

*Pelargonium* × *hortorum*

**Pelargonium flower break virus and tomato ringspot virus:  
Infection trials, symptomatology and diagnosis**

*Pelargonium* × *hortorum*

*Pelargonie-blomsterspætningsvirus og tomatringpletvirus:*

*Infektionsforsøg, symptomudvikling og diagnosticering*

**Niels Paludan and Jens Begtrup**

**Summary**

Many viruses have been found and described in *Pelargonium* × *hortorum*, causing either constant or seasonal leaf and flower symptoms in sensitive cultivars.

These viruses are spread to the next generation as the plants are vegetatively propagated.

By the further cultivation and handling of the plant material, the viruses may spread to even more sensitive cultivars where they may cause serious symptoms.

In order to get a better understanding of the virus diseases in pelargonium investigations concerning symptomatology, infection trial and diagnosis were carried out comprising the two most common viruses occurring in the Danish pelargonium crops, the pelargonium flower break virus (PFBV) and the tomato ringspot virus (TomRV).

Correlation was found between the occurring viruses and the developed symptoms in the sensitive cultivar 'Springtime Irene', the PFBV causing serious leaf and flower symptoms. The symptoms were strongly increased by a simultaneous infection of TomRV.

Single or double infection of PFBV and TomRV caused leaf symptoms, but never flower symptoms in five other cultivars.

Pelargonium line pattern virus (PLPV) and pelargonium ring pattern virus (PRPV) were also found in some of the cultivars as latent viruses, while the pelargonium leaf curl virus (PLCV) was absent in all the plant material.

In 'Springtime Irene' the PFBV and the TomRV can both be diagnosed all the year round by symptomatology, bio-assay or immunosorbent electron microscopy (ISEM), all three methods being for the most part equally sensitive. In other cultivars bio-assay and ISEM are most sensitive.

Using the ISEM-method particles of PFBV were always found in high concentrations the year round, whereas the TomRV occurred in lower concentrations and with seasonal variations.

PFBV spread easily among pelargonium plants with infected sap and contaminated knives.

**Key words:** *Pelargonium* × *hortorum*, pelargonium flower break virus, tomato ringspot virus, infection trials, symptomatology, diagnosis.

## Resumé

Mange virus er fundet og beskrevet i *Pelargonium × hortorum*, hvor de forårsager enten vedvarende eller sæsonmæssige blad- og blomstersymptomer i følsomme sorter.

Disse virus følger med formeringsmaterialet til den næste generation, som regel uden at vise symptomer. Ved den fortsatte kultur og håndtering af plantematerialet kan viruset blive spredt yderligere blandt andet til følsomme sorter.

For at få en større forståelse af virussygdomme i pelargonie blev der gennemført symptomregistrering, infektionsforsøg og diagnostiske undersøgelser omfattende de to almindeligst forekommende virus i danske pelargoniekulturer, nemlig pelargonie-blomsterspætningvirus (PBSV) og tomatringpletvirus (TomRV).

Overensstemmelse blev fundet mellem de forekommende virus og de udviklede symptomer i den følsomme sort 'Springtime Irene', hvor PBSV forårsagede alvorlige blad- og blomstersymptomer. Symptomerne blev betydeligt forstærket ved en samtidig infektion med TomRV.

Enkel eller dobbel infektion af PBSV og TomRV forårsagede bladsymptomer, men aldrig blomstersymptomer i fem andre sorter.

Pelargonie-linjemosaikvirus og pelargonie-ringmosaikvirus blev desuden fundet som latente virus i mange sorter, medens pelargonie-krøllemosaikvirus ikke blev påvist i hele plantematerialet.

I 'Springtime Irene' kan både PBSV og TomRV diagnosticeres hele året igennem enten ved symptomregistrering, indikatorplanter eller immunosorbent elektronmikroskopi (ISEM), hvor alle tre metoder stort set er lige følsomme.

Ved ISEM-metoden blev partikler af PBSV altid fundet i høj koncentration hele året igennem i modsætning til TomRV, der forekom i en lavere koncentration med sæsonmæssig variation.

PBSV blev nemt spredt mellem pelargonieplanter med inficeret saft og kontamineret kniv.

**Nøgleord:** *Pelargonium × hortorum*, pelargonie-blomsterspætningvirus, tomatringpletvirus, infektionsforsøg, symptomudvikling, diagnosticering.

## Introduction

The pelargonium plant is a very popular plant in most European countries, used indoors as a pot plant and outdoors for decorative purposes in gardens and on balconies.

As a vegetatively propagated plant many viruses have arisen over the years.

In greenhouse crops many different viruses have been found where the importance of each virus has changed during the decades due to the epidemiology of the virus, the sensitivity of the cultivar, the cultivation method and traditions (4, 5, 6, 13, 16, 17, 18, 19).

The effect of virus infection has also been described (2,15).

Earlier, before the polyethylene glycol (PEG) was introduced, diagnosis of the viruses in pelargonium was nearly impossible due to the phenols in the plant sap.

The addition of the PEG and the following absorbance of the phenols made it possible to transfer virus infection by bio-assay and improve the sensitivity of serological methods (1,3,14).

Preliminary Danish results concerning spread, symptomatology and diagnosis of viruses in *Pelargonium × hortorum* have shown the following results:

Pelargonium flower break virus (PFBV) and tomato ringspot virus (TomRV) are the two most frequently occurring viruses in Denmark (5,12).

PFBV infected sap from *Tetragonia expansa* has a dilution end point at  $2 \times 10^{-6}$ , a thermal inactivation point at 60°C and a longevity in vitro at 20°C of 32 days (7).

Dry inoculation to young plants of *Chenopodium quinoa* at the 4–6 leaf stages without any pretreatment has been the most sensitive bio-assay method, comprising nine virus isolates, compared with bigger and lightdeprived plants, sap inoculation and grafting to seed plants of the cultivar 'Carefree' (8).

PFBV and TomRV can both be recognized all the year round by the developed symptoms in leaves and flowers, and they can both be diagnosed by dry inoculation to *Chenopodium quinoa* and by the ISEM method. Furthermore the PFBV is easily transmitted amongst pelargonium plants when the material is handled (9,10,11).

The final results from the above mentioned research work carried out over the years 1983–85 at the Institute of Plant Pathology in Lyngby is described in this paper, comprising the following three topics:

1. Virus diagnosis by ISEM all the year round of collected pelargonium plants showing virus-like symptoms.
2. PFBV and TomRV-diagnosis all the year round based on symptomatology, bio-assay and ISEM-assay.
3. Transmission of PFBV amongst pelargonium plants by infected plant sap using sap inoculation and knife.

## Methods

### *Plant material and growth conditions*

The plant material consisted of the following cultivars of *Pelargonium × hortorum* 'Amanda' (A), 'Penny Irene' (PI), 'Springtime Irene' (SI), 'Treasure Chest' (TS) and 'Vesuv' (V).

The plant material was collected as diseased showing more or less vigorous leaf and/or flower symptoms. The SI consisted of ten different isolates of which one was made virusfree by heat treatment and meristem-tip culture.

The plants were grown under normal greenhouse conditions, i.e. 18°C during the day

and 16°C during the night at winter time, and up to 30–32°C during the warmest summer period. The plants were illuminated during the winter season with four hours additional assimilation light to stimulate growth. The supply of water and nutrient was performed by the drip-water method and controlled after the evaporation. The concentration of the nutrient, using the Hornum mixture of macro- and microelements, was one per thousand during the winter time and 1.5–2 per thousand during the summer.

### *Infection trials*

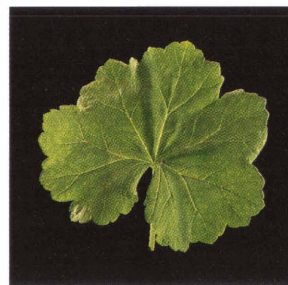
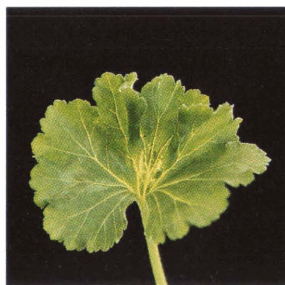
Different methods such as sap inoculation, cutting with knife and grafting have been used in order to infect pelargonium plants and to measure the transmission rate of the virus. In addition to the sap inoculation a phosphate buffer has been used.

Concerning transmission by knife, the knife was contaminated by cutting a PFBV infected plant and then used for harvesting one healthy cutting following by a sterilization (dipping in alcohol and flaming). The cuttings were rooted and the base of each mother plant grown. Observations were made to record virus symptoms developed in the new growth.

### *Bio-assay*

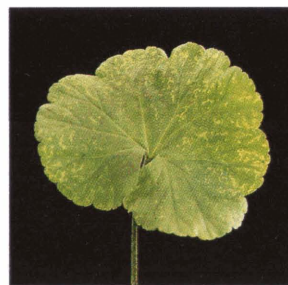
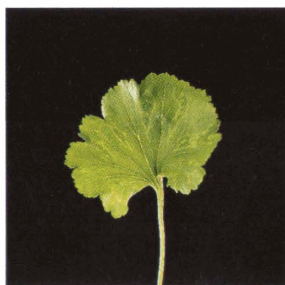
*Symptomatology*: Development of symptoms in pelargonium were recorded regularly through the year, in some cases once a month, comparing younger leaves, mature leaves and the flowers.

*Indicator plants*: Based on earlier experiences dry inoculation to *Chenopodium quinoa* was used comprising carborundum powder (400 mesh) and a phosphate buffer pH 7.6 including 4% polyethylene glycol (M 6000). The dry inoculation was performed from one mature pelargonium leaf which was rolled together and transversally cut, the cut surface being rubbed over the indicator leaves, which were pretreated with the buffer solution and the carborundum powder (Figs 17–18).



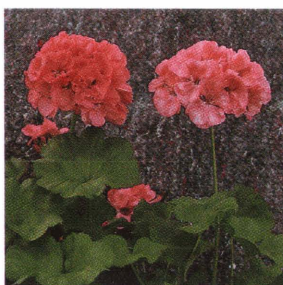
Figs 1–3. *Pelargonium* 'Springtime Irene' infected with pelargonium flower break virus (PFBV) showing deformation and yellow veinbands (1), chlorotic spots and streak along the veins and rugosity (2) and enlarging chlorotic spots (3).

*Pelargonie* 'Springtime Irene' inficeret med pelargonie-blomsterspætningsvirus (PBSV) med deformerede blade og gule nervebånd (1), klorotiske pletter og streger langs nerver og rynkning (2) samt udflydende klorotiske pletter (3).



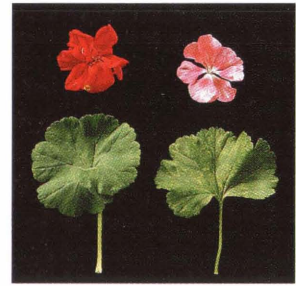
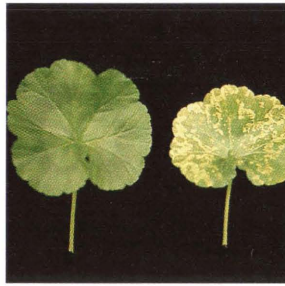
Figs 4–6. *Pelargonium* 'Springtime Irene' infected with both PFBV and tomato ringspot virus (TomRV) showing vigorous leaf deformation (4), chlorotic streaks (5) and chlorotic to yellow streaks and ring figurations (6).

*Pelargonie* 'Springtime Irene' inficeret med både PBSV og tomatringpletvirus (TomRV), med kraftigt deformerede blade (4), klorotiske streger (5) og klorotiske til gule streger og ringdannelser (6).



Figs 7–9. *Pelargonium* 'Springtime Irene' infected with both PFBV and TomRV showing distinct and vigorous flower break and rugosity in the petals (7, 8, 9). healthy plant to the left in Figs 7 and 8.

*Pelargonie* 'Springtime Irene' inficeret med både PBSV og TomRV med tydelig og kraftig blomsterspætning og rynkning i kronbladene (7, 8, 9). Sund plante til venstre i fig. 7 og 8.



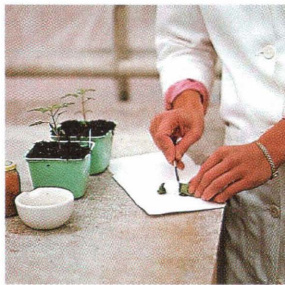
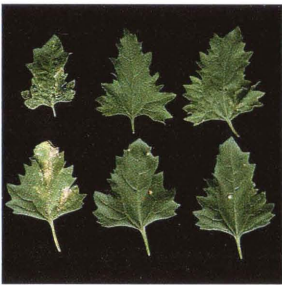
Figs 10–12. *Pelargonium* 'Amanda' infected with both PFBV and TomRV showing yellow figurations partly ringformed in the older leaves (10, 11), and almost symptomless 'Amanda' (infector) and 'Springtime Irene' with vigorous symptoms (indikator) from a grafted plant (12).

*Pelargonie* 'Amanda' inficeret med både PBSV og TomRV med gule figurationer delvis ringformede i de ældre blade (10, 11) og symptomløs 'Amanda' (infektor) og 'Springtime Irene' med kraftige symptomer (indikator) fra en podet plante (12).



Figs 13–15. *Pelargonium* 'Treasure Chest' infected with both PFBV and TomRV showing distinct yellow figurations in older leaves (13, 14) and *Chenopodium quinoa* with local and systemic reaction caused by the 2 viruses (15).

*Pelargonie* 'Treasure Chest' inficeret med både PBSV og TomRV med tydelige gule figurationer i ældre blade (13, 14) og *Chenopodium quinoa* med lokal og systemisk reaktion forårsaget af de to virus (15).



Figs 16–18. *Chenopodium quinoa* with local lesions (bottom row) and systemic symptoms (upper row) caused by PFBV + TomRV, PFBV and TomRV, respectively (16) and dry inoculation performed by a transverse cut of a leaf rolled together (17) and the cut surface rubbed over the indicator leaf (18).

*Chenopodium quinoa* med lokal læsioner (nederste række) og systemiske symptomer (øverste række) forårsaget af henholdsvis PBSV + TomRV, PBSV og TomRV (16) og tørinokulation udført ved et snit på tværs af et sammenrullet blad (17) og gnidning af snitfladen på indikatorbladet (18).

### Immuno-assay

Immunosorbent electron microscopy (ISEM) was used to identify the viruses pelargonium flower break and tomato ringspot (Figs 19–20). The ISEM test was carried out in accordance with the original procedure (7), but 2% polyethylene glycol was added to the usual phosphate buffer (1,14) in order to solve tannin, lignin and other problems in sap from many woody plants generally causing difficulties when electron microscopic grids are prepared for ISEM decoration.

Sap was used from young leaves from plants grown in greenhouses. The results from the ISEM test were based on the concentration (1–3) of decorated virus particles found by scanning of at least 5 squares per grid (400 mesh). The antisera used were kindly supplied by *Renate Koenig*, Braunschweig (TomRV, PLCV), *Olven Stone*, Littlehampton (PFBV), *Nada Plese*, Zagreb (PLPV) and *L. Boss*, Wageningen (PRPV).

### Results

*Virus diagnosis by ISEM all the year round of collected Pelargonium × hortorum plants showing virus-like symptoms*

A collection of diseased pelargonium plants showing more or less virus-like symptoms were tested four times during the year by the ISEM method in order to investigate the seasonal sensitivity, the content of viruses and the occurring symptoms. Apart from the PFBV and TomRV, the following viruses were tested as well: Pelargonium leaf curl (PLCV), pelargonium line pattern (PLPV) and pelargonium ring pattern (PRPV). The results are shown in Table 1.

Pelargonium leaf curl virus was not found in any of the isolates.

The test in August was carried out both in 1984 and 1985 and the results are presented as an average. The deviation in the number of reactions achieved comprising 15 samples was 3 for PFBV, 2

**Table 1.** Virus diagnosis by ISEM all the year round of pelargonium cultivars and isolates showing viruslike symptoms. *Virusdiagnose ved ISEM hele året igennem af pelargoniesorter og isolater med viruslignende symptomer.*

| Cultivar           | No.              | Virus symptoms recorded in March, May and October |         |         | Concentration <sup>1)</sup> of decorated virus particles |      |                    |      |       |      |                    |      | Other viruses found |      |
|--------------------|------------------|---|---------|---------|--|------|--------------------|------|-------|------|--------------------|------|---------------------|------|
|                    |                  | leaves  |         | flowers | PFBV   |      |                    |      | TomRV |      |                    |      |                     |      |
|                    |                  | young   | old     |         | Febr.  | June | Aug. <sup>2)</sup> | Nov. | Febr. | June | Aug. <sup>2)</sup> | Nov. | PLPV                | PRPV |
| 'Amanda'           | 36               | CS  | CS,YR   | 0       | 2  | 1    | 3                  | 3    | 1     | 1    | 1                  | 0    | +                   | 0    |
| »                  | 38               | 0   | YR      | 0       | 0  | 0    | 0                  | 0    | 0     | 0    | 2                  | 0    | +                   | +    |
| 'Penny Irene'      | 53               | CS,YVB,D  | YR      | 0       | 3  | 2    | 1                  | 1    | 0     | 0    | 1                  | 0    | 0                   | +    |
| 'Springtime Irene' | 21               | CS,YS,NS,D  | YS,NS   | WSt,Ru  | 3  | 2    | 3                  | 2    | 1     | 0    | 1                  | 0    | +                   | 0    |
| »                  | 28               | CS  | CS      | WSt,Ru  | 3  | 3    | 2                  | 0    | 0     | 0    | 0                  | 0    | +                   | +    |
| »                  | 40               | CS  | CS      | WSt,Ru  | 3  | 3    | 2                  | 3    | 0     | 0    | 0                  | 0    | 0                   | 0    |
| »                  | 48               | CS,YVB,D  | CS,VB,D | WSt,Ru  | 3  | 3    | 2                  | 2    | 0     | 0    | 0                  | 0    | +                   | 0    |
| »                  | 50               | CS  | YR      | WSt,Ru  | 2  | 3    | 3                  | 1    | 2     | 2    | 1                  | 0    | +                   | 0    |
| »                  | 52               | CS,YVB,D  | YR      | WSt,Ru  | 1  | 2    | 1                  | 3    | 1     | 0    | 1                  | 0    | +                   | 0    |
| »                  | 54               | CS,YVB,D  | CS,VB,D | WSt,Ru  | 0  | 2    | 2                  | 2    | 0     | 0    | 0                  | 0    | 0                   | 0    |
| »                  | 56               | CS  | 0       | WSt     | 0  | 0    | 1                  | 0    | 0     | 0    | 0                  | 0    | +                   | +    |
| »                  | 49 <sup>3)</sup> | 0   | 0       | 0       | 0  | 0    | 0                  | 0    | 0     | 0    | 0                  | 0    | 0                   | 0    |
| 'Treasure Chest'   | 25               | Weak chlorosis                                    | 0       | 0       | 2  | 2    | 2                  | 3    | 2     | 0    | 1                  | 0    | +                   | +    |
| 'Vesuv'            | 47               | CS  | CS      | 0       | 2  | 2    | 3                  | 1    | 0     | 0    | 0                  | 0    | +                   | +    |
| Unknown            | 43               | 0   | YR      | 0       | 0  | 0    | 0                  | 0    | 1     | 0    | 1                  | 0    | 0                   | 0    |
| Total No.          | 15               | No. of plants with positive reactions:            |         |         | 10   | 11   | 12                 | 10   | 6     | 2    | 8                  | 0    | 10                  | 6    |

C: chlorotic, D: deformation, N: necrotic, 0: symptomless, R: ring, Ru: rugose, S: spot, St: streak, VB: vein band, W: white, Y: yellow

<sup>1)</sup> Scale 1–3: Few-many particles

<sup>2)</sup> Average of 2 tests

<sup>3)</sup> Virusfree meristem plant originating from No. 50

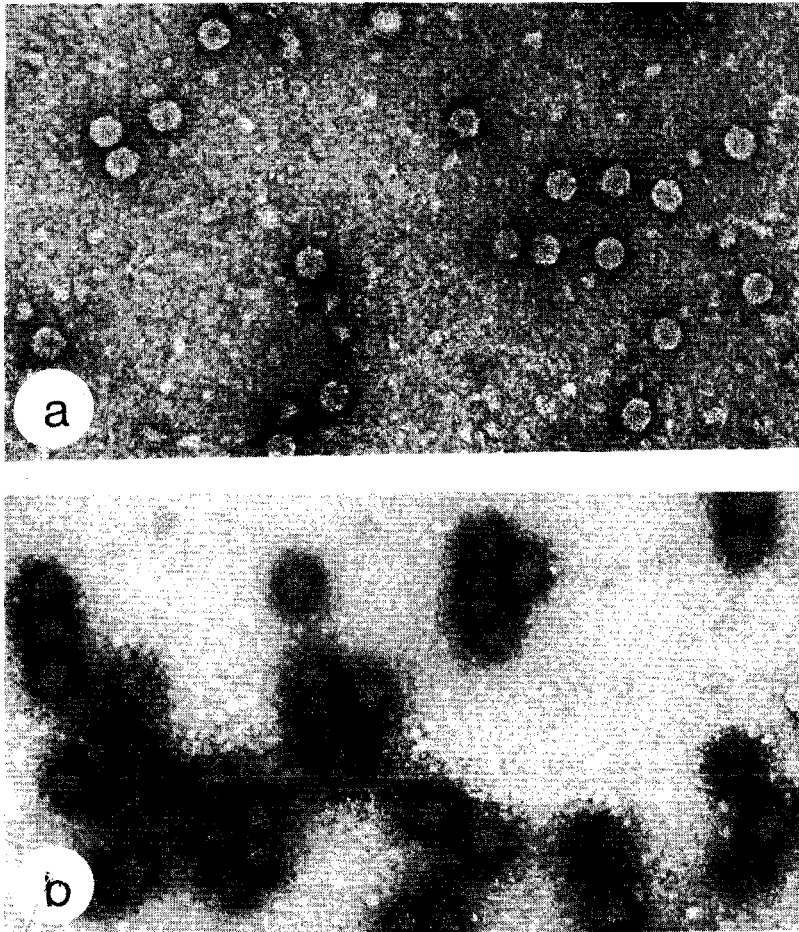


Fig. 19. Pelargonium tested for pelargonium flower break virus with ISEM.

a. trapped particles.

b. trapped and decorated particles.  $\times 140,00$ .

*Pelargonie tested for pelargonie-blomsterspætningvirus med ISEM.*

a. fangede partikler.

b. fangede og dekorerede partikler.  $\times 140.000$ .

for TomRV, 1 for PRPV and 10 for PLPV, the last one indicating very low accordancy and no certainty.

No seasonal variation occurred concerning the PFBV in contrast to the TomRV which was diagnosed most clearly in August and February.

There was a correlation between the viruses found and the symptoms developed in the cultivar 'Springtime Irene', where the PFBV caused seri-

ous symptoms both in leaves (Figs 1-3) and in flowers. A simultaneous infection of PLPV and/or PRPV does not change the existing symptoms, indicating these two viruses as being latent viruses.

In the other cultivars investigated single or double infection of PFBV and TomRV caused leaf symptoms but never flower break symptoms (Figs 10-14).

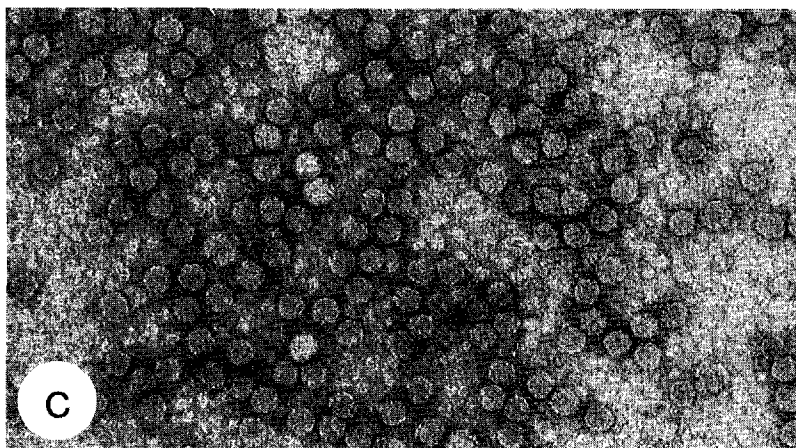


Fig. 20. Pelargonium tested for tomato ringspot virus with ISEM.

c. trapped particles.

d. trapped and decorated particles.  $\times 140,00$ .

*Pelargonie tested for tomatringpletvirus med ISEM.*

c. fangede partikler.

d. fangede dekorerede partikler.  $\times 140,00$ .

Photos: J. Begtrup.

Independent of the cultivars and single or double infections, the TomRV caused the development of yellow rings in six of eight cases in the older leaves (Figs 10–11).

*PFBV and TomRV diagnosis all the year round based on symptomatology, bio-assay and ISEM-assay*

Infection trial was performed with the cultivar

'Springtime Irene' to investigate the correlation between virus infection and the developed leaf and flower symptoms and the possibility of obtaining a certain diagnosis.

Virus-free plants of the cultivar were side grafted with scions from infected isolates with a known content of virus, comprising the mentioned viruses as single and double infection.



After establishment of virus symptoms in the new growth the plants were, all year round, recorded for symptoms in leaves and flowers, tested for virus infections by dry inoculation to

*Chenopodium quinoa*, where local lesions indicated PFBV and systemic reaction TomRV infection and finally diagnosed by the ISEM method.

The results are shown in Table 2, 3 and 4.

**Table 2.** Recording of virus symptoms all the year round of infected pelargonium 'Springtime Irene'.  
*Registrering af virussympotomer hele året igenem af inficerede pelargonie 'Springtime Irene'.*

| Infector/material<br>Virus    | Part       | Recorded symptoms in the months: |        |          |         |          |          |
|-------------------------------|------------|----------------------------------|--------|----------|---------|----------|----------|
|                               |            | February                         | May    | July     | August  | October  | December |
| PFBV <sup>1)</sup>            | Young leaf | CS                               | CS     | CS       | CS      | CS       | CS,D     |
|                               | Old leaf   | 0                                | 0      | CS       | CS,VB,D | CS       | 0        |
|                               | Flower     | -                                | WSt    | WSt      | WSt,Ru  | WSt,Ru,P | WSt,P    |
| PFBV<br>+ TomRV <sup>2)</sup> | Young leaf | CS                               | CS     | CS,YVB,D | CS,D    | YSt,R    | CS,D     |
|                               | Old leaf   | YVB,Ru                           | CS,R   | YS,R     | YS,D    | YS,R     | R        |
|                               | Flower     | -                                | WSt,Ru | WSt,Ru   | WSt,Ru  | WSt,Ru   | P        |
| Virusfree                     | Young leaf | 0                                | 0      | 0        | 0       | 0        | 0        |
|                               | Old leaf   | 0                                | 0      | 0        | 0       | 0        | 0        |
|                               | Flower     | -                                | 0      | 0        | 0       | 0        | -        |

C: chlorotic, D: deformation, N: necrotic, O: symptomless, P: pale, R: ring, Ru: rugose, S: spot, St: streak, Y: yellow, VB: vein band, W: white, -: no record

<sup>1)</sup> Average of 3 repetitions

<sup>2)</sup> Average of 2 repetitions

**Table 3.** Bio-assay all the year round, using dry inoculation to *Chenopodium quinoa* from virus-infected pelargonium 'Springtime Irene'.

*Biotest hele året igennem ved tørinokulation til Chenopodium quinoa af virusinficerede pelargonie 'Springtime Irene'.*

| Infector<br>Virus        | No. of local lesions and systemic reactions in the months: |    |       |    |      |    |        |    |         |    |          |    |
|--------------------------|--|----|-------|----|------|----|--------|----|---------|----|----------|----|
|                          | February   |    | April |    | June |    | August |    | October |    | December |    |
|                          | L  | S  | L     | S  | L    | S  | L      | S  | L       | S  | L        | S  |
| PFBV <sup>1)</sup>       | 12   | 0  | 7     | 0  | 2.5  | 0  | 4      | 0  | 4       | 0  | 5.5      | 0  |
| PFBV+TomRV <sup>2)</sup> | 8.5  | ++ | 4.8   | ++ | 2.5  | ++ | 2.5    | ++ | 7       | +0 | 4.8      | +0 |
| Virusfree                | 0  | 0  | 0     | 0  | 0    | 0  | 0      | 0  | 0       | 0  | 0        | 0  |

L: local lesion, S: systemic reaction (0: none, +: reaction)

<sup>1)</sup> Average of 3 repetitions

<sup>2)</sup> Average of 2 repetitions

**Table 4.** ISEM-assay all the year round of PFBV and TomRV from virus-infected pelargonium 'Springtime Irene'.  
*ISEM-test hele året igennem af PBSV og TomRV af virusinficerede pelargonie 'Springtime Irene'.*

| Infector<br>Virus        | Concentration <sup>1)</sup> of decorated virus particles in the months: |       |       |       |      |       |        |       |         |       |          |       |
|--------------------------|---|-------|-------|-------|------|-------|--------|-------|---------|-------|----------|-------|
|                          | February  |       | April |       | June |       | August |       | October |       | December |       |
|                          | PFBV  | TomRV | PFBV  | TomRV | PFBV | TomRV | PFBV   | TomRV | PFBV    | TomRV | PFBV     | TomRV |
| PFBV <sup>2)</sup>       | 2   | 0     | 2.3   | 0     | 2.7  | 0     | 2      | 0     | 3       | 0     | 2.3      | 0     |
| PFBV+TomRV <sup>3)</sup> | 3   | 2     | 2.5   | 1.5   | 2    | 2     | 2.5    | 1     | 3       | 2     | 2.5      | 1     |
| Virusfree                | 0   | 0     | 0     | 0     | 0    | 0     | 0      | 0     | 0       | 0     | 0        | 0     |

<sup>1)</sup> Scale 1-3: Few-many particles

<sup>2)</sup> Average of 3 repetitions

<sup>3)</sup> Average of 2 repetitions

Pelargonium 'Springtime Irene' infected by PFBV or by PFBV and TomRV can be diagnosed all the year round either by symptomatology, bioassay or ISEM-assay, all three methods being very similar in their sensitivity (Tables 2, 3 and 4).

The symptomatology (Table 2) has shown that PFBV infection always causes development of enlarging chlorotic 2–5 mm spots mainly in the younger leaves (Fig. 3) and flower break as white streaks and rugosed petals in the flowers. In some cases a few leaves are deformed on account of the development of yellowing vein bands starting from the base of the leaf veins (Fig. 2).

All the mentioned symptoms are strongly increased with a simultaneous infection by TomRV (Figs 4–9).

The bioassay (Table 3) showed local lesions from the PFBV infections and systemic mottling from the TomRV infections in *Chenopodium quinoa* (Figs 15–16). However, the TomRV infection was not found in the October and December tests in one of two samples, where the indicator plants failed to develop the systemic mottling.

The ISEM assay (Table 4) showed virus infections in all the tests performed from infected plants – the PFBV and the TomRV in high and low concentrations respectively, independent of the time of year.

#### *Transmission of PFBV amongst pelargonium plants by infected plant sap using sap inoculation and knife*

Transmission trials were performed using virus infected pelargonium sap comprising sap inoculation with five PFBV-isolates and contaminated knife with two PFBV-isolates.

Young healthy pelargonium 'Springtime Irene' plants were used as indicator plants.

Virus symptoms developed in the treated material indicating transmission of the PFBV.

The results showed that the PFBV was transmitted by sap inoculation to all 18 treated plants (100 per cent), and by knife to 2 of 14 rooted cuttings (14.4 per cent) while no transmission occurred to 15 mother plants after the shoot excision.

None of the untreated control plants showed any virus symptoms.

The developed virus symptoms were typical of PFBV infection comprising chlorotic spots some deformed leaves and white streaks in the petals.

#### **Discussion**

The symptomatology is limited to the cultivar 'Springtime Irene' and other sensitive cultivars, which makes the recording of symptoms unsuitable as a general diagnostic method, as also found by Stone (18).

Infection of PFBV causes serious symptoms both as single and double (+ TomRV) infection in 'Springtime Irene', but can also be seen in less sensitive ones. The characteristic symptoms are chlorotic spots, flower break and deformation of a few leaves comprising chlorotic vein clearing (single infection) and also yellowing streaks along the veins at double infection. These latter symptoms have not earlier been described.

Infection of TomRV causes the development of chlorotic yellow rings in the older leaves in single as well as double (+ PFBV) infected plants in six of eight cases, indicating this symptoms as characteristic of TomRV. Single infected plants are mostly symptomless except in the spring, while double infection increases the symptoms and make them visible from May to October. Stone (18) claims that single TomRV infection is symptomless.

Bio-assay and the ISEM-assay are both sensitive and usable in the diagnostic work during the year, and they support each other in a valuable way. If PEG is used, plant sap taken directly from the pelargonium plants can be used for both methods, whereas this will usually cause problems in ELISA and gel-diffusion tests.

The high and stable concentration of PFBV particles shown by the ISEM method may explain why it is easy to diagnose and easy to transmit the PFBV to other pelargonium plants in contrast to the low concentration of TomRV. This is new information and does not correspond with Stone (18).

## Conclusion

PFBV and TomRV have both been found frequently in diseased *Pelargonium × hortorum* cultivars and isolates.

Correlation was found between the occurring viruses and the symptoms in the cultivar 'Springtime Irene' where the PFBV alone caused serious leaf and flower symptoms.

Typical PFBV symptoms in 'Springtime Irene' consisted of 2–5 mm chlorotic spots in the young leaves, a few deformed, rugosed leaves with yellowing vein bands and white streaks in the petals as flower break.

The symptoms are strongly increased by a simultaneous infection of TomRV.

PLPV and PRPV were found as latent viruses in many of the cultivars without causing any additional symptoms.

PLCV was not found in any of the tested cultivars.

PFBV and TomRV in 'Springtime Irene' can both be diagnosed all the year round either by symptomatology, bio-assay or ISEM-assay, all three methods being, for the most part, equally sensitive. However, the recording of symptoms applies only to 'Springtime Irene'.

The bio-assay failed only in the TomRV tests in October and December.

The ISEM method showed a constant high concentration of PFBV particles all the year round. For the TomRV there was found a lower concentration and with a seasonal variation, the highest sensitivity shown during February, April, June and October.

The PFBV was transmitted easily amongst pelargonium plants by infected sap and by contaminated knife.

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