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Occurrence of *Little cherry virus-1* on *Prunus* species in the State of Baden-Württemberg, Germany

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Abstract

A survey on *Little cherry virus-1* (LChV-1) on several *Prunus* species has been performed at four different sites in the State of Baden-Württemberg (BW) between 2003 to 2006. These included a state-run growing site for prebasic and basic material, two commercial nurseries for certified scion or rootstock production and an orchard for cultivar verification testing. A total of 63 varieties of sweet, sour and ornamental cherries belonging to four *Prunus* species (*P. avium* L., *P. cerasus* L., *P. serrulata* Lindl., *P. subhirtella* Miq.) as well as six types of *Prunus*-rootstocks were tested. Ten of the 44 *P. avium* and one of the two *P. serrulata* varieties were partly or totally infected, whereas the *P. cerasus* and *P. subhirtella* varieties and the *Prunus*-rootstocks gave negative results. None of the infected plants showed distinctive disease symptoms.

Dispersal of LChV-1 from the infested *P. avium* trees was not detected in the orchard for cultivar verification testing after a period of five years. A natural dispersal from varieties infected for about 10 years in a nursery for scion production to adjacent healthy varieties was observed only in single cases. There was no indication of any involvement of animal vectors.

Testing of randomly sampled material from some trees of *P. avium* and *P. serrulata* for scion production proved a homogenous distribution of the virus in shoots in autumn.

At the moment studies are conducted to verify the responses of young trees of the sweet cherry variety 'Regina' to experimental inoculation with either LChV-1 or LChV-2 or a mixture of both viruses. First year results indicate that - in complete contrast to LChV-2 - no adverse effects of LChV-1 on the fruit yield, single fruit weight, fruit size and trunk circumference were observed. In mixed infections, LChV-1 seems to attenuate the adverse effects of LChV-2 on fruit yield and trunk circumference.

Keywords: *Little cherry virus-1*, *Little cherry virus-2*, Baden-Württemberg, *Prunus* species, varieties, rootstocks, certification, dispersal, distribution, effects

Introduction

Observations on the presence of Little Cherry Disease symptoms in German cherry fruit production orchards were already reported in the 1980s (Büttner and Graf, 1995). Two different virus types were detected through detailed genome analysis relating the disease to either LChV-1, LChV-2 or a mixture of both (Rott and Jelkmann, 2001). Until a few years ago there was no indication of the presence of Little Cherry Disease in the commercial cherry tree production of Baden-Württemberg. Particularly with regard to certified propagation material there was no justified reason to presume any infestation, since the legally regulated testing for certification with the common indicators did not produce any disease signs. Only after the adoption of the PCR method as a specific technique for LChV-1 and LChV-2 detection and random testing of samples from basic material of some sweet cherry varieties was LChV-1 infection confirmed. Thereafter, the entire stock of prebasic and basic material of sweet and sour cherry in BW were tested, as well as *Prunus*-stocks of other institutions involved in the regional certification system (one nursery for scion and one nursery for rootstock production and an orchard for cultivar verification testing).

Since there is little information on the distribution of LChV-1 in the tree, or the effects of the disease on the pomological properties of cherries e.g. yield, fruit weight, studies were initiated.

Materials and methods

The presence of LChV-1 in varieties of different *Prunus* species was examined in leaf samples (commonly 3-5 leaves/tree as mixed sample). The number of tested trees per variety varied, due to availability, between 2 and ca. 100. For virus detection in rootstocks, rows were divided in sections of typically 20 m. Within one section 20 leaves were sampled and pooled. The number of replicates per type varied between 14 and 27. For the assessment of virus distribution in individual shoots 15 random shoot samples were taken from mid to late October from about 10 years old trees of the varieties 'Hedelfinger Riesenkirsche, type Froschmaul' (*P. avium* L.) and 'Amanogawa' (*P. subhirtella*

Miq.) (3 replicates/variety). The shoots were divided in three sections of equal length. From each part three leaves or buds (if leaves were unavailable) were taken, pooled and analysed.

For comparing the effects of a LChV-1 infection with those of either LChV-2 or a mixed infection, the rootstock 'Gisela 5' was grafted with the sweet cherry variety 'Regina' and inoculated by chip budding (5 replicates/treatment) in 2004. The LChV-1 and LChV-2 inoculum was obtained from the sweet cherry variety 'Kassins Frühe Herzkirsche' (basic material site nearby Stuttgart) and 'Regina' (commercial orchard near Lake Constance), respectively. The infection with either the single viruses or their combination was confirmed by PCR. Fruit yield and further parameters were measured in 2008 for the first time. The single fruit weight and size (diameter) was determined as an average of 10 fruits/tree, respectively. The trunk circumference was measured 30 cm above the grafting point.

For the detection of LChV-1 and -2 total nucleic acid was extracted from leaves or buds using the silica capture method as described by Menzel (2003). RT-PCR for LChV-1 and LChV-2 was performed with primer pairs LCV1 U 16390 / LCV1 L 16809 and LCV2UP2 / LCV2LO2, respectively, according to Rott und Jelkmann (2001) and later extended with primers LCH1_7634F / LCH1_7942R and LCH2_01F / LCH2_03R according to Jelkmann et al. (2008).

Results

Occurrence of LChV-1 in *Prunus* species and varieties: Out of 44 tested varieties of *P. avium* 10 tested positive for LChV-1 (Tab.1). Mostly, all tested trees of a variety proved to be virus positive, in few cases only single trees of a variety were infested. In one case ('Hedelfinger Riesenkirsche, type Froschmaul') a section of trees within an infested row tested negative. The majority of the diseased varieties were identified as being old varieties, e.g. 'Burlat', 'Büttner Rote Knorpelkirsche', 'Große Schwarze Knorpelkirsche' and 'Kassins Frühe Herzkirsche'. Among the remaining *Prunus* species only the variety 'Amanogawa' (*P. serrulata*) tested LChV-1 positive. All six types of *Prunus* rootstocks ('Colt', 'Gisela 3', 'Gisela 5', 'Gisela 6', 'F12/1', 'Piku 4') belonging to different certification categories (prebasic, basic, certified mother plants) were tested virus negative. A part of the samples (scions and rootstocks) was also tested for LChV-2 but there was no evidence for the presence of the virus.

Tab. 1 Number of varieties / types of *Prunus* species from several sites (certified commercial scion and rootstock production, state-run facility for prebasic and basic material, state orchard for cultivar verification testing) found to be LChV-1 infested between 2003 and 2006

Species	No. varieties/types totally tested	LChV-1-positive
<i>P. avium</i>	44	10
<i>P. cerasus</i>	16	0
<i>P. subhirtella</i>	1	0
<i>P. serrulata</i>	2	1
<i>Prunus</i> rootstocks	6	0

None of the virus positive trees showed obvious symptoms indicating a virus infection with LChV-1. The motherplants of some of the varieties were previously submitted to thermotherapy for virus elimination and failed to produce any indication of LChV-1 infestation in subsequent tests with the standard indicators 'Sam' and 'Canindex'. Nevertheless, these varieties proved later to be LChV-1 positive by PCR.

In an orchard for cultivar verification testing comprising about 80 *P. avium* and *P. cerasus* trees (44 varieties), 10 trees belonging to 5 varieties were LChV-1 positive. Diseased trees of the same variety were adjacent but the varieties were randomly distributed in the plot. At the time of planting it was unknown that the trees were already diseased, however backtracking to the origin of the planting material revealed that the mother plants were already LChV-1 infected. The distance between single trees within a row was 2.5 m and between rows 4 m. However, a spread of the virus was not observed after a period of five years. Similar observations were made in a plantation for scion production comprising different varieties grafted on *P. avium* F12/1. Also in this case, LChV-1 infested varieties were placed side-by-side with healthy varieties over a period of 10 years and the infection was, except very few cases, restricted to the originally diseased plants. In the latter site the distance between the trees was 0.8 m and 2.75 m between the rows. Also here the status of virus infection was unknown at the time of planting, but infested varieties could be traced back to the infected mother plants of basic material.

Virus distribution within shoots: Examination of scion producing trees of the varieties 'Hedelfinger Riesenkirsche', type Froschmaul' (*P. avium*) and 'Amanogawa' (*P. serrulata*) revealed that all samples, except an indistinct single case, were PCR positive at the time of sampling. The virus was present in leaves and buds.

Effects of virus infection: Four years after inoculation of the sweet cherry variety 'Regina' with LChV-1 no disease symptoms nor any negative effects on the pomological parameters measured were observed (Tab. 2). In contrast, LChV-2-infection induced significant reduction in fruit yield (-74%), single fruit weight (-44%), fruit size (-23%) and trunk circumference (-46%). In addition, typical symptoms like a reddish-brownish colouring of intercostal fields (in 2009 appearing since July) were recorded. An infection with a mixture of both viruses had intermediate effects on fruit yield and trunk circumference compared to those of LChV-2 alone. However, single fruit weight and fruit size were comparable to LChV-2 infected trees.

Tab. 2 Recorded effects of LChV-1, LChV-2 and a mixture of both on the sweet cherry variety 'Regina' four years after experimental inoculation of trees (first year results).

	Fruit yield/tree (g)	Single fruit weight (g)	Fruit size (mm diameter)	Trunk circum-ference (cm)
Control	950	11.1	25.0	18.0
LChV-1	1215	11.8	25.9	20.7
LChV-2	250	7.3	19.3	9.7
LChV-1+ 2	685	6.5	19.6	15.0

Discussion

The detection of LChV-1 in certified propagation material of various varieties of two *Prunus* species in the State of Baden-Württemberg (BW) was very surprising, since all mother trees were virus tested at earlier occasions. The expression of the typical symptoms, as described in the literature for the Little Cherry Disease, is therefore apparently primarily related to the infection by LChV-2. Symptoms of LChV-1 infection are reported to be milder or even latent (Jelkmann and Eastwell, 2009). The LChV-1-isolate(s) present in BW is obviously not detected when the standard indicators ('Sam', 'Canindex') are used nor could it be eliminated by standard thermotherapy for cherry viruses. Only the use of the PCR assays made its detection possible. As there is no known vector for the transmission of LChV-1, it can be assumed that the dispersal of the virus is via vegetative propagated tree material. Since many diseased varieties are growing already for more than 30 years in the virus-tested cherry stands, it can be assumed that the LChV-1 virus does exist in the State of BW the same time. The locally certified rootstock stands, however, are free of LChV-1 since no proof of infection was obtained until now. In Poland, however, there is evidence for LChV-1 in the rootstock cultivar 'Gisela' (Komorowska und Cieślińska, 2004). The fact that within five years, no dispersal of LChV-1 in the orchard for cultivar verification testing was observed, can be explained by a lack of vector transmissibility or the absence of a potential vector. The observed cases of virus transmission to neighbouring healthy varieties in a scion producing nursery after a 10 years period can be probably attributed to root transmission. The narrow tree distance and growth properties of the used rootstocks of *P. avium* F12/1 strongly support root expansions into the adjacent rhizosphere.

A homogeneous distribution pattern of the virus within the aerial parts of trees for scion production can be assumed at least for two varieties in autumn due to positive testing of all sampled shoots. Because of the periodical strong pruning of trees in spring, virus particles are likely to move apically due to shoot re-growth thereby reaching a homogeneous distribution in autumn or possibly earlier. The risk of failing to detect the virus when mixed samples are collected in autumn is most likely very low.

The preliminary results of the experimental infection trials using the sweet cherry variety 'Regina' with LChV-1 showed that the infection had no effects on the pomological parameters studied. Therefore, it is likely that the isolate of LChV-1 used for inoculation, contrary to LChV-2, is only weakly virulent or avirulent. Surprisingly, the infection with a mixture of the two viruses seems to attenuate the effects of LChV-2 on fruit yield and trunk circumference. This trial is ongoing and hopefully results in the years to come contribute to a further understanding of the interaction.

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