The majority of viruses belonging to the listed genera can be transmitted by mechanical inoculation. The reaction of test-plants eliminates such viruses as *Blueberry red ringspot virus*, *Cestrum leaf curling virus*, *Petunia vein clearing virus* and *Tobacco vein clearing virus* which are not transmitted in mechanical manner [3, 6].

For further identification of virus DAS-ELISA test were used. No positive reaction with antiserum to CaMV was obtained. According to results of DAS-ELISA we can exclude infection of *P. bifolia* by CaMV and serologically related viruses: *Carnation etched ring virus*, *Dahlia mosaic virus*, *Horseradish latent virus* and *Strawberry vein banding virus* [3].

In conclusion, host assay of sap samples extracted from infected plants of *P. bifolia* approved its infectivity. EM studies confirmed the presence of virus-like particles in infected plant tissues. The isometric morphology and size range allows to suggest that the viral pathogen of *P. bifolia* can be a representative of family *Caulimoviridae*. The cases of similar virus infection of orchids are not described in literature data. However, there are not much investigation about spreading viruses in natural ecosystems. Further work will be focused on identification of the virus infecting *P. bifolia*.

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J. Polák

BIOTECHNOLOGICAL (GM) CROPS IN THE WORLD AND EUROPE, PLUM HONEYSWEET RESISTANT TO PPV, RESEARCH IN THE CR: THE PAST, THE PRESENT AND THE FUTURE

Быстрое увеличение площадей посевов ГМ-культур, делает их наиболее высокоадаптивными посевными культурами в истории сельского хозяйства. Здесь представлены результаты девяти лет тестирования слив клона С5 (трансформирован капсидным белком вируса шарки сливы) при высокой и постоянной инфекционной нагрузке PPV-Rec, как самостоятельного агента, так и в сочетании с вирусами карликовости чернослива и кольцевой хлоротичности яблони.

Fast increasing hectarage of GM crops makes them the highly adopted crop technology in the history of agriculture. Results of nine years testing of plum clone C5 (transformed with the Plum pox virus coat protein) under the high and permanent infection pressure of PPV-Rec alone and in combinations with Prune dwarf virus, and Apple chlorotic leafspot virus both from graft inoculation and natural aphid vectors are presented.

Introduction. Research in genetic engineering started in late seventies (Colwell et al., 1985). The first genetically modified (GM), syn. biotechnological (Biotech) crops were commercialized in 1995 (cotton, company Monsanto; potato, company Syngenta). 1.7 million hectares of Biotech crops (cotton, corn, potato) were planted in 1996 already (USDA-APHIS Biotechnology Permits Database, available at htpp://www.nbiap.vt.edu/ (March 2001). Increase in hectarage to 160 million hectares in 2011 (Fig. 1) makes Biotech crops the fastest adopted crop technology in the history of agriculture. The International Service for the Acquisition of Agri-biotech Applications (ISAAA) is every year publishing "Global status of commerialized Biotech/GM crops". The last one was published as ISAAA Brief No. 43 in 2011 (James C., 2011). The objective of this Brief is to provide information and knowledge to the scientific community and society on Biotech/GM crops and its contribution to global food, feet, fiber and fuel security, to sustainable agriculture.

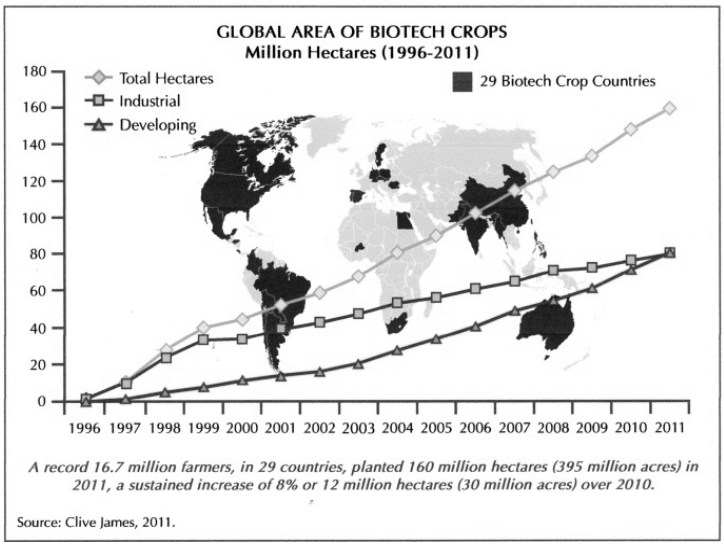

Fig. 1. Global area of biotech crops

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Of the 29 countries planting Biotech crops in 2011 (Fig. 2), 19 were developing countries and 10 were industrial countries. 60% of the world's population live in countries planting Biotech crops. The US is the lead producer of Biotech crops with 69 million hectares. US is followed by Brazil with 30.3 mil. ha, Argentina (23.7 mil. ha), India (10.6 mil. ha), and Canada (10.4 mil. ha). Developing countries grew close to 50% of global Biotech crops. Another five countries, China, Paraguay, Pakistan, South Africa, and Uruguay each grew more than 1 million hectares. Biotech soybean remains with 75.4 million hectares (47% of global Biotech area) the dominant crop, followed by Biotech maize with 51 million hectares, Biotech cotton 24.7 mil. ha (Fig. 3), and Biotech canola 8.2 mil. ha.

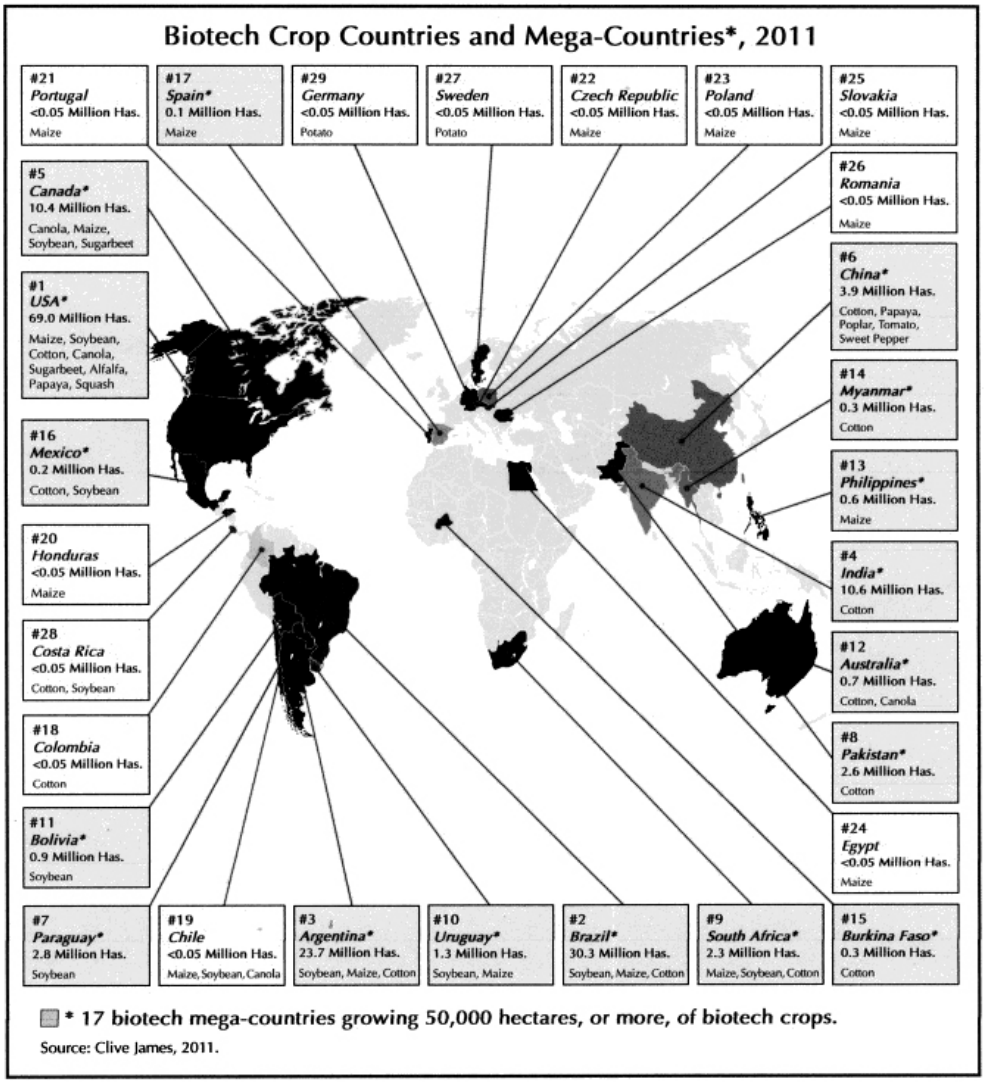

Fig. 2. Global map of biotech crop countries and mega-countries in 2011

Fig. 3. Biotech cotton in grown in Africa

The only 0.114507 million hectares of Biotech crops were planted in Europe. Six EU countries, Spain, Portugal, Czechia, Poland, Slovakia, and Romania planted 114490 ha of Bt maize. Sweden (15) and Germany (2) planted 17 ha of Biotech starch potato cv. 'Amflora'. Spain as the first European country is with 97326 ha of Bt maize on the 17. place. In the Czech Republic the Bt maize was grown on 5091 ha in 2011. European policy in relation to GM crops is scandal. In October 2011, forty one leading Swedish biological scientists in a strongly-worded open letter to politicians and environmentalists asked to revise European legislation to allow society to benefit from Biotech crops. A contingent of scientists from the United Kingdom endorsed the Swedish petition.

Biotech crops are strongly contributing to food security. This was achieved from 1996 to 2010 (in five years) by: increasing crop production and value by US 78 billion dollars, providing a better environment, by saving 443 million kg a.i. of pesticides; in 2010 alone reducing CO_2 emissions by 19 billion kg, equivalent to taking 9 million cars off the road, saving 91 million hectares of land, and helping 15 million farmers, the poorest people in the world. From the point of food security European policy is going against humanity in the world.

Plum (*Prunus domestica* L), clone C5 transformed with the *Plum pox virus* (PPV) coat protein (CP) was obtained by Scorza et al. (1994). Clone C5 (cv. 'HoneySweet' at present) was proved to be highly resistant to PPV under glasshouse conditions (Ravelonandro et al., 1997). Field tests were conducted in Poland (Malinowski et al., 1998), Romania (Ravelonandro et al., 2002), Czech Republic (Polák et al., 2005), and in Spain (Malinowski et al., 2006). The all experiments confirmed the resistance of clone C5 to PPV infection. A trial of high and permanent infection pressure of PPV-Rec alone and in combinations with *Prune dwarf virus* (PDV), and *Apple chlorotic leafspot virus* (ACLSV) was initiated in the Czech Republic and published (Polák et al., 2008a; 2008b). Summarized results of nine years testing of plum clone C5 under the high and permanent infection pressure both from graft inoculation and natural aphid vectors are presented. The transgenic plum trees were evaluated during the years 2003-2011.

Materials and Methods.

Field trial, transgenic plum trees, inoculation of viruses.

Plum clone C5 buds were grafted onto virus-free rootstocks of St. Julien in 2002, and 55 trees of P. domestica clone C5/St. Julien were obtained. Eleven trees inoculated with PPV-Rec, PPV-Rec + ACLSV, PPV-Rec + PDV, PPV-Rec + ACLSV + PDV, control non-inoculated trees were obtained and a plantation established (Fig. 4). PPV-Rec, ACLSV, and PDV infected buds were allowed to grow throughout the period of evaluation. The transgenic clone C5 part of each tree was under a very strong inoculum pressure.

Fig. 4. Plantation of HoneySweet trees in CR in 2011 (Orig. J. Polák)

Evaluation of leaf and fruit symptoms, quality of fruits.

The all trees were evaluated every year from May to September for the presence of viral symptoms in leaves (2003-2011). Fruit symptoms were evaluated in July and August 2010 and 2011 before and at ripening. In 2010 and 2011 overall fruit uniformity, attractiveness, weight, length, width and fruit thickness, flesh thickness, fruit shape, skin colour, flesh colour, flesh firmness, flavour, flesh freeness, total soluble solids, total titratable acidity, stone size, weight and stone/flesh ratio, and dry weight of fruits harvested from trees of clone C5 inoculated with PPV-Rec, and unicoculated control trees of clone C5, and 'Stanley' were recorded.

Serological detection of viruses.

ELISA testing of leaves was performed every year in June. Fruits were evaluated in August 2010. Polyclonal antibodies raised against PPV, ACLSV, and PDV (Bioreba, Switzerland) were used in DAS-ELISA (Clark and Adams, 1977). Leaf samples were extracted in phosphate-buffered saline. The relative concentration of PPV-Rec was determined by semiquantitative DAS-ELISA in samples prepared from symptomatic leaves in June 2005 and 2007, and in samples prepared from fruits in August 2010. The relative concentration of PPV protein was established by determining the lowest dilution of leaf or fruit sample with positive reaction in semiquantitative DAS-ELISA (Albrechtová et al., 1986).

Detection of viruses by Reverse Transcription-Polymerase chain reaction (RT-PCR).

100 mg of ground leaf or fruit tissues were used for total RNA extraction by using RNeasy Plant Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the procedure recommended by the manufacturer. PPV-Rec was detected by RT-PCR using primer pair mD5/mM3 as described by Šubr et al. (2004). For PDV and ACLSV the primers as described by Jarošová and Kundu (2010) were used.

RESULTS AND DISCUSSION. No PPV symptoms appeared in the leaves of transgenic plum clone C5-cv. 'HoneySweet' trees in the first year after inoculation with PPV-Rec by graft inoculation. PPV symptoms appeared only in the leaves that emerged from infected buds (IB). Mild diffuse spots and rings appeared two years after inoculation in some basal leaves of cv. 'HoneySweet' trees inoculated with PPV-Rec, and in those inoculated with virus

combinations PPV-Rec + ACLSV, PPV-Rec + PDV, and PPV-Rec + ACLSV + PDV (Polák et al., 2005). PPV presence was confirmed by ELISA and RT-PCR. A reduction of symptoms was observed beginning in the third year after virus inoculation. PPV symptoms were observed only in several basal leaves and symptoms were milder in each year (Polák et al., 2008a). Further reduction of PPV symptoms was observed during the vegetation period from June to September. No differences in the intensity of PPV leaf symptoms among different virus combinations were observed in the years 2003-2011. No symptoms of PDV and ACLSV appeared during the vegetative periods of 2003-2011. PDV was not detected by ELISA in transgenic parts of trees inoculated with PPV-Rec + PDV and PPV-Rec + PDV + ACLSV. The presence of PDV was inconclusive by RT-PCR. PDV was detected by ELISA and RT-PCR only in leaves growing from the IB. ACLSV was detected by ELISA and RT-PCR in leaves of transgenic parts of trees inoculated with PPV-Rec + ACLSV and PPV-Rec + PDV + ACLSV. No symptoms of PPV, PDV, and ACLSV appeared in the leaves of non-inoculated control trees of cv. 'HoneySweet' throughout the experiment. PPV, PDV, and ACLSV were not detected in control trees by DAS-ELISA and RT-PCR. The growth of control trees was more vigorous in comparison with trees inoculated with PPV, and combinations with PDV and ACLSV. The severe PPV symptoms which appeared first in 2003 in IB leaves growing from buds infected with PPV-Rec appeared again every year (2004-2011) with the same intensity.

The relative concentration of PPV-Rec in symptomatic leaves of cv. 'HoneySweet' determined by semiquantitative DAS-ELISA fluctuated from $1.56x10^{-2}$ to $9.76x10^{-4}$ in 2005, and from $5.0x10^{-1}$ to $7.81x10^{-3}$ in 2007. There were no significant differences in relative concentration of PPV among combinations of inoculated viruses.

Pomological evaluation of external and internal characteristics of fruits harvested from non-graft-inoculated cv. 'HoneySweet' trees, cvs. 'Stanley', and 'Domácí švestka' trees, and from cv. 'HoneySweet' trees growing nine years under the high and permanent infection pressure of PPV-Rec, PPV-Rec + PDV, PPV-Rec + ACLSV, PPV-Rec + ACLSV + PDV demonstrated the high quality of 'HoneySweet' fruits.

Two-year results indicate that the characteristics of 'HoneySweet' fruits harvested from control virus non-inoculated trees are well within the range of the characteristics of control cultivars 'Stanley' and 'Domácí švestka' and are of higher quality in some characteristics. Fruit harvested from 'HoneySweet' trees inoculated with PPV-Rec + ACLSV + PDV, PPV-Rec + PDV, PPV-Rec + ACLSV, and PPV-Rec were comparable with fruits from control healthy 'm 'HoneySweet' trees indicating that there was little, if any, effect of the virus inoculations on fruit quality of 'HoneySweet'.

'HoneySweet' plum trees resistant to PPV remained virus-free under natural aphid-vectored infection pressure throughout this nine-year study. Graft inoculated trees were exposed to a very high infection pressure with IB being allowed to reach the size of 20-30% of the supporting 'HoneySweet' tree (Fig. 5a, b). Under this high and permanent virus pressure 'HoneySweet' trees showed PPV symptoms and positive serological and molecular tests on some basal leaves only, and symptoms subsided during the growing season. ACLSV infection did not appear to affect PPV symptoms and PDV infection could not be detected in 'HoneySweet' throughout the course of the study despite graft inoculation. The evaluations of fruit quality of graft inoculated and un-inoculated 'HoneySweet' trees maintained nine years under the high and permanent infection pressure of PPV, ACLSV, and PDV confirmed not only the high resistance of 'HoneySweet' to PPV, but also suggested that 'HoneySweet' fruit maintain their quality and healthful properties when exposed not only to PPV but also to ACLSV and PDV. Clone C5, resistant to PPV, was deregulated as plum 'HoneySweet' in the U.S.A. in 2010. The meeting of U.S. International Research team for 'Honey Sweet' plum in Prague, May 2011 decided to submit an application for deregulation of plum cv. 'HoneySweet' in European Union. Members of International team are scientists form USA, France, Spain, Poland, Romania, Bulgaria and the Czech Republic. The Czech Republic is represented by Ass.Prof. Jaroslav Polák, DrSc. and Dr. Jiban Kumar from Crop Research Institute in Prague. International team under the leadership of Dr. Ralph Scorza, U.S.A. and Dr. Michel Ravelonandro, France is setting up the application for deregulation of 'HoneySweet' in EU. Crop Research Institute, Prague, and the Czech Republic are supposed to submit the application to EFSA. J. Polák is coordinating and setting up results of research on plum 'HoneySweet' in Europe. The growing of plum 'HoneySweet' in the Czech Republic will be principal contribution not only for fruit growers, but also for producers of plum brandy. It is unique opportunity to establish PPV free orchards and to grow high quality fruits.

a b

Fig. 5. HoneySweet tree:
a – with non-transgenic PPV infected bottom part. (Orig. J. Polák)
b – with cutted away non-transgenic PPV infected bottom part. (Orig. J. Polák)

The future is growing of high quality and productive crops resistant to plant diseases and pests, tolerant to herbicides, to ensure food security and to prevent hunger of people in the world. Genetic engineering will make possible to obtain high quality crop cultivars today very susceptible to diseases, highly resistant in the future, e.g. plum 'Domácí švestka' highly resistant to PPV.

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J. Polák

RECOVERING OF FRUIT CULTIVARS FROM PLANT VIRUSES AND PHYTOPLASMAS IN THE CZECH REPUBLIC: THE PAST, THE PRESENCE, AND THE FUTURE

Ликвидация вирусов растений из растительного материала, особенно для экономически важных культур для Чехии, которые входят в систему сертификации, было основным заданием данных исследований. Представлены результаты оздоровления культур сливы, абрикоса и персика, пораженных вирусом шарки слив, а также культур яблонь, груши и черешни, инфицированных вирусами.

The task of research was to eliminate plant viruses from the basic plant material, especially from the Czech economically important cultivars included in the system of certification. Topical results of recovering plum, apricot, and peach cultivars infected with PPV, and apple, pear and sweet cherry cultivars infected with several economic important viruses are presented.

Research of *in vitro* cultures of plants and the elimination of viruses in plants started in the Czechoslovakia fifty years ago (Svobodová J., 1962; 1964; 1965) in the Institute of Experimental Botany CZAS. First woody plants were recovered in 1972 (Svobodová-Santilliová J., 1972). Thermotherapy of fruit crops continued in the Research and Breeding Institute for Pomology (RBIP) in Holovousy (Janečková M. et al., 1978; Janečková M. and Blattný C. jun., 1980; Janečková et al., 1985, Janečková M., 1988). *Plum pox virus* (PPV) infected plum mother plants of maintenance breeding situated in the open field of Breeding Station Turnov, nord-east Bohemia in the eighties. Practically the all plum cultivars were PPV infected. Technical isolate for mother plants of stone fruits was established in the Research and Breeding Institute for Pomology (RBIP) in Holovousy still in the eighties. Some of especially Czech cultivars were recovered from PPV, and other economically important viruses (Fig.1). Janečková M. (1993) eliminated PPV, *Prunus necrotic ringspot virus* (PNRSV), and *Prune dwarf virus* (PDV) by thermotherapy using combination *in vivo* and *in vitro* cultures. Janečková (1995) applied thermotherapy *in vivo* with bud cultures *in vitro* to eliminate apple viruses. System of certification of virus free status of fruit trees in nurseries was established at the beginning of 21 century in the Czech Republic. Technical and space isolates for apple, pear, cherry and plum cultivars were situated to RBIP Holovousy, for apricot, peach, and almond cultivars to the Faculty of Horticulture (FH) Lednice, Mendel's University Brno. The third technical isolate was established in CRI Prague-Ruzyně. This isolate is divided into two parts, the first part for disease plants and collection of viruses for control purposes, the second one for healthy woody indicators and governmental reserve of basic plants of recovered cultivars.

Fig.1: Fruit of peach cv. Fortuna, severe susceptible to PPV, severe rings and malformations. (Orig. J. Polák)

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