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

EVI DELTEDESCO*, MAGDALENA NIEDRIST, SABINE OETTL

Laimburg Research Centre, Laimburg 6 - Pfatten (Vadena), 39040 Auer (Ora), BZ, Italy

*Corresponding author. E-mail: evi.deltedesco@laimburg.it

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.

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 (Poláček 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. (Koziel 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 (Predajiña 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-Kilic, 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/Vinschga 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
Detecting cherry viruses in South Tyrol (Italy)

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:

\[
\text{mean value} + 3 \times \text{standard deviation} + 10\% 
\]

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 ChemiDoc™ 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 ampiclons 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 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 |
|------|--------------------|
| A    | 9                  |
| B    | 9                  |
| C    | 9                  |
| D    | 9                  |
| F    | 9                  |
| G    | 9                  |
| H    | 9                  |
| I    | 9                  |
| Positive trees | 11/15    |
| Overall proportion infected | 15.3/20.8 |

| Site | ACLSV | PNRSV | PDV |
|------|-------|-------|-----|
| A    | 0/0   | 0/3   | 3/3 |
| B    | 0/0   | 0/2   | 1/2 |
| C    | 0/4   | 0/2   | 2/2 |
| D    | 8/7   | 0/1   | 0/3 |
| F    | 0/0   | 1/2   | 0/2 |
| G    | 1/1   | 0/4   | 0/3 |
| H    | 0/1   | 0/6   | 0/6 |
| I    | 2/2   | 1/1   | 3/3 |
| Positive trees | 2/21 |
| Overall proportion infected | 2.8/29.2 |

| Site | ACLSV | PDV |
|------|-------|-----|
| A    | 3/3   |
| B    | 1/2   |
| C    | 2/2   |
| D    | 0/3   |
| F    | 0/2   |
| G    | 0/3   |
| H    | 0/6   |
| I    | 3/3   |
| Overall proportion infected | 12.5/33.3 |
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 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 |

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% |
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 diagnostic 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.

**LITERATURE CITED**

Adams M., Antoniw J., Bar-Joseph M., Brunt A., Candresse T., … Fauquet C., 2004. Virology Division News: The new plant virus family Flexiviridae and assessment of molecular criteria for species demarca-
tion. *Archives of Virology* 149: 1045–1060.

Anderson P.K., Cunningham A.A., Patel N.G., Morales F.J., Epstein P.R., Daszak P., 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology & Evolution* 19: 535–544.

Aparicio F., Myrta A., Di Terlizzi B., Pallás V., 1999. Molecular Variability Among Isolates of Prunus Necrotic Ringspot Virus from Different Prunus spp. *Phytopathology* 89 (11): 991–999.

Babini A.R., D'Anniballe A., Fini P., Grillini P., 2014. Le piante di ciliegio minacciate dai virus. Servizio Fitosanitario, Regione Emilia-Romagna, Agricoltura (dicembre). Available at https://agricoltura.regione.emilia-romagna.it/fitosanitario/doc/pubblicazioni#articoli

Barba M., De Sanctis F., Cupidi A., 1985. Distribuzione dei virus del Mandorlo nell’Italia centrale/Distribuzione di Almond viruses in Central Italy. *Phytopathologia Mediterranea* 24: 267–269.

Barba M., Ilardi V., Pasquini G., 2015. Chapter Three - Control of Pome and Stone Fruit Virus Diseases, in: Loebenstein, G., Katis, N.I. eds, *Advances in Virus Research*. Academic Press: 47–83.

Bariana H., Shannon A., Chu P., Waterhouse P.M., 1994. Detection of five seedborne legume viruses in one sensitive multiplex polymerase chain reaction test. *Phytopathology* 84: 1201–1205.

BIOREBA, 2011. Technical information, Simple ELISA Data Analysis. Available at: https://www.bioreba.ch/saas/CustomUpload/374O357O340O370O356O369O35O0321O360O366O369O356O353O352O350O32O0326O/Simple_ELISA_Data_Analysis.pdf

Catalano L., 2013. Focus sulla cerasicoltura italiana. *Kar- pòs Magazine*: 79–96.

FAO. 2022. FAOSTAT, Crops and livestock products. February 02, 2022 revision. Available at: https://www.fao.org/faostat/en/#data/QCL. Accessed May 03, 2022.

Fiore N., Fajardo T.V.M., Prodan S., Herranz M.C., Aparicio F., … Sánchez-Navarro J., 2008. Genetic diversity of the movement and coat protein genes of South American isolates of Prunus necrotic ringspot virus. *Archives of Virology* 153: 909–919.

Gamper M., 2010. Kirschenanbau in Südtirol 11/2010. *Fachmagazin des Südtiroler Beratungsringes*: 399–400.

Gamper M., 2013. Das Kirschenjahr 2013 11/2013. *Fachmagazin des Südtiroler Beratungsringes*: 356–357.

Gasparro M., Milella R.A., Alba V., Giannandrea M.A., Caputo A.R., 2019. Seasonal dynamics and spatial distribution of main Grapevine viruses in field-grown grapevine cultivars. *European Journal of Plant Pathology* 155: 193–205.

Gospodaryk A., Moročko-Bičevska I., Püopoly N., Käle A., 2013. Occurrence of stone fruit viruses in plum orchards in Latvia, Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences. *Sciendo*: 116–123.

Hadidi A., Barba M., Candresse T., Jelkmann W., 2011. Virus and virus-like diseases of pome and stone fruits. *American Phytopathological Society*.

Honjo M.N., Emura N., Kawagoe T., Sugisaka J., Kamitani M., … Kudoh H., 2020. Seasonality of interactions between a plant virus and its host during persistent infection in a natural environment. *The ISME Journal* 14: 506–518.

Hu D., Wang L., Jiang X., Wang N., Gu L., 2014. The RT-PCR identification and sequence analysis of *Apple chlorotic leaf spot virus* from apple cultivars in Jiaodong Peninsula, China. *Biotechnology & Biotechnological Equipment* 28: 238–241.

Hull R., 2013. Plant Virology. Academic press.

Kamenova I., Borisova A., Popov A., 2019. Incidence and genetic diversity of *Prune dwarf virus* in sweet and sour cherry in Bulgaria. *Biotechnology & Biotechnological Equipment* 33: 980–987.

Katsiani A.T., Maliogka V.I., Candresse T., Katis N.I., 2014. Host-range studies, genetic diversity and evolutionary relationships of ACLSV isolates from ornamental, wild and cultivated Rosaceaeous species. *Plant Pathology* 63: 63–71.

Knapp E., da Câmara Machado A., Pühringer H., Wang Q., Hanzer V., … da Câmara Machado M.L., 1995. Localization of fruit tree viruses by immuno-tissue printing in infected shoots of *Malus* sp. and *Prunus* sp. *Journal of Virological Methods* 55: 157–173.

Koziel, E., Otulak-Koziel K., Bujarski J.J., 2020. Modifications in Tissue and Cell Ultrastructure as Elements of Immunity-Like Reaction in Chenopodium quinoa against *Prune Dwarf Virus* (PDV). *Cells*: 9: 148.

Kummert J., Vendrame M., Lepoiivre P., Steyer, S., 2001. Development of routine RT-PCR ELOSA tests for fruit tree certification. *Acta Horticulturae*.

Liu P., Li Z., Song S., Wu Y., 2014. Molecular variability of *Apple chlorotic leaf spot virus* in Shaanxi, China. *Phytoparasitica* 42: 445–454.

Mandic B., Matić S., Rwahnih M.A., Jelkmann W., Myrta A., 2007. Viruses of sweet and sour cherry in Serbia. *Journal of Plant Pathology*: 103–108.

Marbot S., Salmon M., Vendrame M., Huwaert A., Kummert J., … Lepoiivre P., 2003. Development of real-time RT-PCR assay for detection of *Prunus necrotic ringspot virus* in fruit trees. *Plant Disease* 87: 1344–1348.
Martelli G., Candresse T., Namba S., 1994. Trichovirus, a new genus of plant viruses. *Archives of Virology* 134: 451–455.

Martini K., 2021. Süßkirschenanbau in der Welt 11/2021. *Fachmagazin des Südtiroler Beratungsringes*: 5–7.

Matic S., Myrta A., Minafra A., 2007. First report of little cherry virus 1 in cherry, plum, almond and peach in Italy. *Journal of Plant Pathology* 89: 75.

Mekuria G., Ramesh S.A., Alberts E., Bertozzi T., Wirthsenoh M., Segdley M., 2003. Comparison of ELISA and RT-PCR for the detection of *Prunus necrotic ring spot virus* and prune dwarf virus in almond (*Prunus dulcis*). *Journal of Virological Methods* 114: 65–69.

Menzel W., Zahn V., Maiss E., 2003. Multiplex RT-PCR-ELISA compared with bioassay for the detection of four cherry viruses. *Journal of Virological Methods* 110: 153–157.

Myrta A., Di Terlizzi B., Savino V., Martelli G., 2003. Virus diseases affecting the Mediterranean stone fruit industry: a decade of surveys. Virus and virus-like diseases of stone fruits, with particular reference to the Mediterranean region. *Options Méditerranéennes: Série B. Études et Recherches* 45: 15–23.

Myrta A., Savino V., 2005. Virus and virus-like diseases of cherry in the Mediterranean region, *V International Cherry Symposium, SHS Acta Horticulturae* 795: 891–896.

Noorani M.S., Awasthi P., Sharma M.P., Ram R., Zaidi A.A., Hallan V., 2013. Simultaneous detection and identification of four cherry viruses by two step multiplex RT-PCR with an internal control of plant nad5 mRNA. *Journal of Virological Methods* 193: 103–107.

Oliver J.E., Freer J., Andersen R.L., Cox K.D., Robinson T.L., Fuchs M., 2009. Genetic Diversity of *Prunus necrotic ringspot virus* Isolates Within a Cherry Orchard in New York. *Plant Disease* 93: 599–606.

Osman F., Al Rwahnih M., Rowhani A., 2016. Real-time RT-qPCR detection of cherry rasplf virus, cherry green ring mottle virus, cherry necrotic rusty mottle virus, cherry virus a and apple chlorotic leaf spot virus in stone fruits. *Journal of Plant Pathology* 99: 279–285.

Öztürk Y., Çevik B., 2015. Genetic Diversity in the Coat Protein Genes of *Prune dwarf virus* Isolates from Sweet Cherry Growing in Turkey. *Plant Pathology* J 31: 41–49.

Paduch-Cichal E., Sala-Rejczak K., Lewko J., 2007. The reaction of *Prunus avium* clone F12/1 plants inoculated with PNRSV isolates from different species of *Prunus* and rose plants. *Phytopathology*. Pol 46: 13–23.

Pirazzoli C., Palmieri A., 2019. Aspetti produttivi e di mercato dell’attuale cerasicoltura, rivista frutticoltura e ortofloricoltura 3/2019. *Frutticoltura*: 10–14.

Polák J., Svoboda J., 2006. The reliability of detection and the distribution of *Apple chlorotic leaf spot virus* in pears in the Czech Republic. *Horticulturae* 33: 7–10.

Prajdaňa L., Sihelská N., Benedíková D., Šoltys K., Candresse T., Glasa M., 2017. Molecular characterization of *Prune dwarf virus* cherry isolates from Slovakia shows their substantial variability and reveals recombination events in PDV RNA3. *European Journal of Plant Pathology* 147: 877–885.

Rana T., Chandel V., Hallan V., Zaidi A.A., 2011. Expression of recombinant *Apple chlorotic leaf spot virus* coat protein in heterologous system: production and use in immunodiagnosis. *Journal of Plant Biochemistry and Biotechnology* 20: 138–141.

Rubio M., Martínez-Gómez P., Marais A., Sánchez-Navarro J., Pallás V., Candresse T., 2017. Recent advances and prospects in *Prunus* virology. *Annals of Applied Biology* 171: 125–138.

Sánchez-Navarro J., Aparicio F., Rowhani A., Pallás V., 1998. Comparative analysis of ELISA, nonradioactive molecular hybridization and PCR for the detection of prunus necrotic ringspot virus in herbaceous and *Prunus* hosts. *Plant Pathology* 47: 780–786.

Sánchez-Navarro J., Aparicio F., Herranz M., Minafra A., Myrta A., Pallas V., 2005. Simultaneous detection and identification of eight stone fruit viruses by one-step RT-PCR. *European Journal of Plant Pathology* 111: 77–84.

Sánchez, R.P., Corts, R.M., Benavides, P.G., Sánchez, M.A.G., 2015. Main viruses in sweet cherry plantations of Central-Western Spain. *Scientia Agricola* 72: 83–86.

Savino V., Digiardo M., Martelli G., Di Terlizzi B., 1991. *Plum pox virus* outbreaks in Apulia and Basilicata (southern Italy). *XV International Symposium on Fruit Tree Diseases* 309: 125–128.

Song G.q., Sink K.C., Walworth A.E., Cook M.A., Allison R.F., Lang G.A., 2013. Engineering cherry rootstocks with resistance to *Prunus necrotic ring spot virus* through RNA i-mediated silencing. *Plant Biotechnology Journal* 11: 702–708.

Spiegel S., Rosner A., Tam Y., Zilkah S., Faingersh E., … Krizbai L., 1997. Detection of *prune dwarf virus* in sweet cherry in Israel. *XVII International Symposium Virus and Virus-Like Diseases of Temperate Fruit Crops* 472: 249–256.

Strange R.N., Scott P.R., 2005. Plant disease: a threat to global food security. *Annual Review Phytopathology* 43: 83–116.
Tsai C., Daugherty M., Almeida R., 2012. Seasonal dynamics and virus translocation of Grapevine leafroll-associated virus 3 in grapevine cultivars. *Plant Pathology* 61: 977–985.

Wang J., Zhai Y., Zhu D., Liu W., Pappu H.R., Liu Q., 2018. Whole-Genome Characterization of *Prunus necrotic ringspot virus* Infecting Sweet Cherry in China. *Genome Announcements* 6/9: 1–2.

Watpade S., Raigond B., Pramanick K.K., Sharma N., Handa A., Sharma U., 2013. Simultaneous detection of *Apple Chlorotic Leaf Spot Virus* and *Apple mosaic virus* in crab apples and apple rootstocks by duplex RT-PCR. *Scientia Horticulturae* 164: 88–93.

Wei T., Lu G., Clover G., 2008. Novel approaches to mitigate primer interaction and eliminate inhibitors in multiplex PCR, demonstrated using an assay for detection of three strawberry viruses. *Journal of Virological Methods* 151: 132–139.

Yardimci B.C.N., Culal-Klllc H., 2011. Detection of viruses infecting stone fruits in Western Mediterranean region of Turkey. *Plant Pathology Journal* 27: 44–52.

Yu Y., Zhao Z., Jiang D., Wu Z., Li S., 2013. A one-step multiplex RT-PCR assay for simultaneous detection of four viruses that infect peach. *Letters in Applied Microbiology* 57: 350-355.

Zago M., 2003. Erfahrungen mit Süßkirschen in Südtirol 6/2003. *Fachmagazin des Südtiroler Beratungsringes*: 185–187.

Zago M., Ropelato E., 2009. Grenzen und Risiken beim Anbau von Süßkirschen in Höhenlagen 11/2008. *Fachmagazin des Südtiroler Beratungsringes*: 360-362.