OCCURRENCE OF GRAFT-TRANSMISSIBLE VIRUS DISEASES OF THE STRAWBERRY IN THE CZECH REPUBLIC

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Summary. – Strawberry virus diseases were monitored using a leaf graft bioassay in 17 cultivars of strawberry *Fragaria ananassa* Duchesne in the Czech Republic. *Fragaria vesca* indicator clones revealed after grafting several symptoms of strawberry mottle, crinkle, vein banding, and mild yellow edge as well as of mixed virus infections. Isometric virus-like particles (VLPs) ranging from 21 to 50 nm in diameter and flexuous filamentous VLPs (12 by 600–1400 nm) were observed by electron microscopy in negatively stained crude sap preparations. Our results confirmed the complexity of virus diseases of the strawberry and represent the first report on the detection of strawberry mild yellow edge disease (SMYED) and of filamentous particles in this crop in the Czech Republic

Key words: grafting; strawberry; mottle, crinkle, vein banding, mild yellow edge; viruses; virus-like particles; electron microscopy

Introduction

The cultivated strawberry (*F. ananassa* Duch.) is an important fresh market and processed fruit crop cultivated in many parts of the world (Converse *et al.*, 1988). Strawberry yields and fruit quality are greatly influenced by the photoperiod, temperature, rest period, diseases, pests, soil conditions, and fluctuations in humidity and soil moisture (Maas, 1998). The strawberry is infected with many viruses and mycoplasma-like organisms, recently renamed phytoplasmas. Currently, 29 such pathogens are known and probably more will be found. A few of these pathogens kill infected strawberry plants, but majority of them reduce productivity while inducing no distinct symptoms. Leaf graft bioassay onto sensitive *F. vesca* and *F. virginiana* indicator clones is universal and the most common method for detection of strawberry virus diseases. Although recent developments in molecular biology and in plant virus serology have provided rapid, accurate and sensitive techniques (Converse *et al.*, 1988; Leone *et al.*, 1995; Petrzik *et al.*, 1998; Posthuma *et al.*, 2001), these could not be used as diagnostic tools to replace the standard bioassays. Until the present gaps in the methodology for laboratory detection of major strawberry virus and virus-like pathogens are closed, determination of the virus status of strawberry planting material will continue to depend on leaf grafting (Converse, 1987; Converse *et al.*, 1988; Spiegel and Martin, 1998).

A research program on the occurrence of strawberry viruses in commercial plantations and nurseries in the Czech Republic has been started in 1991. Results of detection of graft-transmissible virus diseases of the strawberry are reported in this paper.

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Abbreviations: DAS-ELISA = double antibody sandwich ELISA; ISEM = immunosorbent electron microscopy; JY = June yellows; SCV = strawberry crinkle virus; SVBV = strawberry vein banding virus; SMV = strawberry mottle virus; SMYEAV = strawberry mild yellow edge associated virus; SMYED = strawberry mild yellow edge disease; SMYEV = strawberry mild yellow edge virus; SPMYEV = strawberry pseudo-mild yellow edge virus; VLPs = virus-like particles

Materials and Methods

Origin of plants. Strawberry cvs. Elista (1), Elsanta (2), Elvíra (2), Kama (2), Korona (14), Redgauntlet (25), and Senga Sengana (2) originated from a strawberry plantation in Planá, southern Bohemia. Cvs. Induka (2) and Senga Sengana (2) were from strawberry plantations in Lhenice, southern Bohemia and in Jesenice, central Bohemia, respectively, Cvs. Adriana (2), Bounty (1), Dagmar (2), Dukát (2), Induka (2), Karmen (4), Lidka (7), Ostara (2), and Zefýr (1) were from nurseries in the Breeding Station in Turnov, eastern Bohemia. Cvs. Goliáš, (7) and Senga Sengana (3) were from a private garden in Třísov, southern Bohemia. They were examined by leaf grafting onto sensitive indicator clones for virus indexing. All *F ananassa* plants used for grafting gave in preliminary double antibody sandwich (DAS-ELISA) and sap inoculation of herbaceous host plants negative results for the presence of mechanically transmitted viruses (Honetšlegrová and Špak, 1995). *F ananassa* cvs. Senga Sengana and Redgauntlet cultivated from meristem tissues were used as healthy controls Indicator clones *F vesca* UC4 and UC6, and *F virginiana* UC10, UC11 and UC12 were kindly supplied by National Clonal Germplasm Repository, Corvallis, ORE, USA. Indicator clones *F vesca* EMK, EMC, UC1, UC2, UC4, UC5, UC6, FV-72 and Bologna, and *F vesca* var. *semperflorens* Alpine were kindly provided by M. Erbenová, Research and Breeding Institute of Pomology, Holovousy, Czech Republic The plants were cultivated in sterilized soil in insect-proof, temperature-controlled greenhouses with supplemented light under photoperiod of 16:8 (L:D) hrs.

Leaf grafting. A modified petiole-insert leaflet grafting technique (Frazier, 1974 a) was used. For improvement of the transmission efficiency and the shortening of the incubation time until the appearance of symptoms in the indicator, all leaves except the grafted ones were removed from the indicator clone at the time of grafting. Central leaflet of indicator plant was removed and replaced by a grafted donor leaflet. In general, 2 leaflet grafts per 1 indicator plant were made. At least 20 indicators were used per 1 *F ananassa* plant examined. The grafted plants were held in a moist chamber for 3 weeks, then placed in a greenhouse and evaluated periodically for symptoms development for 1 year.

Electron microscopy Crude saps from leaves of examined strawberries and healthy controls were negatively stained with 2% uranyl acetate and examined in a Philips 420 transmission electron microscope.

Immunosorbent electron microscopy (ISEM). An antiserum against strawberry mild yellow edge virus (SMYEV, species *Strawberry mild yellow edge virus*, genus *Potexvirus*), kindly provided by Dr. W. Jelkmann, Institut fuer Pflanzenschutz im Ostbaum, Dossenheim, Germany (Kaden-Kreuziger *et al* , 1995) was used for ISEM. The antiserum was diluted 1:1000 and 1:50 in 0.1 mol/l sodium/potassium phosphate buffer pH 7.0 for trapping and decoration, respectively. The grids were incubated in the diluted antiserum for 5 mins, rinsed with 40 drops of the phosphate buffer, transferred to leaf homogenate and incubated at 20°C overnight. The grids were rinsed with 50 drops of distilled water before negative staining. The same electron microscope as mentioned above was used.

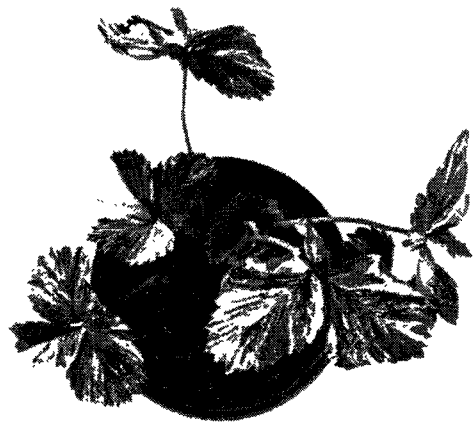

Fig. 1
Sectorial leaf mottling on *F. ananassa* cv. Korona affected with June yellows

Results and Discussion

Symptoms on various strawberry cultivars

Different distortion was observed on cultivated strawberries during inspection in 1991–1995. Virus-like symptoms ranged from mild mosaic, chlorosis, mottle, rings, streaks, leaf roller, deformities, reddening and necrosis of leaves to stunting and decline of plants. Plants with nutrient deficiency symptoms and common symptoms of herbicide injury (foliar deformities, vein clearing and marginal and interveinal chlorosis or necrosis) were detected in inspected strawberry plantations (Crandal, 1987). Chlorotic or white variegation of leaves known world-wide as June yellows (JY) (Wills, 1987) was observed on strawberry cvs. Induka, Korona, Lidka and Redgauntlet (Fig. 1).

Transmission of strawberry diseases by leaf graft bioassay

The most important virus diseases of the strawberry in the world are thought to be mottle, crinkle, vein banding and SMYED (Converse, 1987, Spiegel and Martin, 1998). There are only a few reports on them in the Czech Republic prior to 1990. Erbenová (1981) has been the first in testing strawberry cultivars by a leaf-graft bioassay and in determining symptoms similar to those caused by strawberry mottle virus (SMV), strawberry crinkle virus (SCV, genus *Cytorhabdovirus*, family *Rhabdoviridae*) and strawberry vein banding virus (SVBV, genus *Caulimovirus*, family *Caulimoviridae*). Polák and Bezpalcová (1987) have found

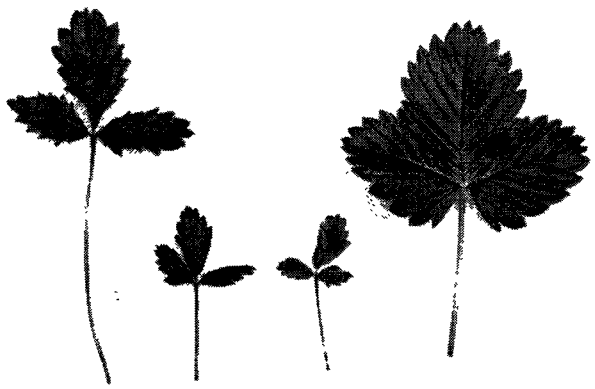

Fig. 2
Chronic symptoms of strawberry mottle on *F. vesca* semperflorens Alpine leaves
Infected (left) and control (right) leaves

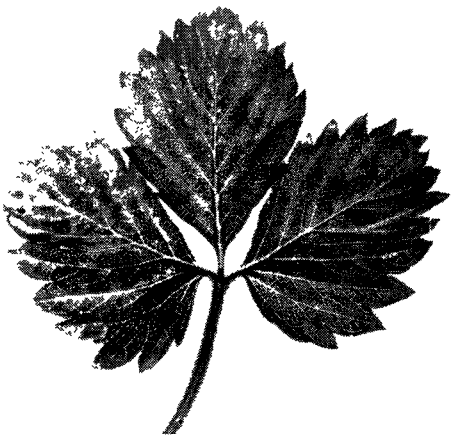

Fig. 3
Chlorotic vein banding pattern on *F. vesca* clone UC6
Diagnostic symptoms of SVBV disease.

infection with SMV in productive populations of strawberry common and more widespread than that with SCV. They did not observe symptoms of SVBV disease and SMYED in the examined plant material. Mechanical transmission and multiplication of three SMV isolates (2 from the Netherlands and 1 from the Czech Republic), characterization of some physical properties and attempts to purify the strawberry mottle disease agent was accomplished by Polák *et al.* (1991).

Grafting onto *F. vesca* clones during this investigation revealed symptoms of strawberry mottle, crinkle, vein banding and SMYED in single as well as in mixed virus infections. Thirty-three of 85 strawberry plants tested (38.8%) gave positive reactions on the graft-inoculated *F. vesca* clones. On the basis of our results, the best *F. vesca* indicator clones for manifestation of strawberry virus diseases seem EMK, EMC, UC5, UC6 and Alpine. No symptoms were observed on *F. virginiana* clones UC10, 11 and 12, although they have been recommended especially for detection of the pallidosis disease agent and SVBV (Frazier, 1974b; Frazier and Morris, 1987; Fulton, 1987).

We found no correlation between the presence of certain graft-transmissible virus diseases and the symptoms in commercial *F. ananassa* plants. Some apparently normal strawberry plants gave positive results after grafting onto indicators, and also VLPs were observed by electron microscopy. On the other hand, detection of strawberry viruses by grafting of leaves from some plants with virus-like symptoms failed. This finding is in agreement with those of Spiegel and Martin (1998), who have reported that many viruses do not induce distinct symptoms in commercial cultivars, and often the only indication of infection is loss of vigour, stunting, lowered yields and general "running out" of a cultivar.

Strawberry mottle. Leaflet grafting from 13 *F. ananassa* cvs. (number of plants) Goliáš (1), Korona (1), Redgauntlet (9) and Senga Sengana (2) onto indicators revealed symptoms resembling those caused by strawberry mottle disease (Mellor and Krczal, 1987). Most of donor plants seemed apparently healthy (cvs. Goliáš (1), Senga Sengana (2) and Redgauntlet (7)), two had leaf mosaic (cvs. Korona and Redgauntlet), and on one plant of cv. Redgauntlet symptoms of JY were observed. Although SMV is thought to be the causal agent of strawberry mottle disease, this virus has not yet been identified, characterized, recognized and classified (Van Regenmortel *et al.*, 2000). Isometric particles ranging from 14 to 37 nm in diameter have been described for SMV isolates by different authors (Polák and Jokeš, 1992; Hepp and Converse, 1990; Kitajima *et al.*, 1971; Leone *et al.*, 1995; Martin and Spiegel, 1998). Numerous attempts to purify SMV have met with very limited success. Therefore, no antisera or cDNA probes specific for SMV are yet available. Moreover, it is possible that typical symptoms of the disease are caused by more than one virus (Martin and Spiegel, 1998; Fránová-Honetšlegrová and Erbenová, 1999).

In our experiments, symptoms of mottle and mosaic appeared on the youngest leaves of graft-inoculated *F. vesca* clones EMK, EMC, UC1, UC2, UC5, UC6, FV 72, Bologna and Alpine usually within 7–15 days after grafting. The affected leaves were irregular in shape and reduced in size, with some leaflets narrow at the base and shortened petioles (Fig. 2). Three of the isolates (cv. Redgauntlet) were considered mild strains of strawberry mottle disease. The

Fig. 4
Symptoms of SCV disease
Severe epinasty of young leaves and lesion symptoms (arrow) on petiole of *F. vesca* semperflorens Alpine (a) Chlorotic spotting, vein chlorosis and shape deformation on leaf of *F. vesca* clone FV 72 (b)

others revealed more severe symptoms including epinasty, necrosis, reddening of petioles and dwarfing of plants. The indicators runnered sporadically and the runner plants produced roots very slowly, if at all.

Strawberry vein banding. Symptoms corresponding to those described earlier for SVBV disease (Frazier, 1955; Prentice,1952) were observed on *F. vesca* indicator clones (Fig. 3) after leaf grafting of 11 *F. ananassa* cvs. (number of plants) Goliáš (2), Kama (1), Lidka (1) and Redgauntlet (7). Donor plants of cvs. Goliáš (2), Kama (1) and Redgauntlet (3) did not show symptoms of virus infection. Chlorotic pattern was obvious on 1 plant of cvs. Lidka and Redgauntlet, while leaf mosaic was expressed on 3 plants of cv. Redgauntlet.

All 3 symptom types which characterize the strawberry vein banding disease, namely leaf curl, vein banding and necrosis (Frazier and Morris, 1987), were detected on indicators in our experiments. Usually, a combination of leaf curl (epinasty of midribs and twisting of leaflets) and chlorotic banding along main veins revealed indicators after grafting from *F. ananassa* plants. These results implicate that different strains of SVBV or other causal agents were probably present in the donor plants. Only the isolate transmitted from cv. Redgauntlet and the isolates from 2 plants of cv. Goliáš developed vein banding and necrotic symptoms without additional epinasty, respectively. The first symptoms developed 13–21 days after grafting and were most severe on 2–4 youngest leaves. Subsequently, group of symptomless leaves occurred. Usually, slight chlorotic vein banding of main veins reappeared in the spring. The best symptom expression was observed on *F. vesca* clones EMK, UC6, EMC and Alpine.

Strawberry crinkle. Two isolates of SCV, one of the most damaging viruses infecting strawberries (Frazier *et al.*, 1987; Zeller and Vaughan, 1932), were transmitted from *F. ananassa* cv. Korona with rolling and deformation of leaves and cv. Lidka with leaf mosaic and dwarf. Chlorotic spotting of the lamina, vein chlorosis, angular epinasty and distortion of leaflets, lesions on stolons and petioles (Fig. 4)

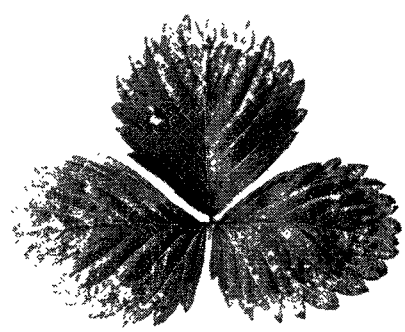

Fig. 5
Marginal chlorosis on *F. vesca* clone EMK infected with SMYEV disease

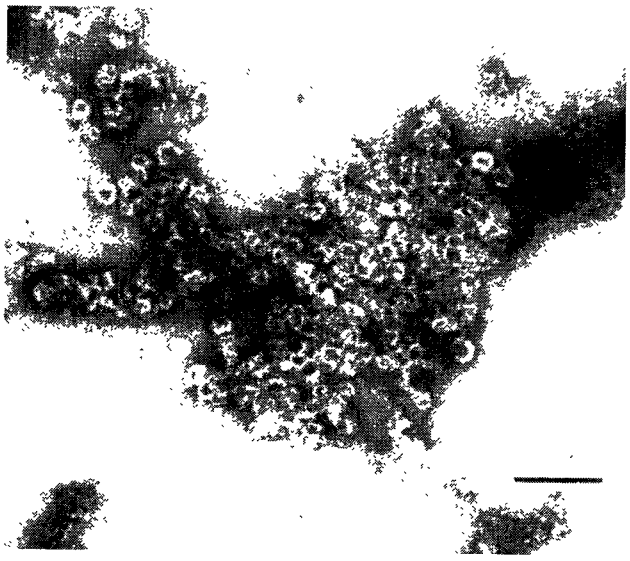

Fig. 6
Cluster of isometric VLPs of about 21 nm in diameter
Electron microscopy, negatively stained preparations from *F. vesca* clone EMK showing severe symptoms of SVBV disease Bar = 100 nm.

and also petal-streak symptoms were observed on *F. vesca* clones EMK, UC5, FV72 and Alpine.

SMYED. On the basis of symptom expression on graft inoculated indicator clones, the agent of SMYED (Jelkmann *et al.*, 1990, Martin and Converse, 1982) was transmitted from plants of 7 *F. ananassa* cvs. (Bounty (1), Dukát (1), Goliáš (3), Korona (1) and Redgauntlet (1)). All donor plants were symptomless except of cv. Korona, where leaf mosaic, dwarfing and necrosis were manifested.

The inoculated plants of *F. vesca* clones EMK, EMC, UC5, Bologna and Alpine revealed epinasty and net-vein chlorosis on the youngest leaves 10–18 days after grafting. During later stages of infection, the chlorotic flecking changed into vein necrosis and the older leaves showed premature senescence (Converse *et al.*, 1987). Marginal chlorosis of leaves (Fig. 5) described for SMYED by Yoshikawa *et al.* (1984) was observed on *F. vesca* clones EMK and Alpine after grafting from cv. Korona. Transmission of the SMYED agent(s) onto *F. vesca* indicators represents the first report on SMYED in the Czech Republic.

Electron microscopy

The hypothesis about the presence of single or mixed virus infection was confirmed by electron microscopy. VLPs were observed in 29 negatively stained crude sap preparations from 33 examined *F. ananassa* plants, but more often in those from corresponding symptom-bearing *F. vesca* indicators. No VLPs were observed in healthy controls.

Flexuous filamentous particles (12 by 600–1400 nm) (Fig. 8), always in conjunction with isometric ones, were observed in crude sap preparations originating from 12 *F. ananassa* plants. They were mostly present in graft-inoculated indicators with vein banding symptoms (7 samples), in 2 preparations from *F. vesca* with mottle and in 3 samples from indicators with symptoms of SMYED. According to our best knowledge, the only filamentous virions described from strawberry have been SMYEV, a potexvirus (Jelkmann *et al.*, 1990), and strawberry pseudo-mild yellow edge virus (SPMYEV, species *Strawberry pseudo-mild yellow edge virus*, genus *Carlavirus*)

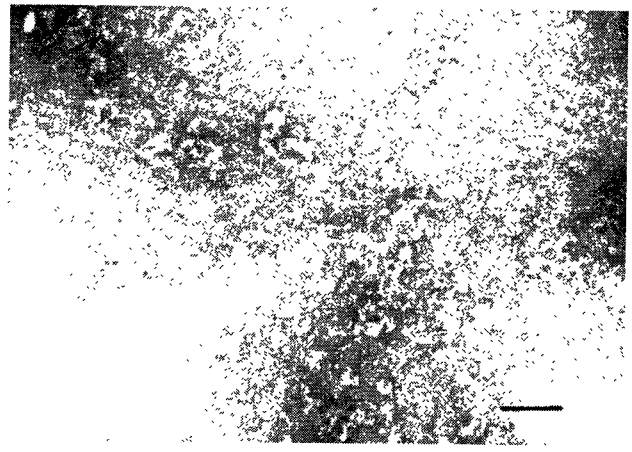

Fig. 7
Single isometric VLPs of about 25 nm in diameter
Electron microscopy, negatively stained preparation from *F. vesca semperflorens* clone Alpine showing symptoms of SMYEV disease Bar = 100 nm

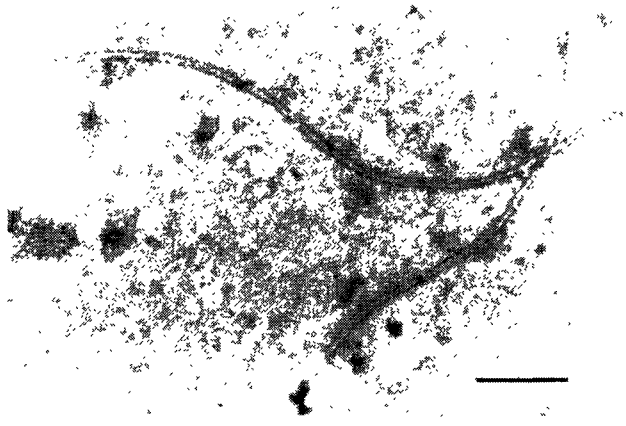

Fig. 8
Filamentous particles from *F. vesca* clone EMC with SVBV disease symptoms
Bar = 200 nm

(Yoshikawa and Inouye, 1986). However, no particles were decorated in ISEM tests using the antiserum against a German isolate of SMYEV (data not shown), suggesting the possibility that SPMYEV may occur in the Czech Republic.

Isometric VLPs ranging from 21 to 50 nm in diameter were detected in preparations originating from 19 *F. ananassa* plants. Caulimovirus-like particles (40–50 nm in diameter) were present in *F. vesca* indicators predominantly with SVBV disease symptoms (5 samples). They were sporadically detected also in crude sap from indicators with dominant mottle and SMYED. These particles were thought to be SVBV virions. Subsequently, this hypothesis was confirmed by dot blot hybridization (data not shown). All samples which revealed presence of caulimovirus-like particles reacted positively with a non-radioactive probe against an American isolate of SVBV (Mráz *et al.*, 1998). Isometric particles ranging from 21 to 37 nm in diameter (Fig. 6) could probably be virions of SMV or strawberry mild yellow edge associated virus (SMYEAV, species *Soy bean dwarf virus*, family *Luteoviridae*) or some of other strawberry viruses (Converse, 1987; Spiegel and Martin, 1998).

Summing up, we found no relationship between the presence of some kind of VLPs and the symptoms on indicators or strawberry cultivars. In most samples (21 of 29), a mixture of different particles (virions) was demonstrated. Moreover, although the diagnostic symptoms of SCV (lesions on petioles and stolons) were expressed on graft-inoculated plants, rhabdovirus-like virions, which are considered to be the causal agent of this disease, were not detected in crude sap probably due their low concentration in plant material. Previously, bacilliform virions have been observed only in partially purified strawberry preparations (Fránová-Honetšlegrová and Erbenová, 1999).

Our results confirmed high complexity of strawberry virus diseases. Additional studies using classical and also new molecular methods are necessary to clarify the etiology and epidemiology of these diseases. The virus isolates transmitted to *F. vesca* indicator clones during this investigation could serve as initial source for these studies.

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