	0	100
Myrcene	terpenoid	51.3	13	97
Myrcene + (E)-ß-farnesene	terpenoid + pheromone	50.4	15	98
Ionol + Tween-20	formulation carrier	51.5	13	95
Liquid-(E)-ß-farnesene	pheromone	41.3	30	101
Dust-(E)-ß-farnesene	pheromone	53.8	9	97
Dust	pheromone carrier	63.0	-6	98
Imidacloprid-immerse	insecticide	67.4	-14	98
Imidacloprid-spray	insecticide	37.4	37	101
Deltamethrin	pyrethroid	12.0	80	99
Zetamethrin	pyrethroid	20.2	66	101
Rapessed (HEA'))	plant oil	43.7	26	91
Rapessed (LEA'))	plant oil	67.4	-14	99
β-pinene polymer	anti-transpirant	34.9	40	99
Luxan oil H + deltamethrin	mineral oil+pyrethroid	5.9	90	96

* HEA = high erucic acid rate; LEA = low accid rate.

Table 3: Effect of polymer-web (17 g/m^2) cover and mineral oil + pyrethroid sprays on the primary infection of tulip breaking virus and on the flower-bud quality of *Lilium* c.v. Star Gazer in 1993.

Treatment	Period in weeks	Spray frequency	% TBV in plantings of April 15	June 1	% distorted buds ') in plantings of April 15	June 1
Untreated	-	-	16.2	18.3	0.7	0.7
Polymer web	4	-	16.5	5.0	1.0	0.5
Polymer web	8	-	7.0 ")	18.5	0.1	0
Polymer web	12	-	0	18.0	1	0
Oil/pyrethroid	4	7 days	8.5	10.0	1	1
Oil/pyrethroid	4	3/4 days	1.5	5.0	5	0
Oil/pyrethroid	8	7 days	3.5	5.5	25	26
Oil/pyrethroid	8	3/4 days	1.5	5.0	31	26
Oil/pyrethroid	12	7 days	1.5	3.5	20	35
Oil/pyrethroid	12	3/4 days	0	3.5	34	4

') Data courtesy E.A.C.M. Brooymans and B.J. Kok (BRC-Lisse); ") Fleece was torn erractically with a few holes.