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Review Article

Citruses in Croatia – cultivation, major virus and viroid threats and challenges

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Abstract – Although Croatia is not often perceived as a citrus growing country, citrus species have been planted in the Croatian southern coastal part for centuries. Citrus had always been important as a source of vitamins and micronutrients for local consumption, but with the introduction of Satsuma mandarins (*Citrus unshiu* Marc.) citrus production started its commercial development. The Croatian coast is probably the northernmost commercial citrus growing area globally with several agroecological constraints influencing citrus production. However, Satsuma mandarins, the most cold-tolerant citrus of economic interest, are successfully cultivated resulting in an excellent quality of fruits that easily finds a market niche. Unfortunately, with the import of plant material in the last century, the simultaneous introduction of viral and subviral pathogens occurred. The most important are *Citrus tristeza virus* and *Citrus exocortis viroid*. As certain rootstock-scion combinations are tolerant to virus or viroid infections and display no obvious symptoms, these pathogens have been continuously spreading by plant propagation. Molecular and biological analyses revealed the existence of pathotypes not believed to be endemically present in the Mediterranean region. Their high intra-isolate genetic variability and frequent coinfections suggest that this geographical region presents a sort of a biological enclave of potentially threatening pathogens. Their transmission from this agroecological niche to areas where sensitive rootstock-scion combinations are common presents a serious risk to citrus production, especially when global warming and the resulting migration of efficient vector species are considered. Comprehensive pathogen monitoring is important for improving citrus production and the prevention of pathogen transmission.

Keywords: *Citrus unshiu*, *Citrus exocortis viroid, Citrus tristeza virus*, East Adriatic coast, phytopathology, Satsuma mandarin

History of citrus cultivation

Citrus is one of the genera within the family of angiosperms Rutaceae, subfamily Aurantioideae, containing some of the economically and nutritionally most important subtropical fruit tree species cultivated worldwide. The related genera are *Fortunella*, *Poncirus*, *Eremocitrus* and *Microcitrus* (Wu et al. 2018) mostly encompassing species important as ornamentals or rootstocks in the cultivation of palatable citrus species. Citruses originated in the southeast foothills of the Himalayas and probably underwent two speciation events. The first radiation across Southeast Asia coincided with the onset of dryer climatic period some eight million years ago, while the second one across the Tanaka line to Australasia occurred some five million years ago (Wu et al. 2018). Citrus cultivars are usually sexually compatible even amongst related genera, but many display partial apomixis characterized by the development of somatic (nucellar) embryos favoured in germination and plantlet development. Consequently, apomixis fixes new genotypes resulting in phenotypes comprising interspecific hybrids and admixtures commonly categorized as new species (oranges, limes, lemons, grapefruits, etc.). Moreover, the frequency of bud mutations and vegetative propagation practice (e.g. grafting) also contributed to the variability and geographical speciation of species within the *Citrus* genus (Wu et al. 2014, 2018, Inglese and Sortino 2019). As a result of the biology and complex domestication of citrus, the taxonomy of this genus is still confusing. The latest genomic studies indicate that the principal progenitors of modern citrus plants are citrons (*Citrus medica* L.), pommelos (*C. maxima* (Burm.) Merr.) and mandarins (*C. reticulata* Blanco). Whilst citrons and pommelos

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are regarded as pure species, contemporary studies (Wu et al. 2014, Curk et al. 2015, Garcia-Lor et al. 2015, Wu et al. 2018) have revealed that almost all modern mandarins are introgressed by *C. maxima* genome fragments.

Citrus cultivation in coastal Dalmatia, including the islands, is known from the 15th century (Kaleb 2014). The first plants of citron, lemon (*Citrus limon* L.), sour orange (*Citrus aurantium* L.), and later on, sweet orange (*Citrus sinensis* (L.) Osb.), were introduced from Sicily, Portugal, and other Mediterranean countries by sailors. They mostly came as seedlings and for a long time were grown only as garden trees propagated by seeds. The first citrus nursery was founded in the village of Čibača near Dubrovnik in 1908, while the commercial cultivation of citrus, mainly the Satsuma mandarin (*Citrus unshiu* Marc.), began in the 1930s with the first introduction from Japan. Thirty years later, in the 1960s, the project of land reclamation in the Lower Neretva Valley enabled the establishment of an economically significant citrus foundation block there (Gatin 1978, Gatin et al. 1983, Škorić et al. 2002, Kaleb 2014). More than 110 types of citrus, among them 35 varieties of Satsuma mandarins, were collected over the years from Georgia (in 1965 and 1980), Corsica (1972 and 1973), California (1978) and Japan (1981). The pivotal role in the introduction of easily adaptable and the most profitable citrus varieties was played by Ž. Gatin who worked on the advancement of citriculture in the Neretva River Valley as well as in other citrus growing regions of ex-Yugoslavia. He proposed measures for the significant increase of citrus production (Gatin 1978, Gatin et al. 1983). In 1991, after the beginning of the war in Croatia, Gatin (1992) pointed to the urgent need for the removal of the citrus foundation block from the Neretva region to save it from potential devastation. Unfortunately, the citrus foundation block was lost, and the citrus industry development was slowed down by the war (1991-1995). In 2007, in the scope of a project financed by the Croatian Ministry of Science, Education and Sports, a new, much smaller, basic citrus mother block was formed at the Institute for Adriatic Crops and Carst Reclamation in Split (Hartl-Musinov et al. 2006, Rošin et al. 2009). The plantation represented 25 varieties and, in total, 78 basic mandarin, lemon, orange and grapefruit mother trees. The establishment of such a plantation followed the clonal selection and the formation of Satsuma collection orchard consisting of 410 superior plants of 12 varieties and 49 clones (HITRA-TEST, 2009).

According to the most recent published data from the Croatian Bureau of Statistics (Anonymous 2017), Satsuma mandarin cultivation in Croatia covers approximately 1,700 ha. This area is constantly increasing and it is estimated that there are more than 1,000,000 mature productive mandarin trees. Although systematic efforts are lacking, in the last 20 years there have been some developments concerning the introduction of new varieties, conservation of citrus germplasm as well as studying and improving the sanitary status of citrus.

Ecological constraints

Citruses in Croatia are cultivated between 42°23' and 44°56' north, the northernmost commercial citrus-growing area in Europe and probably in the world. Even though the entire Adriatic region is characterized by a Mediterranean climate, the distinguishing characteristic of this area are winter temperatures below 0°C. This, together with low temperatures in late autumn, during the maturing period and in the beginning of spring when the vegetation starts, may have adverse effects on citrus production. The only citruses that can be grown in these conditions is the Satsuma mandarin, the most cold-tolerant mandarin of commercial value. Because of a low total heat requirement, most Satsuma cultivars ripen earlier than oranges and other mandarin varieties (Gatin 1978, Bakarić 1983, Gatin et al. 1983). Its early ripening season is an additional advantage in avoiding the potential winter freezing of the fruits. Another agroecological constraint is the irregular distribution of precipitation. Two thirds of precipitation occurs during the autumn/winter period and drought very often takes place during summer and late spring (Gatin 1978).

Citrus can be cultivated in different types of soils in Croatia, but the main constraint is the high active lime content, which often causes the appearance of chloroses. Regarding climatic and pedological conditions, citrus growing is possible in five areas of the country (Fig. 1), although its commercial cultivation is restricted to a narrow coastal part, from the town of Trogir to the Konavle region, and to the Dalmatian islands (Fig. 1). The largest and the most important citrus growing area is the Neretva River Valley (Figs. 1, 2) with approximately 70% of all citrus trees in Croatia. The only vari-

Fig. 1. Citrus growing areas in Croatia (framed numbers). 1 – Dubrovnik area including the islands of Hvar and Vis, 2 – Neretva River Valley area, 3 – Split area: coastal part and the islands of Brač, Šolta and Drvenik, 4 – Lošinj-Zadar group of islands, 5 – the west coast of Istria.

Fig. 2. Citrus orchards in the Lower Neretva Valley.

ety that can be cultivated in all five growing areas is the Satsuma mandarin, whilst the cultivation of lemons, oranges and other citruses is possible only in warmer microclimatic locations such as areas of Dubrovnik, Neretva River Valley and the surroundings of Split including the islands.

The first rootstock considered in citrus cultivation was sour orange, mainly due to high drought tolerance and *Phytophtora* resistance, but because of its cold sensitivity most trees were damaged in the great frost of 1956. Gradually, it was replaced with *Poncirus trifoliata* (L.) Raf., a rootstock exhibiting better resistance to low temperatures. Nowadays, sour orange is used sporadically as well as citrange (*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.), rough lemon (*Citrus jambhiri* Lush.) and Cleopatra mandarin (*Citrus reshni* Hort. ex Tan.). The variety of citrus cultivars in Croatian orchards has been continuously changing. Based on the latest Official cultivar list (Anonymous 2019), the main Satsuma cultivars are 'Kawano Wase', 'Chahara', 'Zorica Rana', 'Owari', 'Kuno', 'Saigon' and 'Okitsu'. The main lemon cultivars are 'Lisbon', 'Meyer', 'Meyer Improved' and 'Eureka'. The most distributed orange cultivars are 'Washington Navel', 'Skaggs Bonanza Navel' and 'Tarocco'. Many other cultivars of these and other citrus species are represented in Croatia, but usually only in small collections or in home gardens.

Italy and Greece are the closest countries with sizable citrus industries. Due to the more favourable citrus growing conditions they have a more versatile assortment of cultivars. In view of this, the restrictive agroecological niche of the Croatian coast was cleverly utilized in promoting the cultivation of Satsuma mandarins. They had the best chance of being marketed due to the prevalence of other fruit types in the closer Mediterranean markets. Local producers are oriented mostly towards Satsuma varieties that have early and midseason ripening periods, but the cultivation of extra-early ripening (September) varieties is preferred due to their higher market value. Such an example is 'Zorica Rana', a high quality extra-early Croatian selection of Satsuma mandarin (Fig. 3). It is a locally obtained bud mutant from a Japanese early cultivar 'Kawano Wase' selected by Ž. Gatin and collaborators (Škorić et al. 2002, Ozimec et al. 2015). 'Zorica Rana' quality is reflected in its excellent organoleptic characteristics that distinguishes it among other cultivars of approximately the same maturing period. Numerous studies (Gatin 1978, 1992, Gatin et al. 1983) provide information on the pomological characteristics of citrus cultivars, their yield and profitability. Also, comparative research into pomological features by Rošin (2004) resulted in some practical advice regarding the growing of extra-early Satsumas. In general, all Satsuma cultivars are recognized by the domestic market and their production completely covers its requirements. Moreover, the Satsuma is successfully exported to neighbouring countries, where it faces almost no competition.

Fig. 3. A fruit bearing tree of local extra-early ripening Satsuma mandarin (*Citrus unshiu* Marc.) 'Zorica Rana' in contrast to a quick declining tree (circled).

Citrus diseases

Fruit quality is directly related to climate constraints and management practices, but also to the phytosanitary status of the plants. A number of bacterial, fungal, nematode, viral and viroid pathogens, as well as pests, affect the growth and yield of citruses all over the world (Roistacher 1991). The detection and characterization of predominantly viral and viroid agents that potentially have the most devastating effects have been conducted in Croatia. The most prevalent and most destructive citrus viral pathogen is *Citrus tristeza virus* (CTV) causing tristeza disease. It is transmissible by aphids or grafting and causes three main syndromes: (i) the decline of infected trees, (ii) stunting and leaf yellowing of seedlings and (iii) stem pitting resulting in lower yield and fruit quality (Roistacher 1991).

There are currently eight viroids recorded to infect citruses as sole pathogens or in mixed infections. Viroids are small circular RNA molecules with no protein coding capacity. Those of the *Pospiviroidae* family replicate in the cell nucleus and have no hammerhead activity (Hadidi et al. 2017). Mixed infections with two or three viroid species are rather common. The citrus is a vegetatively propagated long living fruit tree. As viroids are easily transmitted by vegetative propagation or mechanically, repeated introductions are usual. *Citrus exocortis viroid* (CEVd) is the longest known citrus viroid. It causes the exocortis disease of citron, lemon, orange and lime scions grafted on trifoliate or trifoliate hybrid rootstocks resulting in bark shelling of the rootstock, tree stunting and deformed fruits (Duran-Vila and Semancik 2003). Another important citrus pathogen is *Hop stunt viroid* (HSVd) previously termed *Citrus cachexia viroid* for its ability to cause cachexia or, as earlier termed, xyloporosis disease. The disease symptoms are discoloration, gumming and browning of phloem tissues, wood pitting and bark cracking. Mandarins, some mandarin hybrids and kumquats are quite sensitive to it (Duran-Vila and Semancik 2003). There are also non-cachexia variants in this genus of viroids, such as *Citrus viroid IIa* (CVd-IIa), CVdII-b and CVd-IIc. Nevertheless, CVd-IIa may cause milder symptoms like mild bark cracking on trifoliate rootstock, reduced canopy volume while improving yield, whereas CVd-IIb and CVd-IIc can be agents of chlorosis, gumming and bark pegging. Other viroids with potential to cause pathological changes in citrus, either alone or in complexes, are *Citrus dwarfing viroid* (CDVd) previously named *Citrus viroid III*, *Citrus bark cracking viroid* (CBCVd) formerly known as *Citrus viroid IV* (CVd-IV), *Citrus bent leaf viroid* (CBLVd) previously termed *Citrus viroid I* (CVd-I), as well as *Citrus viroids V*, *VI* (formerly CVd-OS) and *VII* (Duran-Vila and Semancik 2003, Hadidi et al. 2017, Chambers et al. 2018).

There is no adequate chemical or biological treatment for viral and subviral pathogens and it is necessary to eradicate infected trees to keep the diseases under control. Unfortunately, their symptom-based identification is not reliable because some of the listed symptoms can be induced by different microbes, frost or nutritional deficiencies. Additionally, the type and intensity of the expressed symptoms depend on molecular characteristics of the pathogen (virus strain, viroid nucleotide variant), but also on the rootstock-scion combination. Different hosts may be more or less susceptible to, or even tolerant to, a certain virus or viroid or a specific variant. In the case of CTV, most commercial citrus species are susceptible when grafted on sour orange. However, when grafted on *P. trifoliata,* they remain symptomless. By contrast, citrus trees are particularly sensitive to CEVd when grafted on *P. trifoliata* but express no symptoms when sour orange is used as a rootstock. As most propagation in Croatia had been done on *P. trifoliata* as a rootstock, the bark cracking symptoms on the rootstock, indicative of viroid infection, appeared often on the lemon (Fig. 4a). Thus, it became obvious from biological data that the trees introduced before the establishment of the citrus foundation block were contaminated by CEVd and possibly other citrus viroids. This was later confirmed by molecular analyses (Škorić 2000).

Italian authors Davino and Catara (1986) first reported the presence of CTV in ex-Yugoslavia, though CTV probably had been introduced in the country with Satsuma mandarins imported from Japan in the early 1930s. The infection remained symptomless and initially went undetected probably because over 90% of Satsuma trees were grafted on *P. trifoliata*. Subsequently, the infected material was introduced into Croatian nurseries, and most of the Satsuma trees originating from them (Šarić and Dulić 1990) sustaining further CTV dissemination.

Recent research

Research conducted in the last 25 years focused mainly on the detection and epidemiology of CTV and citrus viroids, their biological and molecular characterisation, as well as the sanitation of the Croatian extra-early selection 'Zorica Rana'. The selected citrus samples from the coast and islands were at first tested for the presence of viroids. Besides biological tests on citron 'Arizona' 861-S1, a citrus biological indicator very sensitive to viroids, other herbaceous indicators were used (Roistacher 1991, Duran-Vila and Semancik 2003). Viroid complexes were purified from the total plant RNA and analysed by sequential polyacrylamide gel electrophoresis (sPAGE, Semancik 1991). The resulting bands were molecularly characterized (Škorić 2000). All tested lemon samples harboured CEVd in complex with CVd-II and CDVd (CVd-III) variants. One of the mandarin samples contained CVd-IIb and CDVd variants, whereas one orange sample was infected with a CVd-IIa variant as a sole viroid. Apart from the highly pathogenic variants of CEVd in the tested Croatian citruses, the other detected citrus viroid variants had low pathogenic potential. Only one tested citrus sample (one out of three mandarin varieties tested) at the time was found to be free of viroids (Škorić 2000, Škorić at al. 2000, 2001).

The pilot CTV molecular characterisation study resulted in finding severe stem pitting (SP) strains in more than 25% of tested field samples (Černi et al. 2005). Stem pitting strains were confirmed in two orange cultivars ('Fukumoto

Navel' and 'Washington Navel'), Satsuma mandarin 'Ichimaru' and potential rootstock *Citrus wilsonii* Tanaka. They displayed high molecular similarity (more than 98%) to severe SP strains from Japan, California and India. Since it was believed that severe SP strains did not naturally exist in the Mediterranean region, this finding was quite surprising. However, it confirmed the hypothesis of CTV introduction with infected plant material and its continuous spreading within the country. No evidence of epidemiologically important vector transmission has been demonstrated in Croatia, hence, it seems that the vegetative propagation represents the main route of CTV transmission in this area.

Further CTV characterisation encompassed samples not only from Croatia, but also from other East Adriatic citrus growing countries, Montenegro and Albania. It confirmed the presence of SP strains, but also revealed the presence of other severe strains causing quick decline and seedling yellows syndromes. Precisely, CTV variants from six out of seven defined phylogenetic groups were detected. This points to the East Adriatic as a reservoir region of severe CTV strains in the Mediterranean basin (Černi et al. 2009). The pathogenic potential of detected CTV variants was simultaneously corroborated biologically (Fig. 4b, c) by graft inoculation on indicator sweet orange plants. Around 50% of positive samples were infected with one of the severe CTV strains, with most of them classified as SP inducing strains. These strains are probably the most threatening in our agroecological niche because, unlike other CTV induced symptoms, stem pitting may appear on trees grafted on *P. trifoliata* rootstock (Niblett et al. 2000).

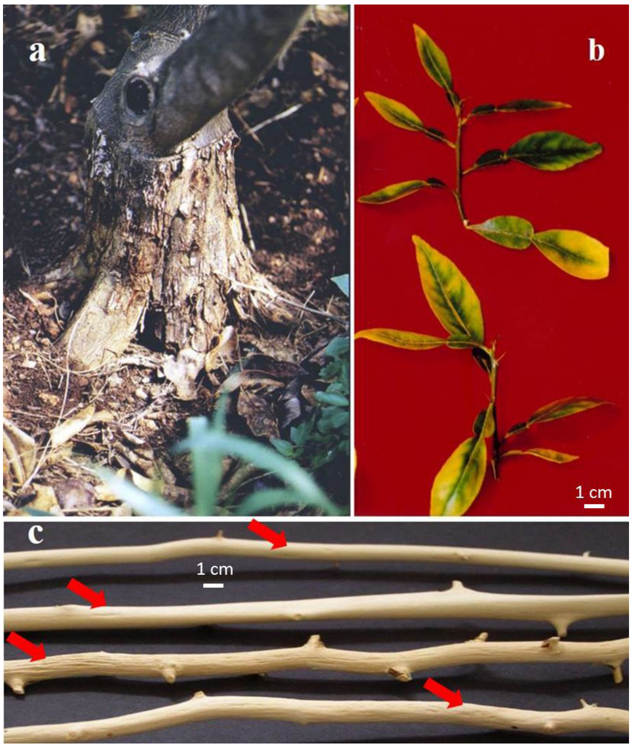

Fig. 4. Viroid and virus induced symptoms on citrus: a – bark shelling caused by *Citrus exocortis viroid* on *Poncirus trifoliata* (L.) Raf. rootstock of lemon (*Citrus limon* L.) on the island of Brač, Dalmatia, b – leaf chloroses induced by *Citrus tristeza virus* (CTV) on experimental *Citrus wilsonii* Tanaka, c – CTV induced stem pitting symptoms on citrus indicators. The number of pits (red arrows) increases with the virulence of stem pitting inducing CTV strain.

Along with the observation of high CTV infection rate (over 60%) in Croatia, a quite heterogeneous within-isolate genetic population structure was observed in almost all samples. Moreover, coinfections with different CTV strains and recombination events between them were not exceptions (Černi 2009, Černi et al. 2009). It is known that stochastic evolutionary events during the virus transmission may change the virus population structure and consequently influence the virulence of the isolate (Li and Roossinck 2004, Ali et al. 2006). This often results in altered symptom expression, sometimes leading toward more severe variants with potentially serious economic implications. In Croatia, this phenomenon has been observed in *C. wilsonii* seedlings that were examined as potentially new rootstock adaptable to cold climate and calcareous soils. After its grafting with buds from the symptomless 'Fukumoto Navel' orange, different *C. wilsonii* seedlings developed different types of symptoms. They varied from mild to severe. The coinfection with three different CTV strains was confirmed in the source orange tree. The analysis of virus population structure therein confirmed the genetic shift induced by the graft transmission bottlenecks. This unintended CTV evolution experiment (effects had been observed fortuitously during grafting compatibility experiment) clearly demonstrated that CTV symptom expression depends on the virus within-isolate genetic structure and that severe symptoms, like stem pitting and seedling yellows, may be linked to sequence variants whose population is low (Černi et al. 2008). As minor virus variants are usually overlooked by the routine screenings, shifts toward more severe isolates probably happen during plant propagation in infected nursery trees.

To better understand the influence of certain CTV variants on disease development and correlate molecular and biological data, genetically pure isolates, the representatives of different phylogenetic groups, were biologically characterised on a panel of plants including both standard indicators and locally interesting Satsuma cultivars grafted on *P. trifoliata* rootstock. After inoculation, syndromes of quick decline, seedling yellows and stem pitting varied in severity, as observed in different indicator hosts. Fortunately, their most severe forms were absent in Satsuma plants (Hančević et al. 2013a). This could also contribute to the sustainability of Satsuma production, as orchard losses associated with CTV infection have not been reported, in spite of the high CTV infection rate and the presence of all CTV strain combinations within field plants. The same cannot be established for *C. wilsonii* whose biological response to the same CTV pure isolates was evaluated. In these experiments *C. wilsonii* was revealed as a potential indicator for the SP type of CTV-strains but not as a suitable rootstock in spite of its great generic rootstock qualities (Hančević et al. 2013b). Different genetic CTV variants showed different symptoms along with different potential to cause early systemic infection in host plants, thus giving us an opportunity to detect more dangerous CTV-isolates in the field faster and easier (Hančević et al. 2016) using the equally reliable ELISA (Enzyme Linked Immunosorbent Assay) and DTBIA (Direct Tissue Blot Immunoassay) tests for diagnostic purposes (Hančević et al. 2012).

Deeper insight into the processes in plants associated with CTV infections were further studied in Mexican lime plants (*Citrus aurantifolia* (L.) Swing.), the main biological indicator of tristeza disease and therefore the best model for screening plant changes. From the physiological and biochemical point of view, the changes in 10-year-old Mexican lime plants infected with CTV were related with stress enzyme level, macro and micro nutrient status and some slight physiological changes. Fortunately, observed changes were of a smaller scale than expected letting us believe that plant defence mechanism could have been suppressed or adjusted due to the long-term coexistence of CTV and the plant (Hančević et al. 2018). With such experiments, the protocols for successful biological and laboratory characterization of CTV isolates of different pathogenicity were adopted and knowledge gained about the triggered defence mechanisms and the practical consequences for citrus production.

Notwithstanding this, the production of virus-free plants is necessary for healthy and fruitful citriculture and for that purpose *in vitro* techniques represent the most successful approach for virus eradication. *In vitro* based sanitation methods, including micrografting combined with thermotherapy, have been successfully introduced for efficient CTV eradication in 'Zorica Rana' (Hančević et al. 2009). This was a prerequisite for beginning a complex process of producing certified planting material (Hartl-Musinov et al. 2006). Although this prerequisite was fulfilled, we still have no complete scheme for producing certified Satsuma plant material in Croatia. It is not only the acreage of Satsuma but also that of 'Zorica Rana' that has increased. This may be interpreted as citriculture in Croatia gaining a new momentum, but the new era should employ sanitized citrus germplasm and modern citriculture practices in order to remain profitable and sustainable.

Conclusions

Citrus cultivation in Croatia is dominated by the most cold-tolerant citrus, the Satsuma mandarin (Anonymous 2017). Despite the restrictive agroecological conditions, the comparative advantages of the Satsuma has been well exploited focusing on earlier ripening varieties. 'Zorica Rana', a local selection of an extra-early Satsuma 'Kawano Wase' (Škorić et al. 2002, Ozimec et al. 2015) is an example of a fruit tree well adapted to the local conditions and highly coveted by growers as a high cash crop. Citrus species, like many other long-living and vegetatively propagated cultivated plants, are riddled with different pests and diseases. Recent high throughput sequencing analyses (Černi, unpubl. data) of *C. wilsonii* sample enabling sequence-unbiased detection of various pathogens confirm this notion. Virus and viroid diseases are particularly important as effective treatments are not available and the best results are achieved with certified pathogen-free material and good practice including disease prevention (Roistacher 1991). Although plant sanitation procedures involving thermotherapy and shoot-tip grafting are available, their success depends on many variables. Those include essentially all aspects of the interactions amongst scion, rootstock and the complex of pathogens infecting citruses and generally determining the form and severity of disease syndromes in plants. In addition, the adaptation of sanitized plantlets to specific growing conditions plays a role in obtaining high quality germplasm (Roistacher 1991, Hančević et al. 2009).

Once planted, even as clean material, plants are exposed the different types of invasions and infections. Many harmful plant viruses and some bacteria are transmitted by insects. A sobering example in citrus cultivation is the emergence of the brown citrus aphid *Toxoptera citricidus* (Kirkaldy 1907) in Portugal and Spain in the new millennium (Ilharco et al. 2005). This is the most efficient aphid vector of CTV, the most dangerous citrus virus, the most harmful strains of which have been recorded in Croatia and further down the East Adriatic coast (Stamo et al. 2000, Černi et al. 2005, Papić et al. 2005, Delić et al. 2013). Molecular investigations of CTV and other virus and virus-like pathogens not cultivable in axenic media are essential for elucidating their pathogenic potential as a first step in the assessment of their impact in the orchards (Hančević et al. 2013a, b, 2016, 2018). The presence of CTV stem pitting strains would have gone undiscovered if not for the molecular analyses (Černi et al. 2005, 2009) as the particularities of citrus growing in Croatia allow for weak or non-existing disease symptoms on *Poncirus trifoliata* rootstocks. Thus, the East Adriatic coast with its citrus trees serves as a reservoir for the most harmful CTV strains threatening the citrus industry in closer Mediterranean countries if the vector *T. citricidus* continues to spread and reaches the Adriatic coasts (Černi et al. 2009). An isolated epidemic in the south of Italy some years ago (Davino et al. 2005) suggests a CTV severe strain outbreak is a plausible scenario, regardless of the transmission route.

Viroids are important citrus pathogens easily transmissible mechanically (Roistacher 1991, Duran-Vila and Semancik 2003). Their occurrence is common in Croatian citruses, including lemons, oranges and Satsuma mandarins. They have been found mostly as complex mixtures of different citrus viroid species with CEVd as almost an indispensable member (Škorić 2000, Škorić et al. 2000, 2001) suggesting their long presence in our groves. The sanitation procedures available for viruses are effective against viroids as well. As in the case of 'Zorica Rana' (Hančević et al. 2009), these procedures should be implemented in the germplasm production of other interesting citrus varieties.

Only by adopting modern methods in the detection of plant pathogens and the production of citrus germplasm along with the modern and sustainable agronomic practices will this new momentum apparently gained in the Croatian citriculture lead to a new and more important citriculture era.

Dedication

This review paper is dedicated to the memory of Dr. Živko Gatin (1923 – 2018), an agronomist, citriculturist, photographer and publicist. He was an erudite scientist who selflessly shared with us his knowledge on citrus history and cultivation. His scientific curiosity and sharp observation skills were indispensable for many aspects of our research in citrus pathology whilst his integrity and scientific rigor made us better scientists and human beings.

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