

Distribution of Apple Stem Grooving Virus in Apple Trees in the Czech Republic

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Abstract

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From 1997 to 2000 the distribution of *Apple stem grooving virus* (ASGV) in selected intensive apple orchards was investigated at six sites in the Czech Republic. Detection of ASGV was by ELISA. The virus was found to be commonly distributed; it was detected in over 50 % of the tested trees. Some cultivars were infected up to 100 %, while several were found to be virus-free. A high incidence of infected trees appeared in both older (20 years) and younger (7 to 8 years) orchards. This emphasizes the necessity to initiate the certification of the health condition of nursery material according to the system recommended by the EPPO.

Keywords: apple stem grooving virus; distribution; intensive orchards; apple trees

Apple stem grooving virus (ASGV), a member of the genus *Capillovirus*, has flexible thread-like particles 600–700×12 nm in size (BRUNT *et al.* 1996). No vector of the virus is known. It infects apple and pear trees, being spread by graft transmission. As woody indicators the apple cultivar Virginia Crab (e.g. DE SEQUIERA 1967) or *Pyronia veitchii* were used in the past, but the incubation periods of ASGV in these indicators were too long, lasting a minimum of six months. Later HOWELL and MINK (1995) found an indicator that reacts within 2 or 3 weeks, i.e. *Malus micromalus*, clone GMAL 273.

For diagnosis of ASGV, mechanical transmission to the herbaceous indicators *Chenopodium quinoa* and *Phaseolus vulgaris* can be used (FUCHS 1979). Immunoenzymatic determination of ASGV, which enabled quick and reliable detection of the virus in trees, was reported by FUCHS *et al.* (1979). The method of sampling to detect ASGV by ELISA was investigated by JANEČKOVÁ and PLUHAŘ (1988).

ASGV is a cosmopolitan virus (NÉMETH 1986) infecting many commercial apple cultivars all over the world. Its occurrence in Czechoslovakia was demonstrated in the 1970's (SEIDL & KOMÁRKOVÁ 1977). ASGV causes considerable losses in yield of apple trees (CAMPBELL *et al.* 1978). The losses increase when apple-trees are infected simultaneously with two or more so-called latent viruses. For instance, in China WU *et al.* (1998) showed

mixed infection by ASGV and apple chlorotic leafspot virus (ACLSV) which occurred in 80–100% of the trees, with a subsequent decrease in yield of about 40%. Similar severe ACLSV infestations of apple trees, including the main cultivars of intensive orchards, was reported by POLÁK *et al.* (1997) from the Czech Republic.

Further research was focused on studies of the distribution of ASGV in selected younger and older intensive apple orchards at six localities of the Czech Republic.

MATERIAL AND METHODS

Plant Material: Apple cultivars of seven intensive apple orchards at six selected localities were tested for the occurrence of ASGV (with age of orchard; cultivars tested):

1. Locality Horoměřice; 20 years; Český ráj, Discovery, Golden Delicious, Idared, James Grieve, Mantet, Spartan, Stark Earliest, Vistabela;
2. Locality Křinec-Sovenice; 7 years; Bohemie, Elstar, Gloster, Golden Delicious, Idared, James Grieve Red, Jonagold, Jonagored, Ontario, Průsvitné, Rubín, Šampion, Vanda;
3. Locality Litoměřice-Gízov; 20 years; Bohemie, Gloster, Golden Delicious, Goldspur, Idared, Jonagold, McIntosh, Melrose, Rubín, Spartan;
4. Locality Loučeň-Mcely; 20 years; Bohemie, Julie, Melrose;

5. Locality Loučeň-Studce; 15 years; Gloster, Zvonkové;
6. Locality Tismice; 20 years; Elstar, Rubín;
7. Locality Tismice; 8 years; Gloster, Idared, Starkrimson.

Sampling of Material: Sampling of material was carried out in the orchards three times: in winter (from the end of November till the end of January), before spring in March, and in spring (April, May) after leaves had appeared. Four randomly selected shoots were taken from the crown of each tree. Shoots collected in winter and before spring were placed first in a cold room and during March transferred to room temperature. After the leaves appeared, a sample of newly expanded leaves from each tree was used for determination of ASGV by means of ELISA or stored frozen at -26°C and investigated later. From shoots taken in spring, samples of young leaves were prepared from the top leaves of four selected shoots of each tree and analyzed immediately after sampling.

Determination of ASGV by ELISA: Determination of ASGV in apple cultivars was carried out with commercial kits from Loewe Biochemica. IgG and IgG conjugated with alkaline phosphatase were diluted 1:200 with 0.05M carbonate bicarbonate buffer pH 9.6. Determination was done by the double sandwich method, by direct DAS-ELISA (CLARK & ADAMS 1977). 0.5 g of fresh or frozen leaves (see Sampling of Material) was homogenized in 10 ml of extraction buffer pH 7.4 in a hand homogenizer (fa Bioreba) in a polyethylene bag. After homogenization the samples were filtered through cheese cloth and 0.2 ml of each was pipetted into the wells of ELISA microplates at 4°C . For each sample two wells were used. Each plate contained two wells with positive and negative controls and extraction buffer. After enzyme substrate was added and incubation for 30–60 min the color reaction was measured using a MR 5000 photometer (Dynatech).

RESULTS AND DISCUSSION

Occurrence of ASGV was demonstrated by ELISA in all tested orchards and in most apple cultivars.

In the 20-year-old orchard at Horoměřice, ASGV occurred in all nine cultivars (Table 1). All tested trees of the cultivars James Grieve and Stark Earliest were found to be infected. Out of a total of 138 trees tested, 82 were ASGV positive (59%).

In the 7-year-old orchard at Křinec-Sovenice, ASGV was found in all 12 cultivars tested: Bohemie, Elstar, Golden Delicious, Idared, James Grieve Red, Jonagold, Jonagored, Ontario, Průsvitné, Rubín, Šampion and Vanda. Out of 97 trees tested, ASGV was detected in 61 trees (63%). For results see Table 2.

When comparing the results from the 7- and 20-year-old orchards it appears that the health condition of both, though growing at different localities, was approximately

Table 1. Occurrence of ASGV in a 20-year-old intensive apple orchard at Horoměřice

Apple cultivar	Number of trees	
	tested	infected
Český ráj	10	6
Discovery	10	3
Golden Delicious	19	8
Idared	20	11
James Grieve	10	10
Mantet	10	5
Spartan	19	18
Stark Earliest	20	20
Vistabela	20	3
Total	138	82 (59%)

the same. In the 20-year-old orchard, some cultivars were completely infected (James Grieve, Stark Earliest), while others (Český Ráj, Golden Delicious, Idared, Mantet) had about 50% infection. Only in cultivars Discovery and Vistabela was there a low level of infection. In the 7-year-old orchard, a high incidence of infection was found in cultivars Gloster, James Grieve Red, Šampion and Jonagold, and a medium incidence in cultivars Golden Delicious and Rubín. Interesting results were obtained in tests of the cultivar Bohemia at this orchard. The trees had come from two different sources; those from one source were free of ASGV, those from the other were infected up to

Table 2. Occurrence of ASGV in a 7-year-old intensive apple orchard at Křinec-Sovenice

Apple cultivar	Number of trees	
	tested	infected
Bohemia (Thoř)	8	0
Bohemia (Holovousy)	13	6
Golden Delicious	13	6
Gloster	10	8
James Grieve red	13	11
Rubín	13	8
Šampion	13	12
Elstar	3	1
Idared	1	1
Jonagold	4	4
Jonagored	1	1
Ontario	1	1
Průsvitné	2	1
Vanda	2	1
Total	97	61 (63%)

50%. The results indicated partial or total possible recovery of some cultivars. Partial infection can be caused by imperfect recovery or by budding of recovered cultivars onto partially infected stocks. That may explain the fact that the same cultivar from one source could be virus-free, while from another it was partially infected.

In the 20-year-old orchard at Litoměřice-Gízov, ASGV was found in nine out of ten cultivars tested: Bohemie, Gloster, Golden Delicious, Goldspur, Idared, McIntosh, Melrose, Rubín and Spartan. ASGV was not found in the cultivar Jonagold. Out of 41 tested trees 30 were infected (73%).

In the 20-year-old orchard at Loučeň-Mcely, the cultivars Julie and Melrose were found to be infected. ASGV was not found in the cultivar Bohemie (10 trees tested).

In the 15-year-old orchard at Loučeň-Studce, ASGV was found in the cultivar Gloster but not in Zvonkové (10 trees tested).

In the 20-year-old orchard at Tismice, ASGV was found in the cultivar Rubín but not in Elstar.

In the 8-year-old orchard at Tismice, ASGV was detected in the three cultivars tested: Gloster, Idared and Starkrimson.

Altogether 371 trees grown in seven intensive apple orchards were tested by ELISA for incidence of ASGV. The virus was detected in 200 trees (54%). High incidences of ASGV-infection were found in both older (20 years) and younger (7 and 8 years) orchards. This suggests that the health condition of intensive orchards is not improving, and that there are serious obstacles to the production of healthy nursery material. Therefore, it is necessary and urgent to introduce a system of certification of the health condition of nursery material according to the recommendations by the EPPO.

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Souhrn

POLÁK J., ZIEGLEROVÁ J. (2001): Rozšíření viru žlábkovitosti kmene jabloně v České republice. *Plant Protect. Sci.*, **37**: 1–4.

V letech 1997–2000 byl proveden průzkum rozšíření viru žlábkovitosti kmene jabloně, *Apple stem grooving virus* (ASGV), ve vybraných intenzivních sadech na pěti lokalitách v České republice. ASGV byl ve stromech jabloní zjišťován pomocí metody ELISA. ASGV je v intenzivních sadech jabloní široce rozšířen a byl stanoven ve více než 50 % testovaných stromů. Některé odrůdy jabloně jsou virem 100% infikovány, na druhé straně bylo zjištěno několik odrůd prostých ASGV. Vysoká infekce stromů

jabloně ASGV byla zjištěna ve starších (dvacetiletých) i mladších (sedmi- a osmiletých) sadech jabloní. Výsledky ukazují naléhavost zavedení systému certifikace zdravotního stavu školkařského materiálu podle doporučení EPPO.

Klíčová slova: virus žlábkovitosti jabloně; rozšíření; intenzivní sady; jabloně

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