Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Occurrence of cherry viruses in South Tyrol (Italy) by comparing growth periods in two consecutive years

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Summary. Sweet cherries (*Prunus avium* L.) are important as fruit crops, and can be affected by numerous viruses. An investigation on the occurrence of the three most common viruses of sweet cherry was carried out in commercially managed orchards in South Tyrol (Italy). The incidence of apple chlorotic leaf spot virus (ACLSV), Prunus necrotic ringspot virus (PNRSV) and prune dwarf virus (PDV) was investigated using enzyme-linked immunosorbent assays (ELISA) and the reverse transcriptase-polymerase chain reaction technique (RT-PCR) in spring 2018 and 2020, and during the summer and autumn of 2020. All three viruses were detected in the surveyed orchards. Comparative analyses showed that detection was more effective with RT-PCR than with ELISA, especially for detecting PNRSV and PDV. Mixed infections were detected in all the surveyed orchards. The results also showed clear differences between and during host growth periods, likely due to a variable virus concentration in the host trees.

Keywords. Apple chlorotic leaf spot virus (ACLSV), Prunus necrotic ringspot virus (PNRSV), Prune dwarf virus (PDV), sweet cherry, *Prunus avium*.

OPEN ACCESS

Citation: E. Deltedesco, M. Niedrist, S. Oettl (2022) Occurrence of cherry viruses in South Tyrol (Italy) by comparing growth periods in two consecutive years. *Phytopathologia Mediterranea* 61(2):395-404. doi:10.36253/phyto-12807

Accepted: June 15, 2022

Published: September 15, 2022

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Arnaud G. Blouin, Institut des sciences en production végétale IPV, DEFR, Agroscope, Nyon, Switzerland.

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INTRODUCTION

Italy has among the worlds' greatest growing areas of sweet cherries (*Prunus avium* L.). In 2018, this country ranked fifth, with a growing area of 29,010 ha, after Syria Arab Republic (30,317 ha), United States of America (34,400 ha), Chile (39,645 ha), and Turkey (82,729 ha) (FAO, 2022). Almost 90% of Italian sweet cherries are cultivated in the regions of Puglia, Campania, Emilia Romagna, and Veneto. However, sweet cherry production does not have particular temperature requirements for fruit ripening, so cultivation in altitudes up to 1,300 m a.s.l. is possible (Zago, 2003; Gamper, 2010). Hence, cherry cultivation can be an economically important supplementary crop for small farmsteads in Alpine regions.

New cherry plantings require substantial investments for protective nets against hail, birds, and insects, as well as for rain covers (Gamper, 2013). Nevertheless, good returns can be achieved for premium quality cherries

produced in South Tyrol. These result from favourable market situations in late summer, when cherries grown in warmer cultivation areas are no longer being offered, and growers from the alpine areas can supply markets at profitable prices (Zago and Ropelato, 2009; Gamper, 2010). Therefore, cherry production has increased in the last decade in South Tyrol, the northernmost province of Italy (Pirazzoli and Palmieri, 2019), and is currently producing sweet cherries on approx. 100 ha (Catalano, 2013; Martini, 2021).

Plant viruses can cause many plant diseases characterised by severe symptoms but can also remain latent in host plants (Anderson *et al*., 2004; Strange and Scott, 2005; Hull, 2013). Cherry plants can be infected by at least 29 viruses (Myrta and Savino, 2005; Kamenova *et al*., 2019). Three economically important viruses of commercial cherry cultivars are apple chlorotic leaf spot virus (ACLSV), Prunus necrotic ringspot virus (PNRSV) and prune dwarf virus (PDV) (Menzel *et al*., 2003; Yu *et al*., 2013; Rubio *et al*., 2017).

ACLSV is a member of *Trichovirus* (*Betaflexiviridae*, Martelli *et al*., 1994; Adams *et al*., 2004; Watpade *et al*., 2013), and is known to infect pome and stone fruits. The concentration of ACLSV in infected trees is low and irregular, hence reliable detection is difficult (Polák and Svoboda, 2006; Katsiani *et al*., 2014). Generally, ACLSV-infected trees are symptomless, but can be responsible for cherry necrosis (Liu *et al*., 2014), fruit deformation and discoloration, as well as graft incompatibilities in nurseries (Rana *et al*., 2011; Sánchez *et al*., 2015). However, symptoms and severity depend largely on the infected *Prunus* species and/or the infecting virus strain (Osman *et al*., 2016; Rubio *et al*., 2017). PNRSV belongs to *Ilarvirus* (*Bromoviridae*), and may cause infections of all *Prunus* species (Mekuria *et al*., 2003; Fiore *et al*., 2008). Cherry trees infected with PNRSV can exhibit a wide range of symptoms, which may include foliar mosaics, ring-shaped or spotted chlorotic areas, but symptoms may also be latent (Oliver *et al*., 2009; Sánchez *et al*., 2015). Furthermore, infections with PNRSV may lead to reduced numbers of flower buds, which leads to yield losses of 20 to 56% (Wang *et al*., 2018), and culminates in death of infected trees (Song *et al*., 2013). Like PNRSV, PDV belongs to *Ilarvirus* (*Bromoviridae*) (*Öztürk* and *Çevik*, 2015), and can infect different *Prunus* spp. (Kozieł *et al*., 2020). Depending on environmental conditions, virus strain, and plant host, PDV-infected trees often remain symptomless. This virus may cause chlorosis, yellowing, mosaic, ringspot, necrosis, and malformations on leaves, as well as reduction numbers of fruits (Predajňa *et al*., 2017). Viruses such as ACLSV, PNRSV, and PDV can occur as mixed infections in cherry orchards, and may result in decline, low yields, and unusual fruit disorders, with crop losses of up to 57% (Mandic *et al*., 2007; Yardimci and Culal-Klllc, 2011). ACLSV is only known to be transmitted by grafting and other vegetative propagation techniques. In contrast, PNRSV and PDV can also be transmitted horizontally in pollen and vertically in seeds (Rubio *et al*., 2017).

Phytopathological alterations observed in many Italian cherry cultivation areas in the last two decades have led to detailed studies of the presence of fruit tree viruses (Aparicio *et al*., 1999; Myrta *et al*., 2003; Matic *et al*., 2007; Babini *et al*., 2014). None of these studies included cherry plantings in the South Tyrol province, there is no information on the virus phytosanitary status of this small cultivation area. However, due to the expansion of cherry cultivation and the associated high investment costs in this mountainous area, it is important to obtain information on virus occurrence, to avoid yield losses caused by viruses. Reliable diagnoses of these viruses are also important for maintaining sustainable plant production. This will assist in selection of pathogen-free propagative plant material in nurseries and in orchards used for commercial production.

Routine virus diagnoses in cherry trees are based on enzyme-linked immunosorbent assays (ELISA) and the reverse transcriptase-polymerase chain reaction (RT-PCR) (Barba *et al*., 2015). There are issues with both of these methods that have been outlined elsewhere (Mekuria *et al*., 2003; Noorani *et al*., 2013; Hu *et al*., 2014; Rubio *et al*., 2017). Furthermore, the seasonal dynamics of virus concentrations throughout growing seasons may affect assay reliability (Tsai *et al*., 2012).

The aims of the present study were: (a) to obtain an overview of virus incidence in commercially managed cherry orchards in South Tyrol; (b) to compare ELISA and RT-PCR for routine diagnoses in cherry virus detection; and (c) to assess annual and seasonal fluctuations of concentrations of ACLSV, PNRSV, and PDV in South Tyrolean sweet cherry orchards.

MATERIAL AND METHODS

Sampling procedure

Eight commercially managed orchards were selected in the Val Venosta/Vinschgau valley (South Tyrol, Italy) (Table 1). The first sampling was carried out on each site in spring 2018, during the full bloom host stage (end of April until early May). Flowers were manually sampled from 30 randomly selected trees at each site (n = 240). All samples were tested immediately after sampling for the presence of three viruses (ACLSV, PNRSV, and

Table 1. Main sampling site parameters and sampling periods at each site, for virus assessments in South Tyrol cherry orchards. Site altitudes indicated (meters above sea level; m a.s.l).

Site	Location	Altitude (m a.s.l.)	Planting year	Sampling periods
A	N 46°60'92" E 10°75'47"	730	2017	Spring 2018, spring 2020
B	N 46°60'99" E 10°75'47"	730	2010	Spring 2018, spring 2020
C	N 46°63'28" E 10°64'95"	891	2015	Spring 2018, spring 2020
D	N 44°66'95" E 10°54'78"	918	2008	Spring 2018, spring 2020, summer 2020, autumn 2020
F	N 46°68'48" E 10°53'30"	1,037	2017	Spring 2018, spring 2020, summer 2020, autumn 2020
G	N 46°68'77" E 10°53'71"	1,037	2017	Spring 2018, spring 2020
H	N 46°57'40" E 10°80'19"	1,901	2016	Spring 2018, spring 2020
I	N 46°55'82" E 10°78'14"	1,901	2008	Spring 2018, spring 2020

PDV) using ELISA (Table S2). Nine trees per orchard, including those which were positive in the ELISA results 2018 (except for four trees tested positive for one of the viruses had to be replaced by negative trees, due to agronomical reasons: one tree tested positive for PDV in each of orchards A and D, and one tree tested positive for ACLSV in each of orchards D and F), were used for RNA extractions and were again sampled in spring of 2020 during full bloom at the end of April until early May, depending on the sampling site. To determine seasonal dynamics of the virus distribution in commercially managed orchards, the same nine trees of the field sites D and F, which were already tested in spring (2018 and 2020), were sampled again in summer (August) and autumn (October) 2020.

All samples (flowers, buds, and leaves) were taken randomly from different branches at different heights all around each assessed tree. Each individual sample consisted of either ten flowers in spring 2018/2020 or ten buds and ten leaf discs in summer and autumn 2020. Each sample was pooled, placed in a plastic bag, put into a cooling box, and brought to the laboratory for further processing.

Laboratory analyses

Enzyme-linked immunosorbent assays (ELISA). 0.5 g of single petals per flower were homogenised together with 4.75 mL of extraction buffer in a universal bag (Bioreba AG,) using an automatic homogenizer (Bioreba AG). The ELISA tests were carried out according to the manufacturer's instructions, with the commercially available Double Antibody Sandwich Assay (DAS-ELISA) (LOEWE® Biochemica GmbH). Absorbance values were detected at 405 nm with FLUOstar OPTIMA microplate reader (BMG Labtech). Data analyses were carried out according to the Bioreba AG technical information (BIOREBA, 2011). The cut-off was set-up individually for each plate. Absorbance values were sorted in ascending order and a histogram for each plate was created. In the resulting histograms, negative and background values could be easily distinguished from potential positive values, which were characterized by an abrupt increase in the OD value. The mean value and the standard deviation calculated from values before this abrupt increase were used to calculate the cut-off as:

$$\textit{mean value} + 3 \times \textit{standard deviation} + 10\%$$

Total RNA isolations

Immediately after sampling, petals (spring 2018 and 2020) or buds together with leaf discs (August 2020 and October 2020) were cut into pieces with a scalpel and mixed homogeneously. Approx. 0.2 g of the blended tissues was homogenized using a TissueLyser II (Qiagen), for which adaptors were pre-cooled in liquid nitrogen. RNA was extracted with RNeasy Plant Mini Kits (Qiagen) according to manufacturer's instruction. Extraction vials were cooled on ice between the extraction steps.

RT-PCR assays

One-step multiplex RT-PCR was carried out using the SuperScript™ III One-Step RT-PCR System with the Platinum™ Taq DNA Polymerase kit (Invitrogen by Life Technologies), and the primer pairs ACLSV_s/ACLSV_a, PNRSV_s/PNRSV_a, and PDV_s/PDV_a (Sanchez-Navarro *et al.,* 2005), at final concentration of 0.25 pmol μL^{-1} for each primer. A region of the chloroplast gene *rbcL*, which encodes the large subunit of ribulose bisphosphate carboxylase, was used as internal control. For the *rbcL* gene, primers Rbcl_s/Rbcl_a (Sanchez-Navarro *et al.,* 2005) were used, at final concentrations of 0.05 pmol μL^{-1}. Thermal cycling (Sanchez-Navarro *et al*., 2005) was: an initial cycle of 50°C for 30 min for cDNA synthesis, followed by a denaturation step of the RT enzyme at 94°C for 2 min, 40 cycles each at 94°C for 15 s, 50°C for 30 s, 68°C for 1 min, and a final incubation at 68°C for 7 min. The amplified PCR products were separated on 2%

agarose gels stained with GelRed® Nucleic Acid Gel Stain (Biotium Inc.), and were visually checked using Chemi-Doc™ MP with Image Lab™ v.4.0.1 (Bio-Rad Laboratories Inc.). On each gel, an artificial ladder was included, made from a mixture of amplicons of each target. This mix was prepared by cDNA amplification of reference gene fragments for ACLSV, PDV, PNRSV, and *rbcL*, with the same primers used for the RT-PCR from formerly tested positive cherry petals (Figure S1). Amplicons were cloned into the pJET1.2/blunt Cloning Vector (Thermo Fisher Scientific s.p.a.) according to the manufacturer's instructions. Inserts were amplified and sequenced with vector-specific forward and reverse primers to confirm amplification of the gene fragments by LGC Biosearch Technologies. The quality of the sequencing data was controlled with the software Geneious v.11.1.5 (Biomatters Ltd.), and sequence identity was confirmed by BLASTn search. The sequences of the three viruses were deposited in the NCBI GenBank under the accession numbers OM585596 for ACLSV, OM585598 for PNRSV, and OM585597 for PDV. Appropriate amplicons were purified with the QiaQuick PCR Purification Kit according to the manufacturer's instructions (Qiagen), and were mixed at approximately equal concentrations for the internal controls.

RESULTS

ELISA detection of viruses in 30 randomly selected cherry plants at each orchard site

All three assessed viruses were detected in the sampled cherry orchards in spring 2018 (Table 2). ACLSV was detected in four orchards, and PDV in five orchards, while PNRSV was detected in two orchards. Only orchard H showed no virus infection. The greatest number of ACLSV-positive trees were detected in orchard D in 11 samples. However, no obvious symptoms were observed on cherry trees during the orchard monitoring, except for samples I29 and I30 which were manifesting scattered chlorotic areas on the leaves. Those trees were tested positive for ACLSV (Table S1). In general, 15 (6.3 %) out of the 240 trees tested positive by ELISA for ACLSV, two (0.8 %) for PNRSV, and 11 (4.6 %) tested positive for PDV.

The following results presented focus on selected samples (n = 9 per orchard), as described in the Material and Methods (above).

Comparison of ELISA and RT-PCR detection methods in 2018

When the results of ELISA and RT-PCR were compared, not all samples positively detected by ELISA were positive by RT-PCR, and *vice versa* (Tables 3 and 4). In general, ELISA was less sensitive for detection of the viruses than RT-PCR, regardless of the virus type. However, the largest difference between the results from both techniques was for PDV, followed by PNRSV and ACLSV.

Table 2. Summary of ELISA results for ACLSV, PNRSV, and PDV on 30 randomly selected samples collected in 2018 from commercial cherry orchards in South Tyrol.

Site	No. of trees tested	ACLSV	PNRSV	PDV
A	30	0	0	4
B	30	0	0	1
C	30	0	0	2
D	30	11	0	1
F	30	1	1	0
G	30	1	0	0
H	30	0	0	0
I	30	2	1	3
Positive trees		15	2	11
Overall proportion infected		6.3%	0.8%	4.6%

Table 3. Comparison of results (based on 72 samples) obtained by ELISA and RT-PCR techniques for cherry tree samples collected during spring 2018, presented per orchard and virus. For both ELISA and RT-PCR, petals were used to determine apple chlorotic leaf spot virus (ACLSV), Prunus necrotic ringspot virus (PNRSV), and prune dwarf virus (PDV).

Site	No. samples tested	ELISA/RT-PCR		
		ACLSV	PNRSV	PDV
A	9	0/0	0/3	3/3
B	9	0/0	0/2	1/2
C	9	0/4	0/2	2/2
D	9	8/7	0/1	0/3
F	9	0/0	1/2	0/2
G	9	1/1	0/4	0/3
H	9	0/1	0/6	0/6
I	9	2/2	1/1	3/3
Positive trees		11/15	2/21	9/24
Proportions infected (%)		15.3/20.8	2.8/29.2	12.5/33.3

Table 4. Comparison of diagnostic results obtained from RT-PCR and ELISA for the detection of apple chlorotic leaf spot virus (ACLSV), Prunus necrotic ringspot virus (PNRSV), and prune dwarf virus (PDV). A total of 72 trees were tested by each method in 2018, and again in 2020 (144 test results). Petals were used as starting material. Neg. = negative; Pos. = positive.

Virus	Test	RT-PCR Neg.	RT-PCR Pos.
ACLSV	ELISA Neg.	113	11
	ELISA Pos.	3	17
PNRSV	ELISA Neg.	113	25
	ELISA Pos.	1	5
PDV	ELISA Neg.	104	27
	ELISA Pos.	4	9

Occurrence of mixed virus infections in 2018 and 2020 or both years based on RT-PCR results

Single infections with either ACLSV, PNRSV, or PDV were more predominant than mixed infections by the three viruses. (Table 5). Based on RT-PCR, the most common co-infection was by PNRSV and PDV (16.7%). Mixed infections by ACLSV and PDV were found in two samples (C18 and D08). Mixed infection by ACLSV and PNRSV was detected in only one sample (D13). Mixed infections by ACLSV, PNRSV and PDV were detected twice (A13 and H15).

Annual and seasonal fluctuations of viruses in cherry plants based on RT-PCR

Not all infections determined in 2018 were confirmed in 2020, and *vice versa*. ACLSV was detected in 11 identical samples of both years, PNRSV in four, and PDV in seven (Table S2). Generally, the number of positive samples during full bloom was greater in 2018 than in 2020 in all orchards (Table S2).

Detailed results of the infection rates of orchard D and F for all four sampling times (two years, three different seasons) are shown in Figure 1. The greatest numbers of virus positives were detected in samples taken during the full flowering stage, regardless of year and virus. ACLSV was detected in seven out of nine samples from block D in spring samples of 2018 and 2020. In summer 2020 only five and in autumn 2020 only three samples tested positive for this virus. The same applied for PNRSV and PDV. In orchards D and F, infected samples were detected when sampling was carried out during full bloom (in 2018 and 2020). Three samples tested positive in both years, spring 2018 and 2020 (PDV: D02 and F16; PNRSV: F16), while for the other samples (PDV: D06, D08, F10 and F15 for PDV; PNRSV: D13 and F20), positive infections were detected only during spring sampling in 2018 or 2020. The sample F10 was an exception, as it tested consistently positive for PNRSV throughout the vegetation periods.

Table 5. Number of trees tested positive by RT-PCR for apple chlorotic leaf spot virus (ACLSV), Prunus necrotic ringspot virus (PNRSV), and prune dwarf virus (PDV), either in 2018 and 2020 or both years. Nine trees were tested in each of eight orchards (based on 72 samples).

Site	Single infections			Mixed infections			
	ACLSV	PNRSV	PDV	ACLSV + PNRSV	ACLSV + PDV	PDV + PNRSV	ACLSV + PNRSV + PDV
A	0	2	2	0	0	1	1
B	0	0	0	0	0	2	0
C	4	1	1	0	1	1	0
D	5	0	2	1	1	0	0
F	0	2	1	0	0	2	0
G	1	3	2	0	0	1	0
H	0	1	2	0	0	4	1
I	2	1	3	0	0	1	0
Positive trees	14	10	13	1	2	12	2
Proportion infected	19.4%	13.9%	18.1%	1.4%	2.8%	16.7%	2.8%

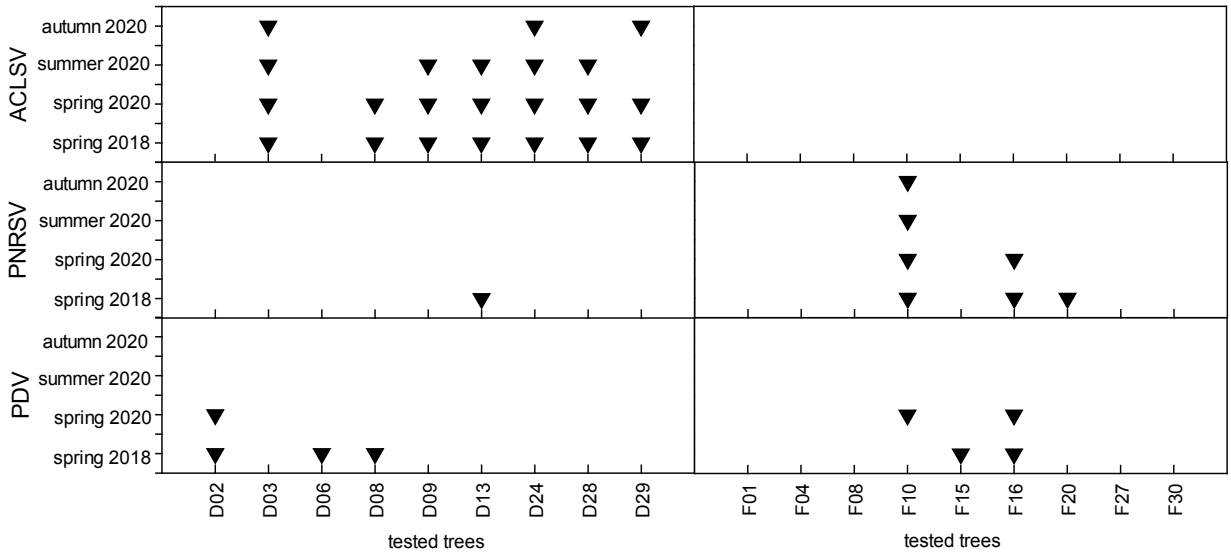

Figure 1. Seasonal fluctuations in detection of virus positive trees using RT-PCR during autumn 2020, summer 2020, spring 2020 and spring 2018 (y-axes for apple chlorotic leaf spot virus (ACLSV), Prunus necrotic ringspot virus (PNRSV) and prune dwarf virus (PDV). Each triangle indicates positively tested trees (n = 9) for orchards D (left) and F (right).

DISCUSSION

This study, using both ELISA and RT-PCR, confirmed the presence of ACLSV, PNRSV, and PDV in commercial sweet cherry orchards in South Tyrol, the northernmost province of Italy (Tables 2 and 3). Previous studies have reported of several *Prunus* species infected with these viruses in Europe and in Italy. These viruses have also been detected in plums, apricots, almonds, peaches, and sweet cherries (Barba *et al*., 1985; Savino *et al*., 1991; Myrta *et al*., 2003; Myrta and Savino, 2005; Paduch-Cichal *et al*., 2007). Recent investigations showed that over 40% of cherries in the Emilia Romagna region of Italy tested positive for ACLSV, PNRSV, and PDV (Babini *et al*., 2014).

Targeted detection of plant viruses relies on diagnosis method and choosing the correct host growth stage for sampling. Routine diagnosis for virus detection in cherry fruit trees is generally performed by ELISA and RT-PCR (Barba *et al*., 2015). In the present study, comparison of ELISA and RT-PCR as diagnostic methods for the detection of cherry viruses resulted in considerable differences, especially for detection of PNRSV and PDV, but less for ACLSV (Table 3). These results were in line with previous studies, which have reported greater infection rates for PNRSV and PDV using RT-PCR than using ELISA (Sánchez-Navarro *et al*., 1998; Yardimci and Culal-Klllc, 2011; Gospodaryk *et al*., 2013). For example, Gospodaryk *et al.* (2013) reported a nearly two-fold greater infection rate for PNRSV and PDV obtained by RT-PCR than by ELISA. These results suggested greater sensitivity for RT-PCR than ELISA, especially for the detection of PNRSV and PDV. Furthermore, Hadidi *et al.* (2011) suggested that *Ilarvirus* particles are unstable in ELISA buffers. However, a few samples detected by ELISA tests were not detected by RT-PCR (Table 4). Nevertheless, as extensively discussed in previous reports, ELISA remains the most widely used approach for detection of viruses in large numbers of samples (Mekuria *et al*., 2003; Noorani *et al*., 2013; Hu *et al*., 2014; Rubio *et al*., 2017). This is probably because the development of a multiplex RT-PCR system can be laborious and time consuming, but is a rapid, reliable, and cost-effective method (Wei *et al*., 2008). RT-PCR has been successfully used for simultaneous detection of several stone fruit viruses, even with low virus titre and in the presence of inhibitors (Bariana *et al*., 1994; Kummert *et al*., 2001). Therefore, focus in the present study was placed on the results obtained with RT-PCR.

Based on RT-PCR results of selected trees (n = 9 per orchard), PNRSV and PDV were both detected in all eight of the surveyed South Tyrolean commercial sweet cherry orchards, while ACLSV was not detected

in two of the orchards (B and F, Table 3). Considering the relatively high number of trees infected with ACLSV, especially at the sampling sites C and D, it is likely that virus-infected plant material was used when establishing these orchards (Figure 1). However, the certified status of the propagation material at purchase is known only for orchards A, F, and H. These orchards were Conformitas Agraria Communitatis (CAC) certified at the time of planting. Unlike ACLSV, PNRSV and PDV can be transmitted by grafting or vegetative propagation techniques and in pollen (Barba *et al*., 2015). Hence, pollen and seed transmission of PNRSV and PDV may have contributed to the occurrence of these in all the surveyed orchards. Viruses with these transmission modes have high distribution potential.

Most surveyed trees (n = 9 per orchard) were infected either with ACLSV, PNRSV, PDV, or with combinations of these viruses (Table 5). Mixed infections of these viruses were reported for cherry orchards in previous studies (Myrta *et al*., 2003; Yardimci and Culal-Klllc, 2011; Gospodaryk *et al*., 2013). In the present study, the combination of PNRSV and PDV occurred most frequently. As suggested by Gospodaryk *et al.* (2013), mixed infections may be the result of the different transmission modes, geographical origins, and grafting, which may contribute to wide distribution of these viruses.

In the present study, virus detection using both ELISA and RT-PCR was found to be variable, with clear differences between and during host growth periods. Detections were maximum during full flowering in spring of both years (2018 and 2020; Table S2 and Figure 1). Uneven distribution of virus in individual trees is a likely explanation for the detection discrepancies between and during growth periods. (Knapp *et al*., 1995; Spiegel *et al*., 1997; Marbot *et al*., 2003). The same phenomenon could also explain differences between sampling years, and influences of weather conditions are also likely to have contributed to the seasonal and annual discrepancies experienced in the present study.

Honjo *et al*. (2020) suggested that in natural systems, the concentration of viruses within hosts may change greatly during growth periods, depending on virus replication and host growth. In the present study, the uneven virus distribution of PNRSV and PDV within trees varied particularly between the two years. For example, only three samples tested positive in spring 2018 and 2020, while for six trees a positive infection was detected at least once, either in spring 2018 or 2020. However, samples F10 (for PNRSV), and D03 and D24 (for ACLSV) showed constant infection status throughout the growth periods. It is likely that the virus concentration was high in these trees, and/or the viruses were uniformly spread throughout the host trees. To obtain reliable results and to prevent false negatives, samples consisted of pooled homogenized tissues, taken from around each tree at different heights. However, even pooling the collected samples from different parts of each same tree, may not sufficiently overcome the effect of uneven virus distribution. Further studies are required to take account of the discrepancies observed. Greater numbers of samples per tree should be examined. Although care was taken to avoid errors during processing samples, human error can never be excluded. Furthermore, no differences in virus prevalence were found for different altitudes or ages of trees, although more studies are required to confirm these results.

CONCLUSIONS

Virus infections can seriously compromise the phytosanitary status of fruit tree nurseries, and commercially managed orchards. In the present study, all three assessed viruses (ACLSV, PNRSV and PDV) were detected in cherry orchards, either by ELISA or RT-PCR. However, RT-PCR was more sensitive than ELISA for detection of these viruses, and especially for PNRSV and PDV. Virus detection was variable and was different during different host growth stages. Future research should include host sampling from different altitudes, orchard ages, host cultivars, and propagation material at time of acquisition, to better understand the observed phenomena.

ACKNOWLEDGMENTS

The South Tyrolean Extension Service for Fruit and Wine Growing, especially Gregor Trafojer and Michael Gamper, supported this study during the sampling campaigns. Dr Valeria Gualandri and Paola Bragagna of Technology Transfer Centre, Fondazione E. Mach, provided information on technical feasibility. South Tyrolean cherry growers gave excellent cooperation within this study. Dr. Markus Gorfer, Austrian Institute of Technology, provided guidance during laboratory work and in proofreading of the manuscript of this paper.

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