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Causal Agents of Stone Fruit Diseases in Slovenia and the Potential for Diminishing Their Economic Impact – a Review

Ivan ŽEŽLINA[1], Mojca ROT[1], Milica KAČ[2] and Stanislav TRDAN[3]

[1]Chamber of Agriculture and Forestry of Slovenia, Nova Gorica Institute for Agriculture and Forestry, Nova Gorica, Slovenia; [2]Department of Food Science and Technology and [3]Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Abstract

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In the last twenty years, stone fruit production in Slovenia has considerably decreased. Acreage with peaches and nectarines has been significantly reduced. Orchard renewal has decreased for various reasons; among them, the frequent occurrence of various diseases is the most significant. These diseases make the economic feasibility of stone fruit production difficult if not impossible. This review addresses the most important stone fruit diseases in Slovenia: European stone fruit yellows, sharka, bacterial leaf spot, bacterioses caused by *Pseudomonas* spp., perennial canker, and Fusicoccum canker. Additionally, this review focuses on the possibilities of reducing the damage caused by these diseases.

Keywords: phytoplasmas; viruses; bacteria; fungi; nectarines; peaches; Slovenia

One of the important agricultural activities in Slovenia is stone fruit production. In 2011, the overall stone fruit production, which included mostly peaches and nectarines (80%), cherries and sour cherries (12.3%), apricots (3.9%), and plums (3.8%), was more than 9541 tons (Statistical Office of Republic of Slovenia 2012). However, in the last several years, stone fruit production (especially of peaches and nectarines) has declined. In 1991, 641 ha were used for peach and nectarine cultivation, but this number declined to 468 ha by 2011. Thus, a 27% reduction in the area used for orchards has occurred over the last 20 years.

However, the orchards of the other stone fruits have not suffered similar reductions in acreage, and production has remained stable. The observed decline in stone fruit acreage is mainly attributable to poor plant health in these orchards, although many fungal diseases (except wood diseases) are manageable and do not pose serious problems to the farmers. Maintaining healthy stone fruit orchards in Slovenia has become particularly challenging due to the emergence of certain new diseases and the greater spread of already prevalent diseases with longer periods of circulation, in addition to the synergism between and succession among the disease agents.

Causal agents of the most important diseases of stone fruits in Slovenia

Several disease agents influence the health of stone fruit, and the most important agents are *Candidatus Phytoplasma prunorum*, *Plum pox virus* (PPV), *Xanthomonas campestris* pv. *pruni* (Smith) Dye, *Valsaria insitiva* (Tode) Ces. & De Not., *Leucostoma persoonii* (Nitschke) Höhn., *Pseudomonas syringae* pv. *persicae* (Prunier et al.) Young et al., and pv. *morsprunorum*

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(Prunier et al.). The intensity of fungal wood diseases has also increased, and the two most important of these diseases are perennial canker caused by *V. insitiva* and *L. persoonii* and Fusicoccum canker caused by *F. amygdali* Delacr. Another very important factor in stone fruit orchards are the interactions between and succession among the more recent and older fungal diseases, such as those observed between bacterial diseases and perennial canker.

New agents of stone fruit diseases

Candidatus Phytoplasma prunorum: the causal agents of European stone fruit yellows (ESFY). ESFY is the most serious disease of stone fruits and is practically unmanageable in orchards of peaches (*Prunus persica* [L.] Batsch), nectarines (*Prunus persica* var. *nucipersica* Dipp.), apricots (*Prunus armeniaca* L.), and plums of Chinese-Japanese origin (*Prunus salicina* Lindl., *Prunus simonii* Carr.). The symptoms of this disease were first recorded in Slovenia in the late 1980s (Seljak & Petrovič 2000) and since then have spread to various locations in the Vipavska dolina (Vipava valley). In 2000, infection in orchards was decisively proven via the molecular analysis of samples taken from these locations (Brzin *et al.* 2001). Subsequently, ESFY symptoms have also been observed in other parts of Slovenia, and consequently, official measures for monitoring and managing this disease at the national level have been introduced.

The most widely spread and commonly observable symptoms of this disease are very early foliation (typical for apricots) and pale leaves with margins turned upwards (Figure 1). The leaves of the affected trees are smaller, and these leaves and ribs (nervures) are thicker and fragile. These plants occasionally flower at unusual times as a response to various stress situations. Trees suffering from ESFY appear sickly, bear less fruit of extremely low quality, and usually die within 2–4 years.

Figure 1. European stone fruit yellows is one of the major diseases in peach, nectarine, and apricot orchards in Slovenia (photo: I. Žežlina)

Phytoplasma causing ESFY is transmitted from diseased to healthy plants through vectors, such as the plum psyllid (*Cacopsylla pruni* Scopoli) and also possibly via the leafhopper *Asymmetrasca decedens* Paoli; transmission from the latter has only been established under laboratory conditions (Pastore *et al.* 2004). Both these vectors are numerous in Slovenia (Ambrožič Turk *et al.* 2011; Seljak & Rot 2013). Because ESFY causal agent can also spread by vegetative propagation, prevention mainly involves the strict control of the health conditions in plantations of stock plants and tree nurseries. Thus, the spread of ESFY rarely occurs with planting material obtained from registered providers and can often be traced back to the use of non-certified planting material (either for inoculation or for planting) (Ambrožič Turk *et al.* 2011).

Thus, to prevent the spread of this disease, controlling the presence of the pathogen in plantations of stock plants and tree nurseries is of utmost importance (Ambrožič Turk *et al.* 2008). Growing healthy plant material under protective nets that prevent contact between stock plants and phytoplasma vectors is beneficial as shown by Fajt *et al.* (2009). Despite the availability of healthy plant material, ESFY is spreading rather quickly, and it is very dangerous, especially for peach and apricot orchards.

In Slovenia, the majority of the infections in orchards can be attributed to high infection pressure that results from the substantial environmental and biological diversity of certain ESFY-tolerant plant species (Carraro *et al.* 2004). This diversity also represents a strong infection potential in the environment (Ambrožič Turk *et al.* 2008). Healthy stock plant material and regular monitoring of planting material are essential for producing healthy plants. Nevertheless, a massive ESFY appearance indicates that such measures are not entirely sufficient and that infection pressure is so pronounced that the phytoplasma can seriously affect the profitability of a newly established orchard in certain years regardless of the use of certified uninfected plants. Available data indicate that up to 80% of the plum psyllid population has been tested positive for ESFY causing phytoplasma (Ambrožič Turk 2011). Thus,

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the costs of stone fruit cultivation must also include the costs of the control of phytoplasma vectors and the removal of diseased trees including their roots.

Recent research has been focused on the development of ESFY-resistant and -tolerant varieties, and such studies are especially important for apricot orchards (Ambrožič Turk *et al.* 2013). Monitoring of the health of apricots in areas where ESFY occurs has revealed that some trees exhibit no disease symptoms despite the infection being confirmed in the laboratory. Some trees also exhibit a so-called "recovery" effect; i.e., after a few years, these trees do not exhibit any signs of the disease despite still being infected. It has also been discovered that in some cases, the disease can be transferred to the progeny (Osler *et al*. 2013). Nevertheless, the use of ESFY-tolerant varieties could be a possible solution for profitable growing of stone fruits in areas in which the infection potential is extremely high (Ambrožič Turk 2011).

Plum pox virus: the causal agent of sharka disease. Although sharka was observed as early as 1915, it was first described only a few years later (Atanasoff 1932). It is one of the most important viral diseases of fruit trees of the *Prunus* genus throughout the world with the exceptions of Australia and New Zealand, where this disease has not been observed. In Slovenia, sharka usually causes problems in plum, peach, and nectarine orchards (Viršček Marn *et al.* 2014).

The symptoms of sharka can be observed on leaves, flowers, and fruits and strongly depend on the species and cultivar of the stone fruit and the vitality and age of the tree. The virus strain and weather conditions also play important roles in the expression of symptoms. In some cases, the symptoms remain latent and can vanish during the growing season. Plums can exhibit characteristic round spots on the leaves in the early spring that may vanish completely by early autumn. Spots in the form of circles and semicircles later become wrinkled. The latter effect can be more pronounced in the cultivars that are more prone to the disease. The fruits exhibit characteristic symptoms at the time of ripening; they become deformed, their taste deteriorates, and they usually drop prematurely. Infected apricot fruits primarily exhibit a deformed surface with rather uneven ripening that results in the deterioration of the taste and consistency and, consequently, a lower general fruit quality. The most characteristic symptom is the spots on the stone, which bear bright margins (Ranković *et al.* 1999; Llacer & Cambra 2006; Rubio *et al.* 2011).

Figure 2. Symptoms of sharka on peaches (photo: I. Žežlina)

Sharka can also be observed very early when the trees are in blossom, and in some varieties of peaches and nectarines, the petals become characteristically spotty. Spots or stripes of brighter colours or discoloration appear on the leaves and may occasionally also appear as two concentric rings. These spots can be observed over the entire plant or only on certain branches. The leaves may also be deformed. The fruits usually have surface discolorations (spots or concentric rings; Figure 2). Because the sharka virus is spread via infected planting material, it can spread over considerable distances. Various species of aphids are also important vectors, and they carry the virus across shorter distances (Wallis *et al.* 2005; Kaya *et al.* 2014).

To prevent the spread of sharka in stone fruit, it is essential to carefully prepare healthy and virus-free planting material. Constant monitoring of the health of propagation material, the elimination of infected plants, and the control of aphid populations are also essential. It is important to mention that the spatial isolation of newly planted orchards from old and infected orchards is an important measure for the control of sharka disease. Growing stock plants under protective nets is a modern method that ensures healthy planting material because it excludes the influence of possible vectors. Importantly, favourable results of the use of such practices have also been documented in Slovenia (Fajt *et al.* 2009). Plum orchards can be saved via the use of sharka-tolerant cultivars, such as the well-known Stanley variety. No completely sharka-resistant varieties of plums are known to exist, and while some hypersensitive tolerance has been observed, only a few related studies have been reported in literature (Neumüller & Hartmann 2008).

Xanthomonas campestris pv. pruni – the causal agent of bacterial leaf spot. The symptoms of

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bacterial leaf spot (*Xanthomonas campestris* pv. *pruni*) in Slovenia were first observed on plums of Chinese-Japanese origin in 1992 in the Vipavska dolina (Vipava valley, Primorje region) (Seljak *et al.* 2002). The disease-causing microorganism was first confirmed in the laboratory in 1999 (Seljak *et al.* 2002). Bacterial leaf spot most likely originated from North America, where it was described for the first time. Nevertheless, it is possible that its area of origin is much wider (Bazzi & Mazzuchi 1980). This bacterial disease is caused by a microorganism that is harmful to all *Prunus* species, and certain peach varieties are the most susceptible (Elegant Lady, Rich Lady, Symphony, etc.), in addition to plums of Chinese-Japanese origin.

The symptoms on peach trees are different from those on plum trees. On peach trees, the symptoms initially appear on the leaves in the form of brownish, irregularly shaped spots that are usually along the main nervure. The infected leaves turn yellow beginning at the top, and in most cases, they fall off prematurely. Diseased twigs exhibit elongated corrosions, and necrosis can develop deep inside the wood, especially in plum trees (Seljak *et al.* 2002). In peaches, the symptoms of the disease initially appear in the form of spots that later progress to necrosis. Damaged spots can consolidate to form larger damaged areas, which drastically diminish fruit quality (Figure 3).

The bacteria can overwinter on fallen leaves, one-year-old twigs or on the damaged parts of older wood. Favourable conditions for infection start at the beginning of blossoming and last until a few weeks after the trees have blossomed. The bacteria infect the leaves through stomata and wounds, and the intensity of the infection largely depends on the weather conditions; infections are more severe in rainy weather and when the plants suffer any harm (e.g., due to rain and hail). The fruits and twigs also become infected through lenticels and injuries. Under favourable conditions, infection can occur throughout the growing season (Ritchie 1995).

Figure 3. Bacterial diseases, such as bacterial leaf spot are common in stone fruit orchards in Slovenia (photo: I. Žežlina)

The spread of this disease over long distances is most likely due to the planting of infected material, and it is very likely that this disease was introduced to Slovenia through the use of such infected material (Seljak *et al.* 2002). In large orchards and over short distances, the disease is primarily spread by droplets (i.e. droplet infection), and the exudate is often carried by wind. The weather conditions in the Primorska region are generally favourable for bacterial leaf spot, although the actual intensity of the infection depends on how long the trees have remained wet and the quantity of rainfall. This disease can also be spread by the tools used for winter and summer pruning. The presence of especially sensitive varieties of peaches and plums in orchards also plays an important part during such infections.

Bacterial leaf spot can be successfully prevented by not planting the most sensitive varieties and using less sensitive or more tolerant varieties. Among the cultivars of plums that are available in Slovenia, many are quite tolerant, and the domestic plum is more or less resistant to this disease (Seljak *et al.* 2002). Importantly, strict adherence to legally enforced phytosanitary measures is essential for successfully combating bacterial leaf spot in the Republic of Slovenia.

Chemical control of this disease is difficult because there are no phytopharmaceutical products that are effective against the bacteria. However, copper fungicides work as effective bacteriostatics and should therefore be consistently sprayed in autumn during the leaf fall and in spring before the flower buds begin to open. These copper fungicides cannot be used during the growing season because they are extremely phytotoxic, especially for the green parts of peach and nectarine plants.

Pseudomonas spp. Bacteria of the genus *Pseudomonas* are very common based on the fact that laboratory analyses of fruit and tree samples have increasingly identified isolates belonging to this genus (Dreo *et al.* 2007). *Pseudomonas syringae* is the most frequently isolated species and is primarily isolated from apple trees. Identification with molecular methods has revealed a very high variability among the strains of this species (Dreo *et al.* 2007).

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Bacterial dieback of peach caused by *P. syringae* pv. *persicae* was firstly observed and reported in France (Vigouroux & Blache 1967). Reports of its occurrence in former Yugoslavia are uncertain (CABI, EPPO 1997), and this species has not been found in Slovenia.

Depending on the weather conditions, in the last few years, apricot fruits in the Primorska region of Slovenia exhibit unmistakable symptoms of bacterial diseases that are probably caused by the *Pseudomonas* genus. BOX-PCR profiles of the isolated bacteria match those of *P. syringae* pv. *morsprunorum* CFBP3801, *P. savastanoi*, and some other pathovars of *P. syringae*. Additionally, because the stone fruit samples also yield other isolates of the *Pseudomonas* genus, it is not possible to accurately determine which of these causes the distinctive disease symptoms (Figure 4).

Preventive measures against bacterial dieback of peach involve the strict use of healthy planting material and the elimination of any infected material from the area (CABI, EPPO 1997). Another important measure is regular use of copper fungicides between autumn leaf fall and spring growth (before the flower buds begin to open) (Dreo *et al.* 2007).

Fusicoccum amygdali – the causal agent of Fusicoccum canker and Valsaria insitiva and Leucostoma persoonii – the causal agents of perennial canker. The incidence of known stone fruit diseases has steadily increased in the last several years. Wood diseases are primarily either Fusicoccum canker (caused by *Fusicoccum amygdali*) or perennial canker (caused by *Valsaria insitiva* and *Leucostoma persoonii*), spread mainly in wet weather conditions, and cause the deaths of a large portion of stone fruit trees each year.

Figure 4. Over the last several years, we have observed spots on apricot leaves and fruits that are probably caused by the *Pseudomonas* genus (photo: I. Žežlina)

Fusicoccum canker was first observed on almond trees in France in 1905 and later in Italy in 1926 (Goidànich 1964). This disease was transferred to Slovenia in the 1960s (Rot 2011) and currently affects peach and almond trees grown in Europe, America, and North Africa.

The fungus infects one-year-old twigs, and the typical symptoms of infection are visible in early spring. The bark of such twigs exhibits shallow invaginations and is reddish brown in colour. Elongated necrotic spots form on the bark, and the margin separating the healthy and diseased tissue is sharp and well defined (Figure 5). The infected bud does not develop, and the necrosis spreads along the twig, which consequently fades and dies. The activity of the fungus is based on the secretion of a toxin called fusicoccin, which increases turgor pressure in the cells that close the stomata. Thus, the stomata remain open, which leads to excessive water loss that causes the plant to begin to wilt (Squire & Mansfield 1974; Rhouma *et al.* 2008). A gum develops at the site of infection.

Low temperatures inhibit and high spring temperatures facilitate the incidence and spread of the infection. The fungus lives like a parasite in wounds and invades the interior of the plant though lesions that appear on the wood during the growing season. Infections are most frequent in autumn and spring when the fungal spores are most abundant. The fungus enters the trees through fresh leaf scars in the fall and stipules, bud scale scars, blossoms, and fruit scars in the spring (Rhouma *et al.* 2008). The pathogen can also infest a plant through young shoots. In addition, the infection can be established during summer through pruning scars, particularly

Figure 5. Symptoms of Fusicoccum canker on a young branch (photo: I. Žežlina)

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during wet weather and in orchards that are irrigated with traditional sprayers over the top of the trees. Thus, the fungus uses all types of injuries to enter the plants regardless the origin of the injury.

Perennial canker is a wood disease of stone fruit trees that is caused by two morphologically similar fungi, *V. insitiva* and *L. persooni*. The disease affects peaches, nectarines, cherries, plums, and apricots. Perennial canker is more harmful than bacterial dieback of peach because the latter affects only a part of the fruit-bearing wood. The damage caused by perennial canker is permanent because it destroys the fruit-bearing wood and reduces the lifespan of the host plant. The symptoms of perennial canker are dry shoots and twigs combined with gumming. Necrosis forms in the centre of the infection site, which has a distinctly bright margin and is clearly distinguishable from the healthy tissue (Figure 6). The dead tissue also turns darker with time. Because gumming is a defence mechanism, it is present from the moment of infection to the complete drying out of the shoot. The leaves on the infected shoot also turn yellow, dry out, and prematurely fall off. If the main shoots and stems are affected, then the characteristic elongated perennial canker wounds are formed, and gum is abundantly secreted.

Perennial canker infections are most frequent in autumn and spring, and the asexual stage of the fungus is most important for the spread of the disease. The *V. insitiva* fungus is more active under wet and cold conditions, while the *L. persoonii* fungus is more active under warm and wet conditions and at temperatures above 15–16°C (Ponti & Laffi 1988). The fungus enters the plant through any site of injury or damage in the wood, such as those due to frost, hail, pruning, cultivation, leaf scars, emerging buds in spring, etc.

Figure 6. Perennial canker symptoms on a peach trunk (photo: I. Žežlina)

Once inside the plant, the fungus begins to secrete toxins that destroy the cambium and the core, and the secretion of rubber-like substances blocks the plant vessels. Perennial canker is an opportunistic parasite and only infects weakened plants, poorly nourished trees, and plants that are already suffering from drought, water stress or other diseases. Because the fungi that cause wood diseases are usually parasites on weak plants and attack weakened trees, keeping the trees in good condition is essential for preventing infection. Most infections occur in autumn or spring and usually occur through major or minor lesions. Therefore, winter pruning must be performed during the dormant period in dry and cold weather when the probability of infection is the lowest. An alternative strategy is to prune as late as possible in spring when longer periods of dry and warm weather are expected. Further, summer pruning should be performed during a dry period. If the orchard is traditionally irrigated (e.g. via spraying above the tree tops), irrigation should be stopped for several days after pruning to provide the lesions time to heal.

From the perspective of protecting trees from various diseases, drip irrigation or the use of micro sprayers is preferable over the traditional methods of irrigation because the latter create conditions that are favourable for the spread of wood diseases. Older or even abandoned peach orchards are important sources of the inoculum and therefore, when possible, new peach orchards should never be planted in the vicinity of old ones.

Interactions between and succession among fungal diseases of wood and bacterial diseases

Because similar weather conditions favour major fungal and bacterial diseases, interactions between and succession among fungi and bacteria play very important roles. Rainfall strongly influences the appearance of fungal and bacterial diseases because both types of diseases are water-borne.

It is well known that certain fungal diseases of wood, such as those caused by *L. persoonii*, can seriously damage young trees only if they cause secondary infections that occur through lesions that are primarily caused by bacterial diseases (Briggs 1995). Combined simultaneous infections by bacteria (*P. syringae*) and

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wood pathogenic fungi (*L. persoonii*) are also frequent. Bacterial infections in trees are often latent and only become apparent when conditions become favourable for the disease development (Hattingh & Roos 1995).

Given the high prevalence of wood lesions caused by perennial canker in stone fruit trees over the years (especially in peaches), interactions and succession between fungal and bacterial diseases has intensified, and this situation is present in extensive and intensive orchards.

Extents of stone fruit diseases and possibilities for diminishing their economic impact

The aforementioned diseases cause significant damage to Slovenian stone fruit orchards. The European stone fruit yellows is the most serious problem in intensive peach and apricot orchards because the losses are apparent even during the first few years after planting, and the disease incidence often reach a point when farming the orchard is no longer profitable in the subsequent years. The disease management is increasingly difficult because the infection pressure in the environment is increasing. Nevertheless, partially satisfactory solutions can be found in new studies of varieties that are tolerant to European stone fruit yellows.

Plum pox virus infections are most serious in plum orchards that consist of cultivars that are susceptible to the virus, and such orchards can even suffer 100% loss. In apricot plantations, sharka disease influences the quality of the fruit, and although this is true for peach orchards, as a rule, damage to the latter is not as drastic. Planting tolerant varieties of plums that are available on the market is only a partial solution, and the use of other agrotechnical measures that contribute to achieving and maintaining the good health of trees in these orchards is essential for controlling both ESFY and sharka. It is also essential that only healthy planting material is used and that all phytosanitary measures, including the removal of diseased trees, are practiced.

The intensity of wood diseases among various stone fruit plantations may vary because the disease incidence is strongly dependent on the technological measures that are adopted and the maintenance of the plantation. When good agricultural practices are followed (e.g. all activities and phytosanitary measures are performed on time and with sufficient care), the problems are much less severe compared to the orchards with insufficient maintenance. Regular and consistent removal of perennial canker lesions, diseased shoots and branches is an absolute "must do". After the lesions have been excised, a fungicidal ointment or a concentrated solution of a copper-based plant protection product should be applied, and the diseased wood should be carried out of the orchard and burned. Tools should also be disinfected after use on diseased plants.

Ensuring the optimum health of the orchard also requires the use of pesticides, although the selection of effective and suitable plant protection products is rather limited. Spraying with copper-based plant protection products is the best available option and should be performed when one-third to one-half of the leaves have fallen off. The application rate should be carefully monitored (one-third or one-half of the label) because copper fungicides can burn young shoots that have yet to form enough wood (Lalancette & McFarland 2007). Spraying at the full recommended rate should be performed only when the tree has shed all of its leaves. Spraying in early spring just before the buds begin to open is also very important. Copper-based plant protection products are effective against both bacterial diseases of stone fruit trees and fungal diseases, such as shot-hole disease of stone fruit trees, which is caused by *Clasterosporium carpophilum* (Lév.) Aderh., and peach leaf curl, which is caused by *Taphrina deformans* [Berk.] Tul. In plantations in which the number of lesions caused by the fungi *V. insitiva* or *L. persoonii* (i.e. the causal agents of perennial canker) is increasing, the application of a copper pesticide mixture prevents or at least restricts the interaction and succession between the fungal wood diseases and bacterial diseases.

CONCLUSIONS

The data from new orchards and stone fruit harvests indicate that the cultivation of stone fruits in Slovenia, especially peaches and nectarines, has stagnated. The reasons for this stagnation include, among others, the phytopathological problems described in this review. Improvement requires modern farming trends, such as introduction of new varieties that are tolerant or resistant to the diseases caused by phytoplasmas and viruses. Good agricultural measures and plant protection strategies have to be introduced and performed. Infectious potential of the mentioned diseases in infected orchards is high, so it

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is very important to plant healthy planting material and eliminate all contaminated material from the orchards and the surrounding area. In addition to these mechanical and other agricultural measures, the plant protection industry has to formulate new products that can be used to successfully prevent or restrict stone fruit diseases. The careful monitoring of disease conditions in stone fruit plantations and compliance with all measures (mechanical and chemical) that are used for the protection of orchards are necessary.

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Corresponding author:

Prof Stanislav Trdan, University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Chair of Phytomedicine, Agricultural Engineering, Crop Production, Grassland and Pasture Management, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia; E-mail: stanislav.trdan@bf.uni-lj.si